REMOVAL OF Ni (II) USING PIGEON PEA POD(*Cajanus cajan*) : EQUILIBRIUM, KINETICS AND OPTIMIZATION STUDIES

A PROJECT REPORT

Submitted by

LENIN. C (Reg. No: 0810204019)
NANCY FLAVIA. C (Reg. No: 0810204027)
RASHIKA. P (Reg. No: 0810204035)
SARAVANAN. S (Reg. No: 0810204039)

in partial fulfillment for the award of the degree

of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY
(An autonomous institution affiliated to Anna University of Technology, Coimbatore)

ANNA UNIVERSITY OF TECHNOLOGY, COIMBATORE
COIMBATORE-641 047

APRIL 2012

ANNA UNIVERSITY OF TECHNOLOGY COIMBATORE, COIMBATORE

BONAFIDE CERTIFICATE

Certified that this project report **"REMOVAL OF NICKEL USING PIGEON PEA POD-EQUILIBRIUM, KINETICS AND OPTIMIZATION STUDIES"** is the bonafide work of **LENIN. C, NANCY FLAVIA. C, RASHIKA. P** and **SARAVANAN. S** who carried out the project work under my supervision.

SIGNATURE	**SIGNATURE**
SUPERVISOR	**HEAD OF THE DEPARTMENT**
Dr. J. ARAVIND	**Dr. A. MANICKAM**
Assistant Professor	Professor and Head
Department of Biotechnology	Department of Biotechnology
Kumaraguru College of Technology	Kumaraguru College of Technology
P. O. Box No. 2034	P. O. Box No. 2034
Chinnavedampatti	Chinnavedampatti
Coimbatore – 641 049	Coimbatore – 641 049

Internal Examiner

External Examiner

ACKNOWLEDGEMENT

We are grateful to our project guide **Dr.J.Aravind**, Assistant Professor (SRG), for his valuable guidance, persistent support, constant motivation, for the successful completion of this project.

We would like to thank the **management** of Kumaraguru College of Technology and **Dr. S. Ramachandran**, Principal for the consistent support.

We express our sincere gratitude to **Dr. A. Manickam**, Professor and Head, Department of Biotechnology for his motivation and encouragement throughout the project.

We thank our review committee members **Mr. M. Shanmugaprakash**, Assistant Professor (SRG) and **Mrs. P. Kanmani**, Assistant Professor for all their valuable suggestions that came at the right time and greatly helped us in completing our project successfully.

We extend our sincere thanks to **Mr. S. Sivamani**, Assistant Professor for his valuable inputs that steered us in the right direction all the way through the project.

Our whole hearted thanks to our Class advisor **Dr. K. Kumaresan**, Assistant professor and our tutor **Mr. P. Muthukumaran**, Assistant Professor for their undivided attention and continual encouragement during the tenure of the project work.

We take this opportunity to thank all the **teaching** and **non-teaching staff** of the Department of Biotechnology, Kumaraguru College of Technology, for all their help, care and guidance throughout the project work.

Finally we thank our parents and siblings for their unambiguous support throughout our project work.

[LENIN. C] [NANCY FLAVIA. C] [RASHIKA. P] [SARAVANAN.S]

TABLE OF CONTENTS

ABSTRACT

Pod of pigeon pea (*Cajanus cajan*), a cellulose rich agricultural residue was investigated for its nickel binding efficiency. The adsorbent exhibited strong adsorption efficiency with equilibrium loading of 250 mg of metal per g of sorbent. The influence of key physico-chemical parameters like contact time, initial metal ion concentration, adsorbent dosage and pH on Ni (II) removal was studied. The equilibrium time was found to be 45 minutes. The optimum Ni (II) removal was obtained at an initial metal ion concentration of 80 mg l^{-1}, pH of 9.0 and an adsorbent dose of 400 mg/100ml. The adsorption data of metal ion have been described by the Freundlich, Langmuir and Temkin isotherm model. Under the optimal conditions selected, the metal ion adsorption equilibrium was best described by the Langmuir isotherm model. The maximum adsorption capacity of NaOH pretreated pigeon pea pod was found to be 250mg g^{-1}. Kinetic models such as pseudo first-order and second order are used to evaluate the mechanism of adsorption of Ni (II) on the adsorbent. The adsorption process follows second order kinetics and the corresponding rate constants, for initial Ni (II) concentration ranging from 20 –100 mgl^{-1}, is found to be 0.179-0.0035 g mg^{-1} min^{-1}. A total of 17 adsorption experimental runs were carried out employing the detailed conditions designed by response surface methodology based on the Box–Behnken design. The analysis of variance (ANOVA) indicated that a second-order polynomial regression equation was the most appropriate for fitting the experimental data. The experimental confirmation tests showed a correlation between the predicted and experimental responses (R^2 = 0.9714). The optimal point obtained was located in the valid region and the optimum adsorption parameters were predicted as an initial Ni (II) concentration of 60 mgl^{-1}, pH value of 9.0 and contact time of 75 minutes. Under these adsorption conditions, a maximum removal of 96.54% of initial metal concentration was demonstrated.

Key words: pod of pigeon pea, biosorption, heavy metal, optimization, isotherm, kinetics, RSM

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND NOMENCLATURE

Abbreviation/Symbol	Description
l	Litre
ml	Milliliter
g	Gram
mg	Milligram
%	Percentage
min	Minute
^oC	Degree Celsius
pH	Potential of hydrogen
Ni	Nickel
q_e	amount of adsorbed metal ions per unit weight of adsorbent at equilibrium (mg/g)
C_e	equilibrium concentration (mg/l)
Q_o	Maximum adsorption capacity (mg/g)
K	Rate constant
b_T	related to the heat of adsorption
B_T	$(RT)/b_T$
R	Universal gas constant
A_T	equilibrium binding constant (l min^{-1})
df	Degrees of freedom
R	Regression coefficient
h	$1/kq_e^2$

1. INTRODUCTION

1.1 Toxic effects of heavy metals

Industrial effluents serve as a major source of Environmental pollution. Among these environmental pollutants, heavy metals have gained relatively more significance due to their persistence, bio-magnification and toxicity. Combined industrial activities of mining, electroplating, tanning, manufacture of fertilizers, etc led to the release of toxic heavy metals into the environment. In spite of this, industrial use of these heavy metals had increased rapidly, over the years. While organic pollutants are biodegradable, heavy metals like Ni, Cr, Cd, etc. are non biodegradable and had contributed to major environmental concern.

The toxic effects of these heavy metals is being felt increasingly by human beings, animals, plants and all living organisms that inhabit the earth. The toxic effects are attributed to their non biodegradability, long biological half lives and capacity to accumulate in human body. Excessive accumulation of heavy metals in agricultural soils, may not only result in soil contamination, but also affect food quality and safety (Muchuweti et al., 2006). The consumption of heavy metal-contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defences, intrauterine growth retardation, impaired psycho-social behaviour, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer. Wong et al. (2002) demonstrated that heavy metals are potentially toxic to crops, animals, and humans when contaminated soils are used for crop production.

1.2 Nickel toxicity

Nickel is a major constituent of effluents from electroplating, fertilizer and paper industries. All nickel compounds, except for metallic nickel, have been classified as human carcinogens by the International Agency for Research on Cancer (IARC).

Uptake of too large quantities of nickel affects human health adversely:

- Higher chances of development of lung cancer, nose cancer, larynx cancer and prostate cancer
- Sickness and dizziness after exposure to nickel gas
- Lung embolism

- Respiratory failure
- Birth defects
- Asthma and chronic bronchitis
- Allergic reactions such as skin rashes, mainly from jewelry
- Heart disorders

Moreover, prolonged exposure to nickel may result in genotoxicity, neurotoxicity, nephrotoxicity, immunotoxicity and reproductive toxicity. Nickel sensitivity, once acquired, appears to persist indefinitely.

1.3 Drawbacks of existing technologies

The existing technologies for removal of metal ion from industrial effluents are very costly. They include ion exchange resin, solvent extraction, electrolytic and precipitation processes, electro dialysis, membrane technology, adsorption using granular activated carbon.

1.4 Background for study

The search for new techniques for the removal of toxic metals directed attention towards biosorption, This method is based on metal binding capacity of various biological materials. Cellulosic agricultural waste materials act as an important source for metal biosorption. The functional groups present in agricultural waste biomass viz. acetamido, alcoholic, carbonyl, phenolic, amido, amino, sulphydryl groups etc. have affinity for heavy metal ions to form metal complexes or chelates. The mechanism of biosorption process includes chemisorption, complexation, adsorption on surface, diffusion through pores and ion exchange etc.

1.5 Motivation for study

Natural materials that are available in large quantities, or certain waste products from agricultural operations are sources of inexpensive adsorbents. Due to their low cost, after these materials have been expended, they can be disposed off without regeneration.

The advantages of biosorption over conventional techniques include low cost, high selectivity, regenerative capacity, no sludge generation, metal recovery and competitive performance. Agricultural wastes are characterized by ready availability, affordability, eco-friendliness and high uptake capacity for heavy metals due to presence of functional groups which can bind metals to effect the removal of heavy metal from effluents.

Severely harmful effects of these heavy metals on environment and human health motivates an extensive study to identify technically and financially feasible methods for effective elimination of these metals. The husk of grains like rice, wheat, Bengal gram and corn had been studied extensively in the past, for their adsorption efficiency. The results showing high efficiencies at low cost triggers further study to estimate the adsorption characteristics of similar low cost agricultural waste products.

Dry matter yield of pigeon pea in pod shells are almost equal to the seed yield. However, pod shells are low in protein and high in fibers. This contains high levels of digestible crude protein, but low levels of digestible energy and sulfur (Whiteman and Norton, 1981).

Present work is aimed at removal of nickel from aqueous synthetic single metal solution using an agricultural by-product, pod of pigeon pea (*Cajanus cajan*) as biosorbent.

1.6 Objectives

- To study the efficiency of bio-resource material (pod of pigeon pea) in removal of heavy metals
- To study the effect of pH, contact time, adsorbent dosage, metal ion concentration on metal adsorption
- Optimization study-RSM

2. LITERATURE REVIEW

2.1 METALS IN WASTEWATER AND THEIR TOXICITY

Effluents from textile, leather, tannery, electroplating, galvanizing, pigment and dyes, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sector contains considerable amounts of toxic metal ions. These toxic metals ions are not only potential human health hazards but also to other life forms. Toxic metal ions cause life-threatening illness including irreversible damage to vital body system (Malik, 2004).

2.2 TOXICOLOGICAL ASPECTS OF HEAVY METALS

Due to their mobility in aquatic ecosystems and their toxicity to higher life forms, heavy metals in surface and groundwater supplies have been classified as major inorganic contaminants in the environment. Even if they are present in dilute, undetectable quantities, their recalcitrance and consequent persistence in water bodies imply that through natural processes such as biomagnifications, concentrations may become elevated to such an extent that they exhibit toxic characteristics (Prabhakar and Rao, 2004).

2.3 CONVENTIONAL METHODS FOR TREATMENT OF HEAVY METALS

The traditional method followed in industries to remove toxic heavy metal wastes is as follows:

A heavy metal (in the form of ion) can be removed from waste water, by adding to the waste water a metal scavenger together with sodium monosulfide (or) sodium polysulfides (or) sodium hydrogensulfide to form a metal ion containing floc. The resulting floc is then removed from the waste water by filtration. The metal scavenger contains at least one carbodithio group or at least one carbodithioate salt group as N-substituents per molecule. The commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction.

The process description of each method is presented below.

- **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 (Johnston *et al.*, 1973).
- **Electrodialysis:** In this process, the ionic components (heavy metals) are separated through the use of semi-permeable ionselective 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 (Pedersen *et al.*, 2003).
- **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 (Hilal *et al.*, 2004).
- **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 (Mier *et al*, 2001).

- **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 (Eccles *et al*, 1995).

The disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal has made it imperative for a cost-effective treatment method that is capable of removing heavy metals from aqueous effluents.

2.4 PHYSICO–CHEMICAL TREATMENT TECHNIQUES FOR WASTEWATER LADEN WITH HEAVY METALS

Ion exchange and membrane filtration are the most frequently applied for the treatment of metal-contaminated wastewater. Ion exchange has achieved a complete removal of Cd (II), Cr(III), Cu(II), Ni(II) and Zn(II) with an initial concentration of 100 mg/L. The results are comparable to that of reverse osmosis, which showed 99% of Cd(II) rejection with an initial concentration of 200 mg/L. Lime precipitation has been found to be one of the most effective means to treat inorganic effluent with a metal concentration of higher than 1000 mg/L. 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 (Wai-HungLo, 2006).

2.5 ADSORPTION OF HEAVY METALS USING COMMERCIAL ACTIVATED CARBON

Batch adsorption and desorption studies were carried out to determine the metal-binding ability of activated carbons. Two commercial activated carbon fibers (ACFs), A-20 and W10-W, were employed to compare the removal performance of poly acrylo nitrile (PAN) derived activated carbons. Influence of oxidation treatment of PAN fiber before steam activation was also determined. Results indicated that steam produced a higher surface area but a lower yield as compared to CO_2. Also, precursors activated by steam showed a greater removal performance. For both activation methods, fiber displayed a better metal-binding ability than coke (Zainni *et al*., 2010).

2.6 BIOSORPTION

Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (Prabhakar and Rao, 2004). Large quantities of metals can be accumulated by a variety of processes dependent and independent on metabolism. Both living and dead biomass as well as cellular products such as polysaccharides can be used for metal removal. Biosorption has advantages compared with conventional techniques. Some of
these have listed below:

- **Cheap**: the cost of the biosorbent is low since they often are made from abundant or waste material.

- **Metal selective:** the metal sorbing performance of different types of biomass can be more or less selective on different metals. This depends on various factors such as type of biomass, mixture in the solution, type of biomass preparation and physico-chemical treatment.
- **Regenerative:** biosorbents can be reused, after the metal is recycled.
- **No sludge generation**: no secondary problems with sludge occur with biosorption, as is the case with many other techniques, for example,precipitation.
- **Possibility for metal recovery** : In case of metals, it can be recovered after being sorbed from the solution.
- **Competitive performance:** Biosorption provides comparatively superior performance than the most similar technique, ion exchange treatment. Ion exchange is, as mentioned above, rather costly.

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms.

2.7 ACTIVATED CARBON PREPARED FROM AN AGRICULTURAL SOLID WASTE AS AN ADSORBENT

Activated carbon was prepared from coirpith by a chemical activation method and characterized. The adsorption of toxic heavy metals from industrial wastewaters onto coirpith carbon was studied. The percent adsorption increased with increase in pH from 2 to 6 and remained constant up to 10. As coirpith is discarded as waste from coir processing industries, the resulting carbon is expected to be an economical product for the removal of toxic heavy metals from industrial wastewaters (Kadirvelu, 2001).

2.8 MICROBIAL DERIVED BIOMASS FOR REMOVAL OF HEAVY METALS

2.8.1 Microbial biomass as a biosorbent

Biomass of *Aspergillus niger, Penicillium chrysogenum, Rhizopus nigricans, Ascophyllum nodosum, Sargassum natans, Chlorella fusca, Oscillatoria anguistissima, Bacillus firmus* and *Streptomyces* sp. have highest metal adsorption capacities ranging from 5 to 641 mg g^{-1} mainly for Pb, Zn, Cd, Cr, Cu and Ni. Biomass generated as a by-product of fermentative processes offers great potential for adopting an economical metal-recovery system (Ahluwalia and Goyal, 2007).

2.8.2 Removal of heavy metals using a brewer's yeast strain of *Saccharomyces cerevisiae*

Brewer's yeast strain was used to remove heavy metals from a synthetic effluent. The separation process was carried out using the flocculation ability of the strain. The yeast strain was able to sediment in the presence of Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Cr^{3+}, which shows that flocculation can be used as an effective separation process for a range of industrial effluents. For a biomass concentration higher than 0.5 g/l, more than 95% of the cells were settled after 5 min. The passage of metal-loaded effluent through a series of sequential batches conducted, after the second batch, Ni^{2+} concentration in solution corresponds to a removal of 91%. A subsequent batch had a marginal effect on Ni^{2+} removal (96%). Therefore, the results obtained suggest that the use of brewing flocculent biomass looks a promising alternative in the bioremediation of metal-loaded industrial effluents (Machado ***et al.,*** 2008).

2.9 PLANT DERIVED BIOMASS FOR REMOVAL OF HEAVY METALS

Cellulosic waste materials can be obtained and employed as cheap adsorbents and their performance to remove heavy metal ions can be affected upon chemical treatment. In general, chemically modified plant wastes exhibit higher adsorption capacities than unmodified forms. Numerous chemicals have been used for modifications which include mineral and organic acids, bases, oxidizing agent, organic compounds, etc. An extensive list of plant wastes as adsorbents including rice husks, spent grain, sawdust, sugarcane bagasse, fruit wastes, weeds and others has been compiled and modified. Some of the treated adsorbents show good adsorption capacities for Cd, Cu, Pb, Zn and Ni (Ngah and Hanafiah, 2007).

2.9.1 Cocoa shell as a biosorbent

The efficiency with which cocoa shells remove heavy metals from acidic solutions was studied and the influence of composition of these solutions on heavy metal uptake efficiency was investigated by Meunier *et al*. (2003). Cocoa shell of different concentrations (5-40 g/l) was used to perform batch adsorption tests in agitated flasks with single-metal solutions (0.25 mM Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn), multi-metal solution (comprised of 0.25 mM of each of the cations above) and an effluent obtained from chemical leaching of metal-contaminated soil. Results from the single-metal solution assays indicated that the fixation capacity of heavy metals by cocoa shells followed a specific order: Pb>Cr> Cd=Cu=Fe > Zn=Co>Mn=Ni=Al. Cocoa shells are particularly efficient in the removal of lead from very acidic solutions (q_{max} = 6:2 mg Pb/g, pH = 2.0 and T = 22 ^{o}C). The maximum metal uptake was reached in less than 2 h. The presence of other metals and cations in solution did not seem to affect the recovery of lead.

2.9.2 Raw rice bran as a biosorbent

The capacity of raw rice bran for chromium and nickel removal from aqueous solution was investigated by Oliveira ***et al.*** (2005). The Langmuir and Freundlich adsorption models were used to describe the adsorption of Ni (II) and Cr (VI). The experimental data and equilibrium data fitted well to Freundlich isotherm model.

2.9.3 Bengal gram husk as a biosorbent

The potential to remove Cr (VI) from aqueous solutions through biosorption using the husk of Bengal gram (*Cicer arietinum)* was investigated by Ahalya ***et al.*** (2005). Batch tests resulted in removal of 99% of chromium in the 10mg/l chromium solution with 1g/mg of biomass at saturation. The experimental data fitted well with Langmuir and Freundlich isotherm models. The maximum adsorption capacity was 91.64 mg Cr (VI)/g of adsorbent at pH 2.0 and contact time of 180 minutes.

2.9.4 Black gram husk as a biosorbent

Black gram husk (*Vigna mungo*) efficiently removed Ni (II) ions from aqueous solution and the efficiency was investigated by Saeed ***et al.*** (2005). The biosorption was well described by Langmuir and Freundlich isotherms. The maximum adsorption capacity of Ni (II) was 19.56mg/g of biomass at a pH of 5.

2.9.5 Black tea leaves as a biosorbent

Adsorption of Cr (VI) on used black tea leaves as a low-cost adsorbent was studied by Hossain and Kumita (2005). Maximum adsorption of Cr (VI) was achieved at initial solution pH of 1.54-2.0, processing temperature of < 50^{0}C and initial chromium concentration of < 150mg/l. The experimental data followed Langmuir isotherm.

2.9.6 Orange waste as a biosorbent

Removal of metal ions by chemically modified adsorption gel prepared from orange wastes was investigated by Dhakal *et al*. (2005). Two types of adsorption gels, Ca^{2+}-form and H^{+}-form gels, were prepared by saponifying orange juice residues with calcium hydroxide and its subsequent acid treatment, respectively. The Ca^{2+} form gel was effective for the complete and selective removal of Pb(II), Cu(II) and Fe(III) compared with other divalent metal ions. The selectivity order for metal ion uptake by the gel is Pb(II)> Fe(III)> Cu(II)> Cd(II)≈ Zn(II)> Mn(II). Among these metal ions, the maximum loading capacities for divalent metal ions such as Pb(II), Cd(II) and Zn(II) was 1.1 mol/kg-dry gel while that for Fe(III) was 1.55 mol/kg-dry gel. The H^{+}-form gel showed a different adsorption profile for Fe(III) compared to the Ca^{2+}-form gel.

2.9.7 Groundnut hull as a biosorbent

The biosorption of lead(II) and chromium(VI) on groundnut hull was investigated by Qaiser *et al.*(2009). The maximum biosorption capacity of lead(II) and chromium(VI) was found to be 31.54 ± 0.63 and 30.21 ± 0.74 mg g^{-1}, respectively. The optimum pH for lead(II) and chromium(VI) removal was 5 ± 0.1 and 2 ± 0.1, respectively. The temperature change, in the range of 20 - 45ºC affected the biosorption capacity. The maximum removal of lead(II) was achieved at 20 ± 2ºC, where as maximum uptake of chromium(VI) was observed at 40 ± 2ºC. The biosorption data was fitted to the Langmuir and the Freundlich isotherm models. The Langmuir model showed better representation of data, with correlation coefficient greater than 0.98. The biosorption followed the pseudo second order kinetics model.

2.9.8 Pectin rich fruit as a biosorbent

The removal of cadmium by pectin rich fruit wastes (derived from several citrus fruits, apples and grapes) was investigated by Schiewer and Patil (2007). Citrus peels were identified as the most promising biosorbent due to high metal uptake and physical stability. The Cd (II) removal reached equilibrium after 30–80 min depending on the particle size (0.18–0.9 mm). Sorption kinetics followed a second-order model. Sorption equilibrium isotherms could be described by the Langmuir model in some cases, whereas in others an S-shaped isotherm, that did not follow the Langmuir isotherm model. The metal uptake increased with pH, with uptake capacities ranging between 0.5 and 0.9 meq/g of dry peel.

2.9.9 Modified coconut husk as a biosorbent

The adsorption capacities of two low cost agricultural wastes in the adsorption of Cr (VI) and Ni (II) ions was investigated by Kehinde *et al.* (2009). The results showed an increase in the adsorption of Cr (VI) with an increase in the mass of adsorbent, with an exception of coconut husk, which consistently had an adsorption of 93-99% irrespective of adsorbent loading. HCl modified coconut husk were found to be more efficient for Cr (VI) and Ni (II) ions.

2.9.10 Palm petiolar felt sheath as a biosorbent

The petiolar-felt-sheath of palm was reported as a new biosorbent for which the removal of heavy metals from contaminated water was investigated by Iqbal *et al.* (2002). Efficient removal of all toxic metals was achieved with selectivity in the order of Pb>Cd>Ni>Cr. The uptake was rapid with more than 70% with an optimum contact time of <15 minutes.

2.9.11 Chestnut shell as heavy metal adsorbent

The influence of initial cation concentration, temperature and pH was investigated to optimize Pb^{2+}, Cu^{2+} and Zn^{2+} removal from aqueous solutions using acid formaldehyde pre-treated chestnut shell as adsorbent. Experiments were planned according to an incomplete 33 factorial experimental design. Under the optimal conditions selected, the metal ion adsorption equilibrium was satisfactorily described by the Langmuir isotherm model. The maximum pre-treated chestnut shell adsorption capacity for Pb^{2+} ions was 8.5mgg^{-1} and the order of cation affinity was $Pb^{2+} > Cu^{2+} > Zn^{2+}$ (Vazquez, 2009).

2.9.12 Raw and modified lignocellulosic materials as a biosorbent

Adsorption of Cr by different low cost materials seems to be a suitable choice for wastewater treatment. Many agricultural by-products have proved to be suitable low cost adsorbents for Cr(VI) and Cr(III) removal from water. Lignocellulosic residues, which include both wood residues and agricultural residues, have adsorption capacity comparable to other natural sorbents, but they have the advantage of very low or no cost, great availability and simple operational process. The recent literature on the use of natural and modified lignocellulosic residues for Cr adsorption, the Cr maximum adsorption capacity and the adsorption mechanism under different experimental conditions has been reviewed (Miretzky and Cirelli, 2009).

2.9.13 Waste coconut buttons as a biosorbent

The removal of heavy metal ions such as Pb(II), Hg(II) and Cu(II) from industrial effluents by activated carbon (AC) derived from waste coconut buttons (CB) as a suitable adsorbent was investigated through batch adsorption process. The effects of initial metal concentration, contact time, pH and adsorbent dose on the adsorption of metal ions were studied. The adsorbent revealed a good adsorption potential for Pb(II) and Cu(II) at pH 6.0 and for Hg(II) at pH 7.0. The experimental kinetic data were a better fit with pseudo second-order equation rather than pseudo first-order equation. The experimental data followed Freundlich isotherm model for the three metals than the Langmuir model. The adsorption capacities of the AC decreased in the order: Pb(II) >Hg(II) > Cu(II) (Anirudhan and Sreekumari, 2011).

2.9.14 Wheat husk as a biosorbent

Large amounts of straw and bran from *Triticum aestivum* (wheat), a major food crop of the world, are produced as by-products/waste materials. This study showed that adsorption of chromium by wheat bran reached to equilibrium after 60 min and after that a little change of chromium removal efficiency was observed. Higher chromium adsorption was observed at lower pHs, and maximum chromium removal (87.8 %) obtained at pH of 2. The adsorption of chromium by wheat bran decreased at the higher initial chromium concentration and lower adsorbent doses. The obtained results showed that the adsorption of chromium (VI) by wheat bran follows Langmuir isotherm equation with a correlation coefficient equal to 0.997. In addition, the kinetics of the adsorption process follows the pseudo second-order kinetics model with a rate constant value of 0.131 g/mg.min (Nameni *et al*., 2008).

2.9.15 Lemon peel as a biosorbent

Batch experiments were performed to study the adsorption of cobalt on lemon peel adsorbent. The maximum adsorption capacity of lemon peel adsorbent for cobalt removal was 22mgg^{-1}. Three simplified kinetic models viz. pseudo-first-order, pseudo-second-order, and Weber and Morris intraparticle diffusion models were tested to describe the adsorption process. Cobalt adsorption on lemon peel adsorbent could be described more favorably by the pseudo-second-order kinetic model. The adsorption process has been found to be exothermic (Bhatnagar *et al.*, 2010).

2.9.16 Tobacco dust as a biosorbent

Tobacco dust was investigated for its heavy metal binding efficiency. The tobacco dust exhibited a strong capacity for heavy metals, such as Pb(II), Cu(II), Cd(II), Zn(II) and Ni(II), with respective equilibrium loadings of 39.6, 36.0, 29.6, 25.1 and 24.5 mg of metal per g of sorbent. Moreover, the heavy metals loaded onto the biosorbent could be released easily with a dilute HCl solution. Changes in the surface morphology of the tobacco dust as visualized by atomic force microscopy suggested that the sorption of heavy metal ions on the tobacco could be associated with changes in the surface properties of the dust particles. The heavy metal uptake by the tobacco dust may be interpreted as metal–H ion exchange or metal ion surface complexation adsorption or both (Qi and Aldrich, 2007).

2.10 OTHER BIOSORBENTS FOR REMOVAL OF HEAVY METALS

2.10.1 Low-cost adsorbents as a biosorbent

The technical feasibility of various low-cost adsorbents for heavy metal removal from contaminated water has been reviewed. Instead of using commercial activated carbon, researchers have worked on inexpensive materials, such as chitosan, zeolites, and other adsorbents, which have high adsorption capacity and are locally available. Low-cost adsorbents have demonstrated outstanding removal capabilities for certain metal ions as compared to activated carbon. Adsorbents that stand out for high adsorption capacities are chitosan (815, 273, 250 mg/g of Hg^{2+}, Cr^{6+}, and Cd^{2+}, respectively), zeolites (175 and 137 mg/g of Pb^{2+} and Cd^{2+}, respectively), waste slurry (1030, 560, 540 mg/g of Pb^{2+}, Hg^{2+},

and Cr^{6+}, respectively), and lignin (1865 mg/g of Pb^{2+}). These adsorbents are suitable for inorganic effluent treatment containing the metal ions. The adsorption capacities of the adsorbents vary, depending on the characteristics of the individual adsorbent, the extent of chemical modifications, and the concentration of adsorbate (Kurniawan *et al*., 2005).

2.10.2 Arca shell as a biosorbent

The biosorption potential of pretreated arca shell biomass for nickel from the artificially prepared solution containing metal was investigated by Dahiya *et al*. (2008). At equilibrium, the maximum total uptake by shell biomaterial was 9.86mg/g of adsorbent.

2.10.3 Fish Scales as a biosorbent

Effective removal of metal ions from industrial wastewater by using fish scales was studied by Mustafiz *et al*. (2003). A series of static tests was performed with 10 g of dried fish scale adsorbent pulverized to micron sizes of 37 or less. Such tests were conducted for lead ions (from lead nitrate solution) at concentrations of 25 ppm, 12.5 ppm, and 6.25 ppm. The dynamic equilibrium results were based on tests on 50 ppm of cobalt chloride solution (flow rate 1 ml/min), followed by 100 ppm of cobalt solution (flow rate 7 ml/min), and then a mixture of cobalt chloride ($CoCl_2$), lead nitrate ($Pb(NO_3)_2$), zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and strontium nitrate ($Sr(NO_3)_2$) solutions. The proposed sorption technique offers an acceptable solution for removal of heavy metal ions from wastewater streams.

High efficiency, high biosorption capacity, cost-effectiveness and renewability are the important parameters making these materials as economical alternatives for metal removal and waste remediation. Since, a number of agricultural wastes like husks of rice, wheat, black gram, black gram has already proved to be potent adsorbents of heavy metals, it triggers further study on such husks.

Present study is aimed at investigating the efficiency of pigeon pea husk as a biosorbent. It also involves optimizing the conditions of pH, time of contact, adsorbent concentration, etc. for optimum adsorption efficiency.

It also involves the optimization of pH, adsorbent dosage, contact time and metal ion concentration for the adsorbent, under study. This in turn, believed to result in reduction of heavy metal toxicity in the environment and human health.

3. MATERIALS AND METHODS

3.1 PREPARATION OF BIOSORBENT

Pigeon pea (*Cajanus cajan*) pod was collected from a legume seed-splitting mill. The pigeon pea pod was washed extensively in running tap water to remove dirt and other particulate matter. It was dried at 40^0C for 24 hours. It was then, finely ground and sieved with a mesh size of 150µm (Ahalya *et al,* 2005).

3.1.1 Choice of appropriate pre-treatment method

The biosorbent was activated using concentrated inorganic acid (H_2SO_4), concentrated organic acid (CH_3COOH), concentrated alkali (NaOH) and the activated adsorbents were subsequently evaluated for their adsorption capacities. The pretreatment which gave the best results was adopted for further studies.

3.1.2 Activation of adsorbent

The finely powdered biosorbent is taken in required quantity and treated with suitable volume of 1M Sodium hydroxide (NaOH) solution. The mixture was filtered and the powdered residue was washed with distilled water, several times to remove any base contents. This filtered biomass was dried in an oven at 60ºC for 6-8 hrs. The dried biomass was stored in air tight containers to protect it from moisture.

3.2 CHARACTERIZATION OF ADSORBENT

The surface morphology of the adsorbent was analyzed by FTIR spectroscopy to determine the surface functional groups, based on which an appropriate activation method is adopted.

3.3 PREPARATION OF SYNTHETIC SINGLE METAL SOLUTION

An aqueous stock solution (1000 mg/l) of Ni (II) ions was prepared using $NiCl_2$ salt (Analytical reagent). pH of the solution was adjusted using 0.5 N HCl or NaOH. Fresh dilutions of 100 mg/l were used for each study.

3.4 BATCH ADSORPTION STUDIES

The biosorption capacity of pigeon pea pod was determined by contacting various concentrations of metal ion solution, on contacting at various pH, on contacting for various contact times and various doses of adsorbent, of 100 ml metal solution in 250 ml conical flasks. While varying one parameter, the others are maintained constant. The mixture was shaken in a rotary shaker at 120 rpm followed by filtration using Whatman filter paper (No.1).The filtrate containing the residual concentration of Ni was determined spectrophotometrically at 465 nm after complexation with Dimethylglyoxime (DMG) (Krishna and Swamy, 2011). Adsorption isotherm studies were carried out. Langmuir, Freundlich and Temkin models were applied to the adsorption.Various parameters like pH, contact time and adsorbent dosage is optimized.

3.4.1 Effect of adsorbent dosage:

The optimal dose is determined by conducting experiment in 5 different flasks containing equal volume of metal solution (100ml) of equal concentration (100mg/l), for uniform contact time (90 min) and pH (8.0) at 30^o C, while loading different doses of adsorbent from 100mg to 500mg.

3.4.2 Effect of pH:

The optimal pH is determined by varying pH from 4 to 12. For this, different flasks containing equal volume of metal solution (100ml) of equal concentration (100mg/l), with uniform adsorbent load (100mg) were agitated for equal contact time (90 min) at 30^oC.

3.4.3 Effect of Contact time:

The optimal contact time is determined by conducting adsorption in 5 different flasks containing equal volume of metal solution (100ml) of equal concentration (100mg/l), with uniform adsorbent loading (100mg) and pH (8.0) at 30^oC, while varying the contact time from 15 minutes to 120 minutes.

3.4.4 Effect of metal ion concentration:

The equilibrium metal ion concentration is determined by experimenting with 5 different flasks containing metal solutions of different concentrations ranging from 20mg/l to 100

mg/l,while loading them with equal amounts of adsorbent (100mg), maintaining uniform pH (8.0) and varying contact time from 15 minutes to 90 minutes at 30^oC.

3.5 ADSORPTION ISOTHERM MODEL EQUATIONS

Adsorption isotherms are useful for finding out the adsorption capacity of the adsorbent, the solute-solution interaction and the degree of accumulation of adsorbate on the surface of the adsorbent. In the present study, the adsorption of metals onto activated carbon was studied by two adsorption isotherm models: Langmuir and Freundlich isotherms (Schiewer and Patil, 2008).

3.5.1 Langmuir isotherm:

The simplest adsorption isotherm is based on the assumptions that every adsorption site is equivalent and that the ability of a particle to bind there is independent of whether or not the adjacent sites are occupied. This is in accordance with the Langmuir model which assumes that:

- the uptake of metal ions occur on a homogeneous surface by monolayer adsorption
- there is no interaction between adsorbed ions
- uniform energies of adsorption are involved
- there is no transmigration of adsorbate in the plane of the surface

The linearized form of Langmuir isotherm is represented by the following equation

$$1/q_e = k/Q_oC_e + 1/Q_o \text{ - - - - - - - - - - } (1)$$

where, q_e (mg/g) is the amount of adsorbed metal ions per unit weight of adsorbent at equilibrium concentration, C_e (mg/l). The Q_o (mg/g) and k (l/mg) are the Langmuir constants related to the maximum monolayer capacity and energy of adsorption, respectively. Langmuir parameters are obtained by plotting $1/q_e$ versus $1/ C_e$.

3.5.2 Freundlich isotherm:

The Freundlich isotherm assumes that:

- the uptake of metal ions occurs on a heterogeneous surface by multilayer adsorption
- the amount of adsorbate adsorbed increases infinitely with an increase in concentration.

The linearized Freundlich model isotherm was applied for the adsorption of Ni(II) and is expressed as Eq.

$\ln q_e = \ln K_F + (1/n)\ln C_e$ - - - - - - - - - - (2)

The K_F and 1/n are Freundlich constants related to adsorption capacity and intensity of adsorption respectively. Freundlich parameters were obtained by plotting $\ln q_e$ versus $\ln C_e$.

3.5.3 Temkin isotherm:

Temkin isotherm assumes that

- The heat of adsorption of all molecules in the layer decreases linearly with coverage due to adsorbent-adsorbate interactions
- The adsorption is characterized by a uniform distribution of the binding energies up to some maximum binding energy

The linearized Temkin model:

$q_e= B_T \ln A_T + B_T \ln C_e$ - - - - - - - - - - (3)

where, $B_T = (RT)/b_T$, T is the absolute temperature in Kelvin and R is the universal gas constant, 8.314 J mol-1 K-1. The constant b_T is related to the heat of adsorption, A_T is the equilibrium binding constant (l min^{-1}) corresponding to the maximum binding energy.

The slope and intercept from a plot of q_e versus ln C_e determines the isotherm constants A_T and b_T.

3.6 ADSORPTION KINETIC MODELS

The study of adsorption kinetics in wastewater is significant as it provides valuable insight into the reaction pathways and into the mechanism of the reaction.

Further, it is important to predict the time at which the adsorbate is removed from aqueous solution in order to design an appropriate sorption treatment plant. Any adsorption process is normally controlled by three diffusive transport processes for the adsorbate:

- ❖ From bulk solution to the film surrounding the adsorbent
- ❖ From the film to the adsorbent surface

- ❖ From the surface to the internal sites followed by binding of the metal ions onto the active sites

It is assumed that the difference between the average solid phase concentration and equilibrium concentration is the driving force for adsorption. So, application of the kinetic model depends only on the initial and final concentrations of the solution at different time intervals. It is incorrect to apply simple kinetic model such as first and second order rate equations to a sorption process with solid surface, which is rarely homogeneous.

Several kinetic models have been proposed to clarify the mechanism of a solute sorption from aqueous solution onto an adsorbent (Meunier *et al.*, 2003):

- ❖ Pseudo first order / Lagergren kinetic model
- ❖ Pseudo second order kinetic model

3.6.1 Pseudo first order / Lagergren kinetic model

The pseudo first order or Lagergren kinetic rate equation for the sorption of liquid-solid system was derived based on solid adsorption capacity. It is one of the most widely used sorption rate equations for sorption of a solute from a liquid solution. According to this model, the overall adsorption rate is directly proportional to the driving force i.e., the difference between initial and equilibrium concentrations of the adsorbate (q_e-q).

Therefore, the pseudo first order kinetic equation can be expressed as:

$dq_e/dt = k(q_e - qt)$ - - - - - - - - - - (4)

where, 'q_e' is the amount of solute adsorbed at equilibrium per unit mass of adsorbent (mg/g), 'q' is the amount of solute adsorbed at any given time 't' and 'k' are the rate constant. By using the boundary conditions and simplifying, the equation:

$\log (q_e - q) = \log q_e – kt/2.303$ - - - - - - - - - - (5)

Where, 'k' can be calculated from the slope of the linear plot between log (qe - q) vs. t for different adsorption parameters such as pH, temperature, adsorbate concentration, adsorbent dose, particle size and agitation speed.

3.6.2 Pseudo second order kinetic model

A pseudo second order reaction model can also be applicable to kinetics of sorption and the equation for this reaction can be shown as:

$dq/dt = k (q_e - q)^2$ - - - - - - - - - - (6)

On integration for boundary conditions when t = 0 to t>0 and q=0 to q>0 and further simplifications, equation:

$t/q = 1/kq^2_e + t/q_e = 1/h + t/q_e$ - - - - - - - - - - (7)

where, $h= kq^2_e$ is known as initial sorption rate where 'k' is rate constant. The plot of t/q_t vs. t at different adsorption parameters will give a linear relationship, which allows for computation of 'q_e', 'k','h'.

3.7 EXPERIMENTAL DESIGN

A full factorial design, which includes all possible factor combinations in each of the factors, is a powerful tool for understanding complex processes for describing factor interactions in multifactor systems. RSM is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously.

The process parameters affecting the removal of Ni (II) by activated adsorbent were studied using RSM combined with the three-level, three-factorial Box–Behnken experimental design as established using Design Expert software (Version 8.0.7.1, StatEase Inc., USA, trial version). The variable input parameters were pH values in the range 4.0–12.0, contact time in the range 15- 120 min and initial metal concentrations of 20–100 mg/l, respectively. The three independent variables were designated as A (pH), B (contact time) and C (initial metal concentration) respectively, for statistical computations. The percentage removal of metal after adsorption was taken as the response of the design experiments.

A total of 17 runs were performed to optimize the process parameters and experiments were carried out according to the actual experimental design matrix. The results were analyzed applying the coefficient of determination (R^2), analysis of variance (ANOVA) and response plots. Employing RSM, the most widely used second-order polynomial equation developed to fit the experimental data and identify the relevant model terms may be written as

$$Y = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_{ii}^2 + \sum \beta_{ij} x_i x_j + \varepsilon \text{ - - - - - - - - - - (8)}$$

where, Y is the predicted response, i.e. the percentage removal for Ni (II), β_0 is the constant coefficient, β_i is the i^{th} linear coefficient of the input factor x_i, β_{ii} is the i^{th} quadratic coefficient of the input factor x_i, β_{ij} is the different interaction coefficients between the input factors x_i and x_j, and ε is the error of the model (Benyounis *et al*. 2005). For this study, the independent variables were coded as A, B and C, and thus the equation could be described as:

$$Y = \beta_o + \beta_i A + \beta_i B + \beta_i C + \beta_{ii} A^2 + \beta_{ii} B^2 + \beta_{ii} C^2 + \beta_{ij} AB + \beta_{ij} AC + \beta_{ij} BC\text{- - - - - (9)}$$

It is important to include the second order model to provide good prediction throughout the region of interest. The second order response surface design is rotatable; this means that the variance of the predicted response is the same at all points. Rotatability is a reasonable basis for the selection of response surface design. Because the purpose of response surface methodology (RSM) is optimization and as the location of the optimum is unknown prior to running the experiment, it makes sense to use design that provides equal precisions of estimation in all directions (Amini ***et al.,*** 2008).

4. RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF ADSORBENT BY FT-IR

The FT-IR spectra of the adsorbent before and after adsorption were used to determine the changes in vibrational frequencies in the functional groups of the adsorbent . The spectra of the adsorbent were measured within the range of 500-4000 cm^{-1}. The FT-IR spectra of the adsorbents display a number of absorption peaks as shown in figures 4.1.1 and 4.1.2, indicating the complex nature of the studied adsorbent. Table 4.1.1 presents the fundamental peaks of native adsorbent and after adsorption of metal solution (Ni).

Table 4.1 FT-IR frequencies of adsorbent before and after adsorption

Adsorbent	O-H stretch	C≡C bond (alkynes)	N-H bond (amines)	C-O stretch
Native	3431	2144	1631	1078
Ni loaded	3427	2132	1627	1049

It can be seen from the table 4.1 that the C-O stretch in the native adsorbent has undergone shift, suggesting that C-O bond participated in adsorption. Similarly the O-H stretch , C≡C bond and N-H bond has undergone shifts showing that they play a significant role in adsorption reaction.

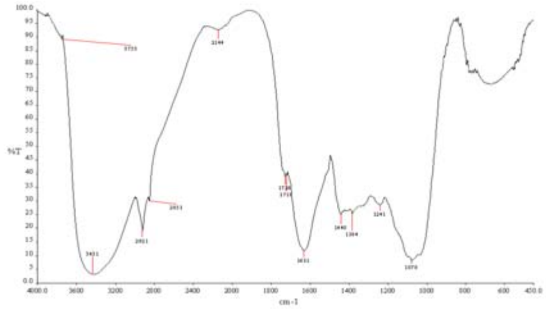


Fig 4.1.1 FT-IR spectra of native adsorbent

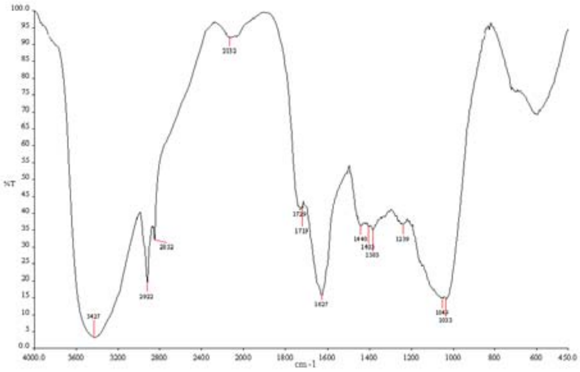


Fig 4.1.2 FT-IR spectra of Ni loaded adsorbent

Thus the spectra show that an observable difference in the functional groups of the adsorbent is due to the reaction of surface molecules to the metal solution. The results obtained were found to be in correlation with the data obtained in the work of Ahalya *et al*., (2005).

4.2 STANDARD CALIBRATION CURVE FOR NICKEL (DIMETHYLGLYOXIME METHOD)

As a first step towards the estimation of nickel, the standard calibration curve is constructed by preparing working standard solutions with concentrations ranging from 20mg/l to 100mg/l. This standard graph can be used to determine the concentration of metal ion in test samples by extrapolation.

Table 4.1 Standard calibration curve for nickel estimation

Reagents	Blank	S1	S2	S3	S4	S5
Working standard solution (ml)	0.0	2.0	4.0	6.0	8.0	10.0
Concentration of working standard (mg/l)	0	20	40	60	80	100
Volume of distilled water(ml)	10.0	8.0	6.0	4.0	2.0	0.0
20% sodium potassium tartarate (ml)	2.0	2.0	2.0	2.0	2.0	2.0
4% ammonium per sulphate (ml)	10.0	10.0	10.0	10.0	10.0	10.0
5M NaOH(ml)	2.5	2.5	2.5	2.5	2.5	2.5
(1+30)HCl (ml)	15.0	15.0	15.0	15.0	15.0	15.0
1% DMG(ml)	0.5	0.5	0.5	0.5	0.5	0.5
Incubate at room temperature for 30 minutes						
Absorbance at 465nm	0.00	0.303	0.590	0.900	1.189	1.529

4.3 EFFICIENCY OF ADSORBENT UNDER DIFFERENT PRETREATMENT METHODS

In order to make the process of adsorption more effective, the adsorbent should be activated suitably. The effect of various activation techniques on biosorption was studied and the maximum removal was achieved with sodium hydroxide pretreated adsorbent.

Table 4.3 Efficiency of pretreatment methods

	Untreated sample	Acetic acid pretreated	NaOH Pretreated	H_2SO_4 Pretreated
Absorbance at 465 nm	0.5095±0.0035	0.595±0.0268	0.328±0.0084	0.4905±0.0275
Percentage removal (%)	66	60	78	74

4.4 EFFECT OF CONTACT TIME

To establish an appropriate contact time between the pretreated adsorbent and metal ion solution, percentage removal of metal ion were measured as a function of time. The graph indicates that the rate of the percentage of Ni (II) removal is higher at the beginning, probably due to the larger surface area of the adsorbent being available at beginning for the adsorption of metals. As the surface adsorption sites become exhausted, the uptake rate is controlled by the rate at which the adsorbate is transported from the exterior to the interior sites of the adsorbent particles. The equilibrium rate of Ni (II) removal was attained after Agitating for 45 minutes. Therefore, equilibrium time of 45 min was selected for all further studies. This is compared to the results obtained by Duvnjak and Al-Asheh, (1998) with pine bark as adsorbent where equilibrium rate of removal was attained at contact time of 24 hours.

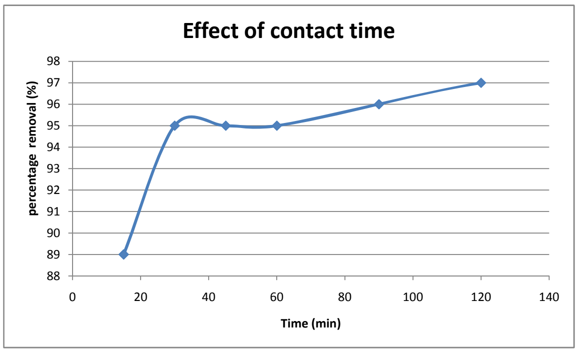


Figure 4.3 Effect of contact time

4.5 EFFECT OF METAL CONCENTRATION

To investigate the optimum equilibrium time, batch experiments were carried out by agitating 100 ml of different concentrations of nickel (II) solutions ranging from 20 mg/l-100 mg/l which are maintained at pH 8.0 and with 100 mg of adsorbent. After equilibrating for different time periods from 15min-90 min, the solutions were filtered and analyzed for Ni (II) content and the percent adsorption in each case was determined. Equilibrium time from the Table 1 was found to be 90 min. At this equilibrium time, the amounts adsorbed were 18.8, 36.8, 54.0, 70.66, 86.0 mg/g for Ni (II) concentrations of20, 40, 60, 80 and 100 mg/l, respectively. The increase in initial Ni (II) concentration decreased the percentage of adsorption and increased the amount of metal uptake per unit mass of the adsorbent in mg/g.

As compared to the adsorption studies conducted by Kehinde *et al.*(2009), using coconut husk as an adsorbent where maximum removal (93%) was demonstrated at an initial metal concentration of 30mg/l, the present study using pod of pigeon pea as an adsorbent reveals a maximum metal removal of 94% at an initial metal concentration of 20mg/l

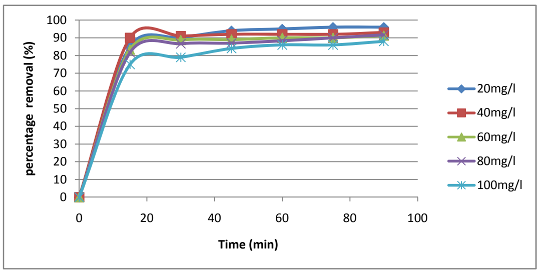


Figure 4.5 Effect of metal ion concentration

4.6 EFFECT OF ADSORBENT DOSAGE

The dosage of adsorbent is a key parameter to control both availability and accessibility of adsorption sites. The results as shown in graph 4.5.1, percentage of removal for 100 mg/L Ni (II) was observed to increase gradually from 88% to 97% with the increase of adsorbent dosage from 0.1 g to 0.5 g and maximum removal was achieved at a dosage of 0.4g. This is comparable to the results obtained by Krishna and Swamy (2011) where maximum removal was demonstrated at a dose of 0.2 g/ 50 ml of metal solution. The observed trend may be due to the fact that higher the dosage of adsorbent greater the availability of surface area and functional groups for metal ions. These chemical groups play an important role in the formation of Van der Waals bonding as the functional groups play the main role in binding metals to the adsorbent during adsorption process. This provides more possibilities for adsorption to occur since there was less competition between metals for the binding sites. However, the adsorption capacity showed a reverse trend to the percentage removal as shown by Fernando *et al*., (2009).

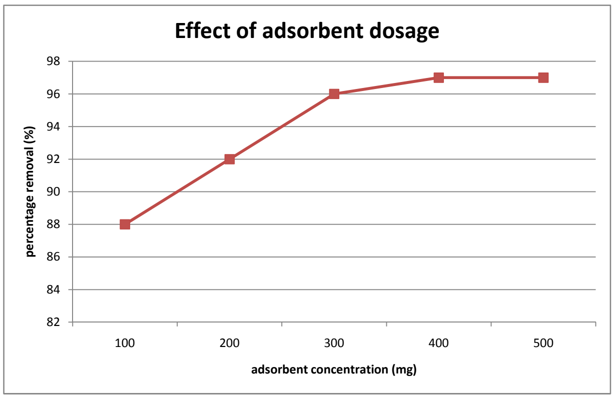


Figure 4.6 Effect of adsorbent dosage

4.7 EFFECT OF pH

In heavy metal biosorption, pH is the most important parameter. The speciation of metals in the solution and the state of chemically active sites is pH dependent. For biosorption of Ni (II), maximum removal was achieved at pH of 9.0. This could be due to an increasing negative charge density on the adsorbent surface. It is apparent that Ni (II) is strongly adsorbed at higher pH values. At pH greater than 8, Ni (II) removal was mostly due to precipitation and not by sorption. Similar results were obtained when mozambi fruit peels was used as biosorbent as investigated by Krishna and Swamy (2011).

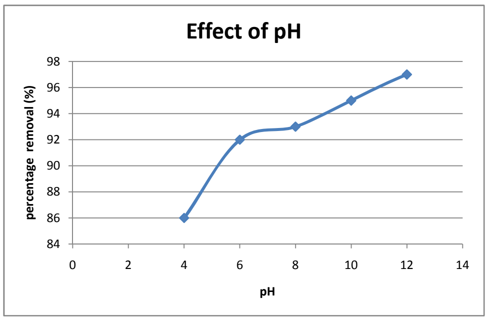


Figure 4.7 Effect of pH

4.8 EQUILIBRIUM ISOTHERMS

The biosorption equilibrium is established, when the concentration of sorbate in bulk solution is in dynamic balance with that on the liquid-sorbent interface. The degree of the sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. Several models are often employed to interpret the equilibrium data. In the present study, the Langmuir, the Freundlich and the Temkin models were utilized to explain the experimental data.

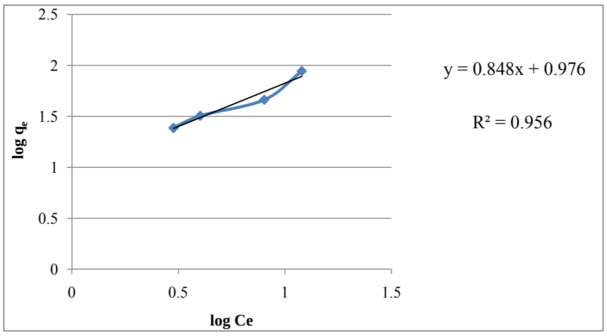


Figure 4.8.1 Freundlich Isotherm

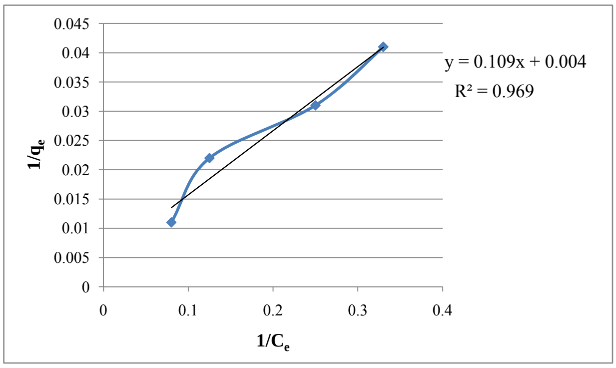


Figure 4.8.2 Langmuir Isotherm

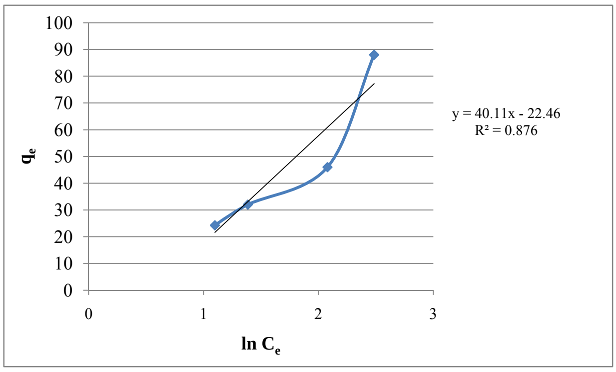


Figure 4.8.3 Temkin Isotherm

Table 4.8.4 Isotherm Parameters

S.No.	Adsorption isotherm	Isotherm parameters		R^2
1	**Freundlich**	K_F	9.462	0.956
		n	0.848	
2	**Langmuir**	K	27.25	0.969
		Q_0	250	
3	**Temkin**	B_T	40.11	0.876
		A_T	0.571	

Figure 4.7.3 indicates the applicability of Langmuir isotherm. The Langmuir constants Q_0 and b evaluated from the slope and intercept are 250 mg/g and 27.25 L/mg, respectively. Q_0 value is greater than the value of adsorption capacity of mozambi fruit peel studied (Krishna and Swamy, 2011). The statistical significance of the correlation coefficient *(*R^2*)* for*1/q*, versus 1/Ce,was the criteria by which the fitting of the data to Langmuir isotherm was tested. It demonstrates monolayer coverage of adsorbate at the outer surface of the adsorbent. The parameters Qo and K have been calculated and the results are represented in Table 4.7.4. It shows that the adsorption of Ni (II) onto pigeon pea pod is a monolayer type one that does not completely cover the surface of theadsorbent. The results are comparable to that of the studies conducted by Yasemin and Zeki (2005) using saw dust.

4.9 ADSORPTION KINETICS

The kinetic studies of adsorption of Ni (II) to pretreated pigeon pea pod was carried out using the pseudo first order and second order models on experimental data and the values obtained are as seen in Tables 4.8.1 and 4.8.2. The regression coefficients obtained from the pseudo first order kinetic graph (Graph 4.8.1) were low. This implies non applicability of the pseudo first order kinetic model to the experimental data of the adsorption of Ni (II) to pigeon pea pod.

The second –order kinetic model kinetics was applied by plotting t/q_t vs. t and this model gave high values of regression correlation co-efficient as seen in Graph 4.8.2. This implies that the mechanism of adsorption of Ni (II) ion on pod of pigeon pea follows second order kinetics. This is compared to the kinetic studies of adsorption of Ni (II) to coconut husk conducted by Kehinde *et al.* (2009) where regression coefficients showed high values for pseudo second order kinetic model (R^2=0.999) indicating its applicability to adsorption.

Table 4.9.1 first order kinetic parameters

Initial concentration (mg/l)	Experimental q_e	Predicted q_e	k	R^2
20	19.6	3.945	0.029	0.981
40	37.2	1.361	0.018	0.799
60	54.75	4.645	0.028	0.739
80	73.34	10.375	0.025	0.924
100	88	18.923	0.035	0.789

`

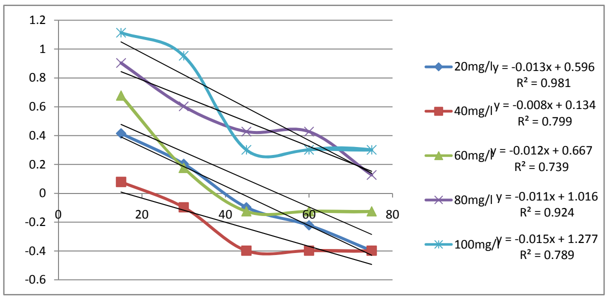


Figure 4.9.1 first order kinetics

Table 4.9.2 Second order kinetic parameters

Initial concentration (mg/l)	Experimental q_e	Predicted q_e	h	k	R^2
20	19.6	20	71.94	0.179	0.999
40	37.2	37.04	83.33	0.061	1
60	54.75	55.56	47.62	0.015	0.999
80	73.34	76.92	40	0.0068	0.999
100	88	90.91	28.57	0.0035	0.999

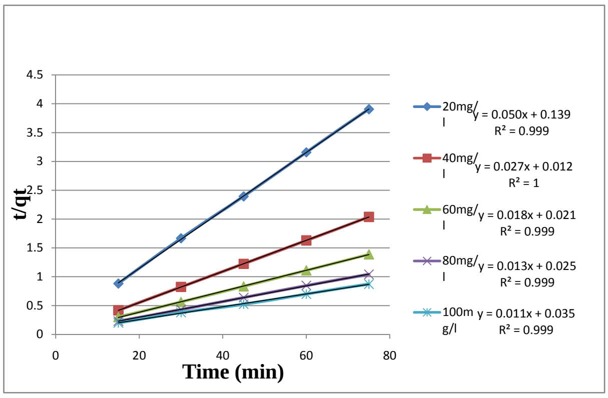


Figure 4.9.2 Second order kinetics

4.10 EXPERIMENTAL DESIGN FOR OPTIMIZATION

4.10.1 Box-Behnken analysis

The best performance of a Box-Behnken design depends on some prior knowledge of the system being optimized. If the values of all the system parameters to be studied are unknown, the projection of the results with the factorial design cannot be optimized. Besides this, the total number of experiments required will be excessively large, making the factorial design very complex. Optimization of biosorption of heavy metals by the classical method is time consuming and expensive for a large number of variables. To overcome this difficulty, the experimental Box-Behnken design under response surface methodology can be employed to optimize the biosorption of heavy metals.

Experiments were performed according to the Box-Behnken experimental design given in Table 4.9.1 in order to search for the optimum combination of parameters for the sorption of nickel using the pretreated adsorbent. The percentage removal of nickel is influenced by the individual effects of combinations of test variables and the results showed a significant variation for each combination. The maximum removal was found to be >78%. This is compared to the results obtained by Rajasimman and Murugaiyan (2011) where maximum removal of Ni (II) by red macro algae was observed to be >63%.

Table 4.10.1 Experimental design matrix with response

Std	Run	Factor1 A:pH	Factor2 B:Contact time (min)	Factor3 C:Metal concentration (mg/l)	% removal Experimental	%removal Predicted
16	1	9	75	60	96.23	96.524
3	2	6	120	60	85.26	85.528
12	3	9	120	100	92.71	93.10625
15	4	9	75	60	96.7	96.524
6	5	12	75	20	92.67	93.33375
14	6	9	75	60	96.48	96.524
17	7	9	75	60	96.98	96.524
1	8	6	30	60	79.96	79.435
8	9	12	75	100	95.74	94.81875
2	10	12	30	60	96	95.732
13	11	9	75	60	96.23	96.524
4	12	12	120	60	90.41	90.935
7	13	6	75	100	88	87.33625
11	14	9	30	100	91.11	92.29875
10	15	9	120	20	89.28	88.09125
5	16	6	75	20	78.19	79.11125
9	17	9	30	20	88	87.60375

4.10.2 Adequacy of the model for Ni (II) removal

A system or process with several variables is likely to be driven primarily by some principal factors and low-order interactions. In the present work, only two-way interactions were investigated. Linear, two-factor interaction (2FI), quadratic and cubic models were used to analyze the experimental data in order to obtain the appropriate regression equations. To determine the adequacy of the models depicting the removal of Ni (II) by pretreated biosorbent, three different tests, such as the sequential model sum of squares, lack of fit tests and the model summary statistics, were carried out and the corresponding results are tabulated in Table 4.9.2.

It can be seen that p values for all the regressions were lower than 0.01 and the quadratic model. This means that at least one of the terms in the regression equation had a significant correlation with the response variable. As a natural log transformation was applied to the experimental data, the interaction of two factors (2FI) and the linear model were suggested to be insignificant using the response surface methodology. From the model summary statistics it can be seen that the "Predicted R^2" of 0.8108 was in reasonable agreement with the "Adjusted R^2" of 0.9714 for the quadratic model. Furthermore, the quadratic model had maximum "Predicted R^2" and "Adjusted R^2" values. The afore-mentioned results indicate that the quadratic model provide an excellent explanation for the relationship between the independent variables and the corresponding response. As compared with the study conducted by Tarangini *et al.* (2009) where highest R^2 of 0.9560 was observed for the quadratic model, it was selected as the most appropriate model for further analysis.

Table 4.10.2 Selection of a satisfactory model for Ni (II) removal

Sequential Model Sum of Squares

Source	sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Mean vs Total	1.413E+005	1	1.413E+_005		
Linear vs Mean	283.534075	3	94.51135833	4.682969	0.0198
2FI vs Linear	41.030525	3	13.67684167	0.617926	0.6191
Quadratic vs 2	214.5076932	3	71.50256441	73.31561	< 0.0001
Cubic vs Quadratic	6.413175	3	2.137725	20.66833	0.0067
Residual	0.41372	4	0.10343		

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value prob>F
Linear	261.9513932	9	29.10571036	281.4049	< 0.0001
2FI	220.9208682	6	36.82014471	355.991	< 0.0001
Quadratic	6.413175	3	2.137725	20.66833	0.0067
Cubic	0	0			
Pure Error	0.41372	4	0.10343		

Model Summary Statistics

Source	Std.Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared
Linear	4.49243	0.519389076	0.408478863	0.247547043
2FI	4.704621	0.594550435	0.351280696	-0.028924077
Quadratic	0.987558	0.98749422	0.971415361	0.810849256
Cubic	0.321605	0.999242131	0.996968525	

4.10.3 Regression analysis

Regression analysis was performed to fit the response functions, i.e. percentage adsorption of Ni (II). The regression models developed represent responses as functions of pH (A), contact time (B), initial metal concentration (C). An empirical relationship between the response and input variables in coded terms is expressed by the following response surface reduced quadratic model equation:

$$Y = 96.52 + 5.4*A + 0.32*B + 2.43*C - 2.72*A*B - 1.69*A*C + 0.080*B*C - 5.12*A^2 - 3.50*B^2 - 2.75*C^2 \text{ - - - - - - - - - - - (10)}$$

This equation reveals how the individual variables or double interactions affected nickel biosorption from aqueous metal solution on pretreated pigeon pea pod as adsorbent. Negative coefficient values indicate that individual or double interaction factors negatively affect nickel biosorption (i.e. removal percentage decreases), whereas positive coefficient values mean that factors increase nickel removal in the tested range. From the equation, it is observed that all individual factors have a positive effect on nickel removal. This is compared to the study conducted by Cordova *et al.* (2011) on lead biosorption, where stirring speed among all factors (temperature, pH, biomass dose) had a negative effect on lead removal.

4.10.4 ANOVA for Response Surface Quadratic Model

The ANOVA suggests that the equation and the actual relationship between the response and the significant variables represented by the equation were adequate. The larger the value of F and the smaller the value of p, the more significant is the corresponding coefficient term (Amini *et al.* 2008; Kalavathy *et al.* 2009). The value of p was lower than 0.05, indicating that the model may be considered to be statistically significant (Kim *et al.* 2003). For the removal of Ni (II) by pretreated adsorbent, the ANOVA results (Table 4.9.4) indicated that the F-value for the model was 61.42, implying that most of the variation in the response could be explained by the regression equation and that the model is significant. The probability $p < 0.0001$ also suggested that the model is significant. In this study, A, C, AB, AC, A^2, B^2, C^2 were significant factors. The other model terms whose p-values are listed as being greater than 0.1000 in Table 4.9.4 were not significant factors. There could be a 0.01% possibility of deviation which could be largely because of noise. "Adequate Precision" measures the signal-to-noise ratio. It is clear that only the "Adequate Precision" value of the quadratic model

(22.989) indicated an adequate signal. Consequently, the quadratic model could be used to navigate the design space.

Table 4.10.4 Analysis of variance

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	539.0722932	9	59.89692147	61.41569	< 0.0001
A-pH	235.5535125	1	235.5535125	241.5263	< 0.0001
B-contact time	0.8385125	1	0.8385125	0.859774	0.3847
C-metal conc	47.14205	1	47.14205	48.3374	0.0002
AB	29.648025	1	29.648025	30.39979	0.0009
AC	11.3569	1	11.3569	11.64487	0.0113
BC	0.0256	1	0.0256	0.026249	0.8759
A^2	110.4087603	1	110.4087603	113.2083	< 0.0001
B^2	51.45376026	1	51.45376026	52.75844	0.0002
C^2	31.91741289	1	31.91741289	32.72672	0.0007
Residual	6.826895	7	0.975270714		
Lack of Fit	6.413175	3	2.137725	20.66833	0.0067
Pure Error	0.41372	4	0.10343		
Cor Total	545.8991882	16			

4.10.5 Diagnostic plots

The data were analyzed to check the normal probability plot and a dot diagram of residuals correlation as shown in Figure 4.9.5.1. The data points on this plot lie reasonably close to a straight line, lending support to the conclusion that A and A^2 were highly significant effects, C, AB, AC, B^2, C^2 were significant effects and that the underlying assumptions of the analysis were satisfied.

The data were also analyzed to check the correlation between the experimental and predicted percentage removal, as shown in Figure 4.9.5.2. The experimental values were the measured response data for the runs designed by the Box-Behnken model, while the predicted values were obtained by calculation from the quadratic equation. It can be seen from Figure 4.9.5.2 that the data points on the plot were reasonably distributed near to the straight line, indicating a good relationship between the experimental and predicted values of the response. The result also suggests that the selected quadratic model was adequate in predicting the response variables for the experimental data. This can be compared to the study by Liu et al. (2011) on methylene blue removal by hydrogel composite where the adequacy of the developed model was in conformance with the diagnostic plots developed.

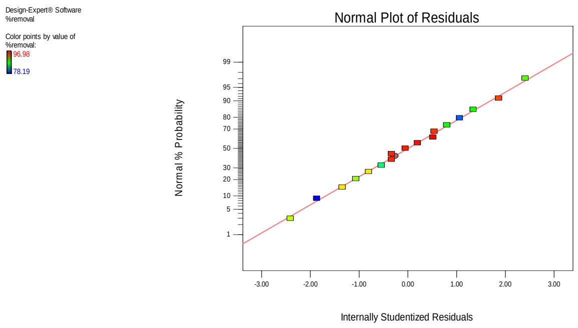


Figure 4.10.5.1 Normal plot of residuals

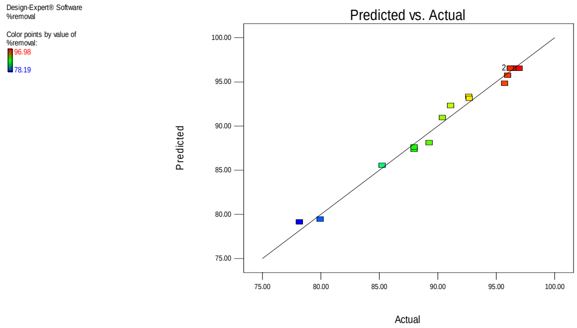


Figure 4.10.5.2 Plot of experimental and predicted values

4.10.6 Response surface contour plot:

Contour plot is a two dimensional representation of the response for selected factors. The contour plots, represented by the projection of the 3D response surfaces in the x–y plane, provide a straightforward determination of the effects of the independent variables on the dependent variable (Wu *et al.* 2009). The response surface contour plots of percentage removal of nickel versus the interactive effect of pH, contact time and initial nickel concentration are shown in Figures 4.9.6.1-4.9.6.3. Each contour plot represents a number of combinations of two test variables with the other variable maintained at zero levels. The maximum percentage removal of nickel is indicated by the surface confined in the smallest curve (circular or elliptical) of the contour plot. In response surface contour plots, the optimum values of variable factors could be analyzed by the saddle point or by determining the maxima formed by the x- and y-coordinates.

The studies of the contour plots reveal the best optimal values of the process conditions which are pH 7.5–9.5, contact time 65-85 min and initial nickel ion concentration

50–75 mg/l as compared with the study conducted by Preetha and Viruthagiri (2006) on copper removal using *Rhizopus arrhizus* where contour plots demonstrated optimal values of process parameters: initial copper ion concentration 50–55 ppm, pH 3.5–4.5, temperature 35–40°C and biomass loading 8–9 g/l.

Figure 4.10.6.1 Response surface contour plot for the effects of pH and contact time

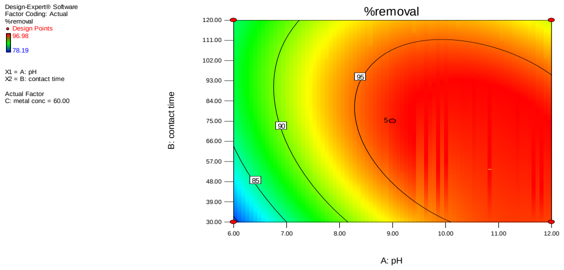


Figure 4.10.6.2 Response surface contour plot for the effects of pH and initial metal concentration

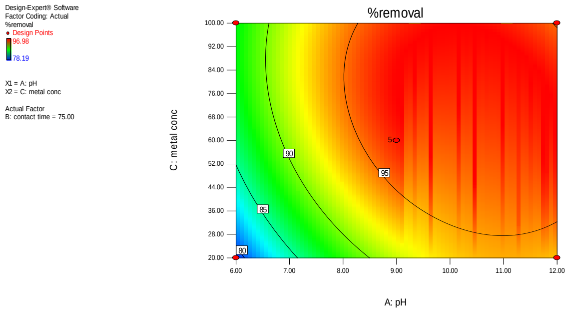
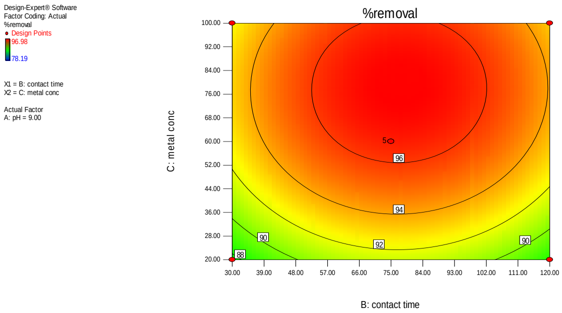


Figure 4.10.6.3 Response surface contour plot for the effects of contact time and initial metal concentration



4.10.7 Numerical optimization

One of the primary objectives of the present study is to find the optimum process parameters for maximizing the adsorption of nickel from aqueous solution. The model capable of predicting the maximum removal showed that the optimum values of the process variables were: a pH value of 9.0, contact time of 75 min and an initial metal concentration of 60 mg/l. Under these conditions, the predicted % removal was 96.524 % which was in good agreement with the experimental value of 96.23 %. This is compared with the study conducted by Preetha and Viruthagiri (2006) on copper removal using *Rhizopus arrhizus* where the optimum values of process variables were Initial copper ion concentration of 53.84 ppm, pH 4.14, temperature 37.75 °C, biomass loading 8.17 g/l and copper removal 98.34 %.

---

5. CONCLUSION

The following conclusions can be drawn based on the investigation of Ni (II) removal by pigeon pea pod. The adsorbent prepared from the pod of pigeon pea appears to be a promising adsorbent for removal of Ni (II) ions from aqueous solution. The study indicated that the pretreated adsorbent produced through chemical activation by using NaOH as an effective material for the removal of Ni (II).The maximum removal of Ni(II) is obtained at an alkaline pH of 9. It is found from the kinetic studies that the adsorption rate of Ni(II) is faster for initial 20 min after which it is saturated. The equilibrium time for Ni (II) adsorption on pigeon pea pod is obtained at 45 min. With the increase of adsorbent amount the percentage removal of Ni (II) increases and the adsorption capacity of pigeon pea pod to adsorb Ni(II) decreases because of availability of more unsaturated adsorption sites. Based on Langmuir isotherm, the maximum adsorption capacity was calculated to be 250 mg/g of adsorbent. Equilibrium studies reveal that Langmuir model provides better solutions and correlation to the adsorption data when compared to Freundlich and Temkin isotherm models. This suggests that the adsorption is limited to monolayer. These results were in agreement with the removal percentage and also the maximum adsorption capacity. The kinetics of biosorption was well represented by pseudo second order kinetic model. The response surface modelling was successfully combined with the three-level three factorial Box–Behnken design to determine the influence of important process parameters, such as the initial metal concentration, the pH and contact time on Ni (II) removal by the adsorbent, over the experimental range was examined. The results indicated that a second-order polynomial regression model was capable of interpreting the experimental data. Through ANOVA by the second-order polynomial regression equation, it was shown that the initial metal concentration and pH had significant effects on metal adsorption. The pod of pigeon pea used to produce adsorbent is locally and abundantly available agricultural waste product. Hence, the adsorbent prepared from the pigeon pea pod would be an economically useful tool and alternative to the commercially available activated carbon in the treatment of effluent containing Ni (II) ions.

REFERENCES

Ahalya, N., Kanamadi, R.D., Ramachandra, T.V. (2005), 'Biosorption of chromium (VI) from aqueous solutions by the husk of Bengal gram (*Cicer arientinum*)', Electronic Journal of Biotechnology, Vol. 8, Issue -3.

Amit Bhatnagar, Minocha, A.K., Mika Sillanpaa (2010) 'Adsorptive removal of cobalt from aqueous solution by utilizing lemon peel as biosorbent', Biochemical Engineering Journal, Vol.48, Issue 2, pp. 181-186.

Ana Fernando, Sofia Monteiro, Filomena Pinto and Benilde Mendes (2009), 'Production of Biosorbents from Waste Olive Cake and Its Adsorption Characteristics for Zn^{2+} Ion', Sustainability, Vol.1, Issue 2, pp. 277-297.

Anirudhan, T.S and Sreekumari, S.S. (2011), 'Adsorptive removal of heavy metal ions from industrial effluents using activated carbon derived from waste coconut buttons', Journal of Environmental Sciences, Vol. 23, Issue 12, pp.1989-1998.

Anne Juul Pedersen, Lisbeth M Ottosen, Arne Villumsen (2003), 'Electrodialytic removal of heavy metals from different fly ashes: Influence of heavy metal speciation in the ashes', Journal of Hazardous Materials, Vol.100, Issues 1-3, pp. 65-78.

Anushree Malik (2004), 'Metal bioremediation through growing cells', Environment International, Vol. 30, Issue 2, pp. 261-278.

Asma Saeed, Muhammed Iqbal, Waheed Akhtar, M. (2005), ‘Removal and recovery of lead(II) from single and multimetal (Cd, Cu, Ni, Zn) solutions by crop milling waste (black gram husk)’, Journal of Hazardous Materials, Vol.117, Issue 1, pp. 65-73.

Bulut Yasemin and Tez Zeki (2006), ‘Removal of metals from aqueous solution by saw dust adsorption’, Journal of Environmental Sciences, Vol. 19, pp. 160-166.

Cerino Cordova, F.J., Garcia Leon, A.M, Garcia Reyes, R.B., Garza Gonzalez, M.T., Soto Regalado, E., Sanchez Gonzalez, M.N., Quezada Lopez, I. (2011) ‘Response surface methodology for lead biosorption on *Aspergillus terreus*’, International Journal of Environment Science and Technology, Vol.8, Issue 4, pp. 695-704.

Eccles, H. (1995), ‘Removal of heavy metals from effluent streams — Why select a biological process?’, International biodeterioration and biodegradation, Vol.35, Issue 1-3, pp. 5-16.

Gonzalo Vázquez, Marcos Calvo, Sonia Freire, M., Julia González-Alvarez, Gervasio Antorrena (2009), ‘Chestnut shell as heavy metal adsorbent: Optimization study of lead, copper and zinc cations removal’, Journal of Hazardous materials, Vol.172, Issues 2-3, pp. 1402-1414.

Helen M Kalavathy, Iyyaswami Regupathi, Magesh Ganesha Pillai, Lima Rose Miranda (2009), ‘Modelling, analysis and optimization of adsorption parameters for H3PO4 activated rubber wood sawdust using response surface methodology (RSM)’, Colloids and Surfaces B: Biointerfaces, Vol.70, Issue 1, pp.35-45.

Hema Krishna, R. and Swamy, A.V.V.S. (2011) ‘Studies on the Removal of Ni (II) from Aqueous Solutions Using Powder of Mosambi Fruit Peelings as a Low Cost Sorbent’, Chemical Sciences Journal, Vol. 2011:CSJ-31.

Hilal, N., Alpatova, A., Verbych, S., Bryk, M., Nigmatullin, R. (2004), ‘Ultrafiltration of water containing natural organic matter: heavy metal removing in the hybrid complexation–ultrafiltration process’, Separation and purification technology, Vol.40, Issue 2, pp. 155-162.

Huan Ma, Wei-Wei Liu, Xing Chen, Yue-Jin Wu, Zeng-Liang Yu (2009), ‘Enhanced enzymatic saccharification of rice straw by microwave pretreatment’, Bioresource Technology, Vol. 100, Issue 3, pp. 1279-1284.

Iqbal, M., Saeed, A., Akhtar, N. (2002) ‘Petiolar felt-sheath of palm: a new biosorbent for the removal of heavy metals from contaminated water’, Bioresource Technology, Vol.81, Issue 2, pp.151-153.

Kadirvelu, K., Thamaraiselvi, K. and Namasivayam, C. (2001), ‘Removal of heavy metals from industrial waste waters by adsorption onto activated carbon prepared from an agricultural solid waste’, Bioresource Technology, Vol. 76, Issue 1, pp. 63-65.

Kirk Johnston, H (1975), ‘Reverse osmosis rejection of heavy metal cations’, Desalination, Vol.16, Issue 2, pp. 205–224.

Mabel Vaca Mier, Raymundo López Callejas, Ronald Gehr, Blanca E Jiménez Cisneros, Pedro J.J Alvarez (2001), ‘Heavy metal removal with mexican clinoptilolite: multi-component ionic exchange’, Water research,Vol. 35, Issue 2, pp. 373-378

.

Malihe Amini, Habibollah Younesi, Nader Bahramifar, Ali Akbar Zinatizadeh Lorestani,Farshid Ghorbani, Ali Daneshi, Mazyar Sharifzadeh (2008), ‘Application of response surface methodology for optimization of lead biosorption in an aqueous solution by *Aspergillus niger*’, Journal of Hazardous materials, Vol.154, Issues 1-3, pp. 694-702.

Manuela D. Machado, Monica S.F. Santos, Claudia Gouveia, Helena M.V.M. Soares and Eduardo V. Soares (2008), ‘Removal of heavy metals using a brewer’s yeast strain of *Saccharomyces cerevisiae* : The flocculation as separation process’, Bioresource Technology, Vol.99, Issue 7, pp. 2107-2115.

Meunier, N., Laroulandie, J., Blais, J.F., Tyagi, R.D. (2003), ‘Cocoa shells for heavy metal removal from acidic solutions’, Bioresource Technology, Vol.90, pp. 255-263.

Miretzky, P. and Fernandez Cirelli, A. (2010) ‘Cr (VI) and Cr (III) removal from aqueous solution by raw and modifiedlignocellulosicmaterials: A review’, Journal of Hazardous Materials, Vol.180, Issues 1-3, pp.1-19.

Mohammad Abul Hossain, Mikio Kumita, Yoshimasa Michigami, Shigeru Mori (2005), ‘Optimization of parameters for Cr (VI) adsorption on used black tea leaves’, Adsorption, Vol.11, No. 5-6, pp. 561-568.

Muhammad Abbas Ahmad Zaini, Yoshimasa Amano, Motoi Machida (2010), ‘Adsorption of heavy metals onto activated carbons derived from polyacrylonitrile fiber’, Journal of Hazardous Materials, Vol. 180, Issues 1-3, pp. 552-560.

Mustafiz, S., Rahaman, M. S., Kelly, D., Tango, M., Islam, M. R. (2003), ‘The application of fish scales in removing heavy metals from energy-produced waste streams: the role of microbes’, Energy Sources, Vol.25, No.9, pp.905-916.

Nameni, M., Alavi Moghadam, M.R., Arami, M. (2008) ‘Adsorption of hexavalent chromium from aqueous solutions by wheat bran’, International Journal of Environmental Science and Technology, Vol.5, No.2, pp.161-168.

Olayinka Kehinde, O., Adetunde Oluwatoyin, T., Oyeyiola Aderonke, O. (2009) ‘Comparative analysis of the efficiencies of two low cost adsorbents in the removal of Cr(VI) and Ni(II) from aqueous solution’, African Journal of Environmental Science and Technology, Vol.3, No.11, pp.360-369.

Oliveira, E.A., Montanher, S.F., Andrade, A.D., Nobrege, J.A., Rollemberg, M.C. (2005), ‘Equilibrium studies for the sorption of chromium and nickel from aqueous solutions using raw rice bran’, Process Biochemistry, Vol. 40, Issue 11, pp. 3485-3490.

Prasad Sudamalla, Pichiah Saravanan and Manickam Matheswaran (2012) ‘Optimization of operating parameters using response surface methodology for adsorption of crystal violet by activated carbon prepared from mango kernel’, Sustainable Environment Research, Vol. 22, No.1, pp. 1-7.

Preetha, B. and Viruthagiri, T. (2006) ‘Application of response surface methodology for the biosorption of copper using *Rhizopus arrhizus*’, Journal of Hazardous Materials, Vol.143, Issues 1-2, pp. 506-510.

Qi, B.C. and Aldrich, C. (2007) ‘Biosorption of heavy metals from aqueous solutions with tobacco dust’, Bioresource Technology, Vol.99, Issue 13, pp. 5595-5601.

Rabindra Prasad Dhakal, Kedar Nath Ghimire, Katsutoshi Inoue (2005), ‘Adsorptive separation of heavy metals from an aquatic environment using orange waste’, Hydrometallurgy, Vol.79, Issues 3-4, pp. 182-190.

Rajasimman, M. and Murugaiyan K. (2011) ‘Sorption of Nickel by *Hypnea Valentiae*: Application of Response Surface Methodology’, International Journal of Civil and Environmental Engineering, Vol.3, No.1.

Sandhya Babel and Tonni Agustiono Kurniawan (2003) ‘Low-cost adsorbents for heavy metals uptake from contaminated water: a review’, Journal of Hazardous Materials, Vol.97, Issues 1-3, pp.219-243.

Sarabjeet Singh Ahluwalia, Dinesh Goyal (2007), ‘Microbial and Plant derived biomass for removal of heavy metals from waste water’, Bioresource Technology, Vol. 98, Issue 12, pp. 2243-2257.

Silke Schiewer, Santosh B. Patil (2008), ‘Pectin-rich fruit wastes as biosorbents for heavy metal removal: Equilibrium and kinetics’, Bioresorce technology, Vol. 99, Issue 6, pp. 1896-1903.

Sudhir Dahiya, Tripathi, R.M., Hegde, A.G. (2008) ‘Biosorption of heavy metals and radionuclide from aqueous solutions by pre-treated arca shell biomass’, Journal of Hazardous Materials, Vol.150, Issue 2, pp. 376-386.

Suleman Qaiser, Anwar R. Saleemi, Muhammad Umar (2009), ‘Biosorption of lead(II) and chromium(VI) on groundnut hull: Equilibrium, kinetics and thermodynamics study’, Electronic Journal of Biotechnology, Vol. 12, No.4.

Tarangini, K., Arvind Kumar, Satpathy, G. R. and Vikas Kumar Sangal (2009) ‘Statistical Optimization of Process Parameters for Cr (VI) Biosorption onto Mixed Cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis*’, Clean, Vol.37, Issues 4-5, pp. 319-327.

Tonni Agustiono Kurniawan, Gilbert Y.S. Chan, Wai-Hung Lo and Sandhya Babel (2006), ‘Physico-Chemical treatment techniques for waste water laden with heavy metals’,Chemical Engineering Journal, Vol. 118, Issues 1-2, pp. 83-98.

Wan Ngah, W.S., Hanafiah, M.A.K.M. (2008), ‘Removal of heavy metal ions from waste water by chemically modified plant wastes as adsorbents: A review’, Bioresource Technology, Vol. 99, Issue 10, pp.3935-3948.

Yi Liu, Yian Zheng and Aiqin Wang (2011) ‘Response Surface Methodology for Optimizing Adsorption Process Parameters for Methylene Blue Removal by a Hydrogel Composite’, Adsorption Science and Technology, Vol.28, No. 10, pp. 913-922.