Science of the Total Environment 550 (2016) 670–675

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Biostimulation of metal-resistant microbial consortium to remove zinc from contaminated environments

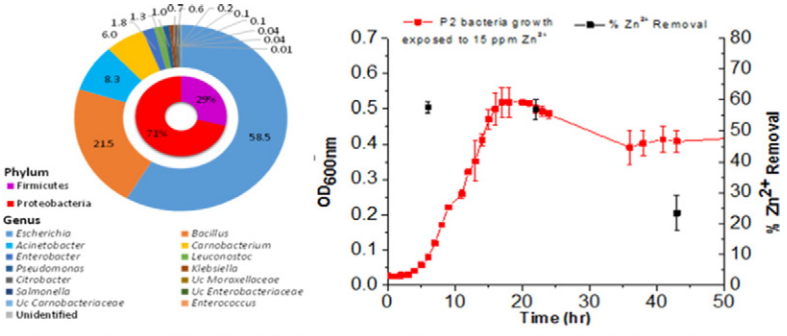
Isis E. Mejias Carpio [a,e,1], Diego Castillo Franco [d], Maria Inês Zanoli Sato [b], Solange Sakata [c], Vivian H. Pellizari [d], Sidney Seckler Ferreira Filho [e], Debora Frigi Rodrigues [a,*]

[a] Department of Civil and Environmental Engineering, University of Houston, Houston, TX, USA
[b] CETESB — Companhia Ambiental do Estado de São Paulo, São Paulo, Brazil
[c] Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP), São Paulo, Brazil
[d] Instituto Oceanográfico. Universidade de São Paulo, São Paulo, Brazil
[e] Departamento de Engenharia Hidráulica e Ambiental, Escola Politécnica, Universidade de São Paulo, Brazil

HIGHLIGHTS

- The consortium biostimulated with glucose presented a diverse bacterial population.
- The consortium represented more than 30% of the species found in the environment.
- The bacterial consortium had high Zn-biomass affinity and Zn removal efficiency.
- Carboxyl, hydroxyl, phosphate and amine groups were responsible for zinc removal.

GRAPHICAL ABSTRACT



Composition of Biostimulated Microbial Consortium

Zinc removal by Biostimulated Microbial Consortium at different growth stages

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 18 January 2016
Accepted 22 January 2016
Available online 2 February 2016

Editor: F.M. Tack

Keywords:
Biosorption
Zinc
Heavy metal
Diversity
Microbial consortium

ABSTRACT

Understanding the diversity and metal removal ability of microorganisms associated to contaminated aquatic environments is essential to develop metal remediation technologies in engineered environments. This study investigates through 16S rRNA deep sequencing the composition of a biostimulated microbial consortium obtained from the polluted Tietê River in São Paulo, Brazil. The bacterial diversity of the biostimulated consortium obtained from the contaminated water and sediment was compared to the original sample. The results of the comparative sequencing analyses showed that the biostimulated consortium and the natural environment had γ-*Proteobacteria*, *Firmicutes*, and uncultured bacteria as the major classes of microorganisms. The consortium optimum zinc removal capacity, evaluated in batch experiments, was achieved at pH = 5 with equilibrium contact time of 120 min, and a higher Zn-biomass affinity (K_F = 1.81) than most pure cultures previously investigated. Analysis of the functional groups found in the consortium demonstrated that amine, carboxyl, hydroxyl, and phosphate groups present in the consortium cells were responsible for zinc uptake.

* Corresponding author.
E-mail address: dfrigirodrigues@uh.edu (D. Frigi Rodrigues).
[1] Isis E. Mejias Carpio: University of Houston Graduate Student.

http://dx.doi.org/10.1016/j.scitotenv.2016.01.149
0048-9697/

1. Introduction

In many bioremediation applications, researchers have investigated diversity and function of environmental microorganisms to improve bioremediation processes (Tiedje et al., 1999; Morris et al., 2002). For instance, studies have correlated biodiversity and operational parameters for biological treatment in wastewater treatment systems (Saikaly et al., 2005; Terahara et al., 2004). Little is known, however, about the impact of biodiversity in heavy metal biosorption reactors with complex environmental microbial communities (Wang and Chen, 2009). Thus, for *in situ* bioremediation it is essential to investigate the role of heavy metal resistant bacterial communities and to identify means to enrich them for metal removal.

Biostimulation of native microorganisms from water and sediment is a realistic approach to grow new biosorbents for heavy metal remediation, but no study so far, has investigated the effects of biostimulation in the diversity of bacteria from aquatic environments involved in heavy metal sorption. Previous studies, with pure cultures in laboratory settings, have found biosorbents, including yeasts, algae, and bacteria, as sustainable options to reduce the heavy metal concentrations in water (Aksu et al., 1998; Moon and Peacock, 2011; Fan et al., 2014). In laboratory settings, especially with pure cultures, the metal removal process may not truly reflect the removal capability under real environmental conditions. Hence, it is important to investigate heavy metal removal under real or simulated environmental conditions with complex microbial communities.

In this study, zinc biosorption removal was investigated, due to the potential toxicity of this metal to humans. The zinc toxicity to humans, for example, is a growing concern because it may impair essential functions of neutrophils and lymphocytes (Fosmire, 1990a). Acute toxicity has been reported in pharmacological dosages of 100–300 mg Zn/day, and in supplements with dosages ranging from 15 to 100 mg Zn/day (Fosmire, 1990b). Thus, for a recommended daily water intake of 2 L, even a 15 ppm concentration of zinc can become toxic.

This work's overarching goal is to investigate the diversity and zinc biosorption behavior of a glucose biostimulated microbial community from a dam in Brazil that receives heavy metal-contaminated effluents. More specifically, this study aims to: i) obtain a metal resistant microbial consortium from the environment by biostimulation; ii) identify the members of the consortium through deep sequencing; iii) compare the consortium diversity obtained *via* biostimulation with the original environmental sample, through 16S rRNA deep sequencing; iv) determine the consortium's optimum growth conditions for Zn^{2+} biosorption; and v) understand the mechanisms of Zn^{2+} biosorption carried out by the consortium.

2. Experimental

2.1. Water and sediment sample collection and characterization

Water and sediment samples were obtained from 'Barragem Pirapora de Bom Jesus' (23°23′31.81″S and 46°59′47.67″W), a dam in the city of São Paulo, Brazil, which acts as one of the reservoirs for the Tietê River. This site is a source of drainage of effluents containing dissolved metals in concentration levels far above the levels permitted by law for discharge. Details of the sample collection are in the supporting information. The Standard Methods and the USEPA Method 3010A protocols were used for the water analysis (USEPA, 1992a; APHA, 2005). For the sediment sample, the USEPA method 3050b was used, which involved digesting the sediment for metal concentration analyses (USEPA, 1996). The metal ions present in the digested samples were identified with an inductively coupled plasma atomic emission spectrometry (ICP-OES Optima 7000DV, PerkinElmer). The P medium (the microbial growth medium) was composed of sediment extract and contaminated water from the dam (Mejias Carpio et al., 2014). Table 1 contains the compositions of the water, the sediment, and the P medium.

2.2. Environmental sample and consortium DNA extraction and analysis

The DNA of the environmental sample (dam) and the biostimulated Pirapora consortium were extracted to identify their population diversity. Genomic DNA from the consortium was obtained from cells grown overnight in a 50 mL volume of P medium at 30 °C. The P medium preparation and the consortium growth conditions are presented in our previously published studies (Mejias Carpio et al., 2014; Rodrigues and Tiedje, 2007). The grown consortium was centrifuged for 30 min at 4000 rpm to obtain a pellet. The pellet was collected and resuspended in a volume of 10 mL of P medium. The bacterial suspension was centrifuged again to collect the pellets. The DNA was subsequently extracted from the pellet with the PureLink® Genomic DNA Mini Kit (Life Technologies). An amount of 500 mg of wet sediment was used to extract the total DNA from the dam. The extraction happened with the PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) (Rodrigues and Tiedje, 2007; Rodrigues et al., 2013). The total DNA concentrations for the consortium and the dam sample were quantified with the NanoDrop ND-1000 (Thermo Scientific, USA). Prior to sequencing, the total DNA extracted were amplified for the 16S genes with 518R and 27F primers. The eubacterial 16S rRNA sequencing was done with a next generation sequencing (NGS) platform, Roche 454 pyrosequencing technology, at ChunLab (Seoul, Korea) (Jeon et al., 2013). Raw sequence files were analyzed using the CLcommunity™ software, version 3.31, and were considered for taxonomy identification. The accession numbers of the sequences deposited in NCBI are SRX672321 and SRX699663.

2.3. Minimum inhibitory concentration and optimum growth conditions for zinc removal

The consortium's minimum inhibitory concentration (MIC) to zinc was investigated by growing the consortium with different zinc concentrations (2.8, 8.8, 20, 26, 48, 71, and 93 ppm) (Mejias Carpio et al., 2014).

Table 1
Physicochemical parameters and metal concentrations of samples collected at the Pirapora Reservoir of the Tietê River.

	Water	Sediment	P medium
Water depth (m)	1	1	–
pH	7.2	6.8	5
Cond (μS/cm)	513	–	–
OD (ppm)	0.86	–	–
T-air (°C)	22	–	–
T-water (°C)	19	–	–
Color	Gray	Black	Amber
Redox	−306	−306	–
Total phosphorous (mg/L for liquids, mg/kg for sediment)	2.39 ± 0.04	1253	6.31 ± 0.06
Inorganic (soluble) phosphorous (mg/L)	0.17 ± 0.04	N/A	3.46 ± 0.12
Total Kjeldahl nitrogen (TKN) (mg/L for liquids, mg/kg for sediment)	3.64 ± 1.20	2248	1.91 ± 0.80
Total organic carbon (TOC) (mg/L for liquids, wt.% for sediment)	11.3 ± 0.5	1.71	428.2 ± 40.3
Metal concentrations	Water	Sediment	P medium
	ppm	ppm	ppm
Al^{3+}	0.92	10,203	ND
Fe^{2+}	1.97	93,238	1.1
Cu^{2+}	0.02	91	ND
Co^{2+}	ND	5	0.3
Cd^{2+}	<0.005	0.93	ND
Zn^{2+}	0.16	328	2.8
Cr^{3+}	<0.02	69	ND
Mn^{2+}	0.21	255	0.4
Pb^{2+}	0.003	44	4.6

ND = not detected.

The plate count method was done in triplicate to determine the inhibitory activity of zinc. The MIC was selected as the zinc concentration at which no cells of the bacterial community grew on the agar plates. From the results of the triplicates, average and standard deviations were calculated.

The best growth phase for zinc removal was determined by exposing the cells in mid-logarithmic, early stationary, and late stationary phases to zinc. Aliquots of 2.5 mL of the consortium culture grown overnight in 'P medium' without metals were transferred to the growth medium containing 22.5 mL at pH = 5. The consortium was then incubated for 48 h at 25 °C and 125 rpm. A volume of 10 mL was then taken in the exponential phase (t = 7 h), early stationary phase (t = 24 h), and late stationary phase (t = 43 h) and exposed to a concentration of 6 ppm of zinc for 120 min. The cells were then removed by centrifugation for 30 min at 4000 rpm for 30 min and the supernatants were analyzed for their zinc concentrations. This experiment was repeated thrice. For the analysis of the Zn^{2+} ions in the supernatant, the supernatant was acid digested on a hot plate and analyzed on an ICP-OES as described by the USEPA Method 3010 A (USEPA, 1992b). Zinc standard curves were prepared in seven different concentrations varying from 1 ppm to 100 ppm.

2.4. Kinetics of Zn^{2+} uptake and isotherm

The evaluation of the zinc uptake kinetic process was determined by calculating the uptake rates with Eq. (S1), from the data found in Table S4:

$$q_t = \frac{(C_t - C_0)V}{1000m}. \quad (S1)$$

In this formula, C_t and C_0 are the final and initial zinc concentrations in mg/L, V is the volume in mL, and m is the bacterial mass in grams.

The isotherm was investigated using the Freundlich isotherm model expressed as:

$$q = K_F C_e^{1/n}. \quad (S2)$$

In this formula, q is the zinc sorption uptake at equilibrium (mg metal removed/g dry cells), C_e is the equilibrium zinc concentration in the liquid phase, K_F is the Freundlich sorption equilibrium constant, which represents the sorption capacity, and n corresponds to the Freundlich constant, which relates to biosorption intensity (Wang and Chen, 2009).

2.5. Determination of 'Pirapora' microbial consortium zinc binding functional groups

The zinc ion binding sites in the biomass was determined using ATR-FTIR. In this investigation, the biomass was exposed to 26 ppm of zinc, then freeze-dried for the ATR-FTIR on a Bomem MB100 containing a DTGS detector. The analyses occurred at 25 °C with a spectrum resolution of 4 cm^{-1} (Mejias Carpio et al., 2014).

3. Results and discussion

3.1. Species compositions of dam microbial community and biostimulated consortium

The dam microbial community was grown in the P medium to simulate environmental growth conditions, since this medium was made with dam water and sediment extract. Glucose was also added to the medium for biostimulation of the native microbial community present in the dam.

The biostimulation allowed growth of a diverse microbial community. The results indicate that the P medium enriched about 30% of the operational taxonomic units (OTUs) identified in the dam (Fig. 1 and Table S1). In the dam sediment and the consortium, the most predominant genus from the *Moraxellaceae* family was *Acinetobacter*. This genus represented 8.3% and 69.7% of the total OTU abundance in the consortium and dam sediment, respectively (Fig. 1). *Acinetobacter* was the only genus with the highest abundance in the dam sediment than in the consortium.

Rarefaction curves (Fig. S2) were obtained to evaluate whether the sequencing was enough to identify all or almost all OTUs in the samples (Heck et al., 1975). In the consortium, the number of OTUs obtained through the sequencing was sufficient to identify all OTUs, since the curve reached an asymptote (Fig. S2). The environmental sample, on the other hand, did not reach an asymptote. This result indicates that not all OTUs were sequenced in the environmental sample. This observation was corroborated by a number of species present in the consortium from the family Enterobacteriaceae, such as, *Escherichia*, *Salmonella*, *Enterobacter*, *Pseudomonas*, *Citrobacter*, and *Klebsiella*, which were not identified in the sequences from the dam (Fig. 1). It is likely that this difference in the composition was caused by the medium composition. Although the P medium contained heavy metals, its zinc concentration was lower than the sediment's but higher than the original water (Table 1). The zinc ions in the medium was kept low to obtain the most diverse microbial consortium with a large range of tolerance for zinc. Because of that, we believe the medium selected and enriched some specific populations from the dam.

The species belonging to the family Enterobacteriaceae were found abundantly in the consortium enrichment. These species are all enteric bacteria and they have been extensively associated with sewage from diverse effluents (Rodrigues et al., 2009). The Barragem Pirapora de Bom Jesus receives waters from the Tiete river, which is polluted with domestic sewage and industrial effluents (Abraham et al., 2007). Thus, the presence of these microorganisms in the consortium, but absence in the dam sequencing data, and the non-asymptotic rarefaction curve of the dam, indicate that the consortium represented more OTUs than we were able to identify (we identified about 30%). It is worth to note that the majority of the genera identified in the consortium have been previously described to either resist or to remove metal contaminations.

For instance, *Bacillus* spp. were abundant in the consortium and is known to sequester heavy metals through extracellular polymeric substance (EPS) production, which contains negatively charged proteins, humic acids, uronic acids, and polysaccharides that can complex with metal ions (Rodrigues and Tiedje, 2007). The second most abundant microorganism detected in the consortium sequencing, and also in the sediment, was *Acinetobacter*. Several species within this Gram-negative genus are known to contain a plasmid that encodes the volatilization of metals (USEPA, 1996). Another Gram-negative genus abundantly identified in the consortium was *Pseudomonas*. This genus is ubiquitously present in metal contaminated sites (Rodrigues et al., 2013), as well as in pristine tropical glaciers (Jeon et al., 2013). Various *Pseudomonas* species, such as *Pseudomonas putida*, have been extensively investigated for metal resistance (USEPA, 1992b; Heck et al., 1975; Rodrigues et al., 2009). *P. putida* has at least four Zn/Cd/Pb efflux transporters to expel metal cations out of the cell (Mejias Carpio et al., 2014). Overall, the genetic composition of the community was 71% Gram-negative bacteria, which is also supported by the FTIR results (Fig. 4). The primary sites for the first metal interaction in the Gram-negative cell wall are the carboxyl and hydroxyl groups (Abraham et al., 2007; Vullo et al., 2008), and were also attributed as the primary metal binding sites in the FTIR results of this study.

Although it is well known that certain species, such as *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., *Acinetobacter* sp., and *Enterobacter* sp. can effectively remove metals in pure cultures, it is still unclear how the biosorption process by these individual species are affected in a consortium. Furthermore, no studies to date used a biostimulated consortium with this diverse composition to estimate metal-biomass affinity or metal removal properties.

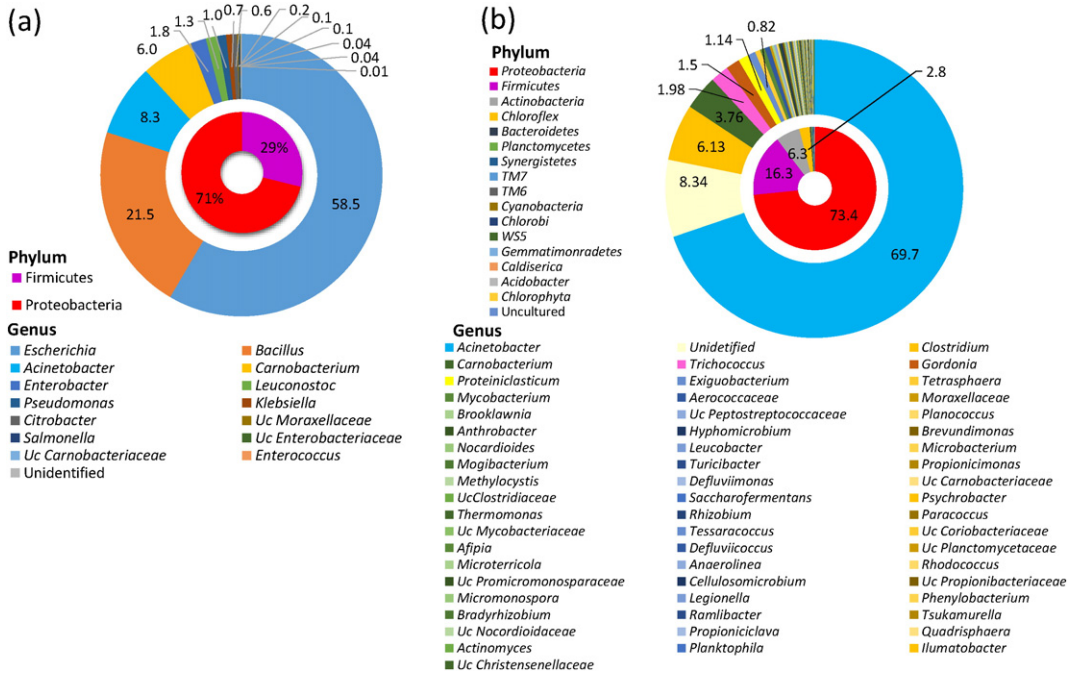


Fig. 1. Composition of (a) consortium and (b) dam populations, expressed in phyla and genera. (Uc = uncultured genera).

3.2. Consortium minimum inhibitory concentration in zinc-rich conditions

After obtaining a biostimulated consortium and determining its diversity, we investigated its zinc tolerance to increasing concentrations of zinc. The MIC result was 48 ppm (Fig. 2 and Fig. S3). The cells in contact to 71 ppm had a growth reduction of about one log when comparing with the control. However, no growth occurred on agar without zinc after exposing the consortium to 93 ppm zinc (Fig. S3).

Other investigations have obtained zinc MIC values of 654 ppm for *Pseudomonas veronii* (Vullo et al., 2008) and *Pseudomonas gladioli* (Piotrowska-Seget et al., 2005), and 523 ppm for *Pseudomonas aeruginosa* (Teitzel and Parsek, 2003). Although zinc MIC values for these microorganisms exceeded the MIC of the present study, these isolates were grown in rich media containing components that can complex with zinc ions. This metal complexation would reduce the toxicity of zinc ions, which would yield higher MICs. Conversely, this consortium was grown in a minimum medium (the P medium) that aimed to simulate some of the water chemistry conditions of the original site, thus, the comparison of the MIC of these pure cultures with the consortium is not really representative, future studies with other consortia under minimum conditions will be necessary for a better comparison.

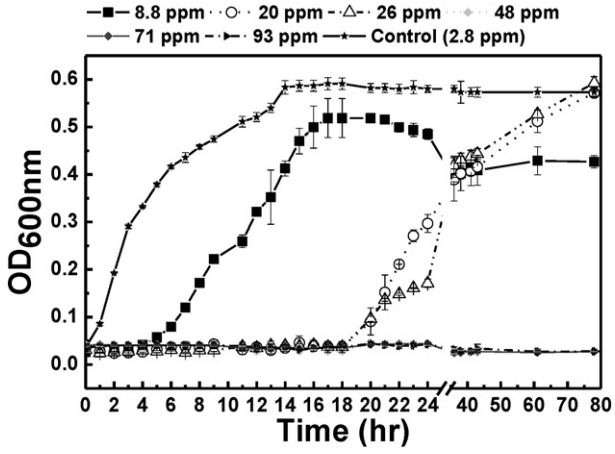


Fig. 2. Microbial consortium growth curves during exposure to $(CH_3COO)_2$ Zn-$2H_2O$ at different concentrations (2.8, 8.8, 20, 26, 48, 71, and 93 ppm) in P Medium.

3.3. Consortium zinc removal capability and mechanisms of adsorption

To confirm that this microbial consortium could remove zinc from the water, we investigated the zinc removal with cells at different growth phases (Fig. 3). The zinc removal mechanisms were further analyzed through FT-IR with the consortium cells grown in the native medium without zinc supplement (control) and in the medium supplemented with 26 ppm of zinc (Fig. 4). The FT-IR analysis allowed us to determine the consortium functional groups binding to the zinc ions.

The rationale to investigate biosorption mechanisms with cells in diverse growth phases was recognized by earlier studies with various microbial species. These investigations reveled that optimum heavy metal sorption can occur in different bacterial growth stages or as dead biomass (Mohamad et al., 2012; Oh et al., 2009). An important aspect of biosorption, then, is to optimize the growth conditions of the microbes to achieve the highest metal removal. For zinc, the biosorption studies are very conflicting and depend largely on the type of cell used (Chen et al., 2005).

In Fig. 3, the consortium presented significant differences in zinc removal at the growth stages investigated. Greater than 50% zinc removal was observed at both mid-log and early stationary phases, but 25% in the death phase. The diverse microbial composition of the consortium could explain this difference in removal. Some species in this consortium could be removing zinc better in the exponential phase, while others in the stationary phase. Potentially, during these two growth phases the removal occurs with metabolically-active and -inactive cells. But, a smaller number of species could be removing zinc in the death phase through adsorption to cellular components, which does not require the cellular metabolism. These differences in zinc removal at the different growth stages are not observed with pure cultures, which typically have just one optimum growth phase for metal removal

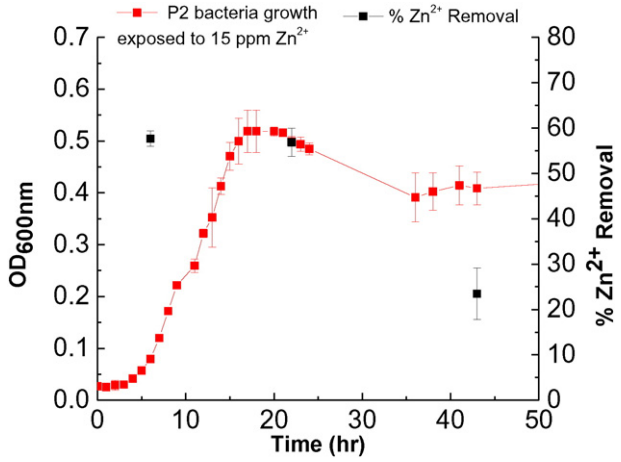


Fig. 3. Sorption of 6 ppm Zn^{2+} by 'Pirapora' consortium at exponential (7 h), early stationary (24 h), and late stationary (43 h) phases. The left axis (curve in red) shows the growth curve with zinc in the medium at OD_{600}. The right axis (black data points) shows the percent zinc removal during each growth phase.

(either exponential or stationary). This finding is essential for bioremediation of zinc contaminated effluents that could constantly face changing environmental conditions that affect microbial growth.

The mechanisms of zinc adsorption to the consortium were probed with FT-IR. By comparing the control consortium with the consortium exposed to zinc, the functional groups involved in binding zinc were identified. In Fig. 4, the control presented a peak at around 1070 cm^{-1}, which matches the C-O stretching vibration of carboxyl groups. This band shifted in the consortium that had contact with zinc, and a new band appeared at 1117 cm^{-1} (gray), which matches the C-O stretching vibration of the carboxyl group bound to Zn^{2+} ions. In the control, we also observed peaks at 1400 cm^{-1} and 3414 cm^{-1}, which depict a tertiary alcohol and a polymeric OH stretch, respectively (Coates, 2000; Schmitt and Flemming, 1998). The peak at 669 cm^{-1} may also be assigned to an OH vibration (Coates, 2000). While the peak at 3414 cm^{-1} and 1400 cm^{-1} did not significantly change with zinc ions. The peak at 669 cm^{-1} moved to 636 cm^{-1}, indicating partial complexation of Zn^{2+} to OH groups. Three new peaks were noticed after the zinc sorption at 947 cm^{-1}, 1007 cm^{-1}, and 1024 cm^{-1}. The peak at 947 cm^{-1} was assigned to phosphate groups, while the peaks at 1007 cm^{-1} and 1024 cm^{-1} may correspond to primary amine vibrations. In the control, the secondary and amine groups were identified at

Table 2
Pseudo-second order kinetics of Zn^{2+} sorption by the consortium.

Metal	Intercept	Slope	v_o mg g^{-1} min^{-1}	q_e mg g^{-1}	k g mg^{-1} min^{-1}	R^2
Zn^{2+}	0.16	0.014	6.2	69.4	0.0013	0.95

1655 cm^{-1} and 1238 cm^{-1}, respectively, and minor changes were observed in these peaks after the zinc sorption. These minor changes suggest a weak binding of the N–H and C–N groups with zinc. These results demonstrated that the main functional groups involved in zinc sorption are carboxyl and hydroxyl, although amine and phosphate groups seem to also bind to zinc to a lesser extent. These functional groups are present in the bacterial cell walls and membranes as part of the proteins, lipoproteins, peptidoglycan, and teichoic acids. Furthermore, these functional groups provide a negative charge to the bacteria, which facilitates interaction with zinc ions that are positively charged (Wang and Chen, 2009).

3.4. Zinc sorption kinetics and isotherm models

The zinc sorption behavior of the consortium biomass as a function of time allowed us to estimate the equilibrium reaction time of the sorption process. The pseudo-second order model was used to determine the kinetics of zinc by the consortium since it is often used to investigate microbial biosorption kinetics (Iqbal et al., 2009; Calero et al., 2011). Details of the kinetic model used are in the supporting information, and the parameters obtained are found in Table 2. The model depicted a R^2 of 0.95, which suggests that most of the variance can be explained with this model. Zinc equilibrium uptake rate occurred after 120 min (Fig. S8).

The sorption mechanisms of the consortium can be better understood with equilibrium isotherm models. These models define the maximum sorption capacity and the metal-biomass affinity, key parameters to find out the quality of any sorbent (Volesky, 2007). The equilibrium point also establishes a relationship between the zinc contaminant and the consortium biomass surface needed to scale-up metal sorption units for bioremediation. We compared the zinc biosorption experimental data to the Langmuir and Freundlich models (Wang and Chen, 2009). The Langmuir model was not a good fit to our data and therefore it is not presented. The Freundlich equilibrium model infers heterogeneous sorption with different active sites (Bohumil, 2003). The Freundlich model for this consortium had a R^2 of 0.89 (Table 3).

The Freundlich model for this consortium indicated a relatively high Zn-biomass affinity ($K_F = 1.81$) than the dead biomass of *P. putida* for

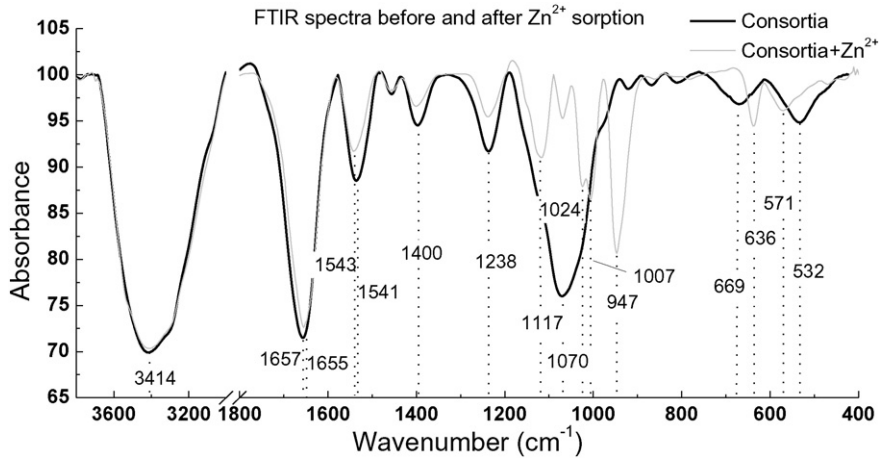


Fig. 4. FT-IR images of the consortium cells with P medium only (control) (black line); and the consortium grown in the P medium enriched with 26 ppm Zn^{2+} for 4 d (gray line).

Table 3
Parameters of the Freundlich equilibrium model for the sorption of Zn^{2+} by the consortium.

Freundlich parameters			
Metal	K_F ($L\ g^{-1}$)	n –	R^2
Zn^{2+}	1.81	0.68	0.89

zinc ions ($K_F = 1.47$) (Chen et al., 2005), and the live biomass of *P. aeruginosa* AT18 for zinc ions ($K_F = 0.002$) (Pérez Silva et al., 2009). The high Zn-biomass affinity was expected since the FT-IR results for zinc depicted strong shifts for the carboxyl and the hydroxyl functional groups (responsible for the zinc binding). These results imply that an environmental microbial consortium can more efficiently adsorb heavy metals than pure cultures. That is, the consortium may hold a greater number or different types of negatively charged functional groups than in pure cultures, which may increase the metal-biomass affinity.

In conclusion, a glucose biostimulated microbial community from the environment allowed the enrichment of a consortium composed of a diverse population of microorganisms. This consortium had more than 30% of the species found in the environment. The results revealed that heavily contaminated environments have bacterial communities with relatively high metal-biomass affinity and metal removal properties. The consortium achieved greater than 50% zinc removal at mid-log and early stationary phases. These traits can allow a microbial community from the environment to serve as a metal biosorbent.

Authors' contributions

Experimental design, metagenomics, MIC, sorption data analyzes, manuscript writing, and proof reading (I.E.M.C. & D.F.R.); DNA extraction from sediment and water samples (I.E.M.C., D.C.F., & V.H.P.); Site selection and collection of sediment and water (I.E.M.C. & M.I.Z.S.); analyses of water, sediment, and P medium (I.E.M.C. & S.S.); Adsorption Freundlich modeling analysis and research support in Brazil (I.E.M.C. & S.S.F.F.).

Acknowledgments

Dr. Isis E. Mejias Carpio is thankful for the scholarships given by the Rotary Foundation (GG #25644) and the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior- CAPES (33002010130PO) to perform part of this research in Brazil. The authors are also thankful for the technical assistance of Dr. Alejandro Caro-Quintero, Dr. Rubens Duarte, Dr. Heloisa Ramos Barbosa, Karina Yoshimoto, Fabio Campos, and the CETESB researchers and technicians for providing the infrastructure for the sample collections.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.01.149.

References

Abraham, W.-R., Macedo, A.J., Gomes, L.H., Tavares, F.C.A., 2007. Occurrence and resistance of pathogenic bacteria along the Tietê River downstream of São Paulo in Brazil. CLEAN – Soil, Air, Water 35, 339–347.
Aksu, Z., Egretli, G., Kutsal, T., 1998. A comparative study of copper(II) biosorption on Ca-alginate, agarose and immobilized *C. vulgaris* in a packed-bed column. Process Biochem. 33, 393–400.
APHA, 2005. Standard Methods. 19th ed. American Public Health Association, Washington, DC.
Bohumil, V., 2003. Biosorption process simulation tools. Hydrometallurgy 71, 179–190.
Calero, M., Blazquez, G., Martin-Lara, M.A., 2011. Kinetic modeling of the biosorption of lead(II) from aqueous solutions by solid waste resulting from the Olive Oil Production. J. Chem. Eng. Data 56, 3053–3060.
Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X., Chen, Y.X., 2005. Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1. Colloids Surf. B: Biointerfaces 46, 101–107.
Coates, J., 2000. Interpretation of infrared spectra, a practical approach. In: Meyers, R.A. (Ed.), Encyclopedia of Analytical Chemistry. John Wiley & Sons Ltd, Chichester, pp. 10815–10837.
Fan, J., Onal, T.O., Rodrigues, D.F., 2014. The synergism of temperature, pH and growth phases on heavy metal biosorption by two environmental isolates. J. Hazard. Mater. 279, 236–243.
Fosmire, G.J., 1990a. Zinc toxicity. Am. J. Clin. Nutr. 51, 225–227.
Fosmire, G.J., 1990b. Zinc toxicity. Am. J. Clin. Nutr. 51, 225–227.
Heck Jr., K.L., Belle, G.V., Simberloff, D., 1975. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology 56, 1459–1461.
Iqbal, M., Saeed, A., Zafar, S.I., 2009. FTIR spectrophotometry, kinetics and adsorption isotherms modeling, ion exchange, and EDX analysis for understanding the mechanism of Cd^{2+} and Pb^{2+} removal by mango peel waste. J. Hazard. Mater. 164, 161–171.
Jeon, Y.-S., Chun, J., Kim, B.-S., 2013. Identification of Household Bacterial Community and Analysis of species shared with human microbiome. Curr. Microbiol. 67, 557–563.
Mejias Carpio, I.E., Machado-Santelli, G., Kazumi Sakata, S., Ferreira Filho, S.S., Rodrigues, D.F., 2014. Copper removal using a heavy-metal resistant microbial consortium in a fixed-bed reactor. Water Res. 62C, 156–166.
Mohamad, O.A., Hatab, S.R., Liu, Z.S., Li, Z.X., Kong, Z.Y., Wei, G.H., 2012. Biosorption and bioaccumulation of Cu^{2+} from aqueous solution using living *M. amorphae* isolated from mine tailings. Mine Water Environ. 31, 312–319.
Moon, E.M., Peacock, C.L., 2011. Adsorption of Cu(II) to *Bacillus subtilis*: a pH-dependent EXAFS and thermodynamic modelling study. Geochim. Cosmochim. Ac. 75, 6705–6719.
Morris, C.E., Bardin, M., Berge, O., Frey-Klett, P., Fromin, N., Girardin, H., Guinebretiere, M.H., Lebaron, P., Thiery, J.M., Troussellier, M., 2002. Microbial biodiversity: approaches to experimental design and hypothesis testing in primary scientific literature from 1975 to 1999. Microbiol. Mol. Biol. Rev. 66, 592–616 (table of contents).
Oh, S.E., Hassan, S.H.A., Joo, J.H., 2009. Biosorption of heavy metals by lyophilized cells of *Pseudomonas stutzeri*. World J. Microbiol. Biotechnol. 25, 1771–1778.
Pérez Silva, R.M., Ábalos Rodríguez, A., Gómez Montes De Oca, J.M., Cantero Moreno, D., 2009. Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum. Bioresour. Technol. 100, 1533–1538.
Piotrowska-Seget, Z., Cycon, M., Kozdroj, J., 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. Appl. Soil Ecol. 28, 237–246.
Rodrigues, D.F., Tiedje, J.M., 2007. Multi-locus real-time PCR for quantitation of bacteria in the environment reveals exiguobacterium to be prevalent in permafrost. FEMS Microbiol. Ecol. 59, 489–499.
Rodrigues, D.F., Sakata, S.K., Comasseto, J.V., Bicego, M.C., Pellizari, V.H., 2009. Diversity of hydrocarbon-degrading klebsiella strains isolated from hydrocarbon-contaminated estuaries. J. Appl. Microbiol. 106, 1304–1314.
Rodrigues, D.F., Jaisi, D.P., Elimelech, M., 2013. Toxicity of functionalized single-walled carbon nanotubes on soil microbial communities: implications for nutrient cycling in soil. Environ. Sci. Technol. 47, 625–633.
Saikaly, P.E., Stroot, P.G., Oerther, D.B., 2005. Use of 16S rRNA gene terminal restriction fragment analysis to assess the impact of solids retention time on the bacterial diversity of activated sludge. Appl. Environ. Microbiol. 71, 5814–5822.
Schmitt, J., Flemming, H.-C., 1998. FTIR-spectroscopy in microbial and material analysis. Int. Biodeterior. Biodegrad. 41, 1–11.
Teitzel, G.M., Parsek, M.R., 2003. Heavy metal resistance of biofilm and planktonic pseudomonas aeruginosa. Appl. Environ. Microbiol. 69, 2313–2320.
Terahara, T., Hoshino, T., Tsuneda, S., Hirata, A., Inamori, Y., 2004. Monitoring the microbial population dynamics at the start-up stage of wastewater treatment reactor by terminal restriction fragment length polymorphism analysis based on 16S rDNA and rRNA gene sequences. J. Biosci. Bioeng. 98, 425–428.
Tiedje, J.M., Asuming-Brempong, S., Nüsslein, K., Marsh, T.L., Flynn, S.J., 1999. Opening the black box of soil microbial diversity. Appl. Soil Ecol. 13, 109–122.
USEPA, Method 3010A: Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by FLAA or ICP Spectroscopy, in, 1992a.
USEPA, Acid digestion of aqueous samples and extracts for total metals for analysis by FLAA or ICP spectroscopy in test methods for evaluating solid waste: physical/chemical methods, in: U.S.E.P. Agency (Ed.) Method 3010A, Washington DC, 1992b.
USEPA, Acid digestion of sediments, sludges, and soils, in: U.S.E.P. Agency (Ed.) Method 3050b, Washington DC, 1996.
Volesky, B., 2007. Biosorption and me. Water Res. 41, 4017–4029.
Vullo, D.L., Ceretti, H.M., Daniel, M.A., Ramírez, S.A.M., Zalts, A., 2008. Cadmium, zinc and copper biosorption mediated by pseudomonas veronii 2E. Bioresour. Technol. 99, 5574–5581.
Wang, J., Chen, C., 2009. Biosorbents for heavy metals removal and their future. Biotechnol. Adv. 27, 195–226.