ORIGINAL RESEARCH PAPER

Microbiology

REMOVAL OF HEXAVALENT CHROMIUM AND OTHER METAL IONS USING *SPIROGYRA AEQUETIALIS*: AS LOW-COST ADSORBENT

KEY WORDS: Spirogyra aequinoctialis, Heavy metals, Cr (VI), Biosorption

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ABSTRACT

The metals in high concentration are always toxic to living organisms. The industrial development is increasing the problem of heavy metal pollution. Each day new chemicals are introduced to environment. Manifestations of heavy metals on humans can vary from sub-clinical forms not directly attributed to heavy metal pollution like lowering IQ of children exposed to chronic lead poisoning to bloody diarrhoea and suppression of urine formation from ingestion of corrosive mercury. Biosorption is an innovative technology that employs inactive and dead biomass for the recovery of heavy metals from aqueous solutions. As an alternative to traditional methods its promising results are now being considered for application by the scientific community. In the recent work is focused on studies on the biosorption of Cr (VI) by dry biomass of Spirogyra and further used for growth independent sorption of Cr (VI). Various parameter influencing the biosorption of Cr (VI) were also studied.

1. INTRODUCTION

The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. In areas polluted as a result of industrial activity concentrations of toxic substances often exceeds the level normally found in soil, waterways and sediments. Heavy metal contamination of industrial effluent is one of the significant environmental problem due to their toxic nature and accumulation throughout the food chain as non biodegradable pollutants. (Akar T. and Tunali S.2005)

Metals are toxic to all living forms. Manifestations of heavy metals on humans can vary from sub-clinical forms not directly attributed to heavy metal pollution like lowering IQ of children exposed to chronic lead poisoning to bloody diarrhoea and suppression of urine formation from ingestion of corrosive mercury. Many heavy metals are known to be carcinogenic (WHO, 1993). Mechanism of metal toxicity can be divided into three categories 1) Blocking of the essential biological functional groups of biomolecules especially proteins and enzymes. 2) Displacing the essential metal ions and biomolecules and 3) modifying the active conformation of biomolecules resulting in the loss of specific activity (Ochiai, 1977).

The conventional physicochemical techniques for removing heavy metals from wastewater involve ionic precipitation, chemical oxidation or reduction, ion exchange, electrochemical treatment, filtration reverse osmosis membrane technology and evaporative technology (Barkat, 2011). However, these techniques have significant shortcomings, which are for instance, low efficiency at low concentration of heavy metals, high capital investment and operation cost and production of sludge (Chong *et. al.*, 2000). Therefore, new technologies are required to reduce toxic heavy metal concentration to environmentally safe levels in cost effective manner.

Biosorption is an innovative technology that employs inactive and dead biomass for the recovery of heavy metals from aqueous solutions. As an alternative to traditional methods its promising results are now being considered for application by the scientific community. In this context research and development of new biosorbent material s has focused especially algae, due to its high sorption capacity and its availability in almost unlimited amount (Klimmek *et. al.*, 2001) It seems likely that algae do not form a homogenous group within the vegetal kingdom. They are divided into several evolutionary pathways "red path way for Rhodophyta, "Brown pathway" for Chromophyta, "Green pathway" for chlorophyta mosses etc. Differences between these types of algae are mainly in the cell wall, where sorption takes place. Green algae are mainly cellulose and a high percentage of the cell wall is proteins bounded to polysaccharides to form glycol proteins these compounds contain several functional groups (amino, carboxyl, sulphate hydroxyl etc.) which play an important role in the biosorption process.

The biosorption process involves a solid phase (sorbent or biosorbent material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate metal ions). Due to high affinity of the sorbent to the sorbate species, later is attracted and removed by different mechanism. The process continues till equilibrium is established between the amount of solid bound sorbate species and its portion remaining in the solution.

The major advantage of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, regeneration of biosorbent and possibility of metal recovery (Kratchovil D. and Volesky B.). The dis advantages are rarely saturation i.e. when metal interactive sites are occupies, metal desorption is necessary prior to further use, the potential for biological process improvement (through genetic engineering) of cells is limited because the cells are not metabolizing and there is no potential for biologically altering the metal valency state (Ahuluwalia and Goyal)

2. MATERIAL AND METHODS

2.1.1 Preparation of Spirogyra biomass and Heavy metal solutions

To study the effect of the different physiological and environmental conditions influencing on percent sorption of Cr (VI) by growth independent *Spirogyra aequinoctialis*, it was isolated from the pond and cultivated. After sufficient growth in BG-11 medium, the broth was filtered and the *Spirogyra aequinoctialis* biomass was washed, dried in hot air oven at 45^{0}C and then crushed to obtain fine powder which was stored sterilized glass bottle with airtight lid by refrigeration during whole studies. During these studies, Stock solution of Cr (VI)-100ppm with different pH values (3, 5,7,9,11) and Stock solution (1:100) of Cu, Cr (VI), Zn, Mn and Ni was used

2.1.2 Effect of various parameters on percent sorption of Cr (VI)

To study various parameters influencing on percent sorption of Cr (VI) by *Spirogyra aequinoctialis* biomass, experiments were carried out in flasks containing 100ml standard Cr (VI) solution. For the optimization of effect of initial concentration of Cr (VI) used was 50-250ppm and inoculated with 1% (w/v) *Spirogyra aequinoctialis* biomass and allowed to react for 1hr. To study the effect of holding time, 100 ppm of Cr (VI) solution was inoculated with 1% (w/v) dried *Spirogyra aequinoctialis* biomass and incubated for varying periods of time (1-5 hrs).

To study the effect of temperature on Cr (VI) sorption, 100 ppm Cr (VI) solution was inoculated with 1 % (w/v) dried *Spirogyra aequinoctialis* biomass and was incubated for 1hr at varying temperature conditions (10-50°C) for 1hr.

To study the effect of initial inoculum concentration 100 ppm Cr (VI) solution was inoculated with various concentrations ranging from 1-5% (w/v), to study the effect of pH of solution on percent sorption ,100ppm Cr (VI)solution with pH 3.0, 5.0, 7.0,9.0 and 11.0 were inoculated with 1%(w/v) *Spirogyra aequinoctialis* inoculum.

2.2 Adsorption isotherms

Adsorption isotherms were applied to the biosorption experiments carried out using *Spirogyra aequinoctialis* biomass. For the percent sorption studies of Cr (VI), Ni^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+},20-200 ppm solution with pH 7.0 was inoculated with 1% (w/v) biomass and incubated at 30°C on a rotary shaker at 100 rpm for varying period of time. After each 30 minutes, results were taken and graph was plotted. The data thus obtained was applied to different adsorption isotherms like Langmuir and Freundlich and the graphs obtained were as follows.

3. RESULTS AND DISCUSSION

From the studies, it was observed and confirmed that, selected *Spirogyra aequinoctialis* biomass as sorbent material was found to be effective. From the results (Figure.1) it was found that increase in retention time showed increase in percent sorption of Cr (VI) in 1 hr retention, metal uptake was 51% and maximum percent sorption (80%) was after 3 hrs of retention time after which it remained constant. The sorption experiment had showed that a fast-initial phase of metal sorption involved which was followed by a slower second phase. Omer *et al.* (1991) observed maximum removal of uranium by *S. cerevisiae* during 60-100 minutes exposure.

From the Figure- 2, increase in Cr (VI) concentration there was increase in percent metal ion sorption by *Spirogyra aequinoctialis* biomass. Maximum percent sorption of Cr (VI) was observed at 200 ppm (79%). Brady and Duncan (1993) also showed similar observations for copper, cobalt and cadmium in *S. cerevisiae*.

From the Figure- 3, it is evident that, maximum Cr (VI) sorption took place at pH 3.0 (72%) followed by pH 5.0 (64%). and then there was decrease in percent sorption. In the biosorption process the pH value affects two aspects of metals i.e. ion solubility and biosorbent total charge, since proton can be absorbed or released. This behaviour will depend on the functional groups present on the algal cell wall. Romera *et. al*. (2007) also observed that optimum sorption pH for Cu^{2+} ranged from 4-5and for lead 3-5.

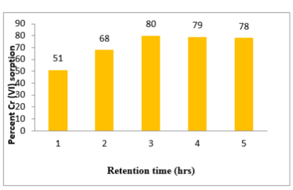


Fig.1: Retention time (hrs)

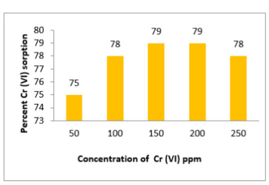


Fig.2: Concentration of Cr (VI) ppm

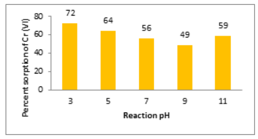


Fig.3: pH

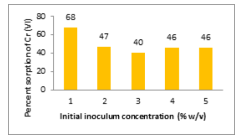


Fig.4: Inoculum oncentration

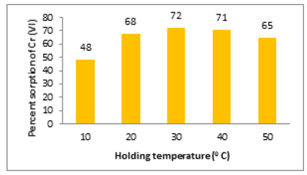
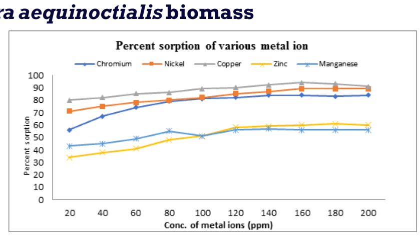


Fig.5: Holding temperature ($^{\circ}$C)

The biomass concentration is also an important variable during metal uptake. It has been observed that electrostatic interactions between cells can be a significant factor in the relationship between biomass concentration and metal concentration. From the Fig.4, it is observed that maximum Cr (VI) sorption was 68% at 1% (w/v) inoculum level and further increase in inoculum was responsible to decrease the percent sorption. At a given metal concentration the lower the biomass concentration in suspension the higher will be the metal sorbed and the higher metal retained by the sorbent unit. Similar observations were made by Mehta and Gaur (2001), who claimed that at a given metal concentration biomass takes more metal ions at lower cell densities than at higher cell densities, it may be due to the higher cell densities can exert a shell effect, protecting the active sites from being occupied by the metal.

From the results shown in Fig.5, it is observed that low and high temperatures showed decrease in percent sorption of Cr (VI), by *Spirogyra aequinoctialis* at 10 $^{\circ}$C the percent sorption was 48% and from 20°C it increased and showed maximum 72% sorption of Cr (VI) at 30° C. There was no significant change in percent sorption (68-71%) between the temperatures 20-40° C. Our observations were similar to Bengtsson *et. al*., (1995) they observed that the uptake of uranium by *Taleromyces emersonii* CRS 81470 was found to remain unchanged between 15 and 60°C.

Figure 6: Percent sorption of various metal ions by *Spirogyra aequinoctialis* biomass



From the Fig. (6) it was observed that the *Spirogyra aequinoctialis* mass had capacity to accumulate metals under study, the maximum metal sorption by the algal mass was found to be of Cu^{2+} (94%) followed by Ni^{2+}, Cr^{6+} and Zn^{2+}. Further it was observed that maximum Cu^{2+} adsorption was at 160 ppm; Cr^{6+} at 150 ppm; Ni^{2+} at 160 ppm; Zn^{2+} at 180 ppm and Mn^{2+} at 150 ppm.

Fig. 7: Langmuir plot for metal ions sorption study *Spirogyra aequinoctialis*

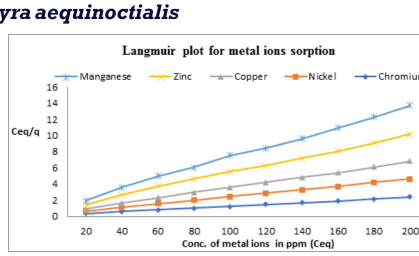
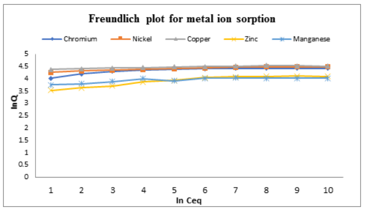


Fig. 8: Freundlich plot for metal ions sorption for *Spirogyra aequinoctialis*



The Langmuir and Freundlich adsorption models were used for mathematical description of adsorption of metal ions under study by the algal biomass and the isotherms constants were determined to find out the adsorption capacity of adsorbent. From the mathematical expressions, from the Fig. (7, 8) and the Table (1) it was observed that the Langmuir isotherm showed straight line and the regression was 0.99 for all metal ions under study. Therefore, this isotherm was more suited.

Table-1: Comparison of values for adsorption isotherms by *Spirogyra aequinoctialis* biomass

Metal ions	Langmuir parameters			Freundlich parameters		
	Slope	Intercept	Regression	Slope	Intercept	Regression
Cr^{6+}	0.01117	0.1304	0.9999	0.173375	3.56032	0.9212
Ni^{2+}	0.01068	0.1133	0.9984	0.107067	3.92753	0.9789
Zn^{2+}	0.01049	0.0644	0.9986	0.071703	4.15528	0.9283
Mn^{2+}	0.01400	0.4712	0.9870	0.28822	2.61299	0.9547
Cu^{2+}	0.016832	0.17592	0.9957	0.130527	3.36032	0.8622

Table-2: Qmax values calculated from the graph for metal ions under study by *Spirogyra aaequinoctialis* biomass.

Metal ions	Qmax
Cr^{6+}	57mg/gm
Ni^{2+}	44mg/gm
Zn^{2+}	12mg/gm
Mn^{2+}	15mg/gm
Cu^{2+}	20mg/gm

The Q max values when calculated using Langmuir isotherms Table-2, it was observed that the used algal biomass showed maximum 57 mg/gm of Cr (VI) sorption followed by Ni^{2+} 40, Cu^{2+} 22 and Zn^{2+} and Mn^{2+} were very less.

The studies carried out using thus *Spirogyra aequinoctialis* confirmed that the biomass possesses metal sorption capacity. Their metal sorption capacity can further be increased by various pre-treatment procedures. Thus, the *Spirogyra aequinoctialis* biomass can be efficiently used to treat factory effluents containing metal ions to recover them.

It can be said that to commercialize the biosorption in wastewater treatment, it is necessary to continue to explore the various aspects relevant to the application, like physicochemical conditions during biosorption, optimization of various parameters, fluid dynamics, various chemical engineering reaction, immobilization of biomass all of which will in turn decide the fate of biosorption. Looking into various advantages offered by different biosorbents, there is a need to have more knowledge of metals and the basic mechanisms involved in order to develop better and effective biosorbents. Further to popularize this waste water technology, critical analysis is essential to attract the industries generating waste water containing metals. Certain strategies have to be formulated to centralize the facility to use the biosorbent technology and recover precious metal ions, regenerate the biomass and reuse it. This will further require an interdisciplinary approach with integration of metallurgical skills along with sorption and wastewater treatment to develop biosorption technology for combating heavy metal pollution in aqueous solution.

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