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Removal of Lead and Mercury from Aqueous Solutions by Pretreated *Rhizopus Stolonifer* Biomass

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Abstract. The effect of different pretreatment processes on biosorption capacity of *Rhizopus stolonifer* biomass to remove lead and mercury was investigated. For this purpose, the biomass was subjected to physical treatments such as autoclaving and gamma irradiation, and chemical treatments such as NaOH, HCl and CTAB. The highest metal uptake values (12.10mgg^{-1} and 9.75mgg^{-1}for Pb and Hg respectively) were obtained by NaOH treated biomass. Kinetics of biosorption, isotherms of adsorption and influence of pH on the biosorption capacity of alkali treated biomass were studied. Analysis by FTIR, SEM and EDX were performed to show interactions between cells and ions. The optimum conditions were pH 8 for Pb and pH 4 for Hg. Maximum biosorption capacities for Pb and Hg were respectively 151, 19 mg g $^{-1}$ and 336, 86 mg g^{-1}. The kinetic is modeled pseudo-order 2 and the isotherm of Langmuir adequately describes the mechanism of biosorption.

Keywords: biosorption, heavy metals Environmental Preservation, Modeling, *Rhizopus stolonifer*.

1. Introduction

Increased industrialization and human activities have a very bad impact on the environment. They contribute to introduction of heavy metals into the aquatic systems. Beyond certain limits, heavy metals are toxic to living organisms and may cause serious hazard to public health. For these reasons the removal of excess heavy metals from wastewaters has generated a considerable interest in recent past [1]. Microorganisms like bacteria, algae, fungi and yeast are known to be very efficient heavy metals removers. But in recent years, the use of dried, not living or pretreated microorganisms seems to be a preferred alternative to the use of living cells because of many advantages: the metal removal system is not subjected to toxicity limitations, there is no requirement for growth media and nutrients, biosorbed metal ions can be easily desorbed and biomass can be reused [2]-[4]. Pretreatment of living cells can be performed using physical or chemical means in order to increase metal biosorption capacity [5], [6].The objective of this study is investigating the use of treated biomass of *Rhizopus stolonifer* as a biosorbent for the removal of Lead and mercury from aqueous solutions, after examination of the effect of different pretreatments on its biosorption capacity.

2. Material and Methods

2.1. Microorganism and growth conditions:

Rhizopus stolonifer was kindly supplied by biology laboratory at sciences university of Boumerdes, Algeria. The microorganism was grown aerobically in agitated Yeast Peptone Glucose nutrient medium.

2.2. Pretreatment of biomass

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After five days of culture, the fungal biomass was harvested by filtration, washed generously with deionized water and pretreated in five (5) different ways as described below:

- Boiled for 15 min in 0.5 N sodium hydroxide solution (40g l^{-1}).
- Agitated in 0,1 N HCl solution at 150rpm for 3 hours at room temperature.
- Agitated in 5% CTAB solution at 150 rpm for 3 hours at room temperature.

After these three treatments, biomass was washed with generous amounts of deionized water till the pH of the wash solution reached neutrality (6.8 – 7.2).

- Irradiated with 10 kGy of Cobalt 60 rays at a rate of 20 Gy/min.
- Autoclaved for 20 min at 120 ℃ and 1 bar of pressure.

After each pretreatment the biomasses were dried at 60 ℃ for 24 hours and ground in a mortar.

2.3. Metal solutions

Lead and mercury solutions were obtained by dissolving an accurate quantity of lead acetate and mercury chloride in deionized water to obtain stock solutions of 1g l^{-1}.

2.4. Biosorption studies

Two aspects of biosorption have been studied. First, the effect of the different pretreatments on the biomass biosorption capacity, for this purpose, 50 mg of each pretreated biomass were introduced to 25 ml of metal solutions containing 20mg l^{-1} of lead or 25 mg l^{-1} of mercury. The reaction mixture was agitated at 150 rpm for 24 hours.

Kinetics of biosorption, isotherms of adsorption and influence of pH on the biosorption capacity of alkali treated biomass were also studied. The concentrations of non adsorbed metal ions by *Rhizopus stolonifer* biomass were determined by means of Atomic Adsorption Spectroscopy (AAS). Analysis by FTIR, SEM and EDX were performed to show interactions between cells and metals ions.

3. Results and Discussion

3.1. Effect of pretreatment of rhizopus stolonifer biomass on biosorption capacity

Fig (1) shows the Pb $^{2+}$ and Hg $^{2+}$ uptake values obtained by treated and untreated biomass.

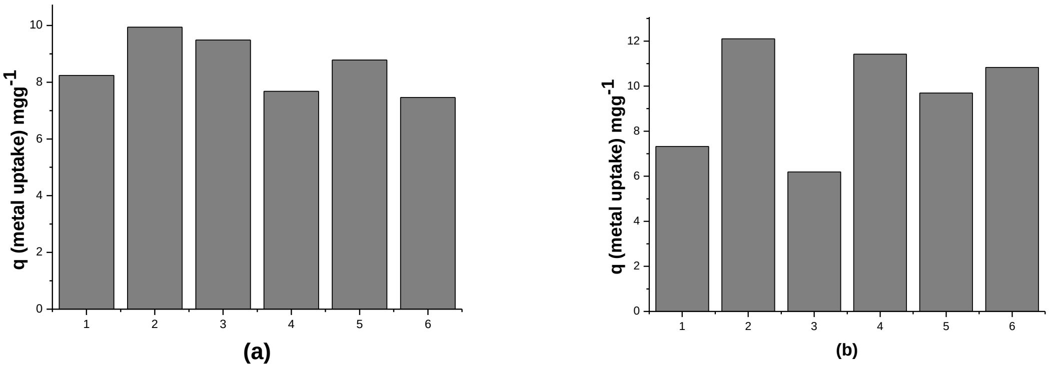

Fig. 1: Effect of different pretreatment on the (a) Pb^{2+} and (b) Hg^{2+} uptake of *R.stolonifer*. (1 Untreated biomass; 2 caustic treated biomass; 3 acid treated biomass; 4 CTAB treated biomass; 5 Irradiated biomass; 6 autoclaved biomass.)

The results indicated that all pretreatments influence the biosorption of Pb and Hg. In comparison with the untreated biomass, alkali treatment improved in a significant way the biosorption of Hg and Pb ions from 7.32mg.g^{-1} (untreated) to 12.10mg.g^{-1} and from 8.24 mg.g^{-1} to 9.75 mg.g^{-1} respectively.

Alkali pretreatment can improve the biosorption of heavy metals due to the fact that it may eliminate lipids and proteins which mask binding sites, liberate certain fungal wall polymers like chitin, which have raised affinity for these ions [5]-[7]. Kapoor and Viraraghavan (1998) [3] observed same results for the biosorption of lead and copper on *Aspergillus niger* pretreated with NaOH, dimethyl sulfoxide or a commercial laundry detergent. Göksungur and al., (2005) [8] improved the biosorption of lead and Cadmium on S*accharomyces cerivisiae* treated with ethanol or NaOH.

3.2. Biosorption study with alkali pretreated biomass

3.2.1. Effect of pH

Figure (2) shows the metal uptake as a function of pH.

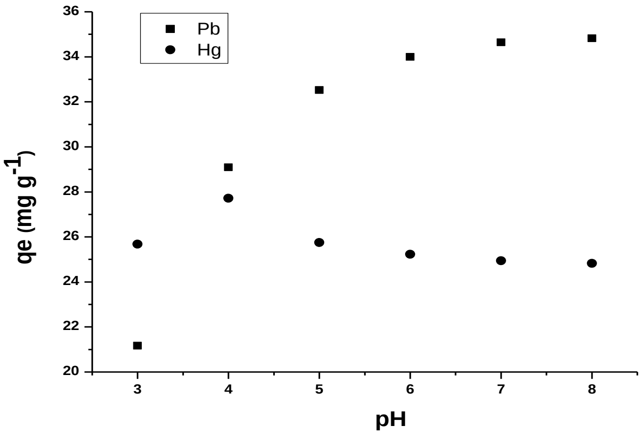

Fig. 2: Effect of pH on the biosorption capacity of Pb and Hg by NaOH treated biomass of *R.stolonifer* (agitation speed: 150 rpm, weight of biomass: 0.005g, initial concentration of Pb and Hg: 35 mg l^{-1} and 30 mg l^{-1} respectively, contact time: 5h).

It is observed that the best result occurred at pH 8 for Pb(II) where 97 % of metallic species were uptaken by biomass with a maximal removal capacity value of 34.82mg.g^{-1}. This value varies little in the range of pH going from 6 to 8. For Hg (II), the best result was obtained at pH 4 with 92.48% of uptake and 27,72mg.g^{-1} of removal capacity. In fact, the availability of sites intended to fix the metal cations is dependent on the pH because in conditions of strong acidity, it occurs a protonation of functional groups making the biosorption of the cations of Pb and Hg impossible. On the other hand, for higher pH values, sites of fixation are deprotonated and available for fixing metal ions. Therefore, the fixation is maximum when the active sites are deprotonated [9], [10].

3.2.2. The effect of contact time: The adsorption of Pb(II) and Hg(II) according to time is represented on figure (3a).

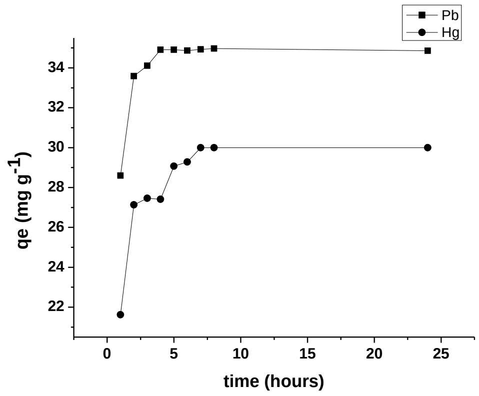

Fig. 3a: Effect of contact time on the biosorption capacity of lead and mercury by NaOH treated biomass of *R.stolonifer* (speed agitation: 150 rpm, biomass weight: 0.005g, initial concentration of Pb and Hg: 35 mg l^{-1} and 30 mg l^{-1} respectively, contact time: 5h).

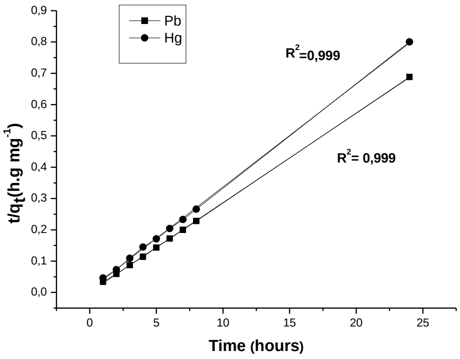

Fig. 3b: Kinetic model of pseudo-second order for the uptake of Pb^{2+} and Hg^{2+} using NaOH treated biomass of *R.stolonifer*

It is observed that the adsorption is very fast for both metallic ions, during the first hour of contact, more than 72 % of Hg and 81 % of Pb were adsorbed. Equilibrium was reached around 5 hours of contact time. A maximal elimination was observed at the end of 7 hours of contact, for Pb (99.78 %) with a capacity of adsorption of 34.92mg.g^{-1} and for Hg (100 %) with a capacity of adsorption of 30 mg. g^{-1}. Similar results were observed in many studies, Kuber et al., (2008) [7] used a NaOH pretreated dead biomass of *Rhizopus oryzae* and obtained a biosorption of 93 % of copper after 10min of contact followed by a balance.

Figure 3 (b) shows the plots of linearized forms corresponding to pseudo-second order kinetic model for the biosorption of the metallic species by treated biomass of *Rhizopus stolonifer*. We can observe that the model fits very well the data obtained, where the correlation coefficients were greater than 0, 99. Calculated qe values of the pseudo-second order kinetic model were closer to experimental values (table1).

Table 1:Pseudo-second order Kinetic parameters for the biosorption of Pb(II) and Hg(II)on treated biomass of *Rhizopus stolonifer.*

adsorbate	**Experimental qe (mg g^{-1})**	**qe (mg g^{-1})**	**K_2 (g mg^{-1}. h^{-1})**
Pb	34.92	35.46	0.25
Hg	30	30.48	0.11

3.2.3. Adsorption isotherms

Figure 4 (a) presents the metallic uptake as a function of the equilibrium concentration. We can see that sorption capacity values increased with the increasing of the initial metal ions concentrations. The obtained results indicate that the Langmuir model [11] describes well the data of lead and mercury equilibrium adsorption by *Rhizopus stolonifer* biomass (fig4b). Small b values (0.045 l.mg^{-1} for Pb and 0.024 l.mg^{-1} for Hg) obtained in this research implied strong binding of metal ions to NaOH treated fungi biomass. The maximum loading capacities obtained were 136.98 mg.g^{-1} for Pb and 341.29 mg.g^{-1} for Hg. These values approximate the experimental ones which are 151.195 mg.g^{-1} for Pb and 336.68 mg.g^{-1} for Hg. In this specific case, the adsorption of lead and mercury occurs on a homogeneous surface by monolayer sorption with interactions between adsorbed molecules.

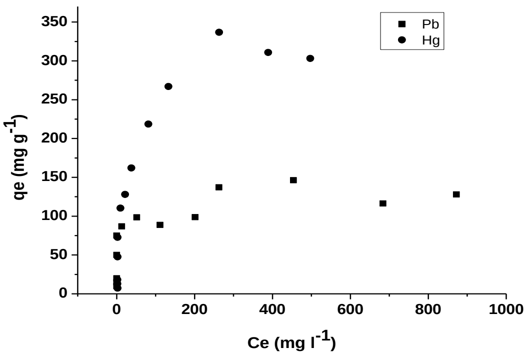

Fig. 4a: Effect of initial concentration of metallic solutions on the biosorption capacity of Pb^{2+} and Hg^{2+} by NaOH treated biomass of *R.stolonifer*. (agitation speed: 150 rpm, weight of biomass: 0.005g, Contact time: 24h).

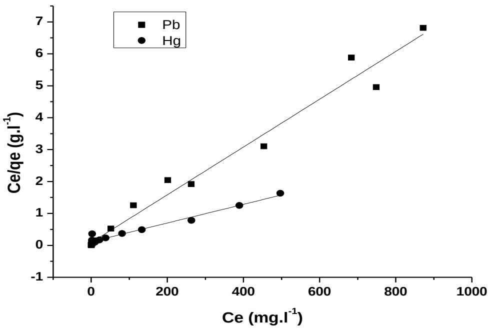

Fig. 4b: Linear transformation of the Langmuir biosorption of Pb^{2+} and Hg^{2+} by NaOH treated biomass of *R.stolonifer.*

3.3. SEM and EDX analysis

EDX spectra obtained before and after Pb and Hg biosorption onto NaOH treated biomass of *Rhizopus stolonifer* are presented in figure (5).

SEM observations have not revealed the presence of any new particles on the surface (photos not showed). However, EDX analysis confirmed the adsorption of metal ions. Characteristic peaks of Pb and Hg appear distinctly and confirm that the biomass has well fixed the metal ions. Several works on the biosorption of metals used these two techniques to highlight the presence of ions on different biomasses, *Plectonema boryanum* [12], *Pseudomonas aeruginosa* [13], *Aspergillus niger* [14] and *Rhodococcus opacus* [15].

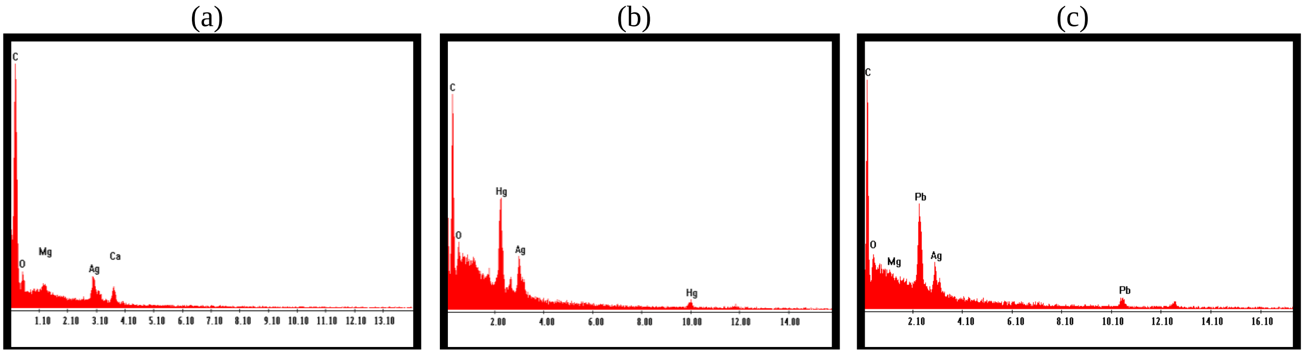

Fig. 5: EDX spectra of NaOH treated biomass of *R.stolonifer* before (a) and after contact with the metal Hg ions (b) and Pb ions(c)

3.4. FTIR spectral analysis

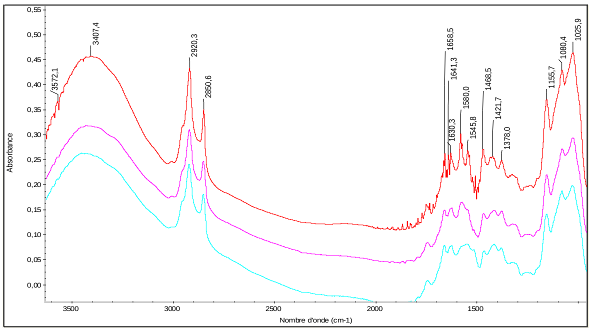

Fig. 6: FT-IR spectra of *Rhizopus stolonifer* biomass: biomass treated with NaOH (upper spectrum), biomass treated with NaOH after contact with Pb ions(middle spectrum) and biomass treated with NaOH after contact with Hg ions (lower spectrum).

The FTIR spectra of NaOH treated biomass in the absence and in the presence of metallic ions in the range of 400-4000cm^{-1} were taken to obtain information on the nature of the possible interactions, they are presented in fig(6). By comparing between metals loaded biomass and that unloaded one, we can observe light differences in the number of wave of the dominant peaks (table2).

Table 2: Wave numbers of dominant peaks obtained from FT-IR transmission spectra of unloaded metals biomass, Biomass +Hg, Biomass +Pb

Sample	**Functional Groups**						
	O-H	**CH_2**	**C=O**	**Amides**		**C-O**	**C-O-C**
Biomass	3407,4	2920,3	1658,6	1545,8	1421,7	1378,0	1080,4
Biomass +Hg	3432,4	2919,4	1658,6	1546,8	1414,6	1379,1	1076,9
Biomass +Pb	3452,0	2920,7	1658,5	1547,1	1421,3	1382,9	1084,1

So bands at 3407 (indicative of –OH groups) moved to 3432 and 3452 for Hg and Pb loaded biomass respectively. A similar light shift was observed for other bands (like bands indicative of C-O and C-O-C groups).These light changes indicated the involvement of these functional groups in the biosorption process [16].

4. Conclusion

The effect of different pretreatments on biosorption properties of *Rhizopus stolonifer* biomass was studied in the present work. NaOH treatment gave the highest increase on biosorption ability among all pretreatment methods. NaOH treated biomass biosorption has been shown to be affected by pH, initial metal concentrations and contact time. The Langmuir isotherm model described appropriately the experimental data.The results indicate that *Rhizopus stolonifer* may be used as an effective and easily cultivable biosorbent for the removal of Pb and Hg from aqueous solutions and that alkali pretreatment is an effective method to improve the biosorption capacity for metal ions.

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Bioaccumulation of Heavy Metals by Non-living *Rhodococcus Erythropolis* B4.

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Abstract. The sorption of lead and mercury ions from aqueous solutions by dead biomass of *Rhodococcus erythropolis B4* was investigated in the batch mode. The influence of initial pH, initial concentration of ions and contact time were studied. The metal concentration was analyzed by Atomic Absorption Spectrophotometry (AAS). Analyses by The Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) were performed to show the interactions between cells and metals ions. Maximum sorption capacities of lead and mercury were found to be 75 $mg.g^{-1}$ and 300$mg.g^{-1}$ respectively. The Langmuir and Freundlich models were applied to the experimental data and the Langmuir model was found to be in better correlation with experimental data. Competitive biosorption experiments were performed with Pb^{2+} together with Hg^{2+}.

Keywords: waste-water treatment; Biosorption; *Rhodoccocus erythropolis*; Modelling; Adsorption; Heavy metals.

1. Introduction

The pollution of the environment with toxic heavy metals is spreading through the world along with industrial progress [1], [2]. The toxicity produced by lowest concentrations of heavy metals ions in industrial wastewaters is a subject of public concerns, since these ions can reach food chain and persist in nature [3]. According to the water standards used in most countries, levels of heavy metals ions in waste waters must be controlled and reduced to permissible limits [4]. Several methods are available for removing heavy metals, such as chemical precipitation, membrane filtration and ion exchange [2]-[5]. Since these traditional methods are often ineffective and/or very expensive when used for removal of heavy metals at very low concentrations, using of microorganisms offers a potential alternative[3], [4]. Biological process for removal of metal ions from liquids can be done by accumulation by viable microorganisms or by adsorption onto dead microorganism's surface [6]. Biosorption by dead biomass is relatively rapid and can be reversible. It involves physicochemical interactions between the metal and functional groups as ketones, aldehydes, carboxyls present on the microorganism's surface [2]-[7]. Among the group of bacteria, we can distinguish Gram positive and Gram negative. Cell wall of Gram negative bacteria which contains peptidoglycan are somewhat thinner and also not heavily cross-linked like the Gram positive ones which contains moreover teichoic acids, thus Gram positive bacteria have more potential binding sites for metal ions and are better biosorbent [8], [9]. The *Rhodococcus* genus, an aerobic gram positive, non motile, mycolate containing and belonging to the class of actinomycetes has a considerable importance in biotechnology applied to the environment, because of its high metabolic diversity and his wide range of enzymatic capacities[1]-[10].

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The objective of this work was the study the adsorption capacity of dead biomass of *Rhodococcus erythropolis B4* strain for lead and mercury, as a function of initial pH, initial metal ions concentrations and contact time.

2. Material and Methods

2.1. Microorganism and growth conditions

Rhodococcus erythropolis B4 strain was grown at 30 ℃ under agitation of 150 rpm, in a liquid medium containing 10 g l^{-1} glucose, 5g.l^{-1} peptone, 3g.l^{-1} yeast extract as well as 3 g. l^{-1} malt extract. Biomass was harvested after 24 h of incubation.

2.2. Metal solutions

Lead and mercury solutions were obtained by dissolving an accurate quantity of lead acetate $(CH_3COO)_2$ Pb, $3H_2O$, and mercury chloride ($HgCl_2$) in deionized water to obtain stock solutions of 1g l^{-1}.

2.3. Batch biosorption experiment

Factors affecting lead and mercury adsorption rate and biosorption capacity by *Rhodococcus erythropolis B4* were examined in a batch system. Kinetics of biosorption, isotherms of adsorption and influence of pH on the biosorption capacity of biomass were studied. The concentrations of non adsorbed metal ions by *Rhodococcus erythropolis B4* biomass were determined by means of AAS. Analysis by FTIR, SEM and EDX were performed to show interactions between cells and metals ions.

3. Results and Discussion

3.1. Effect of pH

The results presented in figure 1 show an adsorption increase with increasing pH values for both metals. At very acidic pH (2 and 3) we observe a low yield of biosorption, about 3% and 5% for Pb and 7% and 17% for Hg. It's only at pH 4 that we observe a significant biosorption increase of about 78% for Pb with a capacity of 139.37 mg g^{-1} and 85% for Hg with a capacity of 115.15 mg g^{-1} respectively. This allows us to say that the biosorption of metals by dead biomass of *Rhodococcus erythropolis* is strongly dependent on pH.

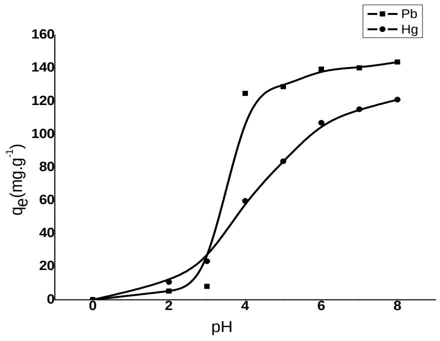

Fig. 1: Effect of pH on the biosorption capacity of Pb and Hg by dead biomass of *Rhodococcus erythropolis* (agitation speed: 150 rpm, weight of biomass: 0.01g, initial concentration of Pb and Hg: 10 mg l^{-1}, room temperature, contact time: 5h)

The Gram-positive cell walls are composed of linear polymers of peptidoglycan covalently tied together around the cell membrane [11]. The peptidoglycan forms a carboxyl and hydroxyl rich giant macromolecule [11]. The presence of these acid groups, especially the carboxylic ones, confers to the surface a very pH-dependent charge [12].

3.2. Effect of contact time initial metals concentration

The results presented in figure 2 show that more than 52 % of Hg and 92 % of Pb are absorbed only after 15 min of contact. The maximal elimination takes place after 5 hours of contact for Pb (97%) with a capacity of 45.90 mg g^{-1} and for Hg (93%) with a capacity of 75.72 mg g^{-1}.

In Fig. 3, it is shown that the increase of initial metal concentrations results in an increase of the biosorption capacity for both Pb and Hg. This behavior is explained by the fact that for high metals concentrations, there are a high number of ions in solution, implying thus a high adsorption capacity. Dursun (2006) [4] reported that the initial metal concentration provides a driving force to overcome mass transfer resistances between the biosorbent and biosorption medium. A stable biosorption capacity is observed beyond 75 mg l^{-1} concentration for Pb and 300 mg l^{-1} for Hg. This stability is due to the insufficient availability of sites for sorption in comparison to the number of molecules to be adsorbed. The phase of saturation is thus attained [13], [14].

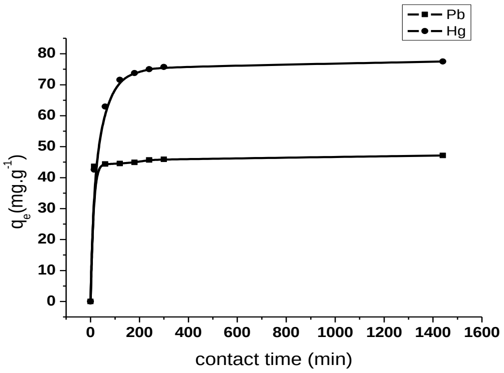

Fig. 2: Effect of contact time on the biosorption capacity of lead and mercury from dead biomass of *Rhodococcus erythropolis* (agitation speed: 150 rpm, biomass weight: 0.01g, initial concentration of Pb and Hg: 10 mg l^{-1}, pH: 5, room temperature).

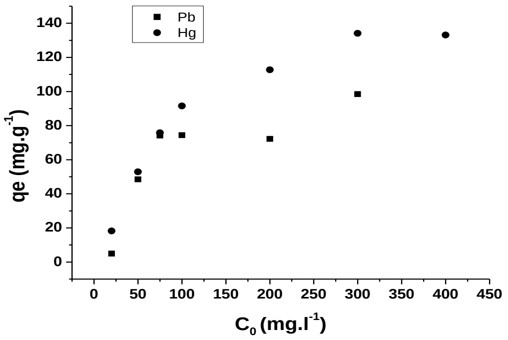

Fig. 3: Effect of initial concentration of metallic solutions on the biosorption capacity of Pb and Hg by dead biomass of *Rhodococcus erythropolis* (agitation speed: 150 rpm, weight of biomass: 0.01g, pH: 5, room temperature, Contact time: 24).

3.3. SEM and EDX analysis

SEM micrographs and EDX spectra obtained before and after contact of the dead biomass of *Rhodococcus erythropolis* with Pb and Hg are presented in figures 5 and 6.

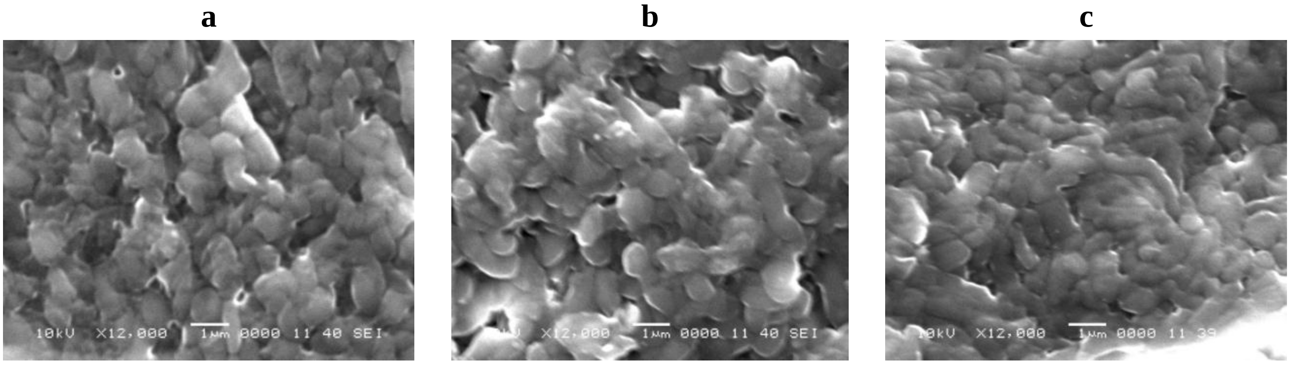

Fig. 4: Micrographs of the dead biomass of *Rhodococcus erythropolis* observed by SEM before (a) and after contact with the metal ions Pb (b) and Hg (c) (x G 12000).

The coccobacillar form of *Rhodococcus erythropolis* cells is well seen by SEM observations in Fig. 4. We distinguish a clear and distinct cluster of morphologically uniform cells (fig. 5a). These micrographs do not clearly reveal the presence of new particles on the cell surface after contact (Fig. 5 b and c). However, EDX analysis confirms the adsorption of metal ions. The characteristic peaks of Pb and Hg appear distinctly and confirm that the biomass has well fixed the metal ions (Fig.6).

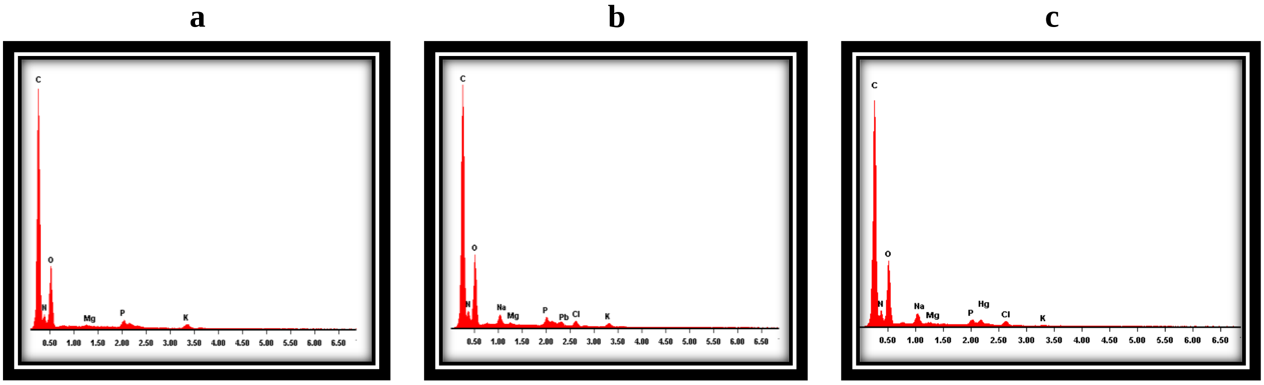

Fig. 5: Dead biomass of *Rhodococcus erythropolis* analyzed by EDX before (a) and after contact with Pb metal ions (b) and Hg metal ions (c).

3.4. FT-IR analysis

The FT-IR spectra of *Rhodococcus erythropolis* before and after contact with Pb and Hg are presented in Fig. 7.

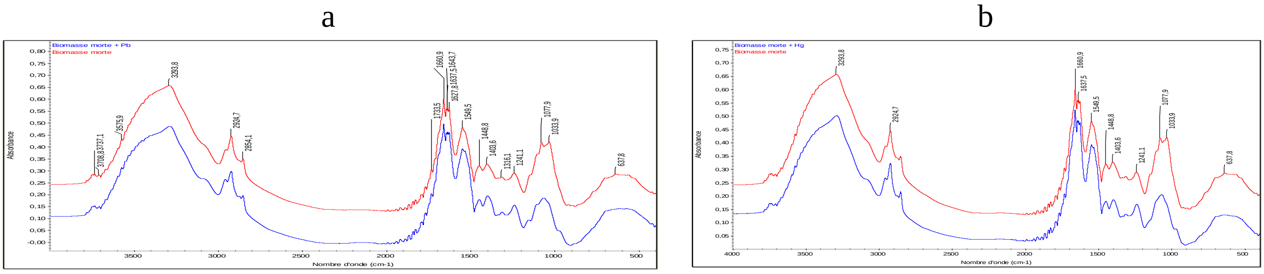

Fig. 6: FT-IR spectrum of dead biomass of *Rhodococcus erythropolis* before and after contact with Pb (a) and Hg ions (b).

The model of the IR biomass spectrum shows a distinct and a strong absorption at 3575 and 3293 cm^{-1} indicative of the existence of OH groups and NH groups [15]. The absorption peak at 2924 cm^{-1} can be assigned to a C–H group and the absorption peak at 1733 cm^{-1} is indicative of the C=O group . The absorption peak at 1077 cm^{-1} can be assigned to a –C–OH group [16] and the peak at 637 cm^{-1} represents the group $C–SH_2$. According to the obtained results, no difference is observed between the *Rhodococcus erythropolis* spectra before and after contact with the metal ions except that the absorbance of the peaks in the Pb and Hg loaded biomass is slightly lower than in the native one which indicate that there is a metal binding process taking place on that surface of the biomass.

3.5. Modeling of adsorption

The equilibrium sorption isotherms are one of the most important data to understand the mechanism of the biosorption (Fig. 8 a and b).

Results (Table 1) indicate that the Langmuir model describes well the data of lead and mercury equilibrium adsorption by dead biomass of *Rhodococcus erythropolis.* Maximum loading capacities obtained are 95,23 $mg.g^{-1}$ for lead and 147, 05 $mg.g^{-1}$ for mercury .These values approximate the experimental ones which are 98, 43 $mg.g^{-1}$ for Pb and 134, 07 $mg.g^{-1}$ for Hg. Lead and mercury adsorption occurs on a homogeneous surface by monolayer sorption with interactions between adsorbed molecules.

4. Conclusion

It is demonstrated that dead biomass of *Rhodococcus erythropolis B4* is a potential candidate for biosorption of lead and mercury from aqueous solutions. The process is pH, initial metal concentration and contact time dependant. Sorption of Pb and Hg on this strain is found to follow a monolayer type of adsorption. The experimental data fit well to the Langmuir isotherm model.

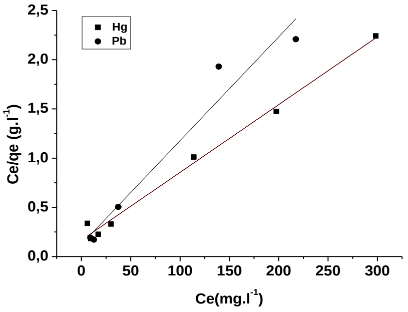

Fig. 7a: Linear transformation of the Langmuir biosorption of lead (●) and mercury (■) by dead biomass of *Rhodococcus erythropolis B4*

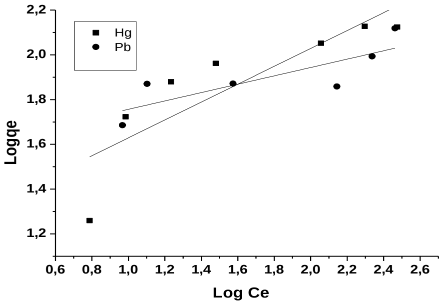

Fig. 7b: Linear transformation of the Freundlich biosorption of lead (●) and mercury (■) by dead biomass of *Rhodococcus erythropolis B4*

Table 1: Adsorption constants obtained from Langmuir and Freundlich models of dead biomass of *Rhodococcus erythropolis B4.*

Adsorbat	Langmuir model			Freundlich model		
	q_{max} (mg. g^{-1})	*b* (l. mg^{-1})	R^2	K_F (mg.g^{-1})	n	R^2
Pb^{2+}	95,23	0,085	0,94	4,75	5,37	0,60
Hg^{2+}	147,05	0,040	0,98	3,38	2,56	0,69

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