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Optimisation of copper ion removal using immobilised red seaweed of *Euchema Cottonii Sp.* in a packed-bed filter via Response Surface Methodology (RSM)

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Abstract. Heavy metal contamination is emerging as a critical environmental concern, particularly in lower to middle-income countries where rapid industrialization, inadequate waste management, and limited regulatory oversight exacerbate the problem. For this reason, the treatment of heavy metal removal by biosorption was studied using a biosorbent derived from the Malaysia local red seaweed of *Eucheuma Cottonii sp*. The primary objective of this study was to optimise the performance of *E.Cottonii sp*. for copper removal via Response Surface Methodology-Central Composite Design (RSM-CCD). The seaweed immobilisation was prepared using 3% (w/v) of sodium alginate. Additionally, the raw and immobilised *E.Cottonii sp*. before and after biosorption were characterised by Scanning Electron Microscopy with Energy Dispersive X-ray spectroscopy (SEM-EDX) for their morphology. The interactive effect of dosage (43-157 g) and flowrate (2-17 L/hr) was further assessed via a packed-bed column filter. Based on the result, immobilised *E.Cottonii sp.* showed promising performance for copper removal. The result indicated that the model fits the data with a coefficient of determination (R^2 = 0.9844). The optimum conditions for a higher removal of copper using immobilised *E.Cottonii sp*. were a dosage = 60 g and a flow rate = 5 L/hr for 51.3% removal. In conclusion, the use of *E Cottonii sp*. as a potential biosorbent for copper removal could pave the way for sustainable environmental technologies.

1. Introduction

Since rapid industrialization, almost all these industrial and municipal effluents have been dumped directly into water bodies without sufficient treatment, causing the water to become highly polluted [1]. There is an increase in toxicity in the water over time because certain heavy metals, such as copper, are non-biodegradable [2]. The primary sources of heavy metals include solid waste and wastewater from the mining, electroplating, printing, dyeing, and metal production industries. Copper is commonly present in wastewater from industrial and agricultural activities[3]. These heavy metals can pose serious threats to the environment and human health due to their non-biodegradability, toxicity, and ease of accumulation in living organisms and the human body[3], [4] .Exposure to heavy metals leads to serious illnesses such as cancer, nervous system damage, and kidney failure.[4]

Heavy metals can be eliminated from wastewater by using conventional methods such as membrane filtration, electrochemical treatment, chemical precipitation, ion exchange and adsorption process[5] [6]. Ion exchange technology is a widely used technique in the industry, although it costs a lot and requires chemicals and resin due to its high removal efficiency and high operation cost [7]. In addition, due to its great effectiveness, simplicity of use, and low cost, membrane technology has become a more useful technology for treating and reusing wastewater in the plating industry[5] . Chemical methods are costly and use a lot of energy. However, these conventional methods will produce by-products that may lead to secondary pollution, be less efficient, costly, require high amounts of energy, and have operational limitations

Biosorption provides an environmentally friendly heavy metal removal approach. Heavy metals are thought to be more effectively and sustainably removed from wastewater using biosorption methods) Biosorption is a subcategory of adsorption in which the sorbent is a biological matrix such as biomass and enables a binding between specific biomass and pollutants. Biosorption utilises the non-living biomass of bacteria, fungus, agricultural wastes, and algae. Due to its low operation cost, simple operation, eco-friendliness with little or no toxic sludge production, and high efficacy even at low metal concentrations, it is often selected for the heavy metal removal[2], [4], [8] .

Seaweed biomass is regarded as one of the most promising biosorbent materials, among others, as it offers low cost , renewability, availability, reusability, lack of production of toxic waste, and high biosorption capability[5], [7], [8], [9], [10]. Seaweed also have better biosorption capabilities towards certain metals compared to commercial biosorbents such as natural zeolite, activated carbon and synthetic ion exchange resin[8], [11]. Furthermore, seaweed have the greatest electrostatic force of attraction for heavy metals in wastewater and have large surface area and strong binding capacity hence promote the highest biosorption efficiency.[8]

Biosorption using seaweed biomass for heavy metal removal has been extensively reported in previous literature. However, the used of Malaysia red seaweed of *E.cottonii sp.* for copper removal has been little studied[9], [12], [13], [14]. *E.cottoni* is red species seaweed normally found along the coast of Asia region[15]. To date, immobilization of algal biomass using natural or synthetic polymeric has limited study in biosorption field [16]. Therefore, this research aimed to treat heavy metals by developing immobilised *E.cottonii sp.* seaweed to be used as a biosorbent, determining the effect of flowrate and dosage by using a packed-bed filter, and finally optimising their performance by using Response Surface Methodology-Central Composite Design (RSM-CCD).

2.Methodology

2.1 Preparation of a copper stock solution.

A copper stock solution with a concentration of 1000 ppm was prepared by dissolving 10 mL of copper standard solution, $Cu(NO_3)_2$ in 990mL of distilled water. Then, 0.1 M of hydrochloric acid (HCl) and 0.1 M of sodium hydroxide (NaOH) were used for pH adjustment of the copper stock solution. The initial concentration of the copper standard solution was determined using a spectrophotometer. Figure 1 illustrates the raw *E.Cottonii sp.*

2.1 Preparation of biomass

2.1.1 Preparation of red seaweed.

E.Cottonii sp. was collected from Kunak, Sabah (4'48'N118°39'E) and transported in dry form to Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA). Once arrived, the *E.Cottonii sp.* was rinsed thoroughly with tap water to remove any remaining contaminants, such as debris or sand. Then, the *E.Cottonii sp*. was washed with distilled water and dried in an oven at 70°C for 24 hours. After drying, the *E.Cottonii sp*. was crushed using a dry mill blender and sieved to produce fine particles of seaweed powder. The seaweed powder obtained was properly stored in a container for further use. Figure 1 shows the species of seaweed, *E.Cottonii sp.*

Figure 1. Raw *E.Cottonii sp.*

2.1.2 Preparation of immobilised E.Cottonii sp.

A 3% (w/v) solution of sodium alginate was prepared and mixed with 0.25 g of seaweed powder to form a gelling solution. The seaweed solution gel was then stirred for 30 minutes using a magnetic stirrer until homogenised. Then, the mixture was dropped into the 3% (w/v) calcium chloride ($CaCl_2$) hardening solution by using a syringe with a diameter of 0.6 cm. The alginate beads were formed and stirred with a magnetic stirrer for 30 minutes. Then, the produced beads were removed from the $CaCl_2$ solution and rinsed with distilled water to remove any remaining $CaCl_2$ beads. The beads were dried with filter paper and exposed to the air for 1 hour. Figure 2 below shows the immobilised seaweed, *E.Cottonii sp.* Accordingly, existing methods of immobilization [16] were adjusted to produce suitable seaweed beads characteristic.

Figure 2. Immobilised *E.Cottonii sp.*

2.2 Characterisation of E.Cottoni sp.
A scanning electron microscope (SEM) LEO 440i, equipped with an energy dispersive X-ray analysis system (SEM/EDX), was employed to determine the porosity of immobilised seaweed and the copper ion distribution on the surface of immobilised *E.Cottonii sp*. This analysis also determined the chemical composition of all samples--raw *E.Cottonii sp*., immobilised seaweed before and after biosorption.

2.3 Biosorption through packed-bed filter
The biosorption process that was conducted via the packed-bed filter was to evaluate the performance of immobilised seaweed, *E.Cottonii sp*., based on the flow rate and dosage. Table 1 listed down various size of packed-bed filter for column study from previous research. For this study, the packed-bed filter column with a 4.3 cm inner diameter and a length of 20 cm was fabricated, as shown in Figure 3. The filter column was filled with the immobilised seaweed of *E.Cottonii sp.* at different dosages of 43g, 60g, 100g, 140g, and 157g. This to allow the porosity at 50% to avoid tightly packed column that limit the copper solution flow [17]. The copper solution was passed through the biosorbent packed-bed column at a controlled flow rate (2-15 L/min), allowing the biosorbent to adsorb the copper ions onto their surface. A filtered solution known as the supernatant was analysed using a spectrophotometer to determine the final concentrations.

Table 1. Various size of packed-bed column

Biosorbent	Height (cm)	Inner Diameter (cm)	Bed height (cm)	Flow rate (mL/min)	References
Humulus Lupulus stem	15	2.4	10	15	[18]
Macroalgae	15	0.5	5	0.3 - 1.1	[19]
Sugarcane baggase	27	10	5,10	5	[20]
Magnetic activated carbon	15	1.3	2-4	4.6-11.4	[21]
Waste-bio material	15	2.0	15,20,45	20-40	[22]
Pinecone shells	20	1.2	0.2-1	1.4-3	[23]
Aloe barbadensis	40	2.0	10	10-30	[24]
Biochar tea waste	30	5.6	30	20	[17]
E.Cottonii sp.	20	4.3	2 -10	33 -333	*This study*

Figure 3. The experimental setup for packed-bed filter (dimension: 20 cm length, 4.3 cm inner diameter)

The percentage of Cu removal was calculated based on Equation 1:

$$Removal, R\ (\%) = \frac{C_i - C_f}{C_i}\ x\ 100 \quad (1)$$

Where C_i is the initial concentration and C_f is the final concentration of copper solution.

2.4 Response Surface Methodology (RSM)

Response surface methodology (RSM) is a technique for optimising independent variables and the response of the process through fitting the experimental data to a second-order equation. RSM Stat-Ease-Design-Expert-version 7.0.0 was used in data analysis and experimental design. RSM-Central Composite Design (CCD) was utilised for optimisation of copper removal using *E.Cottonii sp*. as a biosorbent. In CCD, the numeric factors varied over 5 levels with a centre point, -/+ alpha (axial point), and -/+1 (factorial points), as shown in Table 2. To construct a second-order polynomial quadratic model that represents the interaction between the factors, 13 runs based on a CCD were carried out. The equation can be described as in Equation 2:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i\, x_i + \sum_{i=1}^{n} \beta_{ii} x_{i^2} + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j \quad (2)$$

Where Y is the response (% removal), X_i and X_j represent the factors (flow rate, dosage), β_{ii} represents the coefficient of the quadratic parameter, and β_{ij} represents the coefficient for the interaction parameters.

Table 2. Factor level and experimental range

Factors	ANOVA				
	-alpha	-1 Level	0	+1 Level	+alpha
Flow rate, (L/min)	2.93	5	7.07	15	17.07
Dosage (g)	43.43	60	76.57	140	156.57

The data were analysed using the statistical technique known as analysis of variance (ANOVA) to determine the significance of factors affecting a response variable. The P-values was evaluated to determine the statistical significance. The R -squared (R^2), adj.- R^2, pred.- R^2, C-V%, and Adequate Precision were evaluated to ensure that the model fits the data. A high value of R^2 shows that the model was well predicted for the dependent variable.

3. Result and Discussion

3.1 Characterisation of E.Cottoni sp. using Scanning Electron Microscopy and Energy Dispersive X-ray Analysis (SEM-EDX).

The SEM analysis of raw seaweed, *E.Cottonii sp*., confirmed a rough and porous surface morphology as depicted in Figure 4(a)(b)(c). Salt deposition was the primary cause of the rough surface layer with protuberances. The image of the SEM of immobilised *E.Cottonii sp.* before biosorption showed a heterogeneous and porous surface composed of small particles, as shown in Figure 4(d)(e)(f). These irregular structures facilitate the biosorption process of copper molecules on the biosorbent surface [25]. Figure 5(g)(h)(i) shows the SEM image of *E.Cottonii sp*. after biosorption. The image indicates the formation of macrospores on the biosorbent due to the copper molecules building up in the pores and on the surface binding wall of the seaweed biosorbent. The obvious changes in the surface of *E.*cottonii sp. with formation of macropores demonstrate the efficient biosorption of copper by the seaweed.

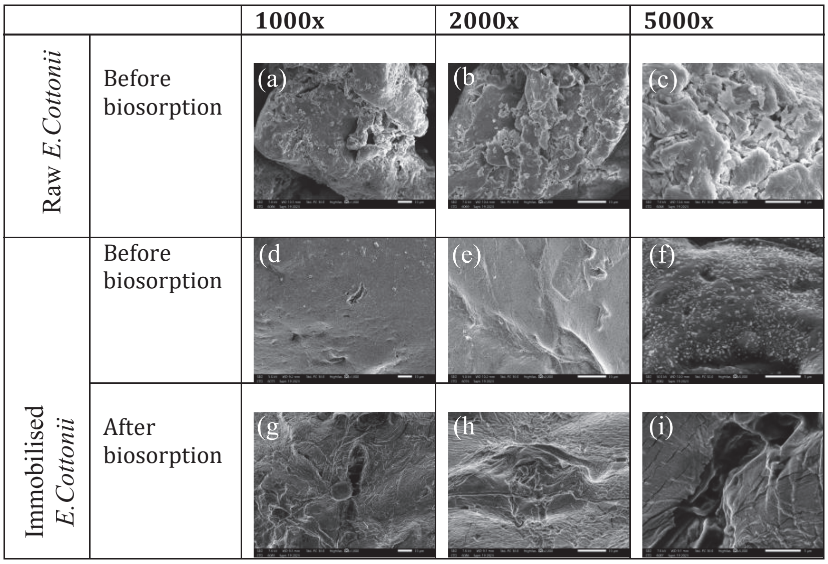

Figure 4. SEM micrograph obtained at various magnification; (a)(b)(c) Raw *E. Cottonii sp.*; (d)(e)(f) Immobilised *E. Cottonii sp.* before biosorption; and (g)(h)(i) Immobilised *E. Cottonii sp.* after biosorption.

3.2 Effect of operational parameters on removal efficiency

3.2.1 Effect of flowrate.

The flow rate was an essential indicator of an adsorption process's effectiveness. The contact times between copper ions and immobilised seaweed were determined by this parameter. The flow rate was varied to 2, 5, 10, 15, and 17 L/hr to determine its effect on the adsorption efficiency. The result demonstrated that as the flow rate decreased from 17 to 2 L/hr, the removal percentage of copper increased from 3.29% to 83.80%. Increasing the flow rate can reduce the contact time between copper and immobilised seaweed and eventually affect the biosorption capacity and removal rate of copper ions. During low flow rate, the contact time was sufficient to achieve equilibrium for adsorption, thereby increasing the contact time between immobilised seaweed and copper ions in the packed bed column[8]. This result shows that the removal of copper increased under slow flow rates because of the longer contact time of immobilised seaweed with copper ions and enhanced adsorption capacity.

3.2.2 Effect of dosage.

The effect of dosage was determined by varying the dosage of immobilised seaweed to 43g, 60g, 100g, 140g, and 157g at an optimised pH of 7.02 with an initial copper concentration of 3.95 mg/L. The results show that as the dosage increased from 43 g to 157g, the copper removal increased from 10.89% to 30.38%. As the dosage of immobilised seaweed increases, the removal of copper also increases because of the increased surface area and binding site for copper ions[26][27]

3.3 Modelling and optimisation

3.3.1 Model fitting.

Following the RSM design, the operating factors and experiment results are presented in Table 3. A total of 13 runs were carried out experimentally in a random order, and the experimental results showed a range from 6% to 80% of copper removal. The regression equation was derived by setting experimental results to a quadratic model. The central composite design (CCD) produced the model regression level ($-\alpha$, 1, 0, +1, and $+\alpha$) second-order polynomial quadratic equation (for p-value ≤ 0.05) as in Equation 3:

$$\text{Copper removal (\%)} = 55.47972 - 8.70630A + 0.7908B - 0.01AB + 0.2345A^2 - 0.00305469B^2 \quad (3)$$

where A indicates the flow rate and B indicates the dosage.

Table 3. Design matrix (CCD) of actual and predicted responses.

Std	Run	Block	Factor 1 A: Flowrate (L/hr)	Factor 2 B: Dosage (g)	Actual Copper Removal (%)	Predicted Copper Removal (%)
12	1	Block 1	10.00	100.00	28	30.4
11	2	Block 1	10.00	100.00	36	30.4
1	3	Block 1	5.00	60.00	50	51.3
2	4	Block 1	15.00	60.00	6	5.1
10	5	Block 1	10.00	100.00	31	30.4
4	6	Block 1	15.00	140.00	7	7.5
3	7	Block 1	5.00	140.00	59	61.7
7	8	Block 1	10.00	43.43	16	16.1
6	9	Block 1	17.07	100.00	6	6.7
8	10	Block 1	10.00	156.57	27	25.1
13	11	Block 1	10.00	100.00	32	30.4
9	12	Block 1	10.00	100.00	25	30.4
5	13	Block 1	2.93	100.00	80	77.6

3.3.2 Analysis of Variance (ANOVA) and lack of fit.

The CCD model's ANOVA results are shown in Table 4. Based on the ANOVA result in Table 4, the quadratic model was applicable because it has a 95% confidence level. The model displayed a low probability value (p-value) of <0.0001, which indicates that the model was significant with only 0.01% noise. The lack of fit value of 0.37 (p – 0.7782) indicates that it was not significant relative to the pure error. The non-significant lack of fit was good because it indicated that the model was fit. In this model, terms A, B, A^2, and B^2 were important, and values greater than 0.1000 indicate that the model terms were not significant.

Table 4. ANOVA results for the designed model of copper removal

Source	ANOVA					
	SS	Df	MS	F-value	p-value	
Model	5595.46	5	1119.09	88.47	< 0.0001	significant
A: Flowrate	5032.64	1	5032.64	397.88	< 0.0001	
B: Dosage	81.64	1	81.64	6.45	0.0386	
AB	16.00	1	16.00	1.26	0.2978	
A^2	239.09	1	239.09	18.90	0.0034	
B^2	166.18	1	166.18	13.14	0.0085	
Residual	88.54	7	12.65	-	-	
Lack of Fit	19.34	3	6.45	0.37	0.7782	not significant
Pure Error	69.20	4	17.30	-	-	
Cor Total	5684.00	12				

R^2: 0.9844; Adj.- R^2:0.9733; Pred.- R^2: 0.9568; Adeq. Precision: 30.005; Std. Dev: 3.56; Mean: 31.00; C.V% 11.47; SS: Sum of Squares; Df: Degree of freedom; MS: Mean Squares.

In addition, the values of R-squared (R^2), adjusted R-squared (adj R^2), and predicted R-squared (pred R^2) were 0.9844, 0.9733, and 0.9568, respectively. These results prove that the generated model has a good ability to predict the removal of copper ions. The relevance of the model was confirmed by the value of adequate precision of 30.005; the desirable ratio of signal to noise should be greater than 4.

3.3.3 Actual versus predicted data.

Figure 5 illustrates the relation between the actual and predicted response values. The close correlation between the actual and predicted values of the model using RSM depicted accuracy of the second-order polynomial quadratic modelling.

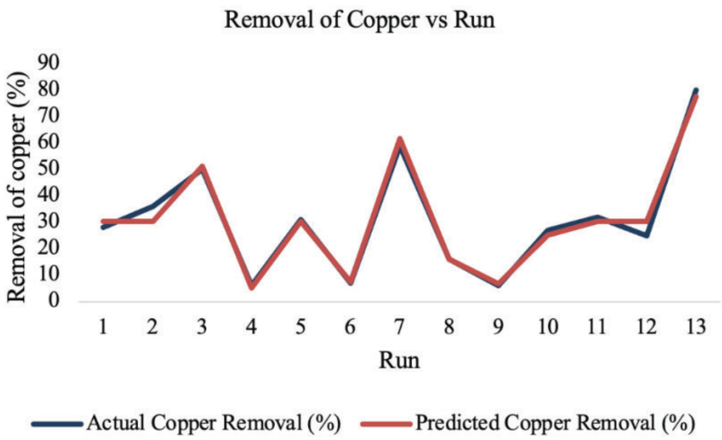

Figure 5. Actual versus predicted values for copper ions removal

3.3.4 Interactive effect of variables.

Figure 6(a)(b) clarify the correlation between independent variables such as flow rate and dosage on the removal efficiency (%) of copper. In addition, the optimum values for the removal of copper were identified. According to Figure 6 (a)(b), the increase in flow rate from 5.00 to 15.00 L/hr decreased the removal rate from 80% to 6%. It shows that the contact times between copper and immobilised seaweed were not enough. At a higher flow rate, the copper ions had less time to interact with the active binding site of immobilised seaweed, resulting in a lower removal rate of copper[28]. Meanwhile, a higher dosage led to higher copper removal rates. However, the removal of copper depended on both flow rates and dosage, with higher dosages and lower flow rates resulting in higher removal rates.

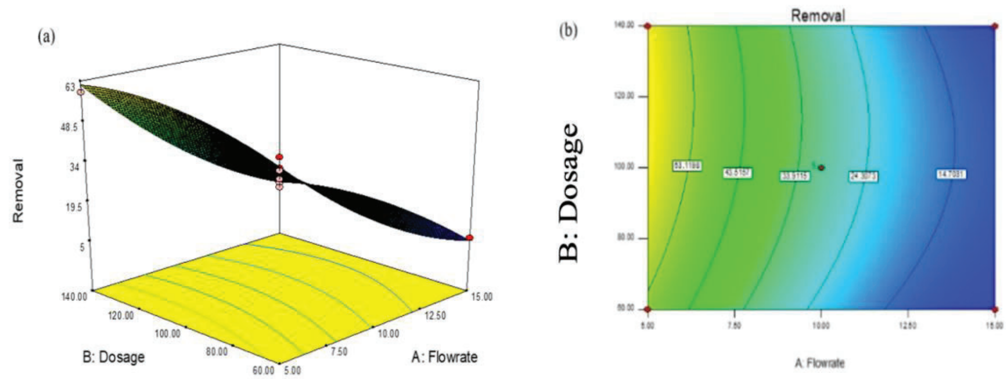

Figure 6 (a)-(b). 3D diagrams and contour plots for copper removal efficiency of flow rate and dosage

3.3.5 Optimisation.
By using numerical optimisation, a maximum predicted removal of 51.3% of copper was obtained, corresponding to a dosage of 60 g and a flow rate of 5 L/hr. By performing similar experiments at the specified optimal conditions, the obtained optimum conditions were verified, as shown in Table 5. This indicates that CCD accurately predicted the actual behaviour of biosorption studies.

Table 5. Validation of optimization process between predictive and experimental work conditions specified by RSM-CCD numerical optimization

Validation	No	Flowrate (L/hr)	Dosage (g)	Copper removal (%)
Predictive	1	5	60	51.26
Experimental Work	1	5	60	52.15
	2	5	60	53.92

The surface morphology of the biosorbent *E.Cottonii sp.* was determined using SEM analysis. From the SEM images in Figure 7, a lot of pores were developed at optimal conditions. The pore diameter varied from 1.29 to 4.102 µm, indicating an effective biosorption of copper ions onto the *E.Cottonii sp.* The abundance of macropores on the surface of the immobilised *E.Cottonii* favourable for efficient biosorption of the copper molecules[10].

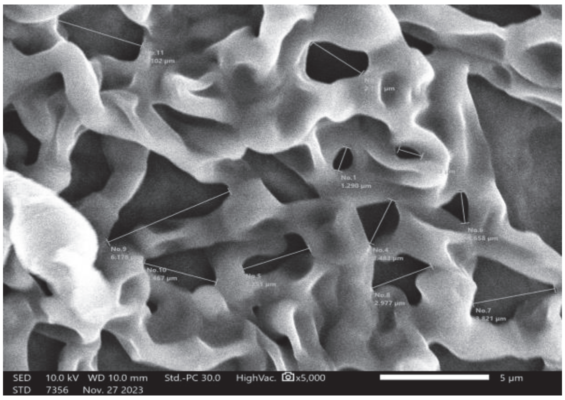

Figure 7. SEM images of *E.Cottonii sp.* after optimisation at optimal conditions of initial concentration of 3.95 mg/L, pH 7.02, flow rate of 5 L/hr, and dosage of 60g.

4. Conclusion
This study demonstrates that immobilised seaweed showed its effectiveness for copper removal, which indicates its potential as a natural and sustainable biosorbent. The packed-bed filter's efficacy was significantly affected by its flow rate and dosage. Analysis of variance (ANOVA) shows that the quadratic models produced were significant, which indicates that the model fits the data with a coefficient of determination (R^2 = 0.9844). The optimisation model predicted that the maximum copper removal of 51.3% can be obtained at optimised conditions (flow rate: 5 L/hr, dosage of biosorbent: 60 g). As a conclusion, *E.Cottonii sp*. is an effective method for copper removal and leads to sustainable environmental technologies. For future work, the study on breakthrough curve should be carried-out to investigate the behaviour of copper biosorption during the operation.

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