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Evaluation of the potential of *Chlorella vulgaris* for the removal of pollutants from standing water

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ABSTRACT

In this study we evaluated the microalga *Chlorella vulgaris* in order to determine the ability of the microalga to remove the highest concentration of contaminants present in stagnant water. A mixing design of the microalgae with the wastewater was carried out for 20 days, with a 12:12 h photoperiod, in a greenhouse. The highest removal efficiencies for heavy metals were ≥ 98% (mainly Cd, Hg, As and Pb) and ≥ 90% for BOD5, COD, nitrates, phosphates, total phosphorus, faecal and total coliforms. Finally, according to the design analysis, it was determined that the optimal microalgae mixture 1:10 achieves the highest DO production and the highest removal, in a cultivation time of 15 days. In conclusion, microalgae demonstrate their capacity for bioremediation of rural domestic wastewater jagüeyes.

Keywords: Jagüeyes, organic load, pollutant, remediation, microalgae.

1. INTRODUCTION

The jagüeyes are lagoons with apparent similarity to lakes, their existence may correspond to any origin, drainage and dimensions. It also indicates that they remain relatively stagnant and are unstable, with variations in water level; they can be temporary or permanent, depending on the rainfall regime. According to Ballut and Monroy (2015), in the municipality of Sincelejo, department of Sucre, Colombia there are 300 units, the actual representation of the jagüeyes corresponds to 0.43% of the municipal territory, which is equivalent on average to one jagüey for every 95 ha. In terms of area, 89.3% are between 0.1 and 1 ha, 5.7% between 1.1 and 2 ha, 2.7% between 2.1 and 3 ha, 1% between 3.1 and 4 ha, 1.3% more than 4.1 ha. This indicates the dependence of rural communities in the municipality of Sincelejo on this type of water storage model and the importance of its study.

Anthropogenic activities generate wastewater with large amounts of pollutants, which are discharged into natural systems, and without prior treatment can generate eutrophication and disturbance of physicochemical parameters such as pH, decrease in photosynthetic activity and thus decrease in dissolved oxygen and increase in biochemical oxygen demand and chemical oxygen demand, among others (El-Kassas and Mohamed, 2014).

Phycoremediation technology has great advantages over conventional methods implemented in wastewater treatment, because it has lower implementation costs 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 use of macro- and microalgae for bioremediation purposes avoids the use of chemicals and the whole effluent treatment process is simplified, reducing sludge formation, making it a very economical and environmentally safe technology (Sivasubramanian et al., 2010). Furthermore, phycoremediation can be implemented for the simultaneous correction of various physicochemical parameters that are out of balance due to pollution such as: pH correction, oxidation of organic matter by enzymatic activities (laccase enzyme) (Vacca et al., 2017), decrease in chemical oxygen demand (COD) by oxygenation of water during photosynthesis (Vacca et al., 2017; Abinandan and Shanthakumar, 2015; Wang et al., 2010), removal of: CO_2, nitrogen, phosphorus (Hoang et al., 2018), hydrocarbons (Vitola et al., 2018; El-Sheekh et al., 2013) and heavy metals, which generate toxic by-products (Benítez et al., 2018; Hernández et al., 2018; Brar et al., 2017; Olguín and Sánchez-Galván, 2012).The use of algae implemented in bioremediation processes avoids the use of chemicals in effluent treatment processes that are with a certain load of pollutants, presenting a considerable reduction in the formation of sludge, consequently the implementation of phycoremediation as a biological alternative framed within bioremediation techniques becomes a very economical and safe emerging technology for the environment (Sivasubramanian et al., 2010). Therefore, the implementation of microalgae, in addition to serving for the remediation of aquatic environments, also has the capacity to generate oxygen, in a very convenient way, since it favors aerobic processes in the symbiotic interaction of bacteria that in turn mineralize the organic matter present in wastewater, eliminating the cost of mechanical aeration.

Based on the above, the efficiency of the microalgae Chlorella vulgaris in the removal of organic, inorganic and biological pollutants present in stored water from jagüey was evaluated.

2. MATERIALS AND METHODS

- **Initial microalgae culture.** The microalgae evaluated was Chlorella vulgaris, provided in a volume of 250 mL by the Germplasm Bank of the microbiological research laboratory of the University of Sucre, with cell densities of 5.20 ± 0.14 106 cells/mL). The culture was continued at 500 mL, 1 L and 5 L, at 5-day intervals in the laboratory. Nutrifoliar culture medium was used in 2.5 L of medium at 4 mM concentration, following label recommendations. 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.

- **Growth curve of *Chlorella vulgaris*.** The growth curve of Chlorella sp. was determined by growing the microalgae in culture medium, aliquots of the microalgae culture were taken daily for 21 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).

- **Wastewater sample.** Water samples were taken from staked waters of jagüey in a rural village located in the municipality of Sincelejo, Colombia.

- **Bioremediation tests.** The tests are carried out in vitro in a glass raceway with the following dimensions: 1 m long by 30 cm wide and 20 cm high. The treatments to be used: control: BBM medium (Bold's Basal Medium) and jagüey wastewater; T1: jagüey wastewater plus 50 cc of Chlorella vulgaris biomass samples concentrated at 106 cells/mL, the tests will be carried out in triplicate.

• **Monitoring of physical-chemical and microbiological parameters.** The parameters that will be measured during the experiment correspond to: Temperature, salinity, dissolved oxygen, pH, Turbidity, total solids, BOD, NO3, phosphorus, faecal and total coliforms, COD and heavy metals (mercury, arsenic, cadmium and lead).

Statistical analysis: The results will be expressed as the mean ± Standard Deviation, an analysis of variance will be carried out, using a completely randomized design, with a 2x3 factorial arrangement; previously determining the normality criterion by means of the Shapiro Wilk test (5%). Significant statistical differences will be determined by Tukey's test ($p < 0.05$). All experiments were performed in triplicate and analyzed in the free version of InfoStat software.

3. RESULTS AND DISCUSIÓN

En la figura 1 se observa el ensayo de biorremediacion en raceway utilizando la especie de microalga *Chlorella vulgaris.*

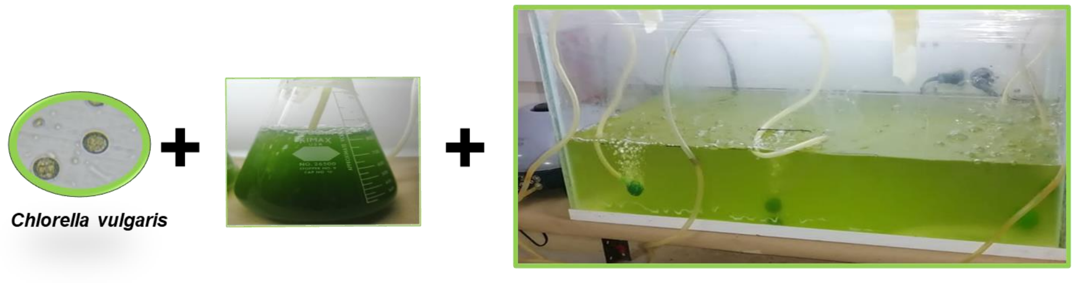

Figure 1. Stages of the bioremediation process of jagüey wastewater using the microalgae *Chlorella vulgaris*.

In figure 2, the growth curve shown by *Chlorella vulgaris* during the removal process is detailed. In the figure, it is observed that C. vulgaris grew up to 17 days and after this time it entered the stationary phase. This shows that the efficiency of this microalgae species for the remediation process would be around 10 to 15 days.

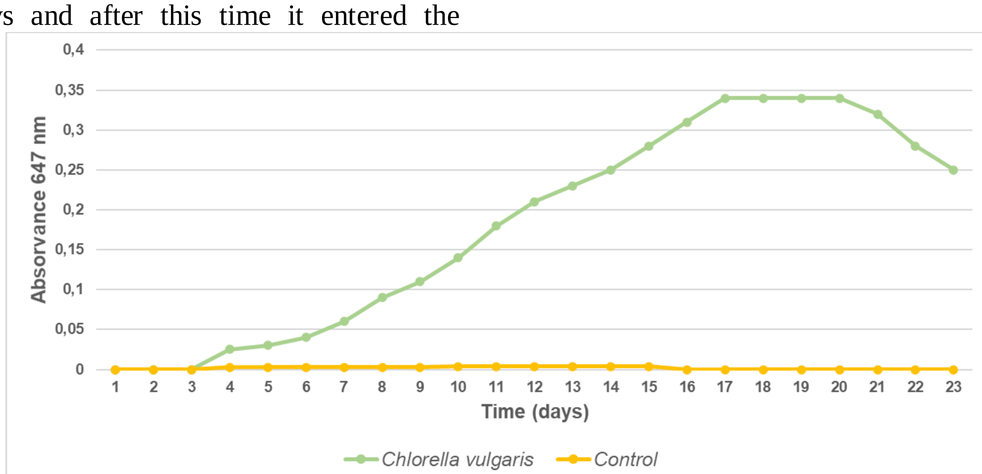

Figure 2. Growth curve of *Chlorella vulgaris* during the process of remediation of stagnant wastewater in jagüey.

The presence of organic and inorganic nutrients exerts a direct effect on microalgae growth. The stagnant wastewater from jagüey showed the presence of nitrates, orthophosphates and total phosphorus (Table 1), both usable by the microalgae consortia during the time of the trial, reducing their concentration (percentage of removal) 15 days after the experiment to ≤ 90 ± ≥ 98.

Table 1. Results of pollutant removal process in jaguey using the microalgae species *Chlorella vulgaris*.

Pollution indicators	***Pre-treatment***	***Control***	***Control***	***Chlorella vulgaris***	***Chlorella vulgaris***
	0 day	**15 days**	**% removal ([Pi-Pf/Pi]* 100)***	**15 days**	**% removal ([Pi-Pf/Pi]* 100)***
pH	**4,4**	**3,2**	decreases	**7,9**	increases
Dissolved Oxygen (In situ)	1,5	1,5	constant	5,58	increases
Turbidity	26,6	26,6	constant	140	decreases
Salinity	0,427	0,427	constant	0,189	decreases
Total Solids	1221	1221	≤ 5,0	81	≥ 90,0
BOD_5	18520	18520	≤ 5,0	2105	≥ 90,0
COD	85,6	85,6	≤ 5,0	52	≥ 90,0
Nitrates	11,5	11,5	≤ 5,0	0,7	≥ 90,0
Orthophosphate	5,47	5,47	≤ 5,0	0,952	≥ 90,0
Phenols	2,3	2,3	≤ 5,0	0,09	≥ 90,0
Total Phosphorus	6,28	6,28	≤ 5,0	1,02	≥ 90,0
Faecal Coliforms	15 000	15 000	≤ 5,0	1	≥ 88,0
Total Coliforms	590 000	590 000	≤ 5,0	21 000	≥ 95,0
Arsenic	1,26	1,26	≤ 5,0	≤ 0,001	≥ 98,0
Cadmium	2,05	2,05	≤ 5,0	≤ 0,001	≥ 98,0
Mercury	3,15	3,15	≤ 5,0	≤ 0,001	≥ 98,0
Lead	1,17	1,17	≤ 5,0	≤ 0,001	≥ 98,0

As can be seen in the table above, when comparing the control with the bioremediation test, it is inferred that this species of microalgae is efficient up to 15 days in removing heavy metals (Cd, As, Hg and Pb) in a percentage of ≥ 98.0 % and other organic and inorganic compounds up to ≥ 90.0 %, similarly the decrease in the presence of coliform bacteria, possibly this last situation is due to the modification of the pH from acidic to basic during the experiment.

According to the results obtained, a pH change from 4.4 to 7.8 was observed in the treatment with *Chlorella vulgaris*, possibly as the microalgae took up nutrients, the pH of the medium increased, the alkaline values indicate the increased photosynthetic activity. It is noted that during the growth of microalgae in high-load lagoon systems, as natural purification systems (a low-cost alternative to conventional systems).

On the other hand, the results obtained by Vitola et al., 2021, indicate significant differences in the biosorption of heavy metals. *Chlorella vulgaris* had the highest biosorption with 94.77 ± 1.63 % for Cd, 92.45 ± 3.95 % for Pb and 81.78 ± 1.36 % for Hg; while Scenedesmus obliquus removed 90.08 ± 2.69 % for Cd, 86.17 ± 1.78 % for Pb and 80.2 ± 5.49 % for Hg. And with respect to 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; while Scenedesmus obliquus 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.

According to studies using microalgae to remove toxic compounds present in wastewater, Chlorella sp., Desmodesmus sp. and Scenedesmus sp. were found to be the most widely used species for wastewater treatment, due to their high capacity to tolerate extreme environmental stress and their potential for biomass and lipid accumulation (Zeng et al., 2015). Dissolved oxygen (DO), on the other hand, is an indicator of the photosynthetic activity of microalgae and the values recorded are higher than those found in equilibrium with air (Su et al. 2012).

4. CONCLUSION

The use of *Chlorella vulgaris* as a biological technique to remediate wastewater used in this research allowed a removal rate of 98% for heavy metals (Cd, As, Hg, Pb) and 90% for inorganic compounds and coliform bacteria, indicating that this species of microalgae has a greater affinity for these heavy metals, which makes it a candidate for implementation as a biological technique for bioremediation of wastewater in the environment.

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