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Biosorption of Mercury and Nickel in Vitro by Microalga *Chlorella sp*. in Solution and Immobilized in Dry Fruit of Squash (*Luffa Cylindrica*)

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Abstract

Objectives: This work aimed to determine the biosorption capacity of Mercury and Nickel by the microalga *Chlorella sp*. present in solution and immobilized in dried fruit of *Luffa cylindrica.* **Methods/Statistical Analysis:** The algal biomass was bioaugmented in photobioreactors with 4mM Agrimins for 18±1 days in constant agitation. For the immobilization of the microalga, the sponge was used as support. The mercury and nickel removal capacity was determined by making daily growth measurements at 647 nm with a UV-vis SpectroquantPharo 300 Merck spectrophotometer. With the results an ANOVA and the Tukey test (p-value≤0.05) were performed in the InfoStat software. **Findings:** The results obtained showed that the microalga adheres to the fibers of the scourer, with an average immobilization of 1.58g of microalga/scourer fragment of 2.5x2.8Cm at 18 days. The results showed significant statistical differences (p-value<0.05) between, microalga in solution, immobilized, Hg and Ni concentration and between the interactions of these factors. Phycoremediation is an efficient removal technique of Hg and Ni present in contaminated water, and is potentialized when algal cells are attached to substrates that provide protection. **Improvements/Applications:** immobilized microalgae are an efficient alternative to remove environmental contaminants, but the type of substrate that contains the biosorbent is a key factor in the success of this biotechnology.

Keywords: *Chlorella*, Bioremediation, Biosorption, Mercury, Nickel

1. Introduction

The presence of heavy metals in water is mainly due to industrial processes such as mining, refining, manufacturing, chemical industry, electronics and metallurgy[1]. These activities are generating wastewater with a large amount of pollutants, which are discharged to natural systems, and without prior treatment can generate eutrophication and disturbance of the physicochemical parameters such as pH, decrease in photosynthetic activity and thus decrease in oxygen dissolved and increased biochemical oxygen demand and chemical oxygen demand, among others[2].

Heavy metals such as: nickel, mercury, copper, lead, chromium, zinc and manganese, etc., are responsible for many ecological and human health damages, because they have the capacity to accumulate and cause serious disorders and diseases[3], cause damage to tissues and organs, malformations and cancer[4] even at trace levels during prolonged exposure are a potential risk for any type of life due to its high toxicity, accumulation and null degradability[1], as well as its great mobility in aqueous systems[5]. For this reason, it is necessary and urgent the removal of metal contaminants from water to avoid their intake[6].

Several techniques have been developed for the removal of heavy metals present in water, such as: extraction, membrane separation, photocatalys is, ion exchange, adsorption and chemical precipitation[7]. However, the chemical extraction those has been used more with

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respect to the other techniques due to the high efficiency of removal in a short time, but has the disadvantage of being expensive and bring adverse effects to the environment[8], while adsorption is a simple, economical, affordable material and the adsorbent can be regenerated[9]. Current technology has allowed the development of high efficiency adsorbents in the removal of heavy metals, although applying this type of material increases the costs of the remediation technique.

Biosorption is considered an alternative technology for the removal of heavy metals from wastewater and the use of algae as adsorbents encourages scientific and technological interest taking into account their great variety, abundance and availability of different species[5]. Therefore, the cultivation of microalgae in wastewater is being widely extended for the elimination of nutrients and control of the physicochemical parameters of these waters, and as a raw material for the production of biofuel[10].

Furthermore, the use of live and non-living microalgae is a low-cost and environmentally friendly technique, which is being increasingly used to eliminate various toxic organic substances such as antibiotics[11,12], phenol and polycyclic aromatic compounds from wastewater[13,14]. However, the non-living microalgae is not affected by the toxicity of the contaminant and requires less space, as well as little maintenance compared to living, while living cells are more efficient when the concentration of contaminants is low[11]. This is due to the fact that, in addition to adsorption, living cells can remove organic substances by various mechanisms, including bioaccumulation, biotransformation or biodegradation[15]. According to the above, the use of biological techniques such as the use of microalgae for the removal of contaminants is an appropriate technique, because it is low cost and of good efficiency. But it is necessary to take into account the factors that affect the biosorption of heavy metals, such as the characteristics of the ions, the concentration, the ionic strength, the temperature, the pH, the contact time and the nature of the sorbent, which define the differences in selectivity and affinity to metal ions[16]. Therefore, this research was aimed at evaluating the sorption capacity of the heavy metals Mercury and Nickel by the microalga *Chlorella sp*. in solution and immobilized in dry fruit of *Luffa cylindrica.*

2. Materials and Methods

2.1 Culture Medium and Growth of *Chlorella sp*

The biomass of *Chlorella sp*. was increased in 2.5 L of culture medium at a concentration of 4 mM Nutrifoliar which supplies the macronutrients K, Mg, S, P, Cl and the micronutrients Fe, Cu, Zn, Mn, B and Mo, was inoculated with a concentration of *Chlorella sp*. of $1x10^6$ CFU and optical density of 0.1 of Abs measured with λ=647 nm[17]. This bioreactor were kept at 28±1°C and in constant agitation to avoid sedimentation of the biomass, with the presence of light for 18±1 days and a photoperiod of 12 hours of light and 12 hours of darkness[18].

2.2 Growth Curve of *Chlorella sp.*

Every day up to 18 ± 1 days aliquots were taken from the microalgal culture and growth measurements were made with a spectrophotometer UV-vis SpectroquantPharo 300 from Merck at a wavelength of 647 nm where the absorbance was measured which is proportional to the concentration of the microalgae in the culture, starting from day 0 with a concentration of 0.1 Abs[17].

2.3 Separation and Washing of Biomass

Once the cells of the microalgae in the phycoreactor entered the stationary phase (18±1 days), the microalga was separated by centrifugation at 4000 rpm for 5 minutes, then successive washes were made with distilled water to dissolve any type of salt present in excess.

2.4 Immobilization of Biomass in *Luffa Cylindrica*

The dried fruit of *Luffa cylindrica*, known by the common name of scouring pad, was used for the immobilization of the microalga, which was cut at the ends to remove the seeds and impurities with successive washes with water and detergent for 30 min[19]. Then cut pieces of scouring pad 2.5±0.2 cm in diameter and 2.8±0.2 cm thick, again washed with distilled water and dried at 70±1°C to be sterilized and transferred to culture medium with 4 mM Agrimins for 24 hours, after this time they were inoculated in the solution of microalga in stationary phase (18±1 days), after which the scourers were removed, washed to eliminate excess microalgae, and the immobilized biomass was determined by the difference of the weight of the scourer before and after immobilization[20].

2.5 Preparation of Synthetic Medium with Heavy Metal

To prepare the synthetic heavy metal media, stock solutions of 5 mg/L were prepared for $HgCl_2$ and $NiCl_2$ analytical grade, Merck brand, in sterile water, and then from these diluted solutions of 300 mL were prepared at the concentrations of 0.50 mg/L, 1.00 mg/L, 1.50 mg/L and 2.00 mg/L.

2.6 Determination of the Removal Capacity of *Chlorella sp*

The removal capacity of heavy metals by *Chlorella sp*. was determined. both in solution and immobilized in scourer, using the aforementioned concentrations of heavy metals, whose removal time was established in 24 hours, after which the microalga biomass was removed by centrifugation, and the removal yields of the metals by analysis of the liquid samples by the atomic absorption technique. All treatments were performed in triplicate.

2.7 Statistic Analysis

A design with 4x2x2 factorial arrangement was made, followed by the Tukey multiple range test to establish the significant differences ($p\text{-value} \leq 0.05$) in the InfoStat software.

3. Results and Discussion

3.1 Growth Curve of the Microalga *Chlorella sp.*

The growth curve of the microalga was performed under mixotrophic growth conditions, using Agrimins as a culture medium at a concentration of 4 mM and an algal concentration of 0.1 Abs. The reactors were incubated for 18 days at 30±1°C and a light intensity of 2000 lux (Figure 1(A)).

Microalgae are photosynthetic organisms that, depending on the conditions in which they are found, can express various metabolic pathways such as autotrophic, heterotrophic and mixotrophic for the generation of carbon and energy[21], the latter being the route that mainly presents the majority of microalgae[22].

During photosynthesis, microalgae use light as an energy source for the synthesis of various compounds, and this depends on changes in the periodicity of light and intensity variations[23], that are considered efficient strategies that stimulate the growth of microalgae and the accumulation of specific biomolecules[24].

3.2 Immobilization of the Microalga *Chlorella sp*. in Dried Fruit of *Luffa Cylindrica*

With the immobilization technique used the effective immobilization of the microalga *Chlorella sp*. in the fibers of the dry loofah fruit (*L. cylindrica*), finding that on average 1.58g of microalga was immobilized in each mop fraction of 2.5x2.8 Cm (Figure 1(B)).

3.3 Determination of the Capacity of Removal of Mercury and Nickel by the Microalga *Chlorella sp*. in Solution and Immobilized

The results fulfilled the ANOVA criteria, which is why we proceeded to perform the analysis of variance indicating that there are significant statistical differences ($p\text{-value}<0.05$) between the factors and their interaction. To find these significant differences, Tukey's multiple range tests ($p\text{-valor} \leq 0.05$) were applied.

With respect to the states of use of the microalga *Chlorella sp*. for the removal of heavy metals, significant statistical differences were found ($p\text{-value}<0.05$), showing the highest average removal of immobilized microalgae in scourer fragments (*L. cylindrica*) with 97.35%. However, the microalga showed a greater than 91% removal of the two heavy metals evaluated (Figure 1(C)).

With respect to the concentrations of heavy metals, no significant statistical differences were found ($p\text{-value}>0.05$) between the concentrations 0.50 mg/L and 1.00 mg/L, while between the concentrations 1.50 mg/L and 2.00 mg/L if there were significant differences ($p\text{-value}<0.05$), being the concentration of 2.00 mg/L the concentration at which the microalga *Chlorella sp*. removed more heavy metals (Figure 1(D)). Therefore, it is proposed to increase the concentrations of the heavy

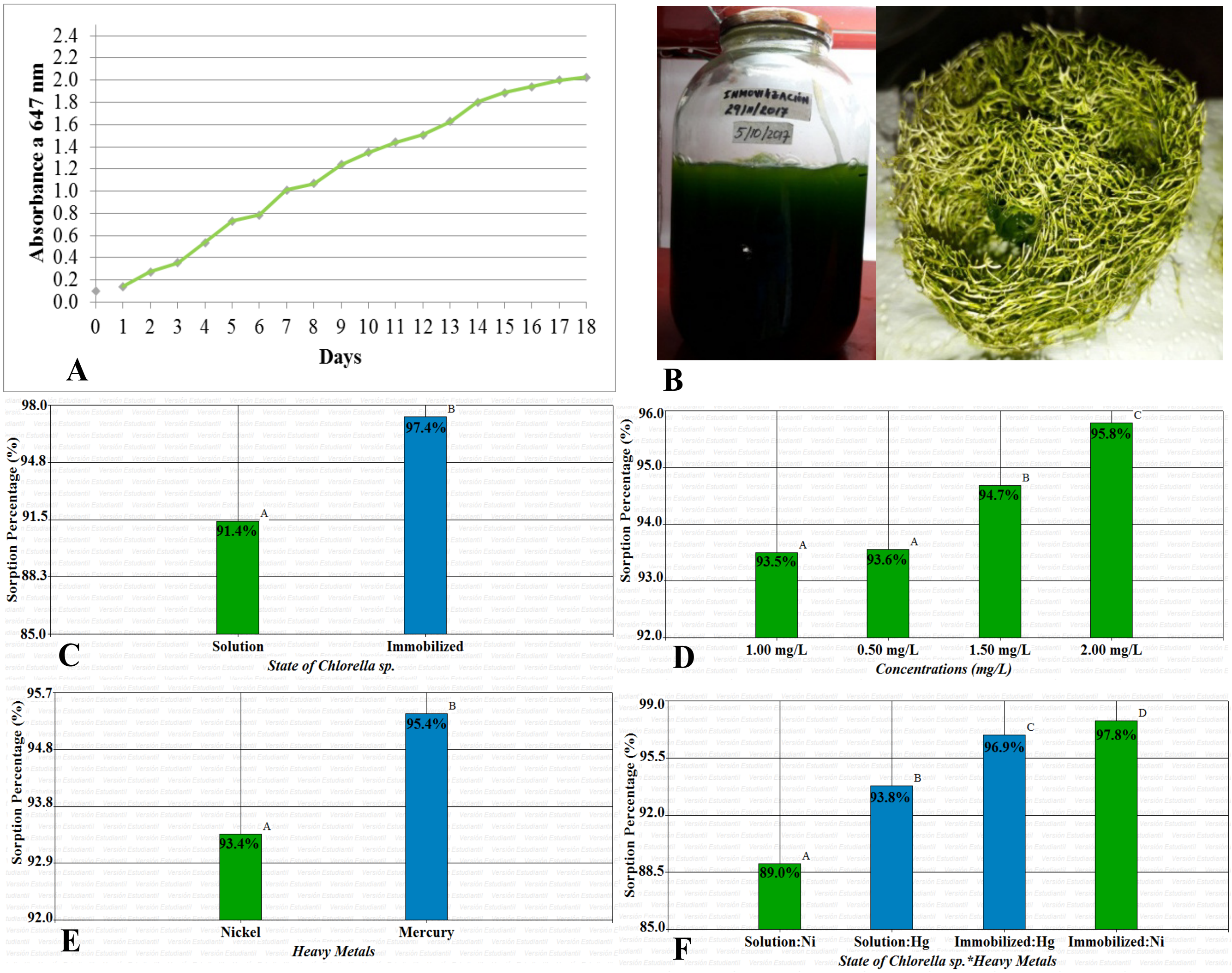

Figure 1. A. Growth curve of *Chlorella* sp. in mixotrophic medium, B. Immobilization of the microalga *Chlorella* sp., C. Tukey test for the removal of heavy metals according to the status of the microalga *Chlorella* sp., D. by concentrations, E. by the type of heavy metal, and F. Interaction between the state of the microalga with the types of heavy metals.

metals evaluated, and to determine the maximum sorption capacity of said microalgae.

In relation to the heavy metals evaluated, significant statistical differences were found (p-value<0.05) between the two heavy metals, with mercury showing the highest average of removal with 95.36%, followed by nickel with a removal of 93.40% (Figure 1(E)).

When the factors of the microalga and heavy metals interact, it was found that the microalga *Chlorella sp*. when immobilized in the dry fruit of *L. cylindrica*, it had the highest removal percentage of heavy metals, reaching 96.92% for removal of mercury and 97.77% removal for nickel. While in the state of solution the highest percentage of removal was for mercury with 93.80% and for nickel of 89.03% (Figure 1(F)). Therefore, for the removal of these heavy metals in aquatic systems, the best way is to use the microalga *Chlorella sp*. immobilized, to guarantee a higher percentage of removal.

While in the state of solution the highest percentage of removal was for mercury with 93.80% and for nickel of 89.03% (Figure 1(F)). Therefore, for the removal of these heavy metals in aquatic systems, the best way is to use the microalga *Chlorella sp*. immobilized, to guarantee a higher percentage of removal.

The contamination of the different environmental compartments with heavy metals by the various human activities is a crucial environmental problem in both developing and developed countries[25], therefore, environmental pollution has positioned itself as one of the most important problems that are affecting society[26] and this pollution is increasing with the passing of days due to the increase in wastewater discharges, and this is accompanied by the flexibility of environmental laws in Colombia, since the maximum levels of contamination allowed by the Ministry of Environment and Sustainable Development for the particular case of Nickel (0.5 mg/L) and Mercury (0.02 mg/L) are well above[27] of the allowed values (Nickel 0.2 mg/L and Hg 0.00003 mg/L) per the Environmental Protection Agency of the United States (USEPA)[28], which indicates the negligence of the Colombian state in matters of environmental protection and human health.

The contamination of water by heavy metal ions is one of the most important environmental problems worldwide[29], since they cause serious environmental problems in aquatic ecosystems and on human beings due to their high toxicity and carcinogenicity[30] and unlike many other pollutants, the removal of heavy metals from the ecosystem is really a challenge since they cannot be degraded by biological or chemical processes and, ultimately, they are indestructible[31] inhibiting the self-purification capacity of water bodies[32].

However, microalgae have developed broad-spectrum intracellular and extracellular mechanisms to counteract the toxicity of heavy metals[31]. Microalgae are photosynthetic organisms that produce peptides with the ability to adhere to heavy metals[33], generating organometallic complexes that neutralize their toxic effect and those housed inside the vacuoles to facilitate cytoplasmic control of these metal ions[34].

In particular, *Chlorella* is a very desirable microalga for the removal of heavy metals, thanks to its high affinity for polyvalent metals, positioning it as a potential biosorbent in the cleaning of wastewater containing dissolved metal ions[35]. Therefore, the use of microalgae as a bioabsorbent of heavy metals is a prevalent technique over the conventional physicochemical processes used in the elimination of toxic heavy metals[31], since its capacity to absorb past metals is much greater than activated carbon, natural zeolite and synthetic ion exchange resin[36].

Further to the ability to remove heavy metals, the biomass of *Chlorella vulgaris* is used as a raw material for the production of biofuels. In addition, this microalgae species also has the potential to biodegrade hydrocarbons from crude oil showing excellent resistance to exposed contaminants, as well as a good remediation capacity and during the experiment the algal biomass increased, indicating a positive effect of the crude on the growth of *C. vulgaris*[37]. It also has the ability to remove the anticancer drug flutamide, showing the improved elimination performance of live microalgae compared to non-living microalgae[38].

4. Conclusions

The wide presence of microalgae in the environment together with their rapid growth determines their suitability in practical applications of bioremediation strategies of heavy metals in wastewater, because they exhibit an important affinity for different metals, and, therefore, are very important, used as biosorbent materials. In this experiment it was proved that the living biomass of *Chlorella sp*. immobilized in dry scourer (*Luffa cylindrica*) is a strategy that potentiates the biosorption of mercury and nickel from aqueous medium.

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6. References

1. Awual MR, Ismael M, Khaleque MA, Yaita T. Ultra-trace copper (II) detection and removal from wastewater using novel meso-adsorbent, Journal of Industrial and Engineering Chemistry. 2014 July; 20(4):2332–40. https://doi.org/10.1016/j.jiec.2013.10.009.
2. El-Kassas HY, Mohamed LA. Bioremediation of the textile waste effluent by Chlorella vulgaris, Egyptian Journal of Aquatic Research. 2014 Oct; 40(3):301–08. https://doi.org/10.1016/j.ejar.2014.08.003.

3. Wang X, Chen J, Yan X, Wang X, Zhang J, Huang J, Zhao J. Heavy metal chemical extraction from industrial and municipal mixed sludge by ultrasound-assisted citric acid, Journal of Industrial and Engineering Chemistry. 2015 July; 27:368–72. https://doi.org/10.1016/j.jiec.2015.01.016.
4. Awual MR, Hasan MM, Khaleque MA, Sheikh MC. Treatment of copper (II) containing wastewater by a newly developed ligand based facial conjugate materials, Chemical Engineering Journal. 2016 Mar; 288:368–76. https://doi.org/10.1016/j.cej.2015.11.108.
5. Gutiérrez-Benítez O, González-Álvarez J, Freire-Leira MS, Rodríguez-Rico IL, Moreira-González AR. Potencialidades de un biosorbente algal para la remoción de metales pesados, Tecnología Química. 2014; 34(1):82–93.
6. Zhao J, Liu J, Li N, Wang W, Nan J, Zhao Z, Cui F. Highly efficient removal of bivalent heavy metals from aqueous systems by magnetic porous Fe3O4-MnO2: Adsorption behavior and process study, Chemical Engineering Journal. 2016 Nov; 304:737–46. https://doi.org/10.1016/j.cej.2016.07.003.
7. Tahmasebi E, Masoomi MY, Yamimi Y, Morsali A. Application of mechano-synthesized azine-decorated zinc (II) metal-organic frameworks for highly efficient removal and extraction of some heavy-metal ions from aqueous samples: A comparative study, Inorganic Chemistry. 2015 Dec; 54(2):425–33. https://doi.org/10.1021/ic5015384. PMid: 25548873.
8. Tang J, He J, Xin X, Hu H, Liu T. Bio-surfactants enhanced heavy metals removal from sludge in the electro-kinetic treatment, Chemical Engineering Journal. 2018 Feb; 334:2579–92. https://doi.org/10.1016/j.cej.2017.12.010.
9. Renu B, Agarwal M, Singh K. Heavy metal removal from wastewater using various adsorbents: A review, Journal of Water Reuse and Desalination. 2017 Oct; 1–33.
10. Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, Ruan RR. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella sp.,* Bioresource Technology. 2010 April; 101(8):2623–28. https://doi.org/10.1016/j.biortech.2009.10.062. PMid: 19932957.
11. Santaeufemia S, Torres E, Mera R, Abalde J. Bioremediation of oxytetracycline in seawater by living and dead biomass of the microalga Phaeodactylumtricornutum, Journal of Hazardous Materials. 2016 Aug; 320:315–25. https://doi.org/10.1016/j.jhazmat.2016.08.042. PMid: 27565856.
12. Xiong JQ, Kurade MB, Jeon BH. Biodegradation of levofloxacin by an acclimated freshwater microalga, Chlorella vulgaris, Chemical Engineering Journal. 2017 April; 313:1251–57. https://doi.org/10.1016/j.cej.2016.11.017.
13. Gao Q, Wong YS, Tam N. Removal and biodegradation of nonylphenol by different Chlorella species, Marine Pollution Bulletin. 2011; 63(5-12):445–51.
14. El-Sheekh M, Ghareib M, El-Souod G. Biodegradation of phenolic and polycyclic aromatic compounds by some algae and cyanobacteria, Journal of Bioremediation and Biodegradation. 2012 Dec; 3(1):1–9. https://doi.org/10.4172/2155-6199.1000133.
15. He N, Sun X, Zhong Y, Sun K, Liu W, Duan S. Removal and biodegradation of nonylphenol by four freshwater microalgae, International Journal of Environmental Research and Public Health. 2016 Dec; 13(12):1239. https://doi.org/10.3390/ijerph13121239. PMid: 27983663, PMCid: PMC5201380.
16. Ahmad A, Bhat AH, Buang A. Biosorption of transition metals by freely suspended and Ca-alginate immobilised with Chlorella vulgaris: Kinetic and equilibrium modeling, Journal of Cleaner Production. 2018 Jan; 171(10):1361–75. https://doi.org/10.1016/j.jclepro.2017.09.252.
17. Infante C, Angulo E, Zárate A, Florez JZ, Barrios F, Zapata C. Propagación de la microalga Chlorellasp. en cultivo por lote: cinética del crecimiento celular, Avances en Ciencias e Ingeniería. 2012 Apr-June; 3(2):159–64.
18. Sánchez E, Garza M, Almaguer V, Sáenz I, Li-án A. Estudio cinético e isotermas de adsorción de Ni (II) y Zn (II) utilizando biomasa del alga Chlorellasp, Inmovilizada, Ciencia UNAL. 2008 April-June;11(2):168–76.
19. Nabizadeh R, Naddafi K, Mesdaghinia A, Nafez AH. Feasibility study of organic matter and ammonium removal using loofa sponge as a supporting medium in an aerated submerged fixed-film reactor (ASFFR), Electronic Journal of Biotechnology. 2008 Oct; 11(4):1–9. https://doi.org/10.2225/vol11-issue4-fulltext-8.
20. Akhtar N, Iqbal J, Iqbal M. Removal and recovery of nickel (II) from aqueous solution by loofa sponge-immobilized biomass of Chlorella sorokiniana: Characterization studies, Journal of Hazardous Materials. 2004 Apr; 108(1-2):85–94. https://doi.org/10.1016/j.jhazmat.2004.01.002. PMid: 15081166.
21. Candido C, Lombardi AT. The physiology of Chlorella vulgar is grown in conventional and biodigested treated vinasses, Algal Research. 2018 Mar; 30:79–85. https://doi.org/10.1016/j.algal.2018.01.005.
22. Candido C, Lombardi AT. Growth of Chlorella vulgar is in treated conventional and bio-digested vinasses, Journal of Applied Phycology. 2016 Feb; 29(1):1–9.
23. Cassuriaga APA, Freitas BCB, Morais MG, Costa JAV. Innovative poly-hydroxybutyrate production by Chlorella fusca grown with pentoses, Bioresource Technology. 2018 June; 265:456–63. https://doi.org/10.1016/j.biortech.2018.06.026. PMid: 29935455.
24. Krzeminska I, Pawlik-Skowronska B, Trzcinska M, Tys J. Influence of photoperiods on the growth rate and biomass productivity of green microalgae, Bioprocess and

Biosystems Engineering. 2014 Apr; 37(4):735–41. https://doi.org/10.1007/s00449-013-1044-x. PMid: 24037038, PMCid: PMC3968445.

25. Asgari B, Ghorbanpour M, Nikabadi S. Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants, Ecotoxicology and Environmental Safety. 2017; 145:377–90. https://doi.org/10.1016/j.ecoenv.2017.07.035. PMid: 28759767.
26. Reyes Y, Vergara I, Torres OE, Díaz M, González EE. Contaminación por metales pesados: implicaciones en salud, ambiente y seguridad alimentaria, Revista Ingeniería, Investigación y Desarrollo. 2016 Nov; 16(2):66–77. https://doi.org/10.19053/1900771X.v16.n2.2016.5447.
27. Ministerio de Ambiente y Desarrollo Sostenible República de Colombia (MADS). Resolución 0631 de 2015 "Por la cual se establecen los parámetros y los valores límites máximos permisibles en vertimientos puntuales a cuerpos de aguas superficiales y a sistemas de alcantarillado público, y se dictan otras disposiciones; 2015. Date accessed 18.04.2015. https://docs.supersalud.gov.co/PortalWeb/Juridica/OtraNormativa/R_MADS_0631_2015.pdf.
28. Nguyen TAH, Ngo HH, Guo WS, Zhang J, Liang S, Yue QY, Li Q, Nguyen TV. Applicability of agricultural waste and by-products for adsorptive removal of heavy metals from waste water, Bioresource Technology. 2013 Nov; 148:574–85. https://doi.org/10.1016/j.biortech.2013.08.124. PMid: 24045220.
29. Hong KS, Lee HM, Bae JS, Ha MG, Jin JS, Hong TE, Kim JP, Jeong E.D. Removal of heavy metal ions by using calcium carbonate extracted from starfish treated by protease and amylase, Journal of Analytical Science and Technology. 2011 July; 2(2):75–82. https://doi.org/10.5355/JAST.2011.75.
30. Bisht R, Agarwal M, Singh K. Heavy metal removal from wastewater using various adsorbents: A review, Journal of Water Reuse and Desalination. 2017 Oct; 7(4):387–19. https://doi.org/10.2166/wrd.2016.104.
31. Kumar KS, Dahms HU, Won EJ, Lee JS, Shin KH. Microalgae-A promising tool for heavy metal remediation, Ecotoxicology and Environmental Safety. 2015 March; 113:329–52. https://doi.org/10.1016/j.ecoenv.2014.12.019. PMid: 25528489.
32. Khan MA, Rao RAK, Ajmal M. Heavy metal pollution and its control through nonconventional adsorbents (1998-2007): A review, International Journal of Applied Science and Technology. 2008 June; 3(2):101–41.
33. Perales-Vela HV, Pe-a-Castro JM, Ca-izares-Villanueva RO. Heavy metal detoxification in eukaryotic microalgae, Chemosphere. 2006 June; 64(1):1–10. https://doi.org/10.1016/j.chemosphere.2005.11.024. PMid: 16405948.
34. Cobbett C, Goldsbrough P. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis, Annual Review of Plant Biology. 2002; 53:159–82. https://doi.org/10.1146/annurev.arplant.53.100301.135154. PMid: 12221971.
35. De-Bashan LE, Bashan Y. Immobilized microalgae for removing pollutants: Review of practical aspects, Bioresource Technology. 2010 Mar; 101(6):1611–27. https://doi.org/10.1016/j.biortech.2009.09.043. PMid: 19931451.
36. Doshi H, Ray A, Kothari IL, Gami B. Spectroscopic and scanning electron microscopy studies of bioaccumulation of pollutants by algae, Current Microbiology. 2006 Aug; 53(2):148–57. https://doi.org/10.1007/s00284-005-0401-7. PMid: 16802205.
37. Xaaldi A, Movafeghi A, Mohammadi-Nassab AD, Abedi E, Bahrami A. Potential of the green alga Chlorella vulgaris for biodegradation of crude oil hydrocarbons, Marine Pollution Bulletin. 2017 Oct; 123(1-2):286–90. https://doi.org/10.1016/j.marpolbul.2017.08.045. PMid: 28844453.
38. Habibzadeh M, Chaibakhsh N, Naeemi AS. Optimized treatment of waste water containing cytotoxic drugs by living and dead biomass of the freshwater microalga, Chlorella vulgaris, Ecological Engineering. 2018 Feb; 111:85–93 https://doi.org/10.1016/j.ecoleng.2017.12.001.