Study of Ni (II) and Diethylketone Removal from Aqueous Solutions Using a Biofilm *of Streptococcus equisimilis* Supported on Vermiculite

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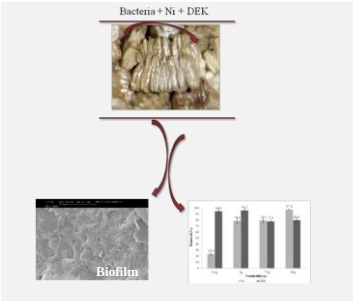

The novelty of this work lies in the development of an environmental-friendly technology to remove diethylketone and Ni (II) from aqueous solutions using the interaction between bacteria and clays. Concentrated biomass of *Streptococcus equisimilis* and vermiculite were used for the removal of both pollutants No published work on the usage of this combination to remove heavy metals and organic solvents from aqueous solutions was acknowledged. The developed work is original and its impact on society and on environment is evident due to the toxicological effects of those substances on the health of living beings.

Background

Contamination of water streams and soils with hazardous compounds has attracted increasing attention in recent years, all over the world. Many of these substances, including heavy metals, aromatic hydrocarbons and ketones are dangerous to humans due to their carcinogenic and toxic properties, their capacity to form toxic intermediates and persistance in the environment [1, 2]. Among the heavy metals that represent a greater environmental impact and concern Hg, Mn, Se, Be, As, Sb, Pb, Cu, Zn, Cd, Ni, Cr, and Co stand out [2]. The last six metals are commonly found in the waste streams from mining operations, tanneries, electronics, electroplating, batteries and petrochemical industries as well as jewellery and textile mill production [3, 4]. Exposure to excessive quantities of heavy metals may lead to bio-accumulation in food chain, toxicity symptoms, disorder in cellular functions and eventually death [1]. For instances, Ni is a carcinogenic, embryotoxin and nephrotoxin element known to be capable of causing several types of acute and chronic health disorders, such as severe damage of lungs and kidney, skin dermatitis, vomiting, nausea, diarrhea, pulmonary fibrosis, chest pain, renal edema, cyanosis, rapid respiration and extreme weakness [5]. Among the organic solvents used, the ketones group stands out because of their extensive and diversified use in numerous industries (e.g. food, chemicals, electronics, pharmaceutical) where they may act as a substrate or as a solvent in the production of drugs, vitamins and cosmetics. One common example is diethylketone (DEK), which despite being used in many anthropogenic activities as a solvent and as an intermediate in the synthesis of pharmaceuticals, flavors and pesticides among others, was subject of very few studies regarding its biodegradability. Exposure to organic solvents, as DEK may cause tachycardia, dizziness, fainting and even cause coma or death in cases of prolonged exposure. In order to control and prevent pollution as well as to protect and improve the quality of the environment, stricter environmental laws were enacted in most countries [6].

Objectives

This works aims the development of an environmental-friendly technology applicable to the treatment of aqueous solutions containing Ni and DEK. In a first stage toxicity assays were performed in batch systems with the aim of investigating the xenobiotic effect of different concentrations of Ni on the growth of the bacteria *Streptococcus equisimilis* and its ability to remove this metal from aqueous solution. In a second stage batch studies combining the use of concentrated *S. equisimilis* and the use of different concentrations of vermiculite to respectively remove and biodegrade Ni and DEK, from aqueous solutions were performed. The main objectives of these last set of assays are 1) to infer and evaluate the ability of this system to remove and biodegrade Ni and DEK, respectively and 2) determine whether these two substances compete for the available sites either on the biomass or on the vermiculite. For the toxicity assays a control with culture media and without nickel was used in order to infer about the normal growth of the bacteria, whereas for the biosorption assays the control consisted in a solution with DEK and Ni and without vermiculite and biomass. The kinetic parameters were estimated using five growth kinetic models for biodegradation of organic compounds: Monod, Haldane, Powell, Luong and Edward and four biodegradation kinetic models: zero order, pseudo-first, pseudo-second and three-half order. The

experimental equilibrium results were analised using the BET, Dubinin-Radushkevich (D-B), the Langmuir and Freundlich adsoprtion isotherms.

Methods

Toxicological assays - effect of different initial concentrations of nickel on the growth of S. equisimilis - A flask (500mL) was filled with 250 mL of autoclaved Heart Brain Infusion (Oxoid CM1135) culture medium. The flask was then inoculated with a pure culture of *S. equisimilis* and an amount of nickel ($NiCl_2$, Carlo Erba Reagents) was added (5-450 mg/L) and left for several days at 37ºC and 150 rpm. At different time intervals, a sample was collected, centrifuged at 13400 rpm for 10 minutes and the OD was measured at 620 nm (T60 UV-Visible Spectrophotometer, PG instruments). The supernatant was used for the determination of Ni concentration. The assays were conducted in duplicate, during 2-3 days, at 25ºC and 150 rpm. The samples were acidified with nitric acid and analysed by ICP-OES (Optima 8000, PerkinElmer).

Batch adsorption assays - The batch adsorption assays were conducted in duplicate in reactors of 2 L, being the final volume 0.85 L. Each reactor was composed by 4-7 $g_{biomass}$/L, Ni (450 mg/L), DEK (3 g/L) and vermiculite (0.1 to 10g). The reactors were rotated at a constant rate of 150 rpm until equilibrium was reached. Previous assays were made to determine the time needed for equilibrium to be reached (5-7 days). Samples of 1 mL were periodically collected, centrifuged at 13400 rpm for 10 minutes, and the supernatant was used for the determination of DEK and Ni concentration. The samples were analysed by GC-MS (Varian 4000) equipped with a flame ionization detector (FID) and mass spectrometry (MS) and by ICP-OES (Optima 8000, PerkinElmer), respectively for DEK and for Ni.

Results

Toxicological assays - effect of different initial concentrations of nickel on the growth of S. equisimilis - Table 1 shows the maximum specific growth rate for the different initial Ni concentrations ranging from 5 to 450 mg/L. It is possible to observe that for concentrations lower than 100 mg/L, the specific growth rate is higher than the specific growth obtained for the control, whereas for higher concentrations this rate starts to decrease to values still positive but more close to the control value. These results suggest that for concentrations lower than 100 mg/L there is a positive stimulation by Ni on the growth of the bacteria tested. The growth kinetic model that best describes the results obtained is the Edward model (Table 2). For the assays conducted with an initial concentration of Ni between 20 and 300 mg/L the removal kinetic model that best describes the experimental results is the pseudo-first order suggesting that under these conditions the process depends on time and sorption sites and not on the concentration of biomass or substrate. For the remaining concentrations the experimental results are best described by the pseudo-second order model suggesting that under these conditions the removal process depends on both the substrate concentration and biomass or the substrate concentration and time. From Figure 1 is possible to observe that as the initial concentration of Ni increases the removal rate decreases slightly for initial concentrations up to 80 mg/L, decreasing more sharply for higher concentrations. These results can be explained by the saturation of the sorption sites when increasing the concentration of Ni and by the decreasing of active uptake by the bacteria due to the inhibitory effect of the metal.

Table 1. Experimental specific growth rate of *S. equisimilis* for the different initial concentrations of Ni.

S_0 (mg/L)	μ_{exp} (h^{-1})
0	0.369
5	0.737
20	0.810
40	0.624
80	1.396
100	0.568
300	0.440
450	0.446

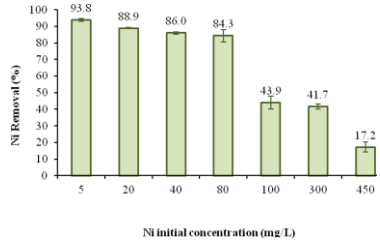

Figure 1. Removal percentage of Ni for the different initial concentrations of Ni tested.

Batch adsorption assays

Regarding the assays performed with concentrated biomass, DEK (3 g/L), Ni (450 mg/L) and vermiculite (0.1 to 10 g) it is observable from Figure 2 that as the mass of clay increases, the removal percentage of Ni also increases reaching the maximum removal value with 10 g of vermiculite, whereas for DEK the maximum removal percentage was obtained for 5 g of

vermiculite (96.2%). For vermiculite masses higher than 5 g the removal percentage decreased significantly (16%).

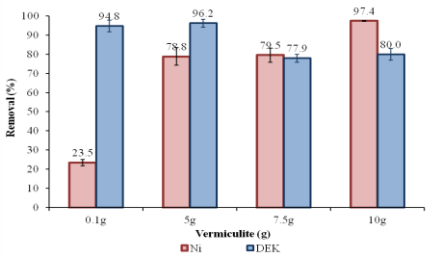

Figure 2. Uptake percentage of Ni and DEK for different initial concentrations of vermiculite.

Table 3. Adsorption isotherm constants for the isotherms adsorption models for Ni (II) onto a *S. equisimilis* biofilm supported on vermiculite.

Model	Langmuir	Freundlich	D-B
q_{max} (mg/g)	23.89	-	-
B (l/mg)	2.24	-	-
R_L	0.02	-	-
K_f (mg/g)	-	21.67	-
N (g/l)	-	51.89	-
Q_{DR} (mg/g)	-	-	16.72
K_{DR} (mol^2/J^2)	-	-	-3.4E-7
B	-	-	-
C_S (mg/l)	-	-	-
R^2	0.993	0.994	0.149

The best fit for the biosorption equilibrium of Ni was obtained with the Freundlich model followed by the Langmuir and Dubinin-Radushkevich model (Table 3). The meaningless value obtained for Q_{max} with the BET model eliminates the consideration of this model to describe the obtained data. The kinetic model that best describes the results obtained for all the assays for both Ni and DEK is the pseudo-second order (R^2>0.9863 and R^2>0.8974, respectively).

Conclusions

It was demonstrated that *S. equisimilis* by itself is capable of efficiently remove Ni up to 100 mg/L (93.8% maximum removal) and that higher concentrations promote the inhibitory effect on the bacteria growth. It was also demonstrated that a system composed by a biofilm of *S. equisimilis* supported on vermiculite is capable of efficiently remove Ni from aqueous solution and also to degrade and/or adsorb DEK. The removal percentage increased with the increase of adsorbent, reaching a maximum value of 97.4% for Ni and 96.2% for DEK. It was also possible to conclude that 1) the presence of DEK does not affect negatively the removal of Ni and 2) the presence of vermiculite increases the adsorption of the metal from the aqueous solution. For the biosorption studies the kinetics and isotherms were well described respectively, by the pseudo-second order and by the Freundlich isotherm model.

Table 2. Growth kinetic parameters obtained for the growth kinetics models used.

Model	μ_{max} (h^{-1})	Ks (g/L)	K_1 (g/L)	K	S_m (g/L)	m	n	R^2
Monod	0.826	0.517	-	-	-	-	-	0.5135
Powell	0.801	0.235	-	-	-	$5.121e^{-5}$	-	0.4929
Haldane	0.893	0.884	$-5.808e^{21}$	-	-	-	-	0.5579
Luong	1.806	12.040	-	-	$-5e^{15}$	-	$1.421e^{13}$	0.8722
Edward	0.751	0.403	-120.600	-256.9	-	-	-	0.9328

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