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Raw Eggshell as an Adsorbent for Copper Ions Biosorption—Equilibrium, Kinetic, Thermodynamic and Process Optimization Studies

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Abstract: The study on the biosorption of copper ions using raw eggshells as an adsorbent is presented in this paper. The influence of different process parameters, such as: initial pH value of the solution, initial Cu^{2+} ions concentration, initial mass of the adsorbent, and stirring rate, on the biosorption capacity was evaluated. The SEM-EDS analysis was performed before and after the biosorption process. SEM micrographs indicate a change in the morphology of the sample after the biosorption process. The obtained EDS spectra indicated that K, Ca, and Mg were possibly exchanged with Cu^{2+} ions during the biosorption process. The equilibrium analysis showed that the Langmuir isotherm model best describes the experimental data. Four kinetic models were used to analyze the experimental data, and the results revealed that the pseudo-first order kinetic model is the best fit for the analyzed data. Calculated thermodynamic data indicated that the biosorption process is spontaneous, and that copper ions are possibly bound to the surface of the eggshells by chemisorption. The biosorption process was optimized using Response Surface Methodology (RSM) based on the Box-Behnken Design (BBD), with the selected factors: adsorbent mass, initial metal ion concentration, and contact time.

Keywords: biosorption; response surface methodology; Box-Behnken design; eggshell; equilibrium; kinetics; thermodynamics; copper ions

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1. Introduction

Wastewater containing heavy metals, that originate from tanneries, batteries, mining and metallurgical operations, chemical manufactories, pesticides, and other sources, has been a major pollutant in the environment for many years. The non-biodegradability and persistent nature of these metals means they tend to enter the food chain and accumulate in the living organisms, causing numerous disorders and diseases [1,2].

Wastewater treatment methods can be classified into five groups, i.e., adsorption-, chemical-, membrane-, electric-, and photocatalytic- based treatments [3].

The adsorption-based separation methods are defined by the properties of the adsorbent, and the working conditions of the process, like temperature, pH value of the solution, adsorption time, etc. The adsorbents can be classified as carbon-based adsorbents, chitosan-based adsorbents, mineral adsorbents, magnetic adsorbents, and biosorbents [3].

Membrane-based filtration and separation is a wastewater treatment method that usually includes ultrafiltration, nanofiltration, microfiltration, reverse osmosis, forward osmosis, and electrodialysis [3].

Chemical-based separation methods for wastewater treatment polluted with heavy metals include precipitation, coagulation and flocculation, and flotation. These methods change the form of the dissolved metal into solid particles, to facilitate their sedimentation [3].

Electric-based separation methods for wastewater treatment include electrochemical reduction, electroflotation, electrooxidation and ion-exchange treatment [3].

Heavy metals are being removed from wastewater on the industrial scale by well-known conventional technologies. However, these conventional technologies have many disadvantages that include high operating costs, incomplete metal removal, continuous input of chemicals, and others. These disadvantages raise the question of finding a new method of wastewater treatment that could become an alternative to the existing conventional technologies, and improve the overall process [1,4].

Adsorption methods are a more suitable processes for wastewater treatment, due to the high metal recovery rate, no sludge production, low economic investments, the ability to regenerate the adsorbent, and many others [1].

In recent years, the scientific community has recognized biosorption as a potential, efficient and economically feasible alternative to conventional technologies for the removal of heavy metal ions from aqueous solutions. The scientific research is focused on examining the possibility of using many industrial and agricultural waste materials as biosorbents [5].

Since inactive biomass is usually used in biosorption processes, the mechanism of metal ions removal is based on adsorption, chelation, ion exchange, complexation, coordination, microprecipitation, electrostatic interaction, or the combination of the beforementioned mechanisms [6].

Many industries that produce and use eggs generate considerable amounts of waste in the form of eggshells. These by-products constitute approximately 6 g/egg, an amount which represents significant waste. Waste eggshells are considered useless and are disposed in landfills without any pre-treatment [7].

The aim of this work is to study the possibility of using waste raw eggshells as an adsorbent for copper ions removal from aqueous solutions, as well as to analyze the specifics of the process and the influence of certain parameters on its efficiency. The use of eggshells as an adsorbent for wastewater treatment could potentially solve two problems. First, it would reduce the amount of waste in landfills, thus directly help industries based on the use of eggs by reducing the costs of their disposal. And, secondly, it would contribute to solving the problem of watercourses contamination with heavy metals (in this case, copper).

The performed analysis in this work include:

- the influence of different process parameters (initial Cu^{2+} ions concentration, pH value of the solution, adsorbent mass and stirring rate) on the biosorption capacity;
- SEM-EDS analysis of the eggshells sample before and after the biosorption process;
- kinetic analysis of the biosorption process;
- equilibrium analysis of the biosorption process;
- thermodynamic analysis of the biosorption process;
- process optimization study by the mean of Response Surface Methodology based on Box-Behnken Design

2. Materials and Methods

Raw chicken eggshells (Figure 1), collected from local households (located in the city of Bor, in eastern Serbia), were washed with distilled water several times, ground, sieved, and the fraction (−1 + 0.4) mm was used for the biosorption experiments.

The eggshells samples were rinsed with 200 mL distilled water, prior to the biosorption experiments, in order to remove the physical impurities.

Biosorption experiments were conducted in batch conditions, using synthetic Cu^{2+} solutions, prepared with $CuSO_4 \cdot 5H_2O$ (p.a.). The concentrations of the solutions varied, based on the specifics of the performed experiment.

pH value of the solutions was adjusted using 0.1 M HNO_3 and 0.1 M KOH.

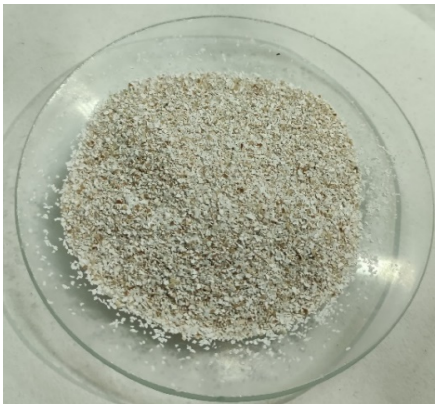

Figure 1. Chicken eggshells sample.

Process parameters, including contact time, initial copper ions concentration, temperature, stirring rate, initial mass of the adsorbent, and initial pH value were adjusted depending on the performed experiment.

All experiments were performed in batch conditions. A spectrophotometer (Spectroquant Pharo 300—Merck, Rahway, NJ, USA) was used to analyze the solutions for the remaining copper ions content. The SEM-EDS analysis was performed on a SEM scanning electron microscope (VEGA 3 LMU, Tescan, Brno, Czech Republic) with an integrated energy-dispersive X-ray detector (X act SDD 10 mm^2, Oxford Instruments, Abingdon, UK).

The biosorption capacity and the adsorption degree were calculated using the following equations:

$$q_t = \frac{c_i - c_t}{m} \cdot V \quad (1)$$

$$AD\% = \left(1 - \frac{c_t}{c_i}\right) \cdot 100 \quad (2)$$

where: q_t is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at time t; c_i is the initial metal ion concentration in the solution; c_t is the metal ion concentration in the solution at time t; m is the adsorbent mass; V is the volume of the solution; $AD\%$ is the adsorption degree.

3. Results and Discussions

3.1. The Influence of Different Process Parameters on the Adsorption Efficiency (Biosorption Capacity)

3.1.1. The Effect of ph Value on the Biosorption Capacity

In order to analyze the effect of the pH value on the biosorption capacity, a series of experiments was performed, using Cu^{2+} ion solutions, of different pH values, ranging from 2 to 5. The pH value of the solutions was adjusted by adding 0.1 M HNO_3 and 0.1 M KOH. The experiments were performed at room temperature, using solutions of initial Cu^{2+} concentration of 500 mg dm^{-3}. The suspension was stirred for 60 min. The obtained results are shown on Figure 2a.

As can be seen from Figure 2a, low pH values of the solution led to a low biosorption capacity. A rise in the pH value of the solution led to a rise in the biosorption capacity. At pH = 2 the biosorption capacity was determined to be around 10.82 mg g^{-1}, while at pH = 5 the biosorption capacity was almost twice as higher (q_t = 21.62 mg g^{-1}). The rise in the biosorption capacity of eggshells at higher pH values of the solution occurs due to the fact that chicken eggshell constitutes of about 95% of calcium carbonate and 5% of organic matter. The calcium carbonate favors precipitation of metal ions, as it dissociates to carbonate and calcium ions. The solubility of calcium carbonate in the eggshells varies, based on the pH level of the solution. Carbonate species appear in solutions as H_2CO_3, HCO_3^- and CO_3^{2-}. The latter two are presumably responsible for the formation of metal

carbonates [5]. Considering the divalent nature of the Cu^{2+} ions in the solution at pH = 5 (Figure 3), it is assumed that the carbonate ions from the eggshell interact with the copper ions to form copper carbonates.

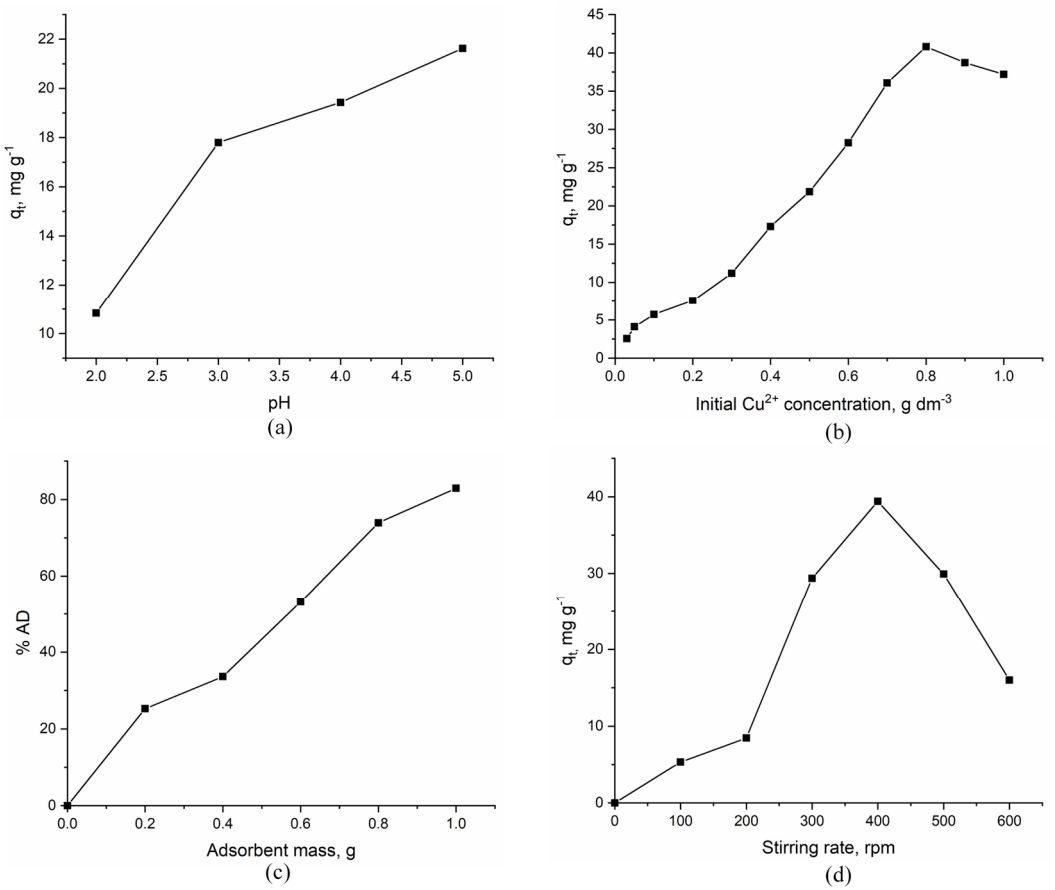

Figure 2. (**a**) pH value effect on the biosorption capacity, (**b**) initial Cu^{2+} concentration influence on the biosorption capacity, (**c**) adsorbent mass effect on the biosorption capacity, and (**d**) stirring rate influence on the biosorption capacity for copper ions biosorption onto chicken eggshells.

3.1.2. The Influence of the Initial Cu^{2+} Concentration on the Biosorption Capacity

The influence of the initial Cu^{2+} concentration on the biosorption capacity was examined by bringing into contact 1 g of chicken eggshell with 0.5 dm^{-3} copper ion solutions of different initial concentrations, ranging from 30 mg dm^{-3} to 1000 mg dm^{-3}, on a magnetic stirrer, for 60 min. The experiments were performed at room temperature. The obtained results are shown on Figure 2b. Figure 2b shows an increase in the biosorption capacity, with the rise in the initial Cu^{2+} ions concentration, up to 800 mg dm^{-3}, where it reaches the maximum value (q_t = 40.79 mg g^{-1}). With a further increase in the initial copper ions concentration, a decrease in the biosorption capacity is noted. It is assumed that this decrease occurs due to the saturation of the adsorbent with Cu^{2+}.

The fact that the adsorption process includes different simultaneous processes, among which are the diffusion in the liquid phase and adsorption in the solid phase, it is assumed that the increase in the initial metal ions concentration leads to an increase in the probability of their contact with the active sites in the structure of the adsorbent. The saturation of the active sites in the adsorbent structure leads to the decrease in the biosorption capacity, with the further increase in the initial metal ions concentration [7].

3.1.3. The Effect of the Adsorbent Mass on the Biosorption Capacity

The effect of the adsorbent mass on the biosorption capacity was determined by bringing into contact 0.5 dm^{-3} copper ion solutions (initial concentration 500 mg dm^{-3})

with different amounts of eggshells, ranging from 0.2 to 1 g. The suspension was stirred on a magnetic stirrer, under room temperature, for 60 min. The results of the performed analysis are shown on Figure 2c. As seen on Figure 2c, the adsorption degree increased from 25% to 82% with the increase in the adsorbent mass from 0.2 to 1 g, due to the higher number of available active sites on the adsorbent structure as a result of a larger amount of adsorbent available [8].

3.1.4. The Influence of the Stirring Rate on the Biosorption Capacity

The influence of the stirring rate on the biosorption capacity was analyzed by performing the following experiment 0.5 g of eggshells was brought into contact with 0.5 dm^{-3} copper ions solutions, and stirred at room temperature using different stirring rates from 100 to 600 rpm, for 60 min. The obtained results are shown on Figure 2d. The results show that the biosorption capacity increased with the increase in the stirring rate, up to 400 rpm, where it reached its maximum value. Further increase in the stirring rate resulted in a decrease of the biosorption capacity.

It is assumed that the increase in the stirring rate accelerates the diffusion of the metal ions through the liquid phase to the surface of the adsorbent, resulting in the rise of the biosorption capacity [9].

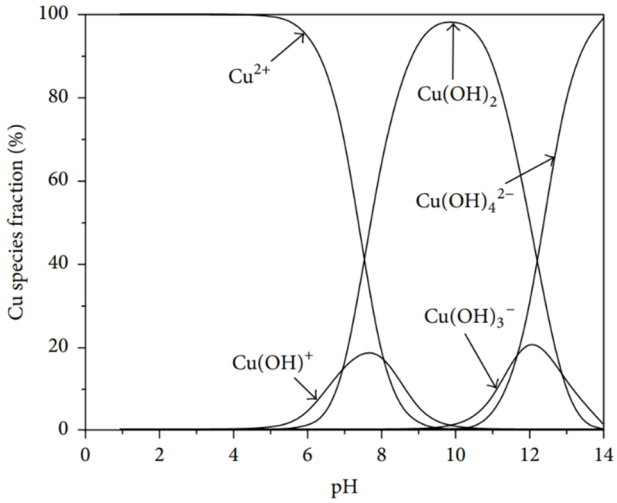

Figure 3. Cu species distribution at different pH values of the solution [10].

3.2. SEM-EDS Analysis

The SEM-EDS analysis of the eggshells was performed before and after the biosorption of copper ions in order to study the surface morphology and texture of the samples. The obtained results are shown on Figure 4.

Figure 4a shows a porous and dense surface structure of the untreated raw eggshell. Figure 4c shows a slight change to the surface morphology, with the surface becoming uneven, rough and heterogeneous, as a result of the incorporation of copper ions inside the structure of the eggshells sample. The interaction of eggshells with Cu^{2+} ions lead to the formation of flake-like deposits on the surface of the adsorbent [11,12].

The EDS analysis was performed by scanning multiple points on the surface of the untreated eggshell as well as the eggshell sample after the adsorption process. The EDS spectrum of the untreated eggshell (Figure 4b) showed peaks for O, Mg, K and Ca, with high O and Ca contents. The obtained spectrum after the adsorption process (Figure 4d) indicates the absence of the Mg peak, while the K and Ca peaks remained but were reduced. A new peak, corresponding to the adsorbed Cu ions appeared. Obtained EDS results indicate that Mg, K and Ca could potentially be exchanged with Cu during the adsorption process.

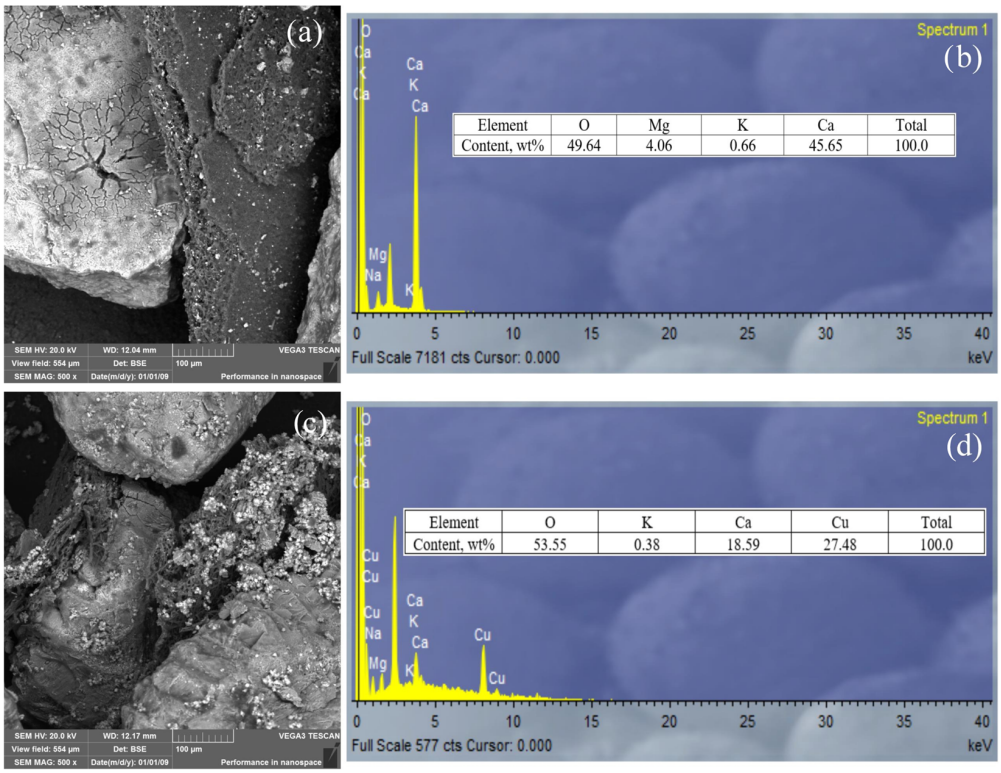

Figure 4. SEM micrographs of eggshells samples before Cu^{2+} biosorption (**a**) and after the biosorption process (**c**), with the corresponding EDS spectra before (**b**) and after the biosorption process (**d**).

3.3. Kinetic Study

Adsorption kinetic data provides insight into the mechanism of the adsorption process, it's rate, as well as information about the step that determines the overall rate of the process. In this work, the experimental data were modeled using the non-linear forms of the pseudo-first order kinetic model, pseudo-second order kinetic model, intraparticle diffusion kinetic model (Weber-Morris model), and the Elovich kinetic model.

In order to obtain the biosorption kinetic data, 50 mL of copper ion solutions (initial Cu^{2+} concentration 500 mg dm^{-3}) were brought into contact with 1 g of eggshells samples, for different process time (ranging from 1 to 90 min). The kinetic analysis is presented in Figure 4 along with the obtained kinetic data which are presented in Table 1.

3.3.1. Pseudo-First Order Kinetic Model

The pseudo-first order kinetic model is often used to describe the kinetics of a sorption process. According to this model, a type of sorbent reacts with one active center in the adsorbent structure, forming a sorption complex [13].

The non-linear form of the pseudo-first order kinetic model can be expressed as:

$$q_t = q_e\left(1 - e^{-k_1 t}\right) \quad (3)$$

where: q_t is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at time t; q_e is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at equilibrium; k_1 is the adsorption rate constant for the pseudo-first order kinetic model (min^{-1}).

The experimental data were fitted using this model (Figure 5), and the kinetic parameters were determined and presented in Table 1.

Table 1. Kinetic model parameters for copper ions biosorption onto eggshells.

Model	Parameters	Values
Pseudo-first order kinetic model	k_1 (min^{-1})	0.018
	$q_{e,exp}$ (mg g^{-1})	22.84
	$q_{e,cal}$ (mg g^{-1})	28.34
	R^2	0.999
Pseudo-second order kinetic model	k_2 (g mg^{-1} min^{-1})	$2.90 \cdot 10^{-4}$
	$q_{e,exp}$ (mg g^{-1})	22.84
	$q_{e,cal}$ (mg g^{-1})	43.26
	R^2	0.982
Intraparticle diffusion kinetic model (Weber-Morris model)	K_i (mg g^{-1} $min^{-0.5}$)	2.311
	C_i	$9.99 \cdot 10^{-24}$
	R^2	0.955
Elovich kinetic model	α (mg g^{-1} min^{-1})	0.603
	β (g mg^{-1})	0.067
	R^2	0.983

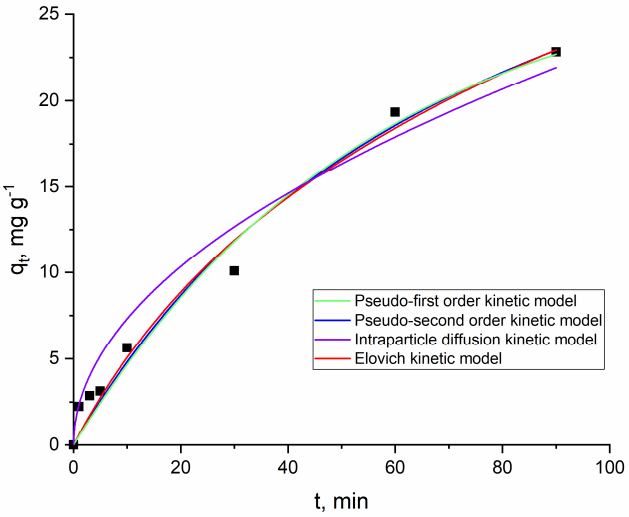

Figure 5. Non–linear kinetic models for copper ions biosorption onto chicken eggshells.

3.3.2. Pseudo-Second Order Kinetic Model

This model assumes that the kinetics of a sorption process simultaneously depends on the number of free active centers on the surface of the sorbent and the concentration of the sorbate in the solution [14].

The non-linear form of this model is given as:

$$q_t = \frac{q_e^2 k_2 t}{1 + k_2 t q_e} \quad (4)$$

where: q_t is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at time t; q_e is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at equilibrium; k_2 is the adsorption rate constant for the pseudo-second order kinetic model (g mg^{-1} min^{-1}).

The biosorption data were modeled using this model, and the results are shown on Figure 5 and in Table 1.

3.3.3. Intraparticle Diffusion Kinetic Model (Weber-Morris Model)

The Weber-Morris model assumes that the adsorption process does not take place only on the surface of the adsorbent, but that diffusion and adsorption inside the adsorbent structure also occur [15].

The non-linear form of the intraparticle diffusion kinetic model is given as:

$$q_t = K_i t^{0.5} + C_i \quad (5)$$

where: q_t is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at time t; K_i is the internal particle diffusion rate constant (mg g^{-1} $min^{-0.5}$); C_i is the boundary layer thickness constant.

The experimental data were fitted using the Weber-Morris model (Figure 5), and the kinetic parameters were determined and presented in Table 1.

3.3.4. Elovich Kinetic Model

The Elovich model is one of the most useful kinetic models for describing chemisorption [16].

The non-linear form of this model is given as:

$$q_t = \frac{1}{\beta} \ln(\alpha\beta t + 1) \quad (6)$$

where: q_t is the adsorbent capacity defined as mass of the adsorbed metal per unit mass of the adsorbent (mg g^{-1}) at time t; α is the starting adsorption rate (mg g^{-1} min^{-1}); β is the parameter that expresses the degree of surface coverage and activation energy for chemisorption (g mg^{-1}).

The obtained corresponding plot and kinetic data for this model are shown on Figure 5 and in Table 1.

The experimental data were fitted using four non-linear kinetic models, i.e., the pseudo-first order kinetic mode, pseudo-second order kinetic model, intraparticle diffusion kinetic model, and the Elovich kinetic model. Based on the obtained kinetic parameters (Table 1), it can be concluded that all the analyzed models show good agreement with the experimental data. However, the pseudo-first order kinetic model has proven to be the best fit for the analyzed data (R^2 = 0.999). Such results suggest that, in theory, copper ions react with active sites inside the structure of the eggshell, forming sorption complexes.

3.4. Equilibrium Study

Adsorption isotherm models are used to analyze experimental data in order to gain information about the mechanism of the adsorption process, it's equilibrium, and the maximum biosorption capacity. In this work, the non-linear Langmuir, Freundlich and Temkin isotherm models were used to analyze the equilibrium of the copper ions biosorption process onto chicken eggshells.

Biosorption isotherm data was obtained by performing the following experiment: 0.5 g of eggshells samples was brought into contact with 50 mL of copper ions solutions, of different initial Cu^{2+} concentrations (in the range from 30 to 400 mg dm^{-3}). The suspension was stirred on a magnetic stirrer for 90 min, assuming that is enough time to reach the equilibrium between phases [17]. The obtained experimental data was fitted using the mentioned non-linear isotherm models, and the results are presented in Figure 6 along with the isotherm parameters in Table 2.

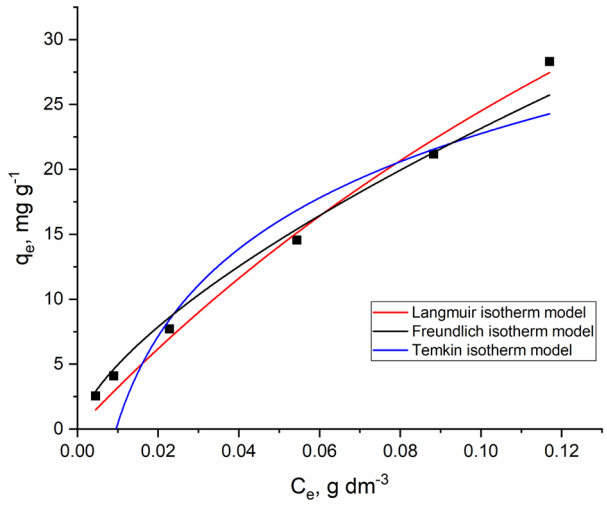

Figure 6. Biosorption isotherm data fitted using non–linear Langmuir, Freundlich and Temkin models.

Table 2. Adsorption isotherm model parameters for copper ions biosorption onto eggshells.

Model	Parameters	Values
Langmuir adsorption isotherm model	K_L (dm^3 mg^{-1})	3.49
	$q_{e,exp}$ (mg g^{-1})	28.3
	q_m (mg g^{-1})	94.59
	R^2	0.989
Freundlich adsorption isotherm model	K_F	108.5
	1/n	0.671
	R^2	0.931
Temkin adsorption isotherm model	B (J mol^{-1})	9.698
	K_T (dm^3 g^{-1})	104.49
	R^2	0.927

3.4.1. Langmuir Isotherm Model

The Langmuir isotherm model assumes that the adsorption process takes place in a monolayer, and that there are a finite number adsorption sites (each site can hold one adsorbate molecule). There is no interaction between the adsorbed molecules, and all adsorption sites are equivalent [18,19].

This model can be expressed as:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (7)$$

where: q_e is the equilibrium biosorption capacity (mg g^{-1}); q_m is the maximum biosorption capacity (mg g^{-1}); *Ce* is the equilibrium concentration of metal ions in the solution (mg dm^{-3}); and K_L is the Langmuir equilibrium constant (dm^3 g^{-1}).

3.4.2. Freundlich Isotherm Model

The Freundlich model is a good representation of sorption processes at low and intermediate concentrations. This model can be applied to non-ideal and multilayer sorption on heterogeneous surfaces [20].

The Freundlich model can be represented as:

$$q_e = K_f C_e^{1/n} \quad (8)$$

where: q_e is the equilibrium biosorption capacity (mg g^{-1}); *Ce* is the equilibrium concentration of metal ions in the solution (mg dm^{-3}); K_f is the Freundlich equilibrium constant ((mg g^{-1}) (dm^3 mg^{-1})$^{1/n}$).

The Freundlich constant n provides insight into the favorability of the adsorption process. When the value of n lays between 1 and 10 (i.e., $1/n$ is lower than 1), the adsorption process is favorable [21].

3.4.3. Temkin Isotherm Model

This model assumes that the adsorption heat of all the molecules in the layer shows a linear decrease with the coverage of molecules, and that adsorption is characterized by a uniform distribution of binding energies, up to a maximum binding energy [18].

The Temkin model is given as:

$$q_e = B \ln(K_T C_e) \quad (9)$$

where: q_e is the equilibrium biosorption capacity (mg g^{-1}); *Ce* is the equilibrium concentration of metal ions in the solution (mg dm^{-3}); $B = RT/b$ is the Temkin constant, which refers to the adsorption heat (J mol^{-1}); b is the variation of adsorption energy (J mol^{-1}); R is the universal gas constant (J mol^{-1} K^{-1}); T is the temperature (K); K_T is the Temkin equilibrium constant (dm^3 g^{-1}).

Based on the correlation coefficients (Table 2), it can be concluded that the biosorption process follows the Langmuir isotherm model, as it showed a very good agreement with the experimental data. This result indicates that there is a homogeneous distribution of active sites on the eggshell surface, while the adsorption process takes place in a monolayer, and that there are a finite number adsorption sites [18,19].

In addition, the Freundlich constant n suggests that the biosorption of copper ions onto chicken eggshells is a favorable process (n is between 1 and 10, i.e., $1/n$ is lower than 1) [21].

The performance of the adsorbent is usually defined by the maximum biosorption capacity. Based on the results in copper removal with various biosorbents reported by other workers (Table 3), it can be concluded that eggshells may play an important role as a cost-effective biosorbent for copper ions removal from aqueous environments.

Table 3. Cu^{2+} ions biosorption on eggshell in comparison with other adsorbents.

Biosorbent	Maximum Biosorbent Capacity (q_m, mg g^{-1})	Work
Eggshell	94.59	This work
Saccharomyces cerevisiae (brewer's yeast)	26.95	[22]
Carbonized sunflower stem	38.05	[23]
Sericin cross-linked with polyethylene glycol-diglycidyl ether	36.17	[24]
Sawdust of deciduous trees	9.9	[25]
Wheat straw	4.3	[17]
Chlorella pyrenoidosa (freshwater green algae)	11.88	[26]
Codium vermilara (codium seaweed)	14.4	[27]
Olive stone	1.96	[28]
Pine bark	11.35	[28]
Chitosan	103	[29]

3.5. Thermodynamic Study

The influence of temperature on the biosorption process was analyzed by bringing into contact 0.5 g of eggshells samples with 50 mL of synthetic Cu^{2+} solutions (initial concentration 500 mg dm^{-3}), at different temperatures (25 °C, 35 °C, and 45 °C). The suspension was stirred for 90 min. The obtained results (Figure 6) are analyzed in order to determine the thermodynamic parameters. The thermodynamic parameters are calculated using the Equations (10)–(13).

$$K_d = \frac{C_A}{C_S} \tag{10}$$

$$\Delta G^0 = -RT \ln K_d \tag{11}$$

$$\ln K_d = \left(\frac{\Delta S^0}{R}\right) - \left(\frac{\Delta H^0}{RT}\right) \tag{12}$$

$$\ln K_d = \left(\frac{-Ea}{RT}\right) + \ln A \tag{13}$$

where: K_d is the thermodynamic equilibrium constant; C_A is the concentration of the adsorbed adsorbate (mg dm^{-3}); C_S is the equilibrium concentration of the adsorbate in the solution (mg dm^{-3}); ΔG^0 is the Gibbs free energy (kJ mol^{-1}); R is the universal gas constant (J mol^{-1} K^{-1}); T is the temperature (K); ΔS^0 is the entropy change (J mol^{-1} K^{-1}); ΔH^0 is the enthalpy change (kJ mol^{-1}); Ea is the activation energy (kJ mol^{-1}).

The change of the Gibbs free energy was calculated using the Equation (11).

According to the Equation (12), the values of the enthalpy and entropy were calculated from the slope and intercept of the plot ln K_D vs. 1/T, which is shown on Figure 7 [20].

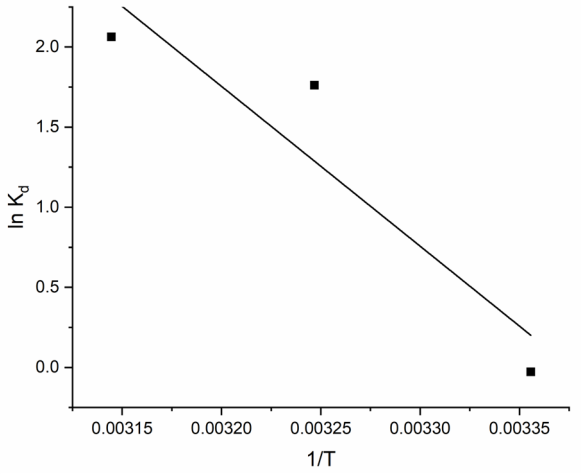

Figure 7. Thermodynamic plot ln KD vs. 1/T for copper ions biosorption onto eggshells.

The activation energy was calculated from the slope of the plot ln K_D vs. 1/T, using the Equation (13).

The thermodynamic parameters are given in Table 4.

Table 4. Thermodynamic parameters for copper ions biosorption onto eggshells.

T (K)	ΔG^0 (kJ mol $^{-1}$)	ΔH^0 (kJ mol $^{-1}$)	ΔS^0 (J mol $^{-1}$ K^{-1})	Ea (kJ mol $^{-1}$)
298	0.07	−9.98	33.68	82.97
308	−4.51			
318	−5.45			

The Gibbs free energy (Table 3) for the copper ions biosorption onto eggshells indicates that the process is spontaneous and favored at temperatures above 25 °C. Negative enthalpy change indicates that the process is exothermic. Positive entropy value indicates that there is an increased randomness at the solid/liquid interface during the adsorption process [30,31].

The activation energy (Ea) value of 82.97 kJ mol^{-1} indicates that the binding of copper ions onto eggshells took place mainly by chemisorption [32].

3.6. Process Optimization Study

The biosorption of copper ions using eggshells was optimized using an experimental design, in order to determine the effects of three selected (independent) variables on the percentage of Cu^{2+} removal (dependent variable). Response Surface Methodology (RSM) represents a set of techniques, which is useful for evaluating the relationships between a number of experimental factors and measured responses [33–35].

The RSM-BBD was applied to optimize the biosorption process using the Design Expert software (version: 22.0.0) [36].

The Box-Behnken factorial design, consisting of 17 experiments, coupled with Response Surface Methodology, was applied with the goal to optimize the biosorption process, comparing three factors: Adsorbent mass (A), initial copper ions concentration (B) and contact time (C). The experimental ranges and their levels in the design are given in Table 5. The experimental design matrix, as well as the response R (adsorption degree) are given in Table 6.

Table 5. Experimental ranges and levels in the experimental design.

Factors	Range Level		
	−1	**0**	**1**
A—Adsorbent mass (g)	0.5	1	1.5
B—Initial metal ion concentration (g/L)	0.5	1	1.5
C—Contact time (min)	10	60	90

Table 6. Box-Behken Design matrix for three factors along with observed response for Cu^{2+} biosorption onto eggshells.

Run	A: Adsorbent Mass (g)	B: Initial Cu^{2+} Ions Concentration (g/L)	C: Contact Time (min)	R: Adsorption Degree (%)
1	1.5	0.5	60	64.54
2	0.5	1	10	9.87
3	1	1.5	10	43.09
4	1	0.5	10	7.02
5	1.5	1	10	11.03
6	0.5	1.5	90	12.6
7	0.5	1	60	25.86
8	1.5	1	90	80.47
9	0.5	1.5	60	7.47
10	1	1	60	54.27
11	0.5	0.5	60	96.16

Table 6. *Cont.*

Run	A: Adsorbent Mass (g)	B: Initial Cu^{2+} Ions Concentration (g/L)	C: Contact Time (min)	R: Adsorption Degree (%)
12	1	1	60	51.14
13	1	1	60	52.16
14	1	1	60	49.67
15	1.5	1.5	60	38
16	1	0.5	90	97.06
17	0.5	1	90	89.74

The correlation between the following independent variables: linear (β_1, β_2, β_3), quadratic (β_{11}, β_{22}, β_{33}), interaction terms (β_{12}, β_{13}, β_{23}) and the response (R), was described by fitting the following polynomial equation [33]:

$$R = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} AA + \beta_{22} BB + \beta_{33} CC + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (14)$$

The obtained results are displayed in Table 6. The biosorption of copper ions onto eggshells can be expressed using the following equation:

$$Y = 46.62 - 1.15A - 20.45B + 26.11C + 15.54A \cdot B - 2.61A \cdot C - 30.13B \cdot C + 6.38A \cdot A - 1.46B \cdot B - 5.22C \cdot C \quad (15)$$

The statistical significance of the applied model was evaluated by the ANOVA analysis and shown in Table 7. The significance of each coefficient is determined by the magnitude of the F-values and p-values (Table 7). The larger the F-value, and the smaller p-value, the corresponding coefficient is more significant. p-values less than 0.0500 indicate high significant regression at 95% confidence level [35].

Table 7. ANOVA analysis for response surface model in relation to Cu^{2+} biosorption onto eggshells.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	
Model	13,714.86	9	1523.87	5.03	0.0224	Significant
A-A	10.58	1	10.58	0.0349	0.8571	
B-B	3346.44	1	3346.44	11.04	0.0127	
C-C	5452.81	1	5452.81	17.99	0.0038	
AB	965.66	1	965.66	3.19	0.1174	
AC	27.20	1	27.20	0.0897	0.7732	
BC	3631.87	1	3631.87	11.98	0.0105	
A^2	171.32	1	171.32	0.5652	0.4767	
B^2	8.93	1	8.93	0.0295	0.8686	
C^2	114.79	1	114.79	0.3787	0.5578	
Residual	2121.64	7	303.09			
Lack of Fit	1571.72	3	523.91	3.81	0.1145	Not significant
Pure Error	549.92	4	137.48			
Cor Total	15,836.50	16				

The model F-value and *p*-value of 5.03 and 0.0224, respectively, indicate that the model is significant. *p*-values lower than 0.05 indicate that model terms are significant. In this study, B (initial Cu^{2+} ions concentration), C (contact time (min)), and BC (initial Cu^{2+} ions concentration combined with contact time (min)) are significant model terms. The suitability of the model was confirmed by the regression coefficients of the predicted and experimental values (R^2 = 0.866 and adj-R^2 = 0.694).

Figure 8 shows the relationship between the experimental responses and the responses predicted by the model.

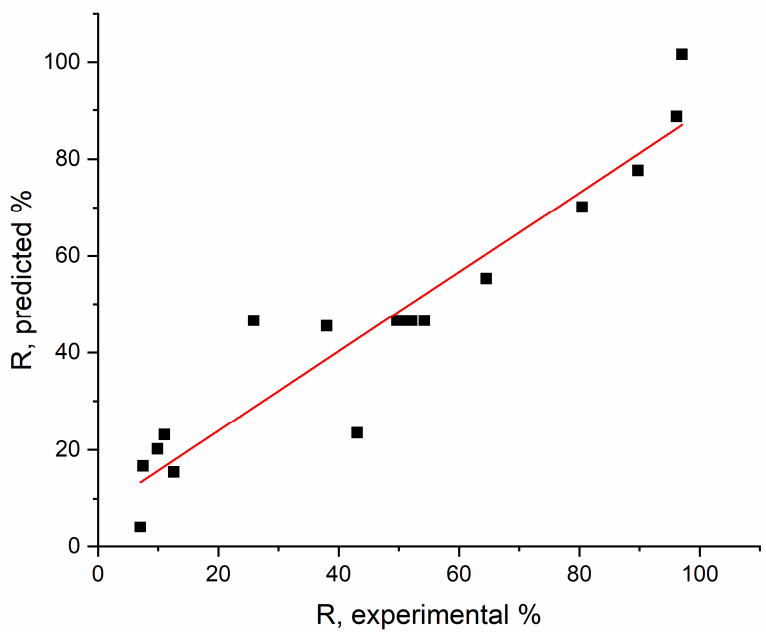

Figure 8. Plot of experimental and predicted responses.

Based on the data shown on Figure 8, and the correlation coefficient (R^2 = 0.897), it can be concluded that there is a good relationship between the experimental and predicted responses.

Response surface plots showing the influence of the analyzed parameters on the adsorption degree (R) are presented on Figures 9–11. Figure 9 indicates that lower initial metal ion concentration combined with lower adsorbent mass leads to a higher percentage of adsorbed metal (ANOVA analysis indicates that the combination of these two factors (A and B) is not significant). Figure 10 indicates that higher adsorbent mass and higher contact time leads to a higher response (metal removal%), while the ANOVA analysis does not classify the combination of these factors as significant. Figure 11 shows the interaction between factors B and C, i.e., the initial metal ion concentration and contact time, respectively. The ANOVA analysis indicates that the combination of these two factors is a significant model term. The corresponding Response surface plot indicates that high contact time and low initial metal ions concentration leads to the highest obtained response (adsorption degree).

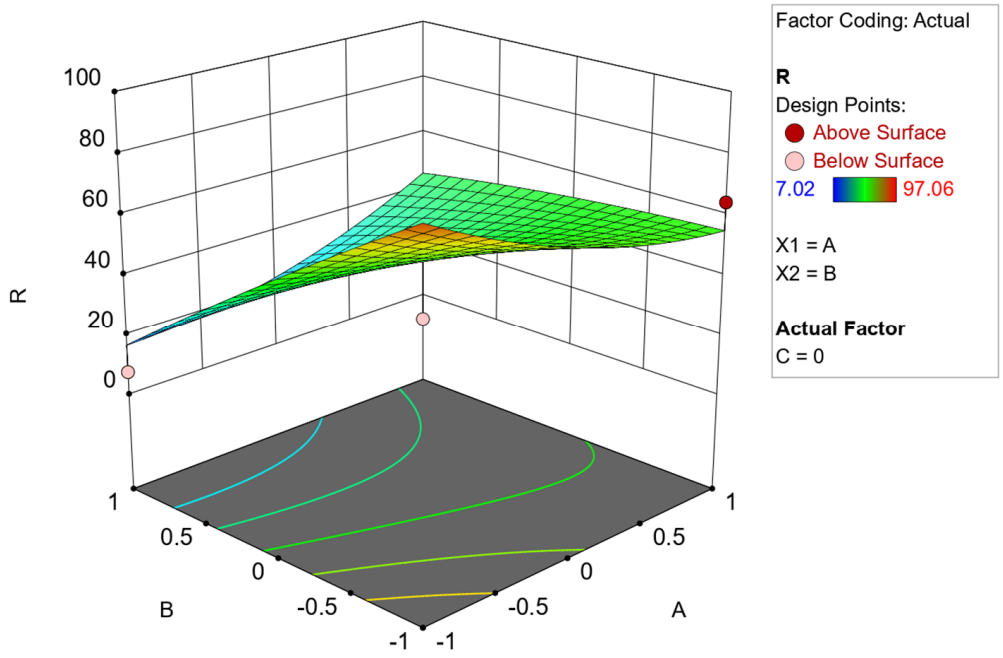

Figure 9. Response surface plot showing the interaction and influence of the adsorbent mass (A) and initial copper ions concentration (B) on the adsorption rate (R).

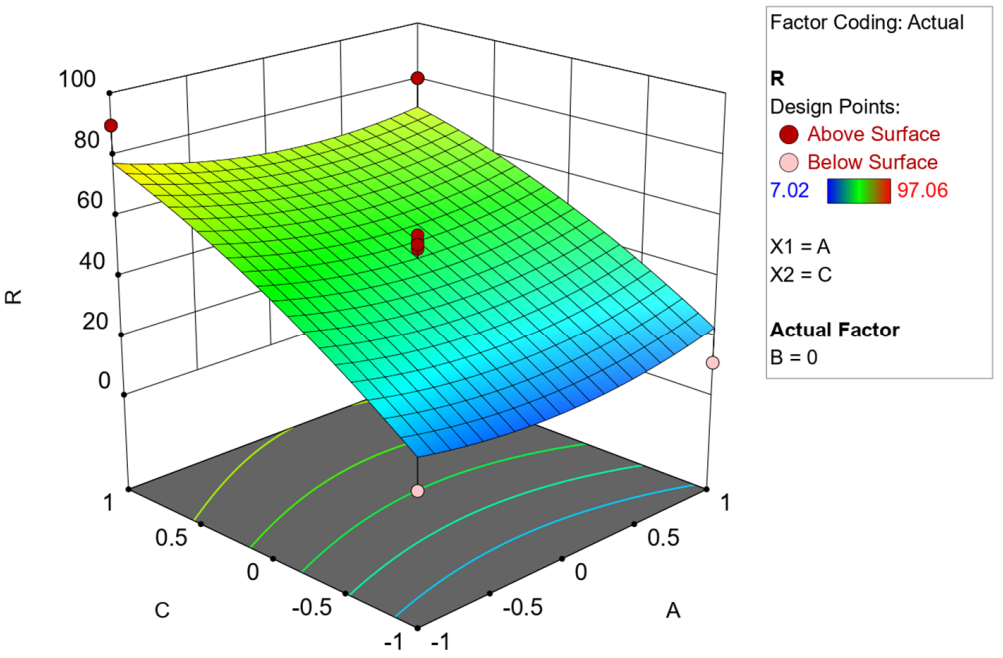

Figure 10. Response surface plot showing the interaction and influence on of the adsorbent mass (A) and contact time (C) on the adsorption rate (R).

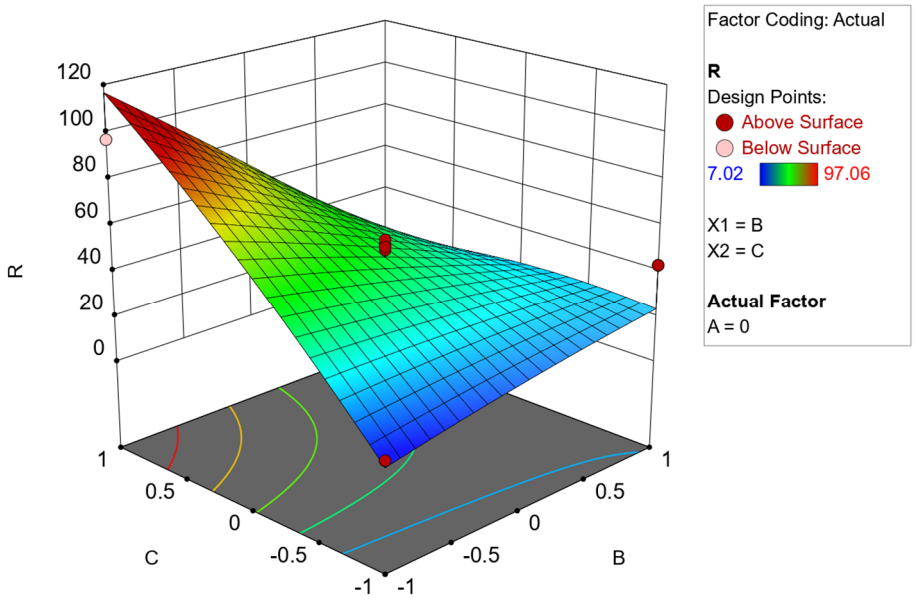

Figure 11. Response surface plot showing the interaction and influence of the initial copper ions concentration (B) and contact time (C) on the adsorption rate (R).

4. Conclusions

Biosorption of copper ions using chicken eggshells as an adsorbent was investigated and presented in this paper.

The influence of different process parameters on the biosorption process was evaluated. The biosorption capacity was found to increase with the increase in the pH value of the solution, reaching its maximum value at pH = 5. The influence of the pH value on the biosorption capacity could possibly be explained by the behavior of the carbonate species that originate from the eggshells (the source of the calcium carbonate) in the solution at different pH values, and their interaction with the divalent copper ions.

The analysis of the influence of initial copper ions concentration showed an increase in the biosorption capacity, with the rise in the initial Cu^{2+} ions concentration, up to 800 mg dm^{-3}, where it reaches the maximum value (q_t = 40.79 mg g^{-1}).

The initial mass of the adsorbent showed a significant influence on the biosorption efficiency. The adsorption degree increased from 25% to 82% with the increase in the adsorbent mass up to 1 g, due to the higher number of available active sites on the adsorbent structure as a result of a larger amount of adsorbent available.

The results of the analysis also showed that the biosorption capacity increased with the increase in the stirring rate, up to 400 rpm, where it reached its maximum value. Further increase in the stirring rate resulted in a decrease of the biosorption capacity.

The SEM-EDS analysis was performed on eggshells samples before and after the biosorption process. The SEM analysis showed a slight change to the surface morphology of the eggshells sample after the biosorption process, to an uneven, rough and heterogeneous nature. This change could be contributed to the incorporation of copper ions inside the structure of the eggshells sample. The interaction of eggshells with Cu^{2+} ions lead to the formation of flake-like deposits on the surface of the adsorbent. The EDS analysis of the eggshells samples before and after the biosorption process indicated that Mg, K and Ca could potentially be exchanged with Cu ions during the adsorption process.

Biosorption kinetics were analyzed using four empirical kinetic non-linear models, namely, the pseudo-first order kinetic model, pseudo-second order kinetic model, intraparticle diffusion kinetic model, and the Elovich kinetic model. The obtained kinetic parameters led to a conclusion that the pseudo-first order best fits the analyzed process, suggesting that copper ions possibly react with active sites inside the eggshell structure, forming sorption complexes in the process.

Three empirical adsorption isotherm models, namely, the Langmuir, Freundlich and Temkin model, in their non-linear form, were used to evaluate the equilibrium of the biosorption process. The performed analysis indicated that the Langmuir model showed the best fit with the experimental data. The Freundlich constant *n* also suggested that the biosorption of copper ions onto chicken eggshells is a favorable process.

The thermodynamic parameters of the biosorption process were calculated. The Gibbs free energy change indicated that the biosorption of copper ions onto chicken eggshell is a spontaneous process, and favored at temperatures above room temperature. The obtained enthalpy and entropy values indicated that the process is exothermic and that there is increased randomness at the solid/liquid interface during the biosorption.

Copper ions biosorption onto eggshells was optimized using Response Surface Methodology, based on Box-Behnken Design. The influence of three parameters (adsorbent mass, initial metal ions concentration and contact time) was investigated. The obtained data indicates that the used model is statistically significant. The data shows that initial Cu^{2+} ions concentration, contact time, and initial Cu^{2+} ions concentration combined with contact time are significant model terms. This model indicated that the optimal biosorption conditions are: adsorbent mass = 1 g; initial Cu^{2+} ions concentration = 0.5 g dm^{-3}; and contact time = 90 min.

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