

The effect of solvents on metal ion adsorption by the alga *Chlorella vulgaris*

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Received 3 March 2004; received in revised form 1 December 2004; accepted 2 December 2004

Available online 5 February 2005

Abstract

The dead dried alga, *Chlorella vulgaris*, was used for metal ion sequestering. The uptake of each of Cu(II), Cd(II), Fe(III), and Sn(IV) from their aqueous solutions decreased upon reuse of the biomass. Introducing mixed ethanol/water (50% v/v) metal ion solutions in batch systems enhanced the metal uptake of the exhausted biomass by 90% for iron, 40% for tin and only 14% for cadmium. In the column system, 20% v/v ethanol/water proved to be superior to acetone/water and isopropanol/water metal solutions in enhancing metal uptake. This increase in uptake offers a new method for regeneration of the algal capacity to remove metal ions from their solutions. Mössbauer results of iron and tin loaded algae from aqueous and mixed ethanol water solutions exhibited the characteristic doublet for (FeCl_4^-) at $0.350\text{--}0.565\text{ mm s}^{-1}$ respectively and the major doublet for inorganic Sn(IV) moieties at -0.217 to -0.365 mm s^{-1} respectively, excluding the possibility of microprecipitation of iron and tin, which is expected at such high concentrations of metals in the alga.

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Keywords: Biosorption; Biomass capacity; Chromatography; Mössbauer; *Chlorella vulgaris*

1. Introduction

Metal uptake by algae and microorganisms is an important phenomenon having many applications that range from sewage treatment (Hughes and Pool, 1989; Oswald, 1990) to precious metal recovery (Greene et al., 1986; Hosea et al., 1986). Several functional groups including carboxylates, hydroxyl, amines, phosphates, sulfates, etc. are involved in the biosorption of metal ions. Ion exchange, complexation, electrostatic attraction and microprecipitation are believed to

be the effective mechanisms. Such diversity in functional groups and mechanisms suggests that by changing working conditions such as pH, solvents, temperature and concentration, the nature of the binding sites and that of the adsorbed metal ion moiety could be altered affecting the capacity of exchange and selectivity towards metal ions (Darbal et al., 1986; Elmahadi and Greenway, 1993; Maquieira et al., 1994, 1996).

Algal biomass (packed into columns) has been used in metal ion sequestering, but batch methods appeared to be much more widely employed for the purpose (Ad-dour et al., 1999; Brady et al., 1999).

In recent years, the dead alga under study, *Chlorella vulgaris* (*C. vulgaris*), has received increased attention in studies on metal ion binding in batch systems; Lopez-Suarez et al. (2000) studied the effect of pH and time

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on the uptake of several metal ions, Aksu (2002) investigated nickel concentration and temperature effects on equilibrium and recommended the method for water treatment, and Aksu and Tezer (2004) studied the effect of pH and temperature on dye biosorption. However, no sorption studies were found on *C. vulgaris* using non-aqueous or mixed solvent metal solutions.

In this paper we report a comparative study of Fe(III), Sn(IV), Cd(II), and Cu(II) uptake by the alga *C. vulgaris* in aqueous and mixed solvent–metal solutions. The use of mixed ethanol/water metal solutions appears to be promising in regenerating the algal capacity to bind these metal ions.

2. Methods

The alga (*C. vulgaris*) was generously provided as a dry green powder by Dr. F. Al-Baz from the Botany Department at the National Research Center, Cairo. It was then washed several times with distilled deionized water, filtered and dried at $\sim 80^\circ\text{C}$ overnight (to a constant weight of the biomass). The dry biomass was sieved to a particle size of 600–355 μm and stored in closed bottles in a desiccator.

FeCl_3 anhydrous (BDH), $\text{Sn}(\text{CH}_3)_2\text{Cl}_2$ (Fluka) AR, $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ (Merck) AR, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (BDH), HCl (Riedl-de-Haen), Absolute Ethanol (Riedl-de-Haen), Isopropanol (Winlab) GRG, and Acetone (Winlab) AR, were used as obtained. Water used in all experiments was distilled deionized water produced by Elgastat Micromeg deionizer.

In batch system experiments, Fe(III) and Cd(II) were determined by atomic absorption spectrophotometry using a Shimadzu A.A-6701F spectrometer according to Vogel (1985). Tin was determined by ICP. In column chromatography experiments, Cu(II) was determined iodometrically as described in Vogel (1985). Columns used for chromatography were 30 cm long and 1.2 cm in diameter. All experiments were performed at ambient temperature ($25 \pm 2^\circ\text{C}$) to match environmentally related conditions.

Iron Mössbauer spectra were recorded on a “Cryophysics” microprocessor controlled spectrometer using a Co/Rh source with activity 25 mCi. The spectrometer was calibrated with iron foil. All experiments were carried out at room temperature. Tin spectra were recorded on a constant accelerator “Cryophysics” spectrometer fitted with a room temperature 5 mCi calcium stannate-119 m sources. Temperature control of sample at 77 K (± 0.1 K) was achieved using continuous flow of liquid nitrogen cryostat linked to a DTC-2 digital variable temperature controller “Oxford Instruments”. Velocity-calibration was based on the spectra of natural iron, and CaSnO_3 was used as the zero velocity reference.

2.1. Column chromatographic method

Three columns were each packed with a 0.5 g of the alga (600–355 μm) using the wet method, where the height of the biomass was about 3 cm, then soaked for at least 2 h in 0.5 M HCl and washed (until free from acid) prior to any exchange process, to eliminate any naturally present metal ions. Ten millilitre of 6.3 g l^{-1} Cu(II) (0.1 M) aqueous solution of copper sulfate was passed through each column and collected at a rate of 0.25 ml min^{-1} . The pH of the initial Cu(II) solution was 4.26. The columns were washed again with water and the adsorbed Cu(II) ions were eluted using 30 ml of 0.5 M HCl solution. The adsorption–desorption process mentioned above was then repeated under the same conditions for nine cycles in each column using the aqueous Cu(II) solution. Starting from the 10th cycle, Cu(II) was adsorbed from solutions having the same copper concentration but in each column a different solvent was used, 20% (v/v) ethanol/water, 20% (v/v) isopropanol/water or 20% (v/v) acetone/water instead of aqueous copper solutions. The adsorption process was repeated for three cycles in each column using 10 ml of the mixed solvent copper solution and desorption using 30 ml 0.5 M HCl.

2.2. Batch method

The alga was protonated by soaking in a 0.5 M HCl solution, washed several times with water, dried at $\sim 80^\circ\text{C}$, and sieved to 600–355 μm particle size.

To test the best ethanol/water v/v ratio for preparing metal solutions, 5.6 g l^{-1} (0.1 M) Fe(III), 33.7 g l^{-1} (0.1 M) Cd(II) and 11.9 g l^{-1} (0.1 M) Sn(IV) solutions were prepared in a mixed ethanol/water in different v/v ratios as solubility permits (100, 80, 60, 40, 20 for Fe(III), 50, 40, 30, 20, 15, 10, 5 for Cd(II) and 100, 80, 60, 40, 20 for Sn(IV)). Initial pH values for these solutions were 1–1.7 for iron solutions, 4.5–5.0 for cadmium, and 1.8–2.15 for tin. In each experiment 0.1 g of the protonated *C. vulgaris* was added to 50 ml of the ethanol/water metal solution in tightly covered vials, and the mixtures were then stirred in an ultrasonic water bath for 10 min, left overnight to attain equilibrium, then filtered and washed. The adsorbed metal ions were then eluted using 50 ml of 1 M HCl and metal ions determined in the eluent.

In order to determine the change in capacity of the biomass upon reuse, three separate experiments were conducted. In each experiment 0.1 g of *C. vulgaris* was soaked in 50 ml of 0.5 M HCl for 2 h, then filtered and washed. To each experiment, 50 ml of the aqueous solution of one of the three metal ions was added; 5.6 g l^{-1} (0.1 M) Fe(III) (in 0.1 M HCl), 33.7 g l^{-1} (0.1 M) Cd(II) or 11.9 g l^{-1} (0.1 M) Sn(IV). The pH values of the initial solutions were 1.11 for Fe(III), 4.44 for Cd(II) and 2.15 for Sn(IV). The mixtures were stirred, left overnight, then filtered and washed. The adsorbed metal ions were

then eluted by soaking in 50 ml of 1 M HCl for 2 h and the metal ions determined in the eluent. This process of adsorption–desorption was repeated for each sample with the same solutions and conditions for eight cycles. Starting from the ninth cycle, the aqueous metal solutions were substituted with 50% v/v metal solutions of the same concentrations (pH values were; 1.57 for Fe(III), 5.06 for Cd(II) and 2.15 for Sn(IV)), then, three adsorption–desorption cycles followed with the same method in tightly covered vials.

2.3. Mössbauer measurements

Samples of 0.5 g protonated alga (in 0.5 M HCl) were suspended in 500 ml of 5.6 g l^{-1} (0.1 M) of each of: FeCl_3 in aqueous and 60% v/v ethanol/water solution, and 11.9 g l^{-1} (0.1 M) $\text{Sn}(\text{CH}_3)_2\text{Cl}_2$ aqueous and 50% v/v ethanol/water solution. Solutions were then mixed in an ultrasonic water bath for 10 min, left under the metal solution overnight. After, samples were filtered, washed, and dried at $\sim 80^\circ\text{C}$, then prepared as finely ground powders to avoid orientation effects. The iron or tin Mössbauer spectra were then recorded relative to iron foil and calcium stannates respectively.

3. Results and discussion

The algal capacity to bind copper in aqueous solution was reduced upon reuse in each column. Introducing

mixed solvent copper solutions imparted an enhancement on the biomass capacity with both 20% ethanol/water and 20% isopropanol/water, but not with 20% acetone/water, see Fig. 1. Samuelson (1963) found that the selectivity coefficient for a given pair of ions is usually greater in solutions that have lower dielectric constants than water. For example, Gable and Storbel (1956) proved that a sulfonic acid resin favors Na^+ over H^+ in pure methanol than in water ($K_{\text{H}}^{\text{Na}} = 3.2$), with a maximum value ($K_{\text{H}}^{\text{Na}} = 17.2$) when using a 75 mol% methanol solution. The same author proved that ethanol–water solvents exhibited higher selectivity coefficient than methanol–water, which our results are in accordance with, where higher selectivity towards copper in the order; ethanol–water > isopropanol–water > acetone–water was observed. In addition, (Wallberg and Andersson, 1999) reported that the use of up to 40% v/v ethanol PCB solution to desorb seawater microorganisms kept the cells intact. As a result, ethanol/water mixed solvent was used in batch experiments.

In batch systems, different ratios of ethanol/water solutions of iron, cadmium and tin were experimented (see Fig. 2). Cadmium and tin uptake increased slightly with increase in ethanol concentration, while iron uptake reached a maximum around 60% then decreased. Accordingly, 50% v/v ethanol/water was used to prepare metal solutions, taking into consideration that cadmium sulfate did not dissolve completely above 50%. In all experiments pH values maintained fairly stable values in solutions of each metal.

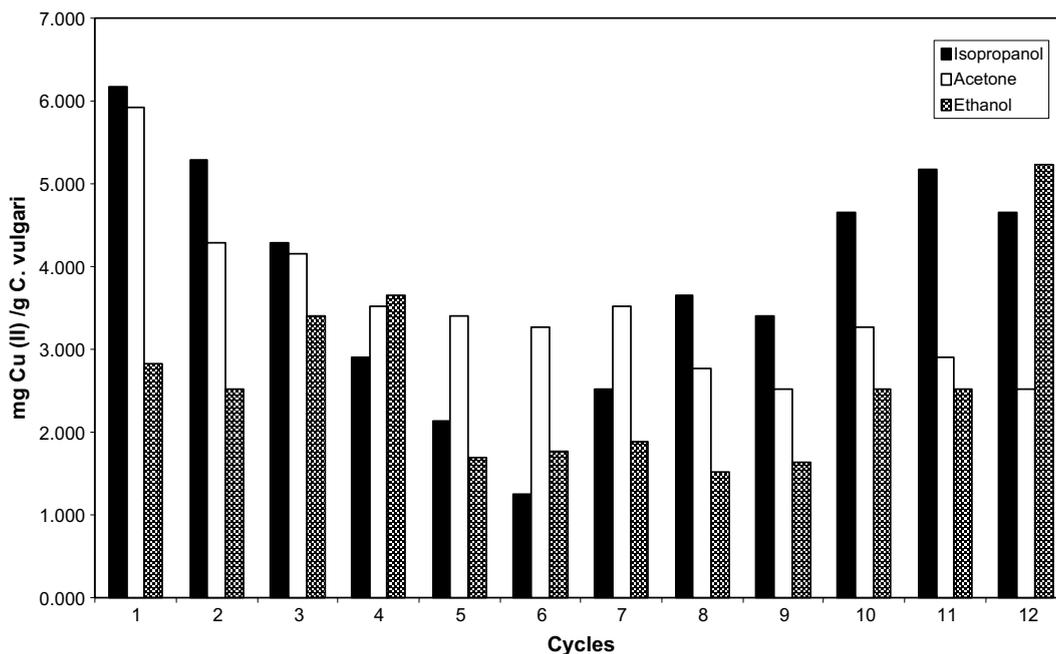


Fig. 1. Regeneration of the algal capacity to bind Cu(II) using different mixed solvents.

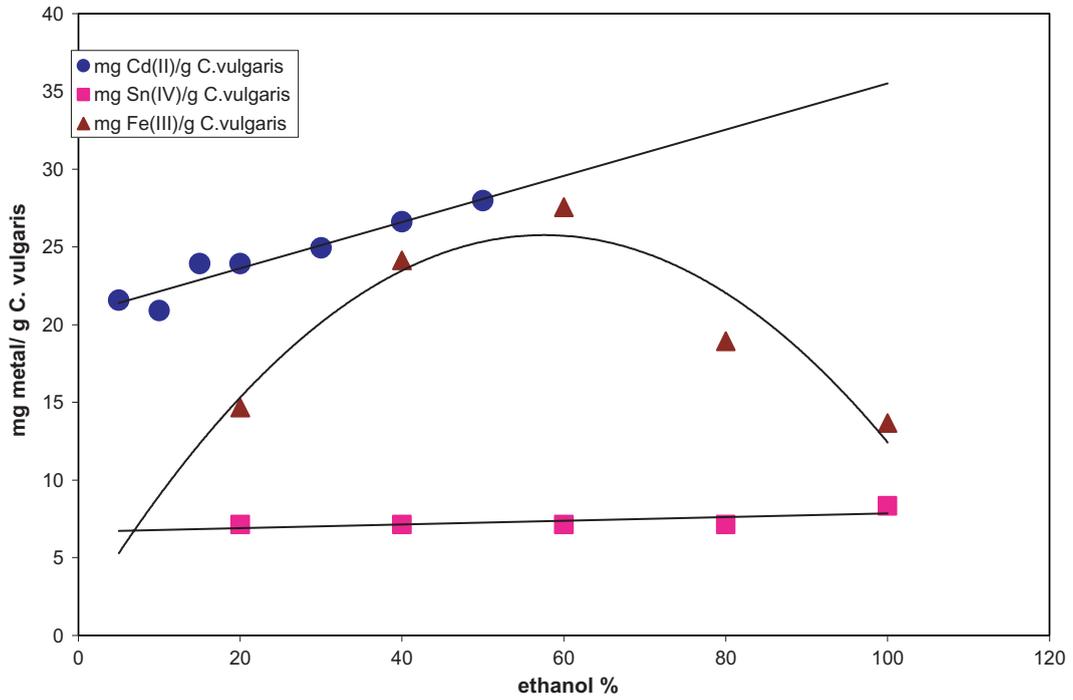


Fig. 2. The effect of ethanol percentage on metal uptake by *C. vulgaris*.

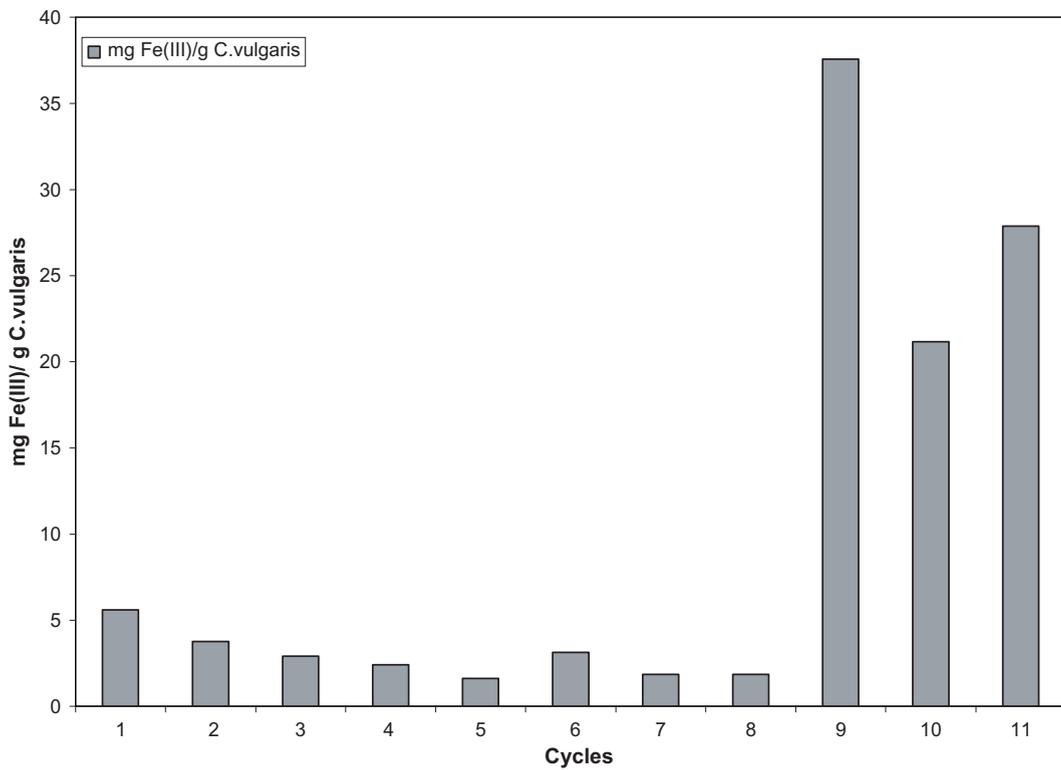


Fig. 3. The effect of regeneration of algal capacity to bind Fe(III) using 50% v/v ethanol/water.

It was reported by Genç et al. (2003) that the biomass was used for five cycles of adsorption–desorption of UO_2^+ using 10 mM HCl with only 2% drop in capacity, but in our experiments, after eight cycles of adsorption of metals from aqueous solutions, and elution with HCl, the capacity of the alga dropped by 67% for iron, 43% for cadmium and 40% for tin. When we started adding the mixed solvent metal solutions to the exhausted biomass at the ninth cycle, the adsorption of iron increased by 90%, tin by 40% and cadmium by only 14% (see Figs. 3–5).

Fluctuations in the uptake of Fe(III), Cd(II) and Sn(IV) were observed, similar to those in Fig. 1 for Cu(II) in the column method. Such behavior could be attributed to alterations in binding sites upon reuse of the algal biomass, and/or stepwise protonation and de-protonation during these processes.

Fourest and Volesky (1996) observed that Cd(II) and Pb(II) uptake by the alga *Sargassum fluitans* increases upon treatment with acetone/water, and was explained as partial purification (extraction) of the “non-sorbing” species, while methanol treatment was reported to decrease uptake. But increased penetration of electrolyte is expected when a less polar solvent is added to a resin in equilibrium with a water solution (Samuelson, 1963),

i.e. the partition of the electrolyte between the solvent inside the resin and the external solvent mixture. Also, ion pair formation may have an influence upon ion exchange equilibrium in mixed solvents.

Generally speaking, the distribution coefficient increases by the incorporation of an organic solvent which may explain the observed increase in uptake by the alga. The addition of the organic solvent as well, brings about a solvation mechanism, which strips the protons from the alga, a phenomenon known as acidity effect on the distribution equilibria, i.e.



where S is the organic solvent. This leaves almost all the sites on the surface of the alga as negatively charged offering a greater chance for metal ions to bind to alga. It is believed then, that as the mixed solvent partially depletes the biomass from protons, the smaller metal ion species of iron has a better chance for binding rather than the larger tin or cadmium ion species.

Such an increase in metal uptake by the biomass in mixed solvents, especially for iron, raises questions regarding microprecipitation. The Mössbauer technique allows the study of the coordination sphere of a metal irrespective of the molecular complexity. The geometry

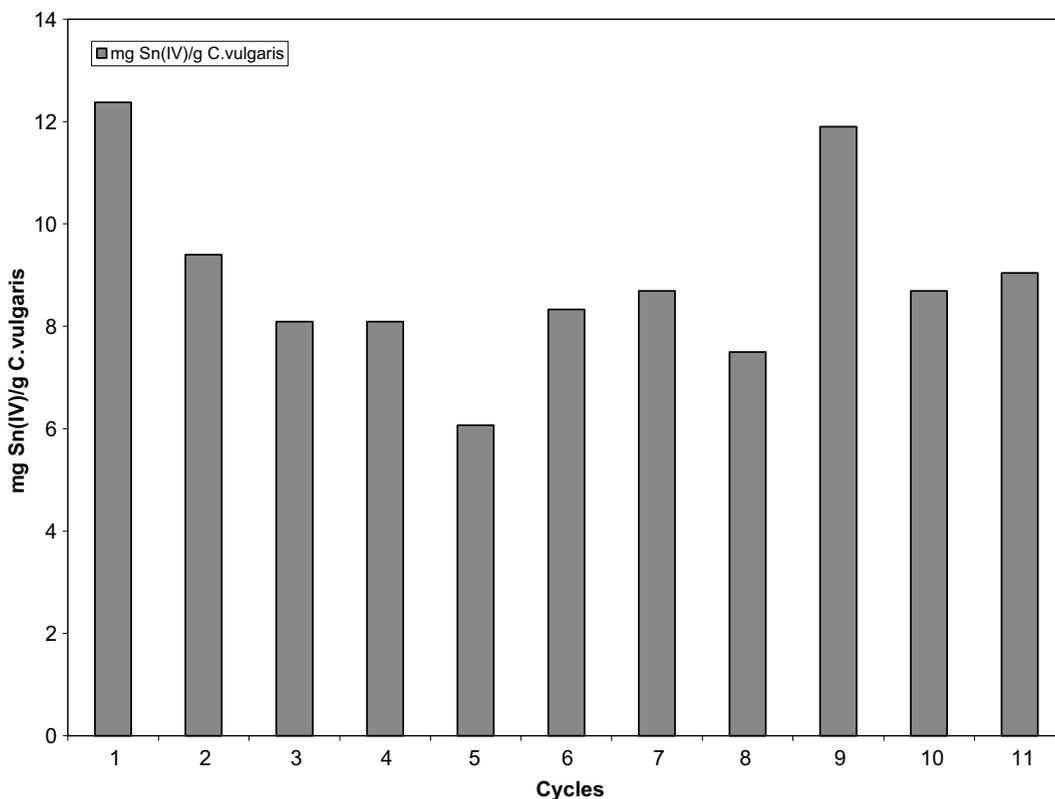


Fig. 4. The effect of regeneration of algal capacity to bind Sn(IV) using 50% v/v ethanol/water.

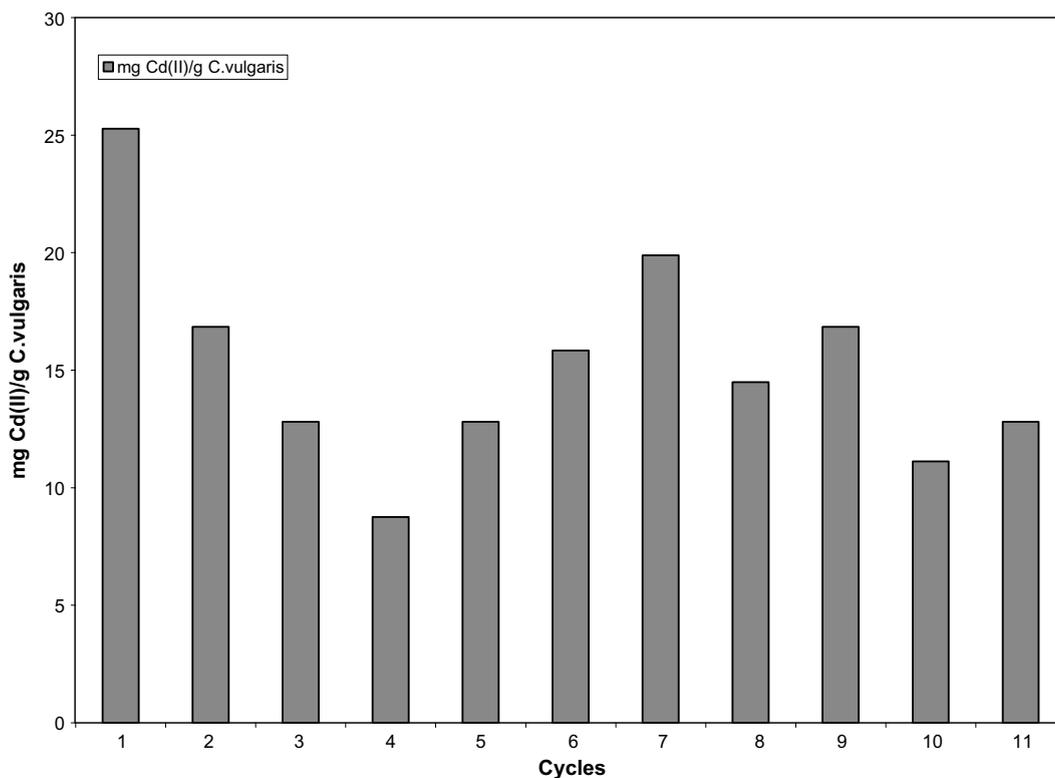


Fig. 5. The effect of regeneration of algal capacity to bind Cd(II) using 50% v/v ethanol/water.

of the environment of iron or tin atoms may be estimated from the experimental parameters of the Mössbauer spectra (isomer shifts (IS) and quadruple splittings (QS)) by fingerprint assignments. The pH of the iron solutions was 1–1.7 excluding chances of hydroxide precipitation. However, the absence of characteristic (IS) of oxides in the iron spectra confirms the theory. But the precipitation of FeCl_3 is still in question. Ionic salts of both divalent and trivalent iron exhibits characteristic isomer shifts. For the sample in aqueous iron solution, the Mössbauer spectrum of the iron-loaded alga showed a doublet with an (IS) of 0.350 mm s^{-1} and (QS) of 0.713 mm s^{-1} assigned to high spin tetrahedral FeCl_4^- entity, and a sextet with IS 0.365 mm s^{-1} and QS of -0.116 mm s^{-1} which is indicative of asymmetry of the polyhedra of the Fe(III) moiety and suggests the presence of different ligands coordinated to iron and/or different modes of coordination. Similar values for the mixed solvent sample were obtained; a doublet with IS 0.369 mm s^{-1} and QS 0.691 mm s^{-1} and a sextet with IS 0.288 mm s^{-1} and QS -0.054 mm s^{-1} . The Mössbauer spectra have not shown the characteristic chemical isomer shift of neither the high spin anhydrous FeCl_3 nor the hydrated $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, both have similar isomer shifts, (IS) 0.53 mm s^{-1} (Ginsberg and Robin, 1963). Hence, we

concluded that no FeCl_3 crystals have accumulated on the surface of the alga or “crystallized” on the already bound molecules, a phenomenon suggested by Beveridge (1978) and Srandberg et al. (1981) to account for the observed non-stoichiometric accumulation of metals on the isolated cell walls.

Sn(IV) in aqueous solutions tend to hydrolyze to give a precipitate of hydrous Sn(IV) oxide, which is not expected in presence of complexing groups on the algal surface. However, tin-containing algae exhibited similar results in both aqueous and mixed solvent samples; a major doublet with an IS of -0.217 mm s^{-1} and QS 0.292 mm s^{-1} in the former, and IS -0.365 mm s^{-1} and QS 0.335 mm s^{-1} in the latter. Both were assigned to coordinated Sn(IV) entity.

Acknowledgement

This work was supported in part by (KACST) and SABIC.

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