

BIOSORPTION OF HEAVY METAL USING MICROALGAE AND CHITOSAN

Project work

Submitted in partial fulfilment of the requirements for the award of
bachelor of science in biochemistry

by

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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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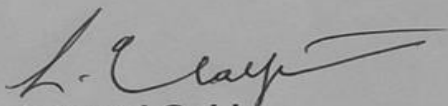
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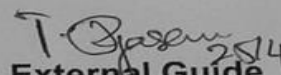
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This is to certify that this Project Report is the bonafide work of **Deepika. C** (41070003). Who carried out the project entitled "**BIOSORPTION HEAVY METAL USING MICROALGAE AND CHITOSAN**" under our supervision from January 2024 to April 2024.


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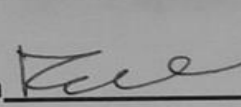
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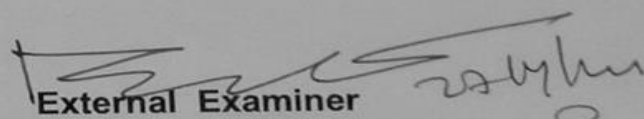
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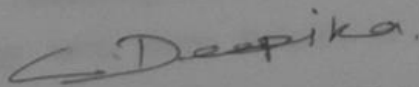
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DECLARATION

I, **C. Deepika** hereby declare that the Project report entitled "**BIOSORPTION OF HEAVY METAL USING MICROALGAE AND CHITOSAN**" done by me under the internal guidance of **Dr. L. Inabathamizh** professor, Department of Biotechnology and External guide **Dr.T. Rajasekar** Assistant professor (Research), Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in B.Sc. Biochemistry.

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ABSTRACT

Metals from industrial and agricultural sectors often end up contaminating surface and subsurface water. The trend to use chitosan, microalgae immobilized alginate beads for removal of metals has been observed in recent years. Current work focuses on development of immobilized materials for the remediation of metals. In these present studies the comparison of efficacy of 3 types of Na-alginate beads namely CAB (Chitosan alginate beads), CCAB (*Chlorella* sp chitosan alginate beads), PCAB (*Picochlorum* sp chitosan alginate beads) are tested for the removal of the following targeted metal Mercury (Hg) The study was performed at initial concentration of 25, 20, 75, 100, 125 mg L⁻¹ for a contact period of 24hrs and at constant adsorbent dose of 5 g mL⁻¹. The treated solution was analyzed in AAS (Atomic Adsorption Spectroscopy) The removal efficiency of beads followed a trend of as CCAB (66%) > PCAB (64%) > CAB (61%). The study shows *Chlorella* chitosan Na alginate bead as the most effective Hg metal removal.

Key word: Heavy Metal, Mercury, Chitosan, Microalgae, Biosorption, Immobilization, Bioremediation, Entrapment, Alginate.

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LIST OF ABBREVIATIONS

Hg	Mercury
CAB	Chitosan-alginate beads
CCAB	<i>Chlorella</i> -chitosan-alginate beads
PCAB	<i>Picochlorum</i> -chitosan-alginate beads
Ci	Initial concentration of metal ions
Cf	Final concentration of metal ions
KNO ₃	Potassium nitrate
NaH ₂ PO ₂ H ₂ O	Sodium hypophosphite
EDTA	Ethylenediaminetetraacetic acid
H ₃ BO ₃	Boric acid
Mncl ₂ .4H ₂ O	Manganese (II) Chloride
Fecl ₃ .6 H ₂ O	Ferric chloride hexahydrate.
Zncl ₂	Zinc Chloride
Cocl ₂ .6 H ₂ O	<i>Cobalt chloride hexahydrate</i>
NH ₄ .6mo ₇ o ₂ 4H ₂ O	Ammonium heptamolybdate tetrahydrate
Cuso ₄ .5 H ₂ O	Copper sulphate pentahydrate
Conc. Hcl	Hydro chloric acid
Vitamin B12	cobalamin
Vitamin B1	Thiamine

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CHAPTER 1

INTRODUCTION

Environmental contamination is a widespread issue that affects both industrialized and developing nations like India. According Indian waste management Report in India 4.3 million tons of hazardous waste are generated annually. Heavy metal is the main hazardous waste. Heavy metal like arsenic, cadmium, chromium, lead, mercury is the main hazardous waste Ecosystems, human health, and biodiversity are all impacted by environmental pollution. "Pollution of physical and biological components of the earth/atmosphere system in such a way" is the definition of environmental pollution. a manner in which typical environmental processes deteriorate. The rising density of human populations and human activities that have deteriorated the earth's surface through the abuse of environmental resources and poor disposal of trash are also strongly linked to the incidence of environmental pollution.

Conventional methods and instruments are insufficient to address the pollution issue. It is necessary to provide a thorough explanation of biotechnology treatments and their potential to increase bioremediation capability. Numerous environmental pollutants are being released as a result of rapid urbanization and industrialization. Because metal contamination is hazardous and tends to bioaccumulate throughout the food chain, it requires extra attention. Among the many drawbacks of conventional methods for extracting valuable metals, radionuclides, and toxic metals from wastewater are the risk of developing hazardous byproducts, high costs, and incomplete metal extraction.

Velkova *et al.*, (2018) There are numerous physical, chemical, and biological techniques for removing metal. Numerous techniques, including chemical precipitation, ion exchange, membrane filtration, flotation, coagulation-flocculation, and electrochemical, can be used to remove heavy metals from wastewater. methods such as electrocoagulation, electrofloatation, and electrodeposition Wang *et al.*, (2011) These systems' drawbacks, meanwhile, include expensive operating and maintenance expenses and secondary contamination from dangerous sludge production. Most heavy metal removal procedures are costly and ineffective at low concentrations (less than 100 mg/L) environmental-friendliness and cost-efficiency at low heavy metals concentration,

Leong *et al.*,(2020) The use of various microorganisms, such as bacteria, microalgae, yeasts, and fungi, in bioremediation of heavy metals has become popular as an alternative to traditional biological processes, like biosorption, bio flocculation, artificial wetlands, etc., which remove metals through adsorption, accumulation, and enrichment mechanisms.

Biosorption is a complex process that occurs through a range of mechanisms, such as ion exchange, surface complexation, adsorption, absorption, and precipitation. Fomina, *et al.*, (2014). Biosorption is a subfield of biotechnology that deals with the removal or recovery of both organic and inorganic substances from solutions using biological material can include seaweed, plant materials, dead or living microorganisms and their parts, natural residues, industrial and agricultural wastes, and seaweeds. Al-Hasawi *et al.*, (2020) cell immobilization techniques, metabolic processes with lower sensitivity to senescence and significant stability over time, were inspired by the attachment of living microorganisms to one another and to solid surfaces Tchounwou, *et al.*, (2020) Biosorption is a cost-effective and environmentally friendly alternative to traditional wastewater treatment processes. Biosorption is a metabolically independent process in which dead microbial biomass can remove and concentrate metal ions from aqueous solutions This research is done by biosorption method using immobilized chitosan and microalgae

1.2 HEAVY METAL TOXICITY

Heavy metals are naturally occurring elements having high atomic weights and densities that are at least five times higher than those of fluids. Because of their many industrial, household, agricultural, medical, and technical applications, they are widely distributed in the environment, which has raised concerns about potential negative effects on the environment and public health. The chemical species, dosage, and route of exposure, as well as the age, gender, genetics, and nutritional status of the exposed individuals, all affect how hazardous they are. Due to their high levels of toxicity, heavy metals such as arsenic, cadmium, chromium, lead, and mercury are important for public health. These metallic elements are known to induce multiple organ damage even at lower concentrations. They fall within the category of systemic toxicants. Tchounwou *et al.*,

(2012) Prolonged exposure to heavy metals can result in a number of illnesses, including cancer, Parkinson's disease, Alzheimer's disease, multiple sclerosis, and muscular dystrophy. Wang *et al.*, (2001) The most dangerous metalloids and heavy metals found in the environment include arsenic (As), chromium (Cr), copper (Cu), chromium (Cr), mercury (Hg), zinc (Zn), nickel (Ni), and chromium (Cr). Animals and humans are significantly impacted by the trophic transmission of these components in terrestrial and marine food webs and chains. Verma., *et al.*, (2023).

1.3 MERCURY (Hg) TOXICITY:

There are three different types of mercury (Hg) that can be found in soil, water, and air: elemental or mineral mercury (Hg^0), mercury that is inorganic (Hg^+ , Hg^{2+}), and organic mercury (methyl or ethyl mercury). Mercury is a common element in switches, lamps, gauges, batteries, and thermometers. Mercury waste amounting to 21,66 kg annually has been seen in Mexico in the form of thermometers. Fluorescent lights are another significant source of mercury leakage. When mercury in these lamps is released, it is in the vapor phase and can reach dangerous room temperatures (Pavithra *et al.*, 2023). Humans are primarily in contact with mercury in three physical forms: methylmercury compounds, mercury vapor, and Hg^0 . In terms of “the most dangerous elements or compounds on Earth, mercury” is listed third by the US Government Agency for dangerous compounds and Disease Registry, after arsenic and lead, which are still being poured into our soil and streams, ingested in our food, and released into our environment. (Rice *et al.*, 2023). It is believed that mercury buildup in the heart is a factor in cardiomyopathy.

1.3 CHITOSAN

The biopolymer is chitosan. A type of linear polysaccharides known as chitosan is composed of varying amounts of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine, GlcNAc) and 2-amino-2-deoxy-D-glucose (glucosamine, GlcN) linked residues. Using an alkaline material like sodium hydroxide, it is extracted from the hard outer skeleton of shellfish, crab, lobster, and shrimp (Fig. 1.1). Aranaz, *et al.*, (2021) Chitin is mostly taken from crustaceans, such shrimp and crabs. It is also present in the exoskeleton of arthropods and as organized crystalline microfibrils in the cell walls of yeasts and fungi.

chitosan produced by deacetylating chitin, the parent polymer. The second most common naturally occurring biopolymer, chitosan has good nontoxic, antibacterial, biocompatible, and biodegradable qualities. Chitosan has been extensively researched for its potential use in the field of wastewater treatment as an excellent chelating agent, biosorbent, and support for other nanomaterials. Recent studies have focused on creating nanocomposite materials using chitosan. Its numerous biological applications include antimicrobial, wound healing, antioxidant, food packaging, anticancer, and biodegradable qualities, all of which have been demonstrated to be beneficial. Not to mention, its low cost. There is a significant amount of hydroxyl and Amino groups can be employed as sorbents to remove heavy metals from wastewater. via ion binding with heavy metals. The use of chitosan in waste water treatment is restricted by a number of factors, including limited mechanical strength, low thermal stability, and sensitivity to pH. Therefore, chitosan can be modified to increase its use. A few areas of interest that have been identified are the modification of polymers to increase their applicability, comprehending the mechanisms underlying the biological activity of chitosan, chitosan derivatives, and chitooligosaccharides, and conducting thorough research on the chitosanolytic and chitinolytic enzymes found in various microorganisms. Kaczmarek *et al.*, (2019) How where Worldwide interest in chitosan is growing due to its industrial applications like sewage disposal, agricultural products, culinary additives, cosmetics, biomedical materials, antimicrobials, separators, and so on. Begum *et al.*, (2021)

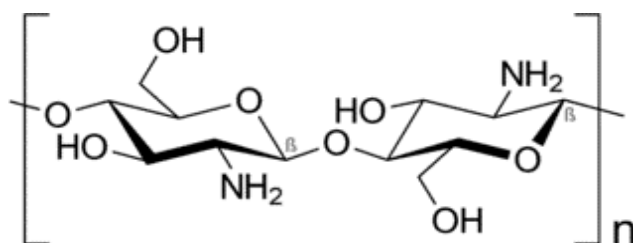


Fig 1.1 chemical structure of chitosan

1.4 MICROALGAE

Microalgae are a diverse group of unicellular and simple multicellular photosynthetic microorganisms that are present in all existing ecosystems on Earth Spain. *et al.*, (2021). recently researchers' attention has been drawn to the topic of microalgae culture because of these plants' capacity to synthesis a wide range of biologically active chemicals, develop biomass quickly, and modify their biochemical makeup in response to particular cultivation conditions. One eukaryotic cell makes up microalgae, which can be found in freshwater or the ocean. Seaweeds have been examined more than microalgae. but they have benefits such as quick development, high photosynthetic efficiency, and the ability to be grown in production settings. Furthermore, the diversity the diversity of microalgae will increase the number of sources of physiologically active components such polysaccharides, proteins, lipids, and pigments. Dolganyuk, *et al.*, (2021) Algae can absorb harmful heavy metals (HMs) from the surrounding water, their concentrations are higher than those in the surrounding water. algae cell wall plays important role in metal biding Given that the microalgal cell wall in particular acts as the initial barrier to the uptake of metal ions, interaction with the cell walls or membranes has been identified as the main route of contact with heavy metals (HM). Kumar *et al.*, (2015) A variety of distinct self-defense mechanisms, including heavy metal immobilization, gene regulation, exclusion, and chelation, as well as antioxidants and reducing enzymes that lower heavy metals through redox processes, are employed by microalgae species to protect themselves against the toxicity of heavy metals.

Priyadarshini *et al.*, (2019) More specifically, the following are the mechanisms by which microorganisms extract metals from solutions: (i) intracellular buildup; (ii) cell-surface sorption or complexation; (iii) extracellular accumulation/precipitation, which can be aided by the use of viable microorganisms; and (iv) additional cellular accumulation. Chugh *et al.*, (2022) Microalgae are oxygen-evolving organisms that absorb large amounts of nitrogen, phosphate, and carbon from wastewater. They may also flourish under a range of climatic and environmental conditions, providing an extra wastewater treatment choice.

Algal remediation of wastewater is economical and environmentally good method of treating wastewater. The use of phycoremediation in wastewater treatment can increase

dissolved oxygen availability and encourage the removal of harmful contaminants and nutrients. Microorganisms, commonly referred to as microalgae, are recognized as "biological purifiers" due to their inherent ability to collect and accumulate nutrients from wastewater while simultaneously sorbing numerous hazardous and persistent contaminants. Additionally, readily able Microalgae assist in the capture of carbon dioxide from the atmosphere, assisting in wastewater treatment and the production of biodiesel. By "sorption," microalgae lessen the HM pollution. both freshwater and marine Microalgae's bioaccumulation phenomena make them highly effective at removing heavy metals (HMs) from contaminated environments. Singh (2021) In addition to eliminating phosphates, nitrates, ammonium, and other organic and inorganic contaminants found in wastewater, microalgae have the ability to absorb and break down long-lasting compounds like heavy metals. In general, biological agents like as bacteria, algae, and fungi are employed in bioremediation to reduce the levels of heavy metals. Compared to fungus and bacteria, the figures show that algae are able to remediate between 15.3 and 84.6% of heavy metals.

1.5 IMMOBILIZATION TECHNIQUE

Since biosorbent lacks sufficient strong structure, immobilization is crucial for its implementation in actual wastewater treatment processes. Consequently, the effective immobilization technique of biomass has been the main focus of recent research on heavy metal biosorption. Many immobilization techniques, such as using polysaccharides or microbes, have been proposed for applications to the biosorbent in order to increase the mechanical strength of freely biosorbent. There are numerous techniques for immobilization, including adsorption, covalent binding, trapping, encapsulation, and crosslinking. Jeon (2011) publications on cell immobilization that date back to 1980 demonstrate the public's interest in this subject.

Opportunities are created by in a range of fields, immobilized microbiological cells, such as environmental pollution prevention. Cell immobilization has numerous benefits over suspended microbe technology, including resistance to harmful substances. The potential of immobilized microbe cells to treat toxic pollutants in industrial wastewater; the concepts, history, and advantages of immobilized cells over suspended cells; the characteristics of support materials; and the principal methods of immobilization, with an

emphasis on cell adsorption for natural immobilization Martins *et al.*, (2013) The final biosorbent particles' mechanical strength and chemical resistance are determined by the carrier and immobilization technique chosen for the biosorption of metal ions from natural and waste water. These factors are crucial for the successful sorption-desorption cycle.

CHAPTER 2

LITRATURE SURVEY

Ayyamperumal *et al.*, (2023) the pervasive presence of heavy metals in various environmental settings and their potential impact on human health and ecosystems. emphasize the widespread distribution of heavy metals due to their industrial, residential, agricultural, and other applications, raising concerns about their toxicity. Highly hazardous are toxic metals including arsenic, cadmium, chromium, lead, and mercury.

Zamora-Ledezma *et al.*, (2021) The review addresses water pollution as a pressing global concern, highlighting the imperative to enhance water quality and mitigate adverse impacts on human and ecosystem health. Industrialization, urbanization, and climate change are identified as key contributors to the proliferation of water pollutants, particularly heavy metals. The paper discusses recent findings concerning heavy metal release, associated environmental and health risks, and available removal technologies and materials. Anthropogenic activities are underscored as the primary source of escalating heavy metal contamination in aquatic ecosystems. Health hazards stemming from prolonged exposure to trace amounts of heavy metals like lead, cadmium, mercury, and arsenic are outlined. Additionally, the review provides insights into various techniques for heavy metal detection and factors influencing contaminant removal. Conventional and non-conventional methods for heavy metal removal are critically evaluated, with a focus on adsorption, nanostructured materials, and plant-mediated remediation, including an overview of commercial products utilized for heavy metal elimination from water. Lastly, the paper emphasizes the imperative for developing efficient heavy metal removal methods, highlighting the potential of nanotechnologies and discussing associated requirements and opportunities.

Raman *et al.*, (2024) discusses the heavy metal pollution in Veeranam Lake, Tamil Nadu, with elevated concentrations found in water bird tissues and organs. The pollution, attributed to industrial effluent and sewage discharge, affects both agricultural lands and drinking water sources, including supply to Chennai.

Sundaram *et al.*, (2020) highlights heavy metal contamination in Korattur topsoil, influenced by nearby industrial activities and urbanization along Tamil Nadu's East Coast. Singh *et al.*, (2001) provides insight into heavy metal concentrations in Yamuna River sediments in Delhi and Agra, emphasizing the significant anthropogenic contribution to metal enrichment, particularly for metals like chromium, copper, zinc, lead, and cadmium.

The field of biosorption is receiving more and more interest in study. The use of biosorption in the recovery of heavy metals has advanced significantly over the past 30 years, but the number of publications devoted to the research of various biomasses has only recently increased. The highest number of biosorption research was recorded by 2021. The most research has focused on bacterial biosorption of heavy metals. Javanbakht *et al.*, (2014) Examines the main biosorption mechanisms and the majority of the functional groups that are involved. Various processes are involved in the biosorption process: complexation, ion exchange, transport across cell membrane, precipitation, and physical adsorption. Finding the functional groups that are in charge of metal binding is crucial to comprehending how metals bind to biomass. Cell walls have been used to characterize the majority of these categories. Metal adsorption is caused by a range of functional sites found in the biosorbent, such as the carboxyl group, imidazole, sulfhydryl, amino, phosphate, sulfate, thioether, phenol, carbonyl, amide, and hydroxyl moieties. These could be useful in improving biosorbents by exchanging functional groups or altering surface reactive sites by surface grafting.

Barquilha *et al.*, (2019) they have used seaweed *Sargassum* sp alginate-based biosorbent for biosorption of Ni^{2+} and Cu^{2+} ions from synthetic solutions and as the result The combination *Sargassum* sp and alginate shows high removal efficiency. So, we can conclude that the combination of species and polymer can show high removal efficiency. Fawzy *et al.*, (2022) the efficiency of *Codium vermilara* for copper ions removal from aqueous solution was studied. Akar *et al.*, (2009) they have combined montmorillonite type clay and the white rot fungus *Trametes versicolor* and investigated for its capacity to eliminate copper (II) ions from solutions tainted with copper. They come to the conclusion that immobilized fungal biomass was a useful tool for continually removing copper ions from real wastewater. These findings suggested that *T. versicolor* immobilized cells in

montmorillonite were a viable substitute for effectively eliminating copper (II) ions from both artificial solutions and actual wastewater.

Bai, J., *et al.*, (2012) they have studied adsorption of uranium from an aqueous solution onto the magnetically modified yeast cell *Rhodotorula glutinis*. initial uranium concentration, and other common cations. Studies of uranium sorption isotherm, kinetics, and thermodynamics on magnetically modified *R. glutinis* were also conducted. According to all of the findings, *R. glutinis* that has been magnetically manipulated may be used as a sorbent to remediate wastewater containing uranium.

Chen *et al.*, (2023) This review aims to investigate the best practice for immobilizing algae for heavy metal bioremediation and to do a thorough analysis of the sustainability of immobilized algae technology Immobilization technology is a green technique that uses a carrier to immobilize algae in order to increase the stability and adsorption effectiveness of the biosorbent. Improving the immobilized algae's adsorption performance was identified through the examination of the parameters influencing the biosorbent's efficiency and the mechanism by which immobilized algae remove heavy metals. Additionally, the life cycle analysis and economic analysis method was applied. This offers a conceptual framework for the real-world implementation of algae bioremediation

Peng *et al.*, (2010) As a result of this research, chitosan-coated magnetic nanoparticles (SICCM), a new magnetic adsorbent, were used to adsorb Cu(II) from a water-based solution utilizing immobilized *Saccharomyces cerevisiae*. When the starting concentration of Cu(II) was 60 mg, the greatest removal efficiency of 96.8% was attained.

Tan *et al.*, (2012) studied Cu(II) removal efficiency from alginate-immobilized *Trichoderma asperellum* in alive and dead forms was analyzed as a function of pH, time, and initial Cu(II) concentrations. The best technique for biosorbing Cu(II) was to use *T. asperellum* cells that had been immobilized and heat-inactivated.

Lieswito *et al.*, (2019) utilizing mixed-microalgae comprising *Chlorella sorokiniana*, *Monoraphidium* sp., and *Scenedesmus obliquus* bound in Na-Alginate, the heavy metal Copper (Cu²⁺) was removed utilizing biotechnology. The study demonstrated the high efficacy of immobilized biosorbent in lowering Cu²⁺ in wastewater, making it a viable and

environmentally responsible substitute for traditional methods of managing heavy metal contamination in water.

Aksu *et al.*, (1990) In a stirred batch reactor, the kinetics of the biosorption of Cu(II), Zn(II), Fe(II), Cr(VI), and Pb(II) ions found in waste water to the green alga *Chlorella vulgaris* were studied. The ideal adsorption temperatures and starting pH values for each of the aforementioned metal ions were found to be 25°C and 55°C in adsorption experiments. 25-35°C and 35-50°C. 55°C and pH ranges of 4.0-4.5, 1.0-2.0, 1.5-2.0, and 5.0, in that order. For the majority of the metal ions, the maximum adsorption rates were obtained at initial concentrations of 200 ppm. Freundlich adsorption isotherms were constructed at 25°C, from which corresponding Freundlich adsorption constants and adsorption degrees were determined.

Waluyo *et al.*, (2020) marine microalgae *Nannochloropsis oculata* to evaluate the bioremediation capacity of lead. Five different types of water samples with lead concentrations ranging from 0.5, 0.7, 0.9, 1.1, and 1.3 ppm were used in this comparison study. By figuring out the % efficiency of Pb absorption by *N. oculata*, data were evaluated. The findings demonstrated that, after seven days of culture, *N. oculata*'s effectiveness in absorbing lead increased with the amount of lead dissolved in the water. Therefore, it can be said that *N. oculata* has the potential to be a bioremediation agent for lead contamination in waterways.

Kwarciak-Kozłowski *et al.*, (2021) investigate in free solution with immobilized calcium alginate Using blank alginate beads and *Chlorella vulgaris* cells, Pb(II) was extracted from municipal wastewater. These results showed that immobilized algal cells in alginate beads, as opposed to freely dispersed cells, may enhance the biosorption of metal ions under investigation. Additionally, under ideal circumstances, the biosorbent has significantly lowered the lead ions in municipal wastewater. Alginate beads with immobilized algae were less effective than free cells. Consistently, high ion removal was achieved.

Rahman *et al.*, (2023) In order to effectively remove harmful heavy metals from contaminated water, this paper proposes a chitin and chitosan adsorbent based on shrimp. that uses a number of processes, acetone washing, deacetylation, acid washing,

and alkaline pretreatment. Examples of this include the adsorption of cobalt (47.82%), nickel (74.50%), arsenic (98.50%), and chromium (97.40%) by chitosan. This method is inexpensive and safe for the environment. Utilizing chitin and chitosan derived from shrimp as a biosorbent could be crucial in mitigating pollution brought on by human activity while also yielding substantial financial gains. In order to improve the use of in order to fully understand the nature of the adsorbent, MSS in the biological remediation of heavy metals from contaminated water may need to investigate the durability and versatility of chitosan.

Mustafa *et al.*, (2021) examined attentively how different strategies are used to remediate various sorts of pollutants that are frequently found in the environment using microalgae. It has been noted Microalgae have the ability to efficiently eliminate pollutants from a variety of sources, such as residential wastewater, agricultural runoff, leather, textile, pharmaceutical, and electroplating industries. In addition, microalgae may utilize the micronutrients in wastewater and attenuate CO₂ during their growth phase.

Cheng *et al.*, (2021) have done adsorption studies on lithium using fabricated chitosan (CS) nanofiber membrane as the results showed that S-CE could adsorb up to 297 mg g⁻¹ of Li⁺ from a high-concentration solution that included 1000 mg L⁻¹ at pH 7.0. Despite the presence of other competing metal ions in the mixed solution, CS-CE maintained a considerable affinity for Li⁺. Furthermore, the highest adsorption capacity of CS-CE for Li⁺ reduced by just 8.8% after five cycle studies, demonstrating the good reusability of CS-CE. Overall findings indicated that CS-CE may be a viable option for Li⁺ recovery.

Sharifi *et al.*, (2023) In this research work they have been combined chitosan with magnetic nanocomposite. Using the coprecipitation approach, chitosan-magnetite nanocomposite (CMNC) was created to extract cobalt and nickel from aqueous solutions. As the results Chitosan-magnetite nanocomposite (CMNC) has been heavy metal adsorption trials indicate that CMNC has high adsorption capabilities of 30.03 mg/g for Ni²⁺ and 53.19 mg/g for Co²⁺ at ideal conditions (pH = 6, contact time = 2 h, adsorbent dosage = 2 g/l). Additionally, the adsorption data had the best fit.

Shao, Z., *et al.*, (2023) The properties of the novel absorbents made using aqueous cast sulfated chitosan/polyvinyl alcohol membranes (SCS/PVA) for the removal of Cu^{2+} and Ni^{2+} ions from aqueous solutions were investigated. Freundlich and Langmuir absorption isotherms were fitted to the experimental data, and a pseudo-second order rate equation was used to describe the uptake kinetics for different concentrations of copper and nickel ions. The results indicate that a SCS/PVA membrane exhibited a higher affinity for Cu^{2+} ions in comparison to Ni^{2+} ions.

Shao *et al.*, (2023) It describes the creation of chitosan-tartaric acid biosorbents (CTBs) to lessen the presence of heavy metals in water and tea drinks, particularly $\text{Cu}(\text{II})$ and $\text{Cd}(\text{II})$. The study focuses on the qualities of CTBs, such as acid resistance, mechanical strength, and surface roughness, as well as their manufacturing process. It also addresses the mechanism and adsorption performance of CTBs, showing that during adsorption they follow the pseudo-second-order kinetic model and the Langmuir isotherm model. According to the study's findings, CTBs removed $\text{Cu}(\text{II})$ and $\text{Cd}(\text{II})$ from water and tea beverages up to 90% of the time with little to no effect on the beverages' quality. All things considered; the study provides fresh perspectives for lowering heavy metal contamination in drinks.

Ivánová *et al.*, (2010) The behavior of gel and dry beads that swell in different acid-base aqueous solutions is discussed in the article. Liquid or dried calcium alginate beads swelled in all basic and acidic solutions; the only exception was very acidic solutions, when the gel beads tended to shrink. Dry beads showed the highest stability since they inflated the least in diluted acidic solutions. Concentrated solutions including sodium and potassium hydroxides completely dissolved both dry and gel beads. The possibility of using immobilized algal biomass in Ca-alginate beads to extract copper ions from aqueous solutions was also investigated. The results of the kinetic trials indicate that gel-immobilized beads are the best alternative for the sorption of copper ions.

Jeon (2011) Since biosorbent lacks sufficient strong structure, immobilization is crucial for its implementation in actual wastewater treatment processes. Consequently, the effective immobilization technique of biomass has been the main focus of recent research on heavy metal biosorption. Many immobilization techniques, such as using

polysaccharides or microbes, have been proposed for applications to the biosorbent in order to increase the mechanical strength of freely biosorbent. Several immobilization techniques, including This study will discuss the following processes: absorption, covalent binding, entrapment, encapsulation, and crosslinking.

Bishnoi *et al.*, (2007) examined the ability of calcium alginate beads without cells and immobilized *Trichoderma viride* biomass for Cr (VI) biosorption. It contrasted the biosorption effectiveness of calcium alginate beads without cells with powdered fungal biomass encapsulated in calcium alginate. The effects of pH, starting metal ion concentration, and removal using calcium alginate beads with and without cells were also investigated.

Chai *et al.*, (2021) highlights the persistent global challenge of wastewater treatment, particularly concerning the removal of heavy metals to safeguard human health. Adsorption emerges as a favored technique due to its adaptability, operational ease, and cost-effectiveness. While for a long time, activated carbon has been the go-to adsorbent for removing heavy metals. challenges such as separation difficulties and high costs hinder its widespread adoption. The paper discusses the emergence of novel materials with competitive advantages, including wide surface area, chemical inactivity, and strength offering promising alternatives for heavy metal ion removal in uses for sewage treatment applications.

Kumar *et al.*, (2015) This comprehensive review underscores the burgeoning interest in microalgal biotechnology as a solution to contemporary environmental challenges. Leveraging inexpensive growth requisites such as solar light and CO₂, microalgae offer a multifaceted approach to eco-friendly technologies, encompassing reducing carbon emissions, providing biofuel, and bioremediation. Microalgae show potential in wastewater bioremediation due to their extensive range of external and internal mechanisms to counteract heavy metal toxicity. Their capacity to multiply and collect heavy metals exceeds that of traditional physicochemical techniques, thus a critical assessment and the creation of economically feasible technologies using interdisciplinary approaches are necessary. In addition to emphasizing well researched metal-microalgae interactions and clarifying the processes of heavy metal treatment within microalgae, the

review offers a thorough assessment of heavy metal remediation from a microalgal perspective.

de Rome *et al.*, (1991) The most effective biological immobilization carrier is alginate. with calcium chloride as crosslinking agent because of its easy the formation of gelatin and strong biological compatibility. sodium alginate is better choice compare to calcium alginate because the exchange of ions mechanism, calcium alginate might expand and dissolve. But major drawback with natural polymeric carrier is diffusion restrictions

Dineshkumar *et al.*, (2024) uses a batch model technique to study the marine microalgae *D. salina*'s capacity for biosorption of lead (Pb). Under ideal outdoor conditions, *D. salina* was isolated, identified, and mass-cultivated. There were experiments carried out in batches. According to the study's findings, *D. salina* is an affordable biosorbent for removing metal from industrial effluents because it has a large number of functional groups that can bind to metal.

Ritu *et al.*, (2024) highlighted the escalating concern of water pollution, particularly due to heavy metals (HMs), stemming from population growth, unplanned urbanization, industrialization, and intensive farming methods. HMs pose significant risks to human health and aquatic ecosystems that result in bioaccumulation and biomagnification The emerging application of microalgae for HM remediation. provides an economical, sustainable, and effective substitute for traditional techniques. Recent developments include improving algal populations and immobilizing algal cells and utilization of microalgal biofilms, along with genetic engineering advancements, have shown promise in augmenting HM uptake by microalgae. This chapter aims to elucidate the sustainable use of microalgae for HM remediation in aquatic ecosystems, exploring mechanisms and recent advancements in phycoremediation for ecosystem restoration in an efficient and eco-friendly manner.

Ordóñez *et al.*, (2023) conducted a thorough evaluation on the metals' biosorption by algae, highlighting the prominence of cadmium, copper, and lead. Cadmium, being the most studied metal with approximately 23% of publications, is primarily investigated due to its environmental toxicity and the imperative need for its removal. This underscores the viability of biotechnological approaches over traditional methods. Following cadmium,

copper (20%) and lead (19%) are also extensively studied, attributed to their widespread dispersion through mining and industrial activities. In contrast, metals like iron (3%) receive comparatively less attention, as their removal via precipitation methods with neutralizing agents is deemed effective and economical, hence lacking sustained motivation for further investigation.

Oehmen et al., (2014) An ion exchange membrane bioreactor (IEMB) was created by adding a cation exchange membrane and a bioreactor containing Hg volatilizing bacteria to treat low concentrations of mercury from drinking water and highly polluted industrial water.

Kusuma, et al., (2024) Complex methods need to be used to remove heavy metals (HMs) like mercury (Hg), which is commonly found in wastewater. Microalgae have gained recognition as a potential biorefinery and bioremediation method for wastewater. Recent research has revealed that using algae as a significant alternative to current wastewater treatment technologies can be achieved. Because microalgae have the advantage of significantly lowering the nutrient level, they have lately been used in wastewater treatment.

Allouche et al., (2021) Currently, a simple synthesis procedure that uses 3% chitosan and 1% *Ulva lactuca* has been suggested to create the hybrid bead needed to extract Hg (II) ions from aqueous solution. According to experimental results, the mass ratio of chitosan to *Ulva lactuca* in a composite bead may significantly influence how effectively mercury is removed.

Huang et al., (2019) Hg(II) immobilization approach: initially, algal cells are cultivated in high-phosphate cultures to modify their surface. Next, P-rich biomass is obtained and used as an adsorbent for increased Hg(II) removal. Finally, the Hg-loaded biomass is charred to prevent phosphate leaching and to immobilize Hg(II).

Goci, M et al., (2021) proposes to evaluate the materials utilized in chitosan-based polymer nanocomposite materials for environmental remediation of mercury pollution because of their multifunctional capabilities, which are favorable for the adsorption of different types of pollutants through wastewater and the atmosphere. These chitosan-

based polymer nanocomposites are also composed of non-toxic, highly porous, biodegradable, biocompatible, and recyclable materials; they are also free of metals, have a high concentration of surface-active sites, are abundant in earth, and have few surface flaws. developments in chitosan modification

CHAPTER 3

AIM AND OBJECTIVES

1. AIM

The aim of this study is to investigate the potential of utilizing chitosan and microalgae biosorbents for the efficient removal of heavy metal mercury from contaminated aqueous solutions. This research aims to explore the biosorption capacity to develop a sustainable and effective method for mercury remediation.

2. OBJECTIVES

- Investigation of the biosorption mechanism of mercury onto chitosan and microalgae biosorbents
- Comparative analysis of the performance of chitosan and microalgae biosorbents with conventional methods for mercury removal.
- Immobilization of chitosan and microalgae for effective removal of mercury heavy metal

CHAPTER 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION AND MASS CULTIVATION OF MICROALGAE:

For this study we have taken commercially available chitosan and microalgae (*Chlorella* and *Picochlorum* sp) bought from Bharathidasan university and for mass cultivation of microalgae. 10ml of mother culture of microalgae (*Chlorella* sp, *Picochlorum* sp) were inoculated separately in two conical flasks containing 250ml of autoclaved sea water and 62.5µl Conway medium for nutrient supply. The culture was maintained under laboratory condition for 15 days. Fig 4.1 For bulk cultivation now grown 250ml of both microalgae cultures were inoculated in two separate tank contain 1L of filtered autoclaved sea water and 250µl of Conway medium. Fig 4.2 (2) This was maintained under airconditioned room at temperature 25°C with aeration pump, light was fixed in tank for faster growth. The incubate condition of microalgae is 12:12 cycle (light: dark) this was maintained for 30days. One liter of seawater with 250µl Conway medium was added to the culture at every 7days intervals. After 30days the culture of microalgae were checked under microscope.

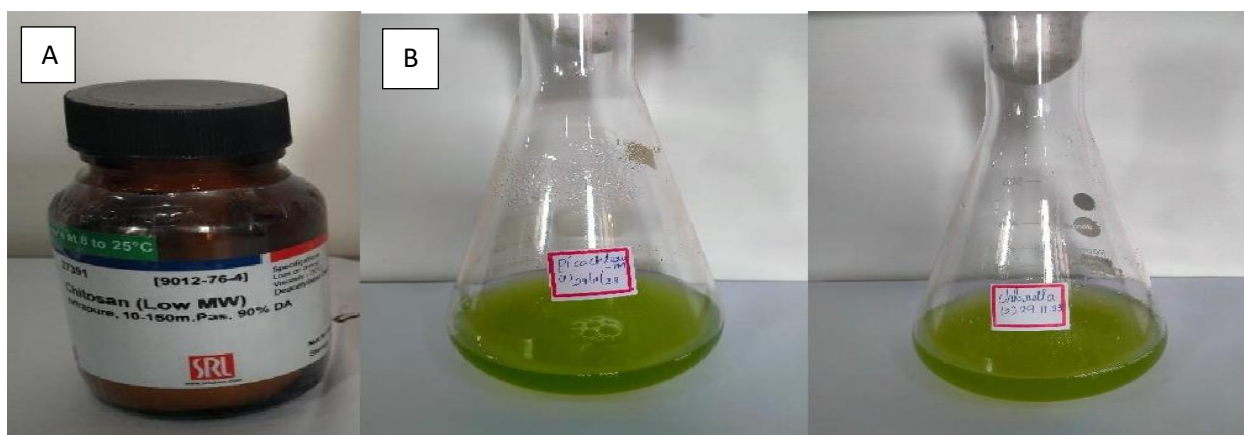


Figure 4.1 A) chitosan B) inoculation in conical flask *Picochlorum* sp & *chlorella* sp



Figure 4.2 Microalgae cultivation in tank

Table: 4.1 Chemical composition of Conway medium

STOCK SOLUTION	CHEMICAL	QUANTITY (gL ⁻¹)
Solution A	Mineral solution	
1.	KNO ₃	100
2.	NaH ₂ PO ₂ H ₂ O	20
3.	EDTA	45
4.	H ₃ BO ₃	33.69
5.	Mncl ₂ .4H ₂ O	0.36
6.	Fecl ₃ .6H ₂ O	1.3
7.	SOLUTION B	0.5ml
DISSOLVE IN DISTILLED WATER- 1L		
TRACESOLUTION (SOLUTION B)	CHEMICAL NAME	QUANTITY (gL ⁻¹)
1.	Zncl ₂	2.1
2.	CoCl ₂ .6H ₂ O	2.09
3.	NH ₂ .6mo ₇ O ₂ 4H ₂ O	0.9

4.	CuSO ₄ .5H ₂ O	2.0
5.	Conc. hcl	Few drop
DISSOLVE IN DISTILLED WATER- 1L		
VITAMIN SOLUTION (SOLUTION C)	CHEMICAL NAME	QUANTITY
1.	Vitamin B12	0.1
2.	Vitamin B1	0.2
DISSOLVE IN DISTILLED WATER - 100ML		

4.2 MORPHOLOGICAL IDENTIFICATION

(A) MICROSCOPIC IDENTIFICATION OF *Chlorella* SP:

Microscopic identification of *Chlorella* sp. was conducted using light microscopy. Cultures of *Chlorella* sp. were mounted on glass slides. The algae cell was observed under light microscopy for their morphological features. Images were captured at various magnifications to assess cellular morphology.

(B) MICROSCOPIC IDENTIFICATION OF *Picochlorum* SP:

Microscopic identification of *Picochlorum* sp. was conducted using light microscopy. Cultures of *Picochlorum* sp. were mounted on glass slides. The algae cell was observed under light microscopy for their morphological features. Images were captured at various magnifications to assess cellular morphology.

4.3 BIOFLOCCULATION METHOD FOR HARVESTING MICROALGAE BIOMASS:

Take 0.1g of chitosan and dissolve in 96ml of distilled water and slowly add 4ml of acetic acid under constant stirring until all chitosan dissolves. Then test the solution for algae flocculation by adding drop by drop till flocculation occurs. And keep the flocculated algae solution for 24hrs after 24hrs algae biomass were settled at the bottom of the beaker and separate using Whatman no.1 filter paper (Fig 4.3). The same procedure follows for *Picochlorum* sp.

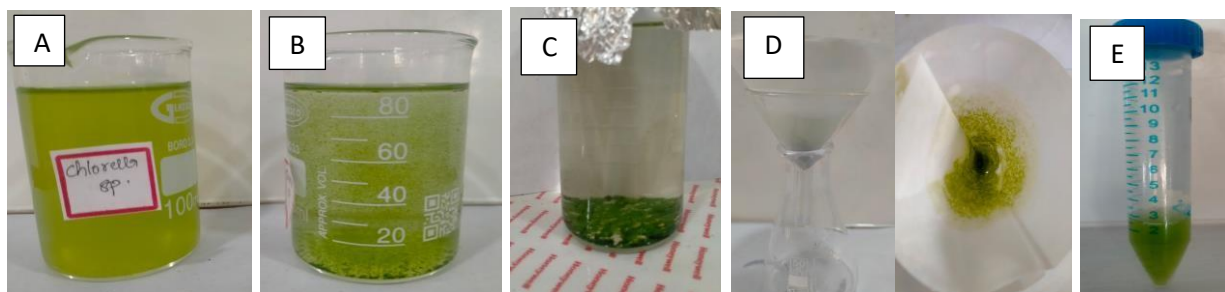


Figure 4.3 A) Before adding flocculant B) after adding flocculant C) after 24hrs D) filtration of algae biomass E) collected algae biomass

4. FORMATION OF IMMOBILIZED BIOSORBENT BEAD:

1. SYNTHESIS OF CHITOSAN - ALGINATE BEAD (CAB):

The chitosan solution is made by dissolving 2g of chitosan powder in 50ml of distilled water stir it well until it completely dissolve. Take another beaker add 0.4g of sodium alginate and 10ml of distilled water stir it well until it completely dissolve using glass rod. The chitosan solution and alginate solution are then mixed and stirred until homogeneous. This solution is then stirred with a stirrer for 24 hours. Now prepare 3% of calcium chloride solution for entrapment method take petri dish transfer prepared calcium chloride solution in petri dish and then mixture of chitosan alginate solution is transferred into petri dish containing calcium chloride solution drop by drop using 20 ml plastic syringe. There will be formation of round bead as soon as drop falls in calcium chloride solution the bead of immobilized chitosan cell is start to form

2. SYNTHESIS *PICOCHLORUM SP*-CHITOSAN- ALGINATE BEAD (PCAB)

Filtrated algae biomass was taken then concentrated species were combined with equal volume of chitosan solution and alginate solution and shaken well to homogenize these ingredients and drop this solution in petri dish containing 3% of calcium chloride solution using 20ml plastic syringe there will formation of round bead

3. SYNTHESIS OF *CHLORELLA SP* -CHITOSAN-ALGINATE BEAD (CCAB)

Filtrated algae biomass was taken then concentrated species were combined with equal volume of chitosan solution and alginate solution and shaken well to homogenize these

ingredients and drop this solution in petri dish containing 3% of calcium chloride solution using 20ml plastic syringe there will formation of round bead

5. WASHING, DRYING AND STORAGING BEADS

Now the formed beads where Leave in calcium chloride solution keep it fridge for 24hrs. after 24hrs filter the beads using strainer. Wash the beads using distilled water. To remove all calcium chloride ions. After washing spread the beads in filter paper using spatula and dry it in hot air oven at 50°C for 3 to 4hrs. store the dried chitosan biosorbent bead at 25°C

6. PREPARATION OF MERCURY HEAVY METAL SOLUTION

Mercury (Hg) solution was prepared at different concentrations 25mg/l, 50mg/l, 75mg/l, 100mg/l, 125mg/l. now add CAB, PCAB, CCAB beads respectively at individual conical flask with each concentration. And set the ph at 7. Now kept the solution at orbital shaker at 100 rpm for 24hrs in 25°C mercury (Hg) solution after treated with biosorbent beads was given to atomic adsorption spectroscopy (ASS) analysis

7. CALCULATION OF REMOVAL EFFICIENCY

After analysis of atomic adsorption spectroscopy, the following equation is used to calculate the removal efficiency for heavy metal mercury (Hg)

$$RE\% = \frac{C_i - C_f}{C_i} \times 100$$

Where,

C_i = initial concentration of Hg

C_f = final concentration of Hg

CHAPTER 5

RESULT AND DISSCUSSION

5.1 MICROSCOPIC IDENTIFICATION OF *Chlorella* sp:

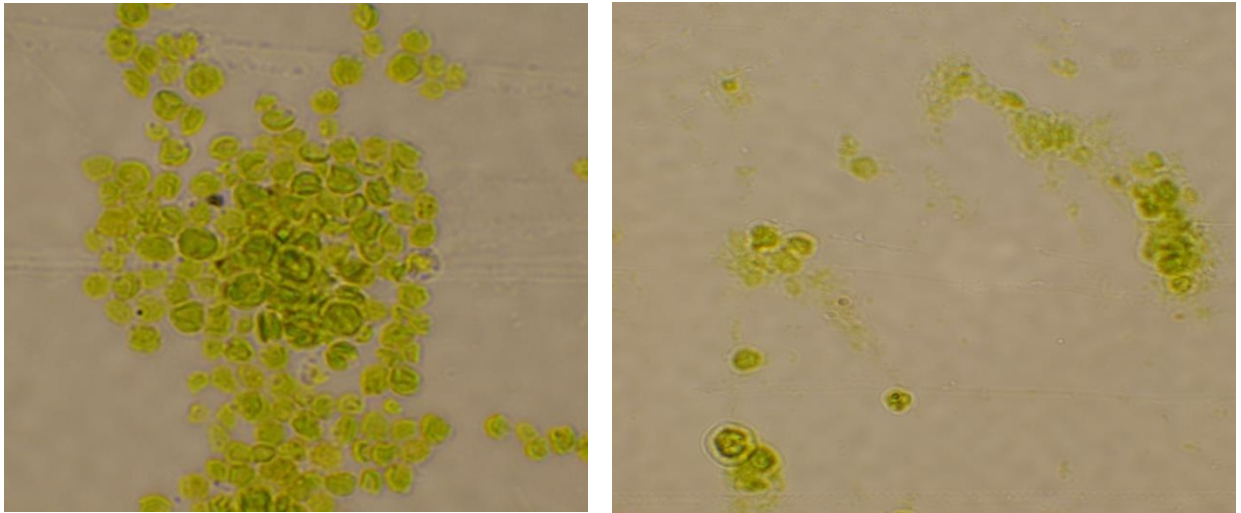


Fig 5.1: The image of chlorella sp of under microscope

MICROSCOPIC INDENTIFICATION OF *Picochlorum* sp

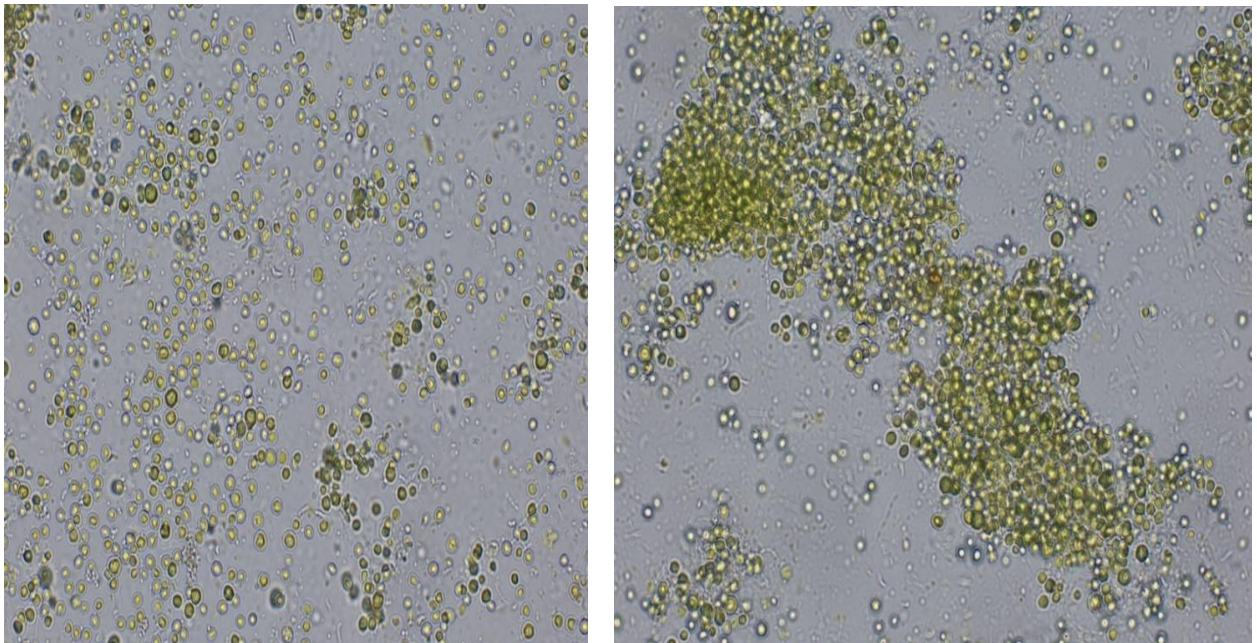


Fig 5.2 The image of Picochlorum sp of under microscope

The traditional classification and identification methods for algae use a set of morphological features to differentiate each species. The identification of morphological study of microalgae species was done after 30 days growth period under light microscope at Fig 5.1,5.2. as the study shows the cell of *Chlorella* and *Picochlorum* sp are spherical shaped and range from 2-10 μ m in diameter. They do not have flagella.

5.2 FORMATION AND DRYING OF BIOSORBENT BEAD

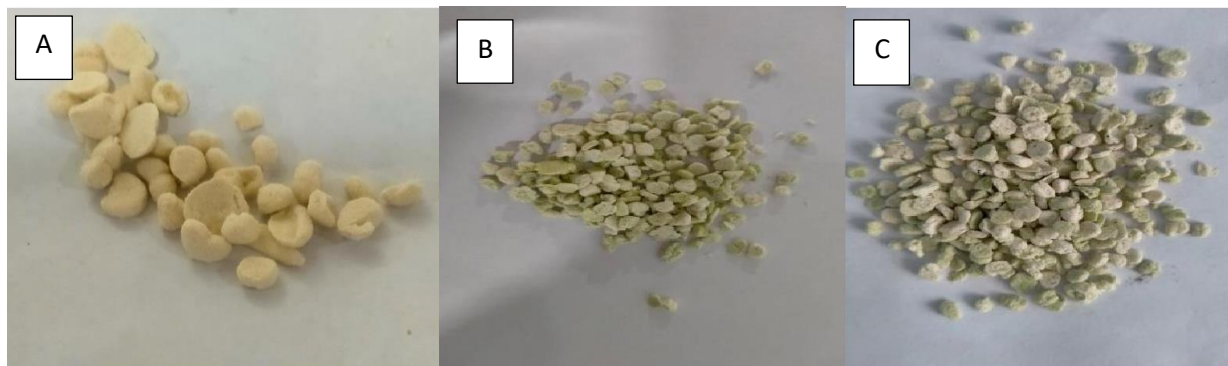


Fig5.3 A) CA Beads

B) PCA Beads

C) CCA Beads

5.3 MERCURY SOLUTION TREATED WITH IMMOBILIZED BEADS

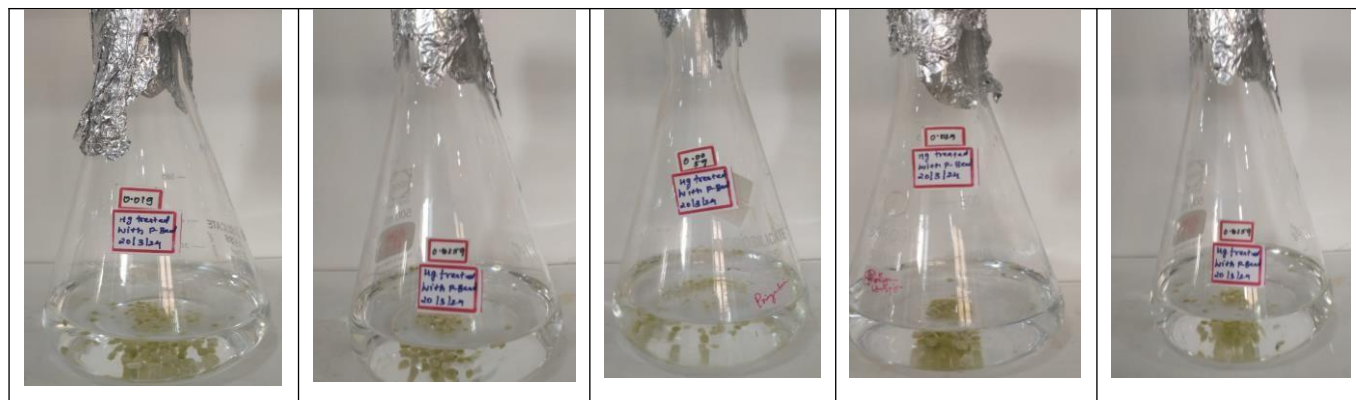


Fig 5.4 Different concentration (25, 50, 75, 100, 125mg/l) of Hg solution were treated with PCA Beads

5.4 CALCULATION OF REMOVAL EFFICIENCY

Table 5.1 Removal efficiency of CAB

Initial concentration (mg/l)	Final concentration (mg/l)	pH	Biosorbent dosage (g/l)	temperature	Contact time	Absorbance	Removal Efficiency %
25	15	7	5	25°C	24hrs	0.032	40
50	24	7	5	25°C	24hrs	0.045	52
75	31	7	5	25°C	24hrs	0.051	58
100	44	7	5	25°C	24hrs	0.064	56
125	48	7	5	25°C	24hrs	0.074	61

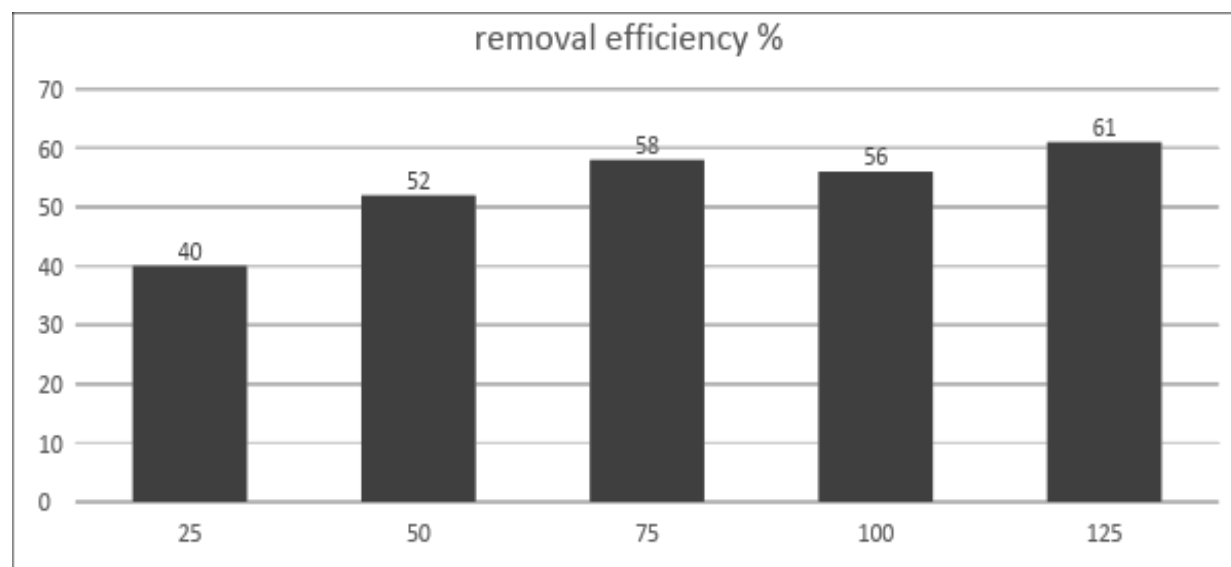


Fig 5.5 graphical presentation of removal efficiency of CAB

Table 5.2 Removal efficiency of CCAB

Initial Concentration (mg/l)	Final concentration (mg/l)	ph	Biosorbent dosage (g/l)	temperature	Contact time	Absorbance	Removal Effency %
25	14	7	5	25°C	24hrs	0.008	16
50	22	7	5	25°C	24hrs	0.012	62
75	28	7	5	25°C	24hrs	0.024	58
100	36	7	5	25°C	24hrs	0.048	61
125	52	7	5	25°C	24hrs	0.061	66

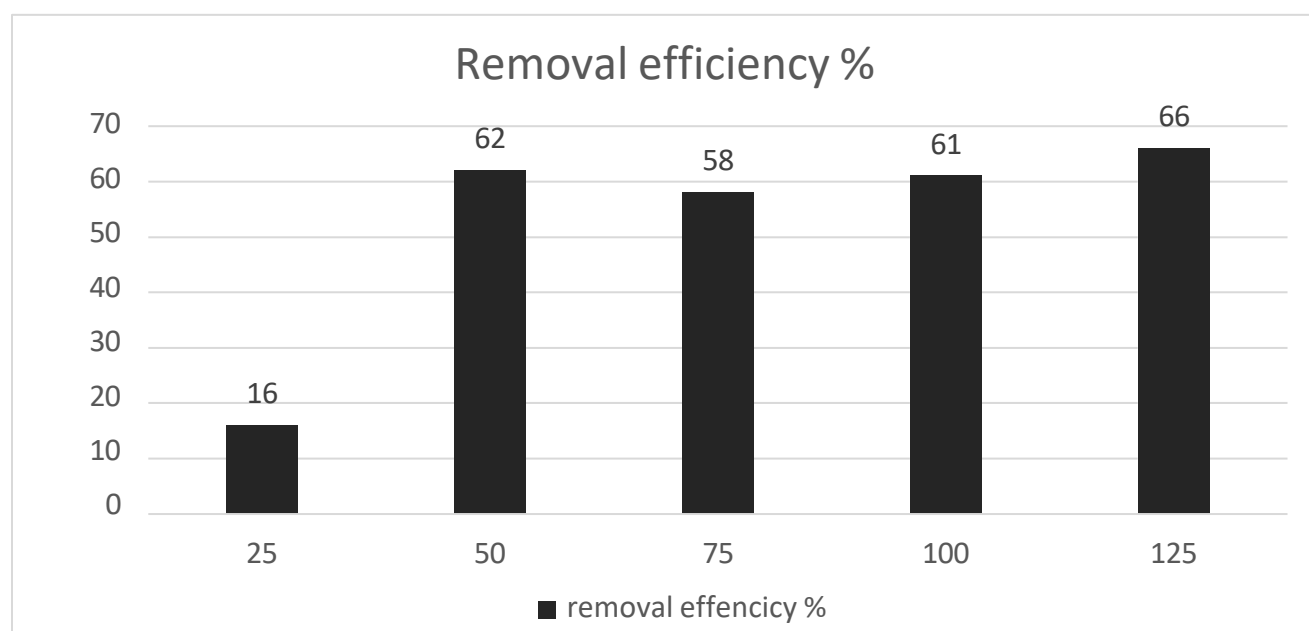


Fig 5.5 graphical presentation of removal efficiency of CCAB

Table 5.3 Removal efficiency of PCAB

Initial concentration (mg/l)	Final concentration (mg/l)	pH	Biosorbent dosage (g/l)	temperature	Contact time	Absorbance	Removal Efficiency %
25	21	7	5	25°C	24hrs	0.016	44
50	19	7	5	25°C	24hrs	0.013	56
75	31	7	5	25°C	24hrs	0.021	62
100	39	7	5	25°C	24hrs	0.034	64
125	42	7	5	25°C	24hrs	0.041	58

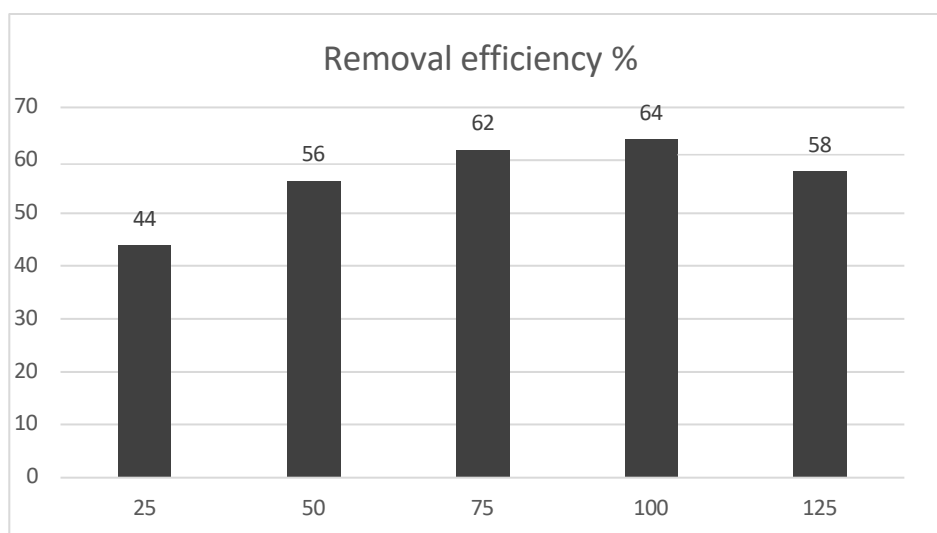


Fig 5.6 graphical presentation of removal efficiency of PCAB

DISCUSSION

Through the result of present study immobilized chitosan and microalgae (*Chlorella* sp *Picochlorum* sp) with sodium alginate beads where shows their ability to treat Hg concentration by using standard concentration (25, 50, 75, 100, 125 mg/l). the Hg

concentration after treated with CAB were (15, 24, 31, 44 ,48 mg/l) respectively with removing ratio (40,52,50,56,61%) for each concentration, respectively. As well as Hg concentration after treated with immobilized CCAB were (14, 22, 28, 36, 52 mg/l) respectively with removing ratio (16, 62, 58, 61, 66%) for each concentration. And Hg concentration after treated with PCAB were (21,19,31,39,42mg/l) respectively with removing ratio (44, 56, 62, 64, 58%) for each concentration, The result means the immobilized CAB and CCAB where shows highest removal efficiency at concentration 125mg/l. PCAB where shows highest removal efficiency at concentration 100mg/l. This study shows that with increase in the initial metal ion concentration (c_i) there tends to be increase in the removal efficiency the c_i is the governing factor as increase in the removal efficiency. Increase the concentration help in overcoming of the mass transfer resistance between the adsorbent used and the bulk fluid phase higher c_i increase the mass transfer driving force, thus increasing the uptake. In addition, increasing initial metal ion concentration increases the number of collisions between metal ion and sorbent. Which enhance sorbtion process Thakur *et al.*, (2021)

CHAPTER-6

6. SUMMARY AND CONCLUSION

The current study emphasize on the ability of biosorbent has been obtained by the immobilization of chitosan and microalgae in alginate. As a result of the presence of more active groups responsible for metal binding shows high efficiency of metal removal beads for the adsorption of mercury in aqueous solution, the removal percentage depends on the initial metal concentration and was found to be decreasing with the increase in concentration. The removal results were varied with all three types of beads which followed the selectivity order of removal efficiency as CCAB (66%) > PCAB (64%) > CAB (61%). the cost benefit while forming a composite material may show promising results in the treatment of heavy polluted effluents from industrial or agricultural sector.

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