

## Hexavalent Chromium Removal *Citrus reticulata* SHELL

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### Abstract

*We studied the Chromium (VI) removal capacity in aqueous solution by the mandarin shell, using the diphenylcarbazide method to evaluate the metal concentration. So, the highest biosorption of the metal (50 mg/L) occurs within 6.5 hours, at pH of 1.0 and 28 °C. According to temperature, the highest removal was observed at 60 °C, in 40 minutes, when the metal (50mg/L) is completely adsorbed. At the analyzed concentrations of Cr (VI), mandarin shell, showed excellent removal capacity, besides it removes efficiently the metal in situ (94.65% removal, 6 days of incubation, 5 g of biomass). After 1 hour of incubation, the studied biomass reduces 1.0 g of Cr (VI) with the simultaneous production of Cr (III); so, it can be used to eliminate it from industrial wastewater.*

**Keywords:** Chromium (VI), mandarin biomass, removal, wastewater, bioremediation

### 1. Introduction

Chromium (Cr) toxicity is one of the major causes of environmental pollution emanating from tannery effluents.

This metal is used in the tanning of hides and leather, the manufacture of stainless steel, electroplating, textile dyeing and as a biocide in the cooling waters of nuclear power plants, resulting chromium discharges causing environmental concerns (Bai and Abraham, 2001). Chromium exists in nine valence states ranging from -2 to +6. From these, only the hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)] have primary environmental significance due they are the most stable oxidized forms in the environment (Smith et. al., 2002).

Both are found in various bodies of water and waste waters. Cr(VI) typically exists in one of these two forms: chromate ( $\text{CrO}_4^{2-}$ ) or dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ), depending on the pH of the solution (Seng and Wang 1994). These two divalent oxyanions are very water soluble and poorly adsorbed by soil and organic matter, making them mobile in soil and groundwater (Smith et. al., 2002).

Both chromate anions represent acute and chronic risks to animals and human health, since they are extremely toxic, mutagenic, carcinogenic and teratogenic (Marsh and McInerney, 2001).

In contrast to Cr(VI) forms, the Cr(III) species: predominantly hydroxides, oxides or sulphates, are less water soluble, mobile (100 times less toxic) (Beszedits, 1988), and 1,000 times less mutagenic (Lofroth and Ames, 1978). The principal techniques for recovering or removing Cr(VI) from wastewater are: chemical reduction and precipitation, adsorption on activated carbon, ionexchange and reverse osmosis, in a basic medium (Park et. al., 2004). However, these methods have certain drawbacks: like high cost, low efficiency, generation of toxic sludge or other wastes that require disposal and imply operational complexity (Sahin and Öztürk, 2005).

In this context, considerable attention has been focused in recent years upon the field of biosorption for the removal of heavy metal ions from aqueous effluents (Volesky and Holan, 1995). The process of heavy metal removal by biological material is known as biosorption. Biomass viability does not affect the metal uptake. Therefore any active metabolic uptake process is currently considered to be a negligible part of biosorption. Various biosorbents have been tried, which include seaweeds, moulds, yeast, bacteria, crab shells, agricultural products such modified corn stalks, (Chen et. al., 2011), hazelnut shell (Cimino et. al., 2000), orange shell (Pérez- Marín et. al., 2007), tamarind shell (Acosta-Rodríguez et. al., 2011).

It has also been reported that some of these biomass can reduce Chromium (VI) to Chromium (III), like tea fungal biomass (Razmovski, and Sciban, 2007); mesquite (Aldrich et. al., 2003), eucalyptus bark (Sarin and Pant, 2006), red roses waste biomass (Shafqat et. al., 2008), and yohimbe bark (Fiol et. al., 2008). The present study is undertaken with following objective: investigate the use of *Citrus reticulata* shell for the biosorption of Chromium (VI) in aqueous solution.

## 2. Experimental

### 2.1 Biosorbent used: *Citrus reticulata* shell

The shell was obtained from the fruits harvested and offered in the marketplace Republic, between the months of November to December in 2011, of the capital city of San Luis Potosi, S.L.P. México. To obtain the biomass, mandarin rind was washed with water trideionized 72 hours under constant stirring, with water changes every 12 hours. Subsequently, boiled 1 hour to remove traces of the fruit was dried at 80 ° C for 12 hours in the oven, ground in blender and stored in amber vials until use.

### 2.2 Determination of hexavalent, trivalent, and total Cr

Hexavalent Chromium and trivalent Chromium were quantified by a spectrophotometric method employing diphenylcarbazide and chromazurol S, respectively (Greenberg et. al., 1992; Pantaler and Pulyaeva, 1985), total Chromium was determined by electrothermal atomic absorption spectroscopy (Greenberg et. al., 1992).

The values shown in the results section are the mean from three experiments carried out by triplicate.

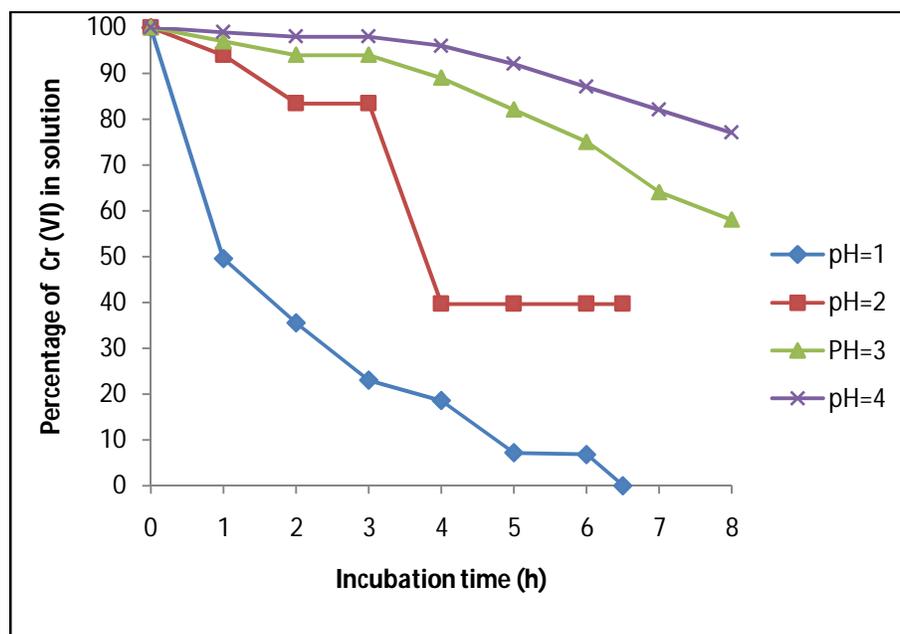
## 3. Results and discussion

### 3.1 Effect of incubation time and pH

Figure 1 shows the effect of the incubation time and pH. The optimum time and pH for Cr (VI) removal was 6.5h and pH 1.0, at constant values of biosorbent dosage (1 g/100 mL), with an initial metal concentration (50 mg/L), and temperature of 28°C. The literature (Pérez-Marín et. al., 2007), report an optimum time of 60 min, for the removal of lead by orange shell, 30 min and 2 hours for the removal of Cr (VI) by the tamarind shell and eucalyptus bark (Acosta et al., 2010; Sarin and Pant, 2006). Changes in the permeability of unknown origin, could partly explain the differences found in the incubation time, providing greater or lesser exposure of the functional groups of the cell wall of biomass analyzed.

Adsorption efficiency of Cr(VI) was observed maximum at pH 1.0 with *C.reticulata* waste biomass. This was due to the dominant species ( $\text{CrO}_4^{2-}$  and  $\text{Cr}_2\text{O}_7^{2-}$ ) of Cr ions in solution which were expected to interact more strongly with the ligands carrying positive charges (Gupt et al., 2001).

This results are like for tamarind shell (Acosta et al., 2010), but the most of authors report an optimum pH of 2.0 like Tamarind shell (Agarwal et al., 2006), eucalyptus bark (Sarin and Pant, 2006), bagasse and sugarcane pulp, coconut fibers and wool (Dakiki et al., 2002), for the tamarind shell treated with oxalic acid (Popuri et al., 2008), at pH of 2.0 and 5.0 for the mandarin bagasse (Zunbair et al., 2008), and almond green hull (Sharanavard et al., 2011).

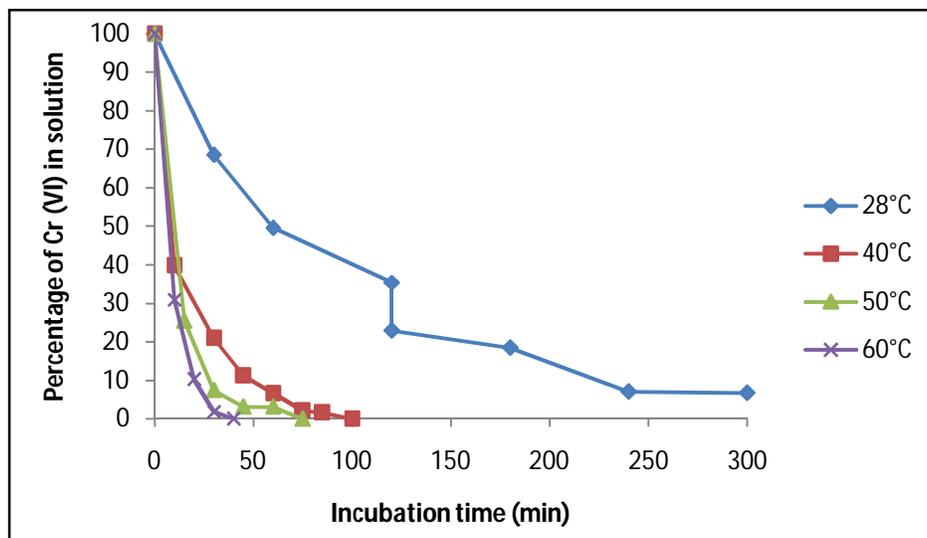


**Figure 1: Effect of incubation time and pH on Chromium (VI) removal by *C.reticulata* shell. 50 mg/L Cr (VI), 100 rpm, 28°C.**

### 3.2 Effect of Temperature

Temperature is found to be a critical parameter in the bioadsorption of Cr (VI) (Figure 2). The highest removal was observed at 60°C. At this point the total removal of the metal is carried out. The results are coincident for tamarind shell with 95% of removal at 58°C and 3 hours (Agarwal et al., 2006), for the adsorption of cadmium(II) from aqueous solution on natural and oxidized corncob (40°C and 5 days)

(Leyva-Ramos et al., 2005), but this are different for the mandarin waste (Zunbair et al., 2008), *Caladium bicolor* (wild cocoyam) biomass (Jnr, and Spiff, 2005), and *Saccharomyces cerevisiae* (Ozer and Ozer, (2003). The increase in temperature increases the rate of removal of Chromium (VI) and decreases the contact time required for complete removal of the metal, to increase the redox reaction rate (Agarwal et al., 2006).



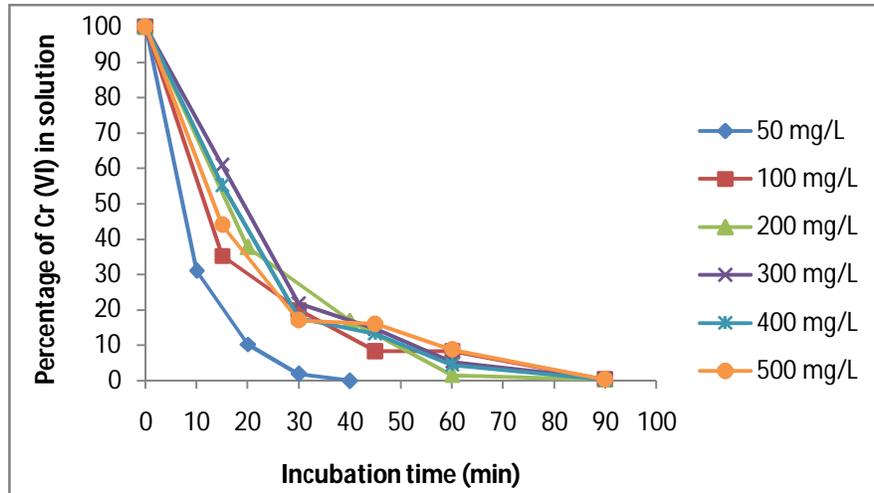
**Figure 2: Effect of temperature on Chromium (VI) removal by *C.reticulata* shell. 50 mg/L Cr (VI), pH 1.0.100 rpm.**

### 3.2 Effect Of Initial Metal Concentration

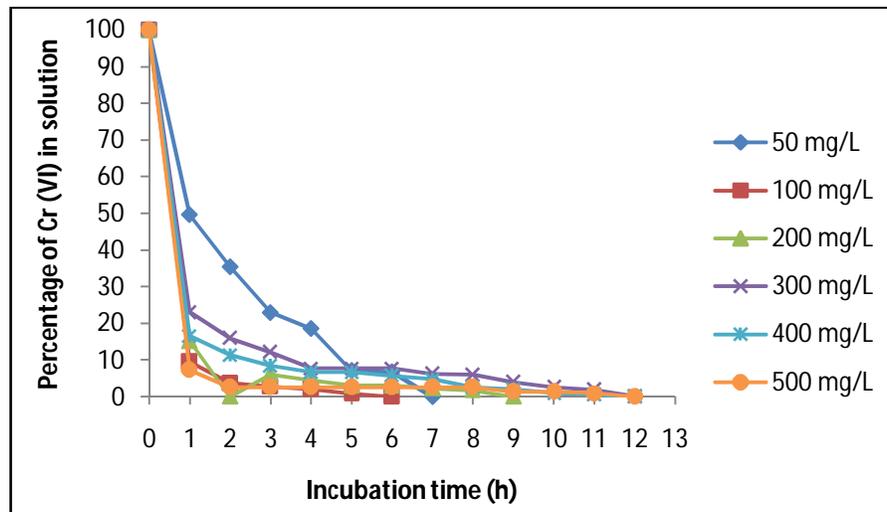
At low metal concentrations (50 mg/L) of biomass studied, shows the best results for removal, adsorbing 100% at 40 min at 60°C, while 500 mg/mL of the metal are removed at 90 min and 12 h at 60°C and 28°C, respectively (Figures 3a, and 3b). Also, we observed the development of a blue-green and a white precipitate, which changes more rapidly at higher temperatures (date not shown). The results are coincident for tamarind shell (Acosta-Rodríguez et. al., 2010;

Agarwal et. al., 2006). With respect to other biomass, most authors report lower removal efficiencies of metal, for example: 45 mg/L for eucalyptus bark (Sarin and Pant, 2006), 13.4 and 17.2 mg/L for bagasse and sugar cane pulp, 29 mg/L coconut fibers, 8.66 mg/L for wool (Dakiki et. al., 2002), 25 and 250 mg/L of chitin and chitosan (Sag, and Y. Aktay, 2002), and 1 mg/L for cellulose acetate (Arthanareeswaran et. al., 2002). The increase in initial concentration of Cr (VI), results in the increased uptake capacity and decreased % removal of Cr (VI). This was due to the increase in the number of ions competing for the available functional groups on the surface of biomass (Agarwal et. al., 2006).

a)



b)

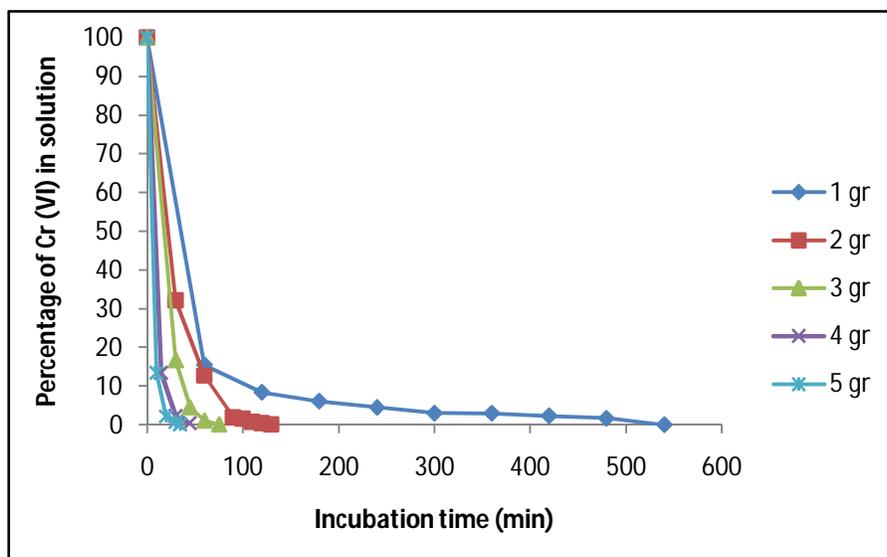


**Figure 3: Effect of initial metal concentration on Chromium (VI) remotion by *Creticulatashell*.pH 1.0. 100 rpm, a)60°C, b) 28°C.**

### 3.3 Effect of Biosorbent Dose

The influence of biomass on the removal capacity of Cr (VI) was depicted in Figure 4. If we increase the amount of biomass also increases the removal of Cr (VI) in solution (100% of removal, with 5 g of biomass at 35 minutes), with more biosorption sites of the same, because the amount of added biosorbent determines the number of binding sites available for metal biosorption (Cervantes et. al., 2001).

Similar results have been reported for modified corn stalks (Chen et. al., 2011), tamarind shell (Acosta-Rodríguez et, al., 2010), and *Mucorhiemalis* and *Rhizopusnigricans*, although latter with 10g of biomass (Tewari et. al., 2005; Bai and Abraham, 2001), but are different from those reported for biomass wastes from the mandarin (gabasse), with an optimal concentration of biomass of 100 mg/L (Zubair et. al., 2008).



**Figure 4: Effect of biomass concentration on the removal of 500 mg/L Cr (VI). (60°C, pH= 1.0, 100 rpm).**

### 3.4 Time Course Of Cr(VI) Decrease And Cr(III) Production

The ability of the *C. reticulata* shell to lower the initial Cr(VI) of 1.0 g/L, and Cr(III) production in solution was analyzed. Figure 5 shows that the shell exhibited a remarkable efficiency to diminish Cr(VI) level with the concomitant production of Cr(III) in the solution (indicated by the formation of a blue-green color and a white precipitate, and his determination for Cromazurol S, date not shown) (Pantaler and Pulyaeva, 1985).

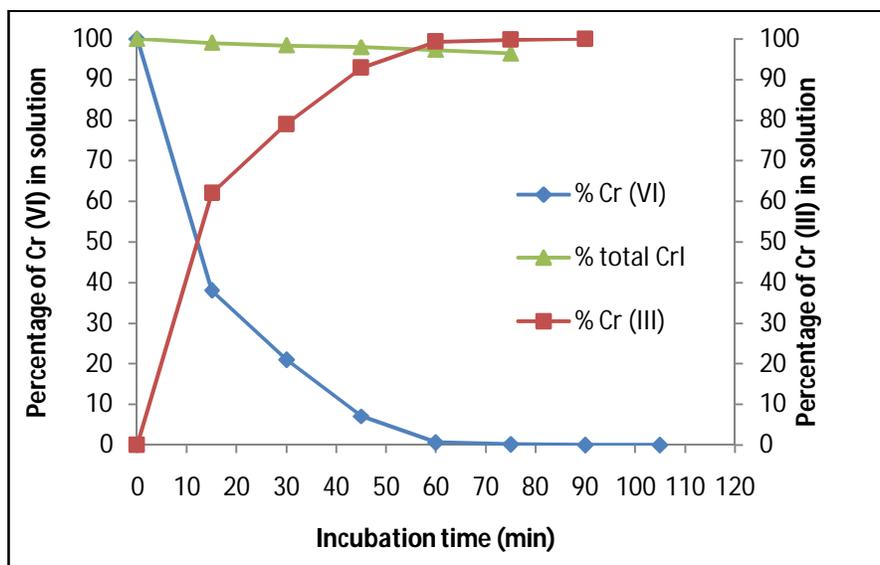
Thus, after 1 hour of incubation, the shell biomass caused a drop in Cr(VI) from its initial concentration of 1.0 g/L to almost undetectable levels, and the decrease level occurred without change significant in total Chromium content. As expected, total Chromium concentration remained constant over time, in solution control. These observations indicate that *C. reticulata* shell is able to reduce Cr (VI) to Cr(III) in solution.

Furthermore, as the mandarin shell contains vitamin C and some carbohydrates, we found that vitamin C and cystine quickly reduce Cr (VI) to Cr (III), and could be very important part in the metal reduction, confirming some reports in the literature (Smith et. al., 2002; Seng and Wang 1994; Acosta et. al., 2012; Xu et. al., 2005, and Yong et. al., 2005). There are two mechanisms by which chromate could be reduced to a lower toxic oxidation state by an enzymatic reaction. Currently, we do not know whether the shell biomass used in this study express and Cr(VI) reducing enzyme(s). Further studies are necessary to extend our understanding of the effects of coexisting ions on the Cr(VI) reducing activity of the biomass reported in this study.

Cr(VI) reducing capability has been described in some reports in the literature (Smith et. al., 2002; Seng and Wang 1994; Sahin and A. Öztürk, 2001; Acosta-Rodríguez et. al., 2010; Aldrich et. al., 2003; Fiol et. al., 2008; Ramírez-Ramírez et. al., 2004; Ramírez-Ramírez et. al., 2004; Park et. al., 2004; Acosta et. al., 2012; and Cárdenas-González and Acosta-Rodríguez, 2011).

Biosorption is the second mechanism by which the chromate concentration could be reduced, because the biomass shell can be regarded as a mosaic of different groups that could form coordination complexes with metals, and our observations are like to the most of the reports in the (Seng and Wang 1994; Smith et. al.,

2002; Sahin and Öztürk, 2005; Acosta-Rodríguez et. al., 2010; Aldrich et. al., 2003; Fiol et. al., 2008; Ramírez-Ramírez et. al., 2004; Park et. al., 2004; Acosta et. al., 2012, and Cárdenas-González and Acosta-Rodríguez, 2011).

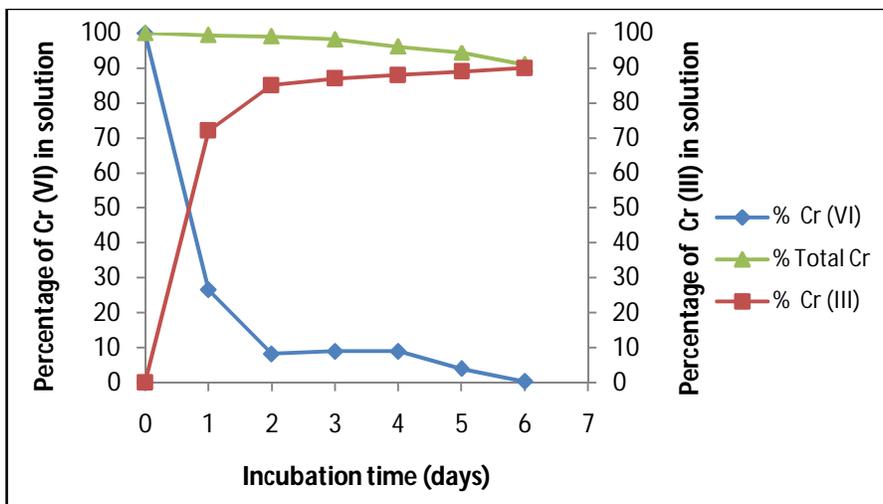


**Figure 5: Time-course of Cr (VI) decrease and Cr (III) production in solution with 1.0 mg/L Cr (VI). 100 rpm, 28°C, pH 4.0**

### 3.5 Removal Of Cr(VI) In Industrial Wastes With *Citrus Reticulata* Shell

We adapted a water-phase bioremediation assay to explore possible usefulness of *C.reticulata* shell for eliminating Cr(VI) from industrial wastes, the biomass was incubated with nonsterilized contaminated soil containing 297 mg Cr(VI)/g, suspended in trideionized water. It was observed that after six days of incubation with the biomass, the Cr(VI) concentration of soil sample decrease 100% (Figure 6), and the decrease level occurred without change significant in total Chromium content during the experiments. In the experiment carried out in the absence of the biomass, the Cr (VI) concentration of the soil samples decreased by about of 18% (date not shown); this might be caused by indigenous microflora and (or) reducing components present in the soil.

The chromium removal abilities of *C. reticulata* shell are equal or better than those of other reported biomass, for example tamarind shell (Acosta-Rodríguez et. al., 2010), *Mammea Americana* (Acosta et. al., 2012), and *Candida maltose* RR1, (Ramírez-Ramírez et. al., 2004). In particular, this biomass was superior to the other biomass because it has the capacity for efficient chromium reduction under acidic conditions. Many of the Cr(VI) reduction studies were carried out at neutral pH (Fukuda et. al., 2008). *Aspergillus niger* also has the ability to reduce and adsorb Cr(VI) (Fukuda et. al., 2008). When the initial concentration of Cr(VI) was 500 ppm, *A. niger* mycelium removed 8.9 mg of chromium/g dry weight of mycelium in 7 days.

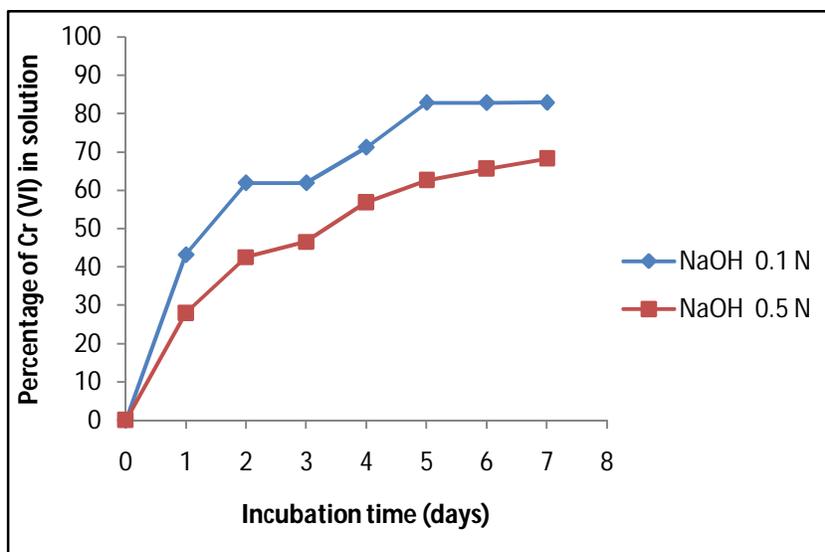


**Figure 6: Removal of Chromium (VI) in industrial wastes incubated with the biomass. 100 rpm, 28°C, contaminated soil (297 mg Cr (VI)/g soil).**

### 3.6 Desorption Of Cr(VI) By Different Solutions

Furthermore, we examined the ability of different solutions to desorb the metal biosorbed (250 mg/L) for the *C.reticulata* biomass, obtaining high efficiency with 0.1 N NaOH and 0.5 N (83% and 62.86% respectively), which are less than reported for desorption of Chromium (VI) with alkaline solutions (100%, pH = 9.5),

1.0 N NaOH (95%) and a hot solution of NaOH/Na<sub>2</sub>CO<sub>3</sub> (90%), respectively, (Singh et. al., 2009; Gupta, and Babu, 2009), and are higher than that reported (14.2%) using 0.2 M NaOH (Wang et. al., 2009). This indicates that binding of metal to biomass is not as strong and that it can be used up to 6-desorption cycles of removal, which further lowers the metal removal process of niches contaminated with it.



**Figure 7: Desorption of Chromium (VI) (250 mg/L) by different solutions (1 g of biomass, 28°C, 100 rpm).**

#### 4.0 Conclusion

The *C.reticulata* biomass complete capacity showed of biosorption and reduction concentrations of 1.0 g/L Cr(VI) in solution after 7 days of incubation, at 28°C, 100 rpm with 1 g of biomass.

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