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GOLD AND SILVER BIOSORPTION KINETICS WITH *ASPERGILLUS NIGER*

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ABSTRACT

The kinetic of biosorption of gold and silver from aqueous media by using a living and dead biomass of fungus *Aspergillus niger* was investigated. For living biomass the adsorption show recoveries up to 80% and 40% for 1 mg/l of ionic gold and silver, respectively, whereas for 3 mg/l the maximum achieved recovery was near 52% and 70% for gold and silver, respectively. From experiments with dead biomass the cumulative recovery for gold was up to 95% and for silver, the achieved recovery, even in a case was 100%. All the metal recoveries occurred during the first 20 hours of contact. With the experimental data both the isotherm model and the suitable kinetic mechanism, which controls the biosorption phenomenon for this research were established. Most of the experimental results fit the adsorption kinetic model proposed by Elovich (also called the intraparticle diffusion model), suggesting that all the active sites are heterogeneously distributed along the biosorbent. Related to the adsorption isotherms for only dead biomass in contact with a metal in solution, the experimental curves are better described by the model of Langmuir, and by the conjugated model of Freundlich and Langmuir, for gold and silver, respectively.

KEYWORDS: Biosorption, biomining, *Aspergillus niger*, Elovich kinetics model, Langmuir-Freundlich isotherm model.

1.   INTRODUCTION

To date, the demand of gold and silver for industrial and medical applications, have increased, beside the fact that the purity of new ore deposits observes a decrease. These two aspects, together with environmental regulations, lead to develop new and more efficient procedures for recovering precious metals from either, conventional or alternative techniques.[1,2]

Ore processing through microorganisms is a novel technique based on the use of non-pathogenic bacteria, leaven, and fungus.[3,4,5] The capacity of some seaweed biomass to remove heavy metals from solutions, provides the basis for innovation recovery technologies, for instance to uptake precious metals dissolved in a liquid media by using fungus *Aspergillus niger*. In these recovery procedures through biosorption, the metals are retained by physicochemical interaction (adsorption, complexation, precipitation, chemical chystalization),

among living and dead biomass and a pregnant solution containing valuable metals.[5,6,7,8]

Pighi et al.[9], Pümpel and Schinner[10], and Gomez and co-workers[11], carried out studies trying to establish the tolerance or adaptability of some microorganisms to solutions from leaching of metals, and certain cyanide concentrations. The main findings from those investigations were the ability of the strain *Aspergillus*, to grow in the liquid media, and the adsorption of ionic metals by these fungus.

In the present investigation, the biosorption capacity of a fungal strain, referred to as *Aspergillus niger* 16404, to recover gold and silver from aqueous media was evaluated. With the experimental results the isotherm model was proposed, together with the suitable kinetic mechanism, which controls the biosorption phenomenon for this research.

2. MATERIALS AND METHODS

Materials and equipment

The strain of *Aspergillus niger* 16404 was obtained from the Center of Research and Advanced Studies (CINVESTAV), of the National Polytechnic Institute in Mexico city. This strain was seeded aseptically in both, test tubes and Petri dishes, with agar-based grown medium: (PDA) potato dextrose agar (P2182, Sigma-Aldrich), and (SDA) Sabourand dextrose agar (S3181, Sigma-Aldrich), placed in incubator (SHELL.LABV, mod. 1515) during 4 days at 26 °C.

Standard solutions of gold and silver were from Perkin-Elmer, used to prepare the solutions with known concentrations of metal.

Samples of dried biomass were analyzed by the following techniques: IR-Spectroscopy (Bruker, mod. Tensor 27); scanning electronic microscopy, SEM, (Jeol, mod. JSM-7600F); Transmission electronic microscopy, TEM, (Phillips, mod. TECNAIF20, 200KeV). A BRANSON 5510 ultrasonic bath was utilized to homogenize the samples, before determining the growth curves of the strain by using a UV-vis Spectrophotometer UNICO, mod. 52100. Metal analysis in the liquid media was done using an Atomic absorption spectrophotometer (PERKIN-ELMER, MOD. 3100).

Fungal biomass production

Living biomass production of *Aspergillus* was in a 350 ml of Sabouraud broth, the inoculums was 3 to 4 cultivations of PDA y SDA. The growing was in incubator during three days (27-29 °C), with agitation of 130 rpm. Dead biomass from living biomass was produced by sterilization using an autoclave at 15 psi, 120 °C, and during 15 minutes. The dry biomass was pulverized using a porcelain mortar.

Experimental procedure for adsorption kinetic studies

Kinetics describes the adsorption velocity of the adsorbate on the adsorbent, and shows this behavior as a function of time. Kinetic studies of removing gold and silver from aqueous solutions was performed using separately living and dead biomass. The initial concentrations of gold or silver were 1 and 3 ppm, and every experiment lasted 50 hours. pH of the media was adjusted adding NaOH or HCl. An Erlenmeyer flask was filled with 1 gram of either, dead or live biomass, and 15 ml of the metal solution. It was agitated at 130 rpm using a shaker bath. The sampling frequency varied from 10 minutes up to 12 hours. Samples were filtered using a cellulose membrane filters (pore size 0.45 µm), placed in a flask kitazato. The filtrates were subsequently analyzed.

Experimental procedure for adsorption isotherm studies

Metal solutions with an initial pH 6.4, and concentrations of gold or silver from 1 to 100 mg/l were used for isotherm studies. For every experiment, 0.2 grams of dead biomass were mixed with 100 ml of the metal solution in an Erlenmeyer flask, and agitated using a shaker bath at 130 rpm at room temperature. According to the results from the kinetic study, the sufficient contact time for each experiment was 20 hours. Like the procedure for the adsorption kinetic study, the samples were filtered, some were diluted, and subsequently analyzed.

3. RESULTS AND DISCUSSIONS

The fungus growth in the Sabouraud broth showed the four stages of development, as showed in Figure 1.
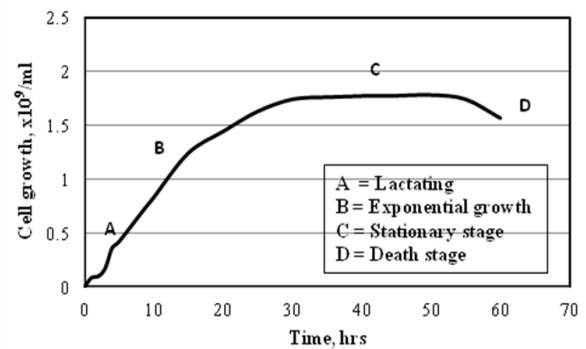

Figure 1: Growth curve of the fungus *Aspergillus niger* in Sabouraud broth.

Fungi adapted well enough to the experimental media, since the same growth was observed for experiments in presence of gold or silver, as it is shown in Figure 2.
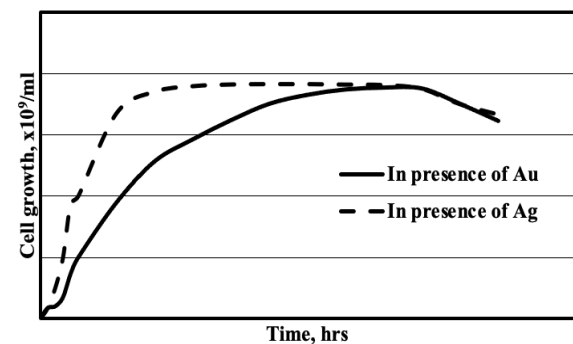

Figure 2: Growth curves of the fungus *Aspergillus niger* in the pregnant solution.

Adsorption kinetics of gold and silver. Living biomass.
Figure 3 shows the experimental results for a metal solution containing 1 and 3 mg/l of gold and living biomass of *Aspergillus niger*. A fast sorption of gold takes place during the first 10 hours, followed by a certain characteristic fluctuations of the stationery phase of growth of the microorganism; then, the recovery of gold from the solution continues, although slowly, until the equilibrium is reached approximately after 30 hours of the experiment, after this time the fungus is in the stage of death, although the recovery still proceeds but with dead biomass; in other words, biosorption occurs since it is independent of cell metabolism.[5]
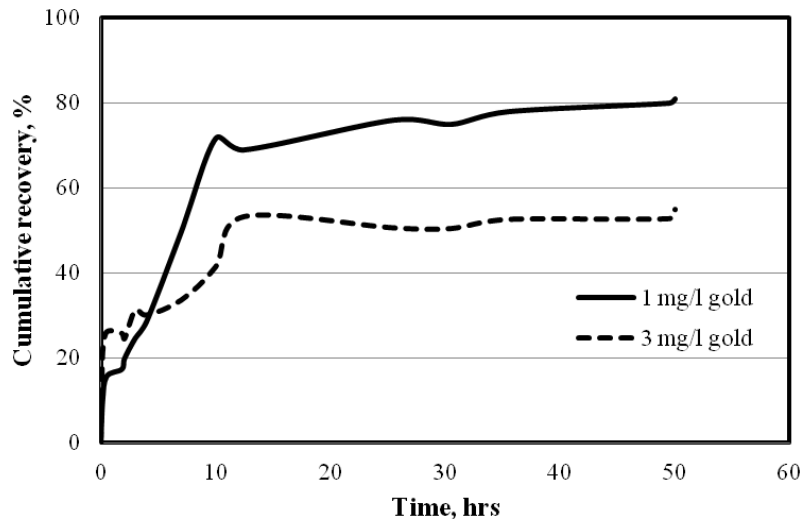
Figure 3: Gold recovery by the fungus *Aspergillus niger*. Living biomass. Initial gold concentrations: 1 and 3 mg/l.

For experiments with initial gold concentration 3 mg/l , the maximum recovery was around 50% suggesting a lack of biomass available for metal adsorption or active sites in the fungus. In the case of silver dissolved in aqueous medium, In general, the adsorption rate is lower compared to gold experiments. On the other hand, and contrary to what was observed in the case of gold, for 1 mg/l of silver the metal recovery is lower than the case of 3 mg/l. As a first approximation, it can be explained in terms of the higher electronegativity (Pauling scale) of gold, being 2.54, whereas for silver is 1.93.
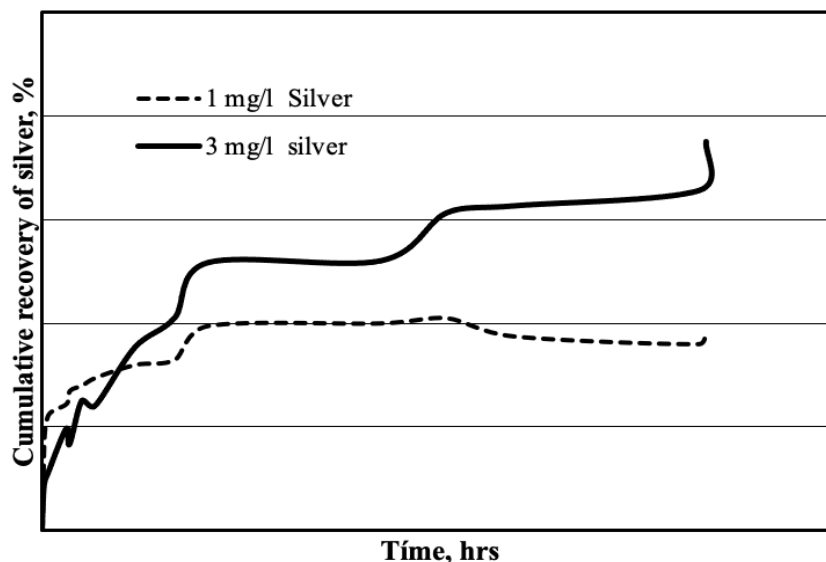

Figure 4: Silver recovery by the fungus *Aspergillus niger*. Living biomass. Initial gold concentration 1 and 3 mg/l.

The structure of the cell wall of *Aspergillus niger*, contains macromolecules such as polysaccharides and proteins with a large number of charged functional groups.[5,12] In order to support the statement that ionic gold or silver can be adsorbed on the *Aspergillus niger* surface, an infrared spectrum was obtained from the biomass, before and after the contact. The spectrum shows several functional groups corresponding to the cellular membrane of the fungus: a stretching vibration mode of –OH is present at 1100-1750 cm^{-1}, and 3000- 3700 cm^{-1}; among 2800 and 3050 cm^{-1} the characteristic bands of the CH_3 and CH_2 groups are detected; this last two functional groups are associated to the fatty acids of membranes or by the presence of some amino acids, and carbohydrates from the nutrients of the agar-based grown medium. It is important to note the disappearance of the peak at 1744 (after the contact), which corresponds to the carboxyl group that is the responsible for the attachment of metal ions.
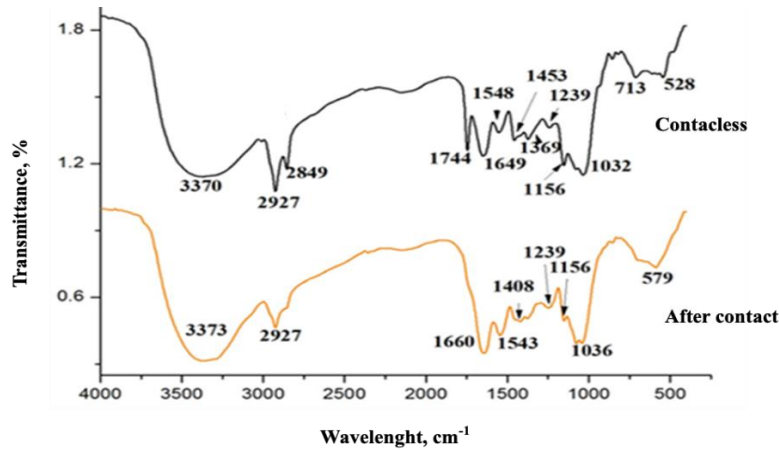
Figure 5: Infrared spectrum of the fungus *Aspergillus niger* biomass before and after the contact with gold in solution.

Adsorption kinetics of gold and silver. Dead biomass.
Figure 6 shows the cumulative recovery of gold by dead biomass of *Aspergillus niger*. The liquid medium contained 1 and 3 mg/l of gold at room temperature. Clearly the metal is adsorbed on the Aspergillus powder, achieving more than 90% of metal recovery during the first 20 hours of the experiment.
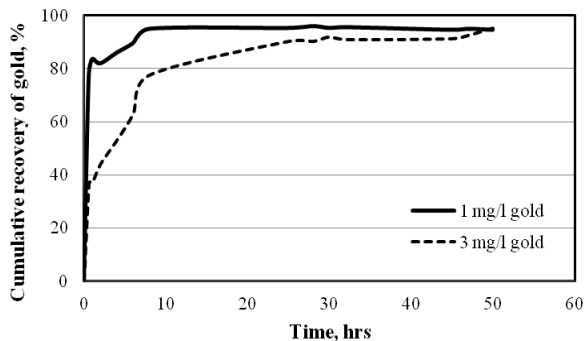

Figure 6: Recovery of gold by dead biomass of *Aspergillus niger*, at room temperature.

Regarding silver recovery as shown in Figure 7, the time for the equilibrium lasted less than 10 hours, and the maximum recoveries were 100%.
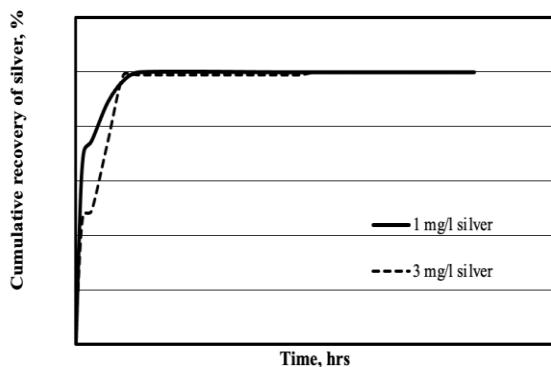

Figure 7: Recovery of 1 and 3 mg/l of silver in liquid media by dead pulverized biomass of *Aspergillus niger*, at room temperature.

Experimental data and the adsorption kinetic models
The adsorption kinetic describes the velocity of capture of the absorbate, controlling the residence time of the dissolved species in the solid-liquid interface.[13,14] The adsorption capacity (q_e) was calculated according to:

$$q_e = \frac{(C_0 - C_e)V}{W}$$

(1)

Where C_0 is the initial metal concentration (mg/l), C_e is the final or equilibrium concentration (mg/l), V is the volume of the metal solution (ml), and W is the weight of the biomass. The mathematical models describing the type of adsorption mechanism are[15]:

The pseudo-first order equation proposed by Lagergren:

$$q_t = q_e \left(1 - e^{-k_1 t}\right)$$

(2)

Ec. (2) takes the linearized form:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303}$$

(2a)

Where k_1 (min^{-1}) is the pseudo-first order kinetic constant, q_t (mg/g) is the adsorption capacity at time t. The pseudo-second order kinetic equation developed by Ho and McKay[14] is as follows:

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

(3)

Being k_2 (g/mg-min) the kinetic constant. This model supposes that the adsorption phenomenon is controlled by chemisorption.

Meanwhile the model derived by Elovich[16], called the intraparticle diffusion model, supposes that all the active sites are heterogeneously distributed along the biosorbent, being such fact the cause of variable activation energy values.

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln t$$

(4)

Where α is the initial velocity of sorption (mg/g-hr), and β is the constant of desorption (g/mg). Figures 8 and 9 show the adsorption capacity (q_t) for the two tested concentrations of gold, graphed against time, for living

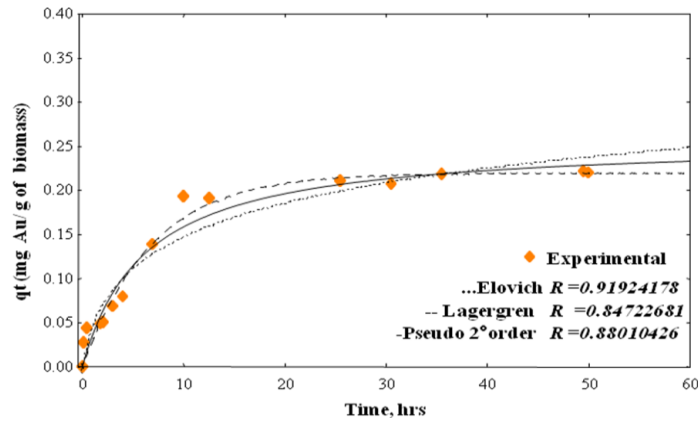biomass, and compared with the three kinetic models described through equations (2a), (3), and (4).

Figure 8: Experimental results adjusted to the adsorption kinetic models, for the case of 1 mg/l of gold. Living biomass.
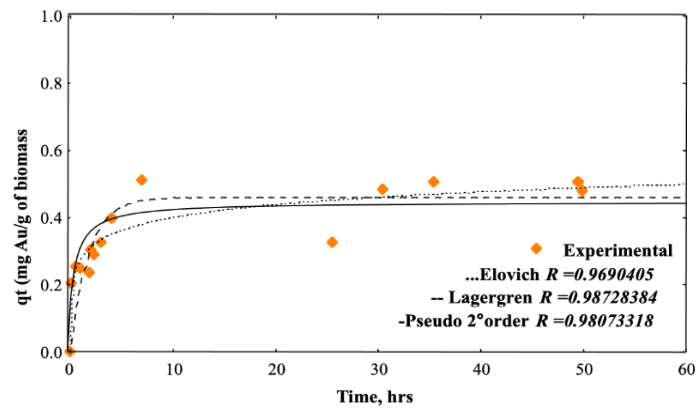

Figure 9: Experimental results adjusted to the adsorption kinetic models, for the case of 3 mg/l of gold. Living biomass.

From these figures, as indicated by the highest values of the correlation coefficient (R^2), the Elovich´s approach fits better to the experimental results of biosorption of 1 mg/l of gold, where the rate limiting-step is caused by the fact that every metal ion is attracted to an active site of the biosorbent. Elovich´s equation has been used in systems highly heterogeneous.[17] For 3 mg/l of gold, the pseudo-first order kinetic equation of Lagergren has the higher correlation coefficient, implying that chemisorptions is the rate controlling mechanism. According to Sheng and co-workers[18], this could indicate that a covalent bond is formed between the metal ion and the biomass.

Figures 10 and 11 show the adsorption capacity against contact time for the system with 1 and 3 mg/l of silver, for living biomass.
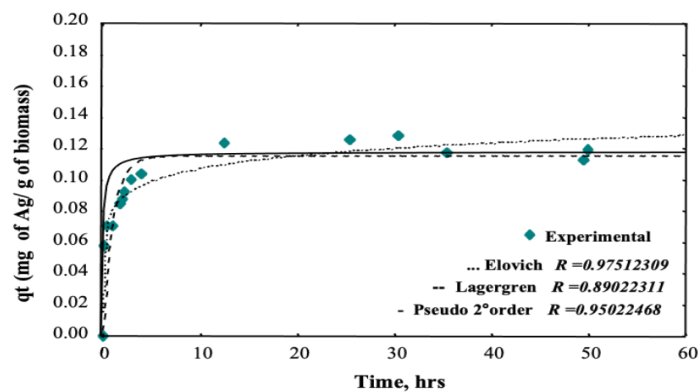

Figure 10: Kinetic models compared to the experimental results for contact among living biomass and 1 mg/l of silver.
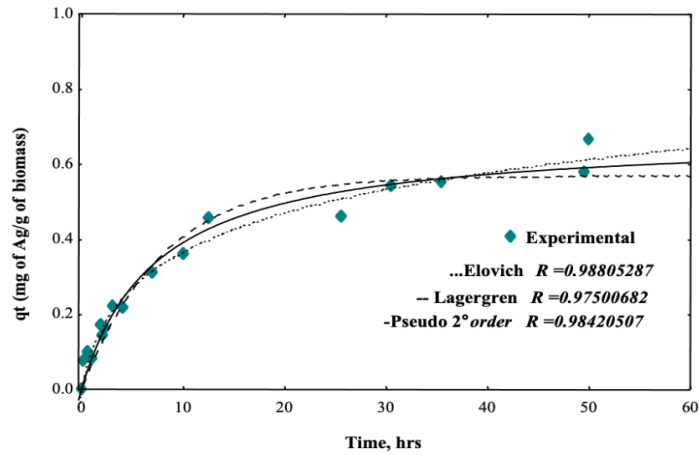
Figure 11: Kinetic models compared to the experimental results for contact among living biomass and 3 mg/l of silver.

The experimental data for the two silver concentrations fit the Elovich´s model; although, for contact with 3 mg/l, the correlation coefficient values of Elovich and pseudo-second order kinetic equations are very close.

Table 1 shows a comparison of kinetic parameters for pseudo-first order, pseudo-second order, and the intraparticle mechanism, for systems of contact between gold or silver in solution and living biomass, at room temperature.

Table 1: Calculated parameters for the three adsorption kinetic models included in this research. Contact among gold or silver ions and living biomass.

mg/l of metal	Pseudo-first order (Lagergren)			Pseudo-second order (Ho and McKay)			Intraparticle model (Elovich)		
	k_1, 1/hr	q_e, (mg/g)	R^2	K_2, 1/hr	q_e, (mg/g)	R^2	α (1/hr)	β (mg/g)	R^2
Au, 1	0.1466	0.2198	0.8472	0.3133	0.2576	0.8801	0.0686	17.1647	0.9192
Au, 3	0.5607	0.4593	0.9872	1.2447	0.4688	0.9807	8.2563	18.2691	0.9690
Ag, 1	0.1152	0.9786	0.8902	9.6691	0.1182	0.9502	10.6323	84.7273	0.9751
Ag, 3	0.1258	0.5695	0.9570	0.1007	0.6776	0.9842	0.1421	6.2023	0.9880

From Table 1 and for 1 mg/l of gold, the values of the adsorption rate parameters (K_i, K_2, and α) are very similar; nevertheless, the difference of the correlation coefficients indicates that the pseudo-second-order model is that controlling the system, and it is possible to infer that the active sites in the biomass are heterogeneously distributed and the dominant mechanism is the chemisorption.

Regarding the experimental results with powder of dead biomass in contact with 1 mg/l of gold, from Figure 12 is observed that the three kinetic models accurately predict the mechanism of adsorption, corresponding the maximum correlation coefficient value to the model proposed by Elovich. In the case of 3 mg/l, also the intraparticle model fits better the experimental values.
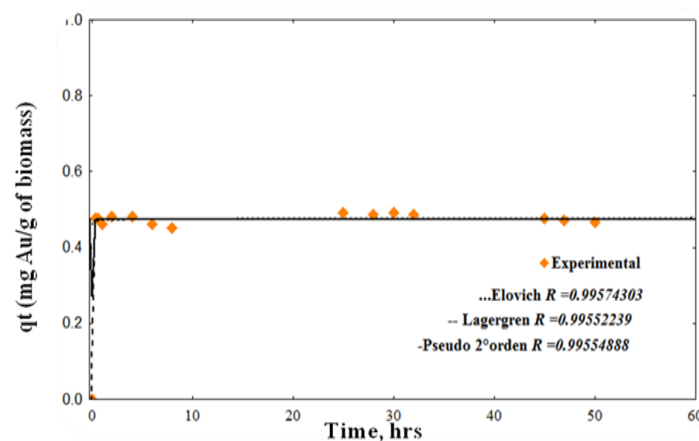

Figure 12: Adsorption capacity of powder of dead biomass in contact with 1 mg/l of dissolved gold.
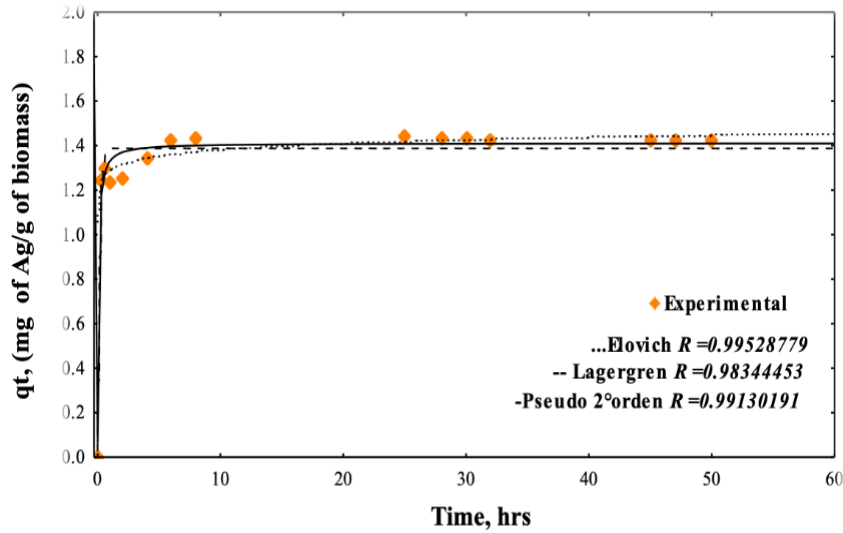
Figure 13: Adsorption capacity of powder of dead biomass in contact with 3 mg/l of dissolved gold.

The kinetic adsorption curves for the same conditions as figures 12 and 13, but for silver ion, shows that for 1 mg/l, the mechanism of sorption is well described by the pseudo-second order model (covalent bond takes place), while in the case of 3 mg/l of silver concentration, the controlling mechanism is that proposed by Elovich.
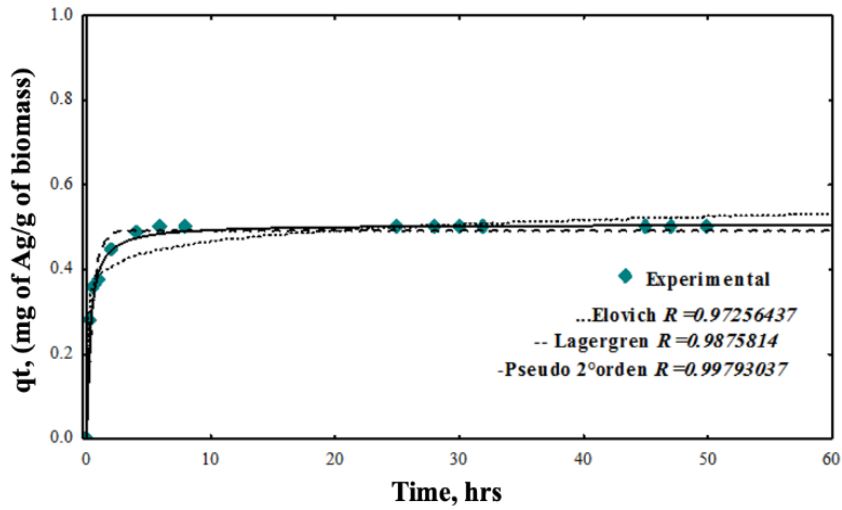

Figure 14: Kinetic adsorption curves for the case of a solution with 1 mg/l of silver ion. Dead biomass.
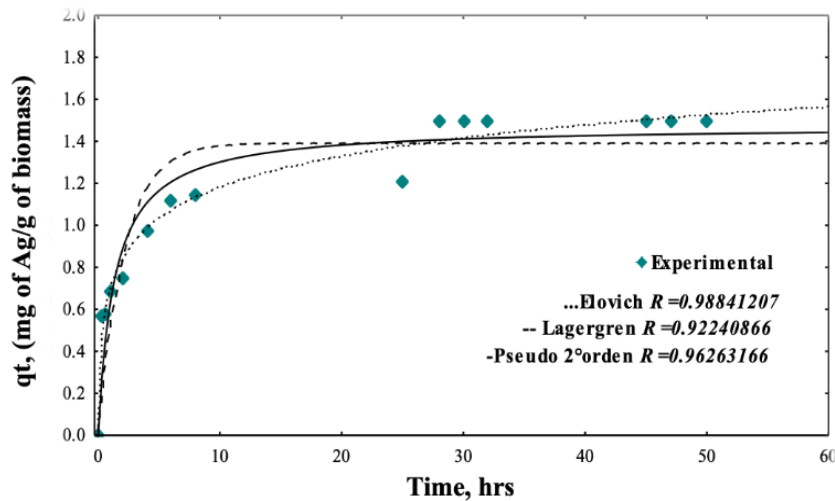

Figure 15: Kinetic adsorption curves for the case of a solution with 3 mg/l of silver ion. Dead biomass.

Table 2 includes the calculated kinetic parameters for systems with dead biosorbent in contact with gold or silver in solution.

Table 2: Calculated parameters for the three adsorption kinetic models included in this research. Contact among gold or silver ions and dead biomass.

mg/l of metal	Pseudo-first order (Lagergren)			Pseudo-second order (Ho and McKay)			Intraparticle model (Elovich)		
	k_1, 1/hr	q_e, (mg/g)	R^2	K_2, 1/hr	q_e, (mg/g)	R^2	α (1/hr)	β (mg/g)	R^2
Au, 1	58.273	0.4742	0.9955	464.88	0.4748	0.9955	1.5020	5.6725	0.9957
Au, 3	6.4131	1.3873	0.9834	50.861	1.4111	0.9913	2.7177	2.4710	0.9952
Ag, 1	0.4926	2.0230	0.9875	3.5761	0.5072	0.9979	1535.39	27.724	0.9725
Ag, 3	0.4661	1.3919	0.9224	0.2558	1.4738	0.9626	5.3982	4.6773	0.9884

Adsorption isotherms of dead biomass
In this part of the research only dead fungal biomass was experimented, because of the best recovery results. Rodríguez and Cortés[19] mention than according to Giles (see figure 16), the shape of the isotherms is related to the mechanism of adsorption, the nature of the adsorbate, and the surface characteristic (electrical) of the adsorbent. From the above, the shape of isotherms corresponds to the L type and the group IV.
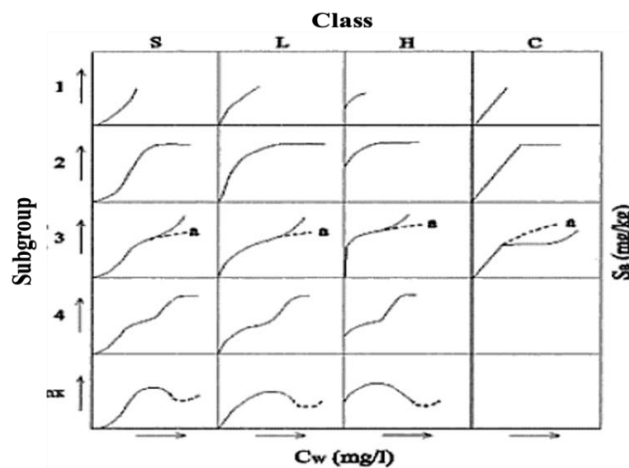

Figure 16: Isotherms classification according to Giles as reported by Rodríguez and Cortés.[19]

According to figure 16, the shape of the isotherm curves for gold and silver dissolved separately in de-ionized water (see Figure 17), corresponds to the L type and to the group 4. The form of the curves indicates a great affinity between the metal ions and the adsorbent for low metal concentrations. The slope of the curve decreases with the concentration of the dissolved metal, because as the adsorption proceeds, the difficulty in finding active sites by the metal decreases. Figure 17 shows the isotherm curves for gold and silver dissolved separately in de-ionized water.
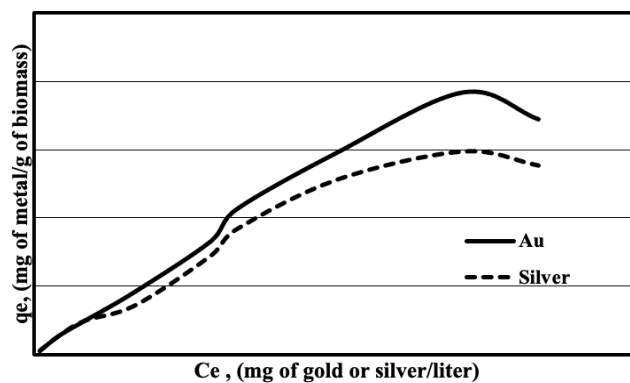

Figure 17: Biosorption capacity (q_e) of gold and silver by the dead biomass *Aspergillus niger*, as a function of the equilibrium concentration in the solution (C_e).

Regarding the traditional models describing the maximum adsorption capacity of sorbents [14, 20], Langmuir assumes that the adsorbent presents homogeneous active sites, the adsorption occurs only in a monolayer, and there is not interaction between the adsorbed molecules. Mathematically this model is represented by:

$$q_e = \frac{Q_0 \, b \, C_e}{1 + b \, C_e}$$

(5)

Where Ce is the concentration of solute (mg/l), Q_0 the amount of solute adsorbed by unit of mass of the adsorbent (mg/g), and b is the Langmuir constant (l/mg), that is the adsorption energy.

Meanwhile Freundlich derived an empirical model trying to describe the non ideal adsorption mechanism, and supposes that the multilayer adsorption occurs. This model is expressed as follows:

$$q_e = K_F \, C_e^{1/n}$$

(6)

Where K_F and n are the constants of the system itself. The experimental information was analyzed through the program STATISTICA 7.

Figure 18 shows the correlation analysis of the experimental data regarding three traditional models describing the maximum adsorption capacity of sorbents; the adsorption isotherm is for dead biomass in contact with gold in solution.
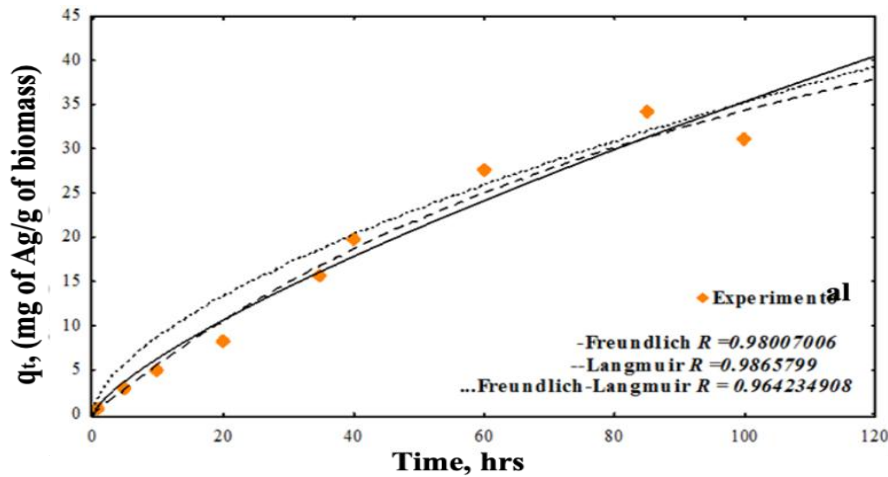

Figure 18: Regression analysis according to three mathematical models relating the maximum adsorption capacity of sorbents. Dead biomass in contact with gold in solution.

As can be observed the experimental results fit better to the model of Langmuir, establishing that the metal is adsorbed homogeneously by the biosorbent.

The adsorption isotherm in the case of silver shows through Figure 19 that the best fit corresponds to the conjugated model of Freundlich and Langmuir. As can be inferred, the silver adsorbs heterogeneously on the surface of the fungus, may be in multilayers, decreasing the amount of captured silver by the *Aspergillus niger*.
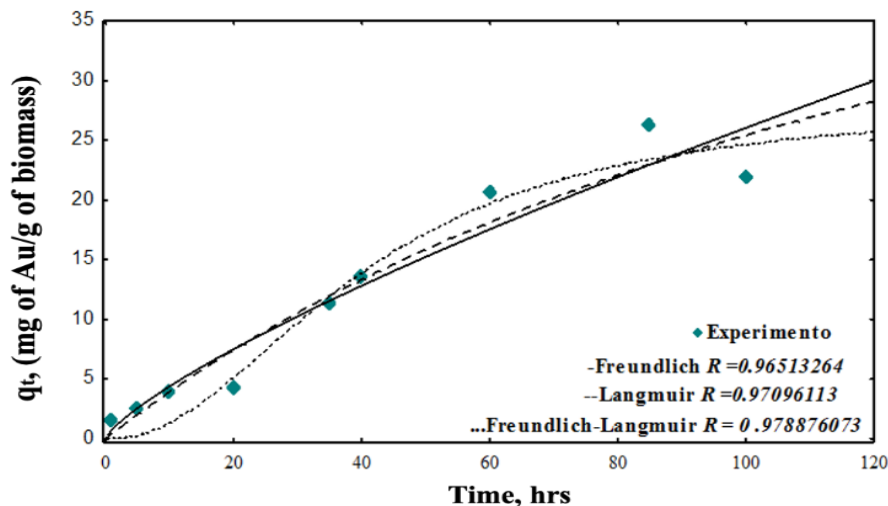

Figure 19: Regression analysis of experimental data of adsorption capacity of silver in solution by dead biomass of *Aspergillus niger*.

4. CONCLUSIONS

Experimental results after contact of Aspergillus niger with solutions containing ionic gold or silver show recoveries up to 80% and 70% for gold and silver, respectively, for living biomass. For dead biomass the recovery values of both metals were higher than 90%. All the metal recoveries occurred during the first 20 hours of contact.

Most of the experimental results fit the adsorption kinetic model proposed by Elovich (also called the intraparticle diffusion model), suggesting that all the active sites are heterogeneously distributed along the biosorbent.

Related to the adsorption isotherms for only dead biomass in contact with a metal in solution, the experimental curves are better described by the model of Langmuir, and by the conjugated model of Freundlich and Langmuir, for gold and silver, respectively.

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