BIOREMEDIATION OF HEAVY METALS USING MICROBIAL BIOMASS

By
MONIKA KUBAVAT
(16MCHE07)

DEPARTMENT OF CHEMICAL ENGINEERING
INSTITUTE OF TECHNOLOGY
NIRMA UNIVERSITY
AHMEDABAD-382481
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BIOREMEDIATION OF HEAVY METALS USING MICROBIAL BIOMASS

Major Project

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For the Degree of

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In
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(Environmental Process Design)

By

Monika Kubavat

(16MCHE07)

Guided By

Dr. Sanjay S. Patel

Dr. Chandrakanth R. Gadipelly

NIRMA
UNIVERSITY
INSTITUTE OF TECHNOLOGY
NAAC ACCREDITED 'A' GRADE

DEPARTMENT OF CHEMICAL ENGINEERING
INSTITUTE OF TECHNOLOGY
NIRMA UNIVERSITY
AHMEDABAD-382481
MAY-201

Declaration

This is to certify that the thesis comprises my original work towards the degree of Master of technology in Environmental Process Design at Chemical Engineering Department, Institute of Technology, Nirma University and not been submitted elsewhere for a degree. Due acknowledgement has been made in the text to all other material used.

MONIKA KUBAVAT
(16MCHE07)

Undertaking for the Originality of the Work

MONIKA KUBAVAT (16MCHE07), gives undertaking that the major project entitled **“BIOREMEDIATION OF HEAVY METALS USING MICROBIAL BIOMASS”** submitted by me, towards the partial fulfillment of the requirements for the degree of Master of Technology in Chemical Engineering (Environmental Process Design) at Chemical Engineering Department, Institute of Technology, Nirma University, Ahmedabad is the original work Carried out by me and I give assurance that no attempts of plagiarism has been made. I understand, that in the event of my similarity found subsequently with any published work or any dissertation work elsewhere, it will result in severe disciplinary action.

Monika Kubavat

Date:

Place: Ahmedabad

Endorsed by

Dr. Chandrakanth R. Gadipelly
Manager – Research Scientist
Speciality Inorganic Chemicals
R&D Centre, Vadodara Manufacturing Division
Reliance Industries Limited,
Vadodara – 391346 Gujarat

Certificate

This is to certify that the Project Report entitled "**BIOREMEDIATION OF HEAVY METALS USING MICROBIAL BIOMASS**" submitted by **Ms. Monika Kubavat (16MCHE07)**, towards the partial fulfillment of the requirements for the award of Degree of Master of Technology in Chemical Engineering (Environmental Process Design) of Nirma University is the record of work carried out by her under my/our supervision and guidance. The work submitted has in my/our opinion reached a level required for being accepted for examination. The results embodied in this major project work to the best of my/our knowledge have not been submitted to any other University or Institution for award of any degree or diploma.

Project Guide/Guides:

Dr. Chandrakanth R. Gadipelly
Research Guide
Manager – Research Scientist
Speciality Inorganic Chemicals
R&D Centre, Vadodara Manufacturing Division
Reliance Industries Limited,
Vadodara – 391346 Gujarat

Professor Sanjay S. Patel
Research Guide
Professor, Department of Chemical Engineering
Institute of Technology
Nirma University
Ahmedabad

Dr. Jayesh Ruparelia
Professor, Head of Department
Chemical Engineering Department
Institute of Technology
Nirma University

Dr. Alka Mahajan
Director,
Institute of Technology
Nirma University

Examiners: **i. ___________________________**

ii.___________________________

iii. ___________________________

iv. ___________________________

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Monika Kubavat..

Table of Contents

LIST OF FIGURES

LIST Of TABLES

Chapter 1

Introduction

INTRODUCTION

Rapid urbanization and industrialization have resulted large quantities of aqueous effluent generation which contains harmful pollutants and some of them are having high level of toxicity. These pollutants and harmful matter releases leas to perturbing of natural ecological conditions. Which are likely to harm human health. Due to increasing concern for the safety and preservation of environment all joint and cooperative efforts are committed to clean up the polluted and contaminated water for the concern of human health and ecology.[1]

Contribution of metals has been dramatically increased due to mining and industrial revolution. These metal included waste is sometimes discarded directly onto land or into streams. [3] Metals are toxic and hazardous in nature for human health and biodiversity.

Various physicochemical and biological processes are generally used for the removal of these pollutants from industrial waste water effluents before releasing into environment. For treating adsorptive pollutants like heavy metals and dyes several conventional methods are available, which are chemical precipitation, ion exchange, filtration, chemical oxidation or reduction, RO membrane electrochemical treatment. [1]

There were some lacking points for conventional technologies like high operational cost, complexity in operation, less environment friendly etc. which gave ignition to alternative option to treat toxic metals. Currently using microorganisms as biosorbents is a new trend of research which is cost effective method with environment friendly approach. It is a potential alternate to treat the heavy metals from industrial effluents.

The research and further development of biosorption phenomena provide a basis for a whole new technology aimed at the removal of various pollutants or the recovery of valuable resources from aqueous systems or effluent wastes. Nowadays biosorption is one of the main components of bioresource technology and environmental technology. [4] Microbes have been widely used in the process of environmental clean-up and are known as bioremediators. Ability of microbes to absorb metal ions from aqueous solutions either as dead or living biomass as well as derived products has been exploited. Physico-chemical mechanisms of removal, which may be encompassed by the general term ‘biosorption”, include adsorption, ion exchange and entrapment which are features of living and dead biomass as well as derived products. In living cells, biosorption can be directly and indirectly influenced by metabolism [1]

1.1 Biological treatment technologies:

Biological treatment technologies are available for remediation of metals-contaminated sites. These technologies are commonly used for the remediation of organic contaminants and are beginning to be applied for metal remediation

Biological treatment exploits natural biological processes that allow certain plants and microorganisms to aid in the remediation of metals. These processes occur through a variety of mechanisms, including adsorption, oxidation and reduction reactions and methylation.

Heavy metal, present in different types of industrial effluents, is responsible for environmental pollution. Traditionally, metal removal was made by chemical precipitation.

Biosorption is a process in which solids of natural origin are employed for binding heavy metals. It is a promising alternative method for treating industrial effluents, mainly because of low cost and high metal binding capacity

Bioaccumulation is an active metabolic process driven by energy from a living organism and requires respiration it occurs by absorbing contaminants which are transferred onto and within the cellular surface. Bioaccumulation is a slow and nutrient dependent while Biosorption is a quick process independent of presence of nutrients. It is also dependent on the culture condition and growth medium conditions the activation energy for this is about 63 KJ/mol, Corresponds to a biochemical process.

1.2 Bioremediation:

It is the use of microorganisms to destroy or to break down hazardous substances or waste material into less toxic or nontoxic substances. It uses naturally occurring substance like microorganisms (i.e. Bacteria (aerobic and anaerobic) Fungi Actinomycetes (filamentous bacteria). Bioremediation can effectively degrade organic constituents present in environment recently, biological remediation process have also been devised to either precipitate effectively immobilize inorganic pollutants such as heavy metals

1.2.1 Bioremediation Mechanism:

- A technology that encourages growth and reproduction of indigenous microorganisms (bacteria and fungi) to escalate biodegradation of organic constituents in the standard zone to remove pollutants from environment
- Generally requires a mechanism for stimulating and maintaining the activity of microbes, e.g, addition of electron acceptor (oxygen, nitrate), nutrients (phosphorus, nitrogen), and energy source (carbon).
- Organic chemical provide: electrons-source of cell building material, carbon, electron donor (source of energy cells catalyze oxidation of organic chemicals), causing transfer of electrons from organic chemicals to some electron acceptor

Biodegradation is one type of modification to the structure of organic chemical element in biologically catalyzed way. However this conversion can be via metabolic pathways and does not confirms the decrease in toxicity. For example, mineralization is one type of biodegradation, which is defined as transforming an inorganic substance from its organic constituent, rendering the harmless nature of the primary substance. Transformation is any metabolically prompted change in chemical structure or composition of a compound is defined as transformation [5]

Bioremediation belongs to the application of microbes to degrade pollutants which are cause for risk to environmental ecosystem and human body. Bioremediation works typically containing the actions of various microorganisms working in sequence or parallel for completing the process of degradation. Generally in situ and ex situ both approaches are used for remediation. The versatile nature of microorganisms of degrading a wide range of contaminants gives bioremediation an identification as a soil decontamination technology. However it is in situ and less expensive technique which can decease disruptive engineering approaches, bioremediation is still not a widely accepted process. [6]

George Robinson owns the credit for using microbes in modern bioremediation.in the late 1960s, he used microorganisms for consuming an oil spill on the coast of Santa Barbara. Around 1980s, bioremediation started getting attention for bioremediation of oil spills and other hazardous waste. Specifically, microbes from native soil have a key role of transforming complex substances into simpler one with biogeochemical agents. [6]

Bioremediation technology uses microorganisms for elimination, reduction, transformation, or containment to benign contaminants present in water, soils, sediments and air. Bioremediation is defined as the application pf microbes to destruct or immobilize waste or pollutants. It is detoxifying process which aims mineralization, alteration or transformation of harmful chemical. [7] For a longer time, natural bioremediation was a part of waste water treatment, but intentional and modified applications for hazardous waste reduction was a recent advancement.

Bioremediation mechanism consists of redox reaction, which ought to produce energy within microbial cell during reaction. These reactions involves respiration and other biological actions required for reproduction and cell maintenance. One delivery system is required which can provide an energy source (electron donor), nutrients and an electron acceptor.

Various types of bacterial electro acceptor class may include nitrate- , manganese-, carbon dioxide-reducing, nitrate-, oxygen-, sulfate-, iron (III) and their corresponding redox potentials. Relative dominance of electron acceptor classes can be determined by the indication of redox potential.

According to Venosa et al, (2001) [8], presence of microbes with efficient metabolic capability is important key factor for oil spill bioremediation. Bioremediation accepted mainly for soil and oil spill remediation as the communities grows in such culture and exposed to hydrocarbons become habituated, accepting specific enrichment and genetic changes. The adapted microbial community is able to respond to the exposure of hydrocarbon contaminants within less time and shows escalated bioremediation rates in comparison with communities which having no history of hydrocarbon contamination [9] thus, the ability to separate high numbers of specific oil decomposing microbes from an environment can be taken as proof to the concept that microbes are the most active oil degraders in that ecosystem and can be used as bio-remediatiors to the petroleum polluted sites. [8]

Individual microbial culture can metabolize only up to a limited degree of hydrocarbon substrates, biodegradation of complex components like some heavy metals and petroleum hydrocarbon needs mixture of various microbial groups or consortia working to degrade a broader range of hydrocarbons. This limitation invites modification and advancements to the bioremediation process. This process depends on certain factors like nutrient availability and the optimum presence of other factors that support biological functions [10]

For efficient bioremediation to convert pollutants to harmless element microbes must attack enzymatically. For efficient bioremediation, environmental conditions should be favorable for bacterial growth and activity. Manipulation of some environmental factors which promotes bacterial growth and decomposition [11]

Control and optimization of process is quite complex in nature which is affected by many factors such as the available microbial population. These factors include: degradation capability of the available microbial population; the existence of pollutants to the microbial population; various environment factors such as type of soil, pH, temperature, nutrients and the presence of oxygen or other electron acceptors) [12]

Bacterial colonies can be isolated from any environmental conditions. Bacteria will grow and get adapted to any given surrounding conditions. As they can grow at subzero temperature, in extreme heat, in water, in deserted situations, anaerobic conditions and also in excess oxygen in presence of hazardous substance or any waste collection. The mainly required component for microbial growth are energy source and carbon source. Because the adaptive nature of microorganisms can be helpful for efficient remediation of hazardous waste. According to given surroundings microbes can be classified into some groups as aerobic, anaerobic, Methylotrophs, Lignin lytic fungi.

Aerobic microbes work in the presence of oxygen. Some aerobic microbes identified for their ability to decompose are alcaligenese Rhodococus, Pseudomonas. These are recognized for degradation of hydrocarbon and pesticides, they degrades both alkanes and polyaromatic. Some of them consider contaminant as the main source of energy and carbon.

Anaerobic are the one inhabited in absence of oxygen. These are not widely accepted as aerobic does. Application of these microbes in dechlorination of the chloroform and solvent trichloroethylene (TCE) and degradation of polychlorinated biphenyls (PCBs) in river sediments has gained increasing interest

The white rot fungus Phanaerochaete chrysosporium is one of the Ligninolytic fungi having the capability of degrading a completely distinct range of persistent or toxic environmental contaminants using Common substrates like saw dust, straw or corn cobs.

Methylotrophs is a class of Aerobic bacteria which grows by using methane as a source of carbon and energy. In the aerobic pathway of aerobic degradation, the initial enzyme methane monooxygenase is having a wide range of substrate which is active against a broad range of substances including 1, 2-dichloroethane and the chlorinated aliphatic trichloroethylene.

For remediation or decomposition it is required that bacteria and the contaminants should be in contact. It is not easy task to do, as neither the bacteria nor pollutants are uniformly spread in the soil. Some microbes are mobile and shows a chemotactic response by sensing the pollutant and moves toward it. Other microorganisms like as fungi grow in a filamentous form toward the contaminant.

1.2.2 Nutrients:

Although the available microorganisms in contaminated soil, cannot be in the the required numbers for cannot necessarily be there in the numbers required for decomposition of the site. Growth and activity of microbes must be stimulated. Usually bio stimulation is the addition of nutrients and oxygen to support indigenous microbes. Nutrients are the basic growth elements of life and permit microbes to develop the required enzymes to break down the pollutants. Carbon is basic nutrient element required in higher quantities compared to others like nitrogen, and phosphorus. The nutritional necessity of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1. [13]

Environmental parameters play a key role in bioremediation efficiency. Rate of decomposition is a function of microbial growth and activity. Process can be affected by pH, temperature and moisture. Apart of this parameters when working on soil, there are other parameters regarding soil condition also affects the bioremediation.

1.2.3 Types of Bioremediation:

Various methods are applied based on the degree of saturation and different parameters available. In situ techniques are applied to soil and groundwater at the site with possibly minimal disturbance. Ex situ techniques are applied to Ground water or soil site which is excavated (soil) or pumped (water) at the treating site Bioaugmentation is the techniques which involves addition of microbes having contaminant degradation capability. Bioaugmentation can include isolation of native organisms and ‘mass-cultivating’ same for exposure to contaminated sites. Genetically modified cells are used with contaminants specificity in question for Bioaugmentation processes. There is a series of technology and experimental setup and methodology involved in these studies.

Ex-situ

Ex-situ is “treatment away from contaminated Site”. These method is about excavating or removing polluted soil from ground. Various simpler techniques are covered under Ex-situ bioremediation.

- Land farming is about spreading excavated contaminated soil over priory prepared bed and tilling it periodically until the defined degradation achieved. It is a simple technique. The goal is achieved by stimulation of indigenous biodegradative bacteria and the goal is to stimulate indigenous biodegradative microorganisms and promote their aerobic degradation of pollutants. This treatment is limited to superficial layer of soil up to 10-30 cm depth. Since land farming is the potential method to reduce monitoring and maintenance costs, as well as clean-up requirements, it gained much attention as an economical disposal alternative.

- Composting is one of the simple method in which contaminate soil is combined with non-hazardous amendments like agriculture waste or manure. The presence of organic substance promotes the growth of rich bacterial population and escalated temperature characteristic of composting.

- Bio piles are a hybrid version of composting and land farming. Mainly, engineered cells are developed as aerated composted piles. This is typically designed for controlling physical losses of the contaminants due to problems like leaching and volatilization.

Bio reactor is another method in ex-situ is slurry reactor in which contaminated soil and water are pumped from polluted body. Processing in this reactor involves an e engineered containment system.

A slurry bioreactor is containment vessel and apparatus applied for developing a three-phase (solid, liquid, and gas) mixing condition to enhance bioremediation rate of soil bound and water-soluble contaminants as a water slurry of the polluted soil and indigenous microorganisms able to degrade aimed impurities. Normally due to the closed bioreactor system and surrounding condition the rate and efficiency of biodegradation are enhanced than in situ systems as conditions are more manageable and controllable and hence predictable. Requirement of pretreatment or physical extraction before placing into reactor may be a drawback point. [14]

In-Situ

In general terms, In-situ is treatment at place. This is most opted technique because of its lower cost and less disturbance as it remediates at place without any excavation and transportation of contaminants. In situ treatment is constrained by the depth of the soil which can be effectively treated. There are various mechanism or techniques covered under In-situ process.

Bioventing is about stimulation of native microorganisms in contaminated soil by supplying air and nutrients by through wells. Bioventing applies low air flow rates and gives only the amount of oxygen required for the biodegradation while eliminating volatilization and release of pollutants in to the environment. It simply applied for hydrocarbons and also useful for deep under surface contaminations. In situ biodegradation necessitate supplying oxygen and nutrients by circulation of aqueous solutions through polluted soils for stimulation of indigenous microorganisms to degrade organic contaminants. This technique can be applied for soil and ground water.

Biosparging include the injection of air under pressure below the water table for increasing oxygen concentration in groundwater and for enhancing the biological degradation rate of contaminants by naturally occurring microbes. For increasing the contact between ground water and soil, Biosparging considers increase of mixing in the saturated zone. The ease and low cost of installation of small-diameter air injection point's permits considerable flexibility in the design and construction of the system.

Bioaugmentation as previously mention, for efficient remediation, indigenous microbes needs to add frequently to the contaminated site.

Two factors limiting factors for using added microbial cultures in a soil treatments are.: 1) non - native cultures rarely show comparable performance with an native population for developing and sustaining useful population levels and 2) if the soil treatment unit is well managed than most soils with long-term exposure to biodegradable waste have microbes of indigenous nature which that are effective in degradation.

1.2.4 Advantages of Bio remediation:

Bioremediation is natural process and is that is the main reason of its wide acceptance in public as an affordable waste treatment process for treating contaminated material like soil the conversion residues after the treatment are generally non-hazardous products. Mainly final products include carbon dioxide, water, and cell biomass. Theoretically, bioremediation is applied for the complete decomposition of a wide variety of pollutant substances. Many of them which are legally termed as hazardous can be transformed into harmless products. This way the future liability related to treat and dispose the contaminated material can be eliminated. Instead of conversion of contaminant from one medium to another one, like from water to air or soil, complete degradation of contaminant can be a possible option. Bioremediation can be carried out on site, often without causing a major disturbance of normal activities. This also eliminates the requirement of transportation of waste off site and hence can remove the potential threats to human health and the environment that can arise during transportation. Bioremediation proved economical option in comparision to other conventional methods being used for the same cause. [12-14]

1.2.5 Disadvantages of Bioremediation

With these advantages some disadvantages of bioremediation are there. It is limited to biodegradable substances. All compounds cannot be susceptible to fast and complete decomposition. Sometimes the converted product can be more harmful than parent product. The extrapolation of batch and pilot scale study to full scale operation is quite difficult. Biological processes are generally very particular regarding parameters. Important related parameters are necessary for process to work needs the presence of metabolically capable bacterial populations, suitable environmental growth conditions, and proper levels of nutrients and pollutants. More research is required for developing and engineering further bioremediation techniques which provide appropriate bioremediation efficiency to site having complex contaminants which are not evenly spread in the environment.

1.2.6 Phytoremediation

Phytoremediation is an advanced step in bioremediation. Phytoremediation is a growing technology which uses plant to remove pollutants from water or soil. It is a vegetation based remediation technique. In this, plants works as filter and by grabbing them from soil it metabolize the substance. As bacterial degradation acquired limited success for extensive residual organic and metals pollutants. This Vegetation- based bio remediation is having capability of immobilizing, accumulating and transforming a low level of persistent contaminants. This term "phytoremediation" was occurred in 1991. It shows a promising potential for future remediation techniques generally five types of phytoremediation techniques are found which are classified on the basis of contaminant fate: phytostabilization, phytoextraction, phytotransformation, rhizofiltration, phytodegradation, , also these can be found in combination in future. There are some drawbacks of the method that it is compulsory to include carefully before selecting it for site remediation: it requires long time duration for remediation, another big threat is potential contamination of the vegetation and food chain, and establishing and maintaining vegetation at some sites having high toxic levels is problematic.

1.3 Bio Accumulation

Bio accumulation is a process which permits for binding organic compounds or toxic metals inside cell structure. Bioaccumulation is having an active metabolic process which is driven by energy from living organisms and needs respiration, it derives from cellular surface by absorbing contaminants which are transferred onto and within it. Bioaccumulation is has two stage, initially metal ions get bounded tp the surface of cell. And then when cells have active metabolism system, then the biomass quantity increases. This can happen only when the cell gets suitable growth conditions. And microbes is metabolically active. However initial part is a passive function of metabolism.

Bioaccumulation is a slow and nutrient dependent while Biosorption is a quick process independent of presence of nutrients. It is also dependent on the culture condition and growth medium conditions the activation energy for this is about 63 KJ/mol, Corresponds to a biochemical process.

Apart of all these, bioaccumulation is truly a function of living cells, which needs enough amount of energy and nutrients. Along with that, heavy metals can harm the cell by imposing toxic effect on the cell. All the methods along with bioaccumulation having active cell turned out as comparative expensive and more complex.

It is the use of microorganisms to destroy or to break down hazardous substances or waste material into less toxic or nontoxic substances. It uses naturally occurring substance like microorganisms (i.e. Bacteria (aerobic and anaerobic) Fungi Actinomycetes (filamentous bacteria)

Bioremediation can effectively degrade organic constituents present in environment recently, biological remediation process have also been devised to either precipitate effectively immobilize inorganic pollutants such as heavy metals.

1.4 Biosorption of Heavy Metals

A search for a low-cost and easily available adsorbent has led to the investigation of materials of biological origin as potential metal biosorbents. In recent years, a significant number of studies on the removal of heavy metals from aqueous solutions by non-live, inactive biomass have been conducted worldwide. This approach of wastewater remediation is defined as biosorption, and the non-live biomass used in this approach is defined as biosorbent.

Removal of heavy metals by biosorption is a relatively new and developing technology in the field of water decontamination [15]. Absorbent materials (biosorbents), derived from a suitable agro-biomass, can be used for the effective removal and recovery of heavy metallic ions from wastewater streams [16]. Extensive studies have been commenced in recent years with the aims of finding alternative and economically feasible biosorbents for wastewater and water treatment. At a large scale, economic sorbents can be defined as materials which are abundant in nature or can be found as a by-product or waste from agro-industry, nature and industry, cheap and effective and which normally do not require pre-processing.

Recent studies on the removal of heavy metals using numerous types of biomass/biomaterials are reported in literature. Several mechanisms involve for biosorption of metals that differ qualitatively and quantitatively from species to species, origin, and processing procedure of biomass/biomaterials [17]. Some biosorbents show high heavy metal binding capacity, and the biological material is abundant and obtained inexpensively from the natural environment.

Biomass/biomaterials comprise with several chemical or functional groups such as acetamido, amino, amido, sulfhydryl, sulfate, and carboxyl which could attract and sequester the metals from solution (Gardea-Torresdey et al., 1990; Schiewer and Volesky, 2000; Volesky and Holan, 1995). Mainly ionic interactions and exchanges; formation of complexes between metal cations and ligands contained in the structure of the cell wall biopolymers, and precipitation on the cell wall matrix of biosorbent are the followed complex mechanisms of biosorption of metals (Schiewer and Volesky, 2000).

Pearson’s concept of hard and soft acid and base theory (HSAB) and by Irving-Williams series could be used to explain the binding characteristics of metallic cations during biosorption (Schiewer and Volesky, 2000). Consequently, biosorbents are now being considered as an alternative to ion exchangers or other metal extraction and concentration operations in metal recovery. As such, the study of biosorption is of great importance from an environmental point of view. There are biomass types of various kinds, such as leaves, wood or agricultural residues, waste crustacean biomass-anything renewable that grow, including microbes. Biosorbent is generally ranked in categories of bacterial, fungi, algae, industrial wastes, agricultural wastes, natural residues, and other biomaterials. The main advantages of biosorption are that it is either abundant or wastes from other sectors. Distinctive capabilities of certain types of biomass which adsorb and immobilize heavy metals can be more or less selective. These advantages have made biosorption an alternative cost-effective way for the treatment of metal bearing wastewaters.

Chapter 2

Literature Review

2.1 Background

Environmental pollution has become a prime concern for industrial and developing countries from the middle of last century. Massive human and industrial growth has changes the pristine condition of the environment towards a worse condition. The need to supply the basic needs for humans a massive development in the industrial sector as well as the modern agriculture sector has impacted on the world. These sectors have become sources of pollutants for nature. The pollutants coming from different sources as wastes are gaseous, liquid and solid form. These wastes are been released to the air, discharged to water or buried into landfill which causes further problems to the environment and living creatures.

Heavy metals are abundantly present in nature and are also added to water by human activities such as battery production, electronics, paper and pulp industries, metal fabrication and mining activities, smelting, electrolyzing, drug manufacturing, paint preparation, alloy manufacturing, galvanizing, printing, dyeing, paper making, ceramics manufacturing and inorganic dyestuff preparation (Liu et al., 2008; Sari et al., 2007). The presence of potentially toxic heavy metals in the environment is of very much concern, primarily due to their non-biodegradability and persistence in the environment (Volesky, 1999b). High concentrations of heavy metals in water supplies are undesirable as they may have potentially adverse effects on the health of organisms, the suitability of water for various purposes, the longevity of water and sewer networks, and the aesthetic of the environment (Demirbas, 2008; Vieira and Volesky, 2010). Some metals are known to be able to become concentrated in food chains through bioaccumulation, notably mercury and cadmium (Burger and Gochfeld, 2004). Some metals are harmless and traces are required as nutrients for balanced growth; for example, cobalt, copper, iron, selenium, and zinc are considered nutrients needed for balanced growth. High dosage of heavy metals may cause toxicity that is acute, chronic, synergistic, or mutagenic/teratogenic (Burger and Gochfeld, 2004). The presence of heavy metals at higher levels in surface and groundwater ecosystems also inhibits the growth of aqueous organisms and stops any beneficial use of the water bodies. These metal ions, and their supplementary complexes, could accumulate in the body of fishes and other aquatic organisms, and finally could reach to the human body by bioaccumulation, bio-concentration and bio-magnification through the drink and food chains (Hong et al., 2006; Hu et al., 2007).

2.2 Heavy metals: sources, toxicity and environmental fates

2.2.1. Sources of Heavy Metals

Heavy metals enter the environment through natural and anthropogenic activities. For instance, the weathering of rocks naturally produces heavy metals. On the other hand, the burning of petroleum and non-ferrous metalworking contribute to the emissions of heavy metals in the atmosphere. It is estimated that an average of 332,350 tons of lead (II), 35,370 tons of copper(II) and 131,880 tons of zinc(II) are emitted in the atmosphere each year. Furthermore, the main causes of water pollution are household waste, iron and steel production, metal smelters, metal plating or finishing operations, mining and fertilizer applications. The anthropogenic median annual inputs of lead, copper, nickel and zinc into aquatic systems were reported to be 138,000 t, 112,000 t, 113,000 t and 226,000 t, respectively (Nriagu et al., 2004). The two principal sources of heavy metals in soils are the disposal of ash residues from coal combustion and general disposal of commercial products on land. It is estimated the average annual worldwide emissions into the soils to be 796,000 t for lead; 954,000 t for copper; 325,000 t for nickel and 1,372,000 t for zinc) (Nriagu et al., 2004).

Heavy metal pollution is one of the most important environmental problems today. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous ores, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation etc (Wang and Chen, 2009). Among these, the following four appear as the main priority targets, particularly in the industrialized world (Volesky, 2007):

- acid mine drainage (AMD)-associated with mining operations,
- electroplating industry waste solutions (growth industry),
- coal-based power generation (throughput of enormous quantities of coal),
- Nuclear power generation (uranium mining/processing and special waste generation).

Three kinds of heavy metals are of concern, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) (USEPA, 2002; Wang and Chen, 2009). Global production of lead from mines has reduced from 3.6 million tonnes in 1975 to 3.1 million tonnes in 2004, while global refined lead production has amplified from 4.7 million tonnes to 7.1 million tonnes, because the recycled lead has added to the supply (USEPA, 2002). Lead production is not much by developing countries compared to the production by other developed countries like China, Australia, USA and Canada. They produce about 76% of the world production. Table 4 shows lead production through different countries including developing ones where, the production via recycling of previously used lead is higher for developed countries (60-70% of their lead production) than developing countries (<30%).

Cobalt:

There are certain characteristics found for cobalt that make it such important element in nature like, it has silver-white color, hard and brittle element, and it consider as hard ferromagnetic. It is a member of VIII group in periodic table similar as iron, also it can be magnetized easily. The physical property of cobalt is very much similar to iron and nickel. Cobalt is more active chemically, and can forms many compounds during reaction. It can stay in stable form in air and stay as unaffected by water but it can be slowly affect by dilute acids.

Cobalt has wide application as it is used as a part of many alloys which are used in many parts of machineries, also used as a drying agent for inks and paints, cobalt is an essential part of craft workers and artists’ pallets in form of cobalt blue. Mainly used for catalyzing processes in petrochemical and chemical industries.

Cobalt generally present in environment in various forms in air, soil, water, animals and plants. It may enter and circulate in these various entities by different modes like by wind-blown dust it enters air and water and by rain water when it flows through rock and soil containing cobalt it passes to surface water sources.

Once Cobalt entered the ecosystem it cannot be destroyed. It circulates or react within it by reacting with other particles or adsorbing into water sediments.it generally moves under acidic condition, but most of the time cobalt will deposit in soils and sediments. When it doesn’t mobilize in natural way, the level of cobalt in soil decreases and the plant grows in deficient soil it also become deficient in cobalt and hence the chain goes on.

On the other side, when the concentration of cobalt is higher in the soil, it mobilize to food chain, generally cobalt leads to accumulate in the food chain by the time. Which gradually leads to worst effects to ecosystem. Exposure to cobalt may enhance by skin contact with water or soil containing the same.

Cobalt is required in human body as a nutrient part as it is a part of vitamin B_{12}, which stimulates red blood cell production, However, Cobalt can cause harm to human health if present in high concentration. When too much of cobalt intake can cause asthma or lung effects.

Manganese:

Manganese is one of the naturally found element in earth’s core, it is available in abundant amount in soils, generally present as oxides and hydroxides, and it cycles through its various oxidation states. It principally occurs as pyrolusite (MnO_2), and up to lesser extent as rhodochrosite ($MnCO_3$). It has a pinkish-gray appearance.it has chemically active nature which let it to oxidized easily. It reactive in pure form. It shows some properties alike to iron as it rusts when in contact to water and also get dissolved in dilute acids. Generally it is hard to melt as it is a hard and brittle in nature.

Manganese is released into the environment by industries such as fertilizers, petrochemicals, electro-plating, tanneries metal processing, and mining. Exposure to manganese causes neurotoxicity [21], low hemoglobin levels [22] and gastrointestinal accumulation [23]. However, human activities are also responsible for much of the manganese contamination in water in some areas. Water to be used in the textile, dyeing, beverages and white paper industries should contain less than 0.05 mg/L of manganese.

Manganese element is beneficial for healthy human dietary system and also for other process in human body. As manganese is essential element for human to survive and on the other side it is one of the three essential toxic trace elements. This means that when it is exposed in a defined limit it is useful but when it exceeds the dosage limit it can cause serious damages. There are some intake limits set by Standard agencies like WHO, USEPA and European standards.

Increased concentration may absorbed into gastrointestinal tract and also it can transported through blood by bounding with plasma proteins. It may transfer through breast milk and placenta also. It can accumulate into liver, pancreas, kidney and central nervous system. It may cause acute and poisoning and may turn to chronic and long term effects.

As per WHO Mn consumption value is regulated to 0.5 mg/L (ppm) for drinking water and further reduced to 0.1 ppm on based of some consumer complaints. USEPA states this limit as 0.05 ppm on health based limit.

Manganese in increased concentration can cause swellings of cell walls, leaf's withering and brown patches on leaves. Increased concentration may accumulate into food chain and can create imbalance in it .Between toxic concentration and concentration that cause deficiency, an optimum concentration is defined for the optimum requirements. Laboratory tests done with animals have shown that severe manganese poisoning is able to cause tumor development with animals.

2.2 Conventional Methods

As the removal of heavy metals from waste water has become the priority of the effluent treatment due to stringent rule and regulations. It is mandatory to treat metal loaded waste water before discharging it to environment system.

There are number of various conventional treatments available to treat inorganic effluent. Such unit operation are ion exchange, chemical precipitation, electrode position, complexation, solvent extraction.

Amongst these methods, some methods are commonly used in waste water treatment. From all the methods precipitation is most economical. Still due to economic factors many industries chose chemical procedures to treat heavy metals. But due to complexity addition to wastewater characteristics, efficiency of overall treatment may decrease and besides that the toxic sludge generation is the main issue.

2.3.1 Chemical Precipitation:

Chemical precipitation is the method of precipitating out the dissolved heavy metals by binding them with precipitating reagent. By adding the reagent, it leads to chemical reaction which makes dissolved metals to bind in solid particles. Then by chemical coagulation, these particles get aggregated and removed sedimentation or filtration. This method is generally incorporate with chemical coagulation for heavy metal removal. This method is one of the less expensive and commonly used method amongst all conventional methods. Though it is not the efficient method to treat effluent loaded with high acidic content. Apart of this, it generates a huge amount of toxic sludge which requires chemically stabilized treatment, proper handling and safe disposal. This is a major drawback of this procedure which leads to financial constraints and complex operations.

2.3.2 Coagulation & Flocculation

Coagulation-flocculation Mechanism is based on measurement of zeta potential, which is the criteria to define the electrostatic interaction between pollutants and coagulant-flocculants agents. In coagulation process the net surface charge of the colloidal particles in wastewater is reduced through stabilizing with the help of electrostatic repulsion process. In flocculation process particles size continuously increase to discrete particles by additional collision and interaction of inorganic polymers that are formed through added organic polymers as discrete particles are flocculated into larger particles, removal or separation of them through filtration, straining or flotation. Main drawbacks of this process are, large amount of sludge production, to find the application of chemicals, and transferring of toxic compounds into solid phase.

2.3.3 Ion exchange

Ion exchange is one type of chemical reaction. In this reaction cations or anions having special ion exchanger are applied to remove metal ions from the waste water. In this heavy metal ions are exchanged on solid particle having similar charge ions. This method is efficient to exchange soluble metal ions from aqueous phase to solid phase. .generally synthetic organic ion resins or inorganic zeolites are commonly used as solid ion exchanger particles. Quantity of exchanges of ions is based on distinct number of available ion sites per unit of resin. This is one of the most efficient and used conventional method. Also is a cost effective and easily operational method but besides that its removal is limited to only low concentrated aqueous solution.it needs pretreatment before the process as it is sensitive to the pH of aqueous phase and limited by high acidic nature of effluent.

2.3.4 Electrolysis

Electrolytic method is one of the method to remove heavy metals by using principle based on electricity. In this a metal bearing solution consists cathode plate and an anode, current passes through that aqueous solution. Electron movement from one element to another element generates electricity. Priorly heavy metals are precipitated in a neutralize catholyte or weak acid or as hydroxides. Various electrochemical processes available to treat effluent are electro-deposition, electro-oxidation, electro-coagulation and electro floatation. Electro-destabilization is similar to precipitation method by creating coagulant as hydroxide by electrolytic oxidation and destabilizes pollutants to form flocks. In electro-coagulation process, charged metal ions are removed by reacting with anion in the effluent. These process have comparatively less sludge generation, easy operations and no complex chemical requirements. Lacking point of these method is increased energy demands and comparatively less efficient.

Physico-chemical techniques are the part of conventional methods of removal. Physical separations are basically used for specific forms of metals and some discrete elements. Mechanical screening, magnetic separation, floatation, hydrodynamic separation, electrostatic separation are physical operations to remove metal impurities from effluents. Amongst these methods not all are applicable to aqueous effluents, mainly all are to treat metal contaminated soil or solid phase.

2.3.5 Membrane Filtration

Membrane filtration has gained noticeable attention for removing suspended solid, organic compounds and inorganic contaminants such as heavy metals. Different types of membranes based on different particle size retention can engaged for heavy metal removal. Various membrane filtration techniques based on filter size are nano filtration, ultrafiltration, and reverse osmosis. Ultrafiltration uses permeable membrane for separation. Removal of heavy metals, suspended solids from effluent is a function of pore size (i.e. 5-20 nm) and molecular weight of the elements to be separated. It generally works on 2-5 bar pressure giving around 90% efficiency.

Another hybrid technique, complexation– ultrafiltration, it works using water soluble metal binding polymer in corporation with ultrafiltration. These can be turn as an efficient technology formed by merger of two methods i.e. precipitation and ion exchange.

Reverse osmosis is a pressure based separation. Membrane used in separation is semipermeable which retains metal and allows solvent to pass through. In RO system most of separation occurs in the polymer matrix which works as dense layer of barrier. RO is used in industrial processes as it can remove various types of molecules and ions from the effluent. Efficiency of separation is dependent on concentration of solute and pressure as it uses a diffusive mechanism.

These are the reasons which enhances the opportunity of novel methods and approaches which can provide economically feasible and efficient solution.

Conventional methods for removing heavy metals have numbers of unignorable disadvantages such as incomplete metal removal, high energy demands and generate high amount of toxic sludge which needs proper handling and discharge methods. Upon all this it has to face financial constraints.

Using precipitation method, cause loss of valuable resources and those resources end up dumping in landfills.

Membrane separation process may be considerable for commercial application, high pressure application of membrane separation, i.e. Reverse Osmosis which is having low water permeability and is limited due to high pressure requirement. On the other side ultrafiltration and microfiltration contains a low rejection potential. Apart of this activated charcoal can be feasible option but the cost of adsorbent and its maintenance can be a disadvantage.

Biological methods for removing heavy metals and other pollutants from waste water includes various biological process like activated sludge process, UASB, trickling filter, aerated lagoons, stabilization pond. Microorganisms are a key function for these processes. Activated sludge is widely used treatment option for treating organic effluents. By providing an active atmosphere for growth, microbes break down the complex organic contaminant. Activated sludge unit consist of an aeration tank, in which the “activated sludge” having active microbes, get continuously recycled to the aeration tank provided with aeration system and agitation. Then the broke down solids create bio flocks which settles down in proceeding clarifier. For increasing the rate of degradation of substances one can play with recycle ratio, retention time, microbial population and aerating rate. For efficient heavy metal by biological techniques research have turned into the direction of suspended microbial growth in activated sludge process. Another most used treatment of trickling filter it is one of the best examples of attached microbial growth techniques. This unit consists of coarse media beds of certain designed height (generally keeps between 3-10 feet) effluent water is pumped in upward direction and sprayed onto the medium beds then it gets trickled down through bacterial beds. Throughout this flow the pollutant comes in contact with attached microbial biofilm, microbes degrades the organic matter and the flow to the treated waste water is in downward direction. It get collected at bottom and then passes through sedimentation to remove solids. To increase the removal efficiency, researchers play with different affected parameter like microbial culture characteristics, retention time, design parameters etc. Other methods like aerated lagoon, stabilization ponds are the economical option. They are comparatively slow and having lower efficiency as these methods depends on the sunlight availability, available oxygen, algal growth and bacterial population.

Apart of these widespread biological waste water treatment, other methods to treat heavy metals and trace metals along with various organic, inorganic contaminants are being studied by the current flow of researchers. These methods are accepted as capable technique for soil treatment and then with further researches various application by various branches has occurred. Bioremediation is very wide term which incorporate almost every mechanism involving microbial remediation including, bioaccumulation, bio stimulation, biosorption, etc.

2.4 Petrochemical Process as Source of Pollution

Petrochemical industries are one of the key sector to the global development. It is one of the most complex industrial sector which affects every sphere of life. Our day-to-day usage of plastics soaps and other products provide support to the statement that “petrochemicals is one of the most important part in our daily world”. According to Naderpour N, each year 5% of oil and gas is required for manufacturing of petrochemical products. Amongst the whole chemicals market petrochemicals share around 40 % of all. Petrochemicals acquires a major part in today's society as they are essential for food, clothing, shelter and leisure.

Petrochemicals links with oil and gas sectors in upward flow while it has a connection with pharmaceuticals and other related products from downward side. The petrochemical industry majorly refurbishes feed stocks such as naphtha and natural gas components like ethane, propane and butane through steam cracking or catalytic cracking into petrochemical building blocks i.e. olefins and aromatics. Other than oil and gas industries, petrochemicals are linked with many industrial sectors like polymers, synthetic rubber, plastics, soaps, solvents ,synthetic fibers and detergents, , drugs, fertilizers, explosives, paints, pesticides, and flooring and insulating materials.

2.4.1 Pure Terephthalic Acid

PTA (i.e. pure terapthalic acid) is one of the important bulk monomers used in the production of polyester. Teraphalic acid is also called as para-pthalic acid. It is a basic reactant in the polyethylene terephthalate (PET) process, PET is a polymer which is generally used in plastic bottle and cloths manufacturing. Polyester is produced by reaction of PTA with ethylene glycol as shown in the Scheme 2.The esterification reaction forms a carbon-oxygen bond with elimination of a water molecule, which is removed from the reaction zone to push the reaction forward to get the polymer of desired molecular weight.

Initially terapthalic acid is produced in form of CTA- Crude terapthalic acid and then CTA is get purified to get pure terapthalic acid. CTA is produced by liquid phase oxidation of *p*-xylene with oxygen in acetic acid, Cobalt and manganese salts are used as catalysts along with promoters such as bromide ions in this reaction. Methyl group get oxidized in two respective steps. First, p-toluic acid gets converted from p-xylene and then to crude p-toluic acid along with undesired product 4-carboxybenzaldehyde (4-CBA). It is then purified by one additional chemical step along with physical procedures like filtration, crystallization, centrifugation and washing to obtain the p-toluic acid with the removal of 4-carboxyaldehyde.

To remove that is quite difficult by physical means but by using catalytic hydrogenation in an aqueous phase it can be reverted to form from which it was formed, which is easily removable by above mentioned physical operations.

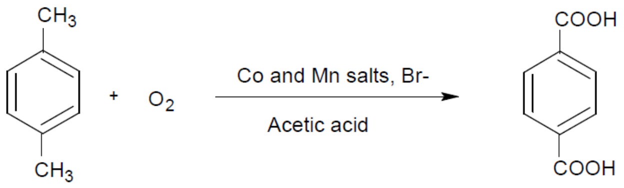


Figure 1 Oxidation of p-Xylene to Terephthalic acid

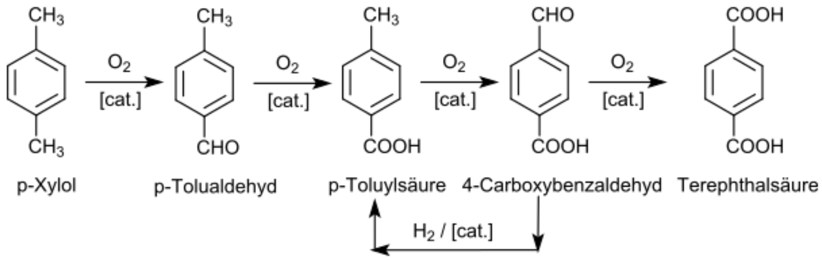


Figure 2 Intermediates reactions in PTA

Major processing steps in purification are:

- para- xylene oxidation (CTA synthesis)
- CTA crystallization
- CTA centrifugation or filtration
- CTA drying
- CTA dissolution
- Hydrogenation (CTA purification to PTA)
- PTA crystallization and filtration
- PTA centrifugation
- PTA drying

Co and Mn are used as co-catalyst in the PTA process in acetic acid medium. After processing of the PTA slurry is pre-treated for purification and in this process a huge amount of Co and Mn enter into the wastewater stream.

Thus, the objective of this project is to remove Co and Mn from wastewater stream by biosorption onto waste biomass generated in effluent treatment plants which is rich in ligno-cellulosic biomass and thus suitable for biosorption.

Chapter 3

Materials and Methodology

3.1 Collection and Pretreatment of Biomass:

Sludge sample was collected from one of the aeration tank of RIL site's ETP plant and biomass was derived by concentrating and centrifuge process of the waste water sample

Collected biomass was thoroughly washed with distilled water and saline solution (i.e. 0.85% NaCl) for the removal of some impurities and for conditioning of biomass. After washing biomass was suspended in (0.85% NaCl solution). MLSS analysis was carried out for determining initial biomass concentration in the collected sludge.

3.2 Equilibrium Assay:

Initial experiment which was carried out was equilibrium study to determine the proof of concept (i.e. biomass is able to intake the metal elements). Experimental run time was considered on maximum basis i.e. 24 Hr., the procedure for the equilibrium assay was derived by reviewing various research literatures.

3.2.1 Procedure:

The experiment was conducted into 250 mL Erlenmeyer flask by adding 10 mL wet biomass into 100 mL solution containing known concentration of Mn (II) and Co (II) metal ions, and agitated for 24 Hr. on a rotary mechanical shaker with controlled rotation speed and temperature to attain equilibrium assay. After that biomass was separated by centrifuging at 11000 rpm for 10 min and supernatant was analyzed by ICP Atomic Emission Spectroscopy (Perkin-Elmer Optima 8300 Model) to determine the metal concentration in it.

3.3 Desorption and Regeneration Study:

Following to Equilibrium Assay desorption and Regeneration study was also carried out. Along with regeneration of biomass, the secondary purpose of this study was to avail mass balance of metal concentration for verification of the fact that the biomass actually take up the metal ions.

3.3.1 Experimental Procedure:

Desorption was carried out for 24 hr. on a rotary mechanical shaker with controlled rotation speed and temperature. In 250 ML Erlenmeyer flask 10 ML metal loaded biomass (which is used previously for equilibrium study). 100 ML of 0.5N HCl was used for the metal leaching process (use of 0.5N HCl was an observational outcome of various review paper. After 24 Hr., the biomass was then washed with distilled water up to neutral pH and then with 0.85% NaCl saline solution for several times to regenerate the metal binding sites and recondition the biomass for making it available for the further cycles. To determine the metal concentration into supernatant it was analyzed by ICP Atomic Emission Spectroscopy (Perkin-Elmer Optima 8300 Model).

Along with equilibrium Assay an experiment was carried out to observe the MLSS growth in biomass. MLSS test was done after equilibrium run and acid leaching procedure. After this analysis one experiment was set up to observe MLSS behavior with respect to time. Equilibrium runs of various time period like 30 minutes, 1 hour, 3 hours, 5 hours and 15 hours were carried out as per previously mentioned procedure. After defined time periods MLSS was carried out for each flask

3.4 Parameter Optimization Study:

Experiments were carried out for Focusing on parameter affecting the efficiency of removal. Parameters studied were contact time, pH and dosage of biosorbent and feed concentration. First parameter which affect the biosorption assay is the contact time of biomass and metal solution.

a. Effect of Contact Time:

For optimizing contact time the time of experimental run 7 Erlenmeyer flask were prepared for experiment. Each flask was containing 100 ML of feed (waste water sample) containing known concentration of metal ions and 10 ML of wet biomass and agitated on rotary mechanical shaker for 6 different time periods. Different time duration were following, 15 min, 30 min. 1 hr., 2 hr., 3 hr. and 4 hr. One blank was kept for 4 hour (100 ml DW + 10 ml biomass) to verify the fact that the biomass itself does not contain any amount of Mn (II) and Co (II) metal ions.

Flask were removed from shaker on defined time period and the separation of biomass was done by centrifugation at rotation speed of 11,000 rpm for duration of 10 min and metal concentration in supernatant was analyzed by ICP Atomic Emission Spectroscopy (Perkin-Elmer Optima 8300 Model).

This biosorption assay was followed by the acid leaching or desorption and regeneration procedure. 7 Erlenmeyer flask were used for the same, each flask was having 10 ML metal loaded biomass and 100 ml of 0.5N HCl. Flask were agitated for 24 Hr. on rotary mechanical shaker on previously defined conditions. After run of 24 Hr. Biomass was separated by centrifuging on defined rotational speed for 10 min and then the separated biomass was washed with distilled water and 0.85% NaCl for conditioning of biomass. And the supernatants were analyzed by ICP Atomic Emission Spectroscopy (Perkin-Elmer Optima 8300 Model).

b. Effect of pH:

For pH study 5 various pH conditions were provided during biosorption assay of feed having known concentration of metal in it, that pH were 3, 5, 7 and 9. In 4 Erlenmeyer flask this pH conditions were provided and procedure was followed same as mentioned in other biosorption assays. After 4 hr. of run time the biomass was separated by centrifugation and the left supernatants were analyzed by ICP Atomic Emission Spectroscopy (Perkin-Elmer Optima 8300 Model) for identifying metal concentration present in it.

c. Effect of Biosorbent Dosage:

For studying this parameter, took 6 Erlenmeyer Flasks and prepared them for biosorption assay by adding 100 ml feed of known metal concentration into that. Biomass added was varied for optimizing the dosage. Various dosage added into flasks were 5 ml, 10 ml, 15ml, 20 ml, 25 ml and 50 ml. and run them for 4 h at optimized pH. As per all previous procedures the biomass was separated by centrifugation and supernatant was analyzed by ICP Atomic Emission Spectroscopy (Perkin-Elmer Optima 8300 Model) to identify metal concentration left in it.

d. Adsorption Isotherm

Of the many adsorption theory isotherm models, the simpler two-parameter models of Langmuir and Freundlich have most often been used in biosorption equilibrium study reports and reference has been made to more complex models. Looking into the classical adsorption theory, additional models are potentially available in order to try to describe the biosorption equilibrium data. We should, however, keep in mind that, as their mechanistic significance for biosorption is absent, augmenting the number of a model's parameters may simply lead to an easier curve-fitting exercise. The Langmuir and Freundlich models can be readily linearized. Thus, one can then determine, from their linearized form and the associated experimental data fitting, the model parameter values pertinent to the specific experimental conditions employed. The availability of biosorption equilibrium model parameters is very useful in the modeling of the performance of engineering reactor configurations for the practical applications of biosorption. Langmuir and Freundlich adsorption isotherms are applied and results are evaluated.

3.5 Continuous Column Study

3.5.1 Preparation of Dry Biomass:

Immobilization of live (wet) biomass with calcium alginate was studied on batch study. Along with this by following various literatures, immobilization of dry biomass was also carried out for comparatively better results than wet biomass immobilization.

Activated sludge flock collected the same way as per previous methods, from an aeration tank of one of the effluent treatment plant. Sludge was washed thoroughly with deionized water and then given saline wash (i.e. 0.85% NaCl) as a part of pretreatment to open the activated sites, then it is again washed with deionized water. Than sludge is concentrated by centrifuging at 5000 rpm for 10 min.

Collected sludge was than dried in hot air oven at 90°C for 24 hr. Completely dried biomass than grinded to powdered form by using a mixture grinder device, powdered biomass used in immobilization was of size of 60 µm, as grinded powder was sieved by a 60 µm sized sieve.

3.5.2 Immobilization Process:

Prepared defined quantity of solutions of 4 % sodium alginate and 3% $CaCl_2$ in two different Erlenmeyer flasks. Added defined weight of dry biomass powder to prepare the solution having 3 % dry biomass in the 4% sodium alginate solution. To avoid lump formation biomass need to be added slowly into alginate solution under continuous stirring system. Stirring must be continued until the homogenous mixture formation occurs. To make sure the even distribution of biomass cells into beads, mixture of alginate and biomass needs to evenly mixed and homogenized. Keep the solution under continuous stirring to avoid the biomass cell to settle down.

To prepare immobilized beads, prepared alginate and biomass mixture was injected drop wise into a vessel having 3 % $CaCl_2$ solution with the help of a hypodermic syringe having 0.5 mm tip diameter. This prepared immobilized beads were cured into 0.1M $CaCl_2$ solution for getting strengthened. After strengthening into $CaCl_2$ solution for 4 to 5 hours, beads were suspended in 2 % glutaraldehyde solution for cross linking of the same for overnight. After cross-linking beads washed thoroughly with deionized water and stored in suitable conditions.

3.5.3 Column Study

In the column experiment, a glass column (1.2 cm internal diameter and 25 cm long) was packed to 9.7 cm with immobilized biomass having desired bio beads. A long column was selected to conduct the studies at various depth of packing such as 20 cm, 30 cm, etc. Co^{2+} and Mn^{2+} ion solutions is allowed to flow from top to bottom of the column at a desired flow rate using a peristaltic pump. The pH of the solution was maintained at 7. The effluent from the bottom of the column was collected at 10 min intervals and the residual Co and Mn ion concentration is measured after filtration using ICP analysis. The column experiment is continued till the effluent concentration reached 99% of the influent concentration.

Figure 3 Experimental set-up for column adsorption study

Chapter 4

Results and Discussion

4.1 BATCH BIOSORPTION STUDY

4.1.1 Proof of Concept

Table 1Analysis for Biosorption Process

Sample Codes	**Mn^{2+} (ppm)**	**Co^{2+} (ppm)**
Initial Conc. In feed	11.43	5.63
After 24 Hr.	0.06	0.072
After Acid leaching	9.061	4.146
% REMOVAL	**99.48**	**98.72**

Initial experiment which was performed with purpose of getting proof of concept was containing the ICP analysis. The ICP results gives conformation of the fact that the biomass being used for the study is sufficiently capable to intake heavy metal ions. The good efficiency of the biomass to sequester the metal ions is being reflected by the percentage removal calculated with respect to the initial concentration metal in the feed.

4.1.2 MLSS Study:

For analyzing the growth of biomass MLSS test was carried out on three different times. This values show the growth in MLSS and indicate growth of biomass by metal uptake.

Table 2 MLSS study result

Sample	I. Wt.(gm)	F .Wt. (gm)	Result (ppm)
initial	0.0894	0.09548	**3020**
After equilibrium study	0.09101	0.09811	**3550**

After acid leachate taste	0.09091	0.09905	**4070**

Table 3 MLSS Study on Time Basis

Flask no.	I.Wt .(gm)	F. Wt. (gm)	Result (ppm)
30 min.	0.08863	0.09472	**3045**
60 min.	0.09087	0.09656	**2845**
3 hr.	0.09066	0.09765	**3495**
5 Hr.	0.08994	0.06913	**3095**
12+ hr.	0.09070	0.09821	**3755**

Study of MLSS with respect to time shows the growth of MLSS with increase in time duration.it also indicates that biomass is in taking the Metal ions and using it in their growth and supports the fact that biomass can alleviate heavy metal concentration

4.1.3 Study of affecting Parameters:

After getting proof of concept, parameter study was started for get optimized condition to achieve the maximum possible removal efficiency. So the analysis results are mentioned of the experiment performed for studying effect of various parameters on metal uptake capacity.

4.1.4 Effect of Contact Time:

Contact time of Biosorbent and feed to be treated needs to be optimized first as it is essential parameter to run the experiment. Contact time is among the most important factors contributing to successful application of an absorber as it indicates the rate at which metals are absorbed. As it can be noticed from the Figure no. 4.4 that the maximum removal efficiency of metal intake is achieved in 4 hrs. of run time. Figure shows the influences of contact time on the changes in the concentration of cobalt, and manganese, throughout the sampling period in the aqueous solution until the equilibrium time is reached.

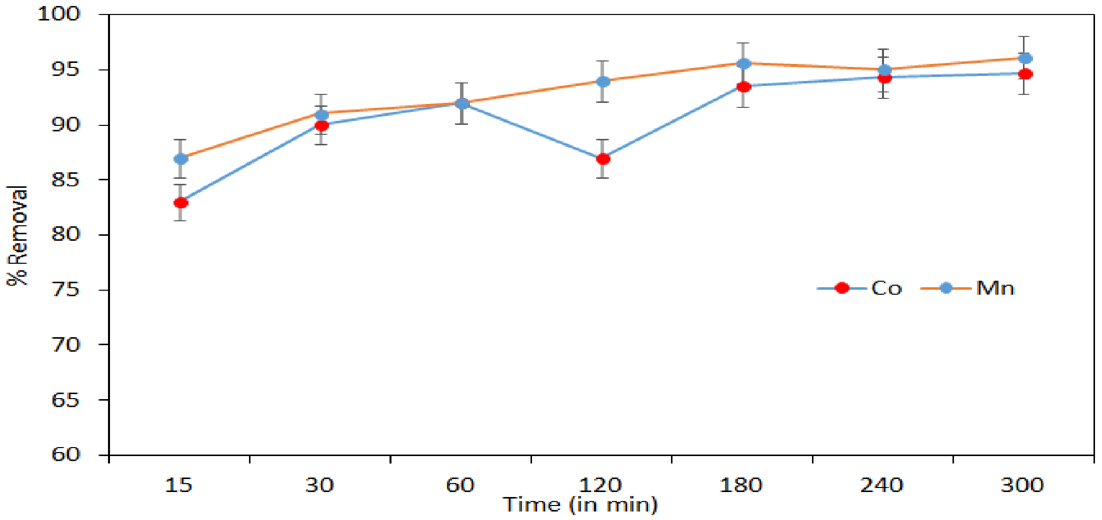


Figure 4

Figure No: 4.1: Effect of Contact Time

As it can be seen maximum adsorption occurs in the initial 20 minutes and then it slowly attains equilibrium. This rapid decrease of metal concentration in the initial phase might be due to interactions with functional groups deployed on the cell surface, while the slow decreasing trend in the second phase is due to intracellular accumulation. Maximum biosorption achieved for Co and Mn was 95% and 97% respectively in 240 min and this is taken as equilibrium time for further studies.

4.1.5 Effect of Initial pH:

Biosorption of metal ions at different pH values (3–9) was conducted at the following conditions: Adsorbent dose 0.5 g/L, 240 min reaction time, feed with initial concentration of Co and Mn as 2.5 and 5 ppm respectively with 200 rpm shaking speed and the results are shown in Figure.

Removal efficiency of Co+2 and Mn+2, was gradually increased with the increase of the pH value from 3 to 7 and then decline at higher pH value owing to the competitive adsorption between hydrogen ion and the heavy metal cation at low pH. Furthermore, the electronegative metal ions possess higher charge density and as a result they easily adsorb onto the biosludge. However, at higher pH value, there is a decrease in removal efficiency of metal ions due to the abundance of −OH ions that cause hindrance effect on the diffusion of metal ions to the organic matter.

It is clear that adsorption capacity of biosludge is based on the pH value and hence the surface charge and the ionization. The optimum pH value is ~7 for maximum adsorption of Co+2 and Mn+2 ions**.**

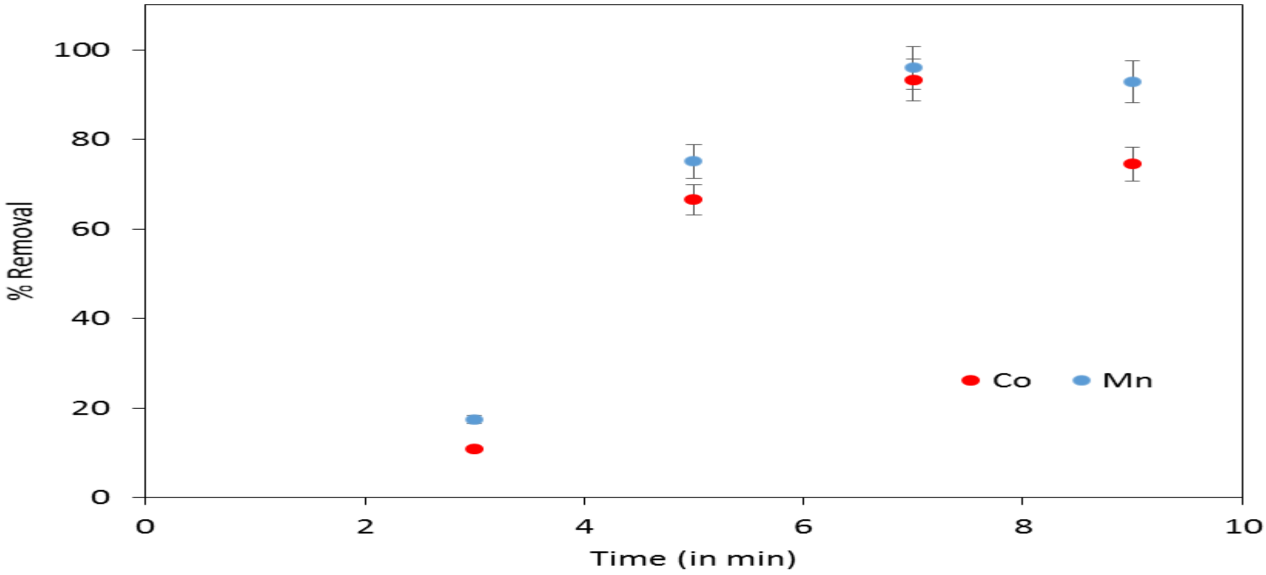


Figure 5 Effect of initial pH

4.1.6 Effect of Adsorbent Dosage

The optimal doses of biosludge 0.25, 0.5, 1, 1.5, 2 and 2.5 g/L to adsorb metal ions $Co^{+2}$and Mn^{+2} were studied by conduction systematic experiments at constant conditions: pH value ~7, for 240 min shaking time, with initial concentration of Co and Mn as 2.5 and 5 ppm respectively with 200 rpm shaking speed.

The results revealed that the metal ions removal efficiency was boosted with the increase of biosludge dose from 0.25 to 2.5 g. Moreover, the Mn^{+2} removal efficiency was the highest and reached to almost 95% within 240 min shaking time. Furthermore, metal ions adsorption capacity decreased with boosting of biosludge dose. There could be many factors contributing to this result. We suggest that the most important one is the adsorption site remaining unsaturated during the adsorption reaction, this is attributed to the non-saturation of the adsorption active sites (Han et al., 2006; Achak et al., 2009). The present results regarding biosludge as efficient adsorbent confirmed that it is efficient for removal of heavy metal ions.

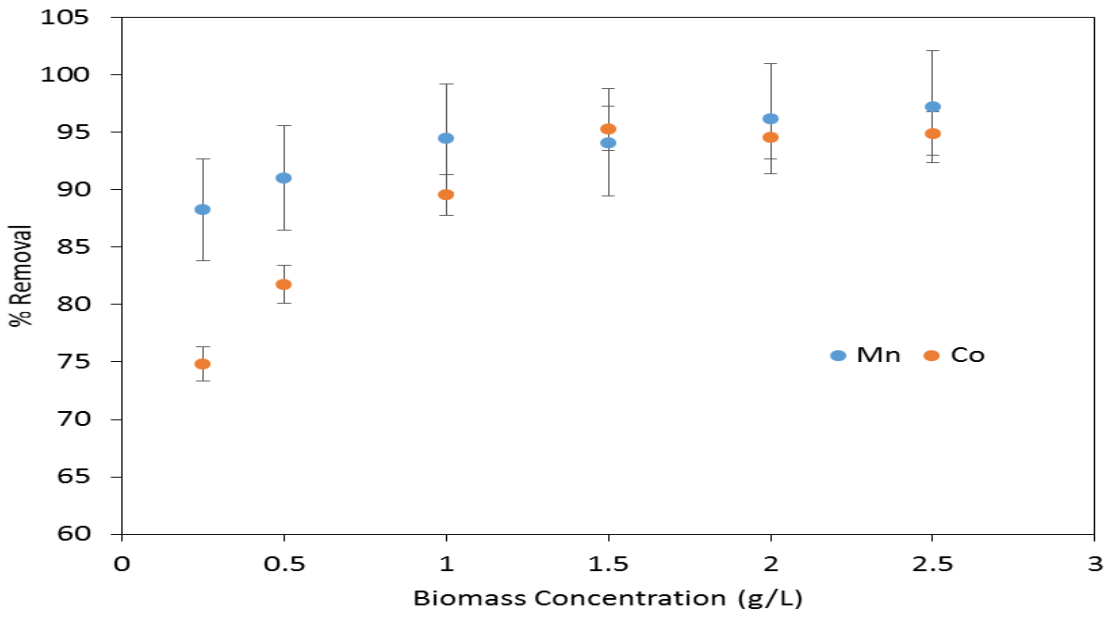


Figure 6 Effect of Adsorbent Dosage

4.1.7 Adsorption Isotherm

(a) Langmuir Adsorption Isotherm

Adsorption isotherm equation represented the relation between the amount of solute adsorbed and the concentration of the solute in the aqueous phase. The adsorption isotherms are evaluated in order to describe the process (Sari and Tuzen, 2009). The Langmuir and the Freundlich adsorption isotherms' equations have been successfully applied to many adsorption processes (Abdelfattah et al.,2016; Khoufi et al., 2008; Aktas and Cecen, 2007; Ozkaya, 2006;Aksu and Yener, 2001) and were adopted in the present study as follows:

The Langmuir equation is based on the assumptions that maximum adsorption corresponds to a saturated mono-layer of adsorbate molecules on the adsorbent surface with uniform distribution of energy level, (Langmuir, 1916). The Langmuir isotherm is defined as:

$$q_e = \frac{q\max \ K_L \, C_e}{1 + K_L \, C_e} \qquad \text{----- (1)}$$

And in linearized form

$$\frac{C_e}{q_e} = \frac{1}{q_m \, K_L} \, \frac{1}{q_m} \times \, C_e \qquad \text{----- (2)}$$

Where q_m and K_L are Langmuir constants related to the sorption capacity, and sorption energy, respectively, C_e is the equilibrium concentration in mg/L, and q_e is the amount of adsorbate adsorbed per unit weight of adsorbent (mg/g). The essential characteristics of Langmuir dimensionless constant separation factor or equilibrium parameter, R_L, which is defined by the following equation:

$$R_L = \frac{1}{1 + C_e \times K1_L} \qquad \text{------ (3)}$$

From the value of R_L calculated using the above expression, the nature of the adsorption process is determined to be one of the following: Unfavorable ($R_L > 1$), Linear ($R_L = 1$), Favorable ($0 < R_L < 1$), Irreversible ($R_L = 0$). The Langmuir isotherm constants and their correlation coefficients R^2 for adsorption of heavy metals ions via biosludge are listed in Table 4

Table 4 Langmuir constants for the sorption of Co2+ and Mn2+ onto biosludge

Metal Ion	Slope	Intercept	Qmax (mg/g)	K_L	R_L	R^2
Co^{2+}	0.164	0.761	6.10	0.22	0.13	0.987
Mn^{2+}	0.070	0.077	14.29	0.91	0.07	0.998

From the regression values obtained t can be clearly seen that the biosorption process fits well into Langmuir adsorption isotherm and also the RL values ranged between 0 and 1 show that the adsorption process in favorable.

(b) Freundlich Adsorption Isotherm

The Freundlich adsorption isotherm, one of the most widely used mathematical descriptions, gives an expression encompassing the surface heterogeneity and the exponential distribution of active sites and their energies (Freundlich, 1906). The Freundlich isotherm is defined as:

$$q_e = C_e^{\frac{1}{n}} \qquad \text{------- (4)}$$

And in the linearized form it is expressed as:

$$\ln q_e = \ln K_F + (1/n) \ln C_e \qquad \text{-------- (5)}$$

Where C_e is the equilibrium concentration in mg/L, q_e = amount of adsorbate adsorbed per unit weight of adsorbent (mg/g). "K_F" is a parameter related to the temperature and "n" is a characteristic constant for the adsorption system under study. The Freundlich isotherm constants (n) and their correlation coefficients R^2 are summarized in Table 5

Table 5 Freundlich constants for the sorption of Co2+ and Mn2+ onto biosludge

Metal Ion	Slope	Intercept	Ln KF	KF	1/n	n	R^2
Co^{2+}	0.206	0.91	0.91	2.5	0.206	4.9	0.764
Mn^{2+}	0.26	1.65	1.65	5.2	0.26	3.9	0.670

The Freundlich isotherm model for adsorption of Co^{2+} and Mn^{2+} fitted less with equilibrium data, presented a moderate R^2 value (ranged between 0.617 and 0.764) (Table 4.5). For both ions' values of "n" between 2 and 10, there is a favorable adsorption behavior.

4.1.8 Conclusion

Looking to the work done so far and the derived results from the lab scale experiments it can be statmented that the selected bio sludge is efficient to remove heavy metals from effluent waste water. By considering this statement and results of metal removal, further study scope are visible to identify and explore more in this study. Further study is aimed to build a continuous operation of biosorpton process by calculating thermodynamic and kinetic parameters.

4.2 CONTINUOUS BIOSORPTION STUDY

4.2.1 Background

To validate the biosorption data obtained under batch conditions, assessment of sorption performance in a continuously operated column is necessary as the biosorbent uptake capacity is well utilized by metals ions in this completely mixed system (Acheampong et al., 2011). In addition the contact time required to attain equilibrium is different under column operation than batch system (Chandra Sekhar et al., 2003a; Vilar et al., 2008). For column operation, the biosorbent is continuously in contact with freshly metals laded wastewater and subsequently, the concentration in the solution in contact with a given layer of the biosorbent in a column changes very slowly. A fixed-bed column is simple to operate and economically valuable for wastewater treatment (Ahmad and Hameed, 2010; Singh et al., 2009; Sousa et al., 2010). Experiments using a laboratory scale fixed-bed column of relatively large volume yield performance data that can be used to design a larger pilot and industrial scale plant with a high degree of accuracy (Chandra Sekhar et al., 2003a).

A continuous removal of Co (II) and Mn (II) from a mixture of artificial wastewater by cabbage waste in a fixed-bed column to determine process design for practical uses are investigated. Further, the breakthrough data obtained for each operating condition were fitted to adsorption model to enable appropriate design and scale up of the biosorption column. The sludge investigated in this study was found to be the most effective biosorbent. The conditions in the column experiments were flow rate, bed depth and initial metals concentration on continuous biosorption.

An acrylic column was designed and constructed for conducting the column experiment. Different conditions are used to evaluate the metal biosorption by immobilized sludge and the experimental results with discussion are as below.

4.2.2 Textural Characterization of prepared Waste Activated Sludge

The immobilized alginate beads were analyzed by polarized optical microscopy (POM) <u>(under progress)</u> and Fourier transform infrared spectroscopy (FTIR) to understand the surface morphology and chemical functional nature of the immobilized beads.

FTIR Analysis

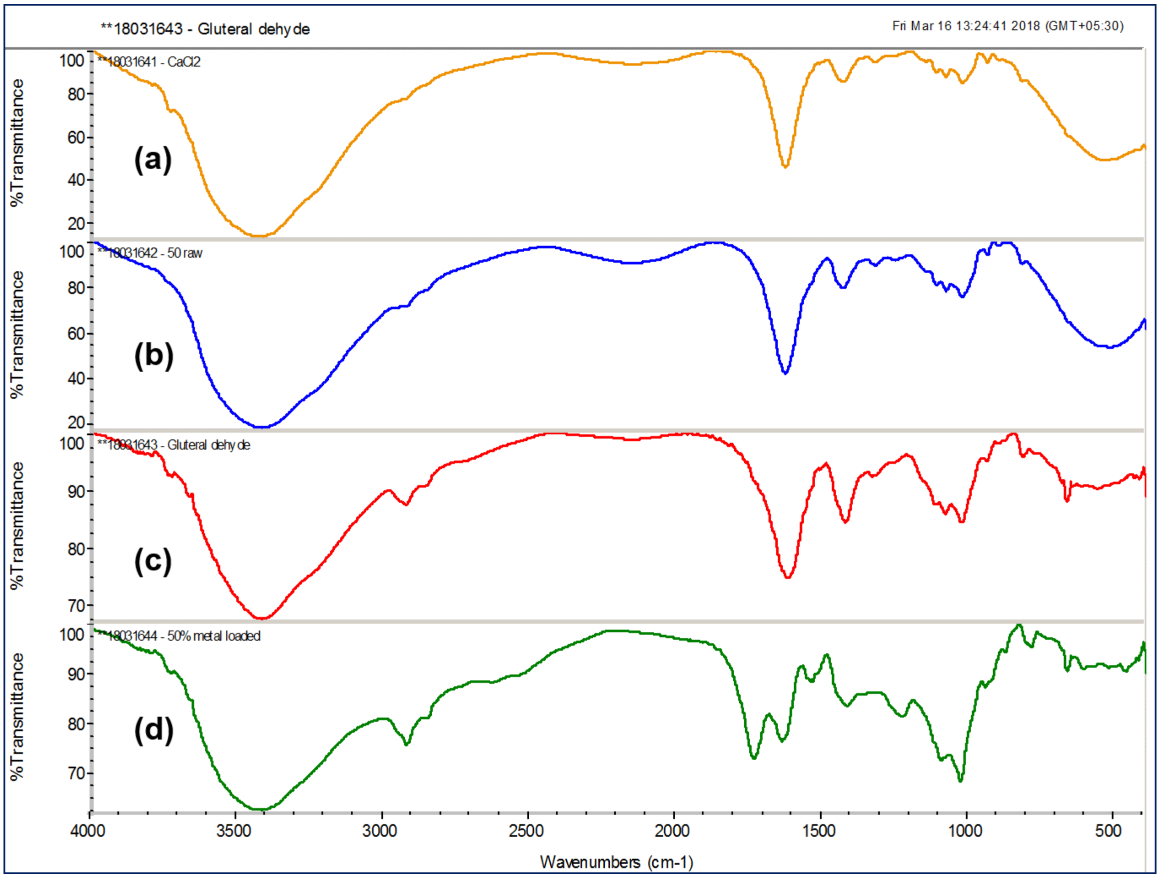


Figure 7 FTIR spectrum of (a) Ca-Alginate bead; (b) Glutaraldehyde cross linked alginate bead; (c) Biomass immobilized-cross liked bead; (d) Metal sorbed onto immobilized bead

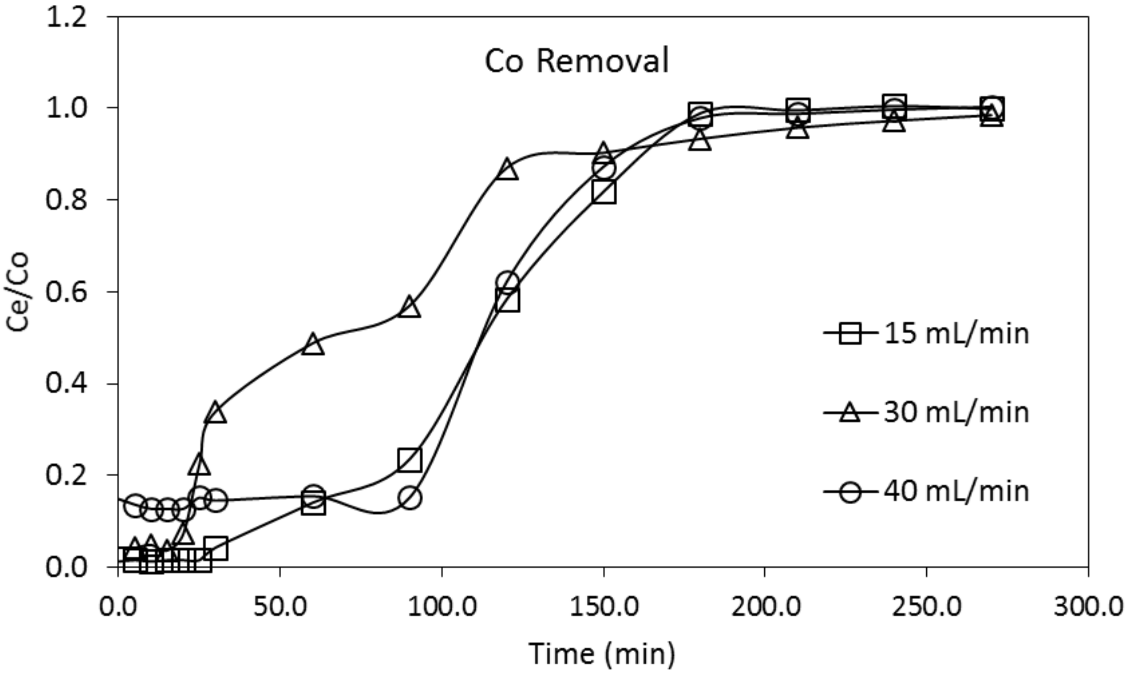
The FTIR spectrum of biomass (Fig. 4a) shows distinct peaks between 3,797.6 and 3,733.4 cm^{-1}, 3418.3 cm^{-1} indicating the presence of OH, NH, and NH_2 groups. At 2,114.11 cm^{-1} asymmetric vibration of CH and 2,860.53 cm^{-1} symmetric vibration of CH. 2,382.17 cm^{-1} vibration of carboxylic acids is indicated. 1,641.48 and 1,552.75 cm^{-1} stretching vibration of C=O and NH peptidic bond of proteins. 1,433.16 cm^{-1} of phenolic OH and CO stretching. The 1,037.74 cm^{-1} band is vibration of C–O–C polysaccharides. The <1,000 cm^{-1} is fingerprint zone which is phosphate and alkyl halides groups. So, it can be concluded that the waste activated sludge has different functional groups of proteins, lipids, polymeric compounds and carboxylic, amine, and amide groups may be responsible for biosorption process.

In Fig 4b the peaks corresponding to NH_2 are not present which might be sue to cross linking of the glutaraldehyde carbonyl groups with amino group. Also it shows strong C=O carboxylic acid vibrational peak. In Fig. 4d there is a change in the intensities of the peaks which might be due to metal interactions with the immobilized beads leading to coordination of metals to the functional group of the alginate matrix. This coordination confirms the sorption of metal ions onto the alginate beads by chemisorption.

4.2.3 Effect of Flow Rate

The column was operated at three flow rates of 15 mL/min, 30 mL/min and 40 mL/min with bed depth of 5 cm, immobilized beads of 25 g. and initial metals concentration of 5 mg/L Co and 10 mg/L Mn were kept constant. The breakthrough curves achieved are presented in Figure 4.5(A) for Co (II) and Figure 4.5(B) for Mn (II).

Figure 4.5 showed that that the breakthrough time as well as the exhaustion time increased with a decrease in flow rate (from 15 to 40 mL/min) for Co and Mn adsorption. The slope of the plots from breakthrough time to exhaustion time increased as the flow rate was increased from 15 to 40 mL/min. It means that the breakthrough curve produced steep slopes with the increased flow rate for all metals adsorption. A higher flow rate resulted in a lower residence time in the column and vice versa (Acheampong et al., 2013). An increase in the flow rate reduced the contact time between metal ions and immobilized beads; and also reduced the volume of effluent efficiently treated before the bed became saturated (Khan et al., 2012; Tunali Akar et al., 2012). Therefore, it decreased the service time of the bed (Figure 4.5 A and B). It demonstrates that breakthrough occurs faster at a higher flow rate for Co ions. It might occur due to bypass of flow in a clod formation at low flow rate.



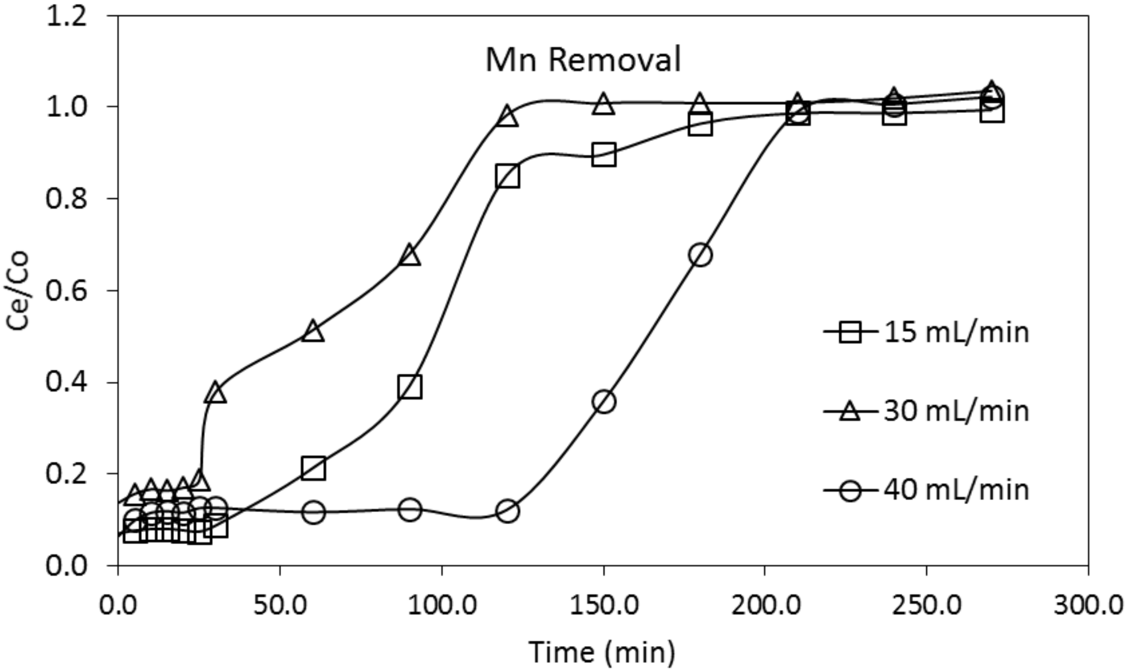


Figure 8 Breakthrough curves for Co and Mn biosorption onto immobilized biomass at different flow rates (bed depth = 5 cm, inlet Co conc. 5 mg/L and Mn 10 mg/L)

4.2.4 Effect of Bed Depth

The bed depth is an important parameter for design a fixed bed column for continuous wastewater treatment system. In this context, three bed depth (5, 10 and 12 cm) are used for removal of multimetals [Co and Mn] from an artificial multi-metal solution. The experimental results are showed in Figure 4.6 (A and B).

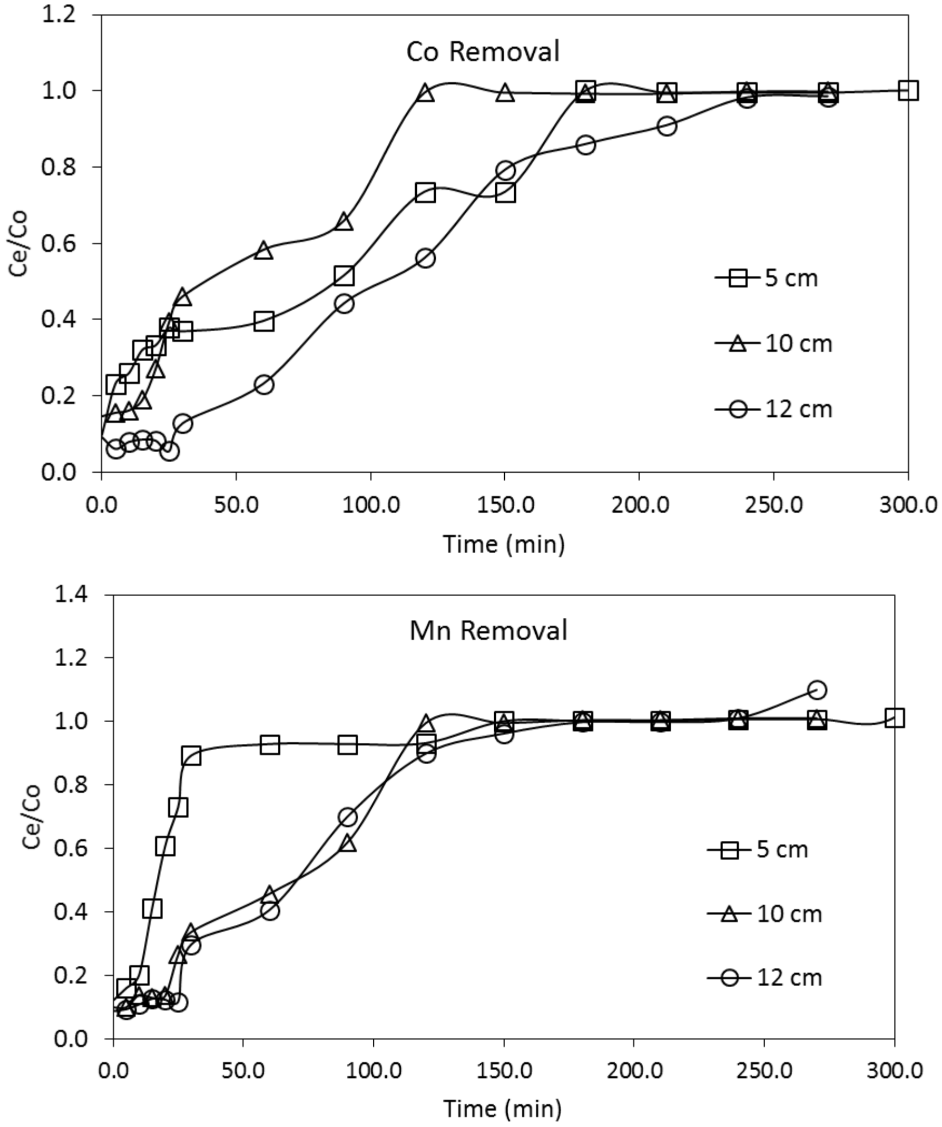
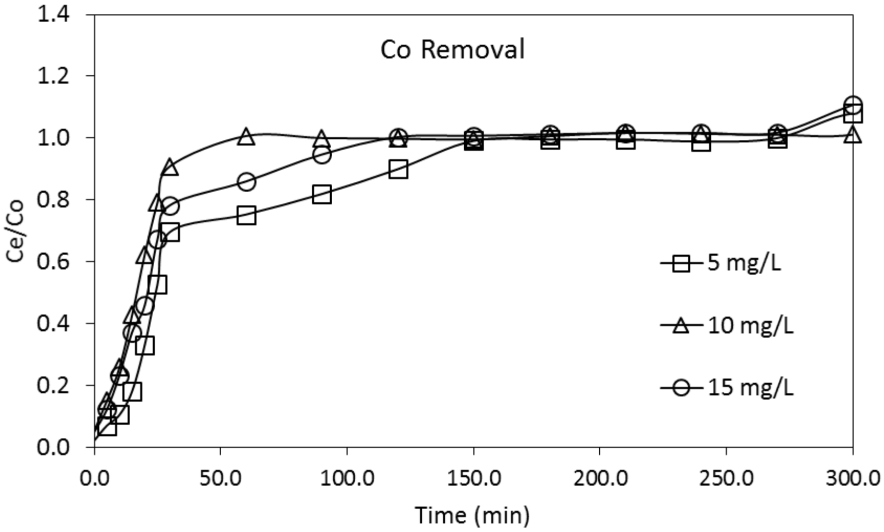


Figure 9 Breakthrough curves for Co and Mn biosorption onto immobilized biomass at different bed height (bed depth = 5 to 15 cm, inlet Co conc. 5 mg/L and Mn 10 mg/L)

The breakthrough curves obtained for Pb(II), Cd(II), Cu(II) and Zn(II) ions biosorption are illustrated in Figure 4.6 (A, B, C and D) for different bed depth of immobilized beads (2.25, 4.5 and 9.0 cm), at a constant linear flow rate of 30 mL/min and metals concentration (5 mg/L Co and 10 mg/L Mn). It is visible from the plots (Figure 4.6) that a characteristic ‘S’ shaped profile generated in ideal biosorption systems. It can be predicted that the breakthrough volume varies with bed depth. Axial dispersion phenomena predominate in the mass transfer and reduce the diffusion of metallic ions when the bed depth is reduced (Malkoc and Nuhoglu, 2006a). The solute (metallic ions) does not have enough time to diffuse into the whole of the adsorbent mass (Taty-Costodes et al., 2005). As observed from Figure 4.6 (A and B), the treated volume considerably decreased from about 31050 to 20700 ml for Co and 28980 to 12420 ml for Mn as the bed depth increased from 5 to 15 cm. At higher bed depth the sludge biosorbent were not dispersed properly in the used flow rate so as the treated volume is reduced (Sousa et al., 2010; Valderrama et al.2010).

4.2.5 Effect of Initial Metals Concentrations

The effect of varying the inlet metals concentration from 5 to 20 mg/l on the shape of the breakthrough curves was studied at a constant biosorbent bed depth (5 cm) and feed flow rate (30 mL/min). The resulting breakthrough curves are presented in Figure 4.7 (A and B) for Co and Mn ions adsorption on to immobilized beads loaded columns.



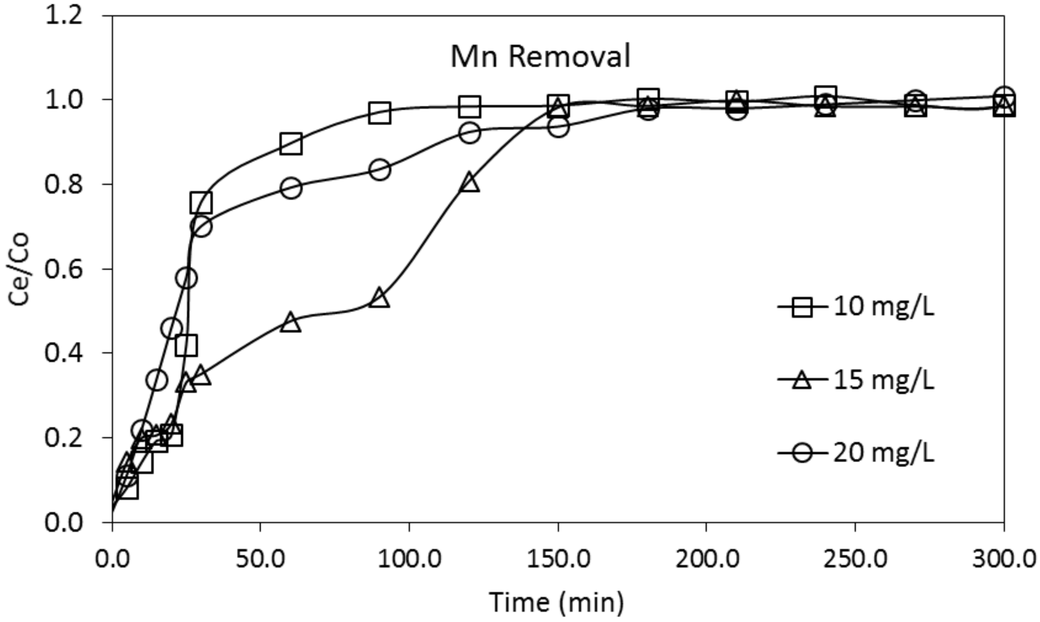


Figure 10 Breakthrough curves for Co and Mn biosorption onto immobilized biomass at different initial concentration (bed depth = 5 cm, inlet Co conc. 5 mg/L and Mn 10 mg/L)

The curves show that the breakthrough time decreased with increasing inlet Co and Mn concentration. The larger the initial concentration (C0) for all metals, the steeper the breakthrough curve and the shorter the breakthrough time (Figure 4.7 A and B). An increase in the inlet concentration reduced the treated volume 31050 ml to 588550 ml for Co and 31050 ml to 625670 ml for Mn before the bed gets saturated. As a high metals concentrations may saturate the biosorbent more quickly, the operation time was decreased. Decreasing the Co and Mn concentration increases the volume of the feed metals solution that can be treated, shifting the breakthrough curve to the right (Figure 4.7 A and B) (Acheampong et al., 2013). The value of Ce/C0 reached near unity at 60 min for Co and 150 min for Mn adsorption when the inlet concentrations were 5, 10 and 20 mg/l, respectively.

4.2.6 Conclusions

It was found that fixed-bed systems achieved a better uptake of Co and Mn by immobilized biomass at a lower metals concentration, lower feed flow rate and lower bed-depth. The suitable service time to breakthrough and metals Co and Mn ions concentration were 60 min and 5 mg/l, respectively. Among the various models applied to describe the metal breakthrough in the column, the BDST model was able to describe the data properly, whereas prediction of the metals uptake capacity by the Thomas model was poor. The adsorption capacities found by Thomas model were higher than batch system. The Yoon-Nelson model predicted the time required for breakthrough well and can be applied to the entire breakthrough curve. Nevertheless, the entire breakthrough curve was best predicted by the Clark model in the higher flow rate and higher bed depth. The design of a continuous fixed bed column treatment system with immobilized biosorbent for Co and Mn laden wastewater can thus be reached using the BDST, Yoon- Nelson and Clark breakthrough models.

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