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MICROALGAE CULTURE FOR BIOREMEDIATION OF MARINE WASTE-WATER

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RESUMEN

Las aguas residuales marinas contienen materia orgánica e inorgánica que pueden causar daños medioambientales. Se han estudiado diferentes métodos de purificación para remover este tipo de contaminantes de los vertidos marinos. Uno de ellos es la fitoremediación. En este trabajo se presentan los métodos experimentales y los resultados de la determinación de los valores de potasio y materia orgánica, entre otros, durante el período de cultivo de un tipo del microalga marina "Nannochloropsis gaditana", que se encuentra de forma natural en la Ría de Vigo. Durante el período de cultivo, se midieron varios parámetros fisicoquímicos como temperatura del agua, pH, DQO, concentración de fósforo disuelto, así como la densidad de microalgas y el peso (materia seca) de las mismas. En estas condiciones, se demuestra que el cultivo de las microalgas puede reducir el contenido en fósforo del agua residual en un 90% en 10 días de cultivo, mientas que la DQO cae un 70% en el mismo período. Además, este tipo de de microalgas se puede emplear como nutriente para animales o personas o la síntesis de biocombustibles.

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INTRODUCTION

Microalgae are considered an important raw material in a number of promising biotechnological applications, as well as offering advantages in reducing the environmental impact of industrial pollution. Consequently, a growing number of studies have focused on investigating primarily processes such as harvesting, strain selection, and obtaining and converting the final products (biodiesel) [1]. However, monitoring the growth of microalgae in cultures is also a key parameter for algal biomass production, mainly to improve the industrial scale. Good crop monitoring could improve production rates both allowing immediate decisions regarding growing medium, e.g. fertilization, harvesting, light intensity and temperature, and avoiding economic losses during optimization process [2]). One of most suggestive application is the treatment of marine wastewater [3] to purify water as obtain biofuels from the cultures.

Unfortunately, an important problem to benefit these cultures is the moment to harvest the microalgae. Methods to estimate biomass concentration are varied, with advantages and disadvantages, depending on the technique. If a classification using offline and online measurements is made, first are slow and invasive of the culture medium, having to extract a sample and analyze it in the laboratory, which complicates monitoring and delays measurement frequencies [4]. With respect to offline methods, dry weight determination has a wide use, but is not easy for small volume systems also containing few cells [5], and microscopic cell counting, due to excessive time consuming and skills of the operator which performs the count.

This paper describes the use of a mobile phone camera as a tool to evaluate the number of microalgae in the water culture of a bioreactor by image processing of pictures taken at different concentrations during microalgae growth (RGB data decomposition). In short, the objective will be check if RGB image analysis of microalgae cultures can result to be easier and more economical techniques to apply for monitoring the decisive parameters, mainly in large-scale production.

EXPERIMENTAL PROCEDURE

Inoculums of *Nanochlorosis Gaditana* from the Algae Collection of the University of Vigo was cultured in a nutrient and artificial medium (lightning 14h per day) at 21 °C. The composition of the nutrient medium could be seen in [6].

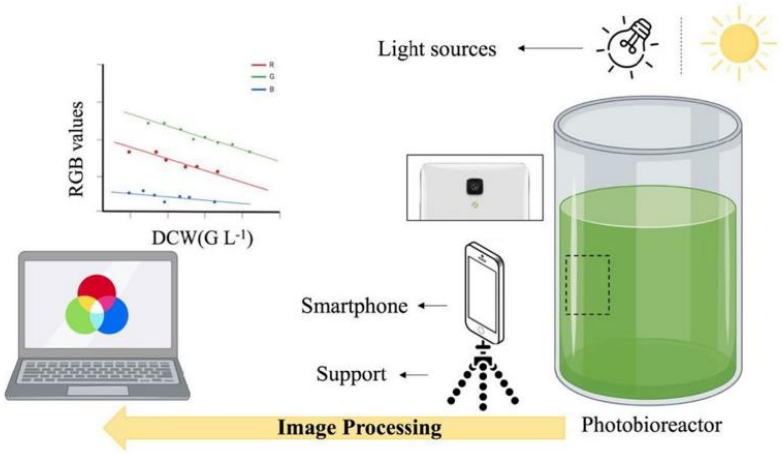
Experiments were performed in cylindrical shape photobioreactors (33 cm diameter and 69 cm height, 5.5 L volume), under light intensity 50 µmol of photons $m^{-2} \cdot s^{-1}$ and 25°C temperature. All reactors were continuously aerated to facilitate the homogenization and pH monitored.

Biomass concentration was determined by weight. After the filtration of a 25 mL of algal suspension through a dried glass fiber filter (Millipore 0.45 µm) with a Sartorius CPA64 balance, samples were washed with 50 mL of distilled water for salt elimination, dried at 105 °C to constant weight, cooled in a desiccator and weighed.

Computer Image Processing and Analysis

To carry out the analysis by image processing, it was necessary to capture digital images of the three types of photobioreactors during the days of cultivation. For this, the digital images were taken with a smartphone camera (Sony IMX214) using the same experimental set (Figure 1) for all the samples.

Figure 1. Experimental set



The sizes of the captured images were 4160×3120 pixels. Then, they were uploaded to a laptop to perform color decomposition by a RGB algorithm, using the **Pillow Python** image processing library. The experimental setup is shown in Fig. 1.

For image processing, a region of interest (ROI) consisting of 120 × 60 pixels were filtered and obtained the RGB color parameters. Thus, each pixel in the image had a R, G and B parameter values between 0 and 255. The mean of all pixels was calculated to be fit with the algal growth values. Both, R, G and B channels were fitted to microalgae mass.

As a contrast, absorbance of the samples was measured in a Jensen Spectrophotometer.

DISCUSSION OF RESULTS

Figure 2 and Table show the result of fitting the microalgae mass (dry cell weight) per volume of culture to the spectrophotometer measured absorbance. As can be seen in the table 1, a very good agreement between mass of microalgae in the culture and absorbance of the culture media was obtained [7] which means that, for *Nannochloropsis Gaditana* culture, absorbance measurements are useful to follow the microalgae growth.

Figure 2. Plot of Absorbance vs. microalgae dry mass for the culture.

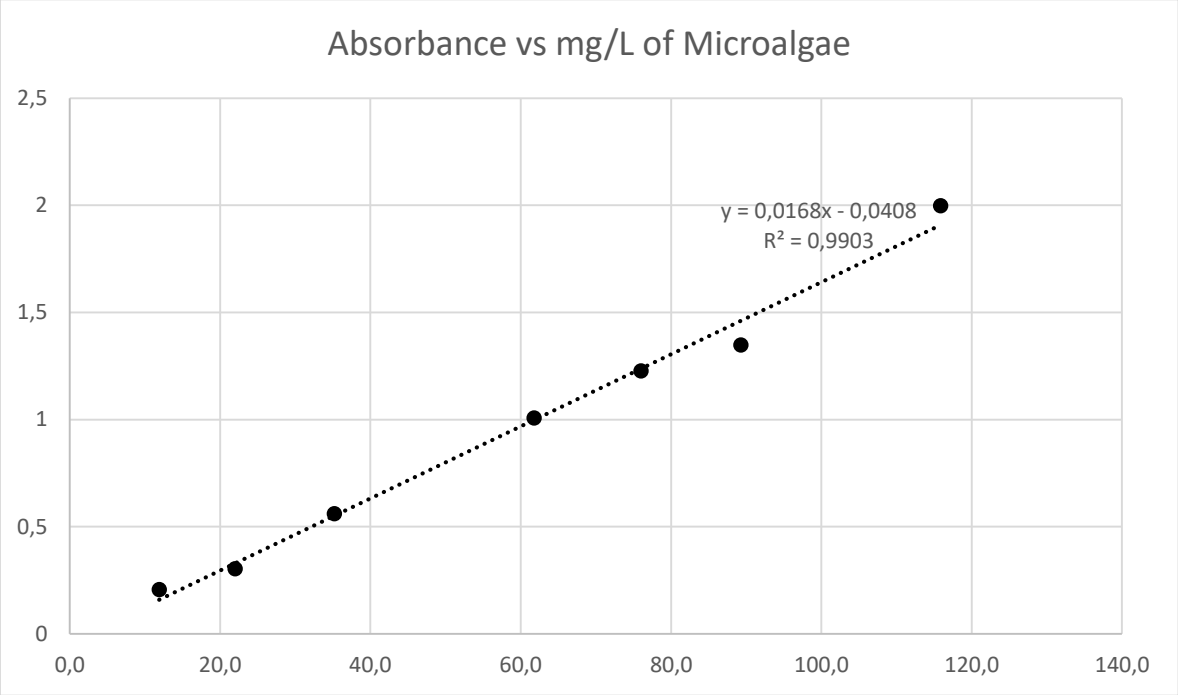


Table 1. Results of the absorbance vs. microalgae dry mass fit.

Slope	**Intercept**	**R^2**
0,0168	0,0408	0,9903

In Figure 3 and Table 2 the values of red, green, and blue and the fit as a function of dry cell weight of *algae* are summarize. It can be observed that the decomposition of the images into reds and greens is more correlated with the data collected in the count. The RGB mean of the image decomposition decreases progressively over time, like the density of cells in the culture. The lower value of parameters means more intense color of the culture (more cell density, results in Table 3. As can be seen in table 4 data, fit shows different correlation coefficients for each color parameter.

Figure 3. RGB parameters image processing decomposition fit for pictures of the samples.

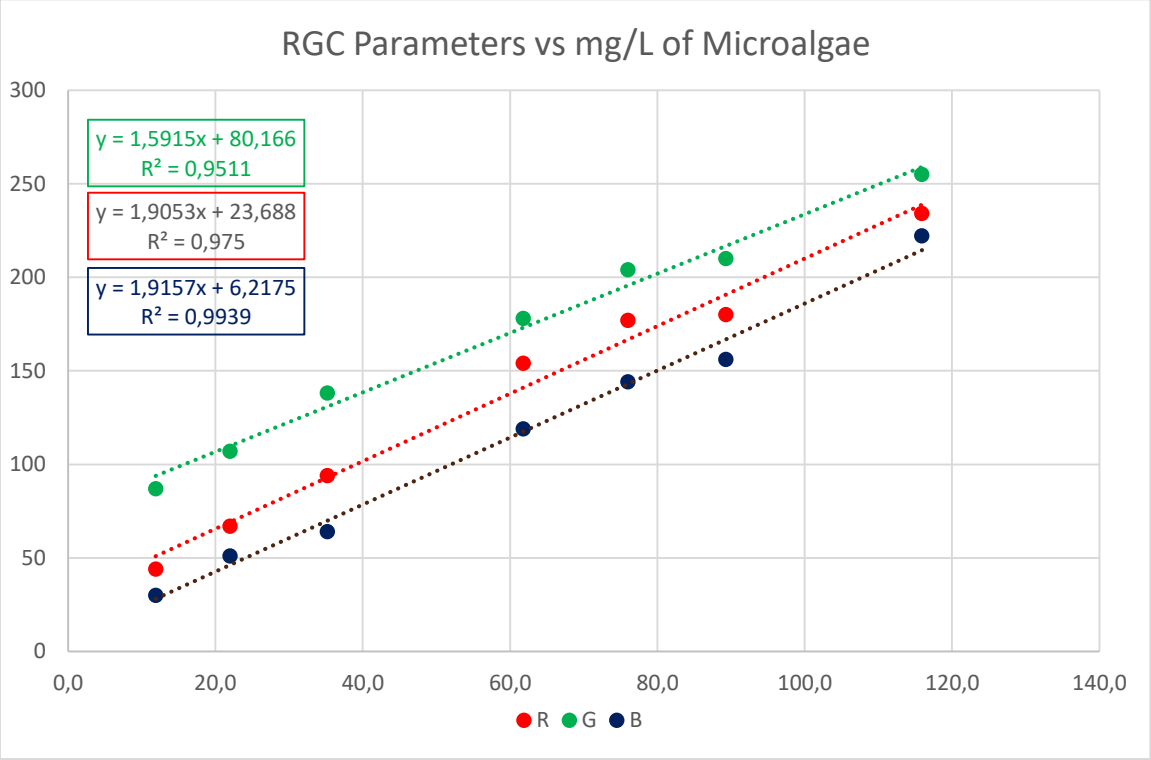


Table 2. Results of dry microalgae mass vs. RGB image decomposition parameters.

	Slope	**Intercept**	**R^2**
R	1,5915	80,166	0,9511
G	1,9053	23,688	0,9750
B	1,9157	6,218	0,9939

From table 1 data, we can state that these are not as satisfactory for monitoring the growth of the microalgae as they were under controlled conditions, because there is a greater dispersion between the values obtained in the image processing. The most accurate decomposition is the blue decomposition, which is contradictory because the *Nannochloropsis Gaditana* natural color is green. Different results appear when we use a linear multivariate fit (table 3), with a correlation coefficient greater than 0.99, which is very similar to the correlation coefficient calculated for the absorbance fit. Other authors have obtained similar results with other microalgae cultures [8].

Table 3. Linear multivariate fit for dry microalgae mass vs. RGB image decomposition parameters.

m_R	m_G	m_B	m_0	R^2
-0,2938242	0,54859421	0,36663323	-34,819049	0,99623638

According to this table, the equation that relations microalgae dry biomass per volume in a *Nannochloropsis Gaditana* culture with the image decomposition of an image taken with a mobile phone camera is:

$$\frac{mg}{L} = m_R R + m_G G + m_B B + m_0$$

In table 4 we present a resume of the results. It can be observed that there are no big differences among the real values of dry microalgae mass those and calculated by the fit. To understand the "color" of the culture it has been included the colored frames of all the samples which should represent the aspect showed by the culture in the photobioreactor.

According to the results of this experiment it promises to be a very innovative and stunning technique, but it must keep in mind that we need to perform previous calibrations for each microalgae, culture media, light conditions and type of culture apparatus (photobioreactor, raceway, etc.).

Table 4. Experimental parameters and fit.

Sample	mg/L	Abs	R	G	B	mg/L calc.	120x60 pixel crop
1	115,9	1,998	234	255	222	117,7	
3	89,3	1,348	180	210	156	84,6	
2	76,0	1,226	177	204	144	77,8	
4	61,8	1,007	154	178	119	61,2	
6	35,2	0,56	94	138	64	36,7	
7	22,0	0,303	67	107	51	22,8	
8	11,9	0,206	44	87	30	10,9	

CONCLUSIONS

The present study demonstrates what following growth of a *Nannochoropsis Gaditana* culture in sea water photobioreactor, a simple analysis of pictures periodically taken with a mobile phone is enough accurate as the absorbance measures made with a laboratory spectrophotometer. On the other hand, alternative methods must be destructive, slow, and costlier both the complex equipment and the use of a larger number of materials, as well as technical staff to constantly supervise the installations.

So, the use of image processing technology in the microalgae cultivation system (simple smartphone and a simple python script) allows to estimate the biomass concentration in situ from digital photographs using the RGB color model. On the other hand, studies on RGB color analysis are limited. But more experimental results will be necessary before all advantages of this technology could be improved as standard method in routine work,

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