Simultaneous copper, cobalt and phenol removal from aqueous solutions by alternating biosorption and biodegradation

K. Tsekova, S. Ganeva, A. Hristov, D. Todorova and V. Beschkov

ABSTRACT

A strategy for removal of heavy metals and phenol from wastewaters is proposed. It involves consecutive cation biosorption by fungi, phenol biodegradation by the yeast association Candida sp. 2326 + Candida sp. 2327 and regeneration. Copper and cobalt removal from aqueous solutions containing 80–120 mg/L phenol by biosorption, using Rhizopus arizicus cells immobilized onto poly(vinyl alcohol), was investigated by conducting a series of batch experiments. The removal efficiencies were 81% for Cu and 5% for Co. The residual concentrations of Cu (1.9 mg/L) and of Co (9.5 mg/L) did not change the biodegradation dynamics of phenol. A quantitative biodegradation of 120 mg/L phenol proceeded within 22 h. After biodegradation of phenol, the removal efficiencies achieved by biosorption after regeneration were 90% for Cu and 44% for Co. It was found that copper and cobalt form positively charged complexes with phenol. This complex formation hinders the retention of Cu and Co by the biosorbent and reduces the uptake of their cations.

Key words | biodegradation, biosorption, cobalt, copper, phenol

INTRODUCTION

The pollution of water and soil by aromatic compounds and heavy metals is a big problem for many industries, e.g., metallurgy, coal burning power plants and oil refineries. Copper, cobalt and phenol are widely used in various industrial applications. Even at low concentrations these pollutants can be toxic to organisms, including humans, because of which they were selected as a model of pollutants often presenting in the wastewaters. Among the various remediation techniques available, that show promise and are economical (Nakamura & Sawada 2000; Li et al. 2006) are the biological treatments. Previously biological treatments for removal of organic compounds and heavy metals have used fungi (Taseli & Gokcay 2005), yeasts (Sheeja & Murugesan 2002; Hamed et al. 2004; Alexieva et al. 2008), consortia of bacteria (Chirwa & Wang 2000) or granular activated carbon with biofilm (Quintelas et al. 2006).

Site remediation is a complex problem because organic and metallic pollutants require different treatment methods (Roane et al. 2001; Sandrin & Maier 2003). The inorganic pollutants in their ionic forms inhibit remediation through interaction with enzymes directly involved in biodegradation (e.g. specific oxygenases or in general metabolism, by binding to the enzymes, sulphhydryl groups) (Nies 1999); thus metal removal or stabilization is the first step for detoxification of a contaminated site (Liu et al. 2001).

Biosorption is an alternative to conventional methods for phenol and heavy metals removal (Quintelas et al. 2006; Thawornraissit & Pakulanon 2007). It uses inexpensive waste biomass, with a low cost of biomass immobilization, and there is the possibility of biosorbent regeneration for simultaneous removal of heavy metals and organic compounds from industrial waste solutions.

The aim of this study is the investigation and development of an innovative process for the simultaneous or consecutive biological removal of heavy metals and phenol. The effect of phenol or its degradation products on metal ion biosorption is studied. Uptake values and removal percentages are quantified.

The biodegradation of phenol by the yeast association Candida sp. 2326 + Candida sp. 2327 as an aerobic degradation by a mixed culture of microorganisms in presence of heavy metals is studied too. Copper and cobalt are selected as model and practically important metals. Batch processes of biosorption of heavy metals and biodegradation

of phenol are studied, using methodology based on the consecutive or simultaneous biological removal of heavy metals and phenol by selected immobilized biomass as a biosorbent and phenol degrading yeast association.

**MATERIALS AND METHODS**

**Biosorption study**

**Fungal strain, culture media, cultivation and biomass immobilization**

The fungus *Rhizopus archizus* from the Collection of the Institute of Microbiology at the Bulgarian Academy of Sciences was used in this study. The strain was routinely maintained on potato/glucose agar slant and stored at 4°C. Subcultures were made every 3 months. Spores from established culture (6–7 days old) were used for the preparation of inoculum.

The liquid growth medium consisting of (g/L): glucose 30; corn steep liquor 40; peptone 10; MgSO4.7H2O 0.5; KH2PO4 10; pH 4.8 was inoculated with a 0.1 mL spore suspension (about 10^6 spores per mL) in 500-mL Erlenmeyer flasks containing 75 mL sterile medium. The flasks were incubated for 18 h at 30°C on a rotary shaker (New Brunswick, USA) at 220 rpm for the development of the fungal inoculum.

The fermentation process was carried out under same conditions with 10% (V/V) inoculum being introduced into the 100 mL sterile liquid medium comprising (g/L): glucose 30; corn steep liquor 45; pH 4.8.

Biomass at the 48th hour of the cultivation was separated by filtration, washed with deionized distilled water and a pre-weighed amount of wet biomass was used as a biosorbent. Synthesis of hydrogel and biomass immobilization onto poly-vinyl-alcohol (PVA) and the structure of the PVA-hydrogel particles have been described in our previous investigations (Tsekova et al. 2008; Vassileva et al. 2009). A scanning electron micrograph of the empty PVA-hydrogel particles is shown in Figure 1. Many folds and cavities are visible. The immobilized biosorbent was cut into pieces of approximately 2×2×2 mm³ size and quantity of 0.3 g/L as dry weight was used in the experiments. The specific area of the biosorbent particles was about 30 cm⁻¹.

**Biosorption equilibrium**

Biosorption experiments were carried out in batches in 500-mL Erlenmeyer flasks. Pre-weighed biosorbent samples (immobilized biomass) with concentration of 1.3 g/L (dry weight) were examined. Each sample was added to 100 mL binary solution, containing 10 mg/L each of heavy metal ions (Cu(II) and Co(II)), prepared by dissolving the corresponding sulfate salts in bi-distilled water. Biosorption studies were carried out at different initial phenol concentrations from 40 to 120 mg/L. The mixtures were agitated at 220 rpm on a rotary shaker for 30 min at 30°C. Then the content of the flasks was separated by filtration using a membrane filter system (Cominig). Control samples with no added biomass were used as blanks. Each experiment was repeated three times.

**Batch biodegradation study**

**Microorganisms, culture media and cultivation**

Yeast association isolated from polluted water biocoenoses and cultivated for long time in the presence of phenol as a sole carbon and energy source are used in this investigation. The yeast association consisted of two members: *Candida sp.* 2326 and *Candida sp.* 2327, and is registered as a Bulgarian patent (Hristov & Abajieff 1996). The initial inoculum of the investigated association was prepared after 5 days of incubation on solid media, containing (g/L): (NH4)2SO4 – 1.0; K2HPO4 – 1.0 and re-suspended in liquid medium to obtain desired optical density (OD) (about 0.45 E). The liquid medium was prepared by adding the same mineral salts at the same concentrations to the filtrate obtained after biosorption of the heavy metals in the presence of 120 mg/L phenol. This medium was used for investigation of phenol biodegradation at its different initial concentrations.

The biodegradation process was performed in 500-mL Erlenmeyer flasks with 100 mL medium and the cultivation was carried out on a rotary shaker at 200 rpm and 30°C.
Samples were taken periodically and the process was finished when the phenol was consumed.

**Extraction studies**

**Solvent extraction**

Ten millilitres of the filtrate obtained after biosorption was transferred into a 15.0-mL extraction tube. The applied solvent extraction systems and the type of the expected extractable species are shown in Table 1. In all cases the contact time was 5 min. Then the organic phase was directly aspirated into the flame of the spectrometer and the absorption signals were recorded against organic standard solutions for Co or Cu in isobutyl methyl ketone (IBMK).

**Solid phase extraction**

The cation exchange resin Dowex 50-X8 (0.5 g) was placed in 15-mL polyethylene centrifuge tube and treated with 0.01 mol/L ethylenediaminetetraacetic acid (EDTA) followed by distilled water to wash and to put the resin in sodium form. Then the resin was converted into calcium form by shaking with 10 mL 0.1 mol/L CaCl₂ solutions for 5 min. The solution was removed and the resin was several times washed. After centrifuging the water phase was discarded. Then 10 mL of the filtrate after biosorption was added to the resin and the mixture was shaken for 5 min. The concentrations of Co and Cu remaining after sorption were measured against aqueous standard solutions. For assessment of the accuracy of the sorption procedure, the sorbed analytes were eluted with 5.0 mL 0.01 mol/L EDTA.

**Analytical procedures**

The biomass concentration was monitored through measuring optical density of the culture liquid at 540 nm. Phenol concentrations were determined colorimetrically using reaction with 4-aminoantipirine (Greenberg et al. 1992). The data shown are averages from three separate experiments. Variations up to 10% were observed.

The concentration of the metal ions in the filtrates was determined by atomic absorption spectrophotometer (AAS) with an air/acetylene flame, model 2380, Perkin Elmer (Germany). Uptake of metal ions was calculated from a metal mass balance yielding:

\[ q = \frac{V(C_i - C_f)}{m} \]  

where \( q \) is mg metal ions per g dry biosorbent, \( V \) is the reaction volume (L), \( C_i \) and \( C_f \) are the initial and residual metal concentrations (mg/L), respectively, and \( m \) is the amount of dry biosorbent (g).

An aliquot of wet immobilized biomass, followed by drying for 48 h at 85°C, was considered as dry biosorbent to calculate the uptake.

**Reagents**

All reagents and solvents were of analytical-reagent grade. Aqueous standard solutions of Co and Cu for calibration were prepared by appropriate dilution of a stock solution (1 g/L Co, 1 g/L Cu, both in 0.2% HNO₃, BDH (UK) standard solutions for AAS). Standard solutions of Co and Cu

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**Table 1** Extractability of Co(II) and Cu(II) from aqueous solutions in presence of phenol in dependence on the extraction system. IBMK – isobutyl methyl ketone; NaDDS – sodium dodecyl hydrogen sulphonate; TOMACl – tetraoctylmethyl ammonium chloride.

<table>
<thead>
<tr>
<th>Extraction system</th>
<th>Extraction recovery for Co (%)</th>
<th>Extraction recovery for Cu (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(II) + Cu(II) + phenol/IBMK (4.0 ml)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Co(II) + Cu(II) + phenol/2%TOMACl in 4.0 ml IBMK</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Co(II) + Cu(II) + phenol + NaDDS (0.5 ml of 0.001 mol/l in 5.0 ml IBMK)</td>
<td>90 ± 8</td>
<td>86 ± 8</td>
</tr>
<tr>
<td>Co(II) + Cu(II) + NaDDS/IBMK</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

**Table 2** The parameters in the Langmuir isotherm for equilibrium adsorption of Cu²⁺ and Co²⁺ on free cells of *Rhizopus archizus* and immobilized ones on PVA. Correlation coefficients are given.

<table>
<thead>
<tr>
<th>Biosorbent</th>
<th>Cu²⁺ qₑₘₐₓ (mg/g)</th>
<th>b (L/mg)</th>
<th>r²</th>
<th>Co²⁺ qₑₘₐₓ (mg/g)</th>
<th>b (L/mg)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free cells</td>
<td>21.59</td>
<td>0.2956</td>
<td>0.9843</td>
<td>15.15</td>
<td>0.3364</td>
<td>0.9575</td>
</tr>
<tr>
<td>Immobilized cells</td>
<td>10.20</td>
<td>0.1534</td>
<td>0.9998</td>
<td>24.81</td>
<td>0.0141</td>
<td>0.9849</td>
</tr>
</tbody>
</table>
in organic solvent were prepared from multi-element standard oil solution (p.a. Merck) by dilution with IBMK. The organic solvent IBMK and the reagents sodium dodecyl hydrogen sulphonate (NaDDS, p.a. Merck, 0.001 mol/L aqueous solution) and tetraoctylmethyl ammonium chloride (TOMACl, p.a. Merck, 2% solution in IBMK) were used as received. The cation exchange resin Dowex 50-X8 (50–100 mesh, hydrogen form, Merck, Germany) was commercially available. Doubly distilled water was used throughout.

**Reproducibility**

All experiments were performed in triplicate and the data shown are averages from three separate experiments. The relative standard deviation of the data was within 3 and 8%.

**RESULTS AND DISCUSSION**

**Biosorption of heavy metals in the presence of phenol**

The effect of phenol on the cation uptake by the immobilized fungal cells is demonstrated by Figure 2. Copper is more easily adsorbed by the biosorbent in presence of phenol with no effect on copper adsorption at phenol concentrations lower than 80 mg/L. Alternatively, cobalt ions are more sensitive to the presence of phenol, as can be seen from Figure 2. The uptake values for Co decrease with increased phenol concentrations. However, in the control experiments (without phenol) higher values of both heavy metal ions uptake were achieved (9.2 mg/g and 7.4 mg/g in the case of Cu and Co, respectively). The adsorption equilibrium was satisfactorily described by the Langmuir isotherm, as follows:

$$q = \frac{b \cdot c_{eq}}{q_{max} + c_{eq}}$$  

(2)

The isotherm parameters for the studied ions on the free and immobilized cells are given in Table 1. The correlation coefficients are high enough, particularly for the preparations with immobilized particles.

The negative effects in presence of phenol could be due to the competitive access to chemical binding sites when the phenol concentration increases (Thawornchaisit & Pakulanon 2007; Aksu & Akpinar 2000, 2001).

The diagram of residual phenol concentrations (Figure 3) shows that phenol is not retained on the biomass after the metal cation biosorption and hence the active centres of the biosorbent could not be blocked by its molecules.

That is why it was hypothesized that the formation of complexes between heavy metals and phenol produces a negative effect on the biosorption.

**Phenol complexes with heavy metals**

Investigation of the extraction of Co(II) and Cu(II) in the presence of phenol gives information about the possible formation of complexes between phenol and these cations, their charge and nature (Table 1). Assuming the investigated complexes are neutral, a quantitative extraction of Co(phenol)$_2$ and Cu(phenol)$_2$ into organic solvent such as IBMK could be expected.

However, the results presented in the table show that less than 1% of the complexes were extracted by IBMK, and hence they were not neutral. Next, if the complexes have negative charge we would predict a high extraction recovery when a long chain quaternary ammonium salt,
such as TOMACl dissolved in IBMK, is used as positively charged counter ion. The very low extraction rate with TOMACl shows that no negatively charged complexes are formed. High extractability was achieved only with an organic dodecylsulphonate anion, which contributed to the formation of an extractable ion associate complex \([\text{Co(phenol)}]_{nn}^{+} (\text{DDS})_{m}, \text{Cu(phenol)}]_{nn}^{+} (\text{DDS})_{m}\) and indicates that the complexes were positively charged. This conclusion was further confirmed by investigations of the sorption properties of Co(II) and Cu(II) in the presence of phenol using a Dowex 50-X8 cation exchange resin, where quantitative cation exchange was observed for elements in the filtrate after biosorption even when the resin was in Ca (II) form, i.e., the complexes bear a positive charge of +2.

Considering that the coordination number for Co(II) and Cu(II) with oxygen containing ligands is 4, it could be assumed with high probability that, in the presence of phenol, the complexes formed were \([\text{Co(phenol)}]_{4}^{2+}\) and \([\text{Cu(phenol)}]_{4}^{2+}\) (Marinsky & Marcus 1997).

**Biodegradation of phenol in the presence of residual concentrations of heavy metals**

The biodegradation dynamics of phenol in the presence as well as in the absence of heavy metals shows that at the 22nd hour of cultivation the phenol was degraded by 96\% with high degradation activity in presence of heavy metals (cf. Figure 4). The time necessary for 96\% degradation of 120 mg/L phenol in the presence of 9.5 mg/L cobalt and 1.9 mg/L copper ions was found to be within 22 h and this was the same as in the control experiment. In both cases the biodegradation followed second-order chemical reaction kinetics with a correlation coefficient \(r^2 = 0.993:\)

\[
- \frac{dC}{dt} = kC^2
\]

The rate constant \(k\) for the control experiment (without metal ions) was \(8.10^{-5} \text{ dm}^3 \text{ mg}^{-1} \text{ h}^{-1}\), whereas for the experiment in presence of copper and cobalt it was \(k = 6.10^{-5} \text{ dm}^3 \text{ mg}^{-1} \text{ h}^{-1}\). For a comparison, the correlation coefficient \(r^2\) for the first-order kinetics was between 0.84 and 0.88. The comparison of the experimental data with the model results is shown in Figure 4.

The growth of the yeast cells was inhibited in the presence of heavy metals, as is more clearly expressed between the second and fourth hours. After this period, there is no growth observed in the both cases, maybe due to the exhaustion of the main phenol quantity in the media. The specific growth rate for the control experiment was \(\mu \approx 0.04 \text{ h}^{-1}\), whereas it was lower in presence of metal ions, e.g., \(\mu \approx 0.02 \text{ h}^{-1}\). These results are in agreement with some studies that have pointed to the occurrence of an inhibitory effect on the process, even at very low concentrations of heavy metals (Kuo & Sharak Genthner 1996; Fijalkowska et al. 1998). Therefore it is necessary to know how to avoid these inhibitory effects to attain complete phenol biodegradation. Much higher activity was reported by Alexieva et al. (2008) in the case of biodegradation of phenol derivates by *Trichosporon cutaneum*, but cultivation was carried out in the absence of heavy metals.

To assess this problem our strategy involved consecutive cation biosorption, phenol biodegradation and consecutive metal biosorption (resorption). The obtained results for the metal ion concentrations after each of these consecutive steps are shown in Figure 5. After the first biosorption in presence of phenol the concentration of the non-adsorbed Cu and Co were 1.9 and 9.5 mg/L, respectively. Also evident from the plot of biosorption (Figure 5), the biodegradation of phenol does not improve the biosorption of heavy metal considerably. The concentration of the non-retained Cu was only with 0.2 mg/L less (1.7 mg/L Cu), cf. bars 2 and 3. The remained cobalt is 7.8 mg/L, i.e., only 18\% enhancement of the retention grade was observed. Also the heavy metal concentrations after the last resorption show that the biosorption of cobalt is again hampered in presence of biodegradation products of phenol. Even when the resorption was repeated several times, the concentration of Co remained about 5.6 mg/L. It could be assumed that the intermediate products of phenol biodegradation either block the
The authors are indebted to Dr. Jane Aiken for her very useful suggestions.

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