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Biosorption of Cobalt by Using Pseudomonas Aerguinosa Bacterial Strain

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Biosorption of Cobalt by Using *Pseudomonas Aerguinosa* Bacterial Strain

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
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DEDICATION

I’m dedicating this thesis to my parents, Arvind Dharanguttikar and Surekha Dharanguttikar whose countless support made me walk this far.

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ABSTRACT

A study of biosorption of cobalt metal by *Pseudomonas Aerguinosa* gram-negative bacterial strain is presented. The present study is carried out to determine the optimum conditions of cobalt biosorption at ultra-low concentration (ppb range) in aqueous solutions. The receptiveness of cobalt metal on the extracellular surface of bacterial strain was examined by varying the pH, Initial concentration of metal and treatment time. Experimental data showed that effect of pH and treatment time is prevalent in biosorption of cobalt and by increasing both these parameters resulted in the efficient sorption of cobalt on the extracellular surface of *Pseudomonas Aerguinosa*. In some cases, higher initial concentration of cobalt resulted in higher metal removal. However, there is no clear relationship is obtained between efficiency of biosorption and initial concentration of cobalt.

CHAPTER 1: INTRODUCTION

Biosorption emerged as one of the propitious technology for sequestering the toxic metals which are originated from industrial waste water stream as well as natural water. It has been providing valuable insight on building cheap substitute for conventional technologies such as Ion exchange, chemical precipitation and electrochemical treatment. Many micro-organisms are capable to accumulate heavy metals ions from aqueous solutions and it is governed by various physio-chemical mechanisms such as adsorption, ion exchange, complexation and microprecipitation [1]. There are numerous advances has been done in bio remediation technology and more than 13000 scientific papers has been published since last 60 years, yet this process is not commercialized on industrial scale due to the issues related to mechanical resistance and stability of biomass However, with the advent of highly efficient bio-absorbents having high metal capacity, it could be said that it has potential to create market for economic and competitive metal removal technology [9].

Heavy metal exhibits high density as compared to water and it has been deduced that there is a correlation between toxicity and heaviness. It is reported that environmental contamination is largely triggered due to the anthropogenic activities such as mining and smelting operations, Power plants, Textile and Microelectronics industry Usage of metals and metallic compounds in agricultural and domestic purposes. The metal elements having insoluble sulphides and hydroxide and create coloured complexes are considered as heavy metals [2]**.** There has been increase in the amount of toxicity and contamination of various water streams due to non-biodegradable and highly soluble nature of heavy metals in aqueous environment [6]. Most commonly known toxic

metals are comprised of Arsenic(As), Nickle(Ni), Lead(Pb), Cadmium (Cd), Chromium (Cr), Cobalt(Co) and Mercury(Hg) and EPA has set a permissible limit on the presence of these metals to avoid potential health risks to humans [9].

Table 1. EPA permissible limit and health hazards of heavy metals

Heavy Metals	**EPA Permissible Limit (ug/L)**	**Health hazards**
Cobalt	50	Pulmonary hypersensitivity, airway obstruction
Arsenic	50	Corrosive to skin, dermatitis, anorexia, kidney damage.
Mercury	2	Corrosive to skin and eyes, dermatitis, anorexia, kidney
Cadmium	5	Carcinogenic, lung fibrosis, weight loss

Table 1. Continued

Chromium	100	carcinogenic, lung tumors,
Copper	1300	Long term exposure causes irritation of nose, mouth, eyes, headache

Recent developments in wastewater treatments shows that microorganism such as *Microcystis Aeruginosa, E. Coli, Arthrobacter sp.*and *Pseudomonas Aerguinosa* has been successfully used in removing these toxic metals and giving alternative to conventional water treatment technologies as they are creating secondary problems. The conventional technologies used in water treatment are mentioned in the following table[26].

Table 2. Existing metal removal technologies

Method	**Materials Used**	**Disadvantages**	**Advantages**
Chemical Precipitation	$Ca(OH)_2$, NaOH, H2S, rimercaptotriazine, potassium/sodiumthiocarbonate,	1) Disposal of resulting toxic sludge 2) Narrow pH range (8-11)	Simple Process, Relatively Cheap

Table 2. Continued

Ion Exchange	Synthetic Resins, Zeolites, Silicate minerals	1)Sensitive to presence of particles 2)Expensive Resins 3)Zeolites only used on lab scale 4)Depends on Initial metal concentration	Effective and metal recovery is possible
Electrochemical Treatment	Aluminium or Iron electrodes	1)Applicable for high metal concentration	Require Fewer Chemicals
Adsorption	Activated carbons, CNTs	Expensive, Processing difficulties	Works in ppb range.

Table 2. Continued

Membrane filtration: Ultrafiltration, Reverse osmosis, Nanofiltration, Electrodialysis	MEUF, PEUF, Semi permeable Membrane	High power consumption due to pumping pressure, restoration of the membranes.	Suitable for large-scale Industrial practice.

The need of alternative wastewater treatment is emerged due to the strong environmental pressure and government has been continuously enforcing uncompromising regulations to control the metal discharges resulting from industrial operations. The existing technologies which are mentioned above are grappling with several problems such as expensive absorbents, disposing the metal bearing sludge and the toxic waste. The main advantage of using bio-absorbents is that they are available in copious amount in environment and can be used to sequester substantial number of heavy metals. In addition to this, they provide very high efficiency, less chemical or biological sludge, regeneration [26]. However, it is commercialized by only two organizations: 1) AlgaSorb™ where they used Algal biomass encapsulated in silica gel matrix for metal removal 2) AMT-Bioclaim where they used granulated biomass in fluid bed reactor system for the treatment of waste water.

CHAPTER 2: BIOSORPTION

2.1 Biosorption Definition

In biosorption process, the functional groups which are present on the extracellular surface of biomass get bonded with metal ions present in the aqueous solutions. It is a rapid, reversible and both cellular metabolism dependent and independent process [27]. There are mainly two phases are involved in the biosorption process: 1) Solid Phase (bio-absorbent) and 2) Liquid phase (Solvent/Water) in which metal ion (sorbate) is dissolved [10]. In general, metabolism-independent accumulation of metals is much faster than metabolism intracellular uptake of metals. In In the present study, mainly the adsorption phenomenon is addressed in which metal ions are physically bonded on the extracellular surface of Pseudomonas Aeruginosa bacterial strain. The efficiency of the biosorption process is governed by several factors such as pH, salinity, presence of nutrients in aqueous media, Redox potential[4].

2.2 Biosorption Mechanism

The process of binding sorbate onto bio-sorbent is a complex process and it occurred through various mechanism such as Ion Exchange (physical or chemical displacement of bound metal cation), Chelation (ionic or covalent interaction) and complexation. The rate and efficiency of biosorption process depends on the nature of metal ions and absorbents, for instance, the molecular weight, ionic radius and oxidation state of metal affects the biosorption while pH of surrounding media, temperature, concentration of absorbents could potentially change the uptake capacity of bio-absorbent [9].

For the present study, we used '*Pseudomonas Aeruginosa'* Gram-Negative bacterial strain and its extracellular surface is more important in term of its constituents in biosorption process. The cell wall of this strain is comprised of functional groups such as hydroxyl, carboxyl, amino, ester, sulfhydryl, carbonyls and majorly the metal cations are bonded with these functional groups present on the cell wall. The biosorption mechanism by bacterial strains is categorized into three categories based on the location where the metal is absorbed on the bacterial surface which are as follows: 1) Extracellular accumulation 2) Cell Surface sorption and 3) Intracellular accumulation[25]. Cell surface sorption majorly contribute to the bio-sorption process and it includes complexation , ion-exchange , physical adsorption and precipitation . On other hand, transport across cell membrane is carried out through intracellular accumulation and there is no scientific study made on this mechanism[27].

2.2.1 Transport Across the Cell Membrane

Transport of heavy metals through cell membrane of bacteria is metabolism dependents and its scientific investigation is restricted due to the toxicity of heavy metals in the presence of metal concentration, and Hence there are few studies are available in literature which gives strong insight on this mechanism[3]. The transport of heavy metals across the cell membrane is occurred by the mechanism through which metabolically essential ions such as sodium, potassium and conveyed to the cell membrane and it can create dubiety in heavy metal transport system if these metals have same ionic charge and radius as the nutrient ions.

2.2.2 Complexation

The outer wall of *Pseudomonas Aeruginosa* consists of Phospholipids, lipoproteins and lipo-polysaccharides (LPS). These biopolymers contain carboxylic acids and phosphate esters which are predominantly contribute to the cells having negative charge The LPS of PA goes up to 40 nm from the cell wall and linked to oligosaccharide section containing O-Antigen side chains directing in outward directions. These two antigens are further classified into: 1) A Band O-Antigen and 2) B-Band O- Antigen [16].

The A-band O-Antigen is comprised of 20 trisaccharide units of D-rhamnose, on the other hand, B-Band O-Antigen consists of 30-50 trisaccharide units with an amino derivative of mannuronic acid. Both these bands are providing numerous negatively charged ligands when surrounding media is maintained at specific pH. Studies shows that, Complex formation takes place between these active groups and metal ions.

The role of amine groups contributing to biosorption process is given by following reaction scheme:

$$R\text{-}NH_2 + H^+ \longleftrightarrow R\text{-}NH_3^+ \quad (1)$$

$$R\text{-}NH_2 + Co(II)X \longrightarrow R\text{-}NH_2Co(II)X \quad (2)$$

$$R\text{-}NH_3 + Co(II)X \longrightarrow R\text{-}NH_3Co(II)X + H^+ \quad (3)$$

The role of carboxyl groups contributing to biosorption process is given by following reaction scheme:

$$R''\text{-}COOH + OH^- \longleftrightarrow R''\text{-}COO^- + H_2O \quad (1)$$

$$R''\text{-}COO^- + Co(II)X \longleftrightarrow R''\text{-}COO^-\,Co(II)X \quad (2)$$

The role of Hydroxyl groups contributing to biosorption process is given by following reaction scheme:

$$R' - OH + OH^- \longleftrightarrow R'-O^- + H_2O \quad (1)$$

$$R'-O^- + Co(II)X \longleftrightarrow R'-O^-Co(II)X \quad (2)$$

It can be deduced from the above reaction mechanisms that the Nitrogen atom from amine group and oxygen atom from hydroxyl and carboxyl group are bonded with cobalt metal ion, and the efficiency of electron loan pair donation of Nitrogen is more efficient than Oxygen due lower electronegativity of Nitrogen [28].

2.2.3 Ion Exchange

Ion exchange is one of the important mechanisms of biosorption and numerous kinetic studies are carried out to understand the ion exchange mechanism in terms of biosorption. The study carried out by et al with *Sargassum filipendula* biomass shows that metabolically essential ions such as Ca^+, Mg^{2+}, K^+ and Na^+ are involved in cation exchange of heavy metals such as Cd^{2+}, Cr^{3+}, Ni^{2+} and Zn^{2+}. Calcium ion are less likely take part in ion exchange with heavy metals as compared to other lighter ions, because they have higher valency and binding strength making its release from the cell wall restricted for ion exchange [13]. By considering the results of same study, it could be said that the following reaction mechanism is employed with respect to the cobalt uptake of *Pseudomonas Aeruginosa*:

$$2Na_{ads}^+ + 1(Co_{ads})^{2+} \longrightarrow 2Na_{sol}^+ + 1(Co_{ads})^{2+}$$

where, ads and sol refer to adsorbent and solution respectively. Due to the maximum release of sodium ions from the cell wall and similar ionic radius with cobalt, the displacement monovalent sodium is more by divalent cobalt ions making stoichiometry of the system 1:2. It should be taken into consideration that Ion exchange mechanism in terms of biosorption is primarily based on experimental observation and it doesn't solely explain the binding mechanism of heavy metal ions with biomass.

2.2.4 Physical Adsorption

Physical adsorption mainly governed by Van der wall forces, it has been studied that the radionuclides present in the marine aquatic environment are directly accumulated by the marine micro-organisms through physical adsorption[25]. The surface of bacterial cells is complex in terms of chemical composition and structure. Non-covalent interaction polysaccharide layer of bacterial cell wall plays significant role in physical adsorption of metals. The nature of water solvent and its high dielectric constant majorly contribute to the electrostatic attraction between poly-ion and its counter ion. In general, this non-covalent bond formation is not unconstrained of the entities that are present in the system and create an equilibrium with the dominating one depending upon thermodynamic conditions[30].

2.2.5 Precipitation

Bacterial cell wall creates protective layer of excreted polymer to oppose heavy metal poisoning and restrict the ion permeability into cell. This defence mechanism results into the formation of compounds which favours precipitation process. The study carried out on cadmium biosorption shows that, Cadmium is removed by Arthrobacter and Pseudomonas species in which cadmium is associated with moderate levels of sulphur through sulphide precipitation. Similarly, the discharge from mines known as acid mine drainage (AMD) containing heavy metals successfully treated with sulphate reducing bacteria *Desulfovibrio*[20] .

In this process, SRB utilize organic matter for the conversion of sulphate to hydrogen sulphide and these metals will react with the dissolved sulphide to form highly insoluble metals sulphides [20] and it is represented by following reaction scheme.

$$SO_4^{2-} + CH_3COOH + 2\ H^+ \rightarrow HS^- + 2\ HCO_3^- + 3\ H^+ \quad (1)$$

$$HS^- + Me^{2+} \rightarrow MeS + H^+ \quad (2)$$

On the other hand, in uranium biosorption by *Rhizopus arrhizus*, uranium-chitin complex is formed, and it undergoes hydrolysis. The resulting hydrolysis product is eventually precipitated in the cell wall [2]. However, there are really few studies which sheds light on this mechanism in terms of biosorption. It is evident from the literature mentioned that biosorption mechanisms are not only various, but they can also take place simultaneously.

CHAPTER 3: FACTORS AFFECTING BIOSORPTION

There are several factors that affects the rate and efficiency of biosorption process. Some of these factors are dependent on the nature of biomass and metal and others are governed by the environmental conditions of the media in which the biosorption is taking place. The prime factors which influence the biosorption process are as follows:

3.1 pH

pH of the surrounding media is one of the most important parameters of biosorption process. As it mentioned earlier, bio-adsorbent like *Pseudomonas Aeruginosa* consists of weakly acidic and basic groups, and the change in pH deeply affect the nature of binding sites and solubility of the metals as it influences the solution chemistry of metals. The decrease in pH increases the H^+ ions in the solution making cell surface positively charged as shown in the figure 1.

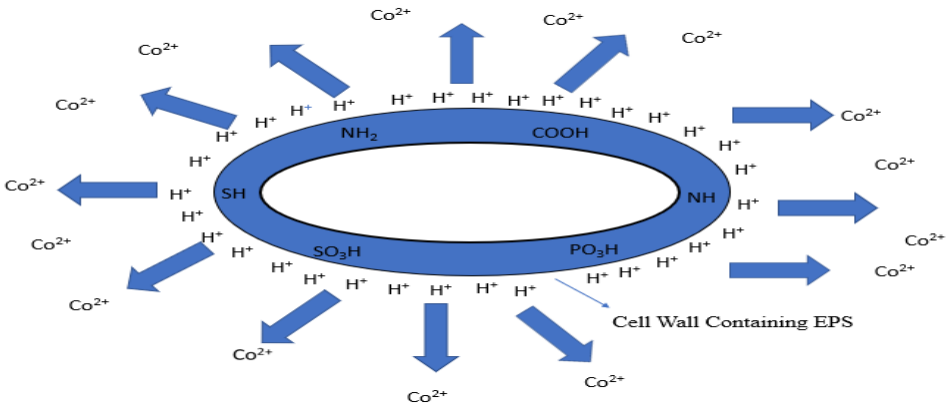


Figure 1. Effect of pH on *Pseudomonas Aerguinosa* biosorption

which restrict the attraction between metals and biomass[1]. Many studies reported that increase in pH favours the biosorption process. For Example, the study carried by Co(II) biosorption shows that uptake capacity at pH = 2 is found to be 4 mg/g while at pH = 7 same adsorbents gave the uptake capacity of 12 mg/g[31] . This increase in uptake capacity is mainly accounted by increase in net negative electrostatic surface charge as the pH is maintained at physiological pH value and Zeta potential measurement studies done by various authors this hypothesis. In the same study, it is observed that Zeta potential values decreased from – 5mV to -25 mV when pH is increased from 2 to 7. This shows the increase in the electronegativity of surface with respect to the pH. In case of Co(II) biosorption the optimum pH is considered between 4-7 by many researchers and it has been suggested that at higher pH (>8) Cobalt is removed by hydroxide precipitation.

3.2 Temperature

In general, Biosorption is an exothermic process and hence the adsorption of metals on biomass decreases with increase in temperature [9]. The change in temperature influence several factors such as: 1) stability of metal ions in the solution 2) cell wall configuration of micro-organism 3) ionization energy of metal-biomass complex. In addition to this, the rate of adsorbate diffusion process across external boundary layer and internal pores of adsorbate particles enhanced with increase in temperature. However, temperature also affects equilibrium capacities which dependent on exothermic and endothermic nature of the process [4]. It has been observed that, when temperature is increased from 0^o to 60^o for Cu(II) and Au(III) biosorption with *C. Pyrenoidosa*, the co-ordination complex formed between metal cations carboxylate ligands is endothermic in nature, while the formation amide ligand complex shown exothermic behaviour [12]. The biosorption of cadmium carried out with *Microcystis Aeruginosa* at 10^oC, 25^oC and 40^oC has shown metal removal of 69 %,99% and 90% respectively. Many biosorption studies carried out at

room temperature shows promising results than the studies carried out temperature greater than 45^{o}. hence the present study is also carried out at room temperature.

3.3 Biomass Concentration

Biomass concentration is one of the most important parameters of biosorption process. If the initial metal concentration is high and biomass concentration is low, then the uptake sites come to be more saturated which results in the inefficient metal sequestration. However, the use of highly concentrated biomass for the uptake of heavy metals having low initial concentration results in poor adsorption, and the reasons behind this are still unexplained [8]. On the other hand, when the *Pseudomonas sp.* concentration is increased from 1mg to 20 mg, the Fe metal uptake is increased from 0.07 ppm to 2.405 ppm. The effect of biomass on biosorption is still not well discovered to provide a strong hypothesis to carry out biosorption studies [7].

CHAPTER 4: BIOSORPTION ISOTHERM MODELS

Various adsorption isotherms are used to quantify the affinity of adsorbate for an adsorbent from biosorption process. Adsorption isotherm is a simple method to examine the efficiency of certain adsorbent for a particular application. The equilibrium relationship between adsorbate concentration in liquid phase and adsorbate concentration in adsorbent particles at given temperature is illustrated by these models.[11].

4.1 Freundlich Model

In 1907, Freundlich and Kuster published the first mathematical equation that fits to an adsorption isotherm and it is given by following empirical formula:

$$Q_e = K\, C_e^{\,1/n}$$

where, K (mg/g) $(1/mg)^{1/n}$ are Freundlich constants corresponding adsorption capacity and adsorption intensity respectively[11].

4.2 Langmuir Model

Langmuir isotherms are widely used to understand the kinetics of biosorption and it is valid for single layer adsorption. It assumes that the energy of adsorption is constant and there is no movement of adsorbate along the adsorbent surface. The Langmuir Isotherm formula is given by following equation:

$$Q_e = Q_m\, b\, C_e / 1 + b\, C_e$$

Langmuir model is based on several assumptions which are given below:

1) The surface of adsorbents is uniform.

2) All binding / adsorption sites are identical

3) The mechanism of all the adsorption is same.

4) Adsorbates are adsorbed at the definite sites on the surface of adsorbent.

Equilibrium parameter can be obtained from Langmuir isotherm equation and it is denoted by R_s.

$$R_s = 1 / 1 + b\ C_o$$

It is also called as separation factors and based on its values the nature of isotherm can be predicted[11].

Table 3. Values of separation factor and type of isotherm

Values of R_s	Nature of Isotherm
$R_s > 1$	Unfavourable
$R_s = 0$	Linear
$0 < R_s < 1$	Favourable
$R_s < 0$	Irreversible

4.3 Temkin Model

The adsorbate and adsorbent interaction is taken into consideration to describe this model. It assumes that the heat of adsorption of all the molecules in layer decrease linearly rather than logarithmic order. It is given by following equation:

$$Q_e = RT \ln (K_T C_e) / b$$

The linearized form of the above equation is given by:

$$Q_e = B_1 \ln K_T + B_1 \ln C_e$$

where, B_1 (KJ/Mol) = RT/b and it constant related to heat of adsorption

K_T (1/mg) = Equilibrium binding constant related to maximum biding energy[11].

4.4 BET Model

This model is developed by Stephan Brunaur, Paul Emmett and Edward Teller which consider the possibility of multilayer formation of adsorbate molecules on the surface of the adsorbent surface. It is an expansion of Langmuir model from monolayer to several molecular layers and it is based on following assumptions:

1) Above the monolayer, all the additional layers equilibrate with the layers below it.

2) Thickness of the layers can be variable and allowed to co-occur.

It is represented by following equation:

$$Q_e = B\ Q\ C_e / (Cs - C_e) [1 + (B - 1) (C_e / C_s)]$$

where, C_s = Saturation concentration of adsorbed component

B = Constant related to the binding energy

Q = Amount of solute forming complete monolayer.

Clearly, it can be said that the adsorption process is described effectively by using these models for bio-sorption phenomenon. However, there are various model are used quantify the adsorption process, the models which mentioned here are prevalent in biosorption studies[11].

CHAPTER 5: MATERIALS AND METHODS

5.1 Microorganism Used for Biosorption Experiments

Pseudomonas Aeruginosa (ATCC® 27853) antibiotic resistant bacterial stain is used for the present study and it is purchased from ATCC which provide wide range biological materials, micro-organisms and bioproducts for research and development purposes. It is a most commonly available rod-shaped bacteria having extensive metabolic diversity and ability to grow in variety of environments and nutrient sources.

5.2 Initialization of Bacterial Growth and Culture Conditions

10 sterile test tubes are prepared containing LB broth(Lennox) medium which is purchased from Sigma-Aldrich and it consists of Tryptone (10 g/L), Yeast Extract (5 g/L) and NaCl (5 g/L). The growth medium is prepared adding 2 g of LB powder to 100 ml of water. The test tubes are autoclaved before and after inpouring the broth to avoid contamination. Additionally, the autoclaved broth is spiked with tetracycline antibiotic to avoid the growth of other microorganisms. The antibiotic is prepared by dissolving 3.125 gm antibiotic powder to 95% ethanol solution. The freeze-dried culture is opened gently according to the instruction and the vial is kept in water bath at normal growth temperature. The vial is removed from the bath and decontaminated the outer surface of vial with 70% ethanol. Then, entire suspension is then transferred to sterile test tubes containing the growth medium (8 ml) and additional test-tubes are prepared by transferring 0.5 ml primary culture to obtain additional secondary cultures. The test-tubes culture is kept at 37^{o} C and monitored their growth in timely manner.

5.3 Analytical Measurement Methodology

Graphite furnace atomic absorption spectrometer is used to determine the concentration of metals in the aqueous solutions. It is a commonly used technique to determine the concentration of very low-level trace metals in variety of samples. Sample preparation for GF-AAS requires special care and the requirements vary according to sample matrix. Due high detection sensitivity of the instrument, standards are scrupulously cleaned and meticulously handled for analytical measurement. All laboratory apparatus beakers, watch glasses, pipettes and volumetric flasks are thoroughly washed three times with DI water and 10 % Liquinox solution to remove initial contamination. After primary cleaning, all the apparatus is filled with 20% v/v Nitric acid and kept it for two days. Furthermore, the Nitric acid is discarded, and all the apparatus rinsed with di water and air dried prior to use.

Calibrating the instrument is very important procedure when the concentration of analyte is very low and normal calibration scheme is employed for all the analytical measurements as described in the manual. Five standards are prepared with 10 ppb, 20 ppb, 30 ppb, 40 ppb and 50 ppb from 1000 ppm cobalt standard which is purchased from Sigma Aldrich. Standards needed to be acidified for calibration and hence all the standards are acidified 2 % v/v Nitric acid. DI water is used as a blank solution and rinse solution is prepared by adding 1 drop of Triton X-100 to 0.5 v/v Nitric acid solution.

For normal calibration, 20 µl of all the standards are dispensed after 5 ul of blank solution in the graphite furnace after filtering with 0.22-micron filter.

Following operating conditions are employed for the calibration as well as Analytical measurements:

Table 4. GF-AAS operating conditions

Step No.	**Temp. (º C)**	**Time (Sec)**	**Gas Flow (L/min)**	**Gas Type**	**Read Command**
1	85	5	3	Normal	No
2	95	40	3	Normal	No
3	120	10	3	Normal	No
4	650	5	3	Normal	No
5	650	1	3	Normal	No
6	650	2	0	Normal	No
7	2300	1.1	0	Normal	Yes
8	2300	2	0	Normal	Yes
9	2300	2	3	Normal	No

The instrument parameters which are used for analytical measurement are given below:

1) Lamp current: 11 mA

2) Spectral bandwidth: 0.2 nm

3) Wavelength of detection: 242.5 nm

4) Maximum Absorbance: 1.1

5) MSR%: 98

5.4 Preparation of Heavy Metal Solution

Cobalt chloride hexahydrate ($CoCl_2.6H_2O$) is used as heavy metal adsorbate throughout the study and it is purchased from Sigma-Aldrich. Ultrapure Deionized water (UDW: 18 MΩ/cm) is used to dissolve metal salt to prepare heavy metal solution. 100 ml of samples are prepared with 20 ppb, 40 ppb, 60 ppb, 80 ppb and 100 ppb analyte concentration. The same samples are then adjusted to different pH ranging from 6 to 10 and pH is adjusted 0.1M HNO_3.

5.5 Preparation of Bio-adsorbent and Biosorption Experiment

Bacterial cultures are incubated for several days at 37^oC and harvested by centrifugation at 6000 rpm for 8 min as shown in the fig.2. The cell density was observed to be 4.0916 gm /L.

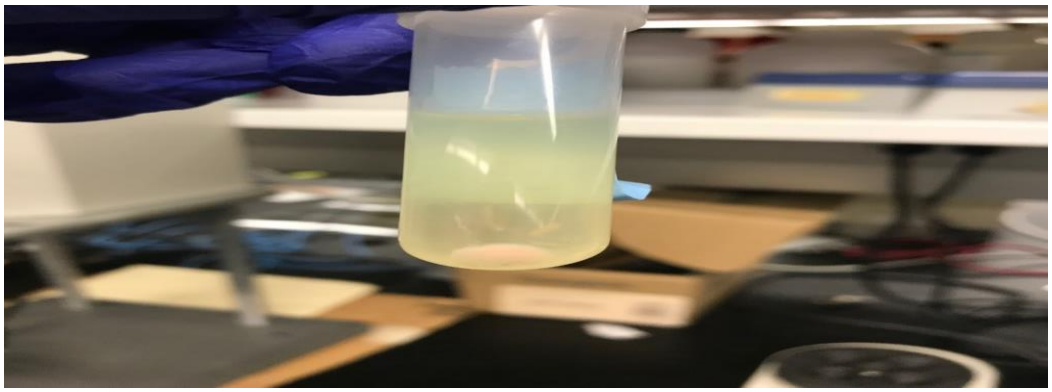


Figure 2. Harvested bacterial pellet

The obtained pellet is then rinsed twice with deionized water to remove the traces of broth. Then, the pellet is suspended in deionized water and dispersed by using vortex mixer. 2 ml of this bacterial suspension is added to metal solution and analytical measurement is taken at specified time intervals.

CHAPTER 6: RESULTS AND DISCUSSION

6.1 Effect of Treatment Time, pH And Initial Metal Concentration on Biosorption

In the present experimental design, the efficiency of this process will be calculated with respect to three variables: 1) pH 2) Initial Metal Concentration and 3) Treatment time.

6.1.1 Effect of pH on Biosorption

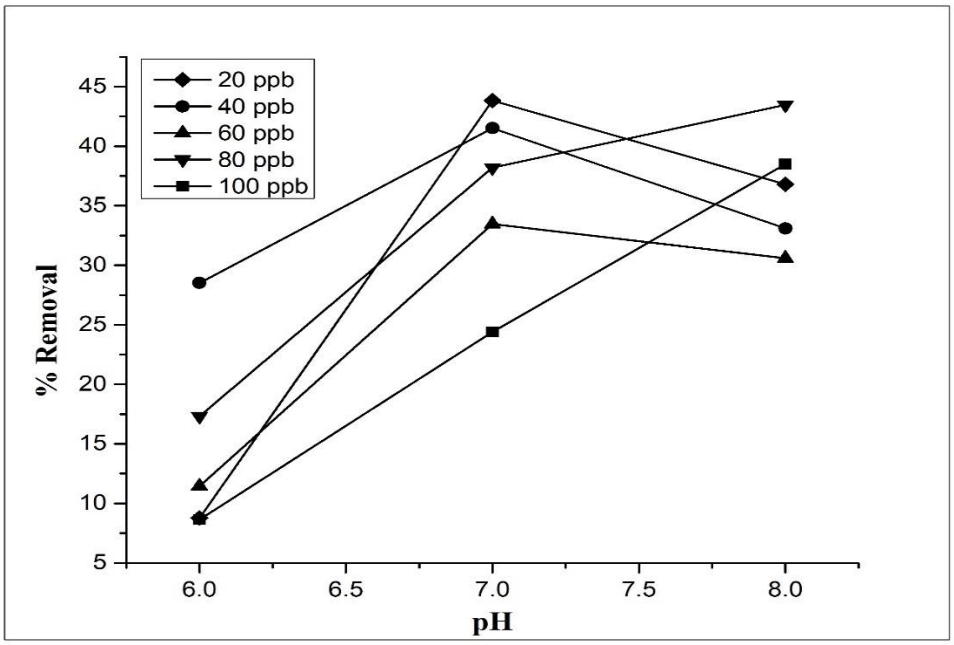


Figure 3. Effect of pH on biosorption

Water streams released from anthropogenic activities with heavy metal contaminants vary significantly in terms of pH. Hence, it is necessary to evaluate the biosorption process at different level of acidity and salinity. The lowest removal of Co^{2+} is observed at pH 6 as shown in the fig.3 Thus, it is clear cobalt adsorption by *Pseudomonas Aeruginosa* is highly affected by the change in pH of the solution. The biosorption efficiency is increased by a mean of 21.34 % when pH is

changed from 6 to 7 and it can be explained by earlier hypothesis that increase in the acidity reduces the metal uptake capacity. On the other hand, when the pH is changed from 7 to 8, the mean % removal is increased by very low amount which is 0.2 %. Hence, for this experimental work the optimal pH is observed is in the range of 7-8.

6.1.2 Effect of Initial Concentration on Biosorption

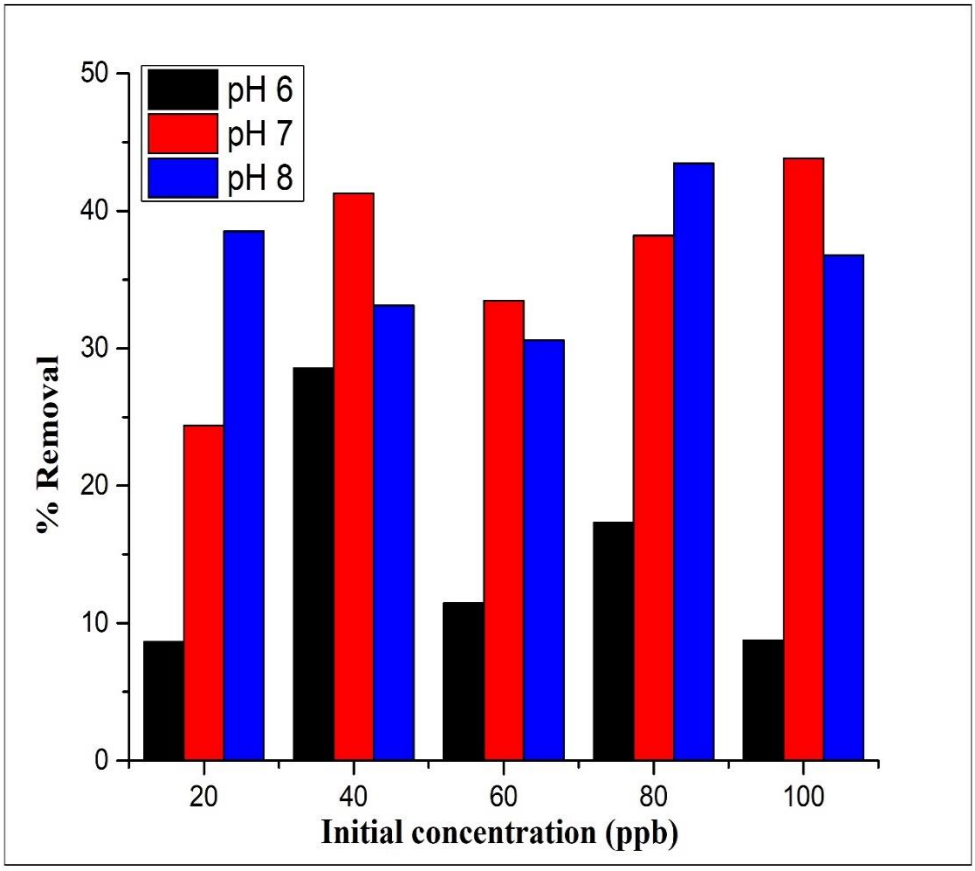
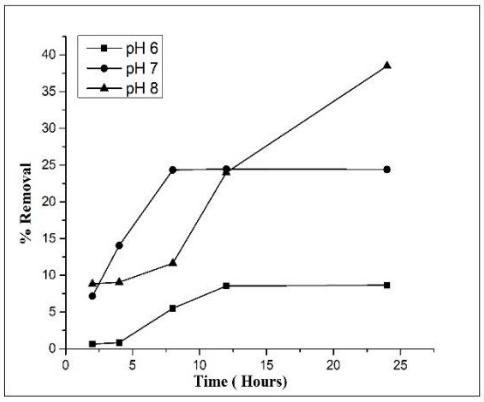


Figure 4. Effect of initial concentration on biosorption

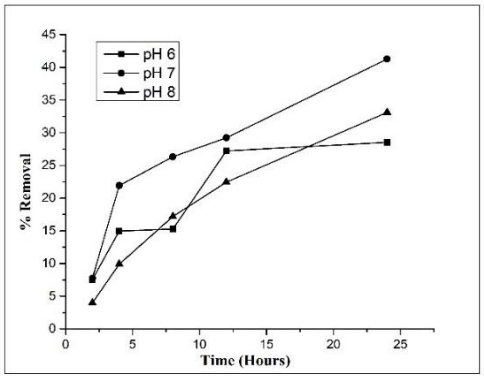
In this experimental work, heavy metal solutions are prepared with various concentration of cobalt such as 20 ppb, 40 ppb, 60 ppb, 80 ppb and 100 ppb and the mean % removal observed for each sample is 23.85%, 34.38%, 25.16% and 32.99 % and 29.38 %. From the results, it can be said there is no clear pattern between initial concentration and biosorption efficiency at such low

concentration. However, literature suggest that the use of highly concentrated biomass for the uptake of heavy metals having low initial concentration results in poor adsorption, and the reasons behind this are still unexplained [9].

6.1.3 Effect of Treatment Time on Biosorption

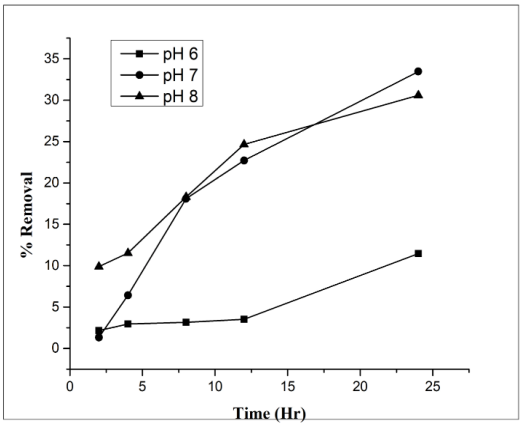


(a)

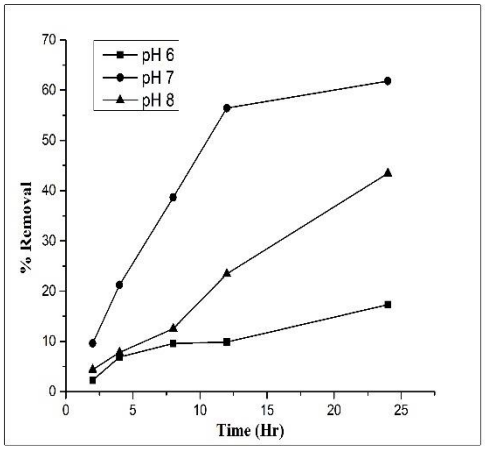


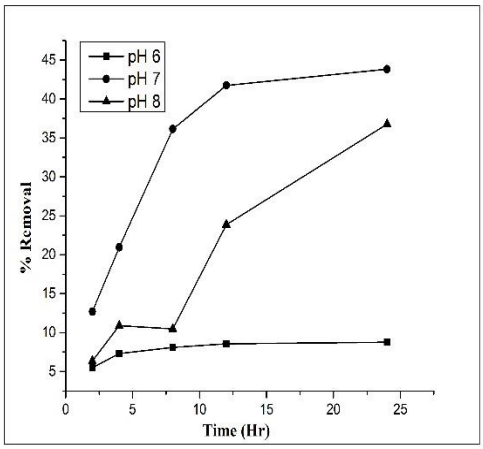
(b)

Figure 5. Effect of treatment time on biosorption of (a) 20 ppb sample (b) 40 ppb sample (c) 60 ppb sample (d) 80 ppb sample (e) 100 ppb sample



(c)





(d) (e)

Figure 5. Continued

From the experimental results , it seems that contact time plays significant role in faclilating the biosorption process. The adsorption process was very slow in the beginning until 4 hours , and the rate of adosrption is maximum in between 4-12 hours. Majority samples shows steady increase in adosrption rate after 12 hours till 24 hours but it is smaller than that to be observed in beween 4-12 hours. This shows that uptake sites come to be more saturated which results in the inefficient cobalt adsorption and reaction is reaching towards equilibrium.

A revised experriment is carried out to make sure the equilibrium is attained after 24 hours of treament time and 100 ppb sample having pH 8 is used for this experimnt. The following graph suggest that there is no large amount of change in % removal of cobalt after 24 hours. There fore , it can be said that equilibrium is achieved around 24 hours.

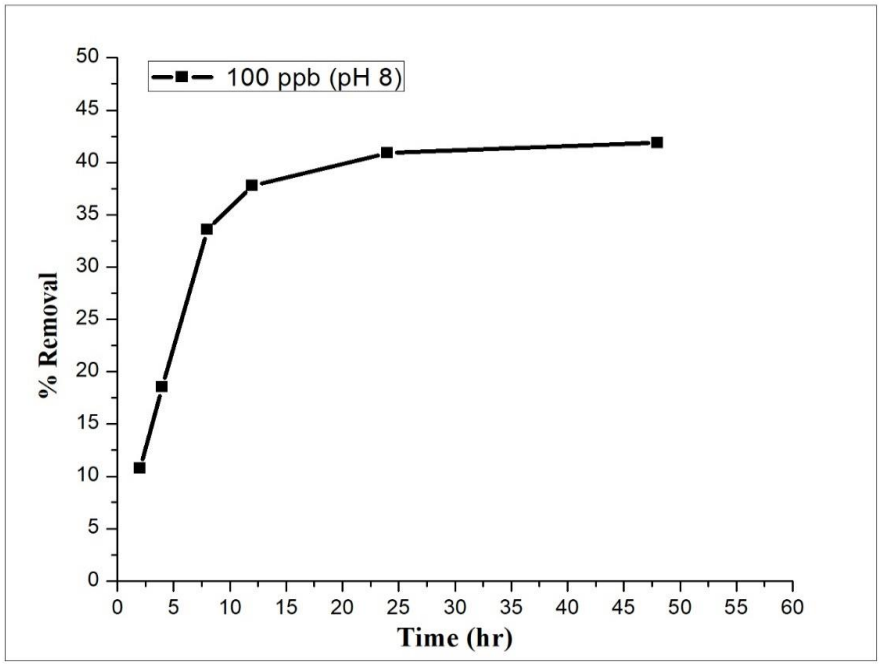


Figure 6. Effect of treatment time on biosorption of 100 ppb sample (Revised)

6.2 Data Analysis

The maximum uptake capacity (Q_{max}) is calculated by by using following equation :

$Q_{max} = (C_o - C_{eq})/ X$

where , C_o = Intial concentration

C_{eq} = Equlibrium Concentration

X = Weight of biomass (g/L)

Table 5. Uptake capacity for samples having pH 7

C_o (µg/L)	C_{eq}(µg/L)	X (g/L)	Q_{max} ((µg/g)
20	18.27	4.0916	0.42
40	28.59	4.0916	2.78
60	53.13	4.0916	1.67
80	66.14	4.0916	3.38
100	91.23	4.0916	2.14

Table 6. Uptake capacity for samples having pH 7

C_o (µg/L)	C_{eq}(µg/L)	X (g/L)	Q_{max} ((µg/g)
20	15.12	4.0916	1.19
40	23.49	4.0916	4.03
60	39.92	4.0916	4.90
80	49.44	4.0916	7.46
100	56.16	4.0916	10.71

Table 7. Uptake capacity for samples having pH 8

C_o (µg/L)	C_{eq}(µg/L)	X (g/L)	Q_{max} (µg/g)
20	12.3	4.0916	3.00
40	26.76	4.0916	6.54
60	41.65	4.0916	10.17
80	45.23	4.0916	11.05
100	63.21	4.0916	15.44

Linear fitting of experimental data is carrired out by plotting the graph between maximum uptake capcity (Q_{max}) vs equilibrium concentration (C_{eq}) and correlatation coeffecients for samples with pH 6 ,pH 7 and pH 8 are obersved around 0.74, 0.95 and 1 respectively.

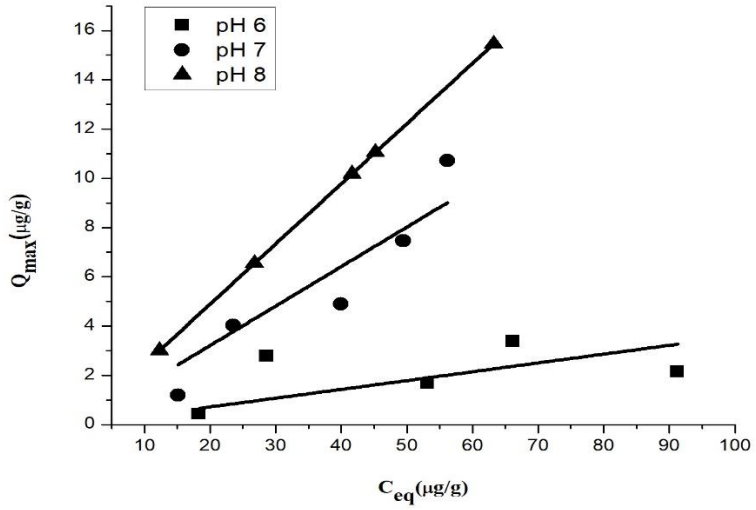


Figure 7. Linear analysis of uptake capacity with respect to equilibrium concentration

6.3 FTIR Characterization of *Pseudomonas Aerguinosa*

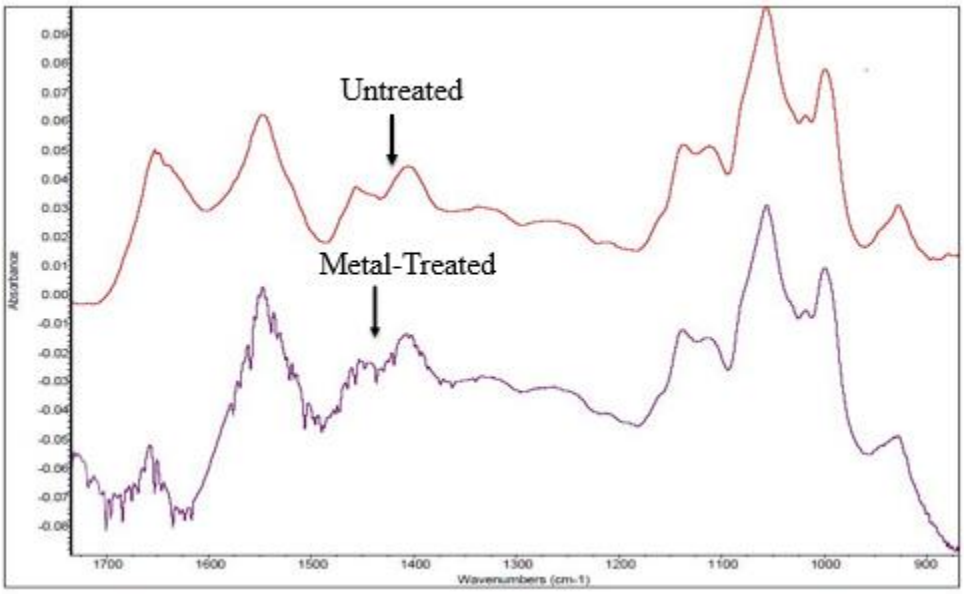


Figure 8. FTIR spectra of metal treated and untreated *Pseudomonas Aerguinosa*

FTIR characterization was carried out to understand the interaction between the functional groups present on the extracellular cellular surface of the *Pseudomonas Aerguinosa* and cobalt metal, it can be understood by the analysing the peaks from the FTIR spectra of bacterial suspension before and after treating with metal ions. Absorption bands between 1650 $cm^{-1}$and 1550 cm^{-1}corresponds to presence of amide groups on the surface of *Pseudomonas Aerguinosa*[29]. By analysing the shifts and changes in the peaks in these bands , it can be said cobalt metal ions was interacted with the amide groups present on the cell wall. However , the obtained FTIR spectra shows very low absorbance which creates the ambiguity in forming mechanistic explanation of cobalt absorption. From the several attempts FTIR characterization , it is observed that the sample analysed under FTIR was not concentrated as required and contain high amount of water resulting into extremely low absorbance. The work is still in the progress to obtain the satisfactory results

for FTIR characterization which enhance the understanding of interaction between functional groups present on the surface of bacteria and cobalt metal ion.

CHAPTER 7: CONCLUSION

In present study, resting cells of *Pseudomonas Aeruginosa* are used study the bioremediation of cobalt metal from the aqueous solutions. There have been really few studies demonstrated on bio-sequestration of heavy metals at ultralow concentration (ppb range), most of the studies has shown successful removal of metals in ppm range. Hence, this study was carried out to determine the lowest concentration of metal that can be removed with bio-adsorbent. The bio-adsorbent used in this study is susceptible to changes in surrounding media, therefore the study was carried out by varying different parameters such as pH, initial concentration of metals and treatment time.

The present study demonstrates that removal of cobalt is very less at pH 6 and the uptake capacity was 2.14 μg/g. This low in uptake capacity resulted due to the acidic nature of the solutions. Increase in a pH by factor of one significantly improved metal removal percentage, at pH 7 the maximum percentage of cobalt is observed for 100 μg/g sample which is 43.84 % and uptake capacity was found to be 15 μg/g. Similarly, when the pH is increased from 7 to 8 removal percentage is increased by 0.2%. At pH 8, the maximum removal of cobalt was 43.46% for 80 μg/g sample and the uptake capacity was 11.05 μg/g . The results obtained from the sample having pH 7 and 8 shows that the decrease in the acidity of the surrounding media and increasing the initial metal concentration results in higher adsorption capacity. However, the mean percentage removal of cobalt with respect to the initial concentration doesn't show a clear relationship between initial concentration and metal removal efficiency. Change in A treatment time has shown notable change in biosorption of cobalt and it can be said that by increasing the contact time

between bio-adsorbent and a metal can significantly contribute to efficient biosorption. Nevertheless, after a certain period binding sites gets saturated with metal resulting in decreased rate of adsorption. In summary, it can be said that *Pseudomonas Aerguinosa* can be effectively used for bioremediation of heavy metals and it has a potential to provide alternative and cost-effective technology in near future.

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