Bioaccumulation of Chromium(III) from Aqueous Solutions of a Leather Wastewater Treatment Plant by *Saccharomyces cerevisiae* Yeast

by

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Abstract

With many industries discharging heavy metals into natural water resources, heavy metals have been found to accumulate in various living organisms which can ultimately threaten human life and pose a big threat to the environment. Thus, in the pursuit of a solution to the above mentioned problem, bioaccumulation has emerged as an interesting option for the removal of heavy metals from wastewater. In this paper, the effectiveness of the yeast *Saccharomyces cerevisiae* in the bioaccumulation of Cr^{3+} has been tested. Also, different factors influencing Cr^{3+} uptake have been discussed. This work has demonstrated that Saccharomyces cerevisiae is an effective Cr^{3+} biosorbent for tannery wastewater. The conditions of use of this yeast to achieve optimal chromium (III) absorption are: i) when a growth of the biosorbent equivalent to a similar concentration of Cr^{3+} is obtained, which contains the residual water that needs to be treated; ii) the smaller the biosorbent is the better the biosorption; iii) the uptake of Cr^{3+} is more efficient when no extra growth medium is added to the wastewater; iv) the longer the exposure period of the yeast to Cr^{3+}, the bigger the Cr^{3+} reduction. Since *Saccharomyces cerevisiae* is an inexpensive, readily available source of biomass, this discovery could be of great use for a low-budget and efficient wastewater treatment system.

Introduction

Industrialization is to a great degree responsible for the contamination of the environment especially water, where lakes and rivers are brimmed with a large number of toxic substances. Compared with other toxic substances, heavy metals are reaching hazardous levels. Their continuous release leads to overconsumption and accumulation. Many industries (leather, fertilizers, pesticide, metallurgy, photography, aerospace, electroplating, mining, iron and steel, surface finishing, energy and fuel production, appliance manufacturing, metal surface treating, electrolysis and electro-osmosis) discharge waste containing heavy metals either indirectly or directly into the water resources.[1] Toxic heavy metals of concern are lead (Pb), mercury (Hg), chromium (Cr), nickel (Ni), arsenic (As), zinc (Zn), copper (Cu), cobalt (Co), cadmium (Cd), and so on. Due to the fact that these metals are not biodegradable, they tend to accumulate in the living organisms and lead to various disorders and diseases which ultimately threaten human life. Bioaccumulation has emerged as an interesting option over conventional methods for the removal of heavy metal ions from effluents discharged from various industries.[2]

Bioaccumulation is an active, metabolism-mediated process where the metal ions accumulate intracellularly in the living cells.[3] As mentioned by Diep et al., bioaccumulation is a natural biological phenomenon where microorganisms use proteins to uptake and sequester metal ions in the intracellular space to utilize in cellular processes (e.g., enzyme catalysis, signaling, stabilizing charges on biomolecules). Recombinant expression of these import-storage systems in genetically engineered microorganisms allows for enhanced uptake and sequestration of heavy metal ions.The process occurs in two steps: firstly, the adsorption of metal ions onto cells, which is quick and identical to biosorption, and the second step is slower and it includes the transport of metal ions inside the cells by active transport.[4] The process of bioaccumulation occurs by cultivating the biomass of a microorganism in a solution that contains the metal that will be accumulated. Since the solution contains the growth medium, the organism begins its metabolic processes and activates the intracellular transport systems for the accumulation of the sorbate.[5] However, the major limitation of the process is that the nutritive medium for growth of the microorganism contains organic carbon sources.[6] Part of the biosorbate accumulates inside the cell which enables the biomass to increase and bind greater amounts of metal ions. The organisms which are capable of resisting high loads of metal ions are best suited for accumulating metal species. They do not possess any mechanisms for blocking the accumulation of metal ions in large quantities. They may possess special mechanisms for synthesizing special intracellular binding regions rich in thiol groups as a response to metal ions in their surviving environment.[7]

Bacteria, microalgae, yeasts and fungi all showed the ability to remove Cr^{3+} in bioacuumulation processes[8] but in this work we were focused on the removal ability of the yeast. Yeasts have been

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Manuscript received June 7, 2020, accepted for publication July 21, 2020.

little studied for use in bioaccumulation processes. However, they are versatile microorganisms since they develop in both aerobic and anaerobic environments, principally the *Saccharomyces* species. In addition to their versatility they show low-cost nutritional requirements, they are safe microorganisms and can be applied both dead and alive.[9]

Saccharomyces cerevisiae is an inexpensive, readily available source of biomass for bioremediation of waste-water.[3] It has been shown to accumulate heavy metals, such as Cr^{3+}, Co^{2+} and Cd^{2+} via two distinct processes. There is an initial rapid accumulation step that is metabolism and temperature independent and is thought to involve cation binding at the surface. This step is followed by a second, much slower, process that is metabolism-dependent and can accumulate larger quantities of cation than the first process. This second process is believed to involve cation internalization into the cell. The uptake system that allows for accumulation of Co^{2+} and Cd^{2+} cations appears to be a general one with only limited specificity, since competition for uptake of cations occurs. The following processes contributing to the mechanism of bioaccumulation includes intracellular accumulation and oxidation or reduction reactions. The process is very complex and depends of several factors (which are almost identical as the factors influencing the cultivation of an organism): initial metal concentration, biosorbent dose, contact time (time the yeast has spent in the presence of the heavy metal), the composition of the growth medium, wastewater content, pH, temperature, the presence of other pollutants (which are growth inhibitors, as well) or other inhibitors. Further investigations demonstrated that yeasts are capable of accumulating other cations such as Cu^{2+}, Mn^{2+} and Ni^{2+} and are superior metal accumulators compared to certain bacteria.[13]

In this paper we have tested if the yeast *Saccharomyces cerevisiae* can be used as a biosorbent to eliminate Cr^{3+} from waste-waters and analyzed how various factors such as composition of medium, biosorbent dose, contact time and adaptation of biosorbent to Cr^{3+} affected the Cr^{3+} absorbtion.

Experimental

Microorganisms and growth conditions

Commercially available yeast *Saccharomyces cerevisiae* was routinely maintained in Erlenmeyer flasks containing YPD broth composed of (g L^{-1}): glucose 20, yeast extract 10 and bacto peptone 20. Erlenmeyer flasks and pipette tips were autoclaved for 20 min at 120°C. Growth medium was inoculated by transferring organisms to 250 mL Erlenmeyer flasks containing 100 mL growth medium and aeration was maintained by shaking at 200 rpm at 25°C. Growth of this medium in the absence of heavy metals was defined as the control run.

Preparation of the Chromium standard solution

A 1000 mg/L commercial stock solution (chromium standard solution 1000 mg/L Cr, chromium (III) nitrate in nitric acid 0.5 mol/L, CR02220100, Scharlau) was used to dose the adequate volume to the 100 mL flask containing the growth media to obtain 0, 5, 10 and 30 mg Cr/L (0.1, 0.5, 1 and 3 mL of commercial stock solution to 100 mL of growth media).

Cr^{3+} uptake experiments with nonadapted and adapted Saccharomyces cerevisiae

To study the bioaccumulation properties of *Saccharomyces cerevisiae*, chromium standard solution 1000 mg/L was added to a 100 mL of growth media. Stock solutions of chromium were prepared and appropriate volumes of stock solution were supplemented to the media to give final Cr^{3+} concentrations of 0, 1, 5, 10 and 30 ppm. The accumulation medium was also inoculated by transferring nonadapted microorganisms. Adaptation to chromium ion was achieved by subculturing the cells at increasing concentration of metal ions corresponding to 1, 5, 10 and 30 ppm of Cr(III). A 2.5 mL of sample taken from the previous culture containing 1 ppm Cr^{3+} ions was used for the inoculation of 100 mL culture medium having 5 ppm metal ions. When the adapted culture reached its exponential growth, the same amount of culture medium (2.5 mL) was used again to inoculate the next 100 mL of culture, which has 10 ppm metal ions. Finally, this procedure was repeated for cultures supplemented with the concentration of 30 ppm of Cr(III). Thus yeast cells exposed to Cr^{3+} in increasing concentrations developed Cr^{3+} resistance. For adapted and nonadapted viable microorganisms, cultures were grown at 25°C in a shaking incubator at a 200 rpm constant stirring rate for a 96 h exposure period. After 96 h the OD660 was analysed for each flask and the amount of yeast cells was calculated. An exact amount of cells were taken from each flask (1,785 × 10^6 cells) and they were used to inoculate 100 mL of wastewater. Cultures were left in the wastewater at 25°C in a shaking incubator at a 200 rpm constant stirring rate for a 96 h exposure period. After 96 h the samples where filtered (90 mm and 0.45 μm pores) and digested. Digestion of yeast samples was performed using a multi-block heater (Lab Line Instruments). Washed yeast cells were digested directly inside the filter plates (100 μL well–1nitric acid, ~88 °C for 40–45 min) using the heating block. After the digestion, the samples were filtered again and analyzed for Cr^{3+} using a Unicam 929 atomic absorption spectrophotometer. The bioaccumulated metal ion amounts were determined as the difference between the initial Cr(III) concentration and concentration in the filtrate.

Cr^{3+} uptake experiments with different biosorbent doses

To study the bioaccumulation properties of *Saccharomyces cerevisiae* with different biosorbent doses, 4 Erlenmeyer flaks containing 100 ml of wastewater each were inoculated with different amounts of

yeast cells (1 mL, 2.5 mL, 5 mL and 10 mL) adapted to 30 ppm of Cr^{3+} to obtain different dilutions of the biosorbent (1:100, 1:40, 1:20 and 1: 10). Cultures were grown at 25°C in a shaking incubator at a 200 rpm constant stirring rate for a 96 h exposure period. After 96 h the samples where filtered (90 mm and 0.45 μm pores) and digested. After the digestion, the samples were filtered again and analyzed for Cr^{3+} using a Unicam 929 atomic absorption spectrophotomete. The bioaccumulated metal ion amounts were determined as the difference between the initial Cr^{3+} concentration and concentration in the filtrate.

Cr^{3+} uptake experiments with different compositions of growth medium

To study the bioaccumulation properties of *Saccharomyces cerevisiae* in different growth medium conditions the growth medium was added to 3 Erlenmeyer flasks containing different amounts of wastewater (100 mL, 75 mL, 50 mL) by adding ultrapure water until achieving a final volume of 100 mL. The wastewaters where inoculated with 2.5 mL of yeast cells adapted to 30 ppm Cr^{3+} and left at 25°C in a shaking incubator at a 200 rpm constant stirring rate for a 96 h exposure period. After 96 h the samples where filtered (90 mm and 0.45 μm pores) and digested. After the digestion, the samples were filtered again and analyzed for Cr^{3+} using a Unicam 929 atomic absorption spectrophotometer. The bioaccumulated metal ion amounts were determined as the difference between the initial Cr^{3+} concentration and concentration in the filtrate.

Cr^{3+} uptake experiments with different contact times

To study the bioaccumulation properties of *Saccharomyces cerevisiae* with different contact time with the Cr^{3+} ions, 4 Erlenmeyer flaks containing 100 mL of wastewater each were inoculated with 5 mL of yeast cells adapted to 10 ppm of Cr (III). Cultures were left in the wastewater at 25°C in a shaking incubator at a 200 rpm constant stirring rate for different exposure periods (1 h, 3 h, 5 h and 24 h). After the specific exposure period finished for each sample, the samples where filtered (90 mm and 0.45 μm pores) and digested. After the digestion, the samples were filtered again and analyzed for Cr^{3+} using a Unicam 929 atomic absorption spectrophotometer. The bioaccumulated metal ion amounts were determined as the difference between the initial Cr(III) concentration and concentration in the filtrate.

Results and discussion

Cr^{3+} uptake experiments with non-adapted and adapted Saccharomyces cerevisiae

It is well known that yeast is readily adapted to new environmental factors.[14] Also, in the literature it is stated that yeasts which grow in a medium containing heavy metals is adapted to them and absorb them more effectively, compared to yeasts grown without the presence of heavy metals.[15] For that reason we decided to test this hypothesis by growing our yeast in the presence of different concentrations of Cr^{3+} (from 0 ppm to 30 ppm) After that we transferred these adapted and non-adapted yeasts to the wastewater and after 96 hours we could see that there are some differences in the Cr^{3+} absorption. In Figure 1 it is noticed that non-adapted yeast diminished the amount of Cr^{3+} by 24% where the yeasts adapted to 10 ppm and 30 ppm diminished the amount of Cr^{3+} by 48% and 47%, respectively. The yeast adapted to 5 ppm achieved the best result in Cr^{3+} uptake and diminished the amount of Cr3+ by 60%. We can conclude that adapted yeast absorb Cr^{3+} more effectively than non-adapted yeast. It is important to notice that the concentration of the Cr^{3+} in the wastewater was between 0.01-5 ppm and we can clearly see that yeast adapted to similar conditions achieved the best results in Cr^{3+} uptake.

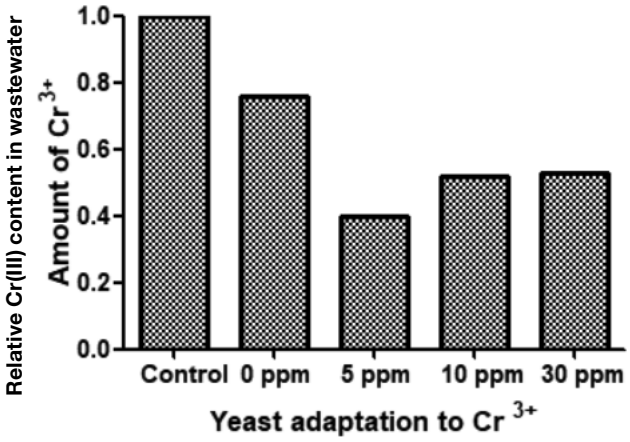

Figure 1. Chromium (III) uptake from wastewater is more effective with adapted than non-adapted yeast.

Cr^{3+} uptake experiments with different biosorbent dose

Once we confirmed that the Cr^{3+} uptake from wastewater is more effective with adapted yeast, we wanted to test how changing the dose of this adapted biosorbent will affect the chromium uptake. In this case, flasks containing 100 mL of wastewater each were inoculated with different amounts of yeast cells (1 mL, 2,5 mL, 5 mL and 10 mL) adapted to 5 ppm of Cr^{3+} to obtain different dilutions of the biosorbent (1:100, 1:40, 1:20 and 1: 10). The results (**Figure 2**) indicate that the amount of yeast in the wastewater is inversely proportional to Cr^{3+} uptake. When the yeast was diluted 10 times, the Cr^{3+} amount was diminished by 21%, on the other hand, when it was diluted 100 times, the Cr^{3+} amount was diminished by 62%. For the dilutions of 20 and 40 times, similar results were obtained as for the 1:10 dilution which are 25% and 26%, respectively. To sum it up, it can be said that the smaller the biosorbent dose is, the better the biosorption. The reason for that can be the fact that yeasts are known to introduce and metabolize Cr^{3+} only in the Log phase of growth which is the phase when cells are growing as fast as they can and that can only happen when there is a small amount of cells in

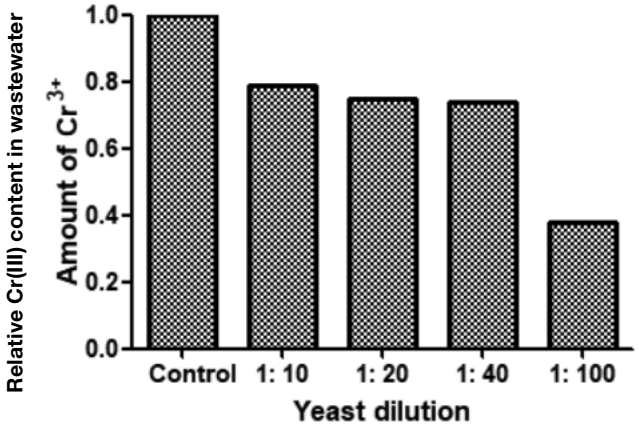

Figure 2. Chromium (III) uptake from wastewater is more effective with a smaller concentration of the biosorbent.

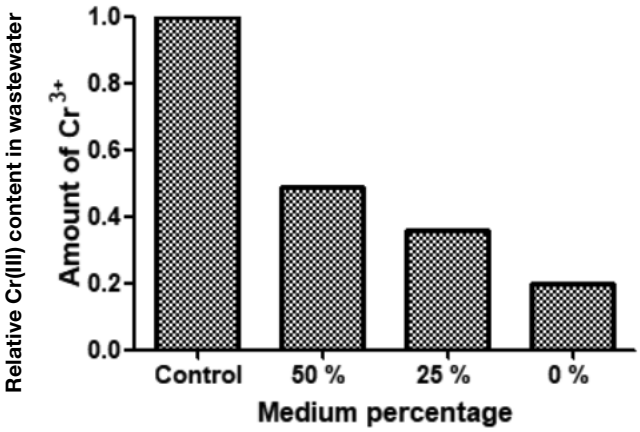

Figure 3. Chromium (III) uptake from wastewater is more effective when there is no extra growth medium added to the solution.

the solution and enough nutrients. As soon as they grow into a large quantity of cells, they start to run down on nutrients and slow down growth therefore also slowing down Cr^{3+} uptake. For that reason it is possible that if the wastewater is inoculated with a big amount of yeast cells they will not grow further and therefore they will not eliminate Cr^{3+} that efficiently.

Cr^{3+} *uptake experiments with different composition of growth medium*

To check how the biosorbent dose affects Cr^{3+} absorption, we decided to test the effect of the composition of the growth medium on the uptake of chromium (III). For this experiment we inoculated different amounts of wastewater (50, 75, 100 mL) with yeast adapted to 30 ppm of Cr^{3+} and added different amounts of medium (50, 25, 0 mL) to obtain a final volume of 100 mL. In Figure 3, it can be seen that the Cr^{3+} uptake is inversely proportional to the amount of growth medium in the wastewater, which means that the more growth medium the smaller is the Cr^{3+} uptake. It can be seen that when the solution is comprised of 50% growth medium and 50% wastewater, the Cr^{3+} amount was diminished by 51%, while there was less medium in the solution (25% and 0%), better results where obtained, which are 64% and 80% less chromium (III), respectively. The reason why this might be happening is that in the medium there is carbon which can also absorb heavy metals like Cr (III), but can't be filtered, so once the samples are digested and analyzed, all the Cr^{3+} absorbed by the carbon from the medium will stay in the solution. To conclude it can be said that the uptake of Cr^{3+} is more efficient when no extra growth medium is added to the wastewater.

Cr^{3+} *uptake experiments with different contact time*

Finally, we wanted to investigate how the exposure duration of the adapted yeast affects the Cr^{3+} uptake from the wastewater. To conduct this experiment, flask containing 100 mL of wastewater each were inoculated with 5 mL of yeast cells adapted to 5 ppm of Cr(III). Cultures were left in the wastewaters for different exposure periods (1 h, 3 h, 5 h and 24 h). As it can be seen in Figure 4, the best results where obtained after 24 h contact time, where the amount of Cr^{3+} was diminished by 39%. Shorter exposure periods such as 1 h and 3 h, showed a Cr^{3+} reduction of 18% and 20 % respectively, while the contact time of 5 h showed similar results as the contact time of 24 h with a Cr^{3+} reduction of 36%. From the results it is obvious that the longer the exposure period of the yeast to Cr (III), the bigger the Cr^{3+} reduction. However, the time required to attain maximum biosorption depends on the type of biosorbent, metal ion, and their combination. The rate of biosorption is rapid initially (within an hour) because all the active sites are vacant and available for metal ion biosorption. But with increase in time the rate of biosorption decreases due to increase in percentage saturation by metal ions remaining in the solution.

The results obtained were quantified in Image J program and normalized to the initial Cr^{3+} concentration.

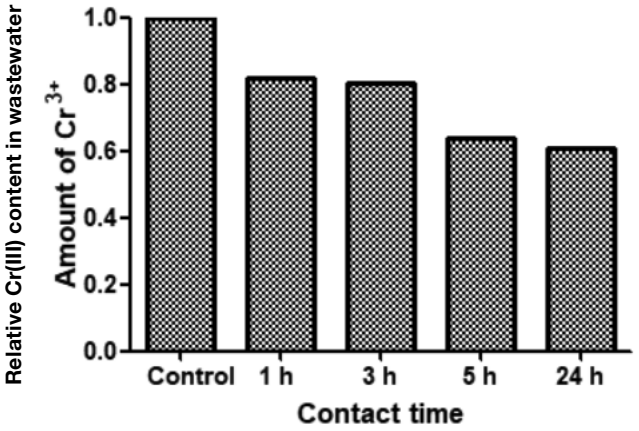

Figure 4. Chromium (III) uptake from wastewater is more effective when the exposure period is longer.

Conclusions

In this study, an alternative way for chemical treatment has been found to reduce the concentration of chromium in the wastewater from the treatment plant of the tanneries of Leather Cluster Barcelona. The application of microorganisms such as *Saccharomyces cerevisae* appears to be a low-cost biotechnological tool. In this paper it has been demonstrated that the yeast *Saccharomyces cerevisiae* can be used as a biosorbent to eliminate Cr^{3+} from wastewater and it has been shown how various factors such as composition of medium, biosorbent dose, contact time and adaptation of biosorbent to Cr^{3+} affect the Cr^{3+} absorbtion. It has been proven that the Cr^{3+} absorption is best in the conditions when the used biosorbent is pre-adapted to a similar concentration of Cr^{3+} like in the wastewater that needs to be treated, when there is a smaller concentration load of the biosorbent in the wastewater, when there is no extra medium added to the solution and long exposure period.

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