

Article

Application of *Saccharomyces cerevisiae* in the Biosorption of Co(II), Zn(II) and Cu(II) Ions from Aqueous Media

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Abstract: Yeast biomass is considered a low-cost material that can be successfully used for the biosorption of metal ions from aqueous solution, due to its structural characteristics. This study evaluates the biosorptive performance of *Saccharomyces cerevisiae* in the biosorption of Co(II), Zn(II) and Cu(II) ions from aqueous media in batch mono-component systems. The influence of solution pH, biosorbent dose, contact time, temperature and initial metal ions concentration was examined step by step, to obtain the optimal conditions for biosorption experiments. Maximum uptake efficiency for all metal ions on this biosorbent was obtained at: pH = 5.0, 4.0 g biosorbent/L, room temperature of 23 °C, and a contact time of 60 min, and these were considered optimal. The equilibrium results were analyzed using Langmuir, Freundlich and Dubinin–Radushkevich isotherm models, while for the modeling of the kinetics data, three models (pseudo-first order, pseudo-second order and intra-particle diffusion) were used. Dubinin–Radushkevich isotherm model and the pseudo-second order model showed the best fit with the experimental data obtained at biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae*. Both maximum biosorption capacities and pseudo-second rate constants follow the order: Co(II) > Zn(II) > Cu(II), suggesting that the structural particularities of metal ions are important in the biosorption processes. Based on the obtained equilibrium and kinetic parameters, the biosorption mechanism is analyzed and the possible applications are emphasized.

Keywords: *Saccharomyces cerevisiae* biomass; biosorption; metal ions; isotherm and kinetics modeling; mechanism



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1. Introduction

Intensive agriculture and industrial development have made the management of environmental pollution caused by hazardous materials an important problem, for which solutions must be sought quickly [1]. Due to their economical and industrial importance, heavy metals have become one of the most serious problems of environmental pollution, mainly because they are persistent, non-biodegradable and can accumulate in food [2–4]. Therefore, a first step in solving the problem related to the pollution of the environment with heavy metals is to find a suitable method to allow for their efficient removal.

Until now, numerous physico-chemical methods, such as precipitation, coagulation–flocculation, ion exchange, membrane processes, electrochemical techniques, adsorption, etc., [5–8], are presented in the literature as possible variants for removing heavy metals from aqueous media. However, these techniques have some important drawbacks (such as unpredictable efficiency, high energy and reagents requirements, laborious stages of operation, high costs, generation of toxic sludge, etc.) [8–10], which have diminished their possibilities for widespread use.

Biosorption is a much more efficient method that can be used to remove many organic and inorganic pollutants, including heavy metals, mainly due to the availability of a wide variety of biosorbents that have low costs and adaptability to different experimental conditions [11–14]. Thus, different types of microorganisms, such as algae, bacteria, fungi, yeasts, etc., [15–17] have proved that they can retain heavy metals from aqueous media by biosorption, and the yield of such processes is closely related to the large number of superficial functional groups of cell walls [16,17].

Saccharomyces cerevisiae is considered a much more convenient biosorbent for the retention of metal ions compared with other types of microbial biomass. This is because it is cheap and easy to obtain in large quantities (as a by-product from fermentation industry), and the growing conditions are not sophisticated and can be easily obtained on a large scale [18,19]. In addition, the chemical composition of this yeast is well known, as well as its high stability over time [20]. All these arguments have led to *Saccharomyces cerevisiae* being considered a model microorganism that has the potential of being used as a biosorbent for the uptake of metal ions in aqueous media, and this use is biologically safe [21].

It is well known that one of the most frequent applications of *Saccharomyces cerevisiae* is in the wine industry. Studies from the literature [22–24] have shown that the fermentation of raw wine (obtained by pressing grapes) in the presence of *Saccharomyces cerevisiae* significantly increases the quality of the final product. It is also known that during the cultivation of grapes, the vine must be treated with various insecticides and fungicides to prevent the occurrence of specific diseases [25]. One of the best known products used for this purpose is Bordeaux juice, which is a mixture based on copper sulfate, but can also contain cobalt or zinc salts. Improper use of this product can cause significant amounts of these heavy metal salts to remain on the surface of grapes even after harvest. From here, there is only one step before pollution of the environment with such heavy metals from the effluents discharged from the wine industry. Even if copper, cobalt and zinc do not have a high toxic potential (compared to lead, cadmium, chromium, etc.), their presence in such effluents above the maximum permissible limit [2,26] has serious consequences on the quality of ecosystems and contributes significantly to environmental pollution. Therefore, *Saccharomyces cerevisiae* added in the fermentation process of grape can act as a biosorbent and can reduce the content of such metal ions (copper, cobalt, zinc), such that the final product is suitable for consumption.

In this research, the influence of experimental parameters (such as solution pH, biosorbent dose, temperature, contact time and metal ions concentration) on the biosorption efficiency of *Saccharomyces cerevisiae* biomass was examined, step by step. The metal ions tested in these experiments were Cu(II), Co(II) and Zn(II) ions, due to their use in vine cultivation. The isotherm equilibrium results were analyzed based on the following isotherm models: Langmuir, Freundlich and Dubinin–Radushkevich, while in the modeling of kinetic data pseudo-first order, pseudo-second order and intra-particle diffusion models were used. The results included in this study indicate the possible use of *Saccharomyces cerevisiae* as a biosorbent for the uptake of Co(II), Cu(II) and Zn(II) ions from aqueous media and open new perspectives for practical applications.

2. Materials and Methods

2.1. Materials

The reagents (copper sulfate, cobalt sulfate, zinc sulfate, NaOH, HNO₃) were obtained from Chemical Company (Iași, Romania), and were used without purifications. Distilled water was used to prepare all aqueous solutions.

The yeast *Saccharomyces cerevisiae*, used as biosorbent in this study, was obtained from Enologia Vason Company (San Pietro in Cariano, Italy). The raw yeast was dried in air at 23 ± 1 °C for 5 days (up to constant weight), homogenized, and stored in desiccators until use.

2.2. Biosorption Experiments

Batch systems were used to perform all biosorption experiments. The effect of experimental parameters: solution pH (2.0–6.5), biosorbent dose (4.0–40.0 g/L), contact time (5–180 min), temperature (10, 23 and 40 °C) and initial metal ions concentration (0.2–2.8 mmol/L) on biosorption efficiency was examined step by step, varying one of the parameters, while the others were kept constant. For each studied metal ion, 25 mL of aqueous solution was mixed with a given amount of yeast in 100 mL conical flasks. All samples were intermittently stirred for a given period of time and then filtered. After filtration, the concentration of each metal ion was measured using AAS spectrometry (AAS Spectrometer NovAA 400P, acetylene/air flame). All experiments were performed in three repetitions (standard deviation = $\pm 2.5\%$), and for the calculations of the biosorption parameters (Equations (1) and (2)), the average values were used:

$$q = \frac{(c_0 - c) \cdot V}{m} \quad (1)$$

$$R = \frac{c_0 - c}{c_0} \cdot 100 \quad (2)$$

where q is the biosorption capacity (mg/g), R is the removal percent (%), c_0 and c are the initial and equilibrium concentration of metal ions in aqueous solution (mg/L), V is the volume of solution (L), and m is the mass of yeast biosorbent used in each experiment (g).

2.3. Isotherm and Kinetics Models

Three isotherm models (Langmuir, Freundlich and Dubinin–Radushkevich (D–R)) and three kinetics models (pseudo-first order, pseudo-second order and intra-particle diffusion) were applied for the modeling of the experimental data. The mathematical equations of these models are presented in Table 1.

Table 1. Mathematical relations of isotherm models and kinetics models used for the analysis of the experimental data [27–30].

Model	Equation	Notations
Langmuir model	$\frac{1}{q} = \frac{1}{q_{\max} \cdot K_L} \cdot \frac{1}{c}$	<p>q is the equilibrium biosorption capacity; q_{\max} is the kmaximum biosorption capacity; K_L is the Langmuir constant; c is the equilibrium concentration of each metal ion; K_F is the Freundlich constant; n is the heterogeneity factor; where q_{\max}^{D-R} is the maximum amount of metal ions retained on mass unit of yeast; β is a constant related to the adsorption energy; ϵ is the Polanyi potential; E is the average biosorption energy; q_t, q_e are the biosorption capacity at time and at equilibrium; k_1 is the rate constant of pseudo-first order kinetics model; k_2 is the rate constant of pseudo-second order kinetics model; k_{diff} is the intra-particle diffusion constant; c is the concentration of each metal ion at equilibrium.</p>
Freundlich model	$\log q = \log K_F + \frac{1}{n} \cdot \log c$	
D–R model	$\ln q = \ln q_{\max}^{D-R} - \beta \cdot \epsilon^2$ $\epsilon = RT \ln(1 + 1/c)$ $E = \frac{1}{\sqrt{-2\beta}}$	
Pseudo-first order	$\log(q_e - q_t) = \log q_e - k_1 \cdot t$	
Pseudo-second order	$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e}$	
Intra-particle diffusion model	$q_t = k_{diff} \cdot t^{1/2} + c$	

The selection of the isotherm and kinetics model that best describes the experimental data was made using the regression coefficients (R^2), calculated statistically.

2.4. Characterization Methods

The compositional and structural particularities of *Saccharomyces cerevisiae* yeast, before and after metal ion retention, were emphasized by FTIR spectrometry, EDX and SEM microscopy. Before characterization, each yeast sample was dried at 23 ± 1 °C in air for 24 h and homogenized. FTIR spectra were recorded with a FTIR spectrometer (Bio-Rad model, 400–4000 cm^{-1} ; KBr pellet technique; resolution of 4 cm^{-1}) and were used to identify the superficial functional groups that are important for the biosorption of metal

ions. The morphology and elemental composition of the *Saccharomyces cerevisiae* surface was examined by energy dispersion X-ray spectroscopy (EDAX) and SEM microscopy (SEM/EDAX Hitach S3000N, 20 kV).

For the estimation of the surface area, 1 g of *Saccharomyces cerevisiae* yeast was mixed with 25 mL of distilled water and stirred for 1 h. Then, 10 g of NaCl was added and pH was adjusted at 4.0. Small volumes of 0.1 N NaOH solution were then added until the pH was raised from 4.0 to 9.0. The surface area ($S, m^2/g$) was estimated using the equation [31]:

$$S = 32V - 25 \quad (3)$$

where V is the volume of the NaOH solution used to increase the pH from 4.0 to 9.0.

3. Results and Discussion

3.1. Main Structural Characteristics of *Saccharomyces Cerevisiae*

The most important structural characteristics of *Saccharomyces cerevisiae* used as a biosorbent in this study were emphasized by recording of FTIR spectra, SEM images and EDAX mapping, and are presented in Figure 1.

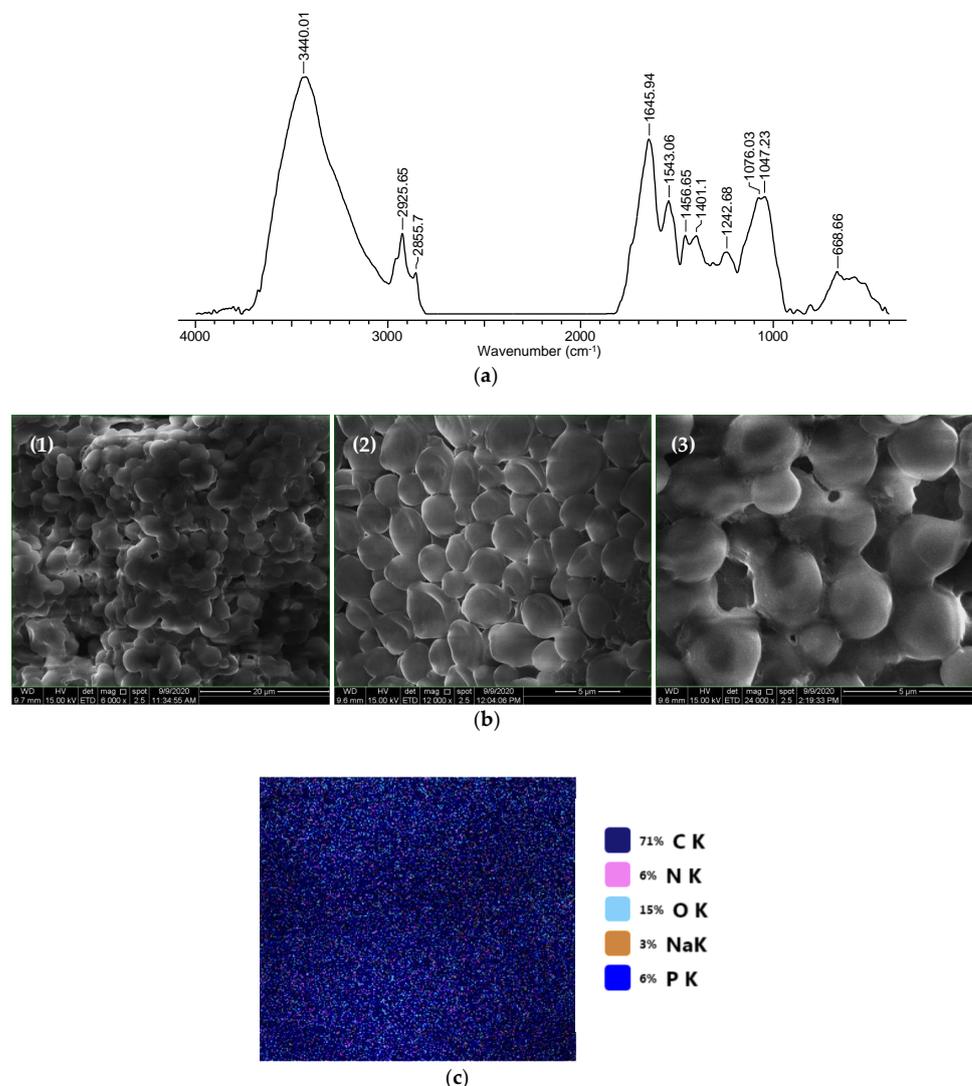


Figure 1. (a) FTIR spectra, (b) SEM image at different magnitudes ((1) 6000 \times ; (2) 12,000 \times ; (3) 24,000 \times), and (c) EDAX map of *Saccharomyces cerevisiae* biomass.

Figure 1a shows that, on the surface of *Saccharomyces cerevisiae*, there is a wide variety of functional groups, most of them with O-donor. The intense band from 3440 cm^{-1} represents the symmetric stretching vibration of a hydroxyl group from the cell wall polysaccharides. The C–H bonds from the saturated hydrocarbon radicals and saturated alcohols are indicated by the bands $2855\text{--}2925\text{ cm}^{-1}$. The band at 1645 cm^{-1} can be attributed to the C=O bond from the carbonyl and carboxyl groups. In addition, the band at 1543 cm^{-1} as well as the bands from $1401\text{--}1456\text{ cm}^{-1}$ are assigned to stretching vibration of the saturated C–O bond from the carbonyl and carboxyl compounds. The band of $1047\text{--}1076\text{ cm}^{-1}$ corresponds to the stretching vibrations of the polysaccharides skeleton (see Figure 1a). All these functional groups can be considered active sites and can determine the efficiency of the biosorption process, as they can be involved in the retention of metal ions from aqueous media. In addition, the rough surface (Figure 1b) and the relative uniform distribution of the functional groups (Figure 1c) are making the access of metal ions to the active sites easy to achieve, which favors the biosorption process. These characteristics together with the rather large surface area ($175.61\text{ m}^2/\text{g}$) make *Saccharomyces cerevisiae* a potential biosorbent for retaining heavy metals through biosorption.

3.2. Establishing the Optimal Conditions

The influence of the important experimental factors (such as solution pH, biosorbent dose, temperature and contact time) has been examined in order to establish the optimal experimental conditions for the studied biosorption processes. The experimental results are illustrated in Figure 2.

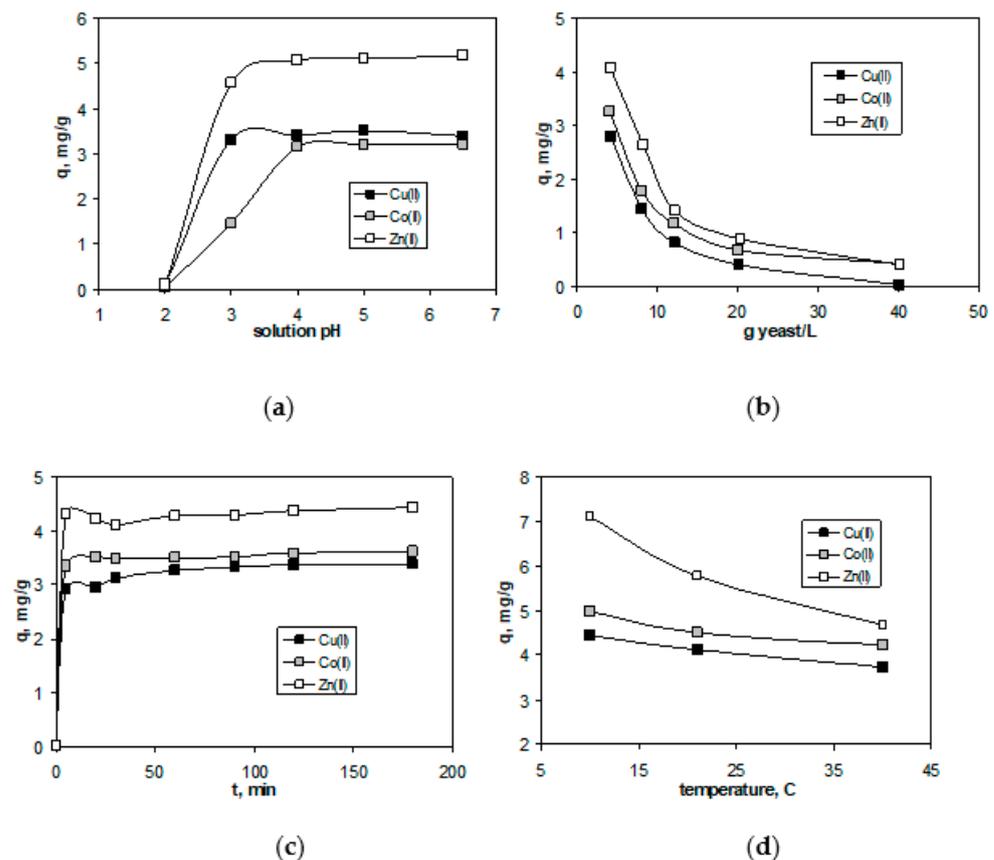


Figure 2. Influence of initial pH (a), biosorbent dose (b), contact time (c), and temperature (d) for Co(II), Zn(II) and Cu(II) ion biosorption on *Saccharomyces cerevisiae*.

It is well known that solution pH is the most important experimental factor which affects biosorption efficiency. In these experiments, the studied range of pH variation was from 2.0 to 6.5, at a constant biosorbent dose (4.0 g/L), temperature ($23\text{ }^\circ\text{C}$), contact time

(24 h) and initial concentration of metal ions (25 mg/L). The experimental results obtained for the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae* (Figure 2a) show that the biosorption capacity increases as the solution pH increases, in all cases. The maximum efficiency of biosorption (74.47% for Co(II), 81.98% for Zn(II) and 75.41% for Cu(II)), is obtained at solution pH of 5.0, and this value was used in all subsequent experimental studies, because it was considered optimal.

The increase in the biosorption efficiency with the increase in the solution pH can be explained considering the competition between metal ions and protons, for the functional groups of *Saccharomyces cerevisiae*. Thus, in acid media (pH = 2.0), protons will bind predominantly to the superficial functional groups of the yeast, because their concentration is much higher (10^{-2} mol/L) than that of metal ions (4×10^{-4} mol/L). As the pH increases (pH = 3.0–4.0), the concentration of protons decreases, the functional groups of the biosorbent remain dissociated, and electrostatic interactions with metal ions are favored. As a result, the efficiency of biosorption processes will improve. When the proton concentration is much lower than that the concentration of metal ions (pH = 5.0–6.5), they no longer influence biosorption efficiency, and the biosorption capacities remain practically constant (see Figure 2a). However, increasing the solution pH above 6.5 can lead to the precipitation of metal ions, and to avoid this secondary process, the experiments were stopped at this value.

The effect of biomass dose on the biosorption efficiency of the studied metal ions (Co(II), Zn(II) and Cu(II)) on *Saccharomyces cerevisiae* is illustrated in Figure 2b. The biosorbent dose was increased from 4.0 to 40.0 g/L, at constant pH (5.0), initial concentration of metal ions (25 mg/L), temperature (23 °C) and contact time (24 h). It can be observed from Figure 2b that the biosorption capacity has the highest value at 4.0 g/L biosorbent dosage (76.05% for Cu(II), 79.01% for Co(II) and 83.37% for Zn(II)), in all the cases. Increasing the biosorbent dose leads to a decrease in biosorption capacity, and this typical variation for biosorption processes is due to the decrease in the ratio between the number of metal ions and the number of binding sites of the biosorbent [31,32]. In addition, increasing the biosorbent dosage in the 4.0–40.0 g/L domain leads to an insignificant improvement (up to 10%) in the removal percentage of metal ions. Therefore, a yeast dose of 4.0 g/L, enough for the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae*, was considered optimal.

Figure 2c shows the variation of the biosorption parameter of *Saccharomyces cerevisiae* for Co(II), Zn(II) and Cu(II) at different values of contact time (0 and 180 min). In this case, the experiments were performed at constant pH (5.0), biosorbent dose (4.0 g/L), temperature (23 °C) and initial concentration of metal ions (25 mg/L). Initially, a rapid increase in biosorption capacities was observed (in the range of 0–30 min), which then slows down, and the equilibrium state is reached after 60 min for each metal ion. After 60 min, there was no further increase in the rate of biosorption, regardless of the nature of the metal ion (Figure 2c), probably because the entire surface of the biosorbent was covered. Increasing the values of q with increasing contact time is a consequence of the dynamic of biosorption processes [33,34]. In the initial moments (at low contact time values), when many of the superficial functional groups of *Saccharomyces cerevisiae* are unoccupied, the binding of the metal ions is performed quickly, regardless of their nature. As the functional groups of *Saccharomyces cerevisiae* are occupied (at high contact time values), it is more difficult for metal ions to find the active centers available for binding, which makes the increase in biosorption capacity much slower. The same values of the biosorption capacities almost obtained after 60 min of contact time is the first indication that the binding of Co(II), Zn(II) and Cu(II) ions is performed predominantly on the surface of *Saccharomyces cerevisiae*, through electrostatic interactions. Therefore, 60 min was considered as the optimum equilibrium time.

The effect of temperature on the biosorption efficiency of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae* is illustrated in Figure 2d. These values were obtained at constant solution pH (5.0), biosorbent dose (4.0 g/L), contact time (60 min) and initial

concentration of metal ions (25 mg/L). The increase in temperature causes a decrease in the biosorption capacity of *Saccharomyces cerevisiae* in all the cases. In addition, this decrease is much more evident in the case of Zn(II) ions than in the case of Cu(II) and Co(II) ions. Although the highest values of biosorption capacity are obtained at 10 °C (see Figure 2d), the differences between them and the values obtained at room temperature (23 °C) are below 10% (9.46% for Co(II), 8.68% for Zn(II) and 6.84% for Cu(II)). Therefore, for economic reasons, the optimal temperature value was chosen as 23 °C (room temperature), and this was used in all experiments.

The rigorous examination of the effect of these experimental parameters showed that the retention of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae* takes place with maximum efficiency when solution pH is 5.0, biosorbent dose is 4.0 g/L, and there is at least 60 min of contact time and room temperature (23 °C). Under these conditions, the removal percentage of metal ions from aqueous solution is higher than 75% (77.17% for Co(II), 82.89% for Zn(II) and 76.93% for Cu(II)), which allows us to say that *Saccharomyces cerevisiae* biomass can be used as an efficient biosorbent for retaining metal ions from aqueous effluents.

3.3. Modelling of Biosorption Processes

For the quantitative evaluation of the biosorption efficiency of Cu(II), Co(II) and Zn(II) ions on *Saccharomyces cerevisiae*, under the conditions established as optimal, it is necessary to model the isotherms and the kinetic curves obtained experimentally. The parameters calculated from the mathematical models will help to establish the utility of this biosorbent in the biosorption of metal ions from aqueous media.

3.3.1. Biosorption Isotherms Modelling

The influence of initial heavy metal concentration on the biosorption efficiency of *Saccharomyces cerevisiae*, under optimal experimental conditions, is illustrated in Figure 3. It can be seen that for each of the studied metal ions, the biosorption capacity of *Saccharomyces cerevisiae* increases with the increase in their initial concentration.

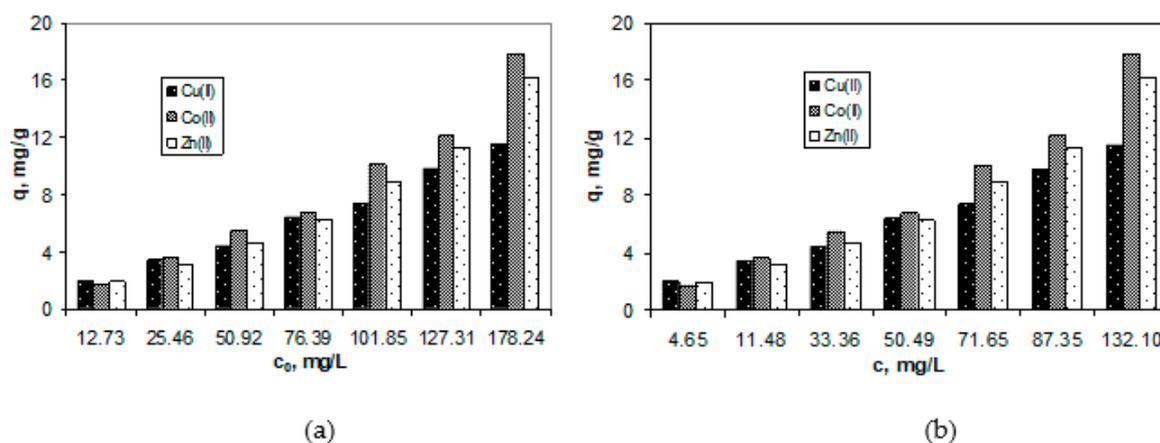
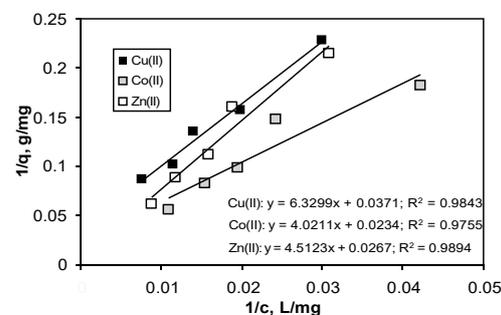


Figure 3. Effect of the initial concentration of heavy metals (a) and biosorption isotherm (b) obtained for the retention of Cu(II), Co(II) and Zn(II) on *Saccharomyces cerevisiae* biomass.

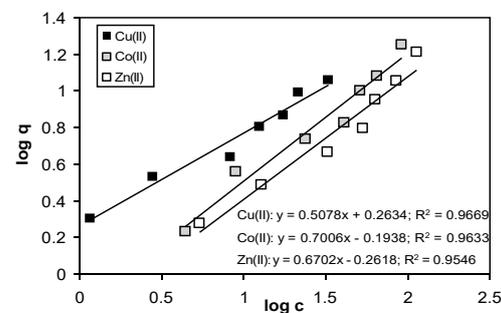
However, the detailed analysis of the variations presented in Figure 3 shows that, if at low concentrations of metal ions (below 75 mg/L), the biosorption capacity of *Saccharomyces cerevisiae* is little influenced by the nature of metal ions, at high concentrations (above 100 mg/L) there are significant differences, and the efficiency of the metal ions removal follows the order: Co(II) > Zn(II) > Cu(II). Variation of the biosorption capacity as a function on the nature of the metal ion (Figure 3) can be explained, taking into account the ratio between the number of metal ions in the aqueous solution and the number of functional groups of the biosorbent. Thus, when the concentration of metal

ions in the aqueous solution is small (low number of metal ions), their binding to the superficial functional groups of *Saccharomyces cerevisiae* is easy. Consequently, the experimental biosorption capacities have close values ($\pm 3\%$), regardless of the nature of metal ions. However, when the number of heavy metal ions is high (high concentration range), their biosorption also involves interactions with the binding centers (functional groups) inside the pores of biosorbent particles. Under these conditions, the small metal ions (as Co(II) (125 pm)) will be able to bind to the inner functional groups (compared with Zn(II) (134 pm) and Cu(II) (145 pm)) [35], contributing to the increase in the values of the biosorption capacities. For example, at the highest initial metal ion concentration (180 mg/L), the biosorption capacity of *Saccharomyces cerevisiae* for Co(II) ions is 19.8% higher than for Zn(II) ions and 32.7% higher than in the case of Cu(II) ions.

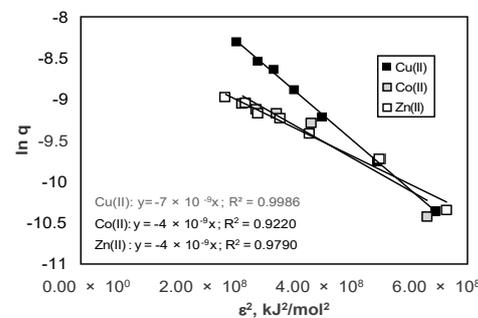
Three models were used for the mathematical modeling of experimental biosorption isotherms, namely: Langmuir, Freundlich and Dubinin–Radushkevich (D–R) models (see Section 2.3). The linear representations of each model for the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae* are illustrated in Figure 4, and the isotherm parameters are summarized in Table 2.



(a)



(b)



(c)

Figure 4. Linear representations of Langmuir (a), Freundlich (b) and D-R (c) isotherm models for the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae* biosorbent.

Table 2. Characteristic isotherm parameters obtained for the biosorption of Co(II), Zn(II) and Cu(II) on *Saccharomyces cerevisiae*.

Isotherm Parameter		Cu(II)	Co(II)	Zn(II)
Langmuir model	R ²	0.9843	0.9755	0.9894
	q _{max} , mg/g	26.95 ± 1.76	42.73 ± 2.03	37.45 ± 1.97
	K _L , L/mg	(5.9 ± 0.3)10 ⁻³	(5.8 ± 0.1)10 ⁻³	(5.9 ± 0.2)10 ⁻³
Freundlich model	R ²	0.9669	0.9633	0.9546
	n	1.97 ± 0.04	1.43 ± 0.08	1.49 ± 0.06
	K _F , L ^{1/n} /g·mg ^{1/(n-1)}	1.8341 ± 0.11	0.6401 ± 0.06	0.5473 ± 0.08
D–R model	R ²	0.9986	0.9220	0.9790
	q ^{D-R} _{max} , mg/g	19.27 ± 0.96	22.89 ± 0.91	20.38 ± 1.02
	E, kJ/mol	8.45 ± 0.51	11.18 ± 0.61	10.98 ± 0.072

Analyzing the regression coefficient values (R²), it can be seen that all three isotherm models (Langmuir, Freundlich and D–R) describe quite well the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae* (Table 2). However, the D–R model can be considered more adequate for describing the experimental data because, compared to the Langmuir model, it allows us to obtain theoretical values of the biosorption capacity (q^{D-R}_{max}, mg/g) closer to the experimental ones.

According to theoretical assumptions of the D–R model [27], during biosorption, metal ions are retained on the biosorbent surface, which is irregular and heterogeneous. Consequently, although *Saccharomyces cerevisiae* has numerous superficial functional groups (see Figure 1), they are in different geometrical planes, which means that not all of them will participate in biosorption processes. This hypothesis is supported by the following observations: (i) much higher values of q_{max} calculated from the Langmuir model (almost double) compared to the experimental ones, suggesting that a much larger amount of metal ions is needed to form the monolayer coverage (see Table 2), and (ii) relatively good correspondence of experimental data with the Freundlich model, indicating that retained metal ions are found in different geometrical planes. In addition, it should be noted that the biosorption processes are favorable, even at high metal ion concentrations, because the values of n parameter, calculated from the Freundlich isotherm model, are higher than the unit for all studied metal ions (see Table 2). Such significant differences between the maximum biosorption capacities calculated from the Langmuir model and the D–R model in the case of the use of *Saccharomyces cerevisiae* for metal ion retention have been reported in other studies from the literature [19–21]. However, the values of maximum adsorption capacity of *Saccharomyces cerevisiae* biosorbent in this study are higher (see Table 2) than the previous reported values (4.73 mg/g for Cu(II) ions [20] or 8.99 mg/g for Zn(II) ions [21]).

In addition, the Langmuir constant (K_L), which is a measure of the affinity between the metal ions and the functional groups of the biosorbent [36], has almost the same value for all studied metal ions (see Table 2). This means that the biosorption of these metal ions involve the same type of interactions, probably electrostatic, and this observation is supported by the values of average biosorption energy (E, kJ/mol), which are between 8 and 12 kJ/mol (see Table 2).

3.3.2. Biosorption Kinetics Modelling

Kinetics modeling of biosorption processes is a useful way to understand the elementary interactions between metal ions and biosorbent [37,38]. Therefore, in order to characterize the kinetics of Co(II), Zn(II) and Cu(II) ion biosorption on *Saccharomyces cerevisiae*, three models (pseudo-first order model, pseudo-second order model and intra-particle diffusion model) were used for the analysis of the experimental results.

The linear representations of each kinetics model (see Table 1) are presented in Figure 5, and the kinetic parameters, calculated for each model, are summarized in Table 3.

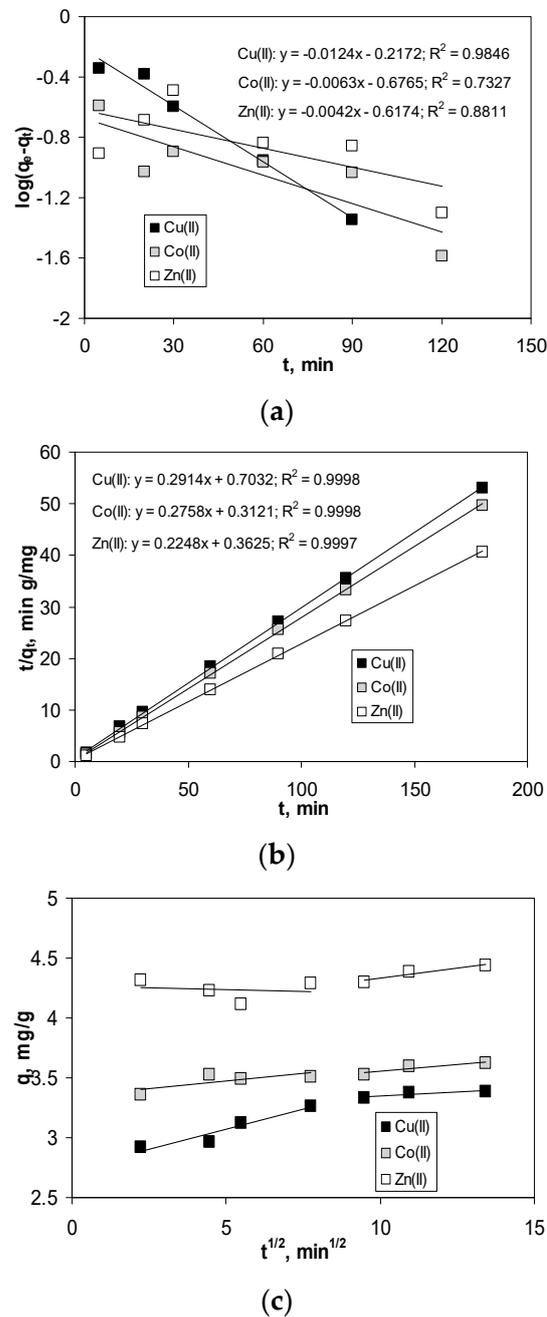


Figure 5. Linear representations of pseudo-first order model (a), pseudo-second order model (b) and intra-particle diffusion model (c) in the case of Co(II), Zn(II) and Cu(II) ions biosorption on *Saccharomyces cerevisiae*.

The high regression coefficients (R^2) and the close values of the biosorption capacities calculated and obtained experimentally (see Table 3) show that the pseudo-second order model best fits the experimental results for the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae*.

Table 3. Characteristic kinetic parameters obtained at the biosorption of Co(II), Zn(II) and Cu(II) ions on *Saccharomyces cerevisiae*.

Kinetic Parameter		Cu(II)	Co(II)	Zn(II)
$q_{e,exp}$, mg/g		3.3887 ± 0.25	3.6203 ± 0.31	4.4363 ± 0.42
Pseudo-first order	R^2	0.9846	0.7327	0.8811
	$q_{e,calc}$ mg/g	0.6065 ± 0.04	0.2106 ± 0.02	0.2413 ± 0.05
	k_1 , 1/min	$(1.24 \pm 0.21)10^{-2}$	$(6.3 \pm 0.33)10^{-2}$	$(0.42 \pm 0.07)10^{-2}$
Pseudo-second order	R^2	0.9998	0.9998	0.9997
	$q_{e,calc}$ mg/g	3.4317 ± 0.28	3.6258 ± 0.19	4.4484 ± 0.31
	k_2 , g/mg min	0.1208 ± 0.02	0.2437 ± 0.04	0.1394 ± 0.03
Intra-particle diffusion model	R^2	0.9037	0.8824	0.8358
	Zone 1			
	c , mg/L	2.7422 ± 0.31	3.3479 ± 0.69	4.2732 ± 0.78
	k_{diff}^1 , mg/g min ^{1/2}	$(6.56 \pm 0.36)10^{-2}$	$(2.52 \pm 0.11)10^{-2}$	$(2.74 \pm 0.14)10^{-2}$
	R^2	0.8526	0.7960	0.9111
	Zone 2			
c , mg/L	3.2144 ± 0.69	3.3321 ± 0.48	3.9929 ± 0.59	
k_{diff}^2 , mg/g min ^{1/2}	$(1.34 \pm 0.33)10^{-2}$	$(2.21 \pm 0.18)10^{-2}$	$(3.37 \pm 0.36)10^{-2}$	

Consequently, the retention of metal ions on this biosorbent is achieved through chemical interactions, which involve two active centers in favorable geometric position [39]. The affinity of metal ions to interact with the functional groups of *Saccharomyces cerevisiae*, described by the rate constant (k_2), shows that Co(II) ions are most easily retained (0.2437 g/mg min), followed by Zn(II) ions (0.1394 g/mg min), and then Cu(II) ions (0.1208 g/mg min) (see Table 3). A particular situation can be observed in the case of Cu(II) ions biosorption, whose kinetics can be described as well by the pseudo-first order model ($R^2 = 0.9846$). This shows that the retention of Cu(II) ions on the *Saccharomyces cerevisiae* surface can be achieved through a single active center [39]. This somewhat different behavior of Cu(II) ions is probably due to their much higher complexing capacity, compared to Co(II) and Zn(II) ions.

In order to show whether the chemical interactions between functional groups of yeast and metal ions are electrostatic or covalent, the FTIR spectra of *Saccharomyces cerevisiae* were recorded before and after the retention of Co(II), Zn(II) and Cu(II) ions (Figure 6).

Figure 6 indicates that after the retention of metal ions, in the FTIR spectrum of *Saccharomyces cerevisiae*, no new absorption bands are present, which indicates that the biosorption of studied metal ions does not involve the formation of covalent bonds. The only notable differences after metal ion biosorption are the shift of the absorption band from 3425 cm^{-1} (corresponding to O–H bonds from alcohols) to higher wave numbers ($3440\text{--}3448 \text{ cm}^{-1}$) and the small shift of the band from 1654 cm^{-1} (attributed to the C=O bond from the carbonyl and carboxyl compounds) to low wave numbers ($1639\text{--}1641 \text{ cm}^{-1}$) (see Figure 6). Therefore, the analysis of FTIR spectra illustrated in Figure 6 allows us to say that the biosorption of Co(II), Zn(II) and Cu(II) ions on the *Saccharomyces cerevisiae* surface is performed predominantly by ion exchange interactions (which are electrostatic), where the hydroxyl and carboxyl groups are predominantly involved. This explains the high rate with which biosorption processes reach equilibrium and the insignificant dependence of these parameters on the nature of the metal ions in the aqueous solution (see Figure 2c). Consequently, the elementary diffusion processes have only the role of bringing the metal ions to the surface of the biosorbent, and they do not intervene in the kinetics of the biosorption processes. This is in accordance with the data presented in Figure 5c, which are clearly grouped into two distinct zones. Moreover, the comparable values of the diffusion constants in the two zones (k_{diff}^1 and k_{diff}^2) show that the diffusion of metal ions inside the biosorbent particle is as easy as their diffusion in the film around them (Table 3). This means that the retention of metal ions to the superficial functional groups has created large spaces on the surface of the biosorbent particles, in which the metal ions can penetrate

during of the biosorption process. This observation is consistent with the results obtained in the modeling of the biosorption isotherm and may explain the fairly good correspondence between the experimental data and the Freundlich model (see Section 3.3.1).

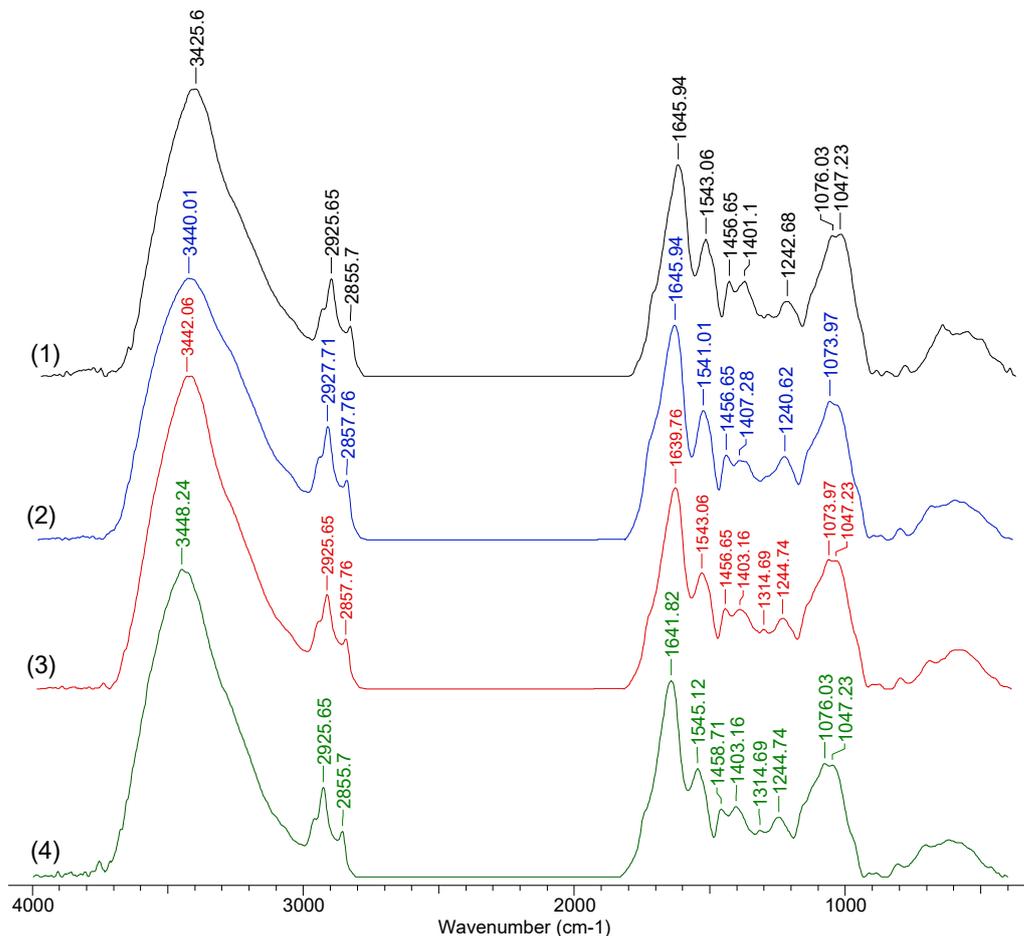


Figure 6. FTIR spectra of *Saccharomyces cerevisiae* before (1) and after biosorption of Cu(II) (2), Co(II) (3) and Zn(II) (4) ions.

3.4. Inside of the Biosorption Processes

Considering the structural characteristics of *Saccharomyces cerevisiae*, biosorption results, isotherms modeling and kinetic analysis of biosorption processes, discussed in detail in the previous sections, a possible mechanism is proposed. The schematic representation of the proposed biosorption mechanism is presented in Figure 7.

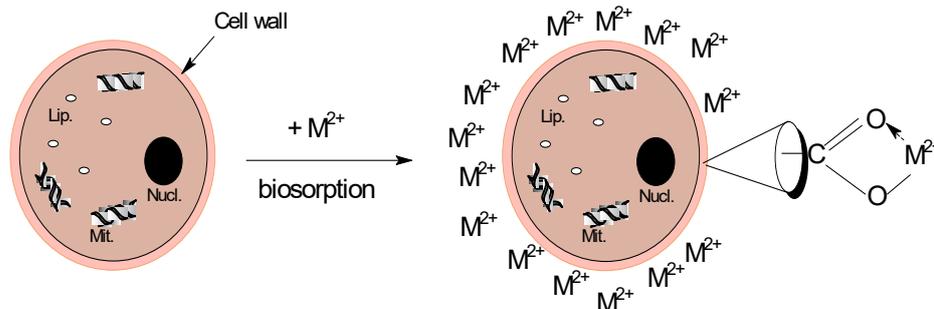


Figure 7. Schematic representation of the biosorption mechanism of metal ions on *Saccharomyces Cerevisiae* biomass (Notations: Mit., mitochondria; Lip., lipid granule; Nucl., nucleus).

It is well known that the cell walls of the *Saccharomyces cerevisiae* yeast mainly contain mannoproteins, β -glucans and chitin [40,41]. These chemical compounds have numerous functional groups, generally with O-donor atoms (such as hydroxyl, carboxyl, carbonyl, etc.), which have been highlighted by the FTIR spectrum (see Figure 1), but also a high mechanical flexibility, due to their polymeric structure. From the perspective of the biosorption processes, the functional groups represent the binding sites of the metal ions from the aqueous solution, and the flexibility of the macromolecules ensures the presence of these functional groups at the solid/aqueous solution interface. Therefore, the metal ions will first interact with those functional groups found outside the interface, and this is evidenced by the high rate of the biosorption processes (see Figure 2), and the close values of the Langmuir constants (see Table 1). However, according to the results of kinetics modeling (see Table 2), the retention of metal ions requires two binding points. To achieve this, the macromolecules in the cell walls can fold to provide geometrically favorable positions for the binding of metal ions. Consequently, some functional groups become unavailable and can no longer participate in biosorption processes. In this way, it can be explained why all three isotherm models (Langmuir, Freundlich and D–R) satisfactorily describe the experimental equilibrium data, and why the maximum biosorption capacities, calculated from the Langmuir model, are much higher than those obtained experimentally (see Figure 3 and Table 1), for all studied metal ions.

These observations clearly show that: (i) the biosorption of Co(II), Zn(II) and Cu(II) ions takes place on the surface of *Saccharomyces cerevisiae* yeast, until the outside functional groups are occupied (according to D–R isotherm model); (ii) during biosorption, the retention of metal ions to the functional groups of the biosorbent takes place mainly through electrostatic interactions (ion exchange), where hydroxyl groups are involved (see Figure 6); and (iii) these interactions require two geometrically favorable binding sites.

All these observations show that the retention of metal ions takes place predominantly at the outer surface of the yeast cells (by biosorption) and do not penetrate them (which means that bioaccumulation has a small share). Therefore, the addition of dry *Saccharomyces cerevisiae* yeast to liquid products to be fermented can significantly reduce the amount of heavy metals in them due to biosorption. However, the evaluation of the efficiency of *Saccharomyces cerevisiae* yeast in such a fermentation process, in the presence of heavy metals, must be examined in detail, and these results will be presented in another study.

4. Conclusions

Saccharomyces cerevisiae biomass was examined as a biosorbent, in this study, for the removal of Co(II), Zn(II) and Cu(II) ions from aqueous solution. The maximum biosorption potential of this biomass was attained at pH of 5.0, 4.0 g biosorbent/L, contact time = 60 min, and room temperature (23 °C), and these conditions were considered optimal. The Dubinin–Radushkevich isotherm model and the pseudo-second order kinetics model best fit the experimental data obtained for the retention of these metal ions from aqueous solution. The maximum biosorption capacities and the pseudo-second order rate constants follow the order: Co(II) > Zn(II) > Cu(II), suggesting that the structural particularities of metal ions play a determinant role in the uptake processes. Detailed analysis of the experimental results shows that the removal of metal ions from aqueous media mainly occurs through biosorption at the external surface of yeast cells and do not penetrate them. Therefore, the addition of dry *Saccharomyces cerevisiae* yeast to liquid products to be fermented can significantly reduce the amount of heavy metals in them due to biosorption. This opens up new perspectives on the practical applicability of *Saccharomyces cerevisiae* yeast in fermentation processes.

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