Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2018**, 7(2), 156-163

Adsorption of Fe^{3+} by a living microalgae biomass of *Scenedesmus obliquus*

Laila Bouzit [1,*], Nohman Jbari [1], Farida El yousfi [1], Nabila Slimani Alaoui [1], Asmae Chaik[2] and Mostafa Stitou [1]

[1] Laboratory of Water, Research and Environmental Analysis, Department of Chemistry, Faculty of Sciences, University Abdelmalek Essaâdi, B.P. 2121, Mhannech II, 93002 Tétouan, Morocco

[2] Laboratory of Microbiology and Molecular Biology, Faculty of Sciences, University Mohammed V, B.P 1014 RP, Rabat, Morocco

Abstract: In this work, the green microalgae *Scenedesmus obliquus* was tested for his Fe^{3+} removal ability in his living state.

To avoid poisoning by heavy metals, the green microalgae *Scenedesmus obliquus* used in the form of paste obtained after incubation, for seven days in the treated wastewater, and centrifugation.

The findings showed that this method achieved total removal of Fe^{3+}in less time, with a lower cost of materials, and with less complexity than the method commonly used which uses absorption.

This method avoids four disadvantages of the current method, which 1) need a long time to achieve total removal of heavy metals, 2) is a slower process which needs greater time and cost, 3) cannot be used for any heavy metal that requires an acidic environment in order to avoid precipitation, and 4) slows the growth of algae and causes mortality of algae due to the acidic environment and prolonged exposure of algae to toxic heavy metal.

The maximum Fe^{3+} removal was estimated to use 16g/l of living algal cells to remove 25g/l of Fe^{3+} with pH=3, T°=30°C and 80tr/min during 20min.

Keywords: wastewater treatment; adsorption; irons; living algae; heavy metals; algae paste

Introduction

Removal of heavy metals such as iron from wastewater is important for health reasons and other reasons. Iron is a chemical element naturally present in soils; iron dissolves in groundwater. However, it can also come from industrial waste or corrosion of metal piping.

Above a certain concentration, iron can have an impact on water aesthetics by changing the taste and smell of water, by staining laundry, and by staining plumbing accessories.

At higher concentrations, iron is considered to be a dangerous micropollutant. It is non-biodegradable and accumulates in living organisms where it can be toxic, increasing the risk of cardiovascular disease, cancer [1], and neurodegenerative diseases [2] such as Alzheimer's disease and Huntington's chorea.

The elimination of iron from water has been the subject of numerous studies. Several methods have been applied such as coagulation, ion exchange, membrane separation, reverse osmosis, solvent extraction, chemical precipitation, electro-flotation, etc [3] . These methods are generally expensive and inefficient, especially when it comes to low ion concentrations (<100mg/L).

More recently, researchers have concentrated on adsorption, which seems to be the most effective and least expensive solution.

Hence the whole panoply of potential adsorbents has been the subject of experimentation. Among the adsorbent, matrices are algae and microalgae.

Iron is an essential element in the growth and development of all living microorganisms, including microalgae. It is involved in many metabolic

*Corresponding author: Laila Bouzit
Email address: bouzitlaila@gmail.com
DOI: http://dx.doi.org/10.13171/mjc72/01809271825-bouzit

Received March 7, 2018
Accepted, April 20, 2018
Published September 27, 201

processes such as respiration, photosynthesis, and the synthesis of some enzymes [4].

The accumulation of heavy metals in algae involves two processes [5]:

Passive uptake

This process is short and irreversible, and it includes:

- *Adsorption*: The first contact between the metal and the biomass takes place in the cell wall. A part of the metal is deposited in the cell wall by adsorption using covalent bonds which form between the metal and the functional groups of the wall. The functional groups have unsatisfied chemical bonds, so they tend to fill these gaps by capturing nearby ions. Passive adsorption is extracellular and metabolism-independent.

- *Diffusion: Simple diffusion* is a transport mechanism which allows molecules to cross freely through the membrane when their concentrations in the extracellular medium exceed those in the intracellular medium.

Facilitated dissemination is a transport mechanism without energy which occurs in the presence of proteins and allows the molecules to pass through the membrane.

Active uptake

Active uptake is a slow process, irreversible, and metabolism-dependent. This process is related to the transport of metal ions across the cell membrane into the cytoplasm.

Researchers have used all of the above processes in investigating removal by algae of heavy metals from waste water destined for reuse.

Several research studies have conducted in recent years in which several species of microalgae have been the subject of investigation either in their inactive states or during growth, for example, the green algae *Spirogyra* [6] and the green microalgae *Chlamydomonas reinhardtii*, *Chlorella pyrenoïdosa*, *Scenedesmus quadricauda* [7], *Scenedesmus obliquus* [8], and *Botryococcus braunii* [9].

Nishikawa showed that algal cells could exhibit structural damage during prolonged exposure to solutions containing heavy metals [10].

From the research findings described above came the idea of using the green microalgae *S. obliquus* in the living state. To avoid poisoning by heavy metals of microalgae, algae cells were centrifuged after seven days of growth in treated wastewater and then used in the form of a paste in adsorption tests.

The objective of this study was to evaluate the adsorption of Fe^{3+} ions by living biomass of the green microalgae *S. obliquus*.

Experimental

In this study, to avoid poisoning of *S. obliquus* by heavy metals, algal cells were incubated for7days in cylindrical photobioreactors, then centrifuged and transferred in the form of a paste to Fe^{3+}solution. It was found that it was possible to work with algal cells as a paste for over 20 min while they remained living. *S. obliquus* showed sensitivity to agitation and temperature: algal cells were destroyed by high levels of agitation and by temperatures exceeding 30°C. Maximum Fe^{3+}removal was estimated to be 16g/L of living algal cells to remove 25g/L of $Fe^{3+}$with pH=3, temperature=30°C, and 80r/min stirring rate over a 20min period.

Microalgae biomass

The microalga used in this work was *S. obliquus* (CCAP 276-3a) obtained from the algae collection at the University of Göttingen (Germany).

S.obliquus is a fresh-water organism. It is a green microalga, unicellular, non-motile, and microscopic. *S. obliquus* exists as a structure (coenobium) of four organisms [11].

S. obliquus generally cultivated in a Rodriquez Lopez (RL) [12] medium rich in nitrogen and phosphorus, the main nutrients of microalgae (Table 1).

The wastewater used in the present work derives from a treatment plant in Tamouda Bay, Fnideq, northern Morocco. It was first subjected to a primary treatment, which is simple decantation allowing the removal of the suspended particles at the origin of the water turbidity. These waters then undergo secondary treatment by activated sludge using aerobic bacteria degrading organic material. They are then placed in an autoclave to eliminate all microorganisms.

The chemical composition of treated wastewater is similar to the composition of RL medium (Table2), hence in this study RL medium was substituted with treated wastewater [13].

The treated wastewater used in this study came from the secondary-treatment output of the wastewater treatment plant in Tamouda Bay, Fnideq, and northern Morocco.

The treated wastewater was first filtered to remove all particles in suspension and then placed in an autoclave to eliminate all microorganisms.

A quantity of 900 mL of the prepared water mixed with 100 mL of algal solution in each of several photobioreactors. The reactor used had 2L capacity (25cm in height and 7.5cm in diameter). The temperature of the culture was regulated using a thermostat (Ultratem 200 P Selecta) in connection with an air pump sterilized by filtration (0.2 µm pore diameter) which allows the injection of air continuously into the medium. Two fluorescent tubes (Osram L40W / 10) provided lighting continuously (24 hours per day, seven days per week).

Preparation of biomass

After seven days (168h) of growth in treated wastewater, the microalgae was separated by centrifugation at 4000 r/min for 20 min with a Hermle Labortechnik GmdH centrifuge and washed with distilled water three times. The biomass then stored in distilled water at 4°C[11]. Before use, the temperature of the biomass raised to 30°C.

The prepared biomass was in the form of a wet paste which then mixed with a synthetic Fe^{3+}solution during the adsorption tests.

Preparation of Fe^{3+}

Iron is generally present in effluents as salts. Hence the iron used in the adsorption experiments (described below) was ferric iron Fe^{3+}, prepared from $FeCl_3$ iron chloride.

Adsorption of $Fe^{3+}$by S. obliquus microalgae

The Fe^{3+} solution used in the majority of adsorption experiments was at a concentration of C_0=50mg/L.

The Fe^{3+} solution(100mL) brought into contact with 0.5 g of the biomass(wet weight), except for the following tests in which some parameters changed: pH test and test for the effect of biomass concentration.

Samples are taken every 5 min over a period of 30 min. These samples are then centrifuged three times at 400r/min for 5min in order to remove the microalgae from the Fe^{3+}solution [11].

All experiments performed twice and the average value taken for calculations.

The adsorption capacity is estimated using the following equation:

$$Q=\frac{(C0-C)*V}{m} \quad \textbf{(Eq1)}$$

Q= adsorbed amount in mg/g

C0= initial concentration of Fe^{3+} in mg/g

C=concentration of Fe^{3+} in mg/g

V= volume of solution in mL

m=mass of biomass in g

The yield of the adsorption R is calculated as follows:

$$\textbf{Yield (\%)} = \frac{(C0-C)}{C0} * 100 \quad \textbf{(Eq2)}$$

In this study, the parameters affecting adsorption were optimized in an orbital agitator (IKA KS 4000 i control), controlling temperature and agitation.

The parameters tested were pH, temperature, agitation, the concentration of the biomass, and effect of the initial concentration.

Analytic method

After the adsorption experiments, the residual concentrations of Fe^{3+} were measured by a spectrophotometer (VARIAN, UV, Visible spectrophotometer) using the colorimetric method of potassium thiocyanate.

Fe^{3+}ions tend to combine with potassium thiocyanate (K^+, SCN6), which allows the formation of the red-colored $[Fe (SCN)]^{2+}$ complex.

$$\mathbf{Fe^{3+} + SCN^- \longrightarrow [Fe(SCN)]^{2+}}$$

Results and Discussion

Culture of microalgae

The growth monitoring of *S. obliquus* done within 168 hours (7days) by comparing the growth in treated wastewater with the growth in the RL synthetic medium. (This comparison only took into account the growth exponential phase.)

We cultivated the microalgae in the Rodriguez Lopez synthetic culture medium and wastewater in order to compare the growth of microalgae in both environments. We noticed that the culture in the wastewater was faster and more profitable (Fig. 1). The composition and characteristics of each environment are presented in Tables 1 and 2.

Samples were taken four times a day and assayed by a spectrophotometer with a wavelength of 685nm (see Fig. 1).

Table 1. Composition of a Rodriguez Lopez medium.

Solution	Concentration of nutrients (mg/l)
NO_3^-	6,200E-01
HPO_4^{2-}	4,372E-02
$H2PO_4^-$	4,850E-03
SO_4^{2-}	9,876E-02
Mg^{2+}	2,433E-02
Ca^{2+}	4,008E-03
Cl^-	7,091E-03
Fe	1,406E-03
Na+	2,325E-02
Mn	5,526E-05
Cu	6,367E-05
B	1,064E-05
Mo	9,936E-07
N	1,407E-01
P	1,567E-02
K	3,910E-01

Table 2. Physical and chemical parameters determined for urban wastewater from secondary treatment.

Solution	Concentration of nutrients (mg/l)
pH	6,64
conductivity	1,63
DBO_5 (mg	21,3
O_2/L)	124,5
DCO (mg O_2/L)	589,1
$N\text{-}NH_4^+$	11,9
$N\text{-}NO_3^-$	1,59
$N\text{-}NO_2^-$	5,13
$P\text{-}PO_4^-$	5,14
P total	

The optimum culture temperature of microalgae in treated wastewater is 30°C [13].

The curve shows that the microalgae grew more rapidly in the treated wastewater than in the medium RL. After seven days the growth has stabilized.

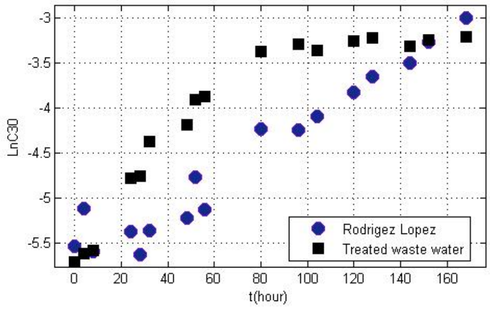

Figure 1. Growth curve of microalgae *Scenedesmus obliquus* in Rodriguez Lopez medium (RL) and in treated wastewater (ER) at temperature = 30 °C

Adsorption of Fe^{3+} by biomass

Influence of pH

The functional groups present on the surface of algal cells give them a negative charge, which promotes the adsorption of cations in favors of anions [14].

At acidic pH, the functional groups preferentially bind with H^+ ions, which prevent the bonding of the metal to the surface.

On the one hand, at acidic pH, the functional groups preferentially bind with H^+ ions, which prevent the bonding of the metal to the surface.

On the other hand, high pH favors the binding of the functional sites with metal cations on the surface cells [15] leading to metal precipitation, in fact, Fe^{3+} precipitates at pH>3.

In this study, the pH was not optimized due to the solubility conditions of Fe^{3+}, which precipitates at pH>3.
Therefore, to avoid any overestimation of the adsorption capacities, all the adsorption tests were carried out by fixing the pH at values below the Fe^{3+}precipitation threshold.

The tests on the influence of pH were performed within a range of pH $\leq$ 3 because it is known that pH > 3 causes Fe^{3+} to precipitate.

The tests were carried out with 50 mL of Fe^{3+}solution at 50 mg/L, 0.2 g of biomass, and a temperature of 25 °C for 30 min.

The results of the experiment are shown in Fig. 2.

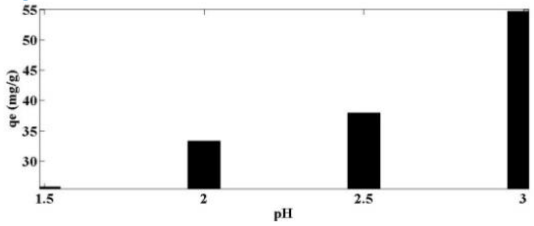

Figure 2. Evolution of Fe^{3+} adsorption by the microalgae *Scenedesmus obliquus* at different pH values (1.5, 2, 2.5, 3). Adsorption conditions: 50mL of Fe^{3+} 50 mg/L, biomass 0.2 g, temperature 25°C, time 30 min.

Contact time

$Fe^{3+}$was adsorbed by the cell membrane passively over a period of 30 min. The adsorption was rapid and 30% of $Fe^{3+}$was eliminated before equilibrium.

After 30min, the equilibrium was affected by passive adsorption, due to the osmotic gradient between the external environment and the intracellular (Fig. 3).

The Fe^{3+}removal reached 45% after 1440 min. This result can be explained by active absorption due to Fe^{3+}transfer inside the cell by carrier proteins (Fig. 4). The results are shown in Figures 3 and 4.

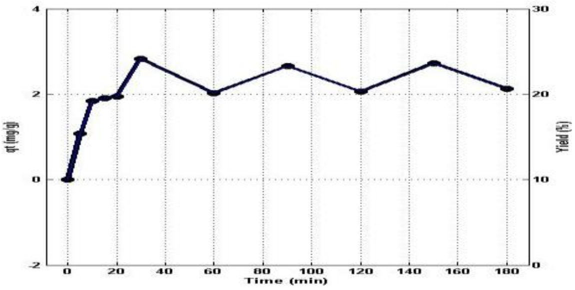

Figure 3. Evolution of Fe^{3+} adsorption by the microalgae *Scenedesmus obliquus* as a function of time (180 min).

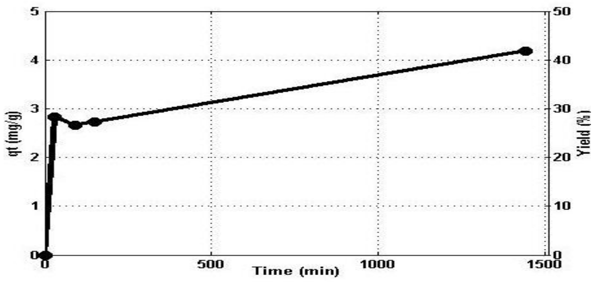

Figure 4. **Evolution** of Fe3 + removal by microalgae *Scenedesmus obliquus* as a function of time (1440 min).

Influence of agitation

Several agitations were tested as shown in Fig. 5.

For low agitations below 80r/min, algal cells were subjected to aggregation phenomena which modify the morphological structure and limit the adsorption [16].

For an agitation of 80 r/min, the adsorption was optimum and reached 60% of Fe^{3+}removal.

By increasing agitation (greater than 80 r/min) protein movements were intense, which decreased the probability of encounter between enzymes and substrates [17].

For higher agitations, the risk of fragmentation of the cell by collision increased. The cells, therefore, were broken and dispersed into the solution [18].

In general, strong agitation of the cells leads to an inhibition of their growth, their metabolism and an alteration of the general morphology (turbolypobiosis) [18].

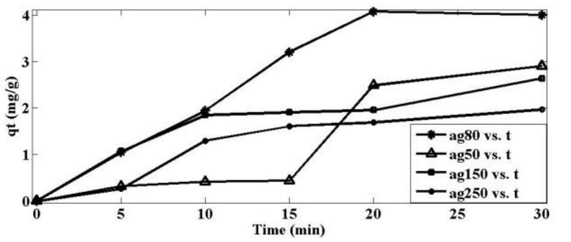

Figure 5. Adsorption of $Fe^{3+}$by *Scenedesmus obliquus* at different agitations 50, 80, 150, 250 r/min.

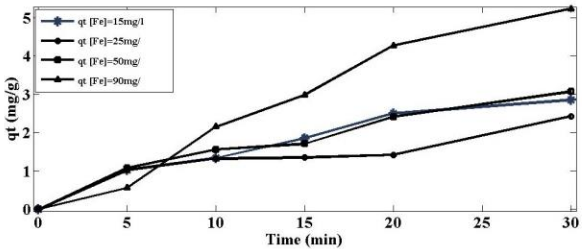

Figure 6. Effect of initial concentration on Fe^{3+}adsorption by *Scenedesmus obliquus*. Different concentrations of Fe^{3+} (15, 25, 50, 90mg/L) are mixed with 0.5g of the biomass

Effect of initial concentration

Various concentrations were tested as shown in Fig. 6.

Adsorption initially increased as the initial concentration of Fe^{3+}in the solution increased. The adsorbed amount tended towards a limit value obtained as a function of the initial concentration for 30 min of contact.

The equilibrium was quickly reached for low Fe^{3+}concentrations.

For $[Fe^{3+}]$<20 mg/L complete adsorption was obtained between 20minand 30min.

For higher concentrations, the equilibrium was reached after 30 min of contact and the adsorption was not complete.

Effect of temperature

Biosorption efficiency of each metal is different for each algae species with different response to the temperature [19].

To understand the influence of temperature on the adsorption of Fe^{3+}, several experiments were carried out at a temperature range of 15°C to 40°C.

The results are shown in Figures 8 and 9.

Below 30°C (Fig. 7), the Fe^{3+} concentration decreased with increasing temperature. In this case, the temperature favored the Fe^{3+} adsorption.

This could be due to an increased number of active sites involved in Fe^{3+} adsorption, an increased tendency of active sites to adsorb the Fe^{3+}, or to reducing mass transfer resistance in the diffusion layer by reducing the thickness of the diffusion boundary layer.

30°C was the optimum temperature of Fe^{3+} adsorption. It was also the optimum temperature of the microalgae growth used in this study, which favored multiplication of active sites and consequently the increase of adsorption [20].

At the same temperature, the equilibrium time was rapidly reached (after 20 min, beginning of fluctuation due to diffusion), which might be due to the reduction of the thickness of the diffusion layer.

Above 30°C (Fig. 8), the Fe^{3+}adsorption decreased with increasing temperature; This might be due to denaturation by the heat of structures responsible of Fe^{3+}sorption [21].

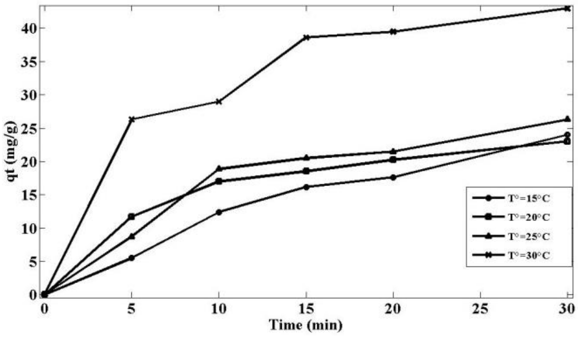

Figure 7. Effect of temperature on Fe^{3+} adsorption by *Scenedesmus obliquus* for temperature<30°C (15°C, 20°C, 25°C, 30°C).

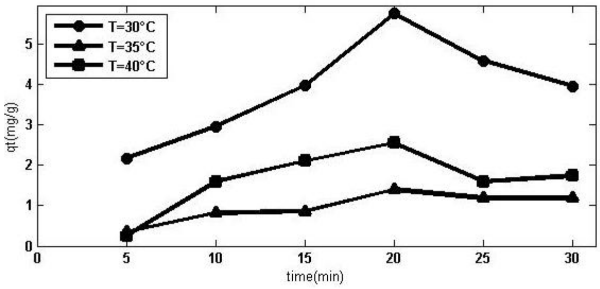

Figure 8. Effect of temperature on Fe^{3+} adsorption by *Scenedesmus obliquus* for temperature>30°C (30°C, 35°C, 40°C)

Effect of biomass concentration

The experiments were carried out after optimization of all parameters in order to determine the minimum mass which gives total Fe^{3+}elimination.

The experiments were carried out with 50 mL of the Fe^{3+}solution at 25 mg/L a temperature of 30 ° C. and an agitation of 80 r/min. All experiments lasted 20 min.

The results obtained are shown in Fig. 9.

The number of metal ions removed from a solution phase was dependent on the algae biomass concentration, and increasing biomass concentrations reduced metal ion uptake per gram of biomass [22].

The Fe^{3+}concentration decreased by increasing biomass concentration. The amount of adsorbed Fe^{3+}depended on the concentration of biomass in the solution because it was directly related to the number of binding sites [23].

With very high biomass concentrations, a decrease in the Fe^{3+}removal was observed. This decrease was due to the partial aggregation of the cells which reduce the availability of the sites [18b].

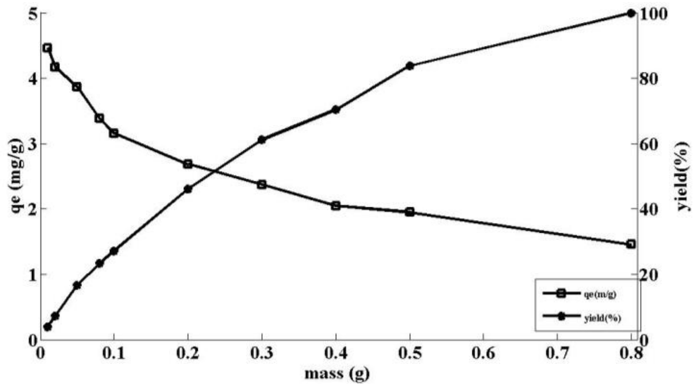

Figure 9. Effect of biomass concentration on Fe^{3+} adsorption by *Scenedesmus obliquus*. Different masses were put in contact with 50mL of the Fe^{3+} solution (50mg/L) over a period of 20 min at a temperature of 30°C at a stirring rate of 80 r/min

Conclusions

Bioprocesses or metal removal by microalgae requires cultivation in synthetic media which are very expensive. In this study, nutrient-rich treated wastewater replaced synthetic media, thus saving the expense of chemicals and approaching a natural experimental environment.

During cultivation, excess iron can cause cell poisoning and consequently stop growth[24]. For this, we used microalgae after the growth phase.

The novel method described in this study promises to provide industrial sites with an effective and efficient method that produces better results while requiring minimum costs, work-intensity, and time.

The optimum conditions for removal can be summarized as follows: temperature of 30°C, agitation of 80 r/min, duration of 20 min, and 16 g/l of biomass corresponding to the total removal of 25 mg/L of Fe^{3+}.

The aim of proving a more effective, efficient method of removing heavy metals from industrial wastewater was achieved concerning Fe^{3+} and *S. obliquus*. The novel method described in this study promises to provide industrial sites with a method that produces better results while being less costly, work-intensive, and time-consuming.

Replication of this study in an industrial environment outside the laboratory will give more importance and efficiency to this work. Moreover, the use of other heavy metals and other commonly used algae species is recommended and will be the subject of future studies.

References

1. EK. Lund, SG. Wharf, SJ. Fairweather-Tait , IT. Johnson, Oral ferrous sulfate supplements increase the free radical-generating capacity of feces from healthy volunteers, Am J Clin Nutr., **2003**, 78, 498.
2. G. Bartzokis, TA. Tishler, MRI evaluation of basal ganglia ferritin iron and neurotoxicity in Alzheimer's and Huntingon's disease, Cell MolBiol (Noisy-le-grand) **2000,** 46, 821-3.
3. FC. Abreu, PN Costa, AM. Brondi, EJ. Pilau, FC. Gozzo, MN. Eberlin , MG. Trevisan, JS. Garcia, Effects of cadmium and copper biosorption on Chlorella vulgaris, Bull Environ Contam Toxicol. **2014**, 93, 405-409.
4. L. Grillet, L. Ouerdane, P. Flis, M. Thi Thanh Hoang, MP. Isaure, R. Lobinski, C. Curie and S.Mari, Ascorbate Efflux as a New Strategy for Iron Reduction and Transport in Plants, J. Biol. Chem. **2014**, 289:2515-2525.
5. S. K.Mehta, J. P. Gaur, use of algae for removing heavy metal ions from wastewater: progress and prospects, Critical reviews in biotechnology, **2005**, 25, 113-152.
6. M. Dirbaz, A. Roosta, Adsorption, kinetic and thermodynamic studies for the biosorption of cadmium onto microalgae Parachlorella sp., Journal of Environmental Chemical Engineering, **2018**, 6, 2302-2309.
7. R. Saavedra, R. Muñoz, ME.Taboada, M. Vega, S. Bolado, Comparative uptake study of arsenic, boron, copper, manganese and zinc from water by different green microalgae, Bioresource Technology, **2018,** 263**,** 49-57.
8. X. Zhang , X. Zhao, C. Wan , B. Chen , F. Bai , Efficient biosorption of cadmium by the self-flocculating microalga Scenedesmus obliquus AS-6-1. Algal Research, **2016**, 16, 427–433.
9. A. Fraile, S. Penche, F. González , M. L. Blázquez, J.A. Muñoz & A. Ballester, Biosorption of Copper, Zinc, Cadmium and Nickel by Chlorella vulgaris. Chemistry and Ecology, **2005,** 21(1):61-75.
10. K. Nishikawa, Y.Yamakoshi, I. Uemura, N. Tominaga, Ultrastructural changes in Chlamydomonas acidophila (Chlorophyta) induced by heavy metals and polyphosphate metabolism, FEMS Microbiology Ecology, **2003,** 44, 253-259
11. Monteiro, M.Cristina, M. L. Paula, Castro , F. Xavier, Malcata, Use of the microalga Scenedesmus obliquus to remove cadmium cations from aqueous solutions, World Journal of Microbiology and Biotechnology, **2009**, 25,1573–1578**.**
12. M. Rodriguez-Lopez, Influence of the inoculums and the medium on the growth of Chlorella pyrenoidosa, Nature, **1964,** 203, 666-667.
13. M E.Martínez Sancho, J.M.Jiménez Castillo, F. El Yousfi, Influence of phosphorus concentration on the growth kinetics and stoichiometry of the microalga Scenedesmus obliquus, Process Biochemistry 1997, 32, 657-664.
14. SJ. Kleinübing, R. Silveira Vieira, MM. Beppu, E. Guibal, M. G. C. da Silva, Characterization and Evaluation of Copper and Nickel Biosorption on Acidic Algae Sargassum Filipendula, Materials Research, **2010**, 13, 541-550.

15. V.K. Gupta, A. Rastogi, A.Nayak, Biosorption of nickel onto treated alga (Oedogoniumhatei): application of isotherm and kinetic models, J. Colloid Interface Sci., **2010,** 342 ,533-539.
16. A. Brenner , A. Abeliovich, Algae in Wastewater Oxidation Ponds, Water Purification, in Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second Edition by A.Richmond, **2013,** chapter 31.
17. N. T. Eriksen, The technology of microalgal culturing, Biotechnology Letters, **2008**, 30, 1525–1536.
18. A. Amanullah, P. Justen, A. David, G.C. Paul, A.W. Nienow and C.R. Thomas, Agitation induced ycelial fragmentation of Aspergillus oryzae and Penicillium chrysogenum, Biochemical Engineering, **2000**, 24, 101-107.
19 (a) C. Monteiro, P.L. Castro, F.X. Malcata, Cadmium removal by two strains of Desmodesmus pleiomorphus cells. Water Air Soil Pollut. **2010**, 208, 17-27.
(b) V.K. Gupta, A. Rastogi, A.Nayak, Biosorption of nickel onto treated alga (Oedogoniumhatei): application of isotherm and kinetic models, J. Colloid Interface Sci., **2010,** 342,533-539. (c) M. Zabochnicka-Swiątek, A.Rygał, The Effect of Biomass (Chlorella vulgaris, Scenedesmus armatus) Concentrations on Zn2+, Pb2+ and Cd2+ Biosorption from Zinc Smelting Wastewater, Inżynieria i Ochrona Środowiska, **2017**, 211-220.
20. (a) S.K. Mehta and J.P. Gaur**,** Use of algae for removing heavy metal ions from treated wastewater: Progress and prospects, Critical reviews in biotechnology, **2005,** 25, 113-152. (b) G. Bayes, S. Raut, V. Patil, R. Lokhande, formation of diazepam-Lanthanides(III) Complexes in the 50-50 volume % ethanol-water solvent system and study of the effect of temperature on the complex formation constants, J. Solut. Chem., **2012**, 41, 241-248. (c) AK. Zeraatkar, H. Ahmadzadeh, AF. Talebi, NR. Moheimani, MP. McHenry, Potential use of algae for heavy metal bioremediation, a critical review, Journal of Environmental Management, **2016**,181, 817-831.
21. M. Dundar, C. Nuhoglu, Y. Nuhoglu, Biosorption of Cu(II) ions onto the litter of natural trembling poplar forest, J. Hazard. Mater., **2008**, 151, 86-95.
22. (a) S.K. Mehta, J.P. Gaur, Characterization and optimization of Ni and Cu sorption from aqueous solution by Chlorella vulgaris, Ecol. Eng, **2001**, 18, 1–13. (b) S. Gokhale, KK Jyoti, SS. Lele, Kinetic and equilibrium modeling of chromium (VI) biosorption on fresh and spent Spirulina platensis/Chlorella vulgaris biomass, Literature Review, **2008**, 99, 3600-8. (c) I. Singleton, P. Simmons, Factors affecting silver biosorption by an industrial strain of Saccharomyces cerevisiae, J. Chem. Technol. Biotechnol, **1996**, 65, 21-28. (d) **E.** Finocchio, A. Lodi,C. Solisio, A. Converti, Chromium (VI) removal by methylated biomass of Spirulina platensis: the effect of methylation process, Chem. Eng. J., **2010**, 156, 264-269.
23. V.O. Arief, K. Trilestari, J. Sunarso, N. Indraswati, S. Ismadji, Recent progress on biosorption of heavy metals from Liquids using low cost biosorbents: characterization, biosorption parameters and mechanism studies,Clean **2008,** 36, 937-962.
24. G.C. Donmez, Z. Aksu**,** Removal of chromium (VI) from saline treated waste waters by dunaliella species, process biochemistry, **2002,** 38, 751-762.