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In Vitro Evaluation of the Growth Potential of Chlorella Sp. and Desmodesmus Sp for Use as A Biological Phycoremediation Agent in Wastewater

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Abstract

The objective of the present study was to evaluate in vitro the growth behaviour of Chlorella sp. and Desmodesmus sp. in a phyco-reactor in a nutritional source with fertilizer and wastewater, artificial light and sunlight to determine the growth conditions for their use as a biological agent to remediate organic load in wastewater in the department of Sucre, Colombia. Chlorella sp. and Desmodesmus sp. were subjected to two nutrient sources (Nutriofoliar 4 Nm fertilizer and wastewater) and light source (artificial light and sunlight) in a phyco-reactor for 20 hours under agitation at 4000 rpm. The results show differences in the growth behaviour of both microalgae according to the nutritional source and the type of light. In the presence of Nutrifoliar the microalgae have a difference in the growth behaviour being higher for Chlorella sp with an exponential phase up to 19 days and a duration of 17 days with respect to Desmodesmus sp, which was 12 days. With respect to the nutritional source wastewater, the same growth behaviour was observed, with the presence of less growth compared to when they were grown in Nutriofoliar, likewise, in both microalgae the exponential phase was observed to be shorter in days when they were grown in wastewater. There is a need to study the growth behaviour of those microalgae species as an alternative to remediate wastewater in the field.

Keywords: *Microalgae, nutritional source, light type, growth.*

Introduction

According to Cezare-Gomes, (2019), microalgae are microorganisms with a wide range of applications, and this is mainly due to their photosynthetic capacity, in which they convert carbon dioxide (CO_2) into bioactive compounds such as pigments, lipids, polyunsaturated fatty acids, carbohydrates, proteins and vitamins. Similarly, as expressed by Torregrosa-Crespo et al. (2018), the metabolites produced by microalgae have received considerable attention, due to their biotechnological applications and their potential beneficial uses in human health, food processing, pharmaceuticals and cosmetics, presenting a high demand in the market.

The above are Su, (2021); Kurniawan et al., (2022); Li et al., (2022); Morillas-Espana et al., (2022), claim that microalgae are the most efficient photosynthetic microorganisms in nature, and their richness in lipids and wide range of active ingredients have important applications in the development of biofuels and bio products. Also, Leong and Chang, (2020); Priya et al., (2022), state that, que the commercialization of microalgae biomass and microalgae fuels, however, is still in its infancy due to high production costs and low productivity. Recent research has revealed that certain microalgae can synthesize complex organic matter using nutrients such as nitrogen and phosphorus and grow metabolically rapidly in wastewater, they are able to remove contaminants from wastewater, such as Zn, Hg, Cd, Cu, U, and Pb, and reduce the nitrogen and phosphorus content of water bodies.

According to Park et al. (2011); Abdel-Raouf et al. (2012), the choice of species to cultivate depends directly on the intended purpose of the resulting biomass (e.g., pigments, food) and/or whether the cultivation is for phycoremediation. The predominant algal species within an open system depend on environmental,

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operational and biological parameters. Light intensity is one of the main parameters to consider in a culture (Contreras-Flores et al., 2003). In the absence of nutrient limitation, photosynthesis increases with increasing light intensity, until the maximum species-specific growth rate is reached at the point of light saturation (Park et al. 2011). Nitrogen is the most important nutrient for microalgae (after carbon) and is incorporated as nitrate (NO_3^-) or ammonium (NH_4^+) (Grobbelaar 2004, Martinez 2008, Abdel-Raouf et al. 2012). It is also a critical factor in regulating the lipid content of microalgae (Park et al. 2011).

For all of the above reasons, the strategy proposed was to evaluate the growth behaviour of *Chlorella* sp. and *Desmodesmus* sp. in a phycoreactor in a nutritional source with fertilizer and wastewater, artificial light and sunlight to determine the growth conditions for their use as a biological agent to remediate organic load in wastewater in the department of Sucre, Colombia.

Materials and Methods

Photosynthetic organism studied. *Chlorella* sp. and *Desmodesmus* sp. were isolated from water bodies of marshes in the department of Sucre. The isolated strains are maintained in a Petri dish in BOLD solid culture medium (according to the protocol proposed by Anderson et al., 2005) under a photoperiod of 12:12 (light: dark) and 27ºC. Once every 25 days, the strains are re-inoculated in Petri dishes with fresh medium and become part of the genomic bank of the Microbiological Research Laboratory of the University of Sucre-Colombia.

Bioaugmentation of microalgae biomass. *Chlorella* sp (*Ch. Vulgaris* - CVLIM 99%) and *Desmodesmus* sp (DsLIM 98 %) were obtained from the germplasm bank collection of the Microbiological Research Laboratory attached to the Agricultural Bioprospecting research group of the University of Sucre. The cells were bioaugmented in a phyco-reactor in Nutrifoliar culture medium (Colinagro 4.0), which contains 200 g/L of total nitrogen, 100 g/L of phosphorus and nutrients such as K, Mg, S, Cl, Fe, Cu, Zn, Mn and Mo (Vitola et al., 2022); this medium was diluted at 2 % v/v in distilled water and sterilized in an autoclave.

Growth curves. Growth of *Chlorella* sp (Ch. Vulgaris - CVLINM 99%) and *Scenedesmus* sp. was carried out in 1-litre phyco-reactors with a working volume of 500 ml of 2% v/v culture medium and sterilized distilled water, a concentration of 1·10^6 cells of initial inoculum, enriched with the different organic nitrogen sources. Microalgal biomass growth was measured every 24 hours for 20 days using a Spectroquant pharo 300 spectrophotometer at a wavelength of 647 nm and compared with the control microalgae assay with the culture medium (Vitola et al., 2022).

Result and Discussion

Figure 1 describes the morphological characteristics of the morpho-genera *Desmodesmus* sp. and *Chlorella* sp. observed under light microscopy at 40X.

***Morphogeny: Desmodesmus sp.* (Chodat) S.S. An, T.Friedl & E.Hegewald, 1999** 1.	**Morphology.** Single cells or colonies of 2 to 16 cells may form linear or lateral or alternate structures; cells are usually ellipsoidal to ovoid with spines either at terminal cell ends or in central cells parietal chloroplast with presence or absence of pyrenoid.	2. 

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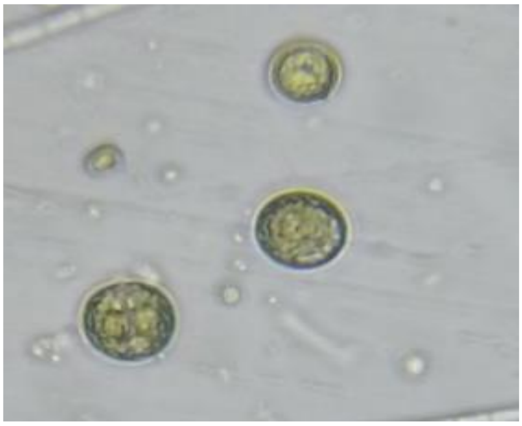
	Distribution. Genus with a cosmopolitan distribution, very abundant in freshwater bodies. (Shubert, E. *et al.*,2015).	3. **Image taken under an optical microscope with a 40x objective.**
Morphogeny: Chlorella sp.**Beyerinck [Beijerinck], 1890, nom. cons.**	**Morphology.** Individual cells spherical, ovoid or ellipsoid in shape, may have mucilage layer, contain parietal chloroplast with pyrenoid surrounded by starch granules. **Distribution.** They can be found in freshwater bodies, in soil or attached to substrates, in symbiosis. (Shubert, E. et al.,2015).	 4. 5. **Image taken under an optical microscope with a 40x objective.**

Figure 1. Morphological characteristics of *Chlorella* sp. and *Desmodesmus* sp.

Figure 2 shows the scheme of the research carried out to evaluate the growth behaviour of *Chlorella* sp. *Desmodesmus* sp. in a phyco-reactor

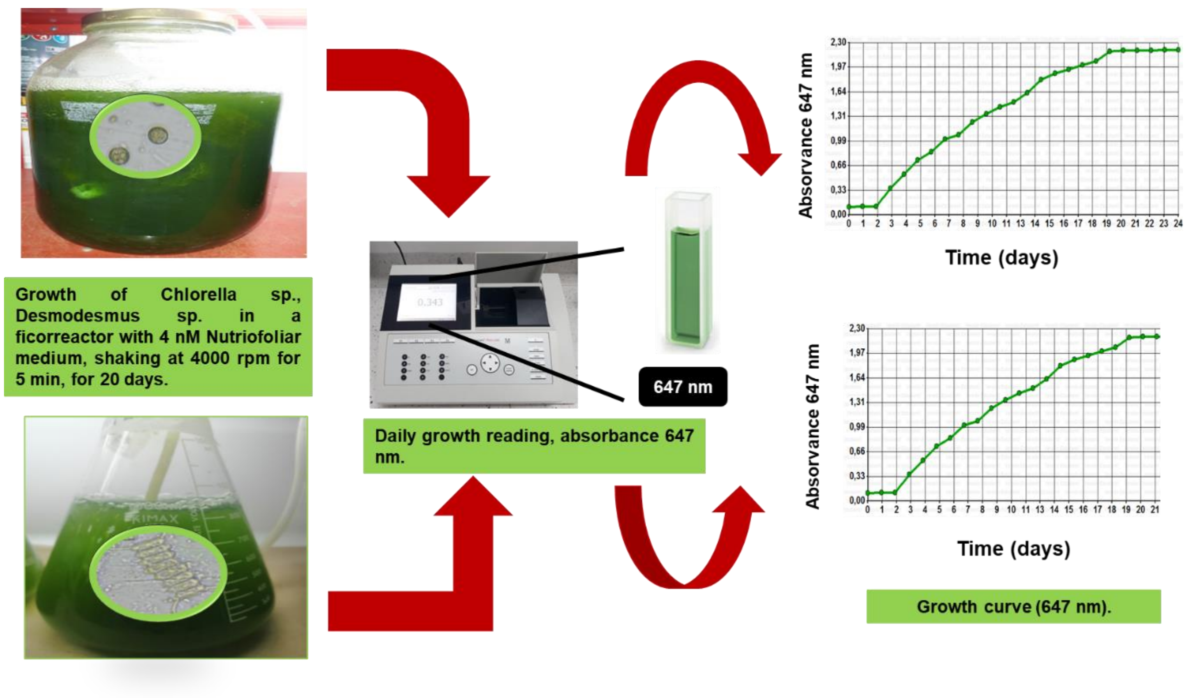


Figure 2. Growth curve of *Chlorella* sp. *Desmodesmus* sp. in a phyco-reactor.

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Figure 3 shows the growth behaviour of *Chlorella* sp. *Desmodesmus* sp. in a 4 nM Nutriofoliar culture medium in a phyco-reactor. The results show that the microalgae *Chlorella* sp. began to grow from the second day of the experiment until 20 days after the start of the experiment, when it reached its maximum growth and began the stationary phase, and then at 23 days after the start of the experiment, it began to decrease in growth. The logarithmic phase lasted from day 2 to day 19, which indicates that it lasts approximately 17 days in the exponential or logarithmic stage.

Comparing the growth behaviour with that of *Desmodesmus* sp., it is observed that this microalga starts the exponential phase from day 6 of the experiment until day 19, where it obtained the highest growth. According to the results, *Desmodesmus* sp. has an exponential phase of 11 days less than *Chlorella* sp., however, the latter microalgae presented higher growth values than *Desmodesmus* sp. under the same nutritional conditions.

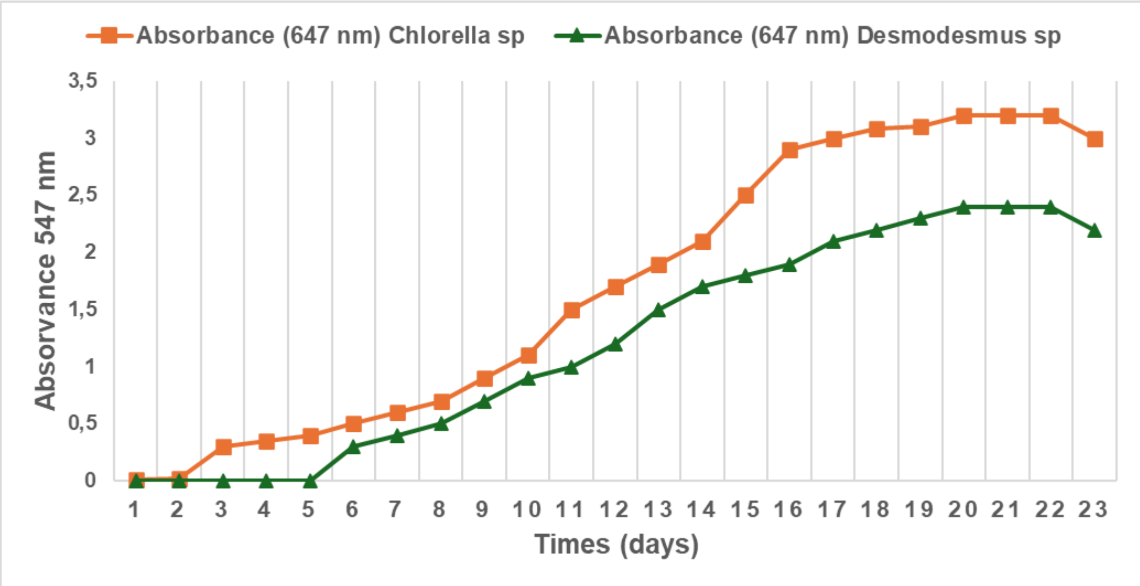

Figure 3. Growth curve of *Chlorella* sp. *Desmodesmus* sp. in 4 nM nutriofoliar culture medium in a phyco-reactor.

So, through a multifactorial experimental design, this study aimed to evaluate the effect of adding triofoliar 4Nm medium (Colinagro 4,0 nM, containing 200 g/L total nitrogen, 100 g/L phosphorous and nutrients such as K, Mg, S, Cl, Fe, Cu, Zn, Mn, B and Mo for 24 h, proposed by Hernández et al., 2018) to the cultivation of two microalgae: *Chlorella vulgaris* and *Desmodesmus* sp.

Figure 4 shows the growth of both microalgae (*Chlorella* sp. and *Desmodesmus* sp.) in the presence of sunlight and darkness (at night) supplemented with wastewater. The figure shows the difference in the growth behaviour of *Chlorella* sp. *Desmodesmus* sp in wastewater using sunlight and darkness during the night. It is observed that both microalgae had a very close and uniform growth behaviour and significant differences were found between both growth behaviours. However, as can be seen when the two microalgae grow in wastewater without the addition of a nutrient source and sunlight, there is a similarity in growth, with a lower exponential phase and entry into the stationary phase from day 17 of the experiment.

According to Su, (2021), the rationale behind carbon, nitrogen, and phosphorous metabolisms in microalgae, which are linked and affect each other, could explain growth using wastewater, as well as N and P concentrations, can be intolerant for many microalgae, so it is imperative to search for sustainable treatments before their cultivation. In addition to effluent characterization, water recycles can be explored between cultivations. Finally, supplementation with specific nutrients might be a solution to enhance performance.

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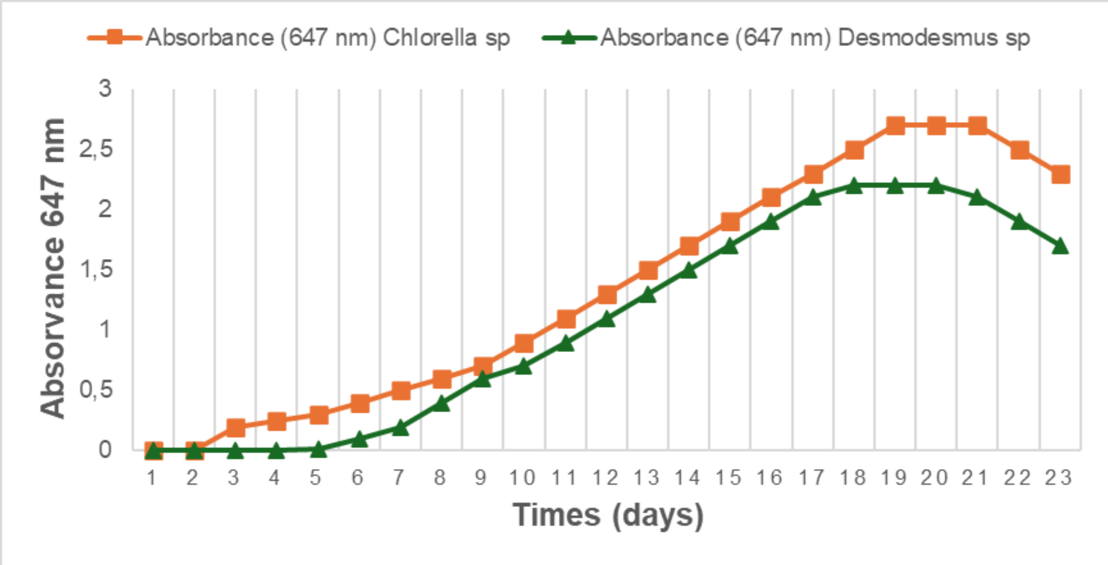

Figure 4. Growth curve of *Chlorella* sp. and *Desmodesmus* sp., inoculated in wastewater using sunlight and photoperiod of darkness in phyco-reactor.

The growth rate increases by irradiance until reaching the threshold of photosynthetic work. Lipids increase as more TAGs are formed; carbohydrates and proteins also increase due to efficient photosynthesis. Thus, microalgal biomass production, in general, is improved by increasing light intensity up to the limit of saturation (stress). Under stress conditions, lipids and carbohydrates are favored (Khan et al., 2018; Morales et al., 2018; Markou et al., 2012).

As stated by Richmond and Hu, (2013), as beforementioned, microalgae can consume carbon from autotrophic (CO_2) or mixotrophic (sugars, acids, alcohols) growth Nitrogen is the second most important nutrient, and its limitation causes a decrease in chlorophylls, an increase in carotenoids, and accumulation of organic carbon both in polysaccharides and oils. Algae can utilize various organic and inorganic N compounds, for example, nitrate, ammonia, or urea. Phosphorus is essential for cell metabolism, including energy transfer and biosynthesis of nucleic acids, DNA, etc., and it is preferably supplied as PO_4^{2-}.

Chlorella sp. distribution and density is correlated with pollutant load, flow rate, temperature, pH and availability of oxygen and light. An important feature of microalgae systems is their versatility, which allows linking different applications within the same process. Microalgae can remove nitrogen and phosphorus from wastewater efficiently and therefore have the potential to play an important remediation role during tertiary wastewater treatment (De La Noue and De Pauw, 1988).

Another important characteristic is their photosynthetic ability to convert solar energy into valuable biomass with an interesting biochemical composition. As such, microalgae could play an important role in solar biotechnology (Guidelines and Bank, 2013). The genus Chlorella is very tolerant and can successfully grow in wastewater as a culture medium (Feng et al., 2011).

A study with C. vulgaris showed uptake of 45-97% nitrogen, 28-96% phosphorus and reduction of chemical oxygen demand by 61-86% from different types of wastewater such as textile, sewage, municipal, agricultural and recalcitrant wastewater (González et al., 1997). It is a pathway for the removal of vital nutrients (nitrogen and phosphorus), carbon dioxide and heavy metals present in wastewater and necessary for their growth. In addition, savings and requirements for chemical remediation and possible minimization of freshwater use for biomass production (Safi et al., 2014). Furthermore, *Chlorella vulgaris* is considered as one of the best microalgae for wastewater bioremediation due to its versatility and low proliferation, thus, its application in water treatment is advantageous, as it is a natural absorption process that avoids the intervention of chemical pollutants.

Desmodesmus is a genus of green algae in the family Scenedesmaceae (see NCBI webpage on Desmodesmus, 2007). It is the only chlorophyll-containing organism known to have caused human infections in immunocompetent individuals. All known cases involved open wounds in freshwater. Desmodesmus is found in the plankton of habitats such as ponds and lakes, especially in eutrophic waters (Hegewald et al., 2017). It is one of the most common types of freshwater plankton (Shubert et al., 2014). It can also be found in soils and biological soil crusts (Johnson et al., 2007).

Different studies have reported an increase in the capacity to assimilate nutrients and in its growth, which has led to the microalgae *Chlorella vulgaris* being used in the development of bioremediation processes, due to its ease of adaptation and its accelerated growth in the environment (Brennan et al., 2010). Desmodesmus is a genus of microalgae of the class Chlorophyceae, order Sphaeropleales, family Scenedesmaceae, subfamily Desmodesmoideae. Microalgae of the genus Desmodesmus are found in freshwater bodies, in wastewater from treatment plants and in water contaminated with heavy metals (Perales-Vela et al., 2006).

Conclusion

In this study the growth behaviour of the microalgae *Chlorella* sp. and *Desmodesmu*s sp., fertilizer with artificial light and wastewater with sunlight and dark period was tested. The results infer that in medium with *in vitro* fertilizer the microalgae have a different growth behaviour being higher for *Chlorella* sp. with respect to *Desmodesmus* sp. When the microalgae were subjected to growth in water supplemented with sunlight a similar growth behaviour was observed for both microalgae, but lower with respect to when fertilizer was used as a culture medium. Use processes such as open field remediation of microalgae require the nutrients present in the wastewater and the presence of sunlight.

The ability to survive in such environments has been explained by their phenotypic plasticity as they can grow as independent cells or as clusters of cells. Their ability to respond to stressful environments makes species of this genus good candidates for biotechnological use.

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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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