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METHODOLOGY FOR EVALUATING THE EFFICIENCY OF NICKEL BIOSORPTION BY *Chlorella vulgaris* IN PHYCOREACTORS

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ABSTRACT
The aim of the present study was to evaluate the Ni^{2+} removal capacity of Chlorella vulgaris in solution and immobilized on Luffa cylindrical for 25 days in phyco-reactors and subsequently subjected to concentrations of 0.5, 1.0, 1.5 and 2.0 mg/L NiCl2 for 24 h in constant light. The immobilized microalgae showed high percentages of 98.0 % Ni^{2+} removal. With respect to the concentrations of nickel, Chlorella vulgaris showed a higher removal at a higher concentration of 2.0 mg/L with 95.8 % removal for nickel, which is possibly due to the fact that the cells of Chlorella vulgaris have a high affinity for divalent Ni^{2+} metals that interact with the functional groups present in the membrane of the microalgae and act as binding and neutralization points for the toxicity of these pollutants. Therefore, phyco-remediation using immobilized *Chlorella vulgaris* is a technique with a high capacity for remediation of Ni^{2+} contaminated waters.

Keywords: nickel, microalgae, removal, suspension, immobilization.

INTRODUCTION
According to (Yin et al., 2019; Gutiérrez-Benítez et al, 2014), biosorption is considered an alternative technology for the removal of heavy metals from wastewater and the use of microalgae as adsorbents encourages scientific and technological interest considering their great variety, abundance, availability of different species and good capacity to absorb heavy metal ions, in addition to their outstanding advantages that include high efficiency, low cost and environmentally friendly (Yin et al., 2019; Vitola et al., 2018). However, the accumulation of these environmental pollutants especially Ni^{2+} in phytoplankton is crucial to understand their pathway in food chains, given their role as primary producers (Garcia et al., 2014; Becker and Bigham, 1995).
The present study consists of the following procedures: initially, the cells of the microalga Chlorella vulgaris were immobilized for 18 d in 2.5x2.8 cm pieces of scouring pad. for 18 d in 2.5x2.8 cm

fragments of scouring pad, then heavy metal biosorption experiments were carried out using synthetic water with concentrations of 0.5, 1.0, 1.5 and 2.0 mg/L $NiCl_2$ in separate treatments for 24 h, using the microalgae Chlorella vulgaris. in free cells at a cell density of 1x106 CFU/mL and immobilized. Finally, the microalgae were removed from each treatment and the nickel concentrations in the water were analyzed by cold vapor atomic absorption spectrophotometry.

MATERIALES Y METDODOS

Bioaugmentation of *Chlorella vulgaris*. The pure microalga *Chlorella vulgaris* was donated by the Microalgae Biotechnology, Applied Physical Chemistry and Environmental Studies Research Group of the Universidad del Atlántico. Bioaugmentation was performed at the Microbiological Research Laboratory of the University of Sucre following the protocol proposed by Hernandez et al., (2018). 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*. The growth curve of Chlorella vulgaris was determined by growing the microalgae in culture medium, aliquots of the microalgae culture were taken daily for up to 24 d 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).

Separation and washing of the biomass. When the microalgae were in stationary phase (18±1 d) the microalgae were separated by centrifugation at a speed of 4000 rpm for 5 min, then successive washes were performed with distilled water to dissolve any excess salt present.

Immobilization of the biomass in *Luffa cylindrica*. The immobilization of the microalgae was done on the dried fruit of Luffa cylindrica, which was cut at the ends to remove seeds and impurities with successive washes with water and detergent for 30 min (Nabizadeh et al., 2008) and following the methodology proposed by Hernandez et al., (2018), after which time they were inoculated in the microalgae solution in stationary phase (18±1 d), after which the straws were removed, washed to remove excess microalgae, and the immobilized biomass was determined by the difference in weight of the straws before and after immobilization (Akhtar et al. , 2004).

Determination of the removal capacity of *Chlorella vulgaris*. The removal capacity of mercury and nickel by *Chlorella vulgaris* both in solution and immobilized on scouring pad, using concentrations of the heavy metals of 0.50, 1.00, 1.50 and 2.00 mg/L, for 25 h at constant light exposure and average light intensity of 2000 lux, after which the microalgal biomass was removed by centrifugation, and the removal yields of the metals were established by analysis of the liquid samples by the atomic absorption technique (Benítez et al., 2018; Hernández et al., 2018). All treatments were performed in triplicate.

Nickel analysis was performed by flame atomic absorption spectrophotometry adjusted to a wavelength of 232 nm, spectral resolution of 0.2 nm, lamp current of 5 mA, air flow rate of 7 L/min and acetylene flow rate of 1.2 L/min (Chávez, 2016).

RESULTS AND DISCUSSION

Growth curve of *Chlorella vulgaris* in phycorreactor

In order to perform the growth curve of *Chlorella vulgaris* in a phyco-reactor, the following protocol must be followed as described in figure 1.

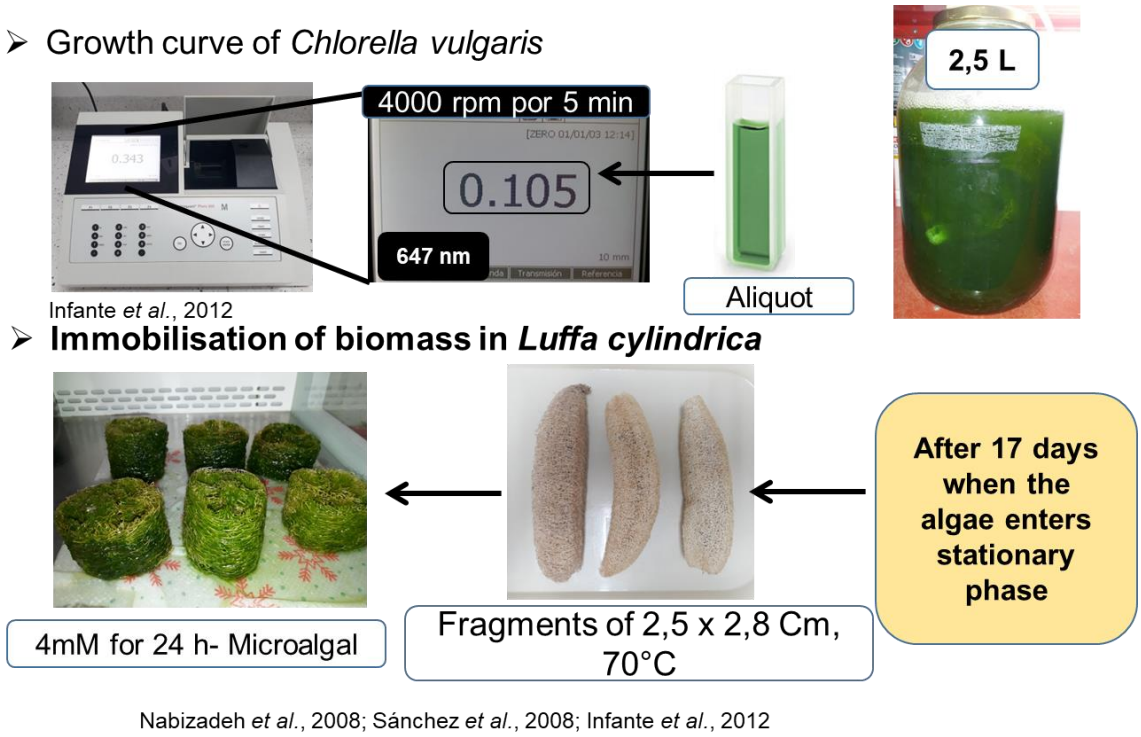
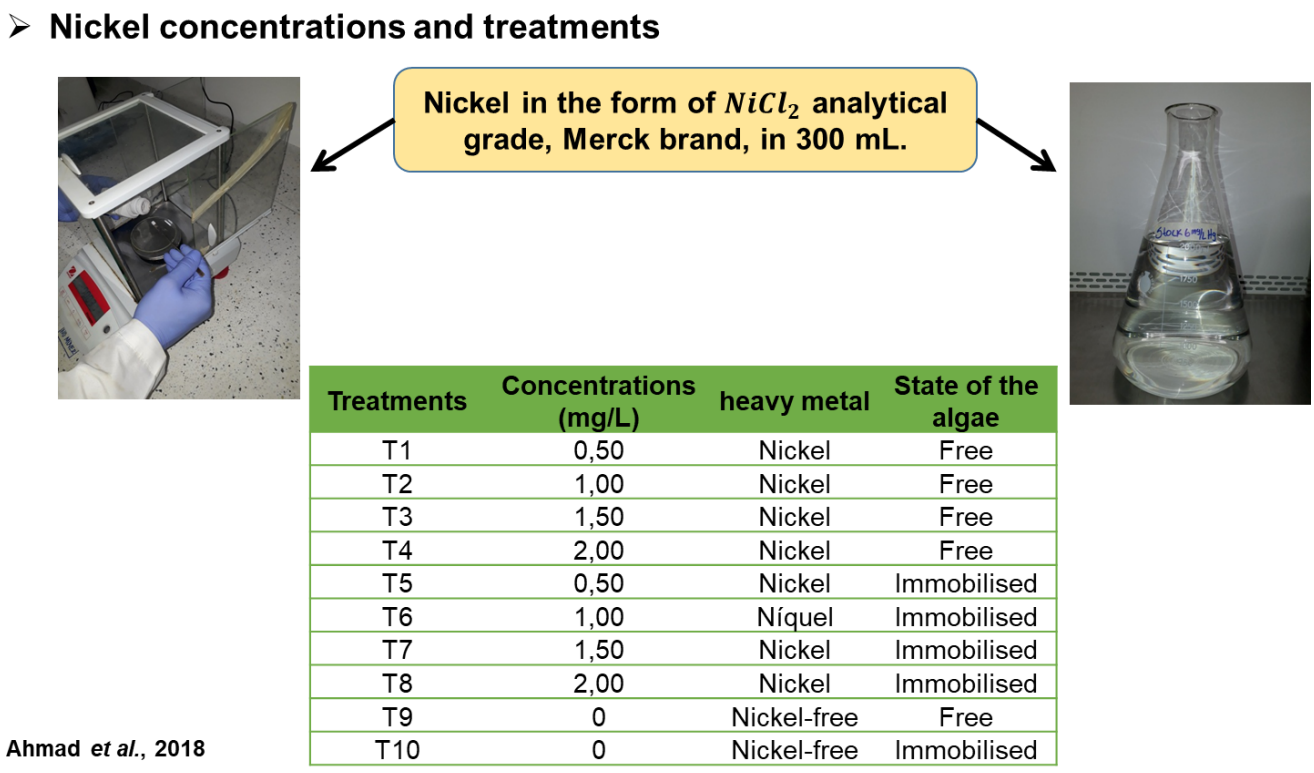


Figure 1. Growth curve of *Chlorella vulgaris* in phycoreactor. Source: Hernandez et al., 2018.

Method for the evaluation of nickel tolerance by *Chlorella vulgaris*.
For the in vitro evaluation of the tolerance capacity of Chlorella vulgaris in the presence of nickel metal, the methodology shall be followed as described in Figure 2.



Treatments	Concentrations (mg/L)	heavy metal	State of the algae
T1	0,50	Nickel	Free
T2	1,00	Nickel	Free
T3	1,50	Nickel	Free
T4	2,00	Nickel	Free
T5	0,50	Nickel	Immobilised
T6	1,00	Níquel	Immobilised
T7	1,50	Nickel	Immobilised
T8	2,00	Nickel	Immobilised
T9	0	Nickel-free	Free
T10	0	Nickel-free	Immobilised

Figure 2. Method for the evaluation of nickel tolerance by *Chlorella vulgaris*. Hernandez et al., 2018.

Determination of the removal capacity of Chlorella vulgaris.

For the in vitro determination of the nickel removal capacity of *Chlorella vulgaris* the following protocol shall be followed as illustrated in Figure 3.

Determination of the removal capacity of *Chlorella vulgaris*.

$$Percentage\ of\ removal(\%) = \frac{C_i - C_f}{C_i} x100$$

Where Ci is the initial heavy metal concentration and Cf is the final concentration after treatment.

Statistical analysis

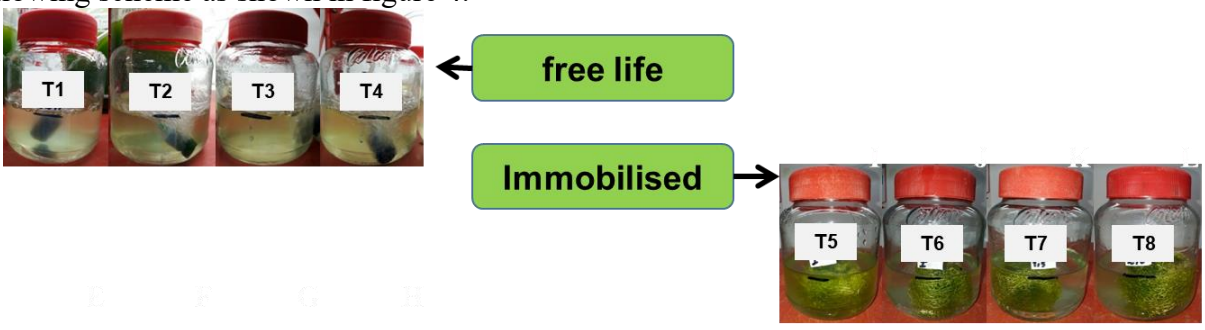
An ANOVA will be performed using a DCA with 2x2x4 factorial arrangement, previously establishing the normality criterion (Shapiro-Wilk), followed by the Tukey rank multiple test to establish the significant statistical differences (p≤0.05) in the free version of InfoStat software.

Ahmad *et al.*, 2018

Figure 3. Scheme of the determination of the removal capacity of *Chlorella vulgaris*. Source: Hernandez et al., 2018.

Determination of the removal capacity of *Chlorella vulgaris*

The determination of the removal capacity of the microalga *Ch. vulgaris* was determined using the following scheme as shown in figure 4.



Treatments	Concentration of nickel	State of the microalgae	Absorvance (647 nm)
T1	0,5	free life	0,06
T2	1	free life	0,15
T3	1,5	free life	0,17
T4	2	free life	0,2
T5	0,5	Immobilised	0,01
T6	1	Immobilised	0,02
T7	1,5	Immobilised	0,04
T8	2	Immobilised	0,05

Figure 4. Scheme to determine the nickel removal capacity of suspended (free-living) and immobilized *Ch. vulgaris*.

Figure 5 shows the results of the in vitro nickel removal capacity of the microalgae Chlorella vulgaris both immobilized and in suspension (free-living).

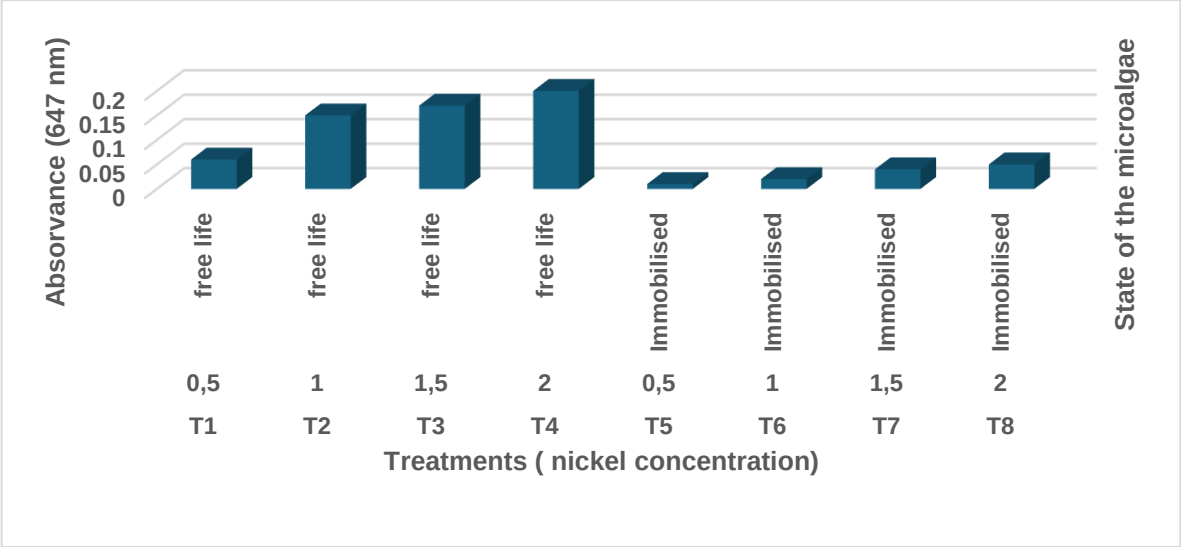


Figure 5. Results of the evaluation of nickel removal capacity using suspended (free-living) and immobilized *Ch. vulgaris*.

According to the results of Sánchez et al. (2008) who evaluated the bioadsorption capacity of Ni2+ and Zn2+ by the microalgae Chlorella sp. immobilized on calcium alginate pellet, achieving the highest removal rates between 0 to 20 min of exposure of the microalgae with removal rates around 99 % for both metals. In addition, demonstrating high percentages of recovery of these heavy metals when the microalgal biomass was subjected to digestion with 0.1 M HCl.

With regard to the results obtained by Hernández, (2018), with respect to the states of use of the microalgae Chlorella sp. for the removal of $Ni^{2+,}$ significant statistical differences were found ($p<0.05$), with higher removal averages being observed for the microalgae immobilized in fragments of scouring pad (Luffa cylindrica) with 97.35 % compared to Chlorella sp. It was observed that the microalgae in solution presented a superior removal of 91 % of the metals evaluated ($Ni^{2+).}$

The results obtained for the removal of Ni^{2+} depending on the presence of the microalgae in suspension and immobilized show that Chlorella vulgaris in the immobilized state in the dried fruit of *Luffa cylindrica* presented removal percentages for nickel of approximately 98.0 % (figure 5).The absorption of nickel is possibly due to the components present in the cell wall of the microalgae such as: proteins, lipids, glycoproteins and carbohydrates such as pectins, xylans, mannans and alginic acid, which provide binding sites for metal ions, thanks to functional groups such as hydroxyl, phosphoryl, carboxyl, sulfuryl, amine, imidazole, sulphate, phosphate, which create high affinity areas for monovalent metal cations such as Ag(I), divalent ones such as Hg(II), Ni(II), Pb(II), Cd(II), Zn(II) or trivalent ones such as Al(III), Fe(III) and Au(III), among others. In addition, they possess molecular mechanisms such as peptide synthesis, which have been inferred to be responsible for the biosorption capacity of heavy metal ions (Kaplan, 2013; de-Bashan and Bashan, 2010; Arief et al., 2008; Sánchez et al., 2008).

CONCLUSION

The Ni(II) removal tests carried out in a phyco-reactor with the microalgae Chlorella vulgaris led to the conclusion that the microalgae removed the highest percentages of nickel with 98.0 %.

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AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

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