*Polish Journal of Chemical Technology, 19, **1**, 1—10, 10.1515/pjct-2017-0001*

Biosorption of nickel (II) and zinc (II) from aqueous solutions by the biomass of yeast *Yarrowia lipolytica*

Sławomir Wierzba

University of Opole, Independent Department of Biotechnology and Molecular Biology, Kardynała Kominka 6a, 40-035 Opole, Poland
**Corresponding author: e-mail: slawomir.wierzba@uni.opole.pl*

This study examined the biosorption process of Ni(II) and Zn(II) from an aqueous solution by dead biomass of *Yarrowia lipolytica*. Optimum biosorption conditions were determined as a function of pH, biomass dosage, contact time, and temperature. The biosorbent was characterized by FTIR, which indicated the participation of hydroxyl, carboxyl, amide and amine groups in the process of binding the metal ions. The results showed that the biosorption processes of both metal ions closely followed pseudo-second order kinetics. The equilibrium data of Ni(II) and Zn(II) ions at 20, 30 and 40°C fitted the Langmuir and Freundlich isotherm models. Langmuir isotherm provided a better fit to the equilibrium data, with a maximum biosorption capacity of the *Y. lipolytica* biomass for Ni(II) and Zn(II) of 30.12 and 44.44 mg/g respectively. The calculated thermodynamic parameters demonstrated that the biosorption of Ni(II) and Zn(II) ions onto the *Y. lipolytica* was feasible, spontaneous and endothermic.

Keywords: biosorption, adsorption isotherms, zinc, nickel, *Yarrowia lipolytica*.

INTRODUCTION

Heavy metals constitute dangerous environmental pollution due to their high toxicity and tendency to migrate and bioaccumulate in the food chain. Zinc and nickel are metals prevalent in wastewater. Sources of zinc include industrial pollution from the production of batteries, paints, textiles, plastics, and from printing plants[1]. Excess levels of this metal cause disorders in the digestive tract, anemia, and impede the absorption of calcium, copper and iron. Zinc concentrations in drinking water should not exceed 5 mg/l[2]. Nickel is present in raw sewage of the galvanizing, paper, refining, metallurgical and fertilizer industries. Excess nickel levels accumulate in the lymph nodes, which may be the cause of many cancers. The content of nickel in drinking water should not exceed 0.02 mg/l[3]. The ever lower limits of heavy metal levels in water and wastewater discharged into the environment require new and efficient methods to remove them. Conventional methods of removing heavy metals from the environment include precipitation, ion exchange, coagulation, adsorption, flotation, reverse osmosis and electrochemical processes. The main disadvantages of these methods include the relatively low efficiency of treatment in wastewater with a low concentrations of metal ions. In addition, their use is energy-intensive and leads to large quantities of secondary impurities[4]. Alternative methods include biotechnological processes such as biosorption or bioaccumulation. They allow the use of cheaper and more efficient materials for sewage and wastewater with low concentrations of impurities (less than 100 mg/l)[5]. The mechanism of binding biomass may take place by biosorption on the biosorbent's surface, bioaccumulation inside cells and chemical conversion of metal ions as a result of metabolic activity. Materials of biological origin are characterized by relatively large sorption capacities and are also much cheaper, especially when using waste materials from various industries (e.g. in food industry).

In recent years, *Yarrowia lipolytica* has been one of the most widely investigated unconventional yeast species, next to species such as *Pichia pastoris*, *P. guilliermondii* or *Kluyveromyces lactis*. *Y. lipolytica* strains are commonly found in nature and are frequently isolated from dairy products, for example cheeses or yogurt, meat products, e.g. sausages and environments rich in fats[6]. These microorganisms are strictly aerobic and have the capacity to be used as a carbon source in a wide variety of substrates (carbohydrates, alcohols, organic acids, alkanes, fatty acids and triglycerides). Physiological and biochemical properties, including the high secretory capability exhibited by *Y. lipolytica*, provided a basis for the biotechnological use of these microorganisms in biosynthesis, biotransformation and biodegradation. Currently, *Y. lipolytica* is used in the synthesis of organic acids, sweeteners, carotenoids, oil and microbiological protein[7]. Due to the high production of a number of enzymes, *Y. lipolytica* is a valuable tool in environmental protection – bioremediation of soil and water contaminated with oil, detoxification of aromatic compounds, biosorption of heavy metals and the treatment of wastewater from the fishing, oil and food industries[3, 7, 8]. The aim of this study was to evaluate the effectiveness of *Y. lipolytica* in removing Ni(II) and Zn(II) from aqueous solutions, to determine the mechanisms of ion binding, to determine the kinetics and equilibrium biosorption process, and the influence of process parameters on performance.

EXPERIMENTAL MICROORGANISM AND PREPARATION OF BIOMASS

Yarrowia lipolytica was sourced from the collection at the Independent Department of Biotechnology and Molecular Biology at the University of Opole, and was stored in YPD medium (2% glucose, 1% yeast extract, 1% peptone, 2% agar, pH 6.0) at 4°C. For the purposes of biosorption determination, *Y. lipolytica* biomass was grown in a 500 ml Erlenmeyer flask containing 100 ml of liquid medium composed of 2% glucose, 1% yeast extract, 1% peptone, and 0.05% $MgSO_4$ (pH 6.0). The culture was then incubated in a thermo-shaker at 120 rpm for 48 hours at 30°C. The yeast cells were harvested

in a stationary growth phase by centrifuging at 5000 rpm for 10 minutes at 4°C . The thus obtained biomass was washed three times with deionized water and dried at 80°C for 24 h. The dried biomass of *Y. lipolytica* was pulverized in a mortar and used in the experiments on Ni(II) and Zn(II).

Preparation of metal solutions

The solutions of Ni(II) and Zn(II) at concentrations of 1000 mg/l were prepared by dissolving appropriate amounts of $NiCl_2 \cdot 6H_2O$ and $ZnSO_4 \cdot 7H_2O$ in deionized water. Other metal ion concentrations were achieved by further diluting the solutions with deionized water. The pH of the metal solutions was determined using 0.1 M NaOH and 0.1 M HCl. Freshly diluted solutions were used for each experiment. All chemicals used were of analytical grade.

FTIR spectroscopy

To identify potential functional groups and the possible binding sites related to Ni(II) and Zn(II) absorption, IR analysis was performed with a FTIR spectrometer (Fourier transform-infrared spectrometer, Nicolet Nexus, USA Nicolet Co.) One milligram (dry weight) of *Y. lipolytica* cells was mixed and ground with 100 mg of KBr (Spectral) in an agate mortar. The IR spectra obtained at 400–4000 cm^{-1} were used to examine the biomass before and after metal-loading (for 24 h in 50 mg/l initial Ni(II) and Zn(II) concentration).

Biosorption experiments

In the biosorption experiments we investigated the effects of pH, quantity of biomass, contact time, initial metal ion concentration and temperature on the biosorption of Ni(II) and Zn(II) by *Y. lipolytica*. All experiments were performed in 250 ml Erlenmeyer flasks containing 50 ml of a solution of known Ni(II) or Zn(II) ion concentration in a thermo-shaker at 120 rpm. The effects of pH ranging from 3 to 8 were tested in solutions at an initial concentration of metal ions of 100 mg/l at 30°C for 60 minutes using 2 g/l of biomass. The effect of the dose of yeast from 0.5 to 3 g/l was determined under similar conditions at pH 5 and 6. The kinetics of the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* were tested at 20, 30 and 40°C for various contact times in the range 10 to 180 minutes in solutions with an initial 100 mg/l concentration of ions, at a biomass dose of 2 g/l and pH of 5 and 6. Biosorption tests of nickel and zinc ions under equilibrium (adsorption isotherm) carried out at temperatures of 20, 30 and 40°C, in concentrations of metal ions ranging from 10 to 300 mg/l for 60 minutes, biomass dose of 2 g/l, and pH of 5 and 6.

Metal ion concentrations in the experiments were determined spectrophotometrically using biosorption cuvette tests Spectroquant® Nickel Cell Test and Spectroquant® Zinc Cell Test (Merck, Germany). Prior to testing the samples were filtered with Whatman filter partitioning membranes (pore size 0.45 μm), diluted with deionized water to the measured range. The test cuvette was measured using a Photolab Spectral spectrophotometer (WTW, Germany).

The amount of adsorbed heavy metal ions per unit biosorbent (mg metal ion/g dry biosorbent) was obtained by using the following expression:

$$q_e = \frac{(C_0 - C_e)V}{M} \tag{1}$$

where q_e is the amount of heavy metal adsorbed onto the unit amount of the biomass (mg/g); C_o and C_e are the concentrations of the heavy metals in the initial and equilibrium solution (mg/l), and after biosorbtion, respectively, V is the volume of the aqueous phase (l) and M is the amount of the biomass (g).

Biosorption kinetics

In order to clarify the kinetics of biosorption of Ni(II) and Zn(II) by the biomass of *Y. lipolytica*, the experimental data have been described using the most popular kinetic models, i.e. the pseudo-first order, pseudo-second order, as well as intraparticle diffusion.

The equation of Lagergren's pseudo-first order[9] after integration takes a rectilinear form:

$$ln(q_e - q_t) = lnq_e - \frac{k_1}{2{,}303}t \tag{2}$$

where q_e and q_t (mg/g) are the amounts of metal ions sorbed at equilibrium time (mg/g) and t (min), respectively, and k_1 is the rate constant of the equation (l/min). The sorption rate constants (k_1) can be determined experimentally by plotting $\ln(q_e - q_t)$ vs. t.

The sorption data were also analyzed in terms of a pseudo-second order mechanism proposed by Ho and McKay[10]. This model is based on the assumption that the adsorption follows second order chemisorptions and predicts the behavior over the whole range of concentration, and is in agreement with an adsorption mechanism being the rate controlling step[1].

The pseudo-second order model can be expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \tag{3}$$

where k_2 (g/mg · min) is the rate constant of the second-order equation, q_t (mg/g) is the amount of biosorption time t (min) and q_e is the amount of biosorption equilibrium (mg/g).

The initial sorption rate is[11]:

$$h = k_2 q_e^2 \tag{4}$$

The slopes and intercepts of plots versus were used to calculate the second-order rate constants k_2 and q_e.

In order to examine the mechanism of Ni(II) and Zn(II) biosorption by the biomass of *Y. lipolytica* the intraparticle diffusion model of Weber and Morris was used[12]:

$$q_t = k_{id} t^{0.5} + C_{id} \tag{5}$$

where q_t (mg/g) is the amount adsorbed at time t (min) and k_{id} (mg/g · $min^{0.5}$) is the rate constant of intraparticle diffusion. C_{id} is the value of intercept which gives an idea about the boundary layer thickness, i.e. the larger the intercept, the greater the boundary effect.

Finally, it is possible to determine the sorption energy by fitting the kinetic constant (k_2) from the pseudo-second order model at different temperatures to the Arrhenius equation[13]:

$$k_2 = k_o \cdot exp(\frac{-E_A}{RT}) \quad (6)$$

where k_o is the temperature independent factor in (g/mg · min), E_A is the activation energy of biosorption in (kJ/mol), R is the gas constant (8.314 J/mol · K) and T is the solution temperature in (K).

Biosorption isotherm models

The description of the equilibrium of Ni(II) and Zn(II) biosorption by *Y. lipolytica* was based on the Langmuir[14] and Freundlich[15] models. The Langmuir model represents one of the first theoretical treatments of nonlinear sorption and suggests that uptake occurs on a homogeneous surface by monolayer sorption without interaction between the adsorbed molecules. In addition, this model assumes uniform energies of adsorption onto the surface and no transmigration of the adsorbate[11]. The Langmuir isotherm is represented in the following equation:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad (7)$$

where q_e is the equilibrium metal ion concentration on the sorbent (mg/g), C_e is the equilibrium metal ion concentration in the solution (mg/l), q_{max} is the monolayer sorption capacity of the sorbent (mg/g), K_L and is the Langmuir sorption constant (l/mg) related to the free energy of sorption. Eq. (7) is usually linearized to obtain the following from:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{K_L q_{max}} \quad (8)$$

The essential characteristic of the Langmuir isotherm can be expressed in terms of dimension less constant separation factor for equilibrium parameter R_L, which is defined by[16]:

$$R_L = \frac{1}{1 + K_L C_o} \quad (9)$$

where C_o is the highest Ni(II) and Zn(II) concentration (mg/l).

The Freundlich isotherm is a nonlinear sorption model. This model proposes a monolayer sorption with a heterogeneous energetic distribution of active sites, accompanied by interactions between adsorbed molecules. The general form of this model is:

$$q_e = K_F C_e^{\frac{1}{n}} \quad (10)$$

where K_F is a constant relating the biosorption capacity and $\frac{1}{n}$ is an empirical parameter relating the biosorption intensity, which varies with the heterogeneity of the material. The logarithmic form of Eq. (10) is:

$$lnq_e = lnK_F + \frac{1}{n} lnC_e \quad (11)$$

Thermodynamic parameters

In order to describe the thermodynamic behaviour of the biosorption of Ni(II) and Zn(II) ions onto *Y. lipolytica* biomass, thermodynamic parameters including the change in free energy (ΔG_o), enthalpy (ΔH_o) and entropy (ΔS_o) were calculated from the following equations:

$$\Delta G^o = -RT \cdot lnK_c^o \quad (12)$$

where R is the universal gas constant (8.314 J/mol · K) and T is temperature (K), K_c^o is the equilibrium constant obtained from the Langmuir isotherm. The free energy change indicates the degree of spontaneity of the biosorption process, and a higher negative value reflects more energetically favorable adsorption[13].

The apparent equilibrium constant of the biosorption is defined as:

$$K_c^o = \frac{C_a}{C_e} \quad (13)$$

where C_a is the amount of Ni(II) or Zn(II) (mg) adsorbed onto the biosorbent per litre of solution at equilibrium and C_e is the residual metal ion concentration at equilibrium in the solution (mg/l). The equilibrium constant may be expressed in terms of enthalpy change of biosorption (ΔH^o) and entropy change of biosorption (ΔS^o) as a function of temperature[4]:

$$lnK_c^o = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \quad (14)$$

Data analysis

All the experiments were carried out in triplicates and the values reported as mean ± SD. SigmaPlot® was used to fit the kinetics and equilibrium models using nonlinear regression.

RESULTS AND DISCUSSION

FTIR analysis

FTIR analysis was performed to give a qualitative and preliminary analysis of the main functional groups present on the cell wall which may be responsible for Ni(II) and Zn(II) biosorption (Fig. 1).

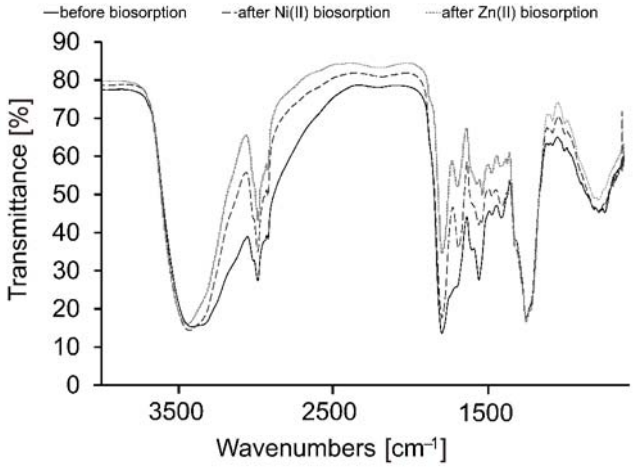

Figure 1. FTIR spectra of. *Y. lipolytica* before and after Ni(II) and Zn(II) biosorption

Fourier transform infrared (FTIR) spectra indicated the presence of hydroxyl, carboxylic, amido and amino groups which are important sites for metal biosorption (Table 1).

The position of the absorption bands in the FTIR spectra is related to the change in energy of particles resulting from the stretching and bending vibration of the atoms. A change within the range of absorption bands of certain functional groups, resulting from the adsorption of Ni(II) and Zn(II) on the surface of *Y. lipolytica* cells,

Table 1. FTIR spectra characteristics of *Y. lipolytica* biomass before and after treatment with heavy metals

Wavelength range [cm^{-1}]	*Y. lipolytica* biomass [cm^{-1}]					Bond	Functional group
	Before sorption	After sorption of Ni(II)	Differences	After sorption of Zn(II)	Differences		
3600–3200	3376	3395	+19	3411	+35	O-H stretching H-bonded	Hydroxyl
3100–2800	2925	2924	–1	2923	–2	C-H stretching	Alkanes
1690–1640 1860–1620	1657	1655	–2	1654	–3	C=O stretching C=C stretching	Amides alkenes
1560–1515	1553	1541	–12	1549	–4	C-N stretching	Amides
1480–1350	1400	1402	+2	1415	+15	C-H bending	Alkanes
1320–1210	1311	1308	–3	1316	+5	C-O stretching	Carboxyl acids
1320–1210 1360–1080	1247	1241	–6	1252	+5	C-O stretching	Carboxyl acids
1360–1080 1050–1150	1075	1072	–3	1072	–3	C-O stretching C-N stretching	Alcohols amines

suggests that these groups are responsible for binding metal ions. A broad band of 3376 cm^{-1} is indicative of the stretching vibration of the hydroxyl groups of alcohols and carboxylic acids[13]. Band shifts after the biosorption process suggest a significant role of the hydroxyl groups in the adsorption of Ni(II) (change +19) and Zn(II) (change +35) on the biosorbent's surface. Strong stretching and bending vibration in the range of 1400 cm^{-1} to 1657 cm^{-1} are characteristic for the presence of C=O and N-H bonds in the amide group of the protein. Significant band shifts on the surface of the yeast cells after biosorption, from 1553 to 1541 cm^{-1} (–12), and from 1400 to 1415 cm^{-1} (+15), indicate that the amide groups of proteins are highly involved in the adsorption of metal ions. The shifts from 1308 to 1316 cm^{-1} and from 1241 to 1252 cm^{-1} indicate stretching vibrations of C-O bonds due to carboxyl groups and may be responsible for electrostatic interactions between positive nickel and zinc ions and the negatively charged carboxylic groups. The shift between 1072 and 1075 cm^{-1} is indicative of stretching vibration of C-O bonds from alcohols and carboxylic acids and C-N bonds from amines.

The transmittance of the peaks in the loaded biomass was substantially lower than those in the raw sample of the yeast biomass. This indicates that bond stretching occurs to a lesser degree due to the presence of Ni(II) and Zn(II), and the following peak transmittance is reduced. These results are consistent with the study of Yin et al.[17] and Ahmad et al.[18] who observed that the biosorption of zinc and nickel ions by the biomass of yeast caused a decrease in transmittance compared to the control.

FTIR spectra of the *Y. lipolytica* biomass implicate hydroxyl, carboxyl, amide and amine groups in the biosorption of Ni(II) and Zn(II). Ahmad et al.[18] showed that the biosorption process of Zn(II) by the biomass of *Candida utilis* and *C. tropicalis* involved hydroxyl, carboxyl, amide and amino groups. Similarly, Shinde et al.[3] observed the participation of hydroxyl, carboxyl, carbonyl and amine groups in the biosorption of Ni(II) by *Y. lipolytica*. Also Asfaram et al.[19] indicated the involvement of the hydroxyl, carboxyl, carbonyl, and amino groups in the biosorption of Ni(II), Zn(II), and Co(II) by *Y. lipolytica* ISF7. The authors also found that nickel ions bound via coordinate bonds with functional groups (amino and carboxyl groups) on the cellular walls of the yeast cells forming complexes. Such complexation is likely the mechanism behind the biosorption of nickel and zinc ions by *Y. lipolytica*.

Effect of initial solution pH

The pH of the solution was one of the most important parameters affecting the efficiency of the biosorption of metal ions from the aqueous solutions. It is directly related with the competition ability of hydrogen ions with metal ions for active sites on the biosorbent surface[20, 21]. Generally, metal biosorption involves complex mechanisms of ion exchange, chelation, adsorption by physical forces, and ion entrapment in inter and intra fibrillar capillaries and spaces of the cell structural network of a biosorbent[22]. FTIR analysis showed that *Y. lipolytica* has many functional groups such as hydroxyl, carboxyl, amide or amino groups involved in potential mechanisms of metal ion binding. Moreover, depending on the pH values of the aqueous solutions these functional groups participated in metal ion bindings. The effect of initial pH on Ni(II) and Zn(II) ion uptake capacity of *Y. lipolytica* was investigated between pH 2–8 at 100 mg/l initial metal ion concentration and a temperature of 30°C. The results are shown in Figure 2. The maximum biosorption of nickel ions and zinc at 14.88 and 17.19 mg/g respectively were observed at pH 6 and 5. Similar results were obtained by Li et al.[23], Liu et al.[21] and Pahlavanzadeh et al.[24] Therefore, the remaining biosorption experiments were carried out at those pH values.

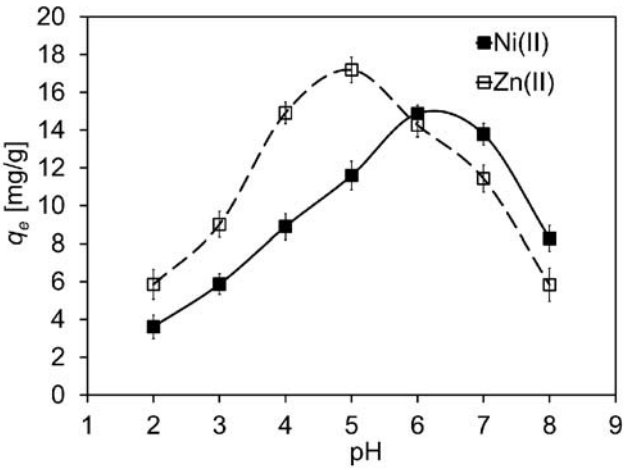

Figure 2. Effect of pH on the biosorption of Ni(II) and Zn(II) onto *Y. lipolytica* biomass (metal concentration: 100 mg/l, biomass dosage: 2 g/l, temperature: 30°C)

The biosorption mechanisms on the yeast cells surface reflect the nature of the physicochemical interaction of the solution. At a highly acidic pH, the overall surface charge on the active sites became positive and metal cations and protons competed for binding sites on the cell wall, resulting in a lower uptake of metal[25]. As the biosorbent surface became more negatively charged as the pH solution increased from 2 to 5, the functional groups of *Y. lipolytica* cells were more deprotonated and thus available for the metal ions. Li et al.[23] and Özer and Özer[22] reported that as the pH increased to 5, more functional groups with negative charge such as carboxyl, amine or hydroxyl became exposed with a subsequent increase in attraction sites to positively charged ions, and thus enhanced the biosorption capacity. At a low pH, cell wall ligands were closely associated with hydronium ions H_3O^+ and restricted the approach of metal cations as a result of the repulsive force[3]. A decrease in biosorption yield at a higher pH (pH>6) is related to the formation of soluble hydroxylated complexes of metal ions (nickel ions in the form of $Ni(OH)_2$, zinc ions in the form of $Zn(OH)_2$)[23]. Similar conclusions were presented by many authors conducting research on the biosorption of nickel and zinc ions using different biosorbents[20, 22, 25].

Effect of biosorbent dose on biosorption

The study, in line with Munagapati et al.[25] and Subbaiah and Yun[26], indicates that the dose of biosorbent is also an important parameter influencing the sorption capacity and the efficiency of metal ion removal from the aqueous solution. The effect of the concentration of *Y. lipolytica* cells in the range of 0.5–3.0 g/l, the sorption capacity of the biomass and the percentage removal of Ni(II) and Zn(II) from the solution, are shown in Figure 3. The maximum biosorption of Ni(II) and Zn(II) was observed at a dose of 0.5 g/l, 37.23 and 42.04 mg/g respectively, while the percentage removal of metal ions from the solution was 39.96% and 48.04%.

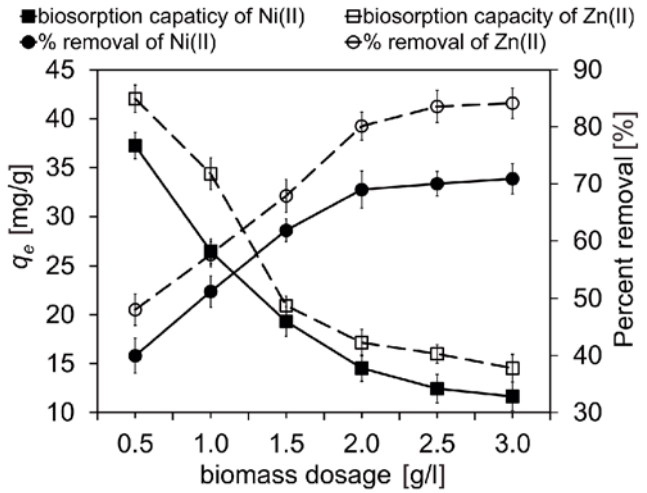

Figure 3. Effect of biomass dosage on biosorption capacity and percent removal of Ni(II) and Zn(II) by *Y. lipolytica* (metal concentration: 100 mg/l, pH: 5.0–6.0, temperature: 30°C).

The precentage of removal of zinc and nickel ions from the solution increased with the increase in biomass concentration, and a dose of 3.0 g/l resulted in a 70.89% and 84.15% decrease with a simultaneous decrease of sorption capacity to 11.65 and 14.53 mg/g. In turn, the sorption capacity of *Y. lipolytica* biomass decreased with increasing concentration of yeast cells. With an increase in biomass concentration the % removal increases because more biosorbent (binding sites) are available for the same amount of cations while specific uptake of metal ions decreased due to lower metal concentration in the solution after a very fast superficial adsorption onto the microbial cells[27]. However, the biomass can undergo different modifications depending on the experimental conditions, such as pH, ionic strength, temperature, metal ion in solution and its same biomass concentration level: for example, the aggregates formed during biosorption may reduce the effective biosorption area[11]. In the case of biosorption of Ni(II) and Zn(II) from a solution of 100 mg/l, the optimal dose of biosorbent 2 g/l, which guaranteed a high yield, respectively 68.99% and 80.12%, with the sorption capacity at 14.54 and 17.15 mg/g. These conclusions are confirmed by the study of Li et al.[23].

Effect of contact time and temperature

The process of biosorption consists in the transport of nickel and zinc ions from the bulk of the solution to the surface of the sorbent. The rate of the process depends first off all on the stages of the mass transfer. The first stage is characterized by the most intense sorption, which is due to the accessibility of the free active sites on the surface of the sorbent and thereby a large concentration gradient that activates the process[1, 17, 19, 28]. In the case of the *Y. lipolytica* biomass, the first stage of rapid transfer of metals to active sites on the biosorbent's surface lasted approx. 20 minutes. The next stage, in which equilibrium was reached, lasted about 60 minutes (Fig. 4).

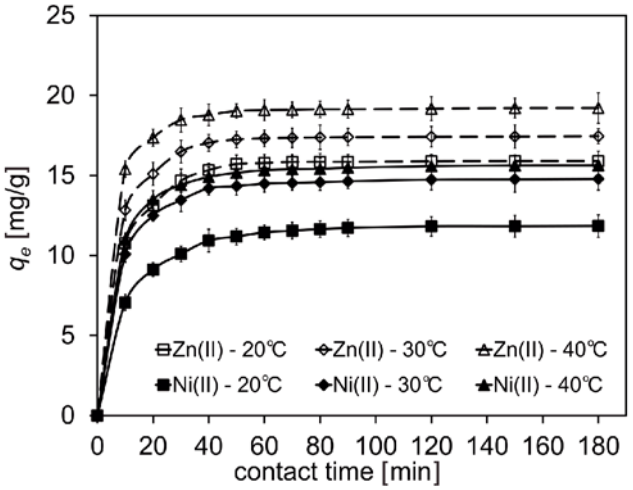

Figure 4. Effect of contact time and temperature on the biosorption of Ni(II) and Zn(II) onto *Y. lipolytica* biomass (metal concentration: 100 mg/l, pH: 5.0–6.0, biomass dosage: 2 g/l)

Therefore, this time value was selected as optimum contact time for sufficient biosorption of the metal ions. The almost same contact time was reported in several earlier works which related with the biosorption of the same metal ions on various biomasses[20, 23].

The rate of mass transfer depends also on the conditions under which the process occurs. Temperature is among the factors that most affect the kinetics of biosorption within the range 20 to 40°C. An increase in temperature

up to 40°C brings about an increase in the rate of biosorption and enhancement of the sorptive capacity[29]. At equilibrium, the maximum biosorption of Ni(II) at 20, 30 and 40°C was 11.48, 14.78 and 15.64 mg/g, respectively and for Zn(II) these were 15.90, 17.45 and 19.21 mg/g. The results indicate that biosorption of Ni(II) and Zn(II) by the *Y. lipolytica* biomass is endothermic. The studies of many authors confirm such an effect of temperature on biosorption[20, 24, 26, 28, 30, 31].

Biosorption kinetics

Biosorption kinetic studies are crucial for describing the adsorbate biosorption rates and for determining the rate-determining step. The pseudo-first order, second order and intraparticle diffusion kinetic models were used in this work. The plots ($q_e - q_t$) of vs. for the pseudo-first order model were not shown as figure because the coefficients of determination for this model at studied temperatures is low (R^2 = 0.896 – 0.944 for the Ni(II) biosorption and R^2 = 0.729 – 0.810 for Zn(II) biosorption, as seen in Table 2). Also, the equilibrium uptake (q_e) values calculated from the pseudo-first order kinetic model did not agree well with the experimental ($q_{e\ exp}$) values. It can be concluded from the R^2 and q_e values that the biosorption mechanisms of nickel and zinc ions onto *Y. lipolytica* biomass does not follow the pseudo-first order kinetic model.

The pseudo-second order model defines the measurements very well with the correlation coefficients very high (> 0.999) (Table 2). The calculated q_e values also agree very well with the experimental data ($q_{e\ exp}$). The data presented in Table 2 indicate that the initial speed of sorption (h) for Ni(II) and Zn(II) increased with an increase in temperature from 20 to 40°C, respectively from 2.155 to 4.955 mg/g · min and 5.285 to 11.61 mg/g · min. At the same time we observed an increase in the rate constant k_2, respectively, from 0.014 to 0.019 g/mg · min and from 0.020 to 0.031 g/mg · min. This means that biosorption occurs more quickly at higher temperatures. Due to the temperature the molecule energy rises and so does the possibility of the molecule-absorbent reaction[29]. Also the amount of nickel and zinc ions adsorbed by the yeast biomass increased when the temperature of the process increased from 20 to 40°C, respectively, from 12.32 to 15.97 mg/g and from 16.29 to 19.46 mg/g (Table 2). However, the equilibrium sorption capacity was little affected by increased temperature. In conventional physisorption systems, increasing temperature usually increases the rate of approach to equilibrium, but decreases the equilibrium capacity[30].

On the basis of these results it can be concluded that the kinetics of Ni(II) and Zn(II) biosorption by the biomass of *Y. lipolytica* proceeded in accordance with the model of the pseudo-second order. Similar conclusions about the kinetics of nickel and zinc biosorption are also presented by Asfaram et al.[19] using *Y. lipolytica* ISF7 biomass, by Suazo-Madrid et al.[31] who used the yeast *Rhodotorula glutinis* as biosorbent, and by Farhan and Khadom[32] using *Saccharomyces cerevisiae*.

Table 2. Kinetic parameters for biosorption of Ni(II) and Zn(II) on *Y. lipolytica* biomass at different temperatures

Metal ion	Temperature [°C]	$q_{e\ exp}$ [mg/g]	Pseudo-first-order			Pseudo-second-order			
			k_1 [1/min]	q_e [mg/g]	R^2	k_2 [g/mg·min]	q_e [mg/g]	h [mg/g·min]	R^2
Ni(II)	20	11.90	0.060	3.079	0.896	0.014	12.32	2.155	0.999
	30	14.80	0.069	2.901	0.944	0.019	15.13	4.380	0.999
	40	15.70	0.055	2.476	0.898	0.019	15.97	4.955	1.000
Zn(II)	20	15.95	0.059	1.792	0.729	0.020	16.29	5.285	0.999
	30	17.50	0.055	1.592	0.746	0.024	17.76	7.728	0.999
	40	19.25	0.058	1.488	0.810	0.031	19.46	11.61	1.000

The straight lines obtained from the plot of versus showed good fit of experimental data with the second-order kinetic model for the different temperatures (20–40°C) (Fig. 5).

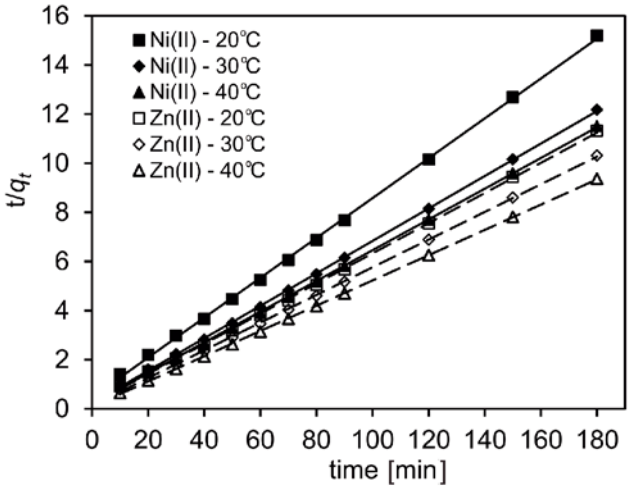

Figure 5. Pseudo-second order kinetic of Ni(II) and Zn(II) biosorption by *Y. lipolytica* biomass at different temperatures (metal concentration: 100 mg/l, pH: 5.0–6.0, biomass dosage 2 g/l)

The energy of activation (E_A) was determined from the slope of the Arrhenius plot of lnk_2 versus $1/T$ according to Eq. (6) to be 11.77 and 16.67 kJ/mol for Ni(II) and Zn(II) respectively (Table 5). These results suggest that biosorption of nickel and zinc ions by the yeast biomass is a process of chemical adsorption. Shinde et al.[3] and Baysal et al.[28] report that the activation energy for the chemical adsorption is usually greater than 4–6 kJ/mol. Positive values of activation energy (E_A) confirm the earlier conclusion that higher temperatures promote the biosorption of nickel and zinc ions by *Y. lipolytica* biomass and the process is endothermic. Equally low results were obtained by Horsfall and Spiff[33] and Uslu and Tanyol[34].

The process of sorption falls into three basic stages: (1) diffusive mass transfer of the component from the bulk through the boundary layer close to the surface of the sorbent, (2) intraparticle diffusion in the pores of the sorbent, (3) binding of the molecules to the active sites in the pores of the sorbent. The binding of the

molecules to the active sites is the fastest stage. It is assumed that this stage does not limit a mass transfer[29].

The Weber-Morris model assumes three stages: external mass transfer and two stages of intraparticle diffusion in the larger and smaller pores until saturation of the surface. The Weber-Morris plot for adsorption Ni(II) and Zn(II) is given in Figure 6. If the intraparticle diffusion is the sole rate determining step, the plots of should be linear and pass through the origin. Non-linearity indicates that intraparticle diffusion is not the only rate-limiting step in the adsorption process. The graph of dependencies q_t from $t^{0.5}$ought to contain the dependency lines. The first linear dependency corresponds to the external surface uptake, the other linear dependency relates to the gradual uptake reflecting intraparticle diffusion as the rate limiting step, whereas the third constitutes a stage during which equilibrium is being attained, when the molecules take up their positions within the pores of the sorbent, the diffusion slows down because of the concentration gradient that decreases with time.

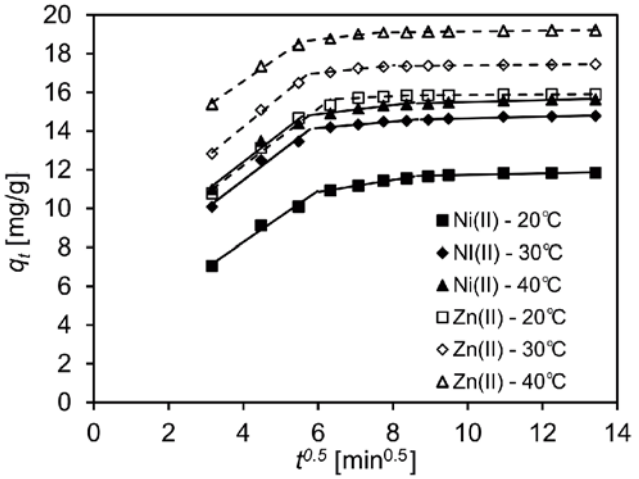

Figure 6. Weber-Morris plots for the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* biomass at different temperatures (metal concentration: 100 mg/l, pH: 5.0–6.0, biomass dosage 2 g/l)

A high value of intercept may imply that the boundary layer significantly affects mass transfer within the sorbent (Table 3). The intercept levels were lowest for the first linear range (2.967–11.23 mg/g), which may mean that this stage takes place quickly and partially inhibits the transfer of mass. The same values are much higher for the other two stages, and in particular for the third stage (11.33–18.93 mg/g). That intercept increases slightly with the increase in temperature for the individual stages of adsorption. The kinetic parameter decreases in particular stages, which confirms the assumption that it is the stage of penetration of the mass in the boundary layer that occurs with the largest rate, then comes diffusion in the pores, and the stage of attaining equilibrium is slowest. Analogous conclusions are presented by Asfaram et al.[19], Munagapati et al.[25] and Witek-Krowiak[29].

Biosorption isotherm models

One of the important aspects for evaluation of the sorption process as a unit operation is the equilibria of sorption. Figure 7 shows the Langmuir plots at different temperature and the constants q_{max} and K_L are tabulated in Table 4.

Another essential parameter of the Langmuir isotherm is R_L. It is reported (Lin et al.[16]) that R_L indicates the shape of the isotherm and nature of the biosorption process ($R_L>1$: unfavorable; $=1$: linear; $0<R_L<1$: favorable; $R_L = 0$: irreversible). The R_L values obtained from this study were 0.140–0.170 to Ni(II) adsorption and 0.155–0.174 to Zn(II) adsorption (Table 4).

Figure 8 shows the Freundlich plots at different temperatures and the constants and are tabulated in Table 4.

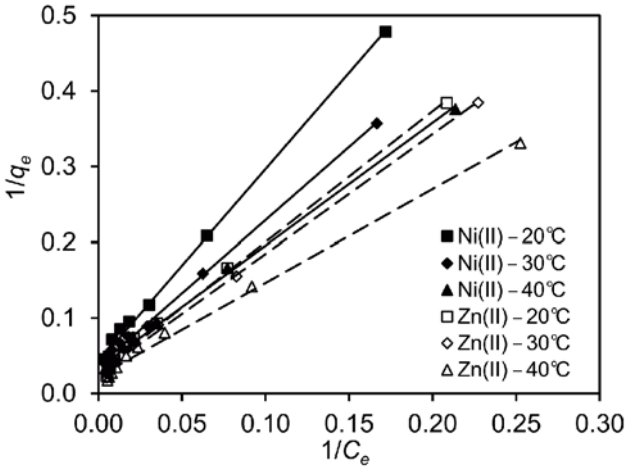

Figure 7. Langmuir isotherm plots for the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* biomass at different temperatures (metal concentration: 100 mg/l, pH: 5.0–6.0, biomass dosage 2 g/l)

As shown in Table 4, experimental data on the isotherms at 20, 30 and 40°C were better fitted to the Langmuir model ($R^2>0.994$) than to the Freundlich model ($R^2<0.993$), suggesting that the former better describes the state of equilibrium of Ni(II) and Zn(II) biosorption by the yeast biomass under the experimental conditions. The maximum sorption capacity of the monolayer increased with increasing temperature (20–40°C)

Table 3. Kinetic parameters of Weber-Morris model

Metal ion	Temperature [°C]	First linear portion			Second linear portion			Third linear portion		
		k_{id} [mg/g · min$^{0.5}$]	C_{id} [mg/g]	R^2	k_{id} [mg/g · min$^{0.5}$]	C_{id} [mg/g]	R^2	k_{id} [mg/g · min$^{0.5}$]	C_{id} [mg/g]	R^2
Ni(II)	20	1.324	2.967	0.984	0.313	8.969	0.982	0.039	11.33	0.809
	30	1.477	5.553	0.972	0.169	13.14	0.970	0.044	14.21	0.893
	40	1.495	6.425	0.965	0.237	13.44	0.946	0.050	14.99	0.903
Zn(II)	20	1.472	6.327	0.979	0.097	15.03	0.999	0.011	15.74	0.879
	30	1.588	7.862	0.996	0.198	15.80	0.938	0.017	17.22	0.910
	40	1.335	11.23	0.993	0.219	17.42	0.907	0.021	18.93	0.947

Table 4. Langmuir and Freundlich isotherm constants and correlation coefficients for the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* biomass at different temperatures

Metal ion	Temperature [°C]		Langmuir			Freundlich		
		q_{max} [mg/g]	K_L [l/mg]	R_L	R^2	K_F [l/mg]	1/n	R^2
Ni(II)	20	24.10	0.017	0.170	0.997	1.200	0.614	0.976
	30	27.85	0.019	0.152	0.997	1.222	0.570	0.966
	40	30.12	0.020	0.140	0.996	1.165	0.619	0.989
Zn(II)	20	36.50	0.016	0.174	0.996	1.067	0.696	0.993
	30	38.17	0.017	0.168	0.995	1.011	0.701	0.989
	40	44.44	0.018	0.155	0.994	1.137	0.727	0.993

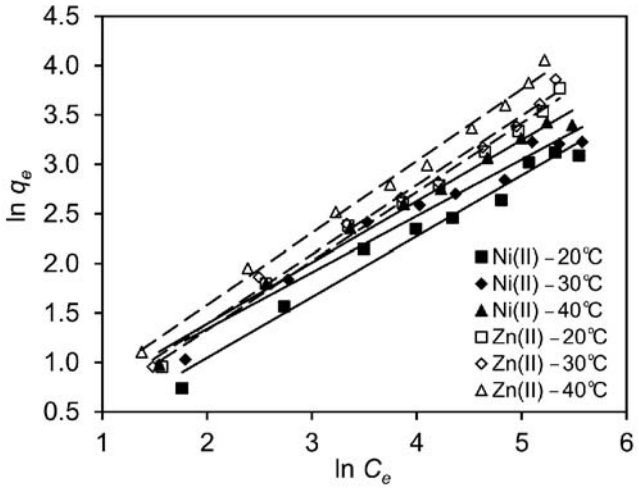

Figure 8. Freundlich isotherm plots for the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* biomass at different temperatures (metal concentration: 100 mg/l, pH: 5.0–6.0, biomass dosage 2 g/l)

from 24.10 to 30.12 mg/g and from 36.50 to 44.44 mg/g, for nickel and zinc ions, respectively. Similar results of biosorption were obtained by Asfaram et al.[19], Özer and Özer[22], Pahlavanzadeh et al.[24], and Chen et al.[35] and Nasernejad et al.[36], using *Y. lipolytica* ISF7, *Saccharomyces cerevisiae*, brown alge *Padina australis*, bacteria *Pseudomonas putida*, and carrot residues as biosorbents, respectively. A much lower maximum sorption capacity was observed by Sari et al.[20] and Celaya et al.[37] who used *Cladonia furcata* and *Thiobacillus ferrooxidans*; the corresponding values were 7.9 mg/g (nickel) and 9.7 mg/g (zinc). On the other hand, a higher biosorption of Ni(II) was observed by Shinde et al.[3] and Suazo-Madrid et al.[31], at 48.3 and 112.9 mg/g, respectively, using *Y. lipolytica* and *Rhodotorula glutinis* as biosorbents. In the case of Zn(II) biosorption, the maximum sorption capacity obtained in the studies by Joo et al.[1] and Li et al.[23] were 83.3 and 75.8 mg/g, respectively, using *Pseudomonas aeruginosa* and *Streptomyces ciscaucasicus*, respectively.

The constant K_L changed in a similar fashion. Its slightly higher values for the biosorption of Ni(II) indicate a higher affinity of biomass for the sorption of these ions, which increased with increasing temperature. The magnitude of K_F and n constants indicated that *Y. lipolytica* had a high adsorption capacity for Ni(II) and Zn(II) ions. Besides, $\frac{1}{n}$ that values of between 0.1 and 1.0 indicate suitable nickel and zinc ions adsorption on anion exchange[11].

Thermodynamic parameters

In this study, the thermodynamic parameters have been calculated using the Langmuir isotherm, i.e. by replacing the equilibrium constant, K_c^o from Eqs. (12) and (14) by the Langmuir isotherm constant K_L, and are given in Table 4. The values of ΔH^o and ΔS^o were calculated from the slope and intercept of the plots of lnK_c^o versus $1/T$ (Fig. 9)[30].

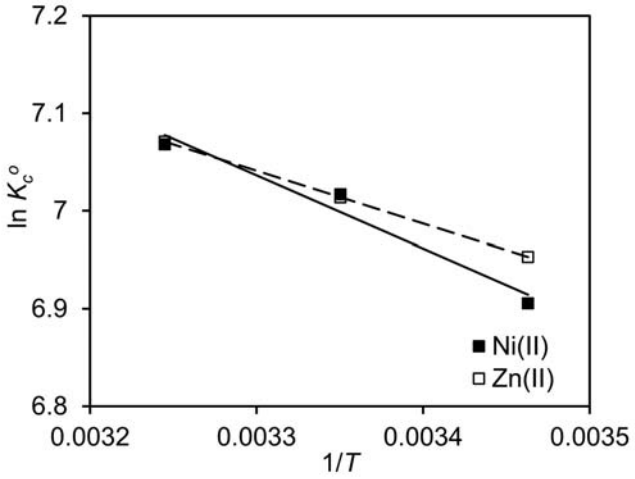

Figure 9. Plot of lnK_c^o vs. 1/T for the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* biomass

The thermodynamic treatment of the sorption data indicates that ΔG^o values were negative at all the temperatures investigated. The negative values of ΔG^o (Table 5) indicate the spontaneous nature of adsorption of metal ion by the biomass and the likelihood of increased spontaneity of the process induced by increased temperature. It is of note that ΔG^o up to –20 kJ/mol are consistent with electrostatic interaction between sorption sites and the metal ion (physical adsorption) while ΔG^o values more negative than –40 kJ/mol involve charge sharing or transfer from the biomass surface to the metal ion to form a coordinate bond (chemical adsorption)[33]. The ΔG^o values obtained in this study for both metal

Table 5. Values of thermodynamic parameters for the biosorption of Ni(II) and Zn(II) by *Y. lipolytica* biomass

Temperature [°C]	Ni(II)				Zn(II)			
	ΔG^o [kJ/mol]	E_A [kJ/mol]	ΔS^o [kJ/mol · K]	ΔH^o [kJ/mol]	ΔG^o [kJ/mol]	E_A [kJ/mol]	ΔS^o [kJ/mol · K]	ΔH^o [kJ/mol]
20	–16.82	11.77	0.079	6.219	–16.94	16.67	0.073	4.490
30	–17.68				–17.67			
40	–18.39				–18.40			

ions are < –20 kJ/mol, indicative that physical adsorption is the predominant mechanism in the sorption process. Asfaram et al.[19] show an analogous mechanism of biosorption of Ni(II), Zn(II) and Co(II) ions using *Y. lipolytica* ISF7 in their study. The positive values ΔH^o (Table 5) of suggests the endothermic nature of Ni(II) and Zn(II) biosorption. The positive values of ΔS^o show that the freedom of metal ions is not too restricted in the biomass confirming a physical adsorption, which is further confirmed by the relatively low values of ΔG^o.

CONCLUSIONS

The obtained results indicate that the optimum parameters of nickel and zinc ion biosorption by the dead biomass of *Yarrowia lipolytica* yeast were as follows: pH at 6.0 and 5.0, and the use of biosorbent at a dose of 2 g/l. Most of the metal ions were adsorbed in the first 20 minutes, and equilibrium was established after 60 minutes. The kinetics of biosorption proceeded in accordance with the pseudo-second order model, suggesting chemisorption in binding the metal ions by the yeast. The good fit of the Weber-Morris model to the experimental data indicates that intraparticle diffusion was not the only limiting stage during biosorption. Increasing the temperature from 20 to 40°C increased the sorption capacity of the biomass, and increased the rate constants. The activation energy values (11.77 and 16.67 kJ/mol) calculated on the basis of kinetic models also suggest the chemical character of the adsorption process. The analysis of the kinetic results revealed that the binding of Ni(II) and Zn(II) ions is a complex and multistage process. The mechanism of biosorption includes both intraparticle diffusion and chemical reactions. The equilibrium of nickel and zinc ion sorption by *Y. lipolytica* was very well described by the Langmuir model (R^2>0.994), and the maximum sorption capacity at 40°C amounted to 30.12 and 44.44 mg/g. FTIR analysis of yeast biomass confirmed the participation of hydroxy, carboxyl, amide and amine groups in the biosorption process, and potential involvement of complexation in the mechanism of binding nickel and zinc. The determined thermodynamic parameters indicate a spontaneous and endothermic nature of the process. Energy ΔG^o values of less than –20 kJ/mol indicate the involvement of physical adsorption in the biosorption of Ni(II) and Zn(II) by the biomass of *Y. lipolytica*. The obtained results confirm the usefulness of the *Y. lipolytica* biomass in the process of zinc and nickel ion biosorption from aqueous solutions. Taking into account the economic and ecological aspects, it seem preferable to use waste biomass obtained during the production of organic acids, sweeteners, or flavor compounds.

LITERATURE CITED

1. Joo, J.H., Hassan, S.H.A. & Oh, S.E. (2010). Comparative study of biosorption of Zn^{2+} by *Pseudomonas aeruginosa* and *Bacillus cereus*. *Int. Biodeter. Biodegr*. 64, 734–741. DOI: 10.1016/j.ibiod.2010.08.007.
2. Mudhoo, A., Garg, V.K. & Wang, S. (2012). Heavy Metals: Toxity and Removal by Biosorption. Lichtfouse, E., Schwarzbauer, J. & Robert, D. (Eds.), *Environmental Chemistry for a Sustainable World: Volume 2: Remediation of Air and Water Pollution* (pp. 379–442). Springer Science+Business Media B.V.
3. Shinde, N.R., Bankar, A.V., Kumar, A.R. & Zinjarde, A.A. (2012). Removal of Ni (II) ions from aqueous solutions by biosorption onto two strains of *Yarrowia lipolytica*. *J. Environ. Manag*. 102, 115–124. DOI: 10.1016/j.jenvman.2012.02.026.
4. Gavrilescu, M. (2010). Biosorption in Environmental Remediation. Fulekar, M.H. (Eds.) *Bioremed. Technol.: Rec. Adv.* (pp. 35–99). Capital Publishing Company. DOI: 10.1007/978-90-481-3678-0_1.
5. Wang, J. & Chen, C. (2009). Biosorbents for heavy metals removal and their future. *Biotechnol. Adv.* 27, 195–226. DOI: 10.1016/j.biotechadv.2008.11.002.
6. Beopoulos, A., Chardot, T. & Nicaud, J.M. (2009). *Yarrowia lipolytica*: A model and a tool to understand the mechanisms implicated in lipid accumulation. *Biochimie* 91, 692–696. DOI: 10.1016/j.biochi.2009.02.004.
7. Bankar, A.V., Kumar, A.R. & Zinjarde, S.S. (2009). Environmental and industrial applications of *Yarrowia lipolytica*. *Appl. Microbiol. Biotechnol*. 84, 847–865. DOI: 10.1007/s00253-009-2156-8.
8. Lanciotti, R., Gianotti, A., Baldi, D., Angrisani, R., Suzzi, G., Mastrocola, D. & Guerzoni, M.E. (2005). Use of *Yarrowia lipolytica* strains for the treatment of olive mill wastewater. *Biores. Technol*. 96, 317–322. DOI: 10.1016/j.biortech.2004.04.009.
9. Lagergren, S. (1898). Zur theorie der sogenannten adsorptiong gelöster stoffe. *Kungliga Svenska Vetenskapsakad. Stockholm: Handlingar. Bihang.* 24(4), 1–39.
10. Ho, Y.S. & McKay, G. (1999). Pseudo-second order model for sorption processes. *Process. Biochem*. 34(5), 451–465.
11. Ertugay, N. & Bayhan, T.K. (2008). Biosorption of Cr(VI) from aqueous solution by biomass of *Agaricus bisporus*. *J. Hazard. Mater.* 154, 432–439. DOI: 10.1016/j.jhazmat.2007.10.070.
12. Weber, W.J. & Morris, J.C. (1963). Intraparticle diffusion during the sorption of surfactants onto activated carbon. *J. Sanit Eng. Div. Am. Soc. Civ. Eng*. 89, 53–61.
13. Blázquez, G., Martín-Lara, M.A., Tenorio, G. & Calero, M. (2011). Batch biosorption of lead(II) from aqueous solutions by olive tree pruning waste: Equilibrium, kinetics and termodynamic study. *Chem. Eng. J.* 168, 170–177. DOI: 10.1016/j.cej.2010.12.059.
14. Langmuir, I. (1918). The adsorption of gases on plane surfaces of glass, mica and platinum. *J. Am. Chem. Soc*. 40(9), 1361–1403**.** DOI: 10.1021/ja02242a004.
15. Freundlich, H.M.F. (1906). Uber Die Adsorption in Losungen. *Zeitschrift For Physikalische Chemie* 57A, 385–470.
16. Lin, Y., Wang, X., Wang, B., Mohamad, O. & Wei, G. (2012). Bioaccumulation characterization of zinc and cadmium by *Streptomyces zinciresistens*, a novel actinomycete. *Ecotox. Environ. Safe*. 77, 7–17. DOI: 10.1016/j.ecoenv.2011.09.016.
17. Yin, H., He, B., Peng, H., Ye, J., Yang, F. & Zhang, N. (2008). Removal of Cr(VI) and Ni(II) from aqueous solution by fused yeast: Study of cations release and biosorption mechanism. *J. Hazard. Mater.* 158, 568–576. DOI: 10.1016/j.hazmat.2008.01.113.
18. Ahmad, M.F., Haydar, S. & Quraishi, A. (2013). Enhancement of biosorption of zinc ions from aqueous solution by immobilized *Candida utilis* and *Candida tropicalis* cells. *Int. Biodeter. Biodegr.* 83, 119–128. DOI: 10.1016/j.ibiod.2013.04.016.
19. Asfaram, A., Ghaedi, M. & Ghezelbash, G.R. (2016). Biosorption of Zn^{2+}, Ni^{2+} and Co^{2+} from water samples onto *Yarowia lipolytica* ISF7 using a response Surface mathodology, and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). *RSC Adv.* 6, 23599–23610. DOI: 10.1039/c5ra27170c.
20. Sari, A., Tuzen, M., Uluözlü, Ö.D. & Soylak, M. (2007). Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (*Cladonia furcata*) biomass. *Biochem. Eng. J.* 37, 151–158. DOI: 10.1016/j.bej.2007.04.007.
21. Liu, Y., Cao, Q., Luo, F. & Chen, J. (2009). Biosorption of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions from aqueous solutions

by pretreated biomass of brown alge. *J. Hazard. Mater.* 163, 931–938. DOI: 10.1016/j.jhazmat.2008.07.046.
22. Özer, A. & Özer, D. (2003). Comparative study of the biosorption of Pb(II), Ni(II) and Cr(VI) ions onto *S. cerevisiae*: determination of biosorption heats. *J. Hazard. Mater.* B100, 219–229. DOI: 10.1016/S0304-3894(03)00109-2.
23. Li, H., Lin, Y., Guan, W., Chang, J., Xu, L., Guo, J. & Wei, G. (2010). Biosorption of Zn(II) by loive and dead cells of *Streptomyces ciscaucasicus* strain CCNWHX 72–14. *J. Hazard. Mater.* 179, 151–159. DOI: 10.1016/j.hazmat.2010.02.072.
24. Pahlavanzadeh, H., Keshtkar, A.R., Safdari, J. & Abadi, Z. (2010). Biosorption of nickel(II) from aqueous solution by brown alge: Equilibrium, dynamic and thermodynamic studies. *J. Hazard. Mater.* 175, 304–310. DOI: 10.1016/j.jhazmat.2009.10.004.
25. Munagapati, V.S., Yarramuthi, V., Nadavala, S.K., Alla, S.R. & Abburi, K. (2010). Biosorption of Cu(II), Cd(II) and Pb(II) by *Acacia leucocephala* bark powder: Kinetics, equilibrium and thermodynamics. *Chem. Eng. J.* 157, 357–365. DOI: 10.1016/j.cej.2009.11.015.
26. Subbaiah, M.V. & Yun, Y.S. (2013). Biosorption of Nickel(II) from Aqueous Solution by the Fungal Mat of *Trametes versicolor* (Rainbow) Biomass: Equilibrium, Kinetics, and Thermodynamic Studies. *Biotechnol. Bioproc. E.* 18, 280–288. DOI: 10.1007/s12257-012-0401-y.
27. Akhtar, K., Akhtar, M.W. & Khalid, A.M. (2008). Removal and recovery of zirconium from its aqueous solution by *Candida tropicalis*. *J. Hazard. Mater*. 156, 108–117. DOI: 10.1016/j.jhazmat.2007.12.002.
28. Baysal, Z., Çinar, E., Bulut, Y., Alkan, H. & Dogru, M. (2009). Equilibrum and thermodynamic studies on biosorption of Pb(II) onto *Candida ablicans* biomass. *J. Hazard. Mater.* 161, 62–67. DOI: 10.1016/j.hazmat.2008,02,122.
29. Witek-Krowiak, A. (2012). Analysis of temperature-dependent biosorption of Cu^{2+} ions on sunflower hulls: Kinetics, equilibrium and mechanizm of the proces. *Chem. Eng. J.* 192, 13–20. DOI: 10.1016/j.cej.2012.03.075.
30. Bueno, B.Y.M., Torem, M.L., Carvalho, R.J., Pino, G.A.H. & Mesquita, L.M.S. (2011). Fundamental aspects of biosorption of lead (II) ions onto a *Rhodococcus oparus* strain for environmental applications. *Miner. Eng*. 24, 1619–1624. DOI: 10.1016/j.mineng.2011.08.018.
31. Suazo-Madrid, A., Morales-Barrera, L., Aranda-García, E. & Cristiani-Urbina, E. (2011). Nickel(II) biosorption by *Rhodotorula glutinis*. *J. Ind. Microbiol. Biot.* 38, 51–64. DOI: 10.1007/s.10295-010-0828-0.
32. Farhan, S.N. & Khadom, A.A. (2015). Biosorption of heavy metals from aqueous solutions by *Saccharomyces cerevisiae*. *Int. J. Ind. Chem.* 6, 119–130. DOI: 10.1007/s40090-015-0038-8.
33. Horsfall, M. & Spiff, A.I. (2005). Effects of temperature on the sorption of Pb^{2+} and Cd^{2+} grom aqueous solution by *Caladium bicolor* (Wild cocoyam) biomass. *Electron. J. Biotech*. 8(2), 162–169. DOI: 10.2225/vol8-issue2-fulltext-4.
34. Usul, G. & Tanyol, M. (2006). Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto *Pseudomonas putida*: Effect of temperature. *J. Hazard. Mater*. B135, 87–93. DOI: 10.1016/j.jhazmat.2005.11.029.
35. Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X. & Chen, Y.X. (2005). Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1. *Colloid Surf. B.* 46, 101–107. DOI: 10.1016/j.colsurfb.2005.10.003.
36. Nasernejad, B., Zadeh, T.E., pour, B.B., Bygi, M.E. & Zamani, A. (2005). Camparison for biosorption modeling of heavy metals (Cr(III), Cu(II), Zn(II)) adsorption from wastewater by carrot residues. *Proces Biochem*. 40, 1319–1322. DOI: 10.1016/j.procbio.2004.06.010.
37. Celaya, R.J., Noriega, J.A., Yeomans, J.H., Ortega, L.J. & Ruiz-Manriquez, A. (2000). Biosorption of Zn(II) by *Thiobacillus ferrooxidans*. *Bioprocess. Eng*. 22, 539–542. DOI: 10.1007/s004499900106.