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Bioremoval of Ni and Cd in the presence of diethylketone by fungi and by bacteria – A comparative study

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ABSTRACT

Two fungi (*Alternaria* sp. and *Penicillium* sp.) and one gram-positive bacterium (*Streptococcus equisimilis*) were used to remove Ni and Cd from aqueous solutions in the presence of diethylketone. Individual toxicity assays were performed at an initial stage to evaluate the xenobiotic impact of the initial concentration of those metals on the growth of the microorganisms and allowed to infer that the growth of *S. equisimilis* is negatively affected by both metals, whereas the growth of both fungi is positively stimulated by the presence of Ni and inhibited by Cd (>40 mg/L). Within the group of microorganisms tested, *S. equisimilis* presented higher removal efficiency (%) and uptake. In a second stage, biosorption assays were performed using aqueous solutions containing Ni, Cd and diethylketone (mixed solutions) and aimed to infer about the overall effect of the initial metal concentrations on the growth and on the sorption capacity of the microorganisms, as well as to evaluate the interaction between the sorbent matrices. It was demonstrated that despite the mixed solution exert a negative effect on the removal process and on the growth of the three microbial cultures, the system is able to decontaminate aqueous solutions with high concentrations of Ni, Cd and diethylketone.

1. Introduction

Many industrial activities have led directly and indirectly to the artificial redistribution of organic and inorganic chemicals into the terrestrial and aquatic environment (Morley and Gadd, 1995). The artificial redistribution of these chemicals has resulted firstly in their increasing release and accumulation into the environment, and secondly, they invariably lead to the development of environmental and public health problems (Işik, 2008; Fereidouni et al., 2009; Flores-Garnica et al., 2013; Khairy et al., 2014; Costa et al., 2015).

Ketones are extensively used in food, chemicals, electronics, paint, rubbers, lubricants and pharmaceutical industries and are generally released into the environment by petrol and petrochemical industries (Gemini et al., 2005). Diethylketone (DEK), as almost all the volatile organic solvents, is dangerous to the aquatic life in high concentrations (Costa et al., 2012, 2015). DEK can react with OH radicals promoting the formation of ozone and other components of the photochemical smog in urban areas (Lam et al., 2012), being persistent in water, soil and air. Chronic exposure to DEK may cause tachycardia, nausea, shortness of breath, dizziness, fainting, coma and death (Costa et al., 2015).

Heavy metals, one of the groups within the inorganic pollutants category, are commonly found in wastewaters from chemical manufacturing, paint and coating, extractive metallurgy (Khairy et al., 2014), metal plating, electroplating, mining, ceramic, batteries, (Işik, 2008; Flores-Garnica et al., 2013). The Agency for Toxic Substances and Disease Registry, of the U.S. Department of Health and Human Services, has designated heavy metals as priority pollutants due to their inherent characteristics as extreme toxicity, tendency for bioaccumulation in the food chain even in relatively low concentrations (Işik, 2008) and inability to be biodegraded, thus causing various diseases and disorders. Cadmium, nickel, copper and cobalt are considered within the more dangerous heavy metals and therefore they are included in the U.S. Environmental Protection Agency's (EPA) list of priority pollutants (Arshadi et al., 2014). Nickel is listed as carcinogenic (group 2B) and has been implicated as a nephrotoxin, an embryotoxin and teratogen element. Acute and chronic nickel exposure can cause several disorders such as chest pain, tightness cyanosis, skin dermatitis and pulmonary fibrosis (Flores-Garnica et al., 2013). Cd, besides playing no constructive role in human-metabolism, may cause severe

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damage in different organs including kidneys, lungs, liver and testis. It may also lead to infertility (Ahmed et al., 1998; Chaudhuri et al., 2014), affect the action of enzymes, impede respiration, transpiration (Ahmed et al., 1998) and induce genomic instability through complex and multifactorial mechanisms, including proteinuria, a decrease in glomerular filtration rate and an increase in the frequency of kidney-stone formation, eventually causing certain types of cancer (group B1) (Khairy et al., 2014).

Although there are several physical-chemical methods for the decontamination of different kinds of pollutants as chemical precipitation, complexation, solvent extraction, membrane processes (Işik, 2008; Fereidouni et al., 2009; Kumar et al., 2011), adsorption on granular activated carbon (Flores-Garnica et al., 2013; Costa et al., 2015), biological processes present several advantages over those methods (Araújo and Teixeira, 1997; Chen et al., 2000; Işik, 2008; Fereidouni et al., 2009; Kocamemi and Çecen, 2009; Zheng et al., 2009; Flores-Garnica et al., 2013; Costa et al., 2015). In this endeavour, biosorption has emerged as an attractive, sustainable, inexpensive and eco-friendly alternative for the treatment of contaminated water with organic and inorganic pollutants (Morley and Gadd, 1995; Aksu, 2005; Quintelas et al., 2012).

The present work aims the development of an eco-friendly environmental technology, applicable to the treatment of aqueous solutions contaminated with diethylketone and/or nickel and cadmium. The ability of three different microorganisms (*Penicillium* sp., *Alternaria* sp. and *Streptococcus equisimilis*) used as biosorbents, to simultaneous decontaminate aqueous solutions containing nickel, cadmium and DEK, as well as the effect of the initial concentration of metal on (i) the microbial growth, (ii) the sorption capacity of these pollutants and (iii) the biological activity after exposure, was accessed. DEK's toxicity towards *Penicillium* sp., *Alternaria* sp. and *Streptococcus equisimilis* is reported in Costa et al. (2014, 2015).

2. Material and methods

2.1. Organisms, culture media and chemicals

The fungi *Penicillium* sp. and *Alternaria* sp. isolated and identified previously (Costa et al., 2015) were used in this work. The bacterium *Streptococcus equisimilis* was obtained from the Spanish Type Culture Collection (University of Valencia). The growth medium employed was Brain Heart Infusion (BHI, OXOID CM1135) with a pH of 7.4. Elementary stock solutions (1 g/L) of cadmium and nickel were prepared by dissolving, respectively, an appropriate amount of $CdSO_4 \cdot 8/3H_2O$ (Riedel-de-Haën) and of $NiCl_2 \cdot 6H_2O$ (Carlo Erba Reagents) in distilled water. All glassware used for experimental purposes was washed with 60% nitric acid and subsequently rinsed with deionized water to eliminate any possible interference by other metals.

The range of concentrations of each metal used in the toxicological experiments was obtained by dilution of the stock solutions and varied between 5 mg/L and 100 mg/L and the main objective was to infer about the toxic effect of each pollutant on the microbial culture. The biosorption experiments were conducted using a mixture of Ni and Cd (1 mg/L to 20 mg/L) and DEK (4 g/L) and aimed to access the sorption capacity of each microbial culture with respect to each pollutant, as well as to infer about the interaction between biomass-pollutant and pollutant-pollutant, in terms of uptake and removal percentages. At the end of all experiments, viability tests were performed to confirm the death or inactivation of the microbial cultures.

2.2. Toxicological assays with metals

Penicillium sp., *Alternaria* sp. and *S. equisimilis* were inoculated separately into 500 mL of autoclaved BHI culture medium (24 h, at 37 °C and 150 rpm - Culture 1). The toxicity experiments were carried out for 24 h at 37 °C and 150 rpm in 250 mL Erlenmeyer flasks containing 125 mL of autoclaved BHI culture medium either with Ni or Cd (5 mg/L to 100 mg/L) and 10 mL of Culture 1. At different time intervals, a sample was collected, centrifuged at 13,400 rpm for 10 min and the OD was measured at 620 nm. The supernatant was used to quantify the concentration of metal over time, by inductively coupled plasma optical emission spectrometry, ICP-OES. A control with each microorganism (microbial control, MC) suspended just in culture medium was used to access the normal growth behaviour of each culture. The assays were conducted during a period of 2 days at 37 °C and 150 rpm. The toxic effect of different initial concentrations of DEK on the growth of all three microorganisms is reported in Costa et al. (2014, 2015).

2.3. Biosorption assays with Ni, Cd and DEK

A set of individual experiments were conducted and aimed to infer firstly about the sorption capacity of all three microorganisms towards Ni, Cd and DEK, and secondly about the effect that this mixture of pollutants exerts in its own removal.

The growth of the three microorganisms was promoted individually, inoculating them in 500 mL of a BHI culture medium for 24 h at 37 °C and 150 rpm. After this period of time, 10 mL of each biomass was inoculated into Erlenmeyer's flasks (250 mL) with a final working volume of 125 mL. Each Erlenmeyer flask contained an aqueous solution with Ni (1 mg/L to 20 mg/L), Cd (1 mg/L to 20 mg/L) and DEK (4 g/L). The Erlenmeyer flasks were rotated at a constant rate of 150 rpm until equilibrium was reached (7 days). Samples of 1 mL were periodically collected, centrifuged at 13,400 rpm for 10 min and the supernatant was used to determine the pollutants concentration. The samples were analyzed by gas chromatography-mass spectroscopy, GC-MS and by ICP-OES, respectively for DEK and for metals. A control with Ni, Cd and DEK was used in order to infer about the influence of the Erlenmeyer flasks walls on the sorption of all pollutants. All the experimental work was done in duplicate. The results presented are an average of both assays. The relative standard deviation and relative error of the experimental measurements were less than 2% and 5%, respectively.

2.4. Analytical methods

2.4.1. Quantification of DEK concentration

Gas chromatography with mass spectrometry (GC-MS) was used to assess the concentration of DEK in aqueous solution and thereby to evaluate the biodegradation capacity of the microorganisms regarding DEK, in the presence of Ni and Cd. The chromatograph was a Varian 4000, equipped with a flame ionization detector (FID) and mass spectrometry (MS). The separations were performed using a Meta Wax column (30 m × 0.25 mm x 0.25 μm). The operating conditions and the retention time are reported at Costa et al. (2015).

2.4.2. Quantification of Ni and Cd concentration

The concentration of Ni and Cd in samples was measured by an ICP-OES (Optima 8000, PerkinElmer). The operating conditions were as follows: RF power: 1300 W, argon plasma flow: 8 L/min, auxiliary gas flow: 0.2 L/min, nebulizer gas flow: 0.5 L/min. For the analysis of nickel concentration, the plasma view was radial and the wavelength used was 221.648 nm, whereas for cadmium, the