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- Biosorption of Cu2+ by *Pseudomonas putida* Immobilized on Loofa Sponge (*Luffa cylindrica* L.)

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RESEARCH ARTICLE

Biosorption of Cu^{2+} by *Pseudomonas putida* Immobilized on Loofa Sponge (*Luffa cylindrica* L.)

Arzuhan SIHOGLU TEPE [a,*], Emine KACMAZOGLU [b], H. Nursevin OZTOP [c], Rana TASKIN [d]

[a] *Gaziantep University, Faculty of Science and Literature, Department of Biology, Gaziantep, TURKEY*
[b] *Inonu University, Faculty of Education, Department of Mathematics and Science Education, Malatya, TURKEY*
[c] *Cumhuriyet University, Faculty of Science, Department of Biochemistry, Sivas, TURKEY*
[d] *Cumhuriyet University, Faculty of Science and Literature, Department of Biology, Sivas, TURKEY*

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ABSTRACT

Cu^{2+} contaminated areas pose a serious health risk for living organisms. In this study, the biosorption of Cu^{2+} by *Pseudomonas putida* immobilized on a loofa sponge (*Luffa cylindrica* L.) was investigated. Effects of the particle size of the loofa sponge, initial pH, temperature, initial Cu2+ concentration, and the stirring speed on the adsorption of Cu^{2+} were examined. Optimum conditions were determined as follows: loofa sponge particle size is 0.42 - 0.85 mm, initial pH is 5.0, the temperature is 30 °C, initial Cu^{2+} concentration is 25 mg/l, and stirring speed is 130 rpm. According to the results of kinetic calculations, Qmax and ro values were determined as 0.394 mg/g, and 0.077 mg/g.min for *P. putida* immobilized on loofa sponge, respectively, while they were found to be 0.096 mg/g and 0.052 mg/g.min for the loofa sponge only. It is thought that Cu^{2+} can be removed effectively from the wastewaters by using *P. putida* immobilized on a loofa sponge.

1. Introduction

Although some heavy metals play an essential role in the metabolic, physiological, and biochemical processes of living things, excessive amounts are toxic (Fashola et al., 2016). Metal toxicity may lead to changes in the structure and function of the enzymes involved in the metabolism. For example, exposure to lead and mercury leads to health problems such as autoimmune diseases, kidney dysfunctions, rheumatoid arthritis, and circulatory and nervous system disorders. In children, exposure to these heavy metals increases cardiovascular diseases and developmental anomalies (Nagajyoti et al., 2010; Wuana and Okieimen, 2011; Wang et al., 2012; Ali et al., 2013; Fashola et al., 2016; Ayangbenro and Babalola, 2017). Cadmium is a mutagen and a carcinogenic heavy metal. It affects the endocrine system and damages the lungs (Nagajyoti et al., 2010; Chibuike and Obiora, 2014; Fashola et al., 2016; Ayangbenro and Babalola, 2017). Chromium causes nausea, vomiting, headache, hair loss, and diarrhea (Cervantes et al., 2001; Barakat, 2011; Mohanty et al., 2012; Ayangbenro and Babalola, 2017). Copper, which is another critical heavy metal, causes headache, nausea, vomiting, anemia, abdominal pain, diarrhea, liver and kidney damages, and metabolic disorders in humans (Nagajyoti et al., 2010; Dixit et al., 2015; Fashola et al., 2016; Ayangbenro and Babalola, 2017).

Various techniques such as adsorption, chemical precipitation, ion exchange, chemical oxidation, or reduction reactions can be used to remove heavy metals from aqueous systems (Siddiquee et al., 2015). However, most of these techniques are not cheap enough to become routine applications. In addition, the formation of unpredictable toxic by-products and the high reactive need are the main disadvantages of these techniques (Ahluwalia and Goyal, 2007). However, in the process known as bioremediation, it is possible to remove heavy metals from aqueous systems using bacteria, algae, fungi, or plants. The use of bacteria in heavy metal removal is a sustainable, eco-friendly, long-term, and low-cost approach (Dixit et al., 2015).

* Corresponding author:
E-mail address: arzuhan.tepe@kilis.edu.tr (A. Sihoglu Tepe)
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Microorganisms exhibit metal removal activity through the processes such as valence transformation, reduction, metal binding (via metallothionein and phytochelatin), volatilization, and vacuole compartmentalization (Sharma et al., 2000; Wu et al., 2010; Siddiquee et al., 2015). The data obtained from understanding the metabolic pathways of microorganisms have been used successfully in manipulating their metal adsorption abilities (Gavrilescu, 2004; Ayangbenro and Babalola, 2017).

Microorganisms have different biosorption capabilities. In addition, the metal binding capacity of bio sorbents depends on pretreatment methods as well as the experimental variables. The bio sorbent to be used in the removal of heavy metals should be made up of rapidly growing and easily harvested organisms and being cheap. If the organism is grown in bioreactors, it should quickly adapt to the changes in bioreactor parameters and the resulting physical and chemical stratification (Fomina and Gadd, 2014). Bacteria are frequently used as bio sorbents because they can be quickly grown under controlled conditions and are resistant to many environmental stress conditions (Wang and Chen, 2009; Fashola et al., 2016). Microorganisms have excellent sorption capacity due to the active chemosorption regions such as teichoic acid in the cell wall and high surface-to-volume ratio (Mosa et al., 2016; Ayangbenro and Babalola, 2017). To date, the efficacy of the heavy metal removal of some species such as Pseudomonas has been tested (Lo et al., 1998; Rajkumar and Freitas, 2008; Andreazza et al., 2012; Li et al., 2014; Qu et al., 2017).

As far as our literature survey could ascertain, some studies investigate the copper adsorption capacity of the loofa sponge (*Luffa cylindrica* L.) (Laidani et al., 2011; Tang et al., 2014; Liu et al., 2017; Zhai et al., 2017). It is also known that some microalgae, such as *Chlorella* and *Synechorococcus*, have also been reported to be used in biosorption studies by immobilizing on the loofa sponge (Akhtar et al., 2003; Akhtar et al., 2004a; Akhtar et al., 2004b; Saeed and Iqbal, 2006). Moreover, *Pseudomonas putida*/goethite composite (Chen et al., 2008) and volcanic matrix-immobilized *P. putida* (Ni et al., 2012) have been used for Cu^{2+} removal. However, immobilization of *P. putida* cells on the loofa sponge for the biosorption of Cu^{2+} has not previously been reported elsewhere. Therefore, the data presented here could be assumed as the first report on this issue.

This study aimed to investigate the biosorption of Cu^{2+} by *P. putida* immobilized on a loofa sponge. For this purpose, effects of the particle size of *L. cylindrica*, initial pH, temperature, initial Cu^{2+} concentration (Co), and the stirring speed on adsorption of Cu^{2+} were examined as the parameters. As a result of the kinetic calculations, optimum conditions for the biosorption of Cu^{2+} were determined.

2. Materials and methods

2.1 Microorganism

P. putida, which is used as bio sorbent, was taken from Prof. Dr. Merih Kivanc as the stock culture.

2.2. Chemicals

Loofa sponge was purchased from a local market in Osmaniye-TURKEY. The entire chemicals used in the study were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Höxter, North Rhine-Westphalia, Germany). The chemicals and solvents were of analytical grade.

2.3. Preparation of microorganism culture

P. putida was cultured overnight at 37 °C in Mueller Hinton Agar (MHA). Then, the strain was suspended in Mueller Hinton Broth (MHB) to give a final density of 5×10^5 cfu/ml and confirmed by viable counts (Sihoglu-Tepe, 2003).

2.4. Immobilization of P. putida cells on loofa sponge

A sponge obtained from matured dried fruits of *L. cylindrica* was used (Sihoglu-Tepe, 2003). The ground loofah sponge was divided into four different particle sizes using a sieve: 0.42 - 0.85, 0.85 - 1.00, 1.00 - 1.40, and 1.40 - 2.00 mm.

The wet weight of the loofa sponges with different particle sizes was measured before and after immobilization of *P. putida* by using the loofa sponge with a dry weight of 1 g, respectively. As a result of the comparison of these values, it was determined that the loofa sponge with a particle size of 0.42 - 0.85 mm could immobilize the microorganism at the maximum rate. Therefore, the experiments were continued with the loofa sponge with a particle size of 0.42 - 0.85 mm. (Sihoglu-Tepe, 2003).

2.5. Experimental system

The MHB media with *P. putida* cells were first placed into a 500 ml Erlenmeyer flask containing 1 g of dry loofa sponge and incubated for 48 hours at 30 °C in a shaker. Then, the loofa sponge with microorganisms was filtered using a Whatman No: 6 filter paper and 3.54 g was taken. Adsorption experiments were carried out using 100 ml of Cu^{2+} solution in the shaker with constant temperature and stirring speed (Sihoglu-Tepe, 2003).

2.6. Experimental conditions

All the experiments were carried out using a loofa sponge with a particle size of 0.42 - 0.85 mm. To measure the Cu^{2+} biosorption capacity of the loofa sponge, initial pH (5.0), temperature (25 °C), initial Cu^{2+} concentration (10 mg/l), and stirring speed (100 rpm) parameters were applied (Sihoglu-Tepe, 2003).

The variables given in Table 1 were examined to determine the parameter with the highest Cu^{2+} biosorption capacity (Sihoglu-Tepe, 2003).

Table 1. The parameters examined to determine the variable with the highest Cu^{2+} biosorption capacity

Parameters	Variables			
Particle size (mm)	0.42 - 0.85	0.85 - 1.00	1.00 - 1.40	1.40 - 2.00
Initial pH	2.5	3.5	4.5	5.0
Temperature (°C)	20	25	30	40
Initial Cu^{2+} concentration (mg/l)	10	25	50	100
Stirring speed (rpm)	50	100	130	150

2.7. Analysis method

At the beginning of the study, the time that *P. putida* immobilized on the loofa sponge was added to the Cu^{2+} solution was determined as t = 0. Subsequently, 5 ml of the sample was filtered through a filter paper at 0., 1., 2., 3., 4., 5., 10., 25., 40., 100., and 160. min and the concentration of free Cu^{2+} in the solution remained after the adsorption process was analyzed. The analysis was performed using a flammable atomic absorption spectrophotometer (FAAS, Unicam 929 Model) (Sihoglu-Tepe, 2003).

2.8. Calculation of the amount of adsorbed Cu^{2+} and adsorption kinetics

The following formula was used to calculate the amount of Cu^{2+} adsorbed:

$$Q = (C_o - C_{end}) \times Vt / M$$

where Q is the amount of Cu^{2+} adsorbed per gram of bio sorbent (mg/g); C_o is the initial Cu^{2+} concentration of the solution (mg/l); C_{end} is the concentration of free Cu^{2+} in the solution remained after adsorption process; Vt is the total volume of the solution (ml), and M is the weight of *P. putida* immobilized on loofa sponge. To obtain the adsorption kinetics, concentration values of the adsorbed Cu^{2+}, which were determined in the presence of a loofa sponge with and without *P. putida* under optimum conditions, were plotted against time, and thus the speed and the maximum biosorption were calculated (Sihoglu-Tepe, 2003).

2.9. Statistical analysis

All tests were performed in triplicate. Data analyses were performed by using the SPSS v22.0 software. The results were presented as mean ± standard deviation (SD) of three parallel measurements. By using Tukey's comparisons with One-way ANOVA, differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

In this study, the adsorption efficiency of Cu^{2+} by *P. putida* was tested using various parameters such as the effects of the particle size of the loofa sponge, initial pH, temperature, initial Cu^{2+} concentration, and the stirring speed. As the first step, bacterial immobilization capacities of the loofa sponge with different particle sizes were determined. Q values obtained from all experiments are given in Tables 2a and b.

3.1. Bacterial immobilization capacities of loofa sponges with different particle sizes

P. putida immobilization capacities of the loofa sponge with different particle sizes are shown in Figure 1. As seen in the figure, it was found that as the particle size increased, the bacterial immobilization capacity decreased. Loofa sponge with the smallest particle size (0.42 - 0.85 mm) immobilized 3.54 g of bacteria, while the immobilization capacity of the sponge with the largest particle size (1.40 - 2.00 mm) was found to be 1.48 g. For this reason, loofa sponge with a particle size of 0.42 - 0.85 mm has been used in the subsequent steps of the study.

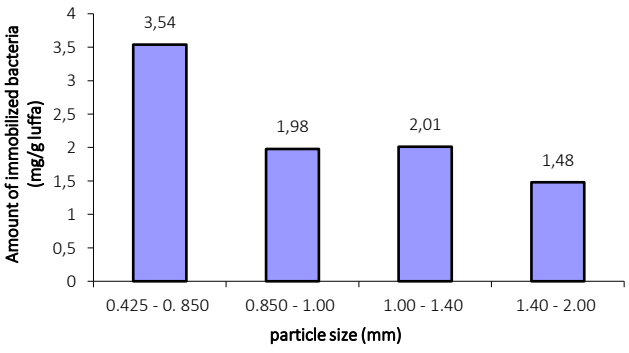

Figure 1. Immobilization capacities of loofa sponge according to different particle sizes (The values given are calculated based on 1 g of dry loofa sponge)

The results obtained from the study carried out with a loofa sponge with a particle size of 0.42 - 0.85 mm were found to be statistically different from the others. It was found that the results obtained from the studies with 0.85 - 1.00, 1.00 - 1.40, and 1.40 - 2.00 variables were not statistically different from each other ($p < 0.05$).

3.2. Effect of the particle size of loofa sponge on the adsorption of Cu^{2+}

At this stage, the time-dependent change in the adsorption capacities of the loofa sponges with different particle sizes was investigated (Experimental conditions; Cu^{2+} concentration: 50 mg/l, temperature: 25 °C, pH: 5.0, stirring speed: 100 rpm). Time-dependent change in Q values (amount of mg metal adsorbed per gram of bio sorbent) obtained is shown in Figure 2a.

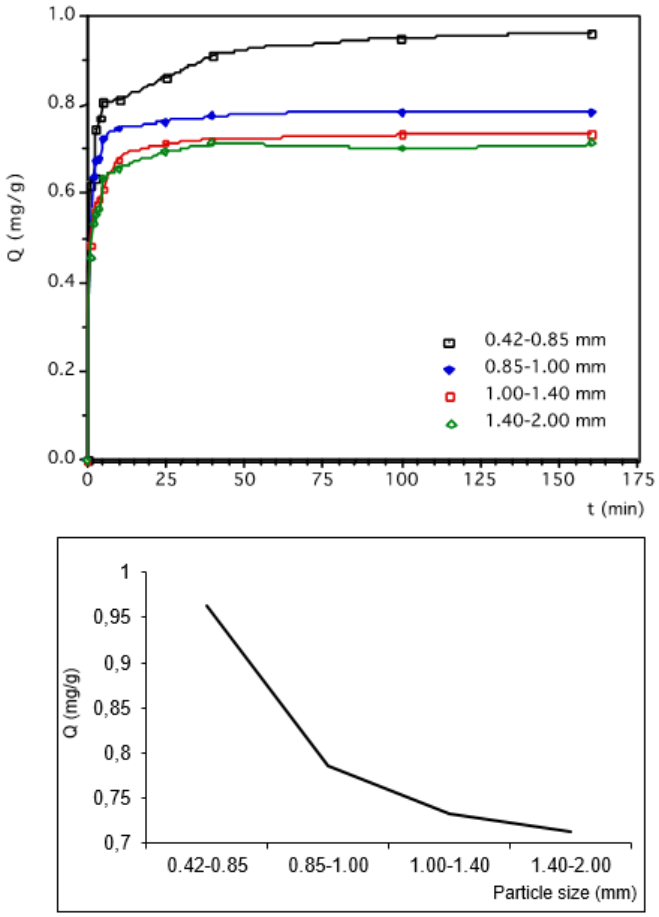

Figure 2. (a) Effect of the particle size of loofa sponge on the adsorption of Cu^{2+} **(b)** Effect of loofa sponge with different particle size on Cu^{2+} adsorption in equilibrium [Cu^{2+} concentration: 50 mg/l, temperature: 25°C, pH: 5.0, stirring speed: 100 rpm]

As seen in Figure 2a, adsorption of Cu^{2+} was found to reach equilibrium between 25^{th}-40^{th} min. Using a loofa sponge with particle sizes of 1.00 - 1.40 and 1.40 - 2.00 mm, the adsorption values were determined as 0.73 and 0.71 mg/g in equilibrium, respectively. The adsorption value in the presence of a loofa sponge with a particle size of 0.85 - 1.00 mm was determined to be 0.78 mg/g.

As a result of the adsorption studies carried out with a loofa sponge with a particle size of 0.42 - 0.85 mm, it was determined that the amount of Cu^{2+} adsorbed per gram of bio sorbent increased rapidly

up to 25th min. The adsorption reached equilibrium after the 40th min. The amount of Cu^{2+} adsorbed in equilibrium for the loofa sponge with a 0.42 - 0.85 mm particle size was determined to be 0.96 mg/g.

As can be seen from Figure 2b, it was found that the loofa sponge with a particle size of 0.42 - 0.85 mm adsorbed the maximum amount of Cu^{2+} in equilibrium than the others having different particle sizes. The loofa sponge followed it with particle sizes of 0.85 - 1.00 and 1.00 - 1.40 mm, respectively. The loofa sponge achieved the lowest adsorption with a particle size of 1.40 - 2.00 mm.

The data presented above were analyzed statistically. The results obtained are similar to those obtained in section 3.1. Here, the results obtained with the loofa sponge with a particle size of 0.42 - 0.85 mm were statistically different from the others ($p < 0.05$).
The efficiency of heavy metal removal is greatly influenced by the organism's cell structure used in biosorption. According to the most general mechanism described, metal removal takes place in two stages. The first is the physical adsorption or ion exchange step, where metal ions are adsorbed by complexing with negatively charged reaction sites on the cell surface and/or substituting with positively charged reaction sites. This step is called passive metal removal and is initially quite fast. Equilibrium occurs shortly after the metal ion contacts with the microorganism, and adsorption isotherms can show the formation of equilibrium. The second step is the active metal removal stage, where chemical adsorption is observed. It progresses more slowly since it depends on metabolic activity (Butter et al., 1998). Ions having higher electronegativity are adsorbed more strongly by the bio sorbent (Allen and Brown, 1995). According to Lo et al. (1998), in the first 3 min of contact with Cu^{2+} and bio sorbent, 75% of Cu^{2+} in the environment is adsorbed. The findings obtained from the present study were found to support this view.

The length of time required to reach the equilibrium in the adsorption depends on the diffusion coefficient of the metal ions and the particle size (Butter et al., 1998). As the particle size decreases, the surface area increases, leading to immobilization of more microorganisms and, hence, more heavy metal adsorption. For this reason, the number of immobilized microorganisms and the adsorption of heavy metals were found to be higher in the loofa sponge with 0.42 - 0.85 mm, which is the smallest particle size studied.

3.3. Effect of different initial pH values on the adsorption of Cu^{2+}

In order to determine the effect of initial pH values on Cu^{2+} adsorption, experiments were performed at pH 2.5, 3.5, 4.5, and 5.0, respectively (Experimental conditions; Cu^{2+} concentration: 10 mg/l, temperature: 25 °C, stirring speed: 100 rpm, loofa sponge particle size: 0.42 - 0.85 mm). Time-dependent change in the amount of Cu^{2+} (mg/g) adsorbed per gram of bio sorbent is given in Figure 3a.

As can be seen from the figure, the adsorption of Cu^{2+} at pH 2.5 reached equilibrium at 10th min. At other pH values, it was determined that the adsorption of Cu^{2+} reached an equilibrium between 25th - 40th min. The adsorption in equilibrium at pH 2.5 was measured as 0.07 mg/g. It is pretty interesting to point out that the adsorption values measured at pH 3.5 and 4.5 were too close to each other in equilibrium (0.17 and 0.18 mg/g, respectively). Maximum adsorption was determined to be 0.22 mg/g at pH 5.0 (Figure 3b). Since Cu^{2+} precipitated as copper hydroxide $(CuOH)^{-}$ at pH values higher than 5.0, higher values than this point have not been tested (Aksu, 2001). As can be understood, the amount of adsorbed Cu^{2+} increased as the initial pH and the time required to reach the equilibrium increases.

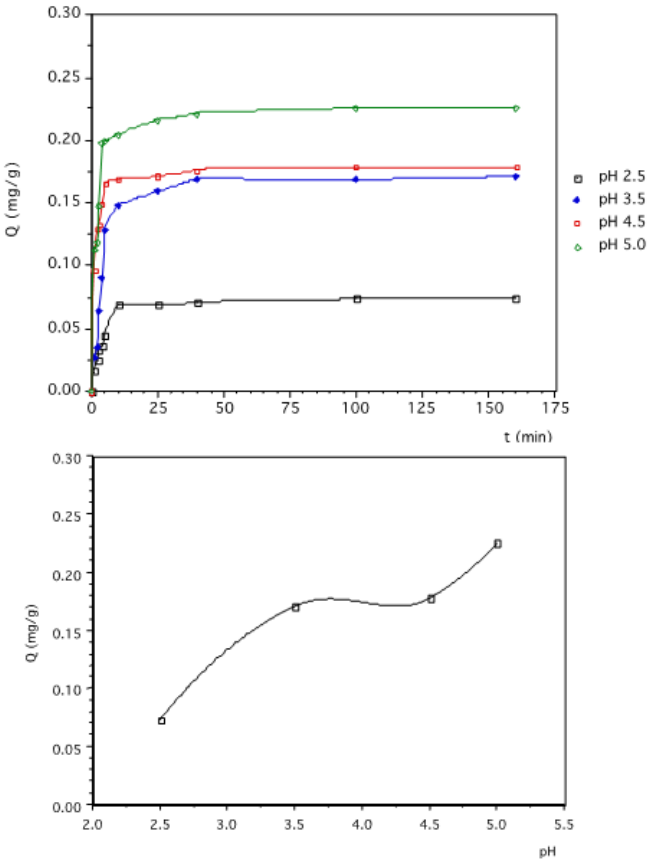

Figure 3. (a) Time-dependent change of different initial pH values on the adsorption of Cu^{2+} **(b)** Effect of different initial pH values on the adsorption of Cu^{2+} in equilibrium [Cu^{2+} concentration: 10 mg/l, temperature: 25 °C, stirring speed: 100 rpm, loofa sponge particle size: 0.42 - 0.85 mm]

The data obtained from pH variables were analyzed statistically. The results obtained at pH 3.5 and 4.5 were not statistically different. However, the biosorption values obtained at pH 2.5 and 5.0 were statistically different from the others ($p < 0.05$).

Several chemical groups (carboxyl, phosphate, amino, sulfate, and hydroxyl groups that show high affinity for metal ions) allow attachment of metal ions on the cell surface (Ahuja et al., 1999). The pH of the medium affects both the functional groups of the bacterial cell wall and the solubility of metals (Say et al., 2001). At low pH (pH 2.0), the amount of H^+ and H_3O^+ increases, limiting the binding of metal ions to the bacterial cell wall (Gadd, 1988). With increasing pH, the metal-binding sites remain empty, and the affinity of the positively charged metal ions to the cell surface increases (Aksu, 2001). In addition, the decrease in the solubility of metal ions at high pH increases the adsorption of these metals by biomass (Guibal et al., 1992).

To the best of our knowledge, some studies are available in the literature concerning the effect of pH on Cu^{2+} biosorption. According to Qu et al. (2017), adsorption was reduced at pH < 5.5 due to physical blocking between montmorillonite and *P. putida*. Li et al. 2004 have

Table 2a. Q values obtained from all the parameters (loofa size, pH, and temperature)

Time (min)	Q values obtained from loofa size variables (mm)				Q values obtained from pH variables				Q values obtained from temperature variables (°C)			
	0.42 - 0.85	0.85 - 1.00	1.00 - 1.40	1.40 - 2.00	2.5	3.5	4.5	5.0	20	25	30	40
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.617	0.456	0.483	0.456	0.016	0.026	0.096	0.112	0.064	0.112	0.134	0.118
2	0.636	0.636	0.559	0.534	0.025	0.035	0.129	0.118	0.076	0.118	0.173	0.132
3	0.745	0.673	0.576	0.552	0.033	0.064	0.132	0.147	0.096	0.147	0.204	0.158
4	0.769	0.678	0.588	0.565	0.036	0.090	0.149	0.197	0.167	0.185	0.211	0.173
5	0.805	0.723	0.610	0.633	0.045	0.128	0.165	0.199	0.180	0.197	0.225	0.182
10	0.811	0.746	0.678	0.657	0.069	0.148	0.169	0.204	0.194	0.204	0.230	0.187
25	0.861	0.763	0.712	0.692	0.069	0.159	0.171	0.216	0.203	0.211	0.235	0.192
40	0.910	0.774	0.722	0.712	0.071	0.169	0.176	0.221	0.208	0.216	0.236	0.216
100	0.950	0.784	0.732	0.702	0.073	0.169	0.178	0.225	0.212	0.221	0.237	0.221
160	0.960	0.784	0.732	0.732	0.073	0.171	0.178	0.225	0.212	0.225	0.237	0.221
220	0.960	0.784	0.732	0.732	0.073	0.171	0.178	0.225	0.212	0.225	0.237	0.221

Table 2b. Q values obtained from all the parameters (Cu^{2+} concentration, stirring speed)

Time (min)	Q values obtained from initial Cu^{2+} concentration variables (mg/l)				Q values obtained from stirring speed variables (rpm)			
	10	25	50	100	50	100	130	150
0	0	0	0	0	0	0	0	0
1	0.118	0.214	0.134	0.127	0.026	0.112	0.166	0.161
2	0.127	0.267	0.339	0.322	0.055	0.117	0.178	0.185
3	0.168	0.288	0.424	0.384	0.072	0.129	0.189	0.190
4	0.174	0.328	0.451	0.402	0.118	0.148	0.199	0.208
5	0.185	0.381	0.452	0.423	0.135	0.161	0.216	0.221
10	0.190	0.425	0.456	0.427	0.142	0.178	0.228	0.226
25	0.199	0.435	0.459	0.434	0.450	0.182	0.230	0.231
40	0.208	0.465	0.461	0.456	0.156	0.186	0.233	0.231
100	0.217	0.475	0.464	0.459	0.165	0.190	0.235	0.236
160	0.217	0.475	0.466	0.459	0.165	0.190	0.235	0.241
220	0.217	0.475	0.466	0.459	0.165	0.190	0.235	0.241

reported that the solution pH strongly affected the biosorption of Cu^{2+}, and the optimum pH value was determined as 5.0. According to a study carried out by Andreazza et al. (2012), *P. putida* displayed high copper resistance in the pH range from 5.0 to 9.0. Similar results were also reported by Andreazza et al. (2010). According to this report, the highest growth and Cu^{2+} biosorption by *P. putida* were recorded at pH 6.0. Additionally, Pardo et al. (2003) and Chen et al. (2005) have reported the optimum pH as 4.5 and 5.0 - 6.0 for Cu^{2+} removal by *P. putida*, respectively. It is possible to increase the number of studies that report the optimum pH level as 4.5 - 5.5 for Cu^{2+} biosorption (Sanchez et al., 1999; Aksu, 2001). It is also possible to find lower results than these pH values in the literature for Cu^{2+} biosorption by *P. putida*. Uslu et al. (2011) have reported the optimum pH level for the bioaccumulation of Cu^{2+} by viable *P. putida* cells as 3.0 ± 0.5.

As can be seen from the literature data given above, the optimum pH range for Cu^{2+} biosorption by *P. putida* is between 4.0 and 6.0 in most studies, and these findings were consistent with the results presented by our research group.

3.4. Effect of temperature on the adsorption of Cu^{2+}

In order to determine the effect of temperature on the adsorption of Cu^{2+}, adsorption values at 20, 25, 30, and 40 °C were investigated (Experimental conditions; Cu^{2+} concentration: 10 mg/l, pH: 5.0, stirring speed: 100 rpm, loofa sponge particle size: 0.42 - 0.85 mm). Time-dependent change in the amount of Cu^{2+} adsorbed per gram of bio sorbent is shown in Figure 4a.

Within the first 5 min, very rapid adsorption has been observed at all the temperatures. The adsorption was determined to reach the equilibrium between 25th - 40th min (Figure 4a). At equilibrium, 0.16 mg/g Cu^{2+} was adsorbed at 40 °C, which tested the maximum temperature. The Cu^{2+} adsorption values at 20 and 25°C were determined

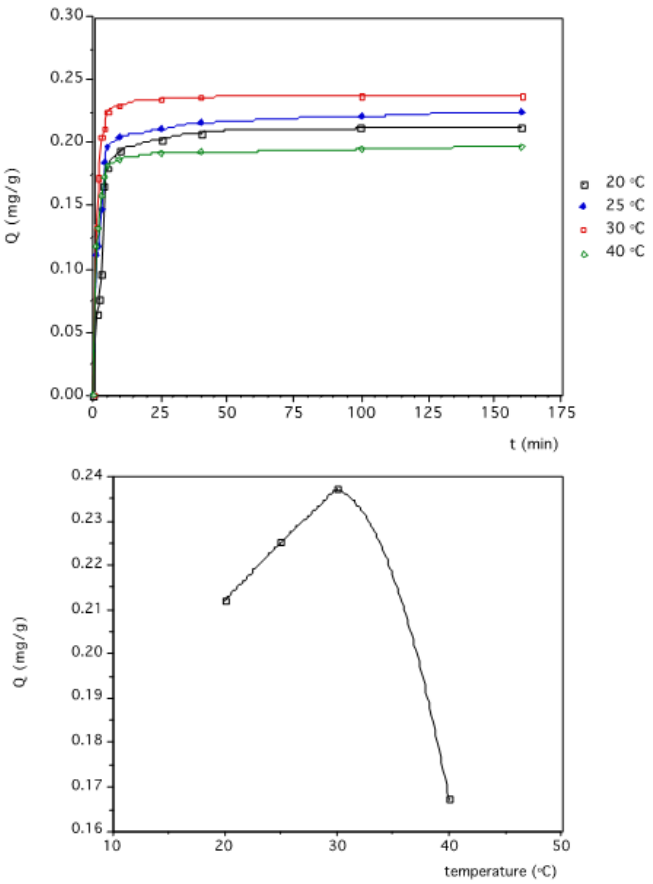

Figure 4. (a) Time-dependent change of temperature on the adsorption of Cu^{2+} **(b)** Effect of temperature on the adsorption of Cu^{2+} in equilibrium [Cu^{2+} concentration: 10 mg/l, pH: 5.0, stirring speed: 100 rpm, loofa sponge particle size: 0.42 - 0.85 mm]

to be 0.21 and 0.22 mg/g, respectively. The highest Cu^{2+} adsorption value was measured as 0.23 mg/g at 30 °C (Figure 4b).

The data obtained from temperature variables were analyzed statistically, and it was found that the results obtained from all variables were not statistically different from each other ($p < 0.05$).

The high rate of adsorption at low temperatures is since Cu^{2+} is physically adsorbed to the microorganism. In physical adsorption, heavy metals are attached to the cell surface components with weak bonds. At high temperatures, these bonds break, and desorption is observed (Ozer and Ozer, 1998).

As can be seen from the data given above, Cu^{2+} adsorption increased up to 30 °C but decreased again dramatically at 40 °C. This finding was found to be compatible with the literature data. According to Andreazza et al. (2012), *P. putida* exhibited high copper resistance in temperatures ranging from 25 °C to 35 °C. According to another report of the same research group (Andreazza et al., 2010), the optimum temperature for growth and Cu^{2+} bio-removal were determined at 30 °C and 35 °C, respectively.

3.5. Effect of initial Cu^{2+} concentration on the adsorption

In order to determine the effect of Cu^{2+} concentration on the adsorption this metal, five different Cu^{2+} concentrations (10, 25, 50, and 100 mg/l) were tested (Experimental conditions; temperature: 25 °C, pH: 5.0, stirring speed: 100 rpm, loofa sponge particle size: 0.42 - 0.85 mm). Time-dependent change in the amount of Cu^{2+} adsorbed per gram of bio sorbent is shown in Figure 5a.

As shown in Figure 5a, adsorption was reached to equilibrium between 25th - 40th min at all concentration values. At equilibrium, the lowest Cu^{2+} adsorption (0.22 mg/g) was measured in the experiment with an initial Cu^{2+} concentration of 10 mg/l. The adsorption values obtained at Cu^{2+} concentrations of 50 and 100 mg/l were relatively close to each other (0.42 and 0.41 mg/g, respectively). At equilibrium, the highest Cu^{2+} adsorption (0.48 mg/g) was determined in the experiment carried out with an initial Cu^{2+} concentration of 25 mg/l (Figure 5b).

The results obtained from the experiments given above were analyzed statistically. The results obtained from the experiments where 50 and 100 mg/l concentrations were tested were not statistically different. However, the results obtained from the experiments in which initial Cu^{2+} concentrations of 10 and 25 mg/l were tested were statistically different ($p < 0.05$).

According to Lister and Line (2001), despite the increase in the total amount of metal adsorbed by the increased starting metal concentration, the percentage of metal that is adsorbed decreases as the metal concentration increases. In higher concentrations, the bio sorbent becomes more saturated with the metal, so the equilibrium is reached in a shorter time (Zulfadhyl et al., 2001). In the present study, the bio sorbent became saturated at higher values than the starting Cu^{2+} concentration of 25 mg/l.

Some other researchers tested the effect of the initial Cu^{2+} concentration on the biosorption too. Chen et al. (2005) reported that metal ion biosorption was increased as the initial metal concentration increased at the optimal conditions. On the other hand, Uslu et al. (2011) reported that an increase in initial Cu^{2+} up to approximately 25 mg/l, the bioaccumulation capacity of *P. putida* increased. In addition to this finding, high Cu^{2+} accumulation yield was determined at low initial metal ion concentrations. This finding supports the results obtained from the present study.

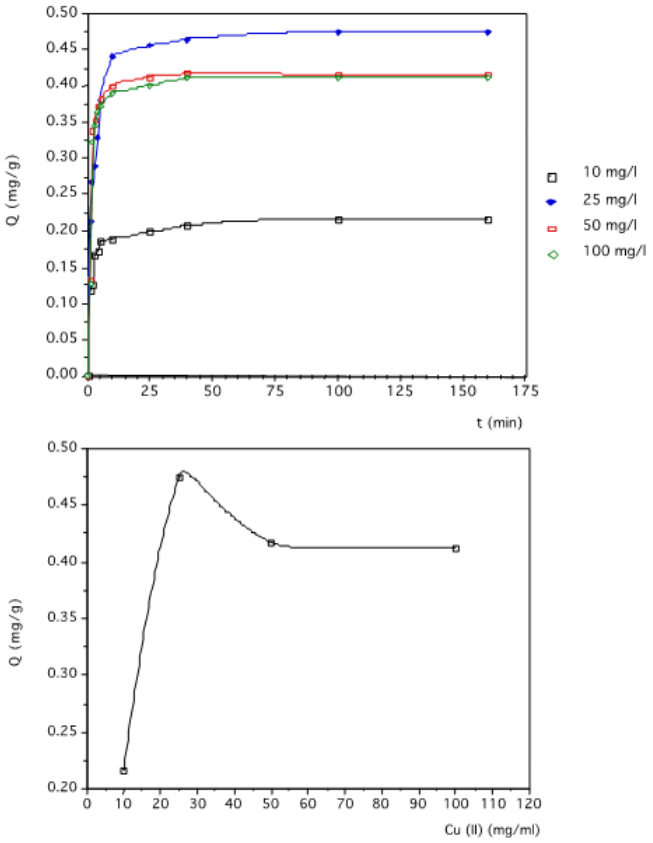

Figure 5. (a) Time-dependent change of initial Cu^{2+} concentration on the adsorption of Cu^{2+} **(b)** Effect of different initial Cu^{2+} concentration values on the adsorption of Cu^{2+} in equilibrium [temperature: 25 °C, pH: 5.0, stirring speed: 100 rpm, loofa sponge particle size: 0.42 - 0.85 mm]

3.6. Effect of stirring speed on the adsorption of Cu^{2+}

In order to determine the stirring speed on the adsorption of Cu^{2+}, stirring speeds of 50, 100, 130, and 150 rpm were tested (Experimental conditions; temperature: Cu^{2+} concentration: 10 mg/l, temperature: 25 °C, pH: 5.0, loofa sponge particle size: 0.42 - 0.85 mm). Time-dependent change in the amount of Cu^{2+} adsorbed per gram of bio sorbent is shown in Figure 6a.

As seen in Figure 6a, Cu^{2+} adsorption reached an equilibrium between 25th - 40th min at all stirring speed values studied. It was observed that within the first 5 min the adsorption took place very quickly. At equilibrium, adsorption of Cu^{2+} was found to be 0.16 mg/g at a stirring speed of 50 rpm. The adsorption value was measured as 0.19 mg/g at 100 rpm. At equilibrium, the adsorption values obtained for stirring speeds of 130 and 150 rpm were found to be relatively close to each other (0.24 and 0.23 mg/g, respectively). As can be understood from the data presented above, it can be said that the most effective Cu^{2+} adsorption occurred at a stirring speed of 130 rpm (Figure 6b).
In biosorption experiments, the stirring speed is an essential factor in increasing the contact of microorganisms with the metals in the solution. However, since biosorption usually involves weak bonds between the active surfaces of the microorganism and heavy metals, these bonds can easily break due to increased mechanical activity at high stirring speeds. As a result, adsorption efficiency decreases at high stirring speeds. As can be seen from the results given above, a higher stirring speed than 130 rpm cause the adsorption efficiency to decrease.

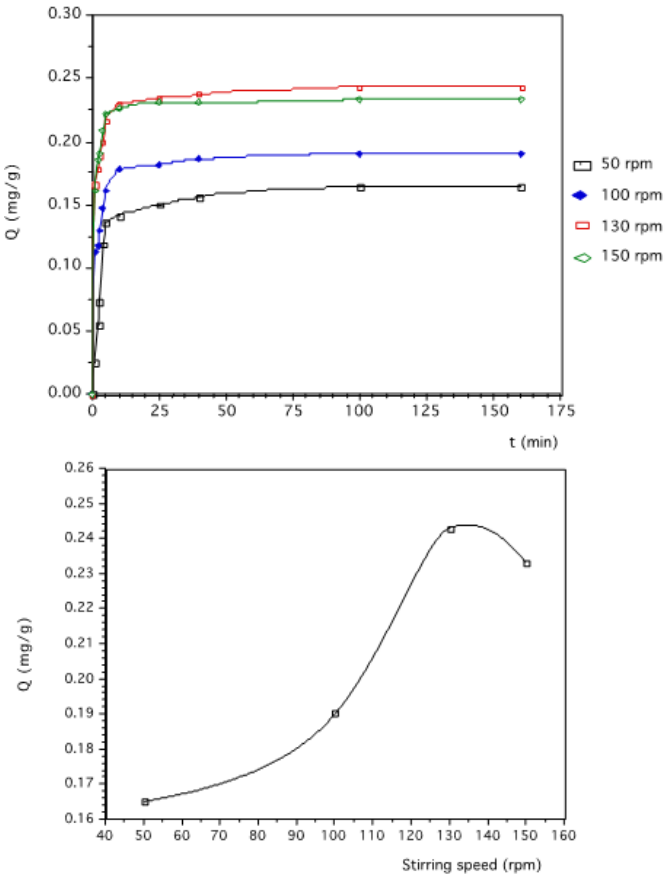

Figure 6. (a) Time-dependent change of stirring on the adsorption of Cu^{2+} **(b)** Effect of different stirring speed values on the adsorption of Cu^{2+} in equilibrium [Cu^{2+} concentration: 10 mg/l, temperature: 25 °C, pH: 5.0, loofa sponge particle size: 0.42 - 0.85 mm]

The data obtained from the experiments given above were statistically analyzed, and the results obtained from the experiments, where 130 and 150 rpm were tested as variables, were found not to be statistically different. On the other hand, the results obtained from the experiments, where 50 and 100 rpm were tested, were statistically different ($p < 0.05$).

3.7. Adsorption kinetics

According to detailed data presented above, it has been determined that optimum conditions for Cu^{2+} adsorption are as follows: Cu^{2+} concentration: 25 mg/l, temperature: 30 °C, pH: 5.0, loofa sponge particle size: 0.42 - 0.85 mm, stirring speed: 130 rpm. To determine the adsorption kinetics under these conditions, experiments were carried out with a loofa sponge containing and not containing *P. putida*.

The adsorbed Cu^{2+} concentration is plotted versus time and is shown in Figure 7a. As can be seen from the figure, the adsorption increases over time, and after a while, it reaches equilibrium. It has been found that the adsorption capacity of the loofa sponge containing bacteria was higher than that of the loofa sponge not containing bacteria. Figure 7b shows the graph of t versus t/Q. This graph shows that the adsorption is compatible with the second-order kinetics [$t/Q = a + bt$ (Q: Cu^{2+} concentration adsorbed at time 't', a: $1/(r_0)$ (the initial adsorption rate, d_c/d_t), b: $1/Q_{max}$ (maximum concentration of Cu^{2+} adsorbed)].

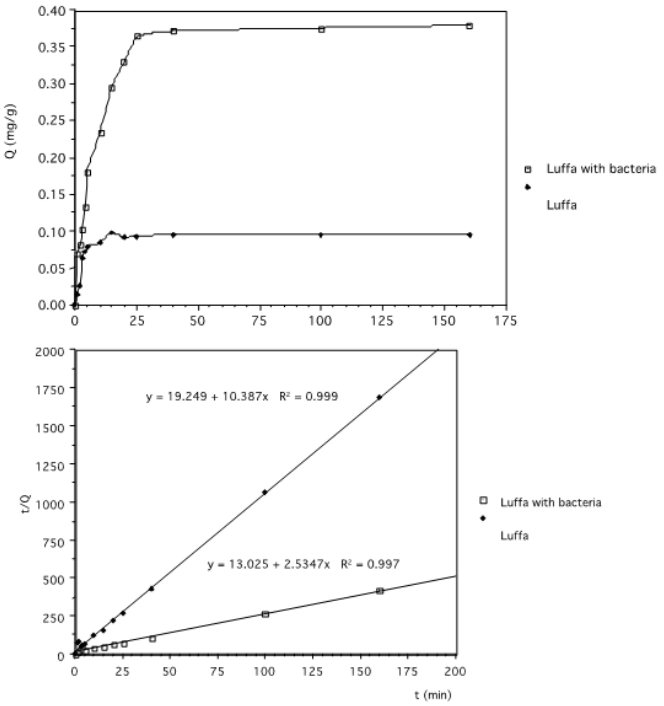

Figure 7. (a) Cu^{2+} adsorption curves for loofa sponge with and without *P. putida* **(b)** Kinetic curves of the Cu^{2+} adsorption for loofa sponge with and without *P. putida* [Cu^{2+} concentration: 25 mg/l, temperature: 30 °C, pH: 5.0, loofa sponge particle size: 0.42 - 0.85 mm, stirring speed: 130 rpm]

Q_{max} and r_o values were calculated from the data presented in Figure 7b. Q_{max} and r_o values were determined as 0.394 mg/g and 0.077 mg/g.min for loofa sponge containing *P. putida*, respectively, while these values were found to be 0.096 mg/g and 0.052 mg/g.min for loofa sponge not containing bacteria. The Langmuir-type adsorption model can describe the process of biosorption. These findings indicate that the microorganism-immobilized loofa sponge adsorbs Cu^{2+} rapidly, and adsorption takes place quite fast.

There are some studies in the literature that support the results obtained from the present study. In a study to investigate the Cu^{2+} biosorption efficiency of *P. putida*, Li et al. (2014) found the biosorption kinetics to be fast, with equilibrium being attained within 120 min. Pardo et al. (2013) studied the biosorption of copper by inactive biomass of *P. putida*. According to the results of this study, a contact time of 10 min was determined to be sufficient to reach equilibrium.

4. Conclusions

Due to the increasing rate of industrialization, heavy metal pollution in the future will create tremendous pressure on aquatic systems. Conventional methods for the removal of heavy metals from wastewater are complex processes that require expensive biochemicals. So, these methods are not sustainable economically. In recent years, the idea of benefiting from microorganisms in removing heavy metals from wastewater has begun to gain importance. Based on the results of this study, it was concluded that Cu^{2+} could be removed effectively from the aquatic systems by using *P. putida* cells immobilized on a loofa sponge under the optimum conditions documented.

Acknowledgments

The data presented here are mainly originated from Mrs. Arzuhan SIHOGLU TEPE's M.Sc. thesis.

Conflict of Interest

The authors confirm that there are no known conflicts of interest.

CRediT authorship contribution statement

Arzuhan Sihoglu Tepe: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing - original draft.
Emine Kacmazoglu: Methodology, Resources, Visualization, Supervision.
H. Nursevin Oztop: Conceptualization, Data curation, Methodology, Supervision.
Rana Taskin: Conceptualization, Data curation, Methodology, Supervision.

ORCID IDs of the Authors

A. Sihoglu Tepe: 0000-0001-8290-9880
E. Kacmazoglu: 0000-0002-9718-6790
H. Nursevin Oztop: 0000-0001-6175-0938
Rana Taskin: 0000-0003-2612-2826

Supplementary file

None.

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