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Influence of cultivation conditions on the bioenergy potential and bio-compounds of *Chlorella vulgaris*

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Abstract

This study aims to evaluate the influence of cultivation conditions on the bioenergy and high value biocompounds contents of *Chlorella vulgaris*. Results show that the use of nitrate rich media, from 170.7 mg/L, favors a faster biomass growth, reaching values above 800 mg/L biomass. In addition, it favors higher pigments concentrations with more emphasis for the cultures with a nitrate concentration of 569 mg/L, where chlorophyll-a and carotenoids reached maximum concentrations of 6 and 2 mg/L, respectively. As regards the lipid content, nitrate deprivation (<28.4 mg/L) favors the accumulation of lipid content by microalgae (around 42%). The use of media with lower iron concentrations (0.5 mg/L) was favorable for obtaining biomass with higher concentrations of chlorophyll-a, at an initial stage, with values varying from 0.2 to 0.6 mg/L. In the tests carried out under mixotrophic conditions (addition of glucose), it was observed that contamination occurred in all the cultures, possibly due to the high concentration of carbon source that had values between 0.5 and 1.5 g/L of glucose, and consequently, growth decreased.

1. Introduction

One of today's society main challenges is to find sustainable ways for obtaining products from renewable resources [1]. In particular, sustainable and ecologically conscious energy can be obtained from renewable resources or from more efficient processes in which concerns its production and use [2]. In this respect, microalgae represent a great potential as an alternative raw material for various products, obtained through a biorefinery process [3].

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Microalgae are microorganisms capable of photosynthetically capture, fix and transform carbon dioxide into organic compounds, thus producing biomass that can be used as a source of energy and food, and even to extract compounds with a diverse range of applications [4,5]. All these characteristics make microalgae the perfect organism for the use of new technologies and as a feedstock for valuable products [6].

Microalgae accumulate different compounds, e.g. lipids, fatty acids, pigments, polysaccharides, proteins and carbohydrates, with different applications, which fit perfectly with the search for new natural ways of obtaining energy, food resources and products of high commercial value such as pigments, supplements and cosmetics [7]. Thus, a biorefinery is the most sustainable way to recover and process them, as different routes are possible and an integrated approach is needed [8].

In a biorefinery all biomass is used, leading to lower production cost and more revenue streams, considering that a broad spectrum of marketable products is obtained through a variety of processes and unit operations [9]. Knowing the biomass biochemical composition, one can correctly select the most adequate processing techniques for obtaining the desired products. Making these processes sustainable is currently one main challenge, allowing implementation of industrial scale biorefineries [10]. Hence, the present study aims to evaluate the influence of cultivation conditions on the bioenergy and high value biocompounds from the microalgae *Chlorella vulgaris*, evaluating different stress scenarios and analyzing how the culture medium composition and the cultivation conditions influence the production of biomass and of other compounds of interest such as pigments and lipids.

2. Materials and methods

The microalgae *Chlorella vulgaris* used in this work was acquired at the SAG library (Sammlung von Algenkulturen der Universität Göttingen — Algae culture collection, University of Göttingen, Germany). Inoculations were initially done in test tubes in 12 ml of BBM — Bold's basal medium, prepared as described in [5].

After two weeks, these cultures were used to inoculate 250 mL flasks, in the proportion of 20% of the volume of inoculum and 80% of the volume of BBM medium. All cultures were kept under the same conditions of temperature, in a controlled climate, with air conditioning at 24 ± 2 °C, aeration by air pumps (BOYU-S4000p), use of filters for the incoming air to the cultures, and lighting with a luminous intensity between 3700 and 3900 Lux, with photoperiods of 12 h/12 h of artificial light.

After obtaining sufficient volume of dense culture, to be able to make multiple replications and begin the trials, the *Chlorella vulgaris* cultures were exposed to three different scenarios of nutritional stress (Table 1), each one with three different nutrient concentrations, for each condition (C1, C2, C3).

Table 1. Scenarios of nutritional stress, using the BBM as the reference medium.

	Glucose ($C_6H_{12}O_6$) (g/L)	Iron ($FeSO_4$) (mg/L)	Nitrate ($NaNO_3$) (mg/L)
BBM reference	–	1.0	56.9
Condition C1	0.5	10.0	170.7
Condition C2	1.0	50.0	569.0
Condition C3	1.5	0.5	28.4

These scenarios were prepared in the same way as the BBM [5], with some changes in the concentrations of glucose, iron and nitrate. For example, in the preparation of cultures with iron stress, a condition (C1) was prepared with 10 times more volume of reagent $FeSO_4$ than in the original BBM medium. Similarly, for the preparation of the nitrate stress media, the amount of $NaNO_3$ was tripled to condition C1, and so on. For the media with iron addition, the pH of the media was controlled with the addition of sodium hydroxide (NaOH) in order to obtain the same pH for the different conditions.

The microalgae growth was monitored at the microscope and evaluated by following the cultures optical density, measured as the absorbance by spectrophotometry, in a UV-1700 PharmaSpec spectrophotometer (at 685 nm of wavelength).

After biomass harvesting, the Chlorophyll-a (Cca), Chlorophyll-b (Ccb) and Carotenoids (Caro) were measured as described in [11] and the total lipid yields were obtained after lipid extraction by using the experimental procedure described in [12].

3. Results and discussion

The microalgae *Chlorella vulgaris* was subjected to three stress scenarios with glucose, iron and nitrate, each one with four different conditions (Reference, C1, C2, C3). Trials were done in triplicate for each concentration and the average values of biomass concentration per day are presented in the growth curves figures. In addition to biomass growth, another parameter evaluated was the concentration of pigments in the microalgae, for the iron and nitrate stress conditions. The absorbance was measured in triplicate and the concentrations of Cca, Ccb and Caro were determined [11]. The lipid content was also evaluated for all the cultures and conditions [12].

3.1. Effect of glucose addition

The first stress scenario considered the addition of glucose at three different concentrations, a condition C1 with glucose concentration in BBM medium of 0.5 g/L, a condition C2 with a concentration of 1.0 g/L and a condition C3 with a concentration of 1.5 g/L. Trials were carried out in triplicate for each concentration resulting in a total of twelve 250 mL flasks (bioreactors). After the addition of glucose to the bioreactors, it was possible to observe the formation of microalgae clusters and that the culture color changed to yellowish, resulting in the contamination and death of the cultures. Fig. 1 shows the growth curves for the three conditions C1 to C3 and Fig. 2 shows the visual appearance of the cultures after the glucose addition. Only for the reference culture, without addition of glucose to the medium, it was possible to observe the biomass growth.
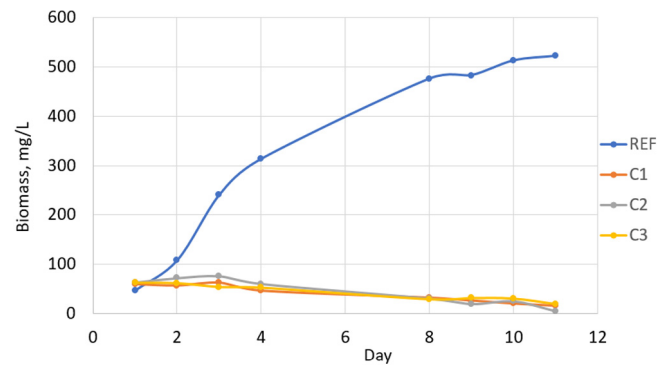

Fig. 1. Growth curves of microalgae *Chlorella vulgaris* for the glucose nutritional stress.
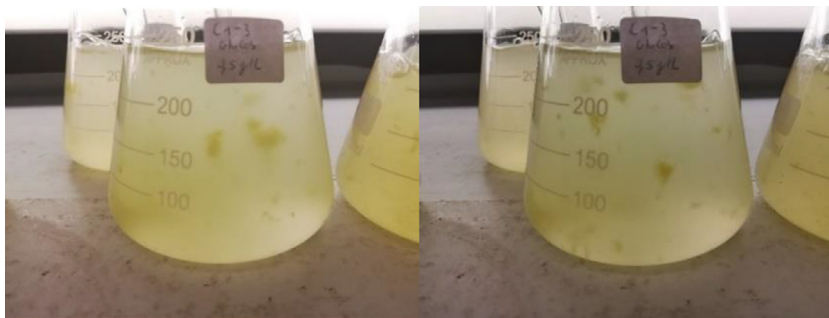

Fig. 2. Cultures of microalgae *Chlorella vulgaris* in 250 mL flasks for the glucose nutritional stresses, showing the formation of microalgae clusters, color change to yellowish, resulting in the contamination and death of the cultures.

3.2. Effect of iron addition

The second stress scenario was with the addition of iron in three different concentrations, a condition C1 with 10 times more FeSO$_4$ (10 mg/L) than the reference medium BBM (1.0 mg/L), a condition C2 with 50 times more

$FeSO_4$ (50 mg/L) and a condition C3 with half the concentration of $FeSO_4$ (0.5 mg/L). Fig. 3 shows a similar exponential growth phase for all the cultures. However, between day 10 and 15 the cultures C1 to C3 reached a growth peak, after which all decayed, while the reference culture continued to grow until day 22, where it reached a peak, but maintained the growth after that.
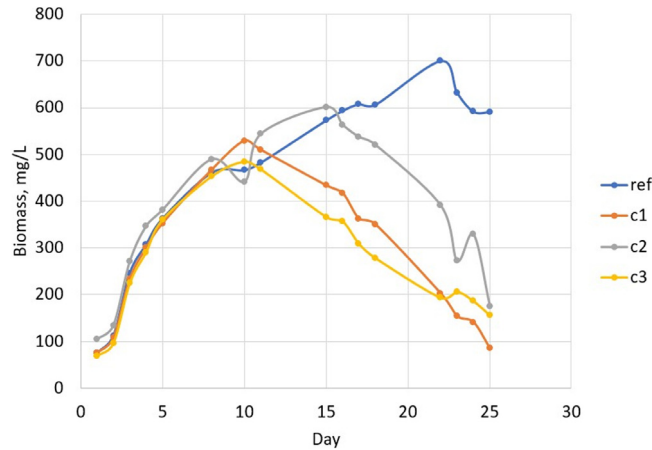

Fig. 3. Growth curves of microalgae *Chlorella vulgaris* for the iron nutritional stress.

Regarding the pigments, Fig. 4 shows an increase in their concentration from day 2 to 4 which depicts the need in the early days to use the photosynthetic capacity of the pigments for the microalgae growth. The concentration of Cca is always higher than those of Ccb and carotenoids Caro, which have similar values throughout the experiment. It is also possible to observe that for Cca in more than half the assay higher specific concentrations are recorded for C3 culture which corresponds to the culture with less iron concentration (0.5 mg/L) reaching values between 0.2 and 0.55 mg/L of Cca. Comparing for the various media with iron and the reference medium it is possible to conclude that the addition of iron does not induce a greater concentration of pigments by the microalgae.
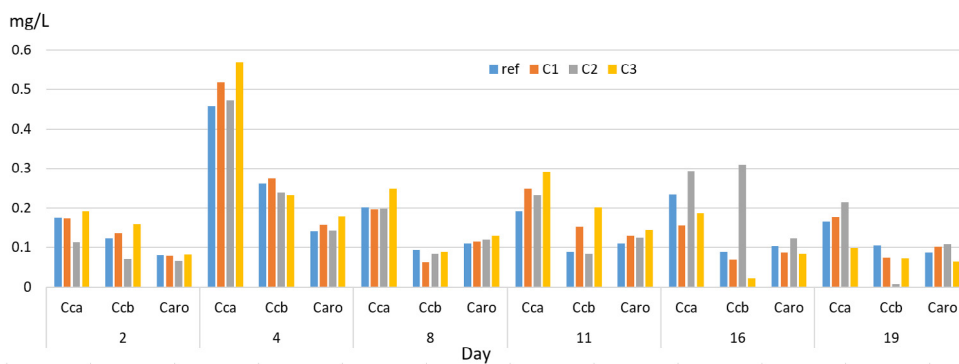

Fig. 4. Concentrations of Chlorophyll-*a* (Cca), Chlorophyll-*b* (Ccb) and Carotenoids (Caro) of microalgae *Chlorella vulgaris* for the iron nutritional stress.

Regarding the lipid content, for conditions C1, C2, C3 and reference it was obtained respectively 16, 12, 15 and 28%. Thus, it is not possible to conclude that the addition or limitation of iron influences the lipid accumulation by microalgae, and as the highest lipid content (28%) was obtained for the reference assay, it is accepted as the best medium with the adequate concentration of iron for a best microalgae growth and lipid content.

3.3. Effect of nitrate addition

The third stress scenario was with the addition of nitrate in three different concentrations, a condition C1 with 3 times the amount of nitrate (170.7 mg/L) compared to the reference medium BBM (56.9 mg/L), a condition C2 with 10 times more nitrate (569.0 mg/L) and a condition C3 with half the amount of nitrate (28.4 mg/L) compared to the BBM reference medium. Fig. 5 shows a better microalgae growth in the cultures with higher nitrate concentration (C1 and C2) than that of the reference culture. In addition to the increased biomass growth, it was also possible to observe a more intense green in the cultures with more nitrate, in particular for conditions C1 and C2 (as shown in Fig. 6).
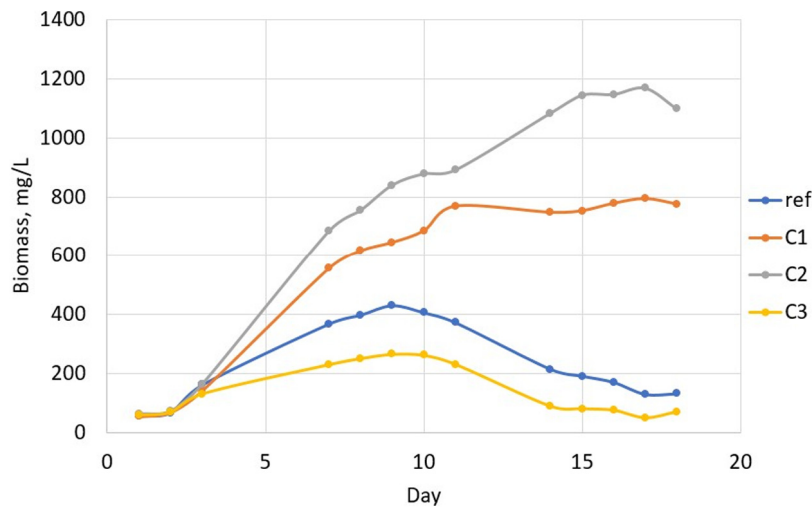

Fig. 5. Growth curves of microalgae *Chlorella vulgaris* for the nitrate nutritional stress.

Fig. 6. Cultures of microalgae *Chlorella vulgaris* in 250 mL flasks for the nitrate nutritional stress (from the left flasks include trials of C1, C2, C2 and C3).

As for the concentration of pigments in the assays with nitrate stress, we can observe in Fig. 7 a high production of Cca for condition C2, with the highest concentration of nitrate in the culture medium. Concerning the concentrations of Ccb and Caro, despite being at much lower concentrations than Cca, the highest concentrations are still recorded for C2, the medium with higher nitrate concentration.
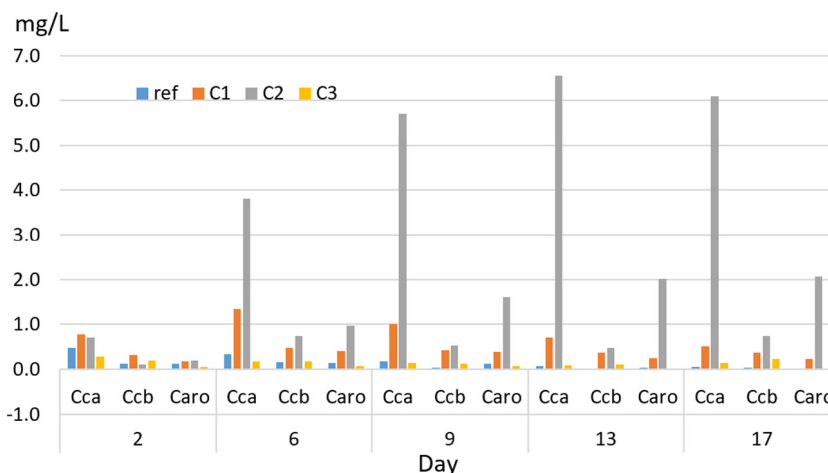
Fig. 7. Concentrations of Chlorophyll-*a* (Cca), Chlorophyll-*b* (Ccb) and carotenoids (Caro) of microalgae *Chlorella vulgaris* for the nitrate nutritional stress.

Regarding the lipid content, for conditions C1, C2, C3 and reference it was obtained respectively 19, 16, 42 and 25%, which shows that the nitrogen deprivation in condition C3 induced the greatest lipid accumulation in microalgae of 42% [13].

4. Conclusion

In this study the microalgae *Chlorella vulgaris* was subjected to stress conditions aiming to evaluate the influence of cultivation conditions on its bioenergy and high value biocompounds contents. Different stress scenarios are evaluated and it is analyzed how the culture medium composition and cultivation conditions influence the production of biomass and of other interesting compounds such as pigments and lipids. It was possible to observe that the use of nitrogen sufficient media favors a faster biomass growth, as well as a higher pigments concentration. Results also showed that nitrate deprivation favors the increase of the lipid content and the use of media with deficient iron concentrations was favorable for obtaining biomass with higher concentrations of chlorophyll-a at an initial stage.

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