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Sustainable Biomass and Biodiesel Production from Microalgae Using Dairy Wastes

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***Abstract*— Microalgae biotechnology can contribute efficiently to promote circular economy concepts in wastes management sector. This study investigated the Algerian microalgae strain of Coelastrella for its efficiency to use cheese whey as source of organic carbon. Biomass production and composition, Fatty acid profile, and biodiesel quality were evaluated. Cells concentration has reached a max of 1.24 x 10^7 in BG11/ Cheese whey medium, compared to 2.75 10^6 cells/ml in BG11 medium. Biomass showed a maximum of 5.75 g/L on the 11th day of culture which is 11 folds of dry biomass obtained in BG11 medium. Local strain achieved a lactose consumption rate up to 70%. Using lactose for the cultivation of *Coelastrella thermophilla var. globulina* enhances lipid content compared to BG11, with a value 20.50%, which represents an increase of 30 % compared to BG11 medium (16.76 %). Fatty Acid profile composition in the BG11/LS culture medium is characterized by the dominance of saturated fatty acids, especially C16:0 and C18:00. Predicted qualities of the biodiesel generally comply with ASTM 6751 and EN 14214 standards.**

Keywords — **Microalgae, Coelastrella thermophilla var. globulina, Biodiesel, Cheese, whey nclude at least 5 keywords or phrases**

I. Introduction

Rising global energy demands and climate crisis has conducted to an unprecedent need for the bio-based circular economy to ensure sustainable development with minimized carbon footprint. Biofuels developed from microalgae biomass offer tremendous potential to fulfil energy demand while contributing to CO2 mitigation [1]. Microalgae have immense potential to drive clean, sustainable bioenergy as a renewable feedstock for biofuels, including bio-char, biodiesel, and biogas, while avoiding competition with food crops [2]. The commercialization of algal derived biodiesel has not yet been realized because the biodiesel production cost is still noncompetitive due to expensive and energy-intensive cultivation, pretreatment, and biodiesel conversion processes [3]. The cultivation of microalgae in media supplemented with organic carbon substrates can significantly increase biomass productivities and overcome the technical constraints associated to CO2 supply[4].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 [5]. This paper aims to evaluate the use of cheese whey as organic carbon source to cultivate an indigenous microalgae Coelastrella thermophilla var. globulina for biomass and biodiesel production. Algal growth (Cells density, dry weight), organic carbon consumption (lactose), biochemical composition of Coelastrella themrophilla var. globulina (carbohydrates, proteins, lipids), fatty acid profile and biodiesel quality were investigated.

II. Materials and methods

Coelastrella thermophilla var. globulina is indigenous microalgae that was isolated from local environment and genetically identified [6]. It was cultivated in batch mode under photoheterotrophic conditions in a torus photobioreactor of 1.3 L effective volume. A mixture of BG11 and Cheese whey (60/40 %vv) was used as growth medium for microalgae under the following conditions: Temperature 25 °C, continuous light intensity

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7000 Lux (LED lamps), inoculum cells density 1.5 10^6 cells/mL, agitation 150 rpm/min, Initial pH 7.5. The culture was lasted for 18 days without pH control.

The cultures were sampled every two days to measure 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 at 105 °C for 2 h. Cells concentration was estimated using Malassez Counting chambers. 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 microalgale biomass was pre-treated prior to biochemical composition determination using sonication for the cells disruption according [7]. After collecting the aqueous extract following sonication, total carbohydrates were estimated by the phenol-sulphuric acid according to [8]. Ash content was determined according to [9], where 20 mg of microalgae biomass was heated in a muffle furnace at 550 °C for 4 hours. The total proteins content was measured using the Lowry method [10]. The lipid extraction from algal biomass was performed using the FOLCH method [11] and readapted by [12] using a solvent of chloroform: methanol (2:1 v/v). Crude lipid was acid-catalyzed transmethylated to FAMEs to identify and quantify fatty acids using gas chromatography (Thermo Scientific TSQ 8000, USA), equipped with a double mass spectrometry detector (GC-MS/MS) and TR-5 capillary column [13]. Biodiesel qualities derived from fatty acids composition such as Saponification number (SN) and Iodine value (IV), Cetane number (CN), Oxidative stability (OS), Cold filter plugging point (CFPP), were estimated using empirical correlations as according [14]. High Heat Value (HHV) was calculated according to [15]. Kinematic viscosity (η) and density (ρ) were calculated according to [16].

III. Results and discussion

A. *Coelastrella thermophilla var. globulina growth and biomass production*

As depicted in 1, the cell growth and dry biomass were significantly increased with the use of BG11/ Cheese whey medium. Cells concentration has reached a maximum of 1.24 x 10^7 cells/ml in BG11/ Cheese whey medium, compared to 2.75 10^6 cells/ml in BG11 medium. Biomass showed a maximum of 5.75 g/L on the 11^{th} day of culture which is 11 folds of dry biomass reached in BG11 medium at the same period. A finale biomass of 1.36 g/L has been obtained for *Nannochloropsis limnetica* cultivated after 8 days of cultivation on autoclaved medium containing 45 g/L of cheese whey powder [17]. Salah et al., have reported a biomass of 3.51-4.92 g/L for *Desmodesmus sp*. using a 50 % of BBM with a content of 10 g/L of lactose after 14 days of cultivation [18].

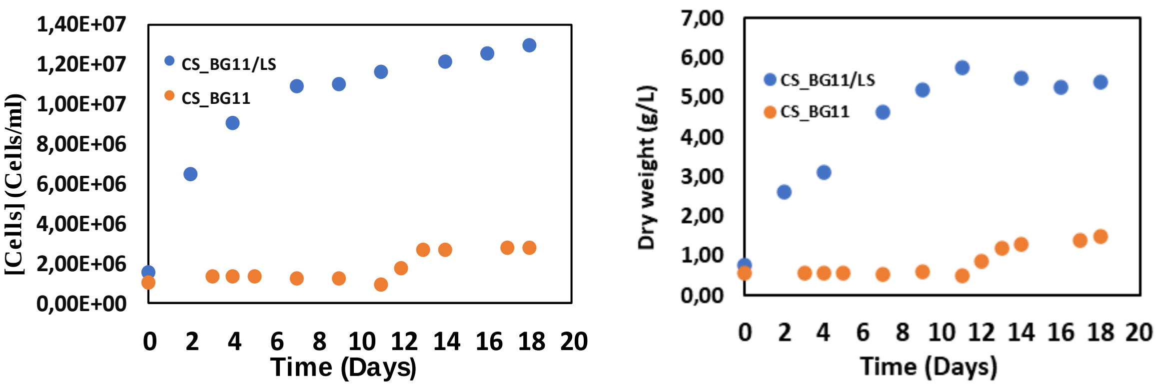

Figure 1: Cells concentration and Dry weight evolution using CW/BG11 (60%/40%) with 50g/L of lactose

It was found that the use of lactose by microalgae is tightly related with the strain's ability to produce β-galactosidase (both intracellular and extracellular), which hydrolyze lactose to glucose and galactose [19]. At our best knowledge there is no studies reporting the use of cheese whey for *Coelastrella thermophilla var. globulina* cultivation. Meanwhile, Thepsuthammarat et al., [20] reported that lactose did not promote and had

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no significant effect on *Coelastrella sp.* KKU-P1 growth due to the lack of the ability to produce β-galactosidase. In this study, we have demonstrated the potential of using cheese whey as carbon source for the photoheterotrophic cultivation of *Coelastrella thermophilla var. globulina*. The lactose consumption is shown in Figure 2 where a consumption rate up to 50% on the 9th of culture is obtained, then a consumption of 70 % on the 18th day, which makes glucose available as carbon source stimulating the biomass production since the 7th day of culture (Figure 1). The total organic carbon (TOC) reduction has been found to be 74.76% of the initial content which confirm the use of lactose by *Coelastrella thermophilla var. globilina*. It is necessary to determine whether lactose is directly assimilated into the biomass as a disaccharide molecule or hydrolyzed by extracellular β-galactosidase into glucose and galactose before being absorbed.

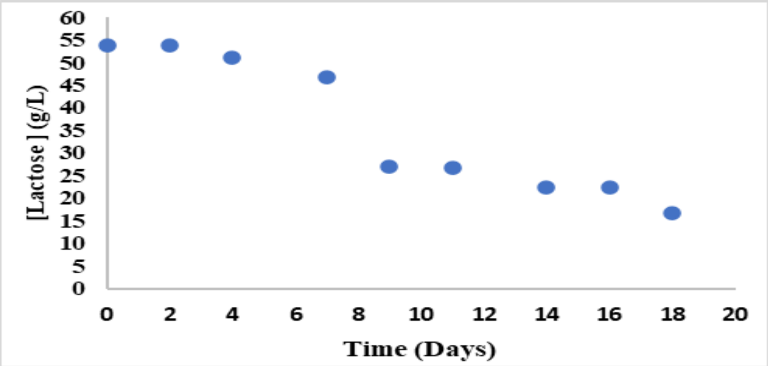

Figure 2: Lactose consumption by *Coelastrella themophilla var. globulina* (50 g_lactose/L)

B. Biochemical composition of Coelastrella thermophilla var. globulina

Biochemical composition of indigenous *Coelastrella thermophilla var. globulina* was carried-out for proteins, lipids, and carbohydrates (figure 3). Protein quantification revealed a content of 37% of dry weight compared to 33.73 % in BG11 medium. The protein content of the Coelastrella sp. genus varies from 18% to 38% of dry weight, according to [7]. Results indicate a decrease in total carbohydrates content of *Coelastrella thermophilla var. globulina* strain which is approximately 29.50% in the CW/BG11 medium compared to 32.56% in the BG11 culture medium. The lipid content in BG11/LS is around 20.50%, compared to 16.76% in the BG11 medium, which represents an increase of 30 %. It has been reported that total lipid content of *Coelastrella sp.* Genus ranges from 17% to 22% and can reach up to 50% of dry matter under nitrogen and phosphorus deprivation, indicating its potential as a lipid-producing strain [21]. Ray et al., 2022 have reviewed many studies and have found that lipid content has been observed to be comparatively higher in microalgae cultivated under mixotrophic mode and resulted in higher algal growth rate [22]. It has been reported a composition of 39.3% proteins, 10.4 % lipids, and 47.2 % carbohydrates for *Desmodesmus sp.* using nano filtered and cheese whey as external source of carbon [23]. [24] have also found a biochemical of *Tetradesmus obliquus* biomass cultivated in cheese whey as 22.53 % proteins, 14.45 % carbohydrates, and 42.39 % lipids.

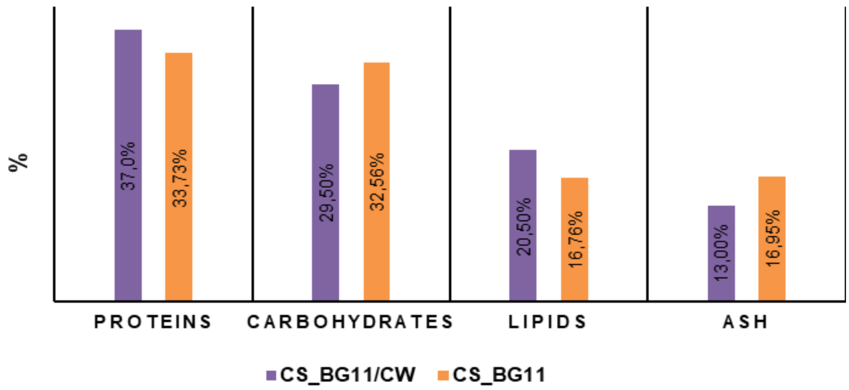

Figure 3: Biochemical composition of in *Coelastrella thermophilla var. globulina*

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C. *Fatty acid composition and derived biodiesel quality*

Table 1 presents the Fatty Acid composition of the *Coelastrella thermophilla var. globulina* strain in the BG11/LS and BG11 culture media, characterized by the dominance of saturated fatty acids, particularly C16:0 and C18:00. The results obtained align with values reported by [20] showing a C18:0 content of approximately 40.78% using molasses as a source of organic carbon. Only one study reporting the fatty acid profile of *Coelastrella thermophilla var. globulina* isolated from an Algerian hot spring [25]. Its FAs profile in BBM culture medium under autotrophic mode was consisted of MUFA (51%), SFA (27%), and PUFA (22%) and rich in C16:0 and C18:1, with values of 21.45% and 35.95% of total fatty acids, respectively. [18] have reported that the major fatty acids were palmitic acid (25.86%), oleic acid (35.31%), and linoleic acid (13.22%) for *Desmodesmus sp* cultivated on combined BBM and cheese whey with lactose concentration of 10 g/L after 14 days of cultivation. [24] have found that fatty acids profile is dominated by palmitic acid (C16:53.92% and oleic acid (18.08%), and Cis-vaccenic acid (14.56%).

As given in table 2, the studied strain of *Coelastrella thermophilla var. globulina* biodiesel meets ASTM D6751 and EN 14214 standards, with a Cetane Number of 61.54, high oxidative stability, compliant iodine value, good low-temperature flow properties, adequate energy content, optimal viscosity, and density within required limits demonstrating its substantial potential for biodiesel applications.

Table 1: Fatty acids composition of microalgae strains

Fatty acid/Sample	CS_BG11/CW	BG11
C16:0	23.78	43.25
C18:0	30.82	10.9
C18:1	7.52	19.86
C18 :2	6.85	12.43
C18 :3	3.27	6.08
C20 :1	2.64	-
C20:4	9.21	-
C22:0	2.39	1.38
C24:0	9.53	1.43
Σ AGS	**66.78**	**59.33**
Σ AGMI	**10.47**	**22.16**
Σ AGPI	**22.55**	**18.51**

Table 2: Predicted properties of biodiesel according to ASTM 6751 and EN 14214 standards.

Parameter/ sample	BG11/CW	BG11	ASTM D6751	EN 14214
Saponification value (mg/g)	188.41	155.90	-	-
Iodine value (gI2/100g)	61.01	51.97		120 Max
Cetane number	61.54	69.62	47 Min	51 Min
Cloud point	7.51	29.72	-	-
Poor point (°C)	1.34		-	-
Oxidative stability	14.24	16.69	3 Min	8 Min
Higher heating value (Mj/kg)	38.28	39.04	-	-
Kinematic viscosity (40 °C; mm2/s)	4.23	4.14	1.9-6.0	3.5-5.0
Density at 20 °C (g/cm^3)	0.840	0.860		

IV. CONCLUSIONS

The potential of using cheese whey as a low-cost organic carbon source for the photoheterotrophic cultivation of microalgae *Coelastrella thermophilla var. globulina* has been investigated and demonstrated in this study. Prospecting and selecting local microalgae species are necessary to assess their biotechnological potential, especially in extreme environments; some of these species may be more beneficial than those

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traditionally exploited, such as thermophilic species. Improving biomass production using a cost-effective substrate is a crucial approach to overcoming cost-related constraints in the development of large-scale microalgae production, especially for low-value-added products like biofuels. The use of this biotechnological tool will contribute to the development of more environmentally friendly industrial processes for the production of eco-friendly products, thereby contributing to the achievement of sustainable development goals.

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