BIOSORPTION OF CHROMIUM (VI) FROM INDUSTRIAL EFFLUENT BY WILD ANDMUTANT TYPE STRAIN OF *SACCHAROMYCES CEREVISIAE* AND ITS IMMOBILIZED FORM

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ABSTRACT

Biosorption of chromium was studied by wild type *Saccharomyces cerevisiae* strain*,* mutant strain, immobilized-wild type and mutant strain. Chromium absorption pattern was observed in all experimental conditions. Hexavalent chromium (VI) was analyzed by diphenyl carbazide method, by oxidizing the trivalent chromium (III). The percentage efficiency of wild type *S. cerevisiae* and its mutant strain, immobilized-wild type and mutant strain were 94.8%, 98.7%, 97.4% and 100% respectively. *S. cerevisiae* mutant strain and their immobilized form was found to be effective in biosorption of chromium (VI) than the wild type forms.

Keywords: Biosorption, chromium, diphenyl carbazide method, Immobilized form, Mutant strain*, Saccharomyces cerevisiae.*

INTRODUCTION

Rapid industrialization and increasing urbanization including technological advancement grossly contaminating our environment by discharging the heavy metals into the effluents and causing severe health hazards to the living beings (Pechova and Pavlata, 2007). Among all heavy metals, chromium is one of the heavy metal pollutant widely used in industries like leather, tanneries, metal finishing, electro plating industry and petroleum refineries (Rodríguez *et al.,* 2004). Tannery wastes contain 79.9 - 249.7 mg/l of chromium (VI). The recommended discharge of chromium (VI) into the water is less than 0.05 mg/l. Above the critical level of chromium is toxic, mutagenic and carcinogenic (Monterio *et al.,* 2002).

The traditional treatment techniques need enormous cost and continuous input of chemicals which becomes impracticable, uneconomical and causes further environmental damage. Hence, easy, effective, economic and ecofriendly techniques are required for fine tuning of effluent treatment (Padmavathy *et al*., 2002). A broad range of bioadsorbent materials like cyanobacteria and microalgae, marine algae, several bacterial species, fungi, yeast and filamentous bacteria were studied for their potential to remove the heavy metals from the solutions and found to be optimistic for treatment of effluents (Ramirez *et al.,* 2000). The ability of the yeasts to adsorb and accumulate metals together with excellent mechanical properties and provide selective sorption of industrial heavy metal ions from polluted waters (Razmovski and Siban, 2008).

The present investigation is mainly focused on the absorption of chromium (VI) from the effluents by using wild type *S. cerevisiae* and its mutant strain, immobilized - wild type and mutant strain and to analyse its percentage efficiency over the absorption of chromium (VI).

MATERIALS AND METHODS

Sample collection and Analysis of chromium (VI)

The tannery effluent was collected from two different tanneries from Dindugal, Tamilnadu. The chromium (VI) content in the samples E01 and E02 were 2612.5 and 2845.7 mg/l and it was measured by Atomic Absorption Spectroscopy. The sample E02 was selected for the present study, because it contains more amount of total chromium (VI).

Microbial culture

The biomass *S. cerevisiae* was revived from dried Baker's yeast and was cultured on yeast peptone agar medium. It was subcultured and incubate it at 30^0C for 48h.

Pretreatment by Acid digestion and Alkali

The organic matter in the effluent was destroyed according to the following procedure for determining the chromium (VI) content in the sample. The mixture of con. Sulphuric acid and nitric acid (1:1) was added to 25 ml of sample and it was boiled on a hot plate at 120^0C until dense white fumes of sulphur trioxide (SO_3) appeared. Dissolve the soluble salts by incorporating 15 ml of 0.5 % v/v nitric acid and allow cooling it. Again the solution was transferred into a 50 ml standard flask and the volume was made up with 0.5% v/v nitric acid (Clesceri *et al.,* 1998).

Estimation of Hexavalent chromium (VI) by oxidation of trivalent chromium (III)

A volume of 0.1 ml of digested sample and standard chromium (Potassium Dichromate) solution ranging from 0.2 to 1.0 ml was taken and appended 3 drops of methyl orange indicator and con. ammonia until the solution began to turn yellow. For the oxidation of chromium (III) to chromium (VI) 5 drops of potassium permanganate is mixed and then the solution is boiled for two minutes until the color turned faded. Then 2 ml of diphenyl carbazide solution was integrated and it was incubated for 10 mts for full color development. Optical Density (OD) was measured at 600 nm to determine the chromium content in the medium (Ahalya *et al.,* 2003).

Chromium tolerance by *S. cerevisiae*

A volume of 50 ml yeast peptone broth containing varying concentration of potassium dichromate such as 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml respectively and inoculated 1ml of overnight culture of *S. cerevisiae* and incubated at 200 rpm in orbital shaker for 5 days. To check the chromate tolerance of cells that survived in medium containing in any of these concentrations by grown the culture on the agar plates with out substrate (Ameri *et al.,* 2008).

Mutagenesis of *S. cerevisiae* by UV Radiation

An amount of 5ml culture was centrifuged at 10,000 rpm for 1 minute and discarded the supernatant. The pellet was suspended with water and it was serially diluted till 10^{-5} dilution. 0.1ml from 10^{-4} and 10^{-5} dilution was placed on yeast peptone agar plates and these plates were exposed to UV-radiation from 5 to 25 minutes. Non-irradiated plates were served as a control to determine the degree of killing (Saifuddin and Raziah, 2007). To screen the temperature sensitive mutants, the irradiated and non-irradiated plates were incubated at 37^0C and 23^0C. If there was no growth in plates incubated at 37^0C, confirmed that they were temperature sensitive mutants (Sumathi *et al.,* 2005).

Immobilization of microbial cells by Sodium Alginate method

S. cerevisiae strain (10% grown cells) were added to 1% sodium alginate slurry and mixed well. The slurry was gently dropped on 0.5M calcium chloride solution and the beads were incubated with 5% sucrose for 10-12 hrs (Debabrata Bera *et al.,* 2006).

Biosorption of chromium

The pH of the tannery effluent was made up to 7.0 with NaOH. Overnight culture (1%) of wild type *S. cerevisiae,* mutant strain and immobilized forms of both were inoculated in an each conical flask containing an effluent. The flasks were kept on rotator shaker at 180 rpm in room temperature for 10 days. The presence of Chromium was estimated by Diphenyl carbazide method at an interval of every 24h for 10 days. OD was measured at 540 nm to determine the concentration of chromium in the effluent (Unnithan *et al.,* 2004).

RESULTS AND DISCUSSION

Analysis of chromium in the effluent

The contaminants and total suspended solids were removed by acid digestion method. When the effluent was subjected to flame atomic absorption spectroscopy, the presence of total chromium was estimated about 2845.7 mg/l. Diphenyl carbazide reagent reacts specifically with hexavalent chromium by oxidizing the trivalent chromium and to form red violet colored complex. According to this method, the total amount of hexa valent chromium present in the E02 sample was found to be 780 mg/l. However, the ability of *P.boergesenli* to biosorb Cr (VI) in a packed column was investigated (Thirunavukkarasu and Palanivelu, 2007).

Determination of Chromium tolerance by *S. cerevisiae*

The growth kinetics of yeast *S. cerevisiae* was investigated with different concentration of chromium and tolerance was measured spectrophotometrically at 600 nm. The observed results were shown in figure 1.

Fig: 1 *Saccharomyces cerevisiae* tolerance to chromate

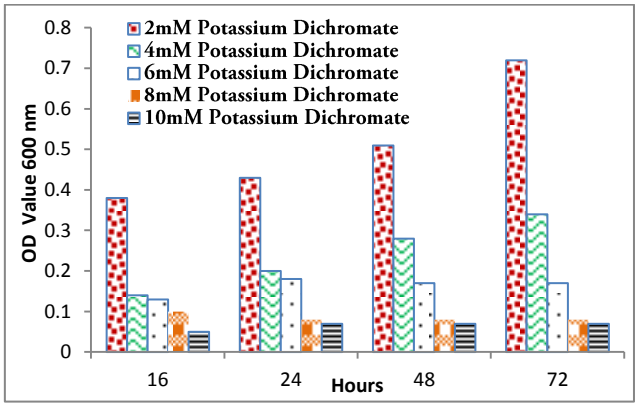


Fig: 2 Comparison of Biosorptive potential Of *Saccharomyces Cerevisiase* mutant Strain and its Immobilized forms

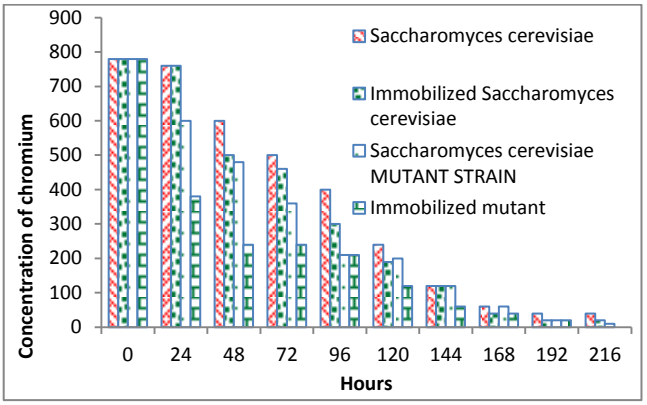
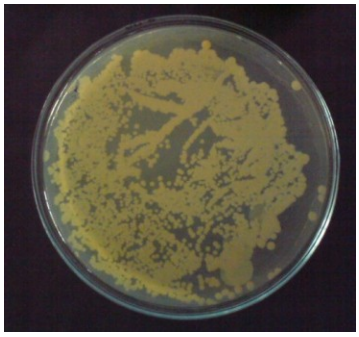
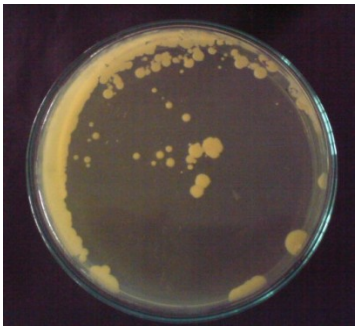


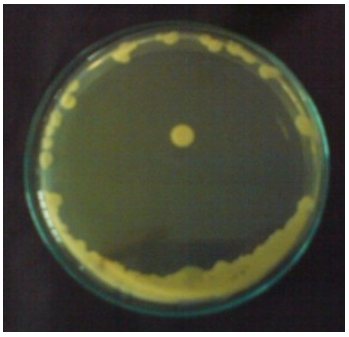
Plate 1 .UV mutagenesis of *Saccharomyces cerevisiae* Non-irradiated *Saccharomyces cerevisiae*



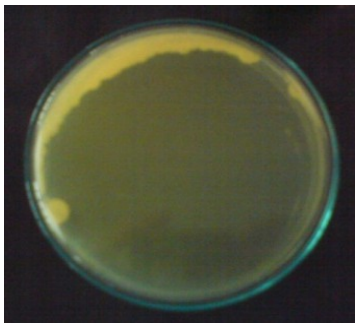
Cells irradiated for 5 minutes



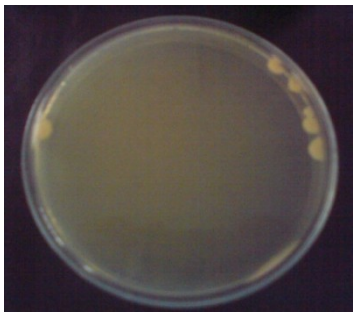
Cells irradiated for 10 minutes



Cells irradiated for 15 Minutes



Cells irradiated for 20 minutes



Mutant strain

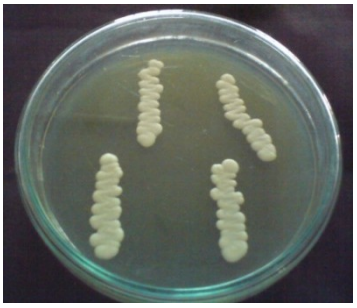
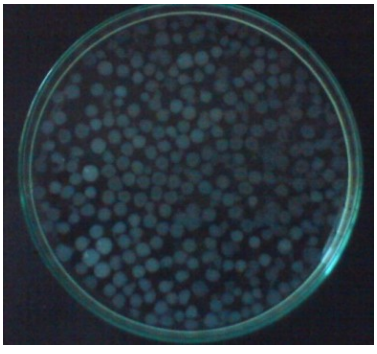


Plate 2: Immobilized beads of *Saccharomyces cerevisiae*



Further, Bukhari *et al*. (2012) developed the convenient analytical method for the determination of chromium and cadmium in tannery wastewater using laser-induced breakdown spectroscopy (LIBS). From the above results, upon incubation of cells for three days, the ability of *S. cerevisiae* to tolerate and accumulate the different concentration of chromium was confirmed by the orange color growth in isolates from 0.2 mg/ml, 0.4 mg/ml chromate concentration , because it could adopt easily and grow rapidly at lower concentration. But at the increased chromate concentration 0.6 mg/ml and 0.8 mg/ml, the growth was observed only at the first day of incubation period. The resistance for the accretion of increased concentration of chromium by the yeast can be caused by deficient sulphate transport and by impaired chromium uptake by the cells as described by (Basu *et al.,* 1997). According to Ramirez *et al.* (2000) the yeast strain of *Candida sp.,* RR1, capable of growing at higher concentration of chromate 0.4 mg/ml was described. The chromate accumulation of this strain was correlated with the ability to reduce chromium at the medium. Further, the biosorption of Cr (VI) ions attained equilibrium at time interval of 240 minutes with maximum removal of 87% at preadjusted initial Cr (VI) concentration of 100 mg/L by *Trichoderma gamsii* (Kavita and Haresh Keharia, 2012).

Mutagenesis of *S. cerevisiae* by UV Radiation

Mutation was carried out in order to analyze whether DNA damage could alter the chromium remedial activity of the organism. Mutagenesis was carried out by exposing the organism to UV radiation. The distinguishable characteristics of radiated and non-irradiated plates were shown in plate 1.

to UV radiation at different time intervals. The colonies which tolerated the UV-radiation were mutated and those mutant colonies were confirmed by temperature sensitive method. When the irradiated plates were incubated at 37^0C and 23^0C, the growth of mutants were delayed for two days at 23^0C, where as 37^0C there was no growth of mutants observed. As described by the researchers (Peter Novick and Randy Schekman 1979), the mutant cells incubated at 37^0C reveals a vast increasing in the number of intracellular membrane bound vesicles which intermediate in the yeast secretary pathway and suggest that exocytosis may contribute the cell surface growth.

Immobilization of microbial cells

Wild type *S. cerevisiae* and its mutant strain were immobilized and it has been reported to be very effective in heavy metal removal. The immobilized beads of *S. cerevisiae* were shown in plate 2.

Immobilized form of *Pseudomonas sp, Streptomyces Sp* and *Citrobactor sp* on PVC has a high efficiency for the removal of heavy metals namely Vanadium, Cadmium, Copper, Lead (Katiyar and Katiyar 1999). The highest value of Cr (VI) uptake by *Bacillus cereus* immobilized in 3% calcium alginate was 92.5% at 25^0C, when initial chromium concentration was 50 mg/l (Debabrata *et al.,* 2006).

Biosorption of chromium

Gradual reduction in the absorbance values were achieved by Wild type *S. cerevisiae,* its immobilized form, mutant strain and its immobilized form and the results were shown in figure 2.

From the above results, the concentration of chromium was reduced from 780mg/l to 40mg/l, 20mg/l, 10mg/l and 0mg/l by wild type *S. cerevisiae* and its immobilized form, mutant strain and its immobilized form respectively.

The percentage efficiency of Wild type *S. cerevisiae,* mutant strain and immobilized forms of both was calculated to be 94.8%, 98.7%, 97.4% and 100% respectively and the results were tabulated in table 1.

Table 1. Efficiency of microbial biosorption of chromium (VI) from tannery effluent in 10 days

Organism	Initial Concentration of Cr mg/l	After Remediation mg/l	Percentage (%)
Saccharomyces cerevisiae	780	40	94.8
S.cerevisiae Mutant strain	780	10	98.7
S.cerevisiae beads	780	20	97.4
***S.cerevisiae* mutant strain beads**	**780**	**0**	**100**

It was concluded that the immobilized mutant strain was the most efficient in absorption followed by mutants. The rate of absorption of chromium by mutant strain was greater when compared with normal wild type *S. cerevisiae.*

A mutated strain of *Bacillus cereus* M1 growing and resting cells is an efficient adsorbent of chromium (VI) removal in dilute solution. Up to 98.08% Cr (VI) removal was possible when initial Cr (VI) concentration were 25-200 mg/l (Subham Paul *et al.,* 2007). The uptake and reduction of Cr (VI) by mutant *Aspergillus niger* and *A*. *parasiticus* was studied (Shugama *et al*., 2012).

Conclusion

The potential of wild type *S. cerevisiae* biomass in mutant and immobilized state for the removal of chromium has been demonstrated. The mutant strain and its immobilized form were found to be effective on absorption of chromium (VI). The percentage efficiency of wild type *S. cerevisiae*, mutant strain, immobilized - wild type and mutant strain were 94.8%, 98.7%, 97.4% and 100% respectively. The result obtained by this study shows that chromium biosorption by immobilized form of mutant strain- *S. cerevisiae* was effective compared to the wild type.

LITERATURE CITED

Ahalya N, Ramachandra TV, Kanamadi RD, 2003. **Biosorption of heavy metals.** *Res.j.chem.environ*. **7**:71-78.

Ameri A, Gholami M, Vaezi F, Mahmkodi M, Moosavi B, 2008. Application and optimization in chromium contaminated waste water treatment of the reverse osmosis technology. *Iran j.Publication.Hlth.* **37**(3): 77-84.

Basu M, Bhattacharya S and Paul AK, 1997. Isolation and characterization of chromium resistant bacteria from tannery effluent. *Bull.Environ.Contam.Toxicol*. **58**:535-542.

Bukhari MM. Awan A, Ishtiaq A. Qazi, and Anwar Baig M, 2012. Development of a Method for the Determination of Chromium and Cadmium in Tannery Wastewater Using Laser-Induced Breakdown Spectroscopy. *Journal of Analytical Methods in Chemistry.* 1-7.

Clesceri JS, Grenberg AE, Eaton AD, 1998. Standard methods for the examination of water and waste water. 20th edition. 3-13, 3-26, 3-36, 3-66.

Debabrata Bera, parimal Chattopadhyay, Lalitagauri Ray, 2006. Chromium (VI) biosorption by immobilized biomass of *Bacillus cereus*. *J.Hazardous Substance Research.* **6**:2-23.

Katiyar SK and Katiyar R, 1997. Microbes in control of heavy metal pollution. *Adv.micro Biotechnol.* **19**:330-344.

Kavita B and Haresh Keharia, 2012. Biosorption Potential of Trichoderma gamsii Biomass for Removal of Cr (VI) from Electroplating Industrial. *Effluent. International Journal of Chemical Engineering*. 1-7.

Monterio, Christina I, Allegra, Sandra, 2002. Determination of total chromium traces in tannery effluent by Electrothermal Atomic Absorption Spectroscopy, Flame Atomic Absorption Spectroscopy and UV- Visible Spectrophotometric methods. *Cetem*: 1-12.

Padmavathy V, Vasudevan B and Dhingara SC, 2002. Biosorption of nickel (II) ions on Bakers Yeast. *Process Biochemistry,* **38**: 1389-1395.

Pechova and Pavlata, 2007. Chromium as an essential nutrient: a review. *Veterinary Medicine.* **52**(1): 1-18.

Peter Novick and Randy Schekman, 1979. Secretion and cell-surface growth are blocked in a temperature sensitive mutant of Saccharomyces cerevisiae. *Proc.Nati.Acad.Sci.* **76**(4):1858-1862.

Ramirez - Ramirez R, Calvo-Mendez C and Avila-Rodri-guez M, 2000. Chromate resistance and resistance and reduction in a yeast strain isolated from industrial waste discharges. *Environ. Eng. Hlth sci***.** 437-445.

Razmovski R and Sciban M, 2008. Iron (III) biosorption by *Polyporus squamosus*. *Afri. Biotechnol.* **7**:1693-1699.

Rodríguez X, Gutiérrez C and Moctezuma Mde G, 2004. Biosorption of chromium (VI) from aqueous solutions onto fungal biomass. *Bioinorg Chem Appl.* 1-7.

Saifuddin and Raziah AZ, 2007. Removal of Heavy metals from industrial effluent Using Saccharomyces Cerevisiae (Baker's Yeast) Immobilized in Chitosan/lignosulphonate Matrix. *J. Appli. sci. Res.* **3**(12):2091-2099.

Shugaba A, Buba F, Kolo BG, Nok AJ, Ameh DA and Lori JA, 2012. Uptake and Reduction of Hexavalent Chromium by *Aspergillus niger* and *Aspergillus parasiticus*. *Petroleum and Environmental Biotechnology.* **3**(3): 1-8.

Subham Paul, Debabrata Bera, Parimal Chattopaphyay and Lalitagauri Ray, 2007. Biosorption of Chromium (VI) by a mutated strain of Bacillus cereus. *J.Hazardous substance Res.* **7**:1-19.

Sumathi Km, Mahimaraja S and Naidu R, 2005. Use of low - Cost biological wastes and vermiculite for removal of Cr from tannery effluent. *Biosource technology*. **96**:309-316.

Thirunavukkarasu E and Palanivelu K, 2007. Biosorption of Cr (VI) from plating effluent using marine algal mass. *Indian Journal of Biotechnology*. **6**: 359-364.

Unnithan MR, Vinod VP and Anirudhan TS, 2004. Synthess, Characterization and Application as a Chromium (VI) Adsorbent of Amino modified Polyacrylamide Grafted coconut Coir pith. *Ind Eng.Chem.Res.* **43**:608-17.

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