African Journal of Biotechnology Vol. 10(55), pp. 11557-11561, 21 September, 2011
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Removal of heavy metals from metal-containing effluent by yeast biomass

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Accepted 6 May, 2011

The aim of this study was to investigate the biosorption of heavy metals, chrome (Cr) and tin (Sn) from metal-containing effluent by waste brewer's yeast. Biosorption of Cr and Sn was studied under batch conditions at a pH value of 6.5. The biomass, non-viable cells of the yeast *Saccharomyces cerevisiae*, is able to adsorb tin from a tin effluent, containing trace amounts of other metals. The uptake proceeded quickly over the first 30 min and slowed down over the following 30 min. Research studies have described this phenomenon of fast initial sorption with a second slower phase. Also, a study has been conducted in this regard and states that initial removal is almost entirely dependent on biosorption of metal cations to the cell wall. The yeast can adsorb both chrome and tin from the respective effluents, but removal of tin is faster initially during the first 40 min. Removal of chrome after 60 min is higher than that of tin at the same time. This can likely be ascribed to the difficulty of removing tin from metal-containing waste water. The yeast, *S. cerevisiae*, in a non-viable state, is able to adsorb chrome and tin from the chrome and tin effluents of a local iron and steel industry.

Key words: *Saccharomyces cerevisiae,* heavy metals, chrome (Cr), tin (Sn).

INTRODUCTION

Many industrial sites are contaminated with toxic trace metals which are then diverted into the environment, leading to pollution of surface and groundwater supplies. Heavy metals have a wide range of industrial applications such as electroplating, metal finishing or tanning and in mining industries. As a result, they are present in many industrial discharges. These heavy metals pose serious environmental implications as they remain mobilized in the food chain and are toxic to the biota (Butter et al., 1996).

The increasing awareness of accumulation of heavy metals in the environment has lead to a quest for new and improved "clean" technologies. Various technologies have been studied and implemented for the removal of heavy metals from liquid effluents. Biosorption, which started to gather importance since the 1980's, has the potential to achieve this (Bakkaloglu et al., 1998).

According to Wang and Chen (2006) *Saccharomyces cerevisiae* has received increasing attention due to its unique nature and capacity for metal sorption. *S. cerevisiae* is one of the most promising biosorbents capable of removing chrome (Cr) (VI) from aqueous solutions (Marmeeva and Podgorsky, 2009).

The source of the raw materials for the new family of biosorbents conveniently is a waste material, as is the case in using by-product biomass from large-scale fermentation processes (Niu et al., 1993; Paknikar et al., 1993; Nemec et al., 1997).

S. cerevisiae is an inexpensive, readily available source of biomass for bioremediation of waste water. It has been shown to accumulate heavy metals, such as cobalt and cadmium via two distinct processes (Norris et al., 1977, 1979). There is an initial rapid accumulation step that is metabolism- and temperature-independent and is thought to involve cation binding at the surface (passive biosorption). This step is followed by a second process that is metabolism-dependent, much slower and can accumulate larger quantities of cation than the first process (active biosorption). This second process is believed to involve cation internalization into the cell (Norris et al., 1977).

Further investigations demonstrated that yeasts are capable of accumulating other cations such as copper, nickel and manganese and are superior metal accumulators compared to certain bacteria (Norris et al., 1979). *S. cerevisiae* was one range of fungi that were

shown to accumulate cadmium cations as well as copper, zinc, lead and cobalt by Huang et al. (1988).

The fact that waste brewer's yeast can accumulate heavy metals has been proven with great success by a number of researchers (Gadd and White, 1993; Brady et al., 1994; Brady and Duncan, 1994; Tobin et al., 1990; Volesky and Holan, 1995; Wilhelmi and Duncan, 1995; Unz and Shuttleworth, 1996; Riordan et al., 1997; Bakkaloglu et al., 1998).

Microorganisms have the ability to actively and passively accumulate metals to levels that are much higher than those found in their immediate environment. There is much interest in the interaction between metals and microorganisms, as well as mobilization of metals by microbes. Unz and Shuttleworth (1996) stated that the capacity of biomass to recover metals from waste water depends on its physical, chemical and biological properties.

Metals are involved in all aspects of microbial growth, metabolism and differentiation. Essential metals, for example, K, Ca, Mg, Cu, Zn, Fe, Co, Mn and those with no essential biological function, for example, Cs, Cd, Pb, Al, Sn and Hg, can be accumulated by microorganisms by non-specific physico-chemical interactions as well as specific mechanisms of sequestration or transport (Rosen and Silver, 1987; Gadd, 1988; Beveridge, 1989a, b).

Silver et al. stated that microorganisms have encountered toxic metals in the environment throughout their evolutionary history, although, it is now mainly a result of industrial activities that ecosystems are subject to contamination by heavy metals, organometal (loid)s and radionuclides (Babich and Stotsky, 1985; Gadd, 1990a).

According to Tsezos and Volesky (1982), Gale (1985) and Beveridge (1989), nearly all biological material has a high affinity for toxic metals and radionuclides. In all microbial groups examined, specific metal-binding proteins and peptides have been recorded, although, most work has concentrated on yeasts (Butt and Ecker, 1987; Winge et al., 1989).

Viable or non-viable biomass can be applied in bioremediation studies, but the advantages of using non-viable biomass include the following: (1) solution toxicity does not affect the biomass' biosorptive capacity; (2) there are no biomass growth requirements to be met; (3) easy to obtain from industrial fermentations.

According to Tobin et al. (1990), immobilised or pelleted biomass offers considerable advantages in terms of handling, solid-liquid separation and ease of scale-up. Freely-suspended microbial biomass has disadvantages that include small particle size, low mechanical strength and difficult biomass/effluent separation. Immobilised biomass particles in packed- or fluidised-bed reactors minimize these disadvantages (Macaskie and Dean, 1989; Gadd and White, 1993).

Heavy metal pollution arises as a result of industrial activities and the pollutants are released into aquatic and terrestrial environments, leading to high concentrations of these metals in the vicinity of the points of exit of the effluents. Abiotic parameters are of importance in determining the fate of these pollutants, but microbiological activity is also of great importance and can account for and/or influence, a number of the environmental fates of these pollutants. Microorganisms are at the beginning and end of almost all food chains and play major roles in almost all biogeochemical cycles. Metal pollutants can be bound or precipitated by microbial products and metabolites, accumulated by cells through non-specific physico-chemical interactions as well as specific mechanisms of sequestration or transport and oxidation-reduction reactions. The main groups of microorganisms involved include bacteria, cyanobacteria, microalgae and fungi (Gadd, 1996). These microorganisms may have been developed by virtue of close association with the surrounding metals in the effluent and slurries, a certain resistance to the metals in question.

Most future biosorbents are discovered by trial and error experimentation. The purpose of the work described here was to determine if non-viable yeast biomass is able to adsorb chrome and tin from metal-containing effluent of a local iron and steel industry.

The effluent contains trace amounts of Mn, Fe, Zn, Ti, Cr, Cd, Sn, Co and Pb and after a trial run it was decided, in collaboration with the industry, to concentrate on removal of Cr and Sn. The main motivation for the direct application of the yeast biomass to the effluent was an economic one. The methods currently in use by the industry, for example precipitation, are rather costly and the purpose of this study was to find a cheaper and more economic way of removing metals from the metal-containing effluents. The choice of biomass fell on brewer's yeast which, being a waste product, has a negligible cost.

MATERIALS AND METHODS

Waste yeast biomass

Waste yeast was obtained from South African Breweries (Alrode branch) and was transported in a 25 l plastic drum. The yeast was recovered by centrifugation at 5 000 *g* for 20 min and subsequently washed three times with ultra-pure water. The cells were resuspended in 5 mmol/l of piperazine-N.N'-bis (2-ethanesulphonic acid) buffer, adjusted to pH 6.5 with tetra-ammonium hydroxide TMAH (PIPES. Sigma. St. Louis. Mo. USA). The cell suspension was filtered and the biomass obtained was dried overnight at 70°C and milled to a uniform size and 0.4 mg dry mass/mmol/l by dilution was used. The same batch of biomass was used throughout the project (Brady and Duncan, 1994).

Preparation of glassware

All glassware was prepared for use by washing with detergent, rinsing, heating in a 1:1 solution of 55% nitric acid/water solution (80°C;12 h), washed with ultra-pure water and heat-dried.

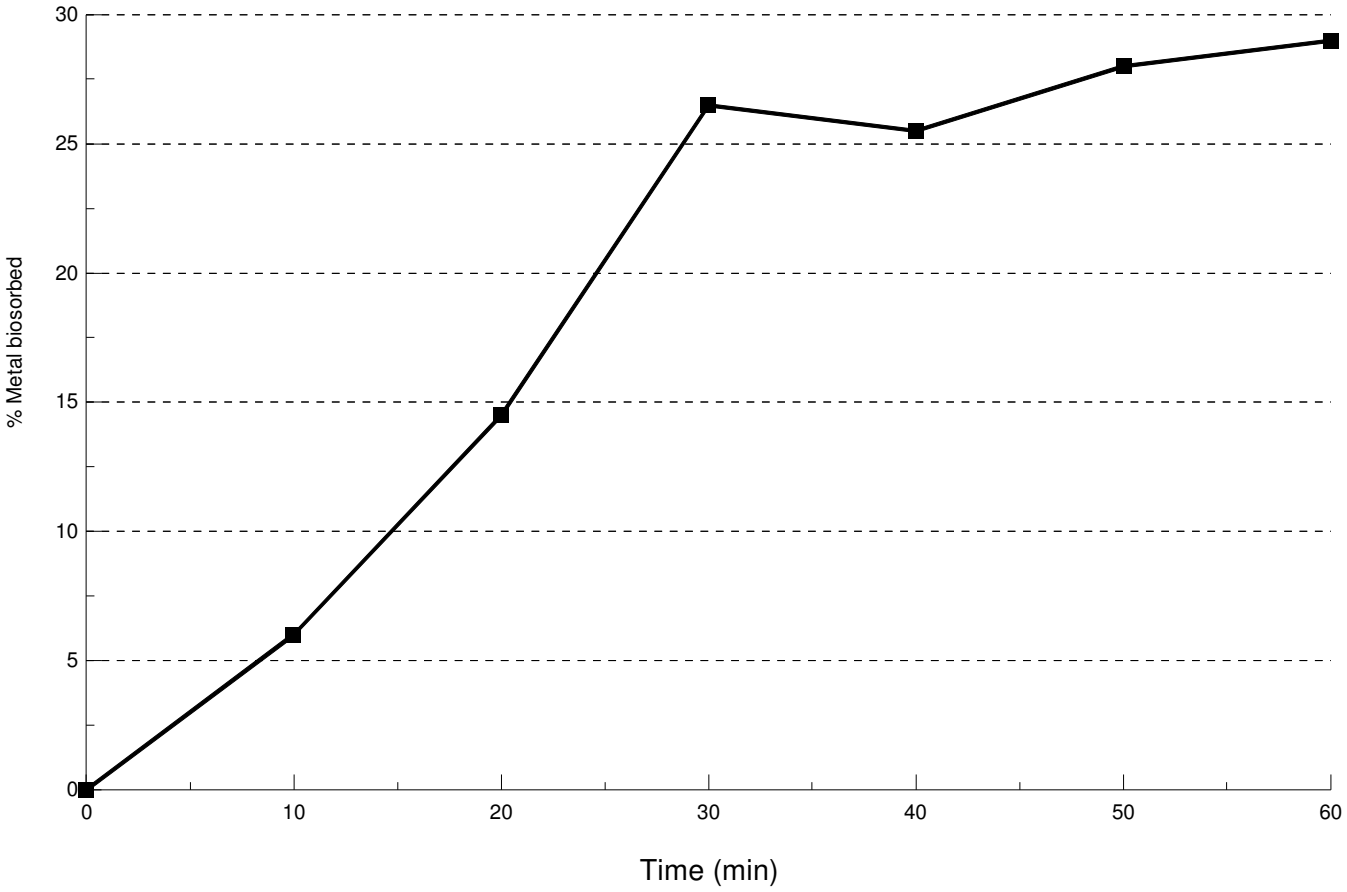

Figure 1. Tin removal by inactive yeast biomass (tin concentration was 0.08 µmol/l).

Effluents used

Two different effluents from a local iron and steel industry were used including tin effluent and chrome effluent.

Biosorption

Treatment of effluent with yeast biomass

Each of the effluents was treated in the same manner: 20 mg dry yeast was added to 50 ml of chrome effluent and shaken in a 250 ml Erlenmeyer flask on a shaker at 110 rpm at 25°C. At time zero and at 10 min intervals until time 60 min, 2 ml samples were withdrawn, using a syringe and transferred to a filtration apparatus fitted with a 25 mm diameter membrane (0.45 Fm, diameter Millipore HA membrane (see results). The filters were washed twice with 5 ml PIPES buffer, removed from the holders and put into glass centrifuge tubes. A volume of 0.2 ml of 55% HNO_3 (analytical grade AECI) was added to each tube containing a filter. In order to release any metal ions associated to cells, the tubes were put into a beaker with boiling water for 60 min. Samples were made up to a final volume of 4 ml with ultra-pure water and centrifuged (1 000 *g* for 10 min). Analyses of both the filtrates and the supernatants were performed by flame atomic absorption for metal content (Brady and Duncan, 1994). The tin effluent was similarly treated. The experiment was done in duplicate. The time span over which the experiment was run was 60 min, according to Brady and Duncan (1994).

RESULTS

Biosorption of tin

It can be seen in Figure 1 that a total percentage removal of 29% tin from the tin effluent, from 0 to 60 min was obtained. A good removal of 26.5% from 0 to 30 min was observed, with a slight decline to 25.5% at time 40 min, after which the removal of tin increased to 29% at time 60 min.

Biosorption of chrome

As seen from Figure 2, uptake of chrome by the yeast occurred maximally until time 20 min. As can be seen from the figure, a total removal of 32%, from time 0 to 60 min, was observed. Initial removal up to 30 min, was steady, after which a decrease was observed from 20.5 to 16.5%. From time 40 min, there appeared a sharp

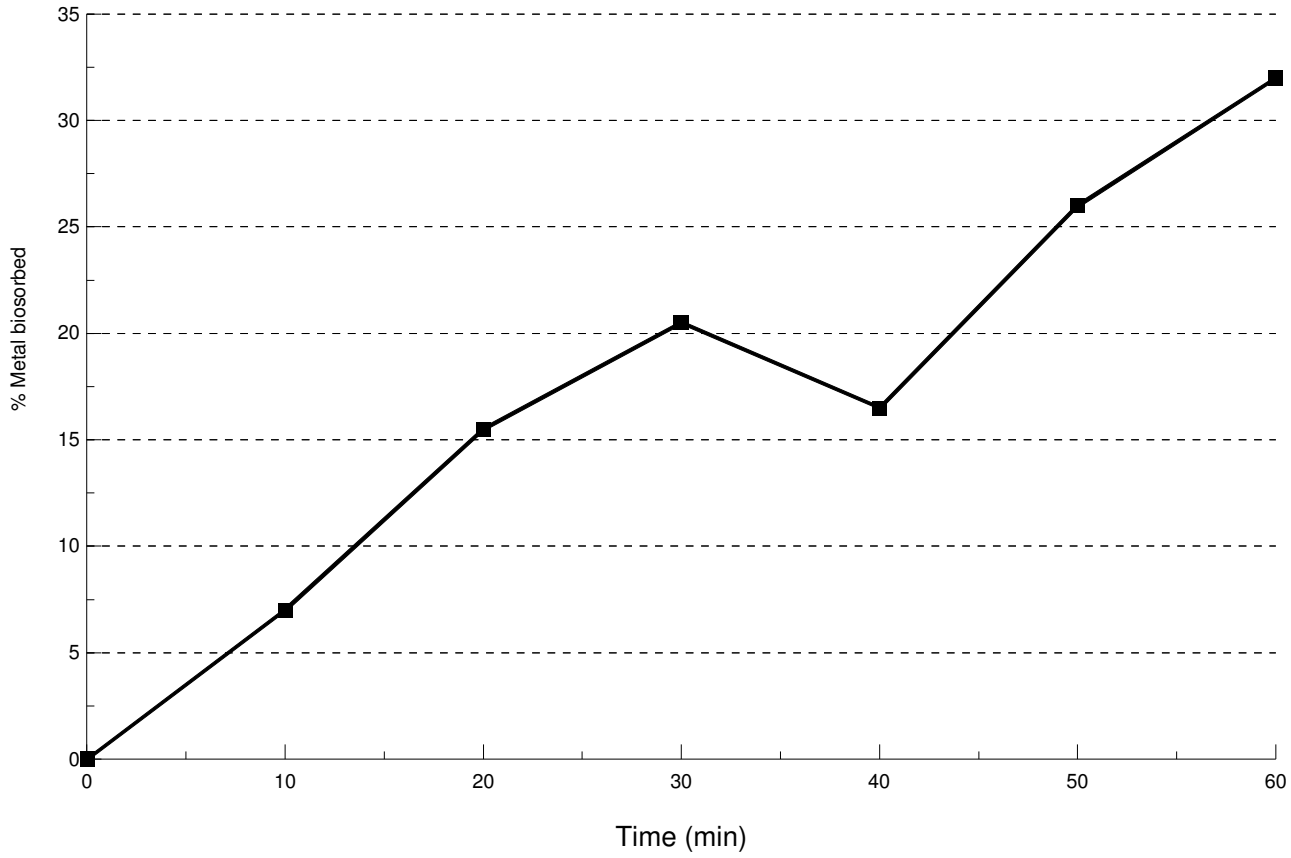

Figure 2. Chrome removal by inactive yeast biomass (concentration of chrome was 0.1 µmol/l).

increase in uptake.

DISCUSSION

According to Gadd and Griffiths (1978), a wide range of organisms has the ability to grow in the presence of high metal concentrations and may be the result of intrinsic or induced features, including specific mechanism(s) of resistance and/or environmental factors that may reduce or eliminate toxicity, for example pH, E_h, inorganic anions, cations, particulate and soluble organic matter, clay minerals and salinity.

Processes such as precipitation, complexation and crystallization of heavy metals and radionuclide species exterior to cells can result in detoxification and many examples of microbial metal deposition are of great significance in biogeochemical cycles (Beveridge, 1989a, b; Ferris et al., 1989; Mullen et al., 1989; Mclean and Beveridge, 1990).

When looking at results from the application of non-viable yeast biomass to metal-containing solutions, biosorption by the inactive biomass compares well with those results (Figures 1 and 2). This is true for both chrome and tin adsorption.

Adsorption at time zero was taken as 0%. A rapid removal of chrome over the first 20 min was seen, with a decrease over the next 40 min. This correlates with findings by Norris and Kelly (1977, 1979), who stated that a second mechanism, probably metal internalization, becomes involved.

The yeast biomass was able to adsorb tin from the industrial effluent. The biomass, non-viable cells of *S. cerevisiae*, is well able to adsorb tin from a tin effluent, containing trace amounts of other metals. The uptake proceeded quickly over the first 30 min and slowed down over the following 30 min. Norris and Kelly (1977, 1979) have described this phenomenon of fast initial sorption with a second slower phase. Duncan and Brady (1994) have conducted a study in this regard and stated that initial removal is almost entirely dependent on biosorption of metal cations to the cell wall.

Conclusion

The inactive yeast biomass can adsorb both chrome and tin from the respective effluents, but removal of tin is faster initially during the first 40 min. This can likely be ascribed to the difficulty of removing chrome from metal-

containing waste water (Volesky and Holan, 1995).

Removal of chrome at 60 min is higher than that of tin at the same time. This study indicates the application of waste yeast for biosorption of tin and chrome from metal-containing industrial effluent.

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