



## Isotherm Models for Chromium Biosorption by Live & Dead Biomass of *Bacillus Subtilis* & *Saccharomyces Cerevisiae*

## KEYWORDS

Biosorption; Hexavalent Chromium; *B. subtilis*; *S. cerevisiae* .

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## ABSTRACT

*Chromium pollution by industrial effluents is one of the significant environmental concerns due to its toxic nature and accumulation in food chain as non-biodegradable pollutant. In this study, live and dead biomass of Bacillus subtilis and Saccharomyces cerevisiae was assessed for its efficiency to remove chromium(VI) from aqueous solutions. The live and dead biomass has the maximum biosorption capacity of 60.02% & 70.80% for B. subtilis and 89.78% & 99.74% for S. cerevisiae at 50ppm initial chromium concentration. The biosorption of Cr(VI) is well described by Langmuir isotherm, which denotes the monolayer adsorption in the experimental conditions. The result indicated that S. cerevisiae has highest metal (Cr) tolerance and biosorption capacity.*

## Introduction

Hexavalent chromium Cr(VI) is widely used in industrial processes (e.g., stainless steel manufacturing, chrome plating, leather tanning, wood treatments, dyes, and pigments). Soluble Cr(VI) can readily cross cell membranes and is quickly taken up by both prokaryotic and eukaryotic cells (Chatterjee and LUO, 2010). The US EPA has set the maximum contaminate level for Cr in domestic water supplies at 0.05mg/l (Baral and Engelken, 2002). Therefore, reduction of Cr(VI) to Cr(III) is an effective way of avoiding its ill effect. There are some conventional physicochemical methods but most of them suffer with cost ineffectiveness and generation of large metallic sludge. Hence, the use of intact microbial cells, either live or dead, or their product as biosorbents of chromium to decontaminate liquid wastes has gained important credibility during recent years (Shrivastava and Thakur, 2003).

Biosorption not only offers an innovative alternative to other remediation approaches, but also allows metals recovery (Viraraghavan and Srinivasan, 2011). The living and non-living cells of microorganisms are reported to remove Cr(VI) from aqueous solutions (Sen and Dastidar, 2007). The advantages of non-living cells over living and resting cells due to the absence of both toxicity limitations and requirements of growth media and nutrients (Ghosh et al., 2013). Present study investigates the chromate removal efficiency of *Bacillus subtilis* and *Saccharomyces cerevisiae* with their live and dead biomass under laboratory conditions. Adsorption isotherm models were also studied.

## Materials and Methods

## Micro-organism and growth conditions

Pure culture of *Bacillus subtilis* and *S. cerevisiae* was obtained from Department of Microbiology, G.B.P.U.A. & T., Pantnagar. Bacterial cell was cultivated in medium containing: soluble Dextrose-20 g/l; yeast extract -2 g/l; peptone -5 g/l; NaCl - 5 g/l. (pH adjusted to 7.2). Cells were inoculated on Petri dishes by scratching and were left over night at 30°C in the incubator. After 48hr of incubation at 30±1°C, biomass was harvested by means of centrifugation at 10,000 rpm for 10 minutes, washed twice with distilled water and then dried for 6 hours at 80°C in

an air oven. The dried biomass was then crushed with a mortar pestle to a fine powder. The powdered biomass was stored in an air tight pack and used for biosorption (Sivaprakash et al., 2009).

The yeast *Saccharomyces cerevisiae* were routinely maintained on a solid yeast peptone dextrose (YPD) medium, comprised of the following (g/L): neutralized bacteriological peptone of 10.0, yeast extract of 5.0, dextrose of 10.0, and agar of 18.0. For experimental purposes, cultures were grown in 150 mL of liquid medium with the same composition (without agar) at 30°C and pH 7.0 with rotary shaking at 150 rpm in 250-mL Erlenmeyer flasks (Blackwell et al., 1998). Remove it from the shaker and heat it in the water bath at 100°C for 10 min for dead yeast biomass.

## Metal Solution

All the reagents were of Analytical Reagent Grade and were prepared in Double Distilled H<sub>2</sub>O. An aqueous stock solution (1000 mg/l) of Cr (VI) ions was prepared using potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) salt. This was used as the source of Cr (VI) in the synthetic wastewater. pH of the solution was adjusted using 0.1 N HCl or NaOH. Fresh dilutions were used for each study (Lokeshwari and Joshi, 2009).

## Cr (VI) Ion Determination

Analysis of heavy metals was carried out from digested effluent using atomic adsorption spectrophotometer (AAS). The percentage of Cr removal due to biosorption was calculated as

$$\% \text{ Cr removal} = [(C_o - C_e) / C_o] \times 100\% \quad (1)$$

Where C<sub>i</sub> and C<sub>e</sub> are the initial and equilibrium concentration of Cr (VI) solution (mg/L), respectively.

## Result and Discussion

## Effect of pH

To examine the effect of pH on the Cr(VI) removal efficiency, the pH varied from 2.0-8.0 as shown on (Fig 1). For live and dead biomass of *B. subtilis* the maximum removal of Cr(VI) 60.09% & 70.08% was observed at pH 3.0. For live

(89.78%) and dead (99.33%) biomass of *S.cerevisiae* the maximum removal of Cr(VI) was observed at pH 4.0.

Higher sorption at lower pH, due to the presence of hydronium ions present on the surface of sorbent, which enhance the attractive forces of Cr(VI) interaction with binding sites of the biosorbent. As the pH increased, however, the overall surface charge on the cells became negative and decreased the biosorption capacity (Ozer and Ozer, 2003).

#### Effect of Temperature

The effect of different temperature regimes showed that the uptake of Cr(VI) from live biomass of *B.subtilis* and *S.cerevisiae* increases upto 30°C and beyond that it decrease as shown on (Fig.2). Similar results have been reported in the biosorption of Cr(VI) from dead biomass of *B.subtilis* and *S.cerevisiae*. At 30°C for live and dead biomass of 60.2% & 70.5% removal was observed in *B.subtilis* and *S.cerevisiae* maximum removal 89.09% & 99.33% was observed. Temperature above 30°C resulted lower sorption efficiency of Cr(VI), which may be due to the damage of active binding sites in the biomass. When the temperature increases and become too high, then the metal sorption capacity decreases due to distortion of some sites present on the surface of the cell available for metal biosorption (Goyal et al., 2003).

#### Effect of Chromium (VI) Concentration

As the Cr(VI) concentration increased, the cellular growth of all the organisms inhibited. The result shows that sorption efficiency decreases with increase in chromium concentration and sorption capacity increases with increase in Cr(VI) concentration upto 200 ppm (Fig.3). In 50ppm maximum removal 60.02% & 70.80% was observed from live and dead biomass of *B. subtilis* and 89.78% & 99.74% was observed from *S.cerevisiae*. At lower concentrations, all metal ions present in the solution would interact with the binding sites and thus facilitated 100% adsorption. At higher concentrations, more Cr ions are left unabsorbed in solution due to the saturation of binding sites. This appears to be due to the increase in the number of ions competing for the available binding sites in the biomass. This phenomenon can be attributed to the decrease in the length of the adsorption zone (Kiran and Kaushik, 2008) and to the fact that by increasing the initial concentration, bonding sites will be saturated faster (Zhang et al., 2011).

#### Analysis of adsorption isotherms

Adsorption isotherms were used to characterize the interaction of each chromium species with the adsorbent. This provides a relationship between the concentration of Cr(VI) in the adsorption medium and the amount of Cr(VI) adsorbed on the solid phase when the two phases are at equilibrium Langmuir and Freundlich adsorption isotherms are the two widely used isotherms.

#### Langmuir Model

The Langmuir model is based on the assumption of surface homogeneity such as equally available adsorption sites, monolayer surface coverage, and no interaction between adsorbed species. The following represents the Langmuir isotherm equation (Langmuir, 1918).

$$C_e/Q_e = 1/Q_0 b + C_e/Q_0 \quad (2)$$

where  $q_e$  is the amount of metal adsorbed (mg/g),  $C_e$  is the equilibrium concentration of solution (mg/L).  $Q_0$  and  $b$  are Langmuir constants indicating adsorption capacity and energy, respectively. The parameters  $Q_0$  and  $b$  have been

calculated and the values including that of the correlation coefficients ( $R^2$ ) are shown in Table 1.

#### Freundlich Model

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal ion binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. The empirical equation takes the form (Freundlich, 1906).

$$\log q_e = \log k + 1/n \log C_e \quad (3)$$

where  $q_e$  and  $C_e$  are the equilibrium adsorption capacity of the biosorbent and the equilibrium concentration in the aqueous solution, respectively.  $k$  and  $n$  are Freundlich constants related to sorption capacity and sorption intensity of adsorbents. The value of  $n$  falling in the range of 1– 10 indicates favorable sorption (Sivaprakash et al., 2009). The adsorption constants and correlation coefficients obtained from the Langmuir and Freundlich isotherms are provided in (Table1). According to Table1 data generally fitted Langmuir adsorption isotherm with higher  $R^2$  value better than the Freundlich adsorption isotherm. The dead biomass showed better affinity to Cr(VI) than live biomass but low capacity may be attributed due to loss of some homogeneous. The best fit of equilibrium data for Langmuir expression confirms the monolayer adsorption occurred over a surface containing a finite number of adsorption sites (Ha-meed et al., 2009).

#### Conclusion

Biosorption process depends significantly on the pH of the solution and is favored at around pH value of 4.0 and 3.0. Comparing the regression coefficient ( $R^2$ ) for the two isotherms, it was found that Langmuir is the best with average  $R^2$  value. Comparative analysis of biosorption data indicated that dead biomass showed better chromium capacity than live biomass due to metabolic entrapment. On the other hand in comparison to live biomass, dead biomass has stronger capacity for heavy metal biosorption due the proton produced during metabolism (Pardo et al., 2003). The maintenance of live microbial population is very difficult due to the requirement of additional nutrient sources and suitable environmental conditions (Malik, 2004). Dead biomass mainly uptake heavy metals in passive mode through adsorption and ion exchange which is independent on energy.

**Table 1  $R^2$  values and equilibrium constants obtained for Langmuir and Freundlich isotherm for biosorption of Cr(VI) at 30°C**

Langmuir isotherm		b	$Q_{max}$	$R^2$
<i>B.subtilis</i>	Live Biomass	0.864	30.3	0.906
	Dead Biomass	0.971	35.7	0.909
<i>S.cerevisiae</i>	Live Biomass	2.330	47.61	0.991
	Dead Biomass	2.641	62.54	0.995
Freundlich isotherm		k	n	$R^2$
<i>B.subtilis</i>	Live Biomass	1.690	6.62	0.952
	Dead Biomass	1.702	7.29	0.968
<i>S.cerevisiae</i>	Live Biomass	1.832	7.39	0.952
	Dead Biomass	1.854	10.75	0.984

Cr(VI) conc. range 50-200 ppm, pH=3, 2 & 4, Temperature=30°C, biomass loading range 4 g/l

Fig (1): Effect of pH on the Cr(VI) uptake by live and dead biomass of *B. subtilis* and *S.cerevisiae*

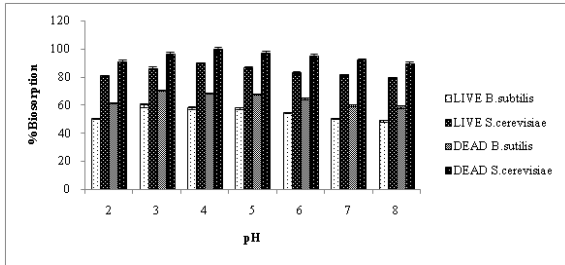


Fig (2): Effect of temperature on the Cr(VI) uptake by live and dead biomass of *B. subtilis* and *S.cerevisiae*

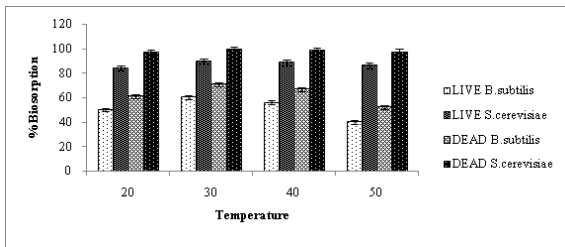
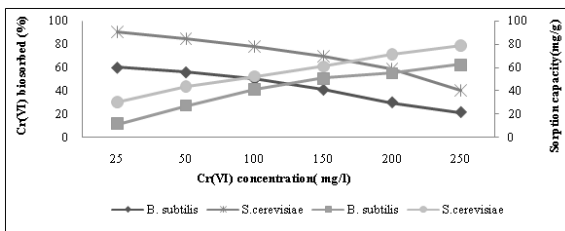


Fig (3): Effect of concentration on the Cr(VI) uptake by live and dead biomass of *B. subtilis* and *S.cerevisiae*



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