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Full Length Research Paper

Reduction of hexavalent chromium by *Rhizopus Oryzae*

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The ability of *Rhizopus* oryzae to reduce Cr^{6+} was evaluated in batch microcosms. The optimum pH of *R. oryzae* growth was between 6.0 and 7.0. The maximum chromium reduction efficiency of 91.15% and biomass growth was achieved at a pH of 7, temperature of 37°C, with an initial Cr^{6+} concentration of 400 ppm and incubation period of 72 h. Monod and Haldane models were used to describe the chromium reduction data and the specific growth rate constant value was calculated as 0.082 and the reduction rate was found to be highest at 400 mg Cr^{6+} /l. The high removal of chromate by *R. oryzae* indicates a feasible, economical technique for chromate removal from industrial wastewater effluents.

Key words: Hexavalent chromium, *Rhizopus Oryzae,* leather tanning, Monod and Haldane models.

INTRODUCTION

Exposure to heavy metals is toxic to the environment, human beings and to animals. Heavy metals include lead, silver, mercury, copper, nickel, chromium, zinc, cadmium and tin. Effluents containing heavy metals are released from a variety of industries such as metal plating, textiles, pulp and paper, leather tanning, metal finishing, pigment and dyes, photography, wood preserving, metallurgical and mineral processing, mining, smelting, rayon fibre production, motor vehicles and air craft industries, etc., For example, the electroplating process generates heavy metals like arsenic, cadmium. Chromium (hexavalent), copper, lead, mercury, nickel, silver, and zinc (Germain and Patterson, 1974; Palmer and Wittbrodt, 1991; Mabbett and Macaske, 2001). The allowable total heavy metal concentration in the wastewater for the electroplating industry is 10 mg/l according to the standards of pollution control board (Jean et al., 2001).

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials (Volesky, 1992). Biosorption can be defined as the ability of biological materials accumulates heavy metals from wastewater through metabolically mediated or Physico-chemical pathways of uptake. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Sakaguchi and Nakajima, 1991; Juwarkar, 1988; Volesky et al., 1993; Kapoor et al., 1999).

The bioremediation and biotransformation methods harness the naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons, polychlorinated biphenyls and metals (Gadd, 1990). Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatics and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into biotransformation and biodegradative pathways and the ability of organisms to adapt to changing environmental conditions (Gadd, 1989; Strandberg et al., 1981; John et al., 1992).

Reduction of hexavalent chromium to trivalent chromium by microorganisms shows significant advantages over physicochemical treatment techniques (Mow and Gadd, 1984). Some of them are low cost, high versatility, high uptake, high tolerance for organics and regeneration. In the present work the ability of *Rhizopus oryzae* towards remediation of Chromium was evolved by characterizing the biotransformation of chromium.

MATERIALS AND METHODS

Microorganism and preparation of media

The *R. oryzae* strain used in the present study was obtained from IMTECH, Chandigarh. The fungal strain was grown in 250 ml Erlenmeyer flask containing 100 ml of media of the following composition; Glucose 1 g; K_2HPO_4 0.5 g; NaCl 0.5 g; $MgCl_2$ 1.0 g;

NH4NO3 0.5 g; yeast extract 0.5 g. The pH of media was adjusted to 7. The flask was incubated in a rotary shaker at 150 rpm at a temperature of 37°C.

Methods

Cr (VI) stock solution was prepared by dissolving 2.829 g Potassium Dichromate in one litre of distilled water. The pH of Cr (VI) solution was adjusted to 7.0 using 0.1M NaOH or 0.1M Hcl. The effect if initial concentration on the rate of Cr (VI) reduction was investigated over a concentration range of 100 to 500 ppm, prepared from the stock Cr (VI) solutions.

The influence of pH on *R. oryzae* growth and chromate reduction were investigated with different pH of medium was adjusted to pH 5.0, 6.0, 7.0, 8.0, 9.0 with 0.1M NaOH or 0.1M Hcl with 400 ppm, 1ml inoculum, incubated at 37°C in 150 rpm.

The influence of temperature on *R. oryzae* growth and chromate reduction were investigated with different temperature of medium was adjusted to 30, 32, 35, 37 and 40°C in the mechanical shaker.

The influence of carbon sources on *R. oryzae* growth and chromate reduction were investigated with different carbon sources (Glucose, Sucrose, Lactose, Sodium Acetate, Tri Sodium Citrate), with 400 ppm, 1 ml inoculum, incubated at 37°C in the mechanical shaker.

Chromium analysis

Three millilitre of samples were withdrawn from the flasks after every 6 h interval, the samples were centrifuged at 6000 rpm for 10 min and the pellet was used for cell density determination by the absorbance at 405 nm. The supernatant analyzed for chromium (VI) concentration. Hexavalent chromium was determined calorimetrically at 540 nm using diphenylcarbazide (DPC) reagent in acetone solution. In a 10 ml test tube, 1 ml of supernatant was mixed with 9 ml of 0.2M sulphuric acid. Then 0.2 ml of freshly prepared 0.25% (w/v) DPC in acetone was added. The mixture was then mixed thoroughly and allowed to stand for 10 min for full color development. The absorbance of the red-violet color formed was measured at 540 nm using distilled water as reference. A colorimetric method, as described in the standard methods, was used to measure the concentrations of the different Cr species (Jean et al., 2001; Sag and Kutsal, 2003; Chihpin and Huang, 1996).

Replication and statistics

Statistical analysis: The experimental data were statistically analysed to find out the influence of various treatments as suggested by Panse and Sukhatme (1985). The critical difference was worked out at 5% (0.05) probability level.

RESULTS AND DISCUSSION

Effect of pH

The effect of pH on the percentage reduction of chromium is shown in Figure 1. With increasing pH beyond 7, the chromium reduction rate decreased, which might be due to osmotic changes and hydrolyzing effect. At higher pH values (8 and 9), a decrease in the uptake of metals and reduction rate was observed. At low pH, considerable amount of chromium reduced may due to the composition between proton(H^+) and chromium and cell wall ligands such as carboxyl, phosphate and amino groups associated with the hydronium ion (H_3O^+) and prevent the chromium uptake. Chromium uptake is protein and enzyme mediated, changes in pH may affect the degree of ionization of proteins confirmation and affects the chromium uptake (Sivasamy, 1988).

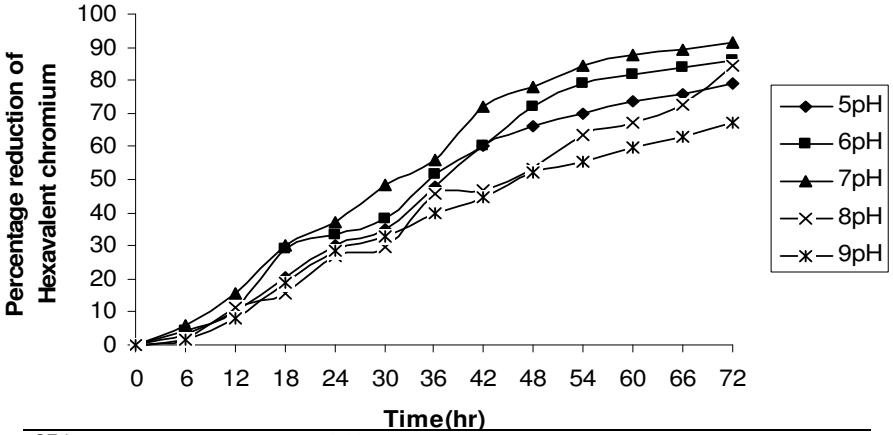
Figure 1 shows the fungal growth with the pH 6 - 7 including that the *R. oryzae* are more susceptible while the chromate concentration exceeded/lowered 400 ppm, the biomass and chromium uptake decreased significantly, because of the inhibitor role of high concentration of chromium for fungal growth. Smooth chromate reduction rate obtained with pH 7 initial concentration 400 ppm and at the temperature at 37°C. At this optimal condition, *R. oryzae* about 91.15% of chromium reduction was obtained in 72 h.

Effect of initial concentration

The chromate reduction exceeded or lowered 400 ppm (Figure 2), the biomass and chromium uptake decreased significantly, because of the inhibitor role of high concentration of chromium for fungal growth. Smooth chromate reduction rate obtained with pH 7 and the initial concentration 400 ppm at the temperature of 37°C. Nouri et al. (2005) reported that the isolate *A. Oryzae* exhibited greater tolerance in different concentration of chromate of 120 – 1080 ppm. The rate of chromium reduction decreased with higher initial concentrations. The metal uptake depends not only on the availability of active sites for metal binding but also on the initial concentration (Prasad and Freitas, 2000; Raji and Anirudhan, 1998; James and Bartlett, 1984; Goyal et al., 2003). At this optimal condition, *R. oryzae* about 91.15% of chromium reduction was obtained in 72 h.

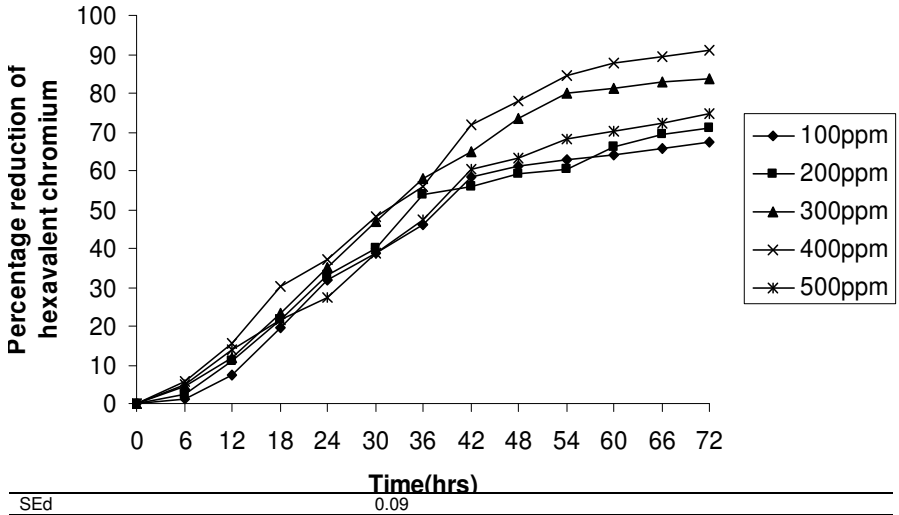
Effect of incubation time on percentage of chromium reduction

The effect of incubation time on percentage reduction of chromate is shown in Figure 3. The result of chromium increased with an increase in incubation time for all the range of initial concentrations studied in the project investigation. Higher percentage reduction of chromium was obtained for the initial concentration of 400 ppm and the reduction was found to be 91.15%. For other initial concentration lesser percentage reduction of chromium was observed. This is may be due to metal uptake ability of cell wall protein composition of fungus (Goyal et al., 2003; Krishna et al., 2004; Wilde and Benemann, 1993;



SEd	0.16
CD(P= 0.05)	0.33

Figure 1. Effect of different pH on chromium reduction at 37°C.



SEd	0.09
CD(P=0.05)	0.19

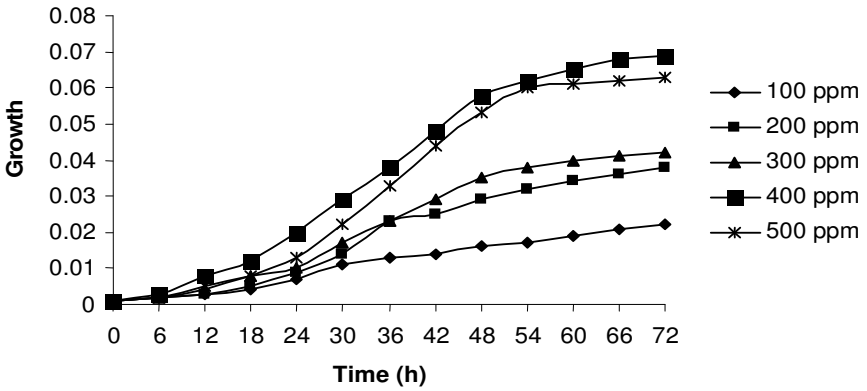
Figure 2. Effect of initial concentration on chromium reduction at pH 7.0 and 37°C.

Pumpel and Schinner, 1993). The role of chromium reduction by fungal strain increased with increase in chromate concentration up to 500 ppm and highest rate of reduction was observed over the initial 72 h of incubation for 400 ppm of chromate.

Growth kinetics

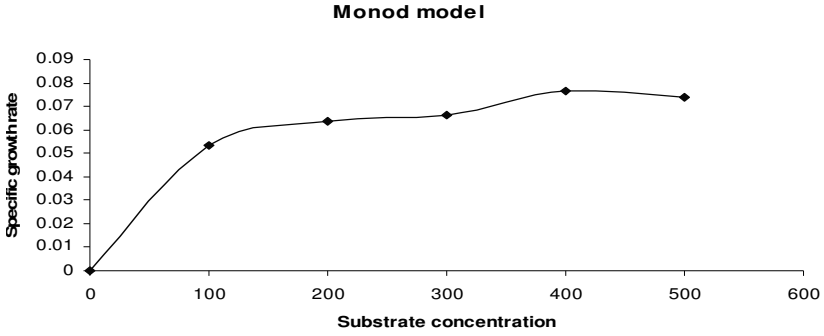
Monod equation: $\mu_g = (\mu_{max}.S)/(K_s+S)$ where μ_g - specific growth rate (h^{-1}), S-Substrate Concentration (mg/l), K_s-half saturation constant (mg/l) and $(1/\mu) = (K_s/(\mu_{max}.S)) + (1/\mu_{max})$. The linearized monod equation is $\mu_g = (0.082.S)/(55.163+S)$.

The biomass data were determined and the respective models were fit as per the mentioned equations. The zone 200 to 500 ppm in Figure 4 is equivalent to the exponential phase in batch culture where substrate concentration is in excess and growth is at maximum specific growth rate. The zone 0 to 200 ppm is equivalent to the deceleration phase of batch. Culture, where the growth of the organism has resulted in the depletion of substrate to a growth limiting concentration, which will not support maximum specific growth rate. If the organism has a very high affinity for the limiting substrate, the growth rate will not be affected until the substrate



SEd; 0.17: CD (P = 0.05); 0.36

Figure 3. Effect of incubation time on percentage of chromium reduction at pH 7.0 and 37°C.



	SEd	CD (P=0.05)
Treatments (T)	1.50	3.03

Figure 4. Monod model for the biokinetics of substrate degradation.

concentration has declined to a very low level (Brady et al., 1994). Thus the deceleration phase for such a culture would be short. The growth rate will be deleteriously affected at a relatively high substrate concentration (Figueria et al., 2006; Gupta et al., 2001; Bai and Abraham, 2003). Inhibition kinetics for the growth of Rhizopus Oryzae was shown in Figure 5.

Haldane's model

$\mu_g = (\mu_{max}.S)/ (K_s+S+ (S^2/K_i))$
Ki-substrate inhibition constant (mg/l)

$(S/\mu) = (K_s/\mu_{max}) + (S/\mu_{max}) + (S^2/ (\mu_{max}.K))$

The above equation is the linearized Haldane's model. The experimental data were fitted in the linearized Haldane's model and shown in Figure 6.

The experimental data were fitted with both the models and the suitable model was determined by best fit.

$\mu g = (0.0809.S)/ (55.67+S+ (S^2/0.220))$

Monod and Haldane models were used to evaluate the data, the specific growth rate constant value was calculated as 0.082 and the reduction was found to be higher at 400 mg/l concentration of hexavalent chromium ion.

Effect of temperature

Figure 7 shows the variation of percentage reduction of chromate ion with temperature. The increase in temperature beyond 37°C decreased the fungal growth and the enzymatic activity. Temperature affects the bioremediation process in presence of fungi cells by influencing enzymatic systems (Volesky and Holan,

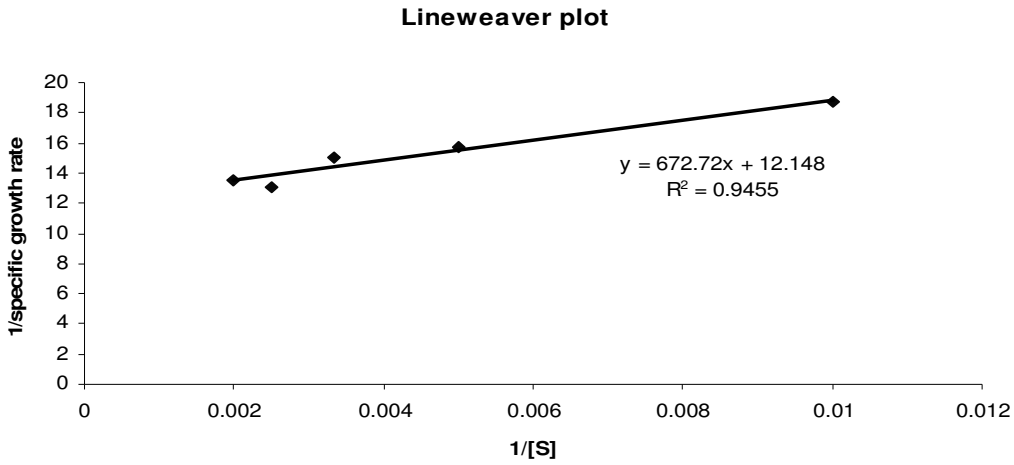


Figure 5. Inhibition kinetics for the growth of *R. Oryzae.*

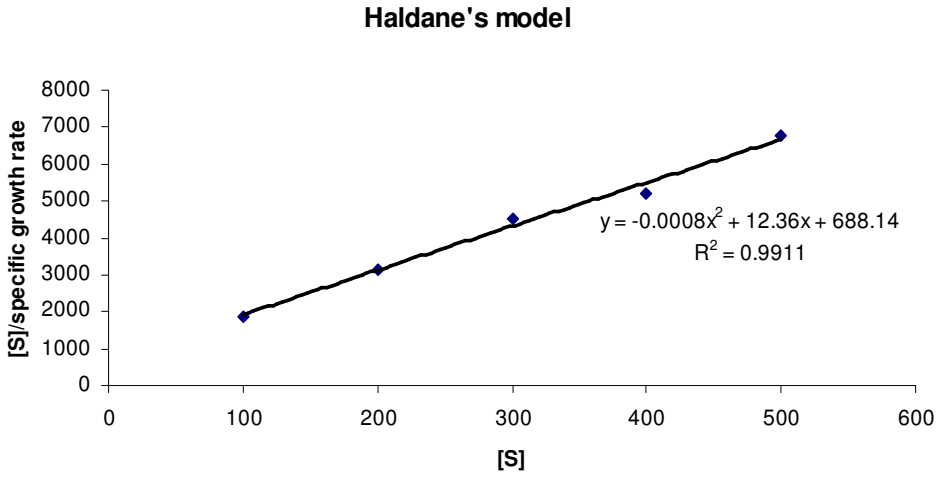
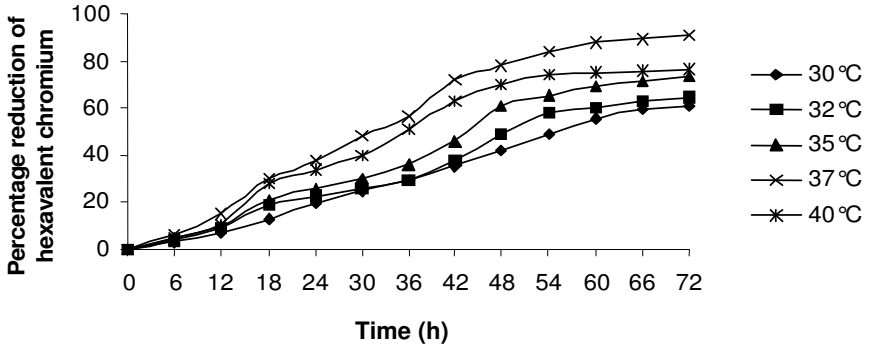
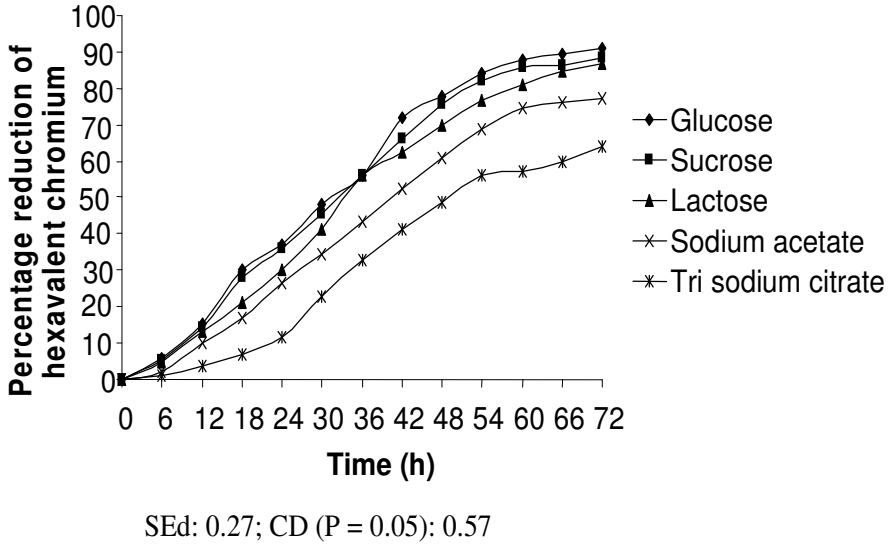


Figure 6. Haldane's model for the biokinetics of substrate degradation.



SEd; 0.16: CD (P = 0.05); 0.33

Figure 7. Effect of different temperature on hexavalent chromium at pH 7.0 and 37°C.



SEd: 0.27; CD (P = 0.05): 0.57

Figure 8. Effect of different carbon sources on chromium reduction at pH 7.0 and 37°C.

1995). Finally, chromate reduction rate obtained at a pH of 7, initial concentration of 400 ppm and at a temperature of 37°C was 91.15%. The reduction of chromate ion increased with respect to temperature upto 37°C and further increases demand the reduction. Therefore 37°C was identified as an optimum temperature for maximum reduction of chromate ion.

Effect of carbon sources

Glucose, sucrose, lactose, sodium acetate and Tri-sodium citrate were used for optimization of carbon source. Batch study was conducted in the Erlenmeyer flasks containing Potassium dichromate (400 ppm) supplemented with mineral salt medium, different carbon sources, that is, glucose, sucrose, lactose, sodium acetate and Tri-sodium citrate and pH adjusted to 7. It was inoculated with *Rhizopus Oryzae* for 72 h at 37°C with shaking in rotary shaker (150 rpm). Sample was removed from the shaker with the time interval of 6 h and chromate reduction was analyzed by UV Spectroscopic method and it was shown in Figure 8.

Glucose has to be catabolized to pyruvate to enter metabolic cycle, where other carbon sources are intermediates of this metabolic cycle. Hence, Glucose would be the best carbon source for fungal growth. Percentage chromate reduction in glucose source was 91.15%, while percentage chromate reduction in presence of sucrose, lactose, sodium acetate, and Tri sodium citrate was 88.33, 86.82, 77.38 and 63.93% respectively.

Conclusion

Chromate is a toxic and carcinogenic agent and it should be removed before disposed to the environment. Its reduction to the less dangerous trivalent form shows to a great solution. The chromium reduction ability of *Rhizopus oryzae* was evaluated at a laboratory scale with optimum pH of 6 - 7. The maximum chromium reduction efficiency of 91.15% and biomass growth achieved at a pH of 7, temperature of 37°C, speed of 150 rpm with an initial concentration of 400 ppm and incubation period of 72 h. Monod and Haldane models were used to evaluate the data, the specific growth rate constant value was calculated as 0.082 and the reduction was found to be higher at 400 mg/l concentration of hexavalent chromium ion. The high removal of chromate by *Rhizopus oryzae* indicates a feasible, economical technique for chromate removal from industrial wastewater effluents.

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