Mihaela Roşca, Raluca-Maria Hlihor*, Petronela Cozma, Elena Niculina Drăgoi, Mariana Diaconu, Bruna Silva, Teresa Tavares and Maria Gavrilescu

Comparison of *Rhodotorula* sp. and *Bacillus megaterium* in the removal of cadmium ions from liquid effluents

DOI 10.1515/gps-2016-0218
Received December 1, 2016; accepted March 22, 2017; previously published online June 3, 2017

Abstract: This study compares the capacity of *Rhodotorula* sp. and *Bacillus megaterium* for Cd(II) removal considering the influence of operating parameters (pH, biosorbent dosage, contact time, temperature, initial metal concentration in solution). The highest Cd(II) uptake of 14.2 mg/g by *Rhodotorula* sp. was exhibited at 30°C, when working at pH 6 and with 5 g/l biosorbent dosage, after 48 h of contact time. In these conditions, a removal efficiency of 85% was obtained. Similar outcomes were obtained for *B. megaterium* (15.1 mg/g, 90%) at 35°C, pH 4 and 3 g/l biosorbent dosage, considered as the optimum set of parameters, equilibrium being achieved for a contact time of 20 min. The possible interaction mechanisms between the biosorbents and Cd(II) were evaluated through point of zero charge (pH_{pzc}), Fourier transform infrared (FTIR), spectroscopy and scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX). Data were modeled using pseudo-first and pseudo-second order kinetic models and Langmuir and Freundlich isotherms models. Further studies considered a modeling approach based on linear regression with Durbin-Watson statistics, while the accuracy and precision of experiments were evaluated by ANOVA.

Keywords: biosorption; Cd(II); microorganisms; modeling; statistical model.

***Corresponding author: Raluca-Maria Hlihor,** Department of Environmental Engineering and Management, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi, 73 Prof. Dr. Docent D. Mangeron Str., 700050 Iasi, Romania; and Department of Horticultural Technologies, Faculty of Horticulture, "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi, 3 Aleea Mihail Sadoveanu, 700490 Iasi, Romania, e-mail: raluca.hlihor@ch.tuiasi.ro
Mihaela Roşca, Petronela Cozma and Mariana Diaconu: Department of Environmental Engineering and Management, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi, 73 Prof. Dr. Docent D. Mangeron Str., 700050 Iasi, Romania
Elena Niculina Drăgoi: Department of Chemical Engineering, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi, 73 Prof. Dr. Docent D. Mangeron Str., 700050 Iasi, Romania
Bruna Silva and Teresa Tavares: Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
Maria Gavrilescu: Department of Environmental Engineering and Management, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi, 73 Prof. Dr. Docent D. Mangeron Str., 700050 Iasi, Romania; and Academy of Romanian Scientists, 54 Splaiul Independentei, 050094 Bucharest, Romania

1 Introduction

Biosorption, a process that uses the ability of naturally occurring biological materials (microorganisms, agricultural wastes, etc.) to remove pollutants from aqueous solutions, has been extensively studied in the past years with high rates of success, especially at laboratory scale. Although there are several studies which confirm the ability of microorganisms to remove heavy metals [1–4], this process is still not applied in large scale in wastewater treatment plants. Even if a number of patents are being released every year, some technical barriers prevent the commercialization of biosorbents, while the mechanism of biosorption in some cases is still difficult to be understood [5]. Considering the increasing industrialization, removal of heavy metals from wastewaters is nowadays a top priority. Based on the abovementioned aspects confirmed by reliable data [2, 3, 6, 7], biosorption is a process with great potential for application for environmental remediation, such as heavy metals removal. Cadmium, one of the most toxic heavy metals, could cause a number of diseases in humans from kidney damage and bone diseases to cancer. Biosorption could be applied as an alternative to conventional methods used for heavy metals removal from wastewaters (such as chemical precipitation, electrochemical treatment, filtration, ion exchange, evaporation, reverse osmosis and membrane technologies) [1, 5, 8].

Although literature in the field highlights a large spectrum of microorganisms used in the removal of heavy metals from aqueous solutions (e.g. *Aspergillus niger*, *Arthrobacter viscosus*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Trichoderma viride*) [2, 3, 9, 10], there is still room for identification of new ones with greater abilities and entrapment characteristics. Moreover, there is a need to study and compare the uptake capacities of different biosorbents, along with investigation and sharing of advanced modeling and optimization procedures to overcome the lack of knowledge for full-scale process application. This study is focused on a complete comparison of two different types of microorganisms, *Rhodotorula* sp., a unicellular pigmented yeast and *Bacillus megaterium*, a Gram- positive bacterium. *Rhodotorula* sp. belongs to the fungal family of *Sporidiobolaceae* (*Phylum basidiomycota*). It is frequently isolated from soil, water, milk, fruit juice, air samples and other products. In addition, this species is also considered as a normal inhabitant of the skin, lungs, urine and feces in humans [11]. By contrast, *B. megaterium* is classified as a microorganism with high phosphatase and ribonuclease activity. The size of the cells of *B. megaterium* is between 5 μm and 7 μm, while a colony of this bacterium reveals the following characteristics: circular, entire, raised, opaque and white aspect. It is found in soil and is considered a saprophyte microorganism. *B. megaterium* has often been used in the laboratory, as an industrial organism which is able to produce a variety of proteins [12–14]. Its high availability makes it the perfect candidate for biosorption.

A study presented by Li and Yuan [15] showed that treated and modified *Rhodotorula* sp. Y11 has also a high potential in cadmium removal from solution (maximum uptake capacity was established at 11.4 mg Cd(II)/g biosorbent). Liu et al. [16] reported that *B. megaterium* dead biomass has a high capacity for removal of Au^{3+} ions from solution. Some 95% of the maximal capacity was reached in the first 5 min of contact time, while the maximum biosorptive efficiency was 99.1%, achieved at pH 3.0 and 30°C, in only 30 min. Based on the results obtained by Miyatake and Hayashi [17], this bacterium is capable of removing arsenic at low concentrations, the maximum biosorption efficiency being 93.2%, at 35°C and pH 7 in 12 h. With respect to Cd(II) removal by *B. megaterium*, only one study describes the uptake capacity for Cd(II) biosorption from aqueous solution considering pH, temperature, initial metal ion concentration and incubation time [18]. These studies highlight the feasibility of *Rhodotorula* sp. and *B. megaterium* in the treatment of different metal fluxes. These biomaterials were chosen as biosorbents since they are natural, easily available, and thus could be considered a low-cost option for the removal of Cd(II) ions. Moreover, from our knowledge, this attempt in comparing two different types of microorganisms (*Rhodotorula* sp. and *B. megaterium*) for the removal of Cd(II) from aqueous solutions, is one of the first to be made.

In this context, a full comparison was performed in the present study to demonstrate the efficiency of *Rhodotorula* sp. and *B. megaterium* dead biomasses in the removal of Cd(II) from liquid effluents. Different parameters influencing the biosorption process were evaluated. In order to elucidate the possible interaction mechanisms, the biosorbents were characterized based on point of zero charge (pH_{pzc}), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX).

Kinetics and biosorption isotherms models were considered for modeling the experimental data (e.g. pseudo-first and pseudo-second order kinetic models, and Langmuir and Freundlich isotherms models).

The implementation of statistical experimental design techniques in biosorption studies enables one to obtain improved product yields, output response closer to target requirements and reduced development time, process variability and overall costs [19]. Moreover, the relatively large number of experiments can be reduced and the interactions among different factors (which are often ignored) are highlighted. When a model captures the key elements of the system, answers to relevant questions for the experimental practice can be found [20]. In addition, predictions of the output parameters can be generated, thus eliminating the need for further experiments. In this framework, a modeling approach based on linear regression with Durbin-Watson statistics provided by the IBM SPSS Statistics software (version 23, IBM Corporation, USA) was considered in order to analyze the experimental results and to estimate the combined effects of the biosorption factors on the removal efficiency and heavy metal uptake. Further, the accuracy and precision of experiments were evaluated by ANOVA.

2 Materials and methods

2.1 Biosorbents: growth and inactivation

Both microorganisms were isolated within the microbiology laboratory of the Department of Environmental Engineering and Management, Iasi, Romania. *Rhodotorula* sp. was isolated from students' hands, while *B. megaterium* was isolated from food products. Identification of microorganisms was made by microscopic observation of the appearance of colonies, cell shape, color and growth on specific

media [21]. *Rhodotorula* sp. was grown in Sabouraud medium containing 20 g/l glucose and 10 g/l peptone in 1 l distilled water, for 72 h, at 28°C and 150 rpm. For the growth of *B. megaterium*, Lysogeny broth medium containing 10 g/l tryptone, 5 g/l yeast extract and 5 g/l NaCl in 1 l of distilled water was used. All chemicals were purchased from Sigma-Aldrich, Germany. The microorganism was grown for 72 h, at 30°C and 150 rpm. Afterwards, both microorganisms were centrifuged at 7000 rpm for 10 min (using a Hettich Mikro 220R centrifuge, Germany), inactivated in an autoclave (Autoclave Trade Raypa AH-21 Clase N, Spain) for 15 min at 121°C, and further dried at 60°C for 48 h. The dried biomass of *Rhodotorula* sp. and *B. megaterium* was crushed and sieved to 125–250 µm particle size and stored in a desiccator until further use.

2.2 Reagents and equipment

All chemicals used in this work were of analytical grade and no further purification was necessary. A stock solution of 1000 mg/l Cd(II) was obtained by dissolving 1.3722 g $Cd(NO_3)_2.4H_2O$ (Riedel-de Haën, Germany) in 500 ml distilled water. The stock solution was diluted with distilled water based on the experiments employed. The pH of the solutions was adjusted by the addition H_2SO_4 1 M and NaOH 1 M (Fisher Scientific, UK), the amount added being negligible (Multi-Parameter ProLab 2000 Meter, SI Analytics GmbH, Germany). Cd(II) concentration in solution was analyzed using an inductively coupled plasma spectrometer (Optima 8000 ICP-OES model, PerkinElmer, Inc., USA).

2.3 Biosorption studies

The biosorption of Cd(II) ions by *Rhodotorula* sp. and *B. megaterium* was studied in a batch system. Experiments were conducted in 200 ml Erlenmeyer flasks with 50 ml Cd(II) solution, in an incubator at 150 rpm. In the investigation concerning the pH influence, the following conditions were considered: working with 5 g/l *Rhodotorula* sp. and 3 g/l *B. megaterium*, at 25°C for 48 h, while maintaining constant a concentration of 50 mg/l Cd(II). The pH ranged from 3 to 7 to avoid a possible precipitation of cadmium ions. The biosorbent dosage varied from 1 g/l to 10 g/l, when investigating the biosorbent dosage influence, keeping a constant pH (pH 6 for *Rhodotorula* sp. and pH 4 for *B. megaterium*) and heavy metal concentration (50 mg/l). After the biosorbent dosage and optimum experimental pHs were established, the following step was to evaluate the influence of contact time (samples were taken at time intervals from 0 min to 48 h) for two different concentrations [50 mg/l and 100 mg/l Cd(II)]. Kinetic studies were conducted in 250 ml Erlenmeyer flasks with 100 ml Cd(II) solution. The influence of initial concentration of Cd(II) in solution on the biosorption process was assessed in the range between 25 mg/l and 300 mg/l. Temperature ranged from 25°C to 45°C. After incubation, samples were centrifuged at 10,000 rpm for 5 min, the supernatants were acidified (in 2%) with nitric acid (69%) (Fisher Scientific, UK) and further analyzed. Control experiments were also conducted for each investigation by considering the same procedure stated above, but in the absence of metal ions. All experiments were carried out in duplicate and the mean average values were used in further analysis. The experimental error was less than 5%.

The uptake of metal ions per gram of biosorbent was calculated using the following expression [Eq. (1)] [22]:

$$q = \frac{C_i - C_f}{m} \cdot V \quad (1)$$

where q is the biosorption capacity (mg/g), C_i is the initial metal ion concentration in solution (mg/l), C_f is the concentration of metal ions in solution at the end of the biosorption process (mg/l), V is the volume of the solution (l) and m is the amount of biosorbent used in the experiment (g).

The removal efficiency (R, %) was calculated using Eq. (2):

$$R\ (\%) = \frac{C_i - C_f}{C_i} \cdot 100 \quad (2)$$

2.4 Characterization of the biosorbents

Characterization of biosorbents is important in understanding the mechanism of heavy metals removal and consisted of the analysis of biomass surface for qualitative assessment of the main functional groups responsible for the metal binding. For this purpose, the point of zero charge (pH_{pzc}) was considered, along with FTIR spectroscopy and SEM-EDX.

2.4.1 Point of zero charge (pH_{pzc}) analysis: For characterization of biosorbents using the analysis of point of zero charge (pH_{pzc}), the experiments were performed by adding 100 mg of biosorbent in 10 ml of aqueous sodium chloride 0.1 mol l^{-1}, with initial pH values from 1 to 6. The pH values were adjusted with sulfuric acid (H_2SO_4 0.1 mol l^{-1}) and sodium hydroxide (NaOH 0.1 mol l^{-1}). After 48 h of contact time at 150 rpm and 25°C, the final pH of the samples was measured. The variations of initial values of pH depending on the final values were plotted and the point at which pH variation was not observed corresponded to pH_{pzc} [23, 24].

2.4.2 FTIR spectroscopy: FTIR spectroscopy was used for the identification of functional groups available on the surface of the biosorbents, before and after biosorption. FTIR analyses enable the identification of different functional groups on the cell wall structure and provide important information related to the nature of the bonds [5]. Approximately 1 mg of biomass was prepared and compressed with 100 mg of dry KBr (Fisher Scientific, UK). FTIR absorption spectra were performed in the wave number range of 4000–500 cm^{-1} with a resolution of 8 cm^{-1} by using a BOMEN MB 104 spectrometer (ABB Bomem Inc., Quebec, Canada).

2.4.3 SEM-EDX: The surface morphology of *Rhodothorula* sp. and *B. megaterium* before and after Cd(II) biosorption was observed using SEM. For determination of elemental composition of biosorbents after the biosorption process, EDX was used. The samples for SEM-EDX (JEOL JSM-7001F, Oxford INCA 250, JOEL Co. Ltd., Japan/Oxford Instruments, UK) observation were first cut into small pieces in accordance with the procedure requirements, before being placed on the sample – stubs. Prior to scanning, the samples were attached to the sample holder and coated with gold using sputter coating (SEM Coating System Machine) [25, 26].

2.5 Biosorption modeling

For modeling the sorption process, Lagergren pseudo-first-order and Ho pseudo-second-order kinetic models and Langmuir and Freundlich isotherm models were initially applied. These models predict the parameters used for designing and modeling of the process and help in elucidating the mechanism involved in Cd(II) biosorption.

The pseudo-first-order model (Lagergren's rate equation) describes the adsorption of the metallic ions from the liquid phase and is one of the most widely used equations. The linearized form of this expression is given by Eq. (3) [27]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{3}$$

where q_e and q_t (mg/g) are the biosorption capacity at equilibrium and at time t (min), respectively, and k_1 (min^{-1}) is the rate constant of the pseudo-first order equation.

The pseudo-second-order kinetic model (Ho model) is related to the assumption that the rate-limiting step may be chemical sorption or chemisorption involving valence forces through sharing or exchange electrons between sorbent and sorbate and is expressed in linear form as Eq. (4) [28]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{4}$$

where q_e and q_t (mg/g) are the biosorption capacity at equilibrium and at time t (min), respectively, and k_2 (g/mg min) is the rate constant of the pseudo-second order equation.

The Langmuir isotherm (1918) considers sorption as a chemical phenomenon and provides information on uptake capabilities that occur on a homogenous surface by monolayer sorption. It can be used for describing equilibrium conditions and to predict the performance of different biosorbents. The non-linear form of the Langmuir isotherm equation can be expressed as follows [Eq. (5)] [27, 29]:

$$q_e = q_m K_L \frac{C_e}{1 + K_L C_e} \tag{5}$$

where C_e (mg/l) is the equilibrium concentration of the metal in solution, q_m (mg/g) is the maximum sorption upon complete saturation at the biomass surface and K_L (l/g) is a constant related to the adsorption/desorption energy.

The Freundlich isotherm relationship is an exponential and an empirical equation; it provides information about the surface heterogeneity and the exponential distribution of active sites and their energies, and it is expressed by the following equation [Eq. (6)] [27, 29]:

$$q_e = K_F C_e^{1/n} \tag{6}$$

where K_F (mg^{1-n}g^{-1}l^n) represents the sorption capacity when metal ion equilibrium concentration is equal to 1, and n represents the degree of dependence of sorption with equilibrium concentration.

In order to determine the biosorption efficiency as a function of the working conditions [pH, temperature, contact time, initial concentration of Cd(II) ions, biosorbent dosage] the biosorption process was modeled using a linear regression approach with Durbin-Watson statistics provided by the IBM SPSS Statistics software (version 23). Taking into consideration that two types of microorganisms were used, the same modeling approach was applied twice, resulting in two different models, one for each microorganism tested.

Further, the accuracy and precision of experiments were evaluated by ANOVA in order to ensure that the determined model predictions follow the experimental data and are statistically significant.

3 Results and discussion

3.1 Influence of pH on biosorption process

The biosorption process of Cd(II) ions from aqueous solutions is influenced by different parameters and one of the most important is the solution pH. The sorption efficiencies and uptake capacities of *Rhodotorula* sp. and *B. magaterium* for Cd(II) ions removal tested for different pH values are shown in Figure 1. It can be observed that changing the pH values between 3 and 7 had an important effect on Cd(II) biosorption. In the case of *Rhodotorula* sp., the maximum removal efficiency was 65%, while the maximum uptake was 5.3 mg/g at pH = 6, for an initial concentration of 50 mg/l Cd(II) ions and 5 g/l biosorbent dosage. For this biosorbent, the data showed an increase of Cd(II) uptake and removal efficiency at pH values from 3 to 5. The uptake capacity of *Rhodotorula* sp. increases from 2.7 mg/g to 5.3 mg/g, while the removal efficiency increases from 40% to 65%. In the case of *B. megaterium*, the optimum pH is 4, with a maximum efficiency of 97% and an uptake capacity of 16.2 mg/g (values obtained for 50 mg/l initial concentration and 3 g/l biosorbent dosage). For pH values between 3 and 4, the removal efficiency increased from 86% to 97%,

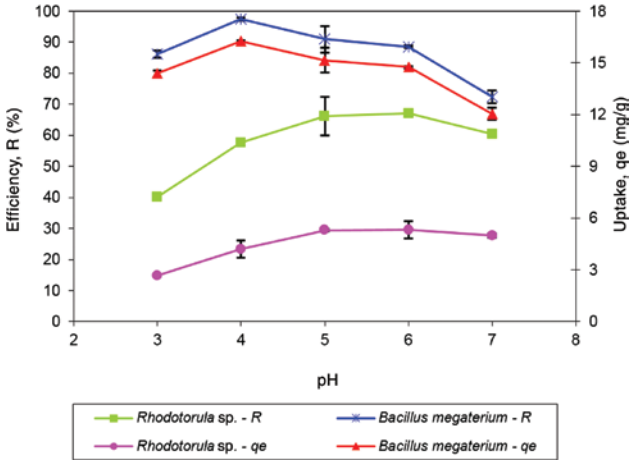

Figure 1: Influence of pH on biosorption of Cd(II) ions by *Rhodotorula* sp. (biomass dosage 5 g/l, temperature 25°C, contact time 48 h, initial Cd(II) concentration 50 mg/l) and *B. megaterium* (biomass dosage 3 g/l, temperature 25°C, contact time 48 h, initial Cd(II) concentration 50 mg/l).

and the uptake rose from 14.4 mg/g to 16.2 mg/g. For pH values higher than 4, the removal efficiency decreased at 72%, and the uptake diminished at 12.0 mg/g. It was not taken into account to maintain a constant pH during the experiments, therefore at the end of the process, it was noticed that the pH increased up to 8 for initial pH values higher than 5, which could be the reason for these results. The decrease of removal efficiencies at higher pH values may be caused by the formation of soluble hydroxylated complexes of Cd(II) ions and their ionized nature; these ions were converted into their hydroxide forms and got precipitated.

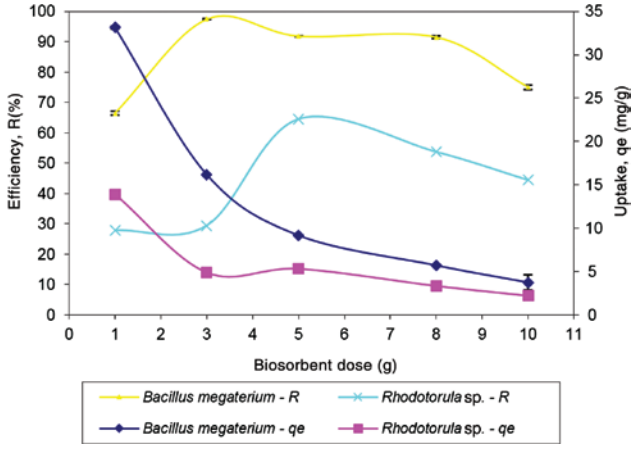

Figure 2: Influence of biosorbent dosage on biosorption of Cd(II) ions by *Rhodotorula* sp. (pH 6, temperature 25°C, contact time 48 h, initial Cd(II) concentration 50 mg/l) and *B. megaterium* (pH 4, temperature 25°C, contact time 48 h, initial Cd(II) concentration 50 mg/l).

3.2 Influence of biosorbents dosage on biosorption process

Another parameter which has an important effect on the biosorption of heavy metals ions is the biosorbent dosage. The studies concerning the influence of biosorbents dose on Cd(II) ions removal by *Rhodotorula* sp. and *B. megaterium* showed that the biosorption capacity of Cd(II) decreased with increasing the biosorbent dosage from 1 g/l to 10 g/l (Figure 2). The maximum uptake of cadmium by *Rhodotorula* sp. was 13.8 mg/g at pH 6 and 33.1 mg/g at pH 4 for *B. megaterium*, respectively (considering 1 g/l biosorbent dosage). The maximum efficiency in the same conditions of pH was 65% for *Rhodotorula* sp. at 5 g/l biosorbent dosage and 97% for *B. megaterium* at 3 g/l biosorbent dosage. The increase in the percentage of the metal ion removal with increase in biosorbent dosage is due to the greater availability of the exchangeable sites or surface area at higher concentration of the biosorbent [30, 31]. Based on the results obtained up to now, further experiments were performed considering 5 g/l biosorbent dosage for *Rhodotorula* sp., and 3 g/l for *B. megaterium*.

3.3 Influence of contact time on biosorption process

For the optimization of the biosorption process, it is necessary to study the influence of contact time on Cd(II) ions removal from aqueous solution. The effect of contact time on Cd(II) removal efficiency and uptake capacity of *Rhodotorula* sp. and *B. megaterium* is shown in Figure 3. It was observed that the percentage of Cd(II)

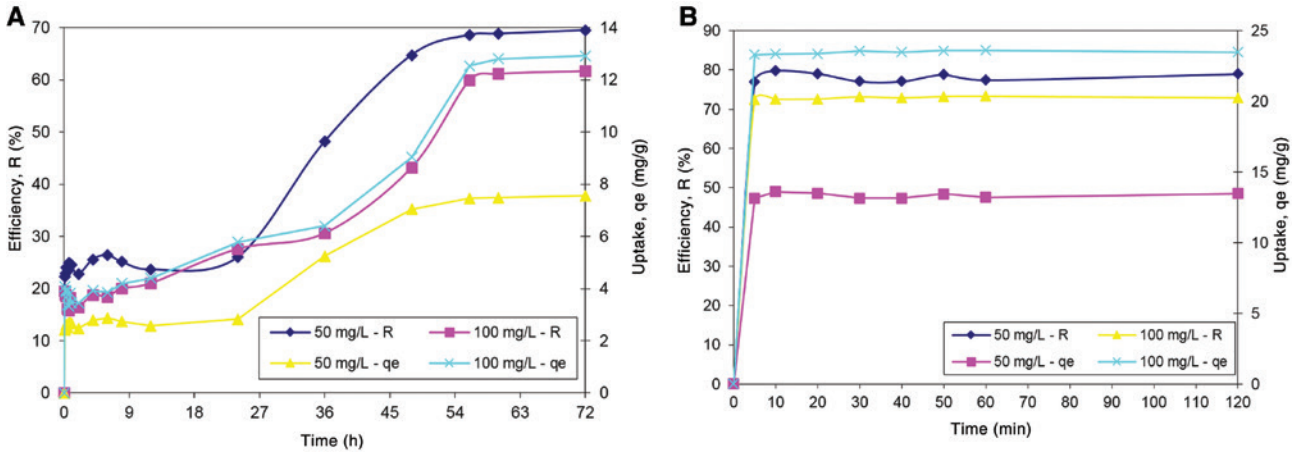

Figure 3: Influence of contact time on biosorption of Cd(II) ions by: (A) *Rhodotorula* sp. (pH 6, temperature 25°C, biomass dosage 5 g/l) and (B) *B. megaterium* (pH 4, temperature 25°C, biomass dosage 3 g/l).

removal is improved with any increase in contact time, the maximum uptake and efficiency for *Rhodotorula* sp. being attained after 48 h, and for *B. megaterium*, in the first 10 min. After this time, it was found that the efficiency of the process did not show a considerable increase [for *Rhodotorula* sp., the maximum efficiency after 72 h of contact time for 50 mg/l initial concentration of Cd(II) ions in solution was 69%, data not shown, while after 48 h of contact time the removal efficiency was 65%, and 80% after 24 h, data not shown, and 79% after 20 min of contact time for *B. megaterium*]. Therefore, the optimum contact time was selected as 48 h for *Rhodotorula* sp. and 20 min for *B. megaterium* in further experiments. Similar results were obtained by Liu et al. [16] for *B. megaterium* dead biomass, when a maximum biosorption efficiency of 95% for Au^{3+} ions removal from solution was attained in 30 min.

3.4 Influence of temperature on biosorption process

Temperature is one of the basic parameters for sorption processes due to the already demonstrated fact that the sorption processes tend to be exothermic and the sorption performances vary with temperature [29]. Biosorption is not considered a strongly exothermic process such as other physical adsorption processes, but the variation of temperature can improve its performance [22, 29]. Studies concerning the influence of temperature on Cd(II) biosorption process of by the selected microorganisms were carried out at four different temperatures (ranging from 25°C to 45°C) for 50 mg/l initial metal solution, and the results are shown in Figure 4. The increase of temperature resulted in the maximum biosorption efficiency and uptake for *Rhodotorula* sp. achieved at 30°C (85% and 14.2 mg/g), and at 35°C for *B. megaterium* (90% and 15.1 mg/g). With a higher increase in temperature, the efficiency of the process decreases for both microorganisms. This could be due to the deactivation of the biosorbent surface, while destructing some active sites on the biosorbent surface caused by bond ruptures or due to the weakness of biosorption forces between the active sites of the biosorbents and the metal ion species [32, 33]. The same behavior of heavy metals biosorption at different temperatures was obtained by Sulaymon et al. [32].

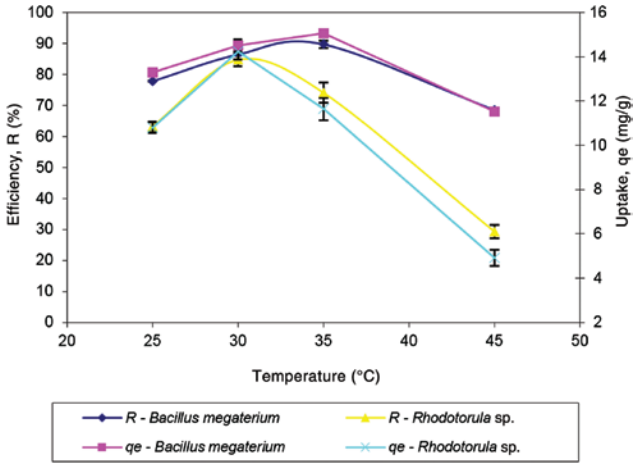

Figure 4: Influence of temperature on biosorption of Cd(II) ions by *Rhodotorula* sp. (pH 6, biomass dosage 5 g/l, contact time 48 h, initial Cd(II) concentration 50 mg/l) and *B. megaterium* (pH 4, biomass dosage 3 g/l, contact time 2 h, initial Cd(II) concentration 50 mg/l).

3.5 Influence of initial concentration of Cd(II) ions in solution on biosorption process

The effect of initial concentration on the removal of Cd(II) ions by the selected biosorbents was studied and the results are given in Figure 5. The analysis of results reveals that the efficiency of Cd(II) removal decreased exponentially with the increase in initial concentration of ions. During the experiments, the capacity of biosorbents for Cd(II) removal was tested for seven different initial concentrations (ranging from 25 mg/l to 300 mg/l). For both types of biosorbents, it was observed that for initial concentrations between 25 mg/l and 100 mg/l, the biosorption capacity increased while biosorption efficiency decreased (from 4.3 mg/g [86%] to 11.2 mg/g [57%] for *Rhodotorula* sp., and from 7.1 mg/g [86%] to 23.8 mg/g [48%] for *B. megaterium*). For an initial Cd(II) concentration of 300 mg/l, the biosorption capacity decreased to 6.2 mg/g (11%) for *Rhodotorula* sp. and to 8.1 mg/g (8%) for *B. megaterium*. The increase of biosorption capacity with increase of initial metal ion concentration indicates that surface saturation of biosorbents is dependent on the initial metal ion concentrations [34]. Similar results concerning the influence of initial ions concentration on the biosorption process were obtained by Ezzouhri et al. [35] for lead removal by *Penicillium* sp.

3.6 Biosorption modeling

3.6.1 Biosorption kinetics

To establish the mechanisms involved in Cd(II) removal by *Rhodotorula* sp. and *B. megaterium*, pseudo-first order

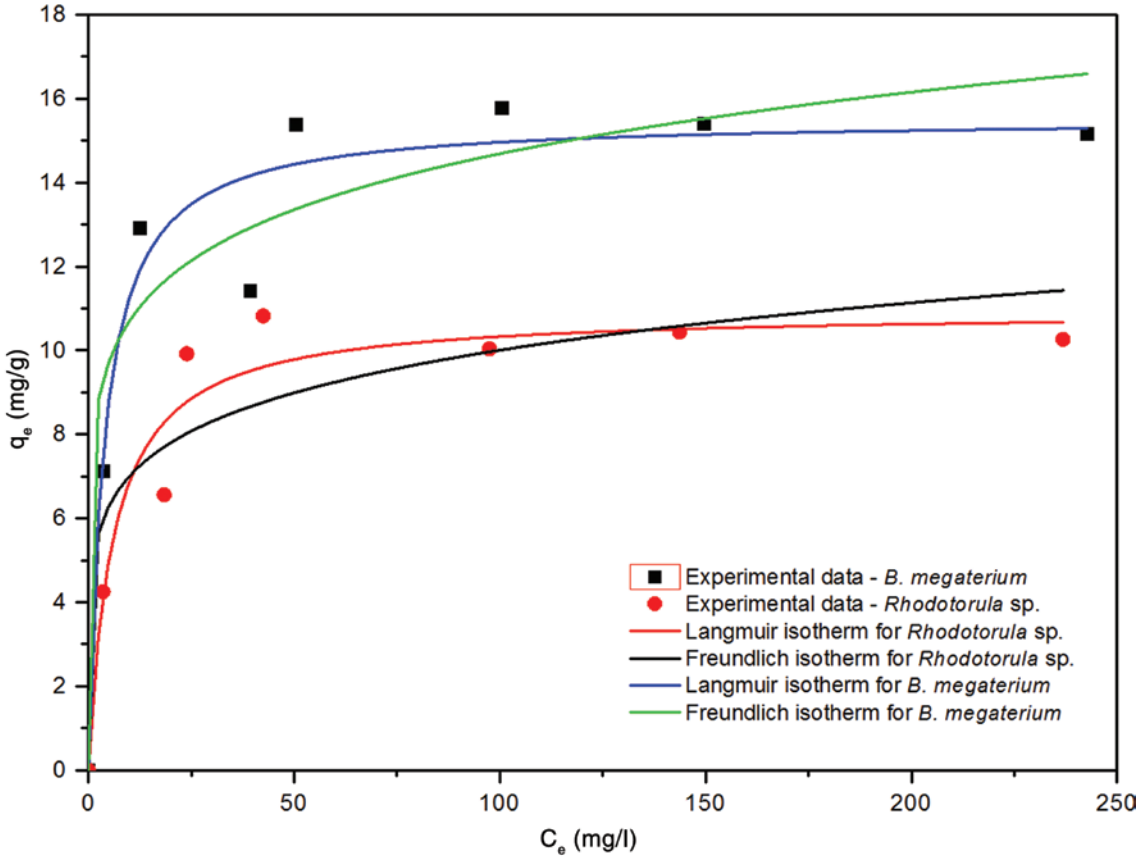

Figure 5: Influence of initial concentration on biosorption of Cd(II) ions by *Rhodotorula* sp. (pH 6, biomass dosage 5 g/l, contact time 48 h, temperature 25°C) and *B. megaterium* (pH 4, biomass dosage 3 g/l, contact time 2 h, temperature 25°C); Langmuir and Freundlich isotherms modeling.

and pseudo-second order adsorption kinetic models were applied. The values of the kinetic parameters are presented in Table 1. Based on the values of correlation coefficients (R^2) the best fitting kinetic model for the selected biosorbents can be observed. For both microorganisms, the ideal model is the pseudo-second order kinetic model with correlation coefficients higher than 0.99. These values provide strong evidence that the process follows this equation. The applicability of the pseudo-second order kinetic model suggested that Cd(II) biosorption by *Rhodotorula* sp. and *B. megaterium* occurs by chemisorption [36, 37].

3.6.2 Biosorption isotherms

Langmuir and Freundlich isotherms provide a relationship between the concentration of cadmium in solution and the amount of cadmium sorbed on biosorbents when

Table 1: Kinetic parameters obtained for Cd(II) removal by *Rhodotorula* sp. and *Bacillus megaterium*.

Concentration	Kinetics	Biosorbent	q_e (mg g^{-1})	k_1 (min^{-1})	R^2
50 mg/l	Pseudo-first order	*Rhodotorula* sp.	4.6	0.00046	0.96
		B. megaterium	0.5	0.00898	0.84
100 mg/l		*Rhodotorula* sp.	9.3	0.00016	0.92
		B. megaterium	6.0	0.00092	0.85
			q_e (mg g^{-1})	k_2 (g/mg min)	R^2
50 mg/l	Pseudo-second order	*Rhodotorula* sp.	2.8	0.164	0.99
		B. megaterium	13.6	0.136	0.99
100 mg/l		*Rhodotorula* sp.	4.1	0.039	0.99
		B. megaterium	23.6	0.210	1

both phases are in equilibrium [38]. For the Langmuir isotherm, the values of R^2 are 0.94 for *Rhodotorula* sp. and 0.95 for *B. megaterium* and the values of K_L are between 0 and 1 for both microorganisms (0.167 l/mg for *Rhodotorula* sp. and 0.264 l/mg for *B. megaterium*, Table 2). These values indicate that the sorption of the metal ion onto biosorbents is favorable [39]. Comparing the correlation coefficients (R^2) obtained, it can be affirmed that the Langmuir isotherm was the best fitting model for Cd(II) sorption onto *Rhodotorula* sp. and *B. megaterium*. This implies that the sorption process takes place at the functional groups on the surface of the biomass, which is regarded as monolayer biosorption [3, 22].

3.6.3 Linear regression with Durbin Watson statistics

The descriptive statistics of the experimental data used to determine the regression models are presented in Table 3.

Table 2: Isotherms parameters obtained for Cd(II) removal by *Rhodotorula* sp. and *Bacillus megaterium*.

Isotherms	Biosorbent	q_m (mg g^{-1})	K_L (l/mg)	R^2
Langmuir	*Rhodotorula* sp.	11.0	0.167	0.94
	B. megaterium	15.5	0.264	0.95
		n	K_F (mg/g(l/mg)$^{1/n}$)	R^2
Freundlich	*Rhodotorula* sp.	6.4	4.911	0.85
	B. megaterium	7.8	7.803	0.90

After applying the linear regression with Durbin Watson statistics, a statistic model is generated. Table 4 shows the linear regression model summary and overall fit statistics are presented.

In Table 4, model 1 corresponds to *B. megaterium* and model 2 to *Rhodotorula* sp. As can be seen from Table 4, both models have a high correlation and a significance lower than 0.05, a fact which indicates that the null hypothesis is negated. The Durbin Watson statistics specify that there is a positive autocorrelation in the samples.

The correlation Table 5 shows the Pearson correlation, significance values and number of cases with non-missing values. In Table 5, biosorbent dosage is denoted with *bd*, time with *ti*, temperature with *te*, initial concentration of Cd(II) ions with *cCd* and process efficiency with *R%*. For *B. megaterium*, there is a strong negative correlation between *R%* and *bd* (–0.86) and a very strong evidence from the significance test that there is a linear correlation between the two ($p < 0.05$). For *Rhodotorula* sp., there is a very strong evidence from the significance test that there is a linear correlation between the *ti* and *R%* and between *bd* and *R%* ($p < 0.005$).

The coefficients of the determined models and a series of statistics related to collinearity are presented in Table 6, where "Unstand. coeff." represent the unstandardized coefficients and "Stand. coeff." the standardized coefficients. The tolerance represents the percentage of variance in a given predictor that cannot be explained by the other predictors and is an indicator of

Table 3: Descriptive statistics of the experimental data.

Independent parameters	Exemplars	Min.	Max.	Mean	Std. deviation
Bacillus megaterium					
pH	37	3	7	4.135	0.631
Biosorbent dosage	37	1	10	3.594	1.572
Time	37	5	2880	832.16	1264.11
Temperature	37	25	45	25.94	3.69
Cd(II) ions conc.	37	24.80	288.18	74.154	49.09
Rhodotorula sp.					
pH	57	3	7	5.912	0.510
Biosorbent dosage	57	1	10	5.03	0.981
Time	57	5	4320	1759.12	1436.79
Temperature	57	25	45	25.614	2.999
Cd(II) ions conc.	57	24.80	288.18	77.57	42.65

Table 4: Model summary.

Model	R	R^2	Adjusted R^2	Std. error of the estimate	Change statistics					Durbin-Watson
					R^2 change	F change	df1	df2	Sig. F change	
1	0.875	0.77	0.73	10.06	0.77	20.22	5	31	0.00	1.10
2	0.891	0.79	0.77	10.33	0.79	39.38	5	51	0.00	1.47

Table 5: Correlation of the determined models.

Model	*R%*	*pH*	*bd*	*ti*	*te*	*cCd*
1						
Pearson correlation						
R%	1.00	0.07	0.27	0.39	0.05	−0.86
pH	0.07	1.00	0.19	0.36	−0.05	−0.12
bd	0.27	0.19	1.00	0.63	−0.10	−0.20
ti	0.39	0.35	0.63	1.00	−0.14	−0.32
te	0.05	−0.05	−0.10	−0.15	1.00	−0.12
cCd	−0.86	−0.12	−0.21	−0.32	−0.12	1.00
Sig. (1-tailed)						
R%	–	0.33	0.05	0.008	0.37	0.00
pH	0.33	–	0.12	0.02	0.37	0.23
bd	0.05	0.12	–	0.00	0.27	0.11
ti	0.008	0.01	0.00	–	0.19	0.02
te	0.37	0.37	0.28	0.19	–	0.22
cCd	0.00	0.23	0.11	0.03	0.22	–
2						
Pearson correlation						
R%	1.00	−0.11	0.11	0.78	0.10	−0.43
pH	−0.110	1.00	0.006	−0.14	0.03	0.12
bd	0.11	0.006	1.00	0.03	−0.007	−0.024
ti	0.78	−0.14	0.03	1.00	0.16	−0.03
te	0.10	0.036	−0.007	0.16	1.00	−0.13
cCd	−0.43	0.13	−0.02	−0.04	−0.13	1.00
Sig. (1-tailed)						
R%	–	0.21	0.20	0.00	0.23	0.00
pH	0.21	–	0.48	0.15	0.39	0.17
bd	0.20	0.48	–	0.42	0.48	0.43
ti	0.00	0.15	0.42	–	0.11	0.39
te	0.23	0.39	0.48	0.11	–	0.15
cCd	0.00	0.17	0.43	0.39	0.15	–

Table 6: Coefficients of the determined models.

Model	Unstand. coeff.		Stand. coeff.	t	Sig.	95.0% Confidence interval for B		Correlations			Collinearity statistics	
	B	Std. error	Beta			Lower bound	Upper bound	Zero−order	Partial	Part	Tolerance	VIF
1												
(C)	109.26	18.19	–	6.01	0.000	72.15	146.37	–	–	–	–	–
pH	−2.56	2.84	−0.08	−0.90	0.37	−8.37	3.24	0.07	−0.16	−0.08	0.87	1.15
bd	0.37	1.37	0.03	0.27	0.79	−2.43	3.17	0.27	0.05	0.02	0.60	1.66
ti	0.002	0.002	0.13	1.08	0.28	−0.002	0.006	0.39	0.19	0.09	0.50	1.98
te	−0.17	0.46	−0.03	−0.36	0.72	−1.12	0.78	0.05	−0.06	−0.03	0.94	1.06
cCd	−0.32	0.03	−0.82	−8.84	0.00	−0.40	−0.25	−0.86	−0.85	−0.77	0.86	1.16
2												
(C)	29.12	21.03	–	1.38	0.17	−13.09	71.35	–	–	–	–	–
pH	2.29	2.76	0.05	0.83	0.41	−3.25	7.84	−0.11	0.12	0.05	0.96	1.04
bd	1.74	1.41	0.08	1.23	0.22	−1.08	4.56	0.11	0.17	0.08	0.99	1.00
ti	0.01	0.001	0.79	12.10	0.00	0.01	0.01	0.78	0.86	0.77	0.95	1.05
te	−0.63	0.47	−0.08	−1.34	0.18	−1.58	0.31	0.10	−0.18	−0.08	0.95	1.05
cCd	−0.21	0.03	−0.42	−6.50	0.00	−0.28	−0.15	−0.43	−0.67	−0.41	0.96	1.04

multicollinearity. When its value is smaller than 0.1, there is a possibility of multicollinearity, which is not the case for the two models.

Based on the unstandardized coefficients, the models for the considered process are given by Eqs. (7) and (8):

$$R\%\ (B.\ megaterium) = 109.260 - 2.566 * \mathrm{pH} + 0.372 * bd + 0.02 * ti - 0.170 * te - 0.325 * cCd \quad (7)$$

$$R\%\ (Rhodotorula\ \mathrm{sp.}) = 29.128 + 2.297 * \mathrm{pH} + 1.742 * bd + 0.012 * ti - 0.633 * te - 0.215 * cCd \quad (8)$$

3.6.4 Analysis of variance

The analysis of variance (ANOVA) was used as a statistical technique to determine the variation among and between groups. This analysis can be applied for comparing three or more groups or variables for statistical significance [19, 40].

The ANOVA Table 7 indicates how well the regression equation fits the data. The value of the Sig. (probability value, $p_{model} > F = 0.00$), which is lower than 0.05, shows that both models are statistically significant for the prediction of the outcome variable. Also, a high F-value (20.22 for *B. megaterium* and 39.38 for *Rhodotorula* sp.) indicates that using the models is better than guessing the mean.

The normal probability plot of the residuals (Figure 6) shows that the residuals are normally distributed, the relationship being approximatively linear, indicating that in the linear regression analysis, there is no tendency in the error term and showing that both equations perform satisfactorily.

3.7 Biosorbents characterization

3.7.1 Point of zero charge (pH_{pzc})

For biosorbents characterization, the value of point of zero charge was initially determined. This value puts

Table 7: ANOVA statistics.

Model	Sum of squares (SS)	df	Mean square (MS)	F-values	Sig.(p > F)
1					
Regression	10,225.72	5	2045.14	20.21	0.000
Residual	3135.99	31	101.16		
Total	13,361.71	36			
2					
Regression	21,004.99	5	4200.99	39.38	0.000
Residual	5440.43	51	106.67		
Total	26,445.42	56			

df, Degree of freedom; p, probability.

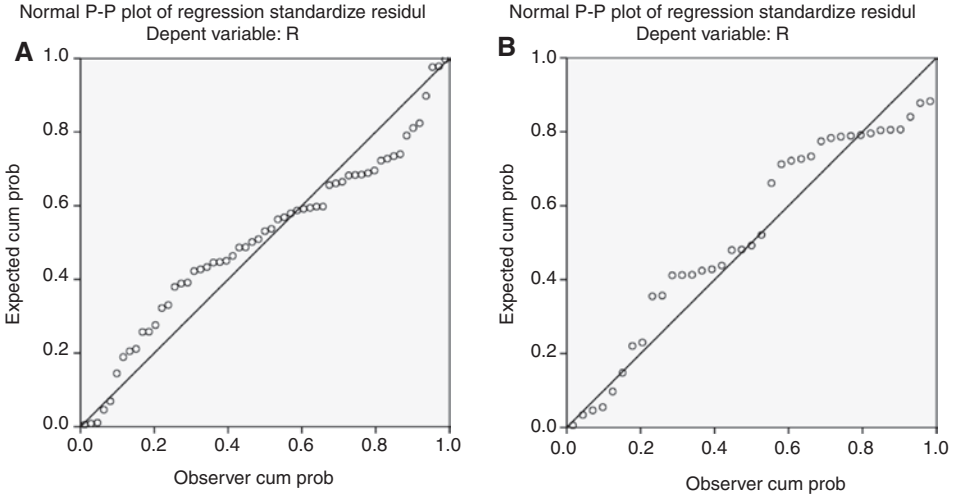

Figure 6: Normal probability plot of regression standardized residual for the dependent variable: (A) *Rhodotorula* sp. and (B) *Bacillus megaterium*.

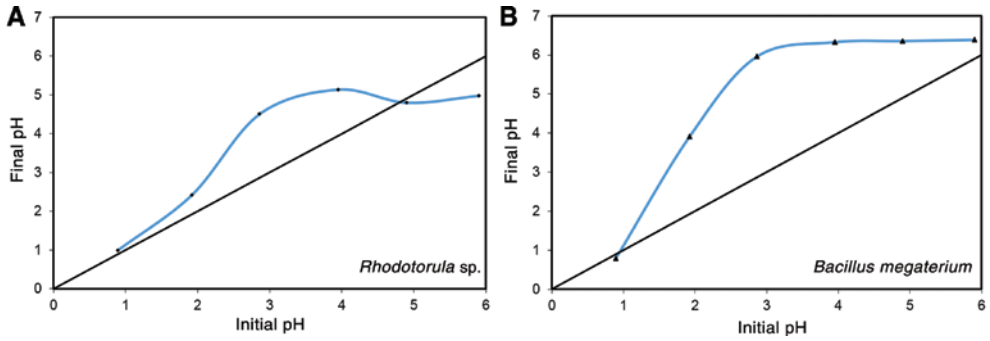

Figure 7: Representation of the pH_{pzc} of (A) *Rhodotorula* sp. and (B) *Bacillus megaterium* using the pH drift method.

into evidence the different surface functional groups with different acid and basic characteristics. This analysis gives information about the possible electrostatic interactions between biosorbents and chemical species of metal. The pH_{pzc} was determined using the pH drift method which was tested as indicated by several authors [23, 24]. The results obtained are presented in Figure 7. The black straight line is used to observe the acidic or basic character of the material surface, and represents the plot of initial pH vs. final pH, for equal values. The intersection of experimental points with the black line represents the point of zero charge (pH_{pzc}). If the experimental points are above the black line, the surface of the biosorbent is negatively charged, while if the points are under the black line, the surface of biosorbent is positively charged. Based on the results obtained, it can be concluded that the surface of the studied biosorbents is negatively charged (pH_{pzc} for *Rhodotorula* sp. is 4.8 and for *B. megaterium* is 0.9) and during the biosorption process, the biosorbent surface attracted the metal ions with positive charges [23, 31].

3.7.2 FTIR spectroscopy

The FTIR spectroscopy provides information about the functional groups present on the surface of the biosorbents. According to Wang and Chen [41], the functional groups related to the biosorption are: hydroxyl, carboxyl, amino, ester, sulfhydryl, carbonyl and phosphate. Also, Michalak et al. [5] and Chojnacka et al. [42] mentioned that the participation in metal ion binding of these functional groups depends on the initial pH values: for values between 2 and 5 carboxyl groups are participating, for pH = 5–9, carboxyl and phosphate groups are involved in the process, and for pH between 9 and 12, the carboxyl, phosphate and hydroxyl (or amine) groups. For *Rhodotorula* sp. biomass, the FTIR spectroscopy (Figure 8A) indicates around 3425 cm^{-1} and 3394 cm^{-1}, the existence of O-H and amine groups. The carboxylic groups are observed at 2923.87 cm^{-1}, 1739.66 cm^{-1} and 1747.38 cm^{-1}, and the absorption from 2854.44 cm^{-1} can be attributed to the stretching vibration of the C-H group. A vibration at 2360.70 cm^{-1} can be attributed to phosphine groups and the vibrations at

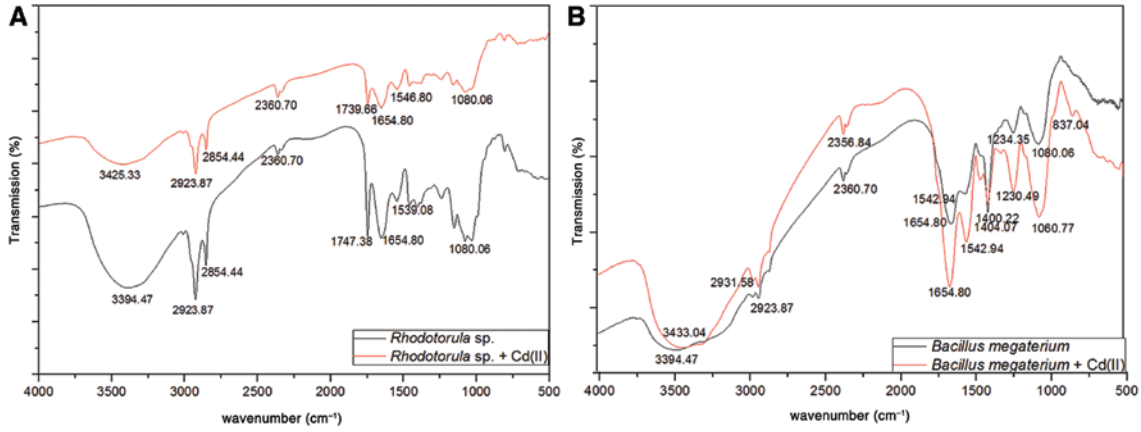

Figure 8: Fourier transform infrared (FTIR) analysis for (A) *Rhodotorula* sp. and (B) *Bacillus megaterium* before and after biosorption.

1654.80 cm^{-1}, 1546.80 cm^{-1} and 1539.68 cm^{-1} are specific for amides. The C-O-C polysaccharides groups are indicated around of 1080 cm^{-1} [43, 44]. The FTIR analysis of the *B. megaterium* before and after Cd(II) biosorption (Figure 8B) showed that on the surface of the biosorbents are present the following functional groups: hydroxyl group or amine (3394.47 cm^{-1} and 3433.09 cm^{-1}), carboxylic (peaks at 2923.87 cm^{-1}, 2931.58 cm^{-1}, 1404.07 cm^{-1} and 1400.22 cm^{-1}), amide (1654.80 cm^{-1} and 1542.94 cm^{-1}), phosphoryl (1234.35 cm^{-1} and 1230.49 cm^{-1}), phosphines (peaks at 2356.84 cm^{-1} and 2360.70 cm^{-1}), CH primarily from lipids (2854.44 cm^{-1}) and C-O-C polysaccharides (1060.77 cm^{-1} and 1080.06 cm^{-1}) [42, 44, 45].

3.7.3 SEM-EDX

To determine the textural characteristics of the *Rhodotorula* sp. and *B. megaterium* before and after the Cd(II) biosorption process, a morphological analysis of the biosorbents was performed. SEM images of the biosorbents before and after metal uptake at 3000× magnification and tension of 5 kV are shown in Figure 9. The comparison of SEM images of the biosorbents shows that during the biosorption process of Cd(II), the cell-surface morphology of the biosorbents suffers significant changes. The *Rhodotorula* sp. cells before exposure were smooth, round and had certain dimensions, but after cadmium ions biosorption, the cells were destroyed, lost their shape and became flat. In the case of *B. megaterium* cells after biosorption, the form was approximately the same but the cells length was shorter. These changes were probably caused by precipitated cadmium ions around the cell surface, linked with their functional groups and the ion exchange process between biosorbents and Cd(II) ions [46, 47]. Similar changes of the biosorbent structure were observed after cadmium removal by green algae, *Ulva lactuca*, as reported by Ghoneim et al. [46].

The EDX analysis was performed in the same time with SEM analysis for the biosorbents after biosorption (Figure 10). This analysis indicates the presence of cadmium ions and provides information about the concentration and distribution of elements in sorbents biomass. The proportions of principal elements that are in the composition of *Rhodotorula* sp. after biosorption are: C (65.8%), O (31.8%), P (1.3%), Cd (0.4%), K (0.4%), S (0.3%) and Mg (0.1%). In the case of *B. megaterium*, the composition includes C (68.3%), O (25%), P (2.5%), Cd (2.5%), Ca (0.3%), K (0.3%), Na (0.3%) and Mg (0.1%).

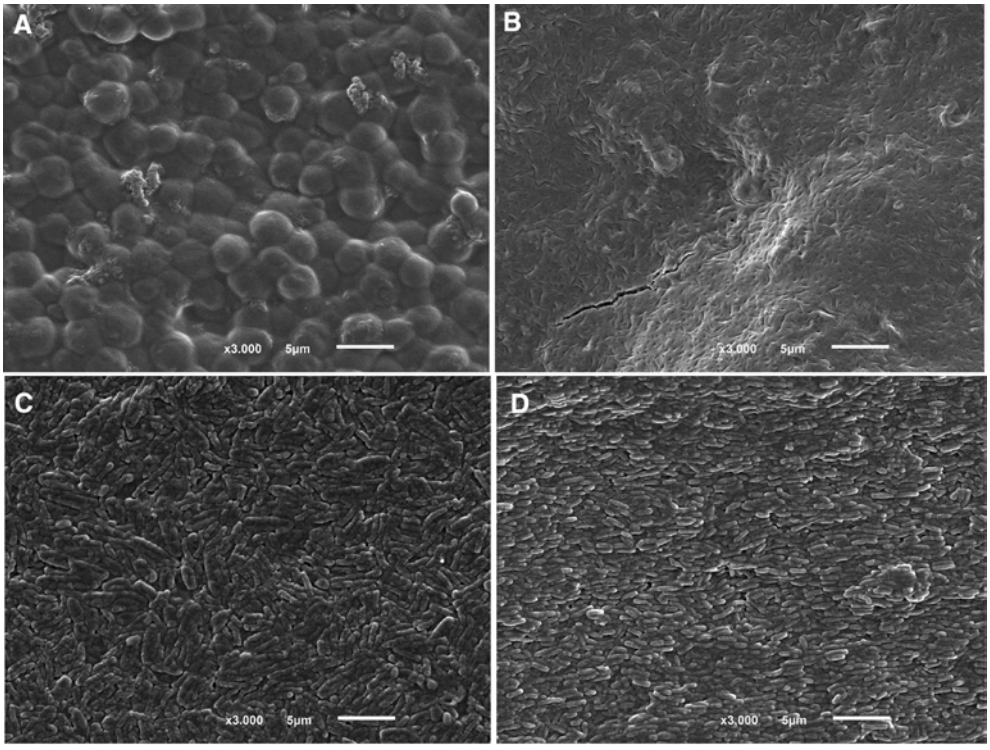

Figure 9: Scanning electron microscopy (SEM) analysis of biosorbents before and after biosorption of Cd(II) ions: *Rhodotorula* sp. (A) before, (B) after; *Bacillus megaterium* (C) before, (D) after.

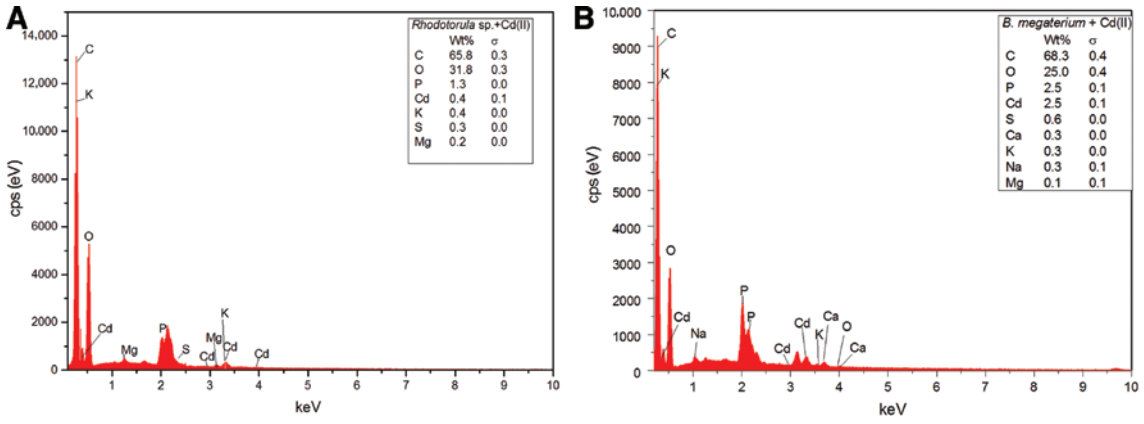

Figure 10: Energy dispersive X-ray microanalysis (EDX) analysis after biosorption of Cd(II) ions by: (A) *Rhodotorula* sp. and (B) *Bacillus megaterium*.

4 Conclusions

The present study clearly demonstrated the applicability of *Rhodotorula* sp. and *B. megaterium* as effective biosorbents for Cd(II) removal from aqueous solutions. Experiments were performed over a wide range of operating conditions [e.g. pH, biosorbent dosage, contact time, temperature and initial Cd(II) concentration]. As expected, the removal efficiency is significantly influenced by the operating parameters. The highest removal efficiency and uptake capacity of *Rhodotorula* sp. biomass for Cd(II) was achieved at 30°C, pH 6 and 5 g/l biosorbent dosage after 48 h of contact time (85% and 14.2 mg/g). Considering *B. megaterium*, the highest values were obtained at 35°C, pH 4 and 3 g/l biosorbent dosage after 20 min of contact time (90% and 15.1 mg/g). These optimal results were achieved at an initial concentration of 50 mg/l Cd(II) in both cases.

Based on high correlation coefficients found for the pseudo-second order kinetic model ($R^2 > 0.99$), there is strong evidence that Cd(II) biosorption by *Rhodotorula* sp. and *B. megaterium* is based on a chemical reaction. This is also confirmed by the applicability of the Langmuir isotherm which indicates that there is a monolayer of metal ions on the surface of the biosorbent. The maximum uptake capacities resulted from Langmuir isotherm modeling are close to the experimental results; 11.0 mg/g for *Rhodotorula* sp. and 15.5 mg/g for *B. megaterium*. The constant related to the adsorption/desorption energy (K_L) was between 0 and 1, which demonstrates that the biosorption of the metal ion onto biosorbents is favorable. The mathematical models developed using experimental data and based on the Durbin-Watson test allowed regression equations to be obtained for the removal efficiency (the correlation coefficient being 0.765 for *B. megaterium* and 0.794 for *Rhodotorula* sp.). The ANOVA showed that the model chosen to explain the relationship between the factors and the response was correct and in very good agreement with the experimental values. Moreover, the normal probability plot of the residuals showed that both equations perform satisfactorily.

The results demonstrate that both microorganisms have similar uptakes and removal efficiencies for Cd(II) ions, when working with an optimum set of experimental conditions. This study can represent a basis for process scale-up, which is a future challenge and direction for further studies.

Acknowledgments: This work was supported by two grants of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI: PN-II-ID-PCE-2011-3-0559, Contract 265/2011 and project number PN-III-P2-2.1-PED-2016-1662, Contract 10/2017 within PNCDI III. The Portuguese team input was performed under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 Programa Operacional Regional do Norte.

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Bionotes

Mihaela Roşca

Mihaela Roşca is currently a PhD student at Gheorghe Asachi Technical University of Iasi, Romania. She holds a Master's degree in Environmental Engineering and Management (2014) and a Bachelor's degree in Environmental Engineering (2012), both from Gheorghe Asachi Technical University of Iasi. Research interests: biosorption and bioaccumulation of persistent pollutants from aqueous solutions, human health risk assessment.

Raluca-Maria Hlihor

Raluca-Maria Hlihor is currently a researcher at Gheorghe Asachi Technical University of Iasi and a lecturer at "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iasi. She holds a PhD in Chemical Engineering (2011), a Master's degree in Environmental Engineering and Management (2009) and a Bachelor's degree in Environmental Engineering (2008), all from Gheorghe Asachi Technical University of Iasi. Research interests: environmental biotechnology, human health risk assessment, life cycle assessment.

Petronela Cozma

Petronela Cozma is currently an Assistant Professor at Gheorghe Asachi Technical University of Iasi. She holds a PhD degree in Environmental Engineering (2013), a Master's degree in Environmental Engineering and Management (2008), and a Bachelor's degree in Environmental Engineering (2007), all from Gheorghe Asachi Technical University of Iasi. Research interests: chemical and biological process engineering, modeling, simulation and process design, integrated pollution prevention and control and sustainable industrial protection.

Elena Niculina Drăgoi

Elena Niculina Drăgoi is currently an Assistant Professor at Gheorghe Asachi Technical University of Iasi. She holds a PhD degree in Chemical Engineering (2013), a Master's degree in Automatic Control and Computer Engineering (2009) and a Bachelor's degree in Computer Science (2008). Research interests: biologically inspired computing (neural networks, evolutionary algorithms, and artificial immune systems) used for monitoring, modeling, and optimization of processes from chemical engineering and bioengineering.

Mariana Diaconu

Mariana Diaconu is an Associate Professor at the Department of Environmental Engineering and Management, Faculty of Chemical Engineering and Environmental Protection, Gheorghe Asachi Technical University of Iasi. Research interests: microbial bioremediation, soil microbiology, water microbiology, microbial physiology and biochemistry, phytotoxicity and ecotoxicology.

Bruna Silva

Bruna Silva is currently a Post-Doc Researcher at the Centre of Biological Engineering, Minho University, Braga, Portugal. She graduated in Biological Engineering in 2006. In 2012, she received her PhD in Chemical and Biological Engineering from Minho University. Research interests: environmental biotechnology, wastewater treatment, catalytic oxidation reactions, design of new catalytic materials for environmental sustainability and characterization of nanostructured materials.

Teresa Tavares

Teresa Tavares is an Associate Professor at the Centre of Biological Engineering, Minho University. In 1991 she received her PhD in Engineering Sciences from Minho University. Research interests: environmental biotechnology and bioengineering, pollution control technologies, wastewater treatment, bioremediation, biofilms, heavy metals removal.

Maria Gavrilescu

Maria Gavrilescu is a Professor at the Department of Environmental Engineering and Management, Gheorghe Asachi Technical University of Iasi, where she teaches courses in chemical and biological process engineering, process synthesis and analysis, integrated pollution prevention and control, and sustainable industrial production and environmental risk assessment and management. She has published numerous books and articles which include over 400 titles.