Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2020**, 10(8), 783-789

Comparative study of the biosorption of Fe^{3+} ion by living and dead biomass prepared from the microalga *Scenedesmus obliquus*

Laila Bouzit [*], Farida El Yousfi, Dorsaf Bouharat and Mostafa Stitou

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

Abstract: The present work compares the biosorption capacity of Fe^{3+} by the living biomass presented in our previous study with dead biomass prepared from the same microalgae after deactivating the cells using temperature (drying). This technique (dry biomass) is the most frequently used in biological processes based on adsorption by microalgae. The influence of different parameters on the biosorption capacity of the two biomasses was studied. The highest efficiency of metal removal was recorded by a live microalgae *Scenedesmus obliquus,* with a removal value of 100% within 20 minutes versus to 43% for dried microalgae within 60 minutes. This work confirms the potential use of a live microalgae *Scenedesmus obliquus* as an efficient technique for removing ions from wastewater.

Keywords: biosorption; microalgae; Fe^{3+} ion; living biomass; dead biomass.

1. Introduction

Environmental pollution is still a topical one, as many industrial activities continue to generate large volumes of wastewater loaded with organic substances and metals with toxic effects. These waters are, in most cases, directly discharged into the natural environment without prior treatment, which is a significant concern for the public authorities because of the consequences of living species and their environments.

Significant investments were made to develop purification systems that guarantee the efficient elimination of pollutants and encourage reusing and recycling industrial water.

The most commonly used decontamination processes are oxidation, filtration, electrolysis, ion exchange, etc. [1]. However, these methods have some disadvantages, such as high energy requirement, generation of toxic sludge, and these methods are only useful for waters with high metals concentrations.

For water with low concentrations (<100), adsorption remains the most suitable technique [2].

For this, the focus was brought on the development of adsorbents. The most used are biological materials based on microorganisms [3], because of the workability and abundance of natural support (algae, microalgae, yeast, etc).

Several research projects have focused on the involvement of microalgal biotechnology in the reduction and elimination of micropollutants.

The first studies concerned dead or metabolically inactive algal cells used after temperature drying and focused on passive biosorption phenomena. Current research is more focused on the removal of metals by living microalgae during the growth phase. Living microalgae are effective in removing nutrients and metals from wastewater. The importance of metals for microalgae is well known: metals are essential elements for the growth and development of all microalgae and are involved in many of their metabolic processes such as respiration, photosynthesis, and the synthesis of certain enzymes.

Microalgae can acquire metals through functional groupings on the cell surface and then catalyzed by specific enzymes. The acquisition is made either by diffusion through the membrane or by passive transport. The active transport then takes over and transmits them inside the cell to integrate them into their specific roles.

Several authors report results on the effectiveness of green microalgae for wastewater treatment. Dirbaz et al. worked on the microalga *parachlorella sp.* [4], Saavedra et al. worked on four green microalgae *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Scenedesmus almeriensis* and *Chlorophyceae spp* [5], Zhang et al. used the microalga *Scenedesmus*

*Corresponding author: Laila Bouzit
Email address: bouzitlaila@gmail.com
DOI: http://dx.doi.org/10.13171/mjc10802009221517lb

Received July 22, 2020
Accepted August 5, 2020
Published September 22, 2020

onliquus [6], and Fraile et al. worked on *Chlorella vulgaris* [7]. The results obtained led to the conclusion that microalgae can quickly grow using wastewater as a culture substrate while ensuring effective removal of nutrients (P and N) and metals.
However, the use of microalgae in the treatment of industrial wastewater presents specific application difficulties, mainly due to the presence of a high concentration of compounds considered toxic for microalgae in these waters, which limits their use in the sector [8]. To remedy these drawbacks, a biosorption process using live microalgae with a new technique has been proposed in our previous study [9].

It highlighted more good possibilities for Fe^{3+} ion removal as a case of metallic micropollutant by proposing a technique for biomass preparation from the living *Scenedesmus obliquus* microalgae that made it possible to avoid the disadvantages cited with a 100% removal of the Fe^{3+} ion is only 20 minutes. This technique consists in recovering the algal cells after the growth phase by centrifugation and using them in the form of a green, moist paste without the usual drying process, thus exploiting all the properties of living algal cells to absorb metals without exposing them to the risk of contamination.

In this work, we will compare the biosorption of the biomass presented in the previous study [9] with another biomass prepared from the same microalgae after deactivating the cells using temperature (drying). This technique (dry biomass) is the most frequently used in biological processes based on adsorption by microalgae.

The objective is to evaluate the biosorption capacity of Fe^{3+} ion by dry or dead biomass prepared from the microalga *Scenedesmus obliquus* and compare it to the biosorption capacity of the same microalga in the living state prepared by the technique proposed in our previously study[1] to validate its effectiveness.

2. Experimental

2.1. Biomass preparation

The microalgae used in this work *Scenedesmus obliquus* is a green, single-celled, immobile, freshwater microalgae that measure approximately 5 to 30 micrometers. It is generally grouped by fours, forming a structure called a cenobe.

Two biomasses prepared from the microalgae *Scenedesmus obliquus* were used in the biosorption of Fe^{3+}, a living biomass B1, and a dead biomass B2. The living biomass B1 has already been studied in the previous study[1] while the dry biomass B2 is presented in the present one.

The two biomasses are prepared as follows:

Living Biomass B1: After the growth phase in treated wastewater (secondary treatment with activated sludge), the microalgae were recovered by centrifugation at 4000 rpm for 20 minutes (in the Hermle labortechnik GmdH centrifuge), and then rinsed three times with distilled water. The recovered biomass is in the form of a wet green paste (moisture = 70%) composed of living algal cells. The biomass is stored in distilled water at 4°C. Before use, it must first be activated at 30°C.

Dry biomass B2: After the growth phase, the microalgae were recovered by centrifugation at 4000 rpm for 20 minutes, rinsed three times using distilled water, and finally dried at 110°C for 24 hours [10]. Before use, the microalgae were crushed with a mortar.

2.2. Preparation of iron solution

Iron is generally present in effluents as salts. In our case, the iron used in biosorption experiments was ferric iron Fe^{3+}, prepared from $FeCl_3$ iron chloride.

2.3. Experimental device

The study of iron biosorption by the microalga *Scenedesmus obliquus* and the influence of different parameters was carried out using the orbital agitator (IKA KS 4000 i control). This agitator allows setting temperature and agitation.

2.4. Analytic method

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

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

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

2.5. biosorption of Fe^{3+} by *Scenedesmus obliquus* microalgae

The purpose of this study is to evaluate the effect of specific experimental parameters on the biosorption of Fe^{3+} ion by the microalgae studied.

Samples are taken every 5 minutes for 180 minutes. These samples are filtered and measured by spectrophotometer.

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

The biosorption capacity is estimated using the following equation:

$$\boldsymbol{Q} = \frac{(\boldsymbol{C} - \mathrm{C0}).\boldsymbol{V}}{\boldsymbol{m}}$$

With:

Q= biosorbed amount in mg/g

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

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

V= volume of solution in mL

m= mass of biomass in g

The yield of biosorption R is calculated as follows:

$$R(\%) = \frac{(C0 - C)}{C0}.100$$

The parameters studied were pH, temperature, agitation, biomass concentration, and initial concentration.

In the biosorption tests, 0.1g of the prepared biomass is mixed with 100mL of the Fe^{3+} solution at 50mg/L.

3. Results and Discussion

3.1. pH effect

The effect of pH was tested by carrying out a series of experiments at different pH levels while respecting the iron precipitation threshold (pH=3 experimentally demonstrated).

The pH values studied are 2; 2.5 and 3.

The variation in the biosorption capacity of the Fe^{3+} ion by dry *Scenedesmus obliquus* microalgae as a function of pH is shown in Fig.1. Fig.2 shows the variation in the biosorption capacity of Fe^{3+} ion by the living microalga *Scenedesmus obliquus* as a function of pH [9].

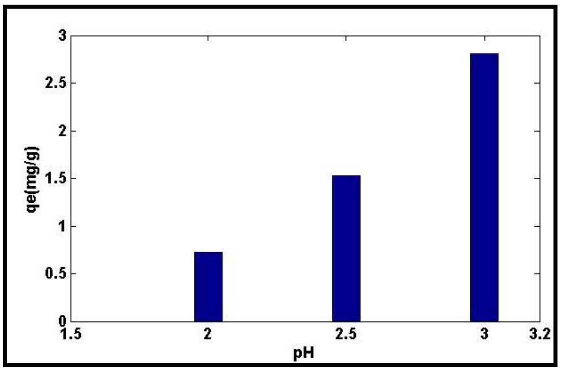

Figure 1. Evolution of Fe^{3+} ion biosorption by the dry Scenedesmus obliquus microalgae at different pH values (2 ; 2.5; 3)

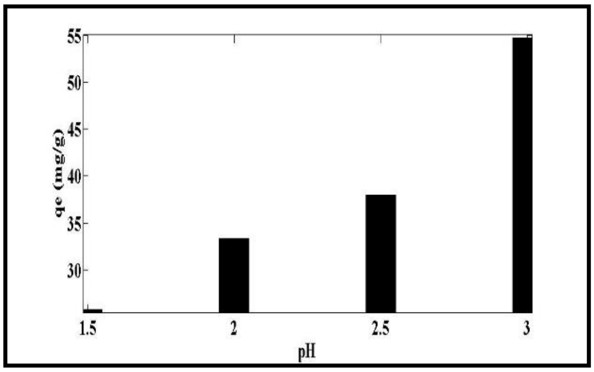

Figure 2. Evolution of Fe^{3+} ion biosorption by the living microalga Scenedesmus obliquus at different pH values (1.5 ; 2 ; 2.5 ; 3) [9]

The two figures show that by increasing the pH, the biosorption of the Fe^{3+} ion by the two biomasses increases and reaches its maximum at pH=3.
Indeed, the functional groups present on the surface of microalgae cells give them a negative charge, promoting the adsorption of cations in favor of anions [11]. In low pH media, the functional groups bind preferentially with H^+ ions, which prevent the binding of the metal to the surface. However, at higher pH, available sites are negatively charged, promoting cation binding [12].
The Fe^{3+} ion biosorption study was not performed for pH over 3 due to the precipitation of insoluble iron hydroxide, which may overestimate the biosorption capacity [13-15].

3.2. Contact time effect

Contact time is the most critical parameter for successfully using a new biosorbent to remove metal ions.

Biosorption is checked at different contact times ranging from 5 minutes to 180 minutes. Fig.3 and 4 show the evolution of the biosorbed amount of Fe^{3+} ion by the dead and living microalgae *Scenedesmus obliquus* as a function of time.

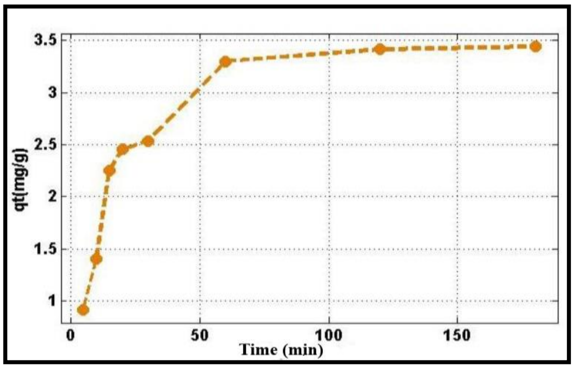

Figure 3. Evolution of Fe^{3+} ion biosorption by the dead microalga Scenedesmus obliquus as a function of time (180 minutes)

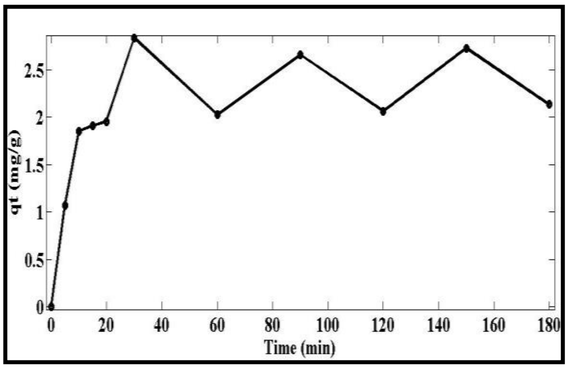

Figure 4. Evolution of Fe^{3+} biosorption by the living microalga Scenedesmus obliquus as a function of time (180 minute) [1]

Fig.3 shows that the process of biosorption of the Fe^{3+} ion by the dead microalga *Scenedesmus obliquus* takes place in two phases:

The first phase is characterized by a rapid biosorption of the Fe^{3+} ion in the first 30 minutes. This can be explained by the abundance and availability of active sites on the surface of the microalgae.

A slower phase follows this phase until equilibrium is reached. In this phase, the progression of occupation and saturation of the active sites makes the biosorption of the Fe^{3+} ion less efficient.

After 60 minutes, the amount of Fe^{3+} ion biosorbed is relatively constant, making it possible to deduce that 60 minutes is the optimal contact time for biosorption of the Fe^{3+} ion by the microalga *Scenedesmus obliquus* where all the sites are occupied.

Analysis of the results of Fe^{3+} ion biosorption by the living microalgae *Scenedesmus obliquus* (Fig.4) show that the Fe^{3+} ion biosorption phenomenon occurs in two phases [1]:

- The first phase is characterized by a rapid fixation of the Fe^{3+} ion after 30 minutes.

- The second phase presents the equilibrium phase. The latter is disturbed by a simple diffusion subject to the chemosmotic gradient between the external medium and the intracellular. This diffusion is responsible for the fluctuations that occur after 30 minutes of biosorption.

3.3. Agitation effect

Agitation consumes energy and affects retention efficiency. Different tests that determine the optimum stirring speed are carried out with different stirring speeds ranging from 50 rpm to 250 rpm, as shown in Fig.5 and Fig.6.

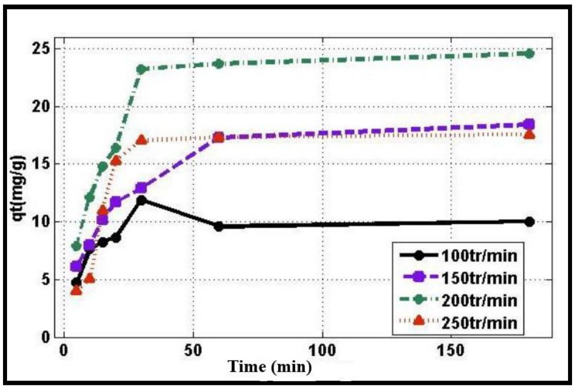

Figure 5. Biosorption of Fe^{3+} ion by the dead microalgae Senedesmus obliquus at different agitations 100, 150, 200, 250 rpm

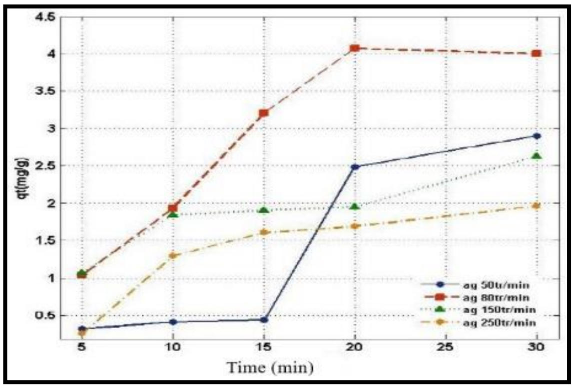

Figure 6. Biosorption of Fe^{3+} ion by the living microalgae Senedesmus obliquus at different agitations 50, 80, 150, 250 rpm

The results of these figures show that the stirring speed influences the retention capacity of the Fe^{3+} ion for both biomass. Regarding the dry biomass (Fig.5), and for low agitation values, diffusion is insufficient, which leads to low biosorption of the Fe^{3+} ion (100rpm).

By increasing the agitation, the degree of mixing increases. When the solution is stirred, the solid particles move with the solution increasing the concentration of metal near their surface.

At high agitation values, the Fe^{3+} ions will not have enough time to settle on the surface of the microalgae. An even higher stirring speed (250 rpm) provides sufficient additional energy to break any bonds that may be formed between the ions and the adsorbent surface [16].

The highest removal capacity was achieved at an optimum stirring speed of 200 rpm.

Concerning biosorption by the living microalga *Scenedesmus obliquus* [1], studies have shown that agitation negatively influences biosorption. By increasing from 80 to 250 rpm, the amount of biosorbed Fe^{3+} ion decreases (Fig.6).

Indeed, for agitation of 80 rpm the biosorption is at its optimum. For low agitation rates below 80 rpm, microalgae are subject to aggregation phenomena modifying the morphological structure and limiting biosorption [17].

When agitation exceeds 80 rpm, protein movement is intense, which decreases the probability of the enzyme meeting the substrate [18].

At higher agitation, there is a risk of cell fragmentation by collision. The cell is thus broken and disperses into solution [12].

In general, vigorous agitation of the cells leads to inhibition of cell growth, metabolism, and alteration of general morphology (turbohypobiosis) [19]. Thus, the optimal agitation in our study is 80rpm.

3.4. Temperature effect

To understand the effect of temperature on the Fe^{3+} ion biosorption by the microalga *Scenedesmus obliquus* we were interested in temperatures ranging from 15 to 40°C.

The effect of temperature on the Fe^{3+} ion biosorption by the dead and live microalga *Scenedesmus obliquus* is illustrated in Fig.7 and Fig.8.

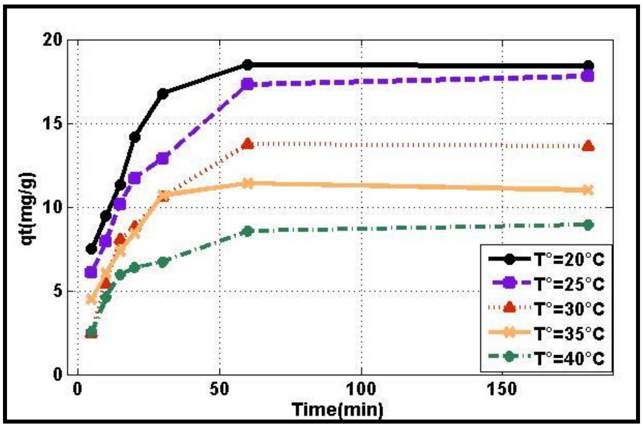

Figure 7. Effect of temperature on Fe^{3+} ion biosorption by the dead microalga Scenedesmus obliquus

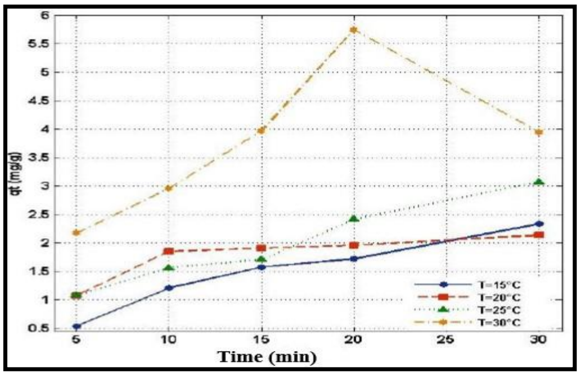

Figure 8. Effect of temperature on Fe^{3+} ion biosorption by the living microalga Scenedesmus obliquus [1] for T°≤ (15, 20, 25, 30°C)

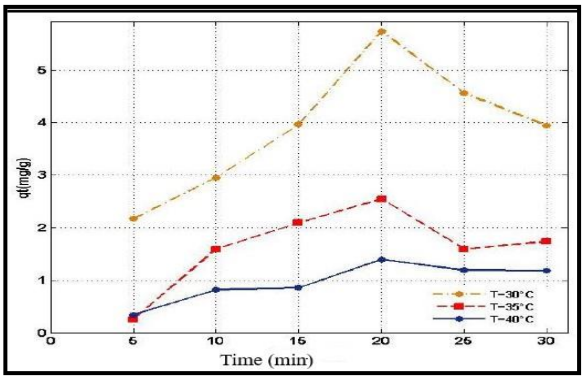

Figure 9. Effect of temperature on Fe^{3+} ion biosorption by the living microalga Scenedesmus obliquus [1] for T°≥(15, 20, 25, 30°C)

The Fe^{3+} ion biosorption by the dead microalgae increases at low temperatures. The decrease in the amount of Fe^{3+} ion absorbed, in high temperatures, maybe due to the destruction of active sites responsible for the biosorption of the Fe^{3+} ion.

The Fe^{3+} ion biosorption appears to be an exothermic phenomenon. As the temperature increases, a decrease in biosorption is observed, which may be due to the destruction of active sites by breaking their surface bonds.

The optimal temperature chosen to conduct the biosorption process is 20°C.

Biosorption by living microalgae [1] shows that the optimal biosorption temperature is 30°C. Biosorbed Fe^{3+} ions increase with increasing temperature, which indicates an endothermic phenomenon.

At the same temperature (30°C), the equilibrium time is quickly reached (after 20 minutes, beginning of fluctuations due to diffusion), which may be due to the reduction in the thickness of the diffusion layer. Above 30°C (Fig.9), the iron adsorption decreased with increasing temperature. This may be due to denaturation by the heat of structures responsible for iron adsorption [20].

3.5. Effect of initial iron concentration

The rate of the Fe^{3+} ion biosorption is strongly dependent on the initial concentration of Fe^{3+} ion in the solution, as shown in Fig.10 and Fig.11. The results shown in the two figures show that the biosorption capacity increased as the initial concentration of Fe^{3+} ion in the solution increased. This increase in biosorption is explained by the rise in the number of ions, which mobilizes a more significant number of biosorption sites.

3.6. Effect of microalgae concentration

In order to optimize the biosorbent amount, different masses (0.002 to 0.05g) of the dead microalgae were stirred for 1hour into 25mL of a solution of Fe^{3+} ion equal to 25mg/L.

The variations in the biosorption capacity of Fe^{3+} ion, against biomass concentration, by the two biomass, dead or alive, are shown in Fig.12 and Fig.13.

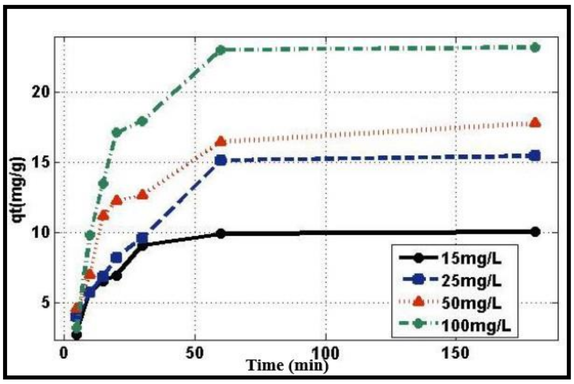

Figure 10. Effect of initial concentration on Fe^{3+} ion biosorption by the dead microalgae Scenedesmus obliquus. Different Fe^{3+} concentrations (15, 25, 50, 100 mg/L) are mixed with 0.1g of the biomass

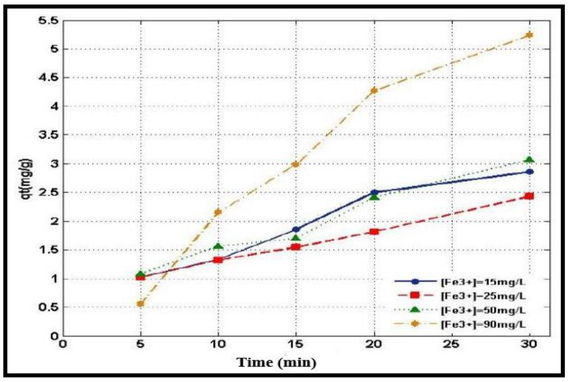

Figure 11. Effect of initial concentration on Fe^{3+} ion biosorption bythe living microalgae Scenedesmus obliquus. Different Fe^{3+} concentrations (15, 25, 50, 90mg/L) are mixed with 0.5g of the biomass [1]

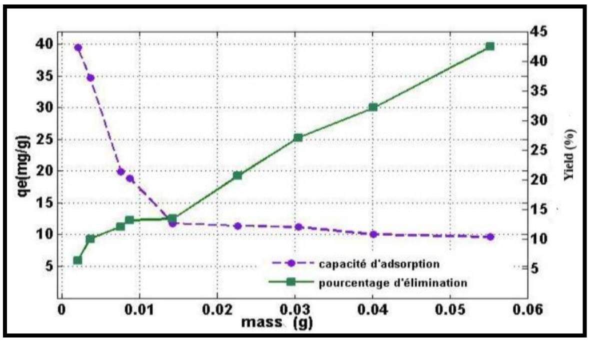

Figure 12. Effect of biomass concentration on Fe^{3+} biosorption by the dead microalgae Scenedesmus obliquus. Different masses were put in contact with 50mL of the Fe^{3+} solution (25mg/L)over a period of 60 minutes at a temperature of 20°C at a stirring rate of 200

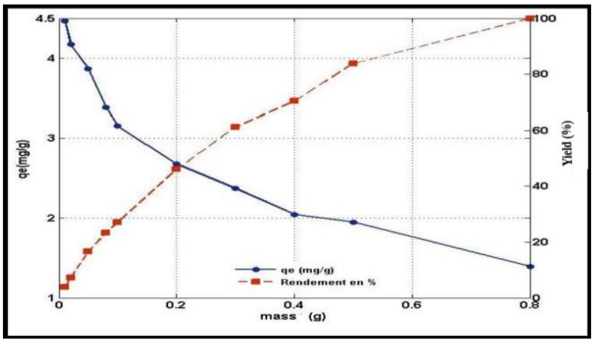

Figure 13. Effect of biomass concentration on Fe^{3+} biosorption by the living microalgae Scenedesmus obliquus. Different masses were put in contact with 50mL of the Fe^{3+} solution (50mg/L)over a period of 20 minutes at a temperature of 30°C at a stirring rate of 80 r/minutes [1]

Based on the presented results, the biosorption rate increases with increasing biomass concentration. This is due to the availability of biosorption sites, which makes biosorption easier.

The maximum biosorption obtained with the dead microalgae was 46% acquired at a concentration of 1g/L of biomass. Biosorption could not be improved due to the precipitation of Fe^{3+}, which occurs under conditions where masses of Fe^{3+} are more significant than 1g/L. Indeed, an increase in the concentration of biomass in solution causes an increase in pH, which causes precipitation of Fe^{3+}. Concerning living biomass [1], studies have shown that the total elimination (100%) of Fe^{3+} ion is achieved with a biomass concentration of 16g/L (corresponding to 3.84g/L dry weight).

4. Conclusion

The comparative study of two biomass of living and dead microalgae showed that the biosorption capacity of the dead microalgae is less efficient, uses a longer biosorption process (60 minutes). It is limited by the precipitation of Fe3+ that occurs when using larger masses of microalgae, preventing the total elimination of this metal.

This study has shown that the retention of Fe3 + by living biomass is faster, at equilibrium reached after 20 minutes, with 100% elimination of the Fe3+ ion.

This study has also revealed certain constraints related to the use of living biomass:

- The equilibrium time is disturbed by internal diffusion responsible for releasing Fe3+ into the external environment.
- The sensitivity to external conditions: temperature and agitation.

The results presented in this study have made it possible to move towards ways of following and on future trials.

- The study of isotherms, kinetics, and thermodynamics for both biomass to understand the biosorption mechanism in both cases.

- Only one species of freshwater microalgae was concerned, it would be relevant for future trials to test other strains abundant in the study area.
- It would also be interesting to extend the biosorption tests to other metals.

References

1- M. E. Goher, A. M. Hassan, I. A. Abdel-Moniem, A. H. Fahmy, M. H. Abdo, S. M. El-sayed, Removal of aluminum, iron and manganese ions from industrial wastes using granular activated carbon and Amberlite IR-120H, The Egyptian Journal of Aquatic Research, **2015**, 41, 155-164.

2- C. H. Yen, H. L. Lien, J. S. Chung, H. D. Yeh, Adsorption of precious metals in water by dendrimer modified magnetic nanoparticles, Journal of hazardous materials, **2017**, 322, 215–222.

3- L. Grillet, L. Ouerdane, P. Flis, M. T. T. Hoang, M.-P. Isaure, R. Lobinski, C. Curie, S. Mari, Ascorbate Efflux as a New Strategy for Iron Reduction and Transport in Plants, Journal of Biological Chemistry, **2014**, 289, 2515-2525.

4- D. Mahboobeh, R. Aliakbar, Adsorption, kinetic and thermodynamic studies for the biosorption of cadmium onto microalgae *Parachlorella sp*, Journal of Environmental Chemical Engineering, **2018**, 6(2), 2302-2309.

5- S. Ricardo, M. Raúl, T. M. Elisa, Comparative uptake study of arsenic, boron, copper, manganese, and zinc from water by different green microalgae, Bioresource Technology, **2018**, 263, 49-57.

6- 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.

7- A. Fraile, S. Penche, F. Gonzále, M. L. Blázquez, J. A. Muñoz, A. Ballester, Biosorption of copper, zinc, cadmium and nickel by *Chlorella vulgaris*, Chemistry and Ecology, **2005**, 21, 61-75.

8- 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.

9- C. Monteiro, P. Castro, F. Malcata, Use of the microalga *Scenedesmus obliquus* to remove cadmium cations from aqueous solutions, World Journal of Microbiology and Biotechnology, **2009,** 25, 1573–1578.

10- L. Bouzit, N. Jbari, N. S. Alaoui , F. El Yousfi, A. Chaik, M. Stitou, Adsorption of Fe^{3+} by a living microalgae biomass of *Scenedesmus obliquus*, Mediterranean Journal of Chemistry, **2018,** 7(2), 156-163.

11- S. Kleinubing, R. Vieira, M. Beppu, E. Guibal, Characterization and Evaluation of Copper and Nickel Biosorption on Acidic Algae *Sargassum Filipendula*, Materials Research, **2010**, 13, 541-550.

12- V. Gupta, A. Rastogi, Biosorption of lead(II) from aqueous solutions by non-living algal biomass *Oedogonium sp*, Colloids Surf B Biointerfaces, **2008**, 64(2), 170-178.

13- H. Ucun, Y. Bayhan, Y. Kaya, A. Cakici, O. Algur, Biosorption of chromium(VI) from aqueous solution by cone biomass of *Pinus sylvestris*, Bioresource Technology, **2002**, 85(2), 155-158.

14- D. Park, Y.-S. Yun, K. Yim, J. Park, Effect of Ni(II) on the reduction of Cr(VI) by Ecklonia biomass. Bioresource Technology, **2006**, 97, 1592-1598.

15- V. Gupta, D. Pathania, S. Sharma, S. Agarwal, P. Singh, Remediation of noxious chromium (VI) utilizing acrylic acid grafted lignocellulosic adsorbent, Journal of Molecular Liquids, **2013**, 177, 343-352.

16- M. Argun, S. Dursun, C. Ozdemir, M. Karatas, Heavy metal adsorption by modified oak sawdust: Thermodynamics and kinetics, Journal of Hazardous Materials, **2007**, 141, 77-85.

17- A. Brenner, Algae in Wastewater Oxidation Ponds, Water Purification, in Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second Edition by A.Richmond, **2013**, chapter 31.

18- N. T. Eriksen, The technology of microalgal culturing, Biotechnology Letters, **2008**, 30(9), 1525-1536.

19- A. Amanullah, P. Jüsten, A. Davies, G. Paul, A. Nienow, C. Thomas, Agitation Induced Mycelial Fragmentation of Aspergillus Oryzae and Penicillium Chrysogenum, Biochemical Engineering, **2008**, 5(2), 109-114.

20- M. Dundar, C. Nuhoglu, Y. Nuhoglu, Biosorption of Cu(II) Ions Onto the Litter of Natural Trembling Poplar Forest, Journal of hazardous materials, **2008**, 151(1), 86-95.