

FACTORS AFFECTING CHROMIUM REMOVAL USING *SACCHAROMYCES CEREVISIAE*

Abd El-Hamid A Hamdy*, Reda F Allam, Sayeda S Mohamed and Abd El-Monem El-Refai

Natural and Microbial Products Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

(Received 16 April 1999; accepted 16 August 1999)

The removal of chromium ions Cr(III) from aqueous solutions using *S cerevisiae* was studied. The influence of some factors affecting biosorption activity such as temperature, pH, weight of dried cells, concentration of Cr(III), time contact between biomass and metal and shaking were examined. Maximum uptake level of Cr (III) ions was attained by using 350 mg biomass and 100 µg Cr(III), in 0.2M phosphate buffer (pH 8.0), 25°C, shaking at 200 rpm.

Key words: Biosorption activity, *S cerevisiae*, Chromium removal.

Introduction

Heavy metals such as chromium, copper, lead, cadmium etc., in waste water are hazardous to the environment. Their toxic effect on ecosystem presents a possible human health risk. The waste water from the dyes and pigments, film and photography, galvanometry and electric metal cleaning, plating and electroplating, leather and mining industries may contain undesirable amounts of chromium ions. Conventional methods for removing chromium ions from waste water such as chemical reduction, electrochemical treatment, ion exchange and evaporative recovery may be ineffective or extremely expensive when the initial heavy metal concentrations are in the range of 10-100 gm⁻³ (Nourbakhsh *et al* 1994; Zagic 1975; Patterson 1977). Recently a process which uses microorganisms for removing heavy metal ions has been proposed (Nourbakhsh *et al* 1994). Inactivated, non-living microbial biomass of several species of bacteria, yeast and algae can serve as a basis for the development of potent biosorbent materials for concentrating and the recovery of strategic or valuable heavy metals, nuclear fuel or radioactive elements. These microorganisms can be highly selective, efficient, cheap and can compete with other methods of accumulation (Volesky 1987).

Yeast cells are capable of accumulating various heavy metals of potential toxicity and of value (Brady and Duncan 1994). Trace elements enter the cell wall or membrane of the biomass. Polysaccharides of the biomass cell walls could provide binding groups including amino, carboxyl, phosphate and sulphate radicals. The amino and carboxyl groups and the nitrogen and oxygen of the peptide bond could be available for characteristic coordination bonding with metallic ions like lead, copper and chromium. Metal ions may also be electro-

statically bonded to unprotonated carboxyl oxygen and sulphate. In addition to these functional binding groups, polysaccharides often have ion exchange properties (Kuyucok and Volesky 1988; Aksu *et al* 1990).

Materials and Methods

All glassware used for metal analysis were washed with detergent, rinsed, soaked in a 2-4% nitric acid bath, then rinsed three times in ultra pure water.

The organism used in this study, *Saccharomyces cerevisiae* (NRRL y567), was obtained from Northern Regional Research Laboratories, Peoria, Illinois, USA. It was grown at 30°C in agitated and aerated liquid media. The medium used composed of (gl⁻¹) glucose 10, yeast extract 3, peptone 5 and malt extract 3. The pH of the feed medium was adjusted by HCl and sodium hydroxide at 5.5. The medium (100 ml) was inoculated with the organism (20 ml of cell suspension) and incubated on rotary shaker (200 rpm) at 30°C for 24 h.

The organism then harvested by centrifugation and washed twice with distilled deionized water (DDW), and dried till constant weight.

Chromium solutions were prepared by diluting a 1.0 gl⁻¹ stock metal ion of chromium chloride solution obtained from Merck. The concentration of the prepared chromium solution was 100 µg ml⁻¹.

Determination of parameters affecting biosorption process.

Effect of pH on chromium removal. 10 ml buffered solution each of citrate, acetate and phosphate containing 100 mg biomass and 100 µg ml⁻¹ of CrCl₃ solution covering pH range from 3-8 were put in 100 ml Erlenmeyer flasks and incubated in shaker incubator (at 200 rpm) for 24 h at 30°C.

*Author for correspondence

Duplicate set of flasks was handled in the same manner. The cell suspensions were centrifuged (at 4000 rpm for 20 min) and then the supernatants were analyzed for Cr(III) as described by Krauter *et al* (1996).

Effect of amount of biomass on chromium removal. Different amounts of biomass (dried) ranging from 25 mg to 500 mg were added to buffered solutions of pH 8.0. 100 µg of Cr(III) were added to each flask. All flasks were placed in incubating shaker (200rpm) at 30°C for 24h. After centrifugation, the suspensions were analysed in the same manner.

Effect of time of contact between metal ions and biomass on chromium removal. Buffered flasks adjusted to pH 8.0 by phosphate buffer containing 100 µg ml⁻¹ of Cr(III), inoculated with 350 mg of biomass were incubated at different time intervals from 0.5 to 30 h. The cell suspensions were centrifuged at 4000 rpm for 20 min. and the supernatants were analyzed for Cr(III) as before.

Effect of chromium concentration on chromium removal. Different concentrations of chromium solutions were used ranging from 25 to 150 ppm.

All flasks were inoculated with biomass (350 mg) and incubated for 14 h at 30°C on rotary shaker (200 rpm). The chromium ions were determined as before.

Effect of shaking on chromium removal. The same experiment was repeated using the best conditions; pH 8.0, dry wt. 350 mg, time contact 14 h, chromium concentration 100 ppm at 30°C are different rpm were used.

Effect of temperature on chromium removal. Inoculated buffered solutions (pH 8.0) with biomass (350 mg) were tested at five different temperatures ranging from 20 to 40°C. Flasks were placed in incubators and allowed to come to the target temperatures then 100 µg ml⁻¹ of CrCl₃ were added. After 14 h. incubation on rotary shaker (200 rpm), suspensions were centrifuged and analysed for Cr(III) (Krauter *et al* 1996).

Analysis. Metal analysis was carried out by Flame Atomic Absorption Spectrophotometry according to the method of Greenberg *et al* (1980).

The maximum absorbance was obtained by adjusting the hollow cathode lamp of chromium at slit 0.7 nm and wavelength 357.9 nm.

Results and Discussion

Effect of pH. *Saccharomyces cerevisiae* biomass was capable of accumulating Cr(III) ions from solutions of chloride salts. pH was probably the major factor determining the quan-

tity of metal ions removed owing to cation competition effects with hydrogen ions. The results (Fig 1) support this assumption. Many buffers such as citrate, acetate and phosphate were used but the phosphate buffer proved to be the suitable one for metal removal (pH range 5.7-8.0). A rapid decrease in Cr(III) removal was noticed at acidic pH below 5.7. A low pH value may increase metal mobility, whereas at near and above pH neutrality, insoluble oxides, hydroxides and carbonates tend to form. Hydroxide formation occurs above pH 5.7. But the quantity of chromium hydroxide formation depends on the competition between hydroxyl groups and cell ligands for the chromium (which in turn is dependent on their molar ratios and affinities for chromium) (Norberg and Rydin 1984).

Effect of amount of biomass. Chromium removal by *S. cerevisiae* from its solutions increased by increasing dry weight (Fig 2). Maximum removal occurred in the range of 250-350 mg. On the other hand, increasing of dry weight up to 500 mg was accompanied by a decrease in chromium ion removal. This probably happened because the sites present on the cell walls of the biomass, (e.g.; phosphate groups, carboxyl groups), were occupied by metal ions and after full saturation, crowding, might have occurred on those sites which resulted in decreasing the metal uptake (Volesky 1987).

Effect of time of contact between metal ions and biomass. Time contact between metal ions and biomass is an important factor affecting metal biosorption. The best residence time for maximum metal adsorption ranged between 14-16 h. Decreasing or increasing the time of contact below

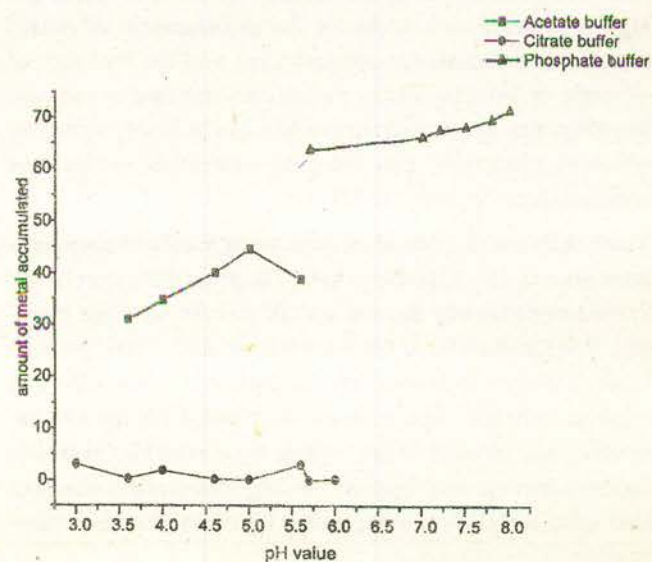


Fig 1. Effect of pH value on metal accumulation.

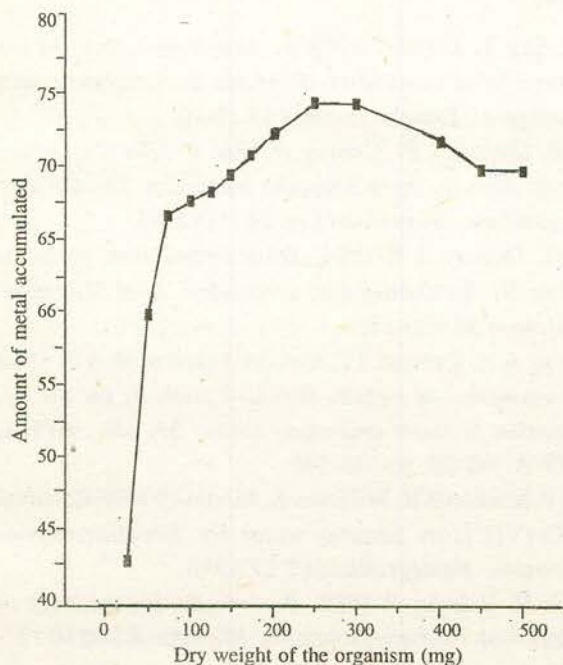


Fig 2. Effect of dry weight on metal accumulation.

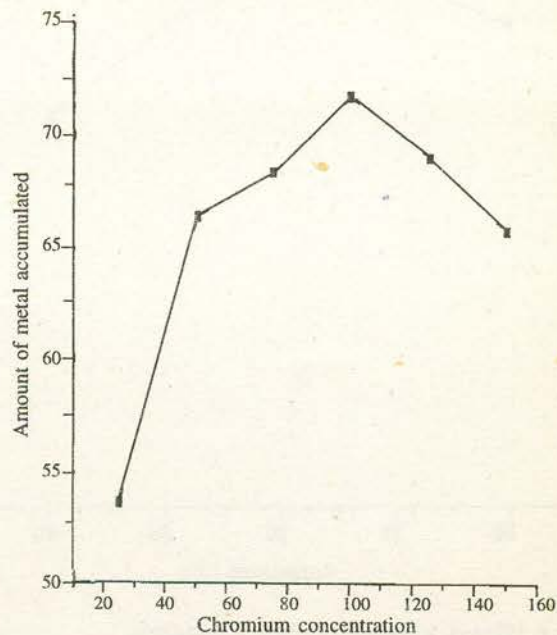


Fig 4. Effect of chromium concentration on metal accumulation.

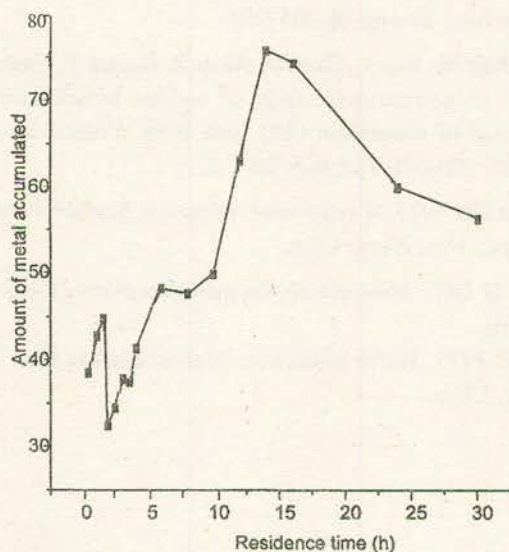


Fig 3. Effect of residence time on metal accumulation.

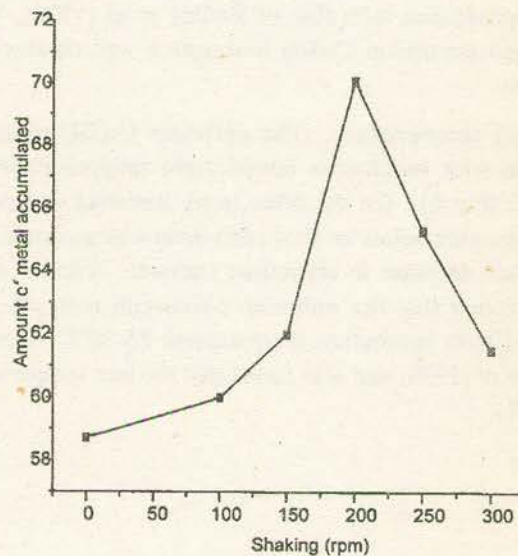


Fig 5. Effect of shaking on metal accumulation.

or above this range led to a decrease in the amount of metal accumulation (Fig 3). Krauter *et al* (1996) used *S. cerevisiae* to remove chromium ions from ground water and attained 70% metal removal after 24 h. On the contrary, other workers (Brady and Duncan 1994) incubated the same organism with the metal solution for 1 h only.

Effect of chromium concentration. In this experiment it was observed that adsorption rates of metal ions to the biomass increased with increasing metal ion concentration up to $100 \mu\text{g ml}^{-1}$ (Fig 4) which was in accordance with that of

Nourbakhsh *et al* (1994) for biosorption of Cr(VI) by *S. cerevisiae*. On the other hand, increasing the Cr(III) concentration up to $150 \mu\text{g ml}^{-1}$ was accompanied by a decrease in the metal uptake. This indicates that after the deposition of metal ions the empty sites were satisfied, and the process of uptake decreased.

Effect of shaking. Biosorption of metal ions by the experimental microorganism was affected by the shaking rate (Fig 5). Maximum metal removal was attained at 200 rpm. Increasing the rpm to 300 and also decreasing the rpm

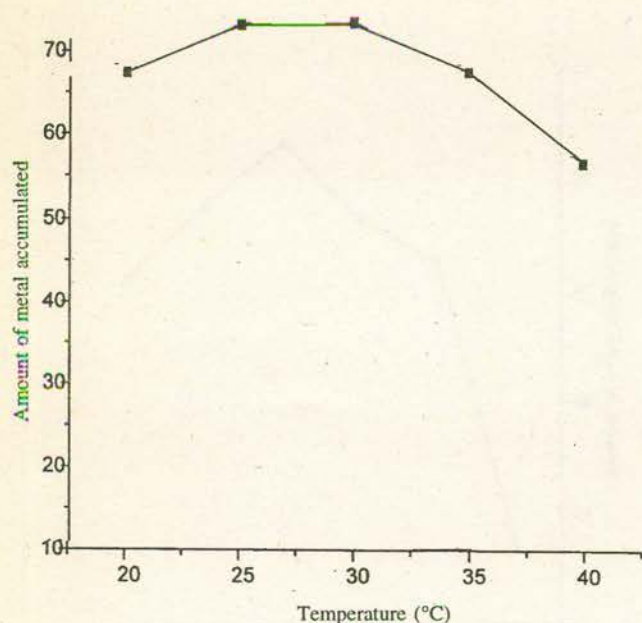


Fig 6. Effect of temperature on metal accumulation.

to 150 led to a decrease in metal removal. These results are in accordance with that of Baillet *et al* (1998), who found that maximum Cr(III) biosorption was obtained at 200 rpm.

Effect of temperature. The optimum Cr(III) removal occurred with incubation temperature ranging between 25-30°C (Fig 6). On the other hand, lowering or raising the temperature below or above this level was accompanied by gradual decrease in chromium removal. Krauter *et al* (1996) found that the optimum chromium removal rate occurred with incubation temperatures 25-30°C whereas baillet *et al* (1998) and also found that the best temperature was 30°C.

References

- Aksu Z, Sag Y, Kutsal T 1990 A comparative study of the adsorption of chromium (VI) ions to *C.vulgaris* and *Z.ramigera*. *Environ Technol* **11** 33-40.
- Baillet F, Magnin J P, Cheruy A, Ozil P 1988 Chromium precipitation by the acidophilic bacterium *Thiobacillus ferrooxidans*. *Biotechnol Lett* **20** (1) 95-99.
- Brady D, Duncan J R 1994 Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* **41** 149-154.
- Greenberg A E, Conners J J, Jenkins, Franson M A H 1980 *Determination of metals. Standard methods for the examination of water and waste water*. 5th edn., APHA, AWWA, WPCF, pp 139-246.
- Krauter P, Martinelli R, Williams K, Martins S 1996 Removal of Cr(VI) from ground water by *Saccharomyces cerevisiae*. *Biodegradation* **7** 277-286.
- Kuyucok N, Volesky B 1988 Biosorbents for recovery of metals from industrial solutions. *Biotechnol Lett* **10** 137-142.
- Norberg A, Rydin S 1984 Development of a continuous process for metal accumulation by *Zoogloea ramigera*. *Biotechnol Bioeng* **26** 265-268.
- Nourbakhsh M, Sag Y, Ozer D, Aksu Z, Kustal T, Caglar A 1994 A comparative study of various biosorbents for removal of chromium (VI) ions from industrial waste waters. *Process Biochem* **29** 1-5.
- Patterson J W 1977 *Waste water treatment*. Science Publishers Inc., New York, USA.
- Volesky B 1987 Biosorbents for metal recovery. *Tibitech* **5** 96-101.
- Zagig J E 1975 *Water pollution*. Marcel Decker Inc., New York, USA.