Biosorption of chromium(VI), nickel(II) and Remazol Blue by *Rhodotorula mucilaginosa* biomass

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**ABSTRACT**

The passive removal of commonly used reactive dye and two heavy metals, from aqueous solutions by inexpensive biomaterial, yeast *Rhodotorula mucilaginosa* biomass, termed biosorption, was studied with respect to pH, initial dye concentration and initial metal ion concentration. The biomass exhibited maximum dye and chromium(VI) uptake at pH 5 and pH 6 for nickel(II) in media containing 50 mg/L heavy metal and 50 mg/L remazol blue. It was found that the highest chromium(VI) removal yields measured were 31.3% for 49.0 mg/l initial chromium(VI) concentrations. The nickel(II) removal yield was 32.5% for 22.3 mg/L. Higher R. Blue removal yields were obtained, such as 77.1% for 117.5 mg/L. The maximum dye biosorption yield was investigated in medium with a constant dye (∼50 mg/L) and increasing heavy metal concentration. In the medium with 48.8, 103.8 and 151.8 mg/L chromium(VI) and constant dye concentration, the maximum chromium(VI) biosorption was 7.4, 9.3 and 17.1%, whereas the maximum dye biosorption was 61.6, 56.6 and 55.9%. The maximum nickel(II) biosorptions in the medium with dye were 38.1, 22.1 and 8.8% at 23.7, 37.7 and 60.1 mg/L nickel(II) concentrations. In these media, dye biosorptions were 93.9, 86.4 and 93.3%, respectively.

**Key words** | biosorption, heavy metal, reactive dye, wastewater treatment, yeast

**INTRODUCTION**

A great number of industries such as textile, printing, paper and pulp, iron-steel, pesticide, paint, solvent, and pharmaceuticals consume large volumes of water, and organic based chemicals. These chemicals show a large difference in chemical composition such as molecular weight, toxicity, etc. In addition, effluents of these industries contain undesired quantities of these pollutants and need to be treated (Lloyd-Jones *et al.* 2004; Aksu 2005). The heavy metal pollution is of greatest concern among the kinds of environmental pollution because of high toxicity and mobility of heavy metals. In addition, colour removal has been the target of great attention in the last few years due to these highly coloured industrial effluents substantially affecting visibility and photosynthesis in the water and being toxic to the aquatic life due to the presence of metals, chlorides and other compounds (Chapman & Kimstach 1996; Slokar & Le Marechal 1997; Mahony *et al.* 2002). Many synthetic dyestuffs and heavy metals are resistant to biological degradation that cannot be biodegraded due to their complex aromatic molecular structures; so, colour and heavy metal removals by traditional biological processes are difficult (Ozcan & Ozcan 2004; Ahmad *et al.* 2010). Lately, many physical and chemical treatment methods including adsorption, filtration, chemical coagulation, precipitation, electrodialysis, membrane separation and oxidation have been used for the treatment of dye and heavy metal containing effluents. Some of these techniques have been shown to be effective, although they have limitations such as overall cost, regeneration problems, limit versatility and secondary pollutants (Ong *et al.* 2005; Crini 2006). Biosorption is an effective technology using inactive and dead biomasses to remove pollutant from aqueous solutions in the absence of metabolic activity necessary for intracellular accumulation (Ileri & Mavittuna 1994; Esposito *et al.* 2002; Davis *et al.* 2005). On the other hand, biosorption represents passive interactions of the cell wall with metal ions. These reactions include adsorption, ion exchange reactions with functional groups at the cell surface, and surface reactions. Metal ion binding sites which are localized at the cell surface include carboxylic, hydroxyl, and phosphate groups of lipids, proteins, and polysaccharides (Beveridge 1989; Yalçın *et al.* 2010).

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In addition, the use of dead biomass appears to be more attractive as compared with live biomass, as heavy metals or other toxic pollutants have little influence on the biosorption process. Besides, there is no requirement for the supplement with any nutrients for maintaining growth of dead biomass. Metal ions, chromium(VI) and nickel(II) were chosen for biosorption studies with regard to their wide and common use in industry and potential pollution impact. Hexavalent chromium from electroplating and similar industries has been reported to be toxic to animals and humans; it is known to be carcinogenic and to create ecological problems (Mungasavalli et al. 2007; Chatterjee et al. 2010). Nickel(II) has been used in many processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, plastics pigments, mining and metallurgical processes then discharged into the environment (Aksu & Dönmez 2003; Gupta et al. 2001). Compared with nickel(IV), nickel(II) is a more toxic and carcinogenic metal. This situation is due to its toxic effects on living systems, so strict limits have been stipulated for discharge of nickel(II) into the environment. R. Blue which was used in this study is a reactive dye. Reactive dyes are typically azobased chromophores combined with different types of reactive groups e.g. chlorotriazine, vinyl sulfone. Reactive dyes are different from the other classes of dyes that bind to the textile fibers, e.g. cotton, through covalent bonds. They are used extensively in textile industries because of their bright colours, simple application techniques and low energy consumption. Therefore their removal is of great importance (Sumathi & Manju 2000; Robinson et al. 2001). A variety of biomaterials have been investigated as biosorbents, such as bacteria (Vijayaraghavan & Yun 2008), fungi (Aksu et al. 2010), algae (Apiratikul & Pavasant 2006), agricultural wastes (Sud et al. 2008), industrial waste (Wang & Chen 2006) and other polysaccharide materials (Crini & Badot 2008). Yeasts are a better crude biosorbent material for the removal of heavy metal ions or dyes because of their unicellular structure and high growth rate (Aksu & Dönmez 2003). In addition, yeast cells can be easily cultivated into inexpensive growth media, and have the potential for bioremediation of wastes at lower pH values (Vasudevan et al. 2003; Malik 2004). Much of the work on the biosorption focusing on the uptake of single pollutants such as cadmium and lead biosorption by the Rhodotorula genus of yeast has been documented (Cho & Kim 2003; Li & Yuan 2006). In practice, wastewaters are polluted with multiple components. For this reason, in this study, a process of biosorption of chromium(VI) and nickel(II) and R. Blue dye onto R. muciloginosa was investigated in medium with an increasing heavy metal and dye concentration and constant dye (approximately 50 mg/L) and increasing heavy metal concentration. These heavy metals and dye were chosen because of their widespread use in Turkish industry with relatively high consumption rate and being quite common pollutants in wastewaters from the textile industry. As little is known of the biosorption of heavy metals and dyes to yeast biomass, the biosorptive properties of the microorganism for heavy metals and dye should be investigated. In addition, the understanding of the biosorptive function of dead R. muciloginosa is essential for the design of a process that makes efficient use of such a biosorbent.

**METHODS**

**Dye and heavy metal solutions**

The stock solutions of chromium(VI) and nickel(II) were prepared by dilution of K₂Cr₂O₇ (Merck) and NiSO₄ (Merck) to a final concentration of 1 g/L of chromium(VI) and nickel(II). The test solutions containing R. Blue reactive dye were prepared by diluting 2 g/L of stock solution of the dye. Appropriate volumes of the stock solutions were added to the media.

**Isolation of yeast**

Samples uptake from textile wastewaters was centrifuged and spread on (0.1 mL) the Petri plates containing molasses media with 50 mg/L R. Blue and 50 mg/L heavy metal (chromium and nickel) and incubated at 30 ± 1 °C. The composition of the growth medium is molasses solution (nearly equivalent to 10 g/L sucrose), 1.0 g/L NH₄SO₄ and 0.5 g/L KH₂PO₄ (Aksu & Dönmez 2003). Agar (15 g/L) was added for plates. The pH of the growth medium was adjusted to pH 5 for nickel(II) and pH 6 for R. Blue and chromium(VI) by dilute (0.01 mol/L) and concentrated (1 mol/L) H₂SO₄ or NaOH solutions. Cells from microcolonies on these plates were isolated and purified and streaked repeatedly on the molasses medium agar plate with R. Blue and heavy metals. The pure cultures were kept at 4 °C and were transferred to molasses media including R. Blue and heavy metals every 3 months.
**PCR and sequencing**

The exponential phase bacterium was used for 18S rRNA gene amplification. PCR was carried out by 25 cycles of denaturation step at 96°C for 10 s, annealing at 50°C for 5 s and elongation at 60°C for 4 min. The primer set used in PCR reaction was the D1/D2 region of large-subunit rDNA sequencing and phylogenetic analysis was carried out as described by Fell *et al.* (2000) and Biswas *et al.* (2001).

**Preparation of biosorbent: Yeast**

Yeast cells were grown in molasses media at pH 5 value at 30 ± 1°C on a rotary shaker for 2 days. The biomass was centrifuged 3.421 g for 10 min (Hettich EBA12). The pellet was exterminated by autoclaving (Alp CL-40M) at 121°C for 15 min. 1.25 g of assassinate biomass was added to the biosorption flasks according to the biomass found in previous bioaccumulation studies (Ertugrul *et al.* 2009).

**Biosorption studies**

The experiments were performed in 250 ml Erlenmeyer flasks containing solutions of different R. Blue concentrations 50, 100, 200, and 400 mg/L, chromium(VI) concentration 50–150 mg/L and nickel(II) concentration 50–100 mg/L in 100 mL water. Samples (3 mL) were taken at the end of 1 h and centrifuged at 3.421 g for 10 min. The supernatant was analyzed for the remaining dye and heavy metal concentration.

**Analytical methods**

A 3 mL sample was taken from each flask, at the end of a 1 h incubation period. Dissolved dye concentrations of R. Blue in the biosorption medium were measured calorimetrically using a spectrophotometer (Shimadzu UV 2001, Japan). The absorbance of the colour of R. Blue was read at 600 nm. The concentration of nickel(II) in the supernatant was determined spectrophotometrically at 540 nm by using sodium diethyl dithiocarbamate as the complexing agent for nickel(II), respectively (Snell & Snell 1959). The concentration of chromium(VI) in the supernatant was determined spectrophotometrically at 540 nm using diphenylcarbazide reagent in acid solution as the complexing agent for chromium(VI). Experiments were conducted in triplicate and the results are the average of triplicate measurements. Finally, the percentage biosorption ($E$, %) and uptake capacity ($Q$, mg/g) were calculated by Equations (1) and (2), respectively.

\[
E = \left(\frac{C_0 - C_f}{C_0}\right) \times 100\% \quad (1)
\]

\[
Q = \frac{(C_0 - C_f)V}{W} \quad (2)
\]

where, $C_0$ (mg/L) and $C_f$ (mg/L) are the initial and residual concentration of metal ion/dye, respectively. $V$ (L) and $W$ (g) are the solution volume and the dosage of biosorbent, respectively.

**RESULTS AND DISCUSSION**

**Effect of initial pH on chromium(VI), nickel(II) and Remazol Blue uptakes**

The pH of metal and/or dye solution plays an important role in the whole biosorption process and particularly in the adsorption capacity, influencing the surface charge of the biosorbent, degree of ionization of the species present in the solution and the dissociation of functional groups on the active sites of biosorbent, and the solution metal and dye chemistries. Optimal pH for the biosorption experiments was found in previous bioaccumulation experiments by Ertugrul *et al.* (2009). According to these experiments, optimal pH values for the yeast cells to remove the pollutants were, pH 6 for only dye and chromium(VI) and dye, and pH 5 for nickel(II) and dye in media containing 50 mg/L heavy metal and 50 mg/L R. Blue. Low pH has been found to favour adsorption of dyes and heavy metals (Bai & Abraham 2001) by the biomass of yeast and fungi.

**Single effect of initial dye and heavy metal concentration on biosorption**

R. Blue biosorption by *R. mucilaginosa* was investigated at different initial dye concentrations between 54.9, 117.5, 173.5 and 365.1 mg/L at pH 6. As shown in Figure 1, maximum dye biosorption yield was 77.1% for 117.5 mg/L. The yeast species was capable of removing more than 50% of the colouring material at 50 mg/L of initial dye concentration. This suggests that the adsorbent capacities of all the biosorbents were most effectively utilized in solutions of this initial dye concentration. Reactive dye biosorption was also reported in previous studies performed with dried yeast cells. For example Aksu & Dönmez (2003) reported...
the biosorption capacities and rates of nine yeast species (S. cerevisiae, S. pombe, K. marxianus, Candida spp., C. tropicalis, C. lipolytica, C. utilis, C. quilliermendii, and C. membranaefaciens) for R. Blue reactive dye from aqueous solutions. Although previous studies have used yeasts for removing reactive dye from effluents, the biosorption of reactive dye by Rhodotorula spp. has not been reported.

Biosorption capacities of the yeast cells at 49, 127.1 and 165.7 mg/L initial chromium(VI) concentrations and 22.3, 47.7 and 62.2 mg/L initial nickel(II) concentrations, are given in Figures 2 and 3. At the end of the experiments, the highest chromium(VI) removal yields measured were 31.3% for 49 mg/L concentrations. The nickel(II) removal yield was 32.5% for 22.3 mg/L. It is clearly seen in Figures 1 and 2 that the R. Blue biosorption yield of the yeast cells was significantly higher than that of chromium(VI) and nickel(II). The previous studies on biosorption indicated that chromium(VI) and nickel(II) are metals with a low affinity for biosorption (Holan & Volesky 1994). The higher dye uptake obtained at acidic conditions may be explained in terms of electrostatic interactions between the surface of R. mucilaginosa biomass and anionic dye (Vijayaraghavan & Yun 2007; Akar & Divriklioglu 2010).

In the experiments with Candida tropicalis and Candida lipolytica, yeasts biosorbed 20 mg/L chromium (VI) at pH 2 with 25 g/L biomass and 81.37%, while for nickel(II) 0.351 mmol/L and 64.6% in 4 h (Yin et al. 2008). However in our studies, we used less biomass and our experiments were more rapid (1 h) than other experiments.

**Simultaneous effect of dye and heavy metal on biosorption**

Dye and heavy metal biosorption by R. mucilaginosa was investigated at different initial heavy metal concentrations at a constant dye concentration. These experiments were repeated at a fixed dye concentration (39.2-51.2 mg/L) to determine maximum dye and/or heavy metal biosorption. The simultaneous effect of dye and heavy metal biosorption of the yeast cells is given in Figures 4 and 5. In the medium containing 50 mg/L dye, the chromium(VI) biosorption stabilized at around 5–17% yield as seen in Figure 4. In the medium with chromium(VI) after 1 h of incubation, the dye biosorption was around 55–61% yield. When the medium contained dye and chromium(VI), the heavy metal uptake decreased with increasing heavy metal concentration. In the medium containing nickel(II) there was about 8–38% nickel(II) and 86–93% dye removal (Figure 5). Contrary to chromium(VI), dye uptake increased with increasing heavy metal concentration. The higher maximum specific heavy metal uptake values for nickel(II) were observed in the medium with dye if compared with
the medium without dye. However, the maximum chromium (VI) uptake values were reduced in the medium. In the same medium, it was observed that maximum dye uptake values decreased when the heavy metal concentrations were increased. Increasing the initial heavy metals concentrations resulted in decreasing the colour removal efficiency for yeast species. This was due to the saturation of the sorption sites on the biosorbent as the concentration of the dye increased. However, higher biosorption yields were observed at lower concentrations of dye.

The single and simultaneous effects of increased initial dye and heavy metal concentrations on maximum specific dye and heavy metal uptake levels after the biosorption period are given in Table 1. The specific dye uptake in the medium without heavy metal increased in parallel to the dye concentration. The highest specific Remazol Blue uptake was 10.2 mg/g at 365.1 mg/L dye concentration. In the medium without any dye, chromium(VI) biosorption increased up to 2.06 mg/g chromium(VI) per g of biomass at 151.8 mg/L initial chromium(VI) concentration, whereas nickel(II) biosorption was only 0.63 mg/g per g of biomass at 47.7 mg/L initial nickel(II) concentration.

In the media containing the dye and heavy metal, the specific heavy metal uptake increased with increasing heavy metal concentration. Higher maximum specific heavy metal uptake values for nickel(II) were observed in the medium with dye if compared with the medium without dye. However, the maximum specific chromium(VI) uptake value was reduced in the medium with dye. In the same medium, it was observed that maximum specific dye uptake values decreased when the heavy metal concentrations were increased.

**CONCLUSIONS**

The aim of this work was to use yeast as a biosorbent for the removal of heavy metals and reactive dye from aqueous
solutions. The biosorption behavior of both chromium(VI), nickel(II) and R. Blue on to R. mucilaginosa was investigated with variations of metal and dye concentrations. This yeast was shown to have a better uptake capacity for R. Blue compared with chromium(VI) and nickel(II). It was found that the highest chromium(VI) removal yields measured were 31.3% for 49.0 mg/L initial chromium(VI) concentrations. The maximum nickel(II) biosorption in the medium with dye was 38.1%, at 25.7 mg/L nickel(II) concentrations. In addition, in these media R. Blue biosorption was 93.9% respectively.

This work illustrated an alternative solution for the management of wastewaters where R. mucilaginosa, fast-growing yeast compared with fungi and algae, could be, to some extent, utilized as biosorbent for the removal of heavy metals and dyes from wastewater.

Conventional technologies to clean up heavy-metal ions and/or dyes from contaminated waters have been utilized, but they remain cost ineffective. From this study, the use of yeast with resistance to heavy metals and/or dyes for the removal of heavy metals and dyes from contaminated waters may be a novel and cost-effective alternative.

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