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Biosorption of Trivalent Chromium from a Model Tanning Solution by Adapted *Aspergillus niger*

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ABSTRACT

Industrial effluents containing metallic species are responsible for environmental degradation which have been prioritised as major inorganic contaminants. Conventional methods are quite expensive resulting in need for cost-effective process for removing heavy metals from discharging effluents. The use of microbial biomass for removal of heavy metals from aqueous solutions (*biosorption*) is one such approach gaining increasing attention. Trivalent chromium ion present in tannery effluents has been the targeted ionic species for removal due to its exceeding limits in industrial discharges (≤ 0.3 ppm as per WHO). At NML, efforts were made for biosorption of trivalent chromium from tannery effluents with Cr (III) concentration in the range 1500-5000ppm. *Aspergillus niger*, obtained from a culture bank has been used in biosorption of trivalent chromium of tannery effluents. The fungal species grown in Czapek Dox Medium and adapted on Cr(III) ions ranging from 10-2000ppm at 2.5 pH and 35°C, was used for biosorption of chromium from a model tanning solution. *A.niger* was used in forms such as live, adapted and pre-treated (autoclaved, alkali-treated) for biosorption at pH 2.5 and 35°C. At Cr(III) conc. of 2000ppm in the aqueous solution, the adsorption efficiency followed the order: alkali treated (52%)>live(38%)>autoclaved dead mass(27%). The varying biosorption capacities may be attributed to exposed metal binding sites in alkali treated fungus causing high biosorption efficiency which also obeyed the sorption isotherm.

INTRODUCTION

Rapid industrialization coupled with exponential growth in population has led to many-fold increase in the utilization and release of chemicals including heavy toxic metals in aqueous systems. Heavy metals like mercury, cadmium, lead, nickel, and chromium are toxic even in extremely minute quantities¹⁻⁴. Of these, chromium is considered as an anthropogenic pollutant produced from industrial sites viz., electroplating, leather tanning, metal finishing, chemical industries and many others. Tanneries are mainly responsible for the release of huge amount of chromium (III) in the environment. Chromium exists in several oxidation states out of which Cr(III) and Cr(VI) are most stable form. High toxicity and potential carcinogenic character of chromium ions are responsible for fixing stringent permissible levels in potable and industrial wastewater are 0.05 and 0.1 mg/l respectively. Physicochemical methods including chemical reduction and precipitation, reverse osmosis, ion exchange and adsorption on activated carbon have been practised for several decades for the removal of toxic heavy metals from the effluent. But, all these methods suffer from severe constraints, such as incomplete metal removal, high reagent or energy requirements, generation of toxic sludge or other waste products that require safe disposal. Some of the treatment methods involve high operating and maintenance cost. There is, therefore, a need for some alternative technique, which is efficient and cost effective. Biosorption could be one such alternative method of treatment. It employs wide variety of biomass for removal of metal ions such as algae, fungi and bacteria, and is poised to emerge as a potential alternative to conventional methods used to decontaminate liquid wastes⁵⁻¹⁴. Biosorption has distinct advantage over conventional methods of treatments: the process does not produce sludge, hence non-polluting, is more efficient and easy to operate. It is particularly appropriate for removal of pollutants from very dilute solutions. Since biosorption often employs dead biomass, thus eliminates the need of nutrient requirement and can be exposed to environments of high toxicity. A major advantage of biosorption is that it can be used in-situ, and with proper design may not need any industrial process operations and can be integrated with many systems in the most eco-friendly manner. The cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures. This gives microorganisms an ability to bind metal cation. The interaction of metal ions and a functional group of microbe depend not only on the nature of biosorbent used but

also on the solution chemistry of the metal to be removed¹⁵⁻²⁰. In this study, a pure culture of *Aspergillus niger* was adapted on various concentrations of Cr(III) in the range 100-2000ppm. These adapted species were subsequently used for optimisation of parameters in biosorption of trivalent chromium from a model tanning solution at 35°C while shaking at 120rpm. The optimized pH was further used as standard parametric condition for Cr (III) biosorption at 35°C.

MATERIALS AND METHODS

Stock model tanning solution

Reagents used were prepared AR grade chemicals in deionised water. A model tanning solution containing Cr (III) was prepared with the following composition:

Sodium sulphate (Na ₂ SO ₄) – 5.0 g/L	Sodium sulphide (Na ₂ S) - 0.2 g/L
Sodium chloride (NaCl) - 25 g/L	Potassium dichromate (K ₂ Cr ₂ O ₇) - 0.001 g/L
Ferric chloride-0.02 g/L	Aluminium sulphate-0.04 g/L
Cr (III) – 2000ppm	

The pH of the solution was maintained to 2.5 by using 10 N H₂SO₄ and 0.1 N NaOH and desired concentration of Cr (III) ion as CrCl₃.6H₂O was prepared from the stock solution.

Microorganism for sorption of chromium

Pure culture of *Aspergillus niger* used in this study was obtained from Microbial Culture Collection Center, IMTECH, Chandigarh, India and was immediately transferred to sterile fungal slants of Czapek Dox Agar media (CZA). Fungal cells were cultivated at 25°C with repeated sub-culturings to ensure the activity of cells. The fungal biomass were transferred to 250 ml Czapek Dox broth containing the components shown in Table-1 and was incubated at 35 °C for 4 days in an orbital shaker at 120 rpm. The pH of the media was adjusted to 7.2. The cell suspension was then separated and stored for subsequent use in biosorption experiments

Table-1: Composition of Czapek Dox medium

CONTENTS	AMOUNT (g/L)
Sucrose	30
Sodium Nitrate	3
Dipotassium Phosphate	1
Magnesium Sulphate	0.50
Potassium Chloride	0.50
Ferrous Sulphate	0.01

Fungal species were used in different forms in the biosorption studies viz., live & adapted, autoclaved and alkali-treated. The pre-treatment was done as follows:

Live and adapted biomass

The Cr (III) adapted fungus was filtered and washed several times with distilled water so as to free it from the media components. It was air dried and 1.0g was used for 100 ml of model solution.

Autoclaved biomass

Known aliquot of adapted *A.niger* was taken in excess and autoclaved at 15 lb and 121°C for 15 min, then it was filtered and washed several times with distilled water to free the biomass from media

components and the biomass obtained was air dried; 1.0g of this was used per 100 ml of model tanning solution.

Alkali treated fungus

The adapted fungal species were boiled in 50 ml of 0.5 N NaOH for 15 min, filtered and washed several times with distilled water. It was then air dried and 1.0g of the alkali treated fungus was used for the experiment per 100 ml of model tanning solution.

Batch biosorption studies

Batch experiments were carried out in Erlenmeyer flasks by adding known volume/weight of fungal biomass in different forms mentioned above in desired concentration of Cr (III) solution was used in biosorption of metal. The flasks were gently incubated at 35 °C and 120 rpm in an orbital motion shaker. Samples were taken from the solution at predetermined time intervals for the estimation of residual Cr (III) ion concentration in the solution.

Samples drawn was treated under ultrasonic vibrations and then centrifuged at 10000 rpm for 5 minutes. The supernatant was filtered using Whatman No.42 filter paper and the biomass retained was washed with a slow stream of deionised water, and again passed through the filter assembly. The net supernatant collected was diluted into volumetric flasks using 5% HCl for estimation of Cr (III) ions by AAS (Model- GBC 980BT). The residual Cr (III) concentration was then calculated from the difference between the total Cr (III) initially added and the present value.

RESULTS AND DISCUSSION

Effect of Adaptation of *A.niger* on Cr (III)

Fungal cells grown in Czapek Dox medium at pH 2.5 and 35°C were adapted to various concentrations of Cr (III) ranging from 100-2000ppm. Adaptation of fungus to the metal ion rendered the cellular metabolism to increase its affinity for that species. Fig.2 (a-d) shows the microscopic observation of fungal cells which infers the growth of biomass and also indicates successful adaptation of *A.niger* at 1000ppm in 96h; the adapted mass in different forms were used for biosorption experiments.

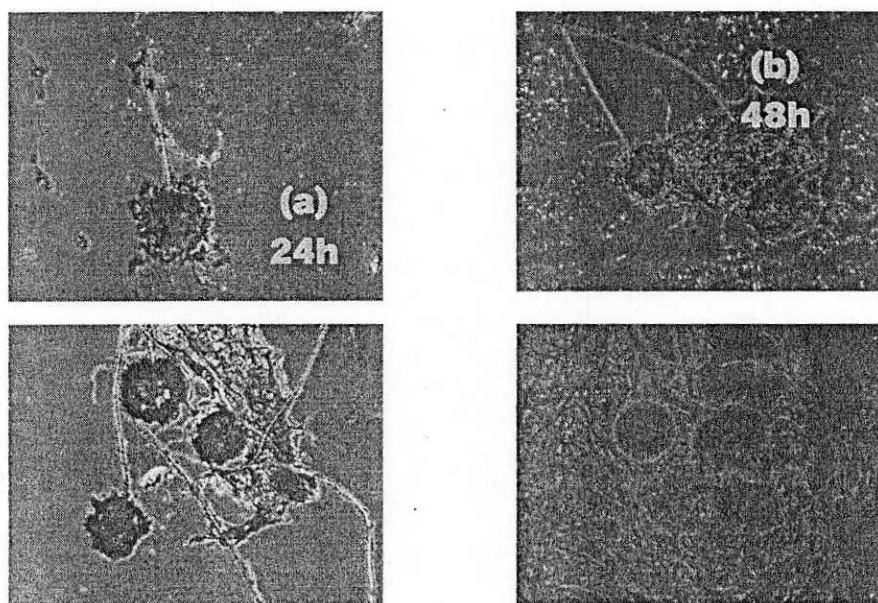


Fig. 2(a-d): Microscopic observation of *A.niger*'s adaptation to 1000ppm Cr (III) at pH=2.0 and 35°C

Biosorption Studies with 1000ppm & 2000ppm Cr (III) concentration

The biosorption of Cr(III) versus time was examined at varying initial Cr(III) concentrations, ranging from 500 to 2000 ppm using different forms of fungal species.

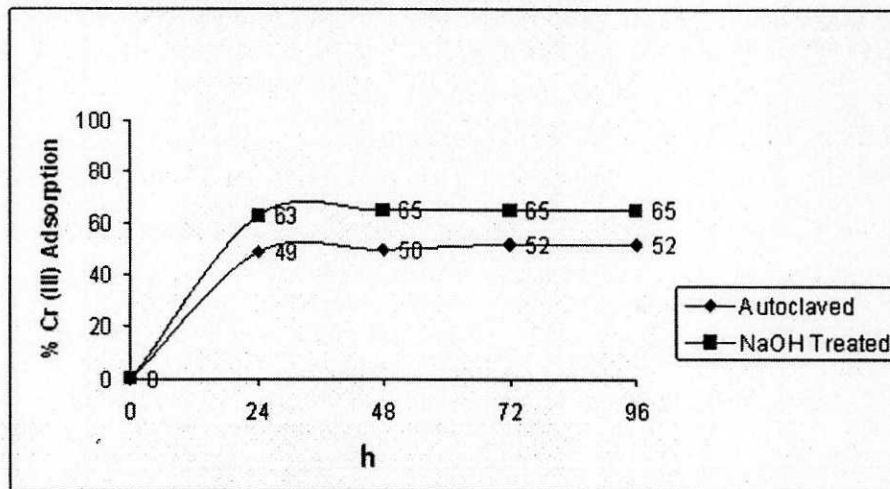


Fig. 3: Biosorption of 1000ppm Cr (III) by Autoclaved / alkali treated *A. niger*

The results presented in Fig.3 show that the dead fungus adsorbed 52% Cr (III) from the feed solution of 1000ppm Cr. The time taken by the autoclaved fungus was 72 h for removing maximum metal, although the uptake beyond 24h was not very different. However, alkali treated biomass was able to remove metal up to 65 % from the same model solution within 48h, the data for 24h was almost near to this value. Here, the alkali treated biomass followed the similar kinetic trend to that of the dead mass, but has proved to be more potent in removing Cr (III) from the solution.

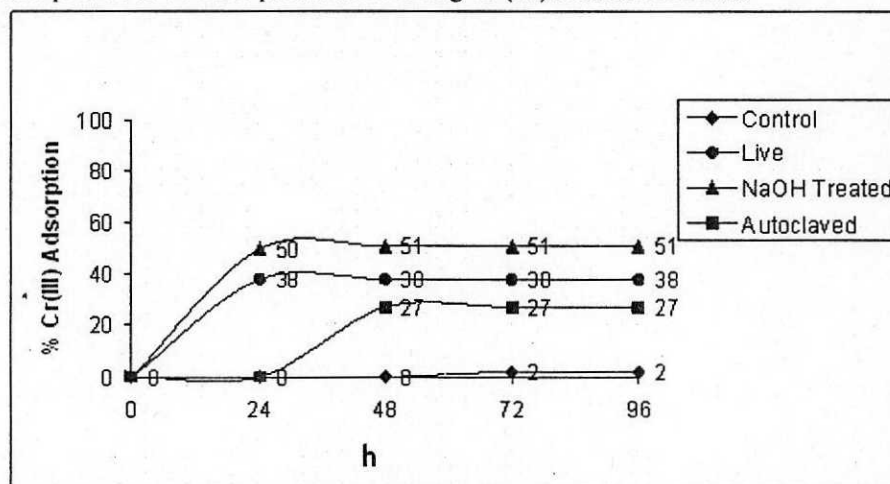


Fig. 4: -Biosorption of 2000ppm Cr (III) using various forms of *A. niger*

The results of biosorption of this metal from a feed solution of 2000ppm Cr(III) with three different pretreatments given to the biomass are shown in Fig.4. It can be seen that the control showed no significant removal of Cr (III) from the solution. The alkali treated biomass removed ~50% chromium within 24h, whereas the live and dead biomass were able to remove only 38% and 27% Cr(III) within 24h and 48h respectively. Thus, the alkali treated biomass was found to be more effective sorbent than the other two pretreated fungal biomass.

Adsorption Isotherms for biosorption of trivalent chromium

The experimental results obtained for the biosorption of chromium on *Aspergillus niger* at room temperature were analyzed using different isotherms.

Langmuir sorption model assumes that the uptake of metal ions occurs on a homogenous surface by monolayer adsorption without any interaction with the sorbed ions species. The model can be represented in the linearised form as:-

$$1/q = [(1/K_L \cdot q_m) (1/C_e)] + [1/q_m] \dots \dots \dots (1)$$

C_e = equilibrium concentration of metal in solution (mg/L)

q = amount of metal sorbed on the surface at equilibrium.

K_L = equilibrium constant related to the affinity of the binding sites for the metal or the Langmuir constant.

q_m = the biosorption capacity (maximum amount of metallic ion sorbed per unit mass of sorbent)

Whereas, *Freundlich model* assumes that the uptake or sorption of metal ions occurs on a heterogeneous surface by monolayer sorption and is described:

$$q = K_f (C_e)^{1/n} \dots \dots \dots (2)$$

$$\text{Log}(q) = (1/n) \log(C_e) + \log K_f \dots \dots \dots (3)$$

Terms in the equation carry the meaning as described above. The K_f and n are Freundlich constants that can be related to the biosorption capacity and intensity respectively.

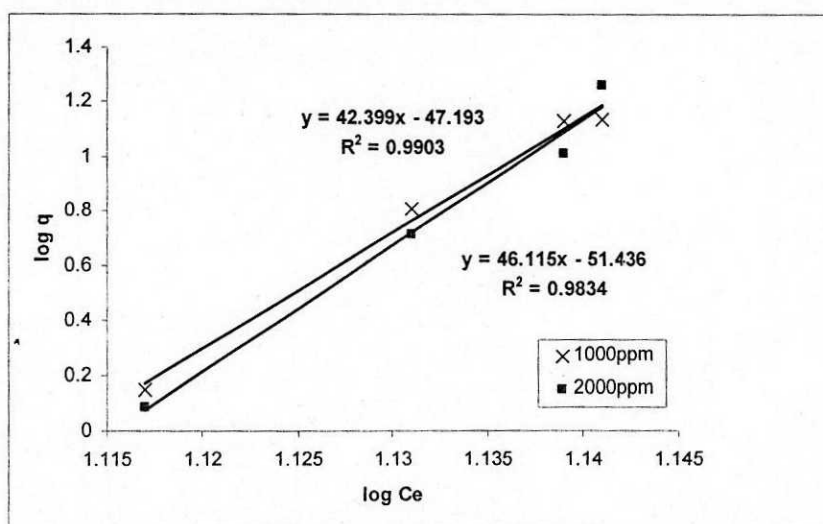


Fig.5: Freundlich Isotherm for Cr (III) biosorption on Alkali Treated *A. niger*

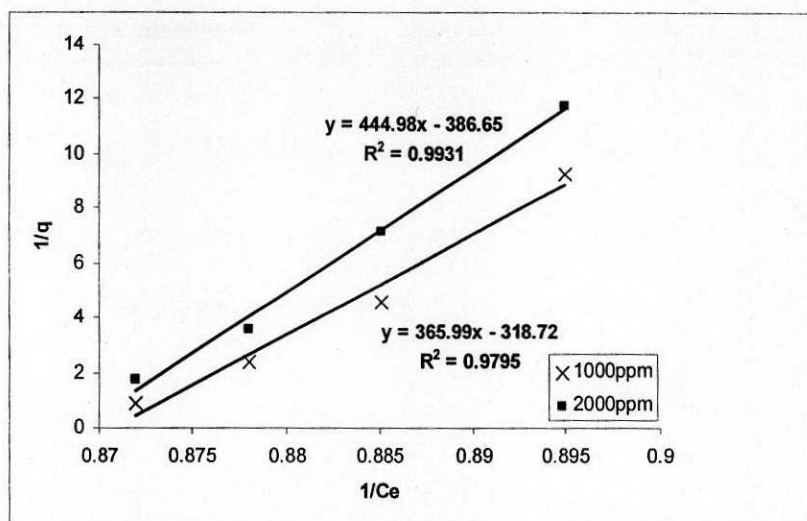


Fig.6: Langmuir Isotherm for Cr (III) biosorption on Alkali Treated *A. niger*

The equilibrium data on Cr (III) adsorption at pH 2.5 and 35°C were fitted into Freundlich isotherm (Fig.5). In case of chromium metal biosorption from a feed of 1000ppm, the sorption data showed good fit to the isotherm with a correlation coefficient value of 0.9903 (Table-2). For the biosorption with 2000ppm Cr (III) feed also, the data fitted well to the Freundlich isotherm showing R^2 value of 0.9834. The value of $1/n$ was found to be 1.15 and 1.21 and the values of K_f are found to be very high indicating a chemical interaction to the binding site of *A.niger* with chromium (III). The equilibrium data on sorption of trivalent chromium at pH 2.5 and 35°C also showed good fit to Langmuir isotherm (Fig.6) with fairly good correlation coefficient for the two feeds. However, very low values of K_L (Langmuir constant) which were found to be 1.12 and 0.08 for 1000ppm and 2000ppm Cr(III) levels respectively indicated limited physical interaction of Cr (III) with the surface of the fungus (Table-2). Thus, the sorption of chromium on *A.niger* was governed mainly by chemical interaction through complex formation as evidenced by high K_f values. The alkali treated *A.niger* might have resulted in exposure of binding sites through release of polysaccharides on the surface which, in turn could react with Cr(III) for complex formation⁷.

Table-2: R^2 values and equilibrium constants obtained for Langmuir and Freundlich isotherm for biosorption of Cr (III)

1000ppm Cr(III)					2000ppm Cr(III)				
Freundlich Model			Langmuir Model		Freundlich Model			Langmuir Model	
R^2	K_f	$1/n$	R^2	K_L	R^2	K_f	$1/n$	R^2	K_L
0.9903	7.3	1.15	0.9795	1.1	0.9834	8.66	1.21	0.993	0.08
	2			2				1	

Kinetics of Cr (III) biosorption

About 1.0g of biomass was taken for sorption of metal [200mL solution of 1000ppm and 2000ppm Cr (III)]. In order to study the kinetics of sorption, Lagergren expressions, (first and second order) were used. The Lagergren first order rate

expression is $(dq/dt) = k_1(q_e - q)$(4) generally described as

Where, q_e and q are the amounts of Cr(III) ion, (mg/g) adsorbed on the sorbent at equilibrium and at time t , respectively and k_1 is the rate constant(/h). Integrating and applying the boundary conditions, $t = 0$ and $q = 0$ to $t = t$ and $q = q_e$ at maximum sorption, equation (4) takes the form:

$$\log (q_e - q) = \log(q_e) - (k_1/2.303)t \dots \dots \dots (5)$$

Sorption data were also fitted to the *second order expression* represented as:

$$(da/dt) = k_2 (ae - a)^2 \dots \dots \dots (6)$$

Where, k_2 is the rate constant of second order sorption (g/mg.h). Integrating and applying boundary conditions $t = 0$ and $q = 0$ to $t = t$ and $q = q_e$, equation (6) can be presented in the linear form as

$$(t/q) = (1/h) + (1/q_e)(t) \dots \dots \dots (7)$$

Where, $h = k_2 q_e^2$ is the initial sorption rate.

The components of rate expression for biosorption of trivalent chromium on *A.niger* are detailed in Table-3.

Table-3: Kinetics of biosorption of chromium [Aqueous Feed- 1000ppm & 2000ppm, Volume of Aqueous Feed- 200mL, pH-2.5, Biomass-1.0g, Temperature-35°C]

Time in h (t)	Residual conc (Ce) [mg/mL]		Cr sorbed (q) [mg/g]		(qe-q)		log(qe-q)		(t/q)	
	1000 ppm	2000 ppm	1000 ppm	2000 ppm	1000 ppm	2000 ppm	1000 ppm	2000 ppm	1000 ppm	2000 ppm
6	0.733	0.0983	1.2	2.9	0.66	1.42	0.032	0.152	5.0	2.068
24	0.457	0.0647	4.4	3.8	0.37	1.11	-0.180	0.045	5.45	6.325
48	0.266	0.0347	4.9	4.4	0.34	0.59	-0.468	- 0.221	9.79	10.90
72	0.325	0.0362	5.1	5.1	0.32	0.37	-0.494	- 0.432	14.11	16.11
96	0.347	0.0374	5.2	5.4	0.31	0.31	-0.508	- 0.508	18.46	17.78

Fig.7 & 8 represent the kinetic plots for the first and second order expression whereas Table-4 shows the values of correlation coefficients (R^2) along with the rate constants for both concentrations.

Table-4: R^2 values and rate constants for 1000ppm and 2000ppm Cr(III) concentrations

1000ppm Cr(III)					2000ppm Cr(III)				
1 st order		2 nd order			1 st order		2 nd order		
R^2	k_1	R^2	k_2	h	R^2	k_1	R^2	k_2	h
0.9149	-0.67	0.9835	0.62	32.83	0.9118	-0.69	0.9844	0.68	34.67

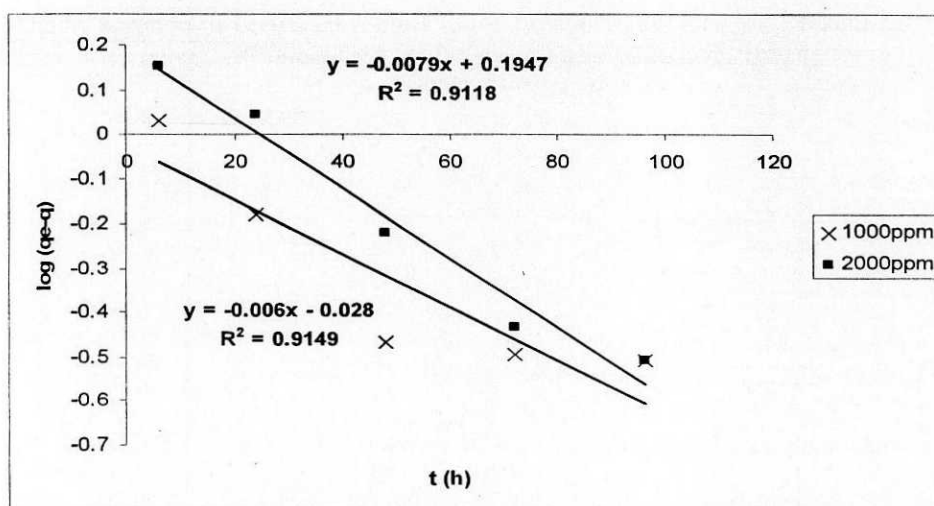


Fig.7: First order kinetics of Alkali treated Biomass for the sorption of Cr (III)

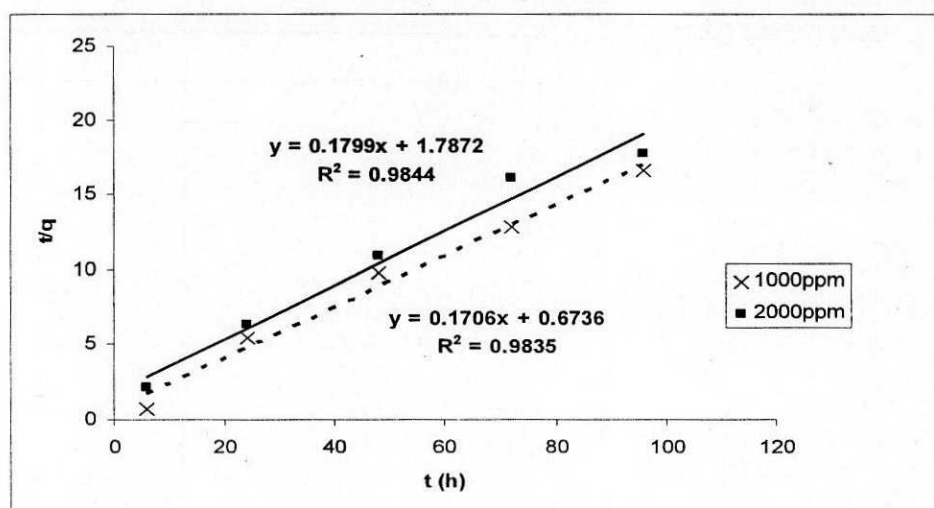


Fig.8: Second order kinetics of Alkali treated Biomass for the sorption of Cr (III)

The values of R^2 for the second order equation were found to be nearly unity as compared to the first order equation for the both concentrations. The very low values (negative) of k_1 for the two aqueous feeds and high values of k_2 showed that the biosorption of chromium (III) on *A.niger* follows second order kinetics at room temperature.

CONCLUSIONS

Adapted *A.niger* with chromium (III) in different forms is found suitable for removing this metal from a model tanning solution. Alkali treated biomass is a better sorbent than live and dead fungus for 2000ppm Cr(III) feed.

For the alkali pretreated biomass, biosorption of ~52% Cr(III) from a feed of 2000ppm concentrations is achieved as against metal removal of 65% from a dilute feed of 1000ppm solution.

Removal of surface impurities, rupture of cell membrane and exposure of available binding sites due to release of polysaccharides for binding Cr(III) chemically through complexation may be the reason for the increased metal uptake by alkali treated fungus. Best fit to Freundlich isotherm further indicates the chemical interaction of Cr(III) with the polymeric substance available on the fugal

surface. Sorption of Cr(III) on *A.niger* follows second order kinetics. Reasons for varying degree of metal biosorption on different forms of the sorbent need to be investigated.

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