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Pre-treatment processes of *Azolla filiculoides* to remove Pb(II), Cd(II), Ni(II) and Zn(II) from aqueous solution in the batch and fixed-bed reactors

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Abstract

Intact and treated biomass can remove heavy metals from water and wastewater. This study examined the ability of the activated, semi-intact and inactivated *Azolla filiculoides* (a small water fern) to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} from the aqueous solution. The maximum uptake capacities of these metal ions using the activated *Azolla filiculoides* by NaOH at pH 10.5 ± 0.2 and then $CaCl_2$/$MgCl_2$/NaCl with total concentration of 2 M (2:1:1 mole ratio) in the separate batch reactors were obtained about 271, 111, 71 and 60 mg/g (dry *Azolla*), respectively. The obtained capacities of maximum adsorption for these kinds of the pre-treated *Azolla* in the fixed-bed reactors (N_o) were also very close to the values obtained for the batch reactors (Q_{max}). On the other hand, it was shown that HCl, CH_3OH, C_2H_5OH, $FeCl_2$, $SrCl_2$, $BaCl_2$ and $AlCl_3$ in the pre-treatment processes decreased the ability of *Azolla* to remove the heavy metals in comparison to the semi-intact *Azolla*, considerably. The kinetic studies showed that the heavy metals uptake by the activated *Azolla* was done more rapid than those for the semi-intact *Azolla*.

Keywords: *Azolla filiculoides*; Activation; Semi-intact; Inactivation; Heavy metals uptake

1. Introduction

Up to now the different methods are used for the removal of heavy metals as important contaminants in water and wastewater. The chemical methods such as precipitation with lime or caustic soda, to effectively decrease of heavy metals to acceptable levels require a large excess of chemicals, which generates volumetric sludge and increases the costs [1]. On the other hand, a number of methods exist for the removal of heavy metals from liquid waste when they are present in high concentrations. These methods, meanwhile, are generally expensive and require frequent service attention, which include methods such as evaporation, electroplating, ion exchange and membrane processes [2].

The biosorption methods are especially considered in the recent decade. The major advantages of the heavy metals adsorption technology by biomass are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials [3,4]. Furthermore, biosorption methods often provide better results than activated carbon and natural zeolites and are comparable to synthetic ion-exchange resins [5].

Adsorbent materials (biosorbent) derived from suitable biomass can be used for the effective removal and recovery of heavy metal ions from wastewater streams [6]. Because of the many problems inherent in maintaining active microbial populations under highly variable conditions of wastewaters, living systems are often unreliable. However, certain types of microbial biomass, even in non-living form, can serve as a basis for development of biosorbent materials for the efficient removal of heavy metals [6,7]. The non-living biosorbents can also be re-used after regenerating the exhausted

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Nomenclature

ACS	*activated by chloride salts* ($CaCl_2$/$MgCl_2$/NaCl)
b	Langmuir constant, sorption binding constant (l/mg)
C_e	heavy metals equilibrium concentration (mg/l)
C_o	heavy metals initial concentration (mg/l)
C_t	heavy metals outlet concentration at desired level (mg/l)
H	depth of adsorption bed (m)
k	rate constant of adsorption (l/mg h)
m	biosorbent dry weight (g)
no. ACS	*not activated by chloride salts* ($CaCl_2$/$MgCl_2$/NaCl)
N_o	adsorption capacity at full saturation of the biomass (mg/l biomass)
q_e	heavy metals uptake (adsorption) by biomass (mg/g dry biomass)
Q_{max}	Langmuir parameter, maximum adsorption capacity (mg/g dry biomass)
SAA	*super-activated Azolla*
SIA	*semi-intact Azolla*
t	service time to breakthrough (h)
U	linear velocity (m/h)
V	suspension volume (l)
$X_{f.el}$	heavy metals final concentration in eluant (mg/l)

Greek letter

ς_{el}	eluant volume (l)

biomass using a suitable eluant [8]. The biomass include bacteria [9], fungi [10], yeast [11], marine algae [12] and others.

Azolla is a small aquatic fern. In fact, it is a symbiotic pair of *Azolla filiculoides* and a heterocystous blue-green alga *Anabaena azollae. Azolla* has been used as a fertilizer in botanical gardens because of nitrogen-fixing capability, therefore has been used for several decades as green manure in rice fields [13]. But, because *Azolla* is capable of colonizing rapidly to form dense mats over water surfaces, imposing negative effects on the aquatic ecology. Controlling its reproduction has been deemed necessary in some *Azolla*-abundant areas like South Africa [14] and the north part of Iran. In this regard, the development of an *Azolla*-based biosorbent for wastewater treatment, especially in developing countries, may benefit both environmental problems, by removing heavy metals from water using this weed [15].

The non-living *Azolla*, has been shown to be able to effectively adsorb hexavalent and trivalent chromium, zinc (II) and nickel (II) from solutions and electroplating effluent [15–17] and gold (III) from aqueous solution [18]. We had also shown that the removal of heavy metals could be increased due to the activation of the non-living *Azolla filiculoides* by the oxidant agent [19].

The initial binding and exchange of heavy metal ions to insoluble constituents in the non-living *Azolla* matrix most probably involves cell wall charged groups (such as carboxyl and phosphate). Pectin is an important polysaccharide constituent of plant cell walls, made of fragments of polygalacturonic acid chains with glycosidic bond α (1→4), which interact with Ca and Mg ions to form a three dimensional polymer [20,21]. K^+ and Na^+ are mostly present in *Azolla* cell as soluble salts [22]. It was shown that as the result of *Azolla* washing by acidic, neutral (distilled water) and alkali solutions were lost the considerable quantities of exchanger ions such as Ca^{2+}, Mg^{2+}, K^+ and Na^+ from cell wall [22]. Using $CaCl_2$ can increase $(-COO)_2Ca$ in the pectin structure of cell wall by exchange of each a Ca^{2+} with two H^+ of neighbor carboxyl groups [21]. This state increases *Azolla* ability for the ion-exchange or removal of heavy metals in the adsorption process. On the other hand, it had been shown that using NaOH in the pre-treatment process increases the Cu^{2+} uptake by yeast cells. It was explained by the removal of protein groups of the cell wall that makes non-adsorbable protein complexes with Cu^{2+} ions [25].

The degree of pectin methylation in the cell wall had been expressed as the relative content of between the quantity of methoxyl groups ($-COOCH_3$) in the chain, as well as the distribution of the carboxyl groups the chain [23]. It had been shown that the demethylation of pectin could be catalyzed by alkali solutions [24]. On the other hand, it was shown that the metal binding carboxyl groups in biomass, including pectin in the cell wall, can be blocked by methylation, viz. with the increasing $-COOCH_3$ groups [27].

The pH values of the solution in the biosorption processes to remove heavy metals by the non-living biomass such as algae and *Azolla* (as waterfern) were selected about 6 as the suitable values and it was shown the adsorption is decreased at the more acidic pHs [8,15,17,18]. These researchers explained that the decrease of adsorption at low pHs is due to the competition between protons and metal ions for the capturing same sites in the biomass cell wall that protons are successful. On the other hand, the higher pH values had not been used due to the rapid precipitating some of the ions such as Pb^{2+}.

The first objective of this work was to study of using NaOH and then $CaCl_2$, $MgCl_2$ and NaCl (individually, binary and ternary combinations) for activation of the non-living *Azolla filiculoides* to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}, separately. The next objective was to study of using HCl, CH_3OH, C_2H_5OH, $FeCl_2$, $SrCl_2$, $BaCl_2$ and $AlCl_3$ as inactivator materials in the pre-treatment (inactivation) process of *Azolla*. On the other hand, the role of HCl, H_2SO_4, CH_3COOH, HCOOH, EDTA, NaOH and Na_2CO_3 as the chemical desorbents and boiling agent as a physical factor was studied to recover these heavy metals.

2. Materials and methods

2.1. Azolla for pre-treatment

Fresh *Azolla* (as the raw living biomass) was collected from the surface of the rice fields, Gilan, north of Iran. In order to do pre-treatment processes of biomass, each 2.0 g of the *Azolla* sample was washed three times with distilled water (each time 100 ml for 30 min) and was air-dried in sunlight. These obtained *Azolla* samples (as the raw non-living biomass) were then sieved to particles of 2.0 mm before use. The all-experimental solutions in the pre-treatment process were also prepared by distilled water at $22 \pm 2\,^{\circ}C$ and agitation rate of 150 rpm. The all pre-treated *Azolla* samples, at last, were dried in oven at 60 °C for 10 h.

2.2. Preparing of activated and semi-intact Azolla filiculoides

Azolla was activated by three following methods: (i) by NaOH (0.2 M), alone, from pH 8.5–12.0. It consisted of five experiments (equal to number of pHs, viz. 8.5, 10, 10.5, 11 and 12). According to this method, the *Azolla* samples, which were activated at pH 10.5 ± 0.2 (as the obtained optimal range, from Fig. 3a and b) were considered as control, (ii) at first by NaOH (0.2 M) at pH 10.5 ± 0.2 (as the first step of activation) and then using $CaCl_2/MgCl_2/NaCl$ individually, and also as binary and ternary combinations (as the second step of activation). It consisted of nine experiments (Fig. 2), (iii) at first by NaOH (0.2 M) from pH 8.5–12.0 (containing five pHs and so five experiments) as the first step of activation process and then using $CaCl_2/MgCl_2/NaCl$ with 2:1:1 molar ratio, respectively (as the obtained optimal ratio from Fig. 2) as the second step of activation process.

The *Azolla* samples, which were activated at pH 10.5 ± 0.2 and then using $CaCl_2/MgCl_2/NaCl$ (2:1:1) were considered as the super-activated *Azolla* (SAA). The uptake ability of the heavy metals by the activated *Azolla*, according to the second method, was compared to that for control to evaluate effect of using $CaCl_2/MgCl_2/NaCl$.

In order to do these activation processes, the *Azolla* samples (2.0 g) were soaked in NaOH solution (0.2 M) at various pHs (from 8.5–12) for 10 h. The *Azolla* samples were then washed three times with distilled water (each time 100 ml for 0.5 min), to remove excess sodium ions. These *Azolla* samples can be used according to the first method of activation (i), as no. ACS (viz. without using the activator chloride salts) in Fig. 3a and b. In the second and third methods of activation (ii and iii), the *Azolla* samples, which were activated by NaOH (according to the first method of activation,) were soaked in 500 ml of $CaCl_2/MgCl_2/NaCl$ solutions as activator chloride salts, as the second step of activation (as ACS, in Fig. 3a and b). The activation by NaOH was considered as the first step of activation in these methods.

These chloride salts were used as individually, and also binary and ternary combinations at the total concentrations of 2 M, at pH 7.0 ± 0.2 for 5 h. The pH values of the solutions were adjusted using 0.1 M NaOH, and 0.1 M HCl. The binary combinations were selected in a 1:1 volume and molar proportion, viz. the concentration of each chloride salts in the samples of $CaCl_2/MgCl_2$, $MgCl_2/NaCl$ and $CaCl_2/NaCl$ was 1.0 M (250 ml for each chloride salts in each samples). The ternary agents were selected in a 1:1:1 volume proportion, viz. 166.6 ml for each chloride salts at the various molar ratios. For instance, the combination of $CaCl_2/MgCl_2/NaCl$ with 2:1:1 molar ratio, respectively, had 1.0 M $CaCl_2$, 0.5 M $MgCl_2$ and 0.5 M NaCl, etc. The activated *Azolla* samples were then washed three times with distilled water (each time 100 ml for 1 min) to remove excess Ca^{2+}, Mg^{2+} and Na^{+} (unadsorbed) from biomass.

In order to prepare the semi-intact *Azolla* (SIA), the biomass samples were soaked in the distilled water with pH 7.0 ± 0.2 (in absence of considerable H^{+} and OH^{-}) for 10 h, without using $CaCl_2/MgCl_2/NaCl$.

2.3. Preparing of inactivated Azolla

The inactivation of *Azolla* was performed by acidic agent (HCl), chloride salts ($FeCl_2$, $SrCl_2$, $BaCl_2$ and $AlCl_3$) and alcoholic agents (CH_3OH, C_2H_5OH). The study method of acidic pHs effect in *Azolla* inactivation by HCl (0.2 M), with and without using $CaCl_2/MgCl_2/NaCl$ (2:1:1), was similar to the method of the activation process at alkali pHs by NaOH, with and without using $CaCl_2/MgCl_2/NaCl$ (2:1:1) (Section 2.2). The use of HCl and $CaCl_2/MgCl_2/NaCl$ were considered as the first and second steps of the pre-treatment processes, respectively. This first step was considered as the inactivation step because as will be seen (Section 3.2.1) the use of HCl decreases the ability of *Azolla* to remove the heavy metals.

The chloride salts as inactivator materials (500 ml, 2 M) were used for each 2.0 g dry *Azolla*, individually. This process was performed during 10 h at pH 7.0 ± 0.2. The pre-treated *Azolla* was then washed three times with distilled water (each time 100 ml for 1 min) to remove the excess Fe^{2+}, Sr^{2+}, Ba^{2+} and Al^{3+} (unadsorbed) from biomass. The uptake ability of the heavy metals by these inactivated *Azolla* was compared to it for SIA.

Azolla was inactivated by alcoholic agents as follows: 2.0 g of dried biomass was packed in a column, refluxed through with 150 ml absolute methanol and ethanol (separately) and 1 ml of 0.1 M HCl for 7 h at a flow rate of 3 ml/min. The treated *Azolla* biomass was then washed with 5 l of 1 mM HCl. Four *Azolla* columns (as alcoholic controls) for each of the heavy metal ions were refluxed for 7 h with distilled water and then washed with 5 l of 1 mM HCl. These inactivated *Azolla* samples and alcoholic controls were used in the batch sorption studies.

Fig. 1 shows the summary of the *Azolla* pre-treatment processes, in this work.

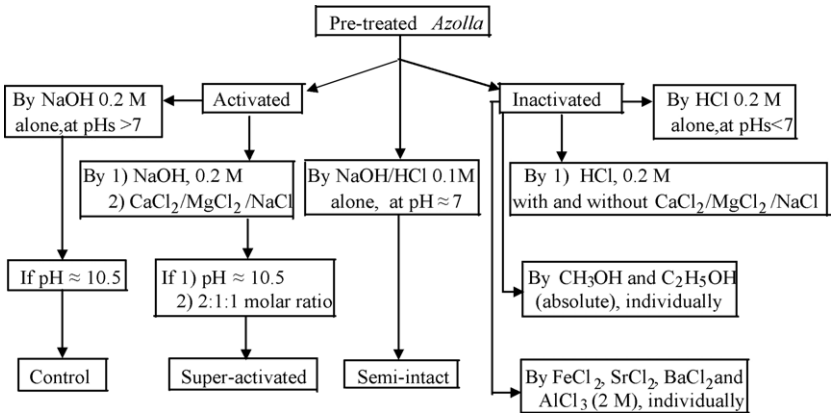

Fig. 1. The summary scheme of *Azolla* pre-treatment processes, in this study.

2.4. Adsorption experiments

The Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} stock solutions were prepared by dissolving their corresponding the salts of $Pb(NO_3)_2$, $CdCl_2{\cdot}2.5\ H_2O$, $NiCl_2$ and $ZnSO_4$ (analytical grade from Merck) in distilled water and standardized by atomic adsorption spectrophotometry.

2.4.1. Batch sorption

For the equilibrium studies (to obtain the adsorption isotherms), a series of flasks (250 ml, as the batch sorption reactors) were prepared containing the heavy metal solutions (100 ml) of known concentrations (C_o) varying from 1.00 to 4.7 mM (each solutions contained one metal ion). The experiments conditions were as follows: the addition of the pre-treated *Azolla* (200 mg) into the each flasks (dose 2.0 g biomass/l), agitating mixtures (150 rpm) for10 h as the adsorption time at $22 \pm 2\,^{\circ}C$ and adjusting adsorption pH at 5.5 ± 0.2 during the equilibrium period. The biomass was removed at last by filtration through a 0.45 μm membrane filter (Millipore) and the filtrate was analysed for ion content (C_e) by atomic absorption spectrophotometry. The other batch sorption studies were similarly done to remove the heavy metals (C_o 1.00 mM), individually.

2.4.2. Fixed-bed sorption

Eight glass columns of internal diameter 25 mm and varying bed heights were used. The columns were packed with 2.0, 4.0, 6.0 and 8.0 g dry super-activated and semi-intact *Azolla* (uniformly milled form) up to the bed heights of 7.8, 15.6, 23.4 and 31.2 cm, respectively. In other words, the mass of each 1 ml of dry *Azolla* at these experiments was 52.27 mg. The feeding each metal solutions (C_o 2.00 mM for each metal ions) were pumped upwards through the column, individually, by a peristaltic pump at pH 5.5 ± 0.2 and $22 \pm 2\,^{\circ}C$. The flow rate and linear velocity were maintained at 12 ml/min and 1.465 m/h, respectively. The heavy metals concentration at effluent (C_e) was measured by atomic absorption spectrophotometry. The difference of these concentrations with the influent concentration appears the metal ions uptake by *Azolla* as fixed-bed.

2.5. Desorption experiments

The solutions of HCl, H_2SO_4, CH_3COOH, HCOOH, EDTA, NaOH and Na_2CO_3 were used at concentrations of 0.1 M as chemical desorbents (eluant). In order to do this study, at first 100 ml of the heavy metals solution (with C_o 25 mg/l, for each metal ions) were prepared in 250 ml flasks, individually. The solutions were incubated with 2.0 g (dry *Azolla*)/l and pH 5.5 ± 0.2 at $22 \pm 2\,^{\circ}C$ with orbital shaking (150 rpm). After 1 h exposure, metals laden biomass were separated by centrifugation (7 min, 2000 rpm) and in order to desorption performance, mixed at 150 rpm and $22 \pm 2\,^{\circ}C$ with the 100 ml eluant for 30 min, individually. To use the boiling process as a desorbent agent, the separated *Azolla* samples by centrifugation were introduced into the jars containing of boiling deionised water (individually) for 30 min, so that the final volumes of suspension were reached to about 100 ml. Temperature range of this mixture was about $101 \pm 1\,^{\circ}C$ during the experiment. The samples were then collected to evaluate metals recovery. The desorbed heavy metals were measured by atomic absorption spectrophotometry.

3. Results and discussion

3.1. Effect of Azolla activation on the heavy metals adsorption

3.1.1. Comparative study of using $CaCl_2$, $MgCl_2$ and NaCl in the activation process (individually, and binary–ternary combinations)

The used *Azolla* samples in this study were activated at first by NaOH (0.2 M) at pH 10.5 ± 0.2. The effect quality of the activator agents was evaluated with due attention to the ability of activated *Azolla* samples by each agents

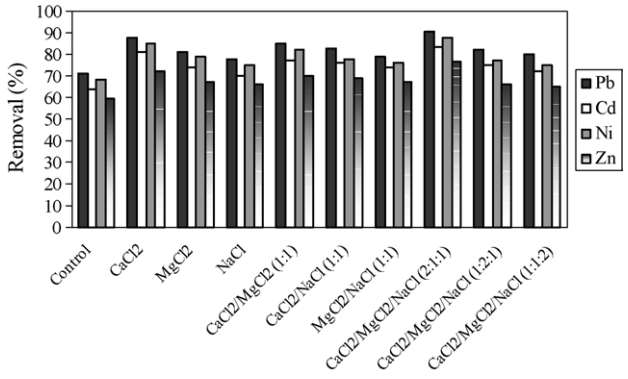

Fig. 2. Effect of using $CaCl_2$, $MgCl_2$ and NaCl in *Azolla* activation process as individually, and binary–ternary combinations on the heavy metals uptake (C_o = 1.00 mM).

to remove the heavy metals, in comparison to those for *Azolla* control (Fig. 2). The removal of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 1.00 mM) by *Azolla* control was determined about 71, 64, 68 and 59%, respectively. The activation ability of these materials is arranged about as: $CaCl_2/MgCl_2/NaCl$ (2:1:1); SAA > $CaCl_2$ > $CaCl_2/MgCl_2$ (1:1) > $CaCl_2/NaCl$ (1:1) > $CaCl_2/MgCl_2/NaCl$ (1:2:1) > $MgCl_2$ > $CaCl_2/MgCl_2/NaCl$(1:1:2) > $MgCl_2/NaCl$(1:1) > NaCl > control. In other words, the ion-exchange ability is according to following arrangement: $Ca^{2+} > Mg^{2+} > Na^{+}$.

The removal of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 1.00 mM) by SAA, in the batch study, was determined about 90, 83, 87 and 76% and/or about 271, 111, 71 and 60 mg/g (dry *Azolla*), respectively.

3.1.2. Studying effect of NaOH and $CaCl_2/MgCl_2/NaCl$ in Azolla activation

As can be seen from Fig. 3a and b, using $CaCl_2/MgCl_2/NaCl$ (2:1:1) as ACS, in the second step of the pre-treatment process increased the removal ability of heavy metals by *Azolla* although partially, in comparison to the non-consumption of chloride salts (as no. ACS), even without using NaOH in the first step of it (viz. at one of the acidic pHs 1, 3, 5 and 6). It can be explained due to Ca^{2+}, Mg^{2+} and Na^{+} replacement instead of the removed ions from *Azolla*. In this study, therefore, the exchanger ions could be lost as result of *Azolla* washing by distilled water (pH 7.0 ± 0.05) in the preliminary stage (prior to activation process) and in the pre-treatment processes at the various pHs.

On the other hand, the increase of used NaOH, alone, in the activation process, viz. without using $CaCl_2/MgCl_2/NaCl$ (no. ACS), had not remarkable effect on the removal of heavy metals in the adsorption process (especially after pH about 8.5).

In all probability, pectin's methylated carboxyls of *Azolla* ($-COOCH_3$) can be converted to free carboxyl groups by NaOH as an alkali agent in the activation process via demethylation, without dissociation of cell wall's three-dimensional structure.

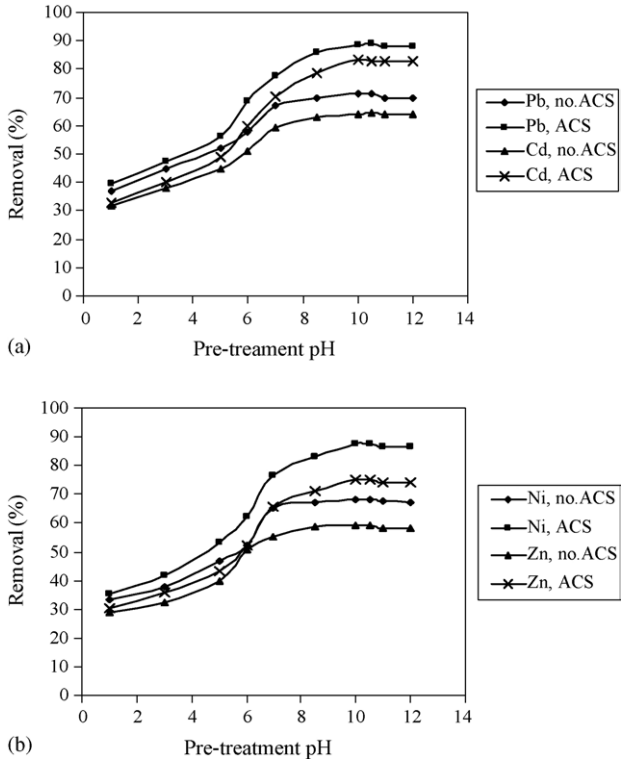

Fig. 3. Effect of using NaOH and HCl with (ACS) and without (no. ACS) $CaCl_2/MgCl_2/NaCl$ (molar ratio of 2:1:1, respectively) in *Azolla* activation and inactivation, respectively, to remove Pb^{2+} and Cd^{2+} (a) and Ni^{2+} and Zn^{2+} (b), C_o = 1.00 mM.

As can be seen from Fig. 3a and b, the increasing carboxyl groups in consequence of increasing NaOH increased heavy metals adsorption, remarkably, when $CaCl_2/MgCl_2/NaCl$ ions as the exchanger ions were existed, adequately, in the second step of activation process.

In other words, using $CaCl_2/MgCl_2/NaCl$ (2:1:1) in the second step of the activation (as ACS) in comparison to non-consumption of chloride salts (as no. ACS) increased the uptake ability of the *Azolla* samples which had been treated at one of the alkali pHs, viz. 8.5, 10 10.5, 11 and 12, as the first step.

Because, the created –COOH groups were converted to carboxylate agents as the important factors of adsorption, in presence of Ca^{2+}, Mg^{2+} and Na^{+}. Whereas the increasing free carboxyl groups alone can not remove the heavy metals, effectively, in the adsorption process.

3.2. Effect of Azolla inactivation on the heavy metals adsorption

3.2.1. Study of acidic agent (HCl)

Fig. 3a and b show that the more consumption of HCl (0.2 M) in the first step of pre-treatment process (from pH 6 to 1), decreased the *Azolla* ability to remove the heavy metals, with and without using $CaCl_2/MgCl_2/NaCl$ (2:1:1) in the second step of pre-treatment (ACS and no. ACS, respectively).

On the other hand, the using $CaCl_2/MgCl_2/NaCl$ (2:1:1) in the second step (as ACS) in comparison to the non-consumption of it (as no. ACS) increased although partially the uptake ability of the *Azolla* samples which had been treated by HCl, in one of the acidic pHs, viz. 1, 3, 5 and 6 (in the first step). It may be explained due to the dissociation of formed three-dimensional structure from pectin to the monomers by the hydrolysis at the acidic pHs, similar to hydrolysis of polysaccharides to monosaccharide [26]. In this case, the heavy metal ions either were not removed or the adsorbed trace quantities during the adsorption process, probably were desorbed from the monomers.

In other words, although, pectin demethylation of the *Azolla* cell wall may be performed by acidic materials (HCl) as the hydrolysis agent (similar to NaOH role), but the dissociation of three-dimensional structure by HCl controls the performance of the *Azolla* treated for heavy metals adsorption. For instance the removal of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 1.00 mM) by this inactivated *Azolla* in absence of $CaCl_2/MgCl_2/NaCl$ at pH 1 ± 0.1 was obtained about 37, 32, 33 and 29%, respectively. While these removals by SIA, were about 67, 59, 65 and 55%, respectively.

3.2.2. Effect of ethanol and methanol

The ethanol similar to methanol can perform the blocking of the metal binding carboxyl groups in biomass, including pectin in the cell wall, by ethylation, viz. with creation and/or increasing $-COOC_2H_5$ groups. As can be seen from Fig. 4, methanol has the more effect on the blocking sites of pectin relative to ethanol, which may be due to the more complete methylation than ethylation. The heavy metals uptake by the inactivated *Azolla* with methanol and ethanol were less than those for the alcoholic control *Azolla*. So that, the removal of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 1.00 mM) by the inactivated *Azolla* with methanol was obtained about 36, 33, 34 and 24%, respectively. While these removals by the inactivated *Azolla* with ethanol was determined about 41, 36, 38 and 31%, respectively. The removals by the alcoholic control *Azolla* at the same conditions were about 50, 43, 40 and 36%, respectively.

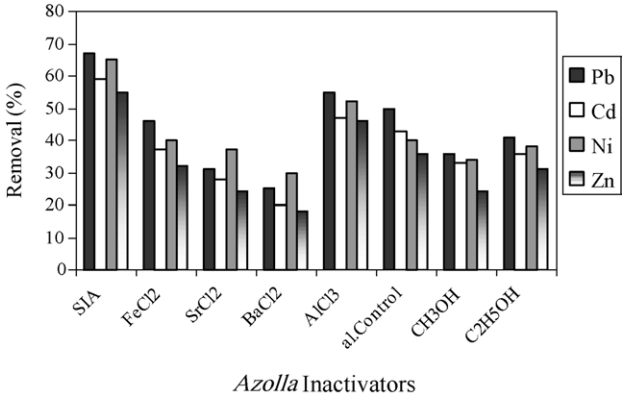

Fig. 4. Effect of using inactivator materials in *Azolla* pre-treatment process to remove heavy metals (C_o = 1.00 mM).

3.2.3. Effect of using $FeCl_2$, $SrCl_2$, $BaCl_2$ and $AlCl_3$, individually

Fig. 4 shows that the removal of heavy metals using the pre-treated *Azolla* by these materials was decreased in comparison to those for the SIA. The quantities of this decrease (as percentage) to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 1.00 mM) due to using each inhibitor are about as follows: ($FeCl_2$; 21, 22, 25 and 23%), ($SrCl_2$; 36, 31, 28 and 31%), ($BaCl_2$; 42, 39, 35 and 37%) and ($AlCl_3$; 12, 12, 13 and 9%), respectively. These results show that Fe^{2+}, Sr^{2+}, $Ba^{2+}$and Al^{3+} have the more affinity to the cell wall sites in *Azolla* relative to Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}. In the meantime, the affinity arrangement of those metal ions to the *Azolla* cell wall is as follows: $Ba^{2+} > Sr^{2+} > Fe^{2+} > Al^{3+}$.

3.3. Adsorption isotherms in the batch reactors

The isotherms can be described by Langmuir equation that is suitable for adsorption by non-living biomass [8]:

$$q_e = Q_{max}\frac{bC_e}{(1 + bC_e)} \quad (1)$$

where q_e is the metals uptake (mmol adsorbed/g dry biomass or mg/g), C_e is the metals equilibrium concentration or unadsorbed (mM or mg/l). Q_{max} (mmol/g or mg/g) and b $(mM)^{-1}$ or (l/mg), are the maximum adsorption capacity and a measure of adsorption energy (sorption binding constant), respectively. The Langmuir equation transforms to the linearized form:

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{(Q_{max}\, b)} \quad (2)$$

that Q_{max} and b are found from the slop and intercept of C_e/q_e versus C_e linear plot such that Q_{max} = 1/slope, and b = (slope/intercept). q_e is given from the following relation:

$$q_e = \left(\frac{C_o - C_e}{m/V}\right) \quad (3)$$

where C_o is the initial concentration of the metal ions (mM or mg/l), m is the biosorbent dry weight (g) and V is suspension volume (l).

Fig. 5a shows the obtained adsorption isotherms by SAA. The Q_{max} values to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} were about 271, 111, 71 and 60 mg/g (dry *Azolla*), respectively. Fig. 5b shows the obtained adsorption isotherms by SIA that the Q_{max} values for these metal ions were about 186, 95, 54 and 48 mg/g (dry *Azolla*), respectively. For instance, the removal of Zn^{2+} in one of the studies by this same type of *Azolla* [15] had Q_{max} of 45.2 mg Zn^{2+}/g (dry *Azolla*). Q_{max} also is 48.4 mg/g (dry *Azolla*) for the removal of Zn^{2+} by SIA in the present study. While due to activation process, the Q_{max} of Zn^{2+} removal by SAA was obtained 60.2 mg/g (dry *Azolla*) in this work.

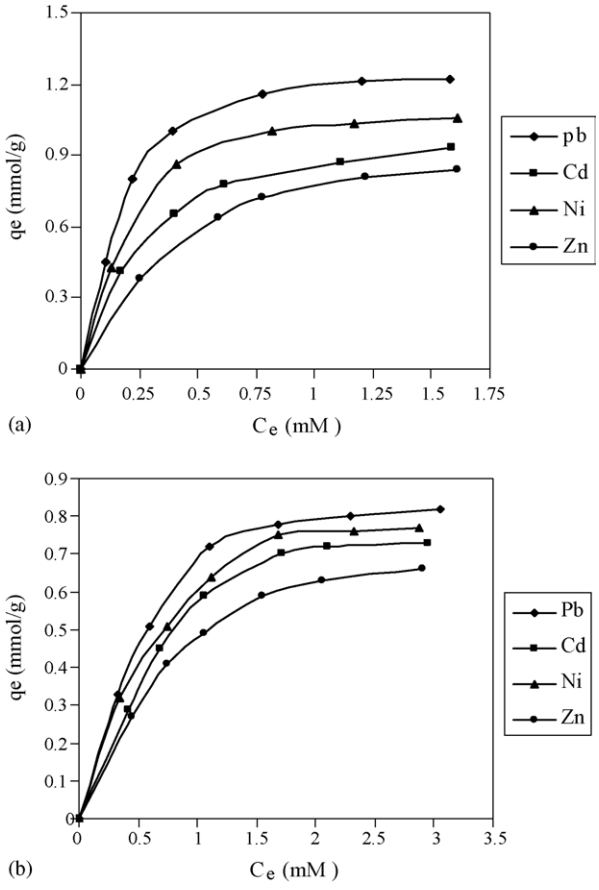

Fig. 5. Adsorption isotherms of heavy metals by super-activated (a) and semi-intact (b) *Azolla* in the batch reactors at 22 ± 2 °C. *Azolla* dose = 2g/l, adsorption pH 5.5 ± 0.2, adsorption time = 10 h.

Table 1 shows the values of Q_{max}, b and correlation coefficients (R^2) for the adsorption isotherms of the heavy metals by SAA and SIA in the present study.

Table 2 shows the values of C_e (mg/l), q_e (mg/g dry *Azolla*) and removal percentage of heavy metals (C_o 1.00 mM) in the adsorption isotherms by SAA and SIA.

3.4. BDST model plots in the fixed-bed reactors

The common model used to correlate service time with other design parameters in column systems is the Bed-Depth-Service-Time (BDST) model [28]. The modified form of this model states that the service time of a column is given by

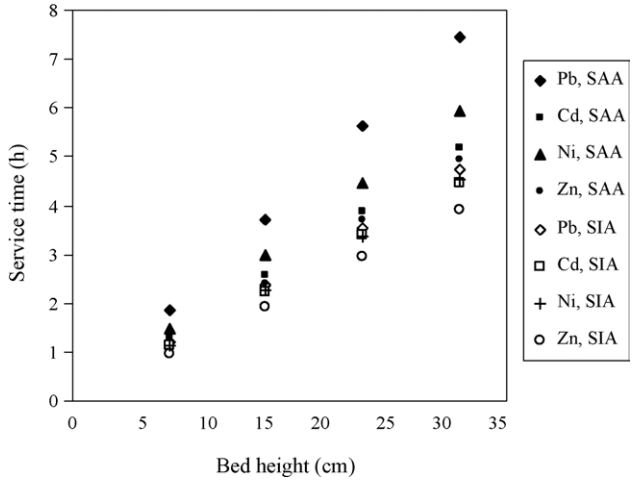

Fig. 6. BDST curves at 50% breakthrough to remove heavy metals by super-activated (SAA) and semi-intact (SIA) *Azolla* in the fixed-bed reactors. C_o = 2.00 mM, flow rate = 12 ml/min, linear velocity = 1.465 m/h, T = 22 ± 2 °C.

$$t = \left(\frac{N_o}{C_o U}\right) H - \left(\frac{1}{kC_o}\right) \ln\left[\left(\frac{C_o}{C_t}\right) - 1\right] \quad (4)$$

where t is service time (h) to breakthrough; N_o is adsorption capacity at full saturation of the biomass (mg solute/l biomass) which is equivalent to Q_{max} in the batch sorption (mmol solute/g biomass); C_o is initial solute concentration (mg/l); U is linear velocity (m/h); H is depth of adsorption bed (m); k is rate constant of adsorption (l/mg h) and C_t is outlet concentration at desired level (mg/l).

By plotting t against H from experimental data, N_o can be evaluated from the slope of the graph and k from the intercept at $t = 0$. In the column operation, the beginning of "breakthrough" is defined as the time, when the effluent concentrations of each metal ion is reached to the limit which can be determined by a suitable detector (such as that which is used in the atomic absorption spectrophotometer). The full saturation point of biomass (breakthrough 100%) is occurred when the concentration of metal ion in the effluent is reached to it in the influent. At 50% breakthrough (C_o/C_t) = 2, the logarithmic term (Eq. (4)) reduces to zero and the expression can be simplified as $t_{50} = (N_o/C_o U)H$. Thus, a t_{50}/H curve is a straight line passing through the origin, provided the data follow the model.

Fig. 6 was obtained for the heavy metals uptake by SAA and SIA at the breakthrough of 50%. The plots of service

Table 1
Langmuir constants of equilibrium isotherms by super-activated and semi-intact *Azolla*

	Super-activated *Azolla*			Semi-intact *Azolla*		
	Q_{max} (mmol/g)	b (1/mM)	R^2	Q_{max} (mmol/g)	b (1/mM)	R^2
Pb^{2+}	1.306	9.182	0.990	0.883	4.602	0.971
Cd^{2+}	0.992	6.968	0.981	0.852	2.368	0.944
Ni^{2+}	1.122	9.690	0.989	0.856	3.017	0.968
Zn^{2+}	0.918	5.370	0.963	0.745	2.271	0.943

Table 2
Equilibrium concentration (C_e), metals uptake (q_e) and removal (%) of heavy metals (C_o = 1.00 mM) in the batch reactor by super-activated and semi-intact *Azolla*

	Super-activated *Azolla*			Semi-intact *Azolla*		
	C_e (mg/l)	q_e (mg/g)	Removal (%)	C_e (mg/l)	q_e (mg/g)	Removal (%)
Pb^{2+}	22.82	92.18 ± 0.10	88.98	68.32	69.43 ± 0.050	67.02
Cd^{2+}	19.28	46.56 ± 0.060	82.84	46.10	33.15 ± 0.030	58.98
Ni^{2+}	7.60	25.55 ± 0.020	87.05	20.58	19.06 ± 0.016	64.94
Zn^{2+}	16.31	24.54 ± 0.020	75.06	29.48	17.95 ± 0.012	54.92

q_e values are as mean ± S.D. (n = 3).

Table 3
Maximum uptake capacities in the fixed-bed reactors (N_o)

	Super-activated *Azolla*		Semi-intact *Azolla*	
	N_o (mmol/g)	N_o (mg/l)	N_o (mmol/g)	N_o (mg/l)
Pb^{2+}	1.350	14635	0.850	9209
Cd^{2+}	0.989	5816	0.809	4756
Ni^{2+}	1.064	3264	0.816	2504
Zn^{2+}	0.882	3018	0.723	2399

R^2 = 0.999.

time against bed height were linear (R^2 = 0.999) indicating the validity of BDST model for the present system. The sorption capacities of the bed per unit bed volume, N_o, were calculated from the slope of BDST plots (for each metal ion and by SAA and SIA, individually), assuming initial concentration, C_o, and linear velocity, U as constant during the column operation. The obtained N_o values (Table 3) were very close to the values obtained for batch equilibrium experiments (Q_{max}).

3.5. Kinetic studies

The kinetic batch experiments were performed by SAA and SIA, individually. As can be seen from Fig. 7, the rate of the heavy metals uptake (100 ml with C_o 1.00 mM) by SAA was rather fast, so that 87–91% of the total uptake was occurred in 25 min, viz. it was determined about 81, 74, 78 and 65% to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}, respectively. While 68–71% of the total uptake was occurred at the same time by SIA, viz. the removal of about 47, 41, 45 and 37% for these metal ions, respectively.

The adsorption of the heavy metals by SAA was also completed after about 4.6 h, while this state was occurred after about 6.1 h by SIA. The removal percentages of these states (at completed sorptions) are shown in Table 2. In other words, the rate of heavy metals uptake both in the initial contact times and at the last parts sorption can be increased with the increasing concentration of $Ca^{2+}/Mg^{2+}/Na^{+}$ as ion-exchanger agents in the three dimensional polymer of SAA cell wall.

3.6. Influence of agitation rate

In order to determine the optimal agitation rate, the uptake of heavy metals by SAA and SIA were evaluated while varying the agitation rate from 0 (without agitation) to 500 rpm. The obtained results from Fig. 8 show that the optimal agitation rate to uptake by both SAA and SIA was in the range 150–200 rpm. This indicates that a shaking rate in the range 150–200 rpm was sufficient to assure that all the cell wall binding sites were made readily available for heavy metals uptake, so the effect of external diffusion on biosorption rate can be ignored in any engineering analysis.

The uptake process by SIA has a more relationship to the agitation rate, in comparison to that for SAA, especially at less than 50 rpm and greater than 400 rpm. The decreased values of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} uptake by SIA at 500 rpm in comparison to those at 150 rpm were about 9, 7, 13 and

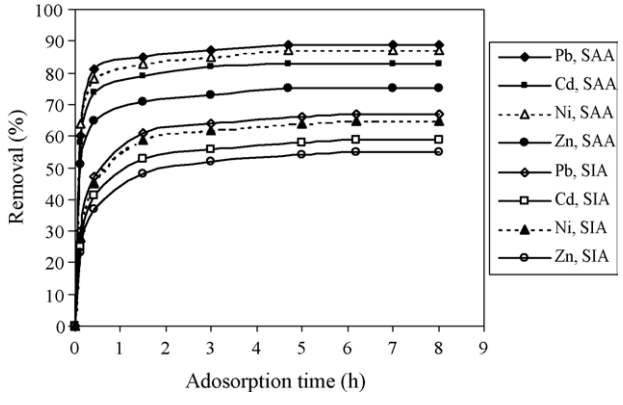

Fig. 7. Kinetics of heavy metals adsorption by super-activated (SAA) and semi-intact (SIA) *Azolla*. C_o = 1.00 mM, *Azolla* dose = 2 g/l, adsorption pH 5.5 ± 0.2.

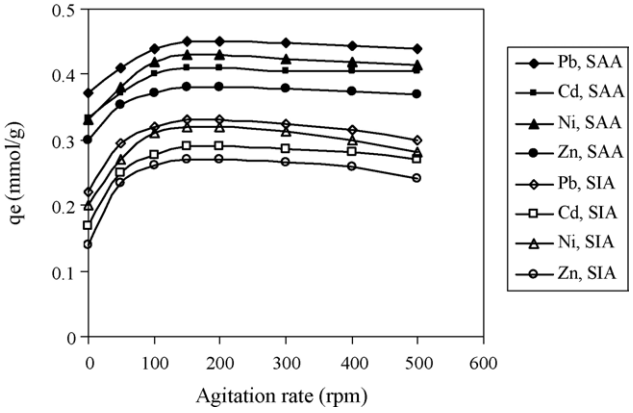

Fig. 8. Effect of agitation rate on the heavy metals (C_o = 1.00 mM) adsorption by super-activated (SAA) and semi-intact (SIA) *Azolla*.

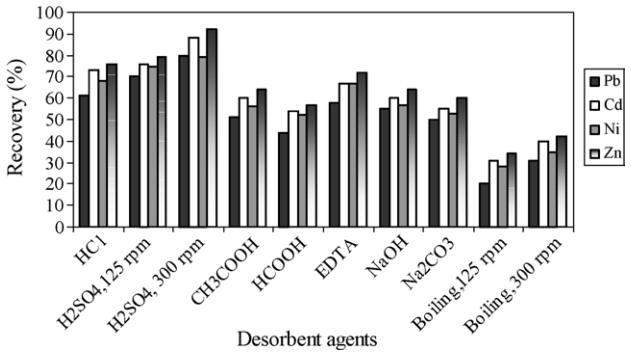

Fig. 9. Effect of desorbent agents in the heavy metals recovery (C_o 25 mg/l).

12%, respectively. It will be shown that the agitation rate is also effective in the desorption and recovery of the metal ions. So the decrease of uptake at the high agitation rates can be due to the desorption which was occurred in the adsorption process.

3.7. Recovery of heavy metals

The relation of heavy metals recovery by eluant (desorbent materials) from biomass is written as:

$$\text{Heavy metals recovery} = \frac{\text{desorbed}}{\text{adsorbed}} = (X_{\text{f.el}}\varsigma_{\text{el}}/m/q_{\text{e}})100 \quad (5)$$

where $X_{\text{f.el}}$ is the heavy metals final concentration in eluant (mg/l); ς_{el} is the eluant volume (l); m is the biosorbent dry weight (g) and q_{e} is the metal uptake (mg/g).

According to Fig. 9, the ability arrangement of used chemical eluants (all 0.1 M) and boiling as a physical desorbent agent to recover the heavy metals from *Azolla* were determined as follows: H_2SO_4 (at 300 rpm) > H_2SO_4 (at 125 rpm) > HCl > EDTA > NaOH > CH_3COOH > Na_2CO_3 > HCOOH > boiling (at 300 rpm) > boiling (at 125 rpm). The agitation rate in other cases was adjusted at 125 rpm. As can be seen, the maximum recovery of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 25 mg/l) was occurred by H_2SO_4 (at 300 rpm) amounting to about 80, 88, 79 and 92%, respectively. The desorption of metal ions by heating can be due to dissolving of pectin similar to thermal hydrolysis of polysaccharides [29].

4. Conclusion

The $Q_{\max}$ and b values (Langmuir constants) to remove heavy metals by the super- activated *Azolla* (SAA, treated at first by NaOH 0.2 M at pH 10.5 ± 0.2 and then by $CaCl_2/MgCl_2/NaCl$ with molar ratio of 2:1:1, respectively) were considerably higher than those for the semi-intact *Azolla* (SIA, treated only by distilled water), at the same conditions. In the batch studies, the $Q_{\max}$ values to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} were obtained 271, 111, 71 and 60 mg/g (dry *Azolla*), respectively, by SAA and obtained 186, 95, 54 and 48 mg/g (dry *Azolla*), respectively, by SIA. The b values to remove these heavy metals for the SAA were obtained 9.182, 6.968, 9.690 and 5.370 (1/mM), respectively, and for SIA obtained 4.602, 2.368, 3.017 and 2.271 (1/mM), respectively.

It was appeared that the ion-exchange ability of the settled activator ions in *Azolla* cell wall with the heavy metal ions is as follow: $Ca^{2+} > Mg^{2+} > Na^{+}$. The maximum uptake capacities of the heavy metals by SAA and SIA in the batch study ($Q_{\max}$) were agree well with those for the fixed-bed study (N_o).

The agitation rate not only affected in the batch adsorption but also was effective in the recovery process. The agitation rate of about 150 rpm was selected as the optimal value for the adsorption process. It was seen that the change of rpm to remove the heavy metals by SAA was more effective than those for the SIA.

The kinetic studies showed that the use of SAA increases the rate of the heavy metals uptake in the all adsorption stages relative to using the SIA.

The removal of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (C_o 1.00 mM) by the inactivated *Azolla* with methanol was obtained about 36, 33, 34 and 24%, respectively. While these removals by the inactivated *Azolla* with ethanol was determined about 41, 36, 38 and 31%, respectively. On the other hand, HCl, CH_3OH, C_2H_5OH, $FeCl_2$, $SrCl_2$, $BaCl_2$ and $AlCl_3$ acted as the inactivator materials in the pre-treatment of *Azolla filiculoides* for the heavy metals adsorption. It was appeared that the inactivator metals affinity to *Azolla* cell wall are as follows: $Ba^{2+} > Sr^{2+} > Fe^{2+} > Al^{3+}$.

The ability of the chemical desobents and boiling at the two agitation rates to recover the heavy metals from *Azolla* were determined as follows: H_2SO_4 (at 300 rpm) > H_2SO_4 (at 125 rpm) > HCl > EDTA > NaOH > CH_3COOH > Na_2CO_3 > HCOOH > boiling (at 300 rpm) > boiling (at 125 rpm).

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