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Biosorption of Copper (II) from Aqueous Solution to *Pantoea agglomerans* Isolated from Water Containing Boron

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Abstract
Heavy metal pollution is an serious environmental problem because they have toxic effects on living organisms. In recent years, the use of microbial biosorbents to remove heavy metal pollution have studied. Many biological materials are used to remove copper ions. In this study, the biosorption capacity of lyophilized *Pantoea agglomerans* for Cu (II) ions were investigated under various conditions (initial pH, contact time, initial heavy metals ion concentration). The monocomponent biosorption data have been analyzed using isotherm (Freundlich and Langmuir) and kinetic models. The highest copper uptake capacity of the biomass was obtained at the initial copper concentration of 250 mgL^{-1} at pH 5.0 and contact time 90th minute. The equilibrium data correlated well with the Langmuir and Freundlich model. It was found that the pseudo-second-order kinetic model fitted the experimentally obtained data. Liyofilized *P. agglomerans* biomass appears to be an inexpensive and efficient biosorbent for the removal of Cu (II) from aqueous solutions.

Keywords: Adsorption; Bacteria; Copper; Freundlich; Heavy metal; Langmuir.

Biossorção de cobre (II) de solução aquosa para de *Pantoea agglomerans* isolados de água contendo boro

Resumo
A poluição por metais pesados é um grave problema ambiental devido aos efeitos tóxicos dos metais pesados sobre os organismos vivos. Estudos recentes têm focado no uso de biossorventes potenciais de base microbiana para remoção de metais pesados. Muitos materiais biológicos para a remoção de íons de cobre já foram empregados. Neste estudo, a capacidade de biossorção e capacidade de *Pantoea aglomerans* liofilizados para íons Cu (II) foram investigados sob várias condições. Sua capacidade de absorção de íons cobre foi determinada em função do pH inicial, tempo de contato, concentração inicial de íons íons Cu (II). Os dados de biossorção de monocomponentes foram analisados usando os modelos isotérmicos de Freundlich e Langmuir. Foram estudados os modelos cinéticos de biossorção de íons de metais pesados nos *P. aglomerados* liofilizados. O pH do cobre mais favorável para remoção foi determinado como 5,0. A maior capacidade de absorção de Cu(II) da biomassa foi obtida na concentração inicial de cobre de 250 mgL^{-1}. Os dados de equilíbrio se correlacionaram bem com o modelo de Langmuir e Freundlich. Verificou-se que o modelo cinético de pseudo-segunda ordem se ajustava aos dados obtidos experimentalmente. A biomassa liofilizada de *P. agglomerans* é um biossorvente barato e os resultados indicaram que ele é eficiente... eficiente para a remoção de Cu(II) de soluções aquosas.

Palavras-chave: Adsorção, Bactérias, Cobre, Freundlich, Metal pesado, Langmuir.

Introduction

The toxic metals coming out because of mining activities cause a significant environmental pollution. The soil is polluted as a result of water mining and energy plants, application of toxic matters containing metals, fertilizers and sludge (Staszewski *et al* 2015). The by-product (wastes) of mining activities may contain high concentration of Cu, Zn, Fe, Mn, Ni, Pb and Cd varying within the range of 1 to 150 g/kg (Mendez *et al*., 2008).

Copper is an essential component of many enzymes and proteins in the body. Therefore, it can affect human health through multiple mechanisms. In addition, studies on the protection of the cardiovascular system, support of bone fracture healing and the development of copper-containing biomaterials with antibacterial effects have increased in recent years (Wang *et al* 2021). Copper is used in many industrial applications. It is found in the structure of fertilizers and pesticides. (Azhar *et al*, 2022)

Besides, certain levels of Cu can be toxic, and has the potential to bioaccumulate in living tissues.

Some microbial biomass can adsorb quite high amounts of metals (Rizvi *et al* 2020). This situation varies depending on the affinity between metal or its ionic forms and binding sites such as lipopolysaccharides, peptidoglycans, and phospholipids in the cell wall of the microbial cell (Siddiquee *et al* 2013, Blaga *et al* 2021). It can also bind galactose, glucose and extracellular polymer substances (EPS) formed by neutral sugar compounds such as small amounts of mannose, xylose, arabinose, rhamnose, fructose and two O-methyl sugars (Mohite, Koli and Patil, 2018; Rizvi *et al* 2020).

In recent years, the emphasis was placed on the development of economic and efficient treatment systems for the removal of toxic compounds from water. The use of microorganisms in this field was addressed (Ayangbenro and Babalola, 2017).

In recent years, many studies have been concerned with economical and efficient treatment processes that remove toxic elements from wastewater. Another promising strategy is the use of microorganisms such as bacteria, fungi or algae that are suitable for binding metal ions in the polymeric matrix (Celebi *et al* 2016, Redha 2020). Biosorption based on live or dead biosorbents has been biotechnologically adopted for the treatment of high volume wastewater containing low concentrations of toxic metals (Davis *et al*. 2003).

In the present study the removal of Cu(II) ions from aqueous solution was investigated using *Pantoea agglomerans* isolated from water containing high amount of boron element.

Materials and Methods

Preparation of Cu(II) solution

Cu (II) ion stock solution was prepared with Cu $(NO_3)_2.3H2O$ (Sigma-Aldrich Chemie GmbH, puriss. p.a., 99-104%) in deionized water. From 1000 mgL^{-1} copper (Cu II) solution, varied from 25mgL^{-1} and 500 mgL^{-1} solutions were prepared in 250 ml volumetric flask for use. Freshly prepared dilutions were used in each experiment.

Preparation of bacteria

Pantoea agglomerans was isolated from Boron pool of Etibor Establishment Eskişehir/TURKEY. Partial 16S rRNA gene sequence and BIOLOG system were used for identification.

After inoculation from stock culture to the nutrient broth, they were incubated (New Brunswick Scientific Innova 44) at 30˚C for 24 hours. They were inoculated to the nutrient agar thereafter and following an incubation at 30˚C for 24 hours, the forming colonies were examined morphologically. *P.agglomerans* form mucoid colonies. The samples taken from here were examined under microscope (Olympus-CHT213E) following gram staining. The appearance of gram-negative bacilli indicates that the culture is pure. They were used in the studies after their purity was determined.

P.agglomerans was inoculated to nutrient broth and incubated at 30ºC for 24 hours. It was transferred from the prepared fresh culture into a 500mL nutrient broth at a rate of 1%, and was incubated in a shaking incubator at 30˚C and 140 rpm for 48 hours. At the end of the incubation period, it was centrifuged at 4˚C and 6000 rpm for 10 minutes. The pellet was washed thrice with sterile distilled water. The collected cells were used in the experiments after lyophilization. Lyophilization process, cultures were frozen in a deep freezer (telstar Igloo) at -20ºC. It was then dried in a freeze dryer (Christ Alpha 1-4) at -38oC under 0.190mbar for 24 hours.

Biosorption studies

The biosorption of Cu^{2+} ions from aqueous solutions onto lyophilized *P. agglomerans* was investigated in batch experiments. Experiments were performed on an orbital shaker operating at 100 rpm. Biosorption experiments were performed in 250mL Erlenmayer containing 50 mg of lyophilized *P. agglomerans* biomass and 50 mL of Cu(II) solution at the desired concentration, pH and temperature. Experiments were performed in duplicate and averaged.

Effect of pH on Cu (II) ion biosorption capacity of *P. agglomerans* was carried out in the pH range from 1.0 to 6.0. Cu (II) solutions at pH levels from 1.0 to 6.0 were prepared in 250 mL volumetric flasks. Then, 50 ml of these prepared solutions were transferred from the 250 ml Erlenmeyers. The pH values of the solutions were adjusted with a pH meter using 1.0 M HCl and 0.1 M NaOH solutions (Yılmaz *et al*. 2010).

Effect of the initial Cu(II) ion concentration on the biosorption capacities of *P.agglomerans* was investigated. The initial Cu(II) ion concentration was determined using Cu(II) ion concentrations between 25mgL^{-1} and 500mgL^{-1}. The effect of temperature on the biosorption capacity of the lyophilized *P. agglomerans* biomass was determined at 20, 30 and 40ºC.

Effect of contact time on the biosorption by *P. agglomerans* biomass was determined. Samples were taken at certain intervals (5th, 10th, 15th, 30th, 60th, 90th, 120th, 150th, 180th and 240th minutes) from the solution prepared in Erlenmeyer flask. After the contact time, the solutions were centrifuged at 5000 rpm for 5 minutes and the supernatant was separated from the pellet. The supernatants were used to determine the concentration of unabsorbed Cu (II) ions. Flame Atomic Absorption Spectrometric Method (Perkin Elmer A. Analyst 800 Model) was used for measurements for unbiosorbed Cu (II) ions in supernatant. Dilution was made prior to the measurements as the device makes measurements within a range of 0.25 mgL^{-1}– 2.0 mgL^{-1}, and the concentrations of the solutions were brought to the range to be measured. Then the amount of adsorbed substance was calculated. The instrument response was periodically checked with a standard copper ion solution.

The equilibrium sorption capacity of the lyophilized *P.agglomerans* biomass at the equilibrium conditions was determined using the mass balance equation:

Equilibrium sorption capacity of lyophilized *P.agglomerans* biomass at equilibrium conditions was determined using the mass balance equation given below:

$$Q_e = \frac{(C_0 - Ce)V}{m} \qquad (1)$$

where *q*e is the amount of Cu(II) ions adsorbed onto *P.agglomerans* (mg /g), *C*o is the initial Cu(II) ion concentration in solution (mg/mL), *C*e is the concentration of non-adsorbed Cu(II) ions in solution (mg/mL), V is the volume of the medium (L) and *m* is the amount of *P.agglomerans* used in the reaction mixture (g).

Adsoption isotherm models

Langmuir and Freundlich isotherms were used for the analysis of biosorption data. The linear form of the Langmuir isotherm model was defined by Langmuir (1918). The Langmuir isotherm model is valid for monolayer adsorption on to surfaces containing a given number of identical sorption sites. The Langmuir equation is expressed as:

$$\frac{c_e}{q_e} = \frac{1}{k_L q_{max}} + \frac{1}{q_{max}} C_e \qquad (2)$$

Where Ce is the equilibrium concentration (mg L-1), qe is the amount of metal ion removed (mg g-1) and qmax maximum uptake capacity (mg g-1), KL is the Langmuir constant.

The Freundlich equation is employed for the sorption on heterogeneous surfaces (Freundlich 1906). The Freundlich equation is generally expressed as:

$$Log\, q_e = Log\, K_F = +\frac{1}{n} log\, Ce \qquad (3)$$

where KF and n are Freundlich constants. Freundlich equilibrium constants were determined from the plot of log q_e versus log Ce, Fig 5.

Kinetic models

Pseudo-first-order kinetic and pseudo-second-order kinetic models were applied to experimental data to elucidate the biosorption mechanism. The pseudo-first-order kinetic model equation is expressed as: Amer *et al* (2015).

$$\mathrm{Log}(q_1 - qt) = \mathrm{Log}q_1 - \left(\frac{k_1}{2.303}\right) t \qquad (4)$$

Where q_t *and* q_t are the amounts of Cu (II) ions (mg/g) adsorbed at equilibrium and at time *t*,

respectively, and k_1 is the first-order rate constant (min^{-1}). The value of k_1 was calculated from the slope of the plot of log(q_1-q_t) versus *t*. The pseudo-second-order kinetic model:

$$\frac{1}{q_1} = \frac{1}{k_2 q_2^2} + \frac{1}{q_2} t \qquad (5)$$

Where q_2 is the maximum adsorption capacity of the biomass (mg/g) for pseudo-second-order adsorption, k_2 is the rate constant for pseudo-second-order adsorption (g /mg/min). The k_2 and q_2 values are estimated from the plot of t/qt against t.

Results and Discussion

In this study, the Cu(II) ion biosorption capacities of the biomass of lyophilized *P.agglomerates* at initial Cu(II) ion concentrations ranging from 25 to 500 mgL^{-1} were investigated. In Figure 1A, the Cu(II) ion adsorption capacities of the lyophilized biomass of *P.agglomerates* are shown as a function of the initial concentration of metal ions in the adsorption medium. The maximum adsorption capacity was reached at 250 mgL^{-1} (Figure 1A). This concentration of the Cu(II) ion solution was used in the further processes of the study. At an initial Cu(II) ion concentration of 250 mgL^{-1}, the amount of adsorbed Cu(II) ions per unit mass of biomass increased.

This situation was thought to be related to the presence of many binding sites for Cu(II) ions at low Cu(II) ion concentrations (Yılmaz *et al*. 2010). However, at concentrations above 250 mgL^{-1}, the adsorption capacity of the biosorbent remained relatively constant (Figure 1A). These findings are likely due to saturation of metal binding sites. In a study, the highest biosorption was obtained for 25 mgL^{-1} Cu(II) ion using immobilized mushroom biomass (Saravanan *et al*. 2021).

When evaluated in terms of pH, the biosorption of Cu (II) ions on P. agglomerates biomass was examined at pH values between 1.0 and 6.0 (Figure). The biosorption capacity of the biosorbent was found to be low at pH 1.0 - 3.0. It was observed that the biosorption capacity of the biosorbent increased significantly when the pH of the medium was increased from 3.0 to 4.0. However, the maximum adsorption capacity was observed at pH 5 (Figure 1B).

In the studies examining the adsorption capacity of Cu (II) ion, it was observed that the biosorption capacity of the ion tended to increase due to the increasing pH value (Yazıcı 2007). When the previous studies on Cu (II) biosorption were examined, it was observed that the maximum biosorption capacity was obtained at pH values between 5.0-5.5, similar to the results obtained in this study (Ozdemir *et al*. 2004, Zheng *et al* 2008, Yilmaz *et al* 2010).

The highest Cu2+ ion adsorption of *E. faecium* was observed at pH 5. Researchers have reported that the adsorption capacity decreases sharply at pH values below pH 4 (Yilmaz *et al* 2010). The optimum pH for the adsorption of Cu(II) ions with *Pantoea* sp.TEM18 was determined as 5 (Ozdemir *et al*. 2004). The maximum adsorption of Cu(II) ions on MBFF19 (EPS) was observed at pH 4.8 (Zheng *et al* 2008).

Maximum Cu(II)ion biosorption capacity with immobilized fungal biomass (*Aspergillus niger* and *Aspergillus flavus*) was observed at pH 5.0 (Saravanan *et al* 2021). The optimum pH value for the biosorption of Cu (II) ions to *Bacillus* FM1 was reported as 5 (Masood and Malik 2011). Sethuraman and Kumar (2011) reported that the highest Cu(II) ion adsorption was obtained at pH 2 with *B.subtilis*. The highest Cu(II) ion adsorption with *P. aeruginosa* and *E. cloacae* was observed at pH 3 and 6, respectively. Researchers attributed the effect of pH on Cu(II) ion adsorption to the interaction between the ions in the solution and the complexes formed on the adsorbent surface.

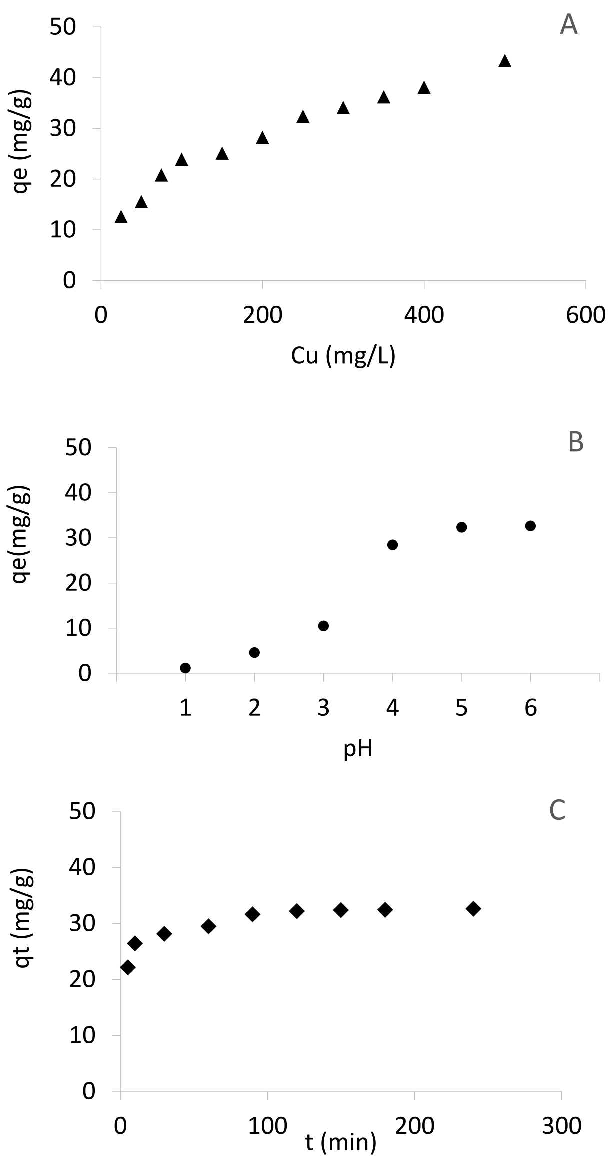

Figure 1. Some factors affecting the Cu(II) ion biosorption capacity of *P. agglomerans*. (A); effect of initial ion concentration (B); effect of PH (C); effect of contact time.

One of the most important parameters affecting biosorption processes is pH. In these processes, pH affects both metal binding sites on cell surfaces and metal chemistry. At high acidic pH, the total surface charge in the active sites becomes positive. As a result, metal cations and protons compete for binding sites on the biosorbent surface, resulting in lower metal uptake (Amer *et al*. 2015). As the pH increases, the more negative the surface of the biosorbent becomes. The electrostatic attraction between the metal ions and the surface of the biosorbent increases. For this reason, the biosorption capacity of metal ions gradually increased. At pH values higher than 6, soluble Cu (II) ions begin to precipitate into hydroxides due to an increase in the concentration of OH^- ions. (Sethuraman and Kumar 2011, Yilmaz *et al* 2015, Amer *et al* 2015).

The temperature of the adsorption medium did not significantly affect the adsorption of Cu(II) ions onto the lyophilized *P.agglomerans* biomass. The qe values obtained at different temperatures (20, 30 and 40°C) were close to each other. For this reason, the experiments were carried out at 30^{o}C.

In experiments to determine the appropriate contact time for copper ion biosorption by lyophilized *P.agglomerates*, experiments were created at an initial copper ion concentration of 250 mgL-1, with contact times ranging from 5 minutes to 250 minutes, while keeping the pH at 5.0. (Figure 1C). As seen in the graphic, *P. agglomerans* could reach at maximum adsorption capacity in 90th minute.

Cu (II) ion binding capacity may depend on the properties of the bacterium. *P. agglomerans* is a Gram-negative bacterium that produces exopolysaccharide (EPS). EPS in the form of capsule or slime layer has different functional groups such as carboxylic, amino, phosphate and sulfhydryl with varying affinity and specificity for metal binding (Ozdemir *et al* 2004). EPS functional groups bind with Cu(II) ions. In addition, proteins and lipids are present in the bacterial cell wall structure. This structure contains functional groups capable of binding to heavy metals (Bennett 2019). The anionic property of EPS is associated with negatively charged ionizable phosphate, carboxylate, acetate, amine and sulfate groups (Liu and Fang 2002, Sun *et al* 2015).

P. agglomerans reached its maximum adsorption capacity in a short time due to its cell structure. Yılmaz *et al*. (2010) reported that a rapid Cu (II) sorption rate was observed in the first 30 minutes with E. faecium. Maximum adsorption capacity with Enterobacter J1 was reached in 100 minutes (Lu *et al* 2006). Zheng *et al*. (2008) reported the Cu(II) ion biosorption of exopolysaccharide (EPS) produced by *Bacillus* F19 as 10 minutes. This time is below our contact time. This is due to the structure of the biosorbent. Mohite, Koli and Patil (2018) reported that *P. agglomerans* had a high uptake of Cu(II) ions.

The purpose of isotherms is to provide an understanding of the adsorption mechanism. Langmuir and Freundlich isotherms were drawn in the light of the information given previously and their suitability was determined by tabulating their parameters (Table 1).

Table 1. Langmuir and Freundlich parameters for the biosorption isotherms of Cu(II) ions from an aqueous solution onto *P. agglomerans* biomass**.**

Langmuir			Freundlich		
q (mg /g)	K_L (L /mg)	R^2_L	n	K_F (L /mg)	R^2_F
46.45	0.59	0.968	3.09	5.89	0.981

As shown in Table 1, the regression correlation coefficients for Cu (II) ions are very high for ions - bacteria systems. When the R values in Table 1 are compared according to the graphics, it was determined that both models were suitable for *P. agglomerans*. This observation indicated that heterogeneous surface conditions as well as monolayer biosorption can coexist under the applied experimental conditions.

Similarly, Lu *et al* (2006) reported that Cu(II) ion biosorption of *Enterobacter* sp J1 was suitable for both models. Yilmaz *et al* (2010) reported that the adsorption of Cu(II) ions to the E. faecium biomass provides a better fit the Freundlich isotherm. Sethuraman and Kumar (2011), reported that the adsorption of Cu(II) ions with *B. cereus*, *P. aeruginosa* and *E. cloacae* biomass conformed to the Freundlich isotherm model.

The Langmuir model can be defined as a monolayer uniform phenomenon that does not interact between metal ions. On the other hand, Freundlich model assumes sorption on a heterogeneous surface.

Figure 2 shows the plots of linearized Langmuir and Freundlich adsorption isotherms.

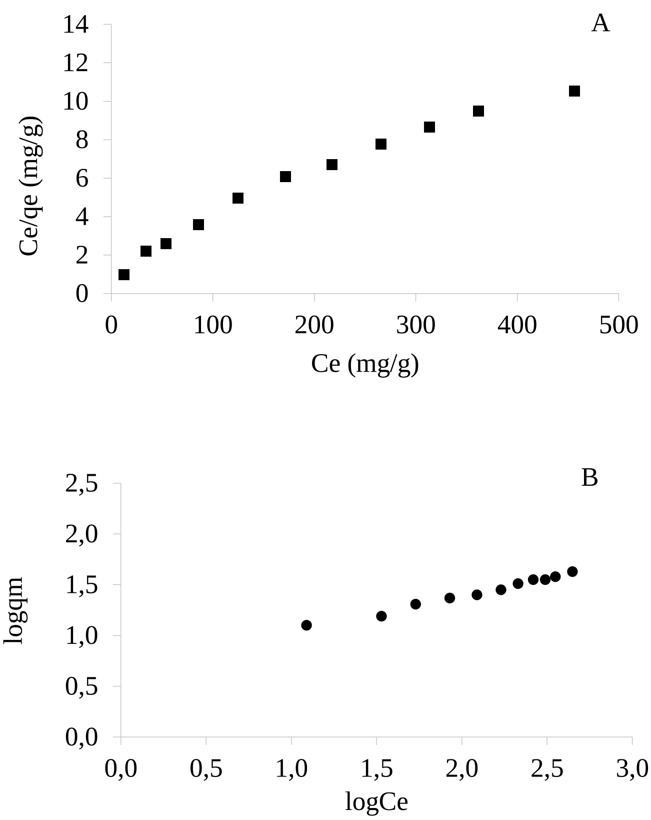

Figure 2. The linearized Langmuir (A) and Freundlich (B) adsorption isotherm plot for Cu(II) biosorption by *P. agglomerans*.

Pseudo-first- and pseudo-second-order kinetic models were applied to experimental data to elucidate the mechanism of Cu(II) biosorption. The parameters of the two kinetic models, the pseudo-primary sequence and the pseudo-second sequence, are given in Table 2. It was found that the pseudo-second-order kinetic model fitted the experimentally obtained data better than the pseudo-first-order kinetic model. The linear regression correlation coefficient R2 was also found to be higher. This high value confirms that the adsorption data are well represented by pseudo-second-order kinetics (Figure 3).

Similar findings have been reported for *Bacillus subtilis*, *Pseudomanas aeroginosa* and *Enterobacter cloace*. The researchers reported that the pseudo-second-order kinetic model matched up satisfactorily with the experimental observation (Sethuraman ve Kumar, 2011).

Table 2. Parameters dependent on 1st and 2nd degree reaction rate equations.

Pseudo-primer-order			Pseudo-second-order		
k_2 (g /mg/ min)	q_2 (mg /g)	r_2^2	k_2 (g /mg/ min)	q_2 (mg /g)	r_2^2
0.0150	2.000	0.1715	0.2500	8.5000	0.9997

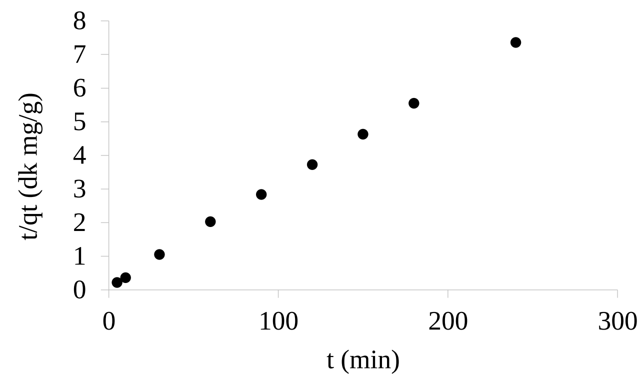

Figure 3. Pseudo-second-order kinetic plot for the biosorption.

Saravan *et al*. (2021) was reported that the biosorption of Cu(II) ions by immobilized fungal (*A. niger*, *A. flavus*) biomass is obtained with a pseudo-first order model. Yilmaz *et al*. (2010) reported that the adsorption of Cu^{2+} ions to *E. faecium* fits the pseudo-second-order kinetic model better than the pseudo-first-order kinetic model.

Conclusion

In this study, the biosorption properties of lyophilized *P. agglomerate* biomass for the removal of copper ions were investigated. Experiments were performed as a function of time, initial metal ion concentration, and pH. Results showed that *P. agglomerans* was a good biosorption medium for Cu(II) ions. The study showed that P. agglomerans has high biosorption efficiency for the treatment of wastewater containing copper ions.

Studies have shown that *P. agglomerans* can be an effective biosorbent for the removal of Cu(II) ions from aqueous solutions. Experiments revealed that the biosorption data have excellent comparability with both the Langmuir and Freundlich isotherms. The pseudo-second order kinetic model, is suitable to depict the absorption process. Lyophilized *P. agglomerans* biomass appears to be an inexpensive and efficient bio sorbent for the removal of Cu(II) from aqueous solutions.

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