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Evaluation the Biosorption Properties of Three *Bacillus* Strains for Cu^{2+} Ions Uptake from Wastewater

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Abstract
Introduction: Toxic metal ion contamination is one of the most important environmental issues in the world. Wastewater of mines and certain industries are known sources of environmental heavy metal contaminations. Copper (Cu) contamination is a common issue. Using biological tools particularly bacterial-derived compounds could be appropriate for bioremediation.
Materials and Methods: In this study, the Cu^{2+} uptake from Chah Musa mine wastewater by three different *Bacillus* species was assessed using the batch equilibrium isotherm. The impact of pH, exposure time, temperature, and the bioabsorbent dose on the adsorption of Cu^{2+} ions was assessed. Also, the kinetics and isotherm models of Cu^{2+} ions adsorption were studied for three different *Bacillus* species.
Results: The results showed that the maximum removal Cu^{2+} ions (99%) was obtained for *Bacillus thuringiensis* biomass. The correlation coefficient value (R^2) of Freundlich isotherm was higher than Langmuir isotherm indicating better metal uptake based on Freundlich model. he rates of adsorption for all bioadsorbant were attained to be conforming to the pseudo-second-order.
Conclusions: According to the current study all three dead Gram-positive *Bacillus* spp. are favorable and effective candidates for the removal of Cu^{2+} from aqueous solution and industrial wastewaters.
Keywords: Biosorption, Microbial Dead Cells; Mine Wastewater; Copper Ion; *Bacillus*
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Introduction

Heavy metal contamination has always been an environmental problem since the introduction of mine and certain petro-chemical, refinery, pulp and paper, fertilizer, steel, textile, plastic, and ceramic industries.[1-3] The most prevalent reported contaminations are due to zinc, chromium, Cu, lead, mercury, cadmium, nickel, cobalt and iron in the wastewater of such industries.[3] Excessive amounts of heavy metals in soil, groundwater and food chains due to inappropriate management of wastewater can have detrimental impact on several organisms in ecosystems including humans, plants and animals leading to the accumulation of heavy metals in the living tissues.[4] The copper ions are the main metal pollutant in the wide range of industries. Copper accumulation in the liver, brain, stomach, heart and skin can cause certain disorders and tissue damages.[5]

There are various physico-chemical strategies such as precipitation, electrochemical destruction, uptake by activated carbon, coagulation, membrane technology, chromatogeraphy etc., to remove the metal ions in industries.[6-8] However, the use of above methods is limited owing to some disadvantages including the production of toxic compounds, remaining unfavorable color and the nonselective uptake of metal ions.[1,9] Alternatively, biological strategies can be used for bioremediation. Both Gram-positive and Gram-negative bacteria, algea, and fungi are common microorganisms having potential abilities to remove heavy metals from the environment.[10] Compared to other methods, microbial treatment is cheaper, much simpler, more accessible and causes regeneration of bioabsorbents leading to more efficient removal of heavy metals.[4,11] Both dead and live microorganisms have been applied for bioabsorption. Dead microorganisms are preferred over the live ones because obviously there is no need for nutrients nor adverse toxic effects on biomass. Bacterial cell wall is the first component that comes into contact with the metal ions. Metal ions can be adsorbed onto or penetrate into the cell wall structure.[12] Since the ion uptake by dead and inactive cells happen extracellulary, functional chemical groups of cell wall play a key role in the biological absorption of metal ions. Regarding the nature of cell walls, there are various functional groups such as carboxyl, phosphonate, amine and hydroxyl.[13] The presence of negatively charged carboxyl groups in the peptidoglycan of Gram-positive bacteria contribute in the attachment of cationic metal ions and their clearance.[14]

In this study, the behavior of three different dead Gram-positive bacteria, *Bacillus thuringiensis*, Bacillus *subtilis* and *Bacillus pumilus* was assessed for the removal of Cu^{2+} from mining wastewater. The impact of experimental conditions incluing, pH, temperature, exposure time, the quantity of bioabsorbant and Cu^{2+} concentration on the uptake of Cu^{2+} was assessed using the batch equilibrium method.

Materials and Methods

Bacteria and Culture Condition

Bacillus thuringiensis (PTCC1385), *Bacillus subtilis* (PTCC1254) and *Bacillus pumilus* (PTCC1529) were purchased from the Industrial Research Organization, Iran. Each microorganism was inoculated in Erlenmeyer flasks containing 500 ml brain-heart infusion broth (BHI, Merck, Germany). The media were incubated in a shaker incubator at 37 °C and 150 rpm overnight. The biomass pellet was obtained using centrifugation at 5,000 rpm for 20 min. To obtain the bioabsorbent powder, the biomass pellet was dried at 60 °C for 24 h and milled to prepare homogenous powder.

Wastewater Collection

The wastewater containing Cu^{2+} ions was collected from Chah Musa region, Semnan, Iran. The Cu^{2+} concentration of wastewater was 460 mg/L. Also, the wastewater contained 50 mg/ml cadmium and 230 mg/L zinc ions.

Biosorption Analysis

To optimize the variables in the Cu^{2+} ion uptake, the biosorption experiments were done according to the batch equilibrium method. To assess the impact of pH, exposure time, temperature, and the bioabsorbent quantity on the adsorption of Cu^{2+} ions, the wastewater stock was diluted 10 times.

Analysis of the Impact of pH, Temperature and Contact Time

To find the optimum values, different pH conditions including 1- 8 were tested. Different temperatures such as 22, 37 and 46 °C were surveyed in the waste solution containing 46 mg/L Cu^{2+} ions and 1 g/L dried biomass powder. After 5 h shaking, the solutions were centrifuged at 12,000 rpm for 5 min and the supernatant was transfered to a tube for further investigations.

Moreover, a wide range of contact times to the biomass including 0-300 min were tested to obtain the equilibrium uptake in the waste solution containing 46 mg/L Cu^{2+} ions and 1 g/L dried biomass powder. After the desired contact time plus shaking, the samples were centrifuged at 12,000 rpm for 5 min and the supernatant was transfered to a tube for further investigations.

Analysis of the Effects of Initial Ion Concentration and Bioabsorbant Dose

To assess the impact of initial ion concentration on the biosorption equilibrium, several dilutions of the ion were prepared serially and was analyzed using 1 g/L biomass. Also, different amounts of bioabsorbent ranging from 0.1 to 0.9 g/L were assessed to evaluate the efficacy of bioabsorbent on equilibrium. To separate the supernatant, all samples were centrifuged at 12,000 rpm for 5 min after 5 h agitation. To measure the Cu^{2+} ion concentration, all samples were analyzed using Pollarogeraph (Metrohm, Switzerland). All tests were carried out three times, the deionized water and the standard solution containing 46 mg/L Cu^{2+} ions without absorbent was examined as controls.

Results

The Effect of pH on Ion Removal

PH is one of the main factors in the removal of Cu^{2+} ion. As illustrated in Figure 1a, the optimum pH for removal of Cu^{2+} ions against all three tested bacterial biomass was around 6. Furthermore, there was a sharp increase in Cu^{2+} absorption above pH 5 to 6 in all cases. There was no noticable absorption at the pH lower than three and higher than six in all conditions.

The Effect of Temperature on Ion Removal

As shown in Figure 1b, three tested bacterial biomasses differ in absorbing behavior at different tempretures. Increasing the temperature from 22 to 37 °C caused an increase of Cu^{2+} absorption by *B. thuringiensis*, but in the case of *B. subtilis* and *B. pumilus*, higher Cu^{2+} removal happened at 22 °C and the temperatures above 22 °C caused decreased absorption

The Effect of Contact Time on Ion Removal

It is observed in Figure 1c that the removal percentage and adsorption capacity of Cu^{2+} ion increased with increasing contact time. The removal rate of all the bioadsorbents were extremely high in the first 30 min. Also, increases of the contact time after 5 min does not have a significant effect on the capacity adsorption but the removal percentage increases with a slight slope.

The Effect of Biosorbent Dose on Ion Removal

As illustrated in Figure 1d, increasing the amount of biosorbent augments the percentage of Cu^{2+} removal. Upon *B. thuringiensis* treatment, increasing the amount of bioabsorbat

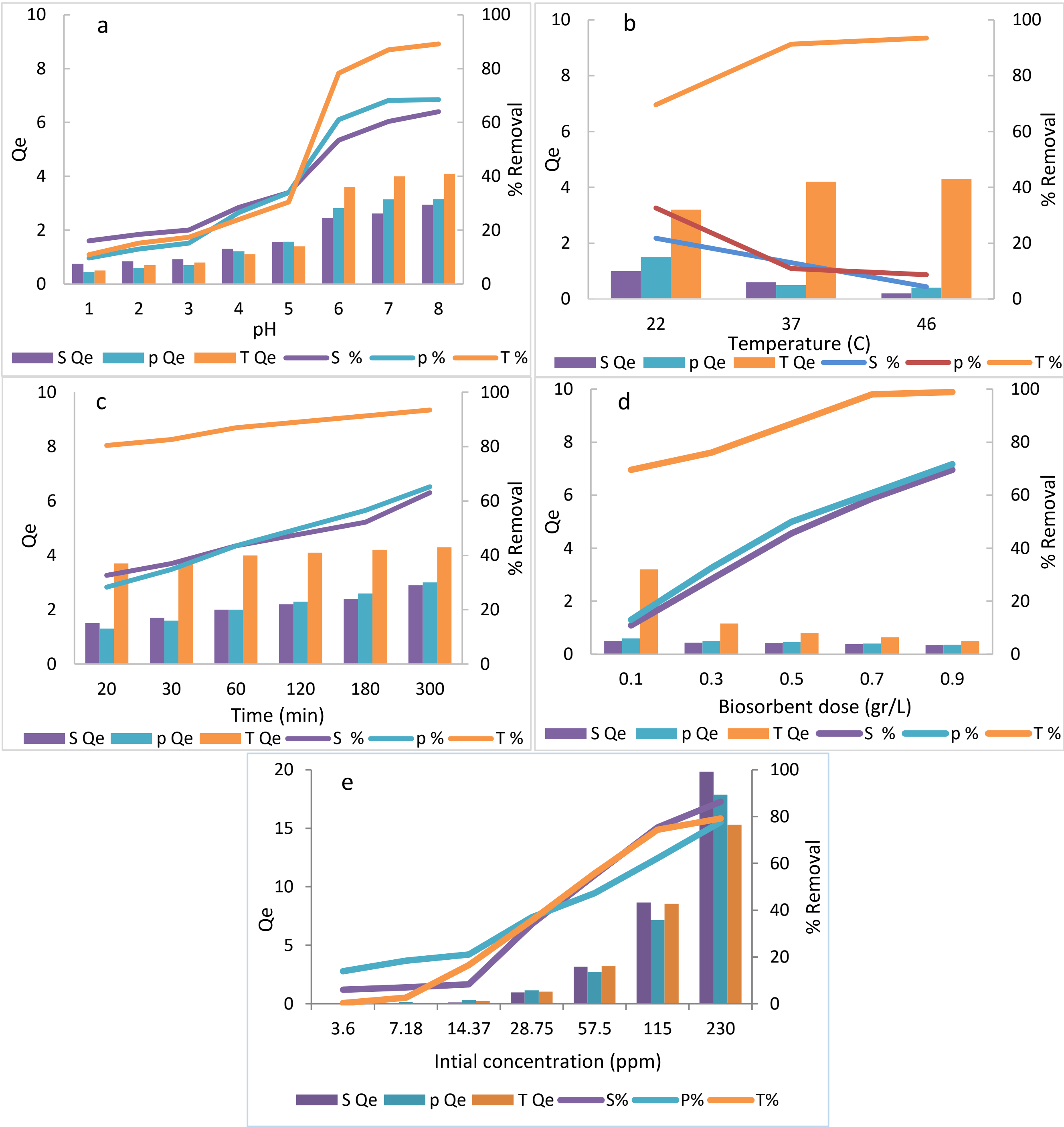

Figure 1. Effects of initial pH (a), temperature (b), bioadsorbent dose (c), contact time (d), and initial concentration (e) on the adsorption capacity and percentage of Cu^{2+} removal.

to 0.7 g/L enhances the ion uptake to 98%, yet the increase of the bioabsorbent dose to 0.9 g/L had no significant effect on ion removal. However, our findings revealed that for *B. subtilis* and *B. pumilus*, increasing the amount of bioabsorbat to 0.9 g/L enhances the ion uptake to about 70%.

The Effect of Initial Ion Concentration on Ion Removal

The amont of initial ion had an important impact on the absorption equilibrium. As shown in Figure 1e, the increase of Cu^{2+} concentration causes higher absorption and uptake of ions by all three tested bacteria having a sharp slope.

Adsorption Isotherm

The assessment of adsorption equilibrium is crucial to find the proper equation for designing and improving the system. Two common adsorption isotherm, named the Langmuir and Freundlich isotherm, were applied to identify the uptake capacity of component by absorbent at equilibrium state with the constant time and temperature. Both described isotherm models along with another isotherm model, Scatchard, were surveyed using different initial concentrations of Cu^{2+} ions ranging from 3.6 to 230 mg/L at equilibrium condition.

Data from the Langmuir equation is appropriate to evaluate the adsorption capacity on the monolayaer surface with similar defined adsorption sites. The Langmuir equation (1) that was used here to calculate the data is presented as follows:

Equation (1): $c_{eq} / q_{eq} = 1/bq_{max} + c_{eq}/q_{max}$

Figure 2 shows the findings fom the Langmuir isotherm analysis for three tested bacteria.

Furthermore, the Freundlich and Scatchard isotherms for all tested bacteria were measured using the equation (2) and (3), respectively:

Equation (2): $\log q_{eq} = \log k_f + 1/n \log c_{eq}$

Equation (3): $q_{eq} / c_{eq} = q_{max} k_b - q_{eq} k_b$

In accordance with outcomes summarized in Table 1, in all experiments, R^2 is between 0 and 1 that indicates the direct but imperfect correlation between absorption level and metal ion concentration. Furthermore, R^2 value of Freundlich isotherm was higher than Langmuir model indicating the metal uptake more efficiently according to Freundlich model.

Adsorption Kinetics

In this section, the kinetic data of Cu^{2+} ion absorption was fitted to pseudo-first-order and pseudo-second-order models. The pseudo-first-order is expressed in equation (4):

Equation (4): $\log(q_e - q_t) = \log q_e - (\frac{K_1}{2.303})t$

Where K_1 is the rate constant of pseudo-first order (min^{-1}) and q_e and q_t (mg g^{-1}) are the adsorption capacity of Cu^{2+} ion at equilibrium and at time t (min), respectively. The results are shown in Figure 2a.

On the other hand, the pseudo-second order is shown as equation (5),[15]:

Equation (5): $\frac{t}{q_e} = \frac{1}{K_2 q_e^2} + (\frac{1}{q_e})t$

The qe, K_2 and the initial adsorption rate (kq_{e2} = h (mg $g^{-1}min^{-1}$)) were calculated by the slope as well as plot intercept of t/q_t versus t and results are shown in Figure 2b.

The R^2 values of pseudo-first-order for all bioadsorbent were lower than the the R^2 values of pseudo- second-order. Based on the R^2 values in the pseudo-second-order, the rates of adsorption for all bioadsorbant were attained to be conforming to the pseudo-second-order with a very good correlation coefficient.

Table 1. Isotherm Model Parameters for Cu^{2+} Ion Absorption from Wastewater

Isotherms	**Langmuir**			**Freundlich**			**Scatchard**		
Parameters	R^2	b	q_{max}	R^2	k_f	n	R^2	k_b	q_{max}
B. thuringiensis	0.813	0.025	6.724	0.9518	8.21×10^{-6}	0.21	0.9922	0.0311	4.74
B. subtilis	0.886	0.017	0.56	0.911	7.44×10^{-4}	0.371	0.998	0.0315	0.492
B. pumilus	0.665	0.028	0.959	0.9031	1.04×10^{-2}	0.563	0.975	0.0179	1.8

Discussion

Recently, the application of microorganisms' biomass and dead biologic materials for removing pollutants such as heavy metals and dyes is on the rise. Bacterial cell wall is the first part encountering metal ions. Metal ions precipitate on the surface of the cell wall or penetrate into the cell wall structure. Therefore, the functional groups including carboxyl, phosphonate, amine and hydroxyl on the surface of cell wall play a key role in the removal of metal ions. Negatively charged carboxyl groups in the peptidoglycan of Gram-positive bacteria actively bind to cationic metals. In addition, amine groups remove cationic metals through chelating metals and absorbing them via electrostatic or hydrogen bonds.[12,13] Actually, pH is one of the main factors in the ion uptake. Generally, high pH levels produce total negative charge of the cell surface due to loss of protons, making it suitable for electrostatic interactions and absorption of metal ions.[16,17] On the other hand, increasing pH levels leads to hydrolysis of metals depending on the type of metal and pH value. In most cases, the absorption occurs in the alkalin pH presenting a problem for evaluating a potential of absorbant owing to metal precipitation.[17] Consistent with previous reports, pH value between three to six is appropriate for bacterial biomass because of the presence of carboxyl groups on the cell surface.[16-18] In pH levels below three, all the anionic groups on the surface of Gram-positive bacteria bind to proton and cannot contribute to metal ion removal.[19]

Furthermore, according to our data, temperature changes in a narrow range between 25-30 °C impacts on absorption by bacterial biomass. Higher temperatures usualy enhance absorption via increasing surface activity and kinetic energy of solvents.[20] However, very high tempreatures decrease the metal absorption due to physical damage of cell surfaces.[21] The optimum temperature for Cu^{2+} absorbing by *B. thuringiensis* was 37 °C, whereas 22 °C was the optimum temperature for the function of *Bacillus subtilis* and *Bacillus pumilus.* In all three tested bacteria in the current study, increase of temperature resulted in attenuated absortion probably due to exergonic reaction and decrease of absorbent capacity.

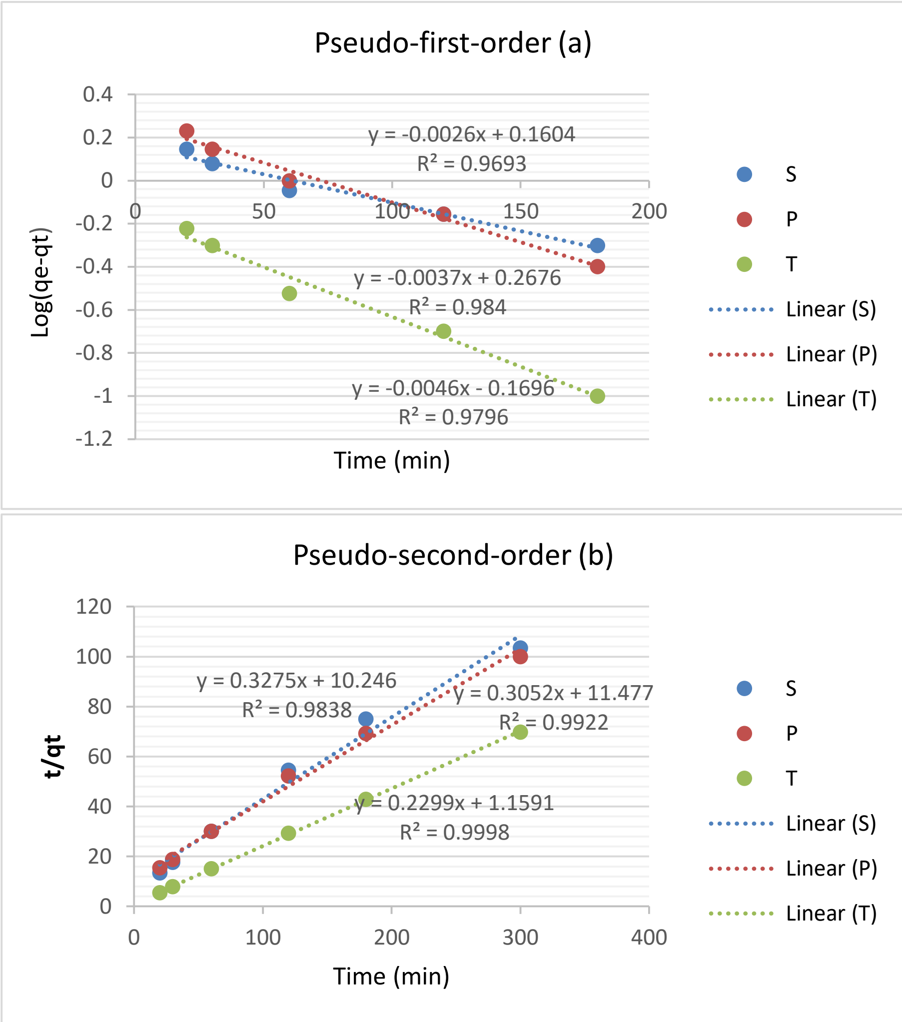

Figure 2. (a) Pseudo-first and (b) pseudo-second; kinetic models of *Bacillus thuringiensis* (**T**) *Bacillus subtilis* (S) and *Bacillus pumilus* (P).

Moreover, the quantity of bioabsorbent remarkably affects the absorption yield.[22] Increase of bioabsorbent concentration leads to enhanced ion removal owing to increasing the absorption surface area and the number of binding sites.[23] Similar to previouce reports, we observed a direct relationship between the quantity of bioabsorbant and the degree of ion removal.[18]

Exposure time to bacterial biomass is one of the most important factors in the absorption equilibrium and is dependent on the nature of bioabsorbants. Removal of metal ion using dead microorganisms is a rapid attachment and a metabolic-independent phenomenon.[24] After 60 min, metal uptake reachs equilibrium. The main reason for the increase of ion upteke over time is a good chance of metal ion collision to binding sites. At the first step, more vacant binding sites is available for the uptake of ions but over time, remaining binding sites cannot easily attach to the ions owing to the forces between the absorbed molecules on the surface of bioabsorbent and liquid phase.[24]

Based on our findings, initial concentration of ion metals directly affects absorption. The initial ion concentration provides a remarkable driving force for overcoming total resistance produced from metal ion mass transfer between the solid and liquid phases.[25-27]

To evaluate the absorption mechanism, bioabsorbant behavior and relationship between the absorption and ion concentration in the equilibrium, three common equilibrium models including Langmuir and Freundlich and Scatchard isotherms were assessed. Langmuir modle is appropriate and valid for survying the simulation of monolayer adsorption on a homogeneous surface.[24,28] Although both classic isotherm models are applied for the prediction of the absorption interaction, the Scatchard plot was used to provide more coherent information concerning interaction between Cu^{2+} ions and the bioabsorbant. The Scatchard plot is associated with the type of absorption interaction.[29] In the present study, the Scatchard plot is linear, thus, there is one type binding site with the equal tendency for metal ions on the bioabsorbent surface. The correlation coefficient acquired for Scatchard and Freundlich models is higher than the Langmuir one. Therefore, herein, the equilibrium equation for Cu^{2+} uptake is more compatible with Freundlich model.

Conclusion

Overall, the dead cells of all three Gram-positive bacteria assessed in this study are favourable and effective candidates for the removal of Cu^{2+} ions from aqueous solution and industrial wastewaters. The yield of ion uptake was dependent to the initial concentration of the ions, exposure time, temperature, pH and bioabsorbent quantity. In addition, the Cu^{2+} ion absorption is more compatible with the Freundlich model.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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