Equilibrium and Kinetic studies on removal of Copper from aqueous solution using Shorea Robusta (Sal Leaves)

A Major Project Report

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BACHELOR OF TECHNOLOGY

in

CHEMICAL ENGINEERING

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REFIFNING AND PETROCHEMICALS

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DECLARATION

I hereby declare that this submission is my own and that, to the best of my knowledge and belief, it contains no material previously written or published by another person nor material which has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement has been made in the text.

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CERTIFICATE

This is to certify that the thesis titled **Equilibrium and Kinetic studies on removal of Copper from aqueous solution using Shorea Robusta (Sal Leaves)** submitted by Bakul Sharma (R900213016), Devansh Sharma (R900213020), Satyam Priyadarshi (R900213040), to the University of Petroleum and Energy Studies, for the award of the degree of **BACHELOR OF TECHNOLOGY** in Chemical Engineering with specialization in Refining and Petrochemicals is a bonafide record of project work carried out by them under our supervision and guidance. The content of the thesis, in full or parts have not been submitted to any other institute or university for the award of any other degree or diploma.

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NOMENCLATURE

Symbol	Description	Dimension
A	Absorbance	Dimensionless
ε	Molar Absorptivity	$mol^{-1}m^2$
C	Concentration	mol $liter^{-1}$ or ppm
L	Cell Length	m
I_o	Intensity of incident light	W m^{-2}
I	Intensity of transmitted light	W m^{-2}
T	Transmittance	Dimensionless
k	Rate constant	Zero order: ppm min^{-1}
		First order: min^{-1}
R^2	Regression Coefficient	Dimensionless

ABSTRACT

The presence of metal ions in final industrial effluent is extremely undesirable. In recent years, the excessive release of heavy metals into the environment is a major concern worldwide. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage. Metals such as copper, zinc, lead, cadmium, arsenic, and chromium (VI) are regarded as toxic. The MCLG for copper is 1.3 mg/L or 1.3 ppm. EPA (Environmental Protection Agency) has set this level of protection based on the best available science to prevent potential health problems. Many methods such as ion-exchange, chemical precipitation, reverse osmosis, adsorption can be used to remove the metal ions, but Biosorption was found to be the most effective, relatively cheaper and less harmful by-product is produced. In order to study the removal of Copper ions from its aqueous solution, a biological adsorbent or biosorbent was used, called Shorea Robusta, commonly known as Sal Leaves. Optimization of contact time and pH was done and the effect of dosage of bio-sorbent, initial metal concentration and size of adsorbent on the amount of metal removed was studied. The maximum removal of copper was attained at pH 8, biosorbent dose of 0.1g per 100 ml of sample, at 180 minutes with metal concentration of 20 mg/L or 20 ppm. With a thorough study of the conducted experiments, it can be concluded that the MCLG of copper (1.3 ppm) can be met with optimization of all the parameters studied.

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CHAPTER 1
INTRODUCTION

The presence of metal ions in final industrial effluent is extremely undesirable. In recent years, the excessive release of heavy metals into the environment is a major concern worldwide. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage. Metals such as copper, zinc, lead, cadmium, arsenic, and chromium (VI) are regarded as toxic. These pollutants are introduced in to the aquatic systems significantly as a result of various industrial operations. Zinc and Copper are the prime metals, which are used in manufacturing processes of galvanizing, sheets for roofing and guttering, metal plating, radiator, dye casting, alloys, chemicals, pigments and finally exits from the industry and mining areas as pollutants in the effluent.

Various techniques like precipitation, adsorption, ion exchange, membrane separation and electrochemical technologies can be used to remove such hazardous metals. However, most of these methods require high capital cost, skilled supervision, requires post treatment, produce toxic byproducts and are not environmentally friendly. Much has been exhibited lately in the use of adsorption technique for removal of heavy metals from industrial waste water. Hence, biomass materials are now a better and economically as well as environmentally friendly substitute. Biosorption is a property of certain types of inactive, dead microbial biomass to bind and concentrate pollutants from aqueous solutions. These materials are typically alive or dead microbial biomass, agricultural by-products and industrial wastes.

Using Ultraviolet and Visible absorption spectroscopy, the presence and concentration of metal ions in an aqueous solution can be determined even when it is in littlest of amount. Absorption measurement can be a single wavelength or over an extended spectral range.

The excessive release of heavy metals has become a worldwide concern in the recent years. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage. Metals such as copper, zinc, lead, cadmium, arsenic, and chromium (VI) are regarded as toxic. Precipitation, adsorption, ion exchange, membrane and electrochemical technologies are the methods being used currently to treat the industrial wastewater and remove the metals present in it. However, these methods either require a high capital cost or require

skilled supervision or produce toxic byproducts. Therefore, a new method which is inexpensive and can efficiently and effectively remove the heavy metals from the industrial wastewater is being investigated. In recent years, Biosorption has proved to be an effective method in removal of heavy metals.

In 1974, the US ruling party, Congress, passed the Safe Drinking Water Act. This law requires EPA to determine the level of contaminants in drinking water at which no adverse health effects are likely to occur. These non-enforceable health goals, based solely on possible health risks and exposure over a lifetime with an adequate margin of safety, are called maximum contaminant level goals (MCLG). Contaminants are any physical, chemical, biological or radiological substances or matter in water.

The **MCLG for copper is 1.3 mg/L or 1.3 ppm**[2]. EPA has set this level of protection based on the best available science to prevent potential health problems.

For most contaminants, EPA sets an enforceable regulation called a maximum contaminant level (MCL) based on the MCLG. MCLs are set as close to the MCLGs as feasible, considering cost, benefits and the ability of public water systems to detect and remove contaminants using suitable treatment technologies. However, because copper contamination of drinking water often results from corrosion of the plumbing materials belonging to water system customers, EPA established a treatment technique rather than an MCL for copper. A treatment technique is an enforceable procedure or level of technological performance which water systems must follow to ensure control of a contaminant. The treatment technique regulation for copper (referred to as the Lead and Copper rule) requires water systems to control the corrosivity of the water.

The regulation also requires systems to collect tap samples from sites served by the system that are more likely to have plumbing materials containing lead. If more than 10 percent of tap water samples exceed the copper action level of 1.3 milligrams per Liter (mg/L), water systems must take additonal steps to reduce corrosiveness.

EPA promulgated the Lead and Copper Rule in 1991, and revised the regulation in 2000 and in 2007. States may set a more stringent regulation for copper in drinking water than EPA.

1.1 BACKGROUND

Industry is a huge source of water pollution, it produces pollutants that are extremely harmful to people and the environment. Many industrial facilities use freshwater to carry away waste from the plant and into rivers, lakes and oceans. Pollutants from industrial sources include:

- **Copper** – If you drink water that contains higher than normal levels of copper, you may experience nausea, vomiting, stomach cramps, or diarrhea.
- **Lead** – This is a metallic element and can cause health and environmental problems. It is a non-biodegradable substance so is hard to clean up once the environment is contaminated. Lead is harmful to the health of many animals, including humans, as it can inhibit the action of bodily enzymes.
- **Mercury** – This is a metallic element and can cause health and environmental problems. It is a non-biodegradable substance so is hard to clean up once the environment is contaminated. Mercury is also harmful to animal health as it can cause illness through mercury poisoning.
- **Heavy metals** from industrial processes can accumulate in nearby lakes and rivers. These are toxic to marine life such as fish and shellfish, and subsequently to the humans who eat them. Heavy metals can slow development; result in birth defects and some are carcinogenic.

Once they enter the food chain, large concentrations of heavy metals may accumulate in the human body. If the metals are ingested beyond the permitted concentration, they can cause serious health disorders. In the near future, the most promising methods to treat such complex systems will be the photo-catalytic ones. They induce both degradation of organic pollutants and recovery of metals in one-pot systems. On the other hand, from the conventional processes, lime precipitation has been found as one of the most effective means to treat inorganic effluent with a metal concentration of >1000 mg/L. It is important to note that the overall treatment cost of metal-contaminated water varies, depending on the process employed and the local conditions. In general, the technical applicability, plant simplicity and cost-effectiveness are the key factors in selecting the most suitable treatment for inorganic effluent.

1.2 OBJECTIVES

- Studying the removal of Copper from aqueous solutions using Sal leaves as adsorbents.
- Optimizing the contact time and pH and studying the effect of dosage of bio-sorbent, initial metal concentration and size of adsorbent on the amount of metal removed.
- Studying the kinetics of Biosorption.

CHAPTER 2
LITERATURE REVIEW

Reverse Osmosis: It is a process in which heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids in wastewater. The disadvantage of this method is that it is expensive.

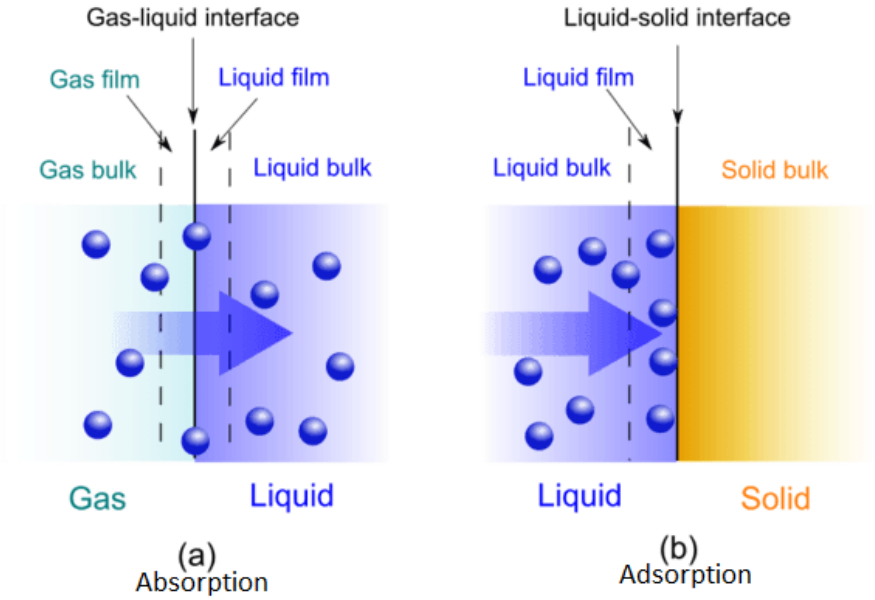
Electrodialysis: In this process, the ionic components (heavy metals) are separated through the use of semi-permeable ion selective membranes. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Because of the alternate spacing of cation and anion permeable membranes, cells of concentrated and dilute salts are formed. The disadvantage is the formation of metal hydroxides, which clog the membrane.

Ultrafiltration: They are pressure driven membrane operations that use porous membranes for the removal of heavy metals. The main disadvantage of this process is the generation of sludge.

Ion-exchange: In this process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. The disadvantages include: high cost and partial removal of certain ions.

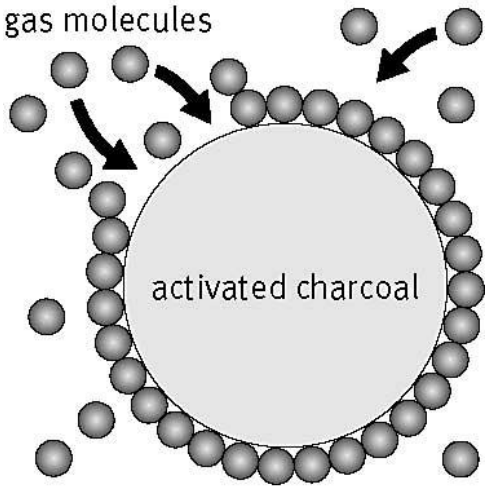
Chemical Precipitation: Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. The large amount of sludge containing toxic compounds produced during the process is the main disadvantage.

Adsorption: Adsorption is the adhesion of atoms, ions or molecules from a gas, liquid or dissolved solid to a surface. This process creates a film of adsorbate on the surface of the adsorbent. Adsorption is a surface phenomenon unlike absorption. Figure 1 shows the difference between the two phenomena.



(Figure 2.1)

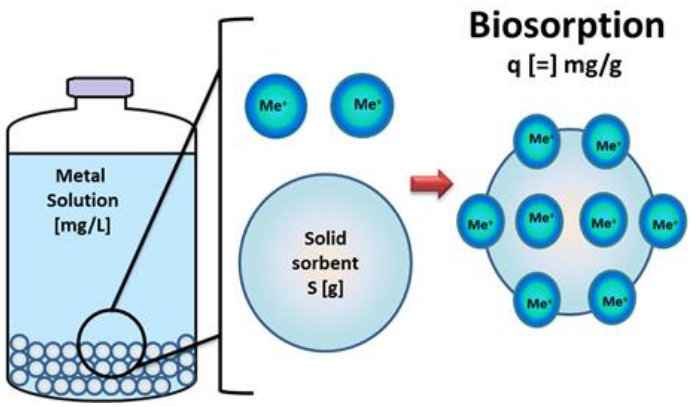
For instance, gas molecules get adsorbed over the surface of activated charcoal in figure 2.



Adsorption on the surface of charcoal

(Figure 2.2)

Biosorption: Biosorption is defined as accumulation and concentration of organic and inorganic pollutants including metals, dyes and odor causing substances, from aqueous solutions by the use of biological materials. Figure 3 below will give a pictorial idea.



Biosorption Process

(Figure 2.3)

Choice of metal for Biosorption process

The appropriate selection of metals for Biosorption studies is dependent on the angle of interest and the impact of different metals, on the basis of which they would be divided into four major categories: (i) toxic heavy metals (ii) strategic metals (iii) precious metals and (iv) radio nuclides. In terms of environmental threats, it is mainly categories (i) and (iv) that are of interest for removal from the environment and/or from point source effluent discharges.

Apart from toxicological criteria, the interest in specific metals may also be based on how representative their behavior may be in terms of eventual generalization of results of studying their biosorbent uptake. The toxicity and interesting solution chemistry of elements such as chromium, arsenic and selenium make them interesting to study. Strategic and precious metals though not environmentally threatening are important from their recovery point of view.

Biosorption Mechanisms

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The Biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria.

According to the dependence on the cell's metabolism, Biosorption mechanisms can be divided into:

1. Metabolism dependent and
2. Non -metabolism dependent.

According to the location where the metal removed from solution is found, Biosorption can be classified as

1. Extra cellular accumulation/ precipitation
2. Cell surface sorption/ precipitation and
3. Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of Biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal.

During non-metabolism dependent Biosorption, metal uptake is by physio-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of Biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988).

In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole, et al. 1994). Further, it may be dependent on the cell's' metabolism if, in the

presence of toxic metals, the microorganism produces compounds that favour the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

Transport across cell membrane

Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically important ions such as potassium, magnesium and sodium. The metal transport systems may become confused by the presence of heavy metal ions of the same charge and ionic radius associated with essential ions. This kind of mechanism is not associated with metabolic activity. Basically Biosorption by living organisms comprises of two steps. First, a metabolism independent binding where the metals are bound to the cell walls and second, metabolism dependent intracellular uptake, whereby metal ions are transported across the cell membrane. (Costa, et.al., 1990, Gadd et.al., 1988, Ghourdon et.al., 1990, Huang et.al., 1990., Nourbaksh et.al., 1994)

Physical adsorption

In this category, physical adsorption takes place with the help of van der Waals' forces. Kuyucak and Volesky 1988, hypothesized that uranium, cadmium, zinc, copper and cobalt Biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells. Electrostatic interactions have been demonstrated to be responsible for copper Biosorption by bacterium *Zoogloea ramigera* and alga *Chiarella vulgaris* (Aksu et al. 1992), for chromium Biosorption by fungi *Ganoderma lucidum* and *Aspergillus niger* .

Ion Exchange

Cell walls of microorganisms contain polysaccharides and bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of K+, Na+, Ca^2+, and Mg^2+. These ions can exchange with counter ions such as CO^2+, Cu^2+, Cd^2+ and Zn^2+ resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky 1988). The Biosorption of copper by fungi *Ganoderma lucidium* (Muraleedharan and Venkobachr, 1990) and *Aspergillus niger* was also up taken by ion exchange mechanism.

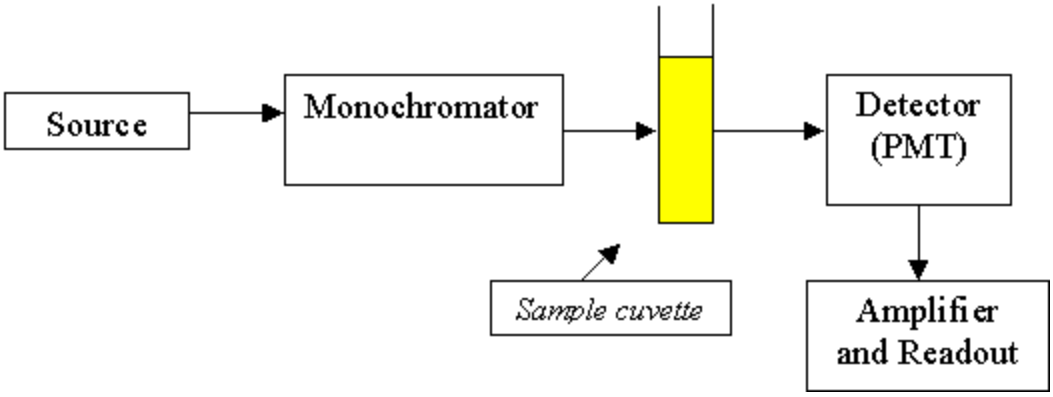
Complexation

The metal removal from solution may also take place by complex formation on the cell surface after the interaction between the metal and the active groups. Aksu et al. 1992 hypothesized that Biosorption of copper by C. *vulgaris* and Z. *ramigera* takes place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae*. Microorganisms may also produce organic acids (e.g., citric, oxalic, gluonic, fumaric, lactic and malic acids), which may chelate toxic metals resulting in the formation of metallo-organic molecules. These organic acids help in the solubilisation of metal compounds and their leaching from their surfaces. Metals may be biosorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers.

Ultraviolet – Visible Absorption Spectroscopy

It is the measurement of attenuation of a beam of light after it passes through a sample or after a reflection from a sample surface. Absorption measurement can be a single wavelength or over an extended spectral range.

A UV-visible spectrometer is a device that displays absorbance of a single wavelength or a narrow band of wavelengths by a sample over a length of radiation path.



Block Diagram of UV Spectrophotometer

(Figure 2.4)

Samples for UV-Visible spectrometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm.

Test tubes can also be used as cuvettes in some instruments. The type of sample container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.

Maximum Contaminant Level (MCL)[1]: The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to the maximum contaminant level goals as feasible using the best available treatment technology.

Maximum Contaminant Level Goal (MCLG): The level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety.

Treatment Technique (TT): A required process intended to reduce the level of a contaminant in drinking water.

Action Level: The concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow.

Maximum Residual Disinfection Level Goal (MRDLG): The level of a drinking water disinfectant below which there is no known or expected risk to health. MRDLGs do not reflect the benefits of the use of disinfectants to control microbial contaminants.

Maximum Residual Disinfectant Level (MRDL): The highest level of a disinfectant allowed in drinking water. There is convincing evidence that addition of a disinfectant is necessary for control of microbial contaminants.

MPL: State assigned maximum permissible level.

Environmental Protection Agency (EPA): EPA was established in December 1970 under United States President Richard Nixon. The EPA is an agency of the United States federal government whose mission is to protect human and environmental health. Headquartered in Washington, D.C., the EPA is responsible for conducting environmental assessment, research and education to create and enforce standards and laws that will promote the health of individuals and the environment. The EPA seeks to protect and conserve the natural environment and improve the health of humans by researching the effects and mandating limits of chemicals and other pollutants. The EPA regulates the manufacturing, processing, distribution and use of chemicals and other pollutants. In addition, the EPA is charged with determining safe tolerance levels for chemicals and other pollutants in food, animal feed and water. The EPA is able to enforce its findings through fines, sanctions and other procedures.

CHAPTER 3
METHODOLOGY

The Biosorption process involves a solid phase (bio-sorbent; biomass material) and liquid phase (solvent; normally water) containing a dissolved species to be adsorbed (bio-sorbate; metal ions).

Biosorption studies will be done using the bio-sorbent as a function of various parameters such as time, dosage of the bio-sorbent, concentration of metal ions, size of the bio-sorbent and pH. To study the effect of various parameters, the following methodology[3] is adopted:

1. The biomass material appropriate for removal of metal ions is selected and subsequently prepared.
2. A stock solution of the metal ions which are to be biosorbed is prepared. All the Biosorption experiments will be carried out using this stock solution.
3. The adsorbent is then added to the solution of the metal ions.
4. The mixture is then kept in a mechanical shaker for a designated amount of time.
5. The sample is then filtered using a Whatman no.42 filter paper.
6. The filtrate samples are then analyzed using UV- Visible Spectrophotometer.

Effect of Contact Time

The effect of contact time on the removal of metal ions is studied by taking samples at regular time intervals and analyzing them under UV-Visible spectrophotometer.

Effect of pH

The effect of pH on the removal of metal ions is studied by varying the solution pH at fixed amount of adsorbent dosage, sorbate concentration and temperature.

Effect of Adsorbent Dose

The effect of adsorbent concentration on the removal of metal ions is studied by varying the dose of adsorbent from 20-100 mg/L at fixed value of pH, temperature, time and sorbate concentration.

Effect of Initial Metal ion Concentration

The effect of initial metal ion concentration on the amount of metal removed is studied using various solutions, with varying concentrations of metal ions. All the solutions should have the same pH, same adsorbent dosage and the experiments should be carried out at the same temperature.

Effect of adsorbent size

The effect of adsorbent size on the removal of metal ions is studied by varying the adsorbent size at fixed amount of pH, temperature, adsorbent dose and sorbate concentration.

Percentage of Biosorption will first be calculated using:

$$\% \text{ of Biosrption} = \frac{(\text{Initial} - \text{final metal concentration})}{(\text{Initial metal concentration})} \times 100$$

Sorption Kinetics[4]

The first order rate equation of Lagergren is one of the most widely used for the sorption of a solute from the liquid solution and is represented as:

$$\ln (C_o - C_t) = \ln C_o - Kt$$

where

$\mathbf{C_o}$ is the initial concentration of metal ions (ppm)

$\mathbf{C_t}$ is the concentration of metal ions at time t (ppm)

K is the first order reaction rate constant (L/min).

The pseudo-first order considers the rate of occupation of adsorption sites to be proportional to the number of unoccupied sites. A straight line of $\ln(C_e - C_t)$ verses t indicates the application of the first order kinetic model.

A pseudo second order equation based on the adsorption equilibrium capacity may be expressed as:

$$\mathbf{t/C_t = (1/K)C_o^2 + t/C_o}$$

where

K is the second order reaction rate equilibrium constant.

A plot of t/C_t verses t should give a linear relationship for the applicability of the second order kinetics.

3.1 SELECTION OF BIOSORBENT

The locally available Shorea Robusta leaves will be used as the adsorbent. It has shown the ability to remove more than 80% of the metal ions from their solutions.

Botanic Description

Shorea robusta is a large, deciduous tree up to 50 m tall and with a dbh of 5 m; these are exceptional sizes, and under normal conditions S. robusta trees attain a height of about 18-32 m and girths of 1.5-2 m; bole is clean, straight and cylindrical, but often bearing epicormic branches; crown is spreading and spherical. Bark dark brown and thick, with longitudinal fissures deep in poles, becoming shallow in mature trees; provides effective protection against fire. The tree develops a long taproot at a very young age. Leaves simple, shiny, glabrous, about 10-25 cm long and broadly oval at the base, with the apex tapering into a long point; new leaves reddish, soon becoming delicate green.

Chemical Analysis

The FTIR analysis of Sal leaves shows the presence of the following functional groups:

Alcohols (-OH)

Carbonyl Group (-C=O)

Carboxylic Group (-COO)

Amine Group (-NH_2)

Elemental Analysis[5]

(Table 3.1)

ELEMENTAL ANALYSIS (%)			
C	H	N	S
46.37	7.096	0.387	0.283

3.2 PREPARATION OF BIOSORBENT[6]

- Fallen Shorea Robusta leaves (Sal leaves) were collected.
- The collected leaves were first washed using distilled water to remove any dirt or sand present on the surface.
- A 0.3M solution of NaOH was prepared and the leaves were then put in this solution.
- The leaves were kept dipped in the NaOH solution for 24 hours.
- The leaves were then dried in sunlight.
- Then the leaves were dried in a Hot air oven, at 60˚C for 90 minutes, to remove any moisture left in the leaves.
- The dried leaves were crushed in a mechanical crusher and then sieved to separate them into different sizes.

Sieving:

- Sieving was done in a sieve shaker available in our Particulate Technology Laboratory.
- The crushed sample was sieved in the mesh sizes 22-36, 36-52 and 52-60.
- 3 main size ranges were obtained as shown in Figure 5.

(a)

(b)

(c)

(**Figure 3.1)**

3.3 SELECTION OF METAL IONS FOR STOCK SOLUTION

After a detailed study of Ultraviolet-Visible absorption spectroscopy, it was found that the attenuation wavelengths of copper and lead ions lie in the visible region. Primary or peak wavelength for the metal ion Cu^{2+} in sulphate solution was found to be approximately 270nm.

Since, copper salts are easily available in our Chemistry Laboratory; we will be preparing the aqueous solution for copper ions.

3.4 CONCENTRATION OF METAL IONS USING UV-VISIBLE SPECTROMETRY[7]

Introduction:

Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory. In many applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness.

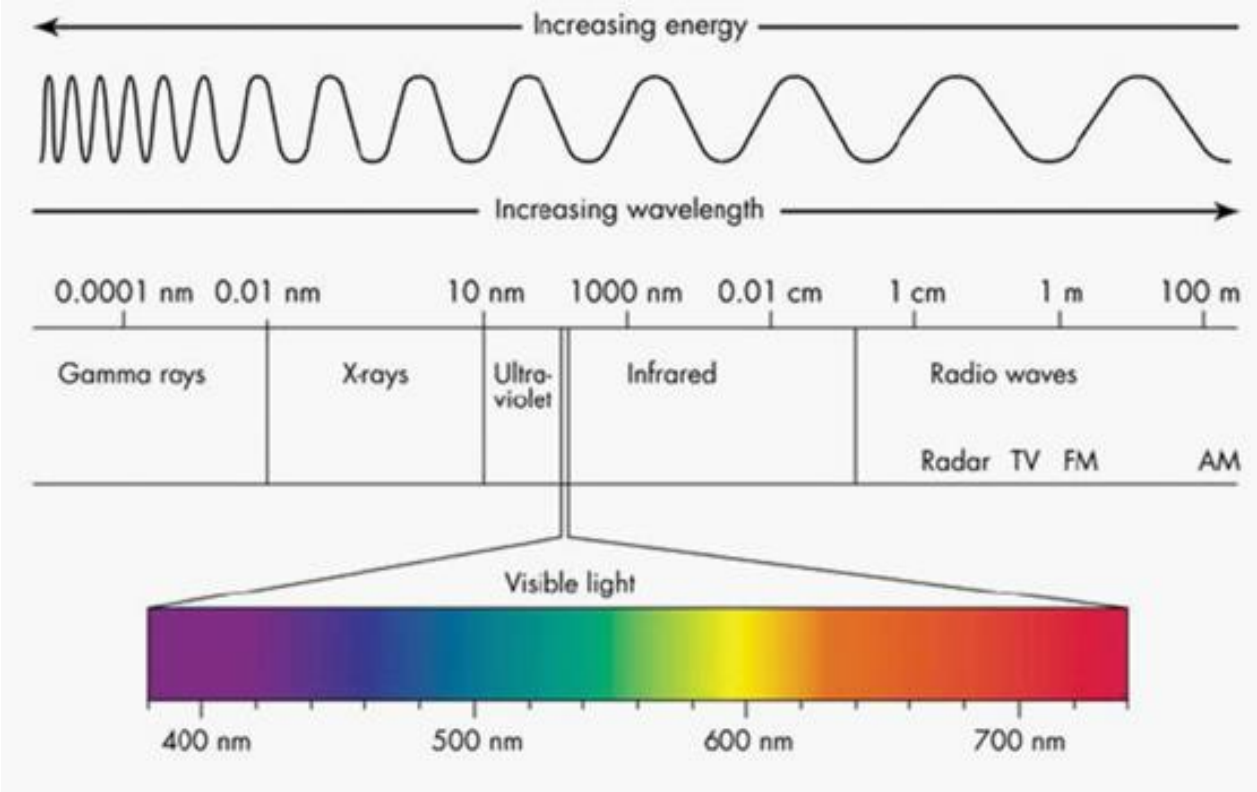
When white light falls upon a sample, the light may be totally reflected, in which case the substance appears white or the light may be totally absorbed, in which case the substance will appear black. If, however, only a portion of the light is absorbed and the balance is reflected, the color of the sample is determined by the reflected light. Thus, if violet is absorbed, the sample appears yellow-green and if yellow is absorbed, the sample appears blue.

However, many substances which appear colorless do have absorption spectra. In this instance, the absorption will take place in the infra-red or ultraviolet and not in the visible region.

The world is full of a multitude of colors that are used for both practical and aesthetic purposes. The colors that are seen when looking at different objects are due to the ability of the compounds in those objects to absorb specific wavelengths of light. In other words, the colors that are seen are the ones that are *not* absorbed. The wavelengths of light that are absorbed are determined by the electrons in a compound. As electrons move around, they can absorb energy and become excited. The energy, and thus the wavelength of light, the electrons absorb is determined by the type of atoms found in the compound and how those atoms are bound together. Different environments for electrons will also determine how much of a particular wavelength of light can be absorbed, a parameter which is reflected in the molar absorptivity of the compound.

Because the color of a species is due to its ability to absorb light, the color should become darker or more intense as the concentration increases. The increase in concentration leads to more electrons in the sample which can then absorb more light at a particular wavelength. Thus, there should be a relationship between the concentration of the compound being studied and its absorbance. This relationship is best determined using a wavelength of light in a region of the

visible spectrum where the maximum absorbance is observed. This wavelength is known as λ_{max} and is most sensitive to the changes in concentration. The spectra is shown in Figure 6.



Electromagnetic Spectrum

(Figure 3.2)

The Beer-Lambert Law[8]:

The Beer-Lambert Law states that the concentration of a substance in solution is directly proportional to the 'absorbance ', A, of the solution.

Absorbance (A) = (constant) x (concentration) x (cell length)

The law is only true for monochromatic light that is light of a single wavelength or narrow band of wavelengths, and provided that the physical or chemical state of the substance does not change with concentration.

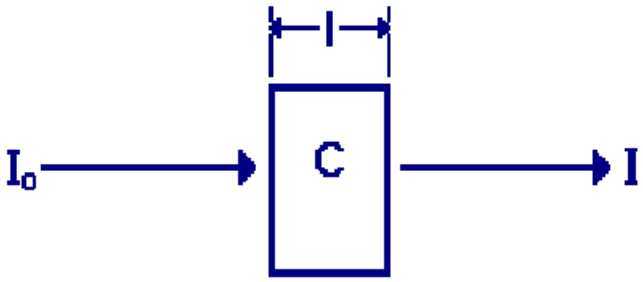
When monochromatic radiation passes through a homogeneous solution in a cell, the intensity of the emitted radiation depends upon the thickness (L) and the concentration (C) of the solution.

$$\mathbf{A = \varepsilon CL}$$

where **A** is the absorbance measured with a UV-Visible spectrometer, **ε** is the molar absorptivity or molar extinction coefficient[9], a constant for the particular solute you are analyzing, **C** is the molar concentration of the solute, and **L** is path length, the distance light travels.

For a single solute, absorbance and concentration are directly proportional if the path length is constant. When a linear trend line analysis is performed on a graph of absorbance vs. concentration, the slope is equal to the molar absorptivity, **ε**, if the path length is 1 cm.

The concentration of the $CuSO_4$ solution after biosrption will be determined by measuring its absorbance with the UV-Visible spectrometer and using the molar absorptivity from our trendline equation to determine its concentration.



Change in intensity of light through a cuvette

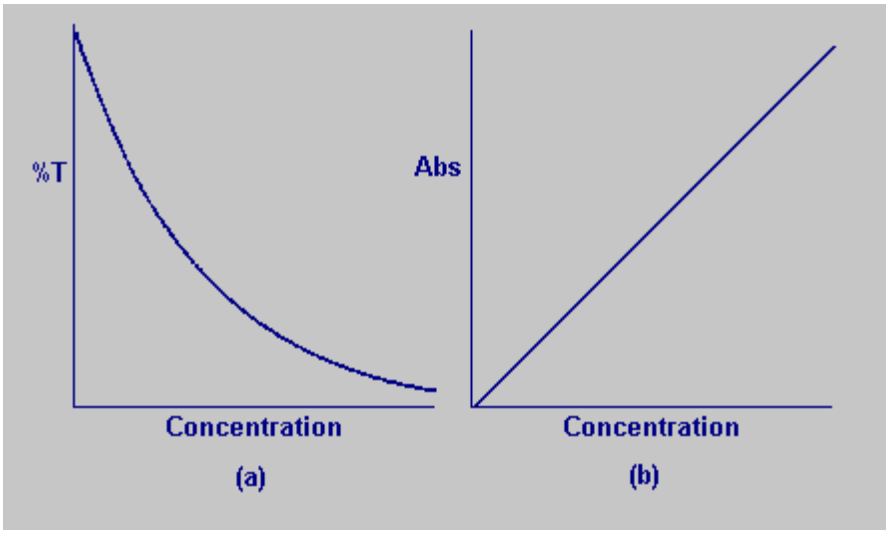
(Figure 3.3)

In Figure 7, 'I_o' is the intensity of the incident radiation and 'I' is the intensity of the transmitted radiation. The ratio 'I/I_o' is called transmittance.

This is sometimes expressed as a percentage and referred to as % transmittance (T).

Mathematically, absorbance is related to percentage transmittance T by the expression:

$$\mathbf{A = \log_{10}(I_o/I) = \log_{10}(100/T) = \varepsilon CL}$$



(a) %T vs concentration (b) Absorbance vs concentration

(Figure 3.4)

If, in the expression **A = εCL**, **C** is expressed in mol l^{-1} and **L** in m, then **ε** is called the molar absorption coefficient. The units of **ε** are $mol^{-1}m^2$. **ε** was formerly called the molar extinction coefficient and concentrations were often expressed as mol l^{-1}, mol dm-^{3} or M and the cell length in cm to give units $mol^{-1}cm^{-1}$, $mol^{-1}dm^3cm^{-1}$ and $M^{-1}cm^{-1}$ respectively.

Limitations of Beer Lambert Law[10]:

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- Deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity.
- Scattering of light due to particulates in the sample.
- Fluorescence or phosphorescence of the sample.
- Changes in refractive index at high analyte concentration.
- Shifts in chemical equilibria as a function of concentration.
- Non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band.
- Stray light.

3.5 PREPARATION OF STOCK SOLUTION

- Stock solution containing copper metal is to be prepared. All the further experiments for different concentration of copper in the aqueous solution will be prepared from this stock solution.

- The stock solution should contain 1000 ppm/liter of copper metal. For instance, if the metal salt available for preparation is copper chloride then, grams of $CuCl_2$ will be calculated to obtain 1000ppm of copper ions per liter.

- Copper Sulphate was the salt available in the chemistry lab; hence the stock solution was prepared using Copper Sulphate crystals.

- 63.5g Cu is present in 159.5g $CuSO_4$. Thus, for preparing a 1000ppm (1g/L) solution of Cu weight of $CuSO_4$ to be used = $\frac{159.5g}{63.5g} \times 1g = 2.52g$

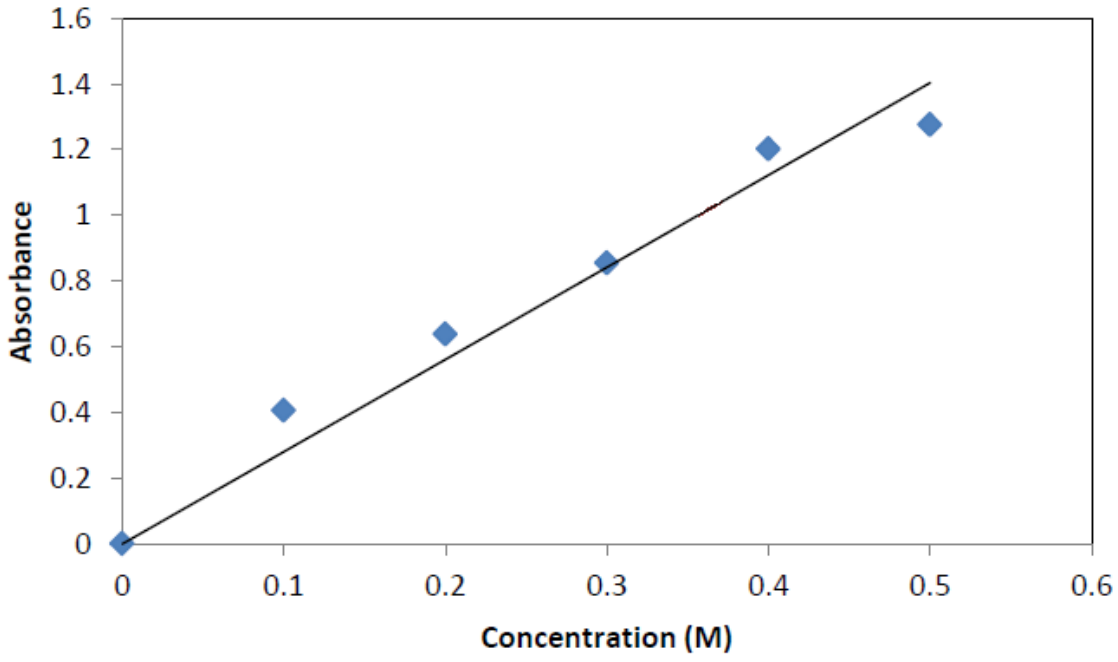
- Further test samples will be prepared from this stock solution by using the formula:

$$\mathbf{C_1V_1 = C_2V_2}$$

- The concentrations needed to be prepared for experiments are 20, 40, 60, 80 and 100ppm per liter of solution.

Procedure:

1. 6 samples will be prepared for analysis by diluting a 1000 ppm $CuSO_4$ stock solution namely 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm.
2. The absorbance these solutions will then be determined using a UV-Visible Spectrophotometer in the range 200-500nm.
3. The maximum absorbance of all the 5 samples will then be calculated which should correspond to nearly the same wavelength for all the samples.
4. A graph of absorbance vs concentration will then be plotted.
5. The slope of the graph will then give the value of **εL** (molar absorptivity x length).
6. The concentration of copper sulphate can then be calculated using this value of molar absorptivity.



Standard Absorbance *vs.* Concentration Graph

(Figure 3.5)

(Table 3.2)

Reference Table for preparation of calibration curve

Concentration of $CuSO_4$ solution for calibration (ppm)	Volume of $CuSO_4$ solution used from 1000ppm stock solution (ml)	Volume of diluting water (ml)	Volume of sample prepared for calibration (ml)

Preparation of calibration curve

- 6 samples of concentration 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm were prepared using the 1000 ppm stock solution.
- The following table gives the volume of stock solution and the volume of diluting water used for the preparation of the above mentioned samples :

(Table 3.3)

Preparation for Calibration Curve

Concentration of $CuSO_4$ solution for calibration (ppm)	Volume of $CuSO_4$ solution used from 1000 ppm stock solution (ml)	Volume of diluting water (ml)	Volume of sample prepared for calibration (ml)
0	0	100	100
5	0.5	99.5	100
10	1	99	100
15	1.5	98.5	100
20	2	98	100
25	2.5	97.5	100
30	3	97	100

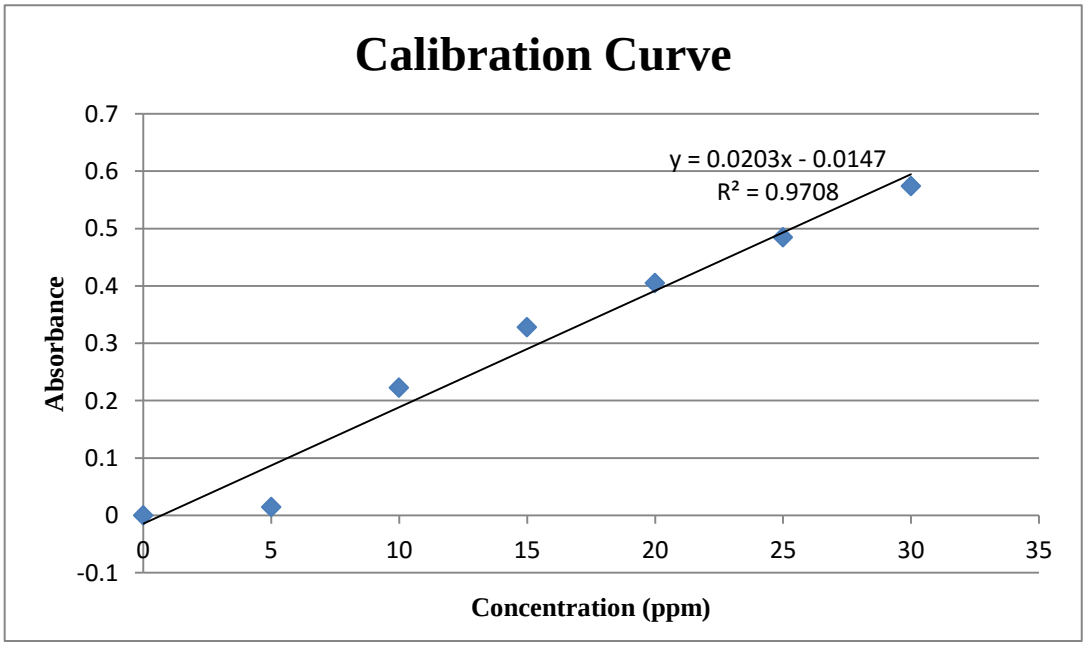
- These samples were then analyzed using a UV-Visible Spectrophotometer in the range 200-500nm to determine the absorbance of the samples.
- The following table gives the maximum observed value of absorbance of the samples and the corresponding wavelengths:

(Table 3.4)

Calibration curve data

Concentration of $CuSO_4$ solution (ppm)	**Maximum Absorbance**	**Wavelength(nm)**
0	0	0
5	0.015	273.5
10	0.223	273.5
15	0.328	270.5
20	0.405	270.5
25	0.485	270.5
30	0.574	270.5

The figure below shows the best fit curve obtained using the above data:



Calibration curve

(Figure 3.6)

3.6 EXPERIMENTAL PROCEDURES AND OBSERVATIONS

3.6.1 Optimization of contact time

- A 100ml solution of 20 ppm $CuSO_4$ was prepared.
- 0.02g of adsorbent of 52-60 mesh size was used.
- Samples were taken after every 15 minutes and were analyzed under UV Spectrophotometer and their absorbance was measured.
- The following table gives the absorbance values for the different samples-

(Table 3.5)

Absorbance at different time (20 ppm, 0.02g, 269.23 microns)

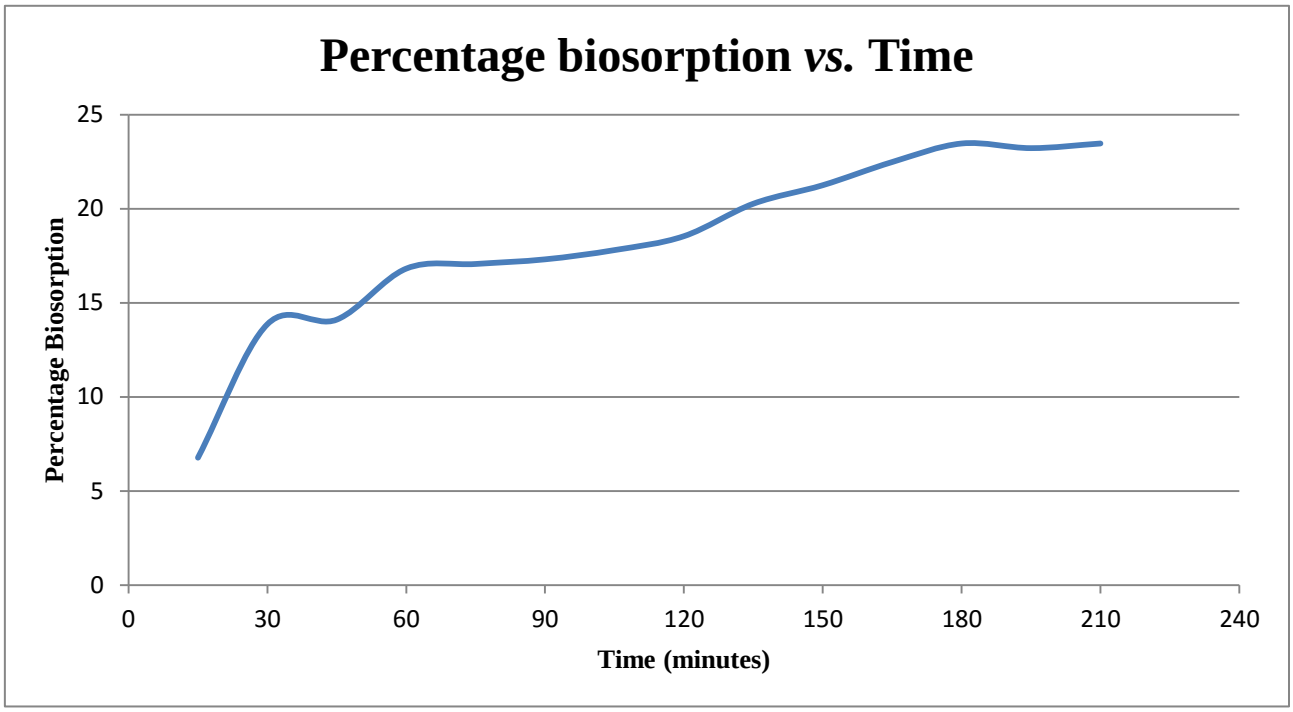
Time	Absorbance
15	0.363
30	0.335
45	0.334
60	0.323
75	0.322
90	0.321
105	0.319
120	0.316
135	0.309
150	0.305
165	0.300
180	0.296
195	0.296
210	0.296

Using the equation of the best fit curve, the concentration values corresponding to the above data were calculated. The following table gives the calculated concentration values and the percentage of Biosorption -

(Table 3.6)

Effect of Contact time on percentage biosorption

Time	Final concentration (ppm)	Percentage Biosorption
15	18.64512	6.7744
30	17.2266	13.86699507
45	17.17734	14.11330049
60	16.63547	16.8226601
75	16.58621	17.06896552
90	16.53695	17.31527094
105	16.43842	17.80788177
120	16.29064	18.54679803
135	15.94581	20.27093596
150	15.74877	21.25615764
165	15.50246	22.48768473
180	15.30542	23.4729064
195	15.35468	23.22660099
210	15.30542	23.4729064



(Figure 3.7)

From the above data it can be concluded that the percentage of Biosorption remains nearly constant after 180 minutes. Thus, the optimum value of time after which the percentage of Biosorption remains constant is **180 minutes.**

3.6.2 Optimization of pH

- To study the effect of pH on adsorption, eleven 20 ppm solutions of $CuSO_4$ were prepared and 0.02g of adsorbent was used.
- To reduce the pH of the solutions 0.01M HCl was used whereas to increase the pH 0.01M NaOH was used.
- The pH meter available in the Chemistry Laboratory was used to measure the pH of the solution.
- HCl or NaOH was added drop wise to the solution until the desired pH was reached.
- Samples were taken from each of the solutions after 180 minutes and their absorbance was measured.
- The following table gives the value of pH and the corresponding absorbance-

(Table 3.7)

Absorbance at different pH (20 ppm, 0.02 g, 269.23 microns and 180 minutes)

pH	Absorbance
2	0.86
3	0.708
4	0.707
5	0.355
6	0.424
7	0.475
8	0.362
9	0.521
10	0.563
11	0.824
12	0.83

Using the standard curve, the corresponding concentration values were calculated-

(Table 3.8)

Effect of pH on final concentration

pH	Final Concentration(ppm)
2	43.08867
3	35.60099
4	31.41379
5	15.55172
6	16.24138
7	15.94581
8	15.10837
9	26.38916
10	28.45813
11	41.31527
12	41.61084

From the above data it can be seen that the lowest absorbance value is obtained for the solution with pH = 8. Thus, the optimum **pH value is 8.**

3.6.3 Effect of adsorbent size on percentage biosorption

- To study the effect of size of adsorbent on percentage Biosorption three 20ppm solutions of $CuSO_4$ were prepared.
- The three adsorbent sizes used were 22-36 mesh size, 36-52 mesh size and 52-60.
- 0.02g of adsorbent was added to each of the three solutions.
- Samples were taken after 180 minutes and their absorbance was measured.
- The following table gives the absorbance values for the three solutions-

(Table 3.9)

Absorbance of final solution for different sizes (20 ppm, 0.02g, 180 minutes)

Mesh Size	Absorbance
22-36	0.336
36-52	0.318
52-60	0.296

The corresponding concentration values and the percentage Biosorption values are as follows-

(Table 3.10)

Effect of average particle size of Biosorbent on Percentage Biosorption

Mesh size	Average particle size (microns)	Final Concentration (ppm)	Percentage Biosorption
22-36	549.24	17.27586	13.62069
36-52	352.56	16.38916	18.05419
52-60	269.23	15.30542	23.47291

From the above data it can be seen that percentage of Biosorption is maximum for 52-60 mesh size. This can be attributed to the increase in surface area as the size of the adsorbent is reduced.

3.6.4 Effect of initial metal ion concentration on percentage biosorption

- To study the effect of initial metal ion concentration on percentage Biosorption five samples of 20ppm, 40ppm, 60ppm, 80ppm and 100ppm of $CuSO_4$ were prepared.
- 0.02g of adsorbent was added to each of the solutions.
- Samples were taken after 180 minutes and were analyzed under the UV Spectrophotometer and their absorbance was measured.
- The following table gives the absorbance of the five solutions after 180 minutes-

(Table 3.11)

Absorbance of final solution at different initial concentration (0.02g, 180 minutes, 269.23 microns)

Concentration	Absorbance
20	0.296
40	0.625
60	0.983
80	1.267
100	1.651

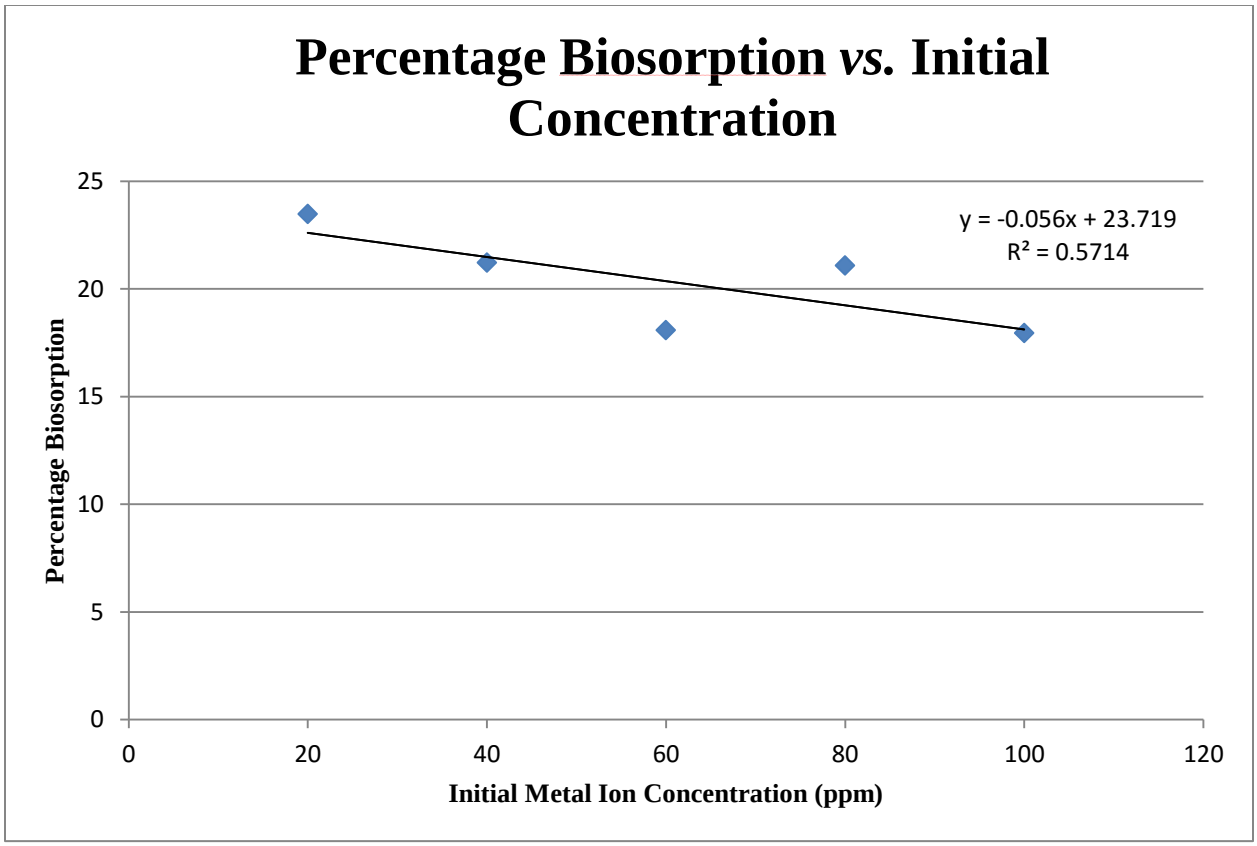
The corresponding concentration values were then calculated from the calibration curve and the percentage of Biosorption was calculated using those values.

(Table 3.12)

Effect of initial metal ion concentration on percentage Biosorption

Initial Concentration of metal ion	Final Concentration (ppm)	Percentage Biosorption
20	15.30542	23.47291
40	31.51232	21.21921
60	49.14778	18.08703
80	63.13793	21.07759
100	82.05419	17.94581

When the initial metal concentration was increased, the percentage Biosorption remained almost same for a particular adsorbent dosage amount.


Percentage Biosorption vs. Initial Concentration
y = -0.056x + 23.719
R² = 0.5714
Percentage Biosorption
Initial Metal Ion Concentration (ppm)
0
5
10
15
20
25
0
20
40
60
80
100
120

(Figure 3.8)

3.6.5 Effect of dosage on percentage biosorption

- To study the effect of dosage on percentage Biosorption five samples of 20ppm $CuSO_4$ were prepared.
- 0.02g, 0.04g, 0.06g, 0.08g and 0.1g of adsorbent was added to the above solutions respectively.
- Samples were taken after 180 minutes and were analyzed under UV Spectrophotometer and their absorbance was measured.
- The following table gives the absorbance of the solution after 180 minutes-

(Table 3.13)

Absorbance at different dosage (20 ppm, 180 minutes, 269.23 microns)

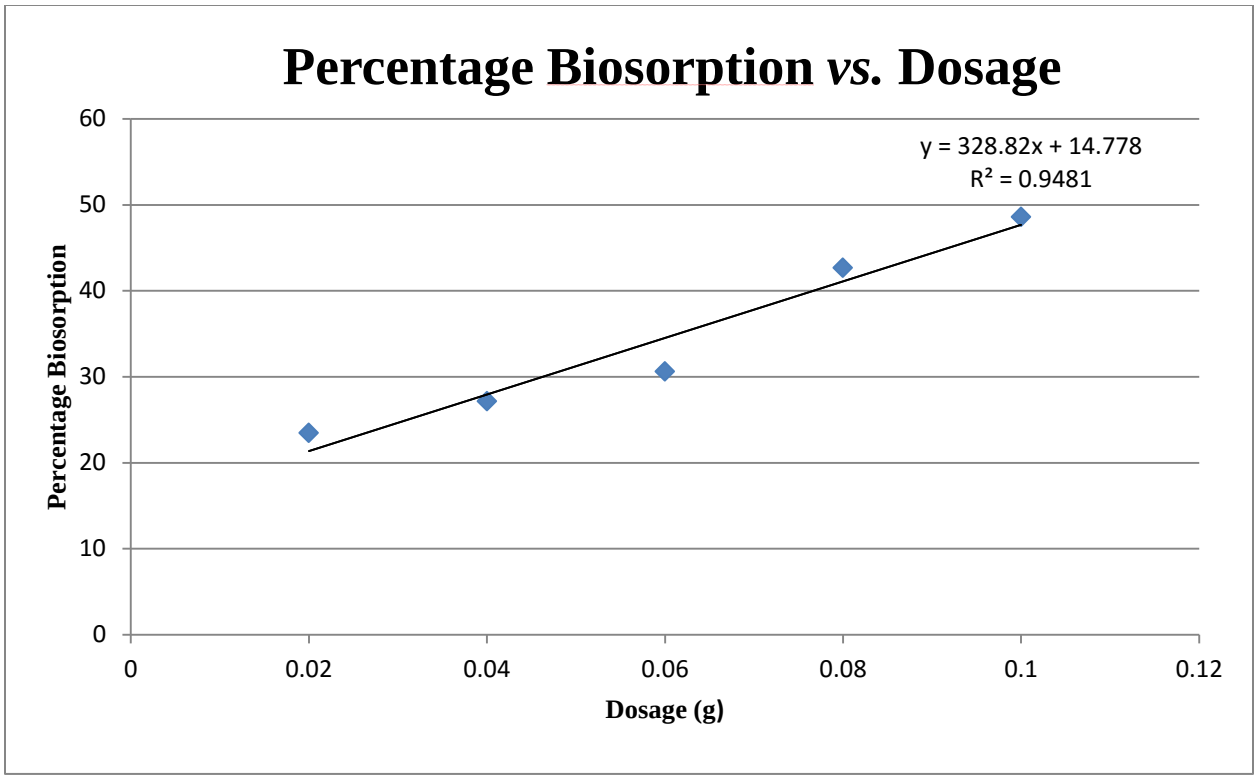
Dosage	Absorbance
0.02	0.296
0.04	0.281
0.06	0.267
0.08	0.218
0.1	0.194

The corresponding concentration values and percentage Biosorption values are as follows-

(Table 3.14)

Effect of biosorbent dosage on percentage Biosorption

Dosage	Final concentration(ppm)	Percentage Biosorption
0.02	15.30542	23.47291
0.04	14.5665	27.16749
0.06	13.87685	30.61576
0.08	11.46305	42.68473
0.1	10.28079	48.59606



(Figure 3.9)

From the above data it can be seen that the percentage Biosorption increases with the dosage of adsorbent. For 0.1g of adsorbent dosage nearly 50% removal was achieved.

3.7 SORPTION KINETICS

- The time was optimized by taking out 10ml samples at 15 minute intervals.
- The samples were then analyzed under the UV Spectrophotometer and their absorbance was found out.
- Corresponding concentrations were then calculated using the calibration chart that was initially prepared.
- The time and concentration readings were then used to find the kinetic model suited for the Biosorption by calculating the rate constant.

First order kinetics:

- The equation

$$\mathbf{C = C_o e^{-kt}}$$

Was used to calculate the k values, where C_o, initial concentration, is equal to 20ppm.

(Table 3.15)

First order Rate constants

S. No.	Concentration (ppm)	Time (minutes)	Rate constant (min^{-1})
1	18.64512	15	0.11537
2	17.2266	30	0.06585
3	17.17734	45	0.04351
4	16.63547	60	0.0297
5	16.58621	75	0.02357
6	16.53695	90	0.01948

- The rate constants had a decreasing trend; hence, first order kinetic model was rejected.

Zero order kinetics:

- The equation

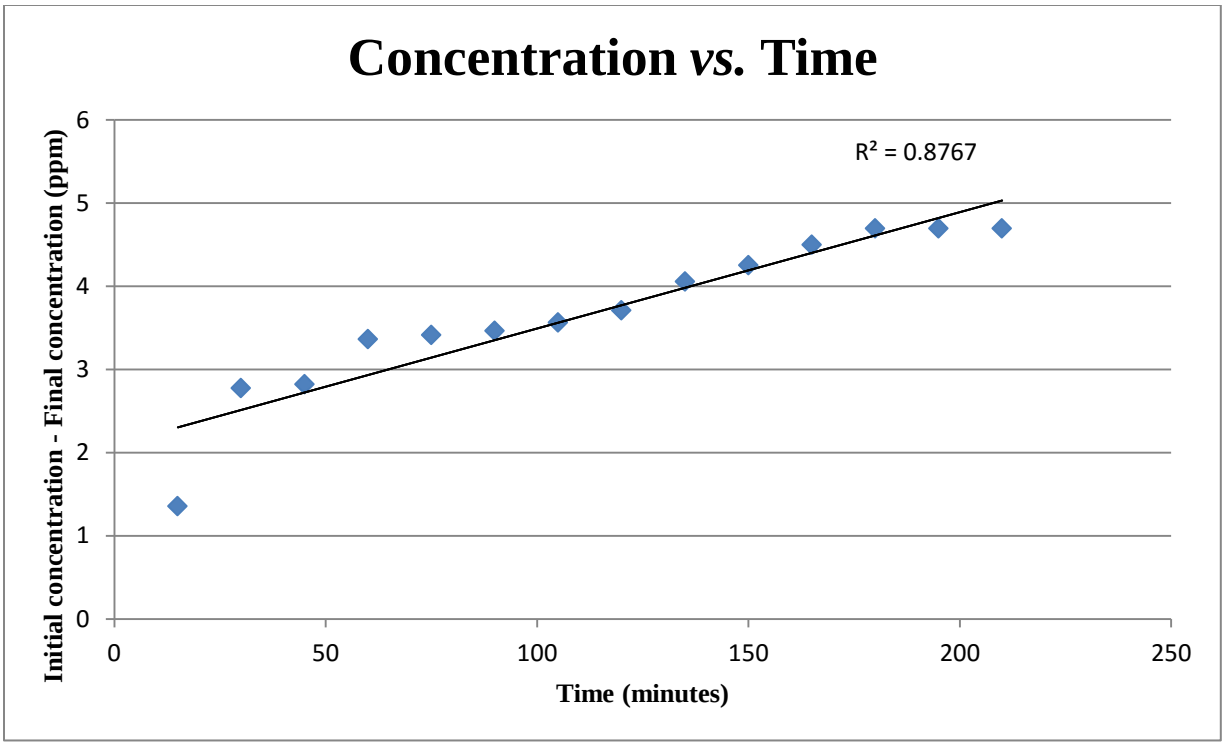
$$C_t = C_o - kt$$

Was used to calculate the k values and a plot was prepared between $(C_o - C_t)$ and t

(Table 3.16)

Zero Order Rate Constants

Time	Absorbance	Final Concentration	Percentage Biosorption	k values (ppm-min^{-1})
15	0.363	18.64512	6.7744	0.0903
30	0.335	17.2266	13.86699507	0.0386
45	0.334	17.17734	14.11330049	0.06272
60	0.323	16.63547	16.8226601	0.05607
75	0.322	16.58621	17.06896552	0.045517
90	0.321	16.53695	17.31527094	0.03847
105	0.319	16.43842	17.80788177	0.03391
120	0.316	16.29064	18.54679803	0.03093
135	0.309	15.94581	20.27093596	0.030031
150	0.305	15.74877	21.25615764	0.02834
165	0.300	15.50246	22.48768473	0.02725
180	0.296	15.30542	23.4729064	0.026081
195	0.297	15.35468	23.22660099	0.02407
210	0.296	15.30542	23.4729064	0.02235



(Figure 3.10)

- A nearly straight line with positive slope was obtained. Thus, the above data fits the zero order kinetic model.
- The rate constant, hence, calculated is **0.039617071 ppm-min^{-1}.**

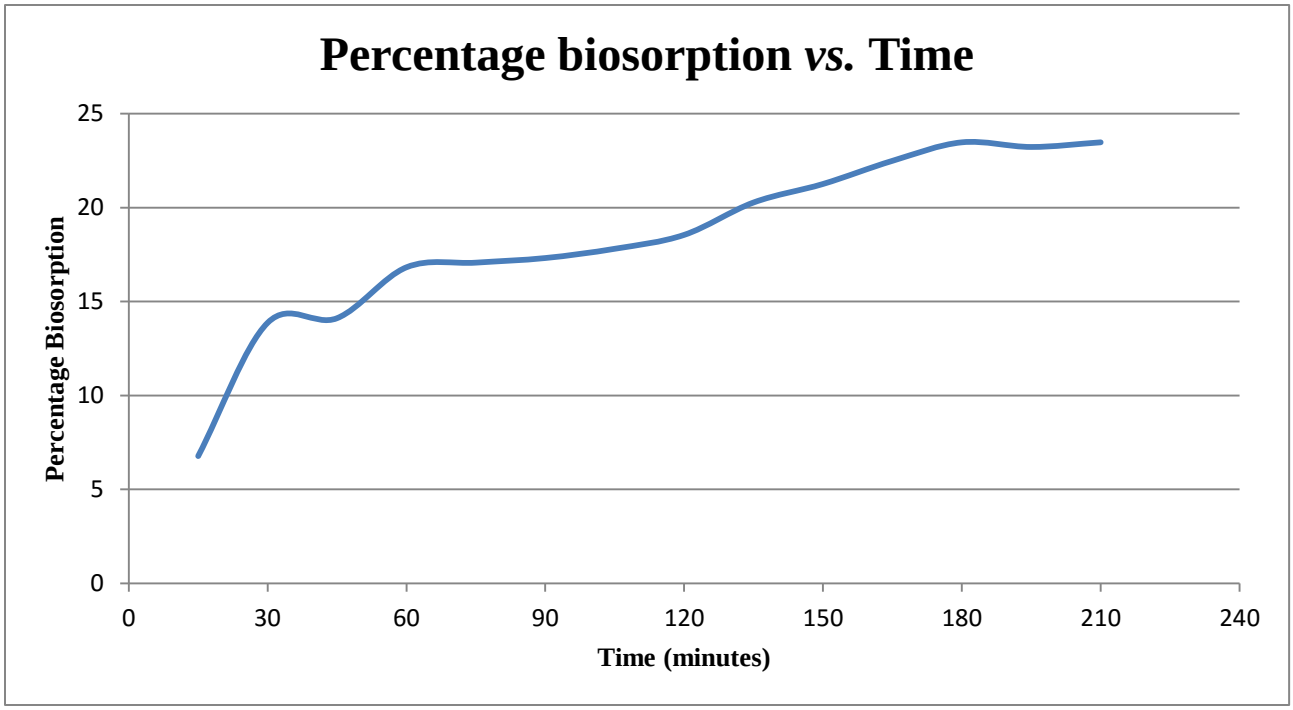
CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Effect of contact time on percentage Biosorption

(Table 4.1)

Time	Absorbance	Final concentration (ppm)	Percentage Biosorption
15	0.363	18.64512	6.7744
30	0.335	17.2266	13.86699507
45	0.334	17.17734	14.11330049
60	0.323	16.63547	16.8226601
75	0.322	16.58621	17.06896552
90	0.321	16.53695	17.31527094
105	0.319	16.43842	17.80788177
120	0.316	16.29064	18.54679803
135	0.309	15.94581	20.27093596
150	0.305	15.74877	21.25615764
165	0.3	15.50246	22.48768473
180	0.296	15.30542	23.4729064
195	0.297	15.35468	23.22660099
210	0.296	15.30542	23.4729064



(Figure 3.11)

While studying the effect of contact time on percentage of Biosorption it was found that after 180 minutes the percentage Biosorption no longer changes appreciably. Thus, the optimum value of time is 180 minutes because after this conducting the experiment for further durations will be uneconomic as it will only lead to added cost while no further Biosorption will occur.

4.2 Effect of pH on percentage Biosorption

(Table 4.2)

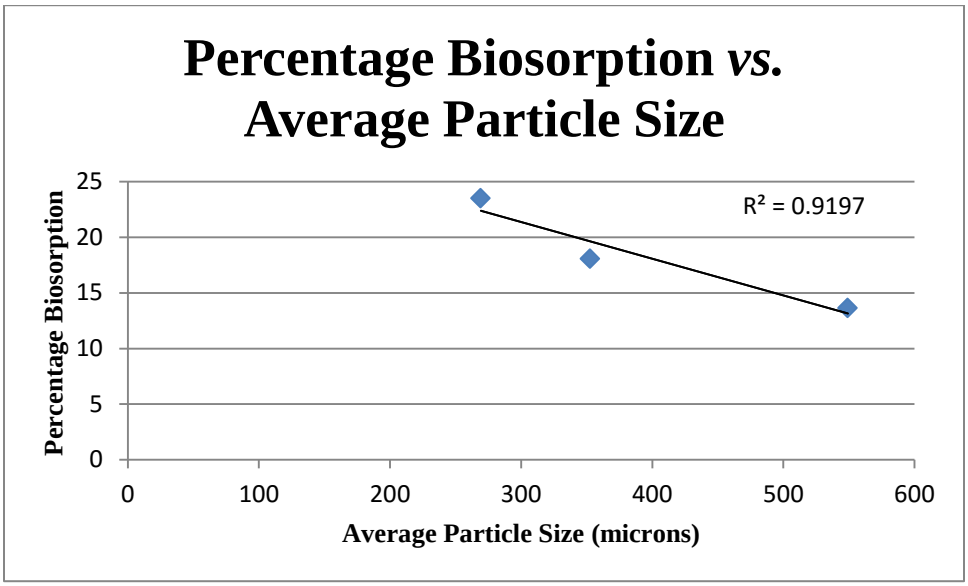
pH	Absorbance	Final Concentration(ppm)
2	0.86	43.08867
3	0.708	35.60099
4	0.623	31.41379
5	0.301	15.55172
6	0.315	16.24138
7	0.309	15.94581
8	0.292	15.10837
9	0.521	26.38916
10	0.563	28.45813
11	0.824	41.31527
12	0.83	41.61084

While studying the effect of pH on percentage of Biosorption it was found that lowest absorbance for the final solution was obtained at a pH of 8. Thus, if the experiment is to be to an industrial process then it should be carried out at a pH of 8 as this will be more economical and will give better results.

4.3 Effect of average particle size on percentage Biosorption

(Table 4.3)

Mesh size	Average particle size (microns)	Absorbance	Final Concentration (ppm)	Percentage Biosorption
22-36	549.24	0.336	17.27586	13.62069
36-52	352.56	0.318	16.38916	18.05419
52-60	269.23	0.296	15.30542	23.47291



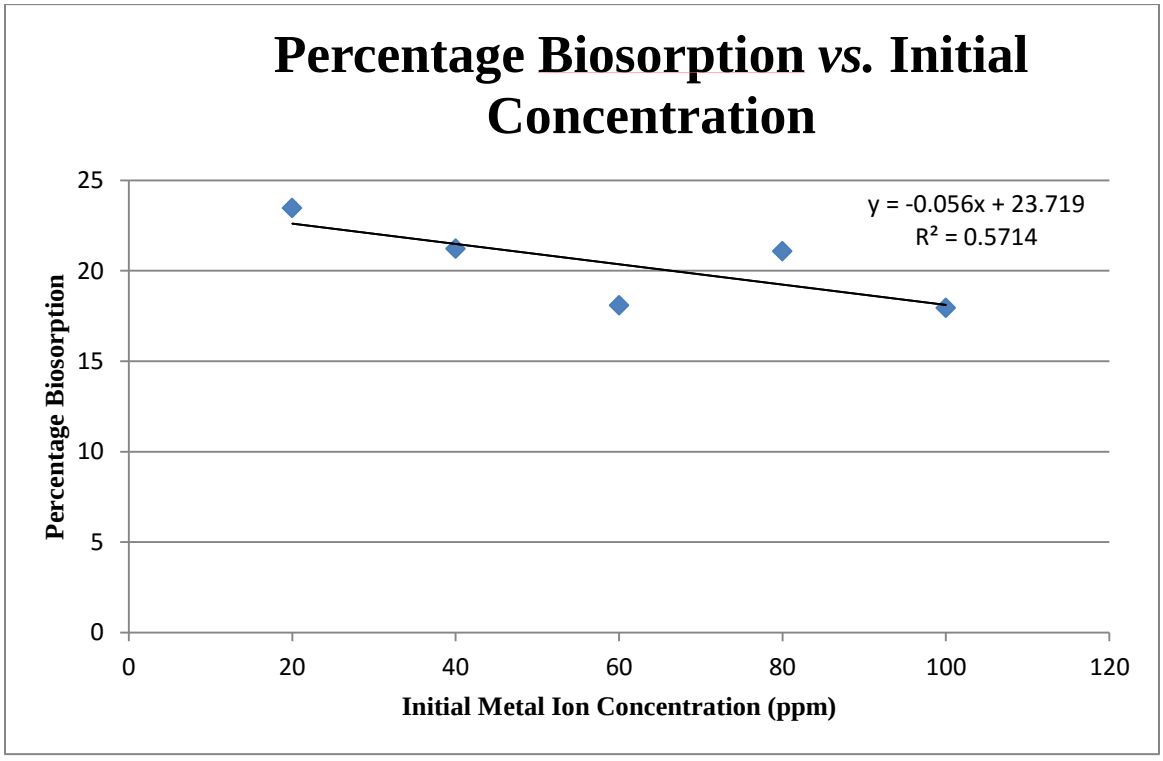
(Figure 3.12)

While studying the effect of the size of biosorbent on the percentage of Biosorption it was found that maximum Biosorption occurs by using the adsorbent of the smallest size. This is due to the fact that the smaller adsorbent particles have a higher surface area and since Biosorption is a surface phenomenon, higher surface area leads to more Biosorption because of the availability of larger number of Biosorption sites.

4.4 Effect of initial metal ion concentration on percentage Biosorption

(Table 4.4)

Initial Concentration of metal ion	Absorbance	Final Concentration (ppm)	Percentage Biosorption
20	0.296	15.30542	23.47291
40	0.625	31.51232	21.21921
60	0.983	49.14778	18.08703
80	1.267	63.13793	21.07759
100	1.651	82.05419	17.94581



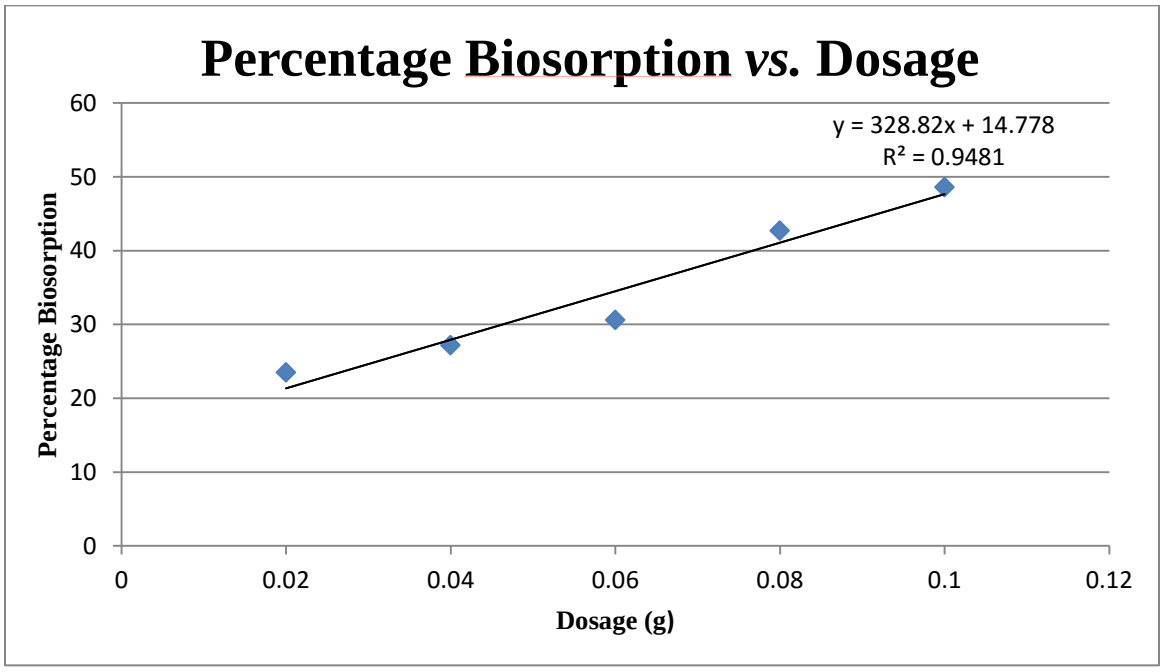
(Figure 3.13)

While studying the effect of initial metal ion concentration on percentage of Biosorption it was found that there was a slight decrease in the percentage Biosorption with increasing initial metal ion concentration.

4.5 Effect of Biosorbent dosage on percentage Biosorption

(Table 4.5)

Dosage	Absorbance	Final concentration(ppm)	Percentage Biosorption
0.02	0.296	15.30542	23.47291
0.04	0.281	14.5665	27.16749
0.06	0.267	13.87685	30.61576
0.08	0.218	11.46305	42.68473
0.1	0.194	10.28079	48.59606



(Figure 3.14)

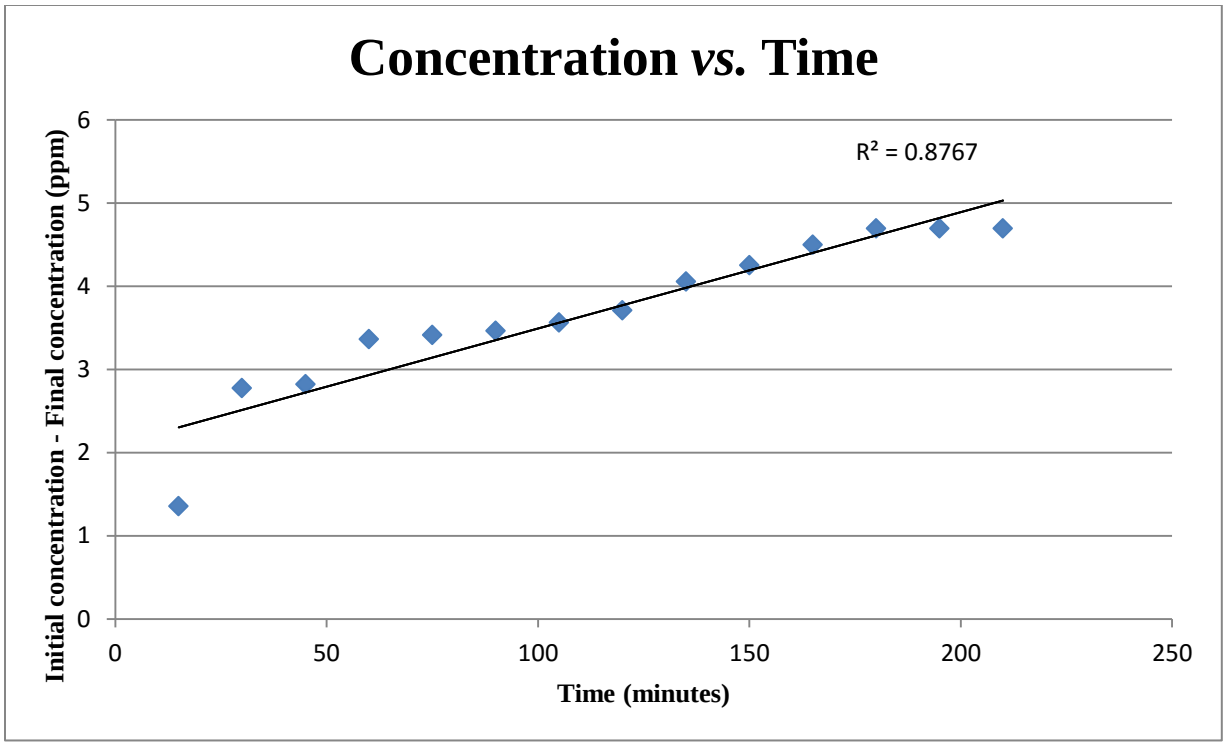
While studying the effect of biosorbent dosage on percentage of Biosorption it was found that as the dosage was increased the percentage of Biosorption also increased. This is due to the added number of available Biosorption sites with increasing dosage.

4.6 Sorption Kinetics

(Table 4.6)

Zero Order Rate Constants

Time	Absorbance	Final Concentration	Percentage Biosorption	k values (ppm-min^{-1})
15	0.363	18.64512	6.7744	0.0903
30	0.335	17.2266	13.86699507	0.0386
45	0.334	17.17734	14.11330049	0.06272
60	0.323	16.63547	16.8226601	0.05607
75	0.322	16.58621	17.06896552	0.045517
90	0.321	16.53695	17.31527094	0.03847
105	0.319	16.43842	17.80788177	0.03391
120	0.316	16.29064	18.54679803	0.03093
135	0.309	15.94581	20.27093596	0.030031
150	0.305	15.74877	21.25615764	0.02834
165	0.300	15.50246	22.48768473	0.02725
180	0.296	15.30542	23.4729064	0.026081
195	0.297	15.35468	23.22660099	0.02407
210	0.296	15.30542	23.4729064	0.02235



(Figure 3.15)

While studying the optimization of time data, the Biosorption kinetics was fitted efficiently into the zero order kinetic model. The rate constant or the slope of the graphical representation of Concentration *vs.* time was found to be **0.039617071 ppm-min^{-1}.**

4.7 Meeting the EPA's Maximum Contaminant Level Goals (MCLG)

The maximum removal of copper was attained at pH 8, biosorbent dose of 0.1g per 100 ml of sample with metal concentration of 20 mg/L or 20 ppm. With a thorough study of the conducted experiments, it can be concluded that the MCLG of copper (1.3 ppm) can be met with the optimization of all the parameters studied, either simultaneously or combination wise.

Increasing the dosage: By increasing the dosage, the percentage Biosorption increases. Hence, in order to achieve 1.3 ppm as the final concentration after Biosorption, a 20 ppm solution has to undergo about 93.5% Biosorption.

48.95606% of Biosorption was achieved using 0.1g of biosorbent and the relation between percentage Biosorption and biosorbent dosage was found to be almost linear. Therefore, by doubling the biosorbent dosage, an approximate of 97.91212% of decrease in copper metal concentration can be achieved. The corresponding final concentration for this percentage decrease is 0.417576 mg/L or 0.417576 ppm, which is well below the MCLG of copper.

Continuous Process: By making the process continuous rather than batch process, it can be ensured that the Biosorption takes place continuously in order to remove the metal efficiently. Continuous process is a continuous flow process where the materials, either dry bulk or fluids that are being processed are continuously in motion, undergoing the physical or chemical reactions, in this case Biosorption.

CHAPTER 5

CONCLUSIONS

- A detailed study of UV – Visible absorption spectroscopy was done and Cu^{2+} ions were found to be in the UV-Visible region.
- For preparation of Copper solution, Copper Sulphate was used.
- 270.5 nm was found to be as the λ_{max} or peak wavelength for copper ions.
- The calibration curve obtained experimentally is in accordance with the standard curve.
- The optimum value of time was found out to be 180 minutes.
- The optimum pH was found out to be 8.
- As the dosage of the adsorbent increases, percentage Biosorption was found to be increasing.
- The larger mesh size adsorbent particles were found to result in less percentage Biosorption.
- When the initial metal concentration was increased, there was a slight decrease in the percentage Biosorption for a particular biosorbent dosage amount and size.
- Analyzing all the data from the various experiments conducted, it was found that the kinetic model suited for this Biosorption process was zero order kinetics.

CHAPTER 6

REFERENCES

1. https://safewater.zendesk.com/hc/en-us/articles/211405328-What-do-MCL-MCLG-and-MRDL-mean-?mobile_site=true
2. https://safewater.zendesk.com/hc/en-us/articles/211402978-4-What-are-EPA-s-drinking-water-regulations-for-copper-?mobile_site=true
3. **E. Rosales1, M. pazos1, M.A. sanromán1 and T. Tavares** Biosorption of heavy metal and dyes: a promising technology leather wastewater treatment
4. **Sharmila. d1 and P. Muthusamy** Removal of heavy metal from industrial effluent using bio adsorbent (*camellia sinensis)*
5. **Surya Narayan Dash, Ramachandra Murthy.Ch.V** Preparation of Carbonaceous heavy metal adsorbent from Shorea Robusta leaf litter using phosphoric acid impregnation. Link: www.ipublishing.co.in/jesvol1no12010/EIJES1028.pdf
6. **Sharmila. d1 and P. Muthusamy** Removal of heavy metal from industrial effluent using bio adsorbent (*camellia sinensis)*
7. **Moriyom Akhter, Muhammad Shah Alam.** Ultraviolet spectroscopy.
8. **Mohammed Junaid Khan**, Raipur. An introduction to UV – Visible spectroscopy.
9. Biology Online. http://www.biology-online.org/dictionary/Molar_absorptivity
10. http://www.tissuegroup.chem.vt.edu/chem-ed/spec/beerslaw.html