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Application of Cross-linked Spirogyra for Biosorption of Lead (II) ion from Aqueous Solution

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ABSTRACT

Lead II ion (Pb^{2+}) is a toxic heavy metal and adversely affects human's health. Spirogyra biomass was pre-treated with dimethyl sulphoxide (DMSO) and cross-linked with epichlorohydrin (EC) for removing Pb^{2+} from aqueous solution. The effects of different variables on Pb^{2+} removal were analyzed using surface response methodology to determine the significant variables and bio sorption capacity. The surface and physical properties of the biomass were characterized using Fourier Transform Infrared (FT-IR) Spectroscopy and Brunauer-Emett-Teller (BET) analyzer. The specific surface area of the cross-linked spirogyra is 86.0 m^2/g which is much greater than 20.5 m^2/g obtained for the grinded raw biomass. The surface functional groups for both raw and cross-linked biomass are the same. The study showed that at low biomass concentration, an increase in initial metal concentration increases the biosorption capacity due to an increase in driving force, which favors the adsorption of the metal ions onto the surface of the biosorbent. A maximum biosorption capacity of 46.9 mg/g was achieved at an initial Pb (II) ion concentration of 500 mg/L and a biomass concentration of 10000 mg/L. On the other hand, biosorption capacity is reduced by high biomass concentration and low initial metal concentration. The model summary indicated that the R^2, R^2 (adj.), and R^2 (pred) are all higher than 70%, indicating a strong fit. At 500 mg/L initial metal concentration, pH 6, 180 min contact time, and 10000 mg/L biomass concentration, a maximum biosorption capacity of 46.9 mg/g was achieved. Under the identical experimental conditions, the model obtained yields a biosorption capacity of 42.3 mg/g which accurately predicts the experimental value. Overall, pre-treatment of spirogyra with DMSO before cross-linking it with EC enhances its surface area and biosorption capacity for removal of Pb^{2+} from aqueous solution.

Keywords: Cross-linked, Spirogyra, dimethyl sulphoxide, epichlorohydrin, Optimization

Introduction

We cannot overstate the importance of clean and affordable freshwater to our everyday activities. For home, agricultural, and industrial purposes, large amounts of pure freshwater are needed. In addition to being essential for sustainable agricultural production, clean fresh water is also needed for household cleaning and cooking. However, the release of untreated or inadequately treated wastewater into water bodies makes it difficult for millions of people to obtain enough affordable clean freshwater. Domestic, agricultural, and industrial effluents substantially contribute to heavy metal contamination in water,

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and the presence of heavy metal ions such as Cr (VI), Cd (II), Pb (II), As (V and III), Hg (II), Ni (II), and Cu (II) above the maximum contaminant level (MCL) poses a number of health risks to humans, including liver failure, kidney damage, gastric and skin cancer, mental disorders, and harmful effects on the reproductive system. (Zhang *et al.*, 2023). Thus, a sustainable method for eliminating heavy metals from wastewater is required.

Various methods are available for removing heavy metals from wastewater, ranging from the conventional techniques such as ion exchange, evaporation, chemical precipitation, membrane separation, chemical oxidation or reduction, electrochemical treatment, and reverse osmosis (Atkinson *et al*., 1998; Sulaymon *et al*., 2013) to the biosorption method (Salam 2019). However, the conventional techniques have drawbacks such as lack of specificity, ineffectiveness at low concentrations (Gray, 1999), and fouling by slightly soluble components in membrane separation (Huang and Koseoglu, 1993). In addition, they require multiple basin configurations, sludge dewatering facilities, highly skilled operators, and large footprints (Zhou *et al.*, 1999). As a result, they are typically uneconomical.

On the other hand, passive biosorption, which involves deactivated (dead) biomass, is potentially cost-effective. Biosorbents are environmentally beneficial and efficient at eliminating both organic and inorganic contaminants from wastewater. They are usually produced from abundant, and low-cost biomass but the use of freely suspended microalgae as biosorbent has some drawbacks such as their small size, low mechanical strength, and difficulty in separating the biomass and effluent (Fanning and Vannice, 1993). These disadvantages have been minimized by using cross-linked biomass (Fanning and Vannice, 1993).

While crosslinking of algae improves its mechanical strength and chemical resistance, it often lowers the metal uptake capacity and slows down the rate of metal removal of the cross-linked biosorbents (Ka´zmierczak *et al*., 1991). Consequently, for efficient metal removal from wastewater, a larger absorber and more time are needed. This could result in unsustainable algal metal biosorption due to high capital and operating costs. This should be improved to make the process economically viable.

Chemical pretreatment prior to crosslinking of biomass has been shown to enhance the adsorption capacity of cross-linked algae (Salam, 2019). Specifically, Liu *et al.* (2009) obtained a significantly higher metal uptake capacity when *Laminaria japonica* macroalgae was pretreated with dimethyl sulphoxide (DMSO) prior to cross-linking it with epichlorohydrin than the non-cross-linked algae. However, to the best of the authors' knowledge, there is no report in the literature on pretreating of spirogyra with DMSO prior to crosslinking with epichlorohydrin.

Therefore, this study investigates the pretreatment of spirogyra with DMSO prior to cross-linking it with epichlorohydrin (EC) in order to enhance its biosorption capacity for removal of Pb (II) ions from aqueous solution. This macroalga was chosen because of its availability, renewability, non-competition with human food, and potential as a low-cost biomass. Moreover, biosorption of Pb (II) from the aqueous solution was optimized using response surface methodology.

2.0 Materials and Method

Materials used in this experiment are green algae (spirogyra species) and aqueous solution of lead Nitrate, Pb $(NO_3)_2$.

2.1 Apparatus

The apparatus used in this experiment are tray oven U.S Standard sieve machines, ceramic mortar and pestle, pH meter, conical flask, measuring cylinder, A set of beakers and pipette, magnetic stirrer, volumetric flask, water bath with shaker, funnels, syringe, electrical mass balance and Whatman filter paper.

2.2 Reagents

Analytical grade reagents such as lead Nitrate Pb $(NO_3)_2$, Sodium hydroxide, Hydrochloric acid, Epichlorohydrin (EC), Dimethyl sulphoxide

(DMSO) and 2-Propanol were used in accordance with standard analytical procedures,

2.3 Sample

Green Algae (spirogyra species), were collected from Suleja River in Niger state (Nigeria). The collected biomass was washed with distilled water to remove the external sand and salt. They were Sun dried in the open place for twenty-four hours (24hr) to remove the free water, then oven dry at 50ºC for eight hours (8hr). The dried sample was crushed with ceramic pestle and mortar to reduce the particle size and sieve to maximum 500µm particle size. The sample was then stored in a plastic bottle at 25ºC.

2.4 Pretreatment and Crosslinking

The method of Liu, *et al.,* (2009) was used for pretreatment and crosslinking the spirogyra biomass. Specifically, a 10g of powdered spirogyra biomass was measured into a flask and mixed with 150ml of Dimethylsulphoxide (DMSO) at 25ºC for 24hr to form a paste. A 20ml of Epichlorohydrin was added to the paste and left at 25ºC for 2hr. A 25ml of 5mol/L NaOH solution was added and stirred at 50ºC for 5hr. After that, it was allowed to cool down to 25ºC. Then it was filtered and washed with 70%, 2-propanol in water (800ml) followed by 0.5 mol/L HCl and finally with 70%, 2-propanol in water (500ml) to a neutral p H. The sample was allowed to dry in an oven at 60ºC overnight (Atkinson *et al*., 1998).

2.5 Biosorption Experiment

A 100 ml of initial metal ion concentration of lead Nitrate Pb $(NO_3)_2$ prepared from the stock solution was added into the conical flask. The solution pH was recorded and adjusted with 0.1 mol/L of HCL and 0.1 mol/L of NaOH. After this, a specified mass of bio sorbent was added to aqueous lead Nitrate. The sample was placed in water bath set at 30 ºC. It was agitated at 200 rpm for a specified time for each experiment. At the end of each batch, the biosorption sample is removed and filtered with Whatman filter paper. The filtrate was analyzed using Atomic Absorption Spectrophotometer (AAS) to determine the final metal ion concentration.

2.6 Analytical Methods

2.6.1 Adsorption capacity

The amount of heavy metal ions adsorbed at equilibrium $q(mg/g) = \frac{(C_o - C_e)V}{m}$

Where Co= initial metal ion concentration (mg/L)

C_e = equilibrium concentration of final metal ion concentration (mg/L)

m = mass of the adsorbent (g)

V = volume of the solution (L).

2.6.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was used to confirm the presence of functional groups in the dry grinding spirogyra and crosslinked spirogyra biomass sample for the removal of Pb^{2+} ion from aqueous solution. The biomass samples were incorporated with KBr pellet and FTIR spectra were measured.

2.6.3 Scanning electron microscopy (SEM)

SEM was used to verify the morphology differences between the dry crosslinked spirogyra biomass and biosorption samples to examine the algal cells surface and to evaluate the Pb^{2+} adsorptions. The images show the pore structures for cross linked biomass and after biosorption of spirogyra. The samples were coated with gold and were examine at different magnification.

2.6.4 Brunauer-Emett- Teller (BET)

BET was used to determine the pore size, pore volume, surface area of crushed dry and Crosslinked spirogyra species. Qantachrome Nova 4200e made in USA was used for the experiment.

2.7 Face-centered central composite design of the experiments

The experiments were designed to investigate the effect of independent variables: initial Pb^{2+} concentration, pH of solution, contact time and biomass concentration on response factor (biosorption capacity). A face-centered central composite design was used. The factors were mapped such that the lowest level setting was -2 ($-\propto$), the midpoint was 0, and the highest level was + 2 ($+\propto$). The total number of runs were 62

where cube points were 32, center points in cube were 14, axial points were 16, and center point in axial was zero. The temperature was held constant at 30 °C for all the experiments, so was not among the RSM variables. The ranges of the independent variables used are listed in the Table 1. Response surface methodology was used in analyzing the results obtained.

Table 1: Ranges of factors used for CCD design

S/N	Factors	lower level	upper level
1	Initial Metal Conc.(mg/L)	10	500
2	pH	2	6
3	Contact time(min)	10	180
4	Biomass Conc.(mg/L)	10,000	20,000

3.0 Results and Discussion

This section highlights the results of the effect of key variables affecting biosorption of $Pb^{2+}$from aqueous solution using a cross-linked spirogyra. The factors (independent variable) were initial Pb^{2+} concentration, solution pH, contact time and biomass concentration while the biosorption capacity was the dependent variable (response factor).

The biosorption capacity of the cross-linked spirogyra was examined in relation to the initial metal concentration, solution pH, contact time, and biomass concentration. Among the four factors and corresponding interactions investigated, it was found that only the initial metal concentration ($p = 0.000$), biomass concentration ($p = 0.000$), and their interaction ($p = 0.000$) significantly affect the biosorption capacity. The finding shows that pH (2–6) and contact time (10–180 min) have no significant effect on the biosorption capacity of the cross-linked spirogyra. The metal removal was rapid, reaching equilibrium before 180 min. The fast kinetics exhibited by the cross-linked spirogyra could be due to the significant increase in the specific surface from 21 m²/g to 86 m²/g (310% increase) and pore volume from 0.014 cc/g to 0.055 cc/g (293% increase) due to pretreatment with dimethyl sulfoxide, while the broad pH resistance is a result of resistance produced by the epichlorohydrin. The final model was improved by eliminating all non-significant components and their interactions. The resulting analysis of variance is shown in Table 2.

Table 3 shows that the R^2, R^2 (adj.), and R^2 (pred) are all higher than 70%, indicating a strong fit. At 500 mg/L initial metal concentration, pH 6, 180 min contact time, and 10,000 mg/L biomass concentration, a maximum biosorption capacity of 46.9 mg/g was achieved. Under the identical experimental conditions, the model in equation 1 yields a biosorption capacity of 42.3 mg/g. The model accurately predicts the response (biosorption capacity of the cross-linked spirogyra) because the percentage error is less than 10%.

Table 2: Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	11281.4	3760.48	76.03	0.000
Linear	2	9774.9	4887.43	98.81	0.000
Initial Conc.	1	9125.0	9125.03	184.48	0.000
Bio Conc.	1	649.8	649.83	13.14	0.001
2-Way Interaction	1	1506.6	1506.59	30.46	0.000
Initial Conc.*Bio Conc.	1	1506.6	1506.59	30.46	0.000
Error	58	2868.9	49.46		
Lack-of-Fit	21	1541.2	73.39	2.05	0.028
Pure Error	37	1327.7	35.88		
Total	61	14150.3			

Table 3: Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.03305	79.73%	78.68%	75.95%

Regression Equation 1 in Uncoded Units

$$Biosorption\ capacity\ \left(\frac{mg}{g}\right) = -7.97+0.149\ X+0.000579\ Y-0.000006\ XY$$

Equation 1

Where:

X=initial metal concentration

Y=Biomass concentration

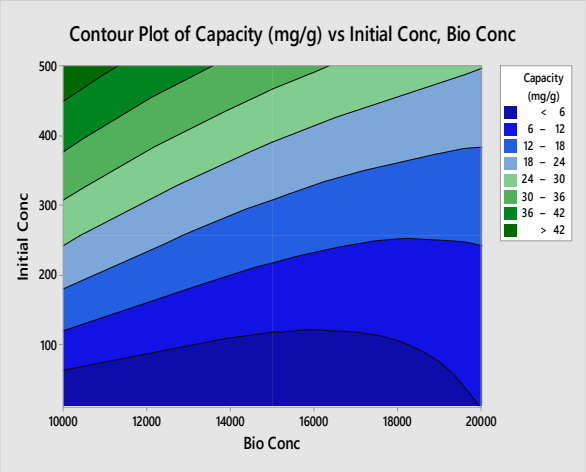

Fig. 1: Contour plot of bio-sorption capacity versus initial metal and biomass concentration

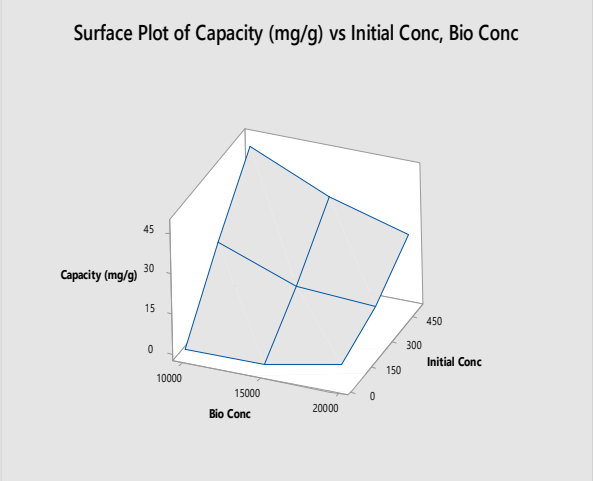

Fig. 2: Surface plot of bio-sorption capacity versus initial metal and biomass concentration

Figures 1 and 2 show the effect of an increase in initial and biomass concentrations on the biosorption capacity of the cross-linked spirogyra to adsorb the lead (II) ions from the aqueous solution. It is clearly shown in figures 1 and 2 that at low biomass concentration, an increase in initial metal concentration increases the biosorption capacity due to an increase in driving force, which favors the adsorption of the metal ions onto the surface of the biosorbent. A maximum biosorption capacity of 46.9 mg/g was achieved at an initial Pb (II) ion concentration of 500 mg/L and a biomass concentration of 10000 mg/L.

Liu *et al.* (2009) biosorbed different heavy metals using cross-linked *Laminaria japonica*. Zn (II) had a maximum of 34.7 mg/g when cross-linked with glutaraldehyde, 45.8 mg/g when not cross-linked, and 81.1 mg/g when cross-linked with epichlorohydrin. Cu (II) reached a maximum of 102.9 mg/g with epichlorohydrin, 55.9 mg/g without crosslinking, and 34.3 mg/g when crosslinked with glutaraldehyde. A maximum of 92.2 mg/g of Cd (II) was achieved when cross-linked with glutaraldehyde, 93.3 mg/g without cross-linking, and 187.7 mg/g with epichlorohydrin. For this investigation, the crosslinked spirogyra with epichlorohydrin produced a maximum biosorption capacity of 46.9 mg/g. This number is lower than the maximum 187.7 mg/g reported by Liu *et al.* (2009) and higher than 34.3 mg/g for glutaraldehyde, which could be caused by difference in heavy metals, macro algae (which may have different binding sites), and experimental conditions.

On the other hand, biosorption capacity is reduced by high biomass concentration and low initial metal concentration. For example, when the biomass concentration was between 10,000 and 20,000 mg/L, a biosorption capacity of less than 6 mg/g was achieved at an initial metal concentration of roughly 50 mg/L. The reason is that a high biosorbent concentration increases the specific surface area and, consequently, the number of active binding sites, leading to increased removal efficiencies (Ka´zmierczak *et al.*, 1991). However, excessive increases in the biosorbent concentration above the optimum value could cause reductions in biosorption capacity, which could be attributed to partial biomass aggregation at high concentrations. Biomass aggregations decrease the net effective specific surface area and effective active binding sites (Ka´zmierczak *et al.*, 1991). This result conforms to a reduction in biosorption capacities with an increase in biomass concentration reported by previous researchers (Lambert *et al.,* 1976; Vasu, 2008)

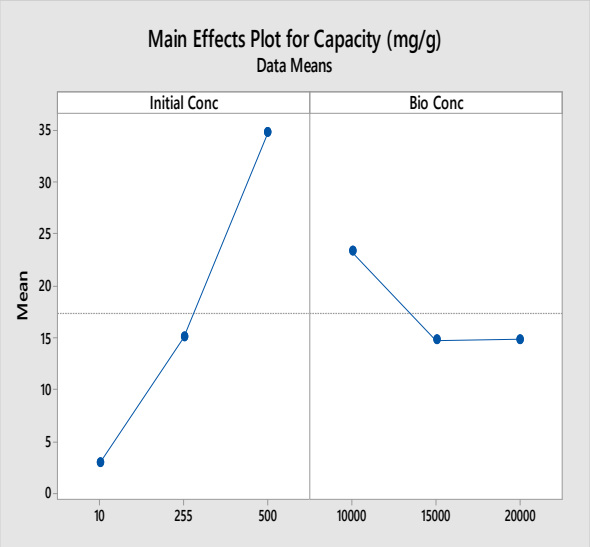

Fig. 3: Main effect plot for bio-sorption capacity

Fig. 3 shows the individual effect of initial metal and biomass concentrations on the biosorption capacity. It is evident that the biosorption capacity increased from 2.5 to 38 mg/g when the initial metal concentration was raised from 10 to 500 mg/L. On the other hand, biosorption ability was significantly reduced when biomass content increased from 10,000 to 15,000 mg/L. However, further increases in biomass concentration from 15,000 to 20000 mg/L caused a low reduction in biosorption capacity. It should be mentioned that agglomeration brought on by high biomass concentration decreases the biomass's ability to effectively use its binding sites to adsorb lead (II) ions.

3.1 FTIR Analysis of dry grinding and cross-linked spirogyra species sample

As can be seen in Fig. 4 and the wave band assignments in Table 4, the raw and cross-linked spirogyra contain the same surface functional groups. This shows that cross-linking does not affect the functional group originally present on the biomass. Thus, the metal binding sites on the biomass surface will not be affected by the cross-linking.

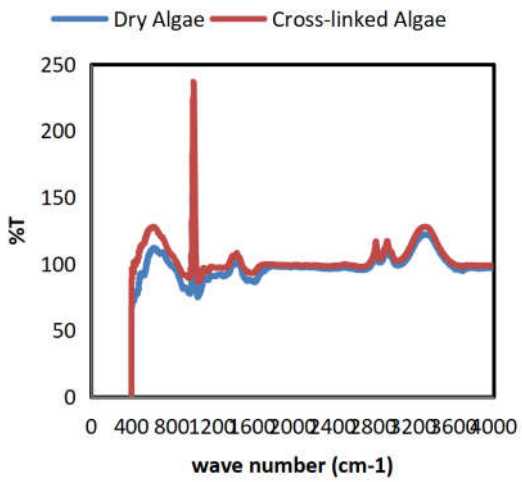

Fig 4: FTIR spectra of raw and cross-linked spirogyra

The wave length peaks were 3200-3500 cm^{-1}, 2900-3000 cm^{-1}, 2800-2900 cm^{-1}, 1400-1500 cm^{-1} and 1030 cm^{-1}. The peak range of 3200-3500 cm^{-1} assigned to O-H group (Dyer, 1965). The O-OH stretching vibrations occur within a broad range or frequencies indicating the presence of free hydroxide groups and bonded O-H peaks of carboxylic acid (Vasu, 2008; Sricharoenchaikul *et al.,* 2008; Dyer, 1965). The 2900-3000 cm^{-1} peak is assigned to C-H stretching of Quinones groups (Fanning and Vannice, 1993; Gómez-Serrano *et al.,* 1999; Ka´zmierczak *et al.,* 1991; Lambert *et al.*, 1976; Vasu, 2008; Sricharoenchaikul *et al.*, 2008). The 2800-2900 cm^{-1} peaks is assigned to C-H stretching and O-H stretching of quinones and phenolic groups (Fanning and Vannice, 1993; Gómez-Serrano *et al.*, 1999; Dyer, 1965). The 1400-1500 cm^{-1}peaks is assigned to C-H stretching of phenolic group (Ferraro and Rein, 1985), and N-H stretching of pyridine-like groups (Przepiórski *et al.*, 2004). The 1030 cm^{-1} peak is assigned to C-O stretching of Ether (Ka´zmierczak *et al.*, 1991; Lambert *et al.*, 1976).

Table 4: FTIR Band assigned for raw and cross-linked spirogyra

Wave length (cm^{-1})	Assignment	References
3200-3500	O-H (stretching).	(Vasu, 2008; Sricharoenchaikul *et al*., 2008; Dyer, 1965)
2900-3000	C-H (stretching).	(Fanning and Vannice, 1993; Gómez-Serrano *et al*., 1999; Ka´zmierczak *et al*., 1991; Lambert et al., 1976; Vasu, 2008; Sricharoenchaikul *et*
2800-2900	C-H (stretching), O-H (stretching).	(Fanning and Vannice, 1993; Gómez-Serrano *et al*., 1999; Dyer, 1965)
1400-1500	C-H (stretching), N-H (stretching),Pyridine-like Group.	(Ferraro and Rein, 1985) Przepiórski *et al*., 2004
1030	C-OH (stretching), Ether (C-O stretching).	Ka´zmierczak *et al*., 1991; Lambert *et al*., 1976

3.2 Scanning Electron Microscopy (SEM) of raw and cross-linked Spirogyra

SEM was used to verify the morphological differences between the cross-linked spirogyra biomass before and after biosorption of Pb^{2+}. Fig. 5 shows SEM micrograph of the cross-linked biomass before biosorption of Pb^{2+} while Fig. 6 shows the SEM micrograph of the biomass after biosorption of Pb^{2+} ions. SEM images have clearly shown that the cross-linked spirogyra biomass samples before and after Pb^{2+} biosorption exhibited different surface morphologies. Cross-linked spirogyra biomass exhibited uniform interconnected structure with a continuous surface before biosorption. However, after biosorption, the walls of biomass have become fragile, irregular surface with the appearance of bright spots due to the accumulation of Pb^{2+} ions.

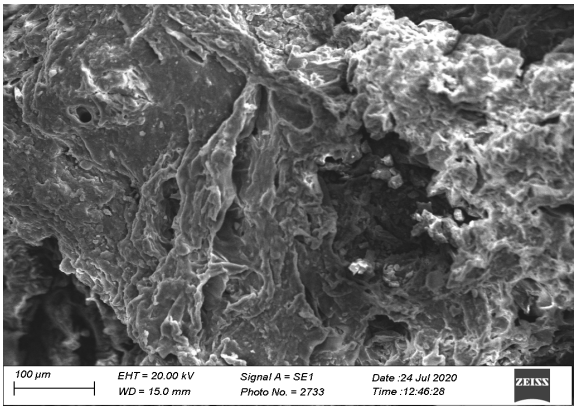

Fig 5: Cross-linked Spirogyra biomass before biosorption

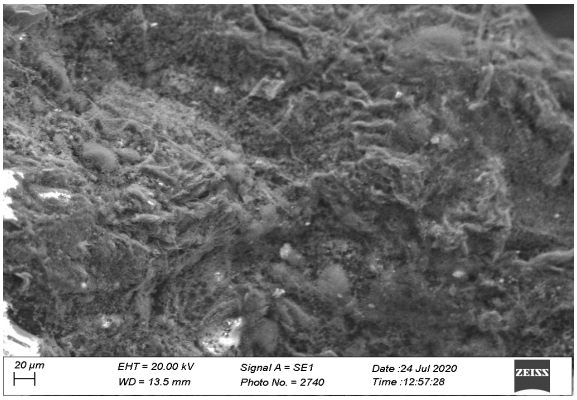

Fig 6: Cross-linked Spirogyra biomass after biosorption

3.3 BET Results Analysis

The specific surface areas were determined for the grinded raw and cross-linked spirogyra. The specific surface area of the dry grinded spirogyra sample was measured to be 21 m^2/g while the cross-linked spirogyra sample was measure to be 86 m^2/g. The specific surface area of the spirogyra increased by 310 % after cross-linking which is quite significant. The pore volume of dry grinded spirogyra samples was 0.014cc/g while that of cross-linked spirogyra sample was 0.055cc/g. Again, cross-linking of the biomass resulted into 293 % increase in the pore volume of the original spirogyra. This substantial increase in the specific surface area and pore volume after cross-linking contributes to high biosorption capacity of the cross-linked biomass. Therefore, pretreatment of the spirogyra with dimethyl sulphoxide before cross-linking it with epichlorohydrin increase the surface area and

biosorption capacity of the cross-linked spirogyra for the accumulation of Pb^{2+} ions during adsorption.

4.0 Conclusion

Spirogyra biomass was cross-linked with epichlorohydrin and successfully used to remove Pb (II) from aqueous solution. Pretreatment of the biomass with dimethyl sulphoxide and cross-linking with epichlorohydrin substantially increase its specific surface area and does not affect the surface functional group.

The biosorption capacity of the cross-linked spirogyra depends only on the initial metal concentration, biomass concentration, and their interaction while the solution pH, contact time cause no significant effect on the biosorption capacity

The model summary shows that the R^2, R^2 (adj.), and R^2 (pred) are all higher than 70%, indicating a strong fit. At 500 mg/L initial metal concentration, pH 6, 180 min contact time, and 10000 mg/L biomass concentration, a maximum biosorption capacity of 46.9 mg/g was achieved. Under the identical experimental conditions, the model in equation 1 yields a biosorption capacity of 42.3 mg/g. The model accurately predicts the response (biosorption capacity of the cross-linked spirogyra) because the percentage error is less than 10%.

Conflict of Interest

I hereby declare that the author has no affiliations or financial involvement with anybody or organization with a financial interest or conflict with the subject or material discussed in this manuscript

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