Removal of Chromium ions using immobilised biomass of *Saccharomyces cerevisiae*

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Abstract

In Kerala around 200 numbers of electroplating industries are functioning. Most of them are small scale industrial units. A number of cases have been noticed that the wastewater is being discharged into the near by drainage/land or water bodies without proper treatment. In this circumstance, it is essential to adopt an economically viable treatment method to remove the toxic heavy metals from the discharging effluents of electroplating industries. Citing previous literatures on the subject, it is observed that biosorbents are highly effective to remove heavy metals from aqueous solutions. Biosorption capacity of immobilised cells of S.cerevisiae was evaluated in the present study. Batch experiments are conducted for Cr (VI) removal. Effect of pH, initial metal ion concentration, contact time and biomass dose in living as well as in dead biomass are carried out. Biosorption efficiency of 73.63% and 87.27% for live and dead biomass were obtained at optimum conditions.

Key words: *Biosorption, chromium, immobilization, S.cerevisiae*

1. Introduction

The phenomenal growth in industries and energy sectors coupled with rapid modernization, under the pretext of providing human luxury and comfort has posed a serious threat to the natural resources and environment. Industries continue to be a potential threat affecting the water. [4] The industrial wastes are mainly responsible for the pollution of rivers. Certain types of organics inorganic and radioactive substances are present in the industrial waste. Some of the industries producing large volumes of acid wastes are steel pickling, viscous rayon and transparent paper producing, manufacture of TiO_2 etc. These waters are potentially harmful to aquatic life. Due to the disposal of industrial effluents to waterbodies, it gets contaminated with acids, alkalies, chemicals, floating materials, suspended matter and other organic matter.

Chromium compounds are widely used by modern industries, resulting in large quantities of this element being discharged into the environment. Water pollution by chromium is of considerable concern, as this metal has found widespread use in electroplating, leather tanning, metal finishing, nuclear power plant, textile industries and chromate preparation this become one of the most important environmental issues.[4] Some of the main uses for chromium compounds are as follows: plastic coating of surfaces for water and oil resistance, electroplating of metal for corrosion resistance, leather tanning and finishing and in pigments and for wood preservatives.

A wide range of technologies are available for the removal of hexavalent chromium from waste water, some of which are well-established methods that have been in practice for decades such as precipitation, co-precipitation and concentration. These processes simply remove chromium from waste waters by reduction, coagulation and filtration. [9] Although these technologies are quite satisfactory in terms of purging chromium and other heavy metals from water, they produce solid residues (sludge) containing toxic compounds whose final disposal is generally by land filling with related high costs and still a possibility of groundwater contamination. From the environmental point of view, removing pollutants from liquid waste water does not solve the problem but transfer it from one phase to another phase. Chromium (III) toxicity to mammals and aquatic organisms appear to be lower compared to Cr (VI), due to generally low solubility of its compounds. [3] [8]

Biosorption can represent a sustainable alternative for metal removal and recovery, based on metal sequestering properties of dead or living biomass. The major advantages of biosorption technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbents materials. Many biological materials can bind heavy metals, but only those with sufficiently high binding capacity and selectivity for heavy metals are suitable for the biosorption process [6] [7].

Djafer et.al., [2] studied the removal efficiency of Chromium using yeast biomass immobilised onto pozzolonic materials. The results indicate that yeast is a better biosorbent of chromium with a removal efficiency of 85-90%. The relationship between yeast surface properties and yeast ability to bind heavy metals was studied by Edyta Kordialik [3]. The study shows that metal adsorption capacity is more for negatively charged yeast cells and biosorption of heavy metals results in the modification of surface properties and hydrophobicity of yeast cells. Hihore et.al.,[5] studied the potential of heat inactivated *Saccharomyces cerevisiae* in the bioremoval and reduction of Cr (VI) form waste water. This study reveals that heat inactivated biomass is capable of reducing Cr (VI) to Cr (III).

In the present study, batch experiments were carried out for a better understanding of Cr (VI) biosorption by using living as well as heat inactivated immobilised biomass of *Saccharomyces cerevisiae* (yeast) and evaluated its biosorption capacity by varying pH, biomass dosage, contact time and initial metal ion. Specific objectives of the study are summarised as follows;

- To determine biosorption of chromium ions from real electroplating effluents.
- To evaluate biosorption by varying pH, biomass and initial metal ion concentrations

3. Materials and Methods

The sample was collected from electroplating unit, Kuravankonam, Trivandrum, Kerala. It was analysed as per the standard methods for the examination of water and wastewater (APHA 2005). [1]

3.1 Preparation of biosorbent

The *Saccharomyces cerevisiae* used was the commercial pressed baker's yeast, obtained from a local supplier. This was maintained on a YEPD (Yeast Extracted Peptone Dextrose) medium. The media was prepared by adding 2.5g of yeast extract, 5g of peptone, 5g of dextrose to 250mL distilled water in Erlenmeyer flask. Yeast cells are added and incubated at 28^{0}C at 125 rpm in an orbital incubator shaker. The content of flask from late exponential growth phase (72hrs) was harvested by centrifugation (2000 rpm, 20 min) at room temperature and the supernatant was decanted. The cell pellet was washed thoroughly with distilled water. For heat inactivated cells these are placed in an oven at 80^{0}C at 24hrs. The dried yeast cells was grounded using a mortar and pestle.

3.2 Immobilisation of biosorbent

Immobilisation of biosorbent via entrapment was performed in the following manner: 4% (w/v) sodium alginate dissolved in distilled water and mixed with 5% (w/v) yeast biosorbent. The mixture should be stirred for 1hr at 30^{0}C. This well mixed alginate slurry is introduced to 4% (w/v) of $CaCl_2$ solution using 10mL syringe and the resultant beads is of 4mm diameter and this was cured for 1hr and washed twice in distilled water and then stored in a refrigerator for later use.

3.3 Metal Solutions

An aqueous stock solution (1000mg/L) of Cr (VI) was prepared using potassium dichromate ($K_2Cr_2O_7$) salt which is used as a source of Cr (VI) in synthetic water. The pH of the solutions is adjusted by using 6N H_2SO_4 and NaOH.

3.3 Batch Studies

The batch adsorption studies were carried out in 250mL Erlenmeyer flasks at 28^{0}C in an incubator orbital shaker at 150rpm. A predetermined amount of biosorbent is mixed with 100mL of metal solution in 250mL conical flasks. After suitable contact time conical flaks are removed and the solutions are filtered using as Whatman filter paper 42 and filtrate is analysed for residual Cr (VI) concentration. All the experiments were done in triplicate. The Cr (VI) ion removal affinity of biosorbent is determined from batch experiments as a function of contact time, pH, biomass and initial metal ion concentrations.

3.4 Estimation of Cr (VI) ions

Chromium analysis is done by spectrophotometric method using 1,5 Di-Phenyl carbazide according to APHA (2005). The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solutions. The reaction is very sensitive and the absorptivity of Chromium is analysed at 540nm wavelength in the spectrophotometer. [1]

Biosorption efficiency (%) was calculated using the following equation

$$E = (C_i - C_r / C_i) \times 100 \quad (1)$$

Reproducibility of experiment was evaluated by conducting statistical analysis using SPSS software.

4. Results and Discussion

4.1 Sample characterisation study

Physico chemical parameters such as pH, Temperature, Turbidity, TSS, BOD, COD, Phosphate, Chromium, Nickel, were analysed. Results obtained are tabulated in table.1

Parameters	Results (mg/L)	Permissible limits for discharging to water body (CPCB)
pH	5.0	6.0 to 9.0
Temperature	30^{0}C	Shall not exceed 5^{0}C above the ambient temperature of the receiving body
Turbidity	180 NTU	-
TSS	2300	100
Phosphate	415	-
Cadmium	0.4	2.0 mg/L
BOD	5036	150 mg/L
COD	12896	400 mg/L
Chromium	5321	0.1 mg/L
Nickel	224	3.0 mg/L

Table 1: Characterisation results

4.2 Optimisation studies

4.2.1 Effect of pH

To study the effect of pH, experiments were conducted with 10gm biosorbent and 50mg/L of initial chromium ion concentration for a contact time of 72hrs at 28^{0}C. The pH is varied from 1 to 10. Biosorption capacity showed an increased result until pH 1 to pH 4 but thereafter it shows a decreasing trend. Figure 1 shows the biosorption capacity of the living and dried biomass obtained from *Saccharomyces cerevisiae* and a maximum biosorption efficiency of 99.54% and 98.63% was observed for pH 1 at 48 hrs. Previous studies on biosorption indicates that pH is one of the most important parameters is to be considered for biosorption process. Increased binding of the chromium ions at lower pH was demonstrated by the electrostatic binding of ions to that of amino groups present in the cell wall.

4.2.2 Effect of contact time

Biosorption capacity for live and dried yeast biomass was studied at different incubation periods of 1hr, 2hr, 24hr, 48hr, and 72hr at pH 1, initial metal ion concentration of 50mg/L, 10gms of biosorbent and a temperature of 28^{0}C. From the investigated incubation time, maximum biosorption efficiency of 95% was observed for live as well as dead biomass at 48 and 24 hrs of incubation. This 24hr is chosen as the best incubation time for further studies. Rate of biosorption was high at the beginning due to large surface area of fungal biosorbent. Once the adsorbent capacity gets exhausted uptake rate is reduced. The difference in biosorption capacity may be due to the ability of organism as well as its cell wall composition leading to difference in interaction of metals with yeast.

4.2.3 Effect of biomass dose

The effect of biomass concentration on Cr (VI) ion removal was examined by varying biomass dosage from 1g to 15g. The present study shows (figure 2) that removal efficiency increased with increase in biomass concentration. Maximum efficiency of 93.18 and 97.72 was obtained for 15gms of live and dead biomass at pH 1and initial metal ion concentration 50mg/L. Biosorbent dosage strongly influences the extend of biosorption. In many instances, lower biosorbent dosages yield higher uptake and lower percentage of removal efficiency. As biomass concentration increases, the uptake of heavy metal by the biosorbent also gets increased, because of increased surface area.

4.2.4 Effect of initial metal ion concentration

Experiments were conducted with varying initial Cr (VI) ion concentrations of 50,100 and 150mg/L under pH 1, 15gms of biomass dosage for 24hrs incubation. An efficiency of 73.63% and 85.90% are obtained for live and dead biomass respectively. From the results obtained it is estimated that that increase in the initial concentrations of Cr (VI) ion reduces the biosorption efficiency (figure3). This substantiates the fact that, at lower concentrations, the ratio of the biosorptive surface of the biomass to the total Cr (VI) metal was high. From the result it is found that maximum efficiency at 50mg/L of initial Cr (VI) ion concentration.

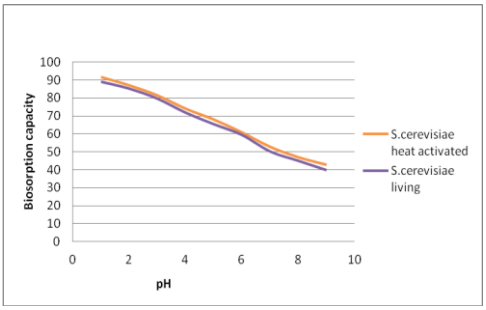

Figure 1: Effect of pH

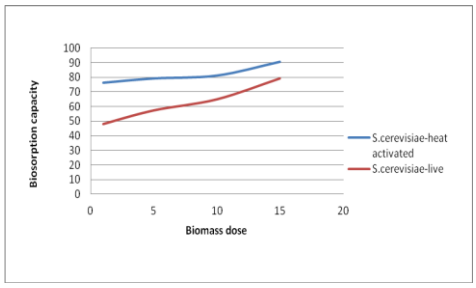

Figure 2: Effect of biomass dosage

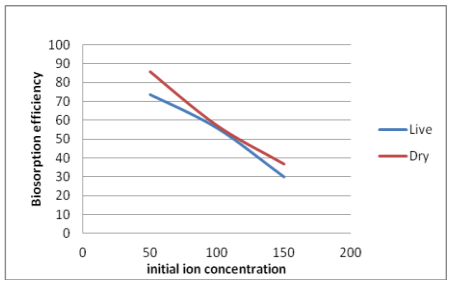

Figure 3: Effect of initial metal ion concentration

Parameters		Pearson Co-relation coefficient (R)
pH	Biosorption (Live)	0.963
	Biosorption (Dead)	0.884
Biomass dosage	Biosorption (Live)	0.899
	Biosorption (Dead)	0.933
Initial metal ion concentration	Biosorption (Live)	0.941
	Biosorption (Dead)	0.937

4.2. Statistical Analysis

Table 2: Co-relation between biosorption and different parameters

5. Conclusion

The present study indicates that, biosorption of chromium ions carried out using immobilised microbial cell of *Saccharomyces cerevisiae* as biosorbent could successfully reduce the Cr (VI) ions form aqueous solutions. This immobilised biomass shows a better removal efficiency of 73.63% and 87.27% for live and dead biomass at an optimum condition of pH, contact time, biomass dosage and initial Cr (VI) ion concentration. Chromium biosorption is highly pH dependent; 50mg/L of Chromium was effectively removed at pH 1 in 24hrs of incubation time by 15gms of yeast biomass. It also gives a better co-relation of 0.937 and 0.941 for live and dead biomass under statistical analysis conducted using SPSS software. It can be concluded that heat inactivated *S.cerevisiae* is an effective and alternative biomass for the removal of Cr (VI) ions from aqueous solutions.

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