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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

plasma view was axial and the wavelength used was 226.502 nm. All calibration solutions were prepared from a Ni and from a Cd stock standard solutions with a concentration of 1 g/L. All samples were acidified with nitric acid before analyses. The instrument response was periodically checked with standard Ni (II) and Cd (II) solutions and a blank (HNO_3 5%).

2.5. Growth kinetics modelling

The kinetics of growth of both fungi was characterized using five different growth kinetic models: Monod (1949), Powell (1967), Haldane (Andrews, 1968), Edwards (1970) and Luong (1987) fitted by linear and nonlinear least squares methods, using MATLAB software.

2.6. Biosorption kinetics modelling

The biosorption kinetics of all pollutants was analyzed using the linearized form of the zero order, pseudo-first order, pseudo-second order and three-half order models (Brunner and Focht, 1984; Khamis et al., 2009; Kumar et al., 2011; Saravanan et al., 2009).

2.7. Statistical analysis

The data were evaluated with one-way ANOVA and the results were considered to be significant if $p > 0.05$ (GraphPad Prism Software, version 5.01, La Jolla, CA).

3. Results and discussion

3.1. Toxicological assays

Alternaria sp. exhibits, without significant variance, similar growth behaviour for all the Ni concentrations tested (Fig. 1), including the microbial control ($p > 0.05$). Maximum removal percentages ranged between 69% and 76% while maximum uptake of 10 mg/g to 254 mg/g were obtained and both tended to increase with the increase of initial concentration of Ni (Fig. S1, see Supplementary material). It was also observable the existence of significant differences in terms of uptake values, for all the different initial concentrations tested, specially for 80 mg/L and 100 mg/L ($p = 0.0012$). Although *Alternaria* sp. presents similar growth profiles (short lag and log phase, Fig. 1) when exposed to Cd, its growth is inhibited for concentrations higher than 40 mg/L ($p < 0.05$). Maximum removal percentages of 29%–67% and uptake values of 3 mg/g to 137 mg/g (Fig. S1, see Supplementary material) were observed. The uptake tended to increase with the increase of Cd concentration and presented significant differences between all the different initial concentrations tested ($p < 0.05$). The growth results obtained for *Alternaria* sp. with Ni and with Cd are both best described by the Haldane model (Table 1, $R^2 = 0.966$ and $R^2 = 0.822$, respectively). This model was originally proposed in 1968 and is used to represent growth kinetics with an inhibitory compound. The critical concentration, $S_{crt,}$ above which the removal rate of the compound decreases due to the self-inhibitory effect (Raghuvanshi and Babu, 2010) was found to be 85.3 mg/L for

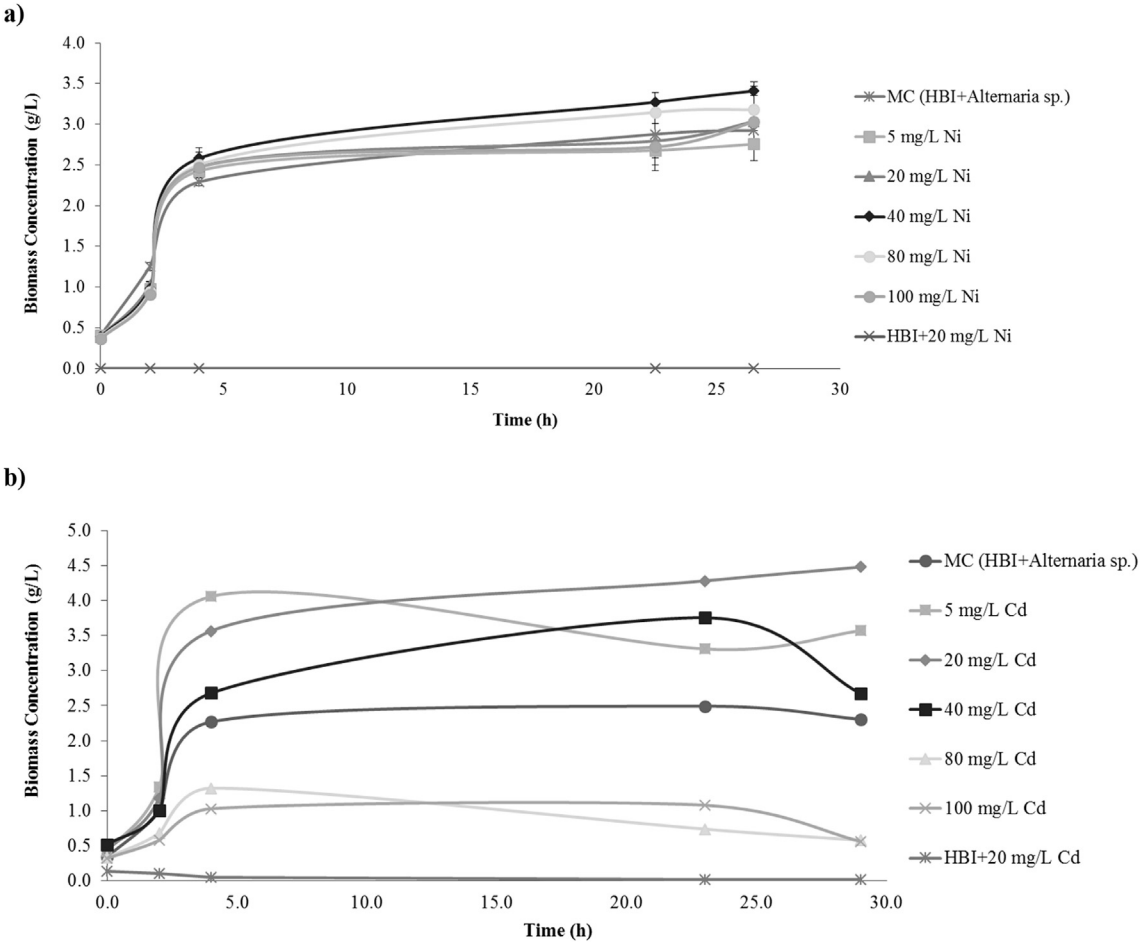

Fig. 1. - Growth profile for *Alternaria* sp. (g/L) when exposed to different initial concentrations of (a) Ni and (b) Cd.

Table 1
Growth kinetic parameters obtained by modelling for *Alternaria* sp., *Penicillium* sp. and *S. equisimilis* in the presence of Ni or Cd.

Microorganisms/Metal	Model	μ_{max} (h^{-1})	Ks (g/L)	K_1 (g/L)	K (mg/L)	R^2
***Alternaria* sp. (Ni)**	**Haldane**	0.533	0.275	26,492	–	0.966
***Alternaria* sp. (Cd)**	**Haldane**	0.107	13.670	562.700	–	0.822
***Penicillium* sp. (Ni)**	**Haldane**	0.851	0.447	530.400	–	0.936
***Penicillium* sp. (Cd)**	**Haldane**	0,287	21.920	15.100	–	0.890
***S. equisimilis* (Ni)**	**Edwards**	0.783	0.169	492.200	1.406×10^{15}	0.970
***S. equisimilis* (Cd)**	**Haldane**	0.374	12.680	20	–	0.999

Ni and 87.7 mg/L for Cd.

The assays conducted with *Penicillium* sp. and Ni showed that the growth profile is identical, not only for all the initial concentrations tested ($p > 0.05$), but also to the growth profile obtained for *Alternaria* sp. when exposed to Ni. As the concentration of Ni increases, the maximum concentration of biomass obtained decreases, always being higher than the one obtained with MC (Fig. S2, see Supplementary material). Maximum removal percentages of 33%–71% and maximum uptake of 7.2 mg/g to 86 mg/g were obtained. Once again, significant differences were observable in terms of uptake values, for all the conditions tested ($p < 0.05$), specially for the highest concentrations (80 mg/L and 100 mg/L, $p < 0.003$). The maximum removal percentage tended to decrease with the increase of Ni concentration, whereas the uptake tended to increase during the first 22.5 h (Fig. 2a and b). When exposed to Cd, the growth profile of *Penicillium* sp. changed dramatically and presented significant variance for all the initial concentrations tested ($p < 0.05$).

For initial concentrations of 20 mg/L and 40 mg/L this fungus growth was faster than the growth in MC. However, when exposed to initial concentrations equal to 5 mg/L, 80 mg/L and 100 mg/L, its growth was significantly inhibited, which may be explained by the

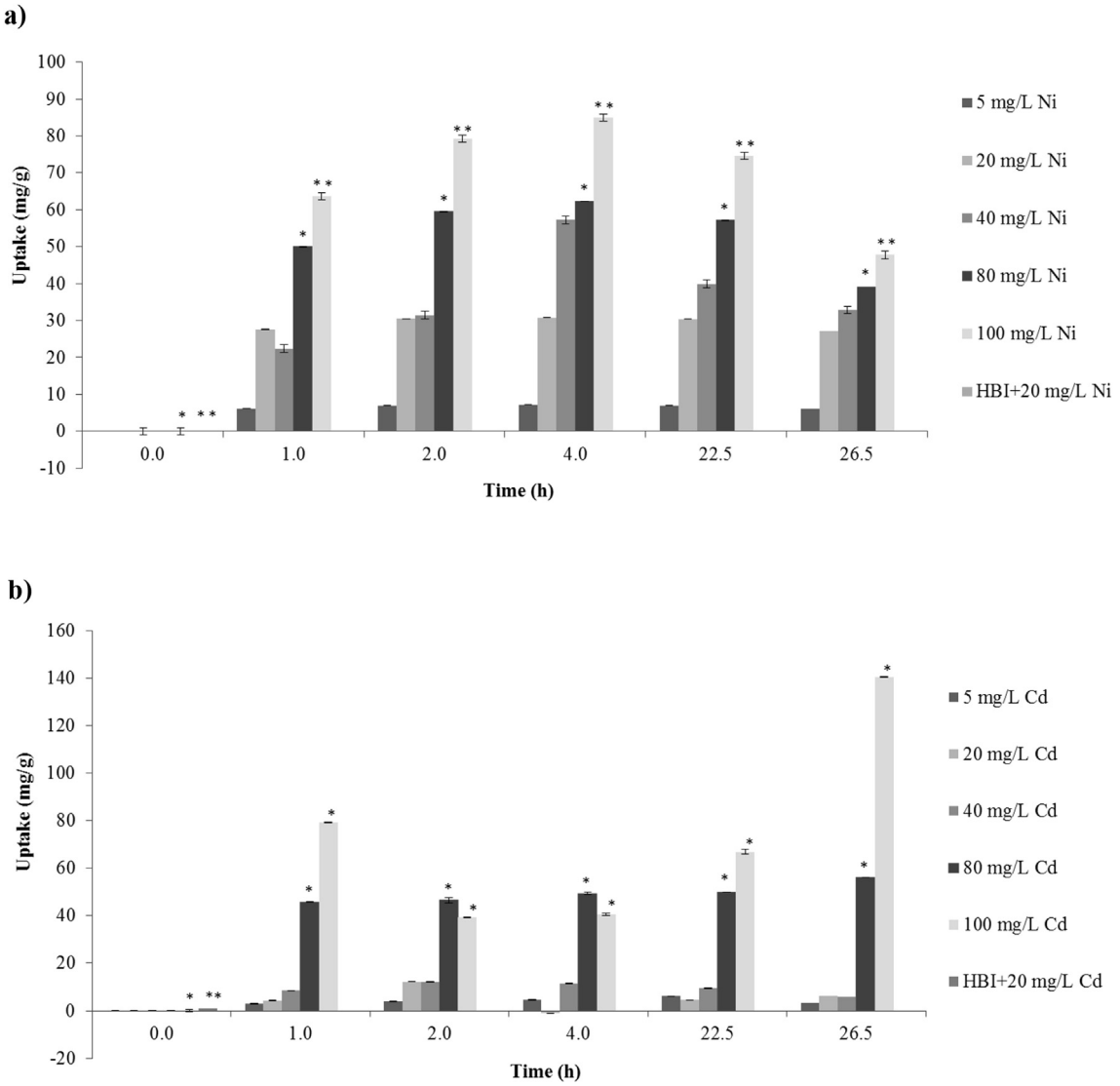

Fig. 2. - Uptake (mg/g) of (a) Ni and (b) Cd obtained for the suspended culture of *Penicillium* sp., when exposed to different initial concentrations of metal (Ni or Cd) (5 mg/L to 100 mg/L) at 37 °C, 150 rpm. * Significant difference between the experimental conditions and the control.

fact that at 5 mg/L, Cd may not exercise any positive effect on the growth mechanisms, whereas for concentrations higher than 80 mg/L, Cd may exhibit a xenobiotic behaviour. Maximum removal percentages of 16%–52% and uptake ranging between 6.2 mg/g and 80 mg/g were observed, revealing once again the existence of significant differences for the conditions tested ($p < 0.05$). The maximum removal percentage was obtained for an initial concentration of 5 mg/L of Cd and the uptake tended to increase with the increase of Cd concentration and through time. The growth results obtained for *Penicillium* sp. when exposed to Ni and Cd are best described by the Haldane model (Table 1, $R^2 = 0.936$ and $R^2 = 0.890$, respectively). The S_{crt} was found to be 15.4 mg/L for Ni and 18.2 mg/L for Cd.

S. equisimilis growth (Fig. S3, see Supplementary material) was significantly inhibited when exposed to initial concentrations higher than 5 mg/L of either Ni or Cd, decreasing to values of about half of those obtained with the MC ($p < 0.05$). Ni maximum removal percentages ranged between 43% and 94%, and the uptake ranged from 3 mg/g to 373 mg/g, showing significant variance in terms of uptake values, for all the initial concentrations of Ni tested ($p < 0.05$). For Cd, the maximum removal percentages ranged between 61% and 88% and the uptake varied between 3.6 mg/g and 155 mg/g, being also observable the existence of significant differences in terms of uptake values for the conditions tested ($p < 0.05$). For both metals, the maximum removal percentage tends to decrease with the increase of initial concentration, whereas the uptake tends to increase (Fig. 3 a and b). The growth results obtained for Ni and Cd are respectively best described by the Edwards model ($R^2 = 0.970$) and by the Haldane model ($R^2 = 0.999$) (Table 1). This corroborates the xenobiotic effect of these metals over the microbial growth. Edwards's model admits the existence of an inhibitory effect which may be caused by the formation of toxic metabolites or by-products, dissociation and/or alteration in the activity of one or more enzymes and by the development of metabolic aggregates (Raghuvanshi and Babu, 2010). The S_{crt} obtained for Cd was 15.9 mg/L.

The viability tests showed that both fungi presented biological activity when exposed to Ni, whereas when exposed to Cd the biological activity was only observed for concentrations lower than 80 mg/L. *S. equisimilis*, on the other hand, was found to be biologically inactive, when exposed to Ni and Cd concentrations higher than 5 mg/L.

Sargassum angustifolium was exposed to Ni and Zn in the assays performed by Ahmady-Asbchin and Jafari (2013). These authors obtained maximum uptake capacities of 0.0417 g/g and 0.0608 g/g of dry *S. angustifolium* for Ni and Zn, respectively. Assays conducted

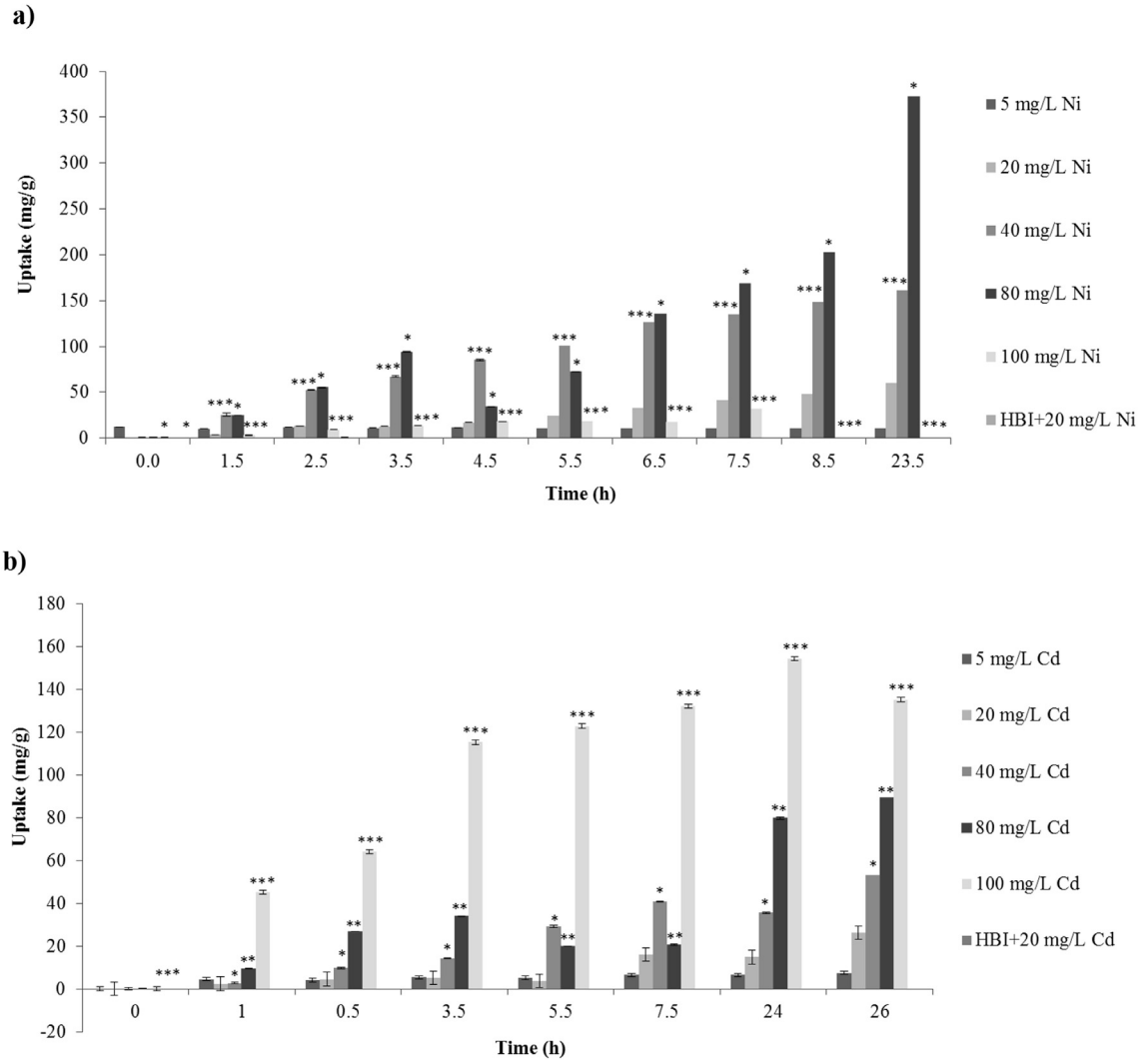

Fig. 3. - Uptake (mg/g) of (a) Ni and (b) Cd obtained for the suspended culture of *S. equisimilis*, when exposed *to* different initial concentrations of metal (5 mg/L to 100 mg/L) at 37 °C, 150 rpm. * Significant difference between the experimental conditions and the control.

by Chaudhuri et al. (2014) with Cd (0.5 mg/L to 3.0 mg/L) achieved maximum removal percentages of 77.07% and 74.47% when using *Lemna minor* and *Spirodela polyrhiza* respectively, as biosorbents. Comparatively, the efficiency of the biosorption matrices herein described appears to be more interesting for the decontamination of Ni and Cd aqueous solutions.

The kinetic data obtained respectively for the removal of Ni and Cd were best described by the pseudo-second order model ($R^2 > 0.985$ and $R^2 > 0.899$ for *Alternaria* sp., $R^2 > 0.927$ and $R^2 > 0.940$ for *Penicillium* sp., $R^2 > 0.927$ and $R^2 > 0.865$ for *S. equisimilis*). This model assumes that the rate limiting step of the overall mechanism is the surface chemisorption, a physicochemical interaction between the two phases and it is usually represented by its linear form (Fig. S4, see Supplementary material). The results obtained indicate that, under these experimental conditions, the rate-limiting step for Ni sorption is the surface chemisorption. The straight-line of t/Q_t (time/amount of substrate removed at time t) versus t plots indicated the good ability of this model to describe the kinetic data. These results suggest that the growth of both fungi are positively affected by Ni and negatively affected by Cd, as opposite to *S. equisimilis* that is negatively affected by both metals. It is, however, extremely important to highlight that despite this sensitivity, the removal percentage as well as the uptake obtained by *S. equisimilis* were significantly higher than those obtained with the fungi. They also suggest that an increase in the initial concentration of metal caused an increase in the uptake (Table 2). This may be explained by the increase of the driving force to overcome all mass transfer resistance between the aqueous and the solid phases (Çelekli and Bozkurt, 2011). Generally, under the same experimental conditions, the sorption data showed a stronger affinity between the microorganism and Ni. This could be due to the smaller size of the ionic radius of Ni (Cd(II) (4.26 Å) > Ni(II) (4.04 Å)) and its higher Pauling electronegativity (Ni(II) (1.91) > Cd(II) (1.69)) (Arshadi et al., 2014).

3.2. Biosorption assays with Ni, Cd and DEK

Fig. 4 a shows the time profile of the growth of the three microorganisms and the removal percentage (%) of DEK (4 g/L), Ni and Cd (5 mg/L). The microbial growth and the removal percentage (%) of all the pollutants, for all the concentrations tested as functions of time presented similar profiles. The biomass of *Alternaria* sp. (Fig. 4a) grew for a period of 25 h, reaching a maximum concentration of 0.39 g/L, comparable to the results obtained with Ni (2.75 g/L to 3.40 g/L) and Cd solutions (0.56–4.48 g/L, respectively). The data also indicates consumption of DEK, even if the growth of *Alternaria* sp. is strongly and adversely affected by the simultaneous presence of the three pollutants. However there were no significant differences in terms of DEK's uptake (or removal), considering the different concentrations of Ni and Cd tested ($p > 0.05$). The increase in the amount of biomass during the first hours, though small, is related to the use of DEK in the cell growth and maintenance, while its decline may be associated with the depletion of the carbon source (DEK) and formation of hazardous conditions, such as accumulation of Ni and/or Cd on the surface and intracellular space of the microbial culture, which may lead to the death of the culture. At this stage of decline, the removal of all three pollutants cannot occur through any biologically active processes (biodegradation, bioaccumulation, etc.) and therefore processes such as sorption are favoured. Table 3 shows that as the initial metal concentration increases, the uptake of Ni and Cd increases, reaching its maximum value for the highest concentration of metal (from 0.08 mg/g to 11.32 mg/g for Ni and from 0.08 mg/g to 8.50 mg/g for Cd). However, the removal percentage of both metals tends to decrease (from 98.4% to 43.8% for Ni and from 98.3% to 32.9% for Cd). This behaviour can be explained by some small variations in the initial concentration of the inoculum employed (±20%) and by the increasing metal concentration itself. At a lower concentration, the ratio between the initial number of moles of metal ions and the available surface area is smaller and consequently, the fractional sorption becomes independent of the initial concentration. However, at higher concentrations, the available sites for sorption become short compared to the number of moles of metal ions present and therefore, the metal sorption percentage becomes dependent upon the initial metal ion concentration. In other words, the sorption process will reach maximum efficiency faster with higher ratio between the number of moles of pollutant and the number of available sites for sorption to occur (Vijayaraghavan et al., 2006). The results obtained indicate that there were significant variances in terms of uptake values, for all the different initial concentrations of Ni and Cd tested ($p < 0.05$) and that *Alternaria* sp. is more sensitive towards Cd than to Ni. It is important to highlight that the sorption of a metal can be explained by several factors such as its ionization state, its electronegativity and by its ionic radius that can facilitate or hamper the penetration of the metal into the polymeric net around the cells. Similar results were obtained by Quintelas et al. (2009) when studying the biosorption performance of an Escherichia coli biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(II). These authors found that the uptake values decrease in the following order: Fe(III) > Ni(II) > Cd(II) > Cr(VI).

The removal of each of the three pollutants was best described by the pseudo-second order model ($0.899 \leq R^2 \leq 1$ for DEK, $0.841 \leq R^2 \leq 0.999$ for Ni and $0.962 \leq R^2 \leq 0.999$ for Cd) and the pseudo-second order constant (K_2) tended to decrease with the increase of metal concentration suggesting that the removal rate of all pollutants decreases over time, due to saturation of the active sites.

Penicillium sp., biomass grew for a short period of 2 h and reached a maximum concentration of 0.31 g/L (Fig. 4b), a significantly lower value compared to the values achieved in the toxicity assays (3.78 g/L to 2.73 g/L), thus revealing that the microbial growth is negatively affected by the simultaneous presence of DEK, Ni and Cd. Once again, the increase of the biomass though smooth, is associated with the consumption of DEK and its decline follows the depletion of the only carbon source, DEK, and the formation of

Table 2
Ni and Cd sorption percentage and uptake obtained with *Alternaria* sp., *Penicillium* sp. and *S. equisimilis* when exposed to an aqueous solution of Ni or Cd (5 mg/L to 100 mg/L).

Alternaria sp.					
Ni Initial concentration (mg/L)	**5**	**20**	**40**	**80**	**100**
Removal (%)	71.07	73.66	69.26	75.51	75.42
Uptake (mg/g)	10.67	43.79	80.45	177.70	253.08
Cd Initial concentration (mg/L)	**5**	**20**	**40**	**80**	**100**
Removal (%)	29.67	64.63	58.06	36.43	43.79
Uptake (mg/g)	3.58	29.39	45.13	56.64	90.80
Penicillium sp.					
Ni Initial concentration (mg/L)	**5**	**20**	**40**	**80**	**100**
Removal (%)	70.62	70.18	49.08	34.56	33.38
Uptake (mg/g)	6.95	30.82	57.28	62.37	85.01
Cd Initial concentration (mg/L)	**5**	**20**	**40**	**80**	**100**
Removal (%)	51.15	26.91	15.56	23.21	45.15
Uptake (mg/g)	6.16	12.24	12.10	56.18	140.58
S. equisimilis					
Ni Initial concentration (mg/L)	**5**	**20**	**40**	**80**	**100**
Removal (%)	87.37	79.75	79.56	61.87	82.83
Uptake (mg/g)	12.40	60.29	161.10	372.64	32.06
Cd Initial concentration (mg/L)	**5**	**20**	**40**	**80**	**100**
Removal (%)	93.76	88.92	85.98	84.31	43.92
Uptake (mg/g)	7.53	26.34	53.16	89.57	154.39

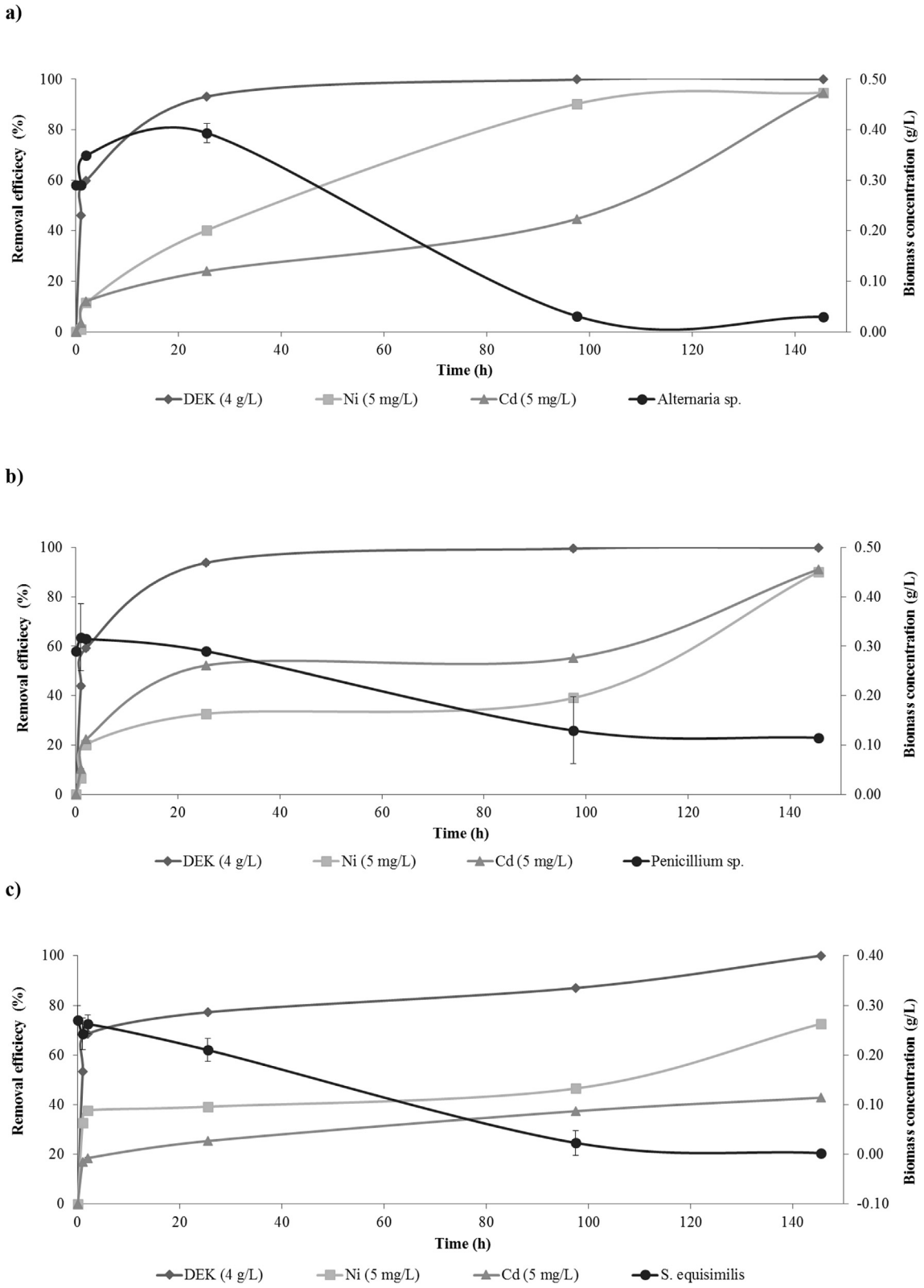

Fig. 4. - Biomass concentration (8 g/L) (a) *Alternaria* sp., (b) *Penicillium* sp., and (c) *S. equisimilis* and removal efficiency (%) as function of time, for an initial concentration of 4 g/L of DEK and 5 mg/L of Ni and Cd (37 °C, 150 rpm).

toxic compounds. The uptake of DEK was not significantly influenced by the different concentrations of Ni and Cd tested ($p > 0.05$), which is a very important advantage in water treatment processes. The uptake increases with the increase of initial metal concentration (Table 3), until it reaches its maximum value (from 0.09 mg/g to 13.06 mg/g for Ni and from 0.09 mg/g to 16.79 mg/g, for Cd).

Table 3
Ni, Cd and DEK sorption percentage and uptake obtained with *Alternaria* sp., *Penicillium* sp. and *S. equisimilis* when exposed to an aqueous solution of Ni, Cd (1 mg/L to 20 mg/L) and DEK (4 g/L).

Alternaria sp.				
Ni initial concentration (mg/L)	**1**	**5**	**15**	**20**
Removal (%)	98.38	94.67	36.84	43.79
Metal uptake (mg/g)	0.08	1.18	2.41	11.32
Cd initial concentration (mg/L)	**1**	**5**	**15**	**20**
Removal (%)	98.27	94.69	48.71	32.88
Metal uptake (mg/g)	0.08	1.18	3.18	8.50
DEK removal (%)	99.10	100	100	99.89
DEK uptake (g/g)	13.91	13.79	44.11	104.55
Penicillium sp.				
Ni initial concentration (mg/L)	**1**	**5**	**15**	**20**
Removal (%)	97.75	90.09	40.80	47.15
Metal uptake (mg/g)	0.09	1.30	2.41	13.06
Cd initial concentration (mg/L)	**1**	**5**	**15**	**20**
Removal (%)	95.08	91.10	55.26	60.63
Metal uptake (mg/g)	0.09	1.31	3.26	16.79
DEK removal (%)	100	100	100	99.93
DEK uptake (g/g)	12.58	14.75	20.28	113.23
S. equisimilis				
Ni initial concentration (mg/L)	**1**	**5**	**15**	**20**
Removal (%)	98.37	72.58	58.92	60.54
Metal uptake (mg/g)	0.96	5.28	7.33	55.65
Cd initial concentration (mg/L)	**1**	**5**	**15**	**20**
Removal (%)	96.90	42.81	42.33	22.15
Metal uptake (mg/g)	0.95	3.11	5.26	20.37
DEK removal (%)	100	99.25	100	100
DEK uptake (g/g)	28.17	39.60	57.97	67.80

Afterwards, the removal percentage of both metals tends to decrease (from 97.75% to 47.15% for Ni and from 95.08% to 60.63% for Cd). The results indicate that there are significant variances in terms of uptake for the different initial concentrations of Ni and Cd tested ($p < 0.05$). *Penicillium* sp. seems to be more sensitive towards Ni than to Cd and therefore the maximum values of removal percentage and uptake achieved are lower. Similar results were obtained by Holan and Volesky (1994) who tested the biosorption capacity of different fungal and wood biosorbents towards Cd, Ni and Pb. For all the four fungal species tested the metals were sequestered in the following decreasing order Pb > Cd > Ni. The biosorption potential of *Phanarochaete chrysosporium* towards Cu(II), Cr(III), Cd(II), Ni(II) and Pb(II) was studied by Yetis et al. (1998), who observed that the sorption capacity follows the order: Pb(II) > Cr(III) > Cu(II) = Cd(II) > Ni(II).

The results are best described by the pseudo-second order model ($0.999 \leq R^2 \leq 1$ for DEK, $0.998 \leq R^2 \leq 1$ for Ni and $0.999 \leq R^2 \leq 1$ for Cd), indicating that the rate-limiting step for the sorption of DEK, Ni and Cd, is dependent on the pollutants initial concentration and on available active sites of biomass (Kumar et al., 2011). The straight-line of t/Q_t versus t plots show the good ability of this model to describe the kinetic data obtained for each pollutant. For each of them, K_2 tended to decrease with the increase of metal concentration, suggesting that the removal rate of all three pollutants decreases over time, caused by the saturation of the active sites on the biomass surface and by the development of inhibitory compounds that will promote death of the microbial culture.

S. equisimilis reached a maximum concentration of 0.27 g/L (Fig. 4c), a value significantly lower when compared either with the values obtained in the toxicity experiments or with the *Alternaria* sp. and *Penicillium* sp. cultures. These results indicate that not only the growth of *S. equisimilis* is adversely affected by the presence of DEK, Ni and Cd, but that *S. equisimilis* is also the most sensitive microorganism tested. The increase of the biomass during the first 2 h, though quite small, can be related to the use of DEK in cell growth and maintenance, while its decrease may be related with the depletion of DEK and subsequent accumulation of toxic substances, which in turn, results in cell death and terminus of the biologically active removal processes. It is important to highlight, that once again the uptake of DEK was not affected by the presence of different concentrations of Ni and Cd ($p > 0.05$). Table 3 shows that the increase in initial metal concentration increases the uptake of both metals, reaching its maximum value for the highest concentration of metal (from 0.96 mg/g to 55.65 mg/g for Ni and from 0.95 mg/g to 20.37 mg/g for Cd), revealing that there were significant variances in terms of uptake values, for the different initial concentrations of Ni and Cd tested ($p < 0.05$). The removal percentages of Ni and Cd tended to decrease (from 98.37% to 58.92% and from 96.90% to 22.15%, respectively) revealing a greater sensitivity towards Cd when compared with Ni. Similar results were obtained by Yetis et al. (1998) and Quintelas et al. (2009). Yetis et al. (1998) studied the sorption potential of *Polyporous versicolor* regarding Cu(II), Cr(III), Cd(II), Ni(II) and Pb(II). Once again the pseudo-second order model was found to be the best model to describe the obtained results ($0.998 \leq R^2 \leq 1$ for DEK, $0.841 \leq R^2 \leq 1$ for Ni and $0.962 \leq R^2 \leq 1$ for Cd), (Fig. 4c). The constant K_2 tends to decrease with the increase of initial concentration of metal, suggesting that the removal rate of all three pollutants decreases over time.

It is important to emphasise that for all the biosorption experiments the removal of DEK was found to be faster within the first hours, after which it progressively slowed down until removal of 100% was reached. This behaviour can be explained by the availability of the biomass and its need to consume nutrients essential for the growth. After this period of time, the removal percentage decreases due to biomass saturation (Costa et al., 2012). It was also found by GC analyses that no metabolites were formed during the process of DEK removal, contrary to what was previously reported by Costa et al. (2015), who studied the biodegradation of DEK, in the absence of metals by these three microorganisms. In the studies conducted by these authors, the degradation of DEK led to the formation of three metabolites identified as methyl acetate, ethyl acetate and 2-pentanone. The absence of metabolites in the current study may be related to the presence of Ni and Cd, which may have influenced the metabolic pathway of DEK employed by the biomass. The viability tests showed that all three microorganisms were biologically inactive, even after successive subcultures in a new culture medium, thus confirming the xenobiotic effect of this mixture.

4. Conclusions

It was demonstrated that the growth of *Alternaria* sp. and *Penicillium* sp. is stimulated in the presence of Ni and inhibited by initial concentrations of Cd higher than 40 mg/L. *S. equisimilis* growth is negatively affected when exposed to Ni and Cd concentrations higher than 5 mg/L, revealing a higher sensitivity of this microorganism towards these two metals. *S. equisimilis* presented the best results in terms of removal efficiency and uptake, thus presenting an advantage over the fungi. It was also observed that (i) an increase in the initial concentration of metal can cause an increase in the uptake, (ii) at the same experimental conditions, the sorption data shows a higher affinity of the three microorganism towards Ni. Although the multi-component solutions exert a strong and negative effect either in the removal process or in the microbial growth, it was possible to infer that system employed is able to decontaminate aqueous solutions, with high concentrations of Ni, Cd and DEK.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2017.02.018.

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