Potential of Various Immobilization Matrices in Heavy Metal Ions Removal Using *Nostoclinckia* beads: A Comparative Study

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Abstract: *This article reports the potential of removal of heavy metal ions of hexavalent chromium Cr(VI) and bivalent Cobalt Co (II) by different immobilization matrices containing dead cyanobacterial biomass of Nostoclinckia. Nostoclinckia was pre-treated by successive washes in distilled water, dried and then crushed to increase the surface area of biomass which was then immobilized in different immobilization matrices like Calcium alginate, polyvinyl alcohol alginate and polyvinyl alcohol alginate glutaraldehyde that was stable and easy to handle. The immobilized beads of Nostoclinckia was used to study the adsorption of metals (Cr VI & Co II) from simulated wastewaters under varying range of contact time (5-180 minutes), pH (1-6) and initial metal ion concentration (10-50mg/l) in a batch adsorption system. Experimental results showed that the different matrices pose impact on the different variable parameters like the optimum pH for biosorption of Cr (VI) ions was found to be 2.0 in alginate immobilized biomass while it was 4.0 polyvinyl alcohol alginate and polyvinyl alcohol alginate glutaraldehyde immobilized biomass. Similarly the maximum biosorption of Co (II) occurred at pH 3.0 in alginate biosorbent while it was 5 in polyvinyl alcohol alginate and polyvinyl alcohol alginate glutaraldehydebiosorbent. Similarly values of optimized contact times and initial metal ion concentrations were different for same biomass concentration immobilized in different matrices. From the results it can be concluded that the immobilization matrices affects the optimum values of different parameters in same conditions. Langmuir and Freundlich adsorption models were applied to describe the isotherms and isothermic constants. Freundlich model was fitted best in case of both metals. Alginate immobilized biomass was suited most for removal of hexavalent chromium (R^2=0.992) while PVA-A is found to have very high adsorption capacity for Co (II) as shown by (R^2=0.9978).*
Keywords: *Alginate, Heavy Metals, Immobilization, Nostoc, Polyvinyl alcohol*

I.   Introduction

The increase in gross domestic product has resulted in increase in pollution and degradation of natural resources. One of the most adversely affected resources is water as various industries are illegally disposing off their effluent either directly or indirectly via sewage system into natural water bodies. These industrial effluents have synthetic compounds, novel toxic chemicals, heavy metals and waste nuclear liquids etc. [1-2]. Contamination of natural streams by toxic heavy metal cations arise because of tannery, leather, electroplating, metal finishing, textiles, dying, paints etc. industries [3-4]. Several heavy metals are carcinogenic and mutagenic [5-6]and shows bioaccumulation through the food chain [7] as they are non-biodegradable hence their level must be reduced to acceptable level before discharging into water bodies [8]. Chromium exists usually in both trivalent and hexavalent forms in aqueous systems. Cr (III) is relatively insoluble and required by microorganisms in small quantities as an essential trace metal nutrient [9], while Cr (VI) is of great concern because of its toxicity. In animals Cr (VI) can cause respiratory problems, a lower ability to fight disease, birth defects, infertility and tumor formation. Due to environmental concern tolerance limit for Cr (VI) for discharge into inland surface waters is 0.1 mg/L and in potable water is 0.05 mg/L [10]. The Ministry of Environment and Forest (MoEF); Government of India has set minimal national standards (MINAS) of 0.1 mg/L for safe discharge of effluent containing Cr (VI) in surface water [11]. After chromium one another metal Cobalt is also of great concern as it is used in many alloys and super-alloys for parts in gas turbine aircraft engines, corrosion resistant alloys, high-speed steels, cemented carbides, in magnets and magnetic recording media, as catalysts for the petroleum and chemical industries, as drying agents for paints and inks. Cobalt is an important constituent of vitamin B12, and is used for treatment of anemia because it stimulates the production of red blood cells. Radioactive cobalt isotopes are also used to destroy tumor in cancer patients. However too high concentration of cobalt may results in asthma, weight loss, vomiting, respiratory hyper sensitivity, dermatitis, nausea, vision problems, heart problems and thyroid damage [12]. Nowadays, there are many methods for metals removal such as chemical coagulation, flocculation, chemical oxidation, photochemical degradation, membrane filtration, ion exchange, gamma ray irradiation, solvent extraction, hydrolysis, aerobic and anaerobic biological degradation

but their overall cost, incomplete metal removal; intensive energy requirement, regeneration problem and secondary pollutant/ toxic sludge generation limit their usages [13-15]. Physico-chemical processes result in transfer of the pollutants from one system to another without degrading it and is highly expensive if the contaminant concentration are in the range of 10- 100mg/L [16]. Considering all these points in the view biosorption could be considered as possible alternative technique for heavy metals removal as it is economically viable and environmentally friendly [17]. Microbial population growing in contaminated environment show adaptation to the toxic pollutants like heavy metals and become metal resistant [18]. Micro-organisms uptakes metal either actively (bioaccumulation) or passively (biosorption) [19-23]. Huge work has been carried out on removal of heavy metals by using microorganisms like algae, fungi and bacteria but little attention has been paid to investigate the effect of operating variables on the metals ion sorption characteristic of microorganisms immobilized in different immobilization matrices. Micro-organisms like fungi, bacteria and algae are available in abundant and can grow easily in contaminated environment, have been studied to see the remediation of heavy metals by live, dead and immobilized biomass [1-2,8,24-28]. Immobilized microorganisms are more suitable for industrial application as compared to free biomass because of better mechanical strength, chemical stability, and repeated adsorption- desorption characteristics [29-30]. Algal biosorbents for metals removal from aqueous solution are in use for quite some time in the form of both live and dead biomass [31-32].

The present study was undertaken with an objective to assess and optimize the role of immobilization matrices in metal ions sorption ability by dead biomass of caynobacteriumNostoclinckia. Different parameters were optimized for their removal by immobilized biomass in different immobilization matrices and kinetic models were also applied on biomass immobilized in different matrices to compare the efficiency of matrices to remove the metal ions.

1.1Alginate

Alginate is natural nontoxic, biodegradable and biocompatible polysaccharide distributed in brown seaweeds. It is a water-soluble most versatile biopolymers used in a wide range of applications as stabilizing agent. Hydrated alginate develops into gel when exposed to calcium ions. Calcium alginate gel shown in Fig 1.1 is used in encapsulating various active compounds. As a consequence, alginate gels have been exploited in food applications for the encapsulation of aroma and essential oils [33-34].
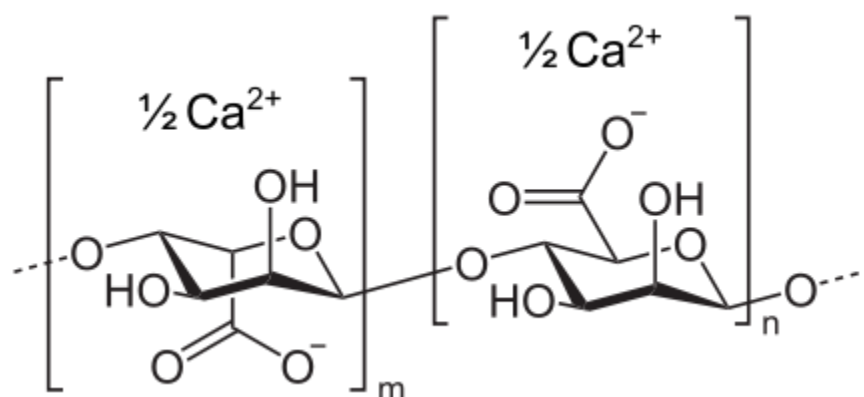

Figure1.1 Chemical Structure Of Calcium Alginate [35]

1.2 Polyvinyl Alcohol

Poly Vinyl Alcohol (PVA) Fig. 1.2 was first prepared by Hermann and Haehnel in 1924 by hydrolyzing polyvinyl acetate in ethanol with potassium hydroxide. Polyvinyl alcohol is an odorless and tasteless, translucent, white or cream colored granular powder. [36]

It is a non-toxic, hydrophilic, synthetic polymer capable of forming hydrogel by chemical cross-linking [37] or freeze–thawing [38]. PVA matrix produced upon freeze–thaw cycles has mainly been investigated for biological applications. These gels are non-toxic and have a high level of mechanical stability [39].

The physical characteristics and its specific functional uses depend on the degree of polymerization and the degree of hydrolysis. When the PVA is dissolved in water, the vinyl alcohol units link up to form chains of about 2,000 units. It is reported that PVA is a good matrix to promote the formation of three-dimensional structure of the beads, strengthen the beads' stiffness and improve the DO inside the beads [40].
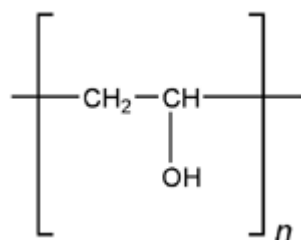
Figure. 1.2 Structure Of Polyvinyl Alcohol

1.3. Glutaraldehyde
Glutaraldehyde is an organic compound, pungent, colorless oily liquid, with the formula $CH_2(CH_2CHO)_2$ shown in Fig. 1.3 and is used to disinfect medical, dental equipment, industrial water treatment and as a preservative.
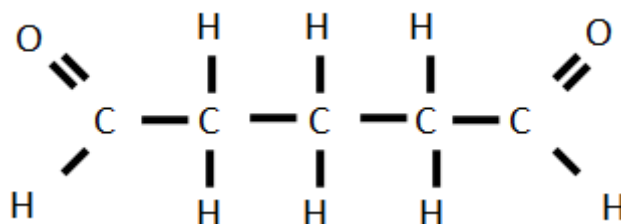

Figure 1.3 Glutaraldehyde [41]

II.   Materials And Methods

2.1 Preparation Of Biomass
Nostoclinckia a filamentous cyanobacteria isolated from contaminated site was taken from department of Environment Science and Engineering, Guru Jambheshwar University of Science and Technology, Hisar, and cultivated in BG-11 medium for 7 days after which it was washed thrice with distilled water and dried in an oven at $80\,^0C$ for one day. It was then finely powdered to increase the surface area of biomass.

2.2 Immobilization Of Biomass
2.2.1. Alginate Gel
Cyanobacterial suspension (7days old culture containing 0.1g dry weight) and 4% sodium alginate were mixed and suspension was dropped into 2% calcium chloride solution via a syringe (5ml) to form the small beads (3mm ± 0.1). The beads were subjected at 4^0C temperature to get polymerize.

2.2.2 Polyvinyl Alcohol Alginate
PVA-alginate slurry (2:1%, W/V) was prepared and biomass powder 0.1 g dry weight of N.linckia was added and stirred. Beads were prepared in 2% $CaCl_2$ with the help of 5ml syringe. After 6 hr of curing, the beads were subjected to three cycles of freezing ($<2^0C$) and thawing (30 ± 2^0C) to get spherical beads.

2.2.3.Polyvinyl Alcohol Alginate Glutaraldehyde
PVA-alginate slurry (2:1%, W/V) was prepared and biomass powdered 0.1 g dry weight of N.linckiawas added and stirred. Add glutaraldehyde into the slurry in the same amount as that of alginate. Give gentle shaking for 10 minutes. PVA-alginate-glutaraldehyde-biomass slurry was then extruded into 2%$CaCl_2$ solution via a syringe to form small beads. The beads were subjected at 4^0C temperature to get polymerize.

2.3 Metal Ions Solution Preparation
Chromium solution was prepared by dissolving 2.828 grams of potassium dichromate (AR Grade) in deionized water and diluted to 1000ml in order to get 1000mg/l hexavalent chromium solution. Different concentrations for the experiments were prepared using this stock solution.
1000mg/l bivalent cobalt solution was prepared by dissolving 3.104 grams of cobalt (II) nitrate (AR Grade) in 1000mL deionized water. This stock solution was used for further dilution for the experiments. The pH of the solution was adjusted using either 0.1 M HNO_3 or 0.1 M NaOH solutions.

2.4. Optimization Studies:
2.4.1 Contact Time Optimization
    100ml culture solution containing 20mg/l metals ion concentration was taken in triplicate and pH was adjusted in between 8-9. Beads containing immobilized biomass were transferred to 250 mL Erlenmeyer flasks containing heavy metals solutions. The flask were shaken at 120 oscillations per minute at light intensity of 3000lux and at constant temp 28 ± 2^0C for different time intervals i.e. 5,10,15,30,45,60,90,120,150 and 180 minutes. The remaining metal concentrations were measured on AAS (atomic absorption spectrophotometer Simazdu 6300AA).

2.4.2 pH Optimization
        100ml culture solution containing 20mg/l buffered metal solutions with pH ranging from 1 to 6 (pH 1, 2,3,4,5 and 6) were used at optimum contact time as determined from the previous experiments. Beads containing immobilized biomass were added to each flask and the solutions were shaken at constant temperature 28 ± 2^0C and speed 120 oscillations per minutes in orbital shaker.

2.4.3 Optimization of Initial Metal Ions
         After optimization of pH and contact time from the above experiments, the initial concentrations of metal ions more suitable for removal by the Nostoc were estimated.
         The beads containing immobilized biomass were added to 100 ml solution of culture medium containing 10-50 mg/l metal ions concentrations at optimum pH and contact time. The flasks were shaken at constant temperature 28 ± 2^0C and speed 120 oscillations per minutes in orbital shaker.

2.5. BiosorptionStudies
        Biosorption studies were conducted in batch mode to evaluate the effect of pH (1-6), contact time (5-180 minutes) and initial metal ion concentration(10-50mg/l) on removal of Cr (VI) and Co (II) from their respective synthetic solution using immobilized biomass. All biosorption experiments were conducted in triplicates using conical flask (250ml) on an incubator cum shaker (120 rpm) at 28 ± 2° C. The amount of metal uptake (mg/g) was obtained by using equation (1).

qe = (Co - Ce) V   (1)
    W

        Where qe is equilibrium uptake (mg/g); Co is initial metal ion concentration (mg/l); Ce is equilibrium metal ion concentration (mg/l); V is the volume of the metal solution (l); W is the mass of the sorbent (g).

2.6 Adsorption Isotherms:
        The biosorption data obtained for the Cr (VI) and Co (II) was analyzed using Langmuir and Freundlich isotherms

2.6.1 Langmuir's Isotherm Model[42] is valid for monolayer adsorption onto a surface containing a finite number of identical sites, which is represented as equation (2).

    Ce/qe = 1/Qob + Ce/Qo   (2)

        Where qe is the adsorbed at equilibrium (mg/g); Ce is the equilibrium concentration (mg/l); Qo and b are the Langmuir constants.

2.6.2 The Freundlich Equation[43] proposes an empirical model that is based on the sorption on heterogenous surface and is represented as equation (3).
Log x/m = log k + 1/nlog Ce   ---------------------------- (3)
        Where k (mg/g) shows adsorption capacity and n represents intensity of adsorption and are known as Freundlich isotherms constants; Ce is the equilibrium concentration (mg/l); x/m is the amount of metal ions adsorbed per unit mass of adsorbent (mg/g) and m is the adsorbent dose (g/l).

III.   Results and Discussion
3.1 Contact Time
        The effect of contact time on removal of metal ions have been shown in Fig. 3.1 a & 3.1 b. Cr (VI) adsorption onto the beads increased sharply with agitation time upto 20 minutes and then equilibrium was attained after 90 minutes, it gradually decreased and remained constant till 180 minutes. The rapid initial removal was likely due to extracellular binding and slow sorption after equilibrium may be due to intracellular binding which tales more time due to mass transfer problems [44-46]. Maximum metal ion removal of 86.6%

occurred at 90 minutes of contact time for biomass immobilized in alginate, 45 minutes for PVA-alginate (86.6%) and 150 minutes in PVA-alginate-glutaraldehyde (86.5%) matrices. From the results it can be concluded that maximum percentage removal is nearly same by immobilized biomass in all the three matrices at different agitation times. Agitation time is very less in case of biomass immobilized in polyvinyl alcohol alginate which reveals that it can remove the same amount of Cr (VI) in very less time as compared to other two matrices.

Kiran et al., [47] have also reported that the maximum removal of Cr (VI) by Lyngbyaputealis HH-15 occurred at 120 minutes of contact time with similar biosorption pattern. Initial rapid sorption of Cr (VI) suggests the involvement of a passive process of metal binding on algal surface that is followed by a gradual and slow state which is likely to be associated with some active energy mediated process. Similar results were obtained byAkhtar[48] and Mohanty[49] that the maximum removal of chromium occurred in the initial stage which gradually decreased and remained almost constant after an optimum period.

It was also observed that the adsorption got slowdown in later stages because initially a large number of vacant surface sites may be available for adsorption and after some time the remaining vacant sites are difficult to occupy due to repulsive forces between the solute molecules of the solid and bulk phase [50-51]

Kiran et al, (2007) [31] observed 60-75% removal of Cr (VI) by immobilized L. putealiswhile in the present study the removal of Cr (VI) is much higher lying between 85-86% by Nostoclinckia immobilized in all the three matrices. Kumar [52] have also obtained similar results for removal of Cr (VI) byPenicilliumjanthinellm.

Biosorption of cobalt (II) metal showed a rapid exponential phase upto the first 60 minutes showing 94-96% removal by algal beads, thereafter it increased slowly and became constant at contact time of 120 minutes in case of biomass immobilized in PVA-alginate (97.5%, Fig.3.1 b) as well as PVA-alginate-glutaraldehyde (97.7%) while in case of biomass immobilized in alginate maximum removal of cobalt occurred at 90 minutes of contact time Fig. 3.1 a (94.7%) after which it decreased which show leakage of adsorbed metal ions and then became costant. Similar pattern of metal biosorption have been observed for other metals also [53-54].

Removal was maximum (97.69%) in PVA- alginate-glutaraldehyde immobilized cells as compared to biomass immobilized in other two matrices. Ajjabi[55] obtained biosorption rate for Cu^{2+} and Zn^{2+} by ChaetomorphaLinum which was fast and most of the process was completed within 120 min. Similarly Garnham[56] showed that the uptake of the Co was maximum at agitation time of 2 h using immobilized biomass of C.salina.
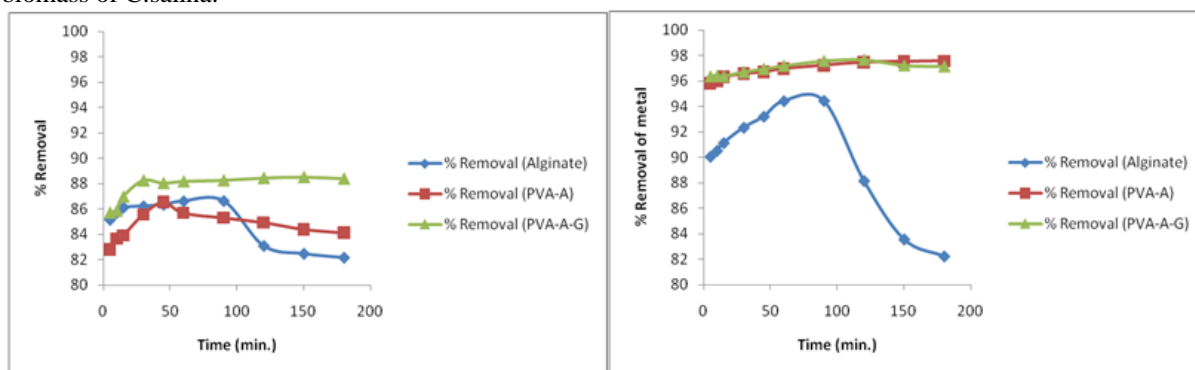

Figure 3.1a Effect Of Contact Time On % Removal Of Cr(VI) And 3.1 B % Removal Of Co(II) By Nostoclinckiaimmobilzed In Three Different Matrices

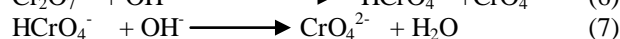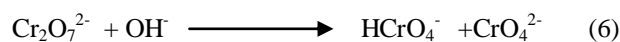### 3.2 Effect of pH

PH affect the percentage of ionization of functional groups present on cell wall of biomass [57-59]. The effect of pH on biosorption has been shown in fig 3.2 a & 3.2 b. It was observed that maximum removal of chromium occurred at pH 2 for biomass immobilized in alginate and pH 4 for other two matrices.

In the range of pH 2.0 to 6.0 , $HCrO_4^-$ and $Cr_2O_7^-$ ions are in equilibrium whereas, at lower pH there is formation of more polymerized chromium oxide species such as $Cr_3O_{10}^{2-}$ and $Cr_4O_{13}^{2-}$ [60] which results in decrease in Cr(VI) removal. As the pH of the solution increases, the number of negatively charged sites increased and positively charged sites decreased. A negatively charged surface site on the adsorbent does not favour the adsorption of anions due to electrostatic repulsion. In acidic medium the equilibrium is shown by equation (4) and (5) as follows:

$$HCr_2O_7 \longrightarrow H^+ + Cr_2O_7^- \qquad (4)$$
$$H_2CrO_4 \longrightarrow H^+ + HCrO_4^- \qquad (5)$$
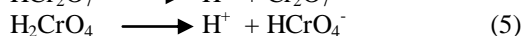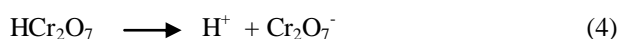
In alkaline medium the equilibrium is as represented by equations (6) and (7) as follows:

$$Cr_2O_7{}^{2-} + OH^- \longrightarrow HCrO_4{}^- + CrO_4{}^{2-} \quad (6)$$
$$HCrO_4{}^- + OH^- \longrightarrow CrO_4{}^{2-} + H_2O \quad (7)$$

The low chromium biosorption at pH 2 may be explained on the basis of active sites being protonated, resulting in a competition between protons and chromium for occupancy of the binding sites. Similar trends in the sorption data have previously been reported for Chromium sorbing onto seaweed biomass of Sargassum[61] and Eckloniaspecies.[62]
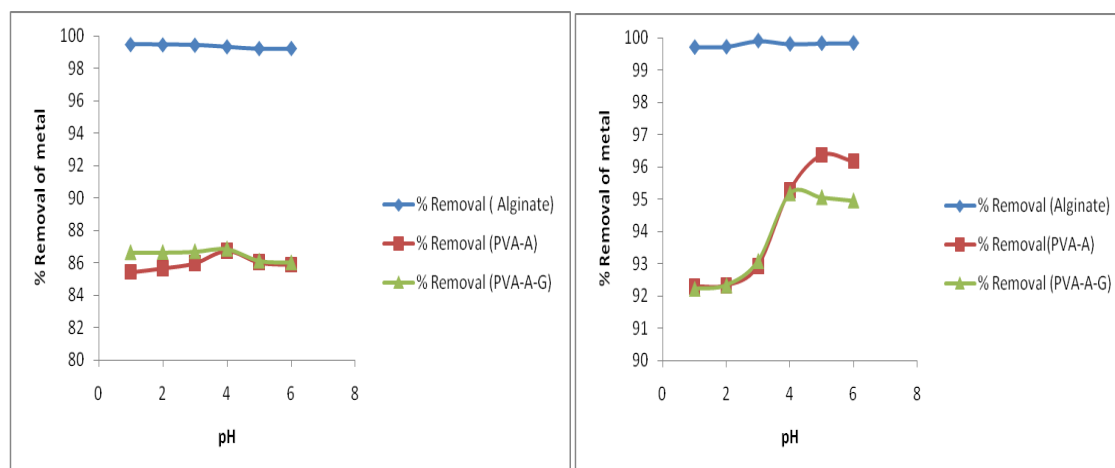

Figure 3.2a Effect of solution pH on the % removal of Cr (VI) and 3.2 b % removal of Co(II) by Nostoclinckiaimmobilized in different matrices

Gardea-Torresdey[63] observed the same value of pH 2 for removal of chromium (VI) by Synechococcus sp. PCC 7942. Similar results were obtained by Tiwari[64] in batch experiments for sorption and desorption of Cr (VI) using Mucorhiemalis. Dacera[65] investigated removal of Cr (VI) and Zn (II) and Ni (II) by A. niger was maximum at pH 4. Similar results were obtained by Kumar [66] in biosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp. Bacillus brevis.

Cobalt (II) Metal removal varied at different pH levels ranging from 2 to 5. It was observed that maximum removal of cobalt was at pH 3 for biomass immobilized in alginate and pH 5 for biomass immobilized in other two matrices.

At pH around 5, Co (II) adsorption capacity leveled off because at low pH values, the concentration of hydrogen protons in the solution far exceeds, these protons competed with the metal ions in forming a bond with the active sites (the functional groups) on the surface of the algae, leaving the metals ions free in solution. These bonded active sites thereafter became saturated and was inaccessible to other cations. When the pH was increased, the competing effect of hydrogen protons decreased and the positively charged metals ions took up the free binding sites. Hence, an increase in the sorption capacity or removal efficiency could be observed. The decrease in sorption efficiency at pH> 5 could be due to the formation of anionic hydroxide complexes and their competition with active sites. These results were in good agreement with findings of previous researchers [67-73] observed themaximumCo (II) removal efficiency occurred at pH 2. Jose [74]obtained the sharpest increase in heavy metal sorption between pH 2 and 3, while around pH 4.5 a plateau is reached. Beyond pH 5 precipitation of heavy metals occur which show the suitability of biosorbent for treatment of acidic waste water.Yalçın[75] investigated maximum biosorption capacity of Cladophoraglomerata at pH 5.0 for Cu (II) and at pH 4.0 for Pb (II) metal ions.

3.3 Initial Metal Ions Concentration

Initial metal ions concentration provides important driving force to overcome all mass transfer resistance of the metal between aqueous solution and the solid phase [76-77]. The effect of initial metal ion concentration on Co (II) and Cr (VI) uptake capacity of immobilized Nostoclinckia biomass was studied at optimized contact time and pH at varying concentrations of metals ions from 10 to 50 mg/L as shown in Fig 3.3 a & 3.3 b. In alginate immobilized cells percent removal of metal ions increased as the initial metal ions concentration increased from 10 to 30 mg/l and then decreased from 30 to 50mg/l. The maximum removal occurred at 30 mg/l in alginate matrix for hexavalent chromium as well as bivalent cobalt and at 10 mg/L by polyvinyl alcohol alginate and polyvinyl alcohol alginate glutaraldehyde immobilized cells for chromium (VI) after that it decreased steadily. Removal of cobalt (II) was maximum at 20mg/L for biomass immobilized in polyvinyl alcohol alginate as well as polyvinyl alcohol alginate glutaraldehyde then decreased from 20 to 50 mg/L.

Similarly Kamra et al, (2007) [32] showed the maximum biosorption capacity of alginate immobilized Nostoccalcicola HH-12 and Chroococcus sp.HH-11 occurred at initial chromium concentration of 20 mg/l for both the strains. Kumar [66] obtained the maximum biosorption of Cr (VI) at 35 mg/l initial metal ion concentration inbiosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp.Bacillus brevis. The optimum biosorption of Cr (VI) occurred at initial chromium concentration of 20 mg/l. Simiarly Kumar [78]   showed that the maximum removal of heavy metal using fungal biomass occurred at a concentration of 30mg/l.
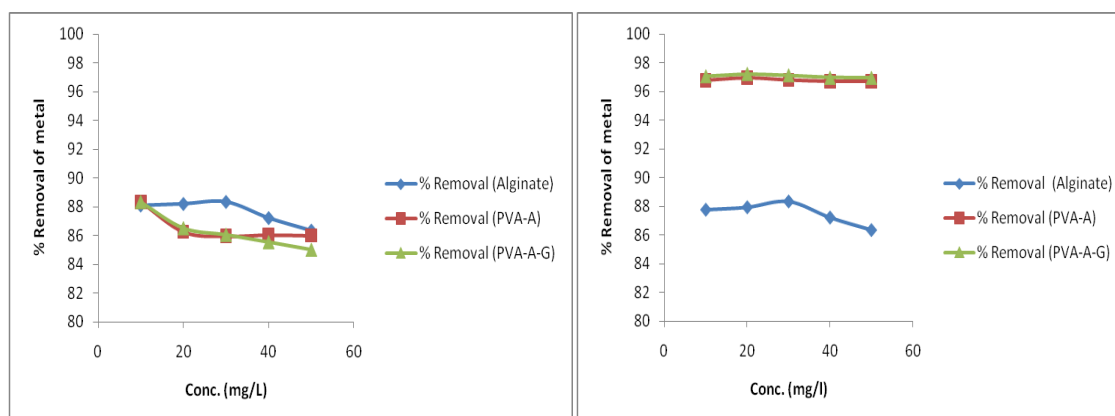

Figure 3.3 a Effect of initial metal concentration Cr(VI) and 3.3 b (CoII) on biosorption potential of Nostoclinckiaimmobilized in different matrices

3.4 Biosorption Studies

Freundlich isotherms was best suited for Cr(VI) as well as Co(II) biosorption. Alginate immobilized biomass was found to be best suited for the removal of Cr (VI) according to adsorption isotherms while Polyvinyl alcohol alginate was good for the removal of Co(II). For hexavalent higher value of K_f (7.57) and n=1.074 indicates high adsorption capacity of biomass per unit weight which is much higher than other biosorbents like Spirogyra sp. (3.75mg/g) [79] Lyngbya sp. (6.42, 1.49) [31] and high intensity of adsorption. High values of correlation coefficient (R^2=0.9926) also supports the suitability of freundlich model.

For cobalt removal the values of $K_f$and n are 30.47 and 1.006 respectively which show the adsorption capacity of biomass per unit of weight and high intensity of adsorption [80]. The situation n> 1 is most common may be due to a distribution of surface sites or any factor that causes a decrease in interaction of adsorbent - adsorbate with increasing surface density [81] and the values ofn lie between 1 and 10 indicates favorable adsorption

[82-84]. In the present study, since n lies between 1 and 10 it indicates the physical adsorption of metal ions onto matrices.

Freundlich model is suited which indicate the heterogenous nature of biosorbent surface [85-86]and hence it show multilayer adsorption of metal ions on the surface of immobilized biomass.

IV.   Conclusion

Three entrapped supports were chosen to immobilize Nostoclinckia. The result of the study revealed that change in solidification material affect the optimum value of the various parameters. Based on the experimental data the following conclusions were drawn:

1. The time required to reach equilibrium for hexavalent chromium removal was found to be 90 minutes in alginate, 45 minutes in PVA- Alginate and 150 minutes in PVA-A-Glutaraldehyde immobilized biomass with 86.6% removal efficiency. Cobalt (II) biosorption was reached at equilibrium at 90minutes in alginate, and 120 minutes in other two matrices with 94.5 to 97.7 % removal efficiency.
2. Alginate immobilized biomass showed the maximum removal of chromium (VI) at pH 2 and cobalt at pH 3 while PVA-Alginate and PVA-A-glutaraldehyde removed the maximum chromium (VI) metal ions at pH 4 and Cobalt (II) at pH 5.
3. Alginate immobilized Nostoc was found to be more efficient than PVA-Alginate and PVA-A-Gltaraldehyde in removing high metal ions concentration up to 30mg/L in both chromium (VI) and cobalt (II).
4. Alginate matrix was found to be best suited for Cr (VI) removal and PVA-Alginate & PVA-A-Glutaraldhyde were best for bio sorption of cobalt(II).
5. Addition of glutaraldhyde to PVA-Alginate does not change the optimization value of parameters up to a large difference.

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