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Microbial biofilm study by synchrotron X-ray microscopy

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HIGHLIGHTS

- We to study bacterial bioremediation by microXRF.
- Dense biofilm may act sequestering metal while protecting bacterial metabolism.
- *Nitratireductor* spp. and *Pseudomonas* spp decreased seawater metal bioavailability.
- Bacterial consortia from polluted areas may be used in bioremediation programs.

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ABSTRACT

Microbial biofilm has already being used to remove metals and other pollutants from wastewater. In this sense, our proposal was to isolate and cultivate bacteria consortia from mangrove's sediment resistant to Zn (II) and Cu (II) at 50 mg L^{-1} and to observe, through synchrotron X-ray fluorescence microscopy (microXRF), whether the biofilm sequestered the metal. The biofilm area analyzed was 1 mm^2 and a 2D map was generated (pixel size $20 \times 20\ \mu m^2$, counting time 5 s/point). The biofilm formation and retention followed the sequence Zn > Cu. Bacterial consortium zinc resistant formed dense biofilm and retained 63.83% of zinc, while the bacterial consortium copper resistant retained 3.21% of copper, with lower biofilm formation. Dehydrogenase activity of Zn resistant bacterial consortium was not negatively affect by 50 mg ml^{-1} zinc input, whereas copper resistant bacterial consortium showed a significant decrease on dehydrogenase activity (50 mg mL^{-1} of Cu input). In conclusion, biofilm may protect bacterial cells, acting as barrier against metal toxicity. The bacterial consortia Zn resistant, composed by *Nitratireductor* spp. and *Pseudomonas* spp formed dense biofilm and sequestered metal from water, decreasing the metal bioavailability. These bacterial consortia can be used in bioreactors and in bioremediation programs.

1. Introduction

Ecosystems of non-consolidated bottom, as soils and sediments, are targets of anthropogenic stressors. Contaminants enter aquatic ecosystems mainly via wastewater, being most prevalent hydrocarbons, solvents, pesticides and metals. Metals, unlike organic pollutants, are non-biodegradable, decreasing drastically the local biodiversity [1,2]. However, among the heavy metals, generally 90% may be sequestered from the water column by the sediment compartment [3]. Once removed from the water column, metals are considered to be unavailable to aquatic biota. Therefore, the pollutant removing process is an important clue for the proposal of remediation for contaminated areas [4,5].

Metals with high bonding strength tend to be associated with molecules from microbial biofilm, and these reactions may lead to the immobilization of metal by biosorption or precipitation of the metal complex [6]. Furthermore, bacterial consortium can develop mechanisms of tolerance or resistance to metals as well as can develop regulatory processes by biochemical and genetic pathways [7].

Microbial biofilm has already being used to remove metals and other pollutants from wastewater. The use of biofilms in bioreactors has shown to be efficient and less cost [8]. The literature describes the use of bacteria in the bioremediation processes of metals. Bacterial resistance have been found as $Zn^{+} > Cd^{2+} > Cu^{2+}$ at concentrations between 0 and 8.0 mg/L [9]. Furthermore, bacteria consortia were able to remove 53%, 49% and 42% of Zn, Cu and Cd, respectively, at concentrations between 0.005 and 0.1 mg/L [10].

However, the main toxic metals concentrations discharged in the environment, such as nickel, iron, zinc and copper, have being at higher concentration, up to 1000 mg L^{-1}[11]. In this context, the

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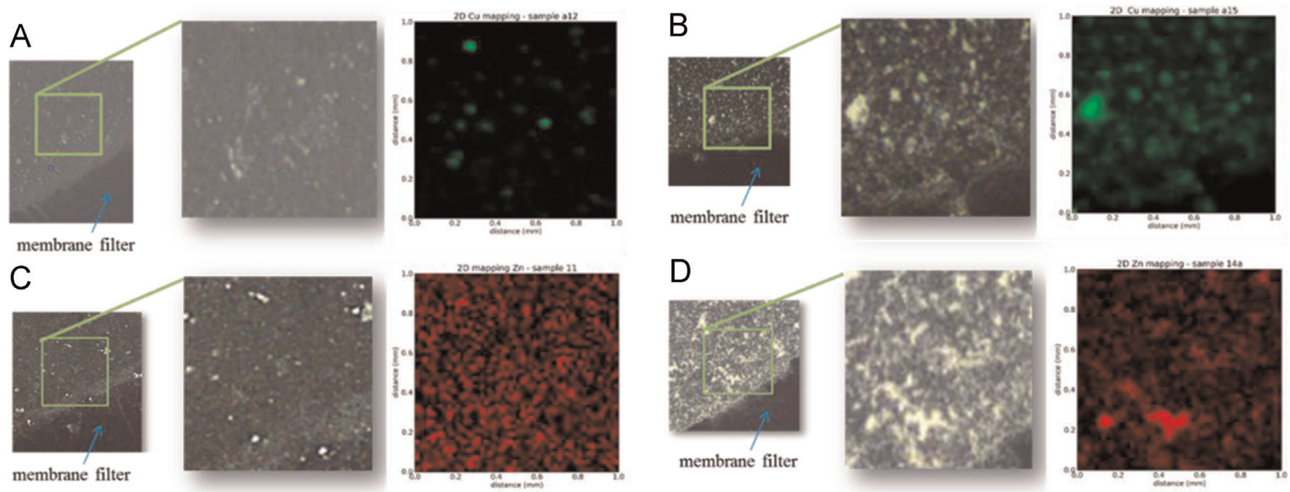

Fig. 1. Maps of synchrotron X-ray fluorescence microscopy (microXRF) of bacterial consortium Cu resistant (A: control; and B: 50 mg L^{-1} of copper sulfate) and bacterial consortium Zn resistant (C: control; and D: 50 mg L^{-1} of zinc sulfate) (area analyzed: 1 mm^2; left two images = optical microscopy; right images = microXRF maps).

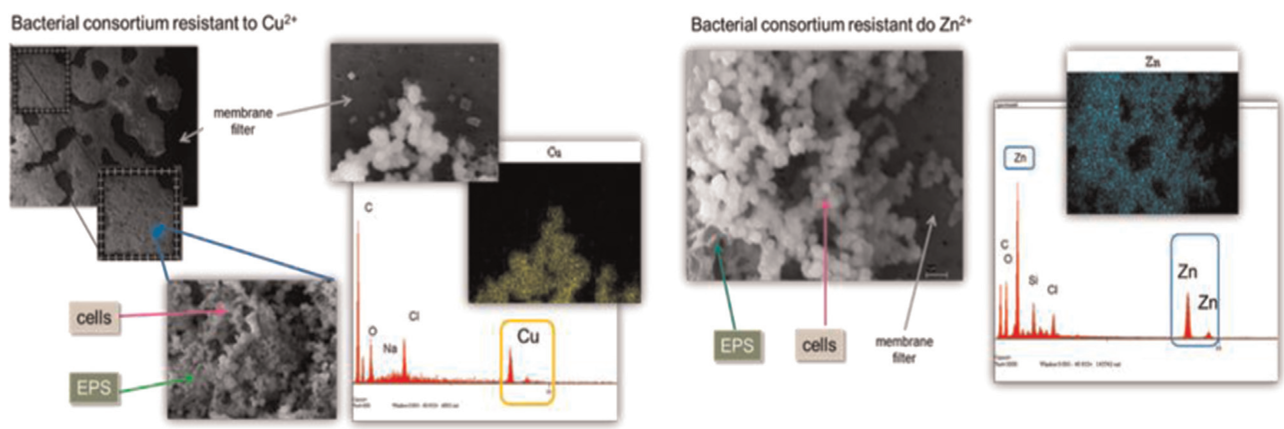

Fig. 2. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) of biofilm formation by bacterial consortia resistant to Cu^{2+} and Zn^{2+} in a salt liquid medium in presence of respective metals at 50 mg L^{-1}. SEM-EDS spectra show the presence of the respective metal in the biofilm.

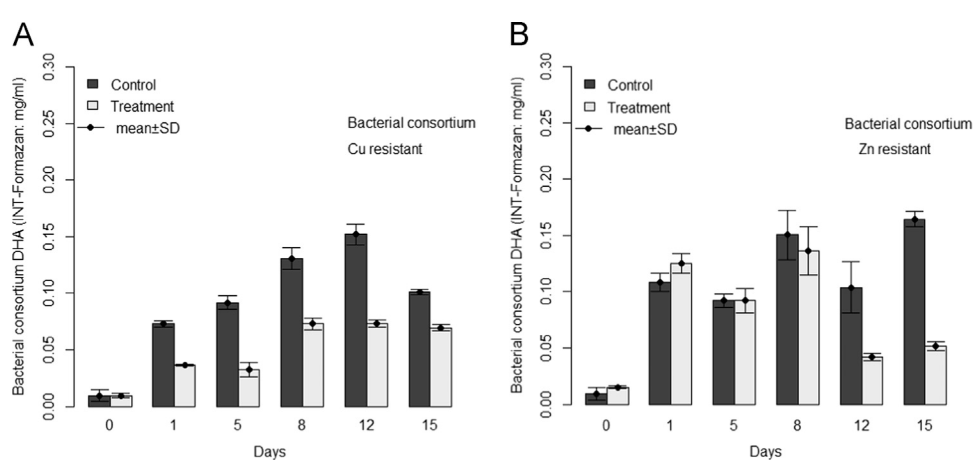

Fig. 3. Bacterial consortia dehydrogenase activity (DHA): (A) DHA of bacterial consortium Cu resistant (treatment = 50 mg L^{-1} of copper sulfate); significant differences among controls and treatments from day 1: $p < 0.001$); (B) DHA of bacterial consortium Zn resistant (treatment = 50 mg L^{-1} of zinc sulfate); non-significant differences among control and treatment from day 1 to day 8: $p > 0.850$).

challenges for bioremediation purpose is to find bacterial consortia resistant to metals which produces a biofilm able to adsorb metals at high concentrations. Thus, our proposal was (1) to find, isolate, identify and cultivate bacteria consortia from mangrove's sediment, test their resistance to Zn (II) and Cu (II) at high concentrations (50 mg L^{-1} for both zinc and copper) and, using synchrotron X-ray fluorescence microscopy and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS),

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