

Technical paper

# Cd(II), Zn(II) and Cu(II) Bioadsorption on Chemically Treated Waste Brewery Yeast Biomass: The Role of Functional Groups

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## Abstract

Here we study the role of functional groups from waste brewery yeast *Saccharomyces cerevisiae* cells in the bioadsorption of Cd<sup>2+</sup>, Zn<sup>2+</sup> and Cu<sup>2+</sup> ions. In order to clarify the role of these functional groups, the brewery yeast was pretreated chemically, thereby helping to determine the mechanisms responsible for binding the target metals. SEM studies were performed to examine the surface microstructure of the adsorbent in pure as well as pretreated forms. The biomass was characterized using FTIR analysis, which indicated that hydroxyl, carboxyl and amid groups are present on the biomass surface. When carboxyl groups were modified by various chemical treatments, the adsorption capacity decreased dramatically, showing that carboxyl groups play a fundamental role in the bioadsorption process. The residual metallic ion concentrations were determined using an Atomic Absorption Spectrophotometer (AAS). Pseudo-first and second-order kinetic models were used to describe the bioadsorption process.

**Keywords:** bioadsorption, heavy metals, *Saccharomyces cerevisiae* yeast cells, functional groups, chemical treatment, SEM and FTIR analysis

## 1. Introduction

The extensive use of heavy metals in industrial activities resulted in a dramatic increase of such residues in wastewaters. Various methods are available to tackle the problem of heavy metal contamination, bioadsorption being one of the most promising applications. The term bioadsorption is used to describe the ability of some microbial biomass (e.g. bacteria, algae, fungi or yeast) to sequester heavy metals from aqueous systems. The advantages of bioadsorption over conventional treatment methods include low cost, high efficiency, the possibility of regeneration of the biosorbent, and metal recovery.<sup>1</sup>

The bioadsorption mechanisms involve several processes, including ion exchange, coordination, complexation, chelation, adsorption, and microprecipitation.<sup>2,3</sup> Metal uptake by bioadsorption is reported to occur through interactions with functional groups native to the biosor-

bent cell wall.<sup>4,5</sup> In the adsorption of heavy metals, the surface chemistry of the biosorbent plays a key role since adsorption is favored by the presence of oxygen-containing functional groups which can vary different according to the nature of the biosorbent.<sup>6,7</sup> The cell walls of *Saccharomyces cerevisiae* consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind metal ions, such as carboxylate, hydroxyl, sulphate, phosphate, and amino groups. In addition to these functional binding groups, polysaccharides often have ion exchange properties.<sup>8</sup> These functional groups are essential for the adsorption of heavy metals due to their chelating attributes. Depending on the chemical activation method, partial oxidation takes place, and the biomass surface becomes rich in a variety of functional groups whose nature and concentration depend on the method of activation, chemicals used and temperature of preparation.

The pretreatment and killing of the biomass either by physical or chemical means (for instance oxidation) or cross-linking are known to improve the biosorption capacity of the biomass.<sup>9,10</sup>

Parvathi and Nagendran<sup>4,11</sup> identified the functional groups in the *Saccharomyces cerevisiae* cell wall by potentiometric titration. These were the carboxyl, phosphate and amino groups.

Different types of *S. cerevisiae* yeast cells (residual brewery waste biomass from brewing industry and pure strain growth in laboratory conditions) in different forms were used to remove heavy metals from aqueous solutions.<sup>12–16</sup> It may be noted that the *S. cerevisiae* yeast go through some kind of modifications during the fermentation process, which provides a better adsorption capacity than its native one.

The objective of this work was to enhance the bioadsorption capacity of the residual waste brewery yeast biomass to remove  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  ions by subjecting the biomass to different types of chemical pretreatments. Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) analysis were used for the extensive characterization and identification of the functional groups present on the yeast cell wall. This analysis gives valuable information concerning the role of functional groups and help to determine the mechanisms responsible for binding the target metals.

Also, kinetic models (pseudo-first and pseudo-second order) for untreated and chemically treated biomasses were taken into account to describe the bioadsorption process.

## 2. Experimental

### 2.1. Biosorbent

The biosorbent, brewery waste biomass (*S. cerevisiae*) was collected after having been used in fermentation processes from Ciuc Brewery (Miercurea-Ciuc, Romania), and transported to the laboratory in plastic containers. The yeast was then washed with bi-distilled water until the pH remained neutral, separated by vacuum filtration and dried in a hot air oven at 80 °C for 24 hours.

### 2.2. Chemicals

#### 2.2.1. Heavy Metal Solution Preparation

The stock metal solutions were prepared by dissolving  $\text{Cd}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$  and  $\text{CuSO}_4 \times 5\text{H}_2\text{O}$  of analytical grade reagent in appropriate amounts of distilled water. All stock solutions were diluted with deionized water to obtain the necessary concentration. The concentration of metal ions in the supernatant fluids was determined using a flame atomic absorption spectrophotometer (SensAA Dual GBS Scientific Equipment, Australia).

#### 2.2.2. Chemical Treatment of the Biosorbent

Two grams of waste brewery yeast biomass were pretreated in different ways as described below:

- 20 mL formaldehyde + 40 mL formic acid were agitated on rotary shaker at 150 rpm for 5 hours, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night, obtaining the amine-methylated biosorbent.
- 65 mL ethanol + 0.6 mL hydrogen chloride were agitated on rotary shaker at 150 rpm for 5 hours, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night, obtaining the carboxyl-esterified biosorbent.
- 50 mL of 40% NaOH solution was boiled for 15 min, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night.
- 10 mL of 10%  $\text{H}_3\text{PO}_4$  solution was boiled for 15 min, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night.
- 10 mL of 10%  $\text{H}_2\text{O}_2$  solution was boiled for 15 min, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night.
- 10 mL of 0.5% commercial laundry detergent solution was boiled for 15 min, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night.
- 75 mL benzene was heated under reflux conditions for 6 hours, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night, obtaining the lipide-extracted biosorbent.
- 10 mL of 2% glutaraldehyde glutaraldehyde solution was boiled for 15 min, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night.
- 50 mL of 1%  $\text{CaCl}_2$  solution was autoclaved for 30 min, centrifuged at 5000 rpm and dried in hot air oven at 70 °C for one night.

After each pretreatment with chemicals the biomasses were washed with generous amounts of deionized water. The NaOH pretreated biomass was washed with deionized water until the pH of the solution was in a near neutral range (pH 4.6–7.2). The dried sample was then ground, using a blender and sieved to pass through a 100  $\mu\text{m}$  mesh size to obtain uniform particle size.

### 2.3. SEM Analysis

Scanning electron microscopy was utilized for characterizing surface microstructures, porosity and fundamental physical properties of the adsorbent. The surface morphology of waste brewery yeast was determined using a scanning electron microscope of the type JEOL, JSM 5510 LV (USA).

### 2.4. FTIR Spectral Analysis

Untreated and pretreated biomass samples were subjected to FTIR analysis. The samples were prepared by

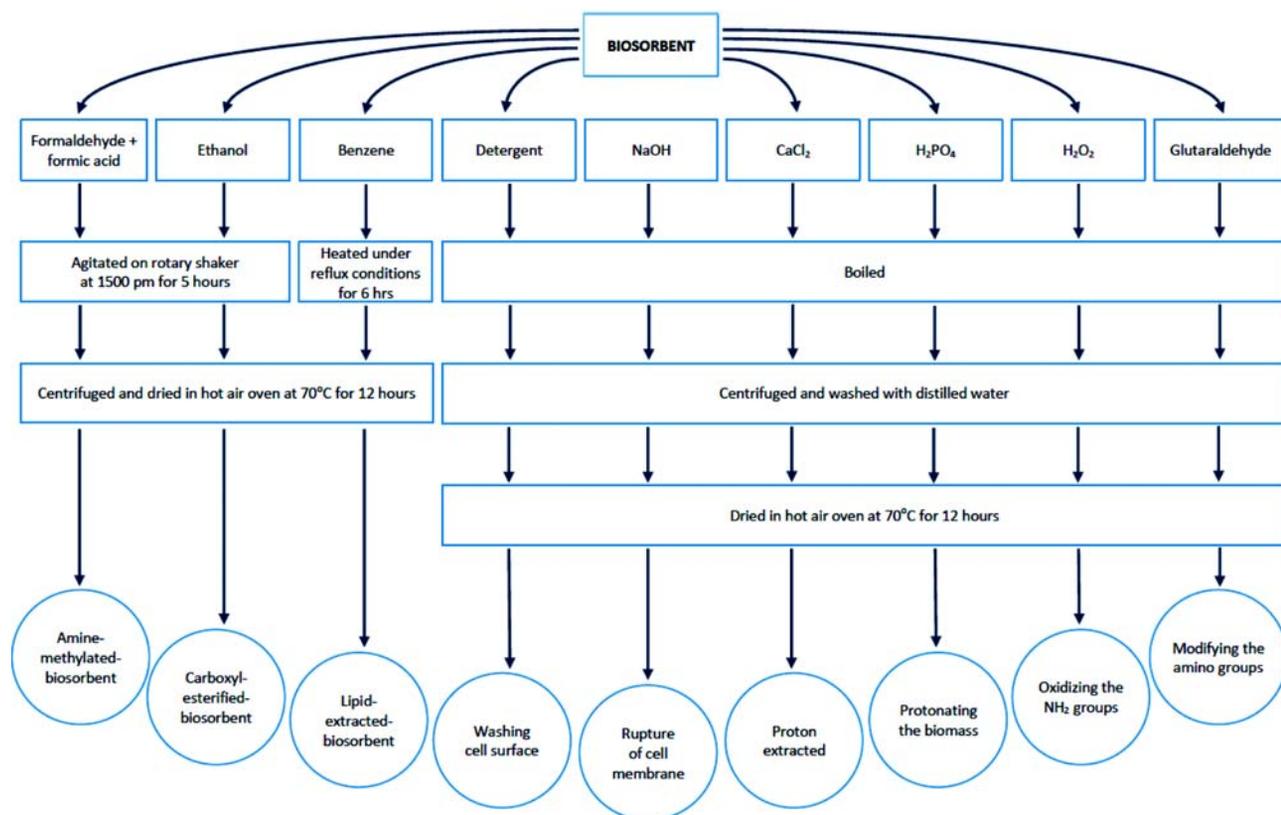


Fig. 1. Schematic representation of the chemical treatments performed on waste brewery yeast.

encapsulating 1.2 mg of finely ground biomass particles in 300 mg of KBr. Infrared spectra were obtained using a JASCO 615 (Japan) FTIR spectrometer, wavelength range 500–4000  $\text{cm}^{-1}$ , resolution 2  $\text{cm}^{-1}$ .

## 2. 5. Metal Bioadsorption Studies

The batch equilibrium method was used to determine the sorption of heavy metals by the tested yeasts. The initial heavy metal concentration was 45 mg  $\text{Cd}^{2+}/\text{L}$ , 50 mg  $\text{Zn}^{2+}/\text{L}$  and 40 mg  $\text{Cu}^{2+}/\text{L}$ , respectively. All bioadsorption experiments were carried out in 250 ml Erlenmeyer flasks containing 0.1 g dried biosorbent in 50 ml of the tested metal ion solution. This suspension was agitated for 4 hours at room temperature on a rotary shaker at 150 rpm. The biomass was separated by centrifugation at 12000 rpm for 5 min and the residual metal ion concentration was measured in the supernatant. In order to determine the exact concentration of metal ions and to establish the evolution of the removal process, samples of 100  $\mu\text{L}$  were collected at different time intervals for up to 240 min.

The amount of the adsorbed heavy metals (at equilibrium) was calculate using the following equation of Volesky and May-Phillips:<sup>17,18</sup>

$$q_e = \frac{(C_0 - C_t) \cdot V}{m} \cdot 1000 \quad (1)$$

where  $q_e$  adsorption capacity at equilibrium (mg/g),  
 $C_0$  is the initial heavy metal concentration (mg/L),  
 $C_t$  heavy metal concentration, at time  $t$  (mg/L),  
 $V = 50$  ml, and  
 $m$  is the quantity of the adsorbent (g).

Experimental data were used to determine the effect of chemical treatments (mechanism of adsorption) over the brewery waste on metal ions bioadsorption and the adsorption capacity under/after adsorption experiments, to establish the equilibrium time, to describe the kinetic models, and to confirm the groups responsible for the metal bioadsorption by FTIR analysis. All experiments were repeated three times, the values presented were calculated using averaged concentration values.

## 3. Results and Discussion

### 3. 1. Characteristics of Adsorbing Material

#### 3. 1. 1. Biomass Characterization

The cell wall of *Saccharomyces cerevisiae* has an elastic structure that provides osmotic and physical protection and determines the shape of the cell. The inner la-

yer of the wall is largely responsible for the mechanical strength of the wall and it also provides the attachment sites for the proteins that form the outer layer of the wall.<sup>19,20</sup> In brewing yeast the electron-transparent inner layer may be as thick as 200 nm.<sup>21</sup>

### 3. 1. 2. SEM Analysis

Scanning electron microscopy was used to examine the surface morphologies of the untreated waste brewery yeast biomass (control) pretreated with NaOH and after Cd<sup>2+</sup> bioadsorption.

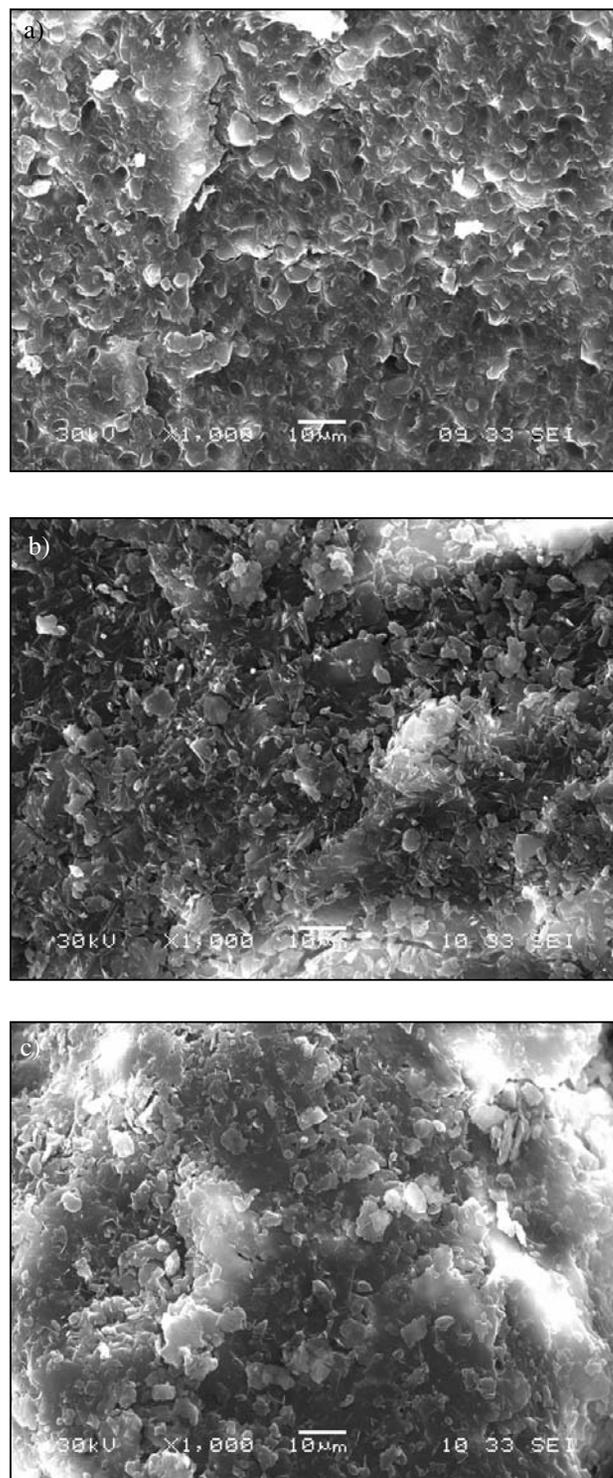
The SEM micrograph shows progressive changes on the surface of the biomass. Comparing Fig. 2a and 2c makes clear that the untreated biosorbent has some cavities in its structure. However, the surface of the biomass is flattened and the cavities disappeared, which is due to metal ions bioadsorption. A decrease in the pore sizes of the biomass is also observable, which may be attributed to the fact that the porous structure plays a role in the heavy metal bioadsorption process. Fig. 2b shows that the biomass surface changes obviously after NaOH pretreatment, having a tendency to form agglomerates and spikes.

### 3. 1. 3. FTIR Spectral Analysis

In order to identify the functional groups responsible for metal adsorption on cells surface FTIR analysis was carried out. The biomasses pretreated with NaOH and esterified were selected for FTIR analysis because these two specific treatments enhanced the Cd<sup>2+</sup> bioadsorption capacities. The identified main peaks for untreated waste brewery yeast biomass before and after Cd<sup>2+</sup> bioadsorption and their assignments are listed in Table 1.

The most significant change was recorded in case of the esterified biomass, where the large and intense band at 3421 cm<sup>-1</sup> and 3188 cm<sup>-1</sup> can be ascribed to the vibration of –OH groups for carbohydrates and –NH groups of proteins and peptides. Therefore, these changes are in concordance with the structural changes. The yeast treated with NaOH, it can be remarked that the shape of this spectra is similar to the untreated yeast, with the peaks situated at the same values. This means that NaOH treatment does not affect the functional groups on the cells' surface. On the contrary, as it will be shown below, this pretreatment facilitates the adsorption of metallic ions by eliminating the impurities from cell wall surface and exposing active binding sites.

In conclusion, the NaOH treatment did not affect significantly the biomass' functional groups. On the other hand, the esterification process induced more significant changes in the –OH and –COOH groups. This latter pretreatment leads to a dramatic decrease in adsorption. This indicates that –OH and –COOH groups play a significant role in the biosorption process.



**Fig. 2.** SEM micrographs of: (a) untreated waste brewery yeast biomass (control), (b) waste brewery yeast pretreated with NaOH, (c) and untreated waste brewery yeast after Cd<sup>2+</sup> bioadsorption.

Other peaks from the IR spectra show only minor modifications, the attribution of these peaks is discussed in detail below. At 2925 cm<sup>-1</sup> and 2368 cm<sup>-1</sup> two low intensity bands appear, characteristic for –CH, –CH<sub>2</sub>, –CH<sub>3</sub>

**Table 1.** FTIR characteristic peaks of untreated waste brewery yeast biomass before and after Cd<sup>2+</sup> bioadsorption.<sup>22–24</sup>

Untreated waste brewery yeast FTIR peaks (cm <sup>-1</sup> )			Assignment
Before adsorption	After adsorption	Differences	
3408	3392	16	O–H stretching / hydroxyl, carboxyl, amide groups
3170	3198	28	N–H stretching / proteins and peptides
2925	2924	1	–CH <sub>2</sub> lipids
2368	2368	–	–CH, –CH <sub>2</sub> , –CH <sub>3</sub> lipids
1653	1653	–	C=O / carboxylic acids groups
1543	1537	6	–O–H stretching / hydroxyl groups
1400	1400	–	–CH <sub>2</sub> stretching from lipids
1235	1235	–	–PO <sub>2</sub> <sup>-</sup> in DNA, RNA and phospholipids
1072	1067	5	–PO <sub>2</sub> <sup>-</sup> from DNA
1048	1047	1	Mannans
833	817	16	Mannans, glucans

lipids. Peaks at 1653 cm<sup>-1</sup> and 1543 cm<sup>-1</sup> can be assigned to elongation vibrations of groups –C=O from different proteins, respectively –N–H and –C–N vibrations of the peptide bond in different protein conformations. The band at 1400 cm<sup>-1</sup> can be attributed to the vibration of –CH<sub>2</sub> group from lipids.<sup>22</sup> The band at 1235 cm<sup>-1</sup> reflects the vibrations of the –N–H bending,<sup>23</sup> while the stretching at 1067 cm<sup>-1</sup> is attributed to phosphonates. The spectra of the region under 1000 cm<sup>-1</sup> showed changes in the degradation of the mannans and β 1-3-glucans, which are widely present on yeast cell wall.<sup>12</sup>

The peak shifts on IR spectra indicate the groups involved in the bioadsorption process. After Cd<sup>2+</sup> bioadsorption, on the large band, a shifting to higher wavelengths can be observed from 3170 cm<sup>-1</sup> to 3198 cm<sup>-1</sup>, and to lower wavelengths from 3408 cm<sup>-1</sup> to 3392 cm<sup>-1</sup>, corresponding to the hydroxyl, carboxyl and amide groups. The valence vibration of –N–H groups has shifted from 1543 cm<sup>-1</sup> to 1537 cm<sup>-1</sup>. A shift from 1067 cm<sup>-1</sup> to 1072 cm<sup>-1</sup> demonstrates the involvement of phosphate groups (mainly from RNA) also present in the biomass. Researchers reported that the modifications occurred in the range of 833 cm<sup>-1</sup> to 817 cm<sup>-1</sup> inform about the participation of mannans in the heavy metal uptake.<sup>24,22</sup>

## 3. 2. Bioadsorption Results

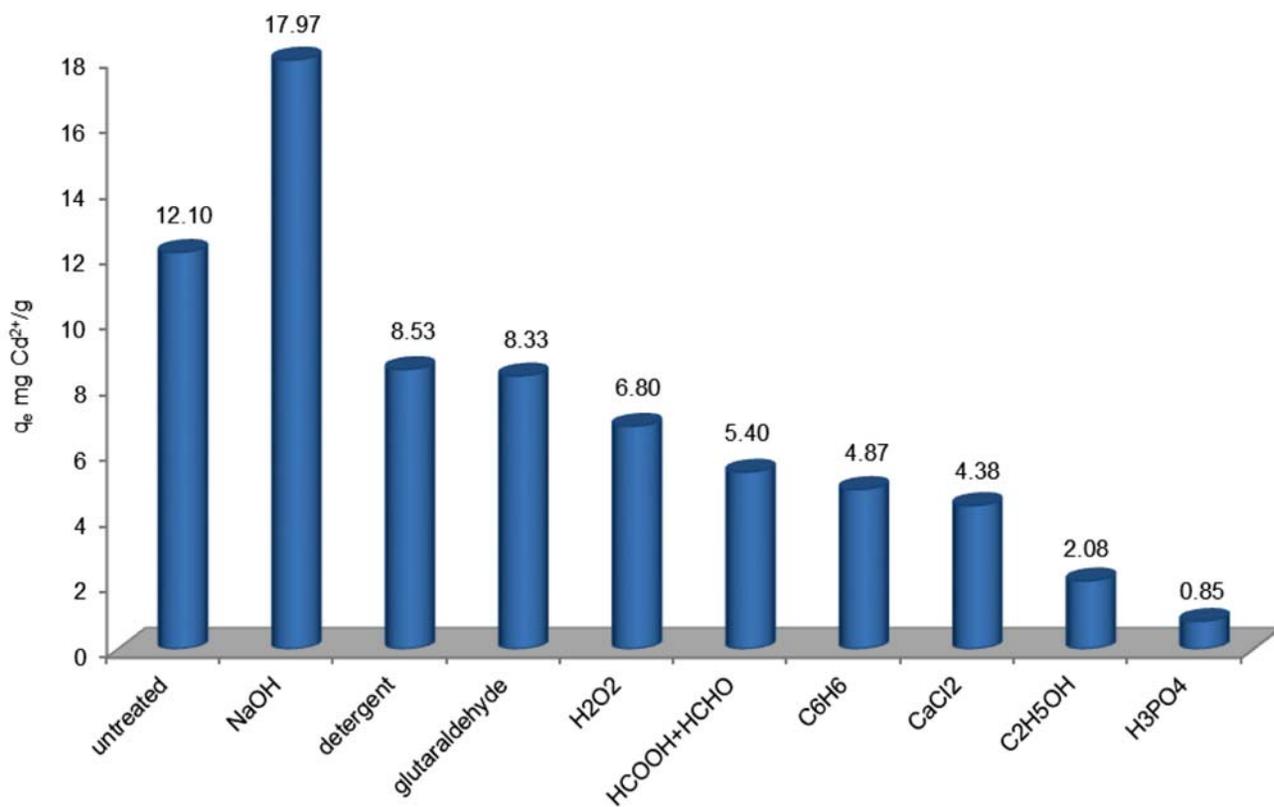
### 3. 2. 1. The Graphical Interpretation of the Metal Uptake

To investigate the effect of pretreatments on the metal uptake of waste brewery yeast biomass, the cells were treated with different chemicals (Fig. 1). It can be noted that the NaOH treated biomass enhances the metal uptake in all cases. Actually, bioadsorption capacity for Zn<sup>2+</sup> is slightly elevated in case of treatment with detergent, benzene, and formaldehyde/formic acid, while treatment with calcium chloride slightly increases bioadsorption capacity for Cu<sup>2+</sup>. When compared with the untreated biomass, the rest of the applied treatments reduce gradually the biomass' bioadsorption capacity.

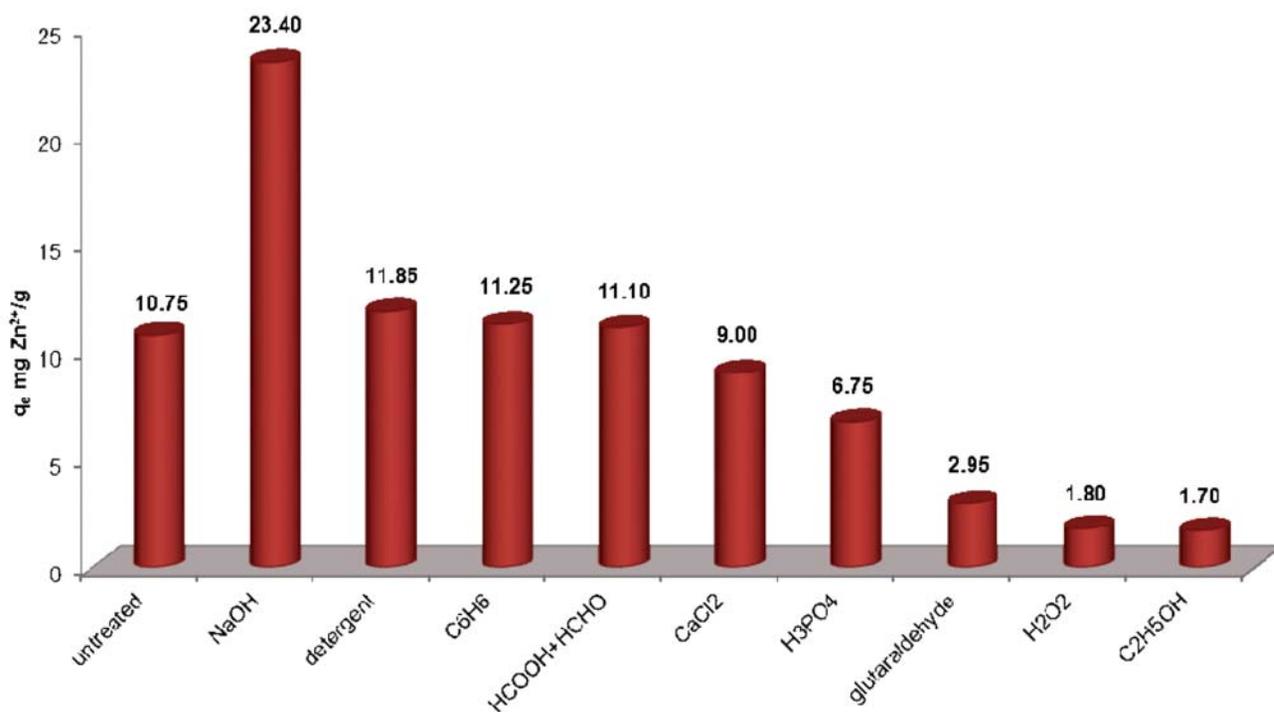
The treatment with NaOH was the only one treatment which increased cadmium adsorption in comparison with the untreated biomass, the maximum value of adsorption increasing from 12.10 to 17.97 mg/g (Fig. 3). Our results confirm that the removal of surface impurities, rupture of cell membrane and exposure of available binding sites after pretreatment may cause an increase in metal bioadsorption. Applying the NaOH treatment, hydrolysis reactions can occur, causing high dissolution of organic substances from the biomass. The hydrolysis reactions can lead to the formation of more carboxylic (–COOH), carboxylate (–COO), and alcohol (–OH), groups in the pretreated biomass, which enhances the cationic biosorption.<sup>25</sup> As Fig. 3 shows, all other treatments applied reduce the Cd<sup>2+</sup> bioadsorption capacity with values between 8.53 mg/g and 0.85 mg/g, evidencing that the functional groups have a certain role in the bioadsorption process. Previous research showed that the alkali treatment of the biomass may destroy autolytic enzymes and remove the lipids and proteins which mask the reactive sites. Besides, the pretreatment could release polymers such as polysaccharides that have a high affinity towards certain metal ions.<sup>26,27</sup>

The highest bioadsorption capacity in Zn<sup>2+</sup> removal was reached with the NaOH treatment (Fig. 4). The bioadsorption capacity of untreated waste brewery yeast biomass increased from 10.75 to 23.40 mg/g. Otherwise, the lowest value was reached in case of the ethanol treatment, when q<sub>e</sub> decreased from 10.75 to 1.70 mg/g. This occurs as a result of the modification of carboxyl groups, which have a significant role in bioadsorption mechanism.

In addition to NaOH treatment, there are three others which increase the adsorption capacity on a smaller scale – from 10.75 mg/g to respectively 11.85 mg/g (detergent), 11.25 mg/g (benzene) and 11.10 mg/g (formic acid/formaldehyde). These positive effects appear probably as a result of the fact that zinc is a metabolic element. The rest of the treatments decrease adsorption capacity, varying between 1.80 mg/g for H<sub>2</sub>O<sub>2</sub> and 9 mg/g CaCl<sub>2</sub>.



**Fig. 3.** Effect of different pretreatments on the Cd<sup>2+</sup> uptake of waste brewery yeast biomass;  $C_i = 45$  mg/L, 0.1 g/100 ml (dry mass per volume), 23 °C, pH 5.6, 150 rpm.



**Fig. 4.** Effect of different pretreatments on the Zn<sup>2+</sup> uptake of waste brewery yeast biomass;  $C_i = 50$  mg/L, 0.1 g/100 ml (dry mass per volume), 23 °C, pH 5.4, 150 rpm.

In case of  $\text{Cu}^{2+}$  (Fig. 5), two treatments enhance adsorption capacity from 17.32 mg/g to respectively 20 mg/g (NaOH) and 19.65 mg/g ( $\text{CaCl}_2$ ). Scientific literature indicates that  $\text{CaCl}_2$  treatment improves copper adsorption capacity as a result of the conversion of active binding sites from  $\text{H}^+$  to  $\text{Ca}^{2+}$ . This substitution may favor the bioadsorption of metals, due to the size of the ions; it should be easier to exchange metal for calcium than for  $\text{H}^+$ .<sup>25</sup>

The lowest adsorption capacity was found by ethanol pretreatment, with  $q_e$  decreasing from 17.32 to 9.82 mg/g. The rest of the treatments also reduced adsorption capacity.

### 3. 2. 2. Adsorption Kinetics

Kinetic studies of metal bioadsorption were developed in order to determine the minimum necessary time to achieve the sorption equilibrium and to evaluate the mechanism of bioadsorption. Two different kinetic models were applied to evaluate the bioadsorption data of  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  onto untreated and pretreated biomass. Pseudo-first (Lagergren) and pseudo-second-order (Ho and McKay) kinetic models were used to correlate the experimental data.<sup>28,29</sup>

Lagergren suggested a first-order equation for the adsorption of liquid/solid system based on solid capacity, which can be expressed as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (2)$$

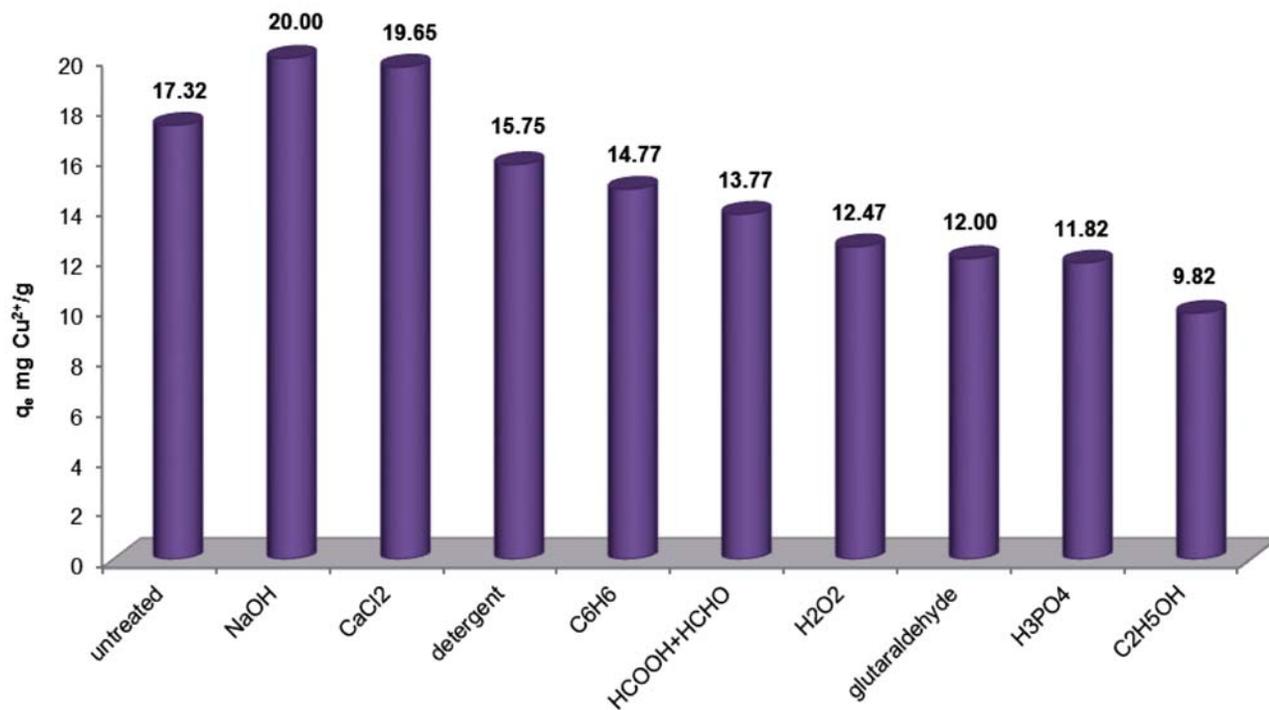


Fig. 5. Effect of different pretreatments on the  $\text{Cu}^{2+}$  uptake of waste brewery yeast biomass;  $C_i = 40$  mg/L, 0.1 g/100 ml (dry mass per volume), 23 °C, pH 4.6, 150 rpm.

Integrating eq. (2) from the boundary conditions  $t = 0$  to  $t = t$  and  $q_t = 0$  to  $q_t = q_t$ , gives:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (3)$$

where,  $q_e$  and  $q_t$  are the amounts of adsorbed metal ions on the biosorbent at time  $t$  ( $\text{mg/g}^{-1}$ ), respectively,  $k_1$  is the rate constant of first-order adsorption ( $\text{min}^{-1}$ ).

In order to determine the rate constant and equilibrium metal ions uptake, the straight line plots of  $\ln(q_e - q_t)$  against  $t$ , eq. (3) were made at different initial cadmium concentrations.

Correlation coefficients obtained for this kinetic model ranging between 0.7120 and 0.8976 led us to conclude that the considered bioadsorption process cannot be classified as taking place according to the first-order model.

Pseudo-second-order equations proposed initially by Ho and McKay are the most simplified and very frequently used kinetic equations. These equations are used to model the adsorption process for a wide range of solute-sorbent systems, including metal ions and natural sorbents, which can be expressed as follows:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (4)$$

where  $k_2$  is the rate constant of pseudo-second-order adsorption, and  $q_t$  and  $q_e$  are adsorption capacities at time  $t$  and equilibrium respectively ( $\text{mg/g}^{-1}$ ).

Separating variables in equation (4) gives:

$$\frac{dq_t}{(q_e - q_t)^2} = k_2 dt \quad (5)$$

Integrating equation (5) between  $t = 0$  and  $t = t$  gives:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \quad (6)$$

If the pseudo-second-order kinetic model is applicable, the plot of equation (6) rearranged ( $t/q_t$  versus  $t$ ) gives a straight line whose slope is equal to  $k_2$  ( $\text{mg/g}^{-1} \text{min}^{-1}$ ). The pseudo-second-order sorption kinetics was also used to fit the experiment data in order to analyze the metal ions bioadsorption on waste brewery yeast (Fig. 6). The values of  $q_e$ ,  $k_2$  and  $R^2$  were calculated and presented in Table 2. The values of the theoretical  $q_e$  for all sorbents (untreated and pretreated) were in good agreement with those obtained experimentally.

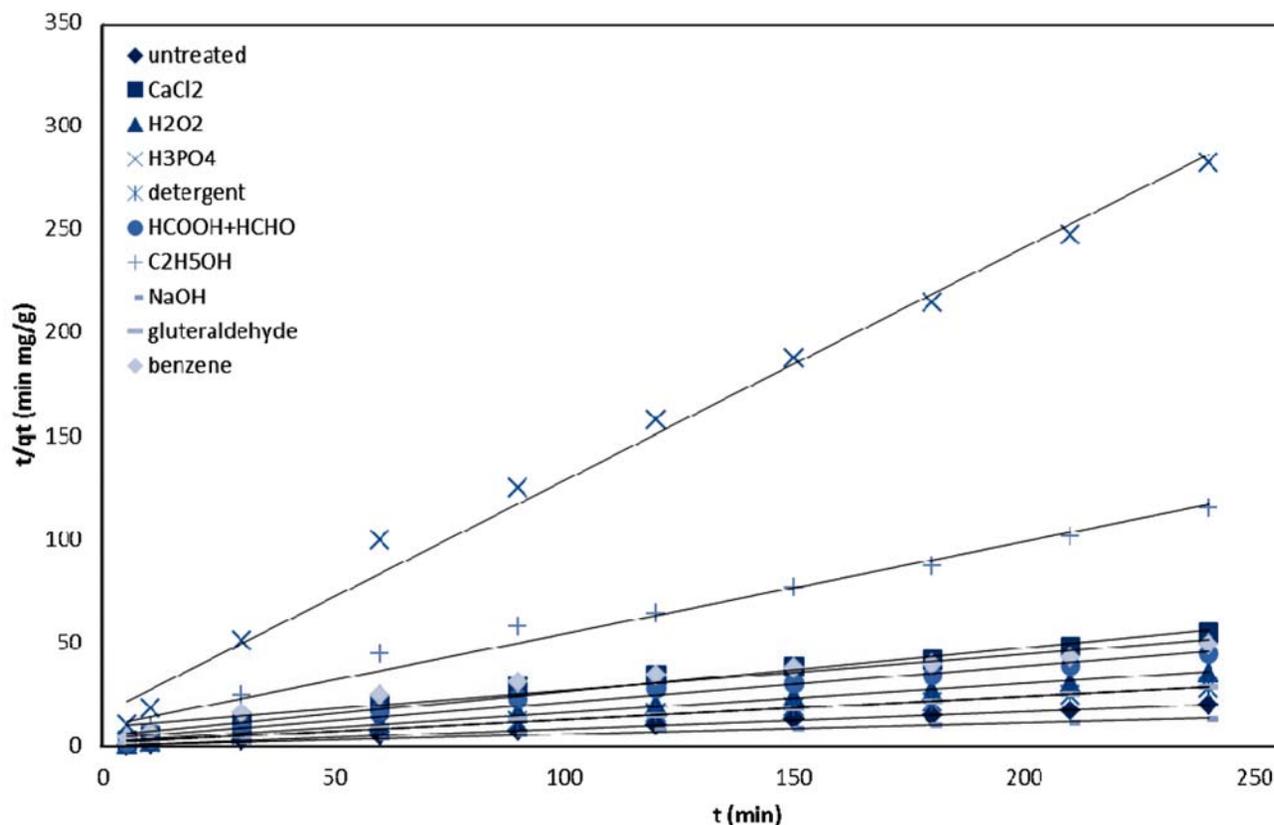


Fig. 6. Plots of the pseudo-second-order kinetic models for  $\text{Cd}^{2+}$  bioadsorption on waste brewery yeast biomass;  $C_i = 45 \text{ mg/L}$ ,  $0.1 \text{ g/100 ml}$  (dry mass per volume),  $23 \text{ }^\circ\text{C}$ ,  $\text{pH } 5.6$ ,  $150 \text{ rpm}$ .

Table 2. Kinetic parameters for  $\text{Cd}^{2+}$  bioadsorption using waste brewery yeast biomass;  $C_i = 45 \text{ mg/L}$ ,  $0.1 \text{ g/100 ml}$  (dry mass per volume),  $23 \text{ }^\circ\text{C}$ ,  $\text{pH } 5.6$ ,  $150 \text{ rpm}$ .

Biosorbent treatment	Pseudo-second-order			
	$q_{e, \text{exp}}$ (mg/g)	$q_{e, \text{calc}}$ (mg/g)	$k_2$ (g/mg min)	$R^2$
untreated	12.1	12.14	$3.04 \times 10^{-2}$	0.9983
$\text{CaCl}_2$	4.38	4.71	$8.56 \times 10^{-3}$	0.9768
$\text{H}_2\text{O}_2$	6.8	7.03	$1.06 \times 10^{-2}$	0.9915
$\text{H}_3\text{PO}_4$	0.85	0.89	$7.71 \times 10^{-2}$	0.992
detergent	8.53	8.93	$7.85 \times 10^{-3}$	0.9922
$\text{HCOOH} + \text{HCHO}$	5.4	5.73	$7.79 \times 10^{-3}$	0.9767
$\text{C}_2\text{H}_5\text{OH}$	2.08	2.25	$2.00 \times 10^{-2}$	0.9832
$\text{NaOH}$	17.97	18.42	$5.20 \times 10^{-3}$	0.993
glutaraldehyde	8.33	8.85	$7.29 \times 10^{-3}$	0.994
benzene	4.87	5.66	$3.34 \times 10^{-3}$	0.9263

**Table 3.** Results from the literature: comparison of different biomasses regarding to Cd<sup>2+</sup>, Zn<sup>2+</sup> and Cu<sup>2+</sup> removal capacity.

Metal	Sorbent material	Bioadsorption capacity (mg/g)	Reference
Cd <sup>2+</sup>	<i>Cladophora fascicularis</i> (untreated)	1	30
Cd <sup>2+</sup>	<i>Saccharomyces cerevisiae immobilized</i> (untreated)	3.78	31
Cd <sup>2+</sup>	<i>Phanerochaete chrysosporium</i> (untreated)	17	32
Cu <sup>2+</sup>	<i>Phanerochaete chrysosporium</i> (untreated)	43.3	32
Cd <sup>2+</sup>	<i>Canoal meal</i> (untreated)	0.642	33
Zn <sup>2+</sup>	<i>Canoal meal</i> (untreated)	0.062	33
Zn <sup>2+</sup>	<i>Scenedesmus quadricauda</i> (untreated)	0.595	34
Cu <sup>2+</sup>	<i>Scenedesmus quadricauda</i> (untreated)	0.765	34
Cd <sup>2+</sup>	<i>Albies alba</i> (untreated)	1.75	35
Cd <sup>2+</sup>	<i>Triticum aestivum</i> (untreated)	8.58	36
Cu <sup>2+</sup>	<i>Triticum aestivum</i> (untreated)	4.16	36
Cd <sup>2+</sup>	<i>Azolla filiculoides</i> (untreated)	86	37
Zn <sup>2+</sup>	<i>Azolla filiculoides</i> (untreated)	62	37
Cu <sup>2+</sup>	<i>Azolla filiculoides</i> (untreated)	48	37
Cd <sup>2+</sup>	<i>Pleurotus florida</i> (untreated)	3.21	38
Cd <sup>2+</sup>	<i>Pleurotus florida</i> (treated with NaOH)	9.76	38
Cd <sup>2+</sup>	<i>Mucor rouxii</i> (untreated)	6.94	26
Zn <sup>2+</sup>	<i>Mucor rouxii</i> (untreated)	4.89	26
Cd <sup>2+</sup>	<i>Mucor rouxii</i> (treated with NaOH)	9.45	26
Cd <sup>2+</sup>	<i>Mucor rouxii</i> (treated with H <sub>3</sub> PO <sub>4</sub> )	0.94	26
Cu <sup>2+</sup>	<i>Neurospora crassa</i> (untreated)	0.54	39
Cu <sup>2+</sup>	<i>Neurospora crassa</i> (caustic treated)	12.28	39
Cd <sup>2+</sup>	<i>Neurospora crassa</i> (treated with NaOH)	9.50	39
Cd <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> (treated with NaOH)	17.97	Present study
Cd <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> (treated with ethanol)	2.08	Present study
Zn <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> (treated with NaOH)	23.4	Present study
Zn <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> (treated with ethanol)	1.7	Present study
Cu <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> (treated with NaOH)	20	Present study
Cu <sup>2+</sup>	<i>Saccharomyces cerevisiae</i> (treated with ethanol)	9.82	Present study

These results indicated that the sorption process of metal ions onto biomass followed the pseudo-second-order kinetics, suggesting that the considered process takes place by chemisorption.

The adsorption capacities of previously reported biomasses for Cd<sup>2+</sup>, Zn<sup>2+</sup> and Cu<sup>2+</sup> bioadsorption are presented in Table 3. It can be observed that the waste brewery yeast *S. cerevisiae* presents good adsorption capacities compared to other low cost adsorbents.

## 4. Conclusions

The bioadsorption properties of the waste brewery yeast *Saccharomyces cerevisiae* biomass were analyzed for Cd<sup>2+</sup>, Zn<sup>2+</sup> and Cu<sup>2+</sup> removal from synthetic aqueous solutions. The biomass was pretreated in various ways in order to study the influence of functional groups on adsorption capacity in the bioadsorption process. Among the pretreatment methods, NaOH treatment was found to be superior to others in case of all metals. However, this study indicates the potential improvement of bioadsorption capacity when using some other chemical agents as well.

The bioadsorption of the metal ions studied is a rapid process and often reaches equilibrium within four hours. The maximum uptake values in case of NaOH treatment were found to be 17.97 mg Cd<sup>2+</sup>/g, 23.4 mg Zn<sup>2+</sup>/g and 20 mg Cu<sup>2+</sup>/g, respectively.

SEM, FTIR analysis and adsorption kinetic models were considered. The FTIR analysis indicated the presence of the hydroxyl and carboxyl groups on the biomass surface, all of which play an important role in the bioadsorption process. When these functional groups were modified, the adsorption capacity decreased dramatically, suggesting that the process takes place mainly by ionic exchange. As the spectra showed, the NaOH treatment did not modify the binding sites on the biomass surface. Therefore, with the digestion of proteins and lipids, the binding points were freely released. This could explain why the bioadsorption capacity disposal of waste brewery yeast (after having been used in fermentation processes) was higher compared to the native *S. cerevisiae* yeast cells. The results above indicate that cadmium, zinc and copper bioadsorption depends on the surface chemistry of the sorbents.

Based on mathematical calculations, we found that the kinetics data describing the bioadsorption process fit-

ted well the pseudo-second-order model. We also determined the parameters of this kinetic model.

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## Povzetek

Proučevali smo vlogo funkcionalnih skupin iz celic *Saccharomyces cerevisiae* odpadnega pivovarskega kvasa pri bioadsorpciji  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  in  $\text{Cu}^{2+}$  ionov. Z namenom, da bi pojasnili vlogo teh funkcionalnih skupin, smo kvas predhodno kemijsko obdelali. Na ta način smo skušali pojasniti mehanizme, preko katerih se na kvas vežejo omenjene kovine. Z uporabo vrstične elektronske mikroskopije (SEM) smo preiskali površinsko mikrostrukturo adsorbenta tako v neobdelani kot v kemijsko obdelani obliki. Biomaso smo analizirali z metodo FTIR spektroskopije, ki je pokazala, da so na površini biomase prisotne hidroksilne, karboksilne in amidne skupine. Ko smo karboksilne skupine spremenili z različnimi kemijskimi obdelavami, se je adsorpcijska kapaciteta kvasa močno znižala, kar nakazuje, da imajo karboksilne skupine pri bioadsorpcijskem procesu zelo pomembno vlogo. Koncentracijo preostanka kovinskih ionov smo določili z atomskim absorpcijskim spektrometrom (AAS). Za opis bioadsorpcijskega procesa smo uporabili kinetični model psevdo-prvega in psevdo-drugega reda.