STUDIES ON BIOSORPTIVE REMOVAL OF HEAVY METAL Ni (II) ION IN A SINGLE COMPOSITE BED EMPLOYING EXTRACELLULAR POLYMER SUBSTANCE

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ABSTRACT

Development in various industries is essential to mankind but also it seems to possess serious threat to the environment and mankind. The effluents are discharged into the land and water bodies which is responsible for the destruction of aquatic creatures. Heavy metals is the major factors responsible for water contamination.. Traditional treatment methods like microfiltration, ultrafiltration, Nano filtration, reverse osmosis etc., are a monotonus and painstaking processes and it also adds on to the capital cost.. Bio sorption is such method which employs living organisms or parts of them to remove metals. Bacteria, fungi and algae are proven to be efficient biosorbents. Extracellular Polymer Substances(EPS) are bacterial secretions which have shown to be a potential biosorbents.. This paper acknowledge on the metal complex formation capability of EPS, extracted from waste water. The advantage of a EPS in the bio treatment of heavy metals is studied with optimization of process parameter. The viability of adsorption and desorption behavior of EPS /sodium alginate(SA) bead prepared in 2:8 ratio for Ni removal has been investigated through batch studies. Optimal condition for adsorption are found to be pH:6.5; adsorbent dose:6g ; initial metal concentration: 75 mg/L and temperature:40°C

Keywords: Biosorbents, Heavy Metal, Adsorption, Extracellular Polymer Substances EPS.

INTRODUCTION

Heavy metal Pollution

Development in different industries is necessary for supporting the needs of growing human population, which has also resulted in the environmental damage and pollution.. Principal heavy metals comprise Nickel (Ni) mercury (Hg) and cadmium (Cd) which are well-thought-out to possess unsafe ecological effects according to toxicological data. Mercury (Hg), Cadmium (Cd), Arsenic (As) Chromium (Cr),Nickel (Ni) are normally considered as heavy metals owing to their toxicity.[1] Arsenic and cadmium causes cancer. Mercury causes mutations which cause genetic impairment, while copper and lead can cause damage to the brain and bone. The major routes by which heavy metal ions get entered into the atmosphere is through various industries like textile, tannery, electroplating, mining , petroleum refining etc.[2], [3]

Traditional and conventional methods which are available for the elimination of heavy metal ions include ozonation, reverse osmosis, ultrafiltration, nanofiltration, chemical precipitation, ion exchange, Membrane separation, electrochemical treatment etc. Though

these methods help in clearing the heavy metals, there exist several limitations like high operating cost, low selectivity, incomplete removal and production of huge amounts of waste [4], [5]

These Processes require integrated technologies for waste water treatment of poisonous heavy metals in order to meet strict environmental standards, to shelter the atmosphere and to save resources. Development of feasible and variety in pertinent technologies that not only clear-out but also recuperate valued components from industrial waste streams is of chief importance.

Biosorption

Biosorption is a quick phenomenon of inactive metal/dye removal by the non-growing biomass/adsorbents[1] via various physiochemical mechanism which includes ion exchange, absorption, adsorption, complexation, chelation, micro precipitation etc. [6], [7]. As a green and sustainable alternatives to conventional technique ,biosorption has gained significant importance in the elimination of dyes and heavy metal ions from waste water even at low concentration. Several kinds of biomass such as fungi, bacteria, yeast, and algae have been inspected which carry out efficient biosorption. Among them, algal biomasses have proven to be more efficient, because of abundant availability, shorter regeneration time and higher metal recovery potentiality[8].

Moreover, algal biomasses have the ability of high metal binding capacities, due to the polysaccharides, proteins, or lipids which they contain on the exterior of their cell walls which in turn contains few functional groups such as amino, hydroxyl, carboxyl, and sulfate, which acts as binding sites for metals. Rcently , biosorption has been well-thought-out as a capable technique for the exclusion of heavy metal ions from industrial effluents. [9], [10].These protein rich algal and fungal biomass endeavored as metal biosorbents have limitations, under moist conditions proteinious materials tend to putrefy., bark along with other tannin-rich materials, chitosan, zeolites, peat moss etc. can serve as low-cost adsorbents.[11]

Extracellular Polymer Substance (EPS)

Extracellular polymer substance (EPS) is a microorganism secreted macromolecule which is abundant in its source. Someresearch paper shows that EPSs has ability to be used as as biosorbents for heavy metals. By changing the ratio of carbohydrate to protein in EPS, it is found that the biofilm exhibits the capacity in removing heavy metals (Cu, Pb and Ni) in waste water. It is also reported that EPS exhibits a great capability to bind multifaceted Pb and Ni. The EPS formed by anaerobic sludge process using sulfate-reducing bacteria was found active in get rid of Cd^{2+} from aqueous solution. [10, 23, 24] The recent study of EPS has given fascinating results .this cost effective biosorbent quiet useful in excretion   of heavy metals Ni ions by using single composite bed.[12],[13]

METHODOLOGY

1. **Selection of microorganism and production of EPS**

1.1. *Selection of organisms*

Alga-bacteria biofilm is collected using a germ-free plastic spatula ,it is then kept in a 200 ml sterized beaker. Immediately the biofilm sample was collected, it is washed with de-ionized water .It was dispersed into suspension by a stirrer.

The bacterium such as Bacillus, Pseudomonas, nitrifying/denitrifying, sulfur reducing bacterial species from the waste water is extracted through serial dilution method and poured into petri dish containing nutrient medium.

1.2. *Culturing of Organisms*

The nutrient medium was sterilized at 121°C for 20 min, cooled to room temperature, inoculated with bacteria was incubated at 37°C for 3 days and the colonies differing in morphological characteristics was selected. Selected bacterium isolates was allowed to grown on Mac-Conkey agar media. The shape and colors of the colonies was scrutinized under the microscope after Gram's staining.

1.3. *Laboratory production of EPS*

Aerobic/sulfate reducing and nitrifying/denitrifying species was grown in laboratory-scale rotating drum biofilm reactors (RDBRs) or Sequencing Batch Reactor (SBR). The reactors was inoculated with sufficient amount of activated sludge from a municipal wastewater

treatment plant. They will be supplied with adequate amount of energy source such as sucrose and other macro nutrients essential for their growth. The walls of the reactors were cleaned every 2 weeks, and the biofilm containing EPS growth was noted down and recovered.[9]

2. Extraction of EPS

2.1. Separation of EPS by Physical and Chemical Methods

Three chemical methods and two physical methods were used to extract EPS from the biofilm.

2.1.1. The following three chemical methods were evaluated for EPS extraction:

Extraction with EDTA – the cell suspension was reacted with 2% EDTA for 3 h at 4°C.

Extraction with formaldehyde – the cell suspension wasreacted with formaldehyde (36.5%) for 1 h at 4°C.

Extraction with formaldehyde plus NaOH – the cell suspension was treated with formaldehyde (36.5%) for 1 h at 4°C and then with NaOH (1M, 4°C, 3 h)

2.1.2.The following 2 physical methods was assessed for EPS extraction:

Extraction with high-speed centrifugation – The cell suspension was centrifuged at 20,000 rpm, 4°C for 20 min. This method was believed not to cause cell lysis. The supernatants will be filtered through a 0.22µm membrane. The filtrate is used as the EPS sample.

Extraction with ultrasonication – The cell suspension will be first subjected to ultrasonication at 40W in an ice bath for 2 min. The ultrasound will be generated by an ultrasound generator. The sonicated cell suspension will then be centrifuged at 20,000 rpm, 4°C for 20 min. The supernatants was filtered through a 0.22µm membrane and the filtrate is used as the EPS sample[10].

3. Analysis of EPS by Advanced Techniques

The extracted EPS will be subjected to the following advanced analytical techniques:

3.1. Quantification of EPS

3.1.1. The extracted EPS is subjected to lyophilization and the amount of extracted EPS will be found by measuring the amount of solid left after lyophilization.

3.1.2. The % amount of carbohydrate in EPS will be found out by the anthrone method using glucose as the standard.

3.1.3. The protein contents and humic substance content in EPS will be measured by Lowry's method and Bradford method using bovine serum albumin and humic acid respectively as standard references and by calculating the absorbance at 660nm.

3.1.4. Uronic acid amount will be measured by the n-hydroxydiphenyl sulfuric acid method using glucuronic acid as the standard reference.

3.1.5. The DNA amount in EPS will be found out by using the diphenylamine colorimetric method which used *E. coli* DNA as the standard.

3.2. Fourier Transform Infrared Spectroscopy(FTIR)

The active functional groupspresent on the surface of EPS will be confirmed using FTIR analysis. Also , FTIR analysis make available information on probable mechanism elaborate in metal ion adsorption.

Before and after samples of The lyophilized or powder dried EPS biosorption process will be analyzed in the range 3500–600 cm^{-1} with a resolution of 4 cm^{-1} by using the Attenuated Total Reflectance (ATR) technique and placing enough sample to cover the diamond sensor.

FTIR creates the absorbance spectra which shows chemical bonds and the molecular structure of the sample material. Absorbance peaks on the spectrum will indicate functional groups (e.g. carboxylic, hydroxyl etc,.)..In FTIR,different functional gps and so the different types of bond absorbs IR radiations at different wavelengths.[14]

3.3 *Preparation of EPS/SA (2:8) bead:*

The EPS/SA bead was prepared by pouring the homogeneous blended mixture of EPS(2g), sodium alginate (8g) dispersed in minimum amount of water prepared at 500 rpm continuous stirring (30 minutes) into 0.2M calcium chloride solution with the help of syringe. After this the formed beads were allowed to dip in 0.2 M calcium chloride solution for few hours,Once this process is over, the beads were allowed to rinse with double distilled water to remove any excess CaCl2 followed by drying for 24 hours.[15]

3.4Adsorbate Preparation:

The effect of various factors such as pH, contact time, adsorbent dose and temperature on the adsorption process can be study by using standard solution (500mg/L) of Ni can be ready by dissolving 0.594g of Nickel Chloride in 1000 ml deionised water. To study the effect of initial metal concentration on adsorption process, the stock solution of Nickel chloride (1000 ppm) was prepared separately and suitably diluted by considering various initial dilution concentrations.

4. Adsorption and Desorption Studies

4.1. Batch adsorption experiments

The extent of adsorption onto EPS/SA bead (2:8) was investigated with Nickel Chloride (500mg/L) using the batch studies by changing the pH, adsorbent dose, contact time, initial metal ion concentration and temperature. Initially the pH of Ni solution was adjusted to the required value (pH=4, 4.5,5, 5.5. 6) by adding 2N NaOH solution for increasing the pH and 1:1 HCl for decreasing the pH. After the adjustment of pH, about 1g of above prepared NCS/SA bead (2:8) was added to 100ml of Nickel Chloride (500mg/L) solution taken in a conical flask. After this ,solution mixture was well agitated   at 30ºC for one hour using orbital shaker at fixed speed of 200 rpm. Once the equilibrium attained, , the adsorbent was filtered using Whattmann filter paper and the filterate collected, analysed using the atomic absorption spectroscopic (AAS) studies. A similar procedure was carried out by varying the adsorbent dose, contact time, metal ion concentration and temperature. The percentage of adsorption of Ni(II) ion was determined by following equation

$$Adsorption \% = \frac{Initial\ metal\ ion\ Conc. - Final\ metal\ ion\ conc.}{Initial\ metal\ ion\ conc.} x100$$

4.2 Desorption Studies

After the adsorption process, the retentate from the filtration will then be treated with acidic/basic solutions and organic solvents for desorption of the heavy metals Desorption of heavy metal is done by using dilute HCl or dilute NaOH, etc., Desorption of metal is done by using Organic solvents such as ethanol, methanol which causes precipitation of metals. Due to change in pH, metals get desorbed from EPS.

The concentration of metals in the treated solvent is determined by spectrometry analysis. Initial concentration of desorption solvent before passing through EPS were determined and compared with solvent concentration containing desorbed metals and dyes after treatment.Metal recovery in this process is 73.6%

5.Result and Discussion

5.1. Effect of Solution pH

As the pH distresses both the adsorbent and adsorbate chemistry in solution , metal ions is mainly affected by the pH of the metal solution [16] . Biosorbents properties get affected by the pH of the solution [17]. By varying the pH of the solution removal of Nickel (II) was analyzed from 4-8, by keeping the other variables as constant. The influence of pH on the adsorption of Ni (II) ions onto the EPS/SA bead was signified in**Fig.1** It was observed from the outcomes presented in the **Fig.1** ,that the Ni ions percentage removal increases by increasing pH from 4 to 6 but afterward it decays. The low pH is the fact behind it , the active sites (OH, NH2) protonation takes place, the protonated H+ ion competes with Ni(II) ions for the available active sites of the adsorbent for adsorption. At pH 4 hence the percentage removal is low. The concentration of H+ decreases with increase in pH , they do not involve in metal ion on adsorption sites. Hence it shows an increased percentage removal (from pH:4-6) [18].A decrease in uptake of the Ni ions was observed, beyond these pH values So pH of 6 is considered as an optimum pH

5.
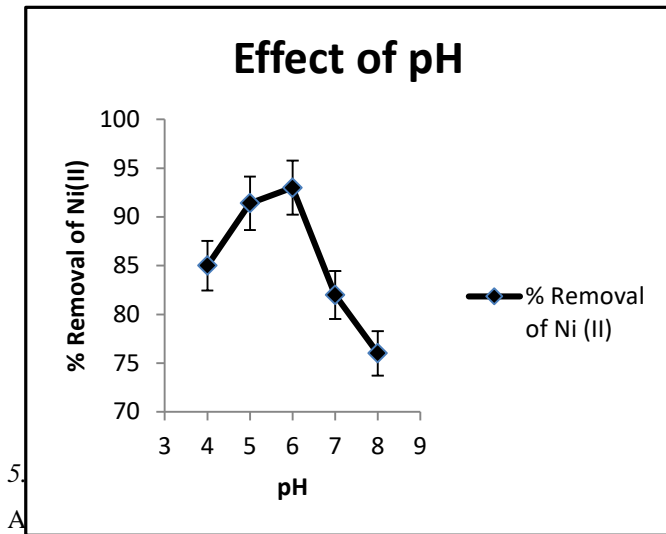
A   effect of adsorbent dose at pH 6.Dose of adsorbent in 100 ml of 500mg/L of Nickel chloride solution was varied from 1g-7g. The effect of adsorbent dose was shown in **Fig. 2** It was been observed through the results that the efficiency increases up to the optimal dosage beyond which the removal efficiency is constant. As more binding sites are available the % removal of Ni with increased adsorbent dose[19] . There remained no appreciable improvement beyond 5 g in the removal of metal ions and this can be indorsed to two causes. Initially at constant Ni ions concentration and volume unsaturation of the adsorption site increase due to increase in the adsorbent dosage and furthermore due to the particulate interface such as accumulation of adsorbents. [19]
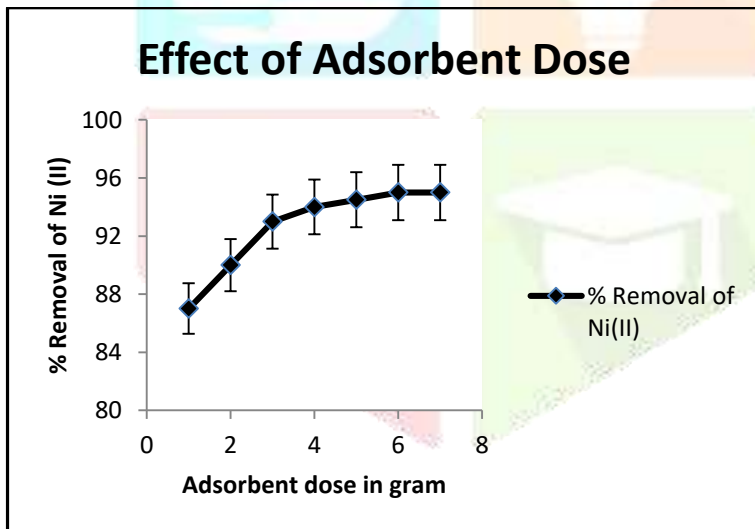

5.3. Effect of contact time

One of the important studies executed during batch adsorption process is the optimization of contact time. Study was carried out by varying the time of contact from 60 min to 360 min on removal of Ni (II) by EPS/SA bead The observed results were presented in **Fig.3** It can be seen form **Fig. 3** that there was a speedy uptake within the first 60 min and adsorption equilibrium was accomplished within 300 min. The percentage deduction of Ni(II) increases from 74.88% to 93.69%. This quick early increase in the deduction of percentage was mainly recognized to more number of vacant accessible adsorption sites and also due to the greater surface area[20]. But beyond a certain amount of contact time, the adsorption sites get shattered and thus lead to decrease in removal of nickel ions[14]

Effect of contact time

% Removal of Ni(II) vs **TIME (MIN)**
— % Removal of Ni(II)
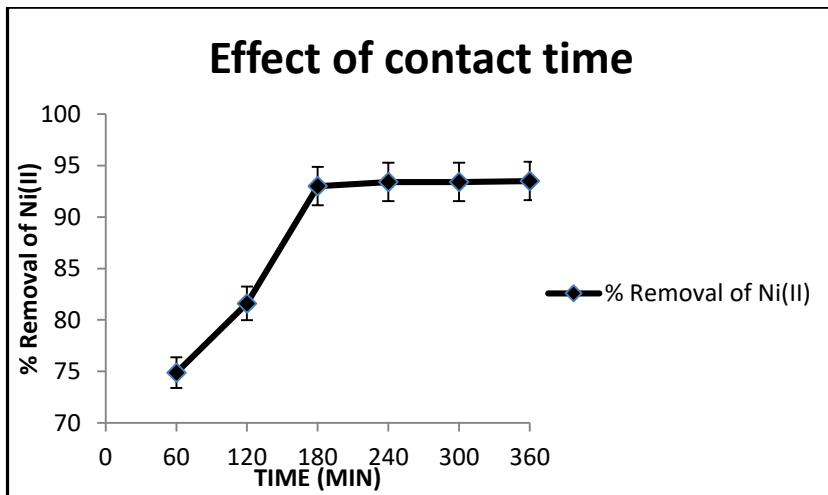
5.4. *Effect of temperature*

The effect of temperature on the adsorption of Ni (II) from aqueous solution by EPS/SA bead prepared in (2:8) ratio was shown in **Fig.4**. The adsorption studies were conducted by wavering the temperatures between 30ºC - 70ºC . The experimental results presented in **Fig.4** show that the percentage of Ni(II) ion acceptance increases with increase in temperature (from 30ºC-50ºC) but afterward it shows a decrease. The early increase with increase in temperature could be accredited to the increased movement of metal ions which lead to quicken the opportunity of their mutual collisions. This will effect in the increased metal ion removal percentage and adsorption efficiency[21] But when the temperature is surpassed beyond 50ºC the decrease in adsorption was witnessed and this might be due to the failing of the requisite forces existing between metal cations and active sites present on the biomass surface [22] and due to the loss of active sites [23]. In supplementary arguments we can say that at greater temperature the desorption speed on the surface quicken which leads to the improved attraction of the metal ion to outflow from the biomass surface to the solution stage and henceforth the reduction in adsorption was originate[24]

Effect of Temperature

% Removal of Ni (II) vs **Temperature (^{0}C)**
— % Removal of Ni (II)
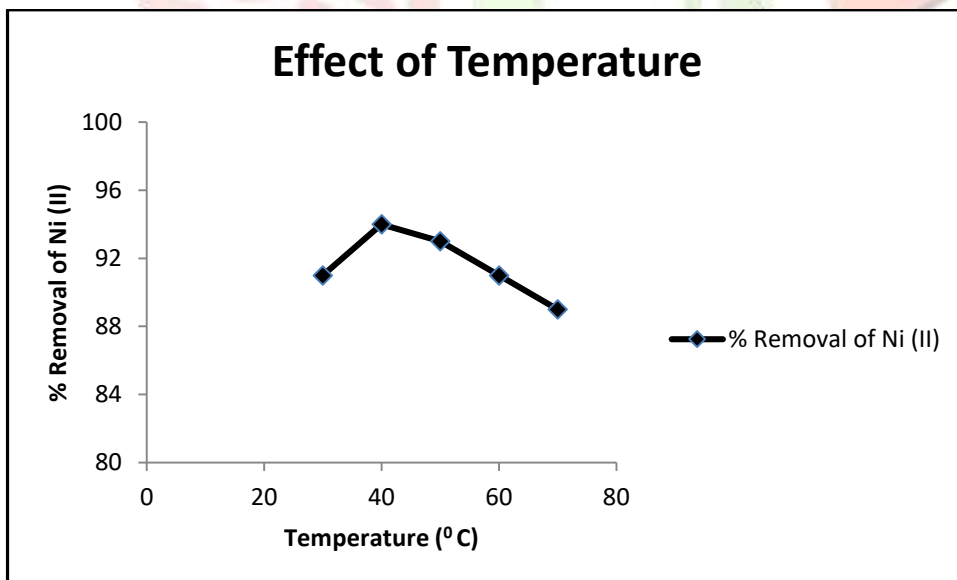
CONCLUSION

EPS represents an efficient and potential class of biosorbents for the removal of metal ions. This was an attempt to use biosorption for waste water treatment. In this study we otimize the the condition for maximum biosorption including optimization of ph , EPS Dose, Temperature, Metal Concentration. In this study Ni removal percentage is 86% . We also tries to focus on selectivity of biomass with

sufficient high biosorption capacity.In future studies analysing behavior of bioadsorbent EPS for use with real industrial effluents and simultaneously analyzing impact of of water quality will be studied.

REFERENCES

1] P. X. Sheng, Y. P. Ting, and J. P. Chen, "Biosorption of heavy metal ions (Pb, Cu, and Cd) from aqueous solutions by the Marine Alga Sargassum sp. in single- And multiple-metal systems," *Ind. Eng. Chem. Res.*, vol. 46, no. 8, pp. 2438–2444, 2007.

2] S. S. Ahluwalia and D. Goyal, "Microbial and plant derived biomass for removal of heavy metals from wastewater," *Bioresour. Technol.*, vol. 98, no. 12, pp. 2243–2257, 2007.

3] Z. Aksu and S. Tezer, "Biosorption of reactive dyes on the green alga Chlorella vulgaris," *Process Biochem.*, vol. 40, no. 3–4, pp. 1347–1361, 2005.

4] S. Amirnia, M. B. Ray, and A. Margaritis, "Heavy metals removal from aqueous solutions using Saccharomyces cerevisiae in a novel continuous bioreactor-biosorption system," *Chem. Eng. J.*, vol. 264, pp. 863–872, 2015.

5] A. J. M. Barros, S. Prasad, V. D. Leite, and A. G. Souza, "Biosorption of heavy metals in upflow sludge columns," *Bioresour. Technol.*, vol. 98, no. 7, pp. 1418–1425, 2007.

6] U. Farooq, J. A. Kozinski, M. A. Khan, and M. Athar, "Biosorption of heavy metal ions using wheat based biosorbents - A review of the recent literature," *Bioresour. Technol.*, vol. 101, no. 14, pp. 5043–5053, 2010.

7] A. Mittal, "Use of hen feathers as potential adsorbent for the removal of a hazardous dye, Brilliant Blue FCF, from wastewater," *J. Hazard. Mater.*, vol. 128, no. 2–3, pp. 233–239, 2006.

8] G. Bayramoglu and M. Y. Arica, "Preparation of a composite biosorbent using Scenedesmus quadricauda biomass and alginate/polyvinyl alcohol for removal of Cu(II) and Cd(II) ions: Isotherms, kinetics, and thermodynamic studies," *Water. Air. Soil Pollut.*, vol. 221, no. 1–4, pp. 391–403, 2011.

9] K. Vijayaraghavan and Y. S. Yun, "Bacterial biosorbents and biosorption," *Biotechnol. Adv.*, vol. 26, no. 3, pp. 266–291, 2008.

10] B. Volesky, "Detoxification of metal-bearing effluents: Biosorption for the next century," *Hydrometallurgy*, vol. 59, no. 2–3, pp. 203–216, 2001.

11] W. S. Wan Ngah and M. A. K. M. Hanafiah, "Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review," *Bioresour. Technol.*, vol. 99, no. 10, pp. 3935–3948, 2008.

12] Z. Zhang *et al.*, "A novel biosorbent for dye removal: Extracellular polymeric substance (EPS) of Proteus mirabilis TJ-1," *J. Hazard. Mater.*, vol. 163, no. 1, pp. 279–284, 2009.

13] A. Jang, S. M. Kim, S. Y. Kim, S. G. Lee, and I. S. Kim, "Effects of heavy metals (Cu, Pb, and Ni) on the compositions of EPS in biofilms," *Water Sci. Technol.*, vol. 43, no. 6, pp. 41–48, 2001.

14] D. Lakherwal, H. P. Singh, and V. K. Rattan, "Kinetic Studies of Adsorption Process of Chromium Ions From Aqueous Solution.," *Int. J. Adv. Res.*, vol. 4, no. 10, pp. 545–550, 2016.

15] K. Y. Show, D. J. Lee, J. H. Tay, T. M. Lee, and J. S. Chang, "Microalgal drying and cell disruption - Recent advances," *Bioresour. Technol.*, vol. 184, pp. 258–266, 2015.

16] E. Ç. Salihi, J. Wang, D. J. L. Coleman, and L. Šiller, "Enhanced removal of nickel(II) ions from aqueous solutions by SDS-functionalized graphene oxide," *Sep. Sci. Technol.*, vol. 51, no. 8, pp. 1317–1327, 2016.

17] W. Pranata Putra *et al.*, "Biosorption of Cu(II), Pb(II) and Zn(II) Ions from Aqueous Solutions Using Selected Waste Materials: Adsorption and Characterisation Studies," *J. Encapsulation Adsorpt. Sci.*, vol. 04, no. 01, pp. 25–35, 2014.

18] J. S. Al-Jariri and F. Khalili, "Adsorption of Zn(II), Pb(II), Cr(III) and Mn(II) from water by Jordanian bentonite," *Desalin. Water Treat.*, vol. 21, no. 1–3, pp. 308–322, 2010.

19] K. Vijayalakshmi *et al.*, "Batch adsorption and desorption studies on the removal of lead (II) from aqueous solution using nanochitosan/sodium alginate/microcrystalline cellulose beads," *Int. J. Biol. Macromol.*, vol. 104, no. Ii, pp. 1483–1494, 2017.

20] D. C. N and S. S, "Biosorption of Lead (II) from aqueous solution and Industrial Effluent by using leaves of Araucaria cookii: Application of Response Surface Methodology," *IOSR J. Environ. Sci. Toxicol. Food Technol.*, vol. 8, no. 7, pp. 67–79, 2014.

21] P. Zhang, W. Ding, Y. Zhang, K. Dai, and W. Liu, "Heavy metal ions removal from water using modified zeolite," vol. 6, no. 11, pp. 507–514, 2014.

22] M. Doula, A. Ioannou, and A. Dimirkou, "Thermodynamics of potassium exchange in calcium bentonite (ca-b)," *Commun. Soil Sci. Plant Anal.*, vol. 26, no. 9–10, pp. 1535–1545, 1995.

23] A. Özer and D. Özer, "Comparative study of the biosorption of Pb(II), Ni(II) and Cr(VI) ions onto S. cerevisiae: Determination of biosorption heats," *J. Hazard. Mater.*, vol. 100, no. 1–3, pp. 219–229, 2003.

24] Z. Aksu and T. Kutsal, "A bioseparation process for removing lead(II) ions from waste water by using C. vulgaris," *J. Chem. Technol. Biotechnol.*, vol. 52, no. 1, pp. 109–118, 1991.