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Biosorption of Heavy Metals by the Bacterial Exopolysaccharide FucoPol

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Abstract: Despite the efforts for minimizing the usage of heavy metals, anthropogenic activities still generate high amounts of wastewater containing these contaminants that cause significant health and environmental problems. Given the drawbacks of the conventional physical and chemical methods currently used, natural biosorbents (microbial cells or their products) arise as promising environmentally friendly alternatives. In this study, the binding efficiency of the polysaccharide secreted by *Enterobacter* A47, FucoPol, towards lead (Pb^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}) cations was demonstrated. FucoPol revealed a higher performance for the biosorption of Pb^{2+}, with a maximum overall metal removal of 93.9 ± 5.3% and a specific metal uptake of 41.1 ± 2.3 mg/gEPS, from a Pb^{2+} solution with an initial concentration of 10 mg/L, by a 5 g/L FucoPol solution. The overall metal removal decreased considerably (≤31.3 ± 1.6%) for higher Pb^{2+} concentrations (48 and 100 mg/L) probably due to the saturation of FucoPol's binding sites. Pb^{2+} removal was also less efficient (66.0 ± 8.2%) when a higher FucoPol concentration (10 g/L) was tested. Pb^{2+} removal efficiency of FucoPol was maximized at pH 4.3, however, it was affected by lower pH values (2.5–3.3). Moreover, the FucoPol's sorption performance was unaffected (overall metal removal: 91.6–93.9%) in the temperature range of 5–40 °C. These findings demonstrate FucoPol's great potential for utilization as a biodegradable and safe biosorbent for treating waters and wastewaters contaminated with Pb^{2+}.

Keywords: biosorption; biosorbent; heavy metals; *Enterobacter* A47; water/wastewater; exopolysaccharide; FucoPol

1. Introduction

Heavy metals are elements naturally found throughout the earth's crust. They have a high atomic number and a density at least 5 times higher than water (1 g/cm^3) [1,2]. Despite being required for several biological processes (i.e., protein, nucleic acids, carbohydrate and lipid metabolism), they are also essential for our industry and daily life. Heavy metal sources include activities such as burning fossil fuels, mining, smelting and electroplating. They are also present in paints, batteries, metal products (e.g., ammunitions and pipes), cosmetics, electronic equipment, ceramics, fertilizers, pesticides, and wood preservatives [3,4]. Despite their widely utilization, heavy metals can cause serious toxicity issues in living organisms [3]. These metals are extremely poisonous and can eventually be lethal, since they inhibit essential metabolic pathways, cause oxidative stress and cellular damages, and can promote mutagenesis [2,4,5]. Co^{2+}, for example, has only one biological function as a component of vitamin B_{12}, nevertheless it can be toxic at high levels. Co^{2+} exposure might occur due to inhalation or ingestion of food and drinking water containing Co^{2+}, and can cause neurological, cardiovascular, and endocrine problems such as cardiomyopathy, vision and hearing impairment, reversible hypothyroidism, and polycythemia [6,7]. Cu^{2+} is a trace nutrient

essential for enzymatic activity and redox reactions due to its capacity to cycle between Cu^{2+} and Cu^{+}. However, this transition to the reduced state can generate hydroxyl and superoxide radicals that can damage living cells [2,4]. Cu^{2+} exposure occurs by ingestion of water and can cause liver and kidney damage, anemia and irritation of the gastrointestinal track [1]. Although Zn^{2+} deficiency causes health problems, Zn^{2+} overexposure can cause cholesterol, anemia, nausea, vomiting, abdominal pain and lethargy [4]. Pb^{2+} is considered carcinogenic and highly toxic, it can bind and inactivate enzymes and proteins, substitute essential ions in the cells (Ca^{2+}, Mg^{2+}, Fe^{2+}, and Na^{+}), and inhibit Ca^{2+} transport. Moreover, Pb^{2+} fastens the formation of reactive oxygen species (ROS) that causes oxidative stress and damages in living cells [3,4,8]. Pb^{2+} is absorbed and distributed into the human tissues after inhalation of Pb^{2+}-contaminated dust or aerosols, and ingestion of contaminated water or food [2,3]. Pb^{2+} poisoning severely affects the nervous system, causing edema, encephalopathy, and brain damage [3]. Even low-level Pb^{2+} exposure is associated with liver/biliary injuries [9]; decreased kidney function and chronic kidney disease incidence [10]; and cardiovascular problems such as hypertension, electrocardiographic abnormalities, left-ventricular hypertrophy, peripheral arterial disease and cardiovascular disease mortality [11]. According to the US Environmental Protection Agency, the maximum concentration of Cu and Zn in drinking water is 1.3 and 5 mg/L, respectively, a much higher value than of Pb (0.015 mg/L), due to their lower toxicity to humans. However, Cu^{2+} and Zn^{2+} posed the greatest risk for aquatic living organisms from an urban lake in China [12].

Moreover, heavy metals are non-degradable and accumulate in the organisms, thus persisting and accumulating throughout the food chain [2,4,13]. Pb^{2+}, for example, due to its environmental persistency, can impact the life course of individuals from the moment they are born. Pb^{2+} exposure during childhood and throughout the years, not only impacts their biological systems, but also has long-term ramifications, such as lower cognitive function and downward social mobility [14,15]. In view of this, the dispersion of heavy metals throughout both terrestrial and aquatic ecosystems has raised many ecological and health concerns.

Conventional physical and chemical methods have been implemented to remove metals from aqueous solutions, namely, ion exchange, reverse osmosis, chemical precipitation, chemical extraction, and electrochemical treatment [4]. However, they have some drawbacks, such as the difficulty for large scale implementation, the generation of undesirable by-products, high costs, and inefficiency to treat effluents contaminated with low metal concentrations [4,16,17]. Consequently, environmental biotechnology is focused on developing novel, attractive and economic alternatives to remove heavy metals from contaminated wastewaters. One of the most promising methods is the biosorption of heavy metals, which utilizes living or dead microorganisms, or products from their metabolism, such as polymers [4,18].

Microbial cells are capable of binding and accumulating metal ions present in water, reducing their bioavailability. This biosorption phenomenon occurs due to the presence of biopolymers, rich in acidic functional groups, in the cellular structures that can bind metallic cations, independently from the cellular metabolism [4]. Biosorption of metal ions by polymers may involve different physical and chemical processes. Physical sorption occurs due to electrostatic and Van der Walls interactions, or due to the imbalance of metal concentration between the surface of the biosorbent and the solution [4,19]. Chemical mechanisms responsible for biosorption are complexation, chelation, coordination, microprecipitation, and ion exchange [4,17].

Numerous extracellular polysaccharides (EPS) secreted by different microorganisms were reported to be effective in metal sequestration. For instance, the EPS produced by *Paenibacillus jamilae* [20], *Bacillus firmus* [21], *Bacillus licheniformis* KX657843 [18], *Herbaspirillium* sp. [22] and *Paenibacillus peoriae* TS7 [23] were capable of removing several heavy metals (e.g., Zn^{2+}, Pb^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}) from aqueous systems. These reports suggest that the use of EPS is a promising solution for heavy metal sequestration and recuperation from water systems. The ability of EPS to adsorb metal cations is due to the presence of negatively charged functional groups (carboxyl, phosphoryl and

hydroxyl groups) in these macromolecules' structures, which can establish electrostatic interactions and bind the positively charged metal ion [4,24]. Moreover, the use of microbial polysaccharides as biosorbents is advantageous since they are eco-friendly and non-toxic, and their production is easy and cost-effective [25]. Compared to the biosorption using living bacterial cells, metal biosorption with EPS has a reduced complexity and no metabolic interferences [4]. Moreover, living cells have nutritional requirements and are affected by abiotic stress factors [24].

FucoPol is an anionic EPS synthesized by the bacterium *Enterobacter* A47 [26]. This high molecular weight EPS is composed of fucose, galactose, glucose and glucuronic acid sugar residues (2.0:1.9:0.9:0.5 molar ratio), and the acyl groups acetyl, pyruvyl and succinyl, that represent 12.3 wt% of FucoPol's dry mass. FucoPol possesses a→4)-α-L-Fucp-(1→4)-α-L-Fucp-(1→3)-β-D-Glcp(1→trimer backbone. The branches, present at position 3 of the first fucose, are composed of an α-D-4,6-pyruvyl-Galp-(1→4)-β-DGlcAp-(1→3)-α-D-Galp(1→trimer, with two pyruvate caps in the terminal galactose, specifically at position C-4 and C-6 [27,28]. FucoPol was employed for the preparation of a bioactive silver nanocomposite [27] and as a coating agent for iron oxide magnetic nanoparticles (MNP). The use of these FucoPol-MNP was evaluated for human antibody purification [29] and as cell labeling nanoprobes for Magnetic Resonance Imaging [30]. The potential of using FucoPol as biosorbent for the removal of heavy metals from water streams was never explored.

This study focused on evaluating the performance of FucoPol for the biosorption of different heavy metals (Pb^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+}). Considering the results, metal toxicity and prevalence, Pb^{2+} was selected for further tests. The effects of FucoPol dosage, initial metal concentration, pH and temperature were explored for Pb^{2+} removal, envisaging the optimization of the biosorption process.

2. Materials and Methods

2.1. FucoPol Production, Purification and Characterization

FucoPol was obtained by cultivation of the bacterium *Enterobacter* A47 (DSM 23139) in a 2 L bioreactor (BioStat B-plus, Sartorius, Germany) using mineral Medium E* with glycerol (40 g/L) as carbon source, as described by Concórdio-Reis et al. [31]. FucoPol synthesis is triggered by imposing growth limiting conditions (i.e., oxygen and nitrogen limitation) concomitant with carbon availability during cultivation [31]. Thus, cultivation was performed under a fed-batch mode (feeding solution: Medium E* supplemented with 200 g/L of glycerol), with controlled temperature (30.0 ± 0.1 °C), pH (7.0 ± 0.02) and dissolved oxygen concentration (10% of the air saturation). FucoPol was recovered from the cultivation broth after 4 days as previously described [27]. The procedure involved dilution of the broth with deionized water (1:10, *v*/*v*) to reduce viscosity, centrifugation (13000×*g*, 45 min) for cell removal, thermal treatment (70 °C, 1 h) of the cell-free supernatant to promote protein denaturation and, finally, centrifugation (13,000× *g*, 45 min) for removal of cell fragments and denatured proteins. For FucoPol purification, the low molecular weight compounds (salts, glycerol, proteins) were removed by diafiltration in a cross-flow module (Sartocon Slide Holder), using a membrane with a surface area of 100 cm^2 and a 100 kDa nominal molecular weight cut-off (Hydrosart ultrafiltration cassette, Sartorius) [27]. During diafiltration, deionized water was added to the retentate to facilitate the diffusion of low molecular weight solutes across the membrane. Finally, the module was operated in ultrafiltration mode, without water addition, to concentrate the treated supernatant (3:1, *v*/*v*), and the solution was freeze-dried to obtain the FucoPol (12.4 g).

FucoPol was characterized in terms of carbohydrate monomers and acyl groups composition, as well as total protein content and molecular mass distribution. For the determination of the sugar and acyl content, FucoPol (1 g/L, 5 mL) was hydrolyzed with 0.1 mL 99% trifluoroacetic acid (TFA) at 120 °C for 2 h. The constituent monosaccharides were identified and quantified in the hydrolysate by HPLC using a Carbopac PA10 column (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), equipped with an amperometric detector, as described by Concórdio-Reis et al. [31]. The analysis was performed at 30 °C with sodium hydroxide (NaOH 4 mM) as eluent, at a flow rate of 0.9 mL/min. The acid

hydrolysates were also used for the identification and quantification of acyl substituents. The analysis was performed by HPLC with and Aminex HPX-87H 300 × 7.8 mm (Biorad, Hercules, CA, USA), coupled to an infrared (IR) detector, using sulphuric acid (H_2SO_4 0.01N) as eluent, at a flow rate of 0.6 mL/min and a temperature of 30 °C.

The protein content was determined by a modified Lowry method: 5.5 mL FucoPol solutions (4.4 g/L) were mixed with 1 mL 20% NaOH and incubated at 100 °C, for 5 min. After cooling on ice, 170 µL of $CuSO_4.5H_2O$ (25% *w*/*v*) were added and the solution was agitated. Afterwards, the solution was centrifuged (3500× *g*, for 5 min) and the optical density was measured at 560 nm. Albumin (Sigma-Aldrich) solutions (0.05–1.0 g/L) were used as protein standards. The average molecular weight (M_w) was determined by size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS), as described by Concórdio-Reis et al. [31]. Briefly, FucoPol solutions (0.2 g/L) were dissolved in 0.1 M Tris-HCl, NaCl (0.2 M), pH 8.09 buffer, which was also the SEC mobile phase. The SEC columns (PL aquagel-OH mixed 8 µm, 30 × 7.5 mm) were equilibrated for 24 h before running the analysis at a flow rate of 0.7 mL/min at 30 °C. In order to follow the purity and molecular mass distribution of the polysaccharide signals from MALS were recorded in parallel and treated with Astra (V 4.73.04). A d*n*/dc of 0.190 mL/g was adopted to calculate the M_w of the FucoPol.

2.2. Preparation of Solutions

Metal solutions (3–100 mg/L) of Pb^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+} were prepared by dissolving the corresponding chloride salts in deionized water: $PbCl_2$ (Sigma, 98%), $CoCl_2.6H_2O$ (Panreac, 98%), $CuCl_2.2H_2O$ (Merck, 99%) and $ZnCl_2$ (Scharlau, 95%). FucoPol solutions were prepared by dissolving the freeze-dried polymer in deionized water at the appropriate concentrations according to the experiments (1–10 g/L).

2.3. Metal Biosorption Experiments

To evaluate the ability of FucoPol for binding metals in aqueous systems, the method described by Maalej et al. [32] was performed, with some modifications. Equilibrium dialysis experiments were carried out by placing dialysis tubing (12–14 kDa MWCO membrane, ZelluTrans/Roth) containing the FucoPol solution (5 mL) in closed glass flasks with 200 mL of the appropriate metal solution. The flasks were placed in an orbital shaker (150 rpm) and kept at 21 °C, for 24 h. Experiments were performed in triplicates. Controls were performed using deionized water in the dialysis tubing instead of the FucoPol solution. Samples of the metal solution (2 mL) were taken before and after the incubation period and the metallic ions were quantified by Inductively Coupled Plasma—Atomic Emission Spectroscopy (ICP-AES) (Ultima, Horiba Jobin-Yvon, France, equipped with a 40.68 MHz RF generator, Czerny-Turner monochromator with 1.00 m (sequential) and autosampler AS500).

2.4. Effect of Dosage, pH and Temperature on Pb^{2+} Biosorption Ability of FucoPol

The effect of FucoPol dosage (1, 2, 3, 5 and 10 g/L) on FucoPol metal binding capacity towards Pb^{2+} was investigated. Moreover, various initial concentrations of Pb^{2+} solutions (3, 10, 48 and 100 mg/L) were tested for a FucoPol concentration of 5 mg/L. The effect of the pH value (2.5, 3.3 and 4.3) was also tested by adjusting the initial pH of the metal solution with HCl (1.0 M) or NaOH (1.0 M). The impact of temperature (5, 21, 30 and 40 °C) in the metal removal abilities of FucoPol was also studied by keeping the orbital shaker with the flasks in an incubator set at different temperatures (21–40 °C) and in a cold chamber (5 °C).

2.5. Calculations

The metal removal efficiency (*M*, %) and the specific metal uptake (*q*, mg/gEPS) were calculated as follows:

$$M = \frac{Cf_{Control} - Cf_{EPS}}{Cf_{Control}} \times 100 \quad (1)$$

$$q = \frac{(Ci - Cf)_{EPS} - (Ci - Cf)_{Control}}{m_{EPS}} \times V \quad (2)$$

where V (mL) is the volume of the metal solution in the flask, Ci and Cf are the metal concentrations before and after equilibrium (mg/L), respectively, and m_{EPS} represents the mass of FucoPol (g).

3. Results and Discussion

3.1. FucoPol Characterization

Metal biosorption has been described for several EPS with different specificities and metal-binding capacities that can be attributed to differences in charge density, attractive interaction, and polymer conformation [20]. The biosorption performance of EPS varies significantly depending on their composition, as the properties of the active functional groups impact the biosorption mechanism [4]. Thus, biosorbent characterization is essential for a comprehensive analysis of the results (Table 1).

FucoPol is a high molecular weight (4.4×10^6 Da) polysaccharide secreted by the bacterium *Enterobacter* A47. It is mainly composed of neutral sugars, fucose, galactose and glucose, and the acidic sugar glucuronic acid (Table 1). The non-carbohydrate moiety comprises the acyl groups acetyl, pyruvyl and succinyl, which account for 12.3 wt% of FucoPol's mass. The presence of non-carbohydrate substituents and ionizable functional groups (carboxyl and hydroxyl groups), which are responsible for the binding capacity of biosorbents [24], in FucoPol's structure drove the interest in exploring its ability to bind heavy metal cations. Specifically, the presence of glucuronic acid residues, together with the pyruvyl and succinyl groups, that confer the biopolymer an anionic character, is relevant for its binding ability towards cations and positively charged molecules. FucoPol sample also has a protein content of 10.8%, which was not completely removed during the purification procedures, probably due to their high Mw [27]. The presence of amine, sulfhydryl and carboxyl functional groups in proteins contribute to the overall negative charge of FucoPol [24]. On the other hand, metals can establish covalent or coordinative interactions with the sulfur- and nitrogen-containing ligands [4], which could also contribute to the metal biosorption capacity of FucoPol.

3.2. Evaluation of the Metal-Binding Ability of FucoPol

The metal-binding efficiency of FucoPol was tested by incubating FucoPol for 24 h in solutions containing 10 mg/L of Pb^{2+}, Cu^{2+}, Zn^{2+} or Co^{2+}. The resulting metal removal efficiency (M) and metal uptake (q) values are presented in Figure 1.

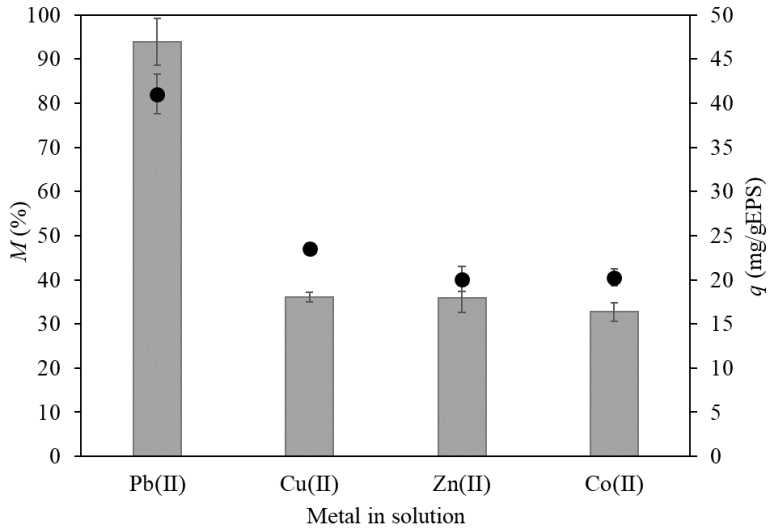

Figure 1. Biosorption of Pb^{2+}, Cu^{2+}, Zn^{2+} and Co^{2+} (Ci 10 mg/L) by FucoPol (5 g/L) in terms of metal removal efficiency (M, ▭) and specific metal uptake (q, •) (temperature 21 °C, incubation time 24 h).

Table 1. Microbial EPS with biosorption capacity towards heavy metals (Man, mannose; Glc, glucose; Gal, galactose; Rha; rhamnose; Fuc, fucose: Fru, fructose; GlcA, glucuronic acid; GalA, galacturonic acid; Pyr, pyruvate; Succ, succinate; Ac, acetate).

Organism	Sugar Composition (wt% or Molar Ratio)	Non-Sugar Residues (wt%)	M_w (Da)	Bisorption Capacity (mg/gEPS or %)	References
Alteromonas macleodii subsp *fijiensis*	GlcA, Glc, Gal, Man and GalA (2.4:1.6:1.4:1.1:1.0)	Protein (4 %)	n.a.	Pb^{2+}: 316 Zn^{2+}: 75	[33]
Bacillus firmus MS-102	Glc, Fru, Man, Gal (12.1:5.7:3.1:1.0) Uronic acids (38 %)	Pyr (6.3 %)	n.a.	Pb^{2+}: 1103 or 98.3% Cu^{2+}: 860 or 74.9% Zn^{2+}: 722 or 61.8%	[21]
Bacillus sp. F19	Man, Glc (1.2:1) Uronic acids (37 %) amino sugars (0.5 %)	Protein (16.4%)	n.a.	Cu^{2+}: 89.6	[34]
Enterobacter A47	Fuc, Gal, Glc and GlcA (2.0:1.9:0.9:0.5)	Protein (10.8%) Ac, Pyr, Succ (12.3%)	4.4×10^6	Pb^{2+}: 108.0 or 93.9% Cu^{2+}: 23.5 or 36.1% Zn^{2+}: 20.1 or 35.9% Co^{2+}: 20.3 or 32.7%	This study
Methylobacterium organophilum	Gal, Man, Glu (3:2:2) Uronic acids (12.4%)	Protein (6.1%) Pyr (5.1%) Ac (0.6 %)	n.a.	Pb^{2+}: 184.2 Cu^{2+}: 200.3	[35]
Paenibacillus jamilae	Glc, Man, Gal, Fuc, Rha (54.6, 25.6, 12.9, 3.8, 3.1%) Uronic acids (28.3%) Aminosugars (2.8%)	Protein (1.5%) Pyr (8.7%) Acetyls (4.13%)	n.a.	Pb^{2+}: 303.0 Cu^{2+}: 12.3 Zn^{2+}: 7.8 Co^{2+}: 20.5	[20]
Paenibacillus peoriae TS7	Fru	-	n.a.	Pb^{2+}: 277.5	[23]
Pseudomonas aeruginosa ATCC-10145	Neutral sugars (30.6%) Uronic acids (2.35%) Aminosugars (0.78%)	Protein (27%)	n.a.	Pb^{2+}: 79.7% Cu^{2+}: 87.4% Zn^{2+}: 80.6%	[36]
Pseudomonas stuteri AS22	Glc, Man, Lactyl rhamnose (1:1.1:0.7)	Lactyl, acetyl and pyruvyl groups	9.9×10^5	Pb^{2+}: 215.6 Cu^{2+}: 0.6 Co^{2+}: 1.4	[32,37]
Rhizobium radiobacter F2 and *Bacillus sphaeicus* F6	Glc, Man, Rha, Gal (10.0:2.1:1.3:1.0)	-	4.79×10^5	Pb^{2+}: 189.3	[38]

FucoPol was able to adsorb all the tested metal species, although with different degrees of efficiency. Considering the overall metal removal efficiency, *M*, FucoPol had a higher affinity for Pb^{2+}, since 93.9 ± 5.3% of Pb^{2+} was removed from the solution. For the remaining tested cations, Cu^{2+}, Zn^{2+} and Co^{2+}, considerably lower values were obtained for 36.1 ± 1.1%, 35.9 ± 3.4% and 32.7 ± 2.1%, respectively (Figure 1). Considering the results, under the tested conditions, the preferential metal adsorption was Pb >> Cu > Zn > Co. When considering the overall specific metal uptake, Pb^{2+} also presented the highest value, 41.1 ± 2.3 mg/g, while Co^{2+}, Zn^{2+} and Cu^{2+} had considerably lower *q* values of 23.5 ± 0.7 mg/gEPS, 20.1 ± 1.4 mg/gEPS, and 20.3 ± 1.0 mg/gEPS, respectively (Figure 1).

The different removal efficiency observed for Pb^{2+} in comparison with Co^{2+}, Zn^{2+} and Cu^{2+} might be attributed to the different charge density of the ions, which is dependent on the cations ionic size [21]. The biosorptive capacity towards different metals can be speculated through the covalent index, calculated as $X^2_m r$, in which the electronegativity (X_m) and ionic radius (r) of the metals are considered. Pb^{2+} has the higher covalent index (6.41), followed by Co^{2+} (2.65), Cu^{2+} (2.64) and finally Zn^{2+} (2.04) [39], which was the preferential sequence of specific metal uptake by FucoPol (Pb >> Co > Cu > Zn). Furthermore, the FucoPol-Pb attraction might have been weaker than with other ions (higher repulsion due to the higher electronic density). This would lead to a less compact FucoPol structure in the presence of the metal. Hence, the same amount of Pb^{2+} occupied less surface area and less negatively charged functional groups than when the other metals were used [21]. This allowed that more Pb^{2+} could bind to FucoPol's molecule within the equilibrium period.

Several studies with other bacterial EPS reported a similar trend, wherein there was a better metal biosorption performance towards Pb^{2+} removal compared to other metals (Cu^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Fe^{2+}). Examples include the anionic EPS synthesized by *P. stutzeri, P. jamilae* and *B. firmus*, in which the order of metal uptake was Pb > Co > Cu, Pb >> Cu > Zn >> Co and Pb > Cu > Zn, respectively [20,21,32]. However, the interaction between EPS and metals highly depends on the structure, composition and surface area of the biosorbent, as well as on the attractive forces and the conformation established between the EPS and the metal [4,20], thus different results might be achieved depending on the characteristics of the EPS. In fact, contrasting results were reported for the EPS produced by *M. organophilum* and *P. aeruginosa*: *M. organophilum* EPS removed preferentially Cu^{2+} over Pb^{2+} (21% and 18%, respectively)[35], while *P. aeruginosa* EPS removed 87.4% of Cu^{2+}, followed by Zn^{2+} (80.6%) and Pb^{2+} (79.7%) [36]. Both EPS had less negative charged groups (e.g., uronic acids, pyruvate and acetate) in their composition than FucoPol and the EPSs that showed preferential removal of Pb^{2+} (Table 1).

Despite the increase in metal concentration inside the dialysis tubing observed in all tests, no insoluble species were formed, which indicated that FucoPol interacted with the metals in solution without chemically converting them into less soluble forms. As so, a biosorption mechanism, or a combination of several mechanisms, must be involved the FucoPol-metal interaction. Possible interaction mechanisms include microprecipitation, ion exchange, coordination, complexation, adsorption, chelation, and electrostatic interactions [4,17].

Based on these results, it was clear that FucoPol has a good performance for Pb^{2+} sequestration. Lead poisoning causes serious health problems in the central nervous system, the kidneys, the gastrointestinal tract and the reproductive system [5]. Moreover, Tchounwou et al. [2] reported that up to 50% of Pb^{2+} adsorbed in adults was due to the ingestion of contaminated drinking water. Therefore, the subsequent experiments focused on the different factors that influenced the adsorption of Pb^{2+} by FucoPol, envisaging the optimization of its use as biosorbent.

3.3. Effect of Initial Metal Concentration on the Pb^{2+}-Binding Ability of FucoPol

The metal-binding performance of FucoPol was evaluated in a range of different initial Pb^{2+} concentrations, 3 to 100 mg/L (Figure 2). This range was chosen since it was described that the conventional methods are inefficient for removal of Pb^{2+} concentrations below 100 mg/L [16,17].

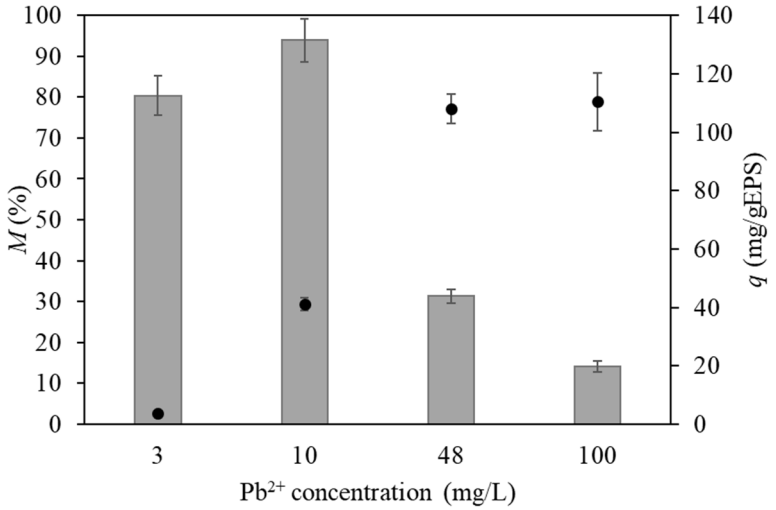

Figure 2. Specific metal uptake (q, •) and removal efficiency (M, ▭) by FucoPol (5 g/L) using different initial Pb^{2+} concentrations (temperature 21 °C, incubation time 24 h).

The results presented in Figure 2 proved that the initial concentration of Pb^{2+} had a significant effect on the Pb^{2+} uptake efficiency of FucoPol. For higher Pb^{2+} concentrations, metal uptake by FucoPol improved greatly. When the concentration of metal was augmented from 3 mg/L to 48 mg/L, the specific Pb^{2+} uptake increased approximately 28 times, reaching a q value of 108.0 ± 5.0 mg/gEPS. However, for metal concentrations of 100 mg/L, the specific uptake had no further significant increase (110.4 ± 9.9 mg/gEPS), probably due to the saturation of the binding sites of the polysaccharide [4,23]. For the highest metal concentrations tested (48 and 100 mg/L), metal uptake reached a plateau value (q_{max}) and FucoPol was saturated, therefore the overall metal removal decreased drastically from the highest value of 93.8 ± 5.3% achieved with 10 mg/L of Pb^{2+} to 14.1 ± 1.3% with 100 mg/L of Pb^{2+}. A similar trend was reported for the EPS produced by a mixed culture of *R. radiobacter* F2 and *B. sphaeicus* F6: when the initial Pb^{2+} concentration increased from 0.1 to 5 mg/L, metal uptake increased 44.5 times, however, metal removal was maintained above 97% for concentrations in the range of 0.1–2 mg/L and decreased for 90% for higher concentrations [38].

3.4. Effect of FucoPol Dosage on the Pb^{2+}-Binding Ability of FucoPol

The effect of the FucoPol dosage on the removal of Pb^{2+} from aqueous solutions (10 mg/L) was measured in terms of metal removal efficiency (M) and specific metal uptake (q), as disclosed on Figure 3.

As shown in Figure 3, the concentration of FucoPol used as biosorbent influenced the metal uptake (q), as well as the overall metal removal efficiency (M). In all experiments, an increase in FucoPol concentration led to an increase in metal removal. These results could be related to the fact that more binding sites were available to adsorb the metal ions in solution [23,40]. Maximal metal removal (93.9 ± 5.3%) was achieved when 5 g/L of FucoPol were used, decreasing when a higher concentration (10 g/L) was tested (66.0 ± 8.2%). Thus, it can be speculated that higher concentrations of FucoPol increase the occurrence of polymer-polymer interaction, reducing the availability of binding sites capable of metal sequestration [21]. Furthermore, higher concentrations of FucoPol cause an increase in viscosity [41], which may affect the diffusion of the metals, thus restraining their interaction with less accessible binding sites. Similar results were reported in the literature for other EPS. For example, for the EPS produced by *P. aeruginosa*, the use of an EPS concentration of 100 mg/L resulted in higher Pb removal rather than the higher dosages tested (1000–10000 mg/L) [36]. Also, for the EPS produced by *P. peoriae* TS7, a maximal Pb^{2+} removal of 89% was obtained with 0.5 g/L of EPS, since both

lower (0.25 g/L) and higher concentrations (up to 2 g/L) led to a significant reduction in the removal efficiency [23].

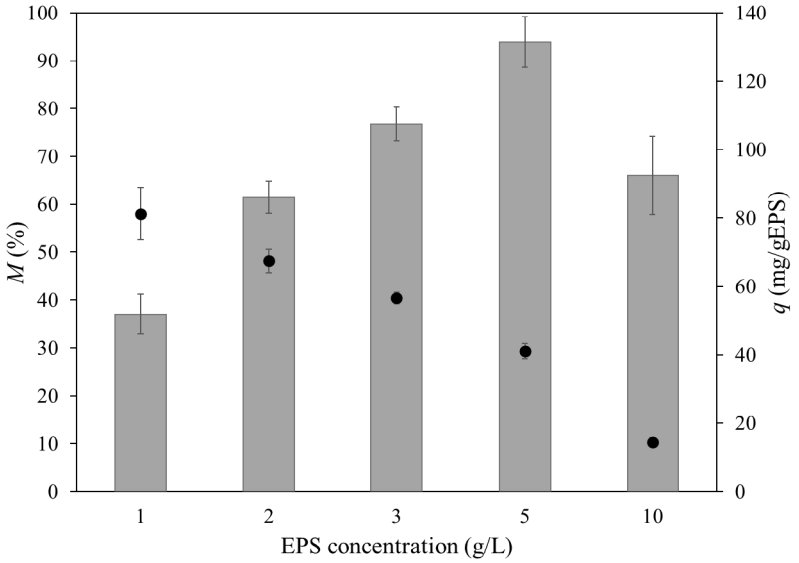

Figure 3. Removal of Pb^{2+} (C_i 10 mg/L) with different FucoPol concentrations. Results presented in terms of metal uptake (q, •) and removal efficiency (M, ▭) (pH 4.3, temperature 21 °C, incubation time 24 h).

An opposite trend was observed for the metal uptake, in which q decreased with the increment of the FucoPol's concentration on the dialysis tubing (Figure 3). When the concentration of FucoPol rose from 1 to 10 mg/L, the metal uptake decreased from 81.3 ± 7.8 mg/gEPS to 14.4 ± 1.2 mg/gEPS. The different profiles of M and q shown in Figure 3 are in line with the results achieved for the biosorption of Cu^{2+} by the anionic EPS produced by *Bacillus* sp. F19. Indeed, an increase in EPS dosage from 500 to 1500 mg/L lead to an increase in removal efficiency from 71.2% to 91,2%, and a reduction in metal uptake from 142 to 61 mg/gEPS [34]. Due to the differences in profiles under the same conditions, the subsequent studies were carried out with 5 mg/L of FucoPol, which was the concentration that maximized M.

3.5. Effect of pH in Pb^{2+} Removal by FucoPol

The pH affects the metal's solubility and the functional groups responsible for the metal-binding capacity since it determines their ionization state (protonated/deprotonated) [1,38,40,42,43]. The influence of this parameter in the metal-binding ability of FucoPol (5 g/L) was evaluated for pH values between 2.5 to 4.3 (the latter was the pH value of the original metal solution), using an initial Pb^{2+} concentration of 10 mg/L (Figure 4). It was not possible to test higher pH-values due to the occurrence of chemical precipitation of the metal above pH 6 [1,44].

As shown in Figure 4, FucoPol had a higher removal efficiency at the maximal pH value tested (4.3). A reduction in pH (3.3 and 2.5) led to a considerable decrease in metal removal (39.8 ± 2.8% and 7.3 ± 2.7%, respectively). These results were probably due to the fact that the acidic groups of FucoPol were protonated, decreasing the negative charges available to interact with Pb^{2+} cations [23,35]. A similar trend was described for other anionic EPS, including the EPS produced by *A. macleodii* subsp *fijiensis* that demonstrated a low binding capacity at pH values below 4, and optimal stable metal uptake values when the pH was between 4.5 and 6 [33]; and for the EPS produced by *B. firmus* MS-102, which optimal Pb^{2+} uptake was found at pH 4.5 [21]. As can be seen in Table 1, these EPSs had polyanionic character due to their presence of uronic acids and acyl substituents in their composition. On the other hand, the EPS that did not present these groups in their composition seemed to be more efficient at neutral pH. Examples include the EPS produced by *P. peoriae*, a fructose

homopolysaccharide [23]; and the EPS produced by a mixed culture of *R. radiobacter* F2 and *B. sphaeicus* F6 that was only composed of neutral sugars (Table 1) [38].

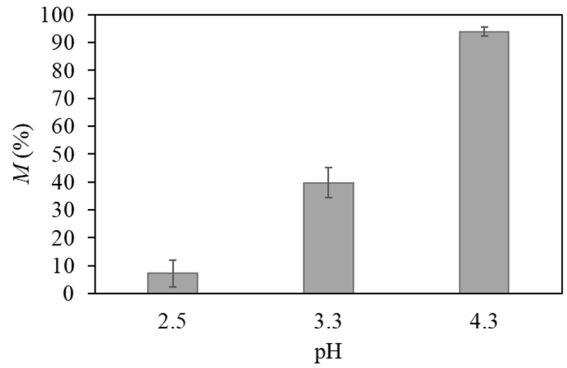

Figure 4. Effect of pH in Pb^{2+} removal efficiency by FucoPol (C_i 10 mg/L, FucoPol concentration 5 mg/L, temperature 21 °C, incubation time 24 h).

3.6. *Effect of Temperature in Pb^{2+} Removal by FucoPol*

Considering that temperature fluctuations occur during the year and that it affects the metal uptake process [45], the effect of temperature in the Pb^{2+} removal abilities of FucoPol was explored in a range of 5 to 40 °C (Figure 5). In literature, temperatures above 35 °C seemed to enhance biosorption due to an increase in surface activity and solute's kinetic energy. Moreover, higher temperatures promote an increase in the diffusion rate of metal though the external layer into the internal pores of the EPS, since its viscosity decreases [4].

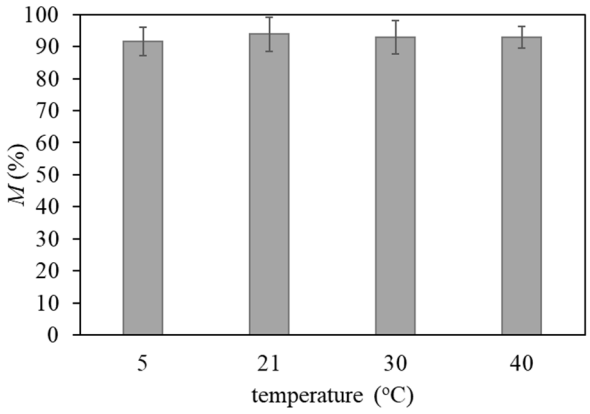

Figure 5. Effect of temperature in the Pb^{2+} removal efficiency by FucoPol (C_i 10 mg/L, FucoPol concentration 5 g/L, pH 4.3, incubation time 24 h).

As Figure 5 demonstrates, the Pb^{2+} removal efficiency of FucoPol was statistically the same in the range of temperatures tested, thus showing that FucoPol is thermostable and suitable to be used at these temperatures. The influence of temperature was not investigated in the majority of the metal removal studies using EPS as biosorbents. Nevertheless, Lin and Harichund [22] reported a similar trend for the *Paenibacillus sp.* CH11 biosorbent, since Pb^{2+} removal by this protein-based exopolymer was not affected by temperature in the range from 4 to 45 °C. Also, the EPS produced by a mixed culture of *R. radiobacter* F2 and *B. sphaeicus* F6 was reported to be efficient within the temperature range of 5 °C to 35 °C [38]. As so, FucoPol could be an effective alternative to be used in the removal of this metal from contaminated wastewaters without temperature control, despite the seasonal variations of this parameter.

3.7. Pb^{2+} Removal by FucoPol: Overall Assessment

FucoPol revealed very promising Pb^{2+}-binding ability, attaining high removal efficiency values (above 90%, for an initial metal concentration of 10 mg/L) at certain conditions, namely pH around 4.3 and temperatures up to 40 °C. Moreover, specific metal uptake values above 100 mg/gEPS were achieved for Pb^{2+} concentrations ≥48 mg/L. The demonstrated Pb^{2+} removal ability of FucoPol is comparable to that of other natural biosorbents reported in the literature, both in terms of removal efficiency (79.7–98.3%) [27,33] and specific metal uptake (16.6–1103 mg/g biosorbent) [21,46,47]. The comparison of biosorbents' performance is difficult because different methodologies are implemented and different experimental conditions (pH, temperature, metal and biosorbent concentration, presence of other contaminants) are used. Therefore, comparing results of different studies must be made with caution. Nevertheless, the values obtained suggest FucoPol might be suitable for the development of environmentally friendly and sustainable processes for the removal and recovery of lead from contaminated waters.

4. Conclusions

The present study demonstrated the capacity of bacterial EPS FucoPol for the biosorption different heavy metals from aqueous solutions. FucoPol was able to remove from solution all the metals tested, though with different removal efficiencies: Pb >> Cu > Zn > Co. Based on these results, metal toxicity and prevalence, Pb^{2+} removal by FucoPol was further studied. FucoPol was highly efficient for Pb^{2+} removal, even at low concentrations. Due to the excellent binding capacity under acidic pH, FucoPol has great potential for use in Pb^{2+} removal from acidic wastewaters, such as those generated by batteries manufacturing. Additionally, FucoPol had high and stable binding activity in a temperature range of 5 to 40 °C, proving it can be used with high efficiency in wastewater treatment throughout the year, despite the seasonal temperature variations.

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