METAL REMOVAL STUDIES USING GOOSEBERRY SEEDS AS A BIOSORBENT

A PROJECT REPORT

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BONAFIDE CERTIFICATE

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ABSTRACT

Gooseberry seed (*Phyllanthus acidus*) was used as an adsorbent to determine its feasibility for the removal of Cr(VI). Various parameters such as pH, temperature, contact time, initial metal concentration and adsorbent dosage were investigated to determine the biosorption performance. Equilibrium was attained within 60min and maximum removal of 96% was achieved under the optimum conditions at pH 2. With an increase in metal concentration from 20 to 100mg/L, Cr(VI) removal sharply decreases from 97 to 88%. Under the optimal conditions, the equilibrium data was very well represented by Langmuir isotherm (R^2=0.992) which accounts for the existence of monolayer adsorption. Langmuir isotherm was followed by Temkin and Freundlich with an R^2 value of 0.990. Langmuir constants K and q_0 was found to be 0.0061(L/mg) and 19.23(mg/g). The adsorption system was found to follow Pseudo second order kinetics with R^2 value of 0.999. A total of 17 trials were run according to the Box Behnken Design created using Design Expert software. Quadratic model had maximum R^2 value (0.9984) and larger F value (1109.92). From the ANOVA table and R^2 value, Quadratic model had been predicted to be the significant model which fitted the best with the generated experimental data. The optimal parameters obtained from the contour plot for the maximum removal of Cr(VI) were initial metal concentration of 60mg/L, pH value of 2, and temperature of 27˚C. Under these conditions, maximum removal of 92% was obtained.

KEY WORDS

Gooseberry seed, Cr(VI), biosorption, Equilibrium, isotherm, kinetics, ANOVA.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND NOMENCLATURE

Abbreviation/Symbol	Description
%	Percentage
A_T	Equilibrium binding constant
b_T	Heat of adsorption
B_T	$(RT)/b_T$
C_e	Metal concentration at equilibrium
C_i	Initial metal concentration
C_o	Final metal concentration
Cm	Centimeter
Cr	Chromium
Df	Degrees of Freedom
G	Gram
K	Constant related to energy of adsorption
k_1	First order rate constant
k_2	Second order rate constant
L	Litre
M	Mass of the adsorbent
mg	Milligram
min	Minute
ml	Millilitre
N	Normality

Pa	Pascal
pH	Potential of hydrogen
q_e	Adsorption capacity
q_m	Theoretical monolayer saturation
q_o	Maximum monolayer adsorption capacity
q_t	Amount of solute adsorbed at any time t
R	Universal gas constant
R^2	Regression coefficient
V	Volume of the metal solution
V_o	Initial adsorption rate
Y	Solute concentration in the solution
°C	Degree Celsius
θ	Fractional occupation
ANOVA	Analysis of Variance
BBD	Box Behnken Design
CV	Coefficient of Variance
D-R isotherm	Dubin- Radushkevisch isotherm
H-J isotherm	Harkins- Jura isotherm
FTIR	Fourier Transform Infrared Spectroscopy
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy

CHAPTER 1
INTRODUCTION

1.1 GENERAL

Rapid industrialization and urbanization have resulted in exponential discharge of industrial effluents and toxic heavy metals in aquatic and agricultural ecosystem (Das et al., 2007). The heavy metals found in these effluents include chromium, copper, nickel, cadmium, arsenic and so on. Chromium is found in mining, tanning, and electroplating industrial effluents and according to US EPA, Chromium is considered to be the topmost priority toxic pollutant. Chromium exists in two states: Cr(III) & Cr(VI). Hexavalent chromium is found to be 100 times more toxic than trivalent chromium. The maximum permissible level of Cr(VI) ion concentration in surface and portable water are 0.1 and 0.05mg/L respectively (APHA; 2005). Thus, it is very essential to make effluents free from Cr(VI) before releasing into the environment.

1.1.2 Toxic Effects of Hexavalent Chromium

Chromium has been used for various applications. It has been used as a pigment in paints and as a mordant in dyeing industries. Chrome plating is another important application where chromium is used to line metals. Though the presence of chromium in trace amounts is good, exposure to larger quantities results in serious effects. Due to its toxicological and carcinogenic consequences, it is likely to cause detrimental health hazards such as dermatitis, shortness of breath, coughing, wheezing, perforations and ulcerations of septum, bronchitis, decreased pulmonary function, pneumonia and other respiratory effects (Rao and Prabhakar, 2011).

1.1.3 Drawbacks behind Existing Technologies

Common technique for the heavy metal removal includes chemical precipitation, ion exchange, oxidation-reduction, membrane filtration, coagulation, floatation, electrochemical methods. The drawback behind these techniques includes high operational cost, high energy requirements, operational complexities and huge sludge deposition. Thus, adsorption has been considered as the best alternative for heavy metal sequestration as it is economical and environmental friendly technique (Salman et al., 2014).

1.1.4 Identification of Biosorbent

In order to make adsorption, an excellent and efficient method, it is vital to identify cost effective and locally available bioresource materials. Agricultural waste residues are considered as important source of biosorbents due to the presence of certain functional groups such as hydroxyl, carboxyl, amino, ester, sulphydryl, carbonyl and phosphor group. Agricultural waste residues such as rice husk, wheat bran, orange peel, watermelon shell, pineapple peel, lemon residues had been extensively studied in past to determine the adsorption efficiency. Bioresource material showing high adsorption capacity and metal selectivity had been suggested as suitable biosorbent for heavy metal sequestration (Nguyen et al., 2013). In this regard, Gooseberry seed (*Phyllanthus acidus*), a novel biosorbent was examined for Cr(VI) binding efficiency. In the present study, various parameters such as pH, adsorbent dosage, contact time, and initial metal ion concentration were investigated in a batch mode for Cr(VI) removal. The suitability of equilibrium isotherm and adsorption kinetics was investigated to understand the adsorption mechanism.

1.1.5 Biosorbent Characterization

Adsorbents were characterized mainly through analytical techniques such as FTIR and SEM. FTIR was carried out to determine the presence of active sites based on the changes in the vibrational frequencies of functional groups in the adsorbent. In the study, spectra of the adsorbent before and after adsorption were measured within the range of 4000 to 400 cm^{-1}.

The surface topology of sample and the porosity was analysed through Scanning Electron Microscope. The images were scanned upto 12,000X with a stable pressure of 110 Pa. The most common method for detection of images in SEM analysis involves excitation of secondary electron by the electron beam. The images can be analysed in both low vacuum as well as high vacuum.

1.1.6 Isotherm Studies

Isotherm study is used to determine the adsorption efficiency and to predict the best model, which fits the generated equilibrium data. Adsorption isotherm shows the equilibrium relationship of solute concentration between the liquid solvent phase and the solid adsorbent phase at a given temperature, pressure, pH, and total solute concentration. They are determined mainly by regression analysis.

In our study, mainly five different kinds of isotherm models were studied (Langmuir, Freundlich, Harkins-Jura, Temkin, and Frumkin isotherm). Langmuir isotherm model is used to quantify the amount of adsorbate that is adsorbed on an adsorbent in terms of partial pressure or concentration at a given temperature. Freundlich isotherm is used to determine the variation of amount of gas adsorbed at a constant temperature. It also determines the heterogenicity. Harkins-Jura model is used to determine the monolayer adsorption and existence of heterogeneous pore distribution. Temkin model is temperature dependent. It determines the relationship between the temperature and binding energy of adsorbate on the adsorbent.

1.2 MOTIVATION

Toxicological consequences of hexavalent chromium on aquatic and agricultural ecosystem motivates an extensive study to identify the technically and economically feasible methods for the effective removal of Cr(VI). In the past, agricultural waste residues such as rice husk, wheat bran, watermelon shell, orange peel, pineapple peel, lemon residues had been extensively studied to determine the adsorption efficiency. The results showing higher efficiencies at low cost triggers further study to identify inexpensive adsorbent for the efficient adsorption of Cr(VI).

Our project work was carried out with an intention of removal of Cr(VI) from simulated waste water using gooseberry seed powder as a biosorbent.

1.3 OBJECTIVES

1. To determine the feasibility of gooseberry seeds as an adsorbent for the removal of chromium.
2. To investigate the effect of pH, temperature, contact time, initial metal concentration and dosage concentration on adsorption performance with adsorption isotherms and modeling.
3. To determine the adsorption kinetics in batch mode.
4. To optimize the processing parameters for Cr(VI) removal by RSM study.

CHAPTER 2
LITERATURE REVIEW

2.1 HEAVY METAL POLLUTION

Heavy metals discharged in the aquatic and agricultural ecosystem due to the rapid industrialization pose a serious threat to human health and environment (Das et al., 2007). The heavy metals found in the effluents include chromium, cadmium, nickel, copper and so on. Conventional methods for the treatment of heavy metals include chemical precipitation, ion exchange, ultra filtration, electro dialysis and reverse osmosis. However, certain factors such as operational cost, energy requirements, operational complexities, sludge deposition should be taken into consideration while selecting the method for the treatment of waste water at large scale (Kundu and Gupta, 2005). Hence, attention has been shifted towards biosorption as it is economical and environmental friendly technique for the sequestration of heavy metals.

Biosorption can be defined as the removal of metal or metalloids from the solutions by the biological materials (Prabhakar and Rao, 2004). Both living and dead biomass as well as cellular products can be used as a biological substrate for the removal of heavy metals. This technique has many advantages when compared with conventional techniques. Some of the advantages have been listed below.

- No sludge generation
- Regenerative : Biosorbents can be reused after desorption
- Cost effective
- Metal selective

2.2 MICROBIAL DERIVED BIOMASS FOR THE REMOVAL OF HEAVY METAL

2.2.1 Algal Biomass as a Biosorbent

Jinsong et al., (2014) reviewed the use of algal biomass as an effective biosorbent for the heavy metal sequestration. In this review, biosorption performance of brown, red and green algae were compared. Out of which biosorption performance of brown algae was found to be the best. The complete adsorption of chromium could be achieved in 20 hours, when chemically modified brown algae (*Sargassum* sp) were used (Yang and Chen, 2008). The theoretical equilibrium model (intraparticle diffusion model) was the best model for describing the adsorption kinetics (Sud et al., 2008).

2.2.2 *Rhizopus nigricans* as a Biosorbent

Emilia et al., (2001) studied "Biosorption of Cr(VI) from aqueous solution by *Rhizopus nigricans*". The process showed maximum removal occurred at an optimum pH of 2. From the study, it had been revealed that adsorption behaviour followed Freundlich and Langmuir isotherm and was found to attain equilibrium within 3 hours at optimum temperature of 45°C within an agitation speed of 120 rpm at an initial concentration of 100mg/L.

2.2.3 *Aspergillus niger var tubingensis* Ed8 as a Biosorbent

Coreno et al., (2014) studied the mechanism of interaction of chromium with *Aspergillus niger* var *tubingensis* Ed8. This paper focuses on the ability of *Aspergillus niger* to capture chromium and its efficiency to reduce Cr(VI) to Cr(III) which is 100 times less toxic than Cr(VI). Biotransformation of chromium was carried out in a bubble column reactor and its reduction rate was found to be 2.62 mg Cr(VI) $g^{-1}hr^{-1}$.

Biotransformation of hexavalent chromium in batch culture was found to be 94% reduction in a medium containing 50 mg Cr (VI) L^{-1}.

2.2.4 *F. vesiculosus* as a Biosorbent

Cobas et al., (2014) carried out an experiment for the biosorption of leather industrial effluents using an eco-friendly adsorbent *Fucus vesiculosus.* The operating variables selected were initial pH, biomass concentration and $CaCl_2$. From the optimization study using Box Behnken Design, biosorption of Cr(VI) was found to be high at pH 4.82, biomass dosage of 1.44g, and pretreated $CaCl_2$ concentration of 0.64M. Also, the biosorption response of Cr(VI) was found to be high with significant increase in operating parameters such as adsorbent dosage concentration and pretreated $CaCl_2$ concentration and pH has a negligible effect. From the coefficient of determination of R^2 value, Pseudo second order was found to be the best fit for describing the adsorption kinetics. From the isotherm studies it has been analysed that Freundlich isotherm fits well with the obtained equilibrium data.

2.2.5 *Pseudomonas aeruginosa* AT18 as a Biosorbent

Batch biosorption experiments were performed to investigate the sorption capacity of *P. aeruginosa* for decontaminating the pollutants from the contaminated site. The effect of pH on biosorption performance reveals that the sorption of heavy metals such as Cr^{3+}, Cu^{2+}, $Mn^{2+}$and Zn^{2+} increased with the increasing pH in the range of 5.5 to 7.5. The observation from the study in comparison with Cr^{3+}, Cu^{2+}, $Mn^{2+}$and Zn^{2+} was that the sorption capacity of *P. aeruginosa* is high for Cr^{3+} with 100% removal in the pH range of 7 to 7.22 and low for Mn^{2+}. Langmuir and Freundlich model were used to evaluate biosorption kinetics. Among them, Freundlich model was found to be the best fit for established equilibrium data (Rosa et al., 2009).

2.2 PLANT DERIVED BIOMASS FOR THE REMOVAL OF HEAVY METAL

2.2.1 Husk of Bengal gram

About 99.9 percent removal of chromium had occurred at an optimum pH of 2 with the agitation speed of 120 rpm and attained equilibrium within 180min when husk of Bengal gram was used as an adsorbent. The adsorption behaviour was found to correlate well with both Langmuir and Freundlich adsorption isotherm (Ahalya et al., 2005).

2.2.2 Tamarind Fruit Shell

Srinivasa et al., (2007) worked on "Biosorption of hexavalent chromium using tamarind fruit shell: A comparative study". In this study, investigations on crude tamarind fruit shell, HCl treated and oxalic acid treated shells were carried out at room temperature. Biosorbents were characterized by FTIR, EDXRF and Porosimetry. The generated results were found to correlate well with both Langmuir and Freundlich adsorption isotherm models. The adsorption kinetics was found to obey Pseudo first order. Maximum removal of chromium had occurred at an optimum pH of 3 and monolayer adsorption capacities of crude tamarind fruit shell, HCl treated and Oxalic acid treated fruit shells were 74.62 mg/g, 140.84 mg/g and 151.51 mg/g respectively.

2.2.3 *Pinus sylvestris*

Pinus sylvestris was employed as an adsorbent for the batch removal of chromium by varying various parameters such as contact time, pH, initial metal ion concentration, agitation speed and temperature. From the results generated, it had been predicted that 80% sorption efficiency was attained at an optimum pH of 1 and equilibrium was reached within

2 hours with agitation speed of 150 rpm at 25°C for an initial concentration of 150 mg/L. From the correlation coefficient values, Freundlich isotherm model was predicted to be the best fit for the generated equilibrium data (Ucun et al., 2005).

2.2.4 Neem Leaf Powder

Gopalakrishnan et al., (2013) carried out a research on "Biosorption of chromium (VI) from industrial effluents using neem leaf". Equilibrium studies were carried out in a batch process by varying various parameters such as agitation time, adsorbent dosage, temperature and initial concentration of chromium. From the correlation coefficient, the adsorption capacity was found to follow Freundlich adsorption isotherm. In this study, equilibrium was achieved within 3 hours.

2.3 ACTIVATED CARBON PREPARED FROM VARIOUS SOURCES

2.3.1 Hazelnut Shell Activated Carbon

Kobya (2003) conducted a research "Removal of Cr(VI) from aqueous solution by adsorption onto hazelnut shell activated carbon: kinetic and equilibrium studies". From the generated results, Pseudo first order kinetics was the best fit and the adsorption behaviour follows Langmuir isotherm. The adsorption capacity calculated from Langmuir isotherm was found to be 170 mg/g at an initial pH of 1 for 1000 mg/L Cr(VI) solution. In this study, thermodynamic parameters were evaluated and the process was found to be endothermic showing monolayer adsorption of chromium. The results revealed that hazelnut shell activated carbon could be utilized as an effective adsorbent for metal removal studies. The maximum adsorption took place in the pH range of 1- 2.

2.3.2 Saw Dust as a Biosorbent

Activated carbon prepared from coconut tree saw dust was used as an adsorbent. Equilibrium studies revealed that adsorption follows both Langmuir and Freundlich adsorption isotherm. The adsorption capacity was found to be 3.46 mg/g at an initial pH of 3 for the particle size ranging from 125- 250 µm. Desorption was favoured by increasing the concentration of NaOH (Selvi et al., 2001).

Baral et al., (2006) conducted a research on "Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust" by varying several parameters such as contact time, pH, amount of adsorbent, concentration of adsorbate and temperature. From the study, it had been revealed that the adsorption kinetics follows pseudo second order and the adsorption behaviour followed Langmuir isotherm. Sawdust was found to be the effective adsorbent and attained equilibrium in 3 hours. In this study, thermodynamic parameters such as standard Gibbs free energy and heat of adsorption were calculated. The calculated values of thermodynamic parameters indicate the exothermic nature of the process. The adsorption capacity was pH dependent and showed maximum removal in the pH range of 4.5- 6.5 at an initial concentration of 5 mg/L.

2.3.3 Tannic Acid Immobilized Powdered Activated Carbon

Xujin et al., (2013) studied the adsorption of Cr(VI) by tannic acid immobilized Powdered Activated Carbon (TA-PAC) from micro polluted water in the presence of Dissolved Humic Acid (DHA). Batch experiments were used to investigate the adsorption capacity of TA-PAC with DHA over a pH range of 3-10. The titration curve of DHA characterization reports that the quantities of total acidic group of DHA, phenol hydroxyls and carboxyl were 10.56 meq/g, 5.76 meq/g and 4.80

meq/g. Chen et al., (2003) reported that the peaks of HA EEM (Excitation Emission spectra) corresponds to humic acid like organics since EX>250 nm and EM>380 nm. It has been investigated that the biotransformation efficiency of Cr(VI) to Cr(III) and the retention of Cr(III) increased over a pH range of 4 to 9 in the presence of DHA. From the correlation coefficient R^2 value, it has been predicted that the Freundlich isotherm is the best fit for equilibrium data obtained from the batch experiments and the maximum adsorption capacity was found to be 5.639 mg/g with DHA and 2.961 mg/g without DHA.

2.3.4 Lignin and Lignin based chars and Activated carbon

A comprehensive review on lignin and lignin based chars and activated carbon as an adsorbent for the removal of heavy metal pollutants from water was done by Suhan et al., (2006). This review highlights the applicability of lignin and its proven ability for adsorption since it is rich in carbon content. The adsorbent characterization studies include FTIR, Temperature Programmed Desorption (TPD), pore size distribution, fractal dimension and pyrolysis. Adsorption capacity of prepared activated carbon from lignin was found to be 604 mg/g for methylene blue and the maximum adsorption was found to be at a temperature ranging from 440 to 550°C. The result revealed that the prepared activated carbon from lignin was considered to be an excellent adsorbent since it requires less energy during regeneration stage. The adsorption capacity was found to be 92.6 mg/g.

2.4 OTHER BIOSORBENTS

The ability of various adsorbents such as brick powder, mango bark dust, mixed algae, chalk powder, ragi seed powder, papaya peel powder, multhani mitti were investigated for the removal of chromium. Among all these, multhani mitti, mango bark dust and mixed algae revealed good results and hence considered to be the effective metal adsorbents. Mixed algae showed 93-98% removal and reached equilibrium within 5 min at an initial concentration of 85 mg/L (Gandhi et al., 2013).

Demirbas et al., (2004) conducted an experiment for the batch removal of Cr(VI) from aqueous solution using low cost adsorbents. The ability of various adsorbents such as Cornelian cherry, apricot stone, almond shell to remove Cr(VI) ions under different experimental conditions were investigated. Adsorption is highly pH dependent and showed maximum removal at an optimum pH of 1. Pseudo second order was found to correlate well with the generated equilibrium data. Among all other adsorbents, almond shell was found to be the effective adsorbent and showed 99% Cr(VI) removal at 25°C.

Treated lemon, orange and sweet lime skin powder were found to be the ideal adsorbents for the removal of Cr(VI) from aqueous solution due to its easy availability and high efficiency. Langmuir adsorption isotherm was employed to evaluate the optimum adsorption capacity. The adsorption kinetics was found to obey pseudo first order kinetics. The maximum adsorption was reported in the pH range of 4.5-6 and attained equilibrium within 3 hours with initial concentration of 30 mg/L. The adsorption process was endothermic in nature (Rane et al., 2010).

Singha et al., (2011) conducted a research on "Cr(VI) ions removal from aqueous solution using natural adsorbents". The ability of eight natural adsorbents for the removal of Cr(VI) from aqueous solution were investigated. Adsorbents chosen for this study includes rice straw, rice bran, rice husk, sawdust, neem bark, hyacinth roots, neem leaves and coconut shell. In this study, detailed FTIR of the adsorbents at the optimized conditions was carried out to identify different functional groups that were responsible for the adsorption of Cr(VI) from aqueous solution. Sorption energy determined from D-R isotherm shows that the adsorption process was chemical in nature. The optimum pH for the maximum removal of Chromium was found to be 1.5 and 2 for husk and other adsorbents respectively.

Meena and Rajagopal (2003) carried out a comparative study on the applicability of various adsorbents such as activated alumina, ion exchange resin, sawdust and treated sawdust for the effective removal of heavy metal. From the study, it had been concluded that percent removal is maximum for treated sawdust followed by powdered activated alumina and anion resin and percent removal is poor for sawdust and sand. Experimental results revealed that the adsorption follows Langmuir isotherm.

Baran et al., (2006) investigated on the comparative studies on adsorption of Cr(VI) ions onto various sorbents such as chitin, chitosan, ion exchangers, Purolite CT-275 (Purolite I), Purolite MN-500 (Purolite II) and amberlite XAD-7. Batch sorption experiments were carried out to investigate the operating parameters such as pH, agitation period, Cr(VI) ion concentration on adsorption performance. From the study, it had been predicted that the optimum pH for Cr(VI) adsorption was 3 for chitin and chitosan. Adsorption of chromium increases with the agitation period. It had been found that Langmuir isotherm was the best fit for the generated equilibrium data for chitin, chitosan, purolite I and purolite II. Freundlich isotherm was the best fit for chitin, chitosan and amberlite XAD-7. In comparison with the other sorbent, the chitosan was revealed to be the potential sorbent as it is readily available and economical.

The ability of cross linked and non-cross linked Chitosan to remove Cr(VI) in aqueous solutions was investigated by Schmuhl (2005). Cr(VI) adsorption behaviour was explained using Langmuir isotherm. The adsorption capacity was found to be 78 mg/g for non cross linked Chitosan and 50 mg/g for cross linked Chitosan and showed maximum removal at an optimum pH of 5. From the generated data, it was clear that adsorption process for both the metals were different. Cr(VI) adsorption followed Langmuir isotherm and was not influenced by stirring speed whereas Cu(II) adsorption followed Freundlich isotherm and was influenced by stirring speed.

Shanmugaprakash et al., (2013) investigated the uptake rate of Cr(VI) from the aqueous solution using defatted pongamia oil cake through optimization methodologies such as RSM and ANN in batch and column mode. The investigation of the performance of the two models indicates that ANN is the best fit for the evaluation of effective parameters such as pH, temperature, initial Cr(VI) ion concentration and adsorbent dosage concentration in comparison with RSM model.

Witek et al., (2013) carried out an experiment on the removal of micro elemental Cr(III) and Cu(II) from the aqueous solution through the application of soybean meal waste. Effects of operating parameters such as pH, initial metal ion concentration, contact time, adsorbent dosage concentration were investigated. The feasibility of soybean meal as a biosorbent was characterized through techniques such as FTIR, XRD and SEM-EDX. Biosorption of chromium and copper were highly pH dependent. Adsorption of Cr(III) and Cu(II) was found to be maximum at a pH of 5. Pseudo second order model provides a better fit for the generated experimental results with correlation coefficient (R^2> 0.99). In comparison with Langmuir and Freundlich model, Sip's model is the best model for describing adsorption kinetics. The results revealed that the soybean meal (SBM) can be employed as a promising low cost adsorbent for the removal of heavy metal pollutant from water and the studies on spent SBM as an animal feed are in progress.

Biosorption of Cr(VI) from aqueous solution using a Hierarchical Porous Carbon (HPC) obtained from pig bone was studied by Shaochen et al., (2013). This paper deals with the study on adsorbent feasibility, adsorption potential, adsorption isotherms and adsorption kinetics. In this study adsorption potential of HPC were compared with the commercial carbon. Also effect of pH, metal ion concentration and temperature on biosorption performances were investigated in this experiment and this study revealed that the adsorption capacity is strongly dependent on pH of the initial solution. Nitrogen adsorption – desorption isotherm of HPC stated that surface area and the total pore volume were larger than commercial pore volume. Adsorption isotherm studies reveals that the Langmuir isotherm fits better to the equilibrium data generated in comparison with the Freundlich isotherm and the Cr(VI) adsorption capacity was found to be 398.40 mg/g at 27°C. From the correlation coefficient R^2 value, it has been found that the adsorption of Cr(VI) onto HPC obeys Pseudo second order. In this regard, HPC can be considered as effective biosorbent for heavy metal decontamination.

CHAPTER 3
MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals

Potassium dichromate used for the preparation of metal solution was obtained from RFCL Limited, New Delhi. Sodium hydroxide pellets was obtained from Leo Chem, Bangalore. 1, 5 Diphenyl carbazide was obtained from Himedia laboratories Pvt. Ltd, Mumbai. Acetone was obtained from SDFCL, Mumbai.

3.1.2 Equipments

Visible spectrometer, Eco testr pH pen, pH meter, Hot air oven and weighing balance.

3.2 COLLECTION AND PREPARATION OF ADSORBENT

Gooseberry seeds (*Phyllanthus acidus*) were collected from Coimbatore, Southern part of Tamilnadu. The seeds were washed under running tap water and were subjected to drying in hot air oven at an optimum temperature of 40°C. Then the seeds were ground and sieved. The finely powdered biosorbent was subjected to washing. After washing with distilled water, the mixture was filtered and dried at 50°C for a period of 1 h. The dried powder was stored in an air tight container to prevent moisture. The dried powder was used as a biosorbent for the experiment.

3.3 METAL ANALYSIS

3.3.1 Preparation of Synthetic Metal Solution

Stock solution of 1000 mg/l was prepared using potassium dichromate crystals. pH of the solution was adjusted using 0.1N NaOH or H_2SO_4. Fresh dilutions of 100 mg/l were prepared from the stock solution for the study.

3.3.2 Chromium Analysis

Chromium was estimated using 1, 5Diphenyl carbazide as a complexing agent spectrophotometrically. Different standards containing less than 100mg/L (20, 40, 60, 80, and 100) were prepared and maintained at pH less than 2. To 10ml of standard, 0.2ml of Diphenyl carbazide was added as a complexing agent. The solution was incubated until red violet color was developed. A blank was prepared for Cr (VI) analysis. Absorbance was measured spectrophotometrically at 540nm. The amount of chromium present in the sample was determined from calibrated curve according to the standard method (APHA method 3500- Cr).

3.3.3 Removal Efficiency

The percentage of Chromium removal (R %) was determined using the formulae:

$$\text{Removal efficiency (R \%)} = \frac{C_i - C_0}{C_i} \times 100 \qquad (3.1)$$

Ci and Co represents the initial and final concentration of chromium metal in mg/L.

3.4 BATCH STUDIES

The adsorption capacity of biosorbent was determined by varying several parameters such as pH, contact time, temperature, adsorbent dosage and initial metal concentration by fixing the volume of metal solution to 100ml. After agitating for 30min in an orbital shaker at 120rpm, the solution was filtered. The residual concentration of chromium present in the filtrate was determined spectrophotometrically using 1, 5 Diphenyl carbazide reagent as a complexing agent at 540nm.

3.4.1 Effect of pH

The experiment was performed by taking 100mg/L of Potassium dichromate solution. The desired pH (2 to 7) was obtained by the addition of either 0.1N NaOH or H_2SO_4 (Shanmugaprakash and Sivakumar, 2013). To this 100ml of solution, 0.1g of adsorbent was added and kept in an orbital shaker rotating at 120rpm. After an equilibration of 30min, OD reading was taken spectrophotometrically at 540nm.

3.4.2 Effect of contact time

The experiment was performed by taking 100mg/L of Potassium dichromate solution. pH was maintained at 2 by the addition of 0.1N H_2S0_4. To 100 ml of solution, 0.5g of adsorbent was added and kept in a shaker at 120rpm. After different equilibration times (15, 30, 45, 60 min), OD readings were taken at 540nm.

3.4.3 Effect of adsorbent dosage

The experiment was carried out by varying the adsorbent dosage from 0.1g to 0.5g. After an equilibration time of 30min, OD readings were taken spectrophotometrically with the complexing agent at 540nm.

3.4.4 Effect of initial metal concentration

The sorption of chromium was investigated with the initial metal concentrations of 20, 40, 60, 80 and 100mg/L (Reddy et al., 2010). The experiment was carried out with 100ml solution of different concentrations with 0.5g of adsorbent. After different time interval, OD readings were taken with the complexing agent at 540 nm.

3.5 ADSORPTION ISOTHERM

Adsorption isotherm is useful in determining the adsorption capacity of an adsorbent. It is used to describe the equilibrium relation between the amount of adsorbate present in the solution and the amount of adsorbate adsorbed in the adsorbent at a given temperature.

Adsorption capacity is given by the empirical relation:

$$q_e = \frac{(C_i - C_0)}{m} \times V \tag{3.2}$$

Where q_e is the adsorption capacity (mg/g), C_i and C_0 are initial and final metal concentration (mg/L), m is the mass of the adsorbent (g) and V is the volume of the metal solution (L). In the present study, adsorption of Cr(VI) onto the biosorbent was investigated by adsorption isotherms.

3.5.1 Langmuir Adsorption Isotherm

Langmuir isotherm is based on the assumption that metal uptake takes place on the homogeneous surface by monolayer adsorption. It involves uniform energies of adsorption. Langmuir adsorption isotherm is given by the empirical equation:

$$\frac{1}{q} = \frac{K}{q_o y} + \frac{1}{q_o} \tag{3.3}$$

Where q is the amount of solute adsorbed per amount of adsorbent (mg/g), y is the solute concentration in the solution (mg/L), K (L/mg) and q_0 (mg/g) are the Langmuir constants related to energy of adsorption and maximum monolayer capacity. By plotting 1/q versus 1/y, Langmuir parameters were obtained (Langmuir 1917).

3.5.2 Freundlich Adsorption Isotherm

Freundlich isotherm model assumes that metal uptake takes place on the heterogeneous surface of an adsorbent by multilayer adsorption. It involves non uniform energies of adsorption. Freundlich adsorption isotherm is given by the empirical equation:

$$\ln q_e = \ln K + \frac{1}{n} \ln y \qquad (3.4)$$

Where q_e is the amount of solute adsorbed per amount of adsorbent (mg/g), y is the solute concentration in the solution (mg/L), K and n are the Freundlich constants related to adsorption capacity and intensity.

By plotting $\ln q_e$ versus lny, Freundlich parameters were obtained. It also determines the heterogeneity of surface pore distribution and the value of n indicates the favorability of adsorption (Freundlich 1906).

3.5.3 Temkin Adsorption Isotherm

Temkin isotherm is based on the assumption that heat of adsorption of all molecules decreases linearly with coverage due to adsorbate-adsorbent interaction. It involves uniform distribution of binding energies upto some maximum binding energy. Temkin adsorption isotherm model is represented by the linearized form of equation:

$$q_e = B_T \ln A_T + B_T \ln Y \qquad (3.5)$$

Where, $B_T = (RT)/b_T$, T is the absolute temperature in Kelvin and R is the universal gas constant. The constant b_T is related to heat of adsorption ($J\ mol^{-1}$), A_T is the equilibrium binding constant ($L^{-1} min^{-1}$). By plotting q_e versus lnY, Temkin constants such as B_T and A_T were determined (Temkin and pyzhev, 1940).

3.5.4 Frumkin Isotherm Model

The Frumkin isotherm equation is given by

$$\ln\left[\left(\frac{\theta}{1-\theta}\right) * \frac{1}{c_e}\right] = \ln K + 2a\theta \qquad (3.6)$$

Where C is the metal concentration at equilibrium (%v/v), θ is the fractional occupation and is given by $\frac{q_e}{q_m}$ (q_e is the equilibrium adsorption capacity(mg/g), K is the constant related to adsortion equilibrium and C_e is the equilibrium concentration(mg/L).

3.5.5 Harkins-Jura (H-J) Isotherm Model

H-J isotherm equation is given by

$$\frac{1}{q_e{}^2} = \frac{B}{A} - \left(\frac{1}{A} * \log C_e\right) \tag{3.7}$$

Where, C_e is the metal concentration at equilibrium (%v/v), q_e is the amount of metal adsorbed per unit mass of adsorbent (mg/g), A and B are H-J isotherm constants.

3.6 ADSORPTION KINETICS

Studies on adsorption kinetics had been carried out to describe adsorption mechanism and diffusion process. The generated data were tested using Pseudo first order and second order kinetics equation.

3.6.1 Pseudo First Order / Lagergren Kinetic Model

This model was derived based on adsorption capacity. According to this model, the overall rate of adsorption is directly proportional to the driving force i.e., the difference between the initial and equilibrium metal concentration (Qiu et al., 2009). The equation can be represented as follows:

$$\frac{dq_e}{dt} = K_1(q_e - q_t) \tag{3.8}$$

Where q_e is the amount of solute adsorbed at equilibrium per unit mass (mg/g), q_t is the amount of solute adsorbed at any time t (mg/g), K_1 is the pseudo first order rate constant (min^{-1}). Integrating the above equation under boundary conditions yields the following equation:

$$\log(q_e - q_t) = \log q_e - \frac{K_1}{2.303} t \tag{3.9}$$

3.6.2 Pseudo Second Order Kinetic Model

According to this model, the driving force (q_e-q_t) is proportional to the available fraction of active site. The equation can be expressed as follows:

$$\frac{dq}{dt} = K_2(q_e - q_t)^2 \qquad (3.10)$$

Integrating under boundary conditions such as q=0 to q>0 and t=0 to t>0 and further simplification yields the following equation:

$$\frac{t}{q_t} = \frac{1}{V_0} + \frac{1}{q_e}t \qquad (3.11)$$

Where $V_o= k_2q_e^2$, V_o is the initial adsorption rate (mg/g min) and k_2 is the pseudo second order rate constant (g mg^{-1} min^{-1}). Adsorption parameters such as V_0, q_e, k_2 can be determined by plotting t/q_t versus t (Ho et al., 2006).

3.7 EXPERIMENTAL DESIGN

RSM is a collection of mathematical and statistical techniques employed for the search of optimum parameters for the maximum removal of Cr(VI). The process parameters affecting the removal of Cr(VI) were studied by means of a three factor, three level Box Behnken experimental design. Experimental design was created using design expert software 9.0.3.1. The main objective behind design of experiment is to optimize a response and to determine the relationship between a response (output variable) and the independent variables (input variables) (Lee et al., 2000; Montgomery, 1997).

Experiments were conducted according to the design created using design expert software, where the changes are made in the input to identify the changes in the response (output). The variable input parameters were pH values in the range 0f 2-6, initial metal concentrations of 20-100mg/L and temperature in the range of 27-57° C. The three independent variables were designated as A (pH), B (Temperature), C (Initial metal concentration) respectively for statistical analysis. Cr(VI) removal percent after adsorption was taken as the response of the design experiments. Three basic steps involved in optimization process are to perform statistically designed experiment, to determine the coefficient estimate, to analyse the response and to check the adequate model (Wang et al., 2010).

A total of 17 trials were run in order to optimize the parameter at which the maximum removal was obtained. Investigations over different tests such as sequential model sum of squares, lack of fit tests, model summary statistics helps in selecting the best fit model for describing the relationship between the response and other influencing independent variable. Regression analysis and ANOVA response were also employed to analyse the result.

In order to fit the generated experimental data and to identify the relevant model terms, the most widely used second order polynomial equation can be represented as follows:

$$Y = \beta_o + \Sigma\beta_i X_i + \Sigma\beta_{ii} {X_{ii}}^2 + \Sigma\beta_{ij} X_i X_j + \in \qquad (3.12)$$

Where Y is the predicted response (the percentage removal of Cr(VI)), β_o is the constant coefficient, β_i is the 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 coefficient between the input factor X_i and X_j and $\in$ is the error of the model (Box and Behnken, 1960). For this study, the independent variables were coded as A, B and C and thus, the equation can be represented as follows:

$$Y = \beta_O + \beta_i A + \beta_i B + \beta_i C + \beta_{ii} A^2 + \beta_{ii} B^2 + \beta_{ii} C^2 + \beta_{ij} AB + \beta_{ij} AC + \beta_{ij} BC \qquad (3.13)$$

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 CHARACTERISATION OF BIOSORBENT

4.1.1 FT-IR Analysis

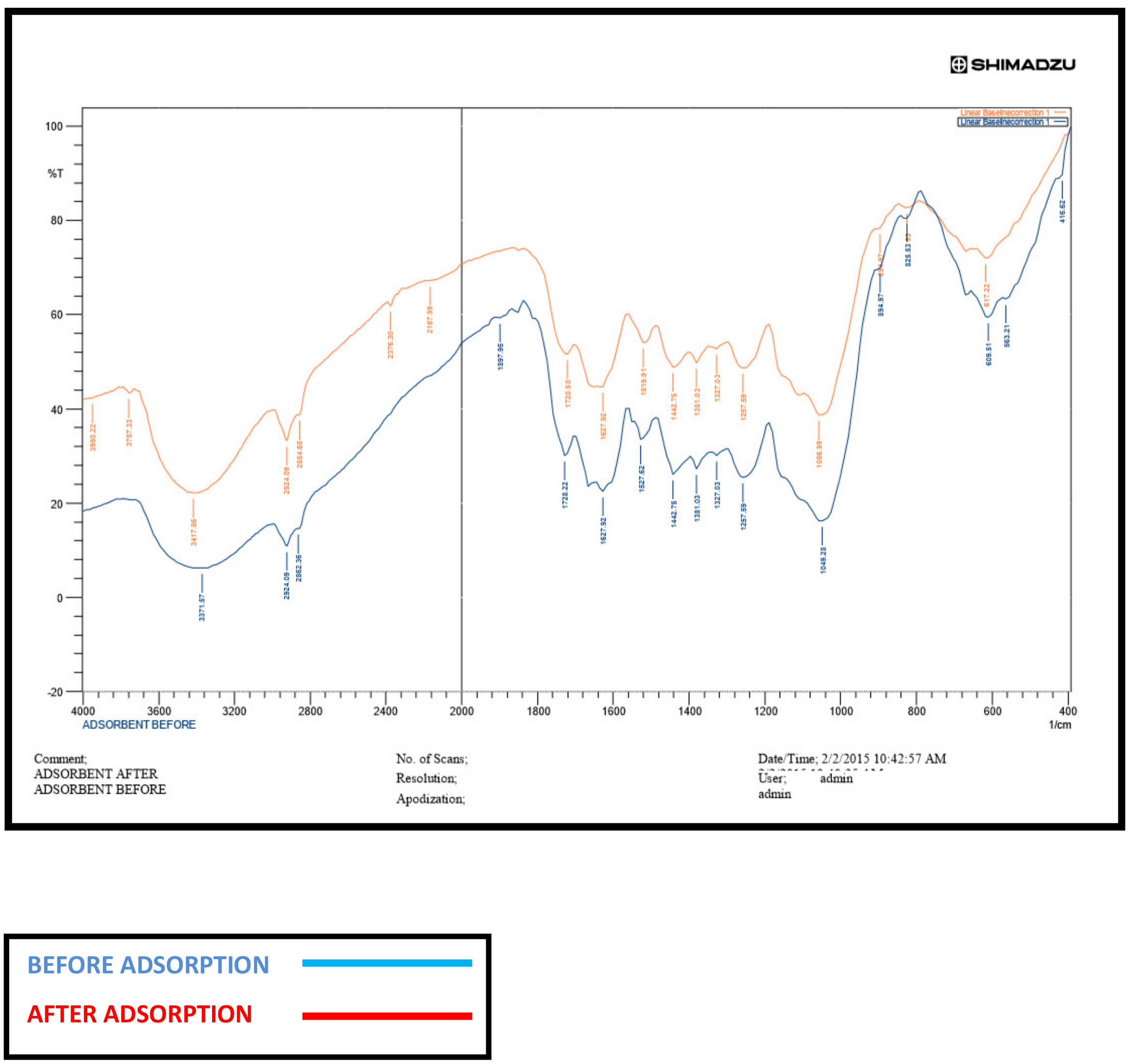
FT-IR analysis was carried out to determine the functional groups present in the adsorbent based on the changes in the vibrational frequencies. The functional groups responsible for adsorption are given in the table 4.1.

Table 4.1 FT-IR spectral characteristics of Gooseberry seed powder before and after adsorption

IR Peak	Before adsorption (cm^{-1})	After adsorption (cm^{-1})	Assignment
1	3317.57	3417.66	-OH Stretch
2	2862.36	2854.65	CH Stretch
3	1728.22	1720.50	C=O Stretch
4	1527.62	1519.91	N-H Bend
5	1049.28	1058.99	C-N Stretch
6	609.51	617.22	C-H Bend

The peaks shift showed that the stretching of O-H group was responsible of the adsorption of Cr(VI) ions onto the adsorbent. It was found that C-N, C=O, C-H stretch, N-H and C-H bend were responsible for the effective removal of Cr(VI) ions.

Similar results were obtained by Muthukumaran and Beulah (2010), where stretching of O-H group was responsible for the adsorption of Cr(VI) using chemically activated *Syzygium jambolanum* nut carbon.



4.1.2 SEM Analysis

The surface morphology of the native and Cr(VI) loaded adsorbents was analyzed using SEM. Surface morphology of the native sorbent was found to be highly porous with different shape and size. The SEM micrograph indicates that there was irregularities and decrease in pore size after adsorption. The formation of white layer on the surface of the sorbent indicates that the sorbent is loaded with Cr(VI) ions. The results obtained were comparable with Das et al., (2013). In this study, surface of the adsorbent was compared before and after adsorption using *Pistia stratiotes* biomass as a biosorbent.

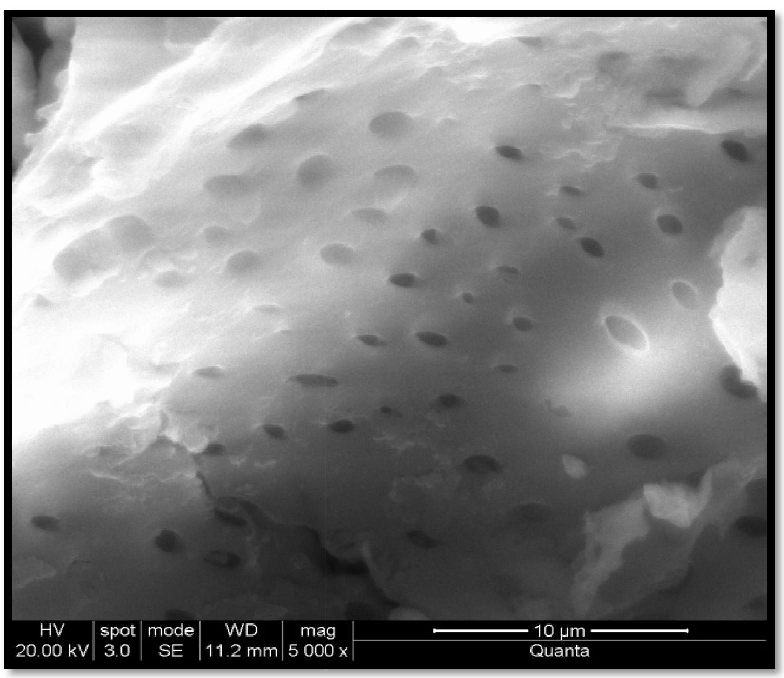


Fig 4.1.1 SEM micrograph of gooseberry seed powder before adsorption

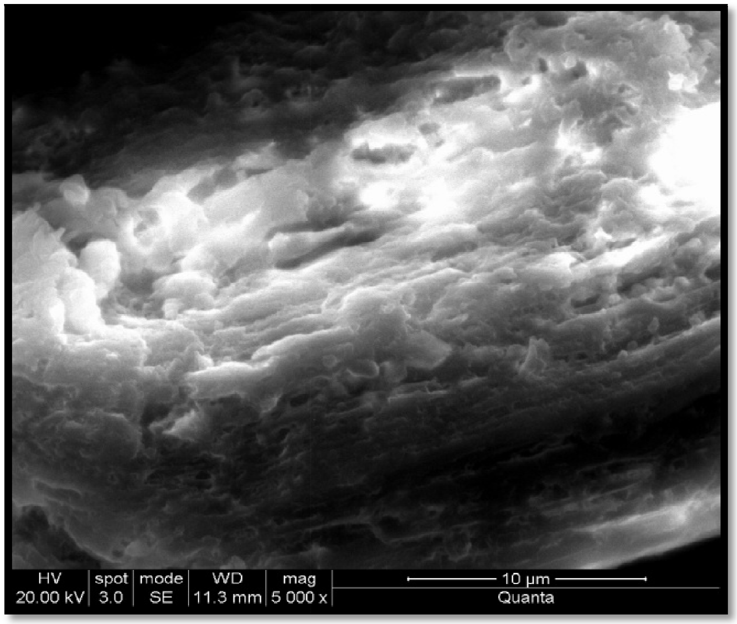


Fig 4.1.2 SEM micrograph of gooseberry seed powder after adsorption

4.2 CHROMIUM STANDARDISATION

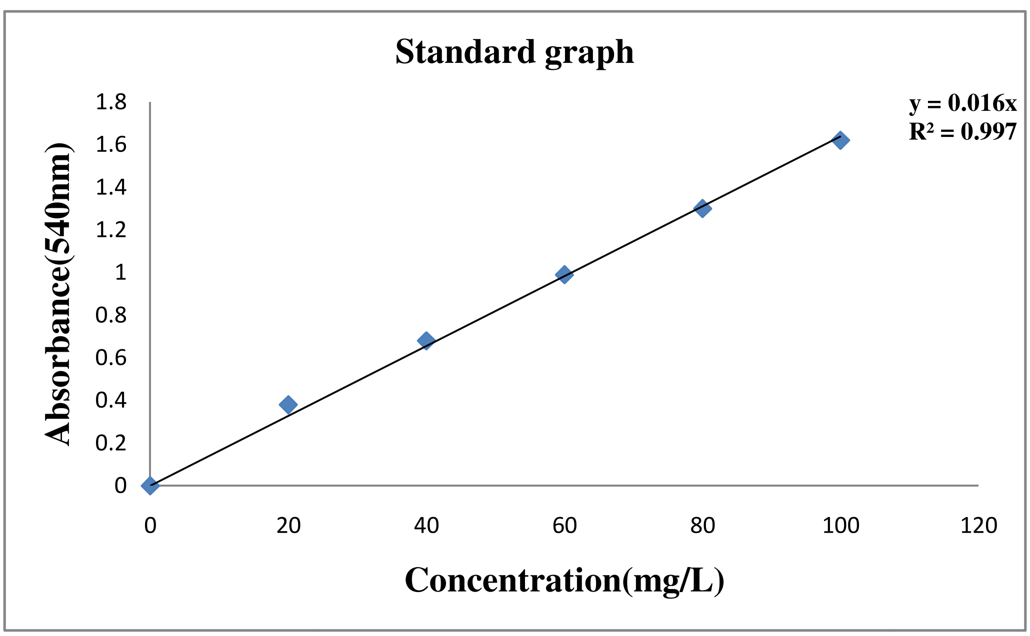


Fig 4.2 Standard graph for Cr(VI)

The standard curve was plotted at different concentrations of Cr(VI). The curve was mainly useful in finding the concentration of Cr(VI) from its absorbance at various experimental conditions for the sample. Cr(VI) was analyzed by Diphenyl Carbazide method.

4.3 BATCH ADSORPTION STUDIES

4.3.1 Effect of pH

pH is the most important parameter in heavy metal biosorption. At aqueous phase, Cr(VI) exist in several anionic forms such as chromate CrO_4^{2-} (P^H>6), dichromate $Cr_2O_7^{2-}$ and $HCrO_4^-$ (pH:1-6). It is clear from the figure 4.3.1 that the removal percentage decreases with an increase in pH from 2 to 7. Maximum removal was obtained at pH 2 indicating the influence of pH in protonation and deprotonation of adsorbent. As the pH is lowered, the surface of the adsorbent becomes protonated and at acidic pH, most of the chromium exists as anionic species. Thereby large number of protons in the adsorbent could easily coordinate with the metal ions present in the solution through electrostatic interaction, whereas the removal percentage sharply decreases with an increase in pH from 2 to7 due to the deprotonation of adsorbent. Hence, the surface of the adsorbent becomes negatively charged and there exists electrostatic repulsion between anionic form of chromium and adsorbent. Similar results were obtained when Eucalyptus bark was used as an adsorbent, where maximum removal was obtained at pH 2 (Sarin and Pant, 2006).

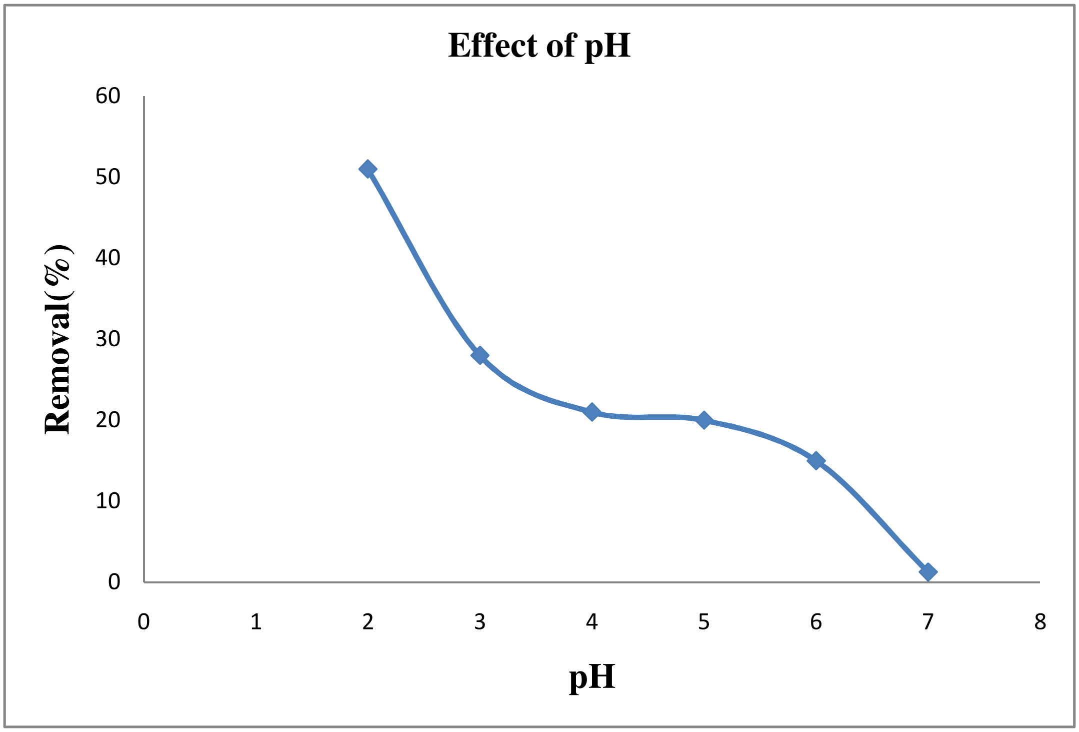


Fig 4.3.1 Effect of pH with 100mg/L metal concentration, adsorbent dosage of 0.1g/100ml, contact time of 30min and speed of 120rpm

4.3.2 Effect of Adsorbent Dosage

Adsorbent dosage plays a major role in adsorption process. From the result, as shown in the Fig 4.3.2, it is understood that, with an increase in adsorbent dosage from 0.1 to 0.5 g, there exists an increase in percentage removal from 51 to 92% and the maximum removal was attained at the adsorbent dosage of 0.5 g. The observed trend may be due to greater availability of surface area and functional groups at higher adsorbent dosage. As a result, electrostatic interaction occurs between the functional groups present in the active site of the adsorbent and the metal ions present in the solution. When activated carbon was used as an adsorbent, 94% removal was attained at an adsorbent dosage of 2g (Devi et al., 2012).

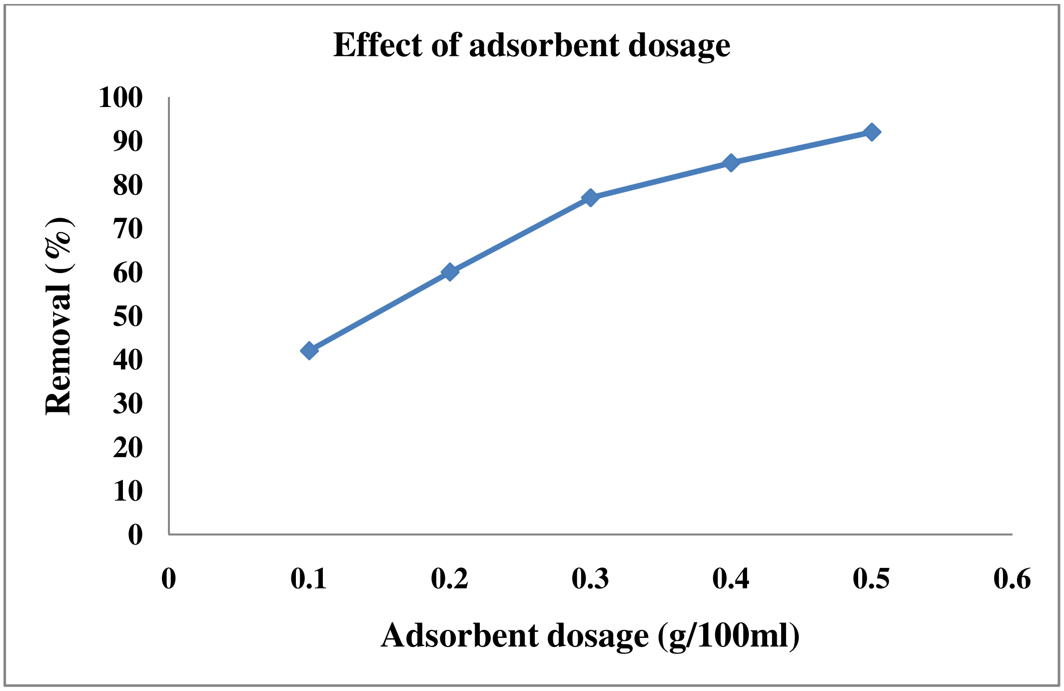


Fig 4.3.2 Effect of adsorbent dosage with metal concentration of 100mg/L with contact time of 30min and speed of 120 rpm at pH 2

4.3.3 Effect of Contact Time

Percentage removal of Cr(VI) ions was measured as a function of time to establish an appropriate contact time between adsorbent and adsorbate. Fig 4.3.3 indicates that the percentage removal of Cr(VI) ions from the solution is higher at the beginning due to greater availability of surface area and functional groups. With an increase in time, it was found that there exists a control in metal uptake rate, since the functional groups present in the surface of the sorbent gets exhausted. It was found that maximum removal was obtained within 30min and equilibrium was attained within 60min. The results obtained were in agreement with Talokar et al., (2011) where maximum removal was described at a contact time of 30min. Thus equilibrium time of 60min was selected for further studies.

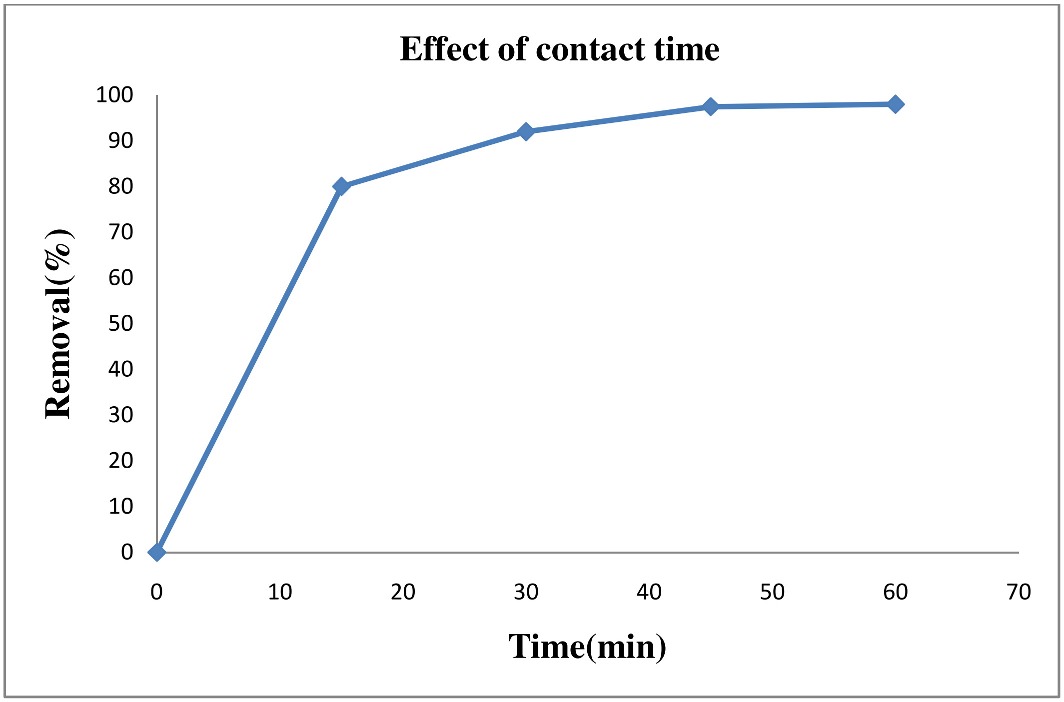


Fig 4.3.3 Effect of contact time with 100mg/L metal concentration, 0.5g/100ml adsorbent dosage, and speed of 120 rpm at pH 2

4.3.4 Effect of Initial Metal Concentration

The experiments were performed by varying the initial metal concentration from 20 to 100 mg/L, while the contact time was varied from 15 to 60min at a constant adsorbent dosage of 0.5g/100ml at pH 2 and an agitation speed of 120 rpm. Cr(VI) removal as a function of contact time and initial metal concentration is given in the fig 4.3.4. It is clear that with an increase in metal concentration, removal percentage decreases with an increase in adsorption capacity. With an increase in metal concentration from 20 to 100 mg/L, percentage removal decreases from 97 to 88. The maximum removal was obtained within 15 minutes, after which there was negligible removal since the surface of the sorbent gets exhausted after the formation of one layer thickness of metal ions and then metal uptake rate is controlled due to the transport of ions from exterior to interior site of the adsorbent. Similar trend was observed with ternary biopolymeric microspheres (Bajpai et al., 2009). The datas generated due to effect of initial metal concentration helps in determining the equilibrium concentration (C_e), adsorption capacity (q_e), metal uptake rate and kinetic characteristics.

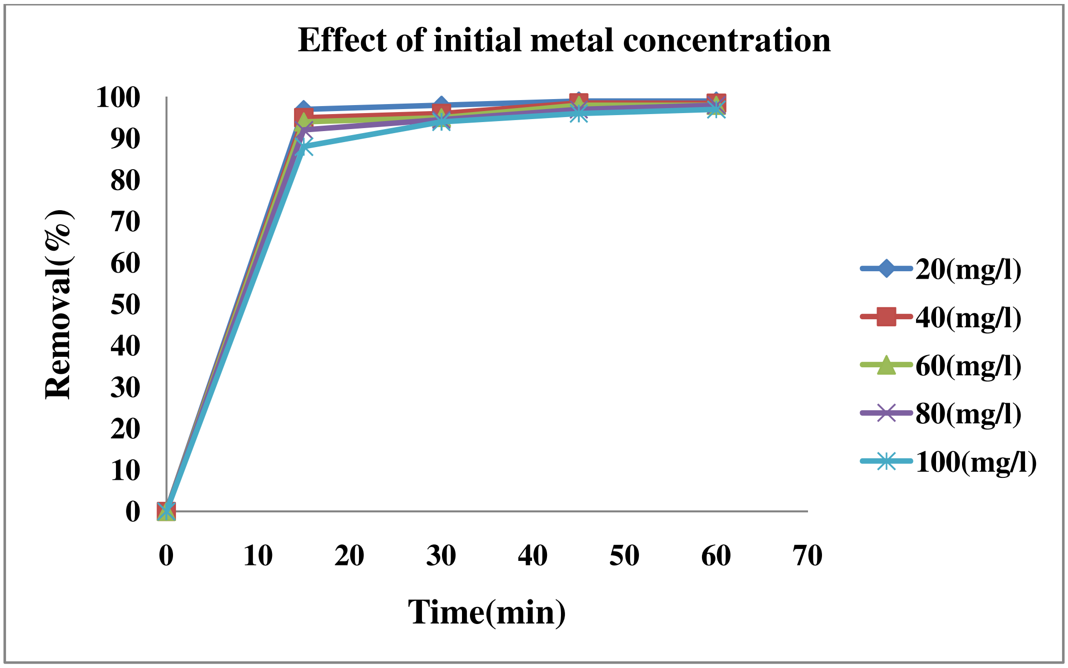


Fig 4.3.4 Effect of initial metal concentration with adsorbent dosage of 0.5g/100ml and speed of 120rpm at pH 2

4.4 EQUILIBRIUM ADSORPTION ISOTHERM STUDIES

Isotherms have been used to describe the equilibrium relationship between the amount of adsorbate present in the solution and the amount of adsorbate adsorbed in the adsorbent at a given temperature. The best equilibrium model was determined based on correlation coefficient. Under the optimal conditions, the metal adsorption equilibrium was very well represented by Langmuir isotherm which accounts for existence of monolayer adsorption on homogeneous surface. Langmuir constants q_0 and k evaluated from the slope and intercept are 19.23(mg/g) and 0.0061(L/mg) respectively with the correlation coefficient of 0.992. The obtained q_0 value is comparable with the adsorption capacity of sawdust (Bhattacharya et al., 2006). R^2 value indicates the strong binding of Cr(VI) ions to the adsorbent. Gooseberry seed powder was found to have better adsorption capacity which indicates the favorability of Langmuir isotherm.

4.4.1 Langmuir Isotherm Model

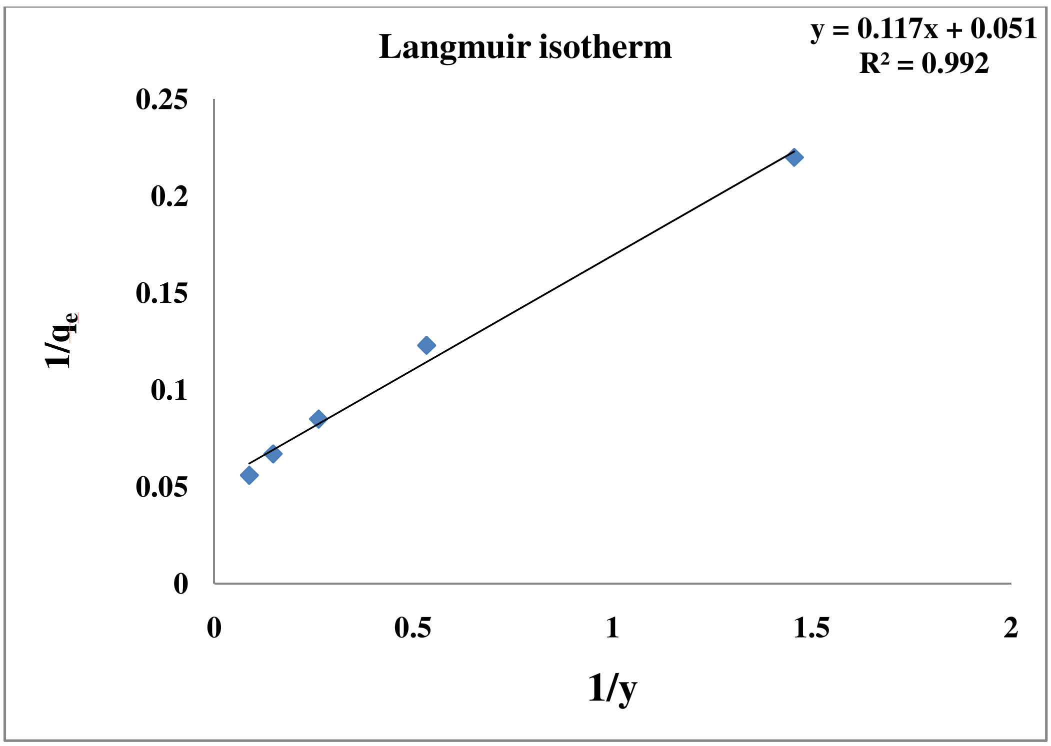


Fig 4.4.1 Langmuir Isotherm Model for Cr(VI) adsorption on Gooseberry seed powder

4.4.2 Freundlich Isotherm Model

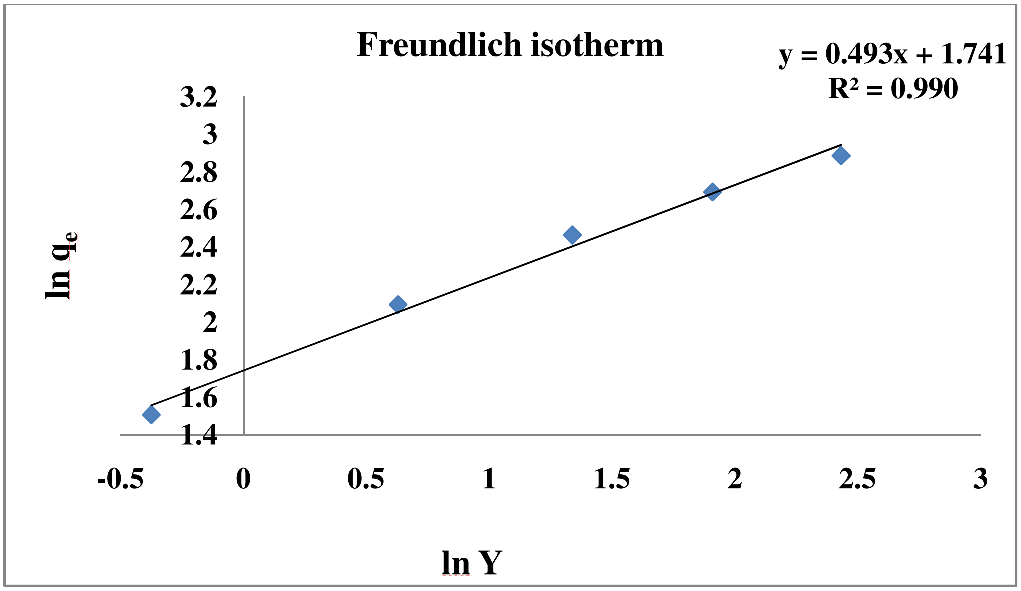


Fig 4.4.2 Freundlich Isotherm Model for Cr(VI) adsorption on Gooseberry seed powder

4.4.3 Harkins-Jura (H-J) Isotherm Model

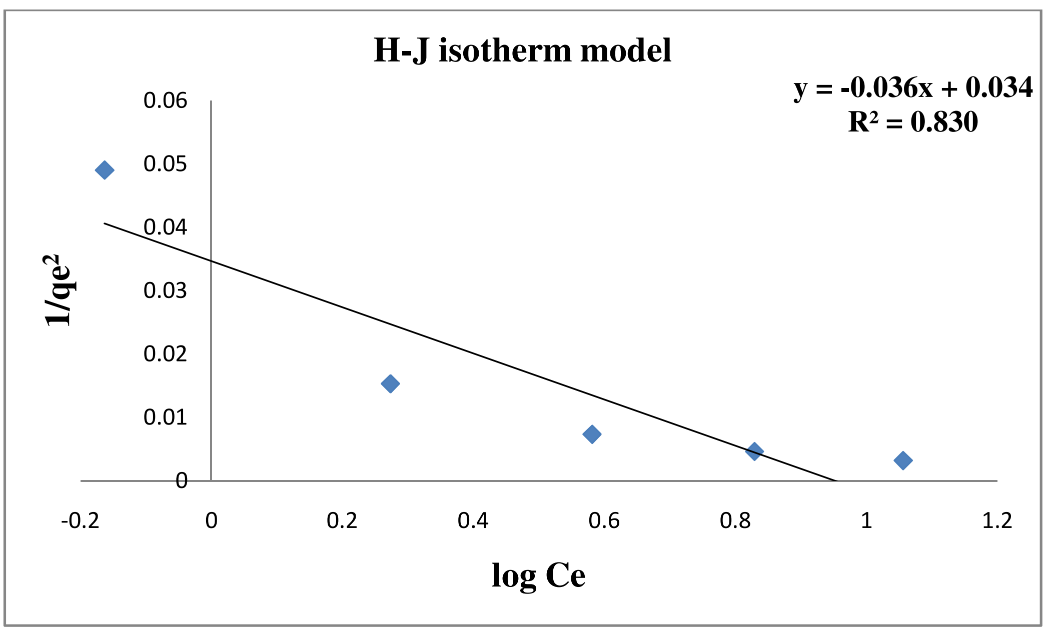


Fig 4.4.3 H-J Isotherm Model for Cr(VI) adsorption on Gooseberry seed powder

4.4.4 Temkin Isotherm Model

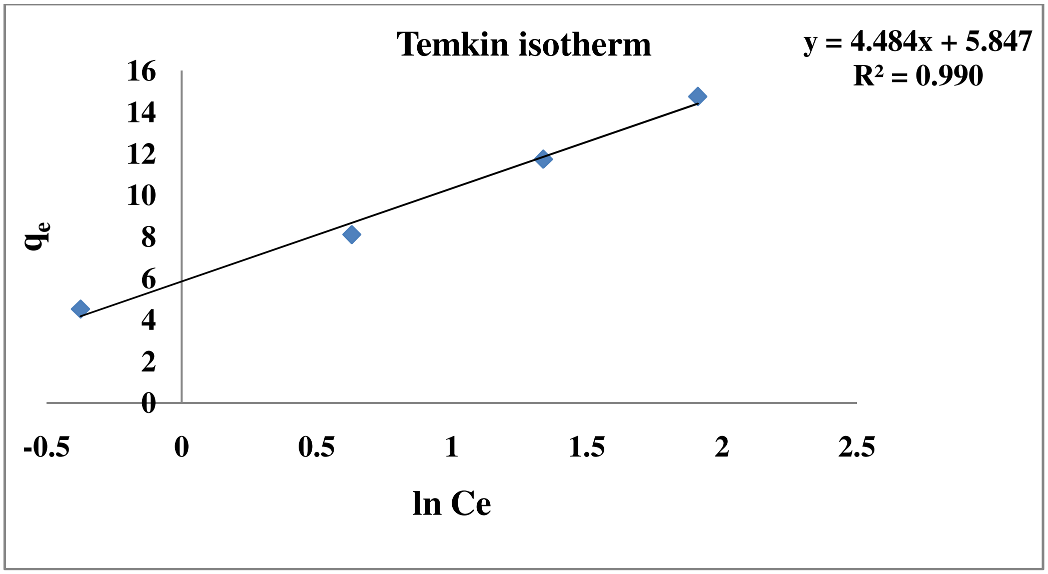


Fig 4.4.4 Temkin Isotherm Model for Cr(VI) adsorption on Gooseberry seed powder

4.4.5 Frumkin Isotherm Model

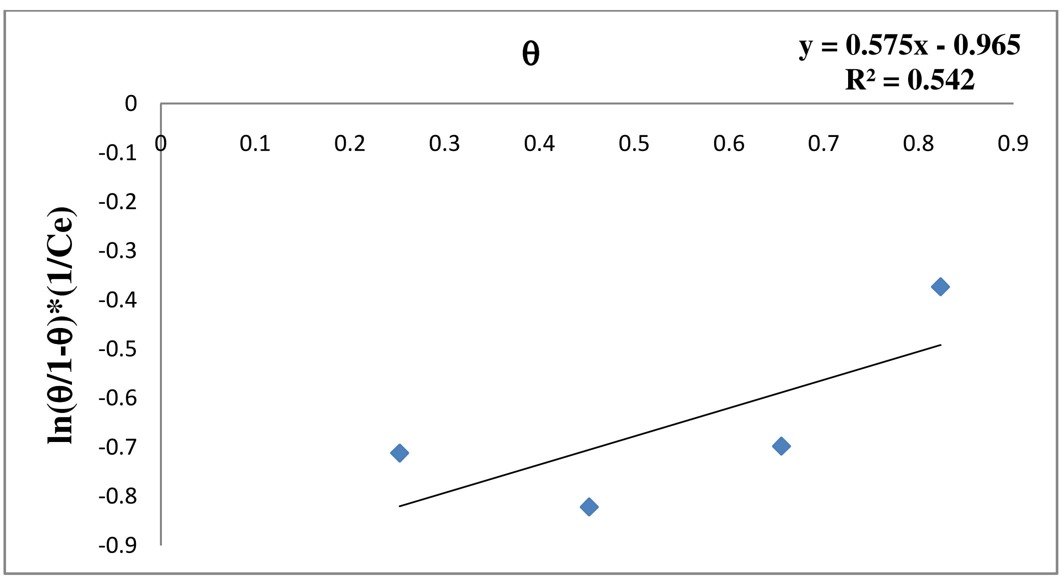


Fig 4.4.5 Frumkin Isotherm Model for Cr(VI) adsorption on Gooseberry seed powder

4.5 EQUILIBRIUM ADSORPTION KINETICS STUDIES

Studies on adsorption kinetics were carried out in order to determine the metal uptake rate. The effect of initial metal concentration was investigated to determine the best kinetic model. Pseudo first order kinetics model were applied by plotting log (q_e-q_t) versus time. The obtained R^2 value was found to be 0.994 and the results obtained were found to be in agreement with the results obtained from the biosorption of Cr(VI) using the husk of Bengal gram (Ahalya et al., 2005).

Pseudo second order kinetics model were applied by plotting t/q_t versus time. From the graph, it is clear that this model gave R^2 value (0.999). This implies that adsorption mechanism obeys pseudo second order. The results obtained were comparable when granular activated carbon was used as an adsorbent (Gholipour et al., 2011).

4.5.1 Pseudo-First Order Kinetics

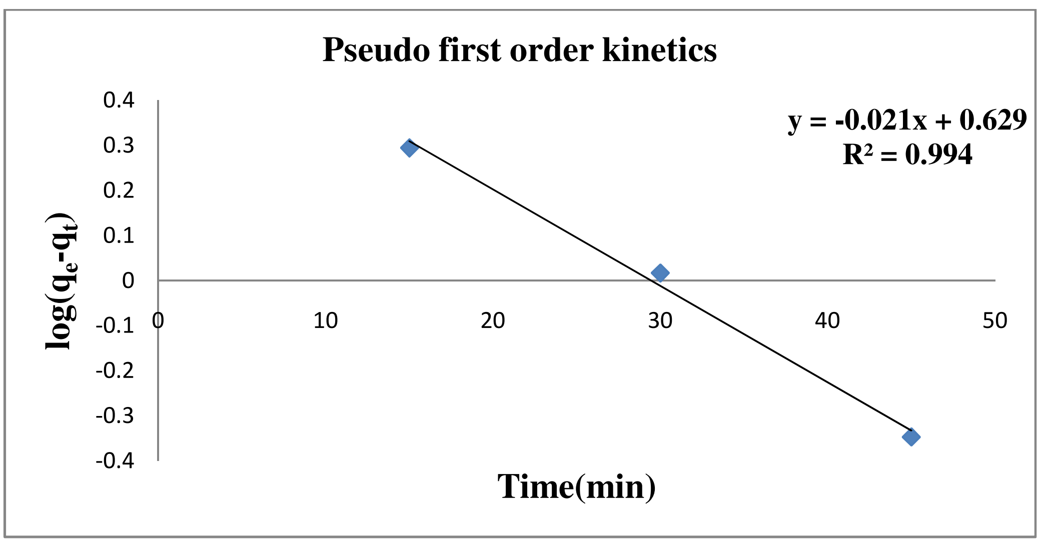


Fig 4.5.1 Pseudo-first order kinetics for adsorption of Cr(VI) on Gooseberry seed powder

4.5.2 Pseudo-Second order Kinetics

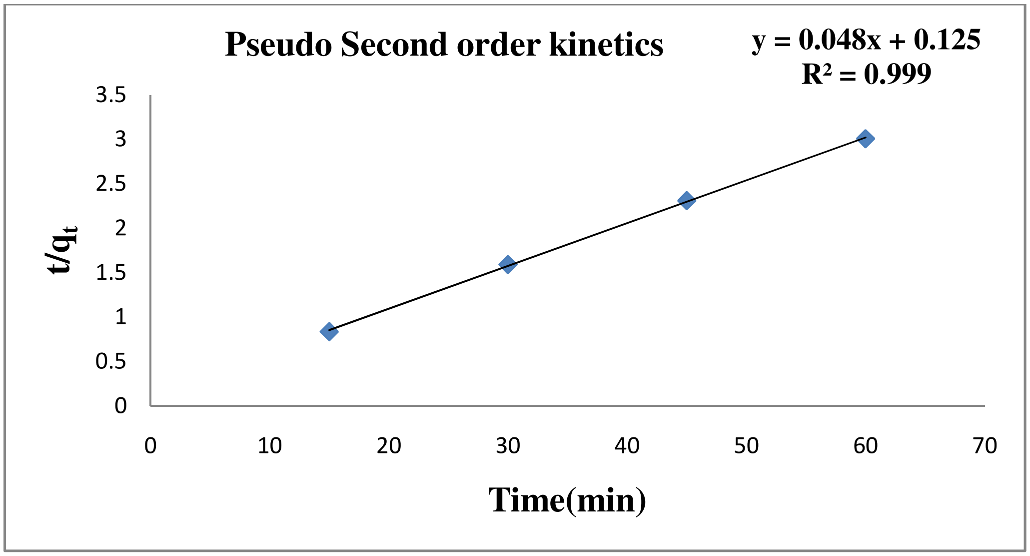


Fig 4.5.2 Pseudo second order kinetics for Cr(VI) adsorption on Gooseberry seed powder

Table-4.4.1 Constants evaluated from various isotherms

ISOTHERMS	PARAMETERS		R^2
Freundlich	K value	5.47	0.990
	n value	2.482	
Langmuir	K value	0.0061	0.992
	q_o value	19.23	
Temkin	A_T Value	5.9	0.990
	B_T Value	5.118	
Frumkin	A Value	0.2875	0.542
	K Value	0.380	
H-J	A Value	27.78	0.830
	B Value	0.944	

Table 4.5.1 Constants evaluated from kinetics

KINETICS	**PARAMETERS**		**R^2**
Pseudo first order	K_1 Value	0.0483	0.994
	q_e Value	18.57	
Pseudo second order	K_2 Value	0.0184	0.999
	q_e Value	20.83	

4.6 Optimisation by Response Surface Methodology

4.6.1 Experimental Design Matrix

Optimisation by classical method is time consuming and expensive. Besides this number of trials required will be large, making the factorial design very complex. In order to overcome such difficulties experimental Box Behnken design can be employed for the optimization of various parameters for the biosorption of Cr(VI) (Ravikumar et al., 2005).

Experiments were performed according to the design created using design expert software to identify the optimum combination of parameters influencing the biosorption of Cr(VI). From the response, it had been concluded that maximum removal of 91.26% was attained with a pH of 2, initial metal concentration of 60mg/L at 27°C.

Table 4.6.1 Experimental Design Matrix with response

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A:pH	B:Temperature	C:Initial metal concentration	Removal percent
			°C	mg/L	%
5	1	2	42	20	89.89
6	2	6	42	20	16.66
9	3	4	27	20	30.71
16	4	4	42	60	23.08
8	5	6	42	100	9.09
4	6	6	57	60	7.69
15	7	4	42	60	25
1	8	2	27	60	91.26
2	9	6	27	60	14.28
12	10	4	57	100	20
17	11	4	42	60	22.22
7	12	2	42	100	86.27
14	13	4	42	60	25
10	14	4	57	20	26.47
11	15	4	27	100	27.27
13	16	4	42	60	23.07
3	17	2	57	60	81.18

4.6.2 Selection of Adequate Model for Cr(VI) removal

A system or process with several variables is likely to be influenced by several external as well as internal parameters and low order interactions. In the present work, only two way interactions were investigated. Investigations on linear, cubic, two factor interaction and quadratic model were done to select the statistically significant model for determining the relationship between the response and the input variables. From the sequential model sum of squares, it can be seen that p value is lower than 0.001 and larger F value (618.98) for quadratic model. Lack of fit tests for quadratic model is found to be not significant with p value of 0.58. Comparable results were obtained by Das et al., (2013), where F value was found to be 0.98. From the model summary statistics, it can be predicted the quadratic model had maximum predicted and adjusted R^2 value. From the above results, it had been concluded that quadratic model provide an excellent explanation for the relationship between response and independent variables.

Table 4.6.2 Selection of Adequate Model for Cr(VI) removal

Sequential Model Sum of Squares	Sum of		Mean	F	p-value
Source	Squares	df	Square	Value	Prob > F
Mean vs Total	22549.08	1	22549.08	-	-
Linear vs Mean	11471.01	3	3823.67	18.83	< 0.0001
2FI vs Linear	9.24	3	3.08	0.012	0.9981
Quadratic vs 2FI	2621.39	3	873.80	618.98	< 0.0001
Cubic vs Quadratic	3.53	3	1.18	0.74	0.5801
Residual	6.35	4	1.59	-	-
Total	36660.60	17	2156.51	-	-

Lack of Fit Tests	Sum of		Mean	F	p-value
Source	Squares	df	Square	Value	Prob > F
Linear	2634.16	9	292.68	184.42	< 0.0001
2FI	2624.92	6	437.49	275.66	< 0.0001
Quadratic	3.53	3	1.18	0.74	0.5801
Cubic	0.000	0	-	-	-
Pure Error	6.35	4	1.59	-	-

Model Summary Statistics	Std.		Adjusted	Predicted	
Source	Dev.	R-Squared	R-Squared	R-Squared	PRESS
Linear	14.25	0.8129	0.7697	0.6643	4737.80
2FI	16.22	0.8135	0.7017	0.2746	10237.01
Quadratic	1.19	0.9993	0.9984	0.9953	66.45
Cubic	1.26	0.9996	0.9982	-	+

4.6.3 Regression Analysis

Regression analysis was performed to fit the response. Regression model developed represents responses as a function of pH (A), Temperature (B) and Initial metal concentration (C). Relationship between response and input variables is represented by the following equation in terms of coded factor.

$$Y = 23.674 - 37.61A - 3.5225B - 26375C + 0.8725A - 0.9875 - 0.775BC + 24.6467A^2 + 0.28175B^2 + 2.15675C^2 \quad (4.1)$$

The equation reveals that the effect of individual variables or combination of several variables over the response (removal percent). Negative coefficient values indicate that individual or double interaction factor negatively affects Cr(VI) biosorption, whereas positive coefficient values indicates that the interaction factors had a positive effect on biosorption process. From the equation, it is clear that all individual factors (pH, temperature and initial metal concentration) had a negative impact on Cr(VI) removal. Similar results were obtained with *Borasus flabellifer* coir powder, where pH and initial metal concentration was found to have negative effect on response (Krishna et al., 2013).

4.6.4 ANOVA for Response Surface Quadratic Model

Analysis of Variance table suggests whether the equation is adequate to describe the relationship between response and other independent variable. The model can be considered as statistically significant, if the value of p is lower than 0.05 with a larger F value (Hamsaveni et al., 2001). From the ANOVA table, it is observed that the quadratic model fitted well with the datas generated and can be considered as statistically significant, since the F value is very large (1109.92) and p value is lesser than 0.0001. In this case A, B, C,A^2,C^2 were considered to be the significant model terms, whereas other terms are not listed as significant factors since their p values were greater than 0.1. The Coefficient of Variance (CV) is the ratio of standard error of estimate to the mean value and considered reproducible once it is not greater than 10%. In our study, CV obtained was 3.26%. Adequate precision value measures signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 90.276 indicated an adequate signal.

Final equation in terms of actual factor is represented as follows:

$$\begin{aligned} Y = {} & 217.10386 - 68.57937\text{pH} - 0.380\text{temperature} - \\ & 0.12529\text{initial metal concentration} + 0.029083\text{pH} * \\ & \text{temperature} - 0.012344\text{pH} * \\ & \text{initial metal concentration} - 1.26250\text{E} - \\ & 003\text{temperatue} * \text{initial metal concentration} + \\ & 6.169\text{pH}^2 + 1.25222\text{E} - 003\text{Temperature}^2 + \\ & 1.34797\text{E} - 003\text{initial metal concentration} \end{aligned} \tag{4.2}$$

Table 4.6.4 Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	14101.64	9	1566.85	1109.92*	< 0.0001
A-Ph	*11316.10*	*1*	*11316.10*	*8016.04*	*< 0.0001*
B-Temperature	*99.26*	*1*	*99.26*	*70.32*	*< 0.0001*
C-Initial metal concentration	*55.65*	*1*	*55.65*	*39.42*	*0.0004*
AB	*3.05*	*1*	*3.05*	*2.16*	*0.1854*
AC	*3.90*	*1*	*3.90*	*2.76*	*0.1404*
BC	*2.30*	*1*	*2.30*	*1.63*	*0.2430*
A^2	*2557.74*	*1*	*2557.74*	*1811.84*	*< 0.0001*
B^2	*0.33*	*1*	*0.33*	*0.24*	*0.6414*
C^2	*19.59*	*1*	*19.59*	*13.87*	*0.0074*
Residual	9.88	7	1.41	-	-
Lack of Fit	*3.53*	*3*	*1.18*	*0.74*	*0.5801#*
Pure Error	*6.35*	*4*	*1.59*	-	-
Cor Total	14111.52	16	-	-	-

*** Significant, # Non significant**

4.6.5 Diagnostic Plot

The generated datas were analysed to determine the correlation between the actual and predicted values as shown in the fig 4.6.5. The experimental values were the obtained results, whereas the predicted values were obtained by calculation from quadratic model equation. From the plot, it is observed that the data points are distributed near the straight line indicating that the quadratic model was statistically significant model in predicting the response variables for the experimental data.

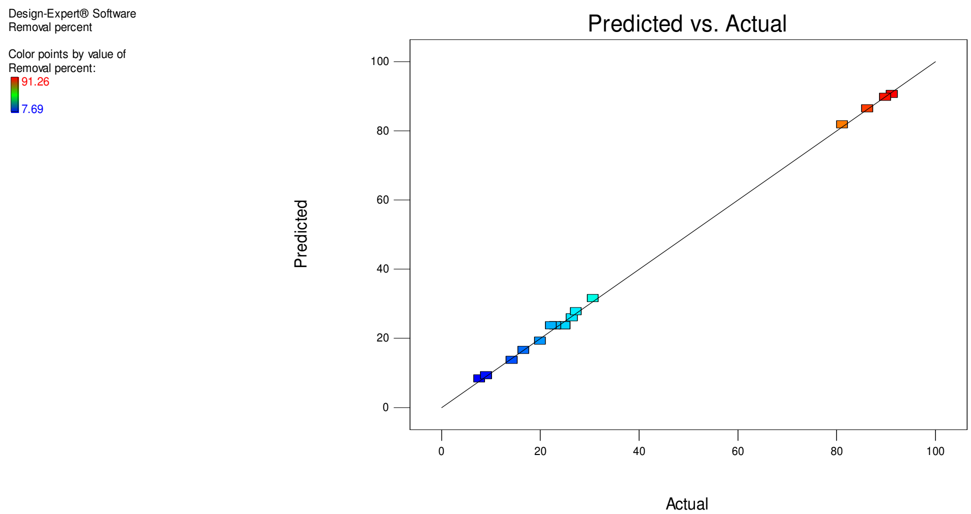


Fig 4.6.5 Plot of actual versus predicted value

4.6.6 Contour Plot

Each contour plot gives the graphical representation of influence of interactive effects of individual parameter over the response. The contour plot may be rising ridges, saddle point, elliptical or circular plot. Circular or elliptical plot indicates that there exists a significant interaction between the operating parameters, whereas saddle point indicates fewer interactions (Kanmani et al., 2013). The maxima formed by the x and y coordinates or the saddle points or the rising ridges in the response contour plots determine the optimum values of the parameter. From the fig.4.6.6.1-4.6.6.3, it is observed that removal percent increases from 7.69 to 91.26 with the decreasing values of pH, temperature and initial metal concentration. The best optimal parameters determined from the studies of contour plot for the biosorption of Cr(VI) were at pH 2, with an in initial metal concentration of 60mg/L at 27°C.

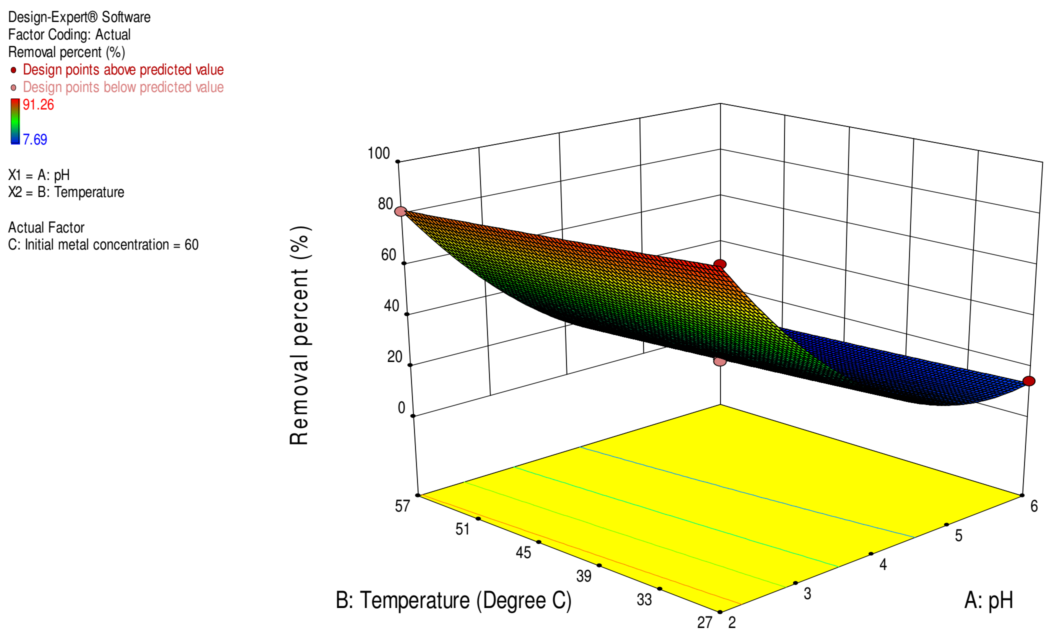


Fig 4.6.6.1 Influence of pH and Temperature on removal percent

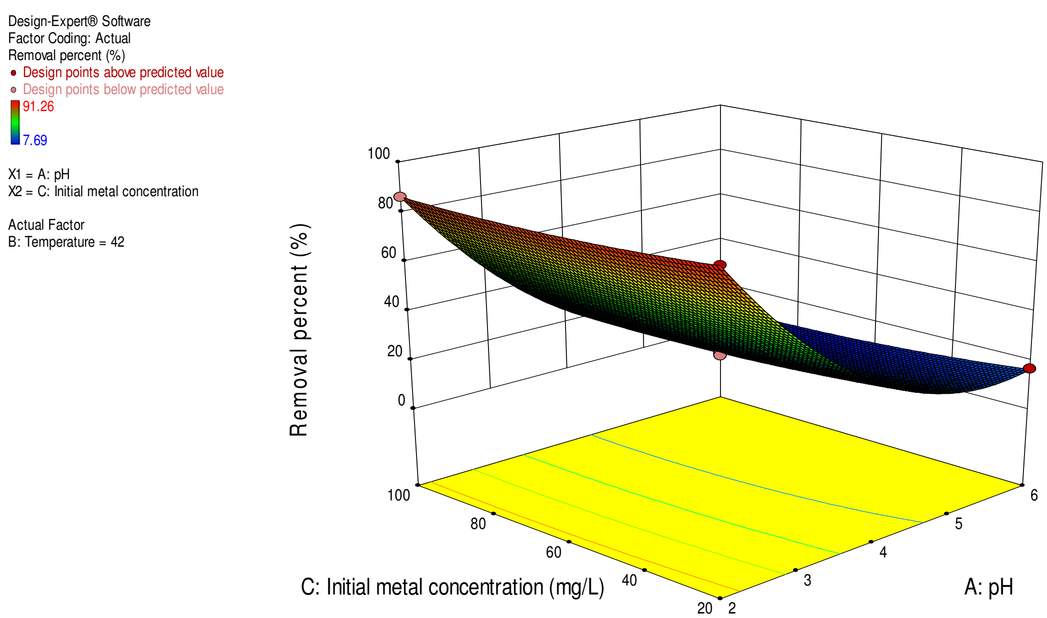


Fig 4.6.6.2 Influence of pH and initial metal concentration on removal percent

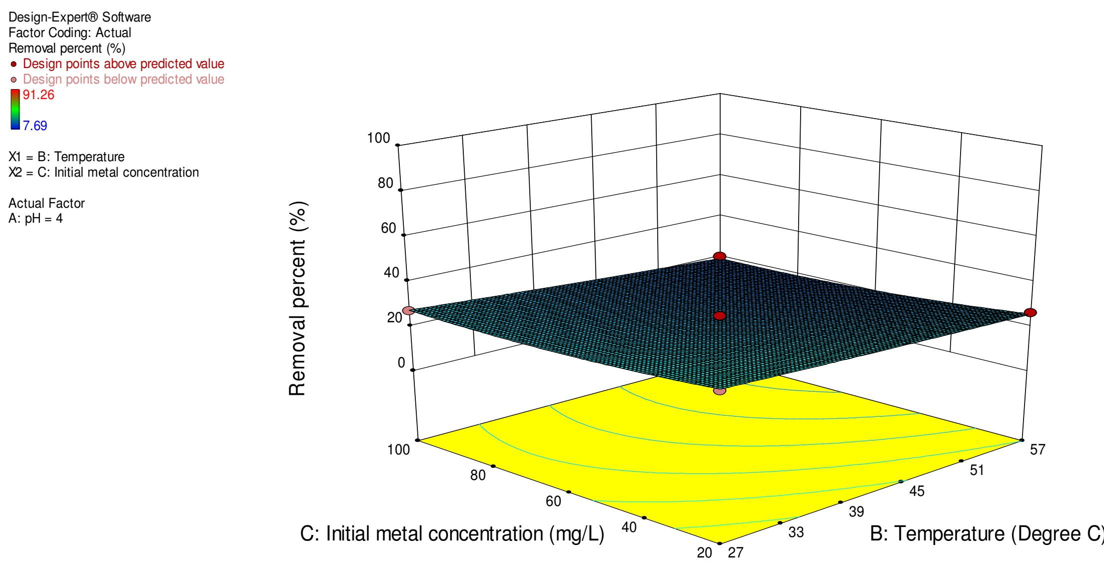


Fig 4.6.6.3 Influence of temperature and initial metal concentration on removal percent

4.6.7 VALIDATION

The results obtained from RSM based experimental trials were validated by carrying out an independent run at a maximum pH of 2 with an initial metal concentration of 60 mg/L at 27°C. A maximum removal of 91.26 percent was attained which validated the design.

CHAPTER 5
CONCLUSION

Following were the conclusions drawn from the investigations on Cr(VI) removal from simulated waste water using gooseberry seed powder as a biosorbent. The adsorbent prepared from gooseberry seed appears to be a promising adsorbent for the removal of Cr(VI) ion from aqueous solution. Maximum removal was obtained at an acidic pH of 2 and equilibrium was attained within 30min. From the study, it was found that removal percentage increases with an increase in adsorbent dosage from 0.1 to 0.5g and maximum removal was attained with an adsorbent dosage of 0.5g. Langmuir isotherm was found to be the best fit for the datas generated with an adsorption capacity of 19.23mg/g and the adsorption mechanism followed Pseudo second order kinetics. The influence of process parameters in adsorptive removal of Cr(VI) was studied using Response Surface Modeling. From the ANOVA table and R^2 value, it had been concluded that quadratic model is the signicant model for describing the relationship between response and input variables. To conclude, the adsorbent prepared from gooseberry seed could be employed as a locally available alternative for the effective removal of Cr(VI) ions from industrial effluents.

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