Enhancing Microalgae Cultivation using Biowastes as Growth Media for High Added-Value Co-Products Generation

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Research Article

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Abstract

This paper aims to study a new growth media using cheese whey and drainage water from agriculture for indigenous microalgae cultivation for value-added product generation. In this context, four combinations are studied beside the BG11 as reference, where BG11/Cheese whey (60/40, *%v/v*), drainage water 100%, drainage water/Cheese whey (60/40, *% v/v*), and Cheese whey 100 % have been used. In this regard, investigated parameters were biomass dry weight, pH variation, total chlorophyll and carotenoid content.

Results showed that used growth media have a significant impact on microalgae culture, particularly in terms of biomass production, pigment content and pH variation. Cheese whey and BG11/Cheese whey mixture presented the best performance for biomass yield. BG11/Cheese whey mixture (60/40, *% v/v*) showed the best impact for total chlorophylls and carotenoid content. Moreover, the mixture, cheese whey/drainage water (60/40, *%v/v*) present a positive effect on pigment content. The use of cheese whey and drainage water lead to enhance the biomass and pigment production.

This study showed that using agro-industrial carbon-rich wastes and drainage water enhanced microalgae biomass and pigment content, thus contributing to pollution abatement. This will contribute to both reducing the cost of production and resources recycling.

Keywords: Indigenous microalgae, Co-products, Carotenoids, Chlorophylls, Biowastes, pollution abatement, resources recycling

1. Introduction

Microalgae is actually considered as a promising biocatalysts to be implemented in the increasing field of "White Biotechnology" mainly to support the production of food, feed, fine chemicals, and diverse "green energy carriers" (Koller et al. 2014). Furthermore, due to its versatile composition, microalgal biomass is presented to be one of the most promising biorefinery feedstock providing alternatives for different areas, such as food, feed, cosmetics and health industries, fertilizers, plastics, and biofuels. Additionally, microalgae can also be integrated into the process of wastewater treatment and CO_2 sequestration (Gorry et al. 2018).

In this regard, pigments production from microalgae have received a particular interest due to its high market values where three major groups are distinguished in microalgae, namely carotenoids, phycobilins, and chlorophylls (Koller et al. 2014), (Ventura et al. 2017). Furthermore, chlorophyll and carotenoids from microalgae are well established, and its global market has been expanding because of its wide application in human healthcare, food industries, and cosmetics. Likewise, carotenoids are commercially available in several forms, including mainly beta-carotene, lutein, lycopene, astaxanthin, zeaxanthin (Mehta et al.,2018 ; da Silva Ferreira and Sant'Anna 2017). Moreover, the addition of pure carotenoids is a common practice as fish feed for some aquaculture species since it is known to improve carcass and antioxidant capacity (Rosas et al. 2019).

However, carbon, nutrient, and water requirements for microalgae cultivation are still a major bottleneck for an efficient and cost-effective large-scale microalgae biomass production (Kannah et al. 2018). Alternatively, agro-industrial carbon-rich wastes (Biowastes) and wastewaters as source of nutrients are representing an interesting approach to boost algae production with a particular interest for high value added co-products such as pigments, to improve the economics and the sustainability of microalgae biorefinery for an efficient integration with industrial process (Salati et al. 2017); León-Vaz et al. 2019). Moreover, further optimizing culture conditions, by selecting organisms that can overcome the limitations imposed by ambient conditions and that present high pigment content could contribute to lower the cost of microalgae biomass in a biorefinery frame.

This paper aims to study a combination mixture BG11 growth media, cheese whey and drainage water issued from agro-industrial activities, with different proportions. These growth media have been used for an indigenous microalga strain cultivation, *Chlorella sp.,* for biomass and its high added-value co-products especially chlorophylls and carotenoids. Therefore, cheese whey and drainage water are used as organic carbon and nutriments sources respectively for microalgae cultivation. Moreover, this study investigates the possibility to integrate microalgae biomass production into an existing industrial sector and wastes recycling to preserve natural resources.

2. Material and Methods

The microalgae used in this study was an indigenous strain of *Chlorella sp*., previously isolated from a local environment and maintained in Erlenmeyer flasks with BG11 medium. Erlenmeyer flasks of 500 mL were incubated on an orbital shaker under 150 rpm continuous agitation

(DaihanScientfic and SHR Digital Shaker, Korea), light intensity of 6000 Lux (add light source) measured on the external surface of the flasks using a photo flux meter (Model Testo 545 GmbH and CO, Germany**)**. The initial cellular concentration was set approximately to 2 x 10^6 cells/mL. The pH of the culture broth was measured using a pH meter (Starter3100, OHAUS corporation, USA).

Four combinations of growth media were studied beside the BG11 growth media as a reference. BG11/Cheese whey (60/40 % vv) [BG11/CW], Drainage water 100% [DW100], Drainage water/Cheese whey (60/40 % vv) [DW/CW], and Cheese whey 100 % [CW100] are carried out for microalgae characterization aiming the production of high benefit co-products. In order to reduce bacteria contamination, the DW was filtered with 0.45 µm vacuum filter holder (Sartorius stedium biotech GMBH-D-37070, Goettingen) and the CW was autoclaved at 120 °C for 20 minutes.

The mixotrophic and autotrophic cultures were kept under continuous illumination. The cultures were sampled every two days to monitor *Chlorella sp.* growth. Biomass concentration was estimated by measuring the optical density at 680 nm with UV/Visible spectrophotometer (Lambda 25 UV-visible spectrophotometer, PerkinElmer life and Analytical, UK). Microalgae cells were washed 3 times with an equivalent volume of distilled water to avoid excess of sugars and salts (centrifuged for 5 min at 4000 rpm between each washing). Culture samples were then dried for 24 h at 70 °C. A good linear regression fit was obtained between the dry weight and optical density measurements at 680 nm (R^2 value of 0.86). The final biomass yield was calculated subtracting the initial biomass used as inoculum and the final biomass at the end of the culture.

The Pigment content determination was carried out according to (Pruvost et al. 2011) using a spectrophotometric method and the following equations are used to determine Chlorophyll a,b and carotenoids.

$$[\text{Chl-a}]\ (\mu g/mL) = -8.0962 \times A_{652} + 16.5169 \times A_{665}; \quad \text{Eq. 1}$$

$$[\text{Chl-b}]\ (\mu g/mL) = 27.4405 \times A_{652} - 12.1688 \times A_{665}; \quad \text{Eq. 2}$$

$$[\text{Carotenoids}]\ (\mu g/mL) = 4 \times A_{480}. \quad \text{Eq. 3}$$

Where: A = the absorbance of the extract measured through 1 cm of solution at 652 nm, 665 nm, 480 nm

3. Results and discussion

3.1 *Chlorella sp.* growth under different growth media

The cultivation was conducted with the objective to investigate the impact of real growth media on the cell growth and adaptation capacity relative to conventional BG11 growth media. *Chlorella sp.* cultivation under different growth media showed a good growth in the CW100 growth media, where the maximum growth is achieved after the 4th day of culture. On the other hand, all growth media showed a general positive effect on the growth of *Chlorella sp.* relatively to reference BG11 media (Figure1). The good growth of *Chlorella sp.* in the CW100 growth media is explained partly by its high content in glucose. In this regard, Girard et al. 2014 found that the microalgae growth is affected by the lactose hydrolysis which makes glucose available as a carbon source in the growth media since the 3rd day of culture thus increasing biomass production. However, Salati et al., 2017 found that total phosphorus content in the growth media was the driving factor affecting algae growth under mixotrophic conditions while carbon availability (BOD_5/COD) did not cause any differences in algae kinetic growth parameters and final biomass concentration.

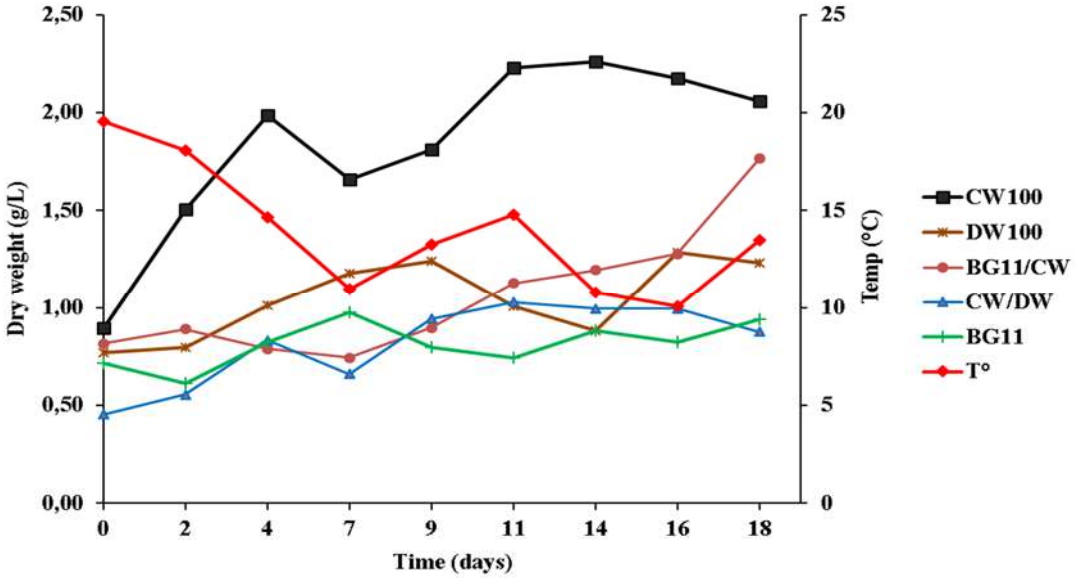

Figure 1: Dry weight evolution under different growth media combination

It should be noticed that culture growth was undertaken under ambient temperature where its range was from 10 °C to 18 °C (winter season). In this regard, temperature is an important factor affecting the microalgae growth and most microalgae prefer an ambient temperature of about 25 °C to 30 °C for maximal biomass production without affecting the photosynthetic

efficiency (Nagarajan et al. 2019). Likewise, temperature was found to have an impact on the biomass production and microalgae growth. This factor could explain the falling of growth since the 4th day due to temperature decreasing where microalgae cultivation was undertaken at the ambient temperature, where it could be seen the variation of biomass production which is decreased for CW100 media when the temperature is about 14 °C (figure 1). In addition, others growth media show a less microalgae growth due to carbon deficiency. In this context, García-Cubero et al. 2018 have found that the highest biomass productivity were noticed in the range of 15–25 °C.

3.2 pH variation during microalgae growth

The pH variation during culture shows a good stability for all growth media. Figure 3 shows a rapid increase in pH to stabilize near to 11 for both BG11 and BG11/CW growth media, while for others pH is stabilized between 9 and 10. Moreover, pH culture evolution indicates the capacity of this local microalgae to support alkaline conditions. In this regard, Vadlamani et al. 2017 have reported that alkaline conditions permit to improve the CO_2 dissolution and to keep an acceptable contamination level avoiding culture failure at large scale.

Chlorella sp. has showed a good adaptation with high value of pH where it went-up rapidly as shown in figures 1 and 2. In comparable conditions of high pH culture, Vadlamani et al. 2017 have found an equivalent biomass productivity in the range of 1.5 to 2 g/L despite low temperature during the culture in the CW100 medium. Likewise, Qu and Miao 2021 have found that microalgae cultivation under alkaline pH of 10 conducts to increase biomass yield of 14.1 % comparatively to pH of 7.5.

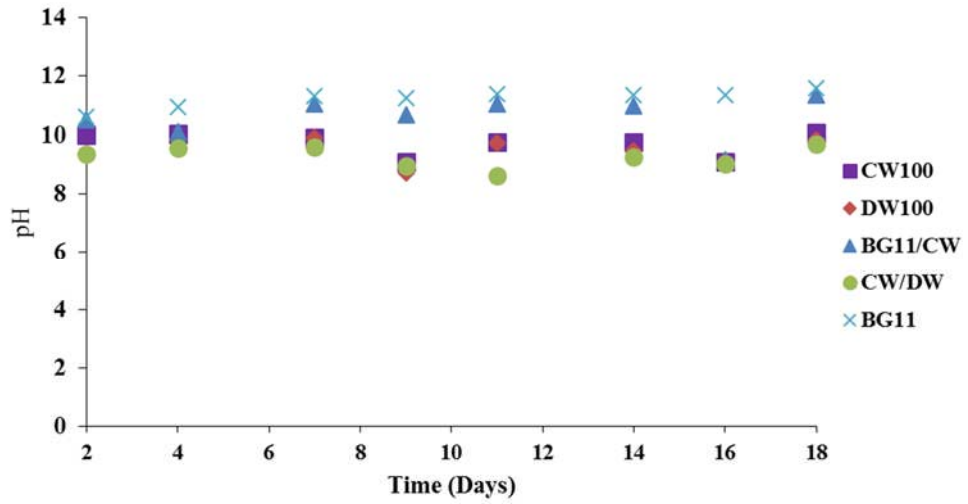

Figure 2: pH variation of different growth media combination

3.3 Final Biomass yield

Obtained results for biomass production expressed in terms of dry weight (g/L) showed a good growth with a high final biomass, which could reach five times more the biomass yield of BG11 growth media. According to figure 3, CW100 culture media showed the best final biomass concentration followed by BG11/CW. This result indicates that the CW100 growth media could be used as an alternative for microalgae cultivation and the *Chlorella sp.* is showing a good use of organic carbon, which presents a good ability for mixotrophic culture.

On the other hand, BG11/CW growth media showed a biomass concentration, better than that obtained using DW100 and CW/DW growth media (figure 3). All the combinations growth media used in this study are presenting the highest biomass production comparatively to BG11 growth media. Final biomass yield in mixotrophic cultivation condition using cheese whey as carbon source was obtained by Girard et al. 2014 to be 3.6 g/L after 13 days of culture using a cheese whey concentration of 40g/L. In this study, the obtained biomass yield in CW100 growth media is 1.16 g/L using a cheese whey concentration of 4g/L. This value may be due to low temperature during the cultivation period, knowing that temperature is a crucial factor for microalgae growth. In fact, a maximum biomass production is obtained for a temperature between 25 °C and 30 °C without affecting the photosynthetic efficiency (Nagarajan et al. 2019).

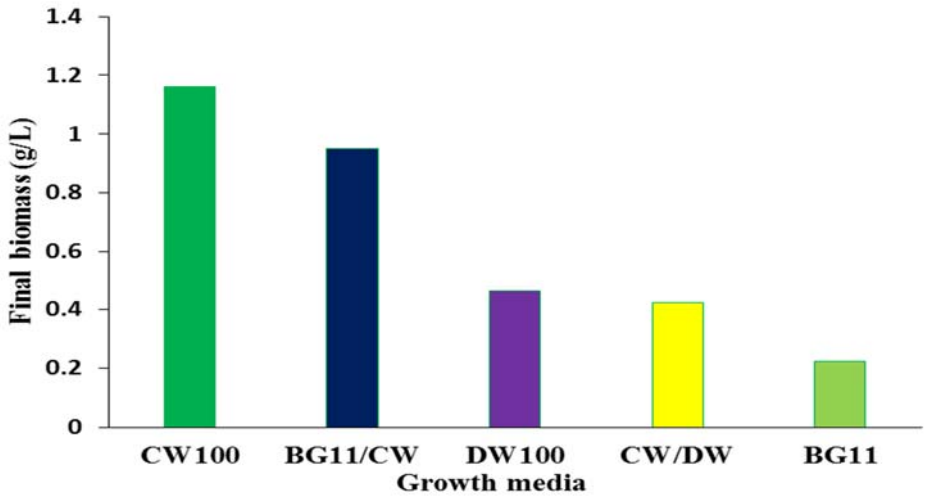

Figure 3: Biomass yield for different growth media combination

3.4 Pigments content determination

3.4.1 Total Chlorophylls

Chlorophyll is one of the valuable bioactive compounds that can be extracted from microalgal biomass. It is used as a natural food coloring agent and has antioxidant as well as antimutagenic properties (Hosikian et al. 2010). As shown in figure 4, total chlorophyll content under different growth media is important where it could reach a maximum of 20-31.58 mg/g dry weight at 9th day for BG11/CW, BG11, DW100, and DW/CW100. However, CW 100 presents a lower content of total chlorophyll. These obtained results are complying with those reported in literature, where total chlorophyll content for *Chlorella sp.* is found in the range of 22.6 to 32.4 mg/g dry weight (Figueroa-Torres, Gonzalo M. et al. 2020). However, *Chlorella vulgaris* has a content of total chlorophylls that varies from 0.3-15.4 mg/g dry weight (Safi et al. 2014).

Many parameters have been reported in order to promote the chlorophyll content in microalgae, including variation in light intensity, culture agitation, changes in temperature and nutrient availability. Nitrogen and phosphorus are essential nutrients for microalgae culture and then critical for the synthesis of chlorophyll molecules. In this regard, a deficiency in these two elements can induce respectively 64% and 67% reduction in chlorophyll content (da Silva Ferreira and Sant'Anna 2017). This could explain the low content of total chlorophylls in CW growth media where the total phosphorus and total nitrogen contents of cheese whey used in this study are 0.092 mg/L and 6.4 mg/L, respectively. The concentration of drainage water in total phosphorus and total nitrogen are 0.3 mg/L. These values are very low comparatively to BG11 growth media where the total phosphorus and total nitrogen are 0.21 g/L and 1.094 g/L, respectively.

High total chlorophyll content is explained by the glucose derived from cheese whey degradation as a carbon source at low concentration (4 g/L) which promotes the biosynthesis of chlorophylls. In addition, it has been found that all glucose could be exhausted within 7 days, which explains the maximum content observed during the 9th day of culture and the decreasing of the total chlorophyll after this period, maintaining its content almost the same with others growth media (Chai et al. 2018).

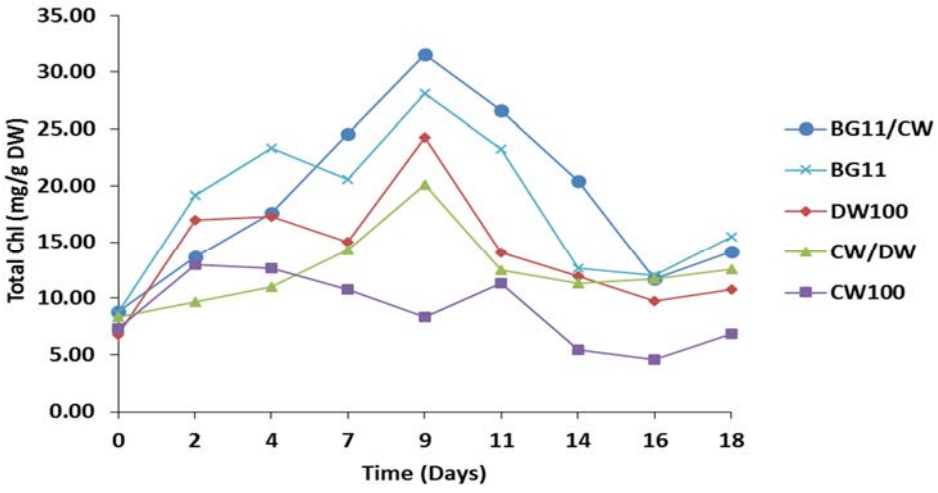

Figure 4: Chlorophylls content under different growth media combination

3.4.2 Total Carotenoids

Figure 5 shows the evolution of total carotenoids content under different growth media. Carotenoids content variation showed practically a good tendency for all growth media since the 7th day, which could reach more than 1% of dry weight even if the temperature is very low. However, it was found that growth media composed of BG11/CW presents the best content after the 7th day which is about 10.56 mg/g dry weight and stabilize around 9 mg/g dry weight. The total carotenoids for other used growth media are varying in a range of 3 to 5.5 mg/g dry weight from the 4th day until the end of cultivation. These obtained values are in the same range of those reported in literature where quantity of carotenoids extracted from *Chlorella sp.* in mixtrophic cultivation was found to be about 6.83 mg/g dry weight (Gong and Huang 2020). Furthermore, total carotenoids of 2.7-8.3 mg/g dry weight for chlorella genus were reported in the literature (Figueroa-Torres, Gonzalo M. et al. 2020).

High content of carotenoids in BG11/CW and BG11 growth media is affected by BG11 nitrogen availability, which is a major essential element for both cell growth and lutein accumulation. This latter is a primary carotenoid under no stress conditions, where a content of 8.39 mg/g of lutein is reported for *Chlorella sorokenia* (Xie et al. 2019). Moreover, it has been reported that increasing in glucose concentration increase lutein content (Minhas et al. 2016). Likewise, glucose derived from cheese whey degradation stimulates the microalgae to produce carotenoids (Chen et al. 2018; Gong and Huang 2020).

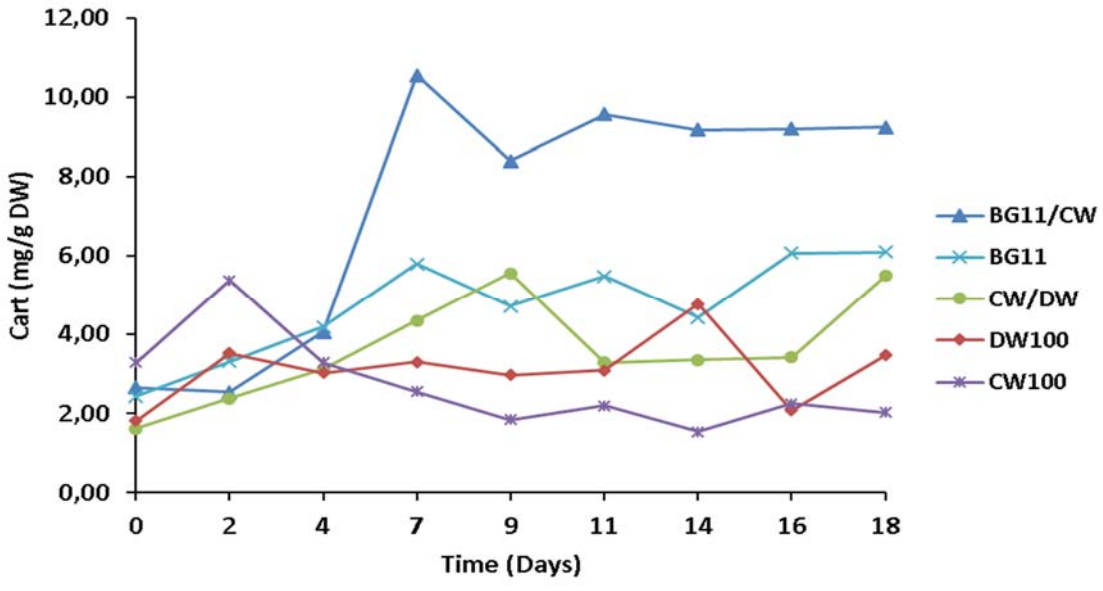

Figure 5: Carotenoids content under different growth media combination

4- Conclusion

Microalgae are considered as excellent candidates for bioactive compounds, yet microalgal residues remaining after the extraction of one or two compounds are usually discarded, which is not economical. The aim of this work was to evaluate new growth media combination issued from waste sources, used for microalgae cultivation for biomass and pigments production in the objective to decrease the cost of production and waste recycling, thus preserving natural resources. In this context, four combinations are studied beside the BG11 as reference, where BG11/Cheese whey (60/40, *%v/v*), drainage water 100%, drainage water/Cheese whey (60/40, *% v/v*), and Cheese whey 100 % have been used.

Results showed that new growth media used for microalgae cultivation have a significant impact on microaglae culture, particularly in terms of biomass production, pH variation support , chlorophylls and carotenoids content. In this regards, the mixture of BG11/Cheese whey (60/40% *vv*) shows the best performance for both biomass and pigments with a dry weight biomass of more than 2 mg/L, final biomass yield of 0.95 g/L, chlorophyll content of 31.6 mg/g dry weight, and total carotenoid content of 9 mg/g dry weight. Moreover, pH variation presents a good stability, which is an important factor for microalgae cultivation.

Using agro-industrial carbon-rich biowastes and drainage water as growth media for chlorophyll and carotenoid production under alkaline conditions represents a promising approach to boost microalgae integration in existing industries in a more sustainable way. Moreover, the use of high-performance strains with a high added-value products potential alongside the use of waste carbon and nutriment rich combination for mixotrophy microalgae cultivation would offer the most economic, environmental-friendly and sustainable algae-refinery. Nevertheless, further optimizing culture conditions by selecting strains is still needed to overcome the limitations imposed by ambient conditions.

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