



Pilot-scale sorption studies of diethylketone in the presence of Cd²⁺ and Ni²⁺

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ABSTRACT

The effect of pH on the sorption capacity of vermiculite towards cadmium and nickel was tested in batch systems and it was shown that the sorption percentages increase with an increase in the mass of vermiculite and with an increase in the initial pH. Maximum sorption percentages were obtained for a pH of 8 and 4 g of vermiculite (86.5% for Cd²⁺ and 86.1% for Ni²⁺, for solutions with 100 mg/L of metal). As a consequence, it was possible to establish a range of optimal pH for biosorption processes, by combining the so determined optimal sorption pH of vermiculite with the optimal growth pH of *Streptococcus equisimilis*, a bacterium used to treat contaminated water. Pilot-scale experiments with a *S. equisimilis* biofilm supported on vermiculite were conducted in closed-loop conditions, aiming to treat large volumes of diethylketone aqueous solutions, eventually containing Cd²⁺ or Ni²⁺. The excellent capacity of this joint system to simultaneously biodegrade diethylketone and biosorb Cd²⁺ or Ni²⁺ was proved. The removal percentage and the uptake increase through time, even with the replacement of the initial solution by new ones. The breakthrough curves that best describe the results achieved for Cd²⁺ and Ni²⁺ are respectively the Adams–Bohart and the Yoon and Nelson model.

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Introduction

Water contamination has become a serious threat to society due to rapid and growing industrialization, increasing use of modern agricultural practices, severe exploitation of natural resources and illegal discharges of wastewater [1,2]. These practices introduce hazardous and persistent substances such as organic and metallic compounds in the environment, which tend to accumulate and deteriorate the different matrices [2]. This problem has been receiving substantial attention, especially since it was established that aquatic organisms can readily incorporate (adsorb, bio-accumulate or biodegrade) those contaminants, which may then enter directly into the human food chain [3]. On a small scale, both organic and inorganic contaminants can safely be removed from any water body and thus not affecting the aquatic communities. However, on a larger scale, the aquatic system is unable, in due time, to effectively remove the contaminants present in it, and thus is severely hampered [1]. In this context, the study of processes able to efficiently and effectively treat wastewater is of utmost importance, as well as the understanding of the contaminants' migration between phases and the factors affecting such phenomena [4].

Industries such as petrol and petrochemical, electronics, paints, pharmaceuticals and food processing use

ketones as solvents, polymer precursors or intermediates in their processes. One such example is diethylketone (DEK), a simple symmetrical dialkyl ketone also known as 3-pentone. The release of DEK into the environment constitutes a threat to living beings since it has high mobility; it is persistent in water, soil and air; it can react with OH radicals, promoting the formation of ozone and other components of the photochemical smog in urban areas [5]; and it can form toxic and photo-toxic intermediates [6].

Cadmium and nickel are two metals commonly found in wastewater from different industries such as electronic devices' manufacturing, printing, paints, oil refining, dyes, pulp and paper, fertilizers, pesticides, steels [7], jewellery, mining, tanneries, textile, batteries, petrochemical and fine chemistry, chemicals [8–10] and healthcare products [11], causing serious environmental and health problems. Cadmium is irritant to the respiratory system and it is able to cause anaemia; to affect the action of enzymes, hampering respiration, photosynthesis, transpiration and chlorosis [8]; to cause cancer; to promote infertility and serious health problems in different organs. In high concentration, nickel is considered a carcinogenic element capable of causing several types of acute and chronic symptoms and illnesses such as nausea and vomiting, skin dermatitis, chest pain, dry

cough, lungs and kidney damage, rapid respiration, pulmonary fibrosis, renal edema, cyanosis and extreme weakness [12].

Techniques such as adsorption on granular activated carbon, air-stripping, oxidation with or without flame, thermal degradation, condensation and incineration, chemical precipitation, reverse osmosis and ion exchange [13,14] are used to remove organic solvents and heavy metal from wastewater. These techniques present, however, several drawbacks such as (a) the emission of nitrogen gases (NO_x), which requires the application of a secondary treatment, with a consequent increase in operational costs and production of contaminated solid wastes, (b) low efficiency, (c) excessive use of chemicals and (d) high costs from operational, maintenance and equipment perspectives [15,16]. In recent years, several studies have demonstrated that biological processes such as biosorption and biodegradation present numerous advantages over the conventional methods mentioned above. These biological processes have an eco-friendly character since they do not generate solid wastes and nitrogen oxides, they present high efficiency and they reduce maintenance and operational costs [16–18].

Volatile organic compounds and heavy metals are considered to be among the high diversity of contaminants present in industrial and domestic effluents [19,20]. Several studies concerning the decontamination of such effluents and its optimization have been conducted, which became of major importance and relevance not only for environmental rehabilitation, but also for its economic sustainability.

A joint system was used in this study that combines the properties of clays and microorganisms to improve the removal of different kind of pollutants from aqueous solutions. The use of joint systems to treat wastewater has proved their efficiency, since they combine the sorption capacity of the clay besides its high cation exchange capacity, large specific surface area, excellent chemical and physical stability [21], with the microorganisms' ability to biodegrade, fix and/or entrap contaminants, due to the presence of several functional groups on the biomass surface (chitin acetamides, phosphate, amino, carboxylic groups, nucleic acids and proteins) [22,23]. This study highlights the kinetics and mechanisms of such processes, metal ions' entrapment and simultaneous degradation of organic molecules by a specific biosorbent easy to maintain, very competitive and with an eco-friendly character.

This research approach aims the framing of the process parameters that will allow the up-scale of a sustainable biotreatment. On the one hand, it will permit the degradation of organic compounds to

harmless molecules by the recourse to ubiquitous bacteria of natural occurrence and on the other hand, it will shift the polluting charge of trace metal ions from aqueous matrices to solid structures that may be further used as catalysts for oxidation in mild conditions [24,25]. In this perspective, the disposal of the used biomass will not generate another polluting problem, and the metal-loaded zeolite will constitute an example of the circular economy as the waste of some industries will be used as a catalyst for some others, in a very competitive breakthrough as environment catalysts tend to be very expensive. The full-scale commercial operation will be quite straightforward as this research aims to deliver a sustainable, low-cost environmental technology targeted at small but locally vibrant industries producing wastewater with low concentrations of solvents and metals. The technology presents attractive advantages due to the reusability of the biomaterial, low operational costs, short operation time and no production of secondary compounds which might be toxic.

Experimental

Support, microorganism and chemicals

Vermiculite is a hydrated magnesium aluminium silicate purchased from Sigma-Aldrich and it was used as a support for the bacterial biofilm establishment and development. It has an average particle diameter of 8.45 μm , a Brunauer, Emmet and Teller (BET) surface area of 39 m^2/g and a porosity of 10%. The bacterium used was *Streptococcus equisimilis* and it was acquired from the Spanish Type Culture Collection, from the University of Valencia (reference CECT 926).

S. equisimilis was grown in sterilized Brain Heart Infusion (Oxoid CM1135) culture medium at 37°C, 150 rpm, for several days. DEK was purchased from Acros Organics (98% pure) and diluted in sterilized distilled water. Individual stock solutions of 1 g/L of cadmium ($\text{CdSO}_4 \cdot 8/3\text{H}_2\text{O}$, Riedel-de-Haën) and 1 g/L of nickel ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, Carlo Erba Reagents) were prepared by dissolving an accurately weighed amount of metal in sterilized deionized water. A multi-element inductively coupled plasma quality control (ICP QC) standard solution purchased from CHEM Lab (QCS-03) (15E) was used periodically to check for the equipment reproducibility. In all the experiments, the concentration of cadmium and nickel was 100 mg/L and it was obtained by dilution of the respective stock solution.

Previous experiments conducted at lab-scale in batch mode to evaluate the effect of concentrations of DEK, Cd^{2+} or Ni^{2+} , on singular or multi-component solutions on the growth of *S. equisimilis* and on the sorption

capacity of *S. equisimilis*, vermiculite and a *S. equisimilis* biofilm supported on vermiculite are reported by our team in Costa et al. [21], Costa et al. [26] and Quintelas et al. [27].

The effect of pH on the sorption of Ni^{2+} and Cd^{2+} by vermiculite – lab-scale experiments

These experiments were conducted in 1 L Erlenmeyer flasks with a working volume of 0.425 L containing 100 mg/L of Cd^{2+} and of Ni^{2+} , different doses of vermiculite (0.1, 2.05 and 4 g) and different initial pH values (3, 5.5 and 8). The Erlenmeyer flasks were incubated at 37°C, in an orbital shaker (150 rpm) until equilibrium was reached. The required time for equilibrium to be reached (24 h for Cd^{2+} and Ni^{2+}) was previously determined. At pre-defined time intervals, samples were collected and centrifuged for 10 min at 1300 rpm (Eppendorf MiniSpin 9056). The supernatant was analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in order to determine the concentrations of Cd^{2+} and Ni^{2+} through time. A blank containing just an aqueous solution of Cd^{2+} and Ni^{2+} was used to assess any sorption by the Erlenmeyer flasks walls or the interactions between the two metals.

Biodegradation of DEK and biosorption of Cd^{2+} or Ni^{2+} by a biofilm supported in vermiculite in open systems – pilot-scale experiments

A compact polycarbonate acrylic column of 22.7 L with an internal diameter of 17 cm and a total height of 100 cm was used as a bioreactor in the pilot-scale experiments. A maximum packing fraction of 1/3 of the bioreactor was filled with vermiculite (700 g). An Erlenmeyer flask (2 L) containing 1 L of Brain Heart Infusion culture medium previously sterilized at 121°C for

20 min was inoculated with *S. equisimilis* and incubated in an orbital shaker for 24 h at 37°C and 150 rpm. The Erlenmeyer flask was capped with a cotton stopper in order to allow passive aeration. The inoculum culture was then inoculated to the bioreactor set-up and was pumped upwards at a flow rate of 250 mL/minute, for 5 days with total recirculation, in order to allow the biomass to attach to the support (vermiculite) and originate a well-developed biofilm. After biofilm formation, the bed was washed out and a 40 L solution (S_1) containing 7.5 g/L of DEK and 100 mg/L of metal (Cd^{2+} or Ni^{2+}) was continuously pumped upwards through the bioreactor with a constant flow rate of 25 mL/minute. For the pilot-scale experiments performed with DEK and Ni^{2+} , the solution S_1 (7.5 g/L of DEK and 100 mg/L Ni) was replaced by new solutions (S_2 and S_3) that contained DEK (7.5 g/L) and Ni^{2+} (100 mg/L). At the end of the experiment, only DEK was added to the solution S_3 (S_4). For the experiments performed with DEK and Cd^{2+} , the solution S_1 (7.5 g/L DEK and 100 mg/L Cd) was replaced by new solutions (S_2 and S_3) that contained DEK (7.5 g/L) and Cd^{2+} (100 mg/L). Samples were collected from the effluent (8 mL) at pre-established times (Collection point, Figure 1), centrifuged at 1300 rpm for 10 min and the aqueous phase was analysed by gas chromatography mass spectrometry (GC-MS) and ICP-OES in order to determine respectively the concentration of DEK and metals through time. At the end of the assays, the bioreactor was washed out, and samples of the effluent and those of the support were inoculated in Petri plates with the Brain Heart Infusion culture medium, in order to access the metabolic activity of *S. equisimilis*. The pH was monitored throughout all the assays. The assays were conducted in duplicate and the results are an average of both duplicates. The relative standard deviation and relative error of the experimental measurements were less than 2% and 5%, respectively.

Analytical procedures

Gas Chromatography and inductively coupled plasma optical emission spectrometry

A GC-MS Varian 4000 equipped with a flame ionization detector, mass spectrometry and a ZB-WAXplus column (30 m × 0.53 mm × 1.0 μm) was used to determine the concentration of DEK in samples. The operational conditions and retention times for DEK and for the internal standard (2-methyl-1-butanol) are described in Costa and Tavares [22].

An ICP-OES (Optima 8000, PerkinElmer) was used to determine the concentration of Cd^{2+} and Ni^{2+} in samples. The operating conditions are as the same as in Costa and Tavares [28]. All the samples were acidified

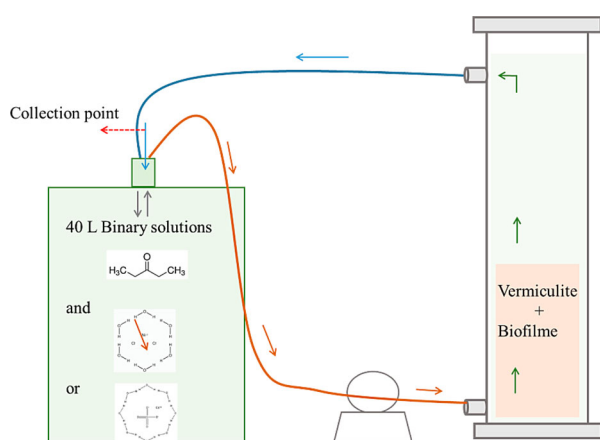


Figure 1. Schematic set-up of the bioreactor column.

with concentrated nitric acid (HNO₃ 69%) and filtered before being analysed. The instrument response was periodically checked with the multi-element ICP QC standard solution (CHEM LAB) and with a blank (HNO₃ 5%).

Characterization of sorbents by FTIR, XRD and SEM analysis

To determine the functional groups involved in the biosorption of DEK, Cd²⁺ and Ni²⁺, infrared spectra of the sorbents, with and without previous contact with the pollutants, were obtained using a Fourier Transform Infrared Spectrometer (FTIR BOMEM MB 104). The operational conditions for the FTIR analysis and for the X-ray powder diffraction (XRD) analyses are described in Costa and Tavares [28]. Scanning Electron Microscopy (SEM) observations of vermiculite with and without previous contact with DEK, Cd²⁺ and Ni²⁺, with and without biofilm, were performed on Leica Cambridge S360 to observe any morphological changes on the sorbents' properties.

Theory and data calculation

Removal kinetics' modelling

The sorption kinetics of all contaminants (Cd²⁺, Ni²⁺ and DEK) were analysed using the linearized form of four different kinetic models: the zero order [29], pseudo-first order [30], pseudo-second order [31] and three-half order [32].

Breakthrough curves' modelling

One of the criteria for a successful design of a fixed-bed column system for adsorption processes is the prediction of the breakthrough curve (concentration–time profile). Adams–Bohart [33], Wolborska [34], and Yoon and Nelson models [35] were used to predict the breakthrough curves.

Results and discussion

The effect of concentrations of DEK, Cd²⁺ and Ni²⁺, in singular and in multi-component solutions, on the growth of *S. equisimilis* and on the sorption capacity of *S. equisimilis*, of vermiculite and of an *S. equisimilis* biofilm supported on vermiculite was previously established at lab-scale and batch mode by our team, as described in Quintelas et al. [26,27] and Costa et al. [21].

Briefly, the relevant results for the present study were the following: *S. equisimilis* growth starts to be inhibited for concentrations of DEK, Cd²⁺ and Ni²⁺ higher than 3.2 g/L, 80 mg/L and 5 mg/L, respectively. Sorption experiments conducted with vermiculite, DEK, Cd²⁺ or

Ni²⁺ (singular sorbate solutions) achieved maximum sorption percentages of 92%, 100% and 95.4%, respectively. In these experiments, the sorption percentages tended to increase with the increase on the mass of vermiculite employed, whereas sorption experiments conducted with vermiculite, DEK and Cd²⁺ or Ni²⁺ (binary-sorbate solutions) attained maximum sorption percentages of 43.6% for DEK and 100% for Cd²⁺ and complete sorption of both sorbates for the assays performed with DEK and Ni²⁺. At lab-scale and in batch mode, the biosorption experiments conducted using an *S. equisimilis* culture suspended in an aqueous solution containing DEK and different concentrations of Cd²⁺ and Ni²⁺ reveal a complete removal of DEK and maximum removal percentages of 96.9% for Cd²⁺ and 98.4% for Ni²⁺. At pilot scale and in batch mode, the biosorption experiments conducted using an *S. equisimilis* biofilm supported into different masses of vermiculite lead to removal percentages (biodegradation and biosorption/ or sorption) higher than 97% for all the contaminants and for all the systems (DEK and Cd²⁺ and DEK and Ni²⁺).

The effect of pH on the sorption of Cd and Ni using vermiculite – lab-scale experiments

In these set of assays, the concentrations of metals (Cd²⁺ and Ni²⁺) were kept constant, whereas the amount of vermiculite employed and the value of the initial pH varied, from 0.1 to 4 g for a mass of vermiculite and from 3 to 8 for pH. It was observed that as the mass of vermiculite employed increased, the sorption percentage of Cd²⁺ and of Ni²⁺ tended to increase as well, whereas the uptake tended to decrease (Table 1). For Cd²⁺, the maximum removal percentages and the uptake values achieved were, respectively, 86.5% and 367.8 mg/g (pH 8), whereas for Ni²⁺ the maximum removal percentages and uptake values obtained were 86.1% and 365.9 mg/g (pH 8).

Table 1. Effect of pH on vermiculite uptake and sorption efficiency of Cd and Ni at lab-scale experiments.

Vermiculite (g)	Ni		Cd	
	Sorption (%)	Uptake (mg/g)	Sorption (%)	Uptake (mg/g)
pH 3				
0.1	11.70	49.71	7.81	33.21
2.05	44.17	9.16	18.08	3.75
4	70.17	7.46	46.28	4.92
pH 5.5				
0.1	47.16	292.27	44.15	196.97
2.05	54.12	9.78	45.21	9.37
4	68.86	5.75	46.35	4.69
pH 8				
0.1	76.57	365.91	63.04	367.77
2.05	85.26	14.94	73.21	15.18
4	86.10	9.06	86.53	6.69

On the other hand, the maximum sorption percentage and uptake tend to increase when increasing the initial pH for the same mass of vermiculite. The concentration of H^+ on the surface sites decreases and the concentration of OH^- increases, leading the clay surface to become negatively charged, resulting in a significant decrease in electrostatic repulsion and thus in an increase in metal sorption [36]. Since the point of zero charge of vermiculite is 2 and the pH at which Ni^{2+} and Cd^{2+} start to precipitate in the form of nickel hydroxide and cadmium sulphide is respectively 7.6 and 8, it is possible to conclude that at pH 3 and 5.5, the decrease on the concentration of metals is due to sorption processes between each metal and the functional groups present on the vermiculite surface (Si–O–Si, H–O–H, CO and OH, for example) and not due to precipitation processes [37]. For the assays conducted at pH 8, no precipitation was observed, allowing the authors to assume that under these experimental conditions the decrease on Ni^{2+} and Cd^{2+} concentration is still due to sorption processes and not to precipitation. It is concluded that the optimal pH to decontaminate aqueous solutions containing Ni^{2+} and Cd^{2+} by vermiculite sorption lies between 6 and 8.

Sorption kinetic modelling

The kinetic model that best describes the results obtained for Ni^{2+} and Cd^{2+} sorption, for all the initial pH values tested (pH 3, $R^2 > 0.98$ for Ni^{2+} and $R^2 > 0.94$ for Cd^{2+} , pH 5.5, $R^2 > 0.97$ for Ni^{2+} and $R^2 > 0.93$ for Cd^{2+} , pH 8, $R^2 > 0.90$ for Ni^{2+} and $R^2 > 0.86$ for Cd^{2+}) is the pseudo-second-order model (Figure 2). The behaviour of the pseudo-second constant (K_2) for Ni and for Cd (except at pH 5.5) was unexpected since it increases with an increase in the vermiculite mass from 0.1 to 2.05 g and then starts to decrease for vermiculite mass of 4 g. These results suggest that the sorption rate of Ni^{2+} and Cd^{2+} increases with an increase in the mass of

vermiculite employed, reaching its maximum with 2.05 g of vermiculite and decreasing when higher amounts of vermiculite are employed [38]. However, as expected, the percentage of metal sorbed increased with an increase in vermiculite. This behaviour can be easily explained due to the decrease in H^+ and the increase in OH^- concentration in a solution, which makes the clay surface to become negatively charged, leading to a decrease in electrostatic repulsion and a consequent increase in metal sorption.

Biodegradation of DEK and biosorption of Cd^{2+} by a biofilm supported in vermiculite in open systems – pilot-scale experiments

Figure 3 shows that the removal (biosorption and/or biodegradation) of DEK, as well as the sorption of Cd^{2+} , are continuous through time and tend to increase after the replacement of the solution S_1 by the solutions S_2 and S_3 . This may be due to microbial growth, which besides increasing the substrate consumption (need for growth and cellular maintenance), also increases the number of active sites of the biofilm used for Cd^{2+} sorption, hampering the saturation of active sites, either of the biomass or of the vermiculite. For DEK, the maximum removal percentages and uptake values obtained were 98.2% and 421.0 mg/g, whereas for Cd^{2+} the maximum removal percentages and uptake values achieved were 87.6% and 5.0 mg/g. From Figure 3 and Table 2, it is possible to observe that the removal percentage achieved for Cd^{2+} is lower than the removal percentage obtained for DEK, which corresponds to the removal of 0.56 mol/g of Cd^{2+} and 36.23 mol/g of DEK. These results may be explained because DEK can either be entrapped by the biofilm or by the vermiculite, but it can also suffer biodegradation by the biofilm. Meena et al. [3] studied the removal of several heavy metals

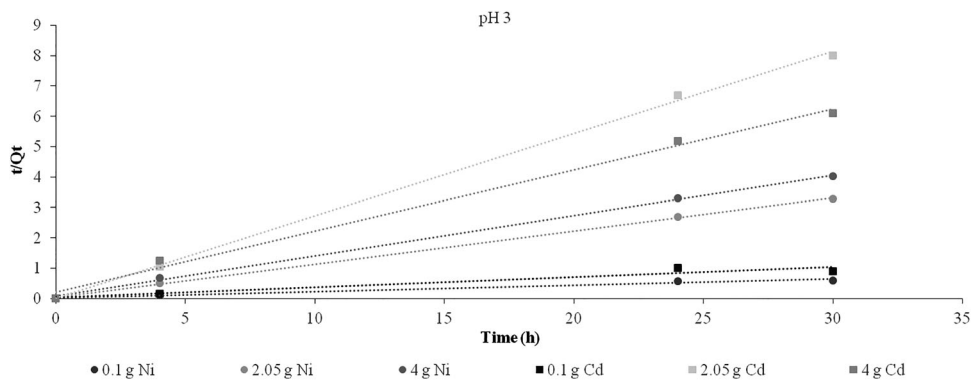


Figure 2. Kinetics modelling of the sorption of Ni^{2+} and Cd^{2+} (100 mg/L) by vermiculite (0.1, 2.05 and 4 g) when exposed to an initial pH 3, at lab-scale.

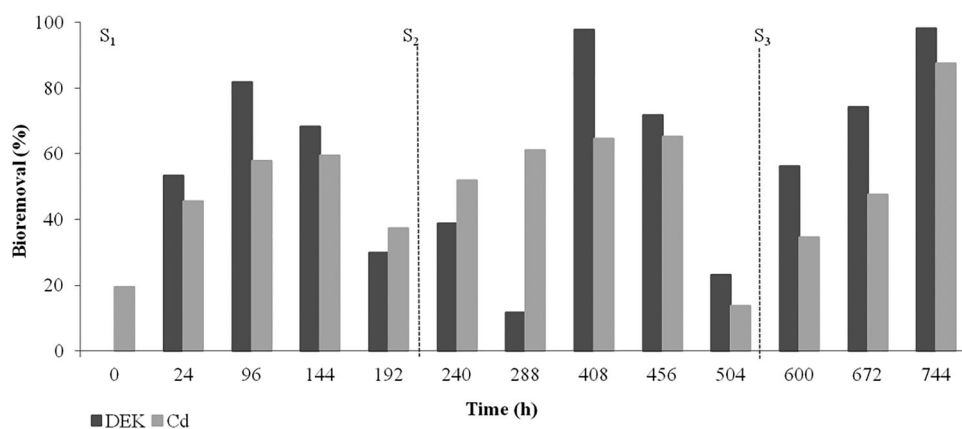


Figure 3. Removal performance (%) by a *Streptococcus equisimilis* biofilm supported on vermiculite for diethylketone (7.5 g/L) and Cd^{2+} (100 mg/L), at pilot plant scale.

from aqueous solutions using carbon aerogel as an adsorbent. These authors obtained Cd removal percentages of about 65%, 72%, 80% and 95% for carbon aerogel adsorbent doses of 5, 8, 10 and 12 g/L, respectively, and an initial concentration of 3 mg/L. Quintelas et al. [39] studied the removal of Cd, Cr, Fe and Ni from aqueous solutions using a *E. coli* biofilm supported on kaolin and for similar initial concentrations of Cd obtained a maximum removal percentage of 71.3%. When comparing the results obtained by these authors, with the results obtained herein, it is possible to corroborate the good performance and capacity of these systems to simultaneously decontaminate aqueous solutions contaminated with Cd^{2+} and DEK. The pH was found to freely range between 6 and 7, which, according to results obtained previously, is within the optimal range for the sorption of these two metals by vermiculite and is close to the optimum pH for the growth of *S. equisimilis* (7.4). This presents a significant advantage since no adjustment of pH is required.

At the end of the pilot-scale experiments, viability tests were conducted and revealed that the *S. equisimilis* biofilm presents biological activity, which is an important advantage in the treatment of wastewater using

microorganisms, since microbial culture resistance allows the continuous removal of the pollutants (organic or mineral) due to biologically active and/or inactive mechanisms.

The results obtained with the GC-MS analysis allowed the elaboration of a degradation mechanism for DEK, where this ketone is firstly degraded into ethyl acetate and then into methyl acetate (Figure 4). At the end of the experiments, no DEK or DEK's metabolites were detected, revealing that this joint system is also able to degrade the intermediates formed during the experiments, an important advantage in the treatment processes of water contaminated with organic compounds.

Breakthrough curves' modelling

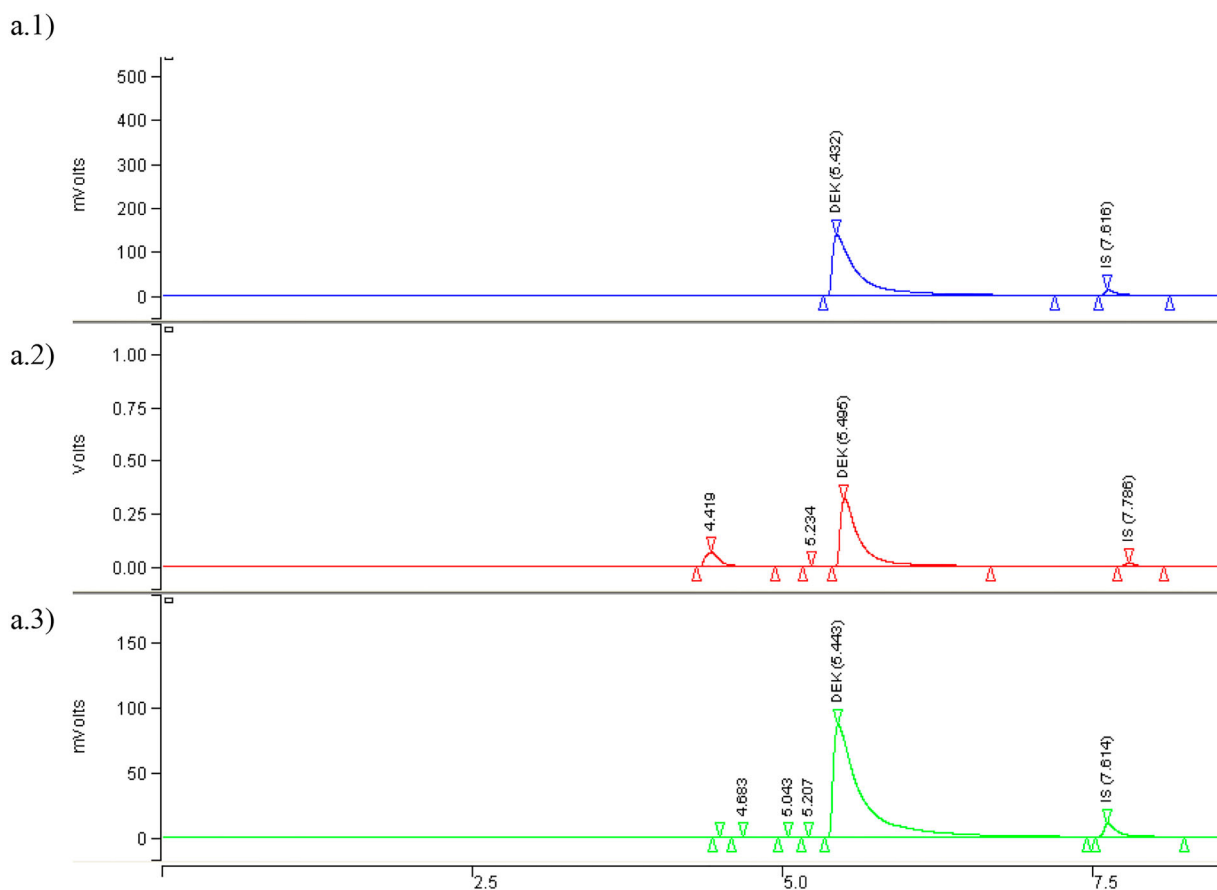
Predicted and experimental breakthrough curves are shown in Figure 5 and all the calculated parameters are shown in Table 3. The results obtained for DEK are properly described by any of the models employed ($R^2 < 0.75$, data not shown). The experimental results obtained for Cd^{2+} are best described by the Adams-Bohart model ($R^2 > 0.80$) and the predicted k_{AB} values for Cd^{2+} are presented in Table 3.

Biodegradation of DEK and biosorption Ni^{2+} by a biofilm supported in vermiculite in open systems – pilot-scale experiments

It is possible to observe in Figure 6 that the removal of DEK is continuous and tends to increase through time (Table 2), whereas the percentage of Ni^{2+} sorbed, despite increasing after replacing the solution S_3 (composed by 7.5 g/L of DEK and 100 mg/L of Ni^{2+}) with solution S_4 (composed 7.5 g/L of DEK), its sorption efficiency is lower.

Table 2. Biosorption performance of the bioreactor column for diethylketone (7.5 g/L) and for Cd^{2+} or Ni^{2+} (100 mg/L).

Time (h)	Maximum biosorption efficiency (%)		Time (h)	Maximum biosorption efficiency (%)	
	DEK	Cd^{2+}		DEK	Ni^{2+}
S_1 (0–192 h)	81.830	59.470	S_1 (0–309.5 h)	46.948	33.641
S_2 (192–504 h)	97.854	65.266	S_2 (309.5–767.5 h)	50.250	57.612
S_3 (504–744 h)	98.236	87.640	S_3 (767.5–1199.5 h)	50.770	38.498
–	–	–	S_4 (1199.5–1942 h)	64.743	51.370
–	–	–	S_4 - end (1942–2374 h)	87.310	67.819



b)

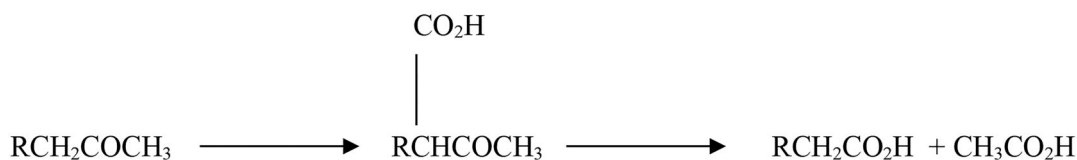


Figure 4. Example of a chromatogram revealing the appearance of several intermediates during DEK's biodegradation by *S. equisimilis* when exposed to 100 mg/L of Cd^{2+} . (a.1) at the beginning of the experiment, (a.2) 41.5 h after the beginning the experiment, (a.3) 983.5 h after beginning the experiment, and (b) proposed anaerobic degradation of diethylketone.

The decrease in Ni^{2+} sorption may be explained by the saturation of the active site present on the biomass surface, not only by the addition of DEK, but also by the continuous formation of by-products, such as methyl acetate and ethyl acetate [26], which may also be used as carbon sources, or sorbed by the cells, competing therefore with Ni^{2+} . The maximum removal percentages and uptake values obtained for DEK were, respectively, 87.3% and 379.0 mg/g, whereas for Ni^{2+} the maximum removal percentages and uptake values achieved were 67.8% and 3.9 mg/g. It is observable that DEK uptake increases continuously through time, probably as a result of the combined action of biodegradation and biosorption processes. Ni uptake also

increases continuously through time, and this may be explained by the conjugation of sorption processes, realized both by the bacteria and by the vermiculite, with continuous bioaccumulation of Ni^{2+} by the bacteria, whose growth increases through time. Meena et al. [3] performed different assays on the removal of Ni^{2+} , among other metals, from aqueous solutions using different masses of carbon aerogel as adsorbent. These authors obtained Ni^{2+} removal percentages of 50%, 60%, 65% and 80% for adsorbent masses of 5, 8, 10 and 12 g/L, respectively, and an initial concentration of Ni^{2+} equal to 3 mg/L. Comparing these results with those described herein, it is possible to infer that apart from the higher removal percentages, the initial

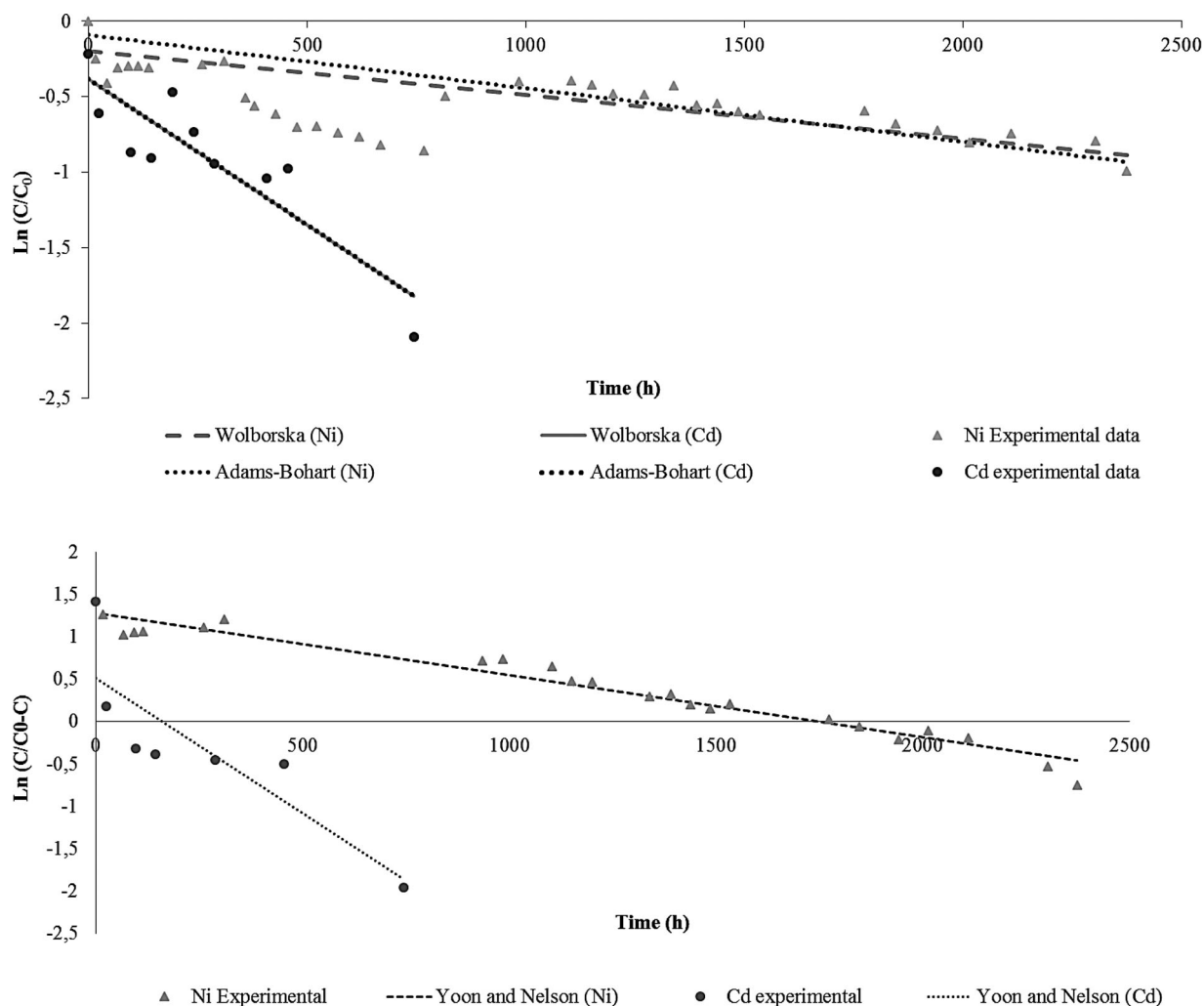


Figure 5. Predicted and experimental breakthrough curves for Cd^{2+} and Ni^{2+} at pilot scale.

concentration of Ni^{2+} is also superior (150 fold), which reveals and corroborates the good performance and capacity of this system to decontaminate Ni^{2+} and DEK aqueous solutions. The pH ranged between 6 and 7, which as mentioned above is in the optimal pH range for the sorption of the two metals by vermiculite and close to the optimum pH for the growth of the biofilm (7.4). After exposure to S_1 , S_2 , S_3 and S_3 -DEK solutions, the column was washed out and different samples of supported biofilm were inoculated in Petri dishes containing fresh culture medium. The growth of *S. equisimilis* revealed to be fast and may be related to the fact that when in the form of biofilm, *S. equisimilis* developed accessible pathways for the substrate and protection mechanisms against hazardous compounds due to the

development of a polymeric matrix that acts as a diffusion barrier, increasing the resistance of the cells against toxic substances. This is an important advantage in the treatment of wastewater using microorganisms.

Once again the results obtained with the GC-MS analysis allowed the elaboration of a degradation mechanism for DEK, where this ketone is degraded into ethyl acetate and into methyl acetate. At the end of the experiments, no DEK or its metabolites were detected.

Breakthrough curves' modelling

Predicted and experimental breakthrough curves are shown in Figure 5 and all the calculated parameters are shown in Table 3. The results obtained for DEK are well described by any of the models employed ($R^2 <$

Table 3. Breakthrough parameters obtained for the pilot scale experiments.

Contaminant	Adams-Bohart			Wolborska		Yoon and Nelson		
	k_{AB} (L/mg h)	N_0 (mg/L)	R^2	β_0 (h^{-1})	R^2	k_{YN} (h^{-1})	τ (h)	R^2
Cd^{2+}	9.953e-5	1.906	0.81	-1.446e-3	0.78	-3.189e-3	160	0.74
Ni^{2+}	-1.186e-3	1.429	0.91	6.420e-5	0.85	7.34e-4	1751	0.96

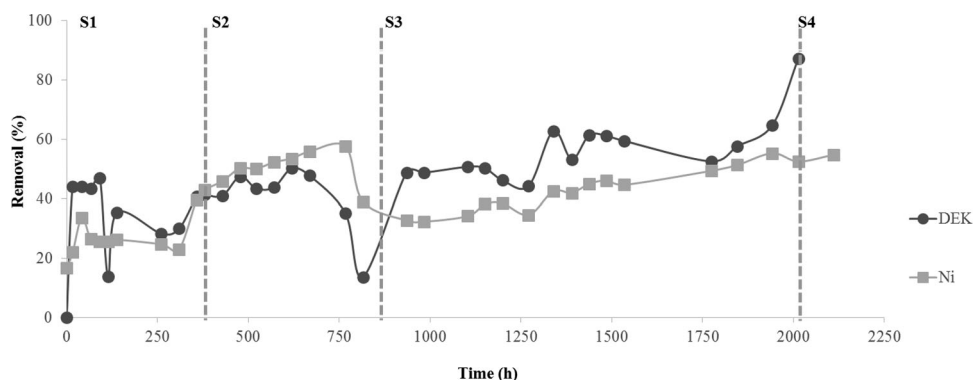


Figure 6. Removal percentage of DEK and Ni²⁺ at pilot plant scale, by a biofilm of *S. equisimilis* supported into vermiculite. S₁ – Solution with Ni²⁺ (100 mg/L) and DEK (7.5 g/L); S₂ – replacement of the solution S₁ for a fresh, similar one; S₃ – replacement of the solution S₂ for a fresh, similar one; S₄ – addition of DEK (7.5 g/L) to the solution S₃.

0.75, data not shown). The experimental results obtained for Ni²⁺ are best described by the Yoon and Nelson model ($R^2 > 0.95$) and the predicted τ values for Ni are presented in Table 3.

FTIR, XRD and SEM analyses

FTIR spectra of (i) loaded and unloaded vermiculite with DEK, (ii) the bacterial biomass, and either in suspension and in the form of biofilm, previously exposed to DEK and Cd²⁺ or Ni²⁺, in the range of 500 cm⁻¹ to 4000 cm⁻¹ were taken to corroborate and determine the presence of functional groups typically accountable for the sorption processes and are shown in Figure 7.

Vermiculite shows a number of sorption peaks that reflects the complex nature of the mineral clays. The band at 1000 cm⁻¹ of the unloaded vermiculite denotes the Si–O–Si stretching, whereas the band at 1635 cm⁻¹ represents the H–O–H in absorbed water bending [40] and the band at 3400 cm⁻¹ represents the OH functional group stretching vibration [41]. Some of these band signals were also identified by several other authors [42,43] on clays and were found to correspond to surface functional groups accountable for the sorption of hazardous compounds [44,45].

These functional groups can interact with the different contaminants used in the present work. Samples of (i) vermiculite exposed to DEK and (ii) of *S. equisimilis* biofilm exposed to DEK (7.5 g/L) and Cd²⁺ or Ni²⁺ (100 mg/L) reveal numerous modifications either on the intensity, on the shape of the peaks and on the disappearance and/or formation of new peaks. After exposure to DEK, bands at 1400 cm⁻¹ and at 2400 cm⁻¹ corresponding, respectively, to C–H bending (–CH₃) and to C≡C and/or C≡N stretching presented important changes, especially the first band that disappeared and the second one presenting higher intensity and different shape. The intensity of the bands detected at 675, 1000, 1650 and 3500 cm⁻¹ and corresponding respectively to C–OH stretching vibrations, Si–O–Si stretching, C=O stretching groups and to the hydroxyl functional group stretching vibration (–OH) was found to decrease significantly. All these modifications may be explicated by the interaction and involvement of the different functional groups present on the sorbent surface with the functional groups (C=O) of DEK. Bands at 2300 cm⁻¹ and at 3500 cm⁻¹ were found to disappear on the samples analysed containing *S. equisimilis* biofilm supported into vermiculite and exposed to DEK and to Cd²⁺ or Ni²⁺. According to Volesky [45], the main

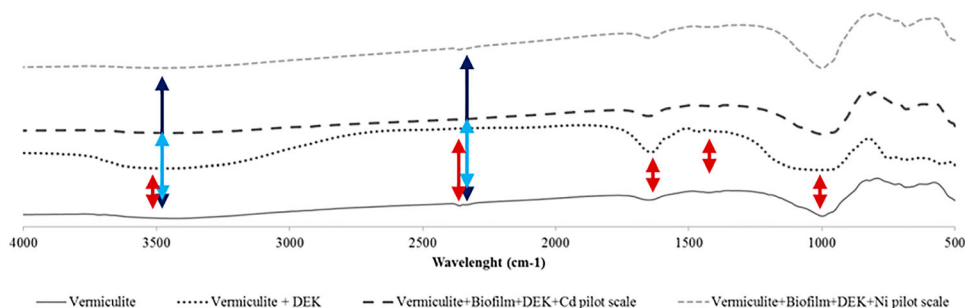


Figure 7. FTIR spectra of different samples: vermiculite unloaded, vermiculite exposed to diethylketone (7.5 g/L), *S. equisimilis* biofilm supported on vermiculite, loaded with diethylketone (7.5 g/L) and 100 mg/L of either Cd²⁺ or Ni²⁺.

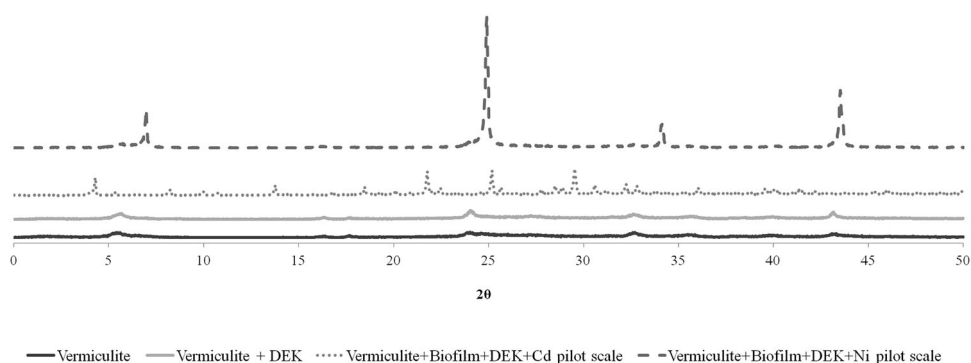


Figure 8. XRD patterns of original and recovered vermiculite.

functional groups responsible for biosorption processes are the imidazole, phosphodiester, amide, sulphonate, hydroxyl, carboxyl, phosphonate and carbonyl groups. Some of these functional groups (phosphate bands at 1237 cm^{-1} and carbohydrate bands at 1070 cm^{-1} next to hydrocarbon sorption bands in the wavelength region between 3000 and 2800 cm^{-1}) [44] are present on the surface of *Streptococcus* sp. and are responsible for the biosorption and biodegradation of hazardous substances such as DEK [26].

The XRD patterns of different samples – (i) loaded and unloaded vermiculite with DEK, and (ii) *S. equisimilis* biomass either in suspension or supported, eventually exposed to DEK and Cd^{2+} or Ni^{2+} – are presented in Figure 8.

The sample of unloaded vermiculite and the sample of vermiculite loaded with DEK exhibited the characteristic pattern of clays, with no evident change in the position of the diffraction peaks for clays, after DEK exposure. This similarity between the two diffractograms suggests a similar crystallinity of vermiculite before and after being exposed to DEK, revealing that no substantial structural modification in the clay occurred. Nonetheless, the samples containing *S. equisimilis* biofilm supported into vermiculite exposed to either DEK and Cd^{2+} or

DEK and Ni^{2+} presented several changes not only in the intensity of the diffraction peaks but also in their position. These changes are an indication of the extensive actions on the vermiculite structure.

Samples of vermiculite without contact to DEK, Cd^{2+} and Ni^{2+} , with and without biofilm were analysed by SEM. SEM analyses confirm (i) that vermiculite exposure (without biofilm) to DEK, Cd^{2+} and Ni^{2+} makes vermiculite surface more glazed and polished, with broken and worn leaves, (ii) the presence of a well-developed biofilm supported on vermiculite (Figure 9) and (iii) the presence of numerous white incrustations on the surface of the sorbent, which, according to Sharma et al. [46], represent the binding of the metal ions to the surface of biomass and are a proof of the biosorption of the metal by the biofilm. Analogous evidence was also found in previous works conducted by Quintelas et al. [27].

Conclusion

The maximum sorption percentages obtained for Cd^{2+} and Ni^{2+} were achieved at pH 8 for 4 g of vermiculite. The pilot-scale experiments demonstrated the excellent capacity of this joint system to simultaneously and continuously biodegrade DEK and biosorb Cd^{2+} or Ni^{2+}

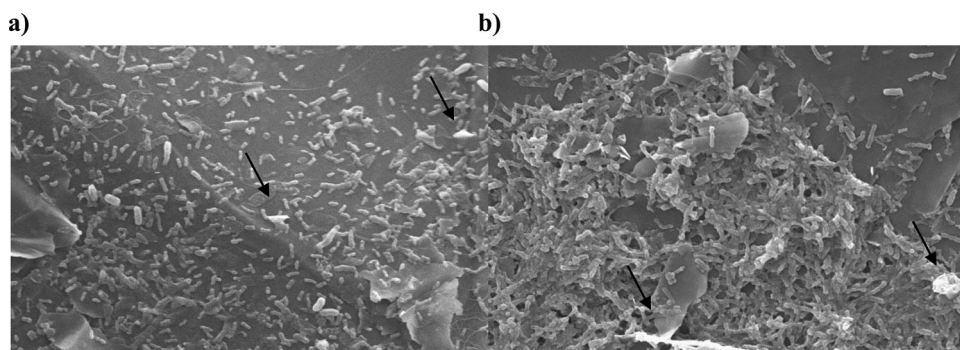


Figure 9. SEM images of *S. equisimilis* supported on vermiculite (700 g) and exposed to diethylketone (7.5 g/L) and (a) 100 mg/L of Cd^{2+} for 456 h, with an amplification of 3000 \times ; (b) 100 mg/L of Ni^{2+} with an amplification of 3000 \times .

without adjusting the pH. The removal percentage and the uptake increase through time, even after replacing the initial solution by new solutions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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