

Use Of Chlorophyll Microorganisms To Improve Physico-Chemical Properties Of Wastewater

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ABSTRACT

In this study, bioremediation with the alga *Chlorella vulgaris* was used for wastewater treatment. The microalgae *Ch. vulgaris* was obtained from the genomic bank of the Agricultural Bioprospecting research laboratory. The tests were carried out in triplicate and control phyco-reactors. Dissolved oxygen increased after 5 days, turbidity had a maximum increase after 10 days and then decreased after 20 days, total solids showed a decrease after 5 days and finally the salinity parameter decreased after 5 days. *C. vulgaris* presented an efficiency in the metabolization of organic compounds, as well as the absorption of these compounds and improved physico-chemical parameters in wastewater for its possible agricultural and livestock use.

Keywords. Wastewater, chlorophyll microorganism, remediation.

I. INTRODUCTION

Wastewater treatment is a priority need worldwide, as it is important to have sufficient and good quality water, which improves the environment, health and quality of life. The use of treated water in agriculture is an increasingly used option in water-scarce regions and growing urban populations with increased demand for irrigation water. Wastewater treatment consists of four stages: pre-treatment, primary treatment, secondary treatment, and tertiary treatment. The discharge of urban wastewater into water bodies introduces high levels of nitrogen and phosphorus leading to eutrophication. Nutrient removal is an important aspect of wastewater treatment as eutrophication is the main cause

of oxygen depletion in aquatic environments, increasing unwanted vegetation, loss of aquatic flora and fauna (Smith and Schindler, 2009).

The use of microalgae in wastewater treatment is supported by studies on the possibility of mass cultivation of microalgae to treat industrial effluents (Gonçalve et al., 2017). The microalgae species *Chlorella vulgaris* has been widely investigated for its high capacity to remove pollutants from wastewater, as well as for the high lipid content of its organic matter (Mata et al., 2010, Maguire-Boyle and Barron, 2014, Elliott et al., 2017). Compared to current conventional processes, microalgae cultivation generates biomass that can be converted into feedstock for the production of

a wide variety of biofuels (Benemann, 2009). The versatility of microalgae means that they are used in different branches of science for different applications.

Microalgae are photosynthetic microorganisms that play key roles in aquatic ecosystems (Moreno-Garrido, 2008) and can be cultivated in open ponds or closed photobioreactors exposed to solar radiation or artificial lighting, whose algal cells are suspended in culture media either by mechanical agitation or by air injection (Kandilian et al., 2017). Microalgae are used as a biological resource in bioremediation techniques, called phycoremediation, to reduce environmental pollutants in wastewater treatment (Mishra et al., 2018), due to their metabolic capacity to remove nutrients, pollutants and heavy metals (Pacheco et al., 2015) as well as to produce secondary metabolites that inhibit the growth of pathogens (Molina-Cardenas et al., 2014).

The microalgae genera most commonly found in untreated wastewater, both industrial and municipal, and are known to be efficient in phycoremediation systems are: *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Micractinium*, *Euglena*, *Ankistrodesmus*, *Oscillatoria*, *Microcystis*, *Nitzschia*, *Navicula* and *Stigeoclonium*, which can work together with cyanobacteria of the genera *Anabaena*, *Limnospira*, *Nostoc*, *Spirulina* and *Aphanizomenon*, bacteria of the genera *Bacillus* and *Pseudomonas*, fungi of the genera *Mucor*, *Aspergillus*, *Fusarium* and *Nigrospora*, and macroalgae of the genera *Lemna*, *Eichhornia*, *Elodea*, *Phragmites*, *Typha*, *Ulva* and *Kappaphycus*, whose natural association increases the removal efficiency of environmental pollutants (Brar et al., 2017).

The species *Chlorella vulgaris* is a spherical unicellular green, microalga without a flagellum, of 2-9 μm in diameter (Hoang et al.,

2018) with the ability to remove significant amounts of nutrients (Nguyen et al., 2016) and can grow under mixotrophic conditions, simultaneously using light and organic compounds as an energy source or assimilating CO_2 , glucose or simple acids as a carbon source (Li et al., 2011). Biosequestration of carbon dioxide from the air is an important remediation factor, as it significantly reduces the levels of this environmental pollutant (Wiesberg et al., 2017). Glucose and serine promote mixotrophic growth with high yields, higher than those obtained under heterotrophic and autotrophic conditions (Li et al., 2011).

Based on the benefits of the microalga *Chlorella vulgaris*, it was proposed as a study to evaluate in vitro the efficiency of this microalga in improving the physical-chemical conditions of dissolved oxygen, turbidity and salinity parameters in wastewater.

2. MATERIALS AND METHODS

2.1 Microalgal biomass. The microalgae used were *Chlorella vulgaris* (CVLINM 99%) which are part of the germplasm bank collection of the Microbiological Research Laboratory of the University of Sucre. Bioaugmentation was carried out with *Ch. vulgaris* cells in logarithmic phase with an initial concentration of 10^6 cells/mL in nutritive culture media

(Colinagro 4.0) containing 200 g/L of total nitrogen, 100 g/L of phosphorus and nutrients such as K, Mg, S, Cl, Fe, Cu, Zn, Mn, B and Mo for 24 h (Hernández et al., 2018).

2.2 Wastewater remediation bioassay. The test was carried out with the waste effluent, 5 flasks of 1 L were placed with the sample, 10 mL of the algae inoculum were added at a concentration of 1.0×10^6 cells/mL and 1 g of sodium bicarbonate for 20 days, from the

water and the biomass obtained in this bioassay, the experimentation and the analysis of the results were carried out in the following pre-treatment stages, 0, 5, 10, 15 and 20 days after the start of the experiment (Vitola et al., 2022). The parameters assessed were: dissolved oxygen, total solids, salinity and turbidity.

2.3 Statistical analysis. The results were expressed as the mean \pm Standard Deviation, an analysis of variance was carried out using a completely randomized design with a 2x3 factorial arrangement. The first factor was the microalgae species *Chlorella vulgaris* and the second, the concentrations of the parameters dissolved oxygen, total solids, salinity and turbidity at 0, 5, 10, 15 and 20 days, previously

determining the normality criterion by means of the Shapiro Wilk test (5%). Significant statistical differences were determined by Tukey's test ($p < 0.05$). All experiments were performed in quintuplicate and analyzed in the free version of InfoStat software.

3. RESULTS AND DISCUSSION

Dissolved oxygen increased after 5 days (figure 1), turbidity had a maximum increase after 10 days and then decreased after 20 days (figure 2), total solids showed a decrease after 5 days (figure 3) and finally the salinity parameter decreased after 5 days as the microalgae species *Ch. vulgaris* interacted with the wastewater (figure 4).

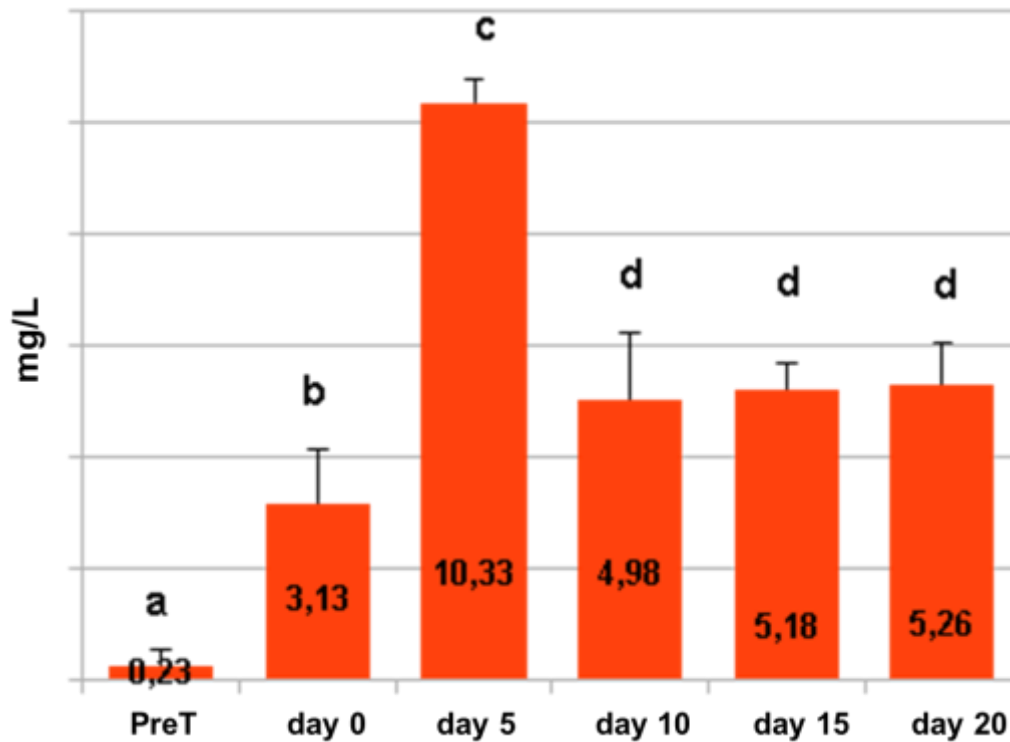


Figure 1. Evaluation of the behaviour of the Dissolved Oxygen parameter in wastewater using the microalgae *Chlorella vulgaris*.

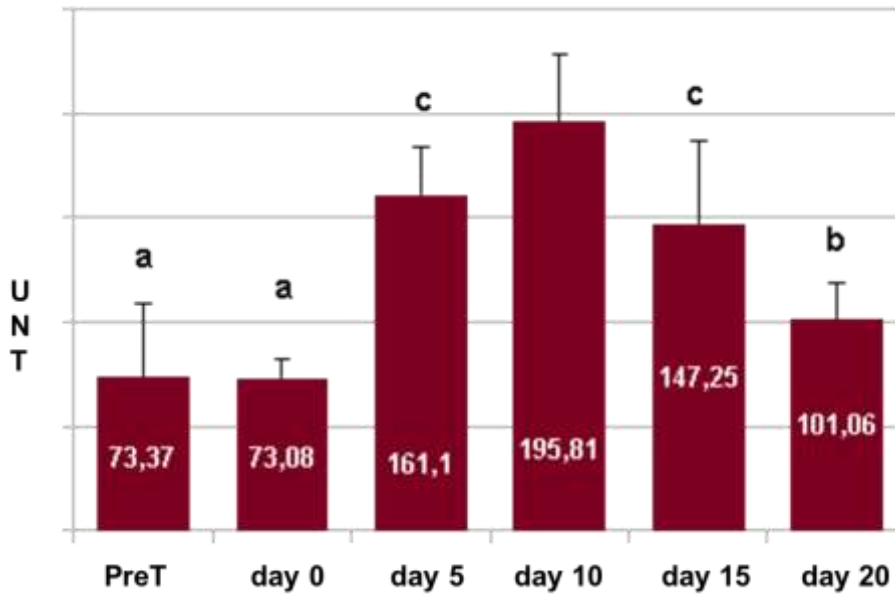


Figure 2. Evaluation of the behaviour of the Turbidity parameter in wastewater using the microalgae *Chlorocella vulgaris*.

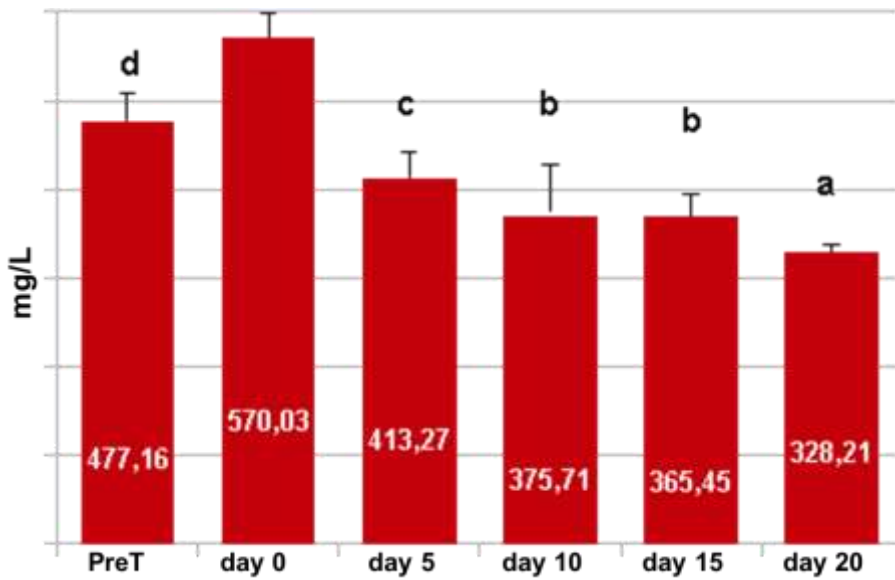


Figure 3. Evaluation of the behaviour of the TDS parameter in wastewater using the microalgae *Chlorocella vulgaris*.

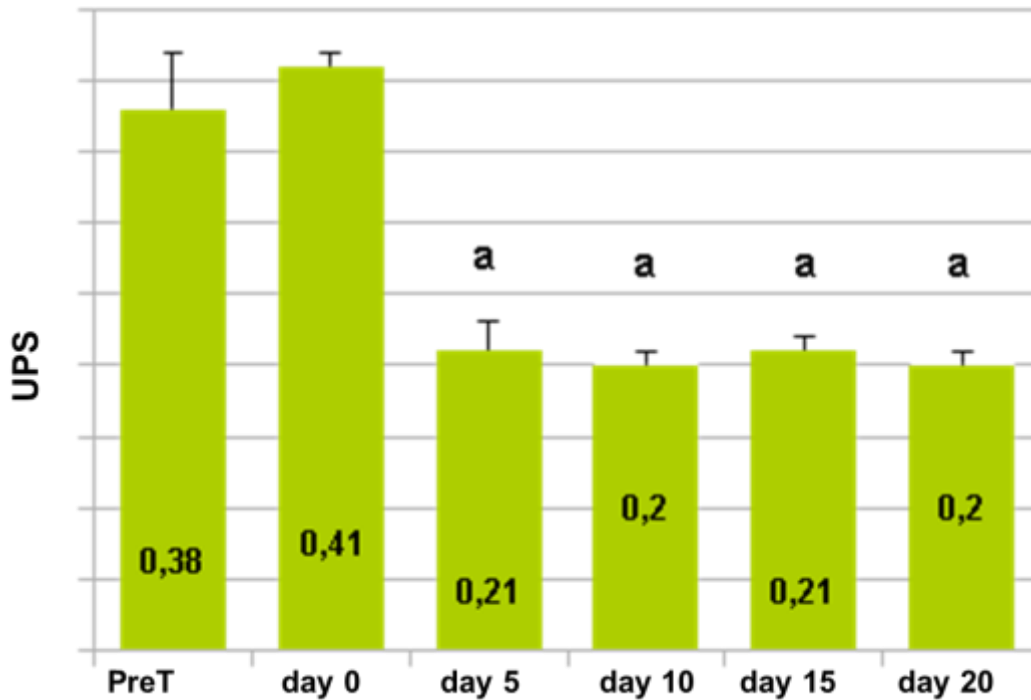


Figure 4. Evaluation of the behaviour of the salinity parameter in wastewater using the microalgae *Chlorella vulgaris*.

Chlorophyll microalgae species *Chlorella vulgaris* acclimatizes to wastewater (López et al., 2019.). *Ch. vulgaris* is economical when incorporated with wastewater treatment owing to its ability to simultaneously accomplish CO₂ bio-fixation, lipid generation, and nutrient or pollutant removal as an alternative nutrient source for microalgae cultivation of *Chlorella vulgaris* (Patel et al., 2020, Moedet et al., 2015).

With regard to the results obtained for turbidity and total solids, significant differences are observed for each of these parameters. Turbidity is an optical property that causes light to be scattered and absorbed, instead of being transmitted. The scattering of light through a liquid is mainly caused by suspended solids. The higher the turbidity, the higher the scattered light, the lower the turbidity in the treatment stage and the higher the turbidity in pre-treatment and 0 days. The results are closely related to the increase of microalgal biomass (among the most important causes of turbidity are: presence of

phytoplankton and algal growths). The presence of total solids was found to behave similarly to the turbidity found in this experiment. The presence of salinity in wastewater can affect the design of wastewater treatment plants significantly, because it can create an inhibitory effect on freshwater biocenosis and affect the settling properties of solids.

The dissolved oxygen content of water is used in the determination of biochemical oxygen demand (BOD). Generally, a higher level of dissolved oxygen indicates better water quality, although high oxygen levels are associated with eutrophication phenomena where the presence of algae generates oxygen levels above saturation values.

4. CONCLUSION

The use of chlorophyll microorganisms has been shown to generate a decontamination of wastewater from the livestock, agricultural and industrial industries and oxidation ponds,

through the production of chlorophyll type a and b, a decrease in total suspended solids and a marked decrease in salinity and dissolved oxygen, favoring olfactory decontamination and preserving the aquifer ecosystem. It was demonstrated that the relative cell growth in *Chlorella vulgaris* cultures with wastewater indicates that these media contain high availability of nutrients, and therefore constitute alternative substrates for its development and use as a bioremediate. *C. vulgaris* presented an efficiency in the metabolization of organic compounds, as well as the absorption of these compounds and improved physico-chemical parameters in wastewater for its possible agricultural and livestock use.

5. AUTHORSHIP CONTRIBUTIONS

All authors have jointly and equally contributed to the argumentation and writing of the manuscript.

6. FUNDING. None.

7. CONFLICT OF INTEREST. None.

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