Enhancement of indigenous microalgae culture using cheese whey as growth media for bioenergy and coproducts production

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Abstract. This study investigates the use of cheese whey to enhance the microalgae cultivation for bioenergy and coproducts in the framework of circular economy and pollution attenuation. A local isolated indigenous *Chlorella vulgaris* strain using a growth medium containing BG11 and cheese whey (BG11/CW) was used. Algae density, dry weight, organic carbon consumption, biochemical composition, fatty acid profile, Total pigments were investigated. The best growth is obtained in the BG11/CW culture media, with a dry biomass and cell density of 2.5 g/L, 6.5×10^7 Cells/ml, respectively. This represents 5 times the dry biomass obtained in the BG11medium (0.45 g/L, 1.68×10^7 cells/ml). Indigenous *Chlorella vulgaris* growth is favored by glycose availability after lactose degradation with a consumption of 62% on the 7th day. Pigments content was improved with an average value of 34.5 mg/gDW and 9 mg/mgDW for total chlorophylls and carotenoids, respectively. *Chlorella vulgaris* cultivation on BG11/CW has showed a high protein content with a value of 46%. Indigenous *Chlorella vulgaris* was able to accumulate a suitable lipid content that could reach 23%, which are rich in C16:00, C18:00, C18:1. This strain is a potential candidate for a sustainable bioenergy and coproducts that could contribute efficiently to promote the circular economy.

1 Introduction

Microalgae have an increasing interest in various sectors as an instruments that are being explored to achieve the target of SDGs , including; renewable energy, food, environmental management, water purification, chemicals production, and healthcare products [1]. Microalgae cultivation has gained significant attention as a sustainable approach for biomass production and the synthesis of value-added products. The availability of cost-effective and nutrient-rich culture media plays a crucial role in achieving optimal microalgae growth and productivity. One potential nutrient source that has gained interest in recent years is cheese whey, a byproduct of cheese production. Cheese whey contains a range of organic carbon compounds, proteins, and minerals, making it a potentially valuable resource for microalgae cultivation [2]. Microalgae-based natural products are the topic of interest in pharmaceutical, food and biotechnological industries, which produce chlorophylls in large quantities, making them important bioactive compounds [3].

The use of cheese whey for microalgae cultivation offers a sustainable and economically viable approach to utilizing this waste product. By providing a nutrient-rich medium, cheese whey can support microalgae growth and biomass production, while simultaneously reducing waste and environmental impact [4]. While the use of cheese whey for microalgae cultivation shows promise, there are challenges to address, such as scaling up production, maintaining stable and consistent nutrient composition, and optimizing the overall process economics. Ongoing research is focused on optimizing the cultivation conditions, exploring new microalgae strains, and developing cost-effective downstream processing techniques.

This study evaluated local isolated indigenous microalgae strain in Algeria, *Chlorella vulgaris*, using a combined growth medium containing BG11 and cheese whey (BG11/CW) with a concentration of 2 g/L of lactose. 100 % of BG11 growth media was used as a control for comparison. In this respect, algal growth (Cells density, dry weight), organic carbon consumption (lactose), biochemical composition of Chlorella vulgaris biomass (carbohydrates, proteins, lipids), fatty acid profile and pigments (Total chlorophylls and carotenoids) were investigated.

2 Material and methods

The microalgae used in this study was an indigenous strain of Chlorella vulgaris, previously isolated from a

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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 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 1 x 106 cells/mL. The pH of the culture broth was measured using a pH-meter (Starter3100, OHAUS corporation, USA).

The mixotrophic and autotrophic cultures were kept under continuous illumination. The cultures were sampled every two days to monitor Chlorella vulgaris growth by measuring cells density and dry weight biomass. Microalgae cells were washed 3 times with an equivalent volume of distilled water to eliminate sugars and salts (centrifuged for 5 min at 4000 rpm between each washing). Culture samples were then dried for 2 h at 105 °C. Cells concentration was estimated using Malassez Counting chambers where cells total number was calculated using the following equation:

Cells Density (Cells/mL) = Total number of cells counted x 105 x DF (DF: Dilution factor) (1)

To monitor the lactose consumption of lactose, the samples are centrifuged at 13400 rpm for 5 minutes and supernatant was recovered and filtered using 0.22-µm syringe filter for analysis by high-performance liquid chromatography Jasco LC Net II/ADC equipped with Eurokat H column (10 µm, 300 × 8 mm) and RI detector (JASCO RI 4030). The mobile phase was 5mM H2SO4, which was eluted at 0.6 mL/min. The column and the refractive index detector were maintained at 60 °C. The standard curve was used to calculate the lactose consumption.

The Pigment content determination was carried out according to (Lee et al., 2010; Ritchie, 2006 ; Pruvost et al. 2011) by centrifuging 2 ml sample at 13400 rpm for 5min and rinsed twice with distilled water. Supernatant was discarded and 1.5 ml of ethanol was added and mixed using a vortex then incubated at 40 °C for 30 min. The following equations are used to determine Chlorophyll a,b and carotenoids.

[Chlorophylle a] (µg/ml) = 0.0604 E630 - 4.5224 E647 + 13.2969 E664 - 1.7453 E691 (2)

[Chlorophylle b] (µg/ml) = - 4.1982 E630 + 25.7205 E647 - 7.4096 E664 - 2.7418 E691 (3)

[Caroténoïdes] (µg/ml) = 4 E480 (4)

Microalgae biochemical composition for total carbohydrate and proteins analysis were estimated by the phenol-sulphuric acid [9] and Lowry [10] methods, respectively, as readapted and described by [11]. Ash content was determined according to [12], [13] where 20 mg of microalgae biomass was heated in a muffle furnace at 550 °C for 4 hours. The total lipids content was estimated by subtracting the percentage of ashes, carbohydrates and crude proteins out of 100%.

3 Results and Discussion

3.1 Chlorella vulgaris growth

As given in the figure 1, the best growth is obtained for the BG11/CW culture media, with a maximum cell density and dry biomass with a value of 6.5 10^7 Cells/ml and 2.85 g/L, respectively. This represents about three folds of the cell density and dry biomass obtained in the BG1 culture media (1.68 10^7 cells/ml and 0.45 g/L).

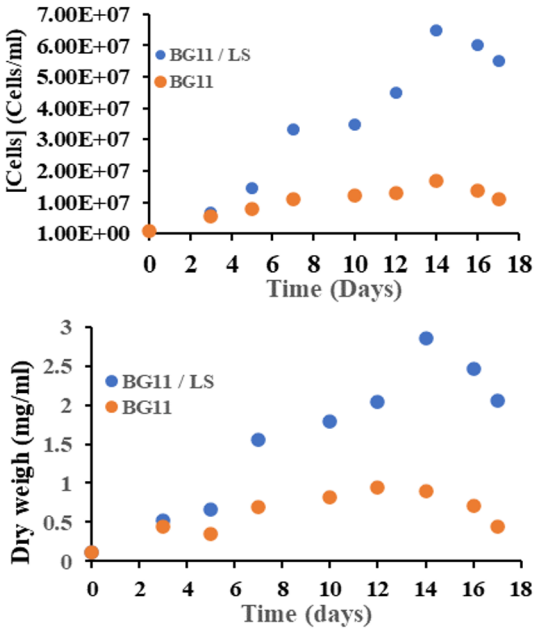

Figure 1: Cells concentration and Dry weight evolution using CW/BG11 with 2g/L of lactose

Lactose degradation in microalgae is tightly connected with the strain's ability to produce β-galactosidase which enables lactose hydrolysis to glucose and galactose [14]. It was demonstrated that mixotrophy mode using cheese whey is the most efficient growth mode for *Chlorella sp* [15] [16]. However, there is a few works reporting the use of cheese whey for *Chlorella vulgaris* cultivation. Obtained results are complying with those reported in scientific literature for other microalgae strains. [17] found a maximum algal biomass yield of 2.44 g/L for *Chlorella pyrenoidosa* using fresh cheese whey wastewater. [18] found that biomass production of *Scenedesmus obliquus* was 2.6 g/L after seven days of cultivation used a mixture of cheese whey and BG11.
High biomass production could be explained partly by indigenous *Chlorella vulgaris* ability to hydrolyse lactose to glucose and galactose with a consumption of 62 % is observed on the 7th day of culture then a total consumption of 100 % on the 12th day, which makes glucose available as carbon source stimulating the biomass production since the 7th day of culture (figure 2).

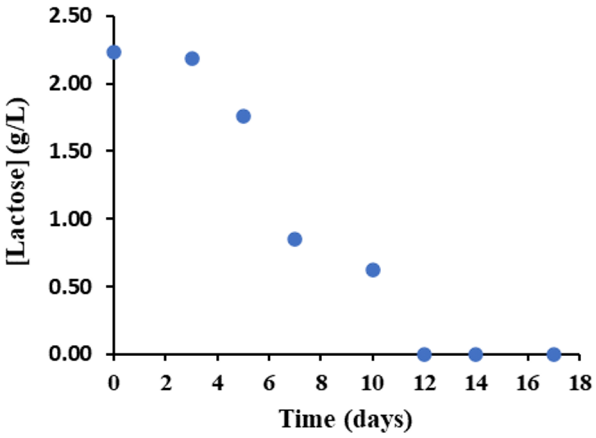

Figure 2: Lactose consumption by *Chlorella vulgaris* along the cultivation process

By contrast, the presence of other nutrients has also reported to have a stimulatory effect where [19] found that total phosphorus was the driving factor for microalgae growth under mixotrophic cultivation while carbon availability did not cause any differences in microalgae kinetic growth parameters and final biomass concentration.

3.2 Biochemical composition

Biochemical composition of indigenous Chlorella vulgaris was carried-out for proteins, lipids, and carbohydrates (figure 3). Proteins content of Chlorella vulgaris cultivated in BG11/CW was found to be of 45.97% versus 34.69 % for BG11. Reported total proteins content in mature *Chlorella vulgaris* represents 42 - 58% of biomass dry weight and varies according to the growth conditions [20]. Relatively high protein content of indigenous Chlorella vulgaris could be explained by growth phase noting that protein accumulation is maximized during the exponential growth and decline in stationary growth phase [21].

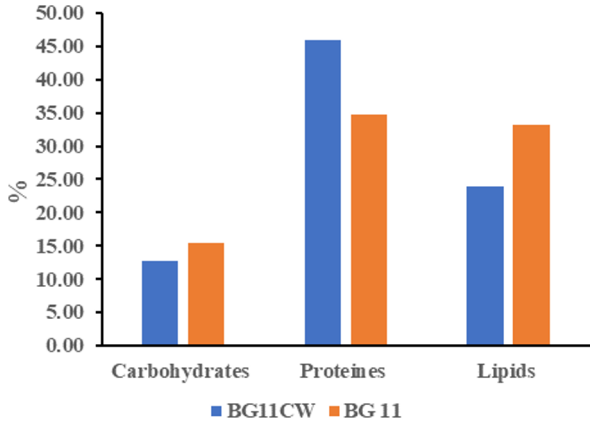

Figure 3. Biochemical composition of indigenous *Chlorella vulgaris*

Furthermore, it is reported that mixotrophic cultivation mode using industry dairy wastes could enhance lipid and protein productivity by reducing the biomass loss in dark hours due to pure respiration [19], [22].

Carbohydrate content of 12.67% were found for Chlorella vulgaris, which is practically similar under BG11 growth media. Reported carbohydrates content for Chlorella vulgaris carbohyrates content were reported to vary from 12-17 % DW [23]. Carbohydrate content depends on the microalgae species and environmental conditions, and can be influenced by various factors such as nutrient limitation, light intensity, salt stress, temperature, and metabolic mode [24].

Lipids content of indigenous Chlorella vulgaris were found to be 23.97% for BG11/CW growth media versus 33.31 % for BG11. An average of lipids content of 14-25% for Chlorella vulgaris was reported in literature under nutrient replete [25], [26]. Lipid content is species dependent. Nevertheless, the major adequate factors for microalgae cultivation are pH and temperature. pH is reported to affect vitally lipid biosynthesis where high pH suppresses the cell division of chlorella species and TAG utilization [27], [28]. Moreover, the indigenous Chlorella vulgaris strain were able to accumulate a suitable lipid content that could reach 23 %, which are rich in C16:00, C18 :00, C18 :1

3.3 Pigments production

As shown in figure 4 the use of BG11/CW improve the pigments productivity where a maximum value of 74 µg/ml and 22 µg/ml, for total chlorophylls and carotenoids, respectively. High total chlorophyll content is explained by the glucose derived from cheese whey degradation as a carbon source at low concentration (2 g/L) which promotes the biosynthesis of chlorophylls. In addition, it has been found that all glucose could be exhausted within 12 days, which explains the maximum content observed during the 14th day of culture and the decreasing of the total chlorophyll after this period. The high pigments content was reported to be influenced positively by glucose supplementation from lactose degradation which promotes the biosynthesis of chlorophylls[29] Total chlorophylls production was evaluated in microalgal biomass cultured under mixotrophic conditions showing an important total chlorophyll content under CW/BG11 growth media is important where it could reach a maximum of 34.65 mg/g dry weight at 10th day (Figure 5). These obtained results are complying with those reported in literature, where total chlorophyll content for Chlorella vulgaris is found in the range of 22.6 to 32.4 mg/g dry weight under autotrophic conditions [30].

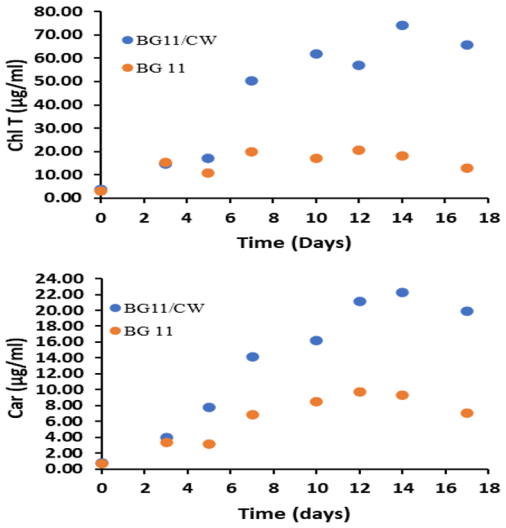

Figure 4. Indigenous *Chlorella vulgaris* Pigments productivity under BG11/CW.

The total carotenoids content was estimated to be 11.77 mg/g dry weight at the 5th day and stabilize around 9 mg/g dry weight. Meanwhile, total carotenoids showed obviously the same content evolution in the BG11 growth media Figure 5. These obtained values are in the same range of those reported in literature where quantity of carotenoids extracted from Chlorella vulgaris in mixtrophic cultivation was found to be about 6.83 mg/g dry weight [29]. Furthermore, total carotenoids of 2.7-8.3 mg/g dry weight for chlorella genus were reported in the literature [30].

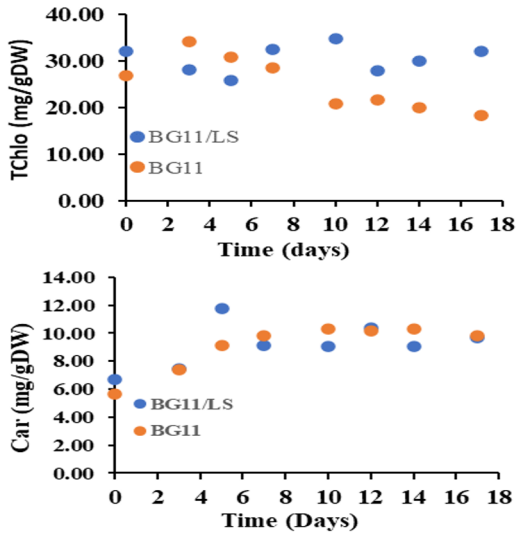

Figure 5. Indigenous *Chlorella vulgaris* Pigments content evolution under BG11/ CW.

Regarding to the biosynthesis of carotenoids, the obtained results are in agreement with those of [22] who found a lower amount of carotenoids in mixotrophic cells of C. vulgaris when compared to cells grown on autotrophic culture. The same conclusions was confirmed by [31]. As depicted in figure, there was no increase in cellular accumulation of total chlorophylls and total carotenoids under mixotrophic condition when expressed as mg/g DW. therefore, for the production of pigments the cells grown mixotrophically could be harvested earlier, due to the faster growth, thereby increasing the annual productivity of pigments, making the process viable and more sustainable.

4 Conclusions

The aim of this work was to evaluate new the effect of using cheese whey rich in lactose on the growth and biochemical and valuable compounds of indigenous *Chlorella vulgaris* isolated locally in Algeria. When compared with the photoautotrophic control culture, mixotrophic microalgae of indigenous Chlorella vulgaris showed higher productivity of biomass, pigments, proteins and lipids.

Using agro-industrial carbon-rich biowastes for microalgae cultivation represents a promising approach to boost microalgae integration in existing industries in a more sustainable way and promote the circular economy, thus contributing to climate change attenuation and natural resources preserving.

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