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RESEARCH ARTICLE

BIODEGRADATION OF HEAVY TOXIC METALS WITH *SACCHAROMYCES CEREVISIAE* BIOSORBENT

[1]Venkata Rao, G., [2,*]Srinivasa Rao, D., [1]Madhusudhana Rao, V. and [2]Sambasiva Rao, K.R.S.

[1]Department of Chemistry, S.R.R. and C.V.R. Govt. College, Machavaram, Vijayawada-520004, Andhra Pradesh, India

[2]Department of Biotechnology, Acharya Nagarjuna University, Gutur-522 510, Andhra Pradesh, India

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ABSTRACT

Heavy metal pollution is one of the most serious environmental problems to be considered at utmost priority. Hence, there is an urgent need to tune all our activities in compliance with environment friendly manner. Many efforts have been made to search for effective and economic techniques for the removal of heavy metals from water. Alternative process is biosorption which utilizes the dead biosorbent for heavy metal removal. This is having many advantages over the other processes. The biosorption has low operating cost and high efficiency in detoxifying very dilute effluents. Keeping in mind the potentiality and availability of the chosen yeast biomass as an adsorbent, the present work has been carried out to come up with more optimal values and conditions for better biosorption of the toxic metals which are under the category of huge volume and low concentration compounds, by that it will become easy to scale up the technology to apply for large scale effluent treatment plants.

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INTRODUCTION

Heavy metals are usually classified as the following three categories: toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.), whose specific weight is usually more than 5.0 g/cm3 (Volesky, 1990a; Bishop, 2002). Due to their mobility in natural water ecosystems and toxicity, the presence of heavy metals in surface water and ground water has become a major inorganic contamination problem (Kim *et al*., 2015). Discharge and treatment of industrial wastewater containing heavy metals are important issues in environmental protection (Amirnia *et al*., 2015). With the development of industrialization and human activities, the discharge of waste and wastewater containing heavy metals to environment has increased. Mine drainage, metal industries, petroleum refining, tanning, photographic processing, and electroplating are some of the main sources of heavy metals (Beszedits, 1983). In addition, domestic effluents, landfill leach-ate, agricultural runoff, and acid rain also contribute to heavy metals in wastewater (Aksu and Kutsal, 1990; Pradhan and Levine, 1992).

Corresponding author: Srinivasa Rao, D.,
Department of Biotechnology, Acharya Nagarjuna University, Gutur-522 510, Andhra Pradesh, India.

Pb, Cd, Ni, and Zn are the heavy metals commonly found in such wastewater discharges. Their concentrations vary with sources. Lead can exist in drinking water from naturally occurring sources (Thippeswamy *et al*., 2014). Man-made sources are the main contributor of lead to drinking water. The primary source is tin-lead solder used in plumbing (Hu *et al*., 2014; Davis, 1990,). Wastewater from battery manufacturing, metal production, acid mine drainage can contain a concentration of 0.5 to 25 mg lead/L; a concentration of 0.02 to 35 mg cadmium/L exists in wastewaters from electroplating and metals finishing plants; up to 130 mg nickel/L can be found in the wastewaters (Martin and Griswold 2009; Patterson, 1985). They are toxic to human and aquatic life. Cadmium and nickel were found to be carcinogenic (Paduraru *et al*., 2015). Exposure to lead will result in harmful effects on liver and gastrointestinal mucous of humans and long term effects include brain and kidney damage, lead and cadmium are classified as priority pollutants (Metcalf and Eddy, 1991). Zinc is an essential element for human nutrition. However, water containing zinc at concentrations exceeding 5.0 mg/L will result in an undesirable astringent taste and may be opalescent and develop a greasy film on boiling. Due to their increasing application and the above immutable nature, the heavy metal pollution has naturally become one of the most serious environmental problems today. The conventional techniques

commonly applied for the removal of heavy metals from wastewater include chemical (precipitation/neutralization) or physical (ion exchange, membrane separation, electro-dialysis and activated carbon adsorption) methods (Beszedits, 1983; Atkinson *et al*., 1998). Apart from the expense, membranes are vulnerable to the attack of microorganisms. An ion exchange process faces the problems of cost at smaller treatment capacity and oxidation of resins by chemicals (Kuyucak, 1997). Therefore, other methods are needed to further treat or "polish" the effluent of low to medium concentrations of heavy metals to meet stringent requirements. Activated carbon adsorption is an effective process in removing organics and heavy metals, but high treatment cost is a concern in many cases. Many studies have been done in laboratories to select the most promising types of biosorbent from extremely large pool of readily available and inexpensive biomaterials, and to probe the mechanism of biosorption. A broad range of materials were selected and compared for their heavy metal binding capacities. These materials include algae, fungi, waste fruit residuals, as well as wastes from food and pharmaceutical industries.

MATERIALS AND METHODS

Chemicals

Heavy toxic metal Chromium (Cr) is chosen for the investigation in the present work. As a source of chromium, "$K_2Cr_2O_7$" is selected. The mentioned chemical as a source for heavy metal is procured from Merck specialties Pvt. Limited, Mumbai, India. All the media components used for the cultivation of the microorganism were procured from Himedia Ltd., India.

Microbial Culture

Present work is focused on the assessing the biosorption ability of *S. cerevisiae* and assessing various important kinetic parameters influencing the rate of biosorption. The mentioned yeast culture is one of the very widely used organisms for various industrial fermentations. The strain used in the present work was obtained from Microbial Type Culture Collection center (MTCC No:177), Institute of microbial technology, Chandigarh, India.

Cultivation of *Saccharomyces cerevisiae*

The cultivation procedures were carried out aseptically. Growth medium and glassware were sterilized at 121 °C in an autoclave for 30 minutes. Freeze-dried *Saccharomyces cerevisiae* culture is revived in sterile water. Activated cultures were transferred to a yeast extract-dextrose agar (YPD) surface for growth. YPD plates were incubated for 2 days at room temperature. The cultures were routinely transferred in a cycle of 10-15 days to fresh YPD plates.

Composition of Yeast extract dextrose agar

Name of nutrient	Quantity (gm/L)
Bacto Yeast Extract	10
Bacto peptone	20
20% Dextrose	100ml
DD. H2O	900ml

Liquid culture media

Revived and activated lyophilized culture of *Saccharomyces cerevisiae* was cultivated in a liquid medium to get the biomass and further to use the biomass in biosorption studies. Yeast peptone glucose extract medium (YPG) was selected for cultivation of yeast cells. Yeast extract, glucose and peptone served to meet requirements of amino acids, nitrogen, carbon and energy sources. 250mL Erlenmeyer flask containing 100mL growth medium was autoclaved for 15 minutes at 121 °C and the medium was allowed to cool down to room temperature. The composition for the YPG medium is

Component	Quantity (g/l)
Yeast Extract	3
Peptone	10
Glucose	20
pH	7.2

Determination of Growth curve

For determination of four phases of growth curve, *Saccharomyces cerevisiae* was grown in above said medium. Based on the time and weight of the biomass, ln (Xt/Xo) and Tt-To were calculated and a graph is drawn by taking ln (Xt/Xo) Vs Tt-To to determine the specific growth rate and generation time. Standard solutions were prepared in the range of 10-50 ppm for chromium and in the range of 50-500 ppm for lead from the respective stock solutions. Prepared working standards were used for estimation by atomic absorption spectroscopy (AAS)

Atomic Absorption spectroscopy

This measures the intensity of the light entering a sample and the light exiting a sample and compares the two intensities. Atomic absorption spectroscopy (AAS) determines the presence of metals in liquid samples. Metals include Fe, Cu, Al, Pb, Ca, Zn, Cd, Ni, Cr and many more. It also measures the concentrations of metals in the samples. Atomic absorption spectrophotometer provides accurate quantitative analyses for metals in water, sediments, soils or rocks. Absorption is measured by the change in light intensity striking the detector and is directly related to the amount of the element in the sample.

Preparation of Biomass for biosorption studies

In the proposed work, the biosorption ability of the chosen organism is assessed for two toxic heavy metals Viz. Cr, and Pb. For this, the biomass is grown in a selective medium till we get the maximum biomass by standardizing and understanding the growth curve of the organism. The grown culture is taken in the three different ways and analyzed for maximal biosorption capacity.

Biosorption of Chromium by *Saccharomyces cerevisiae*

Yeast, one of the model organisms in various molecular biology studies, and one of the very well characterized eukaryotic organisms. Keeping the above said facts and availability of the whole genome sequence and ease of handing

in all experimental to production scale reactions, still it is the leader, and the better choice compared to the other microorganism. This is the organism used for the production of most of the pharmaceutical products to the production of ethanol from various carbon sources. This is the microorganism widely accepted by the public for various applications, and generally considered to be safe. But, in concern to treatment of effluents and removal of toxic metals in large volume and low concentration toxic metals, there is a huge search going on for the past few decades.

Even though, researchers have explored the ability of various organisms for their ability of biosorption of toxic heavy metals, these are not satisfying certain facts in regard to the scaling to the large scale effluent treatment plants. In this connection, the present work is undertaken with the view of optimizing various conditions for better biosorption of chromium and lead by *Saccharomyces cerevisiae.* Yeast cells were grown as said above and taken in three different ways like free cells, dried cells, and pretreated with NaOH. All three different cells analyzed individually for their biosorption abilities and also optimized various parameters like pH, temperature, contact time, initial metal concentration and effect of dosage.

RESULTS AND DISCUSSION

Revival and determination of maximal growth phase of the yeast

As mentioned in materials and methods, the yeast lyophilized culture is procured and revived in the said medium. For determination of maximal growth phase to obtain the best possible biomass for biosorption studies, a growth curve experiment is carried out and identified that 36 hrs is the time hour to get the biomass.

Biosorption studies using *Saccharomyces cerevisiae*

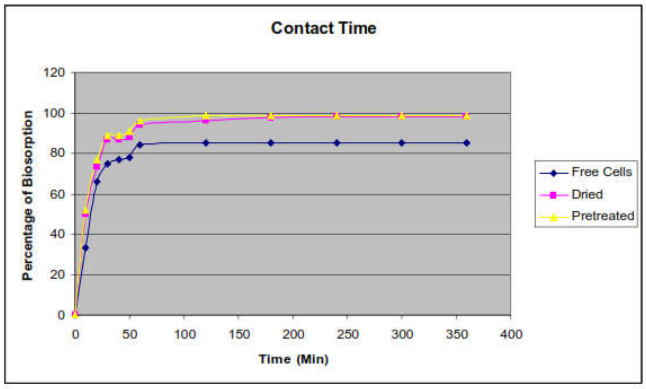
In the investigation carried out, the free cells, cells dried at 100^{o}C, and the cells pretreated with 0.5N NaOH of the yeast species "*Saccharomyces cerevisiae"* were used for the biosorption of chromium and lead. The parameters influencing the biosorption of said metals using the yeast biomass were studied. Furthermore the effects of these parameters are discussed below:

Effect of contact time

The first and fore most important factor considered for analysis was contact time. Here, the biomass in three different forms were taken and carried out the experiment as said in materials and methods. The adsorption experiment of chromium was carried out for different contact times with a fixed adsorbent dose of 1gm/Lt by maintaining pH 7 at 30^{o}C.

The results were plotted in Figure 1, which indicate that maximum sorption attained at 120 min for chromium by all the three forms of yeast biomass. From 30 to 50 min the adsorption is almost constant, but after 50 min there might be multilayer adsorption. Hence the adsorption increased up to 60 min and after that it remained constant.

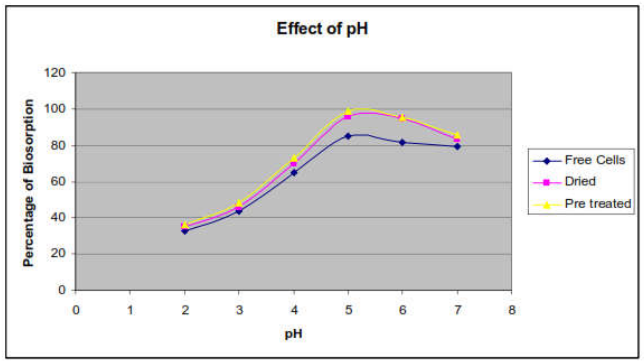
Figure 1: Effect of Contact time on Biosorption of chromium by *S.cerevisiae*



Effect of pH

The most important single parameter influencing the sorption capacity is the pH of the adsorption medium (Goyal *et al.,* 2003). The influence of pH on the percentage sorption of chromium is depicted in the Figure 2. The absorption increased from ~65-70% in all the three different forms of biomass at pH 4 to 98.5% at 5 and significantly decreased with increase of pH. The pH trend observed in this case is shown in Figure 2 from this study we can conclude that at pH 5 for *Saccharomyces cerevisiae* maximum percent of biosorption occurred. The fluctuation beyond this optimum pH 5 was due to decrease of low availability of surface for biosorption at low pH and formation of metal hydroxide and other metal-ligand complexes significantly reduce the amount of metal ions sorbed at high pH (Vijayaraghavan and Yun, 2008).

Figure 2: Effect of pH on Biosorption of chromium by *S.cerevisiae*



Effect of biomass concentration

The influence of biomass concentration on the percentage sorption of chromium is depicted in Figure 3. To achieve the maximum biosorption capacity of the biosorbent for chromium, the biomass concentration which is in three different forms like free cells, dried at 100oC, and pretreated cells with 0.5 N NaOH were varied from 0.1 to 1.5 gm/Lt and it was found that a concentration of 1gm/Lt was adequate for maximum percentage of chromium biosorption under the reported experimental conditions. These findings are shown in Figure 3. It is also seen from this Figure that a further increase in biomass does not affect the biosorption percentage greatly.

This may be due to the unavailability of binding sites to the metal and also due to the blockage of binding sites with excess biomass. In this study it was observed that at 1gm/Lt Concentration showed highest sorption percentages (Vijayaraghavan and Yun, 2008).

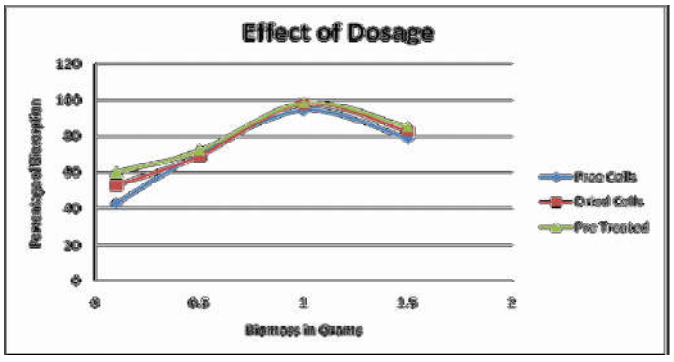
Effect of temperature

In the studies of biosorption using the cells of *Saccharomyces cerevisiae* it was observed that the temperature range between 25°C to 30°C was found to be favorable than that of the lower or higher temperatures. The influence of temperature is depicted in Figure 4. Maximum sorption of around 98% was seen at 28/° C. In these experiments there was an increase in sorption percentage with increase in the temperature till 28°C. A gradual decrease in sorption percentage was observed after that. This is because of the shrinkage of cells at higher and lower temperatures which reduces the surface area of contact (Vijayaraghavan and Yun, 2008). From this we can conclude that the temperature 28°C was favorable for biosorption of chromium using *Saccharomyces cerevisiae.*

Effect of Initial concentration

Like the above said factors, the other one i.e. initial concentration of toxic metal can also influence the rate of biosorption. So, this particular parameter is also analyzed and the experiment was carried out as per the protocol mentioned in materials and methods. Experimental results show that the maximum initial concentration for free cell was 10mg/Lt and the 30mg/lt were the concentration for cells dried at 100°C and cell pretreated with 0.5N NaOH. These results clearly show that there is advantage of using pretreated cells against to the free cells collected from the culture.

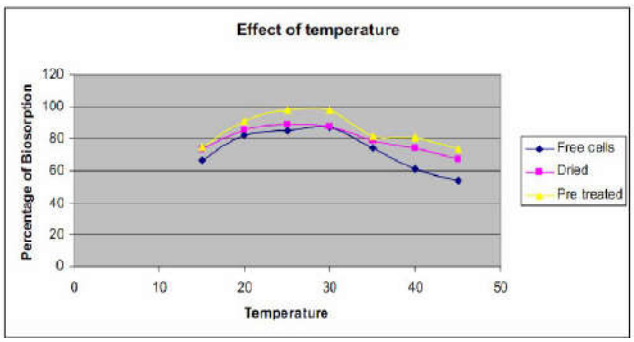
Figure 3: Effect of Dosage on Biosorption of Chromium by *S. cerevisiae*



Conclusion

Mainly biosorption is influenced by factors like pH, Temperature, contact time, initial metal concentration, and biomass dosage. Various experiments were carried out and analyzed the optimal conditions for maximal biosorption. Resultant values have been considered to find out the most suitable adsorption isotherm and identified that Langmuir is the most fitting one among the others. The sorption increased from ~65-70% in all the three different forms of biomass at pH 4 to 98.5% at 5 and significantly decreased with increase of pH.

Figure 4: Effect of temperature on Biosorption of chromium by *S.cerevisiae*



The pH trend observed in this case as shown in graph. From this study we can conclude that at pH 5 for *Saccharomyces cerevisiae* maximum percent of biosorption occurred and 30 to 50 min the adsorption is almost constant, but after 50 min there might be multilayer adsorption. Hence the adsorption increased upto 60 min and after that it remained constant.

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