

Determination of microalgae limiting nutrients in the Green Lagoon “Charco Verde de Los Clicos”, Lanzarote (Canary Islands)

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ABSTRACT

The “Charco Verde de Los Clicos” is a hypersaline green coastal lagoon located at the west coast of Lanzarote Island. It is separated from the sea by a black sand barrier, but it is connected with the sea by underground channels. It is part of a semi-submerged hydro-volcanic building. The surface and volume of the lagoon is 7.700m² and 17.700m³ with a maximum depth of 3.5 m at the middle, being the green color due to high chlorophyll levels. The phytoplankton concentrations found in the lagoon indicates eutrophic characteristics. The aim of this work was to confirming that nitrogen is the main limiting nutrient of the primary production because phosphorus is provided by the volcanic substrate. Water samples from six different points of the lagoon were mixed and incubated as control and three different treatments with N, P and N+P were made with five replicates of each treatment. Biomass growth was measured by optical density and flow cytometry during twenty-two days. Results show that during an 11 days period, nitrogen was the limiting nutrient in “Charco Verde de Los Clicos” because the samples, which had received nitrate, present a biomass increase, contrary to the treatments without addition of nitrate where a biomass decrease was found. When both nitrate and phosphate were added it was observed a higher biomass increase. These results indicate that phosphorus started to be also limiting of primary production when the growth by nitrate concentration was high.

INTRODUCTION

Lanzarote Island is located at 100km off the coast of Africa. Because of its submarine volcanic origin, a hydrovolcanic building denominated “El Golfo” is situated on the west coast of the island. In this area, a beach of black sand is found and associated to it, there is a hypersaline coastal lagoon called “Charco Verde de Los Clicos”. Sand extractions from the southern part of the beach from 1965 to 1996 caused a change in the lagoon volume due to the wave’s entrance. At the present the beach is being restored to recover the 1965 situation [1].

The green lagoon has a 50% of its 1965 volume. It is connected with the sea by undersand filtration and the data of high salinity and nutrients indicates the lagoon show hypersaline conditions and eutrophic conditions. The color of it is due to the presence of high chlorophyll levels, being dominants phytoplankton group chlorophytes, diatoms and dinoflagellates

In aquatic ecosystem the deficiency of nitrogen and phosphorus alter the photosynthesis process. The lack of nitrogen decreases the structural proteins of photosystems and alters the structure of molecules chlorophyll *a*, *b* and *c*. Phosphorus plays an important role in phospholipids and is required in molecules of ATP and NADPH to take CO₂ and elaborate organic matter. In most coastal marine

ecosystem, primary production tends to be nitrogen limited due to be found as atmospheric nitrogen. In several hypersaline coastal lagoons the limiting nutrient is phosphorus. The subject of the study was to know the extension of nitrogen and phosphorus as growth limiting factors over the lagoon’s phytoplankton.

MATERIAL & METHODS

Samples were obtained in March 19th 2015. Five liters of water were collected in small tanks at the same depth (50cm) in six points distributed throughout the lagoon. A volume of three liters of each initial sample bottle was taken and deposited in one 20L tank to have a unique and more homogeneous sample. This new sample was well shaken by hand and one aliquot (500-450ml) was introduced in each autoclaved Erlenmeyer. Flasks were manipulated in a laminar flow chamber and close by a cotton plug covered with aluminum foil and pierced by a glass rod. Flasks received continuous air from a blower pump passed first by a water tramp, the glass rods were join to a 0.2 µm filter, which was connected to air/CO₂ hose, producing circulation into the bottles from a blower pump. Two stock solution of 100 nM NaNO₃ (N) and 100 nM KH₂PO₄ (P) were added to get 4 different nutrient treatments using lagoon water (LW) as control (Table 1).

Table 1. Volume used of each treatment.

Treatments	Control	N	K	N+K
LW (mL)	500	450	450	400
N (mL)	0	50	0	50
K (mL)	0	0	50	50

Five replicates of each treatment were prepared. Samples were under different radiation flux. Absorbance of the samples was measured during 22 days by optical density using a Perkin Elmer Lambda 25 spectrophotometer [2].

RESULTS & DISCUSSION

It is observed that the average deviations of the control (C), phosphate (P) and nitrogen (N) treatments are very similar during all the experiment (Fig. 1; A, B, C). In the N+P treatment, the exponential phase of the growth curve starts at day 9 and ends at day 19. Maximal optical density value measured was 0.09, which coincides with the moment when culture begins the stationary phase (Fig. 1E).

In the C, P and N treatments, neither exponential phase nor stationary phase are differentiated, it remains in adaptation phase with a high growth and a maximum value of 0.0106 in the nitrate samples (Fig. 1F).

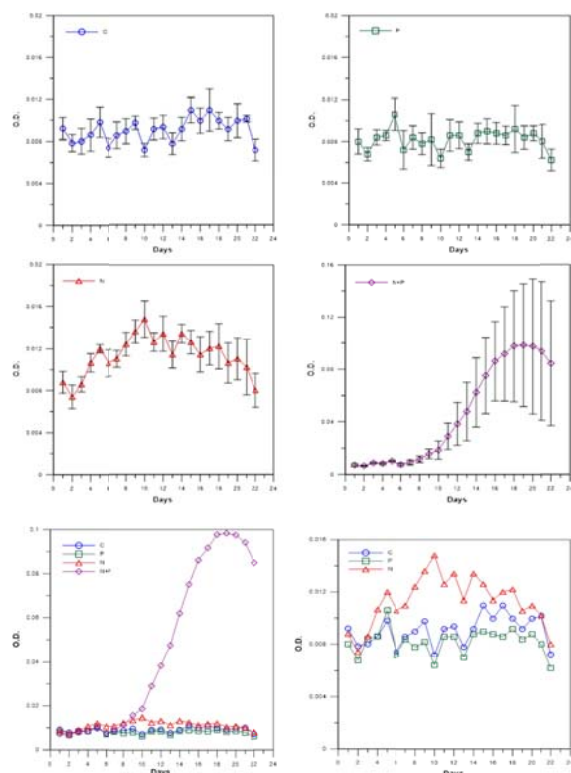


Fig. 1. Growth curves of the different treatments with average deviations realized by optical density.

Otherwise, it gets a distinct behavior in the nitrogen and phosphorus (N+P) treatments, being very low at the beginning and increasing progressively from day 7 (Fig. 1D). The maximum value of average deviation is 0.05 at the day 20 and it starts decreasing after it.

The increase of biomass obtained at optical density and flow cytometry studies in the treatments which had received nitrate addition, with the lack of biomass increment in the non-nitrate treatments, makes evident the role of nitrogen as first limiting factor.

The difference of the growth curves between the N+P treatment and the N treatment is due to phosphate concentration existent in each one. [3] show that when exists an enough nitrate and phosphate concentration, it is produced an increase of the biomass and nitrogen acts like the limiting nutrient, but if the concentration of phosphate is under 0.1 mmol/m^3 , phosphorus is a limiting nutrient with the nitrogen. The C and P treatments have not an increase of biomass probably by the nitrogen low concentration. [1] found a very low concentration of nitrate in the lagoon, similar to obtained in the sea water, and a phosphate concentration three times higher than those found in the sea water near the beach, due to "El Golfo" is rich in phosphorus [3]. Similarly, [4] measured a very low nitrate concentration, under 0.1 mmol/m^3 in January and undetectable in July. Therefore, due to this limiting nutrient concentration is low, it has not produces increases of biomass in these treatments.

We concluded that nitrogen is the first limiting nutrient of the "Charco Verde de Los Clicos" as show the increases of biomass in the lagoon samples incubated with nitrate and the stability or decreased of biomass in the samples without addition of it. When the phosphate concentrations decrease by the phytoplankton absorption, its addition produces an increase of biomass by its role as a secondary limiting nutrient. This must be considered in the restoration plans because add nutrients could be necessary to recovery the biological conditions of the lagoon.

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