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A Selective Bioreduction of Toxic Heavy Metal Ions from Aquatic Environment by *Saccharomyces cerevisiae*

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Abstract: The need to remove or recover metal ions from industrial wastewater has been established in financial as well as environmental terms. This need has been proved financially in terms of cost saving through metal reuse or sale and environmentally as heavy metal toxicity can affect organisms throughout the food chain, including humans. Bioremediation of heavy metal pollution remains a major challenge in environmental biotechnology. Current removal strategies are mainly based on bioreduction of Co^{++}, Ni^{++}, Cu^{++} and Cd^{++} to their metallic forms by *Saccharomyces cerevisiae* in buffered aqueous solution. The rate of biotransformation was significantly influenced by pH of aqueous solution, concentration of biomass and hardness of water. All reaction conditions were optimized and maximum reduction of Co^{++}, Cd^{++}, Ni^{++} and Cu^{++} were observed as 80%, 63%, 50%, and 44% respectively. Unreacted Co^{++}, Cd^{++}, Ni^{++}metal ions were extracted by 8-hydroxyquinoline and Cu^{++} by diethylthio carbamate in $CHCl_3$ at different pH. Furthermore, the concentrations of unreacted metal ions were established spectrophotometrically.

Keywords: *Saccharomyces cerevisiae*, Biotransformation, Heavy metals ions, Bioreduction

Introduction

Water is regarded as the most fundamental and indispensable of all natural resources. The management of water resources is a big challenge for most countries of the world. In India, the need to recycle wastewater is increasing due to the severe shortage of fresh water. Large-scale wastewater treatment constitutes a very important part of the management of water resources. In addition, heavy metals released by number of industrial processes are major pollutants in marine, ground, industrial and even treated wastewaters[1]. Heavy metals can be extremely toxic as they damage nerves, liver, kidney and bones, and also block functional groups of vital enzymes. Cobalt, nickel, chromium, cadmium and their compounds are widely used in electroplating, leather tanning, cement, dyeing, metal processing, wood preservatives, paint and pigments, textile steel fabrication and canning industries. These industries produce large quantities of toxic wastewater effluents[2]. The physical and chemical treatment of wastewater may involve one or more of the following: ion exchange[3], reverse osmosis[4], electrolysis[5], precipitation[6] and reduction[7]. Unfortunately, these methods are expensive and require the use of contaminating products for desorption of metals for cleaning up of the inorganic matrix. Physico-chemical methods presently in use have several disadvantages such as unpredictable metal ion removal, high reagent requirements and formation of sludge and its disposal, in addition to high installation and operational costs[8].

Recently there has been a trend towards the use of biological materials such as fungi, bacteria, yeasts and algae as new sampling and sample clean up approaches[9, 10]. Although most organisms have detoxification abilities (i.e. mineralization, transformation and immobilization of pollutants), play a crucial role in biogeochemical cycles and in sustainable development of biosphere. The use of microbial cells as biosorbents for heavy metals offers a potentially inexpensive alternative compared to conventional methods of heavy metal decontamination from variety of industrial aqueous process streams[11]. Baker's yeast, *Saccharomyces cerevisiae* has been reported to show good sorption characteristics for several metals and its potential utility for bioremediation is acknowledged[12-21].

Recently, Mapolelo and Torto have reported the use of Baker's yeast for trace metal enrichment of metal ions in aquatic environments[21]. However, in order to realize practical application, such studies need further evaluation of time required for sorption as well as other parameters that affect the rate of biotransformation processes. Therefore the present study evaluates a mechanistic approach of bioreduction of toxic heavy metal ions like Co^{++}, Ni^{++}, $Cu^{++}$and Cd^{++} by *Saccharomyces cerevisiae* suspended in aqueous solution and the factors affecting the biotransformation. The effect of pH, water hardness and biomass dose proved to be important for the trace metal ion enrichment process and biotransformation as well.

Experimental

Materials and methods

Cadmium (II) nitrate, cobalt (II) sulphate, nickel (II) sulphate, copper (II) sulphate and zinc (II) sulphate, calcium chloride, acetic acid, sodium acetate, dextrose and chloroform were obtained from s.d. fine, Mumbai, INDIA, as analytical reagent grade materials and were used without further purification. 8-hydroxy quinoline and diethyl thio carbamate were purchased from Aldrich. All dilute solutions were prepared in double-distilled water, with specific conductance equal to $(1.3\underline{+}0.1)\ \mu\Omega^{-1}cm^{-1}$. Stock solutions of Cd^{++}, Co^{++}, Ni^{++}, and Cu^{++} of 0.1mM were prepared. For standardization of EDTA, 0.1 mM standard $ZnSO_4$ solution was prepared. Local brand active dry Bakers yeast was purchased from a grocery shop.

Instrumentation

Spectronic 20 was used to determine concentration of unreacted Cd^{++}, Co^{++}, Ni^{++} and Cu^{++} by solvent extraction. All metal ions were abstracted from artificially prepared aqueous solution by known techniques of solvent extraction. All pH measurements were carried out with locally made digital pH meter. Incubation was carried out in incubator maintained at 25^0C.

Effect of pH on biotransformation

10 mL of pH adjusted (pH 3.0, 4.0, 5.0, 6.0) metal solutions having 0.01mM metal ion concentration were added to the activated culture of 2.0 g of *Saccharomyces cerevisiae* in four separate centrifuge tubes and incubated at 25^0C in incubator for 24 hours. Subsequently the solutions were centrifuged at 5300g for 1minute.10mL of the clear supernatant liquid was separated from each centrifuge tube, diluted to 20 mL with deionised water. The unreacted metal ions were abstracted by 8-hydroxy quinoline / diethylthio carbamate in $CHCl_3$ at different pH and further analyzed spectrophotometrically (Table 1). The procedure was repeated for the optimization of maximum metal ion biotransformation. The reaction conditions were optimized at pH 7.0 with maximum out put of bioreduction. For pH adjustment, 1.0mM of HCl and 1.0mM of NaOH solutions were used.

Table 1. Bioreduction of toxic heavy metal ions by *Saccharomyces cerevisiae*

Metal ions	Initial conc. mM	pH	Ligand in $CHCl_3$	Conc. of unreacted metal ions in mM 10^{-3}	Total bioreduction in % moles
Co^{++}	0.01	3.6	8-Hydroxy Quinoline	2.0	80
Cd^{++}	0.01	4.5	8-Hydroxy Quinoline	3.7	63
Ni^{++}	0.01	3.5	8-Hydroxy Quinoline	5.0	50
Cu^{++}	0.01	8.5	Diethyl thio carbamate	5.6	44

Effect of biomass

Experiments were conducted to study the effect of biomass at 7.0 pH. 0.5 g, 1.0 g, 2.0 g of dry *Saccharomyces cerevisiae*, 2.0g of glucose and 3mL of potassium dihydrogen phosphate buffer (pH = 7.0, 1.0mM) in three separate centrifuge tubes were incubated for several minutes at 30^0C till CO_2 evolution ceased. To the activated culture of *Sacchromycomyces cerevisiae*, the metal ion solution was added in such a way that the strength of the solution became 10^{-2}mM. The procedure was repeated for each metal ion. In order to study the effect of biomass, three sets were prepared. In each set 0.5 g, 1.0 g, 2.0 g of dry *Sacchromycomyces cerevisiae* was added separately and put into a shaker at room temp (200 rpm). The reaction was carried out for a period of 24 hours.

Effect of water hardness

Anhydrous Calcium Chloride (0.0026, 0.0152, 0.0270 and 0.0366 g) was added to 250 mL of 0.1 mM Co^{++}, Cd^{++}, Ni^{++} and Cu^{++} solutions separately in each flask. In each case the pH was adjusted to 7.0 to avoid introducing other potential interferent. 10 mL of 0.01 mM metal ion solution was added to the activated culture of 2.0 g *Saccharomyces cerevisiae*-glucose solution.

The same sampling and analysis procedure described in preceding section was repeated.

Results and Discussion

Effect of pH on biotransformation of metal ions (Figure 1.)

The pH level is one of the most important parameters of biotransformation for metal ions from aqueous solution. Regarding *Saccharomyces cerevisiae*, its high content of ionizable groups such as carboxyl, phosphate, hydroxyl, sulphate and amino on the cell wall polysaccharides make it, at least in theory, liable to influence pH variations. For effective biotransformation, it was evident that at low pH ($pH_{initial} < 7$), the requirement of biomass was significantly increased. The optimal reaction conditions were set at pH 7 with 2.0 g of biomass for effective bioreduction. After completion of reaction, the pH of reaction mixture was changed from 7 to 6.3.

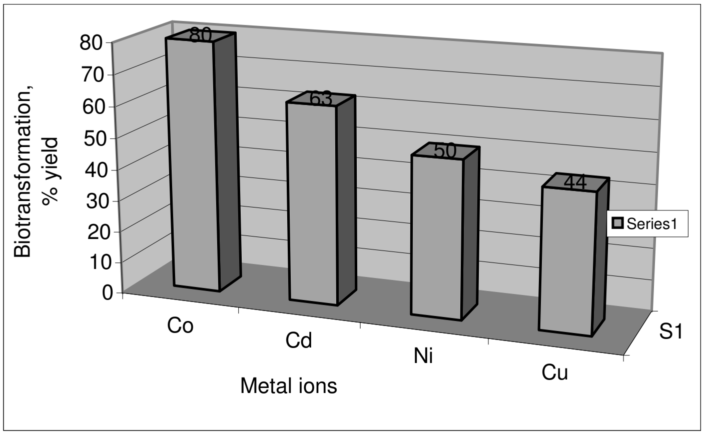

Figure 1. Effect of pH on biotransformation of metal ions
Maximum biotransformation of heavy metal ions at S1 = pH- 7

Effect of biomass (Figure 2)

Analysis of effect of biomass on conversion of metal ions to their metallic form revealed that: at 0.5 g of biomass, the conversion of Co^{++}, Cd^{++}, Ni^{++}, and Cu^{++} was 30%, 23.62%, 18.75%, 16.5% respectively in their metallic forms. At 1.0 g of biomass, the conversion of Co^{++}, Cd^{++}, Ni^{++} and Cu^{++} was 50%, 39.37%, 31.25%, and 27.5% respectively in their metallic forms. At 2.0 g, maximum biotransformation was observed i.e. total conversion of Co^{++}, Cd^{++}, Ni^{++}, Cu^{++} was 80%, 63%, 50%, and 44% respectively in their metallic forms. Thus the reaction conditions optimized for maximum output were at 2.0 g of biomass with pH 7.

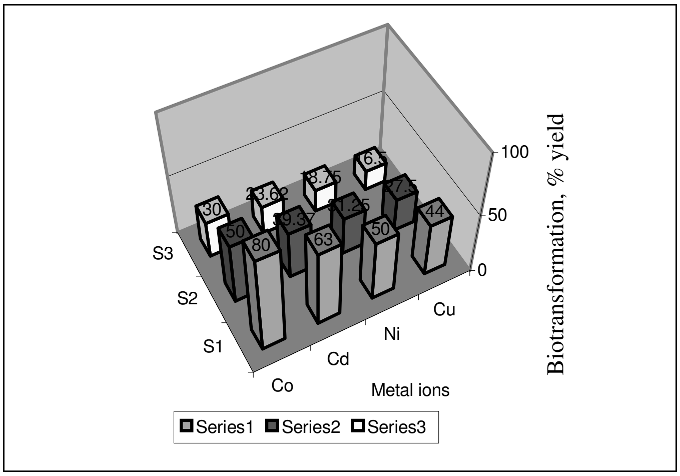

Figure 2. Effect of biomass
S1 = 2.0 g, S2 = 1.0 g, S3 = 0.5 g of *Saccharomyces cerevisiae*

Effect of water hardness (Figure 3)

Experimental findings concerning the effect of water hardness evaluated that the rate of biotransformation of Co^{++}, Ni^{++}, Cu^{++}, and Cd^{++} ions to their metallic forms, in the presence of Ca^{++} was strongly reduced as shown in Figure 3.

- At 0.0026g of CaCO3: % Conversion of Co^{++}, Ni^{++}, Cu^{++}, and Cd^{++} was 70, 42, 35 and 54 respectively.
- At 0.0152g of CaCO3: % Conversion of Co^{++}, Ni^{++}, Cu^{++}, and Cd^{++} was 50, 30, 25 and 38.57 respectively.
- At 0.0270g of CaCO3: % Conversion of Co^{++}, Ni^{++}, Cu^{++}, and Cd^{++} was 26, 12, 10 and 15.42 respectively.
- At 0.0366g of CaCO3: % Conversion of Co^{++}, Ni^{++}, Cu^{++}, and Cd^{++} was 20, 3, 2.5 and 3.85 respectively.

As water hardness increased from 9.45 to 133.09mg of $CaCO_3$/L, the rate of biotransformation decreased. This suggests that there is a competition between calcium ion and other toxic heavy metal ions for bioreduction, shows the contrast effect to heavy metal ion removal in solution. Though in this study, hardness showed contrast effect on bioreduction of Co^{++}, Ni^{++}, Cu^{++}, and $Cd^{++}$and retarded the rate of biotransformation but evidently this technology also reduced the hardness through Ca^{++} reduction.

BOD, COD & SO_4

BOD and COD results were increased significantly. SO_4^{-2} concentration was also reduced.

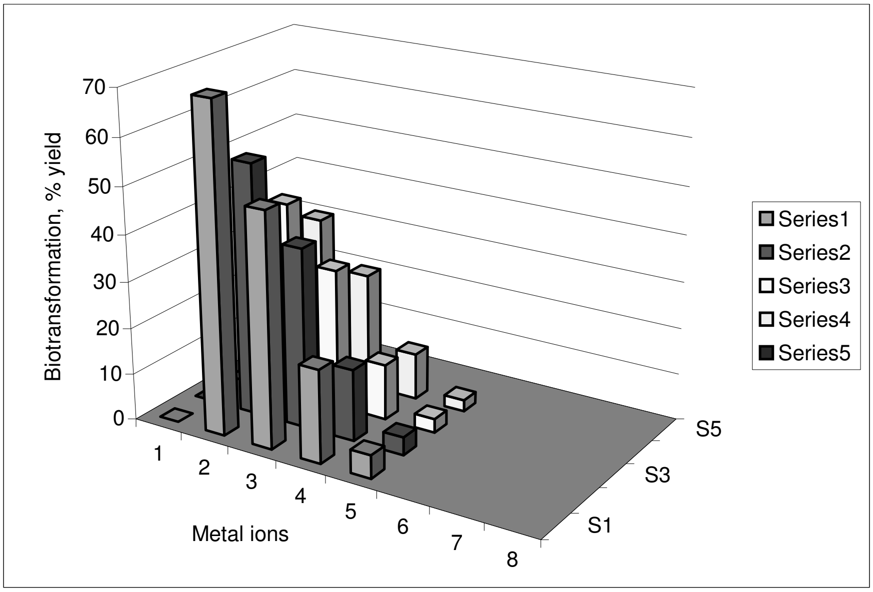

Figure 3. Effect of water hardness
S1 = 0.0026g of $CaCO_3$, 2 = Co^{++}
S2 = 0.0152g of $CaCO_3$, 3 = Cd^{++}
S3 = 0.0270g of $CaCO_3$, 4 = Ni^{++}
S4 = 0.0366g of $CaCO_3$, 5 = Cu^{++}

Conclusion

From the inferred results the following conclusions can be drawn.

- The biomass concentrations, pH of the reaction mixture and water hardness influence the rate of bioreduction of Co^{++}, Cd^{++}, Ni^{++} and Cu^{++} to their respective metallic forms.

- This eco-friendly technique can be scaled for the treatment of metal containing effluent from various industrial establishments, thus preventing the release of hazardous metal ions to water bodies, which can otherwise lead to poisoning of aquatic as well as human life.
- The illustrated technique provides a fruitful area of study on removal of other inorganic and organic impurities from wastewater using *Saccharomyces cerevisiae* as biocatalyst.

Similarly it was evident that the metallic forms were adsorbed on to cell wall of the fungi. Hence the technique is very much important in development of nano particles of metals, which would be useful in bioremediation of wastewater, after recycling and sufficient aeration of water it can be reused.

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