BIOSORPTION OF COPPER USING IMMOBILISED *EUCHEUMA COTTONII SP.* BIOMASS BY USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRAK

Logam berat, kadang-kadang dikenali sebagai logam toksik, adalah satu bentuk efluen industri yang sering dihasilkan oleh sisa kimia, pembuatan, kumbahan, penyaduran elektrik, dan perlombongan. Biosorpsi ialah kaedah kos rendah untuk penyingkiran logam berat dalam air sisa. Tujuan kajian ini adalah untuk menilai keberkesanan rumpai laut tidak bergerak sebagai biosorben penyingkiran kuprum dari segi masa sentuhan awal, dos dan kepekatan awal dan untuk mengoptimumkan prestasi rumpai laut tidak bergerak dengan menggunakan Response Surface Methodology-Central Composite Design (RSM-CCD).) untuk proses biosorpsi yang dijalankan. Untuk membuat larutan akueus kuprum, 150ml CU(NO3)2 telah dilarutkan dalam 850ml air suling untuk mendapatkan 1000 mg/L larutan Cu^{2+}. Sebanyak 20 larian telah dijalankan untuk menghasilkan model kuadratik dalam RSM-CCD. Kuantiti larutan Cu^{2+} yang diperlukan telah dihasilkan dengan mencairkan larutan stok dengan pencairan yang sesuai. Kandungan Cu awal dalam air sisa sintetik ditentukan. Spektrofotometer digunakan untuk menentukan kepekatan akhir logam berat dalam sampel. Rumpai laut akan dialihkan dan dibasuh dengan air suling. Reagen pengaktif, 3g larutan natrium alginat, dihasilkan dalam bikar 1000ml yang telah dibersihkan dan dikeringkan. 0.25g serbuk rumpai laut ditambah ke dalam larutan semasa ia masih dikacau. Kemudian satukan dan ketepikan untuk menyejukkan ke suhu bilik. Menggunakan picagari, campuran kemudiannya disuntik ke dalam larutan kalsium klorida 3g. Akhir sekali, tunggu sekurang-kurangnya sejam sebelum menggunakan penyelesaian. Selepas itu, manik alginat yang telah dirawat ditukarkan kepada $CaCl_2$ dan dicuci dengan air suling. Manik kering rumpai laut yang tidak bergerak didedahkan kepada air selama setengah jam selepas dikeringkan dengan kertas yang ditapis. Eucheuma Cottonii sp. yang tidak bergerak, dikenali sebagai rumpai laut merah, telah digunakan sebagai biosorben untuk mengekstrak logam berat daripada efluen industri. Kepekatan akhir logam berat ditentukan menggunakan spektrofotometer. Selepas pengoptimuman, State-Ease Design Expert model versi 7.0 oleh Ching Liu RSM-Central Composite Design (CCD) telah digunakan untuk eksperimen proses biosorpsi. 20 larian mencipta model linear. Akhir sekali, pengesahan model ANOVA menunjukkan bahawa model ANOVA (R^2= 0.5414, adj-R^2= 0.4554) boleh meramal dengan tepat. Kesimpulannya, Eucheuma Cottonii yang tidak bergerak mungkin merupakan biosorben semulajadi untuk penyingkiran ion kuprum.

ABSTRACT

Heavy metal, sometimes known as toxic metal, is a form of industrial effluent that is often produced by chemical waste, manufacturing, sewage, electroplating, and mining. Biosorption is a low-cost method for heavy metal removal in wastewater. The purpose of this study is to assess the effectiveness of immobilised seaweed as a copper removal biosorbent in terms of initial contact time, dosage and initial concentration and to optimise the performance of immobilised seaweed by using Response Surface Methodology-Central Composite Design (RSM-CCD) for carried out biosorption process. To make the aqueous solution of copper, 150ml of CU(NO3)2 was dissolved in 850ml of distilled water to get 1000 mg/L of Cu2+ solution. A total 20 runs were carried out to generate a quadratic model in RSM-CCD. The requisite quantities of Cu2+ solution was generated by diluting the stock solution with suitable dilutions. The initial Cu content in synthetic wastewater is determined. The spectrophotometer was used to determine the final concentration of heavy metal in the sample. The seaweed will be immobilised and washed with distilled water. The activating reagent, 3g of sodium alginate solution, was produced in a 1000ml beaker that had been cleaned and dried. 0.25g seaweed powder is added to the solution while it is still being stirred. Then combine and set aside to cool to room temperature. Using a syringe, the mixture was then injected into a 3g calcium chloride solution. Finally, wait at least an hour before using the solution. Following that, the treated alginate beads were converted into CaCl2 and washed with distilled water. The dried beads of immobilised seaweeds are exposed to air for half an hour after being dried with filtered paper. Immobilized *Eucheuma Cottonii sp.*, known as red seaweed, has been employed as a biosorbent to extract heavy metals from industrial effluent. The final concentration of heavy metal was determined using spectrophotometer. After optimization, model State-Ease Design Expert version 7.0 by Ching Liu RSM-Central Composite Design (CCD) was used for biosorption process experimental. 20 runs created a linear model. Finally, ANOVA model verification showed that the ANOVA model (R^2= 0.5414, adj-R^2= 0.4554) can accurately predict. In conclusion, immobilised *Eucheuma Cottonii* might be a natural biosorbent for copper ion removal.

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LIST OF SYMBOLS

Cu(NO3)2	Copper Nitrate
Cu2+	Cuprum (II)
Pb	Plumbum
Cu	Copper

LIST OF ABBREVIATIONS

$Cu(NO_3)_2$	Copper Nitrate
RSM	Response Surface Methodology
CCD	Central Composite Design
OFAT	One-Factor-at-Time
Cu^{2+}	Cuprum (II)
$CaCl_2$	Calcium chloride
HCL	Acid hydrochloric
NaOH	Sodium Hydroxide
Pb	Plumbum
Zn	Zinc

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CHAPTER 1

INTRODUCTION

1.1   Background of Study

Wastewater is a combination of commercial, agricultural, industrial, and home uses. Depending on the water sources, wastewater might have different compositions. Three categories of wastewater exist. The first kind is municipal wastewater, or sewage, which comes from towns. Domestic wastewater from homes is the second form of wastewater, while industrial wastewater is the third type.

Sewage is a liquid waste that is created by human actions such as flushing toilets, using the shower, running the dishwasher, and using the sink. Although industrial effluent is excretory, it may also include dangerous poisons or heavy metals. Much of the heavy metal ion is thought to be toxic and carcinogenic since it is not biodegradable, unlike organic pollutants, and it continues to accumulate within a live cell. In industrial wastewater, zinc, lead, silver, copper, cadmium, chromium, iron, and arsenic were the heavy metals that were most commonly discovered. But given that it is regarded as the most valuable and frequently utilized metal in several sectors, including electroplating, plastics, and metal finishing, copper is most frequently discovered at high concentrations in wastewater (Al-Saydeh et al., 2017). Copper is a metal that is extremely poisonous even at low concentrations, thus wastewater that has been contaminated with copper must be cleaned before it is discharged into the environment.

Different traditional treatment methods, including ion exchange, membrane separation, precipitation, coagulation, reverse osmosis, and activated carbon adsorption, can be used to remove heavy metals from industrial wastewater (Arbabi et al., 2016). With its high effectiveness at low levels, cheap cost, lack of extra nutritional requirements, ease of handling, and lack of negative environmental consequences, biosorption has the potential to be a successful approach (Arumugam et al., 2018).

According to Anastopoulos et al. (2015), the cost of biosorbing 10 g of silver (II) onto cellulose phosphate was around RM8.67.

Many studies have paid close attention to the special qualities of marine macroalgae as a sustainable biosorbent up to this point. Their aptitude for biosorption is concentrated on the components of the cell wall, including the lipids, polysaccharides, and proteins that can give a variety of functional groups (Anastopoulos et al., 2015). Additionally, biosorbent may be utilized in sorption systems in both living and non-living forms, although the latter is preferred since it is unaffected by the toxicity of the compounds in wastewater. According to the previous study, more algae than bacteria and fungus and 15.3% more than other types of biomasses were utilized as biosorbent materials for heavy metal removal (Sweetly, 2014).

An organism that produces oxygenic photosynthesis and has chlorophyll is referred to as an algae. Ion-exchange is a crucial factor in the binding of the metal ions by the biomass of algae. Other mechanisms, such as complexion, coordination, electrostatic attraction, or micro precipitation, are also involved in the biosorption process. Additionally, because it is easy to get in the fishing sectors, macroalgae can provide cost-effective heavy metal removal by overcoming some chemical and physical restrictions.

1.2 Problem Statement

Due to the harmful impact that heavy metals may have on the environment and living things, especially when they do not adhere to regulatory standards, heavy metal removal from wastewater is now a very difficult environmental task (Arumugam et al., 2018). Even in small amounts, certain heavy metals are hazardous and carcinogenic. They may quickly build up in living things and are not biodegradable. Heavy metal build-up is a concern to human health and can be transferred to people through the aquatic food chain (Zeraatkar et al., 2016).

The five most concerning heavy metals in the world are lead (Pb), copper (Cu), chromium (Cr), mercury (Hg), and cadmium (Cd), as they can directly impair people's health (Islam et al., 2015). Environmental exposure to cadmium may be caused by human

activities such as the production of building materials, the electroplating industry, municipal garbage, and the incineration and fertilization of industrial waste (Chattopadhyaya et al., 2014). The nervous system, kidneys, and brain can be harmed by lead, a very poisonous element that can build up in human organs. Although copper is a necessary component of nourishment for all living things, over exposure to it can be hazardous (Drahansky et al., 2016).

Ion exchange, coagulation-flocculation, adsorption, and precipitation are frequently used industrial methods to remove heavy metals from water. However, there are a number of restrictions on the heavy metal removal methods. For instance, the ion-exchange approach is only appropriate for very low metal concentrations. High concentrations of heavy metals in wastewater cannot be treated using the precipitation process. Coagulation and flocculation are caused by insufficient removal of heavy metals and the creation of sludge. While certain adsorbents for the adsorption process must be hybridized for maximal binding capacity (Abdullah et al., 2019).

The capacity of algae to adsorb wastewater may be tested using both actual and synthetic wastewater. As the concentration of the solution may be adjusted and it can be concentrated on a specific heavy metal to assess the capacity of algae to adsorb that metal, the study uses simulated wastewater. On the other hand, there hasn't been much research on utilizing native Malaysian algae species to remove heavy metals, particularly red algae (Rhydophyta) species.

1.3   Objectives

Based on the issue description, this research has three aims. The study's aims are as follows:

● To prepare immobilised seaweed to use as biosorbent for copper removal.

● To determine the performance of immobilised seaweed in terms of initial contact time, dosage and initial concentration.

● To optimise the performance of immobilised seaweed by using Response Surface Methodology (RSM).

1.4   Scope of Study

This research recommends treating copper ions using a red macroalga named *Eucheuma Cottonii sp.* from synthetic wastewater. This research looked at the amounts of heavy metals such as copper ions in actual wastewater. One species of red (*E. Cottonii*) seaweed were extensively studied for their biosorption capability.

For the study on the effect of the first initial contact time, dosage and initial concentration, two methods of biosorption process were used which will constitute the study's parameters. This experiment requires numerous procedures, including seaweed preparation and heavy metal preparation. The harvested seaweed was washed and cleaned in preparation for the following procedure, which is oven drying (Memmert, UNB100). After drying, the seaweed was grind using a dry mill blender (Butterfly) and sieving via sieve at size (600μm). Makes the particles small to aid in the immobilisation process. Sodium alginate (Duchefa Biochemistry) is the substance utilized in research for immobilized algae. Also utilized to harden the immobilized beads is calcium chloride (Bendosen). Following the immobilisation procedure, do the biosorption experiment and gather data using a spectrophotometer D5000 (HACH UV-VIS). To prepare the synthetic wastewater, copper standard solution traceable to SRM from NIST $Cu(NO3)2$ in $HNO3$, 0.5 mol/l 1000mg/l Cu Certipur® is the substance used to make the aqueous solution (R&M Chemicals). To change the pH of an aqueous solution, hydrochloric acid, HCL and sodium hydroxide, NaOH (HmbG Chemicals) are utilized.

The effectiveness of immobilized seaweed in terms of contact time, dosage and initial concentration will be evaluated using batch biosorption in One-Factor-At-Time (OFAT) (5 samples) and using model State-Ease Design Expert version 7.0 by Ching Liu of Response Surface Methodology (RSM) (20 samples) for the biosorption experiment.

Figure 1. 1   Dried red seaweed, *Eucheuma Cottonii sp.*

1.5   Significance of Study

This research aims to eliminate copper ions, one of the heavy metals that pollute the environment. Because these heavy metals are not biodegradable, they cannot be eliminated. It indicates that if no quick action is done, pollution will continue to occur, exposing humans or living things to the dangers of heavy metals. They are readily dispersed by the wind, food, water, and air. So, it makes sense for us to do the research and eliminate these heavy metals.

This study is being conducted to demonstrate the efficacy of macroalga, namely *Eucheuma Cottonii sp.*, sometimes known as red algae, as a biosorbent in water treatment. Algae may be found in abundance in aquatic systems, and there are several types of algae. This study has the potential to optimise the utilisation of algae and reap the advantages of algae for the waste water treatment system. Macroalga may also be used in the treatment of genuine waste water.

CHAPTER 2

LITERATURE REVIEW

2.1   Wastewater

Wastewater is water that has been polluted by domestic, industrial, or commercial usage. Water constitutes 99.9% of the makeup of wastewater. Organic debris, microorganisms, and inorganic compounds make up 0.1 percent of wastewater makeup. Wastewater effluents are released into a variety of bodies of water, including lakes, ponds, streams, rivers, estuaries, and oceans. As a consequence, the composition of all wastewaters is constantly changing and extremely variable, making it impossible to define the word in a single way. (Tuser, 2020).

Industrial wastewater is wastewater emitted from industrial and commercial sources that may include hazardous contaminants, endangering the quality of receiving waterways or interfering with publicly owned treatment facilities that will receive such effluent discharges. In general, industrial effluent contains suspended particles, colloidal solids, and dissolved solids. Furthermore, industrial wastewater may have unusually high acidity or alkalinity, as well as high or low amounts of coloured debris. In addition, industrial effluent may include inert, organic or inorganic substances, toxic chemicals, and infectious microbes. Heavy metal pollution may be present in industries such as mosaic, food processing, electrical appliance production, and chemical compound processing (Phadtare & Patil, 2015). Heavy metals must be removed from industrial effluent because they damage water bodies and are hazardous to numerous living forms (Phadtare & Patil, 2015). Heavy metal water pollution creates severe environmental problems due to the potentially dangerous impacts of heavy metals on both people and the environment (Husoon et al.,2013).

2.1.1 Wastewater Regulation

The electroplating business has been identified as a significant polluter of the environment. This business produces contaminants such as heavy or trace metals such as zinc (Parinda Suksabye, 1995). This heavy metal, particularly the zinc variety, is known to be poisonous and carcinogenic. Heavy metal-containing industrial wastewaters are carefully controlled and must be treated before being released into the environment due to their high toxicity. Industrial wastewaters must adhere to the Environmental Quality (Sewage and Industrial Effluents) Regulations 1978. Table 2.1 shows the parameter limit for standard A and standard B. When wastewater is discharged in the upper stream of a river and there is water input to a Drinking Water Treatment Plant, Standard A is used, whereas Standard B is used when wastewater is released downstream of the river. According to the table below, wastewater must have less than 0.05mg/L zinc for both standards A and B.

Table 2. 1   Environmental Quality Act 1974

Parameter A	Parameter B	Unit	Standard	Health Hazard
	(i)	(2)	(3)	(4).
i	Temperature	ºC	40	40
ii	pH value	-	6.0-9.0	5.5-9.0
iii	BOD5 at 20ºC	mg/L	20	50
iv	Suspended solid	mg/L	50	100
v	Mercury	mg/L	0.005	0.05
vi	Cadmium	mg/L	0.01	0.02
vii	Chromium, Hexavalent	mg/L	0.05	0.05
viii	Chromium, Trivalent	mg/L	0.20	1.0
ix	Arsenic	mg/L	0.05	0.10
x	Cyanide	mg/L	0.05	0.10
xi	Lead	mg/L	0.10	0.5
xii	Copper	mg/L	0.20	1.0
xiii	Manganese	mg/L	0.20	1.0
xiv	Nickel	mg/L	0.20	1.0
xv	Tin	mg/L	0.20	1.0
xvi	Zinc	mg/L	2.0	2.0
xvii	Boron	mg/L	1.0	4.0
xviii	Iron (Fe)	mg/L	1.0	5.0
xix	Silver	mg/L	0.1	1.0
xx	Aluminium	mg/L	10	15
xxi	Selenium	mg/L	0.02	0.5
xxii	Barium	mg/L	1.0	2.0
xxiii	Fluoride	mg/L	2.0	5.0
xxiv	Formaldehyde	mg/L	1.0	2.0
xxv	Phenol	mg/L	0.001	1.0
xxvi	Free Chlorine	mg/L	1.0	2.0
xxvii	Sulphide	mg/L	0.50	0.50
xxviii	Oil and Grease	mg/L	1.0	10
xxix	Ammoniacal nitrogen	mg/L	10	20
xxx	Colour ADMI*		100	200
MI – American Dye Manufacturers Institute				

Source: Latif (2016). Treatment of Palm Oil Mill Effluent (POME)

2.2   Heavy Metal

Due to their toxic nature, heavy metals have emerged as the most important contaminant. A major area of worry at the time is the increase in the quantity of heavy metal in our resources, especially since many enterprises are dumping their wastewater effluent, which contains heavy metal, without the proper treatment. When heavy metals come into contact with people in medical, industrial, agricultural, or residential contexts, they may enter the body through food, drink, air, or skin adsorption. If they are not digested by the body and build up in soft tissue, they may become poisonous to humans. Human activities and the natural environment both pollute the environment and its resources because they produce more waste than the ecosystem can handle (Masindi et al., 2018).

Heavy metal generated by a range of enterprises and household waste has lately been identified as a significant contributor to adverse and damaging environmental consequences if not handled properly. Growing worldwide industrialisation has a significant influence on natural water reservoir contamination due to enormous heavy metal discharges in multiple water bodies (Vishal Mishra, 2013). Heavy metal in wastewater has also been a major cause of concern due to its toxicity and detrimental effects on human health. Heavy metals that are not biodegradable will accumulate in biological systems due to bioaccumulation (Norzila Othman et al., 2014). Another equally important style is the caption. All captions for figures, tables and equations are formatted using their respective styles prepared in this template.

Metal concentrations that exceed legal limits create health issues. Because heavy metals are known to accumulate within biological systems, there is still a danger of bioaccumulation and associated chronic toxicity even when metal concentrations do not approach these thresholds (Shamim, 2018). **Table 2.2** depicts the health impacts of many dangerous heavy metals. Among these metals are arsenic, mercury, cadmium, lead, chromium, nickel, zinc, and copper.

Metal plating, pulp and paper manufacturing, mining operations, ceramics manufacturing, tanneries and radiator manufacturing, alloy manufacturing, battery manufacturing, textile and dye manufacturing are all significant sources of harmful

effluents that are increasing in tandem with rapid industrial growth (Muhammad Shafiq et al., 2018).

Table 2. 2 Health effect of various toxic heavy metals

Metal Contamination	Health Hazard
Arsenic	Carcinogenic, producing liver tumours and skin and gastrointestinal effects.
Mercury	Corrosive to skin, eyes, and muscle membrane, dermatitis, anorexia, kidney damage, and severe muscle pain.
Cadmium	Carcinogenic, cause lung fibrosis, dyspnoea and weight loss.
Lead	Suspected carcinogen, loss of appetite, anaemia, muscle and joint pains, diminishing IQ, cause sterility, kidney problem and high blood pressure.
Chromium	Suspected human carcinogen, producing lung tumours and allergic dermatitis.
Nickel	Causes chronic bronchitis, reduced lung function, cancer of lungs and nasal sinus.
Zinc	Cause short-term illness called "metal fume fever" and restlessness.
Copper	Long term exposure cause irritation of nose, mouth, and eyes, headache, stomach-ache, dizziness and diarrhea.

Source: Zwaim (2014)

2.2.1 Source of Heavy Metal in Wastewater Effluent

The two main origins of heavy metals in wastewater are the natural environment and human activity. While human factors include metal cutting, electroplating, and industrial facilities, natural causes include volcanic eruption, soil erosion, and particle aerosols.

Volcanic eruptions are recognized to have hazardous effects on the environment, the climate, and those who are exposed to them. Along with the deterioration of social and environmental circumstances and the emissions of gases (sulphur dioxide, carbon monoxide, and carbon dioxide), other organic materials and heavy metals, including lead, gold, and mercury, copper, zinc, and a variety of others, are also released (Akpor et al., 2014).

It is frequently asserted that water-borne heavy metal pollution contributes to soil erosion. The two primary factors that induce soil erosion are wind and water. Due to erosion, the heavy metal may be swept up and dispersed to the atmosphere during run-off. After a runoff, a lot of heavy metal debris is washed into inadequate drainage systems, where it finally finds its way into nearby rivers (Taiwo et al., 2011).

The electrochemical injection of a thin protective coating onto ready metal surfaces is necessary for metal finishing and electroplating. When this happens, hazardous metals may be released into wastewater effluents. It can be done by rinsing with water, spilling, or removing it from a machine bath. Additionally, it should be highlighted that the cleaning procedure and wastewater treatment might result in large amounts of wet sludge with high levels of hazardous metals.

Additionally, textile factories are recognized as important sources of pollutants containing heavy metals in water. According to others, the dyeing process, which is important in various businesses, is the major source of this. Chromium, lead, nickel, and copper, which are all very dangerous and carcinogenic substances, are included in the materials used in such colouring procedures. In certain cases, nuclear power plants have also been linked to the release of heavy metals like copper and zinc into surface waters. The industrial effluent including heavy metals is discharged into surface water and fresh water sources during operation at nuclear reactors, where substantial amounts of water are consumed for service, which may contaminate aquatic habitats (Akpor et al., 2014).

2.2.2   Copper

A naturally occurring reddish metal called copper may be found in air, rock, soil, and water (Department of Health and Human Services, 2004). Copper is utilized as a heat and electrical conductor. A prominent source of environmental contaminants is copper. The World Health Organization (WHO) declared that copper ions concentration in drinking water should not exceed 2 mg, while the United States Environmental Protection Agency (USEPA) claimed that the permitted maximum of copper ion in industrial effluent is 1.3 mg/L. (Al-Saydeh et al., 2017). Additionally, the Department of Environmental Protection has established 0.20 and 1.0 as the permitted levels for dumping copper under standards A and B. (DOE). However, because it is a crucial

component of the respiratory enzyme complex cytochrome oxidase, copper is an important nutrient for all living things as a trace dietary mineral (Jiwan et al., 2011). The standard copper dischargeable by the various organizations is compiled in Table 2.1. The body uses copper for oxidation-reduction processes, to stimulate hemoglobin production and activity, to harden collagen, to keratinize hair, to affect lipid metabolism, and to affect the characteristics of the myelin sheath that surrounds nerve fibers (Drahansky et al., 2016).

Despite the fact that copper is a necessary metal, over exposure to it can be dangerous. such as Indian Childhood Cirrhosis (ICC), Wilson's disease (hepatolenticular degeneration), which are characterized by copper build-up inside liver cells in addition, inhaling copper will result in gastritis, diarrhea, and nasal mucosal congestion (Drahansky et al., 2016).

Table 2. 3   Acceptable amount of copper ions for the discharge of industrial effluent

Metal Contamination	Health Hazard
United State Environmental Protection Agency (USEPA)	13
World Health Organization (WHO)	2.0
Department of Environmental (DOE)	Standard A-0.20 Standard B-1.00

Standard A- applicable to discharge into any inland water within catchment areas
Standard B- applicable to any other inland water or Malaysia waters

2.2.3   Conventional method for heavy method removal

2.2.3.1   *Ion Exchange*

Ion exchange is based on the reversible exchange of an ion between the solid and liquid phases, in which an ion substituted from solution, electrolyte, or molten salt is exchanged for a similarly charged ion bound to an immobile and insoluble solid substance while maintaining overall electroneutrality (Bashir et al., 2019). Synthetic organic resin and a three-dimensional inorganic matrix are the most often utilised ion-exchange

matrices. The ion-exchange process has a drawback in that it cannot handle high concentration metal solutions because organics and other particles in the wastewater readily contaminated the matrix (Arbabi et al., 2015). Furthermore, ion exchange is non-selective and must function at specified temperatures and pH levels (Masindi et al., 2018).

2.2.3.2 *Chemical Precipitation*

Coagulants such as phosphorus, iron, alum, lime, and other organic polymers are used to achieve chemical precipitation. The precipitation process is used by around 75% of the planting industries as a treatment procedure to remove metal ions from solution (Joshi et al., 2017). It is capable of efficiently treating inorganic wastewater with metal concentrations more than 1000 mg L-1 (ppm). Chemical precipitation, on the other hand, requires a significant number of chemicals to lower the concentration of metal in wastewater to an acceptable limit for disposal. And it is seldom utilised to deal with low metal ion concentrations in wastewater less than 100 mg L-1. Another constraint is the generation of sludge from the process, which need additional treatment, which raises the cost of sludge disposal and has long-term environmental consequences (Pugazhenthiran et al., 2016).

2.2.3.3 *Coagulation-flocculation*

The coagulation-flocculation process is centred on identifying the electrostatic interaction between contaminating and coagulant-flocculant chemicals by calculating zeta potential. The coagulation process reduces the net surface charge of the colloidal particle, allowing it to stabilise through electrostatic repulsion (López-Maldonado et al., 2014). While the flocculation approach constantly increases particle size to discrete particles by collisions and interactions with inorganic polymers formed by the added organic polymers. Filtration, straining, or flotation may be used to remove or separate discrete particles that have flocculated into larger particles. The production of sludge, the use of chemicals, and the transformation of hazardous substances into solid phase are all significant downsides of this approach (Gunatilake et al., 2015).

2.2.4 Adsorption versus Biosorption

The word "sorption" can refer to both adsorption and absorption. The assimilation of a material from one state into another is known as absorption. For example, solids may absorb liquids. In contrast, adsorption is the act of an ion physically adhering to or attaching to the surface of the sorbent. Adsorption, however, is more preferable in the clean-up procedure.

2.2.5 Adsorption

The mass transfer technique known as "adsorption" involves moving an object straight from the liquid phase to the surface of the solid phase after it has been bonded by physical and/or chemical interactions (Al-Saydeh et al., 2017). The most popular and efficient adsorbent is activated carbon. Because of its porous structure, which consists of an interconnected network of macropores, micropores, and mesopores, and because of its large surface area, activated carbon is often utilized in powdered or granular form. According to Davarnejad et al. (2016), the adsorption method is the most popular approach for removing the Cu2+ ion and offers several benefits over other methods due to its straightforward design. Activated carbon does have certain drawbacks, though. Commercial activated carbon is costly, and the cost increases with quality. The cost of thermal and chemical regeneration of used carbon is high.

2.2.6 Biosorption

Adsorption of contaminants by the use of biological materials, or biomass, as the biosorbent, is known as biosorption (Chojnacka, 2010). A physical-chemical process known as biosorption occurs when one material is linked to another. Where the biological matrix's solid surface (sorbent) interacts with metal (sorbate), the concentration of sorbate in the solution decreases (Fomina et al., 2014). The ability of the microbe to adsorb heavy metal ions means that the biomass may be utilized successfully as a sorbing agent for organic materials. Protozoa and bacteria are both present in wastewater system activated sludge. The protozoa are unicellular, eucaryotic, rather large cells without cell walls. Through the protein and lipid-containing membranes on their exterior surfaces, they may

absorb substances. While the cell wall of bacteria is mostly made up of several organic substances, including lipids, amino acids, carboxyl, and acidic polysaccharides.

The majority of studies describe biosorption as the passive or metabolically independent absorption of heavy metals. A dynamic, reversible adsorption-desorption process, the passive process can cause metal ions to attach to the biomass in the solution. Because of the interaction between the metal ion and the functional group of biomasses, the metal molecule can adsorb on the surface of biomass. One of two algal biosorption processes, including (1) ion-exchange, in which the ions Ca, Mg, Na, and K on the algal surface are replaced by metal ions, and (2) complexation of functional groups with metal ions, is involved, according to Abbas et al. (2014). The following mechanism (Babák et al., 2012) covers biosorption.

Table 2. 4   The advantages and disadvantages of biosorption

Advantages	Disadvantages
Low-cost	Early saturation
High efficiency	The potential for biological process improvement
Minimization of chemical and biological sludge	No potential for biological altering the metal valency state.
Regeneration of biosorbent and possibility of metal recovery	

Source: Das et al. (2008)

Numerous factors, including pH, temperature, biomass content, pre-treatment of the biomass, and type of biomass, can have an impact on the capacity of biosorption to remove heavy metals. Research on the factors that might increase biosorption capacity and selectivity has been conducted by Zeraatkar et al. (2016). He said that a deeper comprehension of the contributing factors is necessary to successfully apply algal biosorption technology in the sector.

According to Fomina et al. (2014), biosorption has not proven economically successful, and its conventional use as a cheap and ecologically benign approach of pollution treatment has to be reconsidered. Physico-chemical and biotic modifications

that improve biosorption in terms of selectivity, a kinetic, capacity, and re-use are more expensive and may cause environmental problems. Zabochnicka-Wiatek et al. (2014) contend that biosorption can be employed in addition to the present physical and chemical approaches to clean up heavy metal-contaminated surroundings. The physical immobilization of biomass can be used to increase the effectiveness of metal removal. By washing the biomass with deionized water and desorption agents like NaOH, HCl, and CaCl2, biosorbent may be subjected to regeneration, which is the main benefit of using them (Chen et al., 2012). The biological origin of the materials, cheap cost, removal of sizable amounts of metals, and metal recovery are further benefits of biosorption applications.

2.2.6.1   *Biosorption by immobilization cell*

Immobilized macroalgae are used to remove heavy metals in order to maintain long-term live cell activity within the gel matrix. Prior research has indicated that immobilized cells are more effective in sorbing metals than freely suspended cells (Barquilha et al., 2017). Additionally, employing immobilized algae to remove heavy metals has certain advantages. These advantages include the ability for non-professionals to do it, strong resistance to harmful compounds, high biomass production that is utilized as a byproduct, several immobilizations of microbes, and recovery of biomass from treated wastewater (Ahmad et al., 2018).

Alginate, agar, and carrageenan are examples of natural supporting materials that may be used in the immobilization of biomass. Synthetic supporting materials include polyurethanes, silica gel, and polyacrylamide. Given that synthetic polymers are hazardous to biomass, natural polymers are preferred over them in terms of use. Alginate was often employed for biomass production, including the immobilization of algae. Alginate is a substance made from the sodium salt that is water soluble in algae.

Ahmad et al. conducted research on the biosorption of Fe (II), Mn (II), and Zn (II) ions from aqueous solution using free- and immobilized Chlorella vulgaris (2018). The results indicated that immobilized microalgae were capable of up-taking the highest amounts of Fe (II) 129.83, Mn (II) 115.90, and Zn (II) 105.29 mg/g under ideal conditions. The immobilized Chlorella Vulgaris in alginate beads is a reliable and

advantageous biosorbent material for removing heavy metals from aqueous solutions, according to this study. According to the findings of a different study conducted by Barquilha et al. (2017), immobilized Sargassum sp. is more able to adsorb copper and nickel ions than free-cell Sargassum sp.

2.2.7   Mechanism of biosorption process

Biosorption employs adsorption mechanisms such as ionic, chemical, and physical adsorption. By generating compounds with negatively charged reaction sites, metal ions might be adsorbed on the cell surface. Several ligands discovered on fungal cell walls are hypothesised to facilitate metal chelation. Among them are hydroxyl, carboxyl, phosphate, sulphydryl, and amine groups (Ramachandra et al., 2005).

Biosorption is mediated by the interaction of cell wall functional groups with metal ions. Microbial cell walls are composed of polysaccharides, lipids, and proteins.

These components have a range of functions in metal ion binding, including the creation of metal ion binding sites. Metal ion biosorption is a fast and unaffected by cell metabolic process (Vishal Mishra, 2013).

The biosorption process involves a solid phase (sorbent or biosorbent, adsorbent, biological material) and a liquid phase (solvent, often water) containing a dispersed species to be sorbed (adsorbate, metal or dyes). The degree of adsorbent affinity for the adsorbate determines the distribution of the adsorbate between the solid and liquid phases. Because of the adsorbent's strong attraction for the adsorbate species, it is attracted and bound in a variety of ways. The procedure is continued until the quantity of solid-bound adsorbate species and the amount of solution remaining are determined (Ramachandra et al., 2005).

2.2.8   Living and Non-Living Algae

The deposition of synthetic dye onto living cells and biosorption of dye ions onto non-living algal biomass distinguishes living algae from non-living algae. The mechanism for both live and non-living algae, however, is primarily an ion-exchange process (Monteiro et al., 2012). Non-living algae have lately acquired appeal as a

biosorbent for the biosorption of synthetic dye from aqueous solution. Non- living algae use the biosorption method to remove manufactured dyes. The most significant advantage of biosorption is that a synthetic dye is bonded to the surface of the cell wall, masking metabolic activity. As a result, the met hod is easy and low-cost in comparison to living algae, which require feeding and energy supply (Zabochnicka-wiatek et al., 2014). Aside from that, the benefits of utilizing non-living algae as a biosorbent include better biosorption of synthetic dye than living algae. Furthermore, by washing the biomass with deionized water and desorption agents (NaOH, HCl, and CaCl2), non-living algae can be reused multiple times (Chen et al., 2012). Furthermore, because non-living cells are not influenced by the toxicity of dye ions found in wastewater, they can improve their adsorption capability through chemical and physical pre-treatment (Sulaymon et al., 2013).

Living algae, on the other hand, have a more sophisticated biosorption mechanism than non-living algae, and their biosorption capability can be influenced by environmental conditions. Because adsorption occurs during the growth phase and synthetic dye uptake occurs intracellular. Bioaccumulation is a mechanism that allows living organisms to bind poisonous dyes or chemical compounds into cell structures (Fomina et al., 2014). During bioaccumulation, living algae will absorb the dye ion. There are two stages of bioaccumulation. In the first stage, dye ions bond to the surface of the biosorbent (algae). This process, like the biosorption mechanism, is metabolically passive. The ions are then transported inside the cell. The second step can occur only when the cell is metabolically active. If the conditions are favourable for organism growth to continue in the second stage, the amount of biomass increases (Zabochnicka-wiatek et al., 2014). However, because the synthetic dye is poisonous, there have been few reports of living algae having a low sorption ability (Zeraatkar et al., 2016).

Previous research has concluded that bioaccumulation refers to the process of using living algae, whereas biosorption refers to the usage of non-living algae. Biosorption is a passive process that occurs without any metabolic activities being activated. As a result, non- living algae are used as biomass. While bioaccumulation occurs in living algae, it necessitates nutrients and energy. **Table 2.5** summarizes the contrast of biosorption and bioaccumulation.

Table 2. 5   Comparison of biosorption and bioaccumulation

Characteristic	Biosorption	Bioaccumulation
Cost	Usually, low. Biomass can be obtained from industrial waste	Usually, high. The process occurs in the presence of living cells that have to be supported
pH	A pH of the solution strongly affects sorption capacity of heavy metals	A significant change in pH can heavily affect living cells.
Selectivity	Poor. However, can be increased by modifications biomass Transformation	Better than in the case of biosorption
Rate of removal	Most mechanism occurs at a fast rate	Slower rate than in the case of biosorption. Intercellular
Regeneration and reuse	Biosorbent can be regenerated and reused in many cycles	Due to intercellular accumulation reuse is rather limited

Source: Zabochnicka-Światek et al., 2014

2.2.9   Algae as Biosorbent

A researcher has focused on using algae as a biosorbent because to the ample nutrient demand, high sorption capacity, abundant availability, high surface area to pore volume, lesser volume of sludge to be disposed of, and the possibility for metal regeneration and recovery (Kanamarlapudi et al., 2018). Additionally, it is a cost-effective and environmentally benign method of treating wastewater. Based on their color and colloid composition, algae can be grouped. Green, brown, and red are the three primary hues of algae, which are often seen in the water. Heavy metal ions can bind to the surface of polysaccharides, which are the primary components of algae. Alginate and sulphate make up the majority of the brown algae's active groups. Red algae contain sulphate polysaccharide, though. In the meantime, the cell wall of green algae is mostly composed of polysaccharides coupled with cellulose and protein (Mokhtar et al., 2017). According to prior research, several kinds of algae have been employed to remove heavy metals from aqueous solutions (Al-homaidan et al., 2014; Zakhama et al., 2011; Ibrahim et al., 2016).

2.2.10 Factor affecting biosorption

Biosorption process is affected by following factors:

2.2.10.1   Initial Contact Time

Contact time is the amount of time provided for the biosorption process. Although the period of contact between the biosorbent and the sorbate has no direct influence on biosorption capacity, it may be a limiting factor. Under testing circumstances, increased contact time would enable the biosorbent material to exhibit its maximal biosorption capability. When the biosorbent's maximum biosorption capacity is attained under given circumstances, the binding sites are completely saturated, and increasing contact time has no further impact (Redha, 2020).

2.2.10.2   Dosage

At low biomass doses, the specific absorption of metal ions increased. Using a low biomass dose in complicated polluted water, on the other hand, may increase competition for the biosorbent's binding site, decreasing the biosorbent's biosorption potential. Increased biomass dose minimises competition among metal ions that bind to functional groups when more than one metal ion is present (Redha, 2020).

2.2.10.3   Initial Concentration

A biosorption process's viability and effectiveness are determined not only by the qualities of the biosorbent, but also by the concentration of the metal ion solution. The initial metal concentration acts as a powerful driving force in overcoming all metal mass transfer resistances between the aqueous and solid phases. The influence of heavy metal ion starting concentrations was investigated. There are several elements that might influence the adsorbate concentration impact. The first, and most crucial, is that adsorption sites stay unsaturated throughout the adsorption procedure. The second reason is adsorbent particle aggregation or agglomeration at greater concentrations. Aggregation reduces the overall surface area of adsorbent particles accessible for adsorption while increasing the diffusional route length.

2.3   Biosorbent

For over three decades, activated carbon has been the go-to adsorbent for wastewater treatment. However, activated carbon is still a costly material. Seaweeds, microorganisms (bacteria, fungus, yeast, and moulds), activated sludge, fermentation waste, and other carefully promoted biomasses are examples of biosorbent supplies for the biosorption process. Table 1.4 shows some of the biomass that has been studied for zinc removal from wastewater. Other biomass, particularly zinc, has been researched for its ability to remove heavy metals from wastewater. This biomass contains olive oil waste, rice husk, and seaweed. All of this biomass has zinc adsorption potential. Some of these adsorbents, however, do not have large adsorption capabilities. Biosorbent exhibited wide sources, cheap cost, and fast adsorption.

Table 2. 6   Biosorbent used in Copper (II) removal

Adsorbent	Metal ion removed	References
Palm kernel husk	Cu(II), Pb(II)	Omgbu and Iweanya (1990)
Coconut husk	Cu(II), Cd(II)	Babarinde (2002)
Waste tea leaves	Ni(II), Pb(II), Fe(II), Cu(II)	Ahluwalla and Goyal (2005)
Tea leaves	Pb(II), Cd(II), Cu(II)	Tee and Khan (1988)

Source: Ali. (2012)

2.4   Concluding Remarks

The biosorption method is recommended for eliminating synthetic dye since it may do so cost-effectively as biomass is frequently a biological substance that is simple to cultivate. There are no chemicals used in the biosorption process. As a consequence, it has minimal impact on the environment and may be recycled and used several times. Macroalgae that are not alive are the best biosorbent to use. This is because non-living macroalgae may be applied right away following sample treatment. Adsorption capacity can be increased by physical treatment, such as shrinking the biomass and immobilizing it with calcium alginate.

CHAPTER 3

METHODOLOGY

3.1   Overview

This chapter went through the study's methodology. **Figure 3.1** depicts the research method flow, from material preparation through sample analysis. The next part intended to go through a full explanation of the technique used in the research.

3.2   Research of flow chart

The general procedure for employing immobilized *Eucheuma Cottonii sp*. to biosorber copper ions from aqueous solutions is shown in **Figure 3.1** below. By using a HACH Spectrophotometer DR5000, the concentration of synthetic copper aqueous solution was evaluated. Determine the efficacy of immobilized seaweed in terms of contact time, dosage, and initial concentration using a biosorption research.
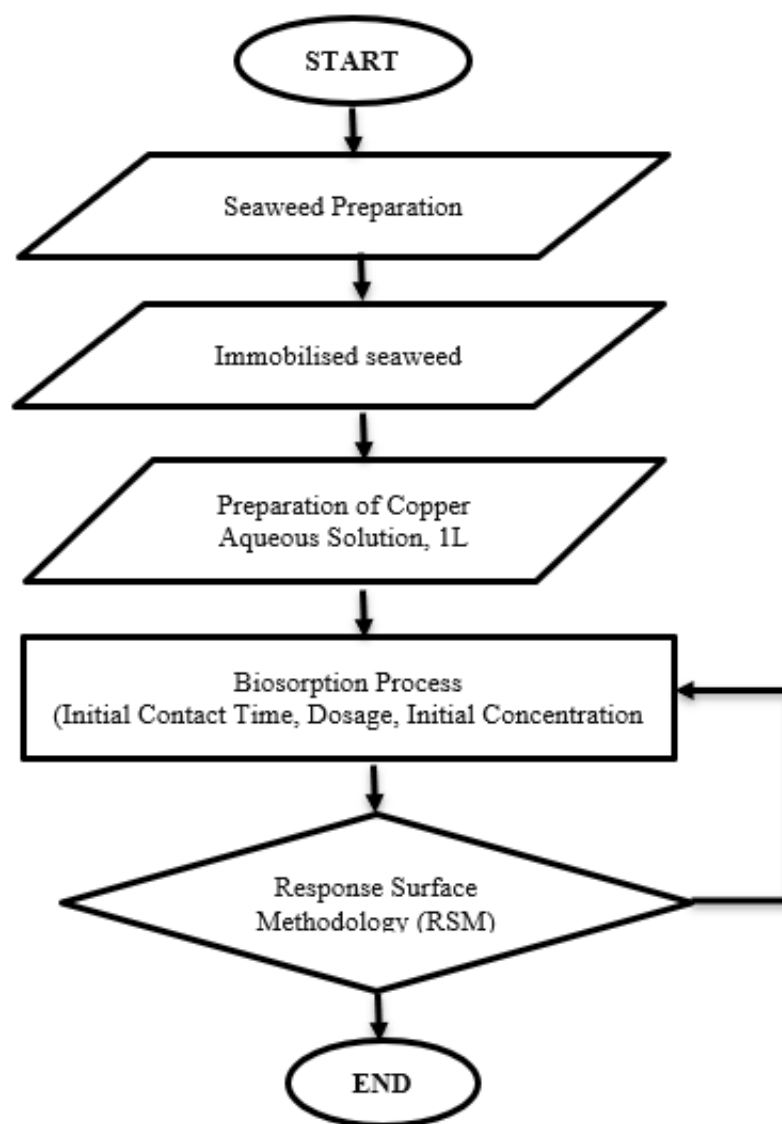
Figure 3. 1   Process flow diagram

3.3   Material

Sodium alginate is the substance utilized in research for immobilized algae (Duchefa Biochemistry). Copper standard solution traceable to SRM from NIST Cu(NO3)2 in HNO3, 0.5 mol/l 1000mg/l Cu Certipur® is the substance used to make the aqueous solution (R&M Chemicals). Also utilized to harden the immobilized beads is calcium chloride (Bendosen). To change the pH of an aqueous solution, hydrochloric acid, HCL and sodium hydroxide, NaOH (HmbG Chemicals) are utilized.

3.3.1 Chemical

This research made use of sodium alginate, calcium chloride, CaCl2, acid hydrochloric, HCL, natrium hydroxide, NaOH, copper standard solution, Cu(NO3)2 and copper buffer pillow reagent for copper testing.

3.3.2 Equipment and apparatus

Table 3. 1   List of the equipment and apparatus used in the experiment

No.	Apparatus	Specification
1.	Sieve	Size: 600μm
2.	Beaker	100ml, 250ml, 500ml
3.	Dry mill blender	Brand: butterfly
4.	Volumetric flask	1000 ml
5.	Measuring cylinder	25 ml, 50ml, 100 ml, 500 ml, 1000 ml
6.	pH meter	Brand: MITTLER TOLEDO **Seven**Compact
7.	Analytical balance	Manufacture: Sartorius
8.	Spectrophotometer	HACH UV-VIS Spectrophotometer DR5000
9.	Spatula	-
10.	Filter paper	540 Harden Ashless, CAT No.145
11.	Rotating Lab Shaker	Rs16000
12.	Centrifuge	1000rpm-4000rpm
13.	Centrifuge Tube	NEST 15/50ml
14.	HACH Sample cell	10ml
15.	Oven	Memmert UNB100

3.4   Experimental Procedure

3.4.1   Preparation of Aqueous Solution

Aqueous solution with concentration 1000ppm was prepared by dissolving 150ml of copper standard solution in 850mL of distilled water. The solution was prepared by using a 1000mL beaker. The 100 ppm of the working solution was prepared by diluting the copper standard solution with distilled water.

3.4.2   Preparation of Red Algae, *Eucheuma Cottonii sp.*

The red algae, *Eucheuma Cottonii sp.* was collected from factor of algae in Sabah. The *Eucheuma Cottonii sp.* were thoroughly soak with tap water to remove irrelevant

debris and salts from the surface of the *Eucheuma Cottonii sp.* before being rinsed with distilled water. Following washing, the biomass was then dried in a Memmert oven (UNB100) at 70 °C for 24 hours. The dried *Eucheuma Cottonii sp.* then grinding in a blender to create particles with an average size of sieve at 600μm and stored in tight plastic bag as shown in **Figure 3.2** (Mata YN et al., 2009).
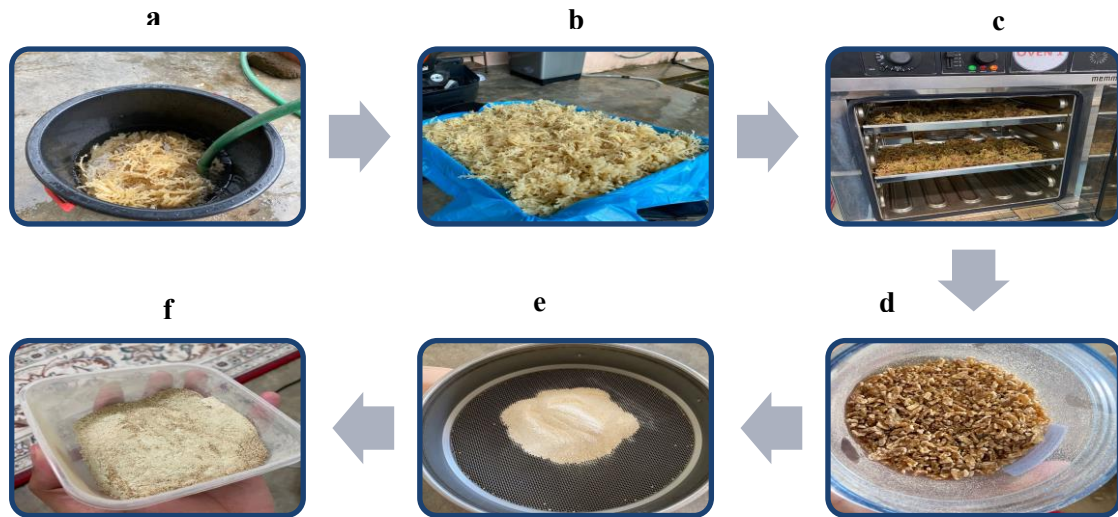

Figure 3. 2   Preparation of seaweed; **a)** Soaking, **b)** Washing, **c)** Drying, **d)** Grinding, **e)** Sieving and **f)** Seaweed powder

3.4.3   Immobilization of Red Algae, *Eucheuma Cottonii sp.*

3% (w/v) sodium alginate was prepared and mixed with 0.25 g of dry algae. The alginate alga mixture was then introduced into a 3% (w/v) calcium chloride (CaCl2) solution by using a syringe. The alginate beads were formed and stirred together with the magnetic stirrer at 3500 rpm and leave for 30 minutes. Then removed the alginate bead from calcium chloride solution and wash with distilled water to remove the remaining CaCl2. The beads were dried by using filtered paper and left it to expose to the air for 1 hour (Ahmad et al., 2018).
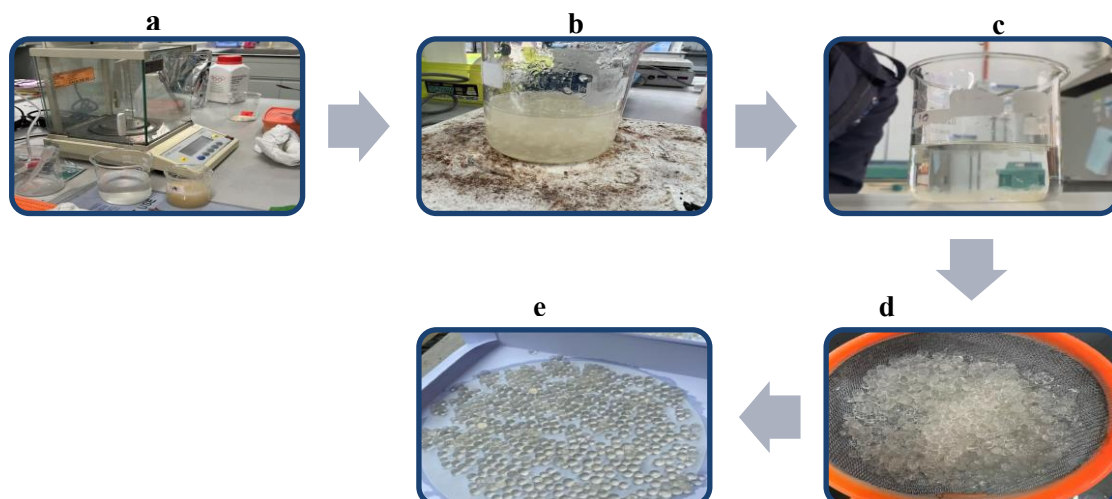
Figure 3. 3   Preparation of biosorbent; **a)** weighing of Sodium alginate, CaCl2, seaweed powder, **b)** Stirring of alginate mixture, **c)** Introducing alginate beads into CaCl2 solution, **d)** Filter the immobilize alginate beads, **e)** Drying of immobilize seaweed by air surrounding.

3.5   Biosorption Experimental Procedure

The biosorption process that is studied in this research is to identify the effectiveness of immobilized algae with sodium alginate. The experiments were undergoing to determine the performance of contact time, dosage and initial concentration. Initially, the pH of the aqueous solution was adjusted with 0.1 M HCl and 0.1 M NaOH. In all contact time experiments, 0.1 g of immobilised *Eucheuma Cottonii sp.* biomass was contacted with 10ml of copper solution at pH 6.97 in a 25 ml glass vial. The mixture was then shaken for 5, 10, 20, 30, and 40 minutes while operating at 80 revolutions in 40 minutes (rpm). Samples were centrifuged at 4000 rpm for 30 minutes after they reached equilibrium, and the supernatant liquid was then analyzed in a spectrophotometer (UV-vis spectrophotometer, DR5000, Arachem) (V. Vijayaragavan er al., 2014). **Figure 3.4** shows the flowchart for the whole biosorption experiment.

Figure 3. 4   pH adjustment

Figure 3. 5   Flowchart of biosorption process

The efficiency of copper removal in percentage can calculate by using **Equation 3.1** (Rahman et al., 2016):

$$\text{Metal Removal, R (\%)} = \frac{c_i - c_f}{c_i} \times 100 \qquad \textbf{(Equation 3.1)}$$

3.5.1 Effect of biosorption condition

The one-factor-at-a-time (OFAT) technique investigates one parameter while leaving the rest. The optimal contact time, biomass dosage, and initial concentration for adsorption were referred to from previous studies and utilised as a reference in this work respectively. A sample cells was used for batch investigations.

3.5.1.1 Effect of initial contact time

The five samples each had 10ml of copper solution with a fixed pH and a biomass dosage of 0.1g immobilised seaweed. Following the addition of the biosorbent, all samples are shaken at 80rpm for 5, 10, 20, 30 and 40 minutes. Finally, the samples are filtered using filter paper and the final concentrations are determined using a spectrophotometer.

Figure 3. 6   Flowchart of process initial contact time was taken

3.5.1.2   *Effect of dosage*

Each of the five samples included 10ml of copper solution with a constant pH of 6.97. Then, in each sample, add 0.1g, 0.2g, 0.3g, 0.4g and 0.5g. After adding the biosorbent, all samples are agitated for 40 minutes using a mechanical shaker set to 80 rpm. Finally, the samples are filtered using filter paper and the final concentrations are determined using a spectrophotometer.

Figure 3. 7   Flowchart of process dosage was taken

3.5.1.3   *Effect of initial concentration*

The five samples each included 10ml of copper solution with a fixed pH and a biomass dosage of 0.1g immobilised seaweed. Following the addition of the concentration to all samples from 1ml, 2ml, 3ml, 4ml, and 5ml. After adding the stock of copper solution, all samples are agitated for 40 minutes Finally, the samples are filtered using filter paper and the final concentrations are determined using a spectrophotometer.
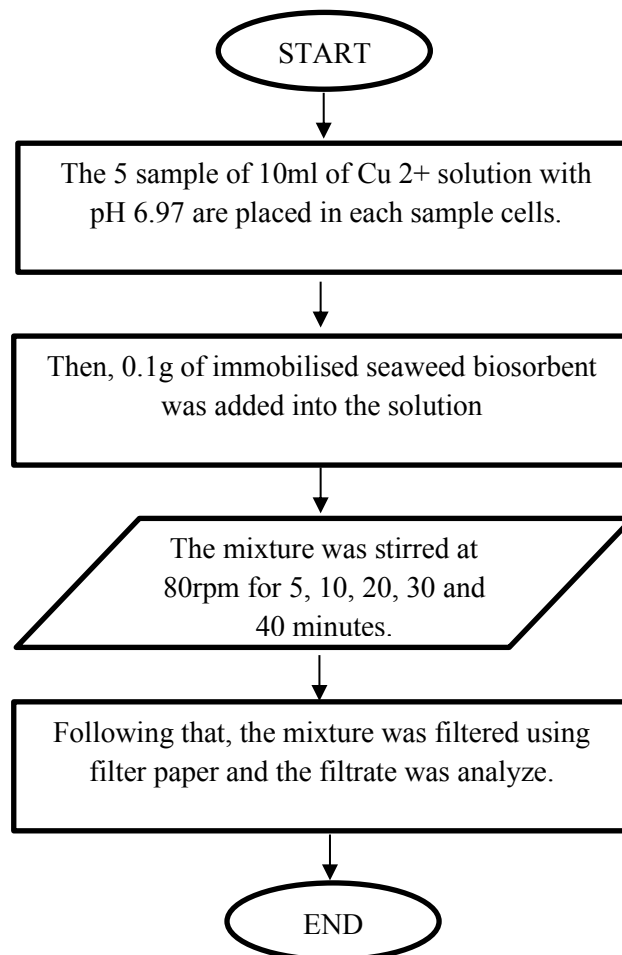

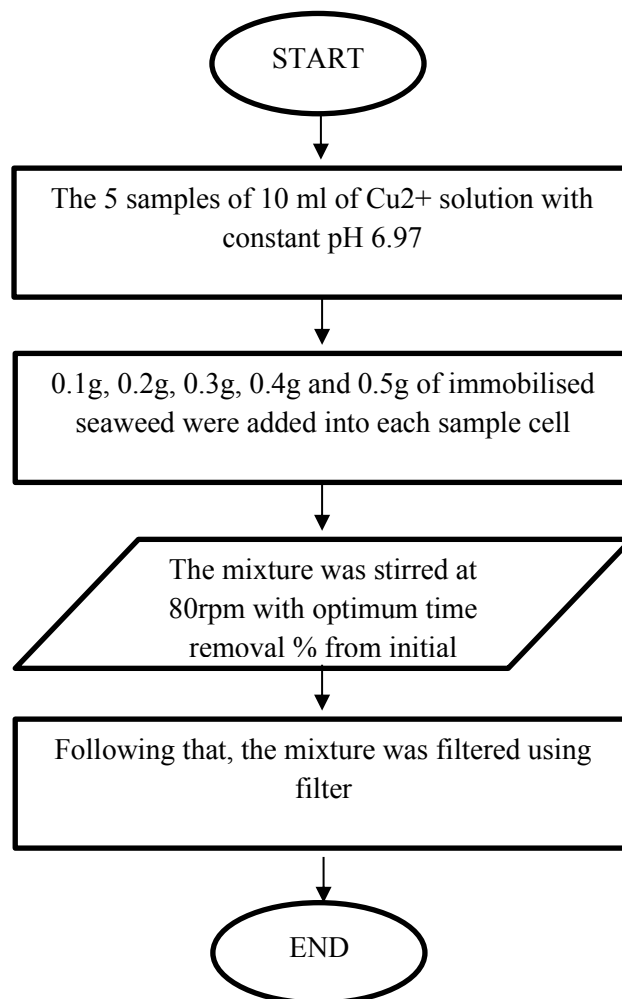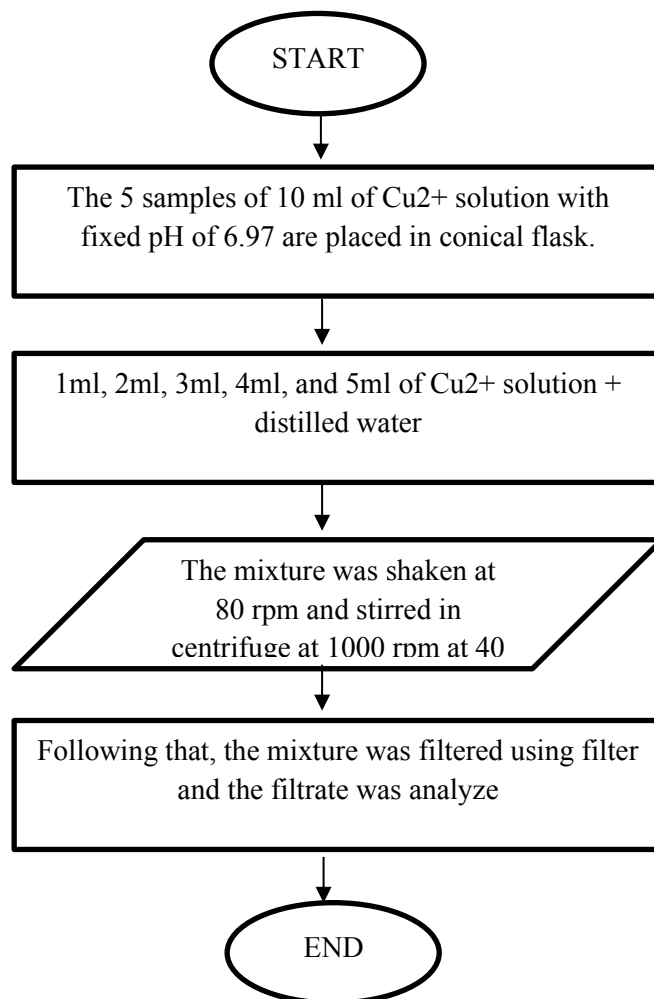**Figure 3. 8**   Flowchart of process initial concentration was taken

3.5.2 Response surface methodology (RSM) for biosorption optimization

Response surface methodology (RSM) is a multivariable optimization tool used to find the optimal response of a process which is a function of several independent variables through fitting the experimental data to a second-order equation. The three major steps of RSM include design of experimental matrix, development of mathematical model and optimization of response of interest

The effect of initial contact duration, dosage and initial concentration on the removal efficiency of immobilised seaweed was investigated in this research. The goal of applying RSM, Stat-Ease-Design-Expert-version 7.0.0 was to determine the best operation in the design space for multi-metal biosorption. A Central Composite Design (CCD), a sort of response surface design, was adopted (Anderson and Whitcomb 2005), which was capable of doing regression analysis on the experimental data and assessing the impact of each variable. A design of 20 experimental runs in five levels (coded values: -, -1, 0, +1, and +) was developed, with four factorial design points (+/-1), four-star points (=2) representing the axial design points (+/-), and six duplicated centre points in **Table 3.2** below.

Table 3. 2   Experimental design for Cu ions biosorption

Factors	Levels			
	-1 Level	**+1 Level**	**-alpha**	**+alpha**
Contact Time, (min)	28.3103	96.6897	5	120
Dosage, (g/L)	0.282428	0.817572	0.1	1
Initial Conc. (mg/L)	10.0675	24.9325	5	30

The range of variables was chosen based on the preliminary single metal studies described in the next section. The Design Expert computer computed coded values of the input variables (or factors) using **(Equation 3.2)**, and the behaviour of the system in this work for competitive metal biosorption with N input factors was evaluated using an **(Equation 3.3)** depicts an empirical quadratic model:

$$Xi = \frac{Xi - Xo}{\Delta X} \quad \textbf{(Equation 3.2)}$$

$$Y = b_0 + \sum_{i=1}^{N} b_i X_i + \sum_{i=1}^{N} b_{ii} X_i{}^2 + \sum_{i=1}^{N-1} \sum_{j=2}^{N} b_{ij} X_i X_j \quad \textbf{(Equation 3.3)}$$

Where Xi and Xj...X_k are variables (or factors), X_0 is the X_i value at the centre point, xi are the coded levels of factors, ΔX is the step change value, Y is the response, and b_0, b_i, b_{ii} and b_{ij} are the coefficients of terms in the regression equation. All batch experiments in this paper were performed in triplicate, and the mean values of three data sets are provided.

3.6   Effect of free cell and immobilization seaweed

0.25 g of freely and immobilised algae were introduced to 50 ml of 25 mg/L of a metal solution in pH 5 and in 50 oC shaker at 250 rpm for 90 minutes to guarantee equilibrium adsorption (Ahmad et al., 2018). The most effective approach will be chosen for the next trial.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Result Overview

In this study, it consists 3 objectives consists of to prepare immobilize seaweed to be used as a biosorbent for copper removal, to determine the performance of immobilised seaweed in terms of initial contact time, dosage and initial concentration and to optimise the performance of immobilised seaweed by using Response Surface Methodology (RSM).

4.2 Effect of Contact Time

By gradually lowering the concentration of copper in the solution, it was possible to calculate the contact time for copper biosorption on immobilized *Eucheuma Cottonii* biomass. A biosorbent dosage of 0.1 g, an initial copper concentration of 10 mg/L, an initial solution pH of 6.0, and a temperature of 29 °C were used in an experiment to determine the impact of contact time on copper ion uptake during a time period of 20–100 minutes (Seyed et al., 2017).

As shown in **Figure 4.1**, the biosorption rate of copper began at 5 minutes (79.92%) and was initially rapid because the majority of the binding sites were free and available for copper biosorption. It took 30 minutes (90%) to reach equilibrium. The later stages of the biosorption process seem to have slowed down in minute of 40 (80.60%) of removal. Because there were many unoccupied binding sites available for copper in the early stages of the biosorption process, as opposed to later stages when biosorption saturation rendered the rest of the unoccupied binding sites in the cell membranes inaccessible (Ho et al., 1999). The maximum copper uptake was attained at 95% in 30 minutes. The findings support earlier research by Md et al. on heavy metal biosorption using *Eucheuma Cottonii sp.* (2015).

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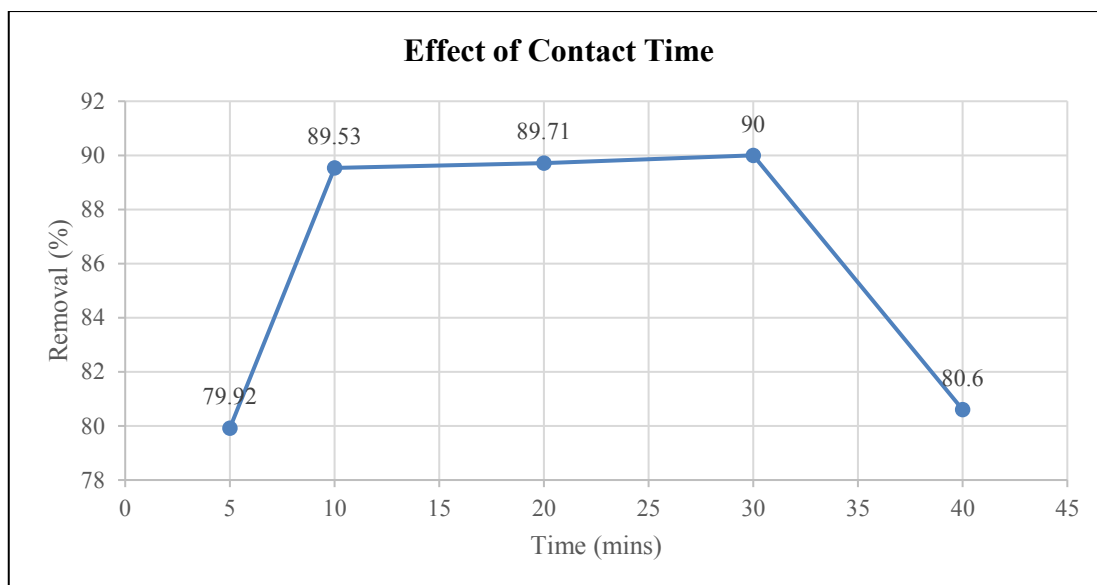
Figure 4. 1 The effect of contact time on copper removal using immobilised *Eucheuma Cottonii sp.* biosorbent

4.3 Effect of Dosage

The effect of *Eucheuma Cottonii* dosage on methylene blue biosorption was investigated using dosages ranging from 0.1 to 0.5 g with an optimised pH of 6.97, temperature 29°C and an initial concentration of 10 ml (Seyed et al., 2017). The dosage of a biosorbent has a significant impact on the extent of biosorption. The uptake capacity increased with decreasing biosorbent dosage, which could be due to interference between the biosorbent's binding sites (Abeer et al., 2019).

Figure 4. 2 The effect of dosage on copper removal; **a)** Blank and sample before biosorption, **b)** Copper solution before biosorption **c)** Blank and sample after biosorption and **d)** Copper solution after biosorption process

The results revealed that the efficiency of copper removal increases with increasing seaweed dosage. An increase in biomass concentration generally increases the biosorbed copper ion due to an increase in biosorbent surface area, which increases the binding sites. For this research, 92.08% copper removal was recorded for 0.1 g/ml seaweed dosage, 89.82% for 0.2 g/ml, 89.26% for 0.3g/ml, 92.48% for 0.4g/ml and optimum removal at 93.09% for 0.5 g/ml shown in **Figure 4.3**. Increased biosorbent dosage, on the other hand, reduces copper uptake. This could be due to the complex interactions of several factors, the most important of which is that at high seaweed dosages, the available copper molecules are insufficient to cover all the exchangeable sites on the seaweed surface, resulting in low copper uptake. Furthermore, increased sorbent dosages will cause interference between binding sites, resulting in low copper uptake. The current study found that 4.75 mg/g of seaweed at 2 g/L increased to 19.4 mg/g at 5 g/L. Vijayaraghavan et al., 2006 was noticed this trend. After analyzing the results, the 5 g/L seaweed dosage was determined to be the most effective at removing methylene blue.
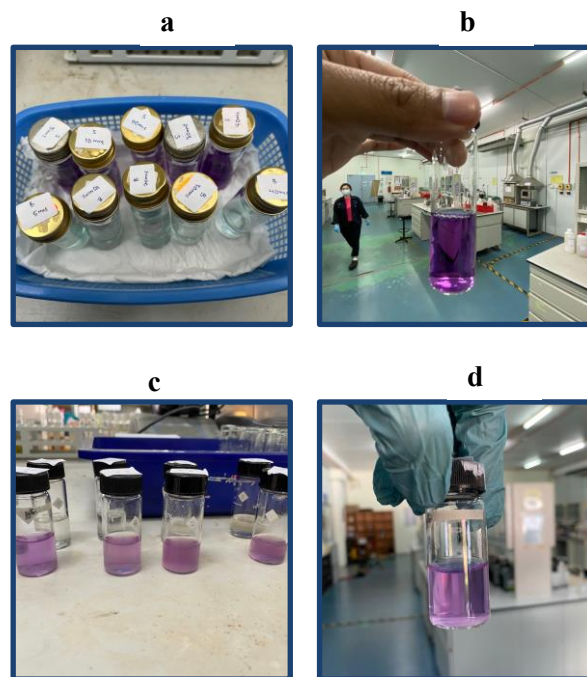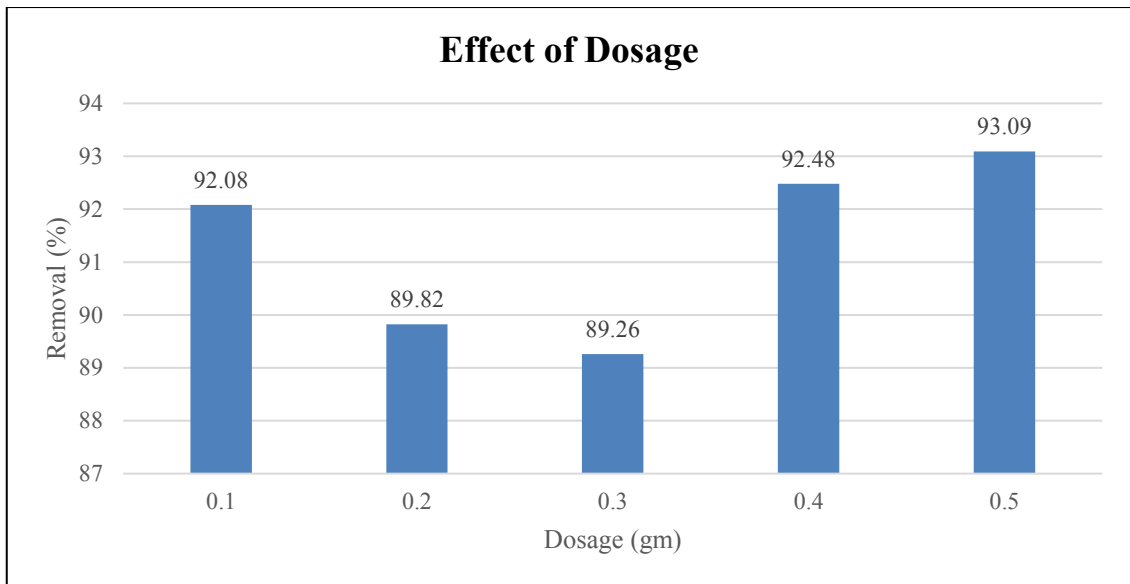
Figure 4. 3   The effect of dosage on copper removal using immobilised *Eucheuma Cottonii sp.* biosorbent

4.4   Effect of Initial Concentration

In 10ml of test solution made using copper stock solution at various beginning copper concentrations (1.0, 2.0, 3.0, 4.0, and 5.0 mg/L), the impact of initial copper concentration on biomass adsorption was examined. Except as otherwise specified, the equilibrium time, dosage, and starting concentration were all maintained constant. After biosorption, it was found that a homogeneous result with a final concentration of 0.37 and a removal of 72.38% had been generated. To get this outcome, a spectrophotometer was employed. This result may be obtained in 40 minutes total. As the starting concentration rose, the proportion of biosorption decreased while the capacity for metal ion adsorption increased. We discovered that although the capacity for metal ion adsorption reduced as the quantity of biomass rose, the proportion of biosorption increased (Rezaei, 2016). A homogenous result was achieved from the final concentration of 0.37 with a clearance of 72.38% after biosorption, it was discovered. With the use of a spectrophotometer, this result was obtained. This result took 40 minutes to get in actual time.
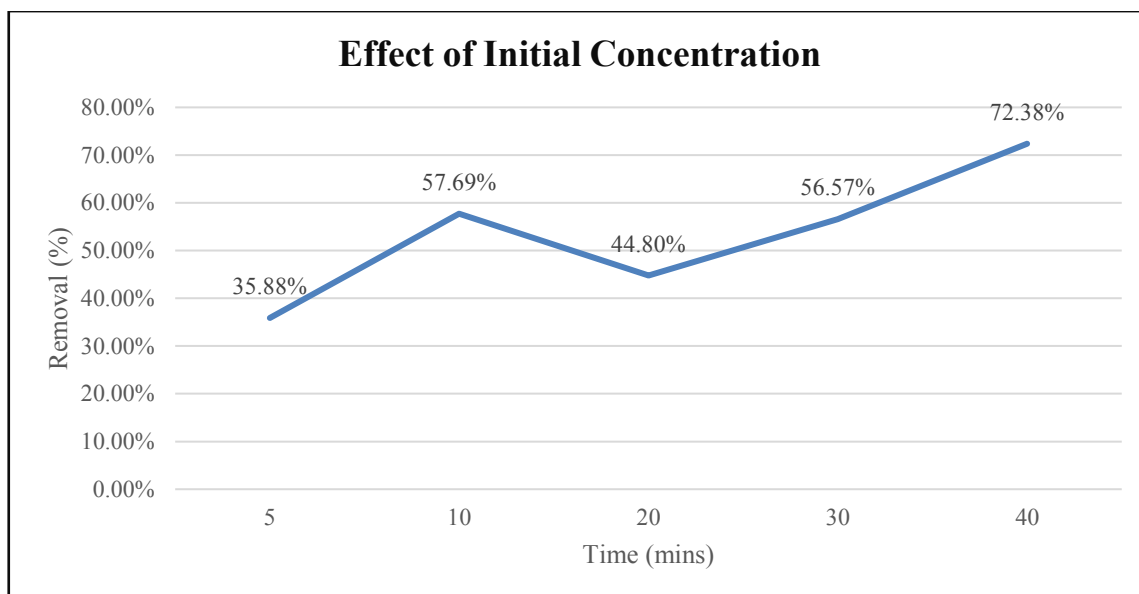
Figure 4. 4   The effect of initial concentration on copper removal using immobilised
Eucheuma Cottonii sp. Biosorbent

4.5   Optimisation using Response Surface Methodology (RSM)

In order to reduce the number of trials required to assess interactions between independent factors and response with the main objective of responding optimally, RSM was employed in this work to develop an experimental design matrix. The parameters were established using the OFAT technique between Low (-1) and High (+1) levels to allow the use of the quadratic model in the study. Customized data ranges and parameter settings were used for RSM analysis.

Central composite design (CCD) was used for the optimization of Cu2+ biosorption onto spent immobilised *Eucheuma Cottonii sp*. by taking input variables as shown in **Table 3.2**. A total of 20 experiments designed by CCD were conducted, and the obtained results were used for the analysis. Complete design matrix generated using CCD with coded variables, response reported from experiments and predicted response for biosorption of Cu2+ onto spent immobilise *Eucheuma Cottonii* is given in **Table 4.1.**

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4.6   Center Composite Design (CCD)

The aim of optimisation is to control the biosorption process in achieving optimum condition at (1) minimal dosage, (2) reasonable condition, and (3) high removal. The experimental data were modelled using CCD for optimisation as CCD is more capable of fitting second-order model (Reza et al., 2019). Three significant variables were studied: initial concentration (mg/L), dosage (g/L) and initial contact time.

Table 4. 1   Numeric factors in Central Composite Design (CCD)

	Name	Units	-1 Level	+1 Level	-alpha	+alpha
A:	Contact time	mins	28.3103	96.6897	5	120
B:	Dosage	g/L	0.282429	0.817572	0.1	1
C:	Initial Concentration	mg/L	10.0675	24.9325	5	30

Table 4. 2   The design (actual) range of contact time, dosage and initial concentration variables input and the percentage of removal (%) for 20 experiments run

Std	Run	Block	Factor 1: A: Contact time mins	Factor 2: B: Dosage g/L	Factor 3: C: Initial Concentration mg/L	Response 1 Removal %
9	1	Block 1	5.00	0.55	17.50	33.78
7	2	Block 1	28.31	0.82	24.93	48.85
16	3	Block 1	62.50	0.55	17.50	56.35
18	4	Block 1	62.50	0.55	17.50	46.35
15	5	Block 1	62.50	0.55	17.50	49.32
13	6	Block 1	62.50	0.55	5.00	52.24
8	7	Block 1	96.69	0.82	24.93	51.75
19	8	Block 1	62.50	0.55	17.50	51.52
11	9	Block 1	62.50	0.10	17.50	52.22
20	10	Block 1	62.50	0.55	17.50	61.25
6	11	Block 1	96.69	0.28	24.93	48.32
4	12	Block 1	96.69	0.82	10.07	72.22
10	13	Block 1	120.00	0.55	17.50	81.52
12	14	Block 1	62.50	1.00	17.50	76.61
14	15	Block 1	62.50	0.55	30.00	57.78
17	16	Block 1	62.50	0.55	17.50	51.25
2	17	Block 1	96.69	0.28	10.07	49.52
5	18	Block 1	28.31	0.28	24.93	31.24
1	19	Block 1	28.31	0.28	10.07	57.75
3	20	Block 1	28.31	0.82	10.07	61.12

4.7   ANOVA for response surface quadratic model

Analysis of variance (ANOVA) was used to determine the significance of each individual process variable as well as the overall model significance. The very low "model P value" (0.0050) and high "lack of ft P value" (0.0788) in the ANOVA results reveal the model's statistical significance. The results also demonstrated that the experimental values and model-predicted values are well-linearized, as indicated by the fact that the value of (R^2adj= 0.4554) is in good agreement with the value of (R^2pred= 0.1980). The ANOVA analysis indicates that the model can be used to explore the design

options for Cu2+ biosorption. A, B, C, AB, and A2 appear to have been significant model terms based on the probable P values of each individual factor and their interactive terms. The combined effects of dosage and initial concentration were more significant than the individual parameter effects, which included initial contact time, dosage and initial concentration biosorbent. The pH square effect has also demonstrated some modest significance for biosorption. Liu et al. (2018) reported comparable outcomes for the biosorption of copper using rapeseed powder.

For this study, predicted that the variables of initial concentration and dosage were highly significant ($p < 0.05$) in biosorption process for both responses. For biosorption capacity, $p < 0.05$ indicates that pH was significant contributing factor in copper biosorption efficiency. The interaction AE, BC, BD and CD were significant at 0.5% probability level.

Table 4. 3   Analysis of variance table (ANOVA) for removal efficiency (R) response

Source	Sum of Squares	df	Mean Square	F value	p-value Prob >F	Significant/Not Significant
Model	15.39.07	3	513.02	6.30	0.0050	Significant
A-Contact time	778.92	1	778.92	9.56	0.0070	Significant
B-Dosage	568.70	1	568.70	6.98	0.0178	Significant
C-Initial Concentration	191.45	1	191.45	2.35	0.1449	Significant
Residual	1303.77	16	81.49			
Lack of fit	1162.11	11	105.65	28.33	0.0788	Not Significant
Pure Error	141.66	5	28.33			
Car Total	2842.84	19				

Figure 4.5 below show that the copper ion removal had increased from 45.21% to 63.88% with the increase of dosage from 0.28 to 0.82 g/L which can be attributed to the higher number of active sites for biosorption process.
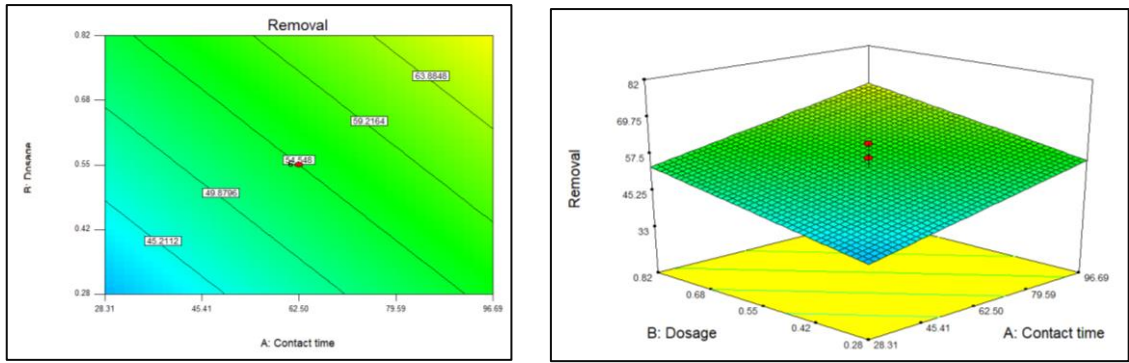
Figure 4. 5   Contour and 3D surface diagram for copper ion removal efficiency as function of 3 parameters

4.8   Numerical Optimisation

Numerical optimisation is design expert software was set to allow optimisation process. In this method, the optimal process was generated by specifying the target value for initial concentration at 10.98mg/L, whilst keeping rest (contact time and dosage) within the range value (Table 4.1). By determining the desirability score 1.0, a maximum predicted copper removal of 88.80% was obtained at optimised conditions. The conduction of six replicates at the specified optimum conditions showed good agreement with predicted results. The obtained optimum conditions were validated by running similar experiments at the specified optimum conditions, and the results were found to be 88.4 +/- 0.4. This confirms the applicability of CCD in predicting the real behaviour of biosorption study.

Table 4. 4   Validation of optimisation process between experimental work and predictive conditions specified by RSM-numerical optimisation

Validation	No.	Initial Concentration	Contact Time	Dosage	Removal (%)
Predictive Experimental work	1	10.98	76.94	0.78	66.60
	2	10.95	79.47	0.73	65.91
	3	12.33	81.17	0.68	64.38

Based on the result of this research, immobilised *E. Cottonii* from red seaweed has been chosen as a potential biosorbent and was used for the entire experiment. The optimisation analysis predicted for copper ion removal was achieved.

CHAPTER 5

CONCLUSION, RECOMMENDATION AND COMMERCIAL

5.1   Introduction

According to the study's purpose, a conclusion has been achieved in this chapter. The project's objectives have been met with success. This chapter wraps up the preceding chapter's findings and discussion. In addition, based on the findings, a few recommendations are made to enhance the efficiency of biosorption in removing Cu from synthetic wastewater by using optimisation of model State-Ease Design Expert version 7.0 by Ching Liu of Response Surface Methodology (RSM).

The interaction of contact time and dosage in this study affected the biosorption of copper removal by red algae, *Eucheuma Cottonii sp*. The main finding is the achievement of copper equilibrium after 40 minutes. Copper uptake was attained at 90% in 30 minutes. The best biosorbent dosage was determined to be 0.5g of immobilised seaweed because it had a high uptake rate and the highest copper removal efficiency (93%). According to the RSM analysis, the biomass cell wall matrix's carboxylate salt, acyl oxygen, and ester carbonyl groups were in charge of the dye ion absorption. According to the study's findings, *Eucheuma Cottonii* immobilised is an excellent choice for the removal of wastewaters containing copper ions.

5.2   Recommendations

There have been numerous studies on copper removal from various sources using various types of biosorbent. However, there are many improvements that can be made to promote an effective method of copper removal. The following is my recommendation for the next project:

1.   As the source dye, use real industrial wastewater. This can validate the biosorbent's ability to remove dye from industrial waste.

2.   A comparison of the dye removal performance of various types of biosorbents. This can be used to identify the most effective biosorbent for dye removal.

3.   Examine the performance of biosorbent desorption and regeneration in removing dye. Regeneration of biosorbent is a critical step in making the biosorption process more cost-effective for industrial use.

4.   For biosorption isotherm studies, use the isotherm model (Henry's isotherm, Dubinin-Radushkevich isotherm, Temkin isotherm, Hill-Deboer model, Langmuir isotherm model, and Freundlich isotherm model).

5.3   Commercial Potential

The cost of the biosorbent is one of the key elements that makes biosorption an economically viable process. This technology can be made more economically viable than traditional ones by using waste or renewable raw materials. Thus, immobilization of algae biomass was essential for the implementation of industrial biosorption. Additionally, biomass algae have a significant capacity to remove heavy metals from aqueous solutions. This can be explained by the fact that algae have a different functional group that is advantageous to heavy metal ions on the cell wall. It is suitable for removing heavy metals.

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APPENDICES

APPENDIX A 1

Process preparation of seaweed *(Euchuema Cottonii sp)*

APPENDIX A 2
Process preparation of Immobilised Seaweed