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Evaluation of the Growth of the Microalga Chlorella Vulgaris at Three Levels of Arsenic Concentrations

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ABSTRACT

The global concern over arsenic contamination in water due to its natural occurrence and human activities has led to the development of innovative solutions for its detection and remediation. Microbial metabolism and mobilization play crucial roles in the global cycle of arsenic. The aim of this work was to evaluate in vitro the growth capacity of the microalga Chlorella vulgaris for 25 days in the presence of arsenic and subsequently subjected to concentrations of 500, 700 and 900 mg/L of NaAsO2. The results show the efficiency of the microalgal Chlorella vulgaris to grow with different behaviours in the three concentrations of mercury in the form of NaAsO2. The data obtained indicate the ability of this microalga to remediate mercury in aquatic environments contaminated with this metal.

KEYWORDS: Contamination, aquatic environment, arsenic, microalgae.

1. Introduction

Arsenic is widely distributed in the earth's upper crust in variable, but generally low concentrations. Arsenic concentrations in soil vary greatly, from 0.1 to over 1000 ppm (Mohsin et al. 2023), it is the 20th most abundant metalloid in the earth's crust occurring naturally at a concentration of 1.5 to 3 mg/m^2.

Arsenic is classified as a class 1 carcinogen by the International Agency for Research on Cancer. The World Health World Health Organization states that the acceptable level of arsenic in drinking water is up to 10 µg/L (Mohsin et al. 2023). drinking water is up to 10 µg/L (Mohsin et al. 2023). Due to its abundance and toxicity, it is essential to control and remove arsenic from water, soil and food.

Several methods have been used to remove arsenic from drinking water, based on the principles of coagulation, filtration, adsorption and reverse osmosis. Chemical oxidants are often added to help oxidise As (III) to As (V). When As (III) is oxidized to As(V), it becomes negatively charged and can rapidly adsorb to iron oxyhydroxides, which help to remove it from contaminated sites (Kumari and Jagadevan 2016). Conventional methods to remediate arsenic-contaminated media have high cost, low efficiency and secondary contamination limitations.

Numerous approaches have now been studied for the development of more economical and effective technologies, both to decrease the amount of wastewater produced and to improve the quality of the treated effluent (Barakat, 2011). Adsorption has become one of the alternative treatments; in recent years, the search for low-cost adsorbents with metal-binding capabilities has intensified (Leung et al., 2000). For this reason, the cultivation of microalgae in wastewater is spreading widely for nutrient removal and control of physicochemical parameters of wastewater, and as a feedstock for biofuel production (Wang et al., 2010).

In addition, the use of living and non-living microalgae is a low-cost and environmentally friendly technique, which is increasingly being used to remove various toxic substances such as heavy metals, antibiotics (Xiong et al., 2017; Santaeufemia et al., 2016), phenol and aromatic-polycyclic compounds from wastewater (El-Sheekh et al., 2012; Gao et al., 2011).

Phycoremediation technology has great advantages over conventional methods implemented in wastewater treatment, because it has lower costs in its implementation and is ecologically viable for aquatic and terrestrial environments (Chabukdhara et al., 2017), in addition to a decrease in energy consumption, the non-implementation of chemicals, the ability to generate photosynthetic oxygen and fix CO_2 (Abinandan and Shanthakumar, 2015; Yao et al., 2015). The aim of the present study was to evaluate the growth behaviours of the microalga Chlorella vulgaris in the presence of three concentrations of arsenic in the form of mg/L $NaAsO_2$.

2. Materials and Methods

Growth of Chlorella vulgaris

The pure microalgae were donated by the Microalgae Biotechnology, Applied Physicochemistry and Environmental Studies Research Group of the Universidad del Atlántico. Subsequently, it was bioaugmented in the Microbiological Research Laboratory of the University of Sucre in 2.5 L of Nutrifoliar culture medium at a concentration of 4 mM, following the recommendations on the label. This culture medium contains as macronutrients K, Mg, S, P, Cl and micronutrients Fe, Cu, Zn, Mn, B and Mo, necessary for normal cell growth. Chlorella vulgaris cells were inoculated in each vessel at a concentration of $1x10^6$ CFU and an optical density of 0.1 absorbance measured with a 647 nm band (Infante et al., 2012). The cultures (Phyco-reactors) were kept under constant agitation to avoid cell sedimentation at a temperature of 28 ± 1°C and presence of light for 24 d and photoperiod of 12 h of light and 12 h of darkness (Sánchez et al., 2008).

Growth curve of Chlorella vulgaris in the presence of Arsenic.

The growth curve of Chlorella vulgaris was determined by growing the microalgae in culture medium with mercury concentrations of 500, 700 and 900 mg/L $NaAsO_2$ daily for up to 25 d. Aliquots of the microalgae culture were taken and growth measurements were made by optical density readings using a Merck Spectroquant Pharo 300 UV-vis spectrophotometer at a wavelength of 647 nm (Infante et al., 2012). The results of microalgae growth at different concentrations of mercury in the form of mg/L $NaAsO_2$ were compared with the behaviours of Chlorella vulgaris without the presence of the metal.

3. Results and Discussion

The results of the present study were based on the growth of the microalgae Chlorella vulgaris, which was subjected to different concentrations of arsenic in the form of $NaAsO_2$. The initial concentration was 100 mg/L NaAsO2 and was increased from 100 to 100 mg/L $NaAsO_2$. According to the results obtained, the growth curve is presented at the maximum concentrations of arsenic in the form of NaAsO2.

Figure 1 shows the growth behaviours of Chlorella vulgaris in the presence of 500 mg/L $NaAsO_2$ compared to the growth of the control (without $NaAsO_2$). The figure shows that Chlorella vulgaris had a growth up to 17 days of 2.1 nm and from 22 days onwards went into dormancy between days 18 and 22 and after this time passed into the death stage. The results also show an adaptation phase in the presence of up to six days 500 mg/L $NaAsO_2$ compared to the growth behaviours of the control microalgae treatment.

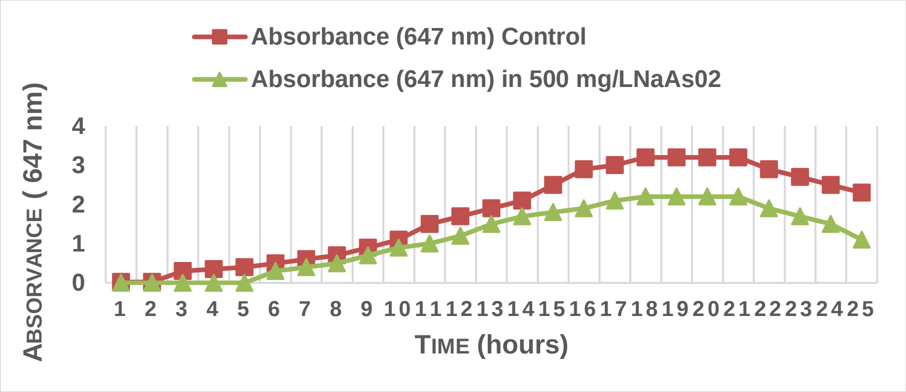

Figure 1. Growth curve of the microalga Chlorella vulgaris in the presence of arsenic in the form of $NaAsO_2$ (500 mg/L).

Figure 2 describes the growth behaviours of Chlorella vulgaris in the presence of 700 mg/L $NaAsO_2$ compared to the control of the same microalgae species without the presence of the metal. The maximum growth shown by Chlorella vulgaris was up to 17 days and from that moment onwards it entered the stationary phase and finally from day 21 it entered the death phase. The results infer growth of the microalgae at concentrations of 700 mg/L $NaAsO_2$.

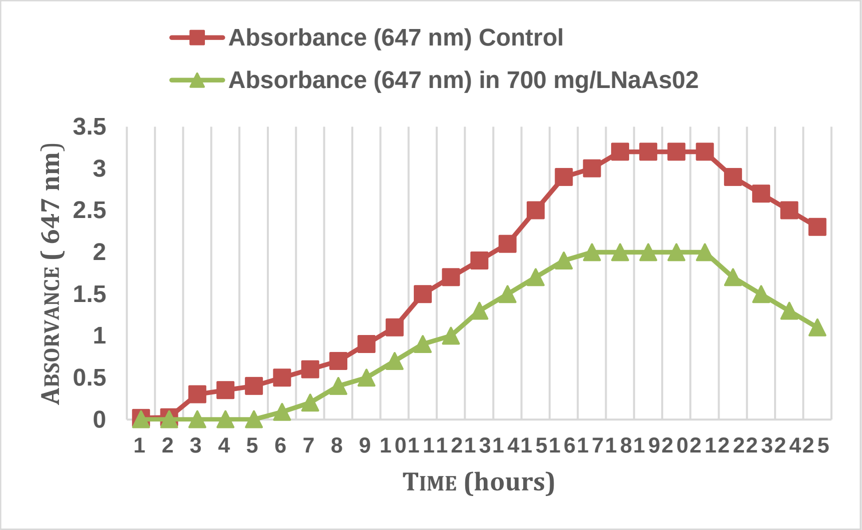

Figure 2. Growth curve of the microalga Chlorella vulgaris in the presence of arsenic in the form of $NaAsO_2$ (700 mg/L).

Figure 3 shows the growth performance of Chlorella vulgaris in the presence of 900 mg/L $NaAsO_2$ compared to the growth performance of the control (without $NaAsO_2$). In figure 3, the growth behaviours of Chlorella vulgaris in the presence of 900 mg/L $NaAsO_2$ compared to the control of the same microalgae species without the presence of $NaAsO_2$ is described. The adaptation phase lasted six days, one day longer than in the 500 and 700 mg/L $NaAsO_2$ concentrations respectively. The maximum growth shown by Chlorella vulgaris was up to 17 days and from that moment it entered the stationary phase and finally from day 21 it entered the death stage. The results inferred growth of the microalgae at concentrations of 900 mg/L $NaAsO_2$.

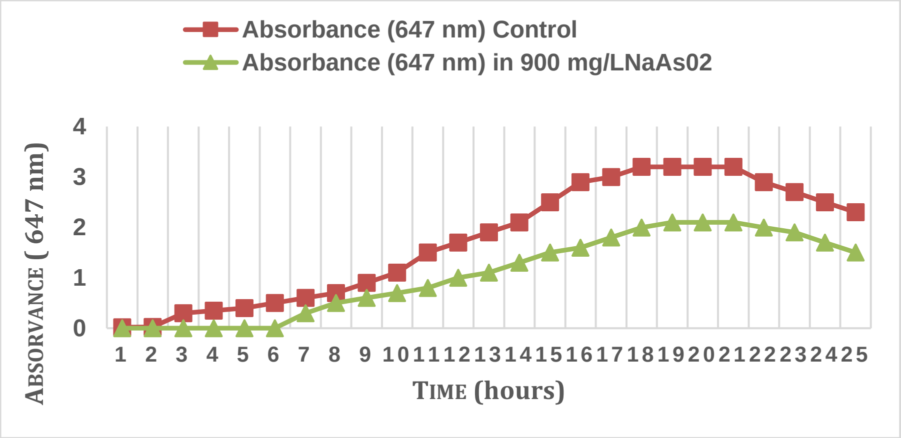

Figure 3. Growth curve of the microalga Chlorella vulgaris in the presence of arsenic

in the form of $NaAsO_2$ (900 mg/L).

Figure 4 describes the capacity of Chlorella vulgaris to absorb arsenic in free and immobilized state. The figure shows that in the free state this microalgae species has the capacity to absorb arsenic.

As demonstrated by (Aksu 2005), Chlorella vulgaris is a green alga which, due to its ability to photosynthesize and its position in trophic chains as a primary producer, makes it an ideal organism for accumulating metals. It has been shown to be able to absorb large quantities of metals, mainly Cr, Fe, Cu, Zn, Pb and Hg. This process is carried out to incorporate metals into its cells in two stages. The first, called biosorption, takes place in a very short time and is similar both in the cell wall and in the whole cell, i.e. both structures introduce Cd, Pb and Cu in a similar time, by means of an ion exchange in which Na, Mg and Ca are displaced in favor of the heavy metals. The metal can then bind to various functional groups such as amines, alcohols, phosphates, hydroxyls. The second stage, called bioaccumulation, requires a longer period of time and unlike the first stage, it is an active process in which it is believed to be involved in the cell's metabolism.

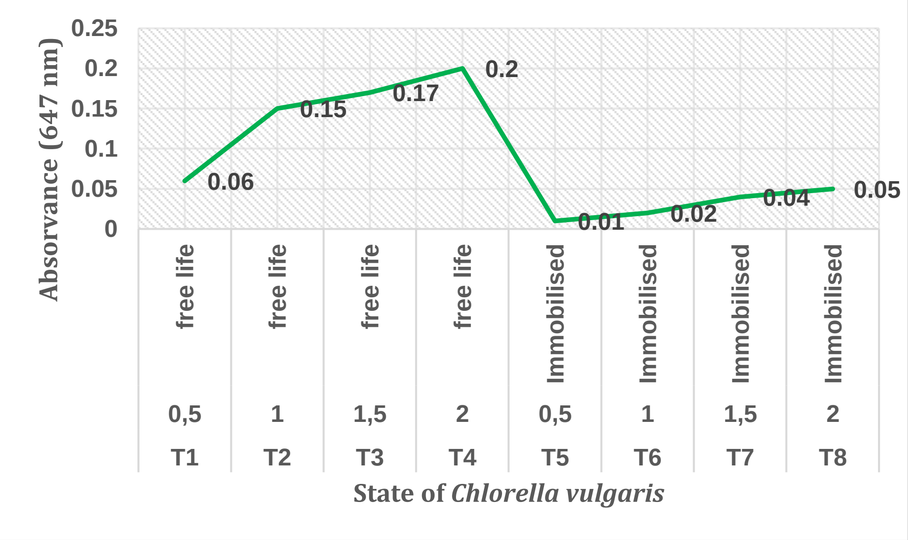

Figure 4. Arsenic biosorption assay in the form of $NaAsO_2$ by Chlorella vulgaris in the free state and immobilized on plant substrate.

Microalgae show affinity for polyvalent metals, which makes them important cleaning agents for water and wastewater containing dissolved metal ions (de-Bashan and Bashan, 2010). Furthermore, Chlorella vulgaris was chosen among 10 microalgae for the removal of dyes from textile wastewater, as it showed higher growth in this medium and higher pollutant removal (Lim et al., 2010).

Comparing the results found in the present study with other similar studies using arsenic, there is a need to present the results found with Chlorella vulgaris as an arsenic remediation alternative, given that there is little scientific information on the subject. Different forms of arsenic enter microbial cell in different ways. No specific transporter systems are evolved in bacterial cells. However, due to its structural analogy with other essential molecules, such as phosphate, toxic arsenic enters the cell through existing transporters (Yin et al. 2022).

In other work with heavy metals other than arsenic, the one reported by Benítez et al. (2018). The results related to the activity of microalgae against different concentrations of mercury, who evaluated the mercury removal capacity of the microalgae Chlorella sp. immobilized on fragments of scouring pad (Luffa cylindrica), finding the highest average mercury removal at 24 h when the microalgae were exposed to a concentration of 3.0 mg/L of $HgCl_2$, although from 8 h of exposure to 4.0 mg/L the removal was higher than 89 %.

The results obtained by Vitola et al, (2022), regarding desorption, Chlorella vulgaris presented the highest averages with 97.29 ± 1.93 % of Hg, 96.86 ± 2.14 % of Cd and 95.48 ± 1.19 % of Pb; in comparison with the microalgae species Scenedesmus obliquus which showed a desorption of 96.74 ± 2.14 % of Hg, 95.15 ± 2.90 % of Cd and 93.82 ± 2.68% of Pb. These results demonstrate that the application of immobilized microalgal biomass for the biosorption of heavy metals is a bioremediation alternative.

4. Conclusions

Microalgae can be a potent solution to the problem of arsenic pollution, offering a viable alternative to conventional physical, chemical, and instrumental methods. The use of Chlorella vulgaris as a biological alternative for heavy metal biosorption techniques is efficient, since the microalgae used in this research grew and showed different growth behaviours against mercury concentrations of 500; 700 and 900 de arsenic in the form of $NaAsO_2$.

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