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Selenastrum Capricornutum a New Strain of Algae for Biodiesel Production

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Abstract: The increasing global demand for biofuels for energy security and to reduce the effects of climate change has created an opportunity to explore new sources of biomass, of which, microalgae is the most promising one. The Laboratory of the Biomass Research Centre (CRB, University of Perugia) is equipped with a photobioreactor that is used to cultivate microalgae under batch conditions. Tests were carried out a temperature of 22 °C and a Photosynthetic Photon Flux Density of 140 $\mu E \cdot m^{-2} \cdot s^{-1}$. Cultures were characterized in terms of biomass produced and lipid fraction distribution. The novelty of this paper is the measure of the fuel properties of *Selenastrum capricornutum*, a new strain for biodiesel production. In particular, after the microalgae have been collected and oil has been extracted, this has been transesterified using a methanol/NaOH solution. The resulting biodiesel has been analyzed with a high-resolution gas chromatograph to determine the concentration of the different methylesters.

Keywords: Microalgae; Photobioreactor; Biodiesel; Lipids; Selenastrum capricornutum

1. Introduction

Biomass and biofuels are key assets for a sustainable, secure and efficient energy system [1–3]. The European Joint Research Centre, in its 2019 report "Sustainable Advanced Biofuels" [4], takes into consideration 15 promising future technologies, such as enzymatic hydrolysis and fermentation (to produce 2G ethanol), catalytic processes to produce alcohols (e.g., methanol to gasoline), aqueous phase reforming of 2G sugars, aerobic fermentation of 2G sugars, anaerobic digestion with pretreatment, gasification with Fischer-Tropsch, gasification with methanation, gasification with syngas fermentation, gasification with catalytic synthesis, fast pyrolysis and catalytic upgrading, HTL and catalytic upgrading, transesterification of waste oils, hydroprocessing of waste oils, co-processing of waste oils, and microalgae. Therefore, microalgae can be seen both as a technology but most of all as a feedstock which can be used in many technologies. Microalgae are microorganisms present in all ecosystems. Due to their simple structure, they are able to grow quickly and live in different environmental conditions. They can be classified into photo-autotrophic, heterotrophic, photo-heterotrophic and mixotrophic. Dealing with cultivation practices, they can be cultivated in two ways: open ponds and photobioreactors. Particularly interesting is the production of transportation fuels through the use of microalgae. This can be done through the following routes [5]: solvent based extraction, anaerobic digestion hydrothermal liquefaction, and hydrothermal gasification.

Microalgae use solar energy to convert CO_2 into carbohydrates, lipids and proteins, therefore biomass production can contribute significantly to biofixation of CO_2 (1 kg of dry algal biomass

utilizes about 1.83 kg of CO_2) [6]. It has been estimated that several million algae species exist compared with about 250,000 species of higher plants [7]. The three most important classes of microalgae in terms of quantity are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae) [8]. Algae, like other plants typically used for the production of biofuels, use photosynthesis to convert solar energy into chemical energy. Considering the yield in oil, microalgae show higher per unit of surface values than other oil crops (approximately 59,000–137,000 L/ha, depending on the lipid content). Table 1 compares the oil yields, the land use and biodiesel productivity of different feedstocks, including microalgae and oil crops [9].

Table 1. Comparison of microalgae with other biodiesel [9].

Feedstock	Oil Content (% Dry Weight)	Oil Yield (L/ha Year)	Land Use (m^2 Year/kg Biodiesel)	Biodiesel Productivity (kg Biodiesel/ha Year)	Cost of Biodiesel Production €/t
Corn	44	172	66	152	n.a.
Hemp	33	363	31	321	n.a.
Soybean	18	636	18	562	0.53 €/L [10]
Jatropha	28	741	15	656	0.99 $/L [11]
Camelina	42	915	12	809	0.75 $/L [12]
Canola	41	974	12	862	0.74–1.5 €/L [13,14]
Sunflower	40	1070	11	946	0.74 [14]
Castor	48	1307	9	1156	1.5 $/kg [15]
Palm oil	36	5366	2	4747	0.10 €/L [13]
Microalgae (low oil content)	30	58,700	0.2	51,927	1.5–4 $/L [16]
Microalgae (medium oil content)	50	97,800	0.1	86,515	
Microalgae (high oil content)	70	136,900	0.1	121,104	

It is evident that microalgae are the ones with higher biodiesel productivity (from 52,000 to 120,000 kg/ha) and requiring a smaller extension of land (49–132 times less than that required by the cultivation of rapeseed and soybean). For the cultivation of algae, the vital elements are light, water, carbon dioxide and nutrients such as nitrogen (N), phosphorus (P) and potassium (K); also silica (Si) and iron (Fe), and other trace elements are important. It is also necessary to reach the right balance between different parameters, such as oxygen, CO_2, pH, light intensity, products and by-products concentrations. In an optimal environment with adequate nutrients, microalgae usually double their biomass in 24 h (3.5 h in the exponential growth phase), because of this they require very short harvest cycles (1–10 days) [17].

On the other hand, from Table 1 it can be seen that microalgae biodiesel production cost is at least double than that of conventional feedstock at the moment. Microalgae contain between 2% and 40% of lipids/oils by weight (Table 2). While the lipids percentages may be different in relation to microalgae strain and culture type, the cultivation of algae can potentially yield 15–70 wt.% of oil (Table 3).

Table 2. Chemical composition of algae on a dry matter basis (%) [18].

Species of Sample	Proteins	Carbohydrates	Lipids	Nucleic Acid
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	-	1.9	-
Scenedesmus dimorphus	8–18	21–52	16–40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra sp.	6–20	33–64	11–21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39–61	14–18	14–20	-
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculata	52	15	3	-
Porphyridium cruentum	28–39	40–57	9–14	-
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	-

Table 3. Oil content of some microalgae strains (%) [19].

Microalgae *Species*	**Oil Content [% Dry wt]**
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schitzochytrium sp.	50–77
Tetraselmis sueica	15–23

Selenastrum capricornutum [20] (see Figure 1) is an unicellular green algae belonging to the class of Chlorophyceae; it has a diameter comprised between 40 and 60 μm.

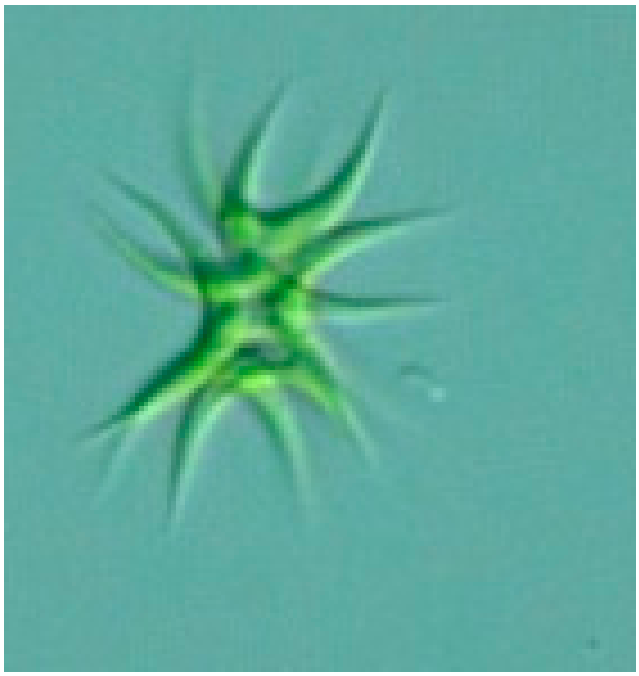

Figure 1. Light microscopy of *Selenastrum sp.* [21].

This species can be easily cultured in laboratories and is readily available commercially. In addition, the growth is rather fast (it doubles its volume even after 72–96 h of incubation) and the species is moderately sensitive to toxic substances. However, few data are available in the Literature [22] and no paper reports the oil yield and its composition, which are useful information to evaluate the potentiality for biofuels production. The purpose of this research is to characterize the oil extracted from *Selenastrum capricornutum* and define the growth conditions. All the experiments have been carried out in duplicates.

2. Materials and Methods

2.1. Microorganisms and Culture Medium

The cultures of *S. capricornutum* were obtained from the laboratory of the Regional Environmental Protection Agency, Perugia Italy (ARPA). The culture medium consists of four nutrient solutions and deionized water at 4 °C:

- Solution 1: $NaNO_3$, $MgCl_2$, $CaCl_2$, H_3BO_3, $MnCl_2$, $ZnCl_2$, CoCl, $CuCl_2$, Na_2MoO_4, $FeCl_3$, Na_2EDTA
- Solution 2: $MgSO_4$
- Solution 3: K_2HPO_4
- Solution 4: $NaHCO_3$

A small amount of microalgae (initial concentration of 100 cells/mL) was inoculated into a 500 mL conical flask and after 10 days transferred into the photobioreactor of 2 L (Figure 2). The algal cultures at 22 ± 1 °C were incubated under continuous cool white fluorescent light with an intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. The algal culture required 3 to 5 days to reach at the exponential growth phase. The cultures were continuously aerated by using an air pump, providing the CO_2 required for algae growth.

Figure 2. Glass batch reactor.

2.2. Photobioreactor

The cylindrical photobioreactor has a total volume of 12 L and it is made of plexiglass (0.193 m ID, 0.75 m high) (Figure 3).

Air is distributed at the photobioreactor bottom by means of a porous ceramic diffuser. The photobioreactor was equipped with lamps, the light irradiance was 140 $\mu E \cdot m^{-2} \cdot s^{-1}$ under continuous cycle. The photobioreactor was inoculated with the culture produced using the 2 L glass flask. Tests lasted about 12 days. The growth of *S. capricornutum* was monitored using a spectrophotometer (Varian Cary 2300, Varian, Palo Alto, California, USA) and deriving cell density from the optical density at 670 nm.

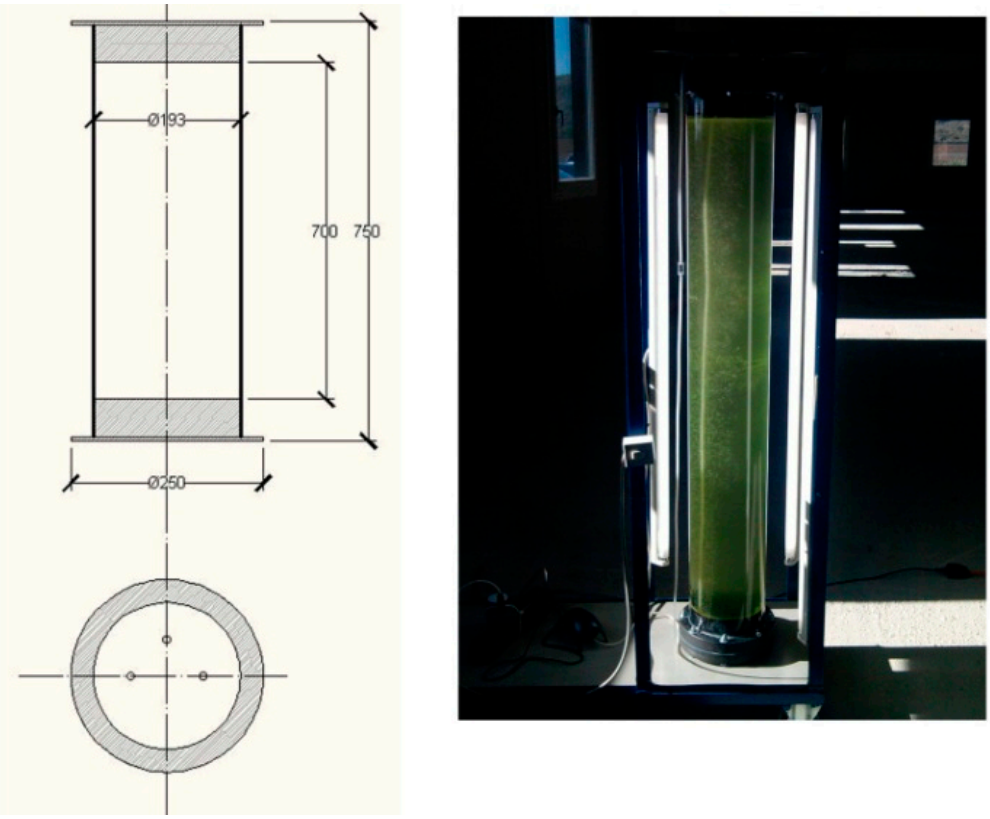

Figure 3. Plexiglass batch Photobioreactor.

2.3. Oil Extraction

After harvesting the biomass, oil extraction can be performed by mechanical methods (crushing, homogenization, ultrasound) or chemical methods (organic solvents, osmotic shock, or enzymatic reactions acidic-basic) [23]. The most accepted extraction method is Soxhlet extraction using hexane as a solvent and with an extraction time of 8–9 h. The oil extracted from the microalgae is different from other vegetable oils as it is rich in polyunsaturated fatty acids, with 4 or more double bonds, which are susceptible to oxidation during storage; this makes it necessary to induce a partial catalytic hydrogenation before conversion to biodiesel [9] where the triglycerides are converted first into diglycerides and monoglycerides and then into esters (biodiesel) and glycerol (by-product) with the use of a reagent (methanol) and a catalyst (NaOH/KOH).

Microalgae were harvested by centrifugation and freeze dried. Total lipid fraction was extracted in hexane for 7 h using a Soxhlet apparatus (Steroglass, PErugia, Italy). Solvents were removed by evaporation and the lipid fraction was weighted. The lipid extracts were transesterified by treatment with methanol/NaOH solution, and the resulting FAMEs were injected into the HRGC-FID system. About 1 g of oil sample was mixed with 2 mL of methanol containing sodium hydroxide, NaOH (10%, g/100 mL) at 65 °C for 60 min under continuous stirring at 100 rpm for 1 h. The obtained Fatty Acid Methyl Esters (FAMEs) were washed with deionized water to remove un-reacted methanol and catalysts. The relative content of FAME was determined. Major fatty acids were identified by comparing their retention times with those of commercial standards (F.A.M.E. Mix, Sigma, St. Louis, MO, USA). The extracted oil was analyzed at the laboratories of the Italian Brewing Research Centre (CERB) at the University of Perugia, using an Agilent Gas Cromatograph (GC), model 6850, equipped with a FID, a capillary inlet system, a DB-23 (60 m × 0.25 mm × 0.25 μm) column, and a model Maestro MPS 2XL multipurpose sampler with a 10 μL syringe (Gerstel Inc., Baltimore, MD, USA). The programmed oven temperature ramp was as follows: 130 °C for 1 min, raised from 130 to 170 °C at 6.5 °C/min, raised from 170 to 215 °C at 2.75 °C/min, 215 °C for 12 min, raised from 215 to 230 °C at

40 °C/min, and held at 230 °C for 3 min. The carrier gas (H_2) flow rate was 1.7 mL/min. The split ratio was set at 50:1. The temperatures of the injector and the detector were 270 and 280 °C, respectively. Peak areas were measured by using an Agilent MSD Chemstation (Agilent, Stanta Clara, California, USA) for HRGC-FID.

2.4. Calculation of Biodiesel Quality Parameters

Once the composition in FAME of the obtained biodiesel has been measured, the concentration of the different FAMEs can be used as input parameter to calculate biodiesel technical characteristics according to what is reported in [24]. Equations to calculate respectively, saponification value, iodine value, Cetane Number, degree of unsaturation, long chain saturated factors, cold flow plugging properties and oil stability index, are the following:

$$SV = \Sigma\ (560 \times F)/MW \quad (1)$$

$$IV = \Sigma\ (254 \times F \times D)/MW \quad (2)$$

$$CN = (46.4 + 5458/SV) - (0.225 \times IV) \quad (3)$$

$$DU = (MUFA, wt.\%) + (2\_PUFA, wt.\%) \quad (4)$$

$$LCSF = 0.1 \times C16 + 0.5 \times C18 + 1 \times C20 + 1.5 \times C22 + 2 \times C24 \quad (5)$$

$$CFPP = 3.1417 \times LCFS - 16.477 \quad (6)$$

$$OSI = 117.9295/x + 2.5905 \quad (7)$$

where SV is the Saponification Value, F = % of each fatty acid, MW= molecular weight of each fatty acid [25], IV is the iodine value, D = no. of double bonds, CN is the Cetane Number, DU is the LCSF is the degree of unsaturation, MUFA stands for Mono Unsaturated fat Acids and PUFA stands for Poly Unsaturated Fat Acids, LCSF is the long chain saturated factors, CFPP is the cold flow plugging properties, OSI is the oil stability index and x = wt.% of oleate (C18:1) + wt.% of linoleate (C18:2).

3. Results

3.1. Microalgae Growth Curves

The growth curves and the optical density curves are shown in Figure 4. Growth is divided in three different growth phases: the lag phase, exponential phase, and stationary phase. The maximum cell density was achieved after 9 days of cultivation. The biomass yield of *S. capricornutum*, at the end of cultivation was about 2.0 g/L for bottle and 2.4 g/L for photobioreactor. The biomass concentration reached at the end of the reported batch tests was acceptable compared to that reported for selected green algae [26] and in satisfactory agreement with the typical data reported in the literature for the majority of algal species. Moreover, the lipid content may be further improved according to Tredici et al. [27]. They reported that nitrogen depletion can double the dry weight content of *S. capricornutum*, as found in many other Chlorophytes.

Algae harvest consists in the removal of large quantities of water to increase the concentration of biomass, and can be conducted in different ways (physical, chemical, biological), depending on the size of the algae (typically in the range of 2–40 μm) [28]. The main collection methods are sedimentation, centrifugation, filtration and ultra-filtration, sometimes preceded by either a step of flocculation (with aluminum and ferric chloride) or flotation. At the end of the tests, algae were harvested by centrifugation at 3000 rpm for 15 min. The cells were washed twice with distilled water after centrifugation. The biomass was dried at 100 °C for 6 h. The dry weight of the algal biomass was determined for the bottle and the photobioreactor. Table 4 Reports the final results for the performed tests.

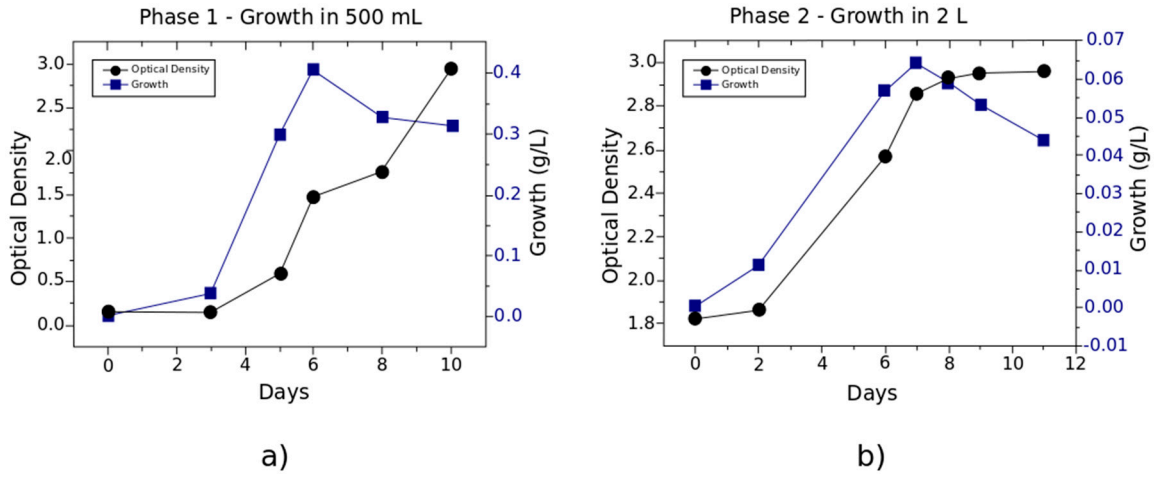

Figure 4. Optical density and growth of *S. capricornutum* cultivated in the glass flask reactor (**a**) and in the photobioreactor (**b**).

Table 4. Biomass productivity of *Selenastrum capricornutum.*

Parameter	Bottle	Photobioreactor
Biomass productivity (g)	2.0 g/L	2.4 g/L
Lipid content (wt.% biomass)	13	17.47
Moisture (wt.%)	90.43	95.67

3.2. Biodesel Cahracterization

The analysis of *S. capricornutum* biodiesel composition shows that C16:0, C18:1, C18:2 ω6 and C18:3 ω3 are the dominant fatty acids; this is typical of freshwater green algae [29,30] and in agreement with the results of Mc Lamon et al. [31]. The methyl esters profiles of the microalgae are presented in Table 5 as detected through GC analysis. The results of the analysis are shown in Figure 5, which highlights the high percentage of palmitic and oleic acid. The total extracted organic content as percentage of dry weight of *S. capricornutum* was 13 wt.%, 17.5 wt.%. Oleic acid (C18:1 cis, ~55%) and palmitic acid (C16:0, ~20%) were observed to be the major components. The heating value of the obtained biodiesel was determined using an LECO Calorimeter AC350, according to UNI EN 14918:2010 Solid Biofuels [32].

Table 5. The composition and content of FAME.

Carbon Number	FAME	Bottle (% of dw)	Photobioreactor (% of dw)
C10:0	Capric acid	0.030	0.040
C14:0	Myristic acid	0.190	0.170
C16:0	Palmitic acid	17.890	19.570
C16:1	Palmitoleic acid	0.180	0.320
C17:0	Heptadecanoate acid	0.240	0.250
C18:0	Stearic acid	1.660	1.260
C18:1	Oleic acid cis	59.530	54.820
C18:2	Linoleic acid	2.690	4.000
C18:2	Linolenic acid n6	0.200	0.300
C18:3	Linolenic acid n3	5.890	6.100
C20:1	Acid C20:1 Cis	0.840	0.550
C20:3	Acid C20:3 n6	0.090	1.190

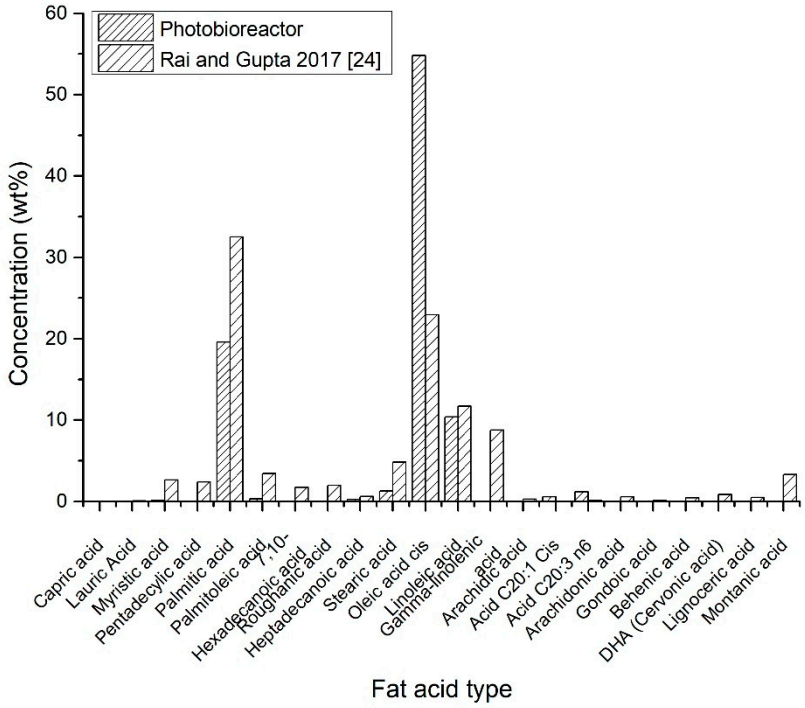

Figure 5. Comparison between Fatty Acid Methyl Esters of *S. capricornutum* obtained in the photobioreactor and Fatty Acid Methyl Esters produced from *Scenedesmus abundans* [24].

4. Discussion

4.1. Biodiesel Quality

In this work, *Selenastrum capricornutum* has been cultivated and the extracted oil has been used to produce biodiesel which has been fully characterized by the point of view of its FAME composition.

Usually, saturated fatty esters have high cetane number and superior oxidative stability, whereas unsaturated, especially polyunsaturated fatty esters have improved low temperature combustion properties. Oleic acid whose ester is known to be a main component of biodiesel amounts to 55% of total fatty acids in *S. capricornutum*. The good concentration of oleic acid (C18:1) ester, represents an interesting compromise solution between oxidative stability and low temperature properties and therefore improves the quality of biodiesel [33,34]. Moreover, the EN 14214 (2004) specifies a limit of 12% in linolenic (C18:3) acid ester for quality biodiesel [29,35,36]; biodiesel produced from *S. capricornutum* in this study showed a concentration of 5.89%/6.10% for C18:3. The heating value of biodiesel from *Selenastrum capricornutum* was 41.03 MJ/kg, which is comparable to that of fossil oil (equal to 42 MJ/kg), Spirulina Platensis [37] and some other microalgae oils [38,39].

S. capricornutun biodiesel composition is compared with the composition of biodiesel obtained from the microalgae *Scenedesmus abundans* [24], as showed in Figure 6.

From Figure 6 it can be seen the higher content of Oleic Acid Methyl Ester in the biodiesel produced from *S. Capricornutum* compared with the biodiesel produced from *Scenedesmus abundans*. The comparison between the contend of saturated, monounsaturated and polyunsaturated acid fats methyl esters is shown in Figure 6.

Biodiesel characteristics are presented in Table 6 and compared with the characteristics of biodiesels obtained from *Scenedemus abundans* [25]. In particular, Saponification Value is indicated as milligrams of potassium hydroxide required to saponify 1 g of oil. It is a measure of the average molecular weight (or chain length) of all the fatty acids contained in the oil. Long chain fatty acids have a low saponification value because they have a lower number of carboxylic functional groups per unit mass, compared to short chain fatty acids.

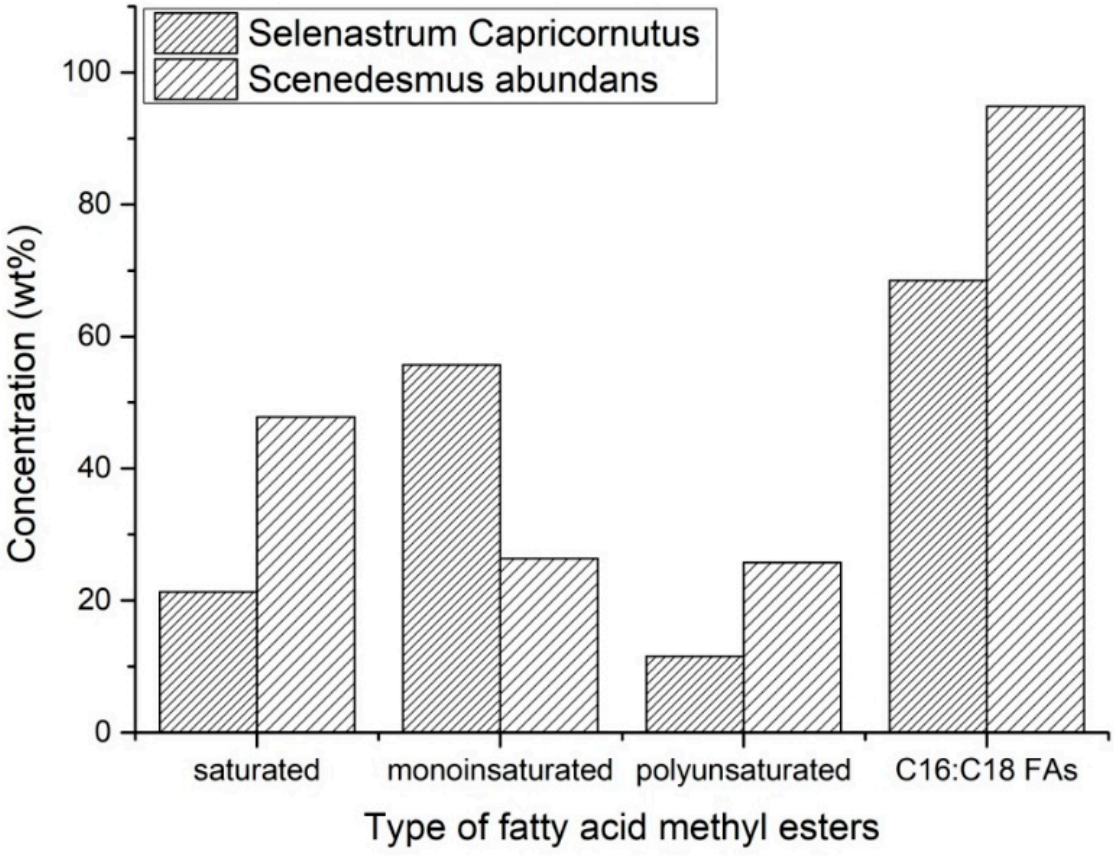

Figure 6. Comparison between saturated, monounsaturated, polyunsaturated fatty acids methyl esters of two microalgae: *Slenastrum capricornutum* and *Scenedemus abundans* [24].

Table 6. Predicted biodiesel properties of *Slenastrum capricornutum* (SC) and *Scenedemus abundans* (SA) [24].

Characteristic	*Selenastrum Capricornutum*	*Scenedemus Abundans*
SV (mg KOH/g oil)	179.90	205.8
IV (I_2 g/100 g oil)	71.98	85.07
CN	60.54	53.68
DU (wt.%)	78.87	76.78
LCSF (°C)	18.48	16.12
CFPP (°C)	41.60	34.17
OSI (h)	4.40	5.99

From the above reported data and considered the characteristics of the oil obtained from *S. capricornutum* we can consider it as a potential new strain to be used for biodiesel production.

4.2. Economic Aspects

An interesting document on costs performance comparison between microalgae biodiesel and biodiesel produced from other vegetable oils (which often are also used for food purposes) is proposed in [40]. The big advantage of microalgae is represented by their higher growth rate; this can push both technical and economic feasibility. Also in this work after demonstrating that the quality of the oil and of the finally produced biodiesel is good, a key aspect still remains the growth rate, given that lipid extraction and transesterification are mature techniques and efficiencies are now higher than 90 wt.%. With this respect we have to consider that biomass productivity in photobioreactos can be lower than that in open ponds. In the work of [40], it is reported that the photobioreactor productivity can vary between 11 and 30 g/m^2/d; this can affect the economic convenience in an important way. Generally,

final productivity of open ponds is higher than that of photobioreactors. The reason is that, inside the photobioreactor, oxygen is also generated and this inhibits microalgae growth. The cultivation phase can contribute to the 70% or more of the total biodiesel cost [10,41,42]. This is why the cost of biodiesel produced from microalgae cultivated in open ponds can be even eight times lower than that of phototbioreactors, as reported in [40]. Cultivation costs can be also reduced using wastewaters as substrate of cultivation to provide nitrogen and phosphorous for free. Additionally, CO_2 emitted by powerplants can be recycled in microalgae production. Based on this data the application of photobioreactor appears to be for a niche market in which integrated production of biofuels and added value products is practiced. Integrated biorefineries where process intensification and energy efficiency are pushed to the extreme can bring the photobioreactor system to be competitive by an economic point of view. While to produce biodiesel with microalgae on a large commercial scale, open ponds appear to be the best and more appropriate solution.

5. Conclusions

A preliminary campaign of tests of cultures of *S. capricornutum* strain in a lab-scale photobioreactor was carried out for biodiesel production. Fatty Acids composition was characterized and the total lipid fraction of the microalgae was in the order of 13 (% of dw) for flask and 17.5 (% of dw) for the photobioreactor. The oil extracted was transesterified to obtain biodiesel. The fatty acid composition of the produced biodiesels shows interesting values, compared with the most common microalgae investigated in the literature. The concentration of oleic acid was higher than the value obtained from most of the microalgae, this can be a very positive feature. Biodiesel technical characteristics were calculated using equations available in the literature. It was determined that the obtained biodiesel has good qualities and can be a promising fuel.

Author Contributions: L.B. is responsible of the realization of the bioreactor, A.P. is responsible of the tests, P.B. is responsible of paper formatting and improvement, F.F. is responsible of designing the bioreactor and coordinating the experimental campaign. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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