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Dowex anion exchanger-loaded-baker's yeast as bi-functionalized biosorbents for selective extraction of anionic and cationic mercury(II) species

Mohamed E. Mahmoud [a,*], Amr A. Yakout [b], Maher M. Osman [b]

[a] Faculty of Medicine, Medical Chemistry Department, King Abdullaziz University, P.O. Box 80205, Jeddah 21589, Saudi Arabia
[b] Faculty of Sciences, Chemistry Department, Alexandria University, P.O. Box 426, Alexandria 21321, Egypt

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ABSTRACT

Dowex anion exchanger-immobilized-baker's yeast [*Dae-yeast*] were synthesized and potentially applied as environmental friendly biosorbents to evaluate the up-take process of anionic and cationic mercury(II) species as well as other metal ions. Optimization of mass ratio of Dowex anion exchanger versus yeast (1:1–1:10) in presence of various interacting buffer solutions (pH 4.0–9.0) was performed and evaluated. Surface modification of [*Dae-yeast*] was characterized by scanning electron microscopy (SEM) and infrared spectroscopy. The maximum metal biosorption capacity values of [*Dae-yeast*] towards mercury(II) were found in the range of 0.800–0.960, 0.840–0.950 and 0.730–0.900 mmol g^{-1} in presence of buffer solutions pH 2.0, 4.0 and 7.0, respectively. Three possible and different mechanisms are proposed to account for the biosorption of mercury and mercuric species under these three buffering conditions based on ion exchange, ion pair and chelation interaction processes. Factors affecting biosorption of mercury from aqueous medium including the pH effect of aqueous solutions (1.0–7.0), shaking time (1–30 min) and interfering ions were searched. The potential applications of modified biosorbents for selective biosorption and extraction of mercury from different real matrices including dental filling waste materials, industrial waste water samples and mercury lamp waste materials were also explored. The results denote to excellent percentage extraction values, from nitric acid as the dissolution solvent with a pH 2.0, as determined in the range of 90.77–97.91 ± 3.00–5.00%, 90.00–93.40 ± 4.00–5.00% and 92.31–100.00 ± 3.00–4.00% for the three tested samples, respectively.

1. Introduction

Elemental mercury and mercuric compounds such as mercury salts, short-chain alkyl mercury compounds, alkoxyalkyl-mercury compounds, and phenyl mercury compounds are all known and characterized by different toxicological properties [1]. Mercury is a non biodegradable species that accumulates in different systems including water components as sediments, water streams in lakes, sea and ocean or even waste water [2]. Bioaccumulation and environmental release of mercury species can lead to a number of undergoing complex chemical and physical transformations [3]. Therefore, a higher degree of concern for direct mercury pollution control and minimization in polluted areas must be taken into consideration owing to the growing attention of mercury threat against human health. This can be accomplished via applications of mercury extraction and recovery techniques which are considered as the most important step for control of mercury pollution. In addition, recovery of mercury is also important from the economical point of view due to a wide range of medical, chemical and industrial mercury applications including dental amalgams, anti-fouling paints, electrolysis and electrochemistry, batteries, fluorescent lamps, catalysts as well as many consumer products such as mercury lamps [4–7].

Several methods were recently applied based on organic or inorganic solid phases or liquid–liquid extraction techniques to remove mercury compounds or species from various matrices via normal or selective extraction and preconcentration. The following are some of these recent published papers, mainly focused on the last five years. A solid phase extraction (SPE) and removal of mercury(II) at trace and ultra trace levels was studied by using 1-(2-thiazolylazo)-2-naphthol (TAN) functionalized activated carbon (AC) [8]. A method based on cloud point extraction (CPE) was used for the preconcentration of mercury, after the formation of a complex with 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol (5-Br-PADAP) and analysis by electrothermal atomic absorption spectrometry (ETAAS) [9]. Preparation of diphenylcarbazone-functionalized silica gel for application to on-line selective solid phase extraction and determination of mercury by flow-injection

* Corresponding author.
E-mail address: memahmoud10@yahoo.com (M.E. Mahmoud).

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spectrophotometry was also reported [10]. A method was developed for the preconcentration of mercury based on the adsorption of its diphenylthiocarbazone complex on a neutral alumina column [11]. On-line preconcentration and determination of Hg(II) in water by using Cyanex 923 as a sorbent followed by cold vapor atomic absorption spectrometry was reported [12]. A method for solid phase extraction and analysis of trace mercury in water by using dithizone modified nanometer titanium dioxide and cold vapor atomic absorption spectrometry was reported [13]. 1,5 Diphenylcarbazide (DPC) functionalized sol–gel silica (DPCSG) were prepared and investigated for the solid phase extraction of Hg(II) from aqueous solution by a batch equilibrium technique [14]. Preparation of xylenol orange functionalized silica gel as a selective solid phase extractor and its application for preconcentration and separation of mercury from water was also reported [15]. The extraction behavior of 1,3-dipropyn-2-yl-oxycalix[4]arene towards mercury(II) ion was examined and reported [16]. A method employing dual competitive ligand exchange by application of two competing ligands, diethyldithiolcarbamate (DEDC) and thiosalicylic acid (TSA), followed by solid phase extraction (CLE-SPE) for complex characterization of inorganic Hg(II) in natural waters was described [17]. New polymeric resins with sulfonamide pendant functions were prepared for the selective extraction of mercuric ions from aqueous solutions [18]. A method was described for physical loading of alumina with thiosemicarbazide and its application in selective preconcentration of mercury(II) from natural water samples [19]. The liquid–liquid extraction of mercury(II) from hydrochloric acid solutions using Aliquat 336 (tri-octyl methylammonium chloride) as extractant dissolved in commercial kerosene Exxsol D-80 was investigated and reported [20]. A new room temperature ionic liquid 1-butyl-3-trimethylsilylimidazolium hexafluorophosphate was prepared and used as a solvent for extraction and preconcentration of mercury followed by cold vapor atomic absorption spectrometric analysis [21]. A method was investigated to show the complete transfer of Hg(II) in 1-alkyl-3-methylimidazolium hexafluorophosphate ionic liquid and the quantitative metal ion partition was found to be strongly dependent both on alkyl chain length and on the working temperature [22].

Biological substrates were also found and characterized by their promising capabilities for metal binding, extraction, preconcentration and speciation. Yeast was successfully used and applied in selective biosorption and preconcentration of a number of metals from different matrices [23,24]. The two main types of yeast are baker's and brewer's yeasts both known as *Saccharomyces cerevisiae* and the difference between them is mainly based on the rate of carbon dioxide, sugar consumption and other waste product creation, as well as the temperature and pH needed for fermentation. However, immobilization of yeast on the surface of solid substrate was found to improve their interaction properties with metal ions. The advantages of such immobilization technique versus direct use of yeast, as previously reported [25], are mainly focused on (i) possible high biomass loadings; (ii) controlled particle size of the immobilized biosorbents; (iii) simple and direct separation of the target analyte; (iv) controlled flow rates; (v) minimization of the clogging problems faced in continuous flow systems; (vi) possibility of recycling and re-using the biomass and finally; (vii) potential applications in both batch and column experiments. The following are some recent publications on the applications of some solid-immobilized-yeast biosorbents. A method was reported for separation and speciation of Cr(III) and Cr(VI) with *Saccharomyces cerevisiae* immobilized on sepiolite followed by determination of both species in water by FAAS [26]. The use of yeast immobilized on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron(III), cobalt(II), manganese(II) and chromium(III) was also reported [27]. Brewer's yeast were magnetically modified using water based magnetic fluid stabilized perchloric acid, characterized by different techniques and applied for Hg(II) biosorption-desorption in batch system [28]. Evaluation of *Saccharomyces cerevisiae* baker's yeast immobilized on silica gel for the speciation of Hg(II) and CH_3Hg^+ was reported [29]. A study was made to evaluate *Saccharomyces cerevisiae* as a substrate for the biosorption and selective determination of Cr(III) and Cr(VI) and arsenic species As(III) and As(V) by a mini-column packed with yeast cells-covalently immobilized-controlled pore glass (CPG) [30,31]. A method was reported for on-line determination of Sb(III) and total Sb using baker's yeast immobilized on polyurethane foam and hydride generation inductively coupled plasma optical emission spectrometry [32]. A solid phase extraction procedure by using *Saccharomyces cerevisiae* immobilized on calcium alginate beads was investigated and reported for determination of Pd in road dust [33].

In this paper we describe a method for immobilization of different mass ratios of baker's yeast on the surface of Dowex anion exchanger [*Dae-yeast*] based on direct and simple surface loading procedure and in presence of controlled buffering conditions. The presented work is mainly directed toward the exploitation of selective metal adsorption capabilities of modified [*Dae-yeast*] biosorbents in the process of mercury extraction. This was aimed in order to explore the straight forward applications of these biosorbents for selective mercury-extraction and recovery from three mercury containing real samples as dental filling waste materials, industrial waste water samples and mercury lamp waste materials.

2. Experimental

2.1. Chemicals and materials

Metal salts are all of analytical reagent grade and purchased from BDH Chemical Company, Poole, UK and Fluka Chemie AG, Switzerland. Metal ions solutions were prepared from doubly distilled water (DDW). Dowex anion exchanger in the chloride form was purchased from BDH Chemical Company, Poole, UK. Buffer solutions (pH 1–7) were prepared from 1.0 M hydrochloric acid to which different volumes of 1.0 M sodium acetate were mixed and the pH-value of the resulting solution was adjusted with the use of a pH-meter.

2.2. Yeast biomass

Baker's yeast supplied by a grocery shop was used in this study. The yeast cells were microbiologically identified as a pure culture of *Saccharomyces cerevisiae* by the Department of Microbiology, Faculty of Science.

2.3. Instrumentation

FT-IR spectra were recorded by using a Nicolet 380-spectrophotometer, Thermoelectro Corporation, USA covering the frequency range 400–4000 cm^{-1}. The pH-measurement of metal ions and buffer solutions were carried out with an Orion 420 and the pH-meter was calibrated against standard buffer solutions of pH 4.0 and 9.2. Metal analyses were determined by Flame atomic absorption spectrophotometric measurements using a Shimadzu model AA-6650 atomic absorption spectrophotometer at the appropriate wavelength. Solaar Unicam 969 AA with cold vapor atomic spectrophotometer was used for determination of low concentration levels of mercury. Inductively coupled argon plasma (ICP) (Thermo Iris Intrepid Inductively Coupled Plasma Spectrophotometer) was used for determination of mercury concentrations in the levels of

($\geq 1\ \mu g\ ml^{-1}$). Scanning electron microscope (JSM-5300, JEOL Ltd.) and an ion sputtering coating device (JEOL-JFC-1100E) were used to obtain the surface images of Dowex anion exchanger-loaded-yeast [*Dae-yeast*].

2.4. Synthesis of Dowex anion exchanger-loaded-yeast [Dae-yeast]

[*Dae-yeast-4*(*1:1*)] was synthesized according to the following procedure. A sample of Dowex anion exchanger (10.0 g) in the chloride form was weighed in a porcelain dish. Another 10.0 g-sample of baker's yeast was weighed and added to Dowex anion exchanger to form a mass ratio (1:1). 20.0 ml of acetate buffer solution pH 4 was then added and all are mixed with stirring for 5.0 min until a homogeneous mixture is formed. The reaction mixture was then left to complete dryness in an oven adjusted to 60 °C. The dry mixture was further homogenized with another 15.0 ml portion of buffer solution pH 4.0 for 5.0 more minutes with stirring and left in an oven to complete dryness at 60 °C. These steps were repeated three more times to obtain a completely homogenized biosorbent of Dowex anion exchanger-loaded-yeast with this assigned structure [*Dae-yeast-4*(*1:1*)]. The same synthetic procedures were applied in presence of distilled water (DW) as well as buffer solutions with pH 7.0 and 9.0 to form [*Dae-yeast-DW*(*1:1*)], [*Dae-yeast-7*(*1:1*)] and [*Dae-yeast-9*(*1:1*)] biosorbents, respectively. The above procedures were also used to synthesize other Dowex anion exchanger-loaded-yeast with different mass ratios of (3:4), (1:2), (3:10) and (1:10) under the same buffering conditions (pH 4.0, 7.0 and 9.0) as well as in distilled water

2.5. Effect of biosorbent mass ratio on the metal up-take properties of [Dae-yeast]

The effect of applied mass ratio [1:1, 3:4, 1:2, 3:10 and 1:10] of Dowex anion exchanger versus yeast on the metal biosorption capacity values determined by the newly synthesized [*Dae-yeast-4*], [*Dae-yeast-7*], [*Dae-yeast-9*] and [*Dae-yeast-DW*] with the same mass ratio was studied for Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II) in distilled water according to the following method. Dowex anion exchanger-loaded-yeast, 100 ± 1 mg, was added to 1.0 ml of 0.1 M-metal ion and 9.0 ml of distilled water in a 50 ml measuring flask and automatically shaken for 30 min. The mixture was filtered, washed with 50 ml DW and the unbound metal ion was subjected to complexometric titration using the proper buffer and indicator and/or atomic absorption analysis.

2.6. Effect of pH and shaking time on the metal biosorption properties

The metal biosorption properties of various synthesized Dowex anion exchanger-loaded-yeast [*Dae-yeast*] for a series of metal ions, viz. Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II) as a function of pH and shaking time were studied by the static technique. The effect of pH-procedure was performed according to this method. 100 ± 1 mg of the Dowex anion exchanger-loaded-yeast [*Dae-yeast*] was added to 1.0 ml of 0.1 M-metal ion and 9.0 ml of the selected buffer solution (pH 1–7) in a 50.0 ml measuring flask and automatically shaken for 30 min. After equilibration, the mixture was filtered, washed with 50 ml DDW and the unbound metal ion was subjected to complexometric titration using the proper buffer and indicator and/or atomic absorption analysis.

The effect of shaking time on the percentage extraction of Hg(II), Cu(II) and Zn(II) was also performed by the static technique. In this method, 100 ± 1 mg of Dowex anion exchanger-loaded-yeast phase was mixed with 1.0 ml of 0.1-M of the selected metal ion and 9.0 ml of buffer pH 7.0 and shaken by an automatic shaker for the selected period of time (1, 5, 10, 20 and 25 min). This mixture was then filtered, washed with 50 ml DDW and the free metal ion was determined as described above.

2.7. Determination of the distribution coefficient and percentage extraction of Hg(II) in presence of interfering ions

100 ± 1 mg of Dowex anion exchanger-loaded-yeast was added to a 50 ml of Hg(II) ($1\ \mu g\ ml^{-1}$) prepared in buffer solution pH 2.0 and distilled water. In these tested samples, the concentration of interfering anions, chloride, nitrate and acetate as well as interfering cations, sodium, potassium and calcium are $1000.0\ mg\ L^{-1}$ each. The mixture was shaken for 30.0 min by an automatic shaker, filtered, washed with water and the filtrate was completed to 100 ml and acidified with 10.0%-concentrated hydrochloric acid solution. Standard and blank solutions were also prepared in a similar way.

2.8. Applications of [Dae-yeast] for selective extraction of mercury from real samples

New modified [*Dae-yeast*] biosorbents were applied for selective extraction of mercury from real sample that are known for the presence of mercury as an effective constituent. The first sample is a dental filling waste material. The extraction procedure was accomplished according to the following method. A sample of 2.5864-g of dental filling waste was dissolved in concentrated nitric acid and the volume was completed to 1.0 L. 40.0 ml of this solution was further diluted to 1.0 L, adjusted to a pH 2.0 and measured by ICP to identify the correct concentration. The latter sample solution was passed over a micro-column packed with 100.0 ± 1.0 mg of modified [*Dae-yeast*] biosorbent with a flow rate of $10.0\ ml\ min^{-1}$. The effluent solution was then subjected to analysis by ICP. The second sample is a waste water from a textile industrial factory. A sample of 250.0 ml of this industrial waste was acidified with concentrated nitric acid to a pH value 2.0. This solution was passed over a mico-column packed with 100.0 ± 1.0 mg of modified [*Dae-yeast*] biosorbent with a flow rate of $10.0\ ml\ min^{-1}$ and the collected effluent solution was then subjected to analysis by ICP. The third sample is a broken mercury lamp in concentrated nitric acid. A sample of 50.0 ml of this solution was diluted to 1.0 L, adjusted to a pH 2.0 and the concentration of mercury was determined by cold vapor atomic absorption spectrophotometry (CV-AA). This volume solution was allowed to pass over a micro-column packed with 100.0 ± 1.0 mg of modified [*Dae-yeast*] biosorbent with a flow rate of $10.0\ ml\ min^{-1}$ and the collected effluent solution was then subjected to analysis by (CV-AA).

3. Results and discussion

3.1. Characterization of surface modification

Scanning electron microscope (SEM) analysis of modified [*Dae-yeast*] biosorbent was performed to compare the surface image of Dowex anion exchanger before and after surface loading of yeast. Fig. 1(i) represents the SEM-image of surface free Dowex anion exchanger showing a homogeneous distribution of spherical and symmetrical particles with particle size in the range of few micrometers. The shape of [*Dae-yeast-4*(*1:1*)] biosorbent has been changed upon loading of yeast as shown in Fig. 1(ii) with the same magnification order with Fig. 1(i). Fig. 1(iii) represents SEM-image of modified [*Dae-yeast-4*(*1:1*)] biosorbent under

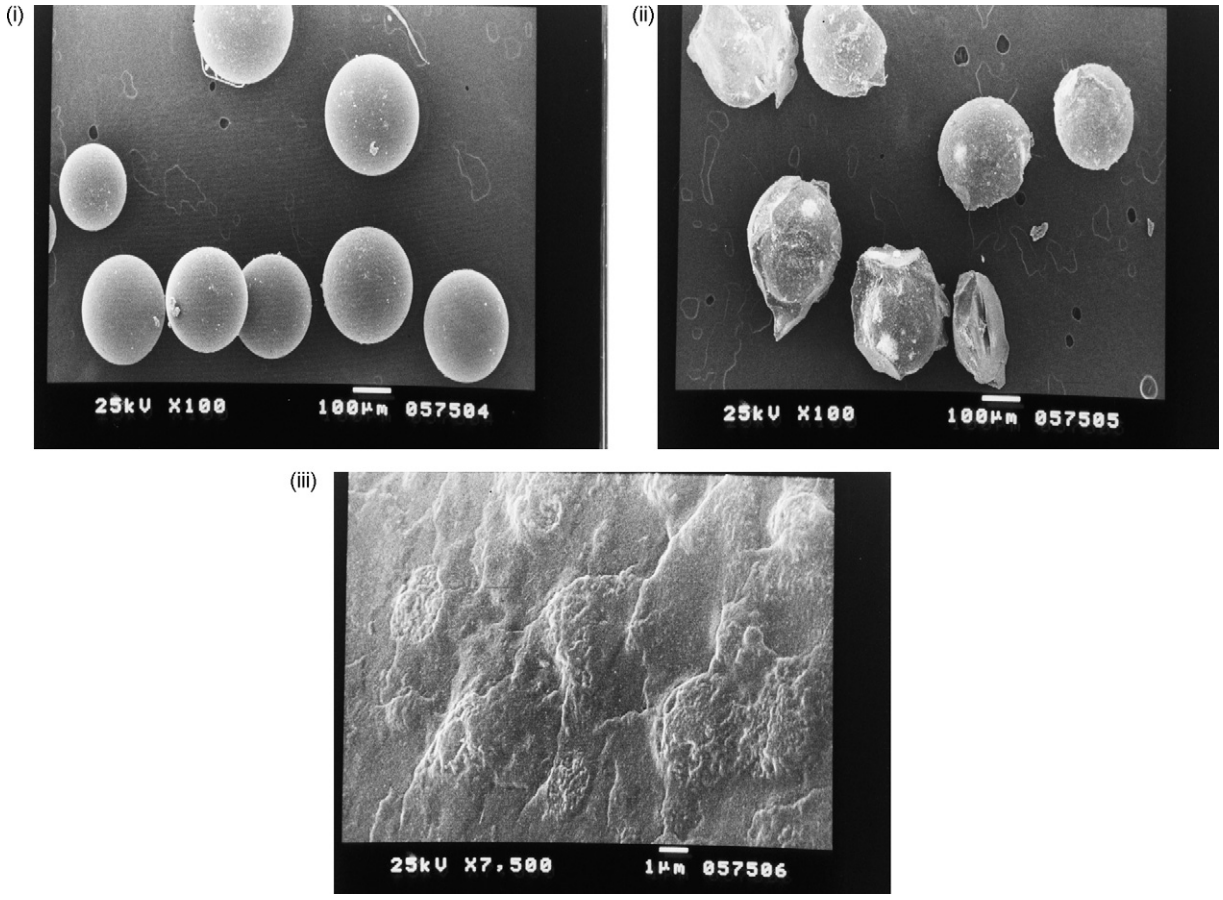

Fig. 1. SEM of Dowex anion exchanger-immobilized-baker's yeast [*Dae-yeast*]. (i) SEM of untreated Dowex anion exchanger magnified 100×; (ii) SEM of [*Dae-yeast-4*(*1:1*)] biosorbent magnified 100×; (iii) SEM of [*Dae-yeast-4*(*1:1*)] biosorbent magnified 7500×.

magnification of 7500 times and clearly shows a homogenous distribution of yeast on the surface of Dowex anion exchanger particles.

The loading percentage of yeast on Dowex anion exchanger for all newly synthesized [*Dae-yeast*] biosorbents were determined based on mass ratio of the surface adsorbed yeast to the added mass in the immobilization procedures. In any buffering condition and for all [*Dae-yeast* (*1:1*)] biosorbents, the loading percentage values were identified in the range of 86–91%. The other loading percentage values were found in the range of 83–90%, 71–75%, 57–63, and 30–35% for all [*Dae-yeast* (*3:4*)], [*Dae-yeast* (*1:2*)], [*Dae-yeast* (*3:10*)] and [*Dae-yeast* (*1:10*)], respectively.

The FT-IR spectra of Dowex anion exchanger, yeast and Dowex anion exchanger-loaded yeast [*Dae-yeast-4* (*1:1*)] biosorbents are shown in Fig. 2 (i–iii). The FT-IR spectra were found to prove the presence of a wide absorption band at 3700–2990 cm^{-1} that may be correlated to the contributions of OH stretching in carbohydrates around 3400 cm^{-1}, NH stretching in proteins and peptides around 3300 and 3080 cm^{-1} and the amide overtone at around 3100 cm^{-1} as previously reported [34]. The absorption region of 2990–2820 cm^{-1} is mainly assigned to lipids and the minor absorption at 2890 cm^{-1} is probably includes the contribution from proteins and peptides. The absorption bands at 1740 cm^{-1}, 1670 cm^{-1} and 1390 cm^{-1} is related to C=O stretching in lipid esters, vibrations of different protein structures and COO^- symmetric stretching in proteins, respectively. The absorption band at 1200 cm^{-1} is mainly related to C—O—C in carbohydrates as previously reported [34].

3.2. Effect of mass ratio on the metal biosorption capacity values

The metal biosorption capacity values expressed in mmol g^{-1} for a series of divalent metal ions, viz. Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II) are compiled in Table 1. These values were determined in distilled water as the contact solution to evaluate the capability of all newly synthesized mass ratios of [*Dae-yeast*] biosorbents to bind and extract the five studied metal ions. It is evident from the listed values that Hg(II) is the most highly extracted metal ion by all of these modified [*Dae-yeast*] biosorbents. The highest mmol g^{-1} biosorption values of Hg(II) were found in the range of 0.847–0.872, 0.931–0.989 and 0.906–970 for [*Dae-yeast*] with mass ratio of (1:1), (3:4) and (1:2) in presence of buffer pH 4.0, 7.0 and distilled water, respectively. However, 0.980, 0.994, 1.009 and 1.078 mmol g^{-1} of Hg(II)-biosorption values were identified for [*Dae-yeast-4*(*1:10*)], [*Dae-yeast-7*(*1:10*)], [*Dae-yeast-DW*(*1:10*)] and [*Dae-yeast-9*(*1:10*)], respectively. The application of buffer pH 9.0 in the synthesis procedure of [*Dae-yeast*] biosorbents was found to show strong effect on minimizing the ability of loaded yeast to bind with the interacting metal ions except in the case of (1:10) mass ratio. The identified mmol g^{-1} values were characterized as 0.455, 0.436, 0.558, 0.460 and 1.078 for [*Dae-yeast-9*(*1:1*)], [*Dae-yeast-9*(*3:4*)], [*Dae-yeast-9*(*1:2*)], [*Dae-yeast-9*(*3:10*)] and [*Dae-yeast-9*(*1:10*)], respectively. One can easily conclude that [*Dae-yeast*] biosorbents with the mass ratio of (1:10) is the highest one in the processes of metal extraction and biosorption. However, the other four mass ratios of synthesized [*Dae-yeast*] biosorbents are experienced with excellent uptake properties judging from the listed values in Table 1.

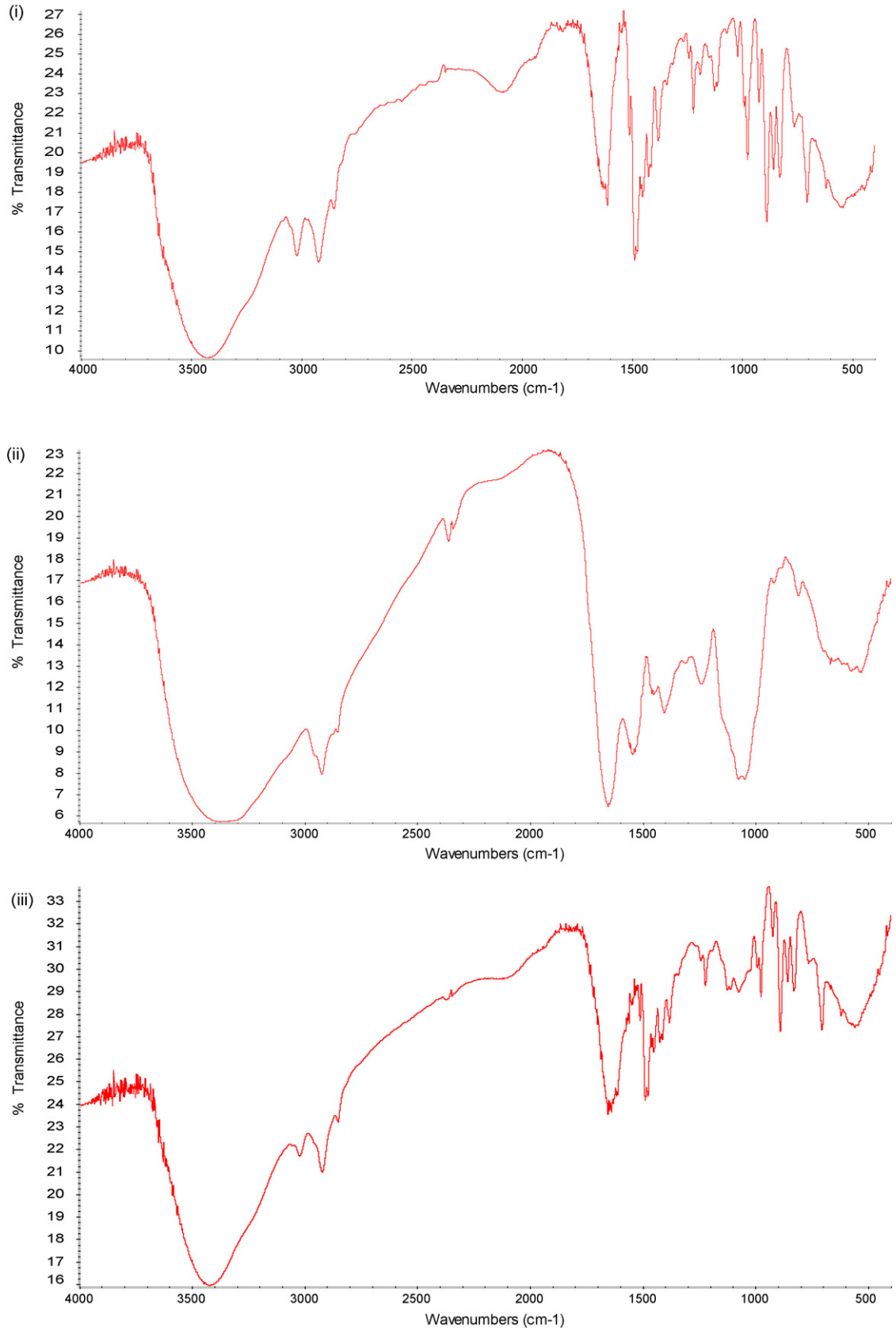

Fig. 2. FT-IR Spectra of (i) Dowex anion exchanger; (ii) yeast; (iii) Dowex anion exchanger-loaded-yeast [*Dae-yeast-4(1:1)*] biosorbent.

Table 1
Effect of mass ratio in synthesized [*Dae-yeast*] on the metal biosorption capacity values.

Phase	Metal Capacity in mmol g^{-1}				
	Cd^{2+}	Hg^{2+}	Pb^{2+}	Cu^{2+}	Zn^{2+}
[*Dae-yeast-4(1:1)*][a]	0.171	0.872	0.225	0.186	0.014
[*Dae-yeast-DW(1:1)*][b]	0.073	0.872	0.240	0.215	0.039
[*Dae-yeast-7(1:1)*][c]	0.142	0.847	0.196	0.245	0.088
[*Dae-yeast-9(1:1)*][d]	0.060	0.455	0.107	0.127	0.034
[*Dae-yeast-4(3:4)*]	0.156	0.931	0.186	0.186	0.034
[*Dae-yeast-DW(3:4)*]	0.191	0.955	0.196	0.196	0.073
[*Dae-yeast-7(3:4)*]	0.161	0.989	0.186	0.210	0.078
[*Dae-yeast-9(3:4)*]	0.078	0.436	0.098	0.132	0.034
[*Dae-yeast-4(1:2)*]	0.215	0.955	0.151	0.176	0.034
[*Dae-yeast-DW(1:2)*]	0.200	0.970	0.171	0.191	0.029
[*Dae-yeast-7(1:2)*]	0.176	0.906	0.161	0.210	0.063
[*Dae-yeast-9(1:2)*]	0.063	0.558	0.098	0.117	0.063
[*Dae-yeast-4(3:10)*]	0.245	0.955	0.127	0.142	0.034
[*Dae-yeast-DW(3:10)*]	0.264	1.014	0.127	0.156	0.063
[*Dae-yeast-7(3:10)*]	0.200	0.989	0.127	0.132	0.063
[*Dae-yeast-9(3:10)*]	0.122	0.460	0.098	0.107	0.073
[*Dae-yeast-4(1:10)*]	0.284	0.980	0.098	0.083	0.044
[*Dae-yeast-DW(1:10)*]	0.279	0.994	0.102	0.107	0.073
[*Dae-yeast-7(1:10)*]	0.240	1.009	0.073	0.093	0.073
[*Dae-yeast-9(1:10)*]	0.107	1.078	0.098	0.098	0.073

[a] [*Dae-yeast-4(1:1)*] means modified phase in presence of buffer solution pH 4.0 with mass ratio of (1:1) for Dowex anion exchanger versus yeast.
[b] [*Dae-yeast-DW(1:1)*] means modified phase in presence of distilled with mass ratio of (1:1) for Dowex anion exchanger versus yeast.
[c] [*Dae-yeast-7(1:1)*] means modified phase in presence of buffer solution, pH 7.0 with mass ratio of (1:1) for Dowex anion exchanger versus yeast.
[d] [*Dae-yeast-9(1:1)*] means modified phase in presence of buffer solution, pH 9.0 with mass ratio of (1:1) for Dowex anion exchanger versus yeast.

The metal biosorption values of the other tested metal ions are low compared to Hg(II) indicating excellent selectivity characters incorporated into [*Dae-yeast*] biosorbents toward mercury. The mmol g^{-1} of Pb(II)-biosorption values were identified in the range of 0.107–0.240, 0.098–0.196, 0.098–0.171, 0.098–0.0.127 and 0.098–0.102 for various synthesized [*Dae-yeast*] biosorbents with mass ratios of (1:1), (3:4), (1:2) and (1:10), respectively. The same interaction trends between Pb(II) and modified biosorbents, as previously outlined for Hg(II), can be noticed concerning the high and low mmol g^{-1} of Pb(II)-biosorption values. Cu(II), Zn(II) and Cd(II) were also found to behave in their interaction processes with all newly modified [*Dae-yeast*] biosorbents in the same manner as described in the case of Pb(II).

3.3. Metal biosorption capacity values in various buffer solutions

The effect of buffer solutions, pH 1.0–7.0, on the metal biosorption properties of various synthesized Dowex anion exchanger-loaded-yeast [*Dae-yeast*] biosorbents for the same series of metal ions, viz. Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II) as a function of pH and shaking time were studied by the static technique. Four [*Dae-yeast*] biosorbents with mass ratios of (1:1) were selected to perform this study and the results are compiled in Table 2. The metal biosorption capacity values of the four tested metal ions viz. Pb(II), Cd(II), Zn(II) and Cu(II) are mainly dependent on the pH value of the contact solution. These metal ions were found to exhibit their minimum mmol g^{-1} values in low buffer solutions with pH in the range of 1.0-2.0 and their maximum values in buffer solutions with higher pH 6.0–7.0. These metal biosorption values prove that the interacting metal ions are directly coordinated via complex formation with some functional groups containing donor atoms such as amino or imide, hydroxyl, carbonyl and carboxylic NH, OH, C=O.

The simple comparison of metal biosorption capacity values determined for Hg(II) with these four tested [*Dae-yeast*] biosorbents versus those listed for other examined metal ions refers to the strong variation in the behavior of mercury for binding with these newly modified biosorbents. It is evident from Table 2 that Hg(II) is highly extracted by [*Dae-yeast-4(1:1)*] with maximum metal up-take of 0.960 mmol g^{-1} in buffer solution pH 2.0. The other three tested biosorbents, [*Dae-yeast-DW(1:1)*], [*Dae-yeast-7(1:1)*] and [*Dae-yeast-9(1:1)*], were also characterized by similar trends as their highest mmol g^{-1} values determined in buffer solutions pH 2.0–4.0. In addition, The other contact buffer solutions were also found to assist the processes of Hg(II)-extraction and uptake by the four tested biosorbents as listed in Table 2. To account for such abnormal behavior of the tested [*Dae-yeast*] biosorbents for binding with Hg(II), one must considers that newly modified biosorbents are characterized by the presence of bi-functionalized characters and these are mainly based on the presence of active functional groups of the yeast, as described in the infrared section, that are acting and behaving as cation exchangers or chelators as

Table 2
Metal biosorption capacity values (mmol g^{-1})[a] in various buffer solutions.

Biosorbent	Metal biosorption capacity (mmol g^{-1}) in various buffer solutions						
	1	2	3	4	5	6	7
[*Dae-yeast-4(1:1)*] *Hg*	0.924	0.960	0.940	0.950	0.942	0.922	0.900
[*Dae-yeast-DW(1:1)*] *Hg*	0.916	0.940	0.960	0.950	0.938	0.950	0.900
[*Dae-yeast-7(1:1)*] *Hg*	0.688	0.860	0.870	0.850	0.796	0.802	0.755
[*Dae-yeast-9(1:1)*] *Hg*	0.654	0.800	0.840	0.840	0.770	0.790	0.730
[*Dae-yeast-4(1:1)*] *Pb*	0.000	0.040	0.095	0.175	0.130	0.180	0.195
[*Dae-yeast-DW(1:1)*] *Pb*	0.000	0.035	0.130	0.160	0.165	0.170	0.240
[*Dae-yeast-7(1:1)*] *Pb*	0.000	0.055	0.065	0.125	0.115	0.115	0.145
[*Dae-yeast-9(1:1)*] *Pb*	0.000	0.040	0.075	0.090	0.100	0.100	0.155
[*Dae-yeast-4(1:1)*] *Cd*	0.235	0.185	0.085	0.070	0.090	0.105	0.030
[*Dae-yeast-DW(1:1)*] *Cd*	0.255	0.155	0.090	0.000	0.050	0.095	0.005
[*Dae-yeast-7(1:1)*] *Cd*	0.225	0.145	0.085	0.060	0.050	0.080	0.020
[*Dae-yeast-9(1:1)*] *Cd*	0.240	0.140	0.080	0.038	0.065	0.050	0.055
[*Dae-yeast-4(1:1)*] *Cu*	0.010	0.030	0.080	0.100	0.100	0.150	0.225
[*Dae-yeast-DW(1:1)*] *Cu*	0.040	0.070	0.040	0.080	0.120	0.180	0.270
[*Dae-yeast-7(1:1)*] *Cu*	0.040	0.020	0.080	0.080	0.110	0.170	0.240
[*Dae-yeast-9(1:1)*] *Cu*	0.030	0.060	0.060	0.110	0.120	0.170	0.210
[*Dae-yeast-4(1:1)*] *Zn*	0.055	0.065	0.060	0.185	0.015	0.085	0.160
[*Dae-yeast-DW(1:1)*] *Zn*	0.035	0.050	0.045	0.045	0.040	0.075	0.065
[*Dae-yeast-7(1:1)*] *Zn*	0.045	0.055	0.055	0.030	0.010	0.085	0.150
[*Dae-yeast-9(1:1)*] *Zn*	0.075	0.105	0.055	0.040	0.015	0.110	0.065

[a] Average values based on triplicate analysis with ±0.005.

well as the anion exchange character due to the presence of chloride moiety in the structure of Dowex anion exchanger. These double characters of already modified [*Dae-yeast*] biosorbents can lead to three different proposed binding processes with Hg(II).

The first proposed mechanism is typically based on anion exchange phenomenon and this can be used to account for the high and strong affinity of Hg(II) toward [*Dae-yeast*] biosorbents in lower pH buffer solutions (1.0–3.0). These buffer solutions are very rich in [Cl^-] ion based on the method of buffer preparation from 1.0 M hydrochloric acid adjusted to the appropriate pH with 1.0 M sodium acetate. This chloride rich medium favors the formation of Hg(II) chloroanionic species in the form of [$HgCl_3{}^-$] and [$HgCl_4{}^{2-}$] which are then subjected to exchange by the negatively charged chloride ion as a part of Dowex anion exchanger. The formation of [$HgCl_3{}^-$] and [$HgCl_4{}^{2-}$] species are in favor of anion exchange mechanism according to the following Eqs. (1)–(4) [19,35,36]. The second proposed mechanism may be suggested to account for the high affinity and selectivity of [*Dae-yeast*] biosorbents toward binding and extraction of Hg(II) in buffer solutions (pH 4.0–5.0) assuming a direct interaction of mercuric chloride with chloride moiety of Dowex anion exchanger to form this ion pair species [*Dae$^+$ [HgCl$_3$]$^-$-yeast*] as given in Eq. (5). The third proposed mechanism is mainly based on the direct chelation process between the active surface functional groups of the yeast with Hg(II) that is favored in higher pH buffer solutions (6.0–7.0) according to Eq. (6).

$$2HgCl_2 + 3Cl^- \rightarrow [HgCl_4]^{2-} + [HgCl_3]^- \quad (1)$$

$$[Dae^+Cl^-\text{-yeast}] + [HgCl_3]^- \rightarrow [Dae^+[HgCl_3]^-\text{-yeast}] + Cl^- \quad (2)$$

$$[Dae^+Cl^-\text{-yeast}] + [HgCl_4]^{2-} \rightarrow [Dae^+[HgCl_4]^{2-}\text{-yeast}] + Cl^- \quad (3)$$

The possible binding process reaction in Eqs. (1) and (2) are in favor of the interaction of the negatively charged mercury species [$HgCl_3$]$^-$ with the positively charged quaternary ammonium moiety of the resin.

$$2[Dae^+Cl^-\text{-yeast}] + [HgCl_4]^{2-} \rightarrow [Dae^+\text{-yeast}]_2[HgCl_4]^{2-} + 2Cl^- \quad (4)$$

The reaction Eq. (4) shows that a possible replacement of two chloride ions with anionic mercury species [$HgCl_4$]$^{2-}$.

$$[Dae^+Cl^-\text{-yeast}] + [HgCl_2] \rightarrow [Dae^+[HgCl_3]^-\text{-yeast}] \quad (5)$$

The binding process of [Dae$^+$ Cl^--yeast] and [$HgCl_2$] in Eq. (5) is mainly based on the direct reaction of [$HgCl_2$] and chloride ion to form the anionic species [$HgCl_3$]$^-$ that replaces the chloride ion into the Dowex molecular part.

$$[\text{Dae-yeast}] + [HgCl_2] \rightarrow [\text{Dae-yeast} \rightarrow HgCl_2] \quad (6)$$

The binding interaction of [$HgCl_2$] to [*Dae-yeast*] in this representation is directly related to coordinate bond formation with yeast functional groups that carry donor atoms as nitrogen, sulfur or oxygen.

These three proposed mechanisms can be used to account for the high selectivity characters incorporated into newly modified [*Dae-yeast*] biosorbents toward selective binding and extraction of mercury species from any solution either acidic, slight acidic or neutral one. The outlined conclusions that may be drown from such study is that by simple selection of the pH-value of contact solution, one can easily separate and isolate Hg(II)-species from other interfering metal ions as Cu(II), Zn(II), Cd(II) and Pb(II). It is also expected to obtain excellent percentage extraction values of Hg(II) in presence of these interfering metal ions if the pH value of contact solution is adjusted to the range of 1.0–3.0.

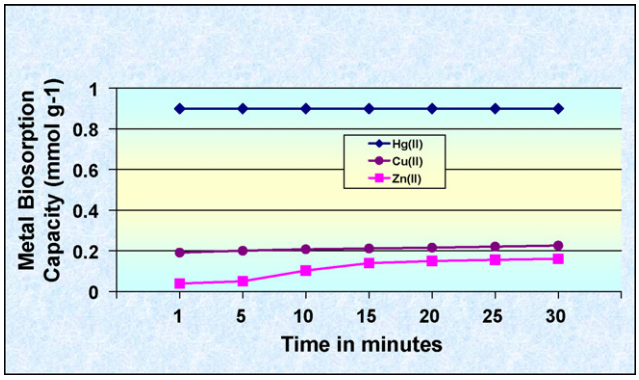

Fig. 3. Effect of shaking time on the metal biosorption capacity of [*Dae-yeast-4(1:1)*] in buffer pH 7.0.

3.4. Effect of shaking time on the metal biosorption capacity values

The effect of shaking time as an important factor in the process of metal extraction by newly modified biosorbents was also studied to evaluate the minimum time to attain maximum metal capacity values [37,38]. The metal biosorption capacity values were determined for three selected metal ions, viz. Hg(II), Cu(II) and Zn(II) under the same buffer solution (pH 7.0) and the selected shaking time intervals are 1.0, 5.0, 10.0, 15.0, 20.0 and 25.0 min. In the case of Hg(II)-extraction by [*Dae-yeast-4(1:1)*], 1.0 min of shaking time was found to afford a complete 100.0% extraction. However, other time values of shaking were found to give the same result. Thus, a range of 1.0–5.0 min can be considered as the optimum minimum time for obtaining a complete and excellent recovery of Hg(II) by [*Dae-yeast-4(1:1)*]. The same results were also collected from the study of shaking time effect on the Hg(II)-biosorption by [*Dae-yeast-DW(1:1)*] indicating a similar behavior of this biosorbent with [*Dae-yeast-4(1:1)*] as previously identified in Sections 3.2 and 3.3. [*Dae-yeast-7(1:1)*] and [*Dae-yeast-9(1:1)*] were found to behave similarly in their binding processes of Hg(II) under the effect of shaking time. The identified percentage extraction after 1.0 min shaking time were found as 88.2% and 95.3%, while the corresponding values after 5.0 min were identified as 94.7% and 100.0% for [*Dae-yeast-7(1:1)*] and [*Dae-yeast-9(1:1)*], respectively.

In the case of Cu(II)-extraction by the same four biosorbents, the maximum percentage extraction values were characterized as 91.1% (at 10.0 min), 75.9% (at 10.0 min), 83.8% (at 5.0 min), 75.4% (at 10.0 min) for [*Dae-yeast-4(1:1)*] [*Dae-yeast-DW(1:1)*], [*Dae-yeast-7(1:1)*] and [*Dae-yeast-9(1:1)*], respectively. Thus, Cu(II)-extraction by these four biosorbents are considered to be relatively slow processes if compared with Hg(II)-extraction under the same experimental conditions of shaking time. The same trends of slow kinetics were also identified in the extraction processes of Zn(II)

Table 3
Distribution coefficient and percentage extraction of Hg(II).

Biosorbent	Contact medium	log K_d	% extraction[a]
[*Dae-yeast-4(1:1)*]	pH 2	3.806	93 ± 2
[*Dae-yeast-DW(1:1)*]	pH 2	3.788	94 ± 3
[*Dae-yeast-7(1:1)*]	pH 2	3.838	93 ± 2
[*Dae-yeast-4(1:1)*]	pH 2	3.722	91 ± 2
[*Dae-yeast-DW(1:1)*]	DW	3.682	91 ± 3
[*Dae-yeast-DW(1:1)*]	DW	3.798	93 ± 2
[*Dae-yeast-7(1:1)*]	DW	3.506	87 ± 4
[*Dae-yeast-9(1:1)*]	DW	3.613	87 ± 3

[a] Values are based on triplicate analysis and in presence of 1000.0 mg L^{-1} each of interfering chloride, nitrate, acetate, sodium, potassium and calcium.

Table 4
Selective extraction of mercury from real samples via micro-column applications.

Sample	Biosorbent	Sample volume (ml)	Initial sample concentration ($\mu g\,ml^{-1}$)	Final effluent concentration ($\mu g\,ml^{-1}$)	Extracted Hg(II) ($\mu g\,ml^{-1}$)	Percent extraction[a]
Dental filling waste	[*Dae-yeast-4*(*1:1*)]	1000	3.500	0.073	3.427	98 ± 3%
	[*Dae-yeast-DW*(*1:1*)]		3.500	0.323	3.177	91 ± 5%
	[*Dae-yeast-7*(*1:1*)]		3.500	0.181	3.319	95 ± 4%
	[*Dae-yeast-9*(*1:1*)]		3.500	0.102	3.398	97 ± 3%
Textile waste water	[*Dae-yeast-4*(*1:1*)]	250	2.000	0.132	1.868	93 ± 4%
	[*Dae-yeast-DW*(*1:1*)]		2.000	0.200	1.800	90 ± 5%
	[*Dae-yeast-7*(*1:1*)]		2.000	0.180	1.820	91 ± 4%
	[*Dae-yeast-9*(*1:1*)]		2.000	0.181	1.819	91 ± 5%
Mercury lamp waste	[*Dae-yeast-4*(*1:1*)]	1000	0.065	0.000	0.000	100 ± 3%
	[*Dae-yeast-DW*(*1:1*)]		0.065	0.004	0.061	94 ± 3%
	[*Dae-yeast-7*(*1:1*)]		0.065	0.005	0.060	92 ± 4%
	[*Dae-yeast-9*(*1:1*)]		0.065	0.005	0.060	92 ± 3%

[a] Values are based on triplicate analysis.

by these four biosorbents. The range of 10.0–20.0 min of shaking time was estimated to provide between 70% and 80%—extraction. In addition 25.0 min of shaking was found to give incomplete Zn(II)-extraction by the studied biosorbents, [*Dae-yeast-4*(*1:1*)] [*Dae-yeast-DW*(*1:1*)], [*Dae-yeast-7*(*1:1*)] and [*Dae-yeast-9*(*1:1*)]. A representation of the effect of shaking time on the metal biosorption capacity values is shown in Fig. 3.

Thus, one can conclude from such study that the four biosorbents are highly selective for Hg(II) under the condition of 1.0–5.0 min as contact time and by selection of the appropriate time one can also be able to experimentally separate and isolate mercury from other interfering ions.

3.5. Determination of the distribution coefficient K_d and percentage extraction of Hg(II) in presence of interfering anions and cations

Determination of the distribution coefficient and calculation of the percentage extraction values for the metal biosorption by newly modified biosorbents are other representations of binding and interaction processes when low concentration levels of metal ions are the case [39,40]. The presence of interfering common anions as chloride, nitrate and acetate as well as common cations as sodium, potassium and calcium can provide more details about the capability of various modified [*Dae-yeast*] biosorbents for selective extraction of Hg(II). Selection of these interfering anions or cations is aimed owing to their expected existence in real water samples as ground, lake, drinking and non-drinking, sea and waste water samples. The concentration of Hg(II) was adjusted in the range of $1.0\,\mu g\,ml^{-1}$ in buffer pH 2.0 and DW as the selected two contact solutions. The concentration of interfering anions is $1000.0\,mg\,L^{-1}$ each and the concentration of interfering cations is also $1000.0\,mg\,L^{-1}$ each. The results of this study as determined by the four modified biosorbents [*Dae-yeast-4*(*1:1*)], [*Dae-yeast-DW*(*1:1*)], [*Dae-yeast-7*(*1:1*)] and [*Dae-yeast-9*(*1:1*)] are compiled in Table 3. The percentage extraction values of Hg(II) in presence of pH 2.0, as the extraction contact medium, determined by the four interacting biosorbents are in the range of 91.33–93.23% ± 2.00–3.00%. The highest extraction values are related to [*Dae-yeast-4*(*1:1*)] and [*Dae-yeast-DW*(*1:1*)] biosorbents and referring to similar trends as discussed and reported in the previous sections. One can conclude that the presence of interfering anions or cations showed insignificant effects in such extraction step owing to similarity in the calculated values of $\log K_d$ and percentage extraction. On the other hand, application of DW as the extraction contact medium was found to give also excellent percentage extraction values of 90.59 ± 3.00% and 92.63 ± 2.00% for [*Dae-yeast-4*(*1:1*)] and [*Dae-yeast-DW*(*1:1*)] biosorbents, respectively. The lowest extraction values of Hg(II) were characterized in the case of [*Dae-yeast-7*(*1:1*)] and [*Dae-yeast-9*(*1:1*)] with 86.51 ± 4.00% and 87.14 ± 3.00%, respectively. Such two low percentage extraction values of mercury may be due to either less selectivity of these two phases for Hg(II) (Section 3.2) or the presence of interfering anions or cations.

3.6. Application of modified biosorbents for selective extraction of Hg(II) from real samples

In order to evaluate the validity of their uses and applications in a direct extraction procedure of Hg(II) from real mercury containing samples, modified [*Dae-yeast*] biosorbents were further used as a packing materials in extraction micro-column applications. Three real matrices were selected to conduct this evaluation step including dental filling waste materials, waste water samples from a textile industrial factory waste materials and mercury lamp waste materials. These samples were treated with concentrated nitric acid and diluted to the volume and concentration that set-up and required by the linear dynamic range of the instrumentation. A micro-column packed with 100 mg of modified biosorbent was used in this extraction procedure. The results of the percentage extraction and recovery values of these three samples are compiled in Table 4. The identified concentration values for these three treated and measured waste samples are 3.500, 2.000 and $0.065\,\mu g\,ml^{-1}$ for dental filling waste, textile industrial waste water and mercury lamp waste materials, respectively. Excellent percentage extraction values were identified and listed in Table 4 and present a good agreement with the previous sections. The dental filling waste as the highest Hg(II)-concentration in the examined sample solution was found to exhibit percentage extraction values in the range of 90.77–97.91 ± 3.00–5.00%. The percentage extraction results of the examined industrial waste water and mercury lamp waste materials were characterized as 90.00–93.40 ± 4.00–5.00% and 92.31–100.00 ± 3.00-4.00%, respectively. The collected results of this extraction procedure are directly referring to the potential applications of newly modified Dowex anion exchangers-loaded-yeast biosorbents for removal and extraction of Hg(II) from any real sample.

4. Conclusion

The presented work affords a number of environmental friendly Dowex anion exchangers-loaded-yeast biosorbents with different mass ratios based on direct loading procedures. Modified biosorbents are all characterized by high incorporated selectivity toward extraction and removal of mercury species from the studied

solutions in presence of any buffering condition in the pH range 1.0–7.0. The excellent capability of these modified biosorbents for extraction and removal of very low or high concentration levels of mercury from contaminated samples, matrices and wastes with mercury species can extend their potential applications for treatment of mercury polluted areas as well as recovery and control of mercury spillage accidental and environmental problems.

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