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MICROBIAL BIOSORPTION OF HG (II), CD (II), AND ZN (II) BY WHOLE CELLS AND THEIR FRACTIONS

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ABSTRACT
Heavy metal pollution has become one of the most serious environmental problems today. Biosorption using microorganisms is the most promise green solution. The present study investigated the biosorption capacity of biosorbents from whole dead cells of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus*, and *Saccharomyces cerevisiae* (bios1– 4, respectively), and biosorbents from their cells fractions (bios 5 – 8, respectively), for Hg (II), Cd (II), and Zn (II). The biosorption characteristics were investigated as a function of contact time, weight of biosorbent, pH, and initial metals ions concentration. It was found that the maximum biosorption capacities (mg/g) were (16, 25, 15), (14, 24, 22), (16, 33, 18), (17, 33, 20), (26, 33, 23), (22, 33, 24), (33, 33, 25), (29, 33, 25) by bios1 – 8, respectively, for (50mg/l) Hg (II), Cd (II), and Zn (II), respectively. These results indicated that cells fractions have higher metals ions biosorption capacities than whole cells, as well eukaryotes more than prokaryotes. Bios 7 was the best at all, with removal efficiency of 100% after 60 min for Hg (II), and 40 min for Cd (II) and Zn (II), at pH 5, and biosorbent weight 1.5g/l for Hg (II) and Cd (II), while 2.0g/l for Zn(II). Desorption efficiency of biosorbents was investigated at various pH values, where it was recovered up to 96% of metals ions at pH 1. Based on the above results, *A. flavus* cells fractions appears to be a more efficient biosorbent for the removal of Hg (II), Cd (II), and Zn (II) from aqueous solution.

KEYWORDS: Biosorption, cell fractionation, heavy metals, microbial biosorbents.

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation *etc*. (Wang and Chen 2009). Various techniques such as precipitation (Rickelton 1998), coagulation (Koukal *et al*., 2003), ion exchange, membrane processing, and solvent extraction (Selatnia *et al*., 2004) have been employed to remove heavy metals pollutants from wastewater. Most of these techniques are ineffective or extremely expensive in terms of energy and reagent consumption, especially when concentrations of dissolved metals are on the order of 1–100 mg/l (Tang *et al*., 2008). Alternative process is biosorption. Biosorption using the biomass of microorganisms (bacteria, fungi, and yeast) is an effective, ecofriendly and economic technology for the removal and recovery metals from wastewater. Biosorbent behavior for metallic ions is a function of the chemical make-up of the microbial cells of which it consists (Volesky and Holan, 1995). The cell wall constituents play a key role in metal sequestering (Myasoedova *et al*., 2007). Such compounds possess numerous functional groups, including carboxylate, hydroxide, amine, imidazole, sulfate and sulfhydryl, with various charge distributions and geometries, so they can selectively bind certain metal ions (Pablo *et al*., 2011). Different cell components allow different charge distributions and geometries, providing to microbial biosorbent the possibility of binding different elements. The cell walls of yeast include a large number of complex organic compounds and their polymers, such as glucan (28%), mannan (31%), proteins (13%), lipids (8%), chitin and chitosan (2%) (Brady *et al*., 1994). Bacteria have a complex membrane that can act as a selective extractant by mimicking the best extraction conditions, usually showing high surface-to-volume ratios and containing abundant potentially active chemosorption sites in their walls. The cell surface hosts multiple functionally and structurally different proteins and they differ considerably from Gram-negative to Gram-positive bacteria (Aller and Castro, 2006). Gram-negative bacteria have a cell envelope composed of a cytoplasmic membrane, a peptidoglycan layer, and an outer membrane with lipopolysaccharides, on the other hand, gram-positive bacteria lack the outer membrane (Beveridge and Graham 1991). The biosorption by fungi have been studied in less extension than other biosorbents like bacteria or yeast. Most fungi have a cell wall consisting largely of chitin and other polysaccharides (Hudler, 1998). A useful technique for studying cell structure and function is cell fractionation, which takes cells apart and separates major organelles and other sub cellular structures from one another. Generally, cells are broken apart as gently as possible; and the mixture, referred to as the cell extract, is subjected to centrifugal force separates the extract into two fractions: a pellet and a supernatant. The pellet that forms at the bottom of the tube contains heavier materials, such as nuclei, packed together. The supernatant, the liquid

above the pellet, contains lighter particles, dissolved molecules, and ions. Cell fractionation enables researchers to prepare specific cell components in bulk and identify their functions (Reece *et al*., 2011). The aim of this study was to test and compare capacities of fractionated and unfractionated microbial eukaryotic and prokaryotic cells, to absorb Hg(II), Cd(II), and Zn(II). As well as, desorption of metals.

MATERIALS & METHODS

Microorganisms

Four microorganisms were used, gram-negative bacterium *Pseudomonas aeruginosa* and gram-positive bacterium *Staphylococcus aureus* were kindly provided by Microbiology Laboratory, King Abdul-Aziz Medical City, Makkah, Saudi Arabia, and their identification was routinely assessed using PHONIX 100BD and MICRO SCAN walk away 9651 apparatus. Fungus, *Aspergillus flavus* was isolated and identified in previous work (Ghanem et al. 2009). *Saccharomyces cerevisiae* was obtained commercially from local store. Bacteria were grown and maintained on nutrient broth medium (NB) (g/l: 10.0 peptone, 3.0 beef extract and 5.0 sodium chloride) at 30ºC and pH 7.4 ± 0.2. Fungus and yeast were grown and maintained on sabouraud dextrose broth (SDB) (g/l: 20 Dextrose, 10 Peptone) at 25ºC and pH 5.7 ± 0.2.

Cells fractionation and biosorbents

Bacteria were inoculated into 300 ml of sterile medium (NB) in 500 ml conical flasks and cultivated on a shaker at 150 rpm for 48 h at 30ºC. Fungus and yeast were inoculated into 300 ml of sterile medium (SDB) in 500 ml conical flasks and cultivated on a shaker at 150 rpm for 5 d at 25◦C. To prepare biosorbents, cells of microbial growth were autoclaved at 121◦C for 15 min, thereafter they were harvested by filtration through whatman No #1 filter paper and washed twice with deionised distilled water. Adequate amounts of the harvested cells were stored at 4◦C to use in the biosorption experiments as a biosorbent from whole cells of *P. aeruginosa* (Bios1), *S. aureus* (Bios2), *A. flavus* (Bios3), and *S. cerevisiae* (Bios4). The residual harvested cells were resuspended in deionised distilled water and homogenized in a blender for 15 min to break them up. Thereafter the resulting mixtures (homogenates) were fractionated by centrifuge at 2000 rpm for 10 min, to get pellets rich in nuclei and cellular debris from different types of used cells. Subsequently the obtained pellets were dried in an oven at 80°C for 48 h, then ground and sieved (1.0 mm mesh) to get the uniform sized particles (powder).They were used in the biosorption experiments as a biosorbent from powder of cells fractions of *P. aeruginosa* (Bios 5), *S. aureus* (Bios 6), *A. flavus* (Bios 7), and *S. cerevisiae* (Bios 8). Cell concentrations in the suspension of the experiments were determined by drying an aliquot onto a pre-weighed aluminum foil container to constant weight at 80 ◦C.

Heavy metals

Standard stock solutions of Hg(II), Cd(II), and Zn(II) at concentration of 1000mg/l were prepared by dissolving $HgCl_2$, $CdCl_2$, and $ZnCl_2$, respectively, in deionized distilled water. Solutions were adjusted to the desired pH values by 0.1 N NaOH and 0.1 N HCl. Metal concentration was determined using atomic absorption spectrophotometer (AAS) (Unicam 929AA).

Biosorption experiments

Batch biosorption experiments were carried out by mixing different weights (0.5–5.0 g/l) of prepared biosorbents (Bios1 – 8) with 100 ml of heavy metals solutions (Hg (II), Cd (II), and Zn (II)) at initial concentration (10–100mg/l) for each metal, at contact time (0–200 min), pH (1–9), temperature (28 ± 2 ◦C), and agitation (150 rpm) in sets of 250 ml conical flasks separately. At the end of the agitation time the residual concentrations of metals in supernatants were measured after centrifugation of the mixtures at 10,000 rpm for 10 min. The values of biosorptive capacity and removal efficiency of metals were calculated as $q = (C_0 - C_1)/M$, and $re\ (\%) = (C_0 - C_1)/C_0 \times 100$. Where q is the equilibrium metal concentration on the biosorbent (mg/g biosorbent), C_0 and C_1 are the initial and final metal concentration (mg/l), respectively, M is the weight of the biosorbent (g). *re* is removal efficiency (%). All biosorption experiments were performed in triplicate and mean values reported.

Desorption of metals

Desorption of metals from previously biosorbents (Bios1 – 8) was performed at pH (1 – 9) using 0.1 N HCl to adjust pH. For the desorption experiments, 0.01 g of previously loaded biosorbent was added to 10 ml of eluent in a 50 ml conical flask and agitated at 150 rpm for 24 h. thereafter the biosorbents were isolated by centrifugation and the eluted metals in the supernatant was measured by (AAS). The desorption efficiency (DE) (%) was calculated according as DE (%) = Amount of metal desorbed /Amount of metal adsorbed × 100

RESULTS & DISCUSSION

Four microorganisms (two prokaryotes and two eukaryotes) were used in this study after special treatment as was mentioned above to prepare eight kinds from biosorbents, they were coded as bios1 – bios8. Their efficiencies to removal of three most toxic heavy metals, namely, Hg(II), Cd(II), and Zn(II) at wide range of contact time, weight of biosorbent, pH, and initial concentration of metal, were investigated.

Effect of contact time

The influence of contact time (0 – 200min) on the biosorption (50mg/l) of Hg(II), Cd(II), and Zn(II) by 1.0g/l of the biosorbents bios1– 8 at pH 6 was separately investigated in sets of experiments. The results are given in Fig. 1a, 2a, and 3a. They revealed that the required time to attain equilibrium between the biosorbent and the metal was 60 min for biosorption of Hg (II) with maximum removal efficiency of 67%, and biosorption capacity of 24mg/g by bios7. The other biosorbents recorded maximum removal efficiency values at same time (60 min) ranged 23 – 51%, as well as biosorption capacity 12 – 26mg/g. While the equilibrium time was 40 min for biosorption of Cd(II) and Zn(II), where bios7 achieved maximum removal efficiency of 83 and 75%, with biosorption capacity 42 and 38mg/g, respectively. The other biosorbents achieved maximum removal efficiency between 40 and 72% in case Cd(II), with biosorption capacity 20 – 36mg/g. In case Zn(II) the values were 39 – 71% and 20 – 36mg/g. After those recorded equilibrium

times, constant of removal efficiency and biosorption capacity was noted in all the cases. Biosorbent of bios7 showed better results at all, this revealed that the uptake of Hg(II),Cd(II), and Zn(II) by bios7 was higher than the others biosorbents , it could be attributed to the increase of binding sites a result of the fractionation of *A. flavus* cells.

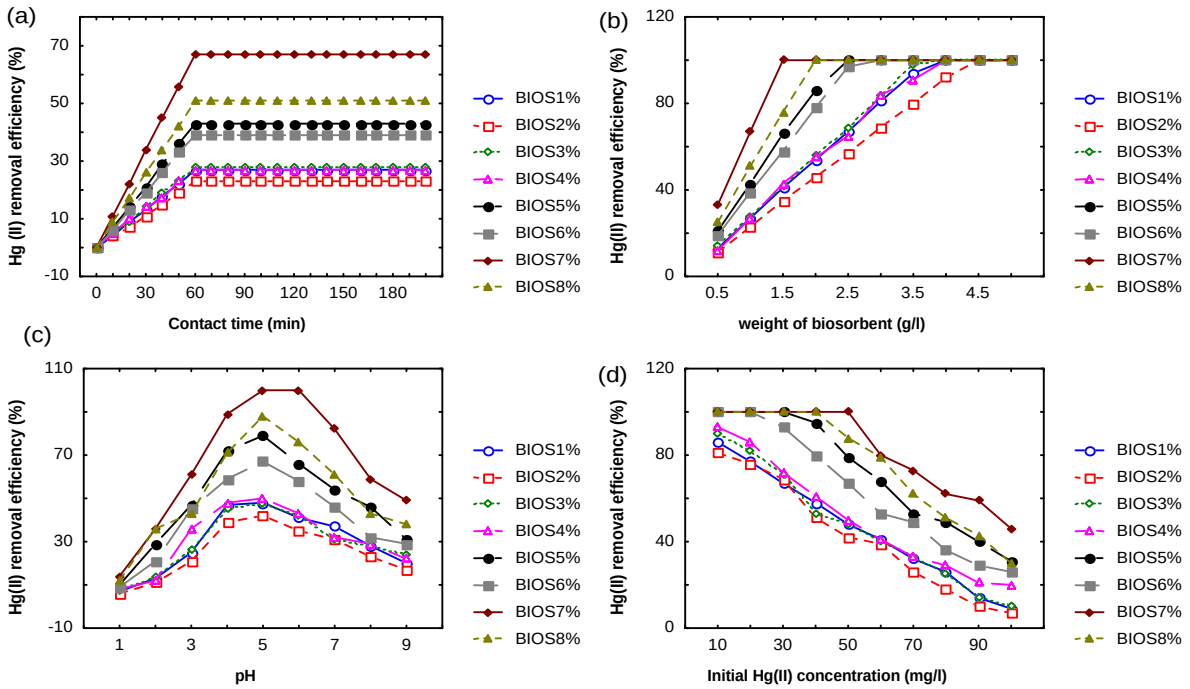

FIGURE 1: (a) Study of contact time for biosorption of Hg (II) by bios1 – 8 (Hg (II) 50mg/l, pH 6.0, weight of biosorbent 1.0g/l). (b) Effect of the weight of biosorbents on Hg (II) removal efficiency by bios1 – 8 (Hg (II) 50mg/l, pH 6.0, 60 min). (c) Effect of different initial pH on Hg (II) removal efficiency by bios1 – 8 (Hg(II) 50mg/l, weight of biosorbent 1.5g/l, 60 min). (d) Effect of initial concentration of metal Hg (II) for removal by bios1 – 8 (pH 5.0, weight of biosorbent 1.5g/l, 60 min).

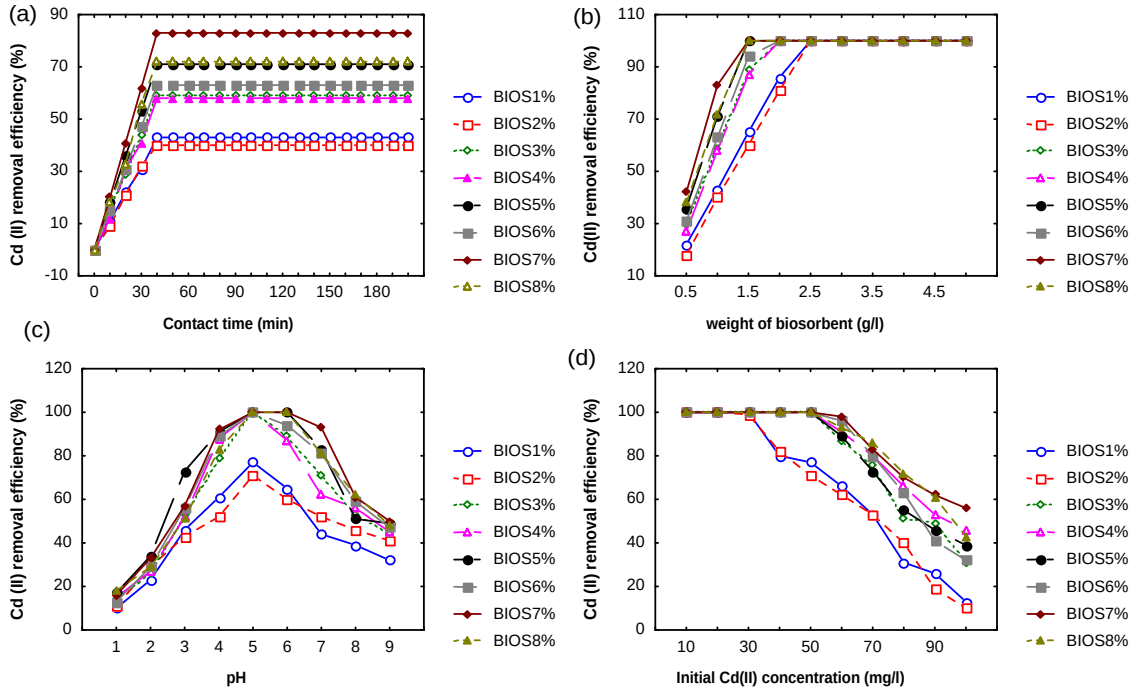

FIGURE 2: (a)Study of contact time for biosorption of Cd(II) by bios1 – 8 (Cd(II) 50mg/l, pH 6.0, weight of biosorbent 1.0g/l). (b) Effect of the weight of biosorbents on Cd(II) removal efficiency by bios1 – 8 (Cd(II) 50mg/l, pH 6.0, 40 min). (c) Effect of different initial pH on Cd(II) removal efficiency by bios1 – 8 (Cd(II) 50mg/l, weight of biosorbent 1.5g/l, 40 min). (d) Effect of initial concentration of metal Cd(II) for removal by bios1 – 8 (pH 5.0, weight of biosorbent 1.5g/l, 4.0 min).

Effect of the biosorbents weights on removal of metals

As shown in Fig. 1b, 2b, and 3b the removal of metal by biomass (bios1 – 8) recorded an increase with increase in the concentration of biomass and reached maximum removal efficiency of 100% in all cases with variance in required weight of biosorbents, this was consistent with the findings of Kahraman *et al.* (2005) by *Phanerochaete chrysopsporium* and *Funalia trogii* for copper. bios7 completed 100% of Hg(II), Cd(II), and Zn(II) removal at weight 1.5, 1.5, and 2.0g/l with biosorption capacity 33, 33, and 25mg/g, respectively, as a best result. While it had been needed to attain 100% of Hg(II), Cd(II), and Zn(II) removal by the others biosorbents, magnitudes of them ranged 1.5 – 4.5g/l, with biosorption capacity varied form 11 to 33mg/g. thus implying that bios7 possess higher affinity for used metals removal, compared to the others biomass. The increase in removal with increase in the biomass weight can be attributed to increased surface area (Palanivel *et al*. 2010).A similar study by Garg et al. (2004) demonstrated that the adsorption of Cr(VI) was dependent on the agro-industrial waste adsorbent dose.

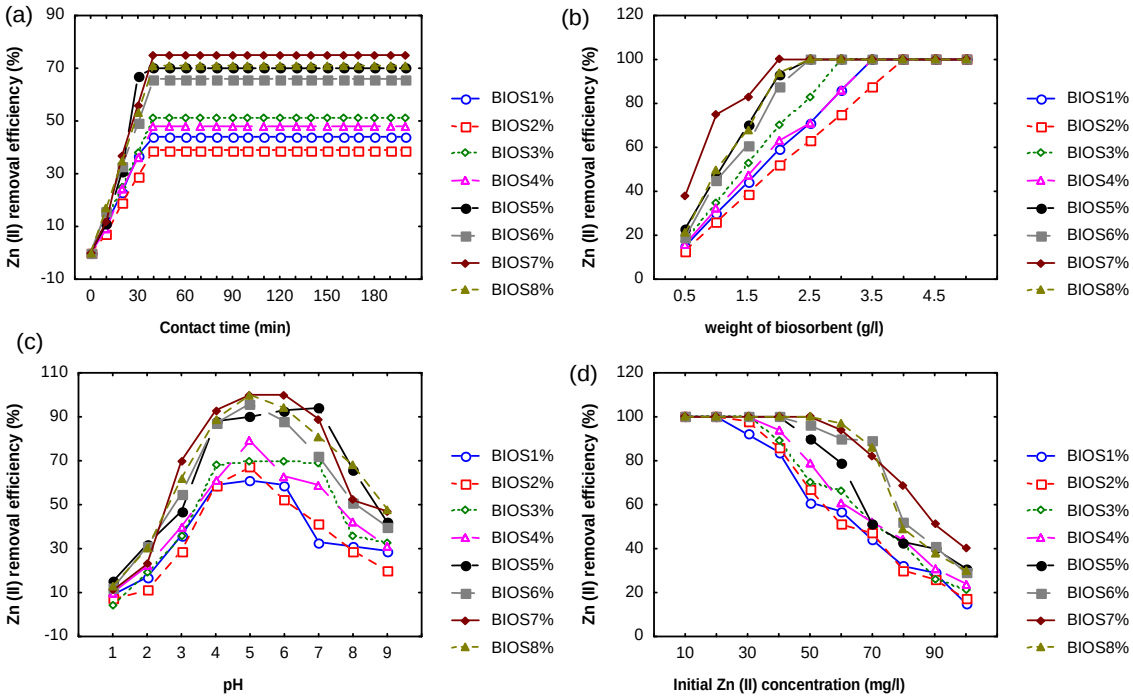

FIGURE 3. (a) Study of contact time for biosorption of Zn(II) by bios1 – 8 (Zn(II) 50mg/l, pH 6.0, weight of biosorbent 1.0g/l). (b) Effect of the weight of biosorbents on Zn(II) removal efficiency by bios1 – 8 (Zn(II) 50mg/l, pH 6.0, 4.0 min). (c) Effect of different initial pH on Zn(II) removal efficiency by bios1 – 8 (Zn(II) 50mg/l, weight of biosorbent 2.0g/l, 40 min). (d) Effect of initial concentration of metal Zn(II) for removal by bios1 – 8 (pH 5.0, weight of biosorbent 2.0g/l, 40 min).

Effect of pH on biosorption

For biosorption of heavy metal ions, pH is one of the most important environmental factors. pH affects on the activity of functional groups, cell surface metal binding sites, property and solution chemistry of the metal ions(Huang *et al.* 2006; Sannasi *et al.* 2006). In this study pH had a significant effect on the removal efficiency of Hg(II),Cd(II), and Zn(II) and biosorption capacities for all biosorbents (bios1 – 8). As seen in Fig. 1c, 2c, and 3c, all removal efficiencies increased with increasing pH and began to decline after reaching maximum removal at optimum pH 5.0. The maximum removal efficiencies (%) (and biosorption capacities mg/g) of Hg (II) ranged 42(14) – 100(33), where bios7 recorded highest value, while lower value was achieved by bios2. Similar results were recorded in case Cd(II) and Zn(II). This is in good agreement with the results obtained by other investigators studying Hg(II), Cd(II), and Zn(II) biosorption by different microorganisms (Matis *et al*., 1994; Mapolelo and Torto 2004; Ozdemir *et al*., 2009; Fei *et al*., 2013). The pH biosorption profiles for various heavy metals may be related to the chemical nature of metal interactions with the biomass. In this study, the lower biosorption capacity at pH values below 5.0 may be due to hydrogen ions that compete with metals ions on the biosorption sites (Chang *et al*., 1997). The increase in biosorption capacity may be related to the ionization of functional groups which serve as the binding sites, while the decrease in biosorption above pH 5.0 might be explained by the reduced availability and solubility of the metal ions with the onset of precipitation of metal hydroxides as has been reported by Sannasi et al. (2000) or attributed to the speciation of the metal, such as the formation of Cd $(OH)_2$ ions that do not adsorb well (Fei *et al*., 2013).

Effect of initial heavy metal ion concentration

The effect of initial metal concentration on the removal efficiency and biosorption capacity of Hg (II), Cd (II), and Zn (II) by all biosorbents (bios1 – 8) is shown in Fig. 1d, 2d, and 3d. All removal efficiencies decreased with increasing initial concentrations but biosorption capacities of biosorbents first increased with increasing the concentration and reached saturation at optimum concentration among 60–70mg/l. At the highest initial Hg (II), Cd (II), and Zn (II) concentrations (100mg/l), the

maximum biosorption capacities were 31, 37, and 20mg/g, respectively, by bios7. Other biosorbents recorded biosorption capacities ranged 5 - 20 mg/g for Hg (II), 7 – 29mg/g for Cd (II), and 7 – 15mg/g for Zn(II). whereas the removal efficiency was 46, 56, and 40% by bios7 for Hg(II), Cd(II),and Zn(II), respectively. While lower values were recorded by the residual biosorbents (Fig. 1d, 2d, and 3d). The results of these experiments indicate that the energetically less favorable sites become involved with increasing the metal ions concentration in aqueous solution (Singha and Das 2011; Fei *et al*., 2013).

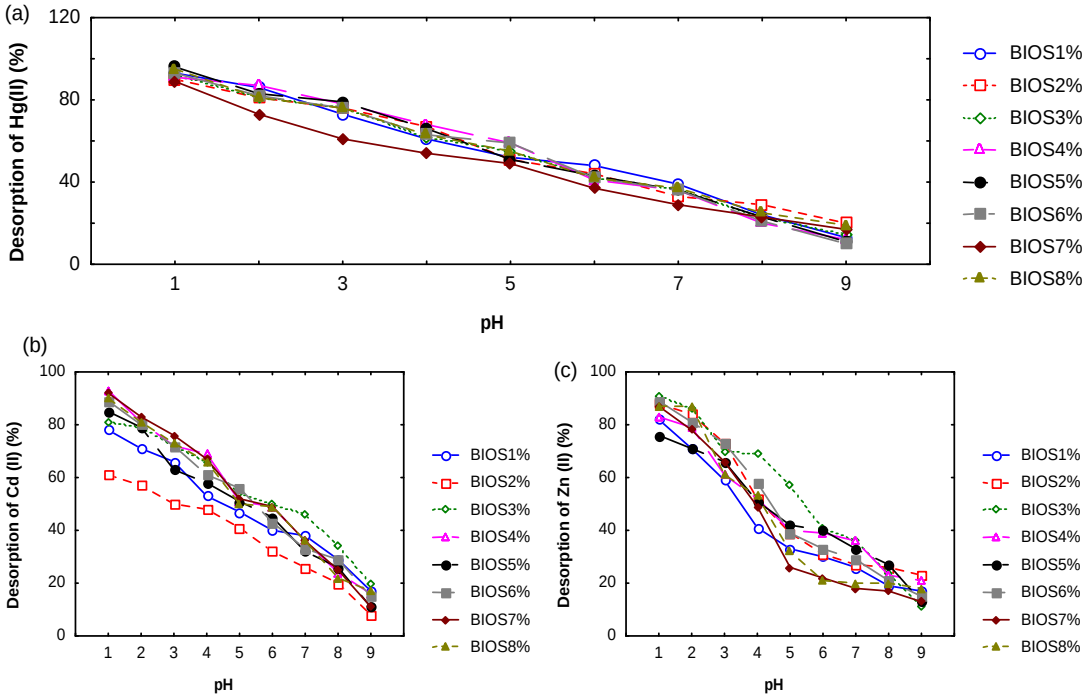

FIGURE 4. Effects of pH on desorption efficiency of (a) Hg (II), (b) Cd(II), and (c) Zn (II) from bios1 – 8.

Comparison of used biosorbents

In this study four microorganisms were used, each one twice, first as whole cells, second as cells fractions after fractionation process. It was clear of the results that cells fractions from each microbe showed efficiency more than whole cells of same microbe. Maximum removal efficiency for Hg (II) (50mg/l) by whole cells of *A. flavus*, *S. cerevisiae*, *P. aeruginosa, and S. aureus*, were, 28, 27, 27, and 23%, respectively, at pH 6 after 60 min. Whereas cells fractions from same microorganisms attained 67, 51, 43, and 39%, respectively, with improving rate 139, 89, 59, and 70%, respectively. Biosorption capacities of the microorganisms have been affected positively after application of fractionation process, within enhancement ratio 67–143%. Similar results were recorded in case Cd(II) and Zn(II). It might be interpreted that cells fractionation process increased surface area ratio to volume, which acted on increasing of metals ions binding sites. The obtained results clearly indicated advantage of filamentous fungus (*A. flavus*), then yeast (*S. cerevisiae*), followed negative bacteria (*P. aeruginosa*), and positive one (*S. aureus*). The result has been interpreted that fungal cell wall content, largely chitin and other polysaccharides, as well as internal organelles are enveloped in membrane, made this biosorbent has more of binding sites and affinity to metals ions. Yeast was second with same characteristics. Advantage of negative bacteria more than positive might be attributed to outer membrane, which has numerous of compounds, work as binding sites.

Desorption efficiency

The availability of effective, low cost and reusable biosorbent is the key to the commercialization of biosorption technology (Utgikar *et al*., 2000); therefore regeneration of biosorbent for repeated uses is a critical issue in practical applications. Desorption of heavy metals from metal-laden biomass was approached through utilizing various elution agents (Kratochvil and Volesky 1998; Chu *et al*., 1997; Lazaro *et al*., 2003) and it appeared that HCl had the best desorption efficiency among the tested chemical reagents. Furthermore, acid treatment did not alter the surface characteristics of the biomass (Huang and Huang 1985) with almost the same binding capacity, except for releasing bound metal from the surface, and, in view of this, several adsorption–desorption cycles could be employed (Davis *et al*., 2003). Accordingly, the present study selected hydrochloric acid as the desorption agent. As shown in Fig. 4a, b, and c. the desorption efficiency increased with decreasing pH. The highest desorption efficiency was recorded at pH 1, with values ranged 89–96% for Hg (II) by different biosorbents, and there were no significant differences in the desorption among them. Similar results were obtained in case Cd(II) and Zn(II). This is consistent to the results obtained by Lin et al. (2012), who reported that 98.11% recovery of Cd(II) could be obtained at pH ≤2 from Cd(II)-loaded biomass of *Streptomyces zinciresistens*.

CONCLUSION

The study showed that improvement of 49–90% in biosorption of Hg (II), Cd (II), and Zn (II) by biosorbents from *A. flavus*, *S. cerevisiae*, *P. aeruginosa, and S. aureus* , after application of cells fractionation processes for them. Biosorbents of eukaryotic cells (*A. flavus* and *S. cerevisiae*) achieved removal efficiency of metals ions and biosorption capacity more than prokaryotic cells (*P. aeruginosa and S. aureus*).

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