INTRODUCTION

The progressive increase in industrialization and human activities translates into an increase in the percentage of waste accumulation worldwide and its release into the environment. The presence of heavy metals in wastewater is increasing with the development of industries such as electroplating, batteries, pesticides, mining, artificial silk, metal washing processes, tanning industry, fluidized bed bioreactors, textile industry, metal smelting, petrochemicals and paper production [Zou et al., 2016; Tjandraatmadja, 2008; Taseidifar, 2017; García-Niño, 2014; Borba 2006]. Unlike organic pollutants present in wastewater, heavy metals are not biodegradable and therefore persist permanently in the environment. Diverse sources are responsible for their excessive presence in ecosystems, whose importance has increased, notably due to the accelerating industrialization in recent decades [Marques et al., 2009].

The dangers of heavy metals in the environment are mainly due to their widespread occurrence and toxicity. Excessive amounts of Cu, Hg, Pb, Cd and Cr in ecosystems are responsible for physiological problems in many species, including humans [Elom et al., 2014; Stankovic et al., 2014]. Due to metal accumulation, a variety of symptoms can appear in organisms, including inhibition of tissue growth and differentiation, organ damage, elevated blood pressure and vascular obstruction, autoimmune diseases, oxidative damage and disruption of DNA methylation, worsening of allergic reactions, as well as sleep and speech disorders, decreased concentration, mood swings, and depression [Qu et al., 2013]. In addition, they can modify the production and function of cellular enzymes [Ageena, 2010]. Some heavy metals play irreplaceable roles as essential substances in mammalian metabolic systems, for example, zinc plays an important role in brain development and intelligence; copper is
a component of hemocyanin in the human body; manganese can promote normal bone growth and development and maintain normal glucose and fat metabolism [White and Broadley, 2009]. Exposure to heavy metals above safe levels can cause serious health damage.

The increasing importance of environmentally friendly and economical methods of heavy metal removal has led to an increased interest in the research and production of adsorbents. Techniques such as adsorption, chemical precipitation, electrochemical technologies, ion exchange and membrane filtration are highly efficient methods for removing toxic elements from water [Fu and Wang, 2011]. The adsorption has the lowest cost and shows unbeatable results in terms of flexibility of operation, effectiveness and efficiency, and has a high quality of the purified product next to the techniques mentioned above. In addition, their reusability after some processes enforces the use of adsorbents [Pan et al., 2009; Zhao et al., 2011]. Adsorption technique, removal of heavy metals from water and wastewater has come to the forefront relative to other techniques. The main reason for the dominance of this technique is its many benefits. [Hua et al., 2012]. Moreover, it is important to choose the most suitable method based on the removal efficiency, addition of chemicals/adsorbents, initial concentration, optimum purification, pH value and other operating conditions that will translate into the efficiency of heavy metal removal under given environmental conditions.

For a lengthy time, adsorption has been regarded as one of the effective methods for pollution control [Reddad et al., 2002; Bradl, 2004]. Easy access, cost-effectiveness and low charge (e.g., the price of adsorption by vermiculite is only 20% of the cost to be incurred when using osmotic membranes), ease of operation, as well as remarkably high adsorption efficiency (e.g., cyanite adsorption efficiency for Cu(II) up to 100%) compared to other technologies, support its application [Ajmal et al., 2001; Keng et al., 2014]. The high efficiency of this technology, up to 100%, in removing heavy metals, also present in trace amounts, also seems to be particularly important [Musyoka et al., 2013]. A crucial aspect of this technology application is the availability of environmentally friendly, low cost and high-efficiency adsorbents. To heavy metals removal from soil and wastewater, activated carbon, zeolite, chitosan and clay, among others, have been developed and studied [Strelko et al., 2004; Kosobucki et al., 2008].

Biosorption is one of the subcategories of adsorption in which a biological matrix acts as a sorbent. This process may be described as the removal or binding of specific desired substances (organic or inorganic, soluble or insoluble) from solution by biological material. Biosorption in the literature is presented through the lens of its efficiency and selectivity, and research using it has focused primarily on the sorption properties of a wide range of natural biomasses in their application to wastewater treatment, especially when the concentration of pollutants is less than 100 mg·l\(^{-1}\) and when other methods are economically impossible or insufficiently effective. The process can be carried out over wide pH and temperature ranges (3–9 and 4–90 °C, respectively). The most optimal particle size are those with a diameter of 1 to 2 mm because the equilibrium state of adsorption and desorption is reached rapidly. Another advantage of the technology is that biological material can be obtained cheaply from agriculture or industrial waste. In addition, compared to conventional methods, biosorption offers the possibility to recover metals, regenerate the biosorbent and minimize chemical and/or biological sludge, as well as does not require the supplying of additional nutrients [Michalak et al., 2013; Costa et al., 2021; Muharrem and Olcay, 2017].

**MATERIALS AND METHODS**

**Filter bed**

Laboratory tests were conducted on a sand bed inoculated with activated sludge, which consisted of three layers. The first layer counting from the top was filter gravel with grain diameter from 2 to 3 mm. This layer accounted for 20% of the total filter fill. Its task was to separate suspended solids present in the sewage flowing into the bed. The second filtration bed layer consisted of a biological part. It amounted to 50% of the total filter filling. To inoculate the biological layer, 200 ml of flocculent activated sludge with a dry weight of 5 kg/m\(^3\) was used, hence the amount of biomass in the biological part was about 1 gram. The bed was adapted to laboratory conditions and worked out for a period of 70 days. It was only after this time that biofilm formation was observed on the filter bed layer. The basis for biofilm formation...
was filter sand with an average grain diameter of about 2 mm. The last layer of the filter bed was the support layer, which was filter gravel with a diameter of 3 to 6 mm. Filter gravel was chosen for biofilm formation because of its homogeneous surface, which has a small number of micropores where contaminants could potentially be trapped. Hence, the sorption process studied occurred mainly in the biofilm layer formed on the surface of individual grains of the filter bed.

**Model wastewater**

The biological filter was fed with model laboratory wastewater prepared to reflect the actual composition of the treated wastewater. The model effluent was prepared using sodium acetate, potassium nitrate, ammonium chloride, and potassium dihydrogen phosphate I and II basic. This composition allowed for carbon, nitrogen and phosphorus compounds in the model wastewater [Ofman and Skoczko, 2018]. The source of Cu ions in the wastewater was CuSO₄. The various components of the wastewater were dissolved in tap water subjected to a carbon bed dechlorination process. Dechlorination of the water was crucial to maintain the parameters of the effluent flowing into the bed. This avoided the conversion of model wastewater constituents from dissolved to suspended form [Ofman and Struk-Sokolowska, 2019]. This regularity was of particular importance in the case of Cu ions, as this approach ensured that the predominant process in the removal of Cu ions was that of sorption on the biological membrane formed on the filter bed. The composition of raw wastewater flowing into the filter bed is shown in Table 1.

**Analytical methods**

The model wastewater used in this study was collected every 24 hours. The experiment was terminated when the concentration of Cu in the treated wastewater sample was equal to that in the treated wastewater. Such a phenomenon was indicative of the depletion of the sorption capacity of the tested bed, which was observed after 26 days. The concentration of copper in raw and effluent from the bed was studied using atomic absorption spectroscopy (ASA) in samples mineralized in nitric acid (HNO₃) with the addition of perhydrol (H₂O₂).

Two equilibrium models, Langmuir and Freundlich [Esmaeili et al., 2008], were analyzed to study the absorption isotherm. In its simplest form, it can rely on the estimation of the coefficients of the constant absorption isotherms. The Langmuir absorption isotherm is often used for the adsorption of a solute from a liquid solution and is often expressed as [Ageena, 2010; Sarbak, 2000]:

\[
q_e = \frac{q_{\text{max}} \cdot b \cdot C_e}{1 + b \cdot C_e}
\]  

(1)

where: 
\( q_e \) – amount of adsorbed metal ions at equilibrium [mg/g]
\( C_e \) – concentration of metal ions in solution at equilibrium [mg/dm³]
\( q_{\text{max}} \) – maximum number of metal ions in the monomolecular layer [mg/g]
\( b \) – Langumir equation constant related to the affinity of the adsorbate to active sites [dm³/mg]

The second type of adsorption isotherm used was the Freundlich isotherm. This is a modified empirical version of the Langmuir isotherm. This type of adsorption isotherm often performs much better than the classical Langmuir isotherm, especially for adsorption on energetically heterogeneous surfaces. In the course of this study, the Freundlich isotherm was used, which takes the following form [Rajczykowski, 2016; Deliyanii, 2007]:

\[
q_e = K_F \cdot C_e^{1/n}
\]  

(2)

where: 
\( q_e \) – amount of adsorbed metal ions at equilibrium [mg/g]
\( K_F \) – Freundlich’s equation constant related to the adsorption capacity [mg/g]
\( C_e \) – concentration of metal ions in solution at equilibrium [mg/dm³]
\( 1/n \) – Freundlich power exponent, indicating the degree of energy inhomogeneity of the adsorbent

**Statistical analysis**

The laboratory results provided the basis for determining the values of the constants included in the Langmuir and Freundlich absorption equations. These values were determined using the
nonlinear regression equation wizard available in the Statistica 13.1 package. The wizard allows for the implementation of a user function and subsequent estimation of the equation parameter constants. The algorithm here is based on running successive approximations to best match the approximated values to the observed values. A least squares function was used for this purpose. The Gauss-Newton method was used to find the values of the equation constants of the Langumir and Freundlich isotherms, and the number of iterations was taken to be 50.

**RESULTS AND DISCUSSION**

Efficiency of Cu ions removal

In the initial stage of the experiment, an increasing removal efficiency of Cu ions from the effluent flowing into the bed was observed (Figure 1). Between the first and third day of the experiment, the Cu ion removal efficiency varied from about 80 to almost 100%. This observation may be due to the fact that the microorganisms present in the biological membrane during the initial phase of the experiment show greater selectivity in capturing metal ions from aqueous solutions and storing them in the cellular structure [Ofman et al. 2021; Zou et al., 2016; Fu and Wang, 2011]. In the following days of the experiment, a gradual decrease in the removal efficiency of Cu ions from wastewater flowing into the bed was observed, which was close to zero on the 26th day of the experiment. It should be emphasized that the Cu biosorption process did not follow a linear course and a slight increase in the efficiency of Cu removal from wastewater was observed periodically. This regularity may be due to the fact that in addition to the biosorption phenomenon, desorption phenomenon may occur simultaneously [Hua et al., 2012; Keng et al., 2014]. As a result of desorption, element ions that have been immobilized in the structure of biomass are released into the aqueous solution. This phenomenon consequently leads to a lower efficiency of ion removal from solution, but on the other hand, in place of the desorbed ions, new ions can attach. At this point, it is important to emphasize that the desorption phenomenon is mainly observed for living biomass [Muharrem and Olcay, 2017; Strelko et al., 2004]. The efficiency with which Cu ions were removed from wastewater flowing into the filter bed in the considered time interval allowed indirectly to determine the full sorption capacity of the bed in relation to the element under study. The changes in the efficiency with which Cu ions were removed from wastewater and the concentration of Cu in the effluent flowing from the bed during the experiment are shown in Figure 1.

At this point, it should be emphasized that the course of the biosorption process depends on many factors, among which stand out the amount of carbon compounds available to the biomass, the pH of the solution and the contact time of the medium with the biosorbent. In the conducted study, an effort was made to keep these conditions constant throughout the experiment. This avoided discrepancies in the results of measuring the concentration of the amount of Cu ions and allowed a more accurate analysis of the process. Additionally, the adopted characteristics of wastewater flowing into the filter bed correspond to the parameters observed in wastewater treated by mechanical-biological processes in conventional activated sludge systems. Hence, the laboratory results obtained may be reflected in the analysis of the course of Cu ion removal from wastewater on real objects. The study indicated that the amount of biomass in the filter was 1 g. Hence, the observed amount of retained Cu in the filter bed can be directly related to this amount. It was observed during the study that the amount of Cu in the biomass of the filter bed was gradually accumulating. The amount of Cu retained was calculated based on the mass balance of this element between the effluents flowing into and out of the bed. The experiment was terminated when the amount of Cu in the effluent flowing out of the bed was similar to that in the inflowing water. In addition, it should be emphasized, this phenomenon may correspond to the achievement of the maximum sorption capacity with respect to Cu. The research work showed that the amount of Cu at which the

<table>
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<th>pH</th>
<th>Temp. [°C]</th>
<th>ChZT Co [mg/dm^3]</th>
<th>N-NH3 [mg/dm^3]</th>
<th>N-NO2 [mg/dm^3]</th>
<th>N-NO3 [mg/dm^3]</th>
<th>P-PO4 [mg/dm^3]</th>
<th>Cu [mg/dm^3]</th>
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<tr>
<td>7.62</td>
<td>20.00</td>
<td>74.00</td>
<td>1.90</td>
<td>1.30</td>
<td>0.06</td>
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full sorption capacity is reached is 10.68 mg/g. The detailed trend of changes in the biosorption process and the amount of retained Cu for each day of the experiment is shown in Figure 2.

**Langmuir and Freundlich isotherms**

The sorption data were subjected to two commonly used isotherm models, namely Langmuir and Freundlich to determine the saturation capacity of each sorbent. It is assumed that an increase in metal concentration in solution results in an increase in adsorption because there are more metal ions to overcome the mass transfer resistance between the aqueous and solid phase (adsorbent) [Vimala and Das 2009].

The Langmuir model (Figure 3) assumes that the sorption process occurs in a single layer and the sorbent surface is homogeneous [Nawrocki and Biłozor, 2000]. The obtained equation was characterized by a coefficient of determination ($R^2$) equal to 0.86, which meant that it reproduced in more than 86% the changes occurring during the sorption of Cu ions on the studied biological bed (Figure 4). Taking into account the value of the coefficient of determination, it can be concluded that the Langumir model does not fully reflect the complexity of the process of biosorption of Cu ions on the analyzed filter bed inoculated with activated sludge biomass.

According to Esmaeili [2014], the Langmuir isotherm fits the experimental absorption data in
most of the works with a high coefficient of determination, $R^2 > 0.99$. In the Langmuir isotherm model, with two parameters, it is assumed that the biosorbent surface is homogeneous and biosorption occurs in one surface layer [Abdelfattah et al., 2016]. The interactions do not occur between adsorbed ions [Li et al., 2017]. All active sites are equal and biosorption is homogeneous [Suzuki et al., 2017]. The value of $n$ in the power of the equation indicates whether the nature of the biosorption is unfavorable, linear, favorable or irreversible [Esmaeili and Beni, 2014]. This model is used for single metal ion systems.

The Freundlich isotherm biosorption model (Figure 5) treats the absorption process as a multidimensional and more complex phenomenon. Hence, in more complex systems such as biological materials, it can give better results in describing this phenomenon. In Freundlich’s equation, the $K_f$ constant describes the temperature conditions under which the adsorption process takes place, while $n$ indicates the intensity with which the sorption process occurs. The value of the $K_f$ constant was 29.79 and the value of the $n$ constant was 1.66. According to other authors’ studies, if $n$ is greater than 1 then adsorption is the dominant process for removing a given contaminant. The obtained model had a coefficient of determination equal to 94%. Hence, compared to the Langmuir model, the Freundlich isotherm
more accurately represents the changes occurring in Cu on the biological bed.

As previously mentioned, the Freudlich isotherm is applicable to systems where the sorption phenomenon can occur in more than one layer. With respect to the research work carried out, the multilayer aspect can be justified by several factors. The first was the design of the filter bed itself, which consisted of filter gravel and microorganism biomass growing on its surface layer. Such an arrangement represents a combination of two matrices capable of immobilizing heavy metals. It should be stressed, however, that the retention of Cu ions on gravel alone was most probably of low efficiency, which is dictated by the very structure of mineral materials whose surface is relatively homogeneous and devoid of micropores. On the other hand, the biological membrane itself is a relatively complex element in terms of describing the biosorption process. The multilayer aspect of heavy metal sorption in a biofilm is dictated by the potential pathways for sorption and ultimate immobilization of the element. Numerous studies indicate [Shuhong et al., 2014; Comte et al., 2006] that the biosorption process occurs with the dominant participation of EPS. EPS is a kind of material used in the exchange of nutrients between groups of microorganisms, but also due to its organic nature, it shows affinity towards heavy metal sorption. It should be noted

![Figure 5. Freudlich isotherm for a biological bed](image)

![Figure 6. Fit of Freudlich isotherm model to observed values](image)
that the biological membrane formed on the filter gravel is significantly formed by EPS. In addition to sorption in EPS, heavy metals can also be retained by microorganisms, both living and dead, that are present in the biological membrane [Zhou et al., 2009].

CONCLUSIONS

1. The study determined that the effective operating time of the biological filter in terms of Cu ion removal was 26 days. The bed retained a total of 10,36 mg of Cu during the test cycle. Considering that 1 g of activated sludge dry weight was used to inoculate the bed, the determined sorption capacity is 10,36 mg of Cu per 1 g of organic dry weight in the bed.

2. The highest Cu ion removal efficiency was observed on the 3rd, 4th and 5th day of the experiment, which could be due to the adaptation of microorganisms in the filter bed to the influent medium in the wastewater.

3. The Langmuir model did not fully reflect the biosorption phenomenon on the filter bed studied. In comparison with the Langmuir model, the Freundlich isotherm more accurately represents the changes occurring during Cu sorption on the biological bed. The Freundlich model was observed to have a coefficient of determination (R^2) of 0.94. The observed differences in the accuracy of representation of the biosorption process by the different isotherms may be due to their properties. The better fit of the Freundlich isotherms indicates that biosorption occurring on a sand bed inoculated with activated sludge is a multilayer system phenomenon.

REFERENCES


