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Study on arsenic biosorption using Fe(III)-treated biomass of *Staphylococcus xylosus*

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ABSTRACT

As(III) and As(V) biosorption using *Staphylococus xylosus* biomass pretreated with Fe(III) solutions were investigated here. Biomass at initial concentration of 7.0 g·l^{-1} was treated with 900 mg·l^{-1} of Fe(III) at pH 3.0, and contact time 1.0 h. Optimum values including pH, biomass concentration and biomass-arsenic contact time, were first investigated and determined at 7.0, 1.0 g·l^{-1} and 30 min for As(III) and 3.0, 2.0 g·l^{-1} and 2.5 h for As(V) respectively. Potentiometric titration of the biomass and FT-IR studies showed that carboxyl groups are mainly responsible for Fe(III) binding, whereas As(III) and As(V) are adsorbed on the biomass surface through interaction with >FeOH and >$FeOH_2^+$ groups. The maximum biosorption capacity was calculated using Langmuir model and found to be 54.35 and 61.34 mg·g^{-1} for As(III) and As(V) respectively. Adsorbed As(III) and As(V) was fully regenerated with 0.09 M HCl at S/L equal to 2.0 g•l^{-1}, showing that Fe(III)-treated biomass can be used effectively as a biosorbent for both forms of arsenic and it can be used for three subsequent adsorption/desorption cycles.

1. Introduction

Arsenic is among the most abundant elements in the earth's crust, it can be naturally found in soil and water, due to leaching from rocks and sediments and also as a result of anthropogenic sources, such as mining and processing sulfide ores, combustion of fossils fuel and use of pesticides [1]. Inorganic and organic arsenic species are found in natural waters whereas inorganic arsenic species are the dominant form in most of the groundwater and surface water sources [2]. Arsenic exists mainly in two inorganic forms, trivalent As(III) and pentavalent As(V) [3]. As(III) can be found in groundwater, whereas As(V) exists in oxygenated surface waters [4]. The presence of arsenic in natural waters is of primary concern, due to its lethal effects, whereas As(III) is 25 to 60 times more toxic than As(V) as well as several hundreds times more toxic than organic arsenic species [5]. Several countries around the world, such as Taiwan, India, Hungary, Mexico, Argentina, Chile and United States are reported to contain high arsenic concentrations in their groundwater [1,6].

The International Agency for Research on Cancer classifies arsenic in Class A of human carcinogens (prone to cancer of the bladder, lungs, skin, kidney, liver and prostate). Due to its acute lethal effects on human health, World Health Organization promulgated the maximum contaminant level (MCL) for arsenic in drinking water not to exceed 10 μg·l^{-1} [7].

In order to keep arsenic limit below 10 μg·l^{-1} several methods have been developed, including coagulation and flocculation, precipitation, adsorption, ion exchange and membrane filtration [7]. However, the standard methods developed to remove arsenic from industrial effluents are often expensive or fail to concentrate arsenic in small waste volumes [8].

Accordingly, there is an urgent need to develop a cost efficient treatment technology capable of separating arsenic from both drinking water and industrial effluents. One method that recently has gained attention is biosorption, where living or dead biomasses, as well as cellular products are used for the removal of metal or metalloid species. In that way, the problem of toxicity can be eliminated whereas no additional cost of nutrient supply and culture maintenance is required [9].

Several biosorbents including plant biomass such as *Moringa oleifera*, or sorghum, as well as bacteria, fungi, yeasts or algae have been used to remove As(III) and As(V) from aqueous solutions [9–12]. The capacity of the biomass used has been increased in some cases, applying several modification methods [13,14]. A chitosan-coated biosorbent has been used successfully for the removal of both forms of arsenic from aqueous solutions [15].

Iron and arsenic may co-exist in natural water sources [6]. Recently iron has been selected for the modification of biomass or other materials since it possesses a natural affinity towards arsenic species, exhibiting high removal efficiencies [16]. Pretreated tea fungus with $FeCl_3$ was found to be an effective biosorbent for As(III), As(V) and Fe(II) removal from ground water samples [12]. In addition, non-viable fungal biomass of *Aspergillus niger*, coated with iron

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oxide showed good removal efficiencies of As(III) and As(V) from aqueous solutions [4].

The main objective of this study was the removal of As(III) and As(V) from aqueous solution using Fe(III)-pretreated biomass of *Staphylococcus xylosus*. The adsorption capacity of *Staphylococcus xylosus* was determined using both Langmuir and Freundlich isotherm models. Potentiometric titration of the biomass along with FT-IR studies were carried out to identify the functional groups on the biomass surface that participate in Fe(III) and arsenic species binding. Desorption studies of As(III) and As(V) along with the extend of Fe(III) leaching were also attempted, whereas biomass reuse was further investigated and the results are reported here.

2. Materials and Methods

2.1. Bacteria and media

Staphylococus xylosus was isolated from contaminated soil in a mining industry near Stratoni, Chalkidiki, Greece and identified according to the criteria described in Bergey's Manual of Systematic Bacteriology [17], by professor E. Litopoulou Tzannetaki in the Microbial Laboratory of Agricultural School of Aristotle University, Thessaloniki, Greece. *Staphylococcus xylosus* was cultivated in Luria-Bertani broth containing 1.0% tryptone, 0.5% yeast extract and 0.5% NaCl (Scharlau Chemie, Barcelona) at 28 °C, using shake flasks in a water bath at 150 rpm (Julabo SW-20C, Germany). Cells were harvested by centrifugation (Kubota 5922, Japan) at 2000 g for 20 min at the static phase of growth after 24 h of incubation and autoclaved (Systec, Germany) at 121 °C for 20 min before their use. Moisture content was determined by drying a pre-weighted amount of the cells in oven (Heraeus, UK) at 100 °C for 10 h.

2.2. Staphylococcus xylosus treatment with iron(III)

Biomass treatment was conducted with Fe(III) solutions at 525 mg·l^{-1} to determine optimal pH, contact time and biomass concentration (dry weight). Cells were suspended in standard solutions of Fe(III), prepared from $FeCl_3$ (Merck, Germany). The effect of pH was investigated in the range of 1.0-7.0 at a biomass concentration of 1.0 g l^{-1} and contact time 24 h. Kinetics of Fe(III) sorption on *S. xylosus* were also studied the first 24 h. Biomass loading was 1.0 g·l^{-1} and pH was adjusted to 3.0 using 0.1 M HCl and 0.1 M NaOH (Merck, Germany). Optimum biomass concentration was also examined in the range of 0.5 to 20.0 g·l^{-1}.

The effect of the initial Fe(III) concentration on the retaining capacity of biomass was studied at 10 to 1000 mg·l^{-1} until equilibrium state is reached. At the end of each experiment, the mixture was centrifuged (2000 g for 20 min) and the remaining concentration of Fe(III) in the supernatant was determined. The experimental data were processed via Langmuir and Freundlich isotherms.

S. xylosus biomass (7.0 g•l^{-1} d.w) was treated with 900 mg•l^{-1} Fe(III) at pH 3.0 and stored in refrigerator (−18 °C) for further use. Moisture content was determined as described in section 2.1, by drying a pre-weighted amount of Fe(III)-treated biomass in oven at 100 °C for 10 h.

2.3. Biosorption studies with As(III) and As(V)

Treated biomass with 900 mg·l^{-1} of Fe(III) was used for the removal of As(III) and As(V) from aqueous solutions. At first, optimum conditions of As(III) and As(V) biosorption were studied similarly with Fe(III) in the previous experiments. The effect of pH was investigated in the range of 2.0-10.0 for As (III) and 1.0-7.0 for As(V) at a biomass concentration of 1.0 g·l^{-1} and contact time of 24 h. Kinetics of As(III) and As(V) sorption on *Staphylococcus xylosus* were studied the first 24 h. Biomass concentration was examined in the range of 0.5 to 5.0 g·l^{-1} for As(III) and 1.0 to 5.0 g·l^{-1} for As(V). Reference solutions were prepared from As_2O_3 and $Na_2HAsO_4{\cdot}7H_2O$ (Merck, Germany) for As(III) and As(V) respectively.

Similarly with Fe(III) biosorption the effect of the initial As(III) and As(V) concentration on its biosorption was studied from 10 to 300 mg·l^{-1}, until equilibrium state is reached. At the end of each experiment, the mixture was centrifuged (2000 g for 20 min) and the remaining concentration of arsenic species in the supernatant was determined. The experimental data were processed also via Langmuir and Freundlich isotherms.

2.4. Langmuir and Freundlich models

Langmuir and Freundlich isotherm equations were used to describe the equilibrium state for single-ion adsorption experiments. The theoretical basis of Langmuir equation (1) relies on the assumption that there are a finite number of binding sites, which are homogeneously distributed over the adsorbent surface of the cells, having the same affinity for adsorption of a single molecular layer and there is no interaction between adsorbed molecules. The mathematical description of the equation is:

$$Q = \frac{Q\,\max \cdot b \cdot C_e}{1 + b \cdot C_e} \quad (1)$$

Where C_e is the metal residual concentration in solution (mg·l^{-1}), Q_{max} the maximum specific uptake corresponding to sites saturation (mg·l^{-1}), and b the biomass-metal binding affinity (l·mg^{-1}) [16].

The Freundlich equation (2) is the empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether the adjacent sites are already occupied or not. This empirical equation has the form:

$$Q = k_f \cdot (C_e)^{1/n} \quad (2)$$

Where, K_f and n are constants for adsorption capacity and adsorption intensity respectively [16].

2.5. Biomass characterization

Potentiometric titration and Infra-Red spectroscopy were used to characterize the type of functional groups and their concentrations on the biosorbent surface after biomass treatment with Fe(III) as well as after sorption and desorption of arsenic species. Potentiometric titration was performed by the addition of 0.1 ml 0.1 N NaOH at 1.0 g·l^{-1} of biomass in deionized water. The pH of the suspension was measured following titrant addition, starting from an initial value of 2.50, until equilibrium state is reached, using a Denver pH-meter with a Mettler Toledo Inlab Easy pH electrode (USA). Inflection points were determined plotting the successive amounts of NaOH added (x-axis) versus dpH/dV (y-axis). The peak location on the x-axis gives the number of biomass acidic groups [18,19]. The amount of NaOH added can be estimated from the following equation (3)

$$[NaOH] = \frac{V \cdot C_{NaOH}}{m_b} \quad (3)$$

Where *[NaOH]* is the amount of titrant added (mmol•g^{-1}), V is the volume of the titrant added (l), C_{NaOH} is the concentration of NaOH used (mmol•l^{-1}) and m_b is the mass of *S. xylosus* biomass (g).

Samples of lyophilized biomass were scanned into transmission mode using an Equinox 55, AXS Bruker (USA) FT-IR spectrophotometer at a wavelength range of 400 to 4000 cm^{-1} and resolution number at 2 cm^{-1}.

2.6. Desorption studies

The effect of varying HCl concentrations between 0.02 and 0.1 M on As(III) and As(V) desorption after biosorption at 200 mg•l^{-1} was studied the first 24 h using 2.0 g·l^{-1} of arsenic loaded biomass. The use of distilled water as a desorbing agent was also examined. The effect of solid to liquid ratio (S/L) ranging from 2.0 to 20.0 g•l^{-1} was investigated and the optimum volume of HCl necessary to dislodge the deposited metal from the biomass was determined.

Kinetics of As(III) and As(V) desorption using 0.09 M HCl were performed, whereas possible Fe(III) leaching was also examined. The efficiency of desorption experiments was expressed as elution efficiency which can be defined as [20]:

$$\textit{Elution Efficiency}\,(\%) = \frac{\textit{Amount of metal eluted}}{\textit{Amount of metal loaded}} \quad (4)$$

Further studies were carried out to determine if the biomass after desorption process could be reused effectively for the removal of As(III) and As(V).

2.7. Analysis.

As(III) and As(V) concentrations were measured using the molybdenum blue colorimetric method at 880 nm [21] and 843 nm [22]. Fe(III) concentration was determined by reading the absorbance at 530 nm of a deep purple colour complex formed between Fe(III) and sodium salicylate (Shimadzu UV-160A, Japan) [23].

All samples were taken in triplicate whereas mean values and deviation bars were calculated using Microsoft Excel 2003. Detection limits were also calculated and found equal to 20, 10 and 30 μg·l^{-1} for As(V), As(III) and Fe(III) respectively, in order to establish better the sensitivity of every method applied here.

3. Results and Discussion

3.1. Biomass treatment with Fe(III)

The first series of biosorption were conducted with raw biomass of *Staphylococcus xylosus* and the results did not show any significant removal of both arsenic species. In an attempt to increase biosorption capacity, *Staphylococcus xylosus* cells were treated with Fe(III) solutions first. Among the parameters affecting the efficiency of biosorption treatment, pH is concerned to be the most critical. Here, at higher than 2.0 pH values, a sharp increase in the percentage of Fe(III) sorption was evidenced probably due to the dissociation of biomass functional groups or their deprotonation [24]. The highest uptake capacities of Fe(III) (99%) were found at pH value of 3.0. At higher than 3.0 pH values, precipitation of Fe(III) occurs, due to the formation of ferric hydroxide [25].

Fe(III) sorption as a function of biomass loading was also studied. The percentage of Fe(III) uptake steeply increased to 90% with a biomass loading up to 7.0 g·l^{-1}. At higher than 7.0 g·l^{-1} biomass concentration, no further positive effect on Fe(III) uptake was observed, probably due to limitations in metal ion mobility.

Fe(III) uptake by *Staphylococcus xylosus* biomass was rapid taking place within a few minutes suggesting very active surface phenomena on the biomass [26]. In our experiments, the employed contact time of biomass and metal was 1.0 h to ensure equilibrium conditions between adsorbed and desorbed forms of arsenic species.

The metal initial concentration provides an important driving force for overcoming the mass transfer limitations of metal ion between the solid and aqueous phase. Here, sorption of Fe(III) from aqueous solutions approached 100% at initial concentrations between 10 and 200 mg·l^{-1}, showing the beneficial use of *Staphylococcus xylosus* biomass in complete detoxification of industrial effluents containing Fe(III). Biomass capacity was increased as the initial Fe(III) concentration increased from 10 to 500 mg·l^{-1} and reached a saturation state at initial Fe(III) concentrations higher than 700 mg•l^{-1}, as shown in Fig. 1. Therefore, the initial Fe(III) concentration was chosen in order to modify the biomass of *Staphylococcus xylosus* and used here after biosorption at 900 mg·l^{-1}, where maximum loading capacity was observed.

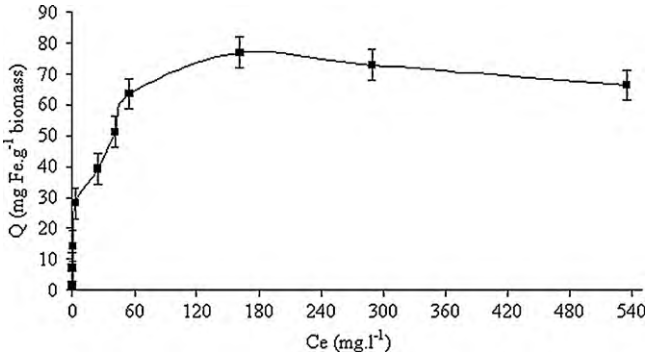

Fig. 1. Effect of initial Fe(III) concentrations on biosorption at biomass concentration 7.0 g•l^{-1}, contact time 1.0 h and pH 3.0.

Experimental data were simulated using the Langmuir model (Q_{max} = 69 mg·g^{-1}, b = 0.24 l·mg^{-1}, R^2 = 0.99), whereas Freundlich model found to be less representative (n = 2.46, K_f = 9.41, R^2 = 0.86). Other biosorbents reported have exhibited Q_{max} values at 37.3 mg·g^{-1} with grape stalks, 15.5 mg·g^{-1} with cork powder and 98 mg·g^{-1} with raw clinoptilolite, showing that *Staphylococcus xylosus* biomass can be included among the most efficient Fe(III) biosorbents and used further as a potential biosorbent system for arsenic removal [27,28].

3.2. Optimum conditions for As(III) and As(V) biosorption using Fe(III)-treated Staphylococcus xylosus cells

Fig. 2a shows the effect of pH on arsenic species biosorption. At higher than 2.0 pH values, As(III) removal was increased and maximum removal was obtained at pH 7.0. According to Vatutsina et al. [16], at pH 7.0, As(III) exists in the form of H_2AsO_3 which can interact with neutral >FeOH via both physical sorption and ligand (ion) exchange (outer- and inner-sphere complexes respectively). At lower than 7.0 pH values, As(III) exists only in the form of H_3AsO_3 which cannot bind with positively charged >$FeOH_2^+$. At higher than 7.0 pH values, $H_2AsO_3^-$ is repelled by >FeO^- anions and therefore lower removal efficiency of As(III) has been obtained.

In the case of As(V), optimum pH value was determined at 3.0. This may be attributed to interactions between predominant positively charged >$FeOH_2^+$ groups and the dominant ionic ($H_2AsO_4^-$) species. According to Vatutsina et al. [16] the main steps involved in As(V) biosorption include first ion exchange between the anion compensating the positive charge of group and arsenate species in the outer-sphere of >$FeOH_2^+$, followed by bond formation between Fe(III) and arsenate (inner-sphere complex). As it can be also seen in Fig. 2a, at pH values 1.0 and 2.0, As(V) sorption percentage is less than 10%, probably due to the absence of interaction between H_3AsO_4 and positively charged >$FeOH_2^+$ groups on the biomass. At higher than 3.0 pH values, the concentration of >$FeOH_2^+$ decreases gradually, causing a reduction in As(V) adsorption [16].

The effect of biomass concentration on As(III) and As(V) biosorption is shown in Fig. 2b. Optimum values of biomass concentration were determined at 1.0 g·l^{-1} and 2.0 g·l^{-1} for As(III) and As(V) respectively. Higher values than 1.0 g•l^{-1} had a lower impact on As(III) biosorption percentage, due to unsaturation of some binding

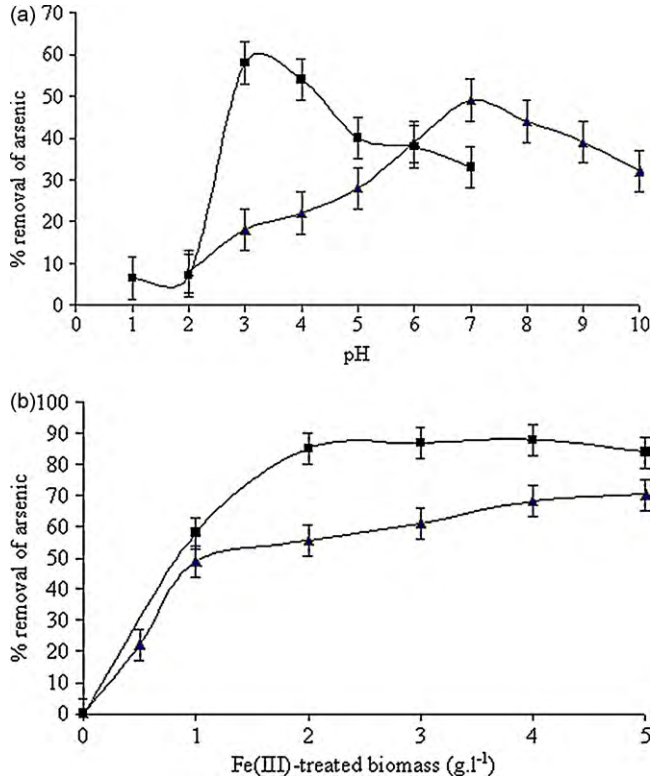

Fig. 2. Effect of pH on As(III) (-▲-) and As(V) (-■-) biosorption at initial concentration 50 mg•l^{-1}, biomass concentration 1.0 g•l^{-1} and contact time 24 h (a) and biomass concentration at initial arsenic concentration 50 mg•l^{-1}, contact time 24 h and pH 7.0 and 3.0 for As(III)and As(V) respectively (b).

sites [29], whereas at higher than 2.0 g·l^{-1} biomass concentrations, no further positive effect on As(V) uptake was observed due to strong limitation on arsenic species mobility.

The removal of As(III) and As(V) as a function of time indicated a biphasic pattern with a rapid initial uptake in both cases (Fig. 3). More specifically, sorption percentage of As(III) and As(V) approached 32 and 64%, within the first 5 minutes, whereas after a period of 30 and 150 min, equilibrium state was gradually reached (49 and 83% for As(III) and As(V) respectively). Therefore, 30 and 150 min were chosen as the optimum contact time to ensure equilibrium state between Fe(III)-loaded *Staphylococcus xylosus* cells and arsenic species in the solution. Slow adsorption kinetics may reflect the relative inaccessibility of the remaining binding sites possibly due to the fast occupation observed by both arsenic forms within the first 5 minutes.

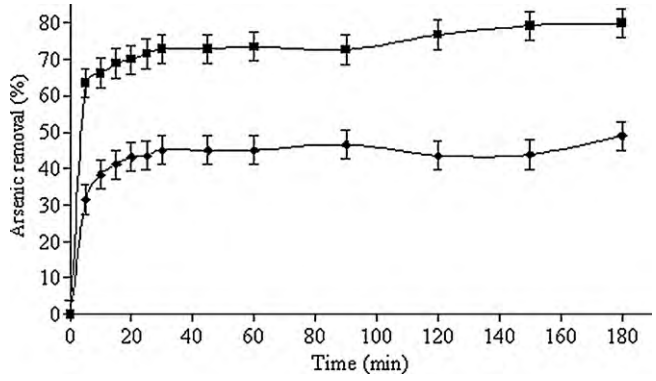

Fig. 3. Kinetics of As(III) (-▲-) and As(V) (-■-) at initial concentration 50 mg•l^{-1}, biomass concentration 1.0 g•l^{-1} and 2.0 g•l^{-1}, pH 7.0 and 3.0 for As(III) and As(V) respectively.

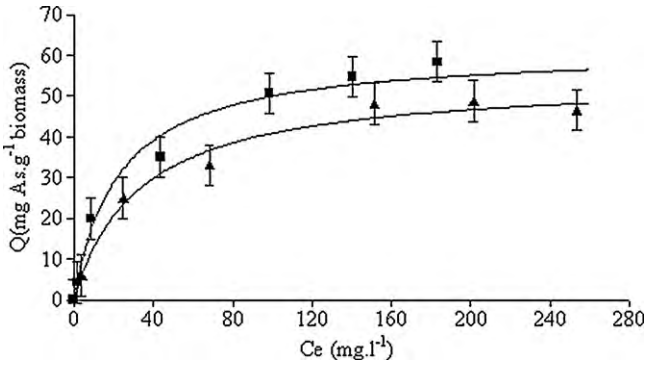

Fig. 4. Effect of initial arsenic concentrations on biosorption at biomass concentration 1.0 and 2.0 g•l^{-1}, pH 7.0 and 3.0 and contact time 30 min and 2.5 h for As(III) (-▲-) and As(V) (-■-) respectively.

3.3. As(III) and As(V) biosorption isotherms-Results interpretation

The equilibrium sorption data for As(III) and As(V) on Fe(III)-treated biomass of *Staphylococcus xylosus* at the optimum conditions previously determined, are given in Fig. 4. At 10 mg·l^{-1} of arsenic species, uptake capacities were obtained at 5.93 and 4.23 mg·g^{-1} for As(III) and As(V) respectively. In both forms of arsenic studied, the amount of adsorption capacity increases upon increasing their concentration and reaches finally a saturation point. This can be attributed to the high availability of As(III) and As(V) ions in the solution/biomass interface, which in turn results in enhanced adsorption capacity. When the surface active sites have been completely covered with arsenic species, the extent of adsorption reaches a limit, which can be described by the maximum biosorption capacity [30].

The experimental results were fitted to both Langmuir and Freundlich isotherms and the obtained parameters are listed in Table 1. Langmuir model was found to be more representative than Freundlich, exhibiting better values of correlation coefficients thus suggesting monolayer sorption and existence of constant sorption energy during the experimental conditions [10].

Although a direct comparison between the examined treated biomass with those obtained in literature is difficult, due to the varying experimental conditions employed, Fe(III)-treated *Staphylococcus xylosus* showed reasonably high sorption efficiency as compared with other adsorbents. More specifically, Al/Fe modified montmorillonite exhibited lower biosorption capacities of As(III) and As(V) at 18.19 and 21.19 mg·g^{-1}, whereas other biosorbents including *Acidithiobacillus ferrooxidedan* BY-3, *Bacillus sp.* strain DJ-1 and agricultural residues exhibited lower values of maximum uptake capacity at 277.22 and 323.85 μg·g^{-1}, 6.14 and 9.8 μg·g^{-1} and 138.88 and 147.05 μg·g^{-1} for As(III) and As(V) respectively [31–34]. However, *Inonotus hipidus* biomass exhibited maximum uptake capacity at 51.9 and 59.6 mg·g^{-1} for As(III) and As(V) respectively, values close to our results [35]. Taking into consideration that *Staphylococcus xylosus* has been previously used for the effective removal of Cd(II) and Cr(VI) in single and binary mixtures [36] and of Ni(II) [37], the results of this study render this strain among the most efficient adsorbents for the removal of toxic ions from aqueous environments.

Table 1
Langmuir and Freundlich constants for As(III) and As(V) biosorption.

	Langmuir constants			Freundlich constants		
	Q_{max} (mg•g^{-1})	b (l·mg^{-1})	R^2	K_f	$1/n$	R^2
As(III)	54.35	0.031	0.998	3.482	0.509	0.950
As(V)	61.34	0.048	0.999	4.377	0.532	0.948

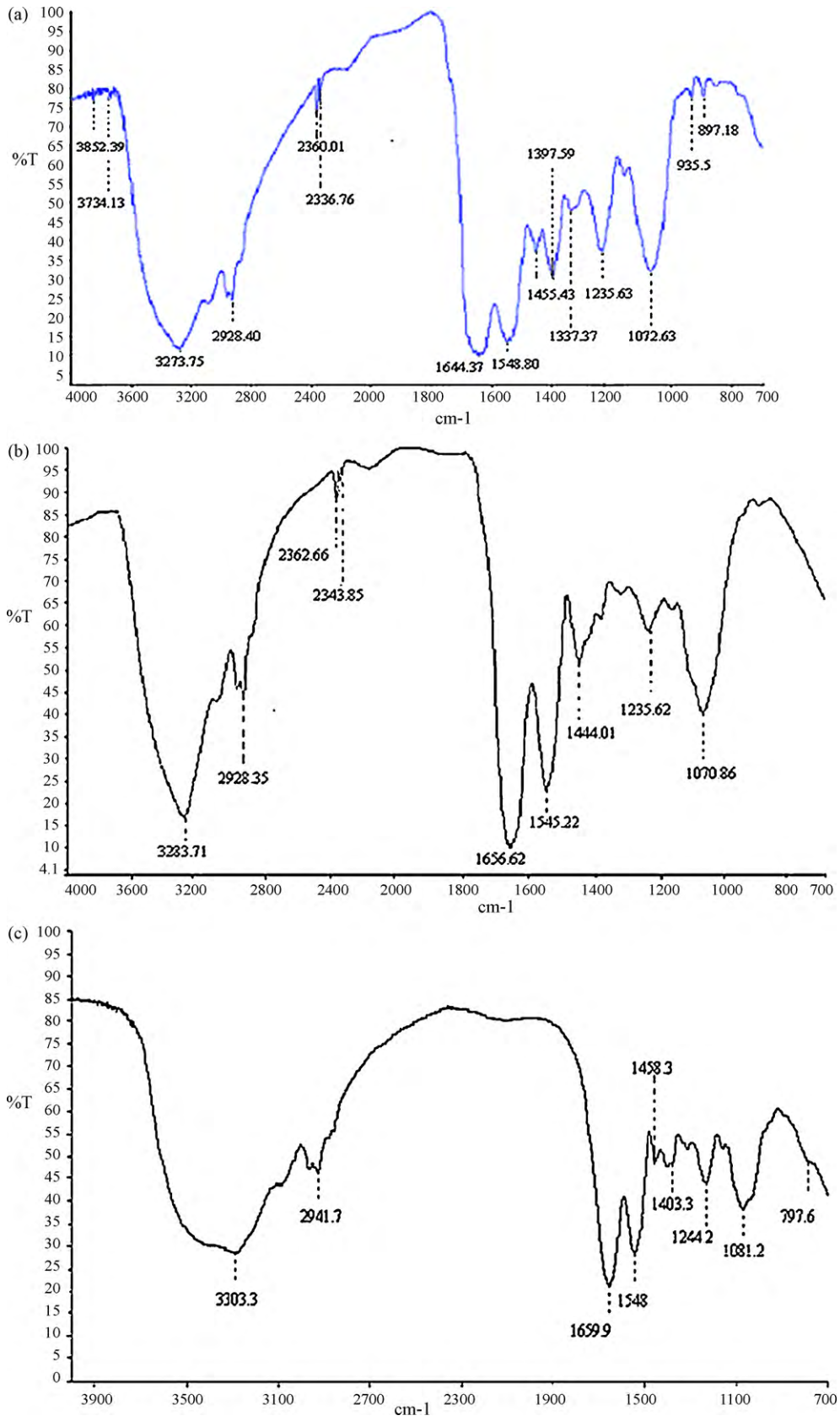

Fig. 5. FT-IR spectra of untreated biomass (a) biomass treated with Fe(III) (b) and Fe(III)-treated biomass followed by As(III) and As(V) biosorption (c) and (d).

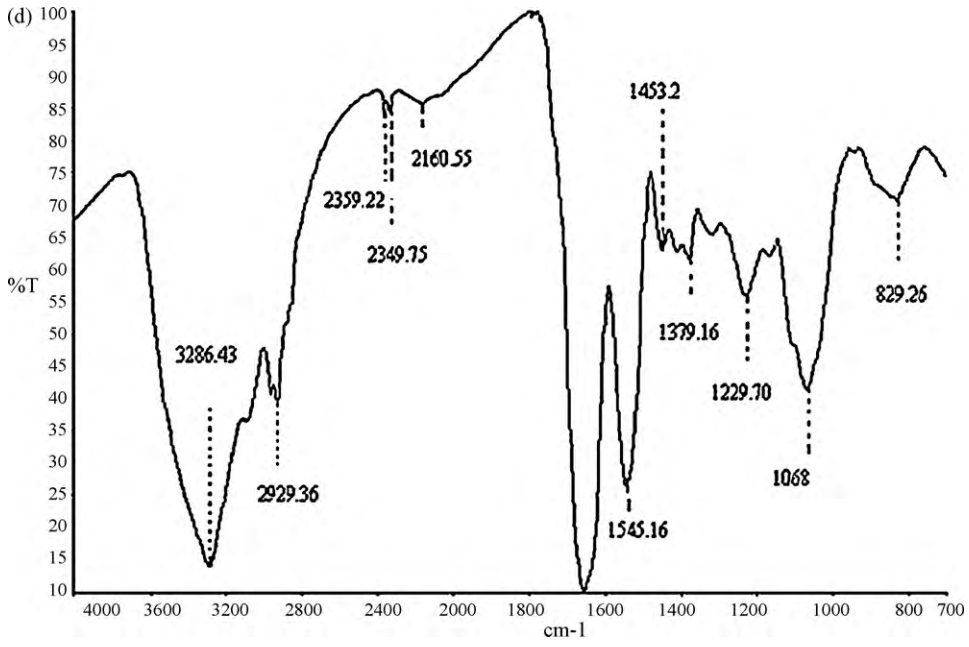

Fig. 5. (*Continued*).

3.4. Potentiometric titration and FT-IR studies

Fig. 5 shows FT-IR spectra of untreated biomass (a), biomass treated with Fe(III) (b) and Fe(III)-treated biomass followed by As(III) and As(V) biosorption (c) and (d) respectively, whereas Table 2 shows the inflection points obtained from potentiometric titration studies as well as their associated groups. Given the fact that biomass is a complicated material, determination of pKa values can confirm to a degree the nature of the organic groups on the surface of the biomass, since many of them have pKa values, which are being overlapped [39]. According to this, the combination of both pKa values and FT-IR spectra can help to a significant degree in the elucidation of the biosorption mechanism, which is attempted here.

More specifically, pKa value at 5.13 of the untreated biomass indicated that carboxyl groups on S. xylosus surface are mainly responsible for Fe(III) binding, since no inflection point at the same pKa value was observed after modification of biomass with Fe(III) [38]. The amount of carboxyl groups (2.4 mmol•g^{-1}) is two fold greater than the maximum iron uptake capacity (1.2 mmol•g^{-1}) indicating that two acidic groups are involved in Fe(III) binding [43]. The participation of carboxyl groups in Fe(III) binding can be further confirmed, from the comparison of FT-IR data between raw and Fe(III)-treated biomass (Fig. 5a and b respectively) where the absorbance at 1397.59 cm^{-1}, responsible for the presence of -OH in the carboxyl groups, is absent in FT-IR spectra of Fe(III)-treated biomass, showing possible binding of iron with these groups. However, we have to mention that this band reappears at pH 7.0 after As(III) biosorption at 1403.3 cm^{-1} (Fig. 5c), since Fe(III) exists in the form of >FeOH and no interaction is involved with carboxyl groups on the biomass.

Interaction between Fe(III) and amine groups seems to be also possible. This is evident by band shifting, observed from 3273.75 cm^{-1} (Fig. 5a) to 3283.71 and 3303.3 cm^{-1} after Fe(III)-treatment and As(III) biosorption respectively (Fig. 5b and c). Higher than 8.0 pKa values indicate also the presence of amine

Table 2
Inflection points of the titration curves and groups quantification on the biomass of *Staphylococcus xylosus*.

	pKa values	Number of groups (mmol•g^{-1})	Functional groups	References
Untreated biomass	5.13 ± 0.1	2.4 ± 0.2	Carboxyl	[38]
	5.71 ± 0.2	2.5 ± 0.3	Phosphoryl	[39]
	8.21 ± 0.1	2.9 ± 0.3	Amine	[40]
Fe(III)-treated biomass	5.78 ± 0.2	2.6 ± 0.3	Phosphoryl	[39]
	8.08 ± 0.1	2.9 ± 0.3	Amine	[40]
As(III)-Fe(III)	6.0 ± 0.2	3.7 ± 0.2	Phosphoryl	[39]
loaded biomass	7.64 ± 0.3	4.1 ± 0.2	Phosphate	[41]
	8.7 ± 0.2	4.5 ± 0.3	Amine	[40]
	9.16 ± 0.1	4.7 ± 0.2	H_3AsO_3	[42]
	9.56 ± 0.3	4.9 ± 0.2	Amine	[40]
As(III) leached biomass	6.0 ± 0.1	3.4 ± 0.2	Phosphoryl	[39]
	8.2 ± 0.2	3.8 ± 0.2	Amine	[40]
	9.56 ± 0.1	4.1 ± 0.2	Amine	[40]
As(V)-Fe(III)	4.7 ± 0.3	2.2 ± 0.2	Carboxyl	[38]
loaded biomass	6.34 ± 0.2	2.6 ± 0.1	Phosphoryl	[39]
	7.01 ± 0.2	2.9 ± 0.2	$H_2AsO_4^-$	[42]
	8.11 ± 0.2	3.3 ± 0.2	Amine	[40]
As(V) leached biomass	5.33 ± 0.2	2.2 ± 0.1	Carboxyl	[38]
	8.31 ± 0.2	3.1 ± 0.2	Amine	[40]
	9.0 ± 0.2	3.3 ± 0.2	Amine	[40]

groups (Table 2) [44]. In addition, amide I band was also shifted from 1644.37 cm^{-1} to 1656.62 cm^{-1} (Fig. 5a and b). The majority of the researchers have used band shifting to describe the interaction between ions in the solution and the groups on the biomass surface [45,46].

As(III) and As(V) presence on the biomass can be confirmed by the pKa values obtained at 9.16 and 7.01 respectively (Table 2), as well as from the bands appeared at 797.6 and 829.26 cm^{-1} (Fig. 5c and d). It has to be mentioned here, that in the case of As(III) a clear band was difficult to be obtained, comparing with the characteristic band of As(V) observed at 829.26 cm^{-1}. This may be attributed to different mechanisms involved in As(III) and As(V) biosorption. Other researchers report that a clear band can be reproduced only in the case of inner-sphere complex formation and not in the case of outer-sphere complex formation where physical sorption is involved [47].

In addition, Fig. 5c shows that As(III) biosorption was followed by a reappearance of the characteristic band at 1403.3 cm^{-1} and the disappearance of the weak bands at 2362.66 and 2343.85 cm^{-1}, indicating the presence of phosphine groups (Fig. 5b) and a possible transformation of the biomass at pH 7.0, in contrary with As(V) [48].

3.5. Desorption of As(III) and As(V) species

Various metals or metalloids associated with biomass waste constitute a valuable source of materials that can be reused in various ways. Desorption process is crucially important, since yields metals in a concentrated form, facilitating their disposal and thus making evident the restore of the biosorbent for effective reuse [49]. Several leaching agents have been used including sodium hydroxide or strong acids [14,50], even though, detailed recovery studies of metal/metalloids resources from various wastes instead of discarding in the landfill sites are limited [20,51].

The arsenic-exposed biomass was treated with HCl solutions at various concentrations. The results showed that at higher than 0.02 M HCl concentrations, desorption of As(III) and As(V) was increased and completed (100%) at higher than 0.09 M HCl. Pokhrel and Viraraghavan [13] have reported lower values of arsenic leaching (47.1 and 67% for As(III) and As(V) respectively) by iron oxide-coated biomass. Also preliminary studies showed that deionized water can be used to some extent, as an alternative means for the regeneration of the biomass, resulting in 28 and 25% of As(III) and As(V) recovery.

An important parameter for metal desorption is the solid to liquid (S/L) ratio defined as the mass of metal-laden biosorbent to the volume of the elutant. Fig. 6 presents the As(III) and As(V) elution efficiency using 0.09 M HCl as a leaching agent at various values of solid to liquid ratio. As it can be seen from this figure, complete

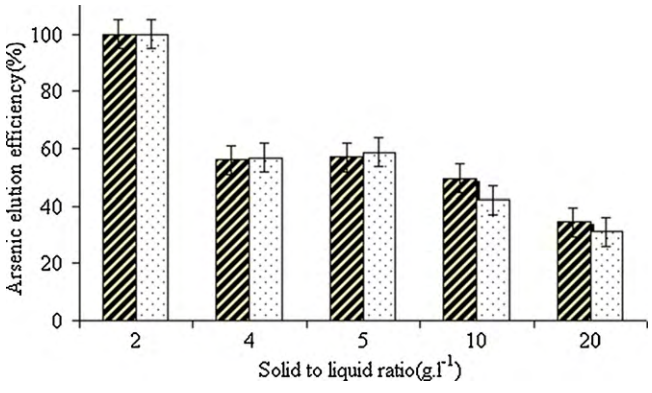

Fig. 6. Effect of Solid to liquid ratio in As(III) (▨) and As(V) (⁘)elution efficiency using 0.09 M HCl.

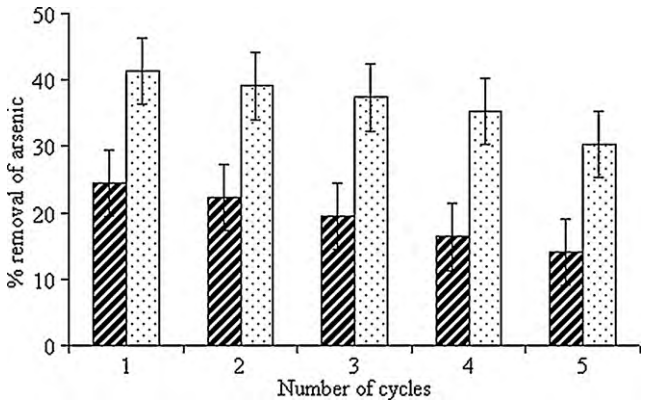

Fig. 7. Effect of subsequent sorption and desorption cycles of As(III) (▨) and As(V) (⁘) in the removal efficiency of Fe(III)-treated biomass at initial arsenic concentration 200 mg•l^{-1}, biomass concentration: 1.0 and 2.0 g•l^{-1} for As(III) and As(V) respectively.

desorption of As(III) and As(V) can be achieved at low values of S/L ratio, whereas a steep decrease in elution efficiency at higher than 2.0 g•l^{-1} values was observed. In general, it is desirable to use the smallest possible eluting volume so as to contain the highest concentration of the metal. However, at the same time, the volume of the solution should be enough to provide maximum solubility for the metal desorbed. In our case, biomass capacity after As(III) and As(V) sorption is high enough and therefore high volumes of 0.09 M HCl are necessary to elute completely the deposited arsenic species. The low values of elution efficiency achieved at high S/L ratio values may be attributed to the limited contact between the biomass and the HCl due to the dense suspension formed [20].

Another parameter affecting the elution efficiency is the concentration factor (CR). Concentration factor can be defined as the ratio between metal concentration in the eluant and metal concentration after the adsorption equilibrium, when process desorption is 100% efficient [20]. High CR is preferred for the overall performance of the sorption process, leading to a higher capacity of the sorbate metal. In our case, complete desorption was achieved at low value of S/L (2 g•l^{-1}), and CR was found to be unity for both arsenic species.

Kinetic studies of As(III) and As(V) desorption showed that in both cases biomass loses half of its content during the first 5 minutes. Complete As(III) and As(V) regeneration was achieved after 30 min and 2 h respectively, whereas Fe(III) leaching follows As(III) and As(V) detachment to some extent, due to double layer of Fe(III)/arsenic species formed on the surface of the biomass. Fast desorption kinetics indicate weak bonds between Fe(III)-treated biomass and arsenic species, similarly observed in biosorption phase (data obtained but not shown) [49].

To reduce the process cost and the dependency of the process on continuous supply of the sorbent, it is important to regenerate the biomass. When recycling process started, Fe(III) leaching was inevitable (in the presence of As(III), Fe(III) loss approached 13 and 97% after 30 min and 24 h respectively, whereas in the presence of As(V), Fe(III) loss approached 38 and 96% after 2 and 24 h respectively) and therefore, biomass was remodified using 250 and 500 mg•l^{-1} Fe(III) for As(III) and As(V) respectively. Regeneration of the biomass could ensure reusability only up to three cycles with both arsenic species at 200 mg·l^{-1}, exhibiting the same uptake capacities (Fig. 7), whereas after the fourth cycle, arsenic removal was reduced significantly to 14 and 31% for As(III) and As(V) respectively, due to biomass deterioration from subsequent acid treatments [52]. According to this, it would be better to use firstly prepared Fe(III)-treated biomass for arsenic biosorption, since the regeneration process is time-consuming and effective only up to three cycles.

4. Conclusions

Arsenic biosorption using *Staphylococcus xylosus* biomass pretreated with Fe(III) solutions was successful with both arsenic species studied, suggesting the potential use of this biomass in detoxification of Fe(III) containing industrial effluents. No significant differences were observed between As(III) and As(V) studied here, concerning the biosorption capacity of the biomass. The attractiveness of biosorption process was enhanced when desorption studies showed full recovery of As(III) and As(V) from the biomass in a short period of time, rendering its safe disposal in the environment. The recycling experiments with Fe(III)-treated *Staphylococcus xylosus* biomass showed that it can be used successfully as an effective biosorbent of As(III) and As(V) in more than one cycles.

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