Silver Uptake and Reuse of Biomass by *Saccharomyces cerevisiae* and *Chlorella emersonii*

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Abstract

Studies were carried out on the recovery of bound silver and reuse of Chlorella emersonii and Saccharomyces cerevisiae biomass for further silver uptake after they were placed in contact with 20mg/l silver for 30 minutes to allow for maximum binding. It was found that 0.16M nitric acid gave the best recovery rates of silver. We conclude that, although both organisms were amenable to reuse in further binding of silver, Chlorella emersonii was more efficient than Saccharomyces cerevisiae. Both organisms lost efficiency in metal uptake after two cycles of silver uptake and reuse of biomass. Metals may be more amenable to recovery and purification following biosorption than through chemical removal which has been reported elsewhere.

Keywords: Biogeotechnology, Silver uptake, Reuse of biomass, *Saccharomyces cerevisiae, Chlorella emersonii*

Introduction

Biogeotechnology is a field in the science of metal extraction from ores, concentrates and solutions with the use of microorganisms and/or their metabolites (Karavaiko *et al*., 1988). Its components are biohydrometallurgy or microbial metal leaching, ore enrichment and biosorption of metals from solutions.

The study of biogeotechnology has significance in pollution control and environmental conservation, reclamation and recycling of scarce metal resources. As the world population grows and standard of living improves, the world-wide demand for minerals will continue and as high grade ores become scarce, lower grade ores in deeper mines will have to be exploited. New mining methods and new processing techniques required for exploitation of these resources will create novel types of environmental problems (Kaas and Parr, 1992). Although legislation has been tightened up, over 50 billion tons of mining wastes have accumulated over the last century. This waste ore consists of gold and silver 2%, lead 3%, molybdenum 1%, uranium 5%, iron 24% and copper 49% (Kass and Parr, 1992). Much of this waste may find its way into the aqueous environment through the action of weathering and run-off. This will continue to be a problem.

Pollution by heavy metals continues to be an increasing environmental problem. Conventional methods such as precipitation, oxidation/reduction, ion exchange filtration, membrane filtration and evaporation can be very costly or inefficient for metal removal from diluted solutions containing from 1 – 100 mg/l dissolved metal (Volesky, 1990). The high cost and the inefficiency of these conventional methods constitute one of the factors that stimulated an interest in alternative metal recovery processes. Some of the alternative metal recovery processes are bioprocesses which have proved to be very efficient and less expansive (Rorrer, 1999). Biosorption which is the ability of some types of inactive, dead microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions, has been reported to be one of the most promising technologies involved in the removal of toxic metals from industrial waste streams from natural waters (Veglio *et al.,* 1997; Zouboulis *et al.,* 1999; Goksungur *et al.,* 2003). The efficiency of biosorption technology has been demonstrated by Goksungur *et al.* (2003) who reported the highest copper ion uptake (120.7mg/l) at pH 4.0, for 198.2mg/l initial copper ion concentration, using waste baker's yeast (*S. cerevisiae*) as a biosorbent. Canterford *et al.* (1978) had earlier reported that the accumulation of the heavy metals copper, zinc, cadmium, and lead by marine planktonic diatom, *Ditylum brightwellii* cultured under laboratory condition, increased with increasing concentration in the medium. It was as a result of the serious consequences of heavy metal pollution of the aquatic environment, as illustrated by Minamata disease in Japan in 1950, that studies of metal uptake by marine organisms (Canterford *et al.,* 1978), were stimulated.

In this study the aim was to examine the possible recovery of bound silver from *Chlorella emersonii*, a green alga, and *Saccharomyces cerevisiae*, yeast, and to further investigate the reuse of the biomass for further accumulation of silver.

Materials and Methods

Cultures: Bakers Yeast (*Saccharomyces cerevisiae*) was obtained from commercial dealers (STK Industries Limited, Lagos). A stock culture of *Chlorella emersonii* (CCAP 211/11N) was obtained from Culture Collection of Algae and Protozoa, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 OLP U.K.

Maintenance of cultures: JM (Jaworski's medium) algal broth described by Thompson *et al.* (1988) and JM agar by the addition of 15g/l of Agar No. 3 (Oxoid) to JM broth were used for the maintenance of algal culture. *Chlorella emersonii* cultures were stored in JM broth and on JM agar slopes at 5^{o}C in a refrigerator and used as required (Droop, 1969). Fresh cultures were prepared by inoculating fresh broth with a loopful of culture and by streaking a fresh

agar slope every two months. The cultures were incubated in natural daylight for two weeks at room temperature before storage. Checks were made for purity using a light microscope. *Saccharomyces cerevisiae* cultures were maintained on malt extract agar and malt extract broth (Oxoid). Growing cultures of *Chlorella emersonii* for use in metal uptake investigations were maintained on a window sill in 20L glass jars (working volume 15L) at room temperature.

Biomass determination: Biomass levels were obtained by dry weight analysis. Cells were washed by suspending them in 10ml distilled water and centrifuged. The cells were resuspended in distilled water after decanting and the procedure repeated to avoid any carry over of salts that might affect the dry weight. The centrifuged cells were added to a dry weighed aluminum dish. This was dried at 105^{o}C until constant weight was achieved on two consecutive weighing (Booth, 1971).

Silver solutions: Standard solutions and stock solutions of silver were prepared using silver nitrate (ANALAR). Stock solution (1000 mgl^{-1}) and standard solutions were prepared using deionised water. Working solutions were made fresh from stock solution as required. To avoid photoreduction, solutions were stored in brown bottles.

Metal analysis: Concentrations of silver were determined using a Varian AA-1275 Series Atomic Absorption Spectrophotometer described in Varian (1979). Metal standards were made up from stock solutions as described above.

Ion selective electrodes: Silver was measured continuously using a silver/sulphide Electrode Model 94-16 ORION (Orion Research Incorporated Products Group). Electrode was calibrated daily before use.

Metal uptake measurements: Investigations were carried out using metal solution (20 mg/l) in 50 ml beakers that had previously been rinsed in distilled water. A suspension of the test organism (2.6mg dry weight in 2 ml) was added to the metal solution and decrease in free ion concentration was measured using an ion selective electrode. To ensure that any decrease in metal concentration was due to the test organism, controls were set up which consisted of metal solution without the test organism. The controls were continuously monitored using an ion selective electrode. To allow for the slight increase in volume caused by introducing the test organism, the controls had an equal amount of distilled water added to them.

Metal recovery: Solutions of metal and organism were centrifuged at 6000 rpm for 10 minutes. The supernatant was analysed for metal concentration using an ion selective electrode. The pellet was resuspended in 20 ml of distilled water, stirred with a glass rod and metal concentration measured.

The above procedure was repeated but with the resuspended pellet having undergone sonication using a MSE Soniprep 150 Ultrasonic Disintegrator which was used at an amplitude of 14 microns (as directed in the instruction manual) for 30 seconds. Metal concentration was determined as above. Concentrated nitric acid (ANALAR BDH Chemicals Ltd Poole, England) digest was used to digest the cell pellets. Biomass was obtained by centrifugation of a known volume of sample, and digesting the cell pellet using concentrated nitric acid, followed by atomic absorption spectroscopy of digests and supernatant (Varian, 1979). Metal was solubilised from biomass using 10ml of 0.8 mol l^{-1} nitric acid. The solution was centrifuged at 6000 rpm for 10 minutes and the metal concentration in the supernatant recorded using an ion selective electrode. Metal was solubilised from biomass using 10 ml of 0.16 mol l^{-1} nitric acid as above.

Biomass reuse: The biomass (2.6mg dry weight in 2 ml distilled water) of each of *Chlorella emersonii* and *Saccharomyces cerevisiae* were placed in contact with 20 mg/l silver nitrate (20ml) for 30 minutes to allow maximum binding. The metal was then washed off using 0.16M nitric acid. The organisms were then placed in fresh solutions of 20mg/l silver nitrate (20ml). This was repeated three times. Estimates were then made of silver bound and removed each time.

Results and Discussion

Recovery of metal was possible by washing, thus opening up the possibility of recycling leading to an economic means of metal recovery. Of the several methods employed to strip bound metal from the *Chlorella emersonii* cell wall, dilute acid gave a better recovery of ionic metal than concentrated acid (Table 1).

Table 1: Removal of bound silver from the surface of *Chlorella emersonii*

Treatment	Bound Metal (µg)	Metal Recovered	
		Actual (µg)	Percentage
Conc. HNO_3	38.6	0.12	0.31
Water	39.2	1.4	3.6
Sonication	39.2	1.4	3.6
0.8M HNO_3	39.2	14.8	37.8
0.16M HNO_3	38.6	16.6	43.0

Although concentrated nitric acid gave the highest ionic silver recovery when *Saccharomyces cerevisiae* was used (Table 2), concentrated nitric acid digest destroyed the biomass and it was, therefore, not possible to reuse the biomass.

Table 2: Removal of bound silver from the surface of *Saccharomyces cerevisiae*

Treatment	Bound Metal (µg)	Metal Recovered	
		Actual (µg)	Percentage
Conc. HNO_3	61.5	17.7	28.8
Water	61	10.2	16.72
Sonication	60.9	9.3	15.26
0.8M HNO_3	61.2	13	21.24
0.16M HNO_3	62	17.3	27.9

Recovery of ionic metal with a water wash was higher with *Saccharomyces cerevisiae* than with *Chlorella emersonii*. It was also observed that, with *Chlorella emersonii,* there was 15% reduction of silver uptake after one wash and 42% after two washes (Table 3). On the other hand, *Saccharomyces cerevisiae*

showed higher rate of reduction in silver uptake after one wash (34%) and a second wash (84%). This implies that the alga, *Chlorella emersonii,* with a lower silver uptake reduction rate, is more efficient than *Saccharomyces cerevisiae* in silver uptake, although both organisms lost efficiency in metal uptake after two washes. Washing with water or sonification resulted in very little removal of metal.

Table 3: The removal and rebinding of silver to *Chlorella* and *Saccharomyces*

Starting Silver (μg silver)	*Chlorella* 20000*	*Saccharomyces* 20000*
1st 30 minute uptake (μg silver)	38	62.5
1st 0.16M HNO_3 wash (μg silver)	17	17.4
2nd 30 minute uptake (μg silver)	32.3	41.3
2nd 0.16M HNO_3 wash (μg silver)	16	18
3rd 30 minute uptake (μg silver)	22	10

**The original silver nitrate concentration used was 20mg/l which is equivalent to 20,000μg/l*

Several microorganisms have been shown to be able to accumulate different metals from solutions. For example, lead and cadmium have been shown to be effectively removed from solutions by species of brown algae such as *Ascophyllum* and *Sargassum* while *Rhizopus* and *Absidia* have been shown to be excellent biosorbents for lead, cadmium, copper, zinc, uranium, etc. (Volesky and Holan, 1995). In this study, the alga, *C. emersonii* and the yeast, *S. cerevisiae*, have been observed to be excellent biosorbents for the uptake of silver as well. The efficiency and rapid uptake of silver by *C. emersonii and S. cerevisiae* indicate that the two organisms met one of the criteria that must be fulfilled by biosorbents for metal processes (Volesky, 1990). Besides, the two organisms were effective under both high and low acidities, with biomass still recovered after some cycles, which is one of the criteria reported by Hughes and Poole (1989). Elsewhere, *S. cerevisiae* was used as a biosorbent for copper ion biosorption when the lowest uptake was 21.1mg/g when the yeast cells were treated with caustic soda, while the highest copper ion uptake of 120.7mg/g was obtained at pH 4.0 for 198.2 mg/l initial copper ion concentration (Goksungur *et al.,* 2003). This agrees with the findings of this study in which 0.31% of silver was recovered from bound silver from the surface of *C. emersonii*, higher uptake of silver with concentrated nitric acid, and low uptake with a more dilute acid (0.16MHN03). Besides, Canterford *et al.* (1978) has reported increased accumulation of zinc, cadmium and lead with increasing concentration of the metals in the medium, by the marine diatom, *Ditylum brightwellii,* under laboratory conditions, and this agrees with the observation made in this study that the alga, *C. emersonii,* is a very efficient biosorbent for silver biosorption. *C. emersonii* and other organisms can easily be immobilized (Trevan and Mak, 1988; Mallick and Rai, 1993), and as dead cells can accumulate metal to the same degree as living cells, contamination by toxic materials is unlikely to affect any process based on biosorption of metals (Goksungur *at al.,* 2003; Arikpo and Eja, 2003). It is concluded that, although *C. emersonii and S. cerevisiae* were amenable to reuse in further binding of silver, *C. emersonii* was more efficient than *S. cerevisiae* in silver uptake.

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