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Biosorption of Cadmium Ions by Unmodified, Microwave and Ultrasound Modified Brewery and Pure Strain Yeast Biomass

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ABSTRACT

The present study investigates the biosorption of cadmium ions from aqueous solution onto unmodified, ultrasound and microwave treated cells of *Saccharomyces cerevisiae*. FTIR analysis was conducted to characterize the biosorbent. Equilibrium and kinetic studies of unmodified RBW (residual brewery waste) cell yeast and DSM 1333 (pure strain) were conducted by considering the effect of initial cadmium ions concentration. Results showed that the ultrasound and microwave treatments decrease the heavy metal uptake compared with the unmodified biomass. Langmuir and Freundlich isotherm models on both unmodified yeast cell types were used to analyze the equilibrium data. It was found that the Langmuir isotherm and pseudo-second-order kinetics models describe better the cadmium ions removal process.

Keywords: Biosorption; Heavy Metals; *Saccharomyces cerevisiae* Cells; Isotherm; Kinetics

1. Introduction

The presence of heavy metals in environment represents a major problem due to their harmful effects over human health and ecosystem. Many of these metals such as iron, manganese, copper, zinc, cobalt are essential nutrients for living organisms, but they become toxic at high levels. Other heavy metals like cadmium and lead exert their toxic effect even at low concentrations. With the rapid development of various industries like mining, metallurgy, electroplating and metal surface treating, wastes containing metals are directly or indirectly dispersed into the environment [1,2]. Cleaning these areas is necessary because of their future use, and their ability to eliminate the environmental impact. Currently, a high number of conventional processes are studied in detail, such as chemical precipitation, ion exchange, electrochemical treatment, membrane technologies etc., with the purpose of removing heavy-metal from wastewaters. Apart from being expensive, these technologies create secondary problems with metal bearing sludge [3]. An answer for this problem is given by one of the most promising techniques for the removal of heavy metals, which is biosorption. Bio-

sorption is a simple and attractive method, economically feasible for the removal of metal ions from wastewaters. Many researchers have tried several inexpensive, easily available materials. Different sorbents have been used for the removal of heavy metal ions such as sawdust [4], zeolite [5], mushrooms [6,7], sun flower [8], algae [9], yeast [10], etc. These materials contain functional groups associated with proteins, polysaccharide, lignin and cellulose as major constituents that play an important role in the metal uptake during sorption processes [11]. An important biosorbent widely investigated for its abilities in heavy metal removal is *S. cerevisiae*. This biomass can be obtained from the brewing industry (residual brewery waste) and/or grown in laboratory conditions (pure strain). It is therefore easily available and provides high removal capacity [12,13]. Several utilization techniques of the waste beer and pure strain yeast were applied to remove heavy metals. Goksungur *et al*. [14] studied the biosorption of cadmium and leads ions by ethanol treated waste baker's yeast biomass. Results showed that the treated yeast had a high biosoption capacity than native yeast. A comparison between acid treated and untreated culture tropical white-rot basidiomycete BDT-13 (DSM

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15396) on chromium (IV) was studied by Trivedi and Patel [13]. It was concluded that the acid treated fungal biomass shows a 100% metal adsorption capacity compared to the untreated biomass with a capacity of only 26.64%. Biosorption of zinc by waste beer yeast was also investigated by Han *et al*. [15], who found that the adsorbed quantity is pH dependent. The capability of heat-treated *S. cerevisiae* was studied by Marcellino *et al*. [16]. This method demonstrated that heat-treated yeast is an efficient biomass in removal of Sb(III) at room temperature. Another form of treatment is the microwave and ultrasound irradiation. Microwaves energy is delivered directly into materials through molecular interaction with the electromagnetic field. Microwaves are electromagnetic waves with frequencies between 300 MHz and 300 GHz. In microwave heating, the transfer of electromagnetic energy to thermal energy is an energy conversion [17]. Microwaves have been applied in many research areas such as medical waste sterilization, organic decomposition and disinfection of food, heating and chemical synthesis. This may be due to the rapid, uniform and selective heating of microwave radiation. Ultrasound is a sound, with a variation of pressure or density with frequencies pitched above human hearing. Ultrasound can be classified according to the frequency level as high frequency and low power (2 - 10 MHz range) and low frequency and high power (20 - 100 kHz) ultrasound which are used for cleaning and welding. Chemical effects of ultrasound do not occur from a direct interaction with molecular species but from cavitation phenomena under formation, growth and implosive collapse of cavities in liquids where large amounts of highly localized energy are released [18,19].

The aim of this study was to investigate the biosorption potential of two types of yeast biomasses unmodified and microwaves and ultrasound modified. RBW (residual brewery waste) and DSM 1333 (pure strain) of residual beer yeast cells were used. The influence of contact time, initial cadmium concentration, microwaves irradiation intensity and ultrasound irradiation time were studied. Also, kinetics and isotherm model of unmodified biomass were considered in order to better describe the biosorption process.

2. Materials and Methods

2.1. Biosorbent

Two types of biosorbents that contain the same strain, namely *S. cerevisiae* were used for experiments. First biosorbent is a residual waste brewery yeast biomass (RBW). The brewery waste biomass was collected from CIUC brewery (Miercurea-Ciuc, Romania) after being used in fermentation processes and transported to the laboratory in airtight special containers. RBW samples

were then washed with bi-distilled water and separated by vacuum filtration, dried in a hot air oven at 80°C for 24 hours. The second biosorbent is DSM 1333 pure strain yeast (DSM) was provided from University of Pécs, Hungary, Medical School, Department of Medical Microbiology and Immunology, in the lyophilized form. The composition of growth medium was Müller-Hinton substrate (3% glucose, peptone, yeast-extra, NaCl, pH = 7). The medium was sterilized by autoclaving at a pressure of 1.5 atm and a temperature of 121°C for 20 minutes. The pure yeast culture was grown in an incubator at 30°C, 200 rpm for 48 h (New Brunswick Scientific). During the growth process, yeast cells number was controlled by measuring the absorbance of the culture. After completing the yeast production, the suspension was centrifuged at 4500 rpm for 30 minutes, and washed two times with sterile PBS (phosphate-buffer solution). Cells were then lyophilized and used in this form for all trials.

2.2. Chemicals

The stock solution, 1 g of Cadmium(II) was prepared by dissolving $Cd(NO_3)_2 \cdot 4H_2O$ salt, in distilled water. The required concentrations were obtained by diluting the stock solution to the different concentrations, 8 - 132 mg/L cadmium. For heavy metals ions biosorption we used only synthetic monocomponent solutions. All chemicals used were of analytical grade.

2.3. Microwave Assisted Irradiation

For microwave irradiation, 1 g of RBW and DSM, respectively were suspended in 6 ml distilled water. The quartz test tube with the suspended samples was inserted in the reactor where it was irradiated at 20 bar pressure, 100°C and 20 to 100 W powers. The microwave irradiated residual brewery yeast biomass (RBW-MW) and DSM (DSM-MW) samples were subsequently dried at 70°C for 8 h. Finally the dried biomass was grinded into fine powder and used for further experiments. The irradiation was made by using a CEM Discover SP microwave reactor.

2.4. Ultrasonic Assisted Irradiation

The ultrasonic irradiation was carried out with an Elmasonic S 15 equipment operating at 37 kHz (ultrasonic frequency). 1.5 g of RBW and DSM biomass was suspended in 8 ml distillated water in a vessel which was then inserted in the thermostated bath of the ultrasonic equipment. The ultrasonic energy was dissipated over samples at 1, 3, 6, 12, 24 min time intervals. The ultrasonic irradiated residual brewery yeast biomass (RBW-US) and DSM (DSM-US) samples were subsequently dried at 70°C for 8 h. Finally, the dried biomass was

grinded into fine powder and used for further experiments.

2.5. Adsorption Experiments

The adsorption experiments were performed in batch condition, contacting 0.1 g biosorbent with 50 ml aqueous solution of cadmium ions at different initial concentrations (8 - 132 mg/L), under stirring (150 rpm), room temperature ($23°C \pm 2°C$) and pH = 6.5. In order to determine the exact concentration of cadmium ions and establish the evolution of the removal process, samples were collected at different time intervals (5 - 240 min). At the end of predetermined time, samples were centrifuged at 10.000 rpm for 5 min, cadmium concentration was determined from the supernatant using an Atomic Absorption Spectrometer (SensAA Dual GBS Scientific Equipment, Australia). In order to evaluate the amount of heavy metals ions retained per unit mass of biomass, the biosorption capacity was calculated using the following equation:

$$q_e = \frac{(C_0 - C_e) \cdot V}{m} \qquad (1)$$

where q_e is the amount adsorbed at equilibrium (mg/g), C_0 is the initial cadmium ions concentration (mg/L), C_e is the equilibrium metal ions concentration (mg/L), V is the volume of the aqueous phase (L), and m is the quantity of the biosorbent (g).
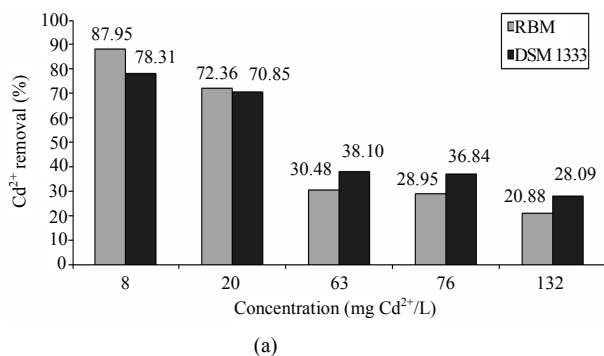
Removal efficiency, E (%), was calculated as ratio between heavy metals biosorbed at time t (mg/L) and the initial cadmium ions concentration (mg/L) [20]:

$$E\,(\%) = \frac{C_0 - C_t}{C_0} \times 100 \qquad (2)$$

3. Results and Discussions

3.1. Effect of Contact Time and Initial Cadmium Ions Concentration

The effect of contact time on cadmium biosorption proc-

ess for RBW and DSM yeast biomasses were studied. It was observed that the biosorbed quantity of cadmium ions increase as the contact time increases, and attains equilibrium in about 240 min (**Figure 1**). Following the evolution in time, the removal of cadmium ions is rapid in the first 10 minutes from the beginning of the experiment.

After that it gradually decreases until equilibrium is reached. This may be due to the large number of adsorption sites available at the beginning, while towards the end of the experiment fewer sites are available for biosorption. Hence, 240 min was chosen as the equilibrium time for each experiment.

The cadmium ions biosorption RBW and DSM yeast biomass is significantly influenced by the initial cadmium concentration in aqueous solution (**Figures 2(a)** and **(b)**). We can observe an increase in the biosorption capacity and a decrease of the removal efficiency with a concentration increase. The concentration provides an important driving force to overcome mass transfer resistance of the metal between aqueous and solid phase.

3.2. Adsorption Kinetics

Kinetics model were applied to data obtained at cadmium removal on RBW and DSM biomass. Pseudo-first- and
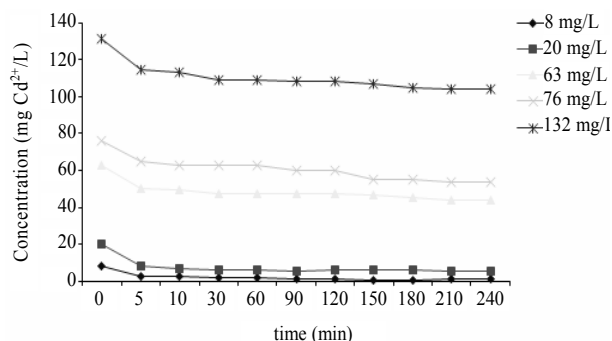

Figure 1. Effect of contact time and initial concentration on the biosorption of Cd^{2+} on RBW biomass; $23°C \pm 2°C$, pH = 6.5, 150 rpm.
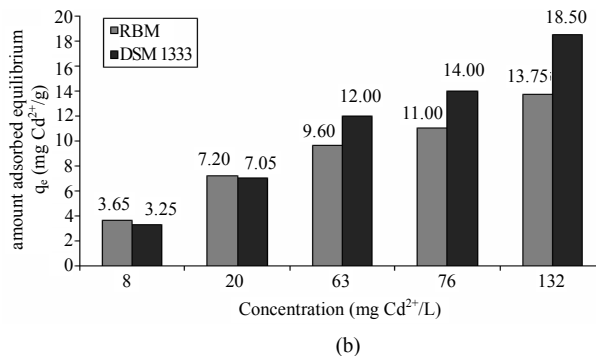

(a)

(b)

Figure 2. Influence of the initial Cd^{2+} concentration over (a) removal efficiency and (b) biosorption capacity of RBW and DSM biomass; $23°C \pm 2°C$, pH = 6.5, 150 rpm.

pseudo-second-order models were used [21].

Linear form of pseudo-first-order kinetic model (Lagergren) is given by equation:

$$\frac{dq_t}{dt} = k_2 \left(q_e - q_t\right)^2 \qquad (3)$$

where q_t and q_e are the amounts of cadmium adsorbed (mg/g) at equilibrium and time t respectively, and k_1 is the rate constant of first-order adsorption (1/min). In order to determine the rate constant and biosorption capacity for cadmium uptake, the straight line plots of $\log(q_l - q)$ against t, were made at different initial concentrations.

Linear form of pseudo-second-order kinetic model (Ho's model):

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \qquad (4)$$

where k_2 is the rate constant of pseudo-second-order ad-

sorption (g/mg min). The values q_e and k_2 are determined from the slope and intercept of the t/q_t versus t plot.

The correlation coefficients (R^2) obtained for the pseudo-first-order kinetic model were low (0.873 - 0.9688) and the calculated q_e values show great differences when compared to experimental values (**Table 1**, **Figure 3(a)**). Therefore, the considered biosorption process cannot be described by pseudo-first-order kinetic model (for neither one of the two biomass samples).

The correlation coefficients for the second-order kinetic model are greater than 0.9900 and 0.9955 for RBW and DSM, respectively. Also, the calculated q_e values are very close to the experimental ones (**Table 1**, **Figure 3(b)**). These results indicate that the cadmium ions adsorption kinetics on the two yeast biomass is better described by the pseudo-second-order kinetic model, suggesting that the considered process takes place mainly as chemisorption.

Table 1. Pseudo-first- and pseudo-second-order rate constants, calculated and experimental q_e values for cadmium ions biosorption on RBW and DSM biomass using different initial concentrations; $C_i = 8 - 132$ mg/L, $23°C \pm 2°C$, pH = 6.5, 150 rpm.

| C (mg/L)	q_e (exp) (mg/g)	Pseudo-first-order			Pseudo-second-order		
		k_1 (1/min)	q_e (calc) (mg/g)	R^2	k_2 (g/mg·min)	q_e (calc) (mg/g)	R^2
RBW							
8	3.65	3.89×10^{-2}	1.56	0.7942	4.69×10^{-2}	3.76	0.9975
20	7.2	2.48×10^{-2}	1.41	0.5747	7.48×10^{-2}	7.19	0.9993
63	9.6	1.63×10^{-2}	4.09	0.6787	0.88×10^{-2}	9.59	0.9834
75	11.00	2.82×10^{-2}	8.52	0.873	0.33×10^{-2}	11.78	0.9588
132	13.76	2.51×10^{-2}	6.83	0.827	0.68×10^{-2}	13.95	0.992
DSM							
8	3.25	3.23×10^{-2}	0.99	0.8057	8.19×10^{-2}	3.27	0.9995
20	7.05	2.63×10^{-2}	3.15	0.8644	1.60×10^{-2}	7.16	0.9955
63	12.00	3.28×10^{-2}	10.97	0.9688	0.25×10^{-2}	13.35	0.9872
75	14.00	2.87×10^{-2}	2.83	0.7334	2.64×10^{-2}	14.00	0.9994
132	18.5	2.58×10^{-2}	13.36	0.8487	0.21×10^{-2}	19.53	0.9524
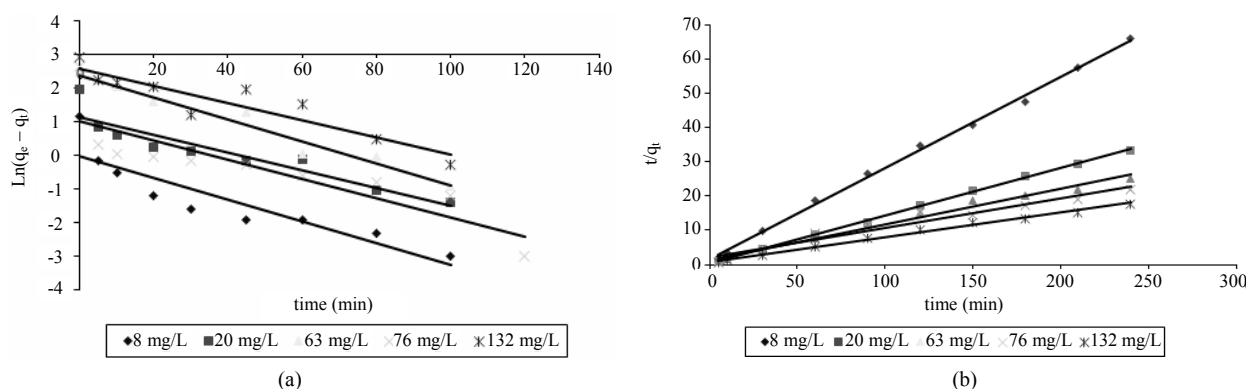
Figure 3. (a) Plots of the pseudo-first-order model for Cd^{2+} biosorption on DSM biomass; (b) Plots of the pseudo-second-order model for Cd^{2+} biosorption on RBW biomass.

3.3. Equilibrium Isotherm Models

Langmuir and Freundlich models were used to determine the adsorption equilibrium between the biosorbent and metal ions.

The Langmuir model assumes that a monomolecular layer is formed when biosorption takes place without any interaction between the adsorbed molecules [22]. Freundlich isotherm is an empirical equation based on a heterogeneous adsorption due to the diversity of adsorption sites or diverse nature of the adsorbed metal ions, free or hydrolysed species [23].

The Langmuir isotherm linear equation is expressed as follows:

$$\frac{1}{q_e} = \frac{1}{q_{max} \cdot b} \cdot \frac{1}{C_e} + \frac{1}{q_{max}} \tag{5}$$

where q_e is the solid-phase adsorbate concentration at equilibrium (mg/g), q_{max} is the maximum adsorption capacity corresponding to the monolayer adsorption capacity (mg/g), C_e is the concentration of cadmium solution at equilibrium (mg/L), and b is the adsorption equilibrium constant that is related to the apparent energy of adsorption. Isotherm parameters q_{max} and b can be obtained from $1/q_e$ versus C_e plot.

The Freundlich isotherm linear equation is expressed by the following equation:

$$\log q_e = \log k + \frac{1}{n} \cdot \log C_e \tag{6}$$

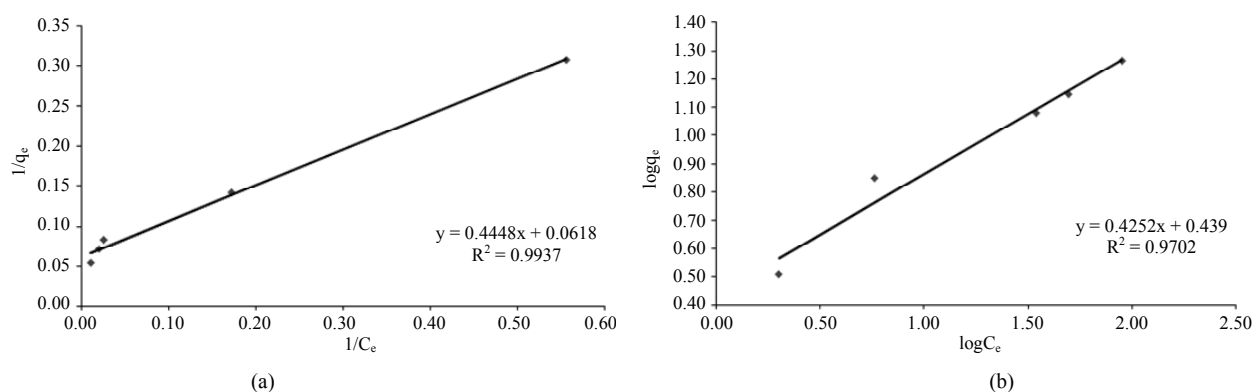where k is related to adsorption capacity, and n is related to intensity of adsorption.

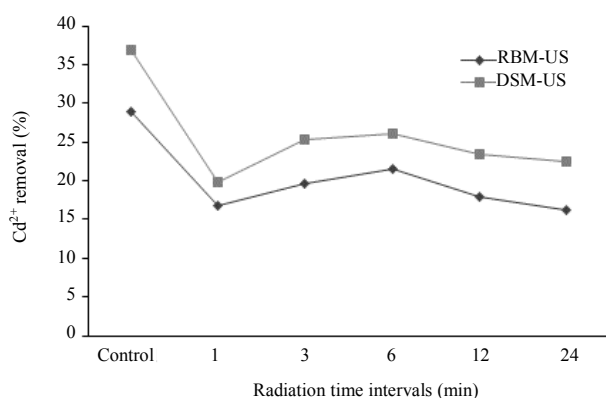The $\ln q_e$ versus $\ln C_e$ plot allows the determination of the Freundlich constants.

The linear plots of the two considered isotherm and calculated coefficients are presented in **Figures 4(a)** and **(b)**, **Table 2**. According to Langmuir isotherm, the monolayer adsorption capacity at equilibrium was 10.83 and 16.18 mg/g for RBW and DSM yeast biomass, respectively. These suggest that the considered biosorbents have high ability to retain cadmium ions from aqueous solutions. To conclude, after having been used in fermentation process, the ability of RBW biomass is smaller than that of the DSM yeast. The relatively high values of k and n Freundlich constants confirm the fact that the two types of yeast biomass have high adsorption capacity and increased adsorption intensity with respect to the considered metal ion. From the calculated regression coefficient values (R^2) can be suggested that the biosorption of cadmium ions onto RBW and DSM biomass are better described by the Langmuir model.

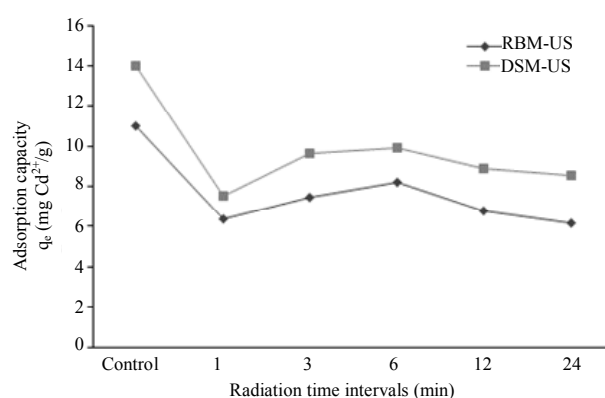## 3.4. Effect of Biomass Treatment on Cadmium Ions Uptake at Room Temperature

The yeast cell wall is composed of two main layers. The outer layer contains heavily glycosylated mannoproteins, whereas the inner layer is mainly constituted of β 1-3-glucans and chitin representing about 50% - 60% of the wall dry weight. Also, the carbohydrate side chains of the cell surface proteins contain multiple phosphodiester bridges [24,25].

| (a) | (b) |

Figure 4. (a) Langmuir plot for Cd²⁺ biosorption on DSM biomass; (b) Freundlich plot for Cd²⁺ biosorption on DSM biomass.

Table 2. Langmuir and Freundlich coefficients calculated using linear regression analysis for cadmium ions biosorption on RBW and DSM biomass; C_i = 8 - 132 mg/L, 23°C ± 2°C, pH = 6.5, 150 rpm.

	Langmuir coefficients			Freundlich coefficients		
	B (L/mg)	q_{max} (mg/g)	R^2	n	K (mg$^{(1 - 1/n)}$L$^{1/n}$/g)	R^2
RBW	0.017	10.83	0.9822	3.83	3.95	0.9604
DSM	0.027	16.18	0.9937	2.35	2.75	0.9702

The results obtained under ultrasonic irradiation are presented in **Figures 5(a)** and **(b)**. The removal capacity of cadmium ions decreases with the increase of contact time in comparison with the control sample (absence of ultrasound). At lower intensity (contact time 1, 3 and 6 min), the implosion of cavity produces moderate modifications in cells structure, therefore, a slow increase was observed in the removal of cadmium ions for both studied yeast biomass. However, a short contact time was enough to reduce the biosorption capacity compared to the control sample. By increasing the time of ultrasound irradiation (12 - 24 min), a major decrease on cadmium uptake was observed. This behavior can be explained by the cavitation processes that affects directly the damages on yeast cell wall.

By microwave irradiation of RBW and DSM biomass cell suspension, a major degradation of complex structure was observed. These cells modifications were observed in reduction of cadmium ions uptake, shown in **Figures 6(a)** and **(b)**. As the figures show, there is a constant decrease in the removal efficiency and biosorption capacity. As a result of the microwave irradiation, the number of viable cells was rapidly reduced or destroyed, decreasing the active sites number on the cell walls. These active sites play an important role in the binding of cadmium ions. Similar results were reported in literature after different chemical treatments were applied. Microwave irradiation on temperatures above 60°C reduces the number of viable cells on *E. coli* [26]. Thermal treatments onto different cell suspensions of *Aspergillus carbonarius* for 2 h at 50°C led to an important decrease of Cu^{2+} uptake [27] or Cr(VI) accumulation by *Neurospora crassa* [28].

This significant modification on cell wall structure of the two types of yeast biomass was confirmed by FTIR spectra analysis before and after ultrasound and microwave modifications (**Figures 7(a)** and **(b)**). Significant changes were observed in the microwave treated DSM,

(a)   (b)

Figure 5. (a) Ultrasound irradiation effect over removal efficiency of cadmium ions biosorption on RBW and DSM biomass; C_i = 8 - 132 mg/L, 23°C ± 2°C, pH = 6.5, 150 rpm; (b) Ultrasound irradiation effect over biosorption capacity of cadmium ions biosorption on RBW and DSM biomass; C_i = 8 - 132 mg/L, 23°C ± 2°C, pH = 6.5, 150 rpm.
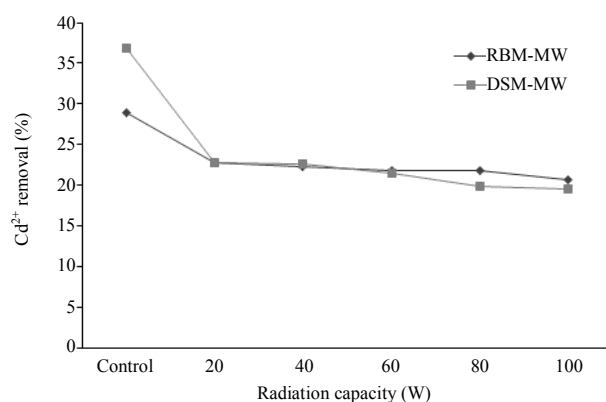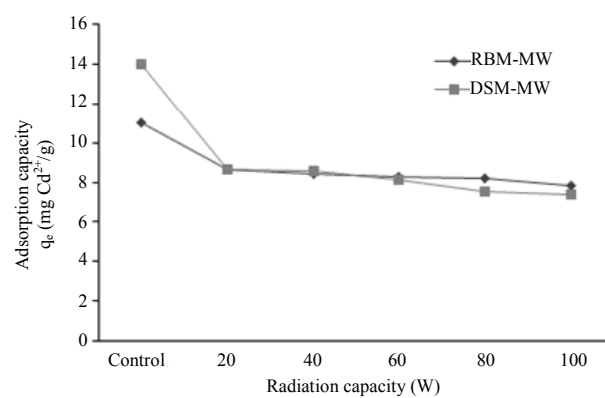
(a)   (b)

Figure 6. (a) Microwave irradiation effect over the removal efficiency of cadmium ions biosorption on RBW and DSM biomass; C_i = 8 - 132 mg/L, 23°C ± 2°C, pH = 6.5, 150 rpm; (b) Microwave irradiation effect over the biosorption capacity of cadmium ions biosorption on RBW and DSM biomass; C_i = 8 - 132 mg/L, 23°C ± 2°C, pH = 6.5, 150 rpm.
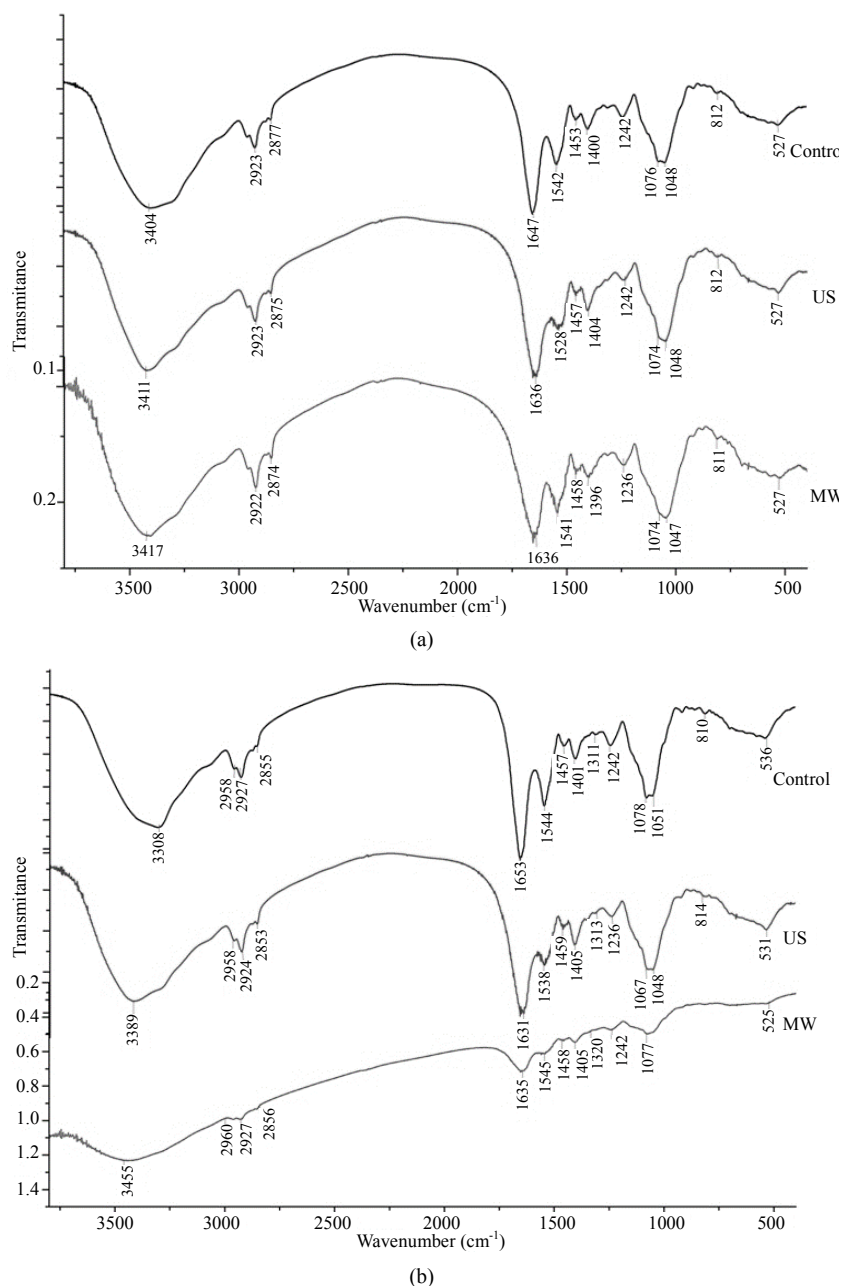
(a)

(b)

Figure 7. (a) FTIR spectra of the ultrasound and microwave modified RBW biomass; (b) FTIR spectra of the ultrasound and microwave modified DSM biomass.

where the spectra show a deformation compared to the control sample. The wide and intense bands at around 3417 - 3389 cm^{-1} showed the most significant changes due to the microwave and ultrasound treatments. These bands can be assigned to the stretching of O-H and N-H of proteins and peptides group from macromolecular association. The 2927 - 2922 cm^{-1} region includes absorption vibrations mainly assigned to lipids -CH$_3$-CH$_2$ groups. These variations can be related to the biochemical changes from the disorganization of the membrane system, especially of the plasmatic membrane [29]. The

weak band at 2877 - 2853 cm^{-1} is assigned to -CH- bond of methylene group. The major bands at 1647 - 1631 cm^{-1} correspond to the amide I (mainly C=O stretching) and amide II (C-N stretching and N-H bending) bands of proteins and peptides. Results show that proteins are partially damaged by the applied treatments. The peaks at 1545 - 1528 cm^{-1} originate from symmetric C=O stretching bands of the carboxylate ion group of terminal amino acid [30,31]. Two wide absorption bands around 1459 and 1453 cm^{-1} are characteristic to proteins and peptides and their intensity increase after treatments due to the

relative increase in lipid content, as well as to protein degradation in transmission spectra [29]. The vibrations from 1242 - 1236 cm^{-1} interval can be assigned to the free amino acids. The behavior of the wide peaks in 1078 - 1047 cm^{-1} range, corresponding to the stretch vibration of C-O in the carbohydrate region, decreased drastically over the microwave treatment on DSM biomass. Furthermore, spectra of <900 cm^{-1} region show significant changes in the degradation of the mannans and β 1-3-glucans which are widely present on yeast cell wall. These results suggest that the degradation of these essential groups following the microwave and ultrasound treatments had a great impact on binding cadmium ions by RBW and DSM biomass.

4. Conclusions

This study presented the results obtained at cadmium ions biosorption on unmodified and ultrasound and microwave modified *S. cerevisiae* cell yeast biomass. Two types of this yeast, RBW (residual brewery waste) and DSM 1333 (pure strain), were subjected to ultrasound and microwave treatments.

The effect of contact time and initial cadmium ions concentration in solution for the two types of unmodified biomass were studied. Following the biosorption process, a contact time of 240 min was necessary to reach equilibrium, depending on cadmium ion initial concentration. Also, high cadmium ions concentration favored the biosorption process. Maximum biosorption capacities of 13.94 and 19.53 mg/g were obtained experimentally, while 10.83 and 16.18 mg/g were obtained from Langmuir model for RBW and DSM, respectively. Equilibrium (Langmuir and Freundlich isotherm) and kinetics (pseudo-first- and pseudo-second-order) of the considered biosorption process were discussed in detail. Equilibrium was best described by the Langmuir isotherm and by the pseudo-second-order kinetics for both types of studied biomass.

Ultrasound and microwave treatments over RBW and DSM biomass showed a substantial decrease in cadmium ion binding compared to the control samples (unmodified biomass). These may be due, as FTIR spectral analysis confirms, to the destruction and damaging of the main important functional groups on the yeasts cell wall. The yeast cell wall components (lipids, proteins, mannans and different types of glucans) play an important role in the process of cadmium ions binding.

5. Acknowledgements

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