UNIVERSIDADE DA CORUÑA
Facultade de Ciencias

Grao en Bioloxía

Memoria do Traballo de Fin de Grao

Optimizing tank culture *of* "sea lettuce" (*Ulva ohnoi,* Ulvophyceae*,* Chlorophyta).

Optimización del cultivo en tanque de "lechuga de mar" (*Ulva ohnoi,* Ulvophyceae*,* Chlorophyta).

Optimización do cultivo en tanque de "leituga de mar" (*Ulva ohnoi,* Ulvophyceae*,* Chlorophyta).

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0. ABSTRACT

Ulva ohnoi is an alga involved in green tides as well as highly used in the aquaculture industry. In this project, we will focus on the optimization of its growth rate and its nutrient absorption by applying different light color conditions through the usage of LEDs positioned at a fixed distance from the culture (28cm). Blue light shows the highest growth rate, followed by white and magenta light. Red light shows the slowest growth rate. Blue and white lights show the highest nitrogen absorption rates and blue light also shows the highest phosphorus absorption rates, whereas red light always shows the lowest nutrient absorption rates. We presume that blue light is the most suitable for the cultivation of *Ulva ohnoi*, being red the worst light color to use for the growth of this type of macroalgae. Blue LED light also shows the lowest net consume values (11.9W/h), which makes it a perfect light source for cultures of *Ulva ohnoi* in the industry, providing the beneficiary with a greater economic benefit.

KEY WORDS: *Ulva ohnoi*, light color, LED diodes, aquaculture

Ulva ohnoi es un alga responsable de las mareas verdes, así como muy usada en acuicultura. En este trabajo nos centraremos en la optimización de sus tasas de crecimiento y absorción de nutrientes mediante el empleo de distintos colores de luz a través del uso de LEDs posicionados a una distancia fija del cultivo (28cm). La luz azul mostró la mayor tasa de crecimiento, seguida por la blanca y la magenta. La luz roja mostró los niveles de crecimiento más bajos. Las luces azul y blanca poseen la mayor tasa de absorción de nitrógeno y la luz azul también presenta la mayor tasa de absorción de fósforo, mientras que la luz roja siempre muestra los valores de absorción de nutrientes más bajos. Podemos asumir que la luz azul es la más adecuada para el cultivo de *Ulva ohnoi*, siendo el rojo el peor color de luz para el crecimiento de este tipo de macroalga. La luz LED azul también muestra los valores más bajos de consumo neto (11,9W/h), lo cual la convierten en la fuente de luz perfecta para cultivos de *Ulva ohnoi* en la industria, proveyendo al beneficiario de un mayor beneficio económico.

PALABRAS CLAVE: *Ulva ohnoi*, color de luz, diodos LED, acuicultura

Ulva ohnoi é unha alga responsable das mareas verdes, así como é moi usada na acuicultura. Neste traballo centrarémonos na optimización das súas taxas de crecemento e absorción de nutrientes mediante o emprego de distintos cores de luz a través do uso de LEDs posicionados a unha distancia fixa do cultivo (28cm). A luz azul amosou a maior taxa de crecemento, seguida pola luz branca e a maxenta. A luz vermella mostrou os niveis de crecemento máis baixos. As luces azul e branca posúen a maior taxa de absorción de nitróxeno e a luz azul tamén presenta a maior taxa de absorción do fósforo, mentres que a luz vermella sempre mostra os niveis de absorción de nutrientes máis baixos. Podemos asumir que a luz azul é a máis adecuada para o cultivo de *Ulva ohnoi*, sendo o vermello a peor cor de luz para o crecemento deste tipo de macroalga. A luz LED azul tamén mostra os valores máis baixos de consumo neto (11,9W/h), o cal a convirte na fonte de luz perfecta para cultivos de *Ulva ohnoi* na industria, proporcionando ao beneficiario dun maior beneficio económico.

PALABRAS CLAVE: *Ulva ohnoi*, cor de luz, diodos LED, acuicultura

1. INTRODUCTION

Aquaculture is one of the most important forms of food production (representing 47% of total fish production and 53% of fish food production) and most of it (64%) is produced on land (FAO, 2019). Nowadays, aquaculture continues to grow, being considered the world's fastest growing food production sector. In 2016, global aquaculture production reached 110 million tons (80 million tons of fish and 30 million tons of seaweed) (FAO, 2018), and since then it has not stopped growing, suffering from a great demand by the consumers.

In order to cope with the evolution of this activity certain measurements are required, such as aquacultural water treatment to avoid dreadful consequences over the plant and animal live around the fisheries. One of the easiest and most effective techniques for these treatments is algae biofiltration. Macroalgae can be used with this intention. They can act as nitrogen and phosphorus traps (del Río et al., 1996). Macroalgae also provide many other benefits like an increase of dissolved nutrients and the removal of ammonium and phosphates (Hernández et al., 2008), the maintenance of a constant pH and the production of biomass that can be used for feed and food, which makes macroalgae biofiltration more efficient than bacterial biofilms (Cahill et al., 2010).

One of the most suitable genera of macroalgae for the biofiltration of seawater is *Ulva* Linnaeus. This genus presents some characteristics that make it outstand other types of algae. *Ulva* is a fast-growing algae that can be cultivated at different temperatures, nutrient concentrations and salinity, which makes it easy to produce high quantities of biomass. *Ulva* can grow at high NH_4^+ and heavy metal concentrations, removing these compounds from the environment by bioaccumulation and producing algal biomass of relatively good quality (Amosu, 2016). The interaction between *Ulva* and other aquatic live has also been studied, showing multitrophic relations between the alga and fish, as well as mollusks and other animals (Hirata, 2002). These characteristics allow us to cultivate *Ulva* in an integrated multitrophic aquaculture system (IMTA), both in open and in recirculating systems (IMTA-RAS). This type of systems are already used in Spain, especially in Galicia (Guerrero & Cremades, 2012).

The growth of *Ulva* in IMTA-RAS can be optimized controlling different properties of the medium. One of the most important parameters to consider is light. It has been shown that microalgae show an optimum growth rate under long wavelength light like red (Tsai et al., 2017), presenting better results under fluorescent conditions rather than under LED light (Satthong et al., 2019). This is probably caused due to the inhibition of photosynthesis under blue light (Luimstra et al., 2018). However, many macroalgae (included *Ulva*) show a maximum growth rate under blue light conditions and present better results with the use of light-emitting diodes (LEDs) rather than fluorescent light (Le et al., 2018). LEDs are a great alternative to tubular discharge lamps, as we can control their intensity and they do not produce any residual heat, contributing to more stable test conditions (Michel & Eisentraeger, 2004).

2. OBJECTIVES

This TFG consists of a study of the optimization of a culture of *Ulva ohnoi* in a simulated IMTA-RAS system, focusing on the influence of different types of light (blue, red, white and magenta). The main objectives of this project are:

-Determination of the growth rate of *Ulva ohnoi* under different kinds of LED lights.

-Determination of the Nitrogen and Phosphorus biofiltration capacity under these lights.

3. MATERIALS AND METHODS

3.1. *Ulva ohnoi*

In these experiments we will be using the exotic species *Ulva ohnoi* Hiraoka & Shimada (Ulvales, Chlorophyta) instead of using some other species of *Ulva* that are more common in the Northwest of Spain, such as *Ulva lactuca*. *Ulva ohnoi* comes from the costs of Japan and due to its high temperature tolerance it possess a high risk of dispersal and settlement that allows it to be present across many geographic regions (Zanolla et al., 2019). This high tolerance to changes in temperature makes it more suitable for its use in the laboratory.

Ulva spp. shows a digenetic, haplodiplontic life cycle with isomorphic alternation of generations. They are also capable of reproducing asexually by fragmentation, producing the green tides (usually under events of eutrophization) (Nakamura et al., 2020; Zanolla et al., 2019).

Ulva ohnoi is conformed of a bright metallic green distromatic laminar thallus with an orbicular, ovate, obovate or, sometimes, irregular shape that is fixated to the substratum by a small disk. The thickness of the blade varies between 30 and 55 μm in the apical-medium zone and between 80 and 90 μm in the basal zone. The thallus usually contains smooth margins, usually with small protuberances known as marginal teeth, visible under the microscope. In the apical zone of the blade we can observe big cells with polygonal or quadrangular shape in which the chloroplast that covers the outer face can contain from 1 to 3 pyrenoids.

3.2. Stock culture

The stock culture of *Ulva ohnoi* used in our experiences was obtained from a wild population recollected in October of 2015 in Isla de Santa Cristina (Huelva, Spain). After its genetic identification (sequencing its *rbcl* gene) the culture has been maintained in suspension in 160L tanks with constant aeration, a temperature of 18ºC ±1,5ºC, a 12:12h photoperiod (light:darkness cycle) and a salinity of 34‰ (compensating the loss of evaporating water by adding fresh water).

3.3. Culture medium

The culture medium was composed of sea water enriched with 20 $mg \cdot L^{-1}$ of Nitrogen (from $NaNO_3$), 1 $mg \cdot L^{-1}$ of Phosphorus (from NaH_2PO_4) and 0,5 mL of a solution composed of different trace elements (Table 1).

Trace elements	g per L
$Na_2EDTA.2H_2O$	14
$FE(NH_4)_2(SO_4)_2.6H_2O$	14
$MnSO_4.4H_2O$	1,6
$FeCl_3.6H_2O$	0,5
$ZnSO_4.7H_2O$	0,2
$CoSO_4.7H_2O$	0,05

Table 1. Composition of the trace element solution used for the culture medium.

3.4. Experimental design

In this experiment we will study changes between different light colors: white, red, blue and magenta. In order to do this, we will grow *Ulva ohnoi* in a medium with a fixed nutrient concentration (mentioned in the previous section) under these light colors. The three experiments that were conducted are:

-Blue Light vs White Light -Blue Light vs Magenta Light -Blue Light vs Red Light

For each light color we will use 8 Falcon® plates that will be kept at a Liebherr® environmental simulation chamber at a temperature of 18 ± 0,2º C (Figure 1). Each of the wells in the plate will contain a 2cm diameter *Ulva ohnoi* disk extracted from the culture with a steel punch (Figure 2). The light that we will be using comes from MaxiLED $\mu mol \cdot m^{-2} \cdot s^{-1}$ and we will expose the algae to a photoperiod of 12:12h (light:dark). The LEDs will be positioned always at the same distance from the culture (28cm), which means that different light colors will have different light intensities that we can measure:

-Blue light: 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (11.9 W) -Magenta light: 420 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (22.4 W)

-White light: 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (28.3 W) -Red light: 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (15.0 W)

The plates will be constantly shaken at 50rpm with a Skyline shaker Dos-20L (Figure 1).

Fig. 1. Skyline shaker with Falcon® plates in the Liebherr® environmental simulation chamber.

Fig. 2. *Ulva ohnoi* disks.

The experiment takes place in 12 days. The first 4 days the biomass suffers a period of acclimation to the new conditions. In the 5th day the wet weight of the *Ulva ohnoi* disks is obtained and the experimental week begins. During this week, all the plates will be rotated clockwise each day to avoid the influence of possible different light conditions and the medium will be changed every 2 days (measuring the concentration of N and P of the replaced medium). The 12th day the experiment will be concluded by measuring the final wet weight of the disks in order to obtain the growth rate.

3.5. Determination of Nitrogen and Phosphorus

The analysis of Nitrogen and Phosphorus was done with a HP 8433 UV-visible spectrophotometer (Figures 3 and 4):

-For the determination of Nitrogen, the APHA (1992) method was used (measuring the absorbance of the nitrate ion at 220nm).

-For the determination of Phosphorus, the Grasshoff et al. (1999) method was used (reduction and linkage of molybdenum and phosphorus, resulting in a colored molecule and measuring its absorbance at 880nm).

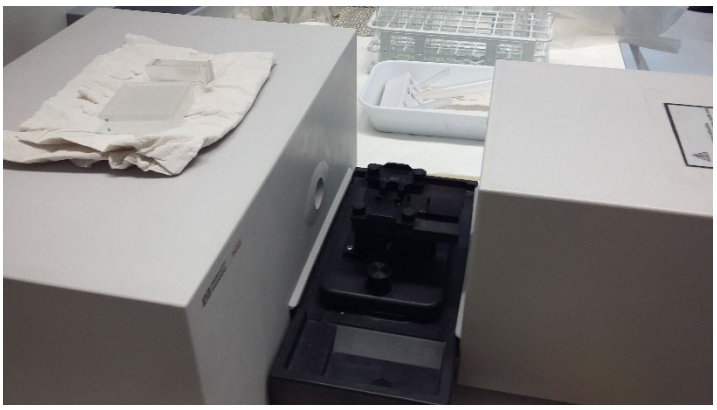

Fig. 3. HP 8433 UV-visible spectrophotometer.

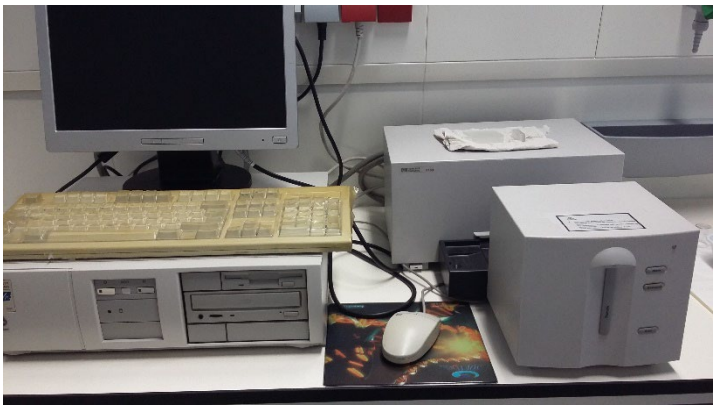

Fig. 4. HP 8433 UV-visible spectrophotometer.

3.6. Determination of growth and biofiltration rates

a) Specific Growth Rate (%·d^{-1}):

$$GR = (100 * \ln(\frac{W_f}{W_o}))/t$$

Where:

-**W_f**: final weight -**W_o**: initial weight -**t**: elapsed time

The wet weight was obtained using a precision balance (Figure 5). Before the disks were weighted, the water excess was retired from them by applying pressure on them with dry filter paper.

b) Removal Rate (mg·L^{-1}t^{-1}):

$$RR = \frac{C_i - C_f}{t}$$

Where:

-**C_i**: initial concentration -**C_f**: final concentration -**t**: elapsed time

This formula must be applied twice, once for the N and again for the P.

c) Removal Efficiency (%):

$$RE = \left(\frac{C_i - C_f}{C_i}\right) \cdot 100$$

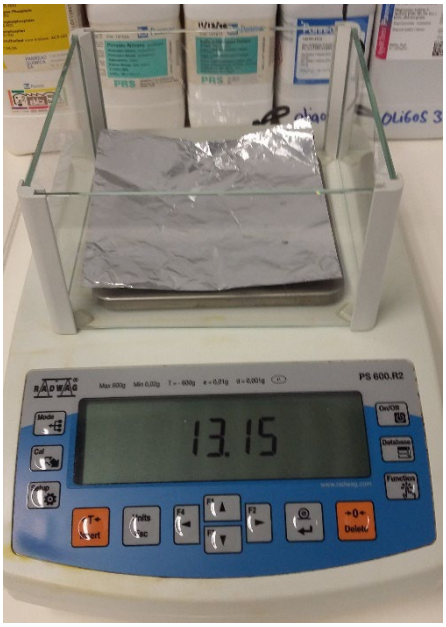

Fig. 5. Precision balance.

3.7. Statistical treatment

After the previous study of the parameters (variance homogeneity and normality hypothesis were fulfilled), the results were obtained through an ANOVA with a significance level of 95%. The data was also contrasted with a Tukey test. The statistical program used to do these tests was Minitab 18, while the graphs were obtained with Microsoft Excel 2010.

4. RESULTS

4.1. Growth Rates

4.1.1 Blue light *vs* White light

Growth rates between blue and white lights (Figure 6) are statistically different (p-value=0.000). Blue light seems to allow the algae to grow faster than white light.

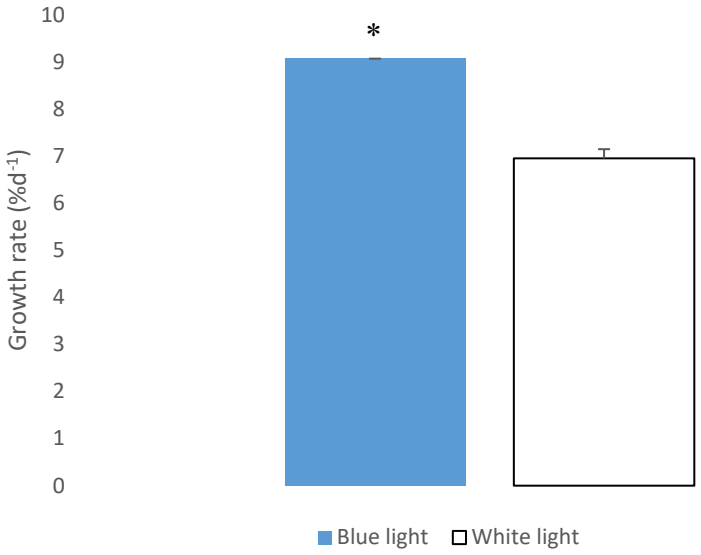

Fig. 6. Comparison of growth rate (GR) with blue and white light.

4.1.2. Blue light *vs* Magenta light

Blue light has statistically higher GR than magenta light (p-value=0.000) (Figure 7).

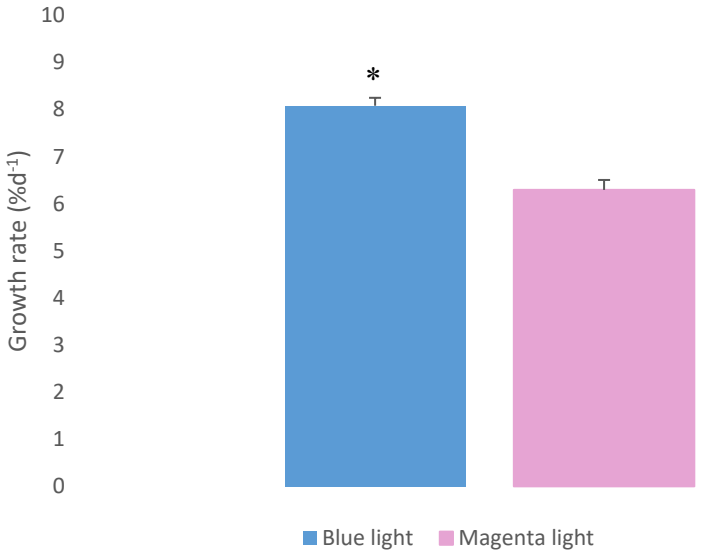

Fig. 7. Comparision of growth rate (GR) between blue and magenta light.

4.1.3. Blue light *vs* Red light

The difference between blue and red light is obvious (p-value=0.000), being the GR higher in blue light than in red light (Figure 8).

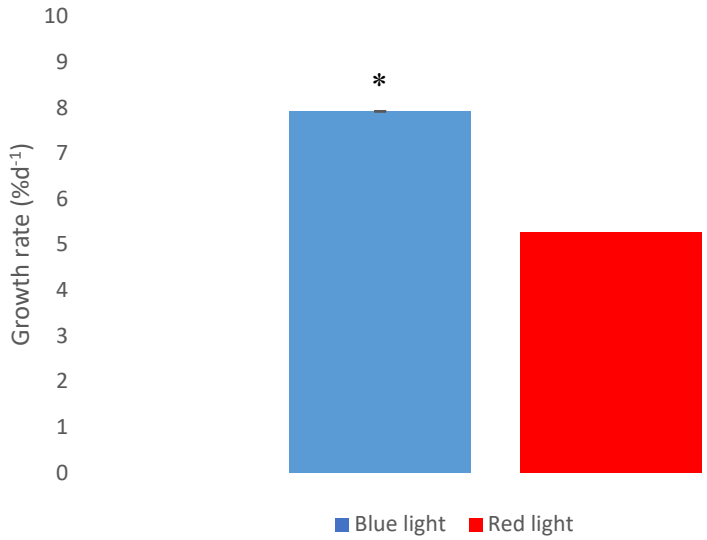

Fig. 8. Comparison of growth rate (GR) between blue and red light.

4.2. N removal rate (RR) and removal efficiency (RE)

4.2.1. Blue light *vs* White light

The Removal Rates in blue light and white light (Figure 9) are not significantly different (p-value=0.119).

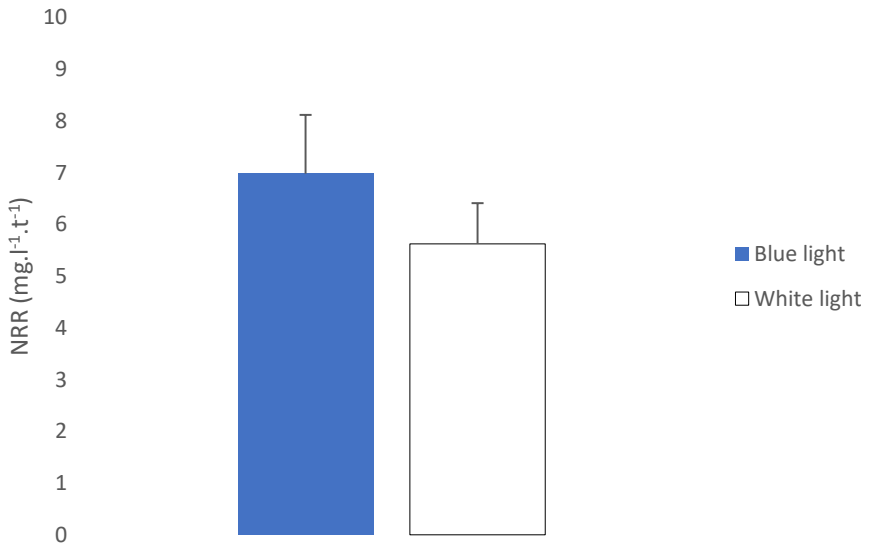

Fig. 9. Comparison of Nitrogen Removal Rates (NRR) between blue and white light.

As for the Removal Eficiency (Figure 10), there is no statistical evidence to prove that blue light and white light have different NRE (p-value=0.119).

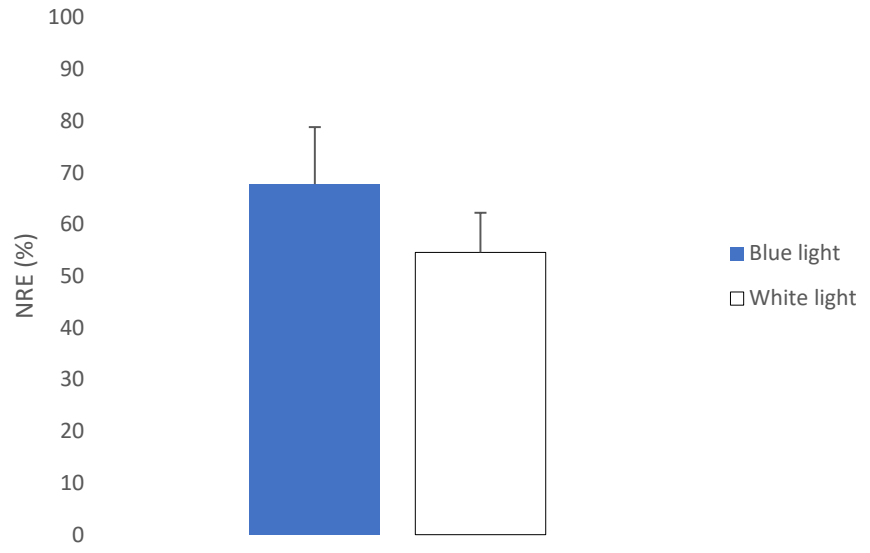

Fig. 10. Comparison of Nitrogen Removal Eficiency (NRE) between blue and white light.

4.2.2. Blue light *vs* Magenta light

The Nitrogen Removal Rates in blue light and magenta light (Figure 11) are significantly different (p-value=0.000), being blue light the one with the highest NRR.

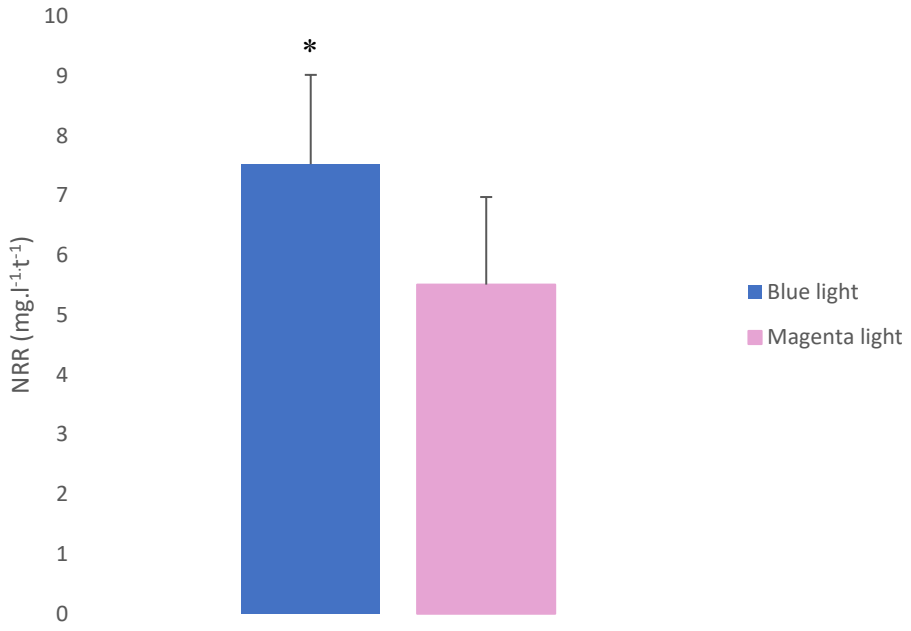

Fig. 11. Comparison of Nitrogen Removal Rates (NRR) between blue and magenta light.

The Removal Efficiency (Figure 12) also shows a significant diference (p-value=0.000) between the different light colors. Blue light has a higher NRE than magenta light.

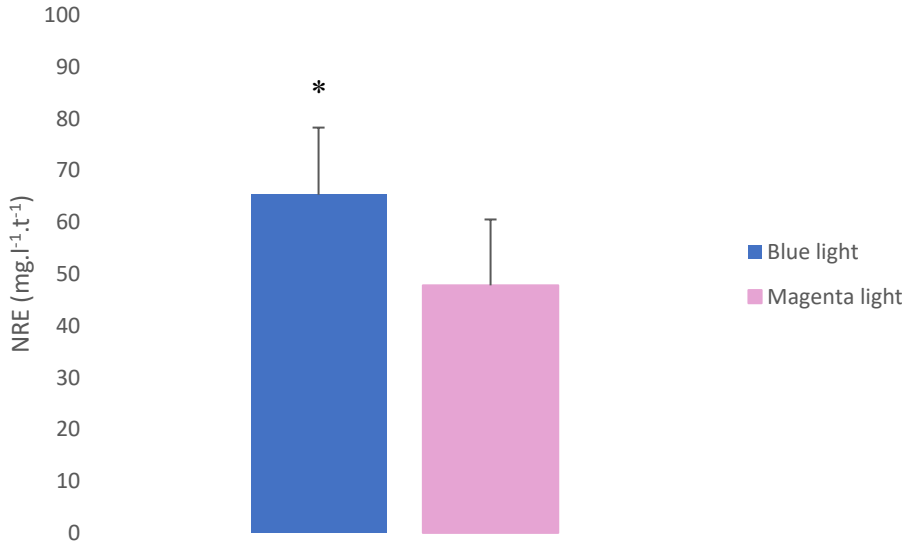

Fig. 12. Comparison of Nitrogen Removal Efficiency (NRE) between blue and magenta light.

4.2.3. Blue light *vs* Red light

The differences in Removal Rates between blue and red light (Figure 13) are statistically significant (p-value=0.000). Blue light has a higher NRR than red light.

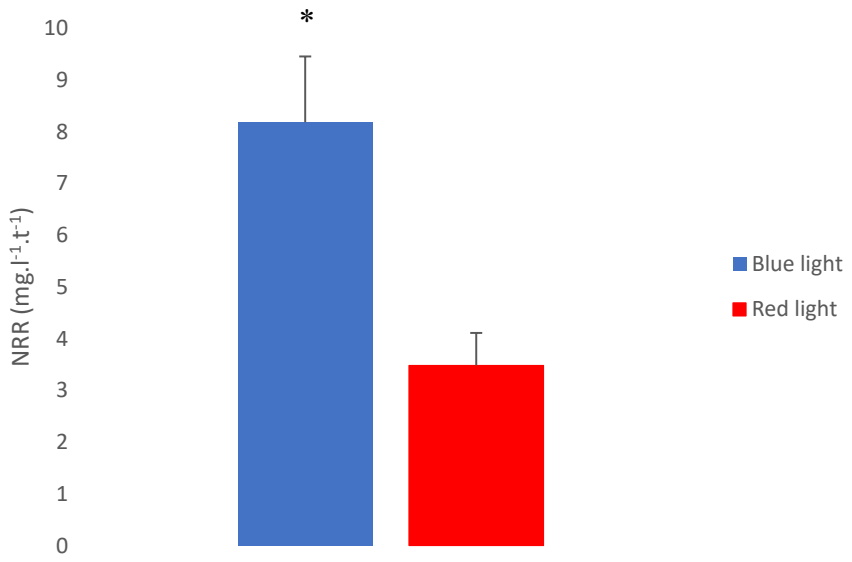

Fig. 13. Comparison of Nitrogen Removal Rates (NRR) between blue and red light.

The Removal Efficiency (Figure 14) is also significant different (p-value=0.000), being higher in blue light than in red light.

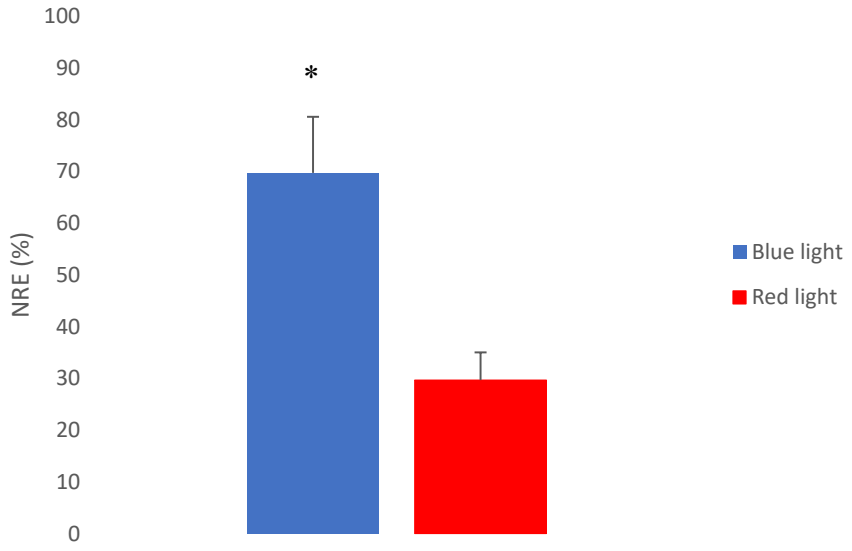

Fig. 14. Comparison of Nitrogen Removal Efficiency (NRE) between blue and red light.

4.3. P removal rate (RR) and removal efficiency (RE)

4.3.1. Blue light *vs* White light

There is a significant difference (p-value=0,000) between the PRR of blue and white light (Figure 15), being blue light the one with the highest PRR.

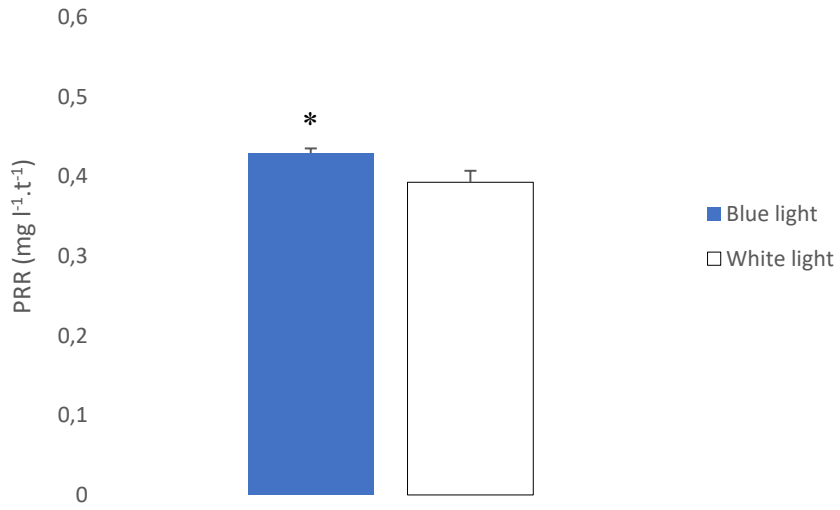

Fig. 15. Comparison of Phosphorus Removal Rate (PRR) between blue and white light.

The Removal Efficiency is also different (p-value=0,000) between blue and white light (Figure 16). Blue light has a higher PRE than white light.

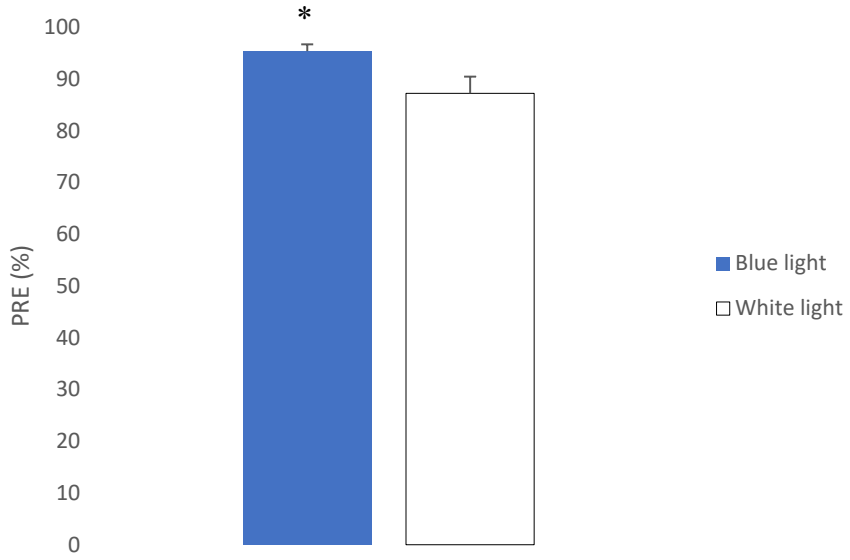

Fig. 16. Comparison of Phosphorus Removal Efficiency (PRE) between blue and white light.

4.3.2. Blue light *vs* Magenta light

The Removal Rate is significantly different (p-value=0,000) between blue and magenta light (Figure 17). Blue light has a higher PRR than magenta light.

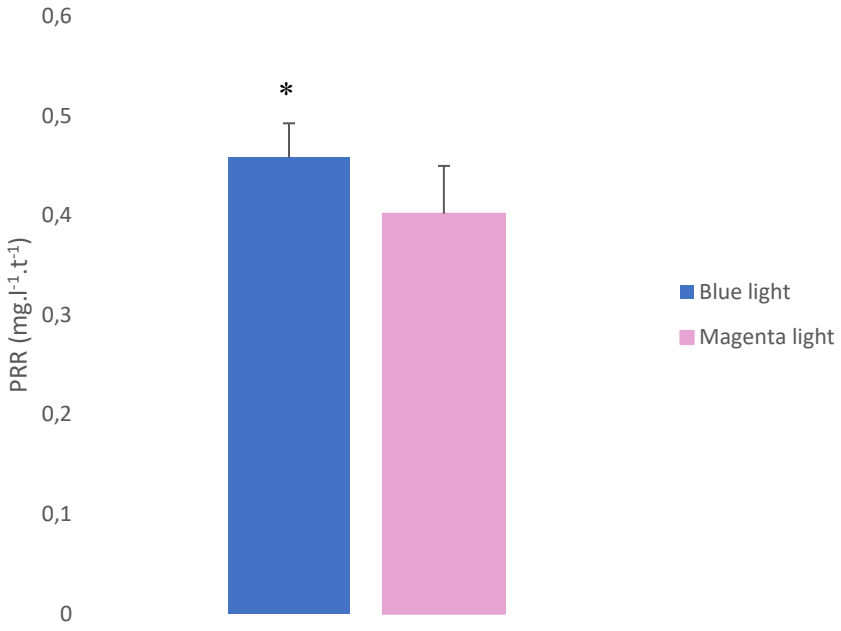

Fig. 17. Comparison of Phosphorus Removal Rate (PRR) between blue and magenta light.

The Removal Efficiency between blue and magenta light (Figure 18) is also statistically different (p-value=0,000). Blue light has a higher PRE than magenta light.

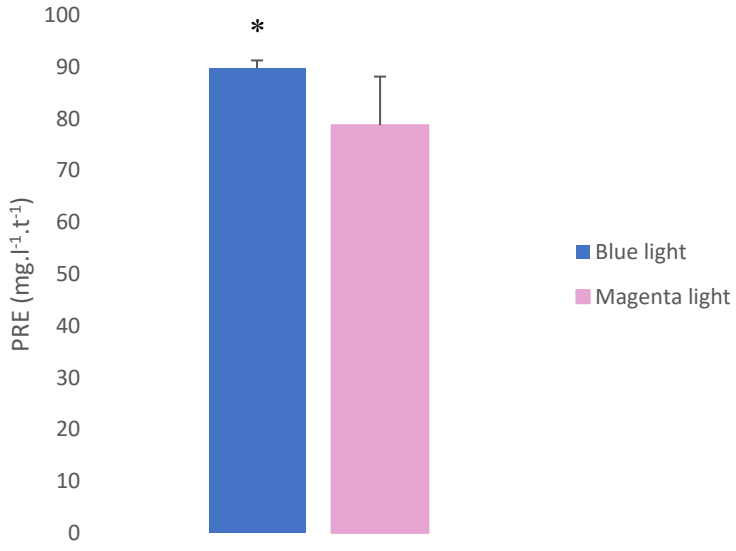

Fig. 18. Comparison of Phosphorus Removal Efficiency (PRE) between blue and magenta light.

4.3.3. Blue light *vs* Red light

The phosphorus Removal Rate is significantly different (p-value=0,000) between blue and red light (Figure 19). Blue light has higher levels of PRR than red light.

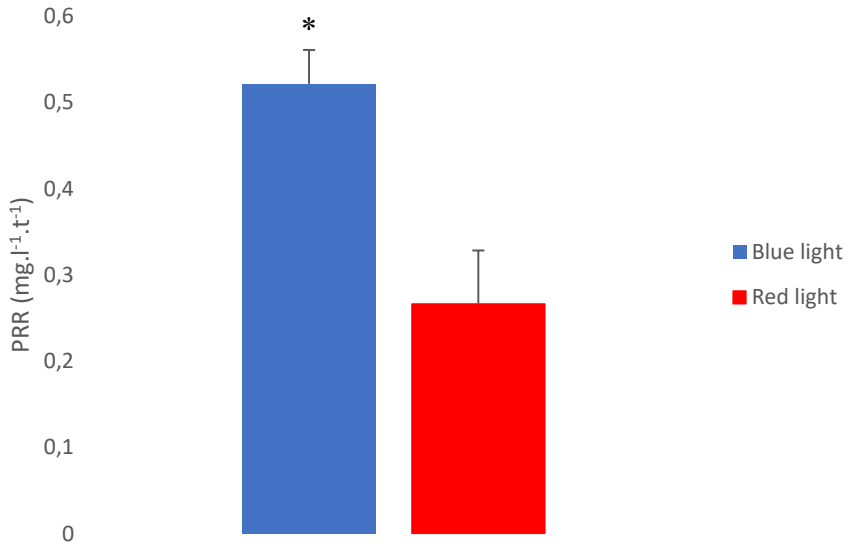

Fig. 19. Comparison of Phosphorus Removal Rate (PRR) between blue and red light.

The Removal Efficiency is also different (p-value=0,000) between blue and red light (Figure 20). Blue light has higher PRE levels than red light.

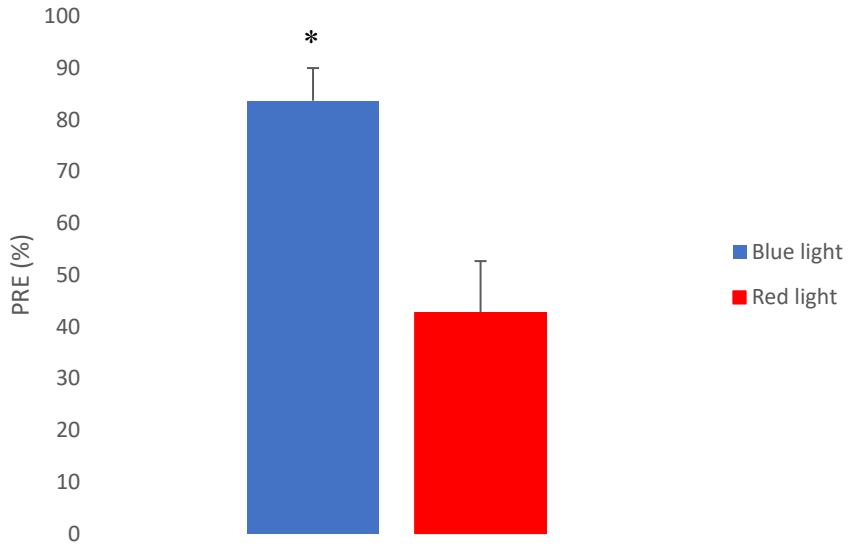

Fig. 20. Comparison of Phosphorus Removal Efficiency (PRE) between blue and red light.

5. DISCUSSION

5.1. Growth rate

There seems to be a clear light type that allows growth more than the others: blue light. *Ulva ohnoi* shows a greater growth rate under blue light conditions and the slowest growth rate under red light conditions. This results are different from those obtained in experiments carried out with microalgae, in which the highest growth was attained under red light conditions (Satthong et al., 2019). However, the results obtained resemble the ones in other experiments with macroalgae like *Porphyra leucosticta* (Soluna Salles & Figueroa, 1996) and *Ulva pertusa* (Le et al., 2018), in which red light shows the slowest growth rate and blue light shows the fastest.

5.2. Nutrients RR and RE

Nitrogen RR and RE acquire the highest value in blue and white light, and the lowest in red light. Phosphorus RR and RE have their highest values under blue light, followed by white light. The lowest PRR and PRE values are also obtained under red light conditions. Another factor to take into account are the microalgae that can be present in the culture tank. Blue light inhibits their growth whilst red light promotes it (Luimstra et al., 2018). This makes blue light not only good for the nutrient removal rates of *Ulva ohnoi* but also a limiting factor for microalgae that grow in the tank.

6. CONCLUSIONS

Blue light condition allows *Ulva ohnoi* to grow faster and to have a greater nutrient removal rate and efficiency, which makes blue light more suitable than other light colors. White light also shows great nitrogen RR and RE, but it does not achieve the same growth rate due to its lower PRR and PRE levels. Another possible reason that would explain the lower growth rate presented into white light conditions could be a photoinhibition effect caused by the high intensity to which the algae were exposed (nearly twice as much as in blue light). Red light shows the lowest levels both in growth rate and nutrient absorption, being the worst light color at the time of creating an *Ulva ohnoi* culture. This biological advantages are also accompanied by greater energy efficiency, as blue light shows the lowest net consumption values (11.9W/h or 0.0119kW·h^{-1}). Therefore, the use of blue light in the industry as a replacement of common white light will cause an increase in the productivity (growth rate) and a decrease in the energy usage in order to obtain this, which can be translated into a higher economic benefit.

Las condiciones de luz azul permiten a *Ulva ohnoi* crecer más rápido y poseer mayores tasas de eliminación y eficiencia de eliminación de nutrientes, lo cual hace a la luz azul más adecuada que otros colores de luz. La luz blanca también presenta buenos RR y RE de nitrógeno, pero no alcanza la misma tasa de crecimiento debido a su menor PRR y PRE. Otra posible razón que explicaría la menor tasa de crecimiento que presenta la luz blanca rente a la luz azul podría ser un efecto de inhibición causado por la alta intensidad a la que las algas han sido expuestas (casi el doble que bajo la luz azul). La luz roja muestra los niveles más bajos de crecimiento y absorcion de nutrientes, siendo el peor color de luz a la hora de cultivar *Ulva ohnoi* de manera industrial. Las ventajas biológicas se ven acompañadas de una mayor eficiencia energética, ya que la luz azul muestra los valores más bajos de consumo neto (11,9W/h o 0,0119kW·h^{-1}). Por lo tanto, el uso de la luz azul en la industria como un reemplazo de las luces blancas comúnmente empleadas resultaría en un aumento de la productividad (tasa de crecimiento) y un descenso del uso de energía necesario para conseguir esto, lo que puede traducirse en un mayor beneficio económico.

As condicións de luz azul permiten a *Ulva ohnoi* crecer máis rápido e posuer maiores taxas de eliminación e eficiencia de eliminación de nutrientes, o cal fai á luz azul máis adecuada que outras cores de luz. A luz branca tamén presenta bos RR e RE do nitróxeno, pero non alcanza a mesma taxa de crecemento e presenta unha menor PRR e PRE. Outra posible razón que explicaría a menor taxa de crecemento que presenta baixo a luz branca podería ser un efecto de inhibición causado pola alta intensidade á que as algas foron expostas (casi o dobre que baixo a luz azul). A luz vermella mostra os niveis máis baixos de crecemento e absorción de nutrientes, sendo a peor cor de luz á hora de cultivar industrialmente *Ulva ohnoi*. As vantaxes biolóxicas venríanse acompañadas dunha maior eficiencia enerxética, xa que a luz azul mostra os valores máis baixos de consumo neto (11,9W/h ou 0,0119kW·h^{-1}). Polo tanto, o uso da luz azul na industria como un reemplazo das luces brancas comunmente empregadas resultaría nun aumento da productividade (taxa de crecemento) e un descenso do uso de enerxía necesario para conseguir isto, o que pode traducirse nun maior beneficio económico.

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