Culture of *Halochlorella rubescens* (Chlorophyceae) as a carotenoid producer: comparison of indoor and outdoor cultivation under stressful growth conditions

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Resumen

Algunas cepas de microalgas bajo condiciones de estrés, por ejemplo, alta intensidad de luz o falta de nutrientes, sintetizan y acumulan carotenoides. En este estudio la Chlorophyta *Halochlorella rubescens* (BEA0069, de la colección del Banco Español de Algas) fue cultivada en recipientes de 5 L en cámara de cultivo (condiciones "indoor"/interior) y en invernadero (condiciones "outdoor"/exterior), con el objetivo de caracterizar el crecimiento y la dinámica de acumulación de clorofilas y carotenoides y así evaluar el efecto de las condiciones de estrés sobre el crecimiento de la especie. La falta de nutrientes tuvo el mayor efecto estresante en los cultivos dentro de la cámara de cultivo, mientras que, en los cultivos de exterior, pese a la mayor radiación solar incidente, incluyendo la radiación UV, combinado con la mayor disponibilidad de nitrógeno para el crecimiento celular causó que los cultivos no alcanzaran la fase estacionaria en el periodo experimental y por tanto la acumulación de carotenoides no aumento de manera significativa.

Palabras clave: *Halochlorella rubescens*, carotenoides, cultivo, limitación de nutrientes, estrés fisiológico, microalgas

Abstract

Some microalgae strains, under severe stress conditions such as high light intensity or nutrient starvation, synthetize and accumulate carotenoids. In the present study, the Chlorophyta *Halochlorella rubescens* (BEA0069, Spanish Bank of Algae) was cultivated in 5-L flasks cultures under indoor (laboratory) and outdoor (greenhouse) conditions, with the objective of characterizing growth performance and carotenoids accumulation dynamic. Chlorophyll and carotenoids extraction and determination were carried out to evaluate the effect of stress conditions on the cultures. Despite the high light intensity for the outdoor cultures nutrient starvation had a higher effect on growth performance and carotenoids synthesis in indoor cultures than those on outdoor ones. The high amount of nitrogen available for the outdoor cultures did not allow them to reach the stationary phase by the end of the study and a major increase in their carotenoid accumulation was not observed.

Key words: *Halochlorella rubescens*, carotenoids, cultivation, nutrient starvation, physiological stress, microalgae.

1- Introduction

Algae are a heterogeneous group of organisms that range in size from tiny unicellular-single cells to giant seaweeds. They are mostly photosynthetic species living in the different aquatic habitats of earth. However, they lack forms and reproductive features of the land plants. The concept of algae includes both photosynthetic protists, eukaryotic, and prokaryotic cyanobacteria (blue-green algae) (Figure 1) (Graham et al., 2009).

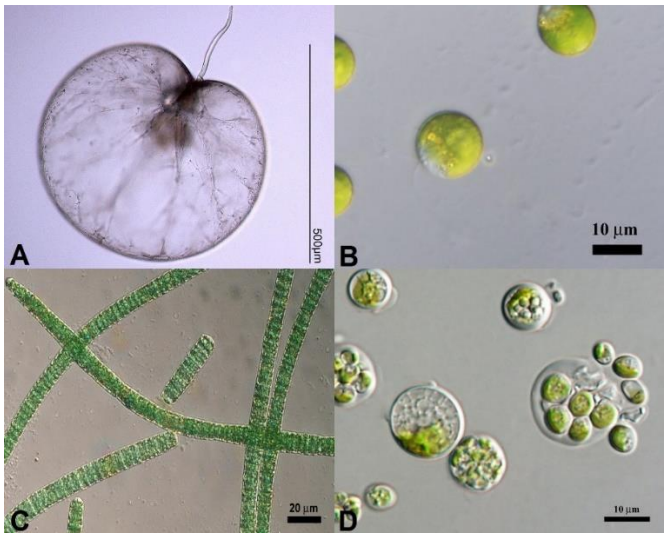

Figure 1: Examples of some microalgae seen with a microscope. A - *Noctiluca scintillans* (A. Slotwinski); B - *Dunaliella salina* (Teodoresco); C - *Arthrospira maxima* (Setchell et Gardner); D - *Chlorella saccharophila* (Krueger).

In order to survive in a highly competitive environment, freshwater or marine algae have developed adaptative and defense strategies that result in a tremendous diversity of metabolic compounds from different biochemical pathways (Cardozo et al., 2007). Pigments (i.e. carotenoids) and/or polysaccharides are some examples of substances produced during or taking part in algal metabolism (Harris, 2014). Many of these substances synthesized by algae have shown potential economic impact in food science, pharmaceutical industry and public health in the last 20 years. Emphasis is given to fatty acids, steroids, carotenoids, polysaccharides, lectins, mycosporine-like amino acids, halogenated compounds, polyketides and toxins. (Cardozo et al., 2007). Microalgae possess a wide range of carotenoids in concentrations that can considerably exceed the concentrations found in higher plants. Besides, microalgae can be cultivated at higher photosynthetic efficiencies and higher growth rates than land plants (Mulders et al., 2013). Interesting features for algae cultivation requires only one-tenth as much land, one-fifth as much water, three fourths as much power,

one-fifteenth as much labor, and one-fourth as much capital as is required by conventional agriculture (Oswald et al., 1967).

Carotenoids such as fucoxanthin and astaxanthin derived from microalgae or plants have received much attention due to their potential health benefits (Vilchez et al., 2011). Under unfavorable growing conditions, it has been reported that some microalgae show the ability to synthesize very high amounts of a complex mixture of secondary carotenoids, especially astaxanthin, canthaxanthin and echinenone (Orosa et al., 2000). Chlorophyll loss is associated to environmental stress and the variation in total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price, 1993).

In the present study, a comparison in relation to the production of certain pigments and metabolites by *Halochlorella rubescens* cultivated outdoor and indoor was carried out to determine which are the conditions for fast accumulation. *H. rubescens* is an eukaryotic microalgae (unicellular) from the Chlorophyceae family. It is a marine species. Cells are fusiform to globose, 3.5-15 µm long (Figure 2). The cell walls are smooth. Cells are uninucleate, becoming multinucleate prior to sporulation. With age cultures become brick red. Asexual reproduction take place by rupture of parental cell wall (Guiry, 2016). *H. rubescens* contains chlorophyll (Chl) *a* and *b* and carotenoids. Chlorophyll absorbs light between 400 and 500 nm which means blue light in the visible range. It was chosen to study the production of carotenoids under light stress and nitrogen availability.

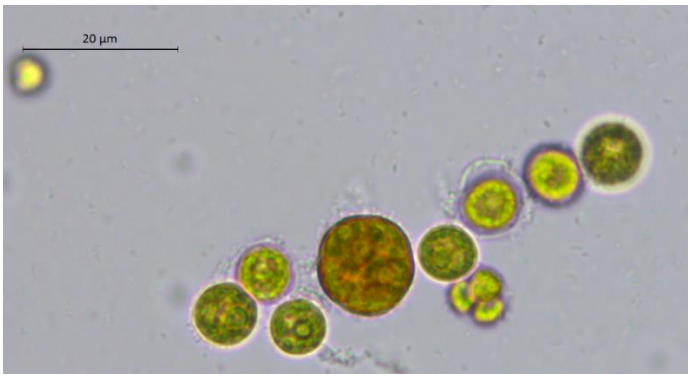

Figure 2: Halochlorella rubescens (Foto: E. Soler - BEA)

2- Materials and methods

2.1- Organism and growth conditions

Halochlorella rubescens (BEA0069) from the collection at the Spanish Bank of Algae, was originally bioprospected in a rocky shore of Fuerteventura (Canary Islands)..

2.1.1- Culture medium

Cultures were prepared in two different mediums for seawater strains: AM (sea water) (Table 1) formulated at the Spanish Bank of Algae, and f/2 medium (Guillard, 1975), modified with extra vitamins (Table 2), for outdoor and indoor cultivation, respectively. For both, indoor and outdoor conditions, cultures were scaled up an acclimated to experimental conditions.

Table 1: AM Medium recipe formulated at the Spanish Bank of Algae

AM Medium

Stock	*Component*	*Stock solution (g/L)*	*Volume per litre of final medium (mL)*
I	Bidistilled water		100
Fe	Na-EDTA	2,5	5
	$FeSO_4 \times 7\ H_2O$	1,66	
II	Seawater		860
A	KNO_3	150	20
B	KH_2PO_4	80	5
C	$MgSO_4 \times 7\ H_2O$	50	5
D	$MnCl_2 \times 4\ H_2O$	0,036	5
	$ZnCl_2$	0,010	
	$Na2MoO_4 \times 2\ H_2O$	0,013	
	$CoCl_2 \times 6\ H_2O$	0,004	

Table 2: f/2 Medium recipe (Guillard, 1975) with extra vitamins.

f/2 Medium

Stock	*Component*	*Stock solution (g/L or mL/L)*	*Volume per litre of final medium (mL)*
I	$NaNO_3$	75g	1
II	NaH_2PO_4 x 1 H_2O	5g	1
III	Na_2SiO_3 x 9 H_2O	30g	1
Trace metals	$FeCl_3$ x 6 H_2O	3,15g	1
	Na_2-EDTA x 2 H_2O	4,36g	
	$MnCl_2$ x 4 H_2O	1mL	
	$ZnSO_4$ x 7 H_2O	1mL	
	$CoCl_2$ x 6 H_2O	1mL	
	$CuSO_4$ x 5 H_2O	1mL	
	Na_2MoO_4 x 2 H_2O	1mL	
Vitamins	Thiamine x 1 HCl (vit B_1)	0,2g	0,5
	Biotin (vit H)	1mL	
	Cyanocobalamin (vit B_{12})	1mL	
Extra vitamins	Cyanocobalamin (vit B_{12})	1mL	1
	Biotin (vit H)	1mL	
	Niacinamid	1mL	
	Thiamine x 1 HCl (vit B_1)	100mg	

Three replicates were prepared for each condition, including 4L of medium and 1L of algae culture, to a final volume of 5L (Figure 3).

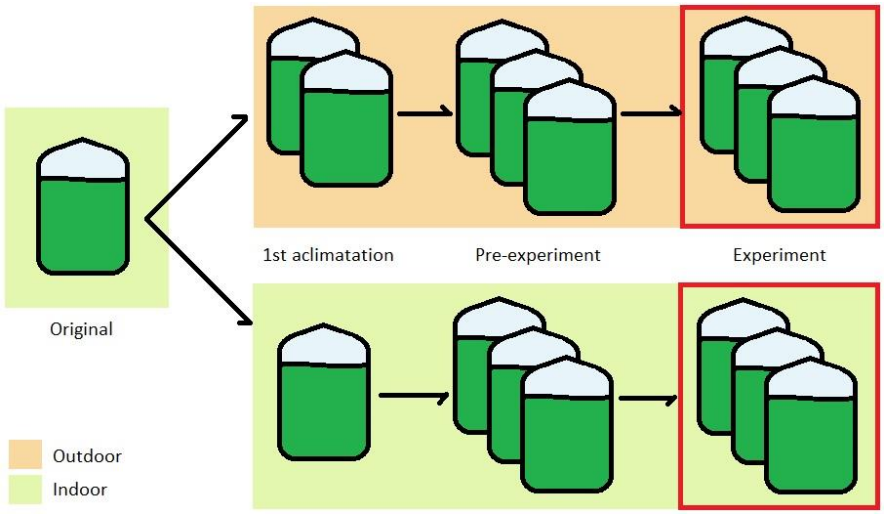

Figure 3: Scale up process from the original culture to the final experiment with a total of 6 replicates of 5L.

2.1.2- Light and temperature

Indoor cultivation conditions were established at a controlled cycle of 18 hours of light (120 µmol m-2 s-1) and 6 hours of darkness (18:6 L:D). Temperature in the culture room was 25 ± 2 ºC. For the oudoor cultures, light and temperature cycle was related to environmental conditions, full sun light (including UV), clouds and other natural phenomena (Figure 4).

Figure 4: Outdoor cultivation (up) and b) the indoor cultures (down)

Temperature for outdoor cultures was measured using a HOBO sensor introduced in a control flask, without algae, under the same conditions during the experimental period (Fig. 5) At the end of the experiment the sensor was removed and data were collected.

Figure 5: HOBO Temperature sensor in a control 5L flask .

2.2- General parameters

Every two days, since the beginning of the experiment until advanced stationary phase, all the data were collected. For that, 10 mL of each culture was sampled for the measurement of pH, photosynthetic efficiency through chlorophyll fluorescence, optical density, quantification of chlorophyll and carotenoids and cell density.

2.2.1- pH

pH was measured with a pH meter (Crison pH25). pH values were maintained around 7.5 by air-CO2 bubbling into the media.

2.2.2- Fv/Fm (photosynthetic efficiency of photosystem II)

Fv/Fm was measured with a Junior PAM equipment (Walz, Germany), which produces blue light absorbed by the chlorophyll, after darkness acclimation of all the samples for at least 15 minutes or more in order to let all the reaction centers to be fully oxidized (open), so they had full capacity of light caption when irradiated. The value of Fv/Fm indicates the level of stress of the algae: in general, (although values can vary among different species), high values (close to 0.8) mean that the organism is not affected and lower values (0.2-0.4) mean it is under stress (Sforza et al., 2012).

2.2.3- Growth

To different methodologies were used to determine the growth rate of the cultures: 1) optical density, measured by a spectrophotometer, and 2) cellular density by cell counts using a Neubauer chamber.

2.2.3.1- Optical density

Regular spectrophotometry was used to measure the growth rate and determine the biomass in cultures of unicellular organisms (Toennies et al., 1949; Griffiths et al., 2011). The amount of light absorbed by a suspension of cells can be directly related to cell mass or cell number (Griffiths et al., 2011). Absorbances at A680 and A750, to remove turbidity, were measured with a Perkin Elmer Lambda 25 Spectrophotometer. Three replicates were used for each sample (Figure 6).

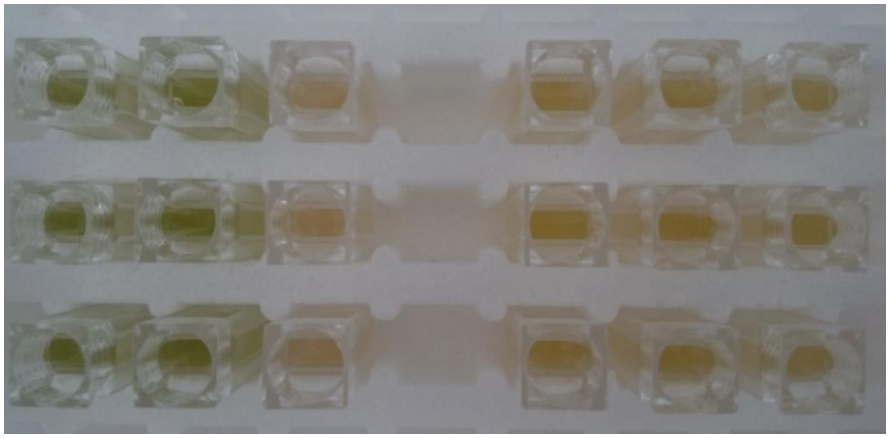

Figure 6: Samples of the outdoor and the indoor cultures, on the left and the right respectively, ready for spectrophotometric quantification of chlorophylls.

2.2.3.2- Cellular density

Cell counts were measured using a Neubauer chamber and a Leica DM2000 microscope. Cellular density was determined every 2 days. A sample was introduced in the chamber and all the cells inside the square mark were counted. With the volume of the chamber and the number of cells the cellular density could be calculated. Depending on the concentration of the culture a previous dilution was made in each sample to facilitate the count.

2.2.3.3- Growth rate and duplication time

For calculating the growth rate (k) and duplication time (g) of the cultures, the following equations were used:

$$k\ (days^{-1}) = \frac{Ln(N2/N1)}{(t2 - t1)}$$

$$g\ (days) = \frac{Ln\ 2}{k}$$

being N1 the value of optical density,at time 1 (t1) and N2 for time 2 (t2). t1 and t2 were the start and the end of the exponential growth phase for each culture (Figure 11).

2.2.4– Chlorophyll and total carotenoids quantification

During the experimental period, chlorophyll a (Chl a), b (Chl b) and total carotenoids (C_{x+c}) were measured in order to observe how nitrogen defficiency and light stress would enhance the carotenoid production (Orosa et al., 2000) over the actual green chlorophyll (basic pigment in *Halochlorella*). To do so, 1,5ml of each culture was placed in a 2ml Eppendorf and centrifuged with a Hettich Mikroliter centrifuge to discard the medium. Then glass beads

and a Penta sonicator were used for breaking the biomass and obtain pigments in 1,5ml of methanol (Figure 7).

Figure 7: Pigments extracted with methanol.

After 1h of darkness at 4ºC this methanol samples were measured with the spectrophotometer to determine pigments concentration. The amount of each pigment was calculated by Wellburn's equations (Wellburn, 1994)

$$\text{Chl } a\ (\mu g \cdot mL^{-1}) = (16.72\ A_{665.2}) - (9.16\ A_{652.4})$$

$$\text{Chl } b\ (\mu g \cdot mL^{-1}) = (34.09\ A_{652.4}) - (15.28\ A_{665.2})$$

$$C_{x+c}\ (\mu g \cdot mL^{-1}) = (1000\ A_{470} - 1.63\ \text{Chl } a - 104.96\ \text{Chl } b)/221$$

Once a week, with the excess of methanol with pigments from the extraction, a wavelength scan was performed (λ=326 to 750nm) from each sample. This would help to determine the variance of all present pigments during the study.

3- Results

The experiment lasted 37 days. During this period the indoor cultures showed a significant change in color from green to orange, suggesting they started to produce relatively high amounts of carotenoids, while the outdoor ones were still dark green and growing by the end of the study (Figure 8). Unfortunately, one of the outdoor replicates (nº 3) died over the weeks, so data from this replicate were omitted.

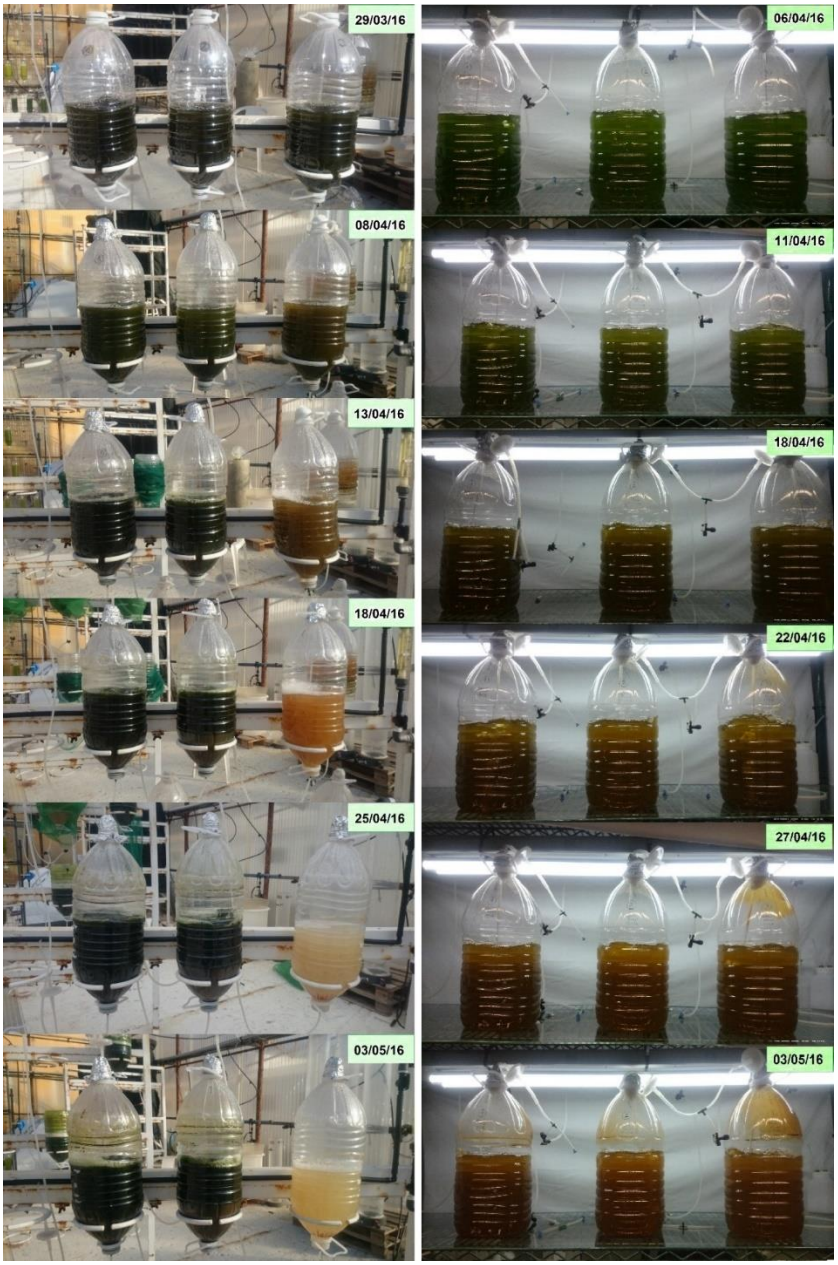

Figure 8: Evolution of the cultures over time. On the left the outdoor system and on the right the indoor one. It can be appreciated how replicate nº 3 of outdoor system slowly died and how the indoor cultures changed from green to orange.

3.1 Growth conditions

3.1.1 - Temperature

Data collected from the temperature (Tª) sensor (Figure 9A) show the daily variation of the temperature during the study. From the tendency line it can be appreciated the increase of Tª from early to late spring, from approximately 21 to 24ºC by the end of the study. Night temperature oscillated from 15 to 18ºC while day temperature had a major oscillation, between 25 to 37ºC. Figure 9B shows the variation in detail for a single day.

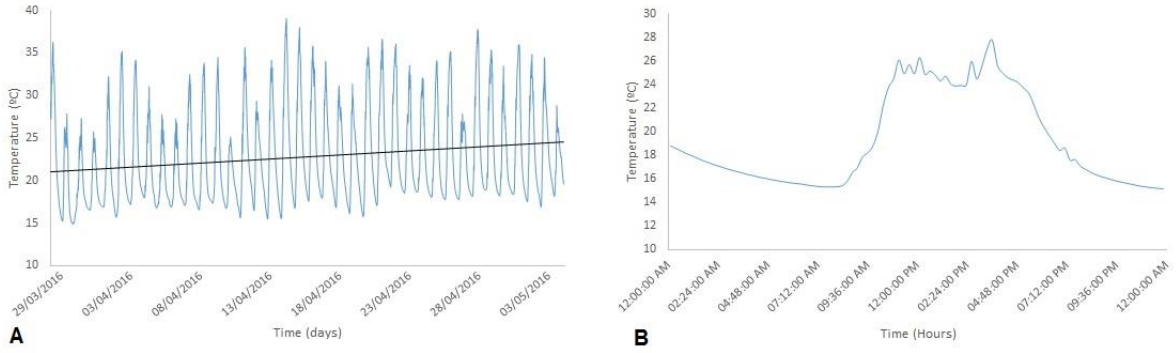

Figure 9: Outdoor temperature data obtained with a HOBO sensor. A - Temperature along the 37 days of study. In blue the daily temperature oscillation and in black the tendency line; B - Temperature for one specific day, as an example.

3.1.2 - pH

pH was stabilized around 7-8 in both indoor and outdoor cultures by injecting CO_2 – enriched air into the cultures. Figure 10A shows the stability of pH along the experiment, with values around 7 and 8.5, except for the first weeks in the outdoor cultures, when pH increased to ~10 due to the temporal lack of CO_2 in the system.

3.1.3 – Fv/Fm

Slowly, all the cultures showed a decrease on their photosynthetic efficiency along the experiment (Figure 10B). The outdoor cultures reached a maximum efficiency of 0.6 and then decreased to 0.4-0.5, while the indoor cultures decreased from 0.45 to approximately 0.3. Fv/Fm values were especially low for the outdoor replicate that died since the beginning of the experiment.

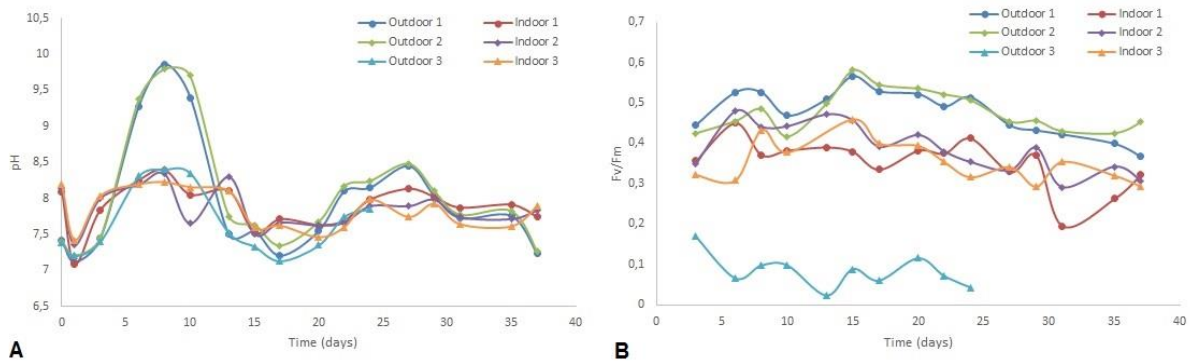

Figure 10: A - pH data and B - Fv/Fm values, along the period of study, for each outdoor and indoor replicate independently.

3.2 - Growth characterization

Two variables were used measured in order to follow the growth of each cu7lture: 1) the absorbance at λ=680 nm due to chlorophyll *a* and 2) cell density (nº cell/mL). With both tracking methodologies, the same result appeared (Figure 11). For the indoor system both optical density and cellular density showed a similar growth curve:,in the first two days an adaptation phase, followed by 18-20 days of exponential growth, then a the stationary phase along 5 to 6 days, and finally the senescent phase for the last few days (Figure 11B). For the outdoor cultures the exponential growth took longer and even could still be growing after the study (Figure 11A). Because of the time the experiment had to end the outdoor system never reached the stationary phase so further investigation could give more data.

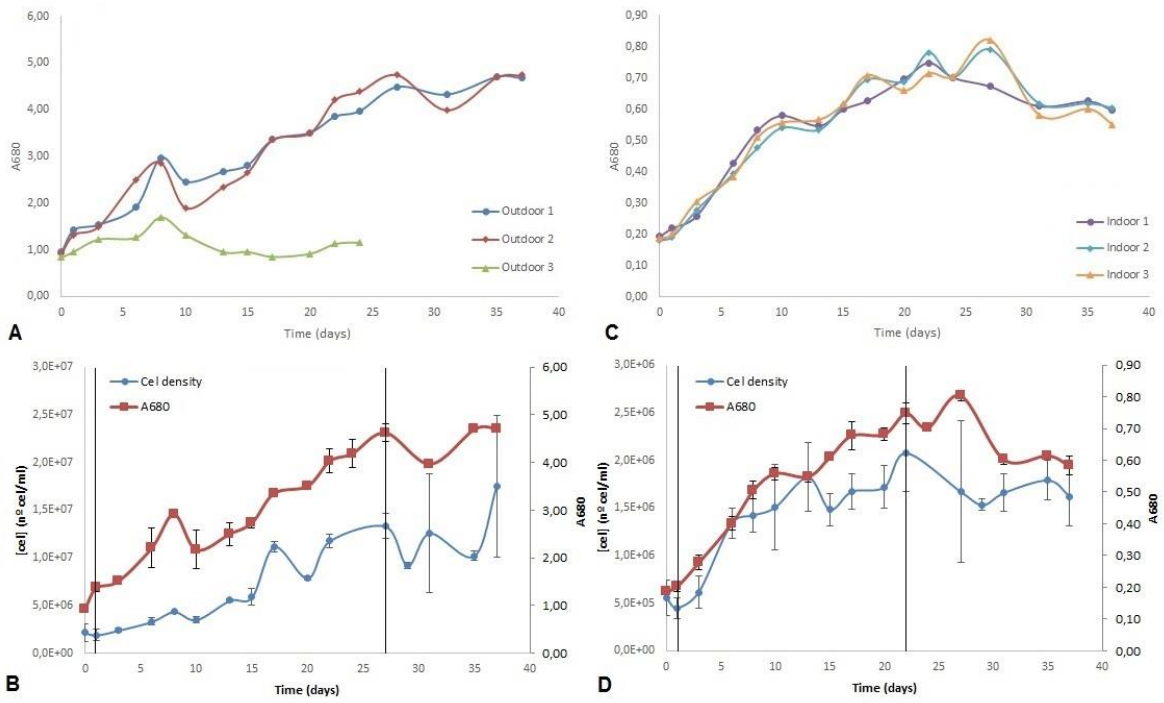

Figure 11: A – Growth curves: A) Absorbance measured at λ=680 nm for outdoor cultures and C) for indoor replicates; B) Mean absorbance measured at λ=680 nm (red line) and mean cell density (nº cell/mL, blue line) for outdoor cultures (n=2). ; D) Red line represents the mean absorbance measured at λ=680 nm and blue line is the mean cell density (nº cell/mL) for indoor cultures (n=3). In both cases, vertical black lines represent the standard desviations and long straight lines show the beginning and the end of the exponential growth.

Comparing the growth rates and duplication times for both systems (Table 3) it is showed how the cultures in the indoor culture system grew faster, with a duplication time of 12 compared with the 14 days needed for the duplication of the biomass in the outdoor system.

Table 3: Growth rate and duplication time for each replicate separately, as well as mean values (± sd) for both outdoor and indoor systems.

	Growth rate ($days^{-1}$)	Duplication time (days)	Mean growth rate (± sd) ($days^{-1}$)	Mean duplication time (± sd) (days)
Outdoor 1	$4.41\ 10^{-2}$	15.731	0.046 (±0.004)	14.875 (±1.211)
Outdoor 2	$4.94\ 10^{-2}$	14.018		
Indoor 1	$5.05\ 10^{-2}$	13.719		
Indoor 2	$5.67\ 10^{-2}$	12.223	0.054 (±0.003)	12.841 (±0.781)
Indoor 3	$5.50\ 10^{-2}$	12.582		

3.3 - Pigment production

Over the length of the experiment, the extraction of Chl and C_{x+t} allowed to observe the increase in the total production of both types of pigments over time as far as the biomass increase (Figure 12). For the outdoor system, the cultures were still growing at the end of the experiment so the concentrations of Chl and C_{x+t} were both still rising (Figure 12A). The contrary happened for the indoor cultures, were a higher carotenoid over chlorophyll concentration could be observed at the end of the growth cycle (Figure 12B).

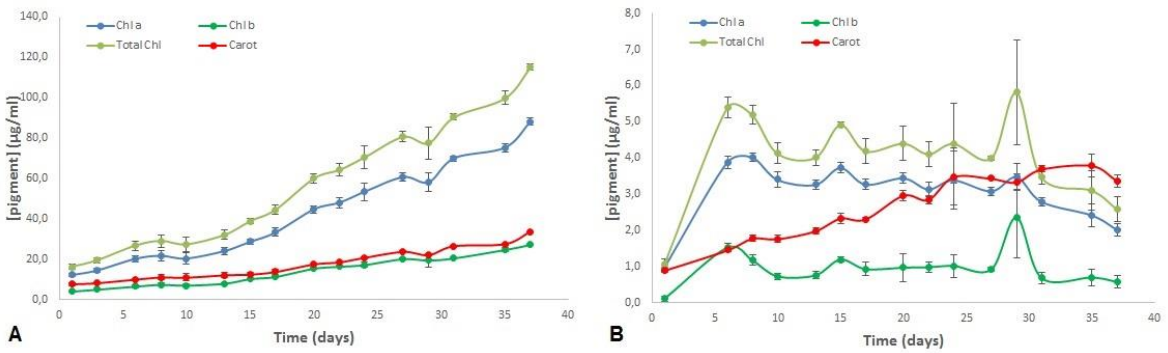

Figure 12: Chlorophylls (a, b and total) and carotenoids concentrations for outdoor (A) and indoor (B) cultivations. Each color line represents a pigment type: Blue – Chl a; Dark green – Chl b; Light green – Total Chl; Red – Carotenoids. Left axis is for chlorophylls concentration and right one for carotenoids. Vertical lines represent the standart desviation.

The wavelength scans showed the presence of this pigments (Figures 13) for four different days during the study. Two principal peaks could be observed in λ=665nm for the chlorophylls and λ=439nm for the carotenoids.

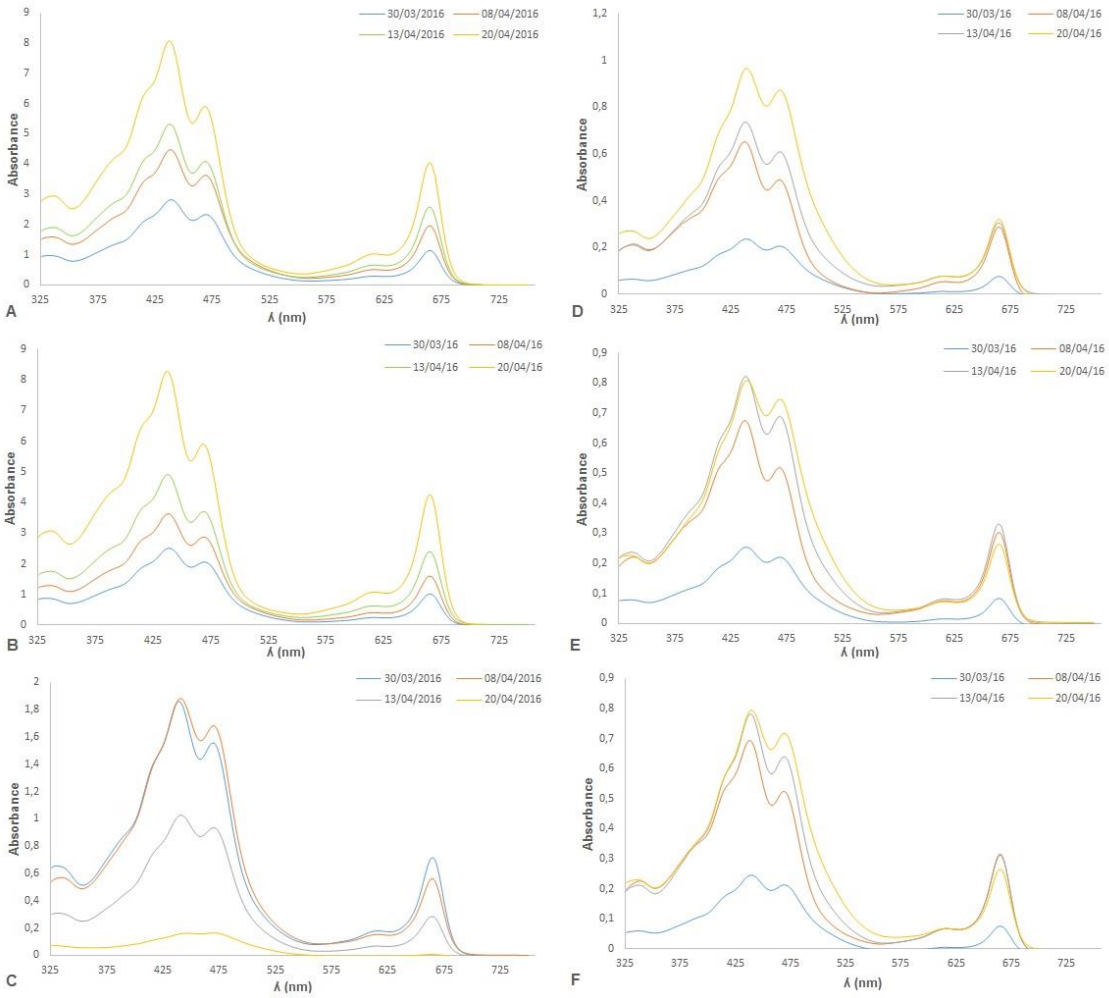

Figure 13: Pigment wavelength scans (326nm to 750nm) for each replicate from both outdoor and indoor cultures at different moments along the period of study. –for outdoor cultures, A – replicate nº1; B –replicate nº2 and C – replicate nº3; For indoor cultures, D – replicate nº1 and E – replicate nº2; F – replicate nº3. The peak at 665 nm represents the chlorophyll a and the peak at 439nm nm represents total carotenoids.

The chlorophyll to carotenoid ratio (Figure 14) gives information about the level of stress for each culture. The outdoor cultures were still growing, producing more Chl a than carotenoids so they showed increasing values of the ratio with time, while the inverse happened with the indoor cultures (tendency to decrease), with higher amounts of carotenoids than chlorophyll.

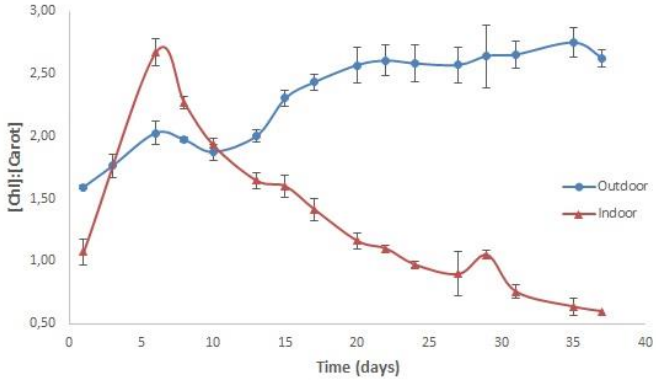

Figure 14: Mean Chl a to C_{x+c} ratio for each culture system during the study. Circles represent the outdoor relation and triangles represent the mean values for indoor cultures. Vertical lines represent the standart desviation.

From pigment contents per cell (pg/cell) (Figure 15A) or per gram of biomass (µg/g) (Figure 15B), the same results can be observed. In both cases, chlorophyll and carotenoids increased for the outdoor cultures, but for the indoor cultures the production of total carotenoids was higher than the production of chlorophyll.

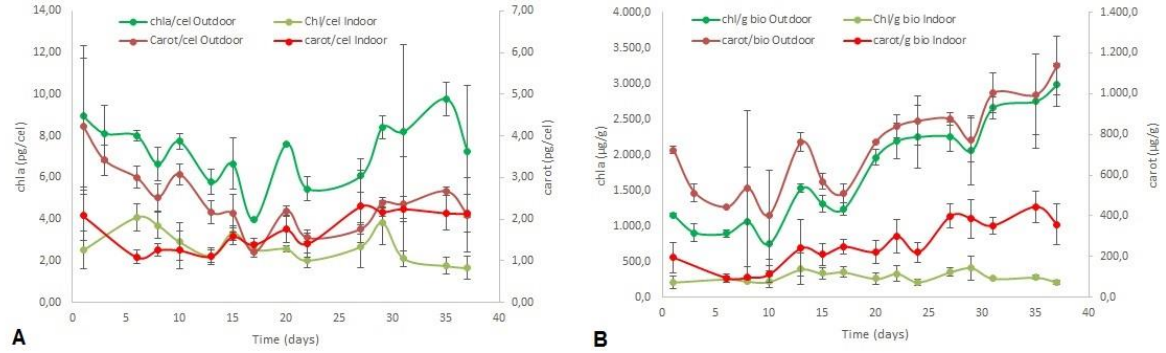

Figure 15: A - Chlorophyll and carotenoids concentration per cell., and. B - Chlorophyll and carotenoids concentration in 1g of fresh biomass. Dark and light green lines for chlorophyll and dark and light red lines for carotenoids. Vertical black lines represent the standard desviation.

4 - Discussion

Halochlorella rubescens, like *Dunaliella salina*, produces carotenoids under stress conditions, such as high light intensity or nutrient starvation (Emeish, 2012). Both indoor and outdoor culture systems allowed algae to fully grow. Although indoor cultures were inoculated from a senescent culture, cells recovered in 2 days and started to produce new carotenoids in a few weeks. On the other hand, the outdoor cultures kept growing until the end of the experiment.

However, a few incidentals occurred. The death of nº3 outdoor sample left this system with just two sets of data. A minor incident was CO_2 lack during the first weeks, luckily it seemed like there wasn't a major effect on the growth of the algae.

Nitrogen is a constituent of chlorophyll a and is incorporated mainly in proteins and nucleoids (Lodish et al. 2004). Taking a look at both mediums used and their concentration of nitrogen, it's appreciable that AM had approximately 40-fold more nitrogen than f/2. Despite the outdoor system was under higher light intensity, the availability of extra N caused the growth of the cultures even after 37 days of experiment. The indoor systems, which grew from a previous stressed culture, with new medium recovered their normal green tonality and again started producing new carotenoids when nitrogen run out. In this case, it seemed that the main factor on carotenoid production was the lack of nutrients, more specifically nitrogen, reaching the stationary phase by nutrient starvation faster.

The photosynthetic efficiency and pigment concentration could be related especially for the indoor cultures. When the algae started to produce carotenoids there was a decrease in their photosynthetic efficiency, indicating the stress suffered by the adverse conditions. Even more, this was also observed in the concentration per cell or g of biomass, especially for indoor cultures where there was a higher increase of carotenoids by the end of the exponential growth (21-22 days). This was also observed with the wavelength scan. The peaks at λ=665nm and λ=439nm represent chlorophylls and carotenoids respectively (Wellburn, 1994). So for the outdoor cultures there was an increase in both pigments and for the indoor ones and increase of the peak in λ=439nm (carotenoids) and a lower increase or even a decrease in the λ=665nm peak (chlorophylls). This result corroborates the major production of carotenoids by the indoor cultures due to higher stress conditions.

Dunaliella salina is currently cultured to produce carotenoids under stress conditions. It showed a maximum accumulation of total carotenoids of 55 pg/cell in Dewalne's medium and 12/12 light/dark photoperiod on 26-day study (Venkatesan et al., 2013). *Halochlorella rubescens*, cultivated in this experiment under outdoor and indoor conditions, with AM and f/2 medium respectively, showed maximum values of 1,77 and 2,31 pg/cells respectively. However, the outdoor cultures never reached the maximum accumulation of total carotenoids due to the lack of time. Although the carotenoids concentration could be higher than that for

Dunaliella, using AM medium the time needed would be much longer, which it's counterproductive.

5 – Conclusion

Halochlorella rubescens produced carotenoids under stress conditions, i.e. nutrient starvation and high light intensity. Two different systems were used for the cultivation of this strain. The outdoor cultures exposed to direct sunlight during the day, with its correspondent daily temperatures and cultivated with AM medium (Higher concentration of nitrogen). The indoor cultures were kept in the laboratory controlled environment, stable light cycle, invariable room temperature and cultivated with f/2 medium (lower nitrogen concentration).

The indoor strains produced carotenoids by the end of the study, however, the outdoor ones were still growing. This shows how in our experiment nutrient starvation (nitrogen deficiency) had a major effect over carotenoids production than high light intensity.

Compared with the well-known carotenoid producer *Dunaliella salina*, *Hallochlorella* accumulated less carotenoids per cell in the same period of time. Further investigation, like increased time of study and same mediums for both outdoor and indoor cultures, could give more information.

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7 – Webgraphy

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Descripción de las actividades realizadas durante la realización del TFT

Para llevar a cabo este experimento hizo falta preparar una serie de elementos previamente. Fotobiorreactores artesanos con garrafas de plástico de ocho litros y mangueras de acuario filia para la aireación. Además, fue necesario aclimatar la cepa elegida tanto en la cámara como en el invernadero.

Una vez empezado el experimento fui 3 veces por semana (lunes, miércoles y viernes) al centro a realizar una serie de medidas. De cada cultivo extraía 10mL, con los que aprovechaba para medir el rendimiento fotosintético (fv/fm), el pH, la densidad óptica y la densidad celular, para controlar la curva de crecimiento, y una extracción de clorofilas y carotenoides. Todo esto durante 37 días, des del 29 de marzo hasta el 5 de mayo.

Con todos los datos almacenados en una hoja de datos de Excel realicé todos los cálculos necesarios para obtener resultados y gráficas donde mostrarlos.

Formación recibida

Toda la formación que recibí a lo largo de mi estancia en el centro fue por parte de los distintos empleados que me ayudaron y explicaron el funcionamiento de las distintas tareas.

Nivel de integración en el centro

Mi integración fue muy buena a lo largo de los 5 meses que estuve en el centro. El trato recibido por parte de la mayoría de empleados fue de los mejores que he tenido nunca en unas prácticas externas. El grupo de trabajadores más cercanos a mi línea del TFT se mostraron des del primer día dispuestos a ayudar en lo que hiciera falta y viceversa.

Aspectos positivos y negativos

Uno de los aspectos más positivos de esta experiencia fue la tutorización diaria por parte de la Dr. Tamara Rodríguez. Todos sus conocimientos, consejos, correcciones e incluso la cantidad de tiempo invertido en mi proyecto fueron de gran ayuda. Además del buen ambiente de trabajo con los demás empleados lo cual facilito mi estancia.

Por otro lado, un aspecto negativo ha sido la duración del proyecto. Si de mí dependiera lo hubiera alargado otro mes más…

Valoración personal

Estoy muy contento con el centro, sus empleados y todos los conocimientos que me llevo. Esta experiencia ha sido la guinda que faltaba para terminar el grado con muy buen sabor de boca. Por mi parte recomiendo a todos los alumnos de la facultad de Ciencias del Mar, interesados en acuicultura vegetal, que realicen sus prácticas y el trabajo de fin de título en el Banco Español de Algas