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Biosorption of Nickel ions by Immobilized and Free Biomass of the Freshwater Alga *Chlorosarcinopsis eremi*

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ABSTRACT

Biosorption is an effective technique for removing heavy metals from the aquatic environment. The aim of this study was to assess the potential of microalgae species *Chlorosarcinopsis eremi* in immobilized and free biomass state for nickel removal. Removal efficiency was studied at pH 7± 2, temperature of 25C˚, initial metal concentrations of 5, 10 and 15ppm, contact time ranging from 0– 8 days, and agitation speed of 120rpm. The highest removal percentages for all coefficients (immobilized biomass, free biomass, and blank alginate beads) were recorded at a concentration of 5 ppm, with values of 62.74, 49.54, and 38.86%, respectively. While, the lowest percentage of removal was associated with concentration of 15ppm for all coefficients, as it reached 50.27, 35.04, and 24.30%, respectively. The relationship was positive between the percentage of removal and increased exposure to the metal ions and reached its maximum on the sixth and eighth days of the experiment, for all coefficients and concentrations. While the highest removal percentage of blank alginate beads was recorded at the second and fourth days of the experiment. Biosorption was analyzed using Langmuir and Freundlich isotherms. Additionally, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analyses conducted before and after biosorption revealed that nickel ions were primarily adsorbed onto the surface of the algae cells during the biosorption process.

INTRODUCTION

Various hazardous pollutants, especially heavy metals, have had a significant impact on the environment. In recent years, heavy metals have become one of the most serious environmental problems due to industrial processes which releases them into natural water. Unlike other pollutants, most heavy metals cannot be brokendown into less toxic or non-toxic compounds and are hence indestructible **(Qader & Shekha, 2023)**. However, they have the ability to accumulate in living tissues that leads to a long term stability. Heavy metals that are released into water bodies through waste have a permanent negative effect on the aquatic ecosystems and inhibit self-purification of the water body **(Mosleh *et al.*, 2021)**. Therefore, complete or partial toxic heavy metals

removal from polluted water prior to discharge into aquatic environs is crucial to prevent potential health and environmental problems and ensure ecosystem sustainability **(Uddin, 2017; Sherlala *et al*., 2018)**.

Nickel is a metal that is widely distributed in the environment and has a wide range of industrial and commercial applications. The main industrial processes that contribute to environment pollution with nickel ions are those used in electroplating, manufacturing of battery and electric– steam power plants **(Jadaa & Mohammed, 2023)**. The waste of these industries is released directly into the environment and pollutes it, as a result, it is readily absorbed by the plants. Consequently, these compounds may become part of the food chain and harm living organisms **(Sreekanth *et al*., 2013)**. High concentrations of nickel cause a number of health issues in human, including asthma, heart disease and cancer **(Jaishankar *et al*., 2014)**.

Various conventional remediation techniques are being used for the removal of heavy metals from contaminated wastewater, including ion exchange, precipitation, electrochemical treatment, filtration, lime coagulation and reverse osmosis **(Dolatabadi & Ahmadzadeh, 2019)**. Though these common methods have numerous disadvantages of expensive operation, high energy needs, high amount of reagents and generation of secondary pollution through the production of toxic sludge that require proper disposal make these ways non-favorable in the long run **(Izionworu *et al*., 2020)**. To address this issue, modern methods such as biotic approaches are employed as sustainable solutions for reducing heavy metal ion content. Moreover, these techniques are easy to be implemented and are environmentally friendly. One of the effective treatment for removing heavy metals ions from polluted water is biosorption **(Kumar *et al*., 2022)**.

Biosorption is a physiochemical process of biological components used to eliminate the accumulation and concentration of heavy metals ions in polluted water by either ionic or covalent bonding; it appears to offer an economically appealing and technically feasible method **(Olawale, 2021)**. The mechanism of biosorption for heavy metals is hypothesized to involve both passive and active transport; the first step is the diffusion of ions into the cell surface, followed by the transport of these ions inside the cell or any cell organelles **(Torres, 2020)**.

Removing heavy metals from polluted water with a modern, efficient technique that uses microalgae is regarded as one of the best option. Since microalgae have multiple active sites (proteins, polysaccharides and lipids) on their cell wall; their adsorption capability is noticeably higher than that of other living microorganism like fungi, bacteria and yeast **(Mustafa *et al*., 2021)**. These sites have multiple functional groups including amine, carboxyl, phosphate and hydroxyl, all of which are involved in the binding of metal ions to a great degree **(Danouche *et al*., 2021)**.

Immobilization of algae live or dead cell are the most significant and practical industrial applications for this sector. This technique has great promise for attaching biomass to materials that are compatible with it, whether natural or synthetic **(Salman *et***

***al*., 2022)**. The main benefits of immobilization are the retention of biomass, the ease of separating the product from the cells, in addition to enhancing biosorption capacity. In terms of stability, activity, selectivity, resistance, separation, purification, and process efficiency, immobilization techniques usually outperform free biomass systems. Various immobilization methods have also already been utilized in laboratory and industrial settings **(Wernke *et al*., 2020)**.

Immobilization simply is a biotechnology that employs natural or artificial means to prevent the free movement of microalgal cells in their original location. This allows the microalgal cells in a restricted area to maintain a desired level of biological activity and be reusable in a water phase system **(Zhuanga *et al*., 2020)**. Immobilized algae usually have higher abilities to remove metal ions due to their enhanced photosynthetic rate, as well as their exchange of ions with the matrix that is immobilized **(Mollamohammada *et al*., 2020)**.

Numerous synthetic (acrylamide, polyurethane, polyvinyl and resins) and natural polymer compounds derived from algal polysaccharides have been employed (alginate, carrageenan, agar and agarose). Additionally, chitosan, an amino polysaccharide derived from chitin, has been employed in experiment. Whichever polymers are utilized, they have to fulfill certain criteria such as phototransparency, nontoxicity, permeability and stability in the culture media **(Bouabidi *et al*., 2019)**.

Most commonly utilized natural polysaccharide gel to cell entrapment is sodium alginate as a carrier. Alginates are a family of unbranched copolymers that are composed of 1–4-linked-b-D-mannuronic acid and a-L-guluronic acid (Fig. 1). Moreover, brown algae are the source of commercial alginates **(Nadersha & Hassan, 2022)**.

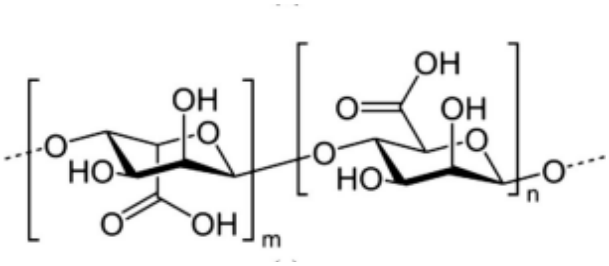


Fig. 1. Alginate structure **(Sahoo & Biswal, 2021)**

The primary goals of this study were to investigate the potential of using both immobilized and free algal cell biomass for eliminating nickel ions from polluted water through biosorption. This research aims to assess the feasibility of utilizing this algae species to purify or mitigate heavy metal contamination in water. Additionally, the study explores how variations in the initial concentration of nickel and the contact time influence the effectiveness of biosorption. The biosorption capacities were evaluated using Langmuir and Freundlich isotherm equations. Finally, the study examines changes

in the algal surface using SEM and EDX before and after exposure to nickel ions to understand their effects on the algal cell surface.

MATERIALS AND METHODS

Chlorophyte alga *Chlorosarcinopsis eremi* (Fig. 2A) was investigated in this study. It was obtained from Algae Research Lab. / College of Science, Basrh university.

The modified CH-10 medium (Al-Aarajy,1996) was used for growing and maintaining algae species. The pH was adjusted to 6.8– 7.2 using 1N NaOH or HCl. Subsequently, 500 mL of the medium in an Erlenmeyer flask was sterilized at 121°C for 20 minutes, then cooled and inoculated under aseptic conditions with 50mL of the algal culture. The cultures were incubated under a cool-white fluorescent illumination with a 16:8 light cycle at 25°C and were stirred by an air pump until a sufficient algal growth was achieved for subsequent laboratory experiments **(Stein, 1975)**.

Immobilizations of algae biomass

Immobilized biomass as a biosorbents were formed as alginate beads by means of ionic polymerization in calcium chloride solution, following the method employed by **Abu Al-Rub *et al*. (2004)**. A certain volume of the algal culture was taken in the stationary phase and concentrated by sedimentation, and the supernatant part was removed. The concentrated algal was washed two times with sterile distilled water, then it was mixed with a 3% (w/v) solution of sodium alginate ($NaC_6H_7O_6$) in a volume ratio of 1:1. After that, this mixture was dropped into a solution of 2% calcium chloride ($CaCl_2 \cdot 2H_2O$) using a separate funnel. When the drops of alginate solution made contact with calcium chloride solution, they gelled and formed beads with a diameter of 4.0± 0.5mm. The beads of immobilized algal were kept in a 2% calcium chloride solution for a minimum of four hours to fully harden. Before use, the beads were rinsed, and stored in sterile deionized water at 4°C (Fig. 2B). A blank set of alginate beads, prepared using the same method but without algae, was also stored in deionized water (Fig. 2C).

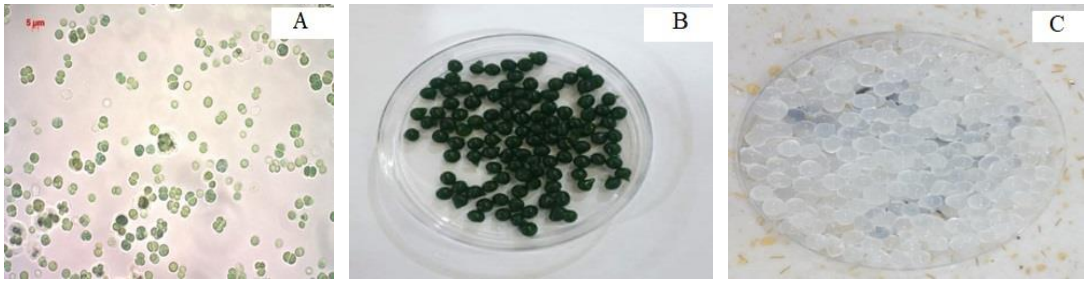


Fig. 2. *Chlorosarcinopsis eremi* species showing: **A.** Free cells; **B.** Immobilized cells, and **C.** Blank alginate beads

Preparation of nickel solution

A stock standard solution of 1000mg/ L for nickel metal ion was prepared by dissolving a known amount of $Ni(NO^3)_2$ powder in a known volume of deionized distilled water. Various concentrations (5, 10, and 15ppm) were then prepared by diluting this stock solution.

Biosorption experiment

The removal efficiency of nickel ions using algae species as biosorbents was assessed in a batch system with initial nickel ion concentrations of 5, 10, and 15mg/ L, a pH of 7 ± 2 and a contact time of 0–8 days at 25°C. The experiments were conducted in 250mL Erlenmeyer flasks containing 100mL of sterilized growth medium. Different concentrations of nickel ions were added to the medium. Separately, 5g of immobilized algae and 10mL of free algae biomass culture were introduced into the medium solution. The mixture was stirred using a shaker for 8 days at 120rpm. As a control, alginate beads without algae were used. Nickel ion concentrations in the culture medium were determined using a flame atomic absorption spectrophotometer.

Removal efficiency analysis and adsorption capacity

Removal efficiency of the absorbed nickel ions by control, immobilize and free algae biomass was determined using the following equation:

$$\text{Removal efficiency (\%)} = C_0 – C_e/C_0 \times 100 \quad \text{.....……..1}$$

Where, C_0 is an initial concentration of metal ions (mg/l), and C_e is a final concentration of metal ions (mg/l) **(Al Prol *et al*., 2017)**.

The adsorption capacity by biosorbent surface was calculated according to the equation as follows:

$$Q_e = (V(C_0 - C_e)) / w \quad \text{……2}$$

Where, Q_e is the biosorption capacity (mg/g); C_0 an initial concentration of metal ions (mg/l); C_e is a final concentration of metal ions (mg/l); V is a volume of the medium (l), and w is a biosorbent weight (g) **(Hashim & Chu, 2004)**.

Biosorption isotherm

Biosorption isotherm study was used to characterize a relationship between the metal ions concentration in the medium solution and the quantity of metal ions adsorbed on the solid-phase when the two phases are at equilibrium. This study utilized two isotherm models: Langmuir and Freundlich. which are frequently employed in data analysis for application involving water treatment.

The Langmuir (eq.3) and Freundlich (eq.4), isotherms were acquired by the use of experimental adsorption data.

$$\frac{Ce}{Qe} = \frac{Ce}{Qmb} + \frac{Ce}{Qe} \quad \text{……3}$$

Where, Q_m is the maximum capacity of biosorption (mg/g) and b Langmuir constant value.

$$\text{Log Qe=log Kf} + \frac{1}{n} \text{log Ce} \quad \text{…….....4}$$

Where, Kf is the Freundlich constant, and n is the Freundlich indicator of adsorption capacity.

Statistical analysis

The results were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) Version 20. The one-way ANOVA was employed to analyze the data with a significance level set at 0.05. Additionally, the least significant difference (LSD) test was conducted to determine the extent of variation in the metal ion removal efficiency of the studied algae.

RESULTS AND DISCUSSION

Effect initial concentration of nickel ions

In the present study, the effect of initial nickel ion concentrations (5, 10, and 15 ppm) on the biosorption process was investigated using immobilized and free algal biomass, as well as blank alginate beads, under optimized conditions of pH and temperature (25°C). Results indicate that the average removal percentages for nickel ions using immobilized *C. eremi* algae were 62.74, 57.18, and 50.27% for initial concentrations of 5, 10, and 15ppm, respectively. In contrast, free algae achieved removal rates of 49.54, 38.62, and 35.04% at the same concentrations. For blank alginate beads, the removal percentages were 38.86, 27.04, and 24.30% for the concentrations of 5, 10, and 15ppm, respectively (Fig. 3).

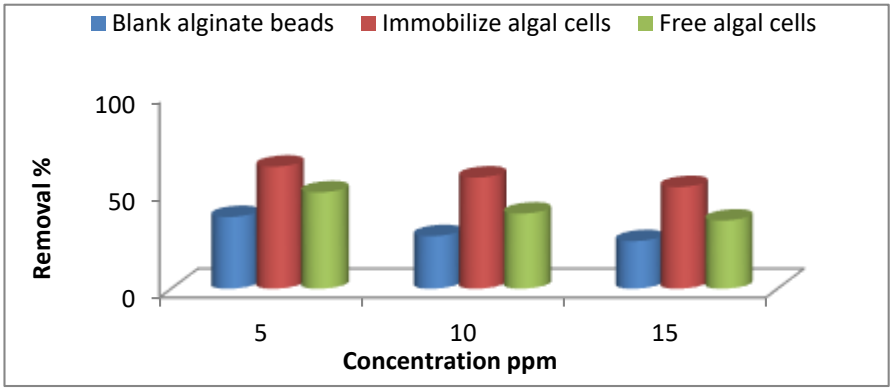


Fig. 3. Effect of nickel concentration to bisorption removal (R%) by *C. eremi*

The results indicated a significant decrease in nickel removal percentage with increasing concentrations ($P< 0.05$). These observations can be attributed to the fact that at low metal ions concentrations, the ratio of active sites on sorptive surface area to the total metal ions available are high, which increases the probability of metal removal. The removal percentage at higher concentration levels shows a decreasing trend **(Banerjee *et al.*, 2016)**. Similar results were recorded in the literature of **Sekhar *et al.* (2003)** and **Kumar *et al.* (2006)**. They reported that at lower concentrations, all metal ions in solution had the capacity to interact with the binding sites, and thus the adsorption percentage was higher than those at higher concentrations metal ions. At higher concentrations, the lower adsorption efficiency can be attributed to the rapid saturation of binding sites, as the amount of biomass remained constant.

As noted by the results, the biosorption efficiency of nickel ions in the immobilized algal cells was significantly ($P< 0.05$) higher than that of the blank beads and free algal biomass. This enhanced sorption by the immobilized algal cells may be explained by the increased number of metal-binding sites after the incorporation of algal cells into Na-alginate beads **(Bayramoglu *et al.*, 2006)**. It was widely reported that immobilization generally increases the biomass's capacity to accumulate metal ions **(Katircioglu *et al.*, 2008; De-Bashan & Bashan, 2010)**. Additionally, **Lee *et al.* (2020)** elucidated that immobilized algal cells have a higher capacity for metal removal compared to free cells.

Effect of contact time

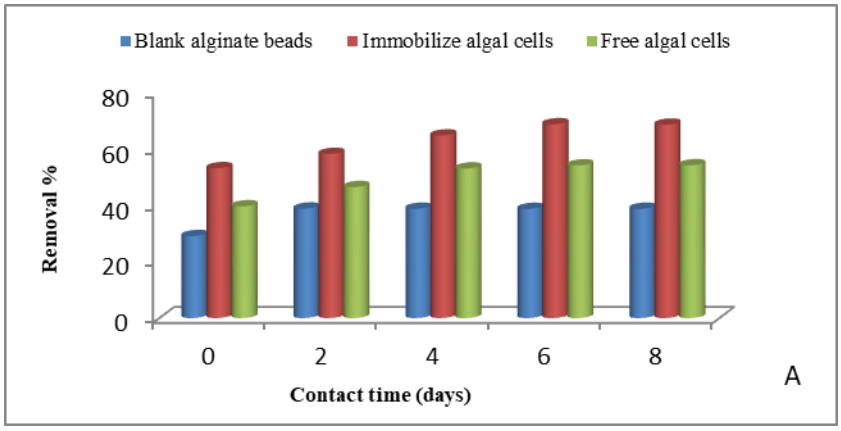
Under optimal conditions, nickel ions biosorption by blank alginate, immobilize and free algae adsorbents were determined as a function of contact time (0 – 8). Study results indicate that contact time has a significant effect ($P< 0.05$) on biosorption efficiency (Fig. 4), and the highest nickel removal rate by immobilized form of algal cells appeared on the sixth day of the experiment for initial concentrations of 5 and 10ppm, respectively, beyond which there was no further removal increase (Fig. 4A, B). While, the highest

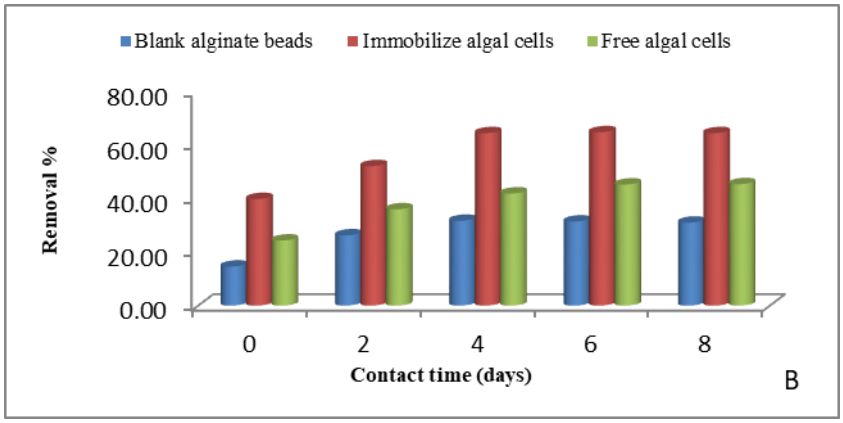
removal rate of initial concentration of 15ppm was observed on the eighth day of experiment. (Fig. 4C).

In addition, the results presented in Fig. (4) demonstrate that the removal efficiency for free algae cells increases as process time increases until day 8 for the initial concentrations of 5, 10 and 15ppm, respectively. No significant difference ($P > 0.05$) was observed between the sixth and eighth days of experiment.

The maximum nickel removal by blank alginate beads was achieved on the second day for the initial concentration of 5 ppm and on the fourth day for the initial concentrations of 10 and 15ppm, respectively (Fig. 4).

The experiment results indicate that nickel sorption by microalgae occurs in two distinct stages. The first stage is characterized by rapid and high adsorption, suggesting passive surface sorption through physical binding on the algal cells or beads surface. This initial fast phase is consistent with the kinetic theory of adsorption, where adsorption occurs quickly at the beginning, followed by a slower phase. The slower stage likely represents intracellular uptake, where heavy metal ions are transported across the cell membrane, possibly involving active metabolic reactions **(Radway *et al.*, 2001)**. These findings are consistent with previous studies on the biosorption of nickel ions from aqueous solutions using *Chlorella vulgaris* algal cells as biosorbents **(Abu Al-Rub, 2004)**.





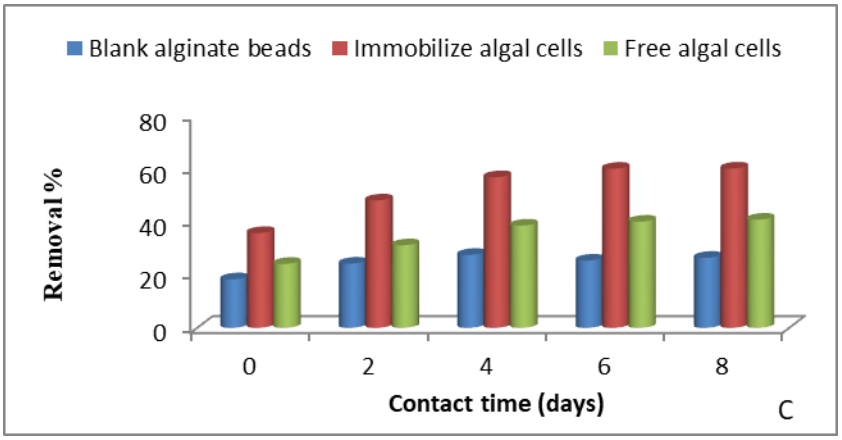


Fig. 4. Effect of contact time on biosorption of nickel by *C. eremi* at **A.** 5ppm; **B.** 10ppm, and **C.**15ppm

Biosorption isotherm

The sorption isotherm models describe the interactions between sorbets and biosorbents surface at constant pH and temperature. The examination of metal ions uptake using isotherm models is a significant study that gives information about nature and capacities of biosorbent surface, sorption process mechanism and design of successful and more efficient method of treatment process **(Sahoo & Prelot, 2020)**. Under the optimal biosorption conditions, the Langmuir and Freundlich models can be applied to analyzed nickel biosorption on the sorbents surface used in this study.

Isotherm models state that the Langmuir isotherm assumes that biosorbent and sorbet are in a state of dynamic equilibrium and that bisorption takes place uniformly and homogeneously on the active sites of the sorbent surface. Conversely, the sorption at a heterogeneous surface is based of the Freundlich isotherm **(Latour, 2015)**.

The modeling results of the Langmuir and Freundlich models for *C. eremi* immobilized and free algae are shown in Figs. (5, 6). Moreover, sorption constants evaluated from the isotherms models with the correlation coefficients(R^2) are listed in Table (1).

Table 1. Parameters for Langmuir and Freundlich isotherm equations for biosorption of nickel by immobilize and free algae species

Algae		Langmuir			Freundlich		
		Qm	b	R^2	Kf	N	R^2
Chlorosarcinopsis eremi	Iimmobilize	2.28	0.052	0.997	57.74	0.740	0.995
	Free	0.049	3.383	0.963	9.1	0.571	0.996

The data presented in Table (1) reveals a high Langmuir isotherm correlation coefficient (R^2) for the immobilized form of *Chlorosarcinopsis eremi* algal cells, indicating that the Langmuir model is suitable for describing the nickel biosorption process in this study. This conclusion is further supported by the data shown in Fig. (5), which illustrate that the sorbent surface is homogeneous. This means that the biosorption sites on the surface are fixed and that the energy distribution across the surface is consistent.

According to the Langmuir model, the biosorption process on the immobilized sorbents is characterized by single-layer adsorption, where the sorption reaches its maximum when all available sites on the sorbent surface are occupied by nickel ions. This model assumes that once a nickel ion occupies a site, no further adsorption can occur at that site, reflecting a finite number of identical sites with uniform energies of adsorption **(Hameed *et al.*, 2007)**.

The high correlation coefficient and the fitting of the experimental data to the Langmuir isotherm suggest that the immobilized algal cells provide a consistent and predictable platform for nickel ion removal, making them a viable option for use in wastewater treatment processes focused on heavy metal removal.

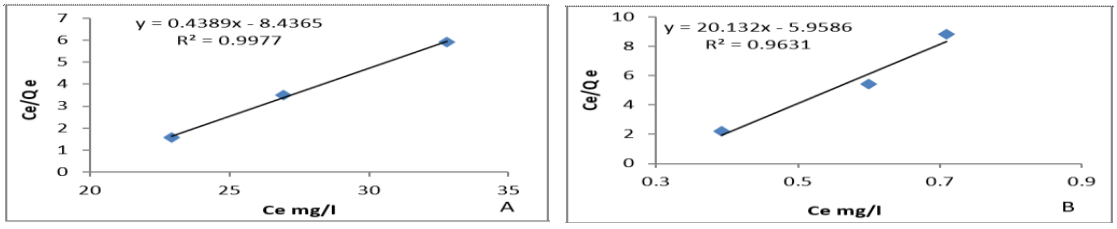


Fig. 5. Langmuir isotherm graphic for biosorption of nickel ions showing: **A.** Immobilize and **B.** Free algae cells by *C. eremi*

The data shows that the Freundlich isotherm R^2 value for the free *Chlorosarcinopsis eremi* cells was higher than that of the Langmuir isotherm, as depicted in Fig. (6). This suggests that the sorption process for the free algal cells follows the Freundlich isotherm, which is characteristic of a heterogeneous surface. The heterogeneity of the surface implies that the free algal cells possess biosorption sites with varied affinities, meaning they have different sites in terms of biosorption energies. This model also indicates that the biosorption process involves multiple layers of adsorption **(Abood & Al-Abdullah, 2023)**.

Additionally, the *n*- values obtained from Table (1) for the Freundlich isotherm model were 0.740 and 0.571 for the immobilized and free algal cells, respectively. These *n*- values indicate that the biosorption process is a chemical process, as the *n*- value is less than 1 **(Ajenifuja *et al.*, 2017)**. This further supports the idea that the biosorption on these algal cells involves complex interactions at the molecular level, making it a viable method for removing nickel ions from contaminated water sources.

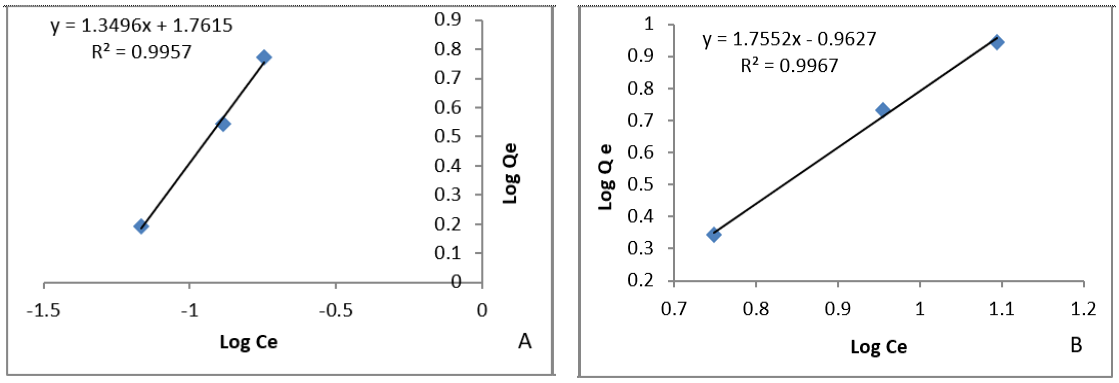


Fig. 6. Freundlich isotherm graphic for biosorption of nickel ions A. immobilize; B. free algae cells by *C. eremi*

Characterization of biomass

Scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDX) studies

SEM attached with EDX analysis was used to identify the effects of metals ions on surface sorbent cells. Examination of algae by electron microscopy was used before and after metal ion adsorption to observe the active sorptive areas on the algal cell surface. Elements or metallic ions that are absorbed on the surface of algae or present within their

structure can be detected using Energy Dispersive X-ray Spectroscopy (EDX) technique **(Al Fakih *et al*., 2011)**.

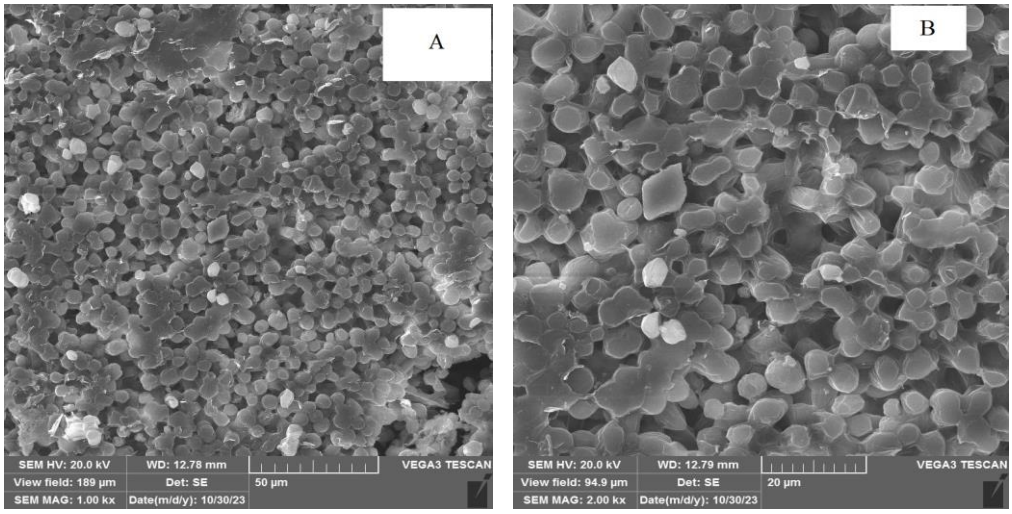


Fig. 7. Scanning electron micrographs of free *C.eremi* untreated with nickel ions at mag at **A.** 1.00kx and **B.** 2.00kx

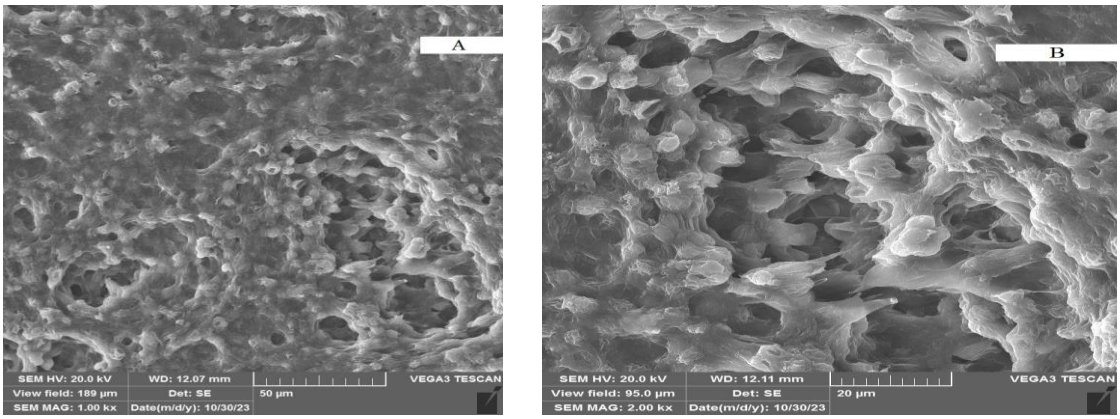


Fig. 8. Scanning electron micrographs of free *C.eremi* treated with nickel ions at mag at **A.** 1.00kx and **B.** 2.00kx (at a concentration of 5ppm)

The images of scanning electron microscopy (SEM) for free *Chlorosarcinopsis eremi* algae exposed to nickel show that the surfaces became relatively rough and irregular, with a fractured appearance and obvious pores (Fig. 8A, B). In contrast, the untreated free algae exhibited a uniformly interconnected structure with a continuous smooth surface (Fig. 7A, B).

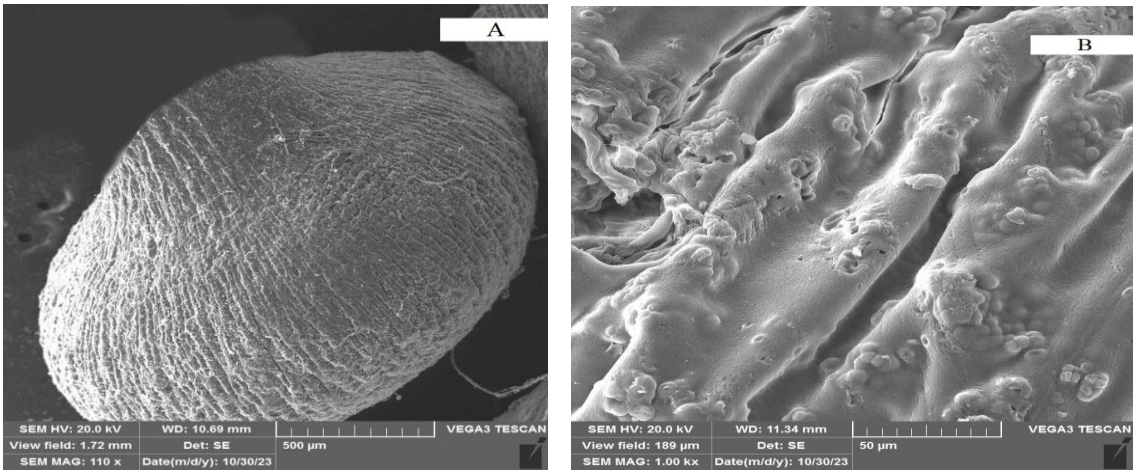


Fig. 9. Scanning electron micrographs of immobilize of *C.eremi* untreated with nickel ions mag at **A.** 110kx and **B.** 1.00kx

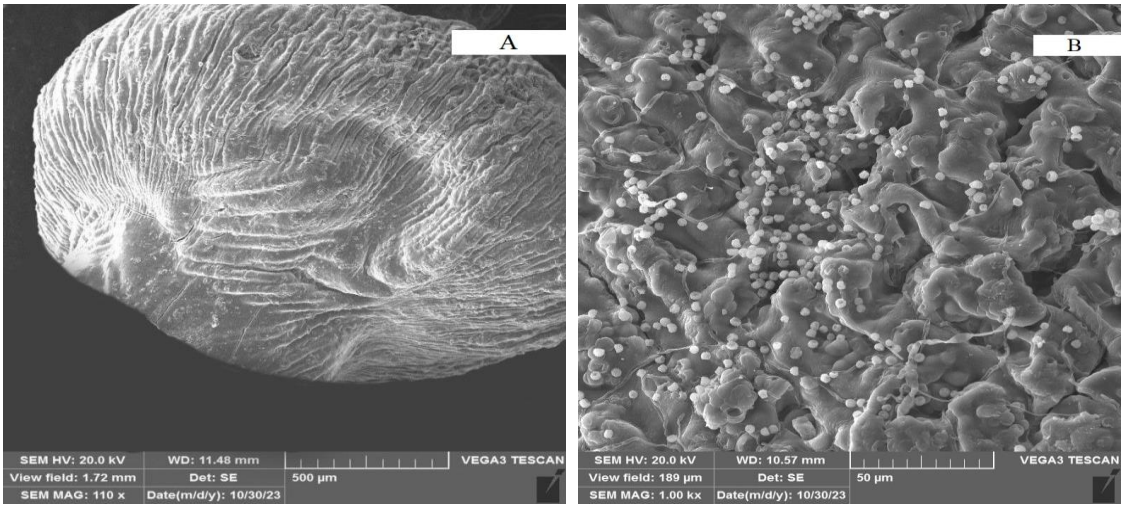


Fig. 10. Scanning electron micrographs of immobilize of *C.eremi* treated with nickel ions mag at **A.** 110kx and **B.** 1.00kx (at a concentration of 5ppm)

The surface morphology of immobilized *Chlorosarcinopsis eremi* algae treated with nickel became relatively rough, with an irregular distribution of crystalline deposits adhered to the algae, making the surface more folded and heterogeneous (Fig. 10A, B). This is in contrast to the surface of untreated immobilized algae cells, which maintained a smoother and more uniform appearance (Fig. 9A, B). These morphological changes could be attributed to the precipitation of nickel ions and their potential linkage with functional groups on the algal surface **(Mehta *et al.*, 2002)**.

According to **Saravanan *et al*. (2011)**, THE formation of significant cross-linkage and the replacement of surface cations with absorbed metal ions are what cause the surface morphological change following metal biosorption. This confirmed the incidence biosorption and accumulation of nickel ions on surface algal cells as compared with untreated ones **(Rasheed & Yahya, 2020)**.

The EDX spectra of both immobilized and free algae biomass treated with nickel ions exhibited clear identifiable peaks at approximately 0.82, 7.45, and 8.25keV, confirming the presence of nickel on the adsorbent surfaces after the adsorption process (Fig. 12A, B). These peaks were not present in the untreated samples (Fig. 11A, B). Additionally, the EDX analysis revealed high carbon and oxygen content on the algae surfaces, with trace amounts of other elements such as sodium, magnesium, chloride, potassium, and calcium both before and after nickel treatment. These elements, naturally occurring in the cell walls of algae, are associated with various functional groups **(Ahmad *et al.*, 2018)**.

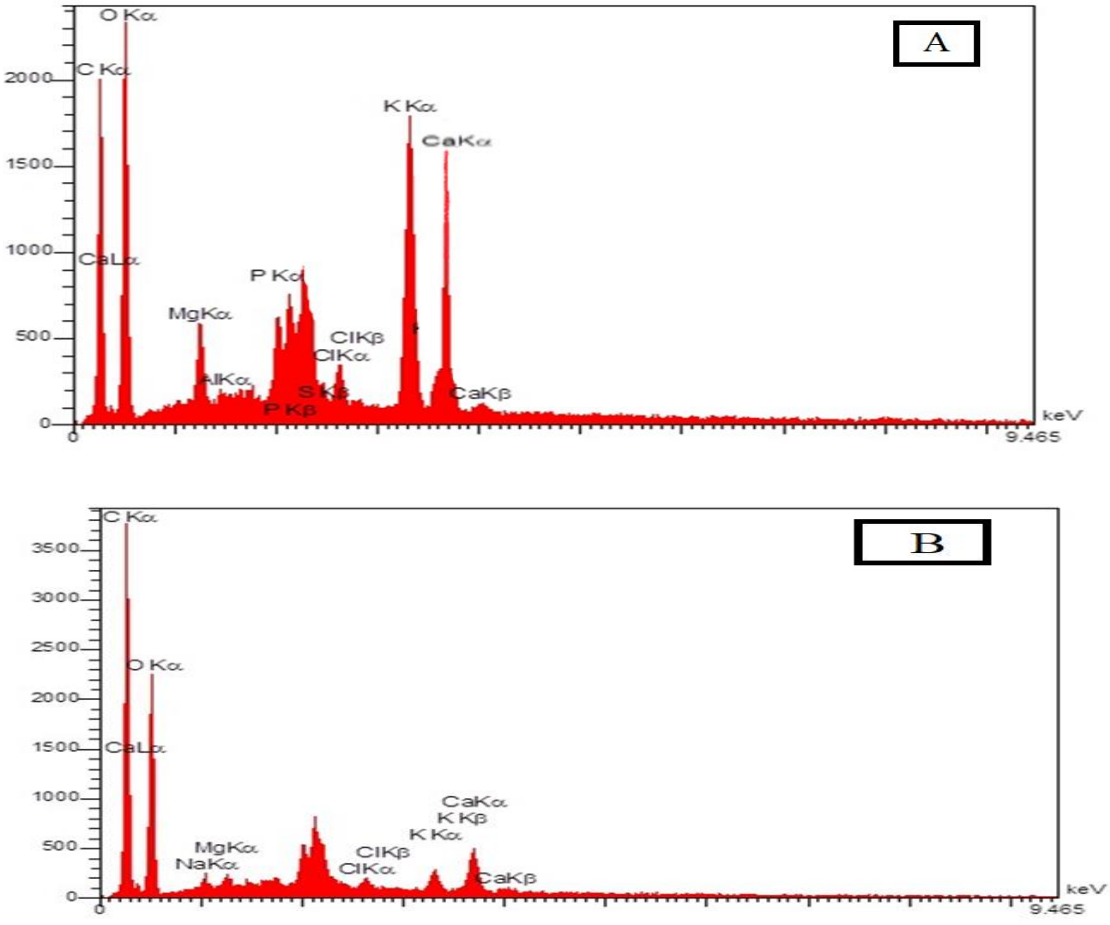


Fig. 11. EDX analyses showing: **A.** Immobilize and **B.** Free of *C.eremi* algae untreated with nickel ions

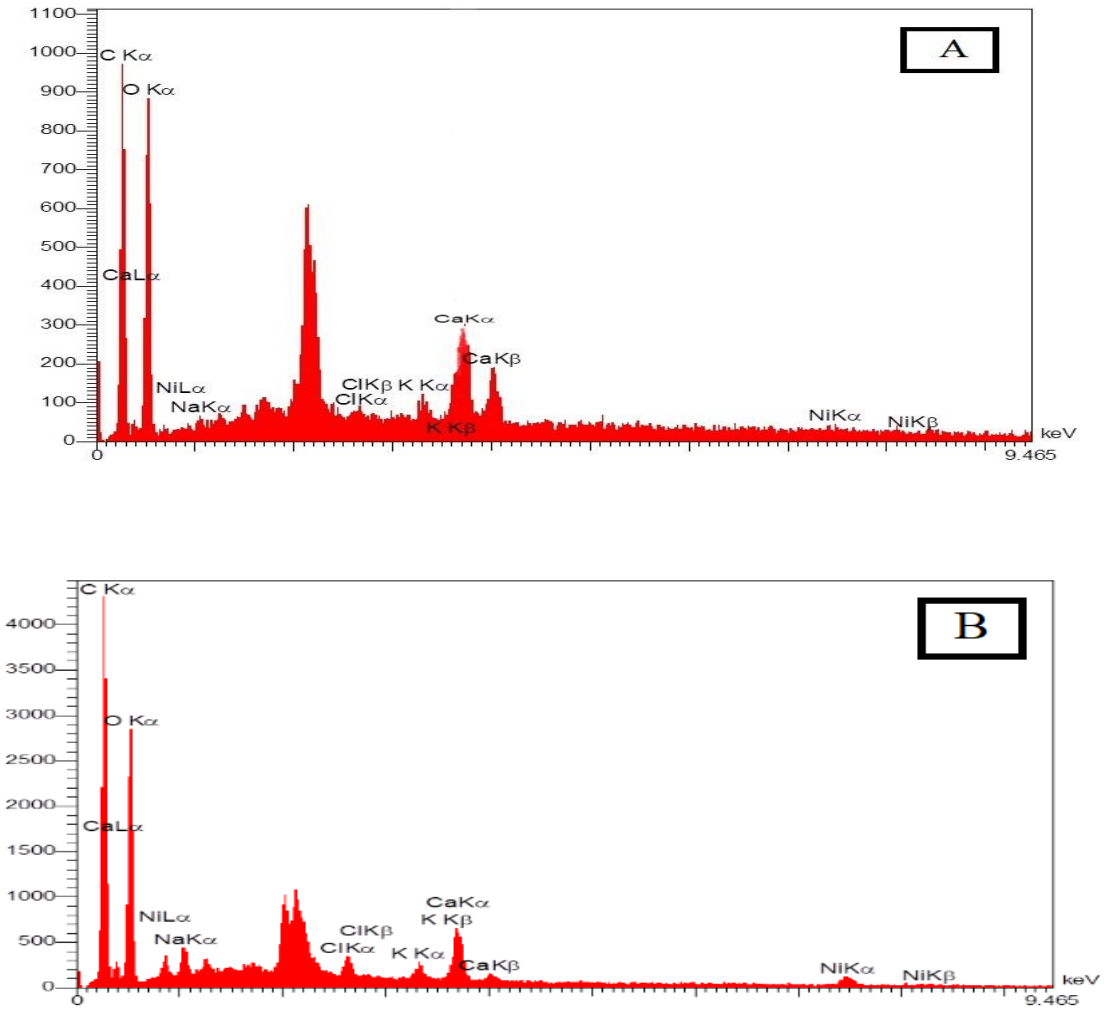


Figure 12. EDX analyses showing: **A.** Immobilize and **B.** Free of *C.eremi* algae treated with nickel ions (at a concentration of 5ppm)

CONCLUSION

The Na-alginate beads, immobilized algae and freely suspended biomass algae of the fresh water algae *Chlorosarcinopsis eremi* were effectively employed as a biosorbing agent to remove nickel ions from the aqueous medium in batch system. The results of the experiment indicate that immobilized algal cells could improve the biosorption of considered nickel ions as compared to freely suspended cells. Factors such as initial concentration of nickel ions and contact time were significantly affecting the biosorption process. The biosorbent was investigated using SEM-EDX before and after biosorbtion process. Biosorption of nickel on immobilized and free *Chlorosarcinopsis eremi* cells has been found to follow the Langmuir and Freundlich isotherm models, respectively. These

results suggest that immobilized algae state offer good biosorbent potential based on a great biosorption capacity. They may thus be considered as an eco-friendly alternative technique for nickel ions elimination from aqueous solutions.

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