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DAMA

SACCHAROMYCES CEREVISIAE AS A BIOSORBENT FOR DETOXIFICATION OF CR (VI)

Kulkarni R. A.[1] **And S. G. Gupta**[2]

[1]**Principal, Government College of Arts and Science, Aurangabad, M. S. India.**
[2]**Director, Government Institute of Science, Aurangabad, M. S. India.**

ABSTRACT

Environment pollution is a constant threat faced by humanity. Industrial effluents entering in surface water are one of the most important sources of contamination adding various toxic metals like chromium, cadmium, nickel etc. Microorganisms have great potential to accumulate these metals and detoxify it. Biomass waste, mainly *Saccheromyces cerevisiae* is generated on a large scale from brewing industry which can be used to detoxify metals. In this paper Saccheromyces cerevisiae is used as a bioremediater. Microbial cells are used as waste non growing biomass and effect of various parameters affecting biosorption was studied. From the adsorption studies it was observed that *S. cerevisiae* is capable of adsorbing 33mg/gm of hexavalent chromium.

KEYWORDS: Biosorption, *Saccheromyces cerevisiae*, Chromium (VI)

INTRODUCTION

Environmental pollution is a constant threat faced by humanity. Industrial effluents entering into the surface water are one of the most important sources of toxic contamination in the environment. Industries effluent contain heavy metal ions such as chromium, nickel, lead, copper, zinc etc. which interfere with metabolism of living environmental systems. Chromium compounds are extensively used in many industries which include tannery, textiles, metal electroplating, paint and pigment industries that adds Cr (VI) to effluent.

Hexavalent chromium at a concentration of 10g/kg of body weight causes liver necrosis, nephritis and even death in human beings (Dikshit *et.al.* 1989).The properties of heavy metals which warrant their reclamation from effluents are there toxicity and commercial value (Kasam and Baecker,1988). Though the conventional methods such as precipitation, ion exchange, evapouration, reverse osmosis have been reported to effectively treat chromium bearing effluents(Chand *et. al.*, 1994) they are expensive and are especially ineffective when the metal ion concentration in aqueous solution is lower than 50mg/ L. Moreover such treatmet produces large amount of sludge to be treated with great difficulties.

Therefore treatment for this waste is important. Microorganisms can remove heavy metal ions from aqueous solution by various mechanisms, which may or may not be related to the metabolic processes of living cells (Norris and Kelly, 1979). In recent years, the process of accumulation of heavy metals by microorganisms was intensely studied. Bacteria, yeast and fungi (Nakajam and Sakaguchi, 1986) as well as algae (Holan *et.al.*, 1993) are being used for metal removal from effluents. Chromium is one of the discharge from the electroplating industries. Hexavalent chromium (Cr^{6+}) due to its water solubility is toxic to living cells so it is important to remove hexavalent chromium from the effluent. Various physiochemical methods include ion exchange, reverse osmosis, precipitation etc.

One of the most ubiquitous biomass type available for bioremediation of metal is yeast. Yeast retains its removal ability for a broad range of heavy metals. *S. cerevisiae* has proved to be use in bioremediation. It is easy to cultivate on large scale. It can be easily grown by unsophisticated fermentation techniques and inexpensive growth media (Kapoor and Virarghavan, 1995) and yield of the biomass is also high. It is generally regarded as safe. Therefore, biosorbent made from *S. cerevisiae* can be easily accepted by the public when applied practically as sorbent to recover metal ions. *S. cerevisiae* is an ideal model organism to identify the mechanism of biosorption in metal ion removal, especially to investigate the interactions of metal-microbe at molecular level. In this paper growth independent Cr (VI)sorption studies were carried out using *Saccheromyces cerevisiae.*

MATERIALS AND METHODS

1. *S. cerevisiae* suspension (Absorbance= 1.0 at 600nm)
2. Cr (VI) stock solution (1000ppm) The metal used for the present investigation was potassium dichromate. The stock metal solution was prepared by dissolving 3.735gm of potassium dichromate in 1000ml distilled water which is further diluted.

A. BIOSORPTION OF Cr (VI) :

Optimization of the important parameters using *S. cerevisiae* was carried out with respect to the

1. Initial metal optimization experiments were carried out using 50-500ppm of metal solution in Erlenmeyer flasks to which 1% (W/V) biomass was added, pH was 7.0 incubated at 30^0C for 30 minutes on a rotary shaker. After retentation the contents were centrifuged at 8000rpm and residul Cr (VI) was analyzed using AAS and percent sorption was calculated.
2. Effect of pH: 100 ml of hexavalent chromium (20ppm) solution with various pH 3,5,7 9 and 11were used
3. Effect of holding time on percent sorption of 20 ppm Cr(VI) containing metal solution inoculated by 1% biomass (*S. cerevisiae*) was studied by varying the holding time at intervals of 30 minutes.
4. Effect of initial biomass of *S. cerevisiae* was calculated using varying concentration of 1-5% biomass.
5. Effect of various temperature was studied where *S cerevisiae* (1%) was inoculated in 100ml of hexavalent chromium (20ppm) with pH 7.0 and they were incubated on shaker at various temperatures 10, 20, 30, 40 and 50^0 c for 3 minutes and analysed for residual chromium

The total Chromium was estimated by using Atomic Absorption Spectrophotometer.

B. ADSORPTION ISOTHERMS AND KINETIC STUDIES:

Adsorption isotherms were appilied to the biosorption experiments carried out using *S.cerevisiae* growth independent percent sorption of Cr (VI) with pH 7.0 at 30°C on a rotary shaker at 100 rpm with 1% (w/v) inoculum concentration for varying period of time. In case of growth independent sorption after each 30 minutes results were taken. The data thus obtained was applied to different adsorption isotherms like Langmuir(1918) and Freundlich (1926)and the graphs obtained were as follows.

Kinetics studies were carried out by growth independent Cr (VI) uptake by *S.cerevisiae*. The 1% (w/v) biomass of *S.cerevisiae* was inoculated in 100 ppm Cr (VI) solution with 7.0 pH. It was incubated at 30°C and after each 30 minutes reaction was terminated by centrifugation at 10,000 rpm and the supernatant was analyzed. The percent sorption of Cr (VI) data thus obtained was used for kinetic studies. The Langergen(1998) kinetic model (pseudo first order) and pseudo second order model was studied.

RESULTS AND DISCUSSION:

A. Growth independent percent sorption of Cr (VI) by *S. cerevisieae*

Biosorptive capacity of metal ions was reported to be related to the ratio of the concentration of initial metal ions to the concentration of the biomass. The percent sorption of Cr (VI) by growth independent *S. cerevisieae* was found to be in between the range of 50-70%

Result in Table 1 indicates the effect of initial concentration of Cr (VI) percent sorption of metals. It was observed that maximum Cr (VI) sorption was 67% at 200ppm and 65% at 1% (w/v) of biomass concentration. Vasudevan *et al.*, (2003) found that equilibrium uptake for Cd^{2+} by the protonated yeast was directly proportional to the ratio of the initial metal concentration to the sorbent mass. Therefore, both aspects cannot be neglected when assessing the influence of concentration of the metal ion and the biomass on biosorption, otherwise error would occur (Schiewer and Volesky, 1995).

Table 1 : Effect of initial Cr (VI) concentration on its sorption by *S.cerevisiae*

Initial metal concentration (ppm)	Percent sorption Cr (VI)
50	45
100	54
150	65
200	67
250	66
300	67
350	64
400	61
450	60
500	60

Optimization experiments showed that metal sorption is a rapid process maximum adsorption of Cr (VI) was 92 % after 90 minutes after that it remained constant (Table 2).The biosorption process of heavy metals by *S. cerevisiae* completed rapidly. The biosorption of metal ions of copper, zinc, lead and uranium by non -growing cells of *S. cerevisiae* is a rapid process and often reaches to equilibrium within few hours. Ferraz, *et. al.* (2004) optimized the sorption time for Cr (III) by *S. cerevisiae* from a brewery company in the sorption and desorption process. Results showed that a 30 minute sorption period was the best option to ensure the metal removal from solution and good recovery from biosorbent.

Table 2: Effect of holding time on precent sorption of Cr (VI) by *S.cerevisiae*

Holding time (minutes)	Percent sorption Cr (VI)
30	55
60	88
90	92
120	92
150	92

Table 3: Effect of pH on percent sorption of Cr (VI) by *S.cerevisiae*

pH	Percent sorption Cr (VI)
3.0	12
5.0	15
7.0	65
9.0	46
11.0	18

The sorption of Cr (VI) as a function of pH indicated in Table 3 showed that maximum sorption of Cr (VI) was at pH 7.0. At higher and lower pH values, the percent sorption gradually decreased. The results were similar as that Zn2+ adsorption by *Mucor hemilis* and *Penicillium chrysogenum* which gets decreased as pH decreases below 4.0 (Fourest *et.al.*, 1994).

Table 4 : Effect of temeperature (C) on percent sorption of Cr (VI) by *S.cerevisiae*

Temperature	Percent sorption Cr (VI)
10	19
20	45
30	60
40	38
50	14

Adsorption reaction are normally exothermic, so biosorption capacity increases with decrease of temperature (Kapoor and Virarghavan, 1997). In this study 60% of Cr (VI) was adsorbed (Table 4) at 30C. The decrease of active binding sites in the biomass (Ozer and Ozer,2003).

Table 5: Effect of inoculum concentration of *S.cerevisiae* on percent sorption of Cr (VI)

Inoculum level % (w/v)	Percent sorption Cr (VI)
1	51
2	62
3	65
4	68
5	69

Effect of inoculum level of *S. cerevisiae* studies (Table 5) showed that if there was an increase in inoculum level there was also increase in percent sorption of metal ions under study. Initial inoculum level of 2-3 % (v/v) gave maximum percent sorption even if there was an increase in percent sorption there was no significant an increase in percent sorption of Cr (VI).

B. ADSORPTION ISOTHERMS AND KINETIC STUDIES:

In the present study growth independent sorption of Cr (VI) by non-growing *S. cerevisiae* cells is presented in Fig.1 This data is further used to the isotherm calculation (fig.2 and 3). It was observed that in Langmuir isotherm a straight obtained indicating that the data fits in this model. Further the regression coefficient study was carried out which showed 0.99 and that the data fits more in Langmuir isotherm. Hence this data is used to predict the maximum sorption of chromium and from it Qmax for Cr (VI) calculated gave 33mg/gm (Table 6).

Fig 1:Percentage sorption of Cr (VI) by growth independent *S. cerevisiae*

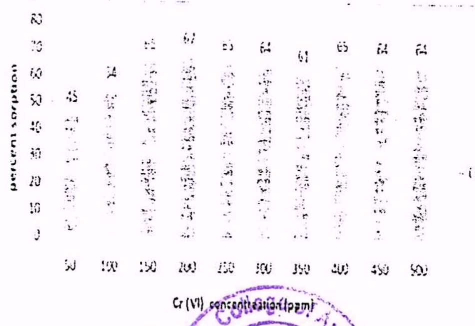

Fig. 2: Adsorption isotherm studies using dead biomass of *S.cerevisiae* (Langmuir)

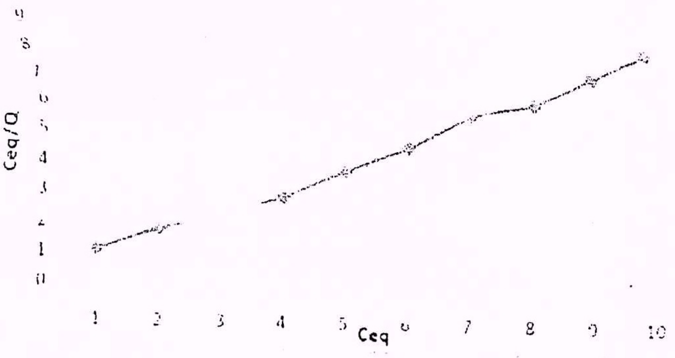

Fig. 3: Adsorption isotherm studies using dead biomass of *S.cerevisiae* (Freundlich)

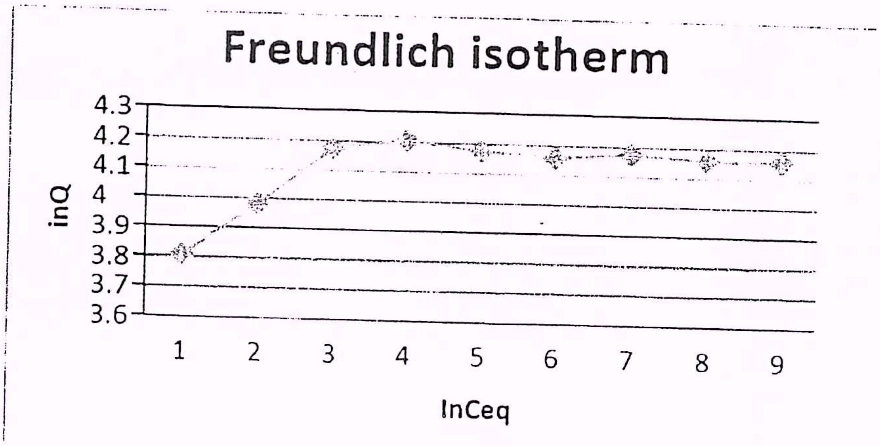

Table 6: Adsorption isotherms parameters for growth independent *S cerevisiae*

Parameters	Langmuir	Freundlich
Slope	0.015139	0.015363
Regression	0.99	0.70
Qmax	33 mg/gm	-

The pseudo first order and second order kinetics model were successfully employed for explaining the kinetic data of adsorption process (Fig.4 and 5) Straight line obtained after plotting Log (qe-qt) vs t and t/qt vs t shows degree of fitness of metal sorption to first and second order rate kinetics model. This is based on the assumption that the adsorption capacity for the metal on the adsorbent is proportional to the number of active sites occupied on the sorbent and metal uptake is by chemisorption. The values of constant of Kd and R^2 were calculated from the plots (Table 7). From the data obtained Pseudo second order was found to be most suitable for adsorption of Cr (VI).

Fig. 4: Growth kinetics of Cr (VI) sorption by Pseudo first order model

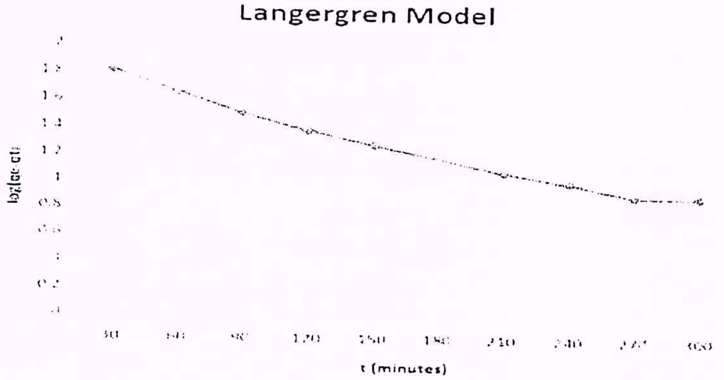

Fig.5: Growth kinetics of Cr (VI) sorption by Pseudo second order model

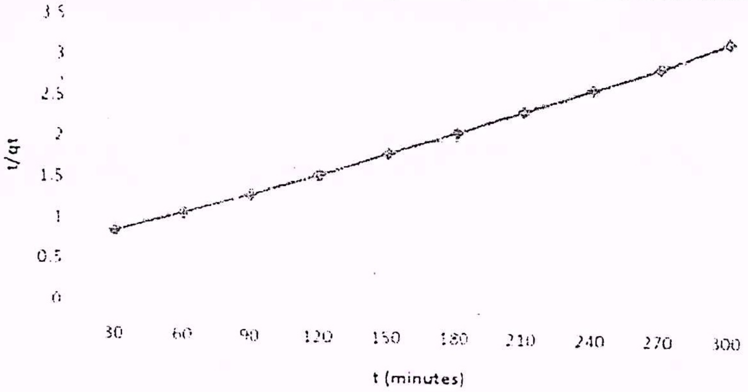

Table 7: Comparison of kinetic model constant for pseudo first order and second order.

Pseudo first order			Pseudo second order		
Adsorbents	**Kd (slope)**	**R^2 (Correlation coefficient)**	**Qe (Slope)**	**K^2 (Intercept)**	**R^2**
Dead biomass of *S. cerevisiae*	0.00343	0.9735	0.008876	0.54093	0.9985

CONCLUSION

Growth independent sorption studies carried out using *S. cerevisiae* showed that free cells appear unsuitable in practical application, largely due to solid/ liquid separation problem. However, Veglio and Beolchini (1997) pointed out that investigation on the performance of free cells for metal uptake can provide fundamental information on the equilibrium of the biosorption process, which is useful for practical application. Meanwhile, flocculation cells have been suggested for biosorption, attempting to overcome the separation problem of free cells (Soares *et.al.,* 2002).

Whether to employ living cells or non-living cells for biosorption is still at arguing state (Suh and Kim,2000). In the early researches on biosorption of heavy metal ions, living cells were used. However, dead cells have been found to have the same or even higher uptake capacity of metal ions, comparing with living cells and dead cells can overcome

PRINCIPAL
Govt. College of Arts & Science
Aurangabad

some limits compared to living cells like nutrition demand, sensitivity to extreme pH value, higher metal ion concentration, etc. Therefore, biosorption studies involving dead/pretreated biomass have dominated during 1980's-90's. However, the limitations of the industrial application of biosorption with immobilized dead cells have been realized from some pilot plants. For example, the cost for producing the required biosorbent with waste biomass was too expensive using immobilized techniques and using various pre-treatment processes. Process of regeneration and re-use on-line is complex and very expensive. For real effluent, the co-existed ions and organic matter in aqueous solution made situation even more difficult and more complex. Hybrid biotechnologies, such as biosorption. Bioprecipitation and bioaccumulation using living cells, even together with physic-chemical process, are suggested in recent year (Tsezos,2001). As a waste microbial biomass from fermentation, study on dead cells of the yeast is also dominant and necessary to apply on commercial level.

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