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Biosorption of Pb (II), Cd (II) and Hg (II) Ions from Model Solutions on Pretreated Waste Bacillus thuringiensis Biomass

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Abstract. In the present study waste pretreated *Bacillus thuringiensis* biomass was applied as an eco-friendly biosorbent for Pb (II), Cd (II) and Hg (II) from model aqueous solutions. Heat inactivated and alkali treated biomass showed ability for removal of metal ions from single solutions in the following order Pb(II) > Cd(II) > Hg(II). It was proved that the major groups involved in biosorption are hydroxyl/amino, alkyl, carbonyl and phosphoryl groups. The influence of different factors as pH, initial sorbate concentration, biosorbent concentration, contact time was evaluated.

Key words: biosorption, toxic metals, *Bacillus*.

Introduction

Heavy metals are one of the major environmental pollutants, which according to their toxicity occupy the second place after the pesticides. Heavy metals originate mainly from industrial activities such as ferrous and non-ferrous metallurgy, ore-mining, ceramic and glass enterprises, as well as those for oil production and processing, thermal power plants using solid and liquid fuels, transport etc. (Ahmed & Ahmaruzzaman, 2016). Some heavy metals such as Cu, Fe, Zn, Mo and other are essential to human life but even at slightly higher concentrations the same metals are toxic. (Smith *et al.*, 2015). Cd, Hg and Pb are characterized by high toxicity, mutagenic and cancerogenic action (Goyer, 2004; Goyer & Clarcson, 2001). The excessive intake of these metals leads to serious health problems. Lead damages central nervous and reproductive systems and also causes psychiatric disorders (Mason *et al.*, 2014). Cadmium causes atrophic rhinopharyngitis, chronic bronchitis, nefropathological damages, osteoporosis and osteomalacia (Bernhoft, 2013). Mercury causes anemia, toxic hepatitis, tremor, vegeto-vessel distony, polyneuropathy and encephalopathy (Patrick, 2002).

According to the United State Environmental Protection Agency (US EPA, 2009), the maximum contaminant level of these metals in drinking water is as follows: cadmium - 0.005 ppm; lead - 0.015 ppm and mercury - 0.002 ppm.

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The conventional methods of removing heavy metals (filtration, precipitation, oxidation, reduction, ion exchange and membrane technologies) are costly and ineffective in case of effluents with low metal concentration, particularly in the range of 1-100 mg dm^{-3} (ZOUBOULIS *et al.* 2004). Due to this fact, the biosorption has focused as an alternative for heavy metal removal (GADD, 2009; SALAM, 2019).

The aim of this study was to investigate the ability of waste pretreated dead biomass of *Bacillus thuringiensis* to remove Pb (II), Cd (II) and Hg (II) from model solutions. The factors that influence biosorption efficiency such as pH, contact time, metal ion concentration and biosorbent dosage were evaluated. Competitive biosorption from binary and ternary solutions was also examined.

Materials and Methods

Biomass

Waste biomass of *Bacillus thuringiensis* used for microbiological production of lipases at laboratory conditions was separated from culture broth by filtration and was washed three times with distilled water.

Pretreatment procedures

The washed biomass was dried a hot air oven at 80 °C for 12 h. The inactivated biomass was stored at 4 °C until further use and referred as a heat treated biomass.

Alkali pretreated biomass was prepared as follows: 5 grams of biomass were suspended in 100 cm^3 1 M solution of NaOH and then the obtained mixture was sterilized for 15 minutes at 121^0 C (GÖKSUNGUR *et al.*, 2005). Bacterial cells were collected by centrifugation at 3000 rpm for 20 minutes. The NaOH treated cells were washed several times with deionized water to remove excess of sodium hydroxide until the pH reached the near neutral range. The pretreated biomasses were dried at 80^0 C to a constant weight, then were powdered in a mortar and pestle and stored at 4^0 C until use and referred as a caustic treated biomass.

Biosorption studies

The stock solutions (1000 mg dm^{-3}) of Pb (II), Cd (II) and Hg (II) were prepared by a weighed quantity of analytical grade $Pb(NO_3)_2$ (Aldrich), $Cd(NO_3)_2.4H_2O$ (Aldrich) and $Hg(NO_3)_2.H_2O$ (Aldrich) in deionized water. Working solutions of different concentrations of each heavy metal were prepared as single solutions by adequate dilution of the initial stock solution. The pH of working solutions were adjusted to required values with 0.1 M HNO_3 or 0.1 M NaOH.

Biosorption experiments were carried out in batch mode using 250 cm^3 Erlenmeyer flasks containing 100 cm^3 single metal solution with desired initial concentration and biomass dose. They were agitated on a shaker for 120 minutes at 250 rpm. Samples were taken in intervals, centrifuged at 3000 rpm for 20 minutes and the liquid supernatant was analyzed for Pb (II), Cd (II) and Hg (II) ions concentration. The batch studies were performed a different experimental condition such as pH, initial metal concentrations (5 – 75 mg dm^{-3}), contact time (5 – 120 min), biomass concentration (1.0 – 4.0 g dm^{-3}). The temperature was maintained constant at 25 ± 0.2^0 C in all studies.

Biosorption from ternary solution

Biosorption from ternary system was studied using a solutions containing 10 mg dm^{-3} of each metal ion. The experiments were carried out at pH 5.0, biosorbent dosage 1 g dm^{-3} and contact time 90 min.

Date analysis

The amount of metal ion sorbed per unit mass of the biosorbent was evaluated by using the following equation:

$$q = (C_0 - C_f) \times V / m, \text{ mg g}^{-1}$$

where C_0 and C_f are the initial and final metal ion concentrations, respectively in mg dm^{-3}; V – the volume of metal solution, dm^3; m – the biomass concentration, g dm^{-3}

The biosorption removal was calculated as follows:

$$R = (C_0 - C_f) \times 100 / C_0, \%$$

All biosorption experiments were done in triplicate. For all graphical represantations, the mean values considered and standard deviations within triolicate were to small to be plotted as error bars (< 1 %).

Analytical Methods

The metal ion concentration in the solution was determined by atomic absorption spectroscopy (AAS) using an atomic absorption spectrometer PinAAcle 900 T (THGA/FLAME) Perkin Elmer.

The main functional groups present on the cell wall of pretreated bacterial biomass *Bacillus thuringiensis* were recorded in a FTIR spectrometer Nicolet iS10 (Thermo Fisher Scientific, USA), equipped with total reflectance sampling accessory (smart iTR). The analysis was carried out at following conditions: 64 scanning, 4 nm resolution, spectral range 4000 – 400 cm^{-1}.

Results and Discussion

Effect of biomass modification

It has been found in a number a studies, that the pretreatment of different kinds of biomass causes an increasing in their removal efficiency towards metal cations. This fact may be due to an elimination the impurities from cell wall surface, an exposure of active-metal binding sites embedded in the cell wall or chemical modifications of the cell wall components (DAS *et al.*, 2007; GÖKSUNGUR *et al.*, 2003; İLHAN *et al.*, 2004; KIROVA *et al.*, 2012; KIROVA *et al.*, 2015a; YAN & VIRARAGHAVAN, 2000).

In our study, as seen on Fig. 1, heat inactivated and alkali treated biomass showed ability for removal of metal ions from single solutions in the order Pb(II) > Cd(II) > Hg(II).

The calculated biosorption capacity for alkali treated cells was 8.35, 7.28 and 6.85 mg g^{-1} respectively. In the case of thermally inactivated biomass, the biosorption capacity decreases with 24.62 % for Pb (II), 27.03 % for Cd (II) and 27.81 % for Hg (II) ions. The calculated values are for 10 mg dm^{3} initial metal concentrations, pH 5.0, biomass concentration 1 g dm^{3} and contact time 120 min.

FTIR analysis

FTIR spectroscopy has been used to identify the functional groups responsible for the biosorption of metal ions and other pollutants (inorganic and organic compounds) on the cell surface of the biomass/ biosorbent. The FTIR spectra of unloaded and Pb (II)-loaded alkali treated biomass were obtained and presented (Fig. 2).

From the Fig. 2A it can be seen that the FTIR spectra present distinct peaks, confirming the complex structure of the cell wall. The broad band situated in the range 3500 и 3100 cm^{-1} does not allow a clear indication of the functional groups, because overlapping of –OH and –NH stretching is possible (MALEKI & MASHINCHIAN, 2011; SIMONESCU, 2012; PAN *et al.*, 2007). The peak at 2929 cm^{-1} is attributed to –CH stretching vibration from alkyl chains. Overlapping of –OH stretching from carboxylic acids with C-H stretching vibrations in the range from 3400 to 2400 cm^{-1} have been reported (VENEAU *et al.*, 2012). The amide I band (stretching vibrations in carboxyl or amide groups) appears at 1644 cm^{-1} and the amide II band (N-H bending vibrations and C-N stretching vibration superposition) - at 1539 cm^{-1}. Sometimes, the C(=O)–O^{-} anti-symmetric stretching vibration in carboxylate so-called $\nu_{C=O}$ (I) appears around 1544.20 cm^{-1} wavenumbers (PAN *et al.*, 2007). The peak at 1402 cm^{-1} can be attributed to N-H bending in amine group. The peak at 1080 cm^{-1} is caused by P=O symmetric stretching vibrations (YUAN *et al.*, 2009). For the lead loaded biomass, the peaks at 3277 and 2929 cm^{-1} almost disappeared, indicating that amine and hydroxyl groups may be involved in the biosorption process. The carboxyl peak observed for unloaded biomass at 1644 cm^{-1} is shifted to 1650 cm^{-1}. The peak at 1539 cm^{-1} (amid II) was weaker and shifted to 1533 cm^{-}. The peak at 1080 cm^{-1} was also weaker and shifted to 1076 cm^{-1}. The peak at 1402 cm^{-1} disappears. From the obtained results it can be concluded that the major groups involved in biosorption are hydroxyl/amino, alkyl, carbonyl and phosphoryl.

Effect of pH

The initial pH of the aqueous solution is the key factor influencing the biosorption process, the surface charge of the biomass and the solution chemistry of metal ions. At low pH values the binding sites of the cell surface are in protonated form resulting in a net positive surface charge, which could in turn, decrease the biosorption of the available positive charged metal ions. In contrast, an increase in pH an opposite effect is observed (FAROOQ *et al.*, 2010).

The effect of initial pH of the solution on the Pb (II) recovery was studied at pH ranging from 2.0 to 5.0 to avoid $Pb(OH)_2$ precipitation, because at pH values higher than 5.0 several low soluble hydroxide species were observed (OZDEZ *et al.*, 2009); for Cd (II) and Hg (II) – from 2.0 to 6.0 (Fig. 3).

The results indicated that the recovery efficiency in all cases is pH dependent. There was an increase in Pb (II), Cd (II) and Hg (II) removal with increasing pH, a plateau was reached in the pH range 5.0 – 6.0 for cadmium and mercury. The calculated removal efficiency at pH 5.0 was 83.5 % for Pb (II); 77.2 % and 68.50 % for Cd (II) and Hg (II) ions respectively, at pH 6.0. Therefore, pH 5.0 was selected for all further experiments. These results were in good agreement with data for the effect of pH on Pb(II), Cd (II) and Hg (II) biosorption reported by other authors (KIROVA *et al.*, 2015a; GÖKSUNGUR *et al.*, 2005; SVECOVA *et al.*, 2006; PAN *et al.*, 2007).

Effect of biomass concentration

Biomass concentration is another important factor affecting biosorption efficiency and the sorption capacity. Efficiency of Pb (II), Cd (II) and Hg (II) removal was examined as a function of biomass dosage (from 1.0 to 4.0 g dm^3).

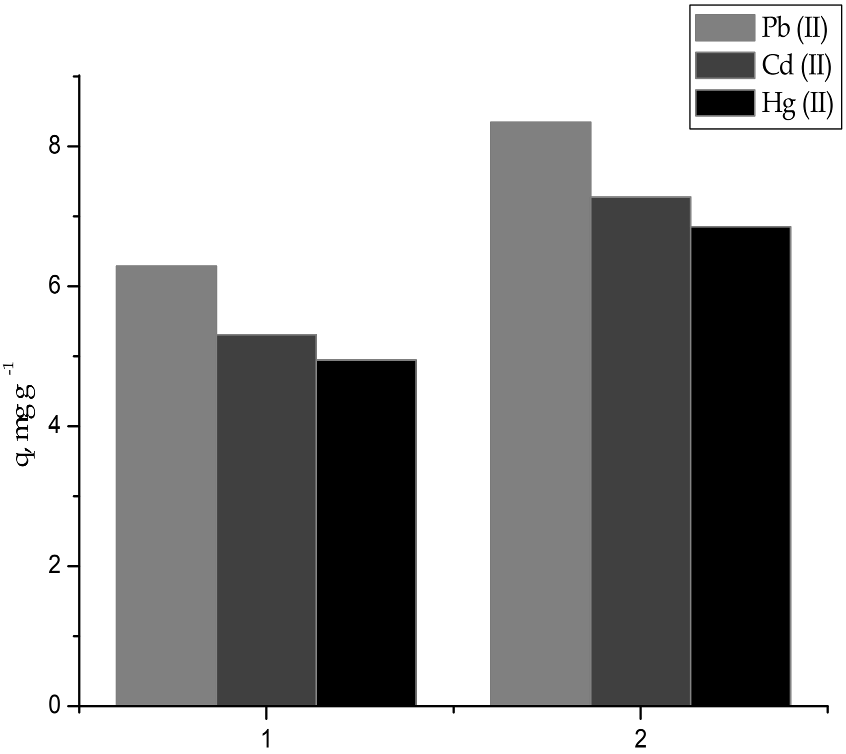

Fig. 1. Pb(II), Cd(II) and Hg(II) uptake of *Bacillus thuringiensis* biomass (1 – heat inactivated, 2 – alkali treated).

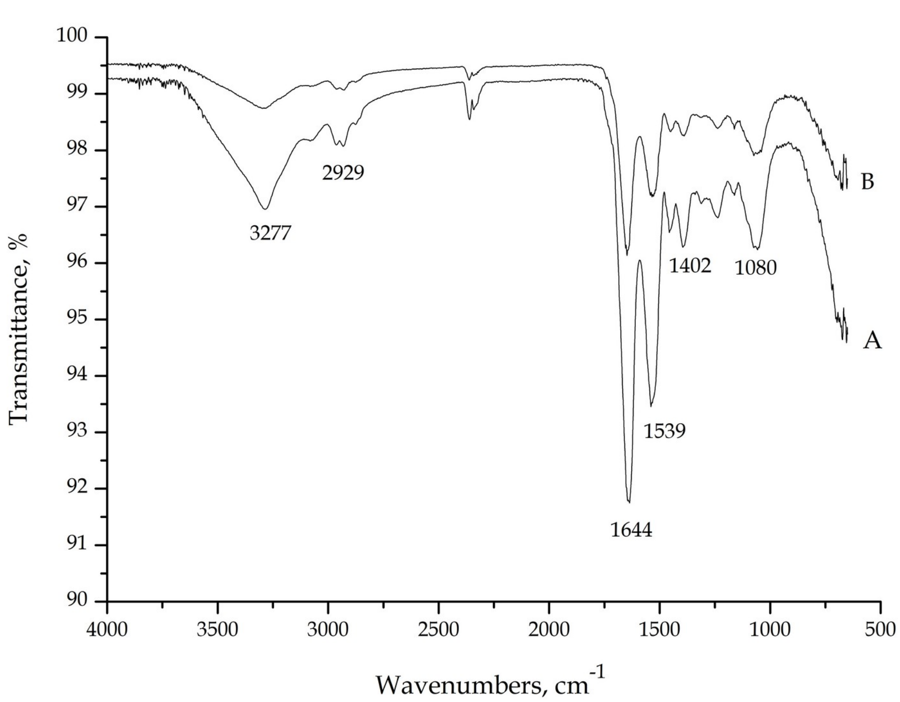

Fig. 2. FTIR spectra of unloaded (2.A) and Pb (II)-loaded (2.B) pretreated biomass.

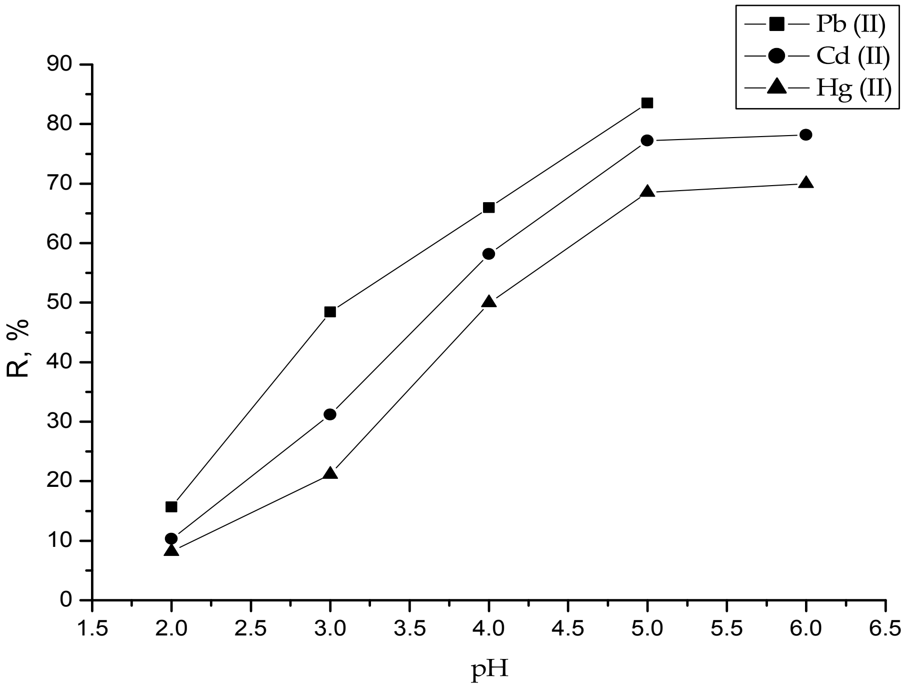

Fig. 3. Effect of pH on the removal efficiency of Pb (II), Cd (II) and Hg (II) ions (initial concentration 10 mg dm^{-3}, biomass dosage 1 g dm^{-3}, contact time 120 min).

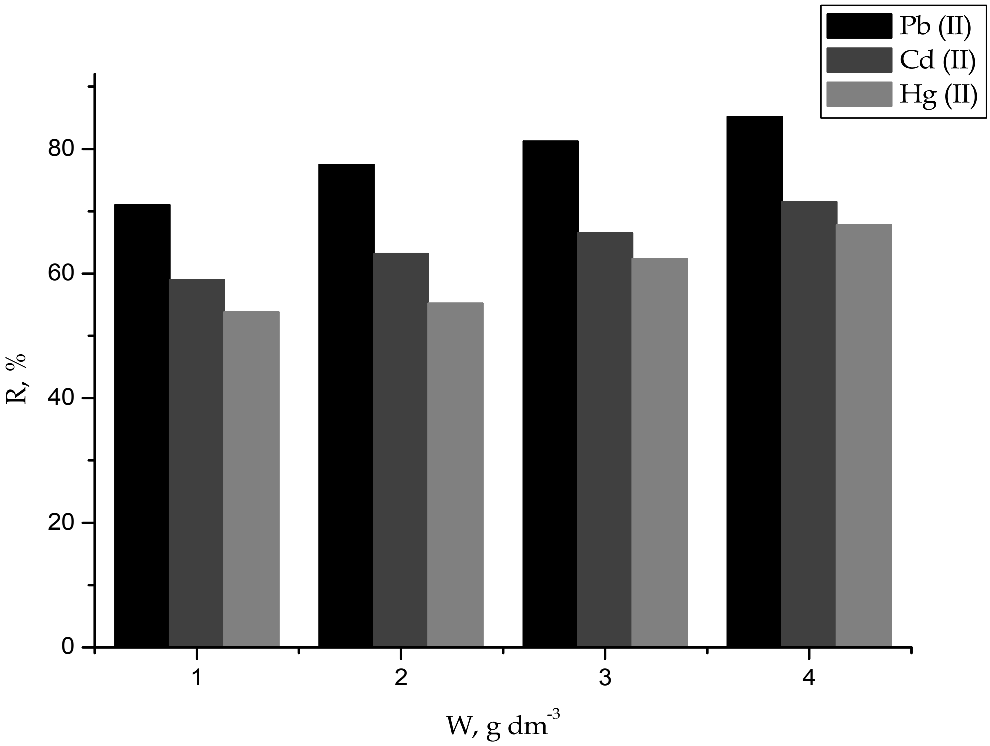

Fig. 4. Effect of biomass dosage on Pb (II), Cd (II) an Hg (II) removal efficiency (initial concentration 25 mg dm^{-3}, pH 5.0, contact time 120 min).

It was observed (Fig. 4, that increasing biosorbent concentration resulted in an increase in removal efficiency for metal ions onto waste pretreated *Bacillus thuringiensis* biomass. The removal efficiency increases from 71.04 % to 85.21 % for Pb (II), from 59 to 71.5 % for Cd (II) and from 53.8 to 67.80 % for Hg (II) ions with the rise of biomass concentration from 1.0 to 4.0 g dm^{3}. The calculated metal uptakes showed a reverse trend. For Pb (II), the biosorption uptake decreased from 17.76 to 5.33 mg g^{-1}.

A high biomass concentration increases the specific surface area and the number of active binding sites leading to increased removal efficiencies, but the metal uptake per gram of biomass decrease (BAI & ABRAHAM, 2001; FRAILE *et al.*, 2005; KIROVA *et al.*, 2015b). A biomass concentration 1 g dm^{3} is used in subsequent experiments.

Effect of initial concentration of metal ions

Biosorption studies with waste pretreated biomass (1 g dm^{-3}) were conducted using solutions containing from 5 to 75 mg dm^{-3} metal ions at pH 5.0 and contact time 120 min. The results are shown on Fig. 5.

The biosorption capacity of the waste pretreated biomass increased with increasing initial concentration of metal ions. A saturation value was observed around 50 mg dm^{-3} for Pb (II), Cd (II) and Hg (II) ions and the calculated uptakes were 19.45, 16.28 and 15.39 mg g^{-1} respectively. Increasing initial metal concentration generally caused a decrease in removal efficiency and this fact could be attributed to the gradual blocking of the free active sites for metal ion binding on the surface of the biomass. At 5 mg dm^{-3} initial metal concentration 95. 6 % Pb (II), 81 % Cd (II) and 65 % Hg (II) were removed. The waste pretreated biomass showed a higher affinity for Pb (II) than for Cd (II) and Hg (II) ions. Similar results have been reported in other studies (KIROVA *et al.*, 2015a; TODOROVA *et al.*, 2019; TÜZÜN *et al.*, 2005).

Effect of contact time

Contact time is one of the important factors affecting the efficiency of biosorption, but contact time depends on the experimental conditions – type of biomass, particle size, type of metals, etc. Biosorption of Pb (II), Cd (II) and Hg (II) ions by waste alkali pretreated biomass was studied with different contact times (5 – 120 min).

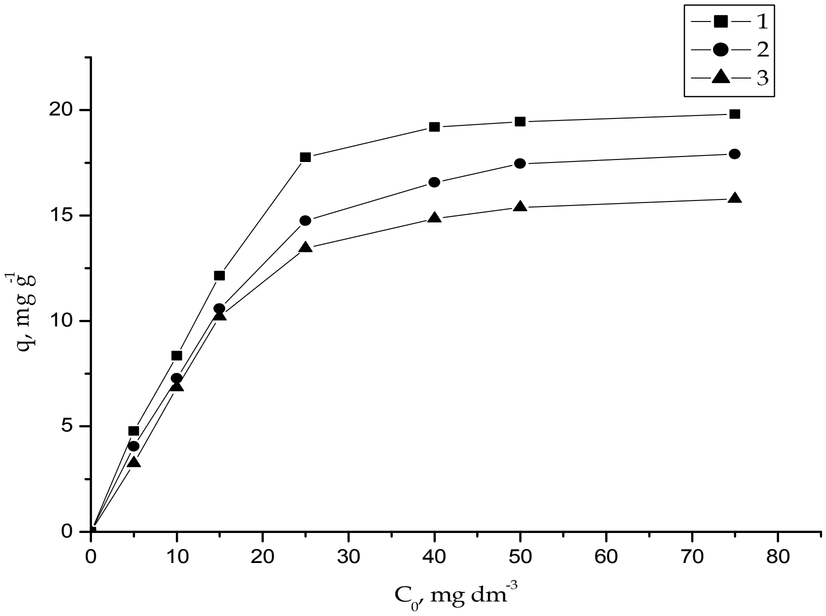

Fig. 5. Effect of initial metal concentration of metal uptake (1 – Pb (II), 2 – Cd (II), 3 – Hg (II)).

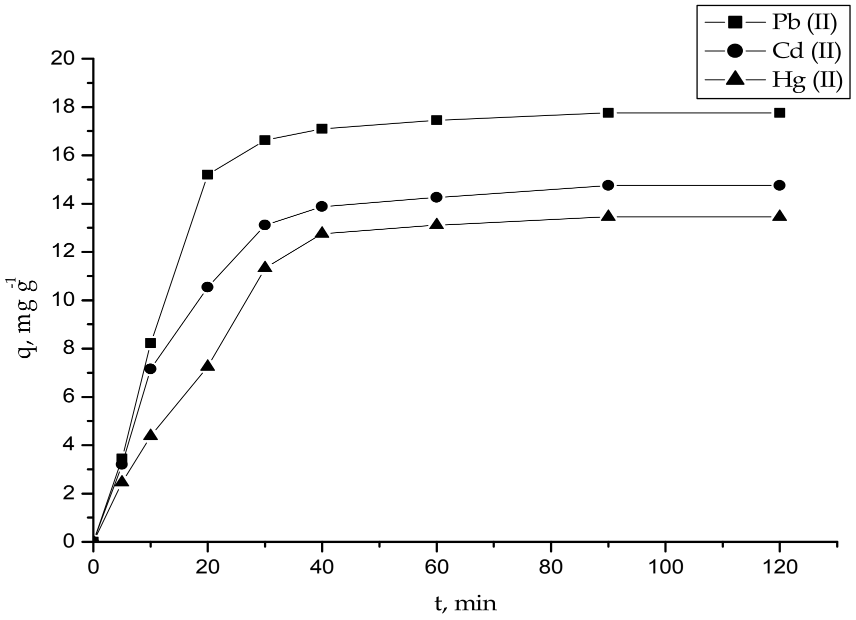

Fig. 6. Effect of contact time on the biosorption (pH 5.0, biosorbent dose 1.0 g dm^{-3}, intial concentration 25 mg dm^{-3}).

As seen on Fig. 6, the biosorption for each of the studied metal ions can be divided into two stages. The first stage was shorter (about 30 min) and characterized with higher biosorption speed and faster increase in the metal uptake. For second stage, a slower increase in metal uptake were observed. Equilibrium was reached at 90 min. At the end of the first stage the removal efficiency was 66.48, 52.44 and 45.32 % for Pb (II), Cd (II) and Hg (II) ions.

WANG (2012) found that the biosorption of Cd (II), Zn (II) and Cu (II) on *Saccharomyces cerevisiae* biomass was rapid for first 15 min and equilibrium was nearly reached after 90 min. OVES *et al.*, (2013) studied the biosorption of five heavy metals (Ni, Pb, Cu, Cd and Cr) by *Bacillus thuringiensis* strain OSM29. They observed that the initial sorption rate was highest at the beginning and moved to equilibrium within half hour.

Biosorption from ternary solutions

Industrial effluents are usually composed of several different metal ions and for this reason biosorption from ternary metal system was studied. The biosorption uptakes of the caustic pretreated biomass were 8.02, 5.31 and 4.51 mg g^{-1} for Pb (II), Cd (II) and Hg (II) respectively. In the ternary system, the metal uptake was reduced for Pb (II) with only 3.95 %, for Cd (II) and Hg (II) – with 27.08 and 34.16 %, respectively, compared to the single metal solutions. The caustic pretreated biomass demonstrated the highest affinity to Pb (II) ions. Similar results were reported in other studies (KIROVA *et al.*, 2015a; TÜZÜN *et al.*, 2005). This fact can be explained with the larger ionic radius (1.19 Å), electronegativity (2.33) and covalent index (6.46) for Pb (II) ions, comparing to Cd (II) and Hg (II) ions (KIROVA *et al.*, 2015a; PEREIRA *et al.*, 2011; SALAM, 2019; SENGIL & OZACAR, 2009).

DUTTA *et al.* (2016) studied the competitive biosorption of Cd(II), Pb(II), and Cu(II) ions on EDTA-treated baker's yeast cells was studied by using artificially prepared wastewater containing 100 mg dm^{-3} of each metal at pH 5. A significant decrease in Pb(II), Cd(II) and Cu(II) uptake was observed (from 98.93, 21.12 and 13.28 mg g^{-1} to 20.40, 1.20 and 6.55 mg g^{-1}, respectively). GÖKSUNGUR *et al.*, (2003) observed also reduction in the uptake using ethanol – treated yeast cells. PARASZKIEWICZ *et al.* (2009) studied the removal efficiency of Pb(II), Cd(II) and Zn(II) ions on pretreated waste biomass *Curvularia lunata*, from single, binary and ternary solutions. They observed that the presence of Pb(II) significantly interfered in the sorption of zinc and cadmium ions. About 3-fold decrease in zinc and cadmium removal was detected in binary and ternary systems as compared with single solutions of Zn(II) and Cd(II). The removal efficiency of Pb(II) from single, binary (Pb +Zn, Pb + Cd) and ternary solutions was calculated as follow: 81.9, 79.2, 72.1 and 80.6 %. As seen the studied bisorbent of pretreated waste biomass of *B. thuringiensis* exceeded other microbial biosorbents as effective sorbent for competitive heavy metals removal from ternary solutions.

Conclusions

Alkali treated waste biomass from *Bacillus thuringiensis* can be applied as effective biosorbent for heavy metals removal from aqueous solutions. The waste pretreated biomass showed a higher affinity for Pb (II), followed by Cd (II) and Hg (II) ions. The highest removal capacity for Pb (II) was reached at optimal process parameters pH 5.0, biomass dosage 1 g dm^{-3} and contact time 90 min. The studied biosorbent is prospective because it successfully removes heavy metals not only from single but even from ternary solutions, which means that is is effective at model conditions closer to real waste waters. Additional studies for application of waste B. thuringiensis biomass in immobilized form for heavy metals removal are in progress.

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