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Fatty Acid Profile of Microalgal Oils as a Criterion for Selection of the Best Feedstock for Biodiesel Production

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Abstract: Microalgae are considered to be potentially attractive feedstocks for biodiesel production, mainly due to their fast growth rate and high oil content accumulated in their cells. In this study, the suitability for biofuel production was tested for *Chlorella vulgaris*, *Chlorella fusca*, *Oocystis submarina*, and *Monoraphidium* strain. The effect of nutrient limitation on microalgae biomass growth, lipid accumulation, ash content, fatty acid profile, and selected physico-chemical parameters of algal biodiesel were analysed. The study was carried out in vertical tubular photobioreactors of 100 L capacity. The highest biomass content at 100% medium dose was found for *Monoraphidium* 525 ± 29 mg·L^{-1}. A 50% reduction of nutrients in the culture medium decreased the biomass content by 23% for *O. submarina*, 19% for *Monoraphidium*, 13% for *C. vulgaris* and 9% for *C. fusca* strain. Nutrient limitation increased lipid production and reduced ash content in microalgal cells. The highest values were observed for *Oocystis submarina*, with a 90% increase in lipids and a 45% decrease in ash content in the biomass under stress conditions. The fatty acid profile of particular microalgae strains was dominated by palmitic, oleic, linoleic, and linoleic acids. Nutrient stress increased the amount of saturated and unsaturated fatty acids affecting the quality of biodiesel, but this was determined by the type of strain.

Keywords: microalgae; biomass; lipid; fatty acid profile; biodiesel; nutrient limitation; stress conditions

1. Introduction

In the transport sector, biofuels are an alternative to fossil fuels, which are environmentally harmful [1]. Increasing attention on biofuels and technologies of their production was also caused by the global fuel crisis [2].

According to the type of feedstock used in production, there are three main generations of biofuels [3]. The first and second generations of biofuels use food crops, oil crops, and plant biomass from non-food crops and from forestry as substrates. Therefore, their use is related to high competition of plant production and area of arable land [4]. A feasible alternative are third-generation biofuels derived from algal biomass [5].

Microalgal biomass contains considerable amounts of molecules suitable for biofuel production, but also for food supplements, fertilisers, pigments, etc. [6,7]. The potential for energetic use of microalgae is very high, e.g., for the production of biogas, bioethanol, bio-

hydrogen, bio-oil or biodiesel [8,9] currently produced mainly from soybean oil, rapeseed oil, sunflower oil, palm oil [10].

Microalgae are promising candidates for industrial production. Their cultivation does not compete with food crop production and can be carried out using not only freshwater, but also salt water, saline water and different kinds of wastewater [11,12]. Compared to plants, they are characterised by a high photosynthetic rate [13]. Under suitable conditions, their growth rate and the yield of biomass are higher than those of terrestrial plants [14]. The yield of microalgal oil is several times higher than the best oil producing crop [15].

Although algal biofuels have many advantages, they have still not been commercialised. A significant barrier is the excessively high production costs compared to fossil fuels [16], including the production of biomass, its dewatering and the extraction of valuable cellular components [6]. These components include proteins, carbohydrates and lipids [17]. Microalgal oil is a good feedstock for biodiesel production [18], but its content in biomass is often not sufficient for industrial application [19]. There are species of microalgae that accumulate large amounts of lipids, but their growth rate is generally very slow [20]. The lipid content varies according to species of microalgae and culture conditions [21]. Average values range from 20% to 70% of the cell dry weight [22,23]. There are some strategies to increase the natural lipid content in algal cells. This can be realised by genetic engineering [24] or by modifying growing conditions by changing light intensity [25], carbon dioxide content [26], low temperature [27], high light intensity [28], high salt and iron concentrations in the culture medium [29,30], salinity levels [31] or limiting nutrient availability [32]. Microalgae for biofuel production should be characterised by a high cellular lipid accumulation capacity [33]. The application of suitable species will have a beneficial effect on the cost-effectiveness of biofuel production [34].

When assessing the potential of microalgae for biodiesel production, not only the amount of lipids in their cells is important, but also the content of the relevant fatty acids (FAs) [22] as direct precursors for third-generation biofuel production. The fatty acid profile of biodiesel is identical to the profile of the oil used in their production [35]. The increase in lipid content is determined by changes in biosynthetic pathways leading to the formation and accumulation of lipids mainly as a triacylglycerol (TGA) [36]. Microalgal lipids can be divided into neutral lipids, including saturated (SFA) and monounsaturated fatty acids (MUFA), representing the main fatty acids in triacylglycerols, and polyunsaturated fatty acids (PUFA) enriched in polar lipids [37]. The fatty acid profile (FA) of microalgae may also include *iso-* and *anteiso-*branched FAs, methyl-branched FAs, 3-Hydroxy and amide FAs [38].

Triacylglycerols can be converted to fatty acid methyl esters by a transesterification process [39]. In this process the fatty acid composition of the feedstock is not changed [40]. The content of each FAs group in the oil is significant for the quality of the biofuel [22], therefore knowledge of the FA profile can be useful in the selection of algae species for fuel applications. As well as the lipid content, the fatty acid composition can also be modified by changing the concentration of carbon dioxide [41] or by nitrogen (N) deficiency [42].

The most important features determining the suitability of a feedstock for biofuel production are its energy properties and parameters indicating the quality of engine combustion [43]. For biodiesel, these are the heating value (HHV) and the cetane number (CN). In addition, the rheological parameters of biodiesel such as density (D) and kinematic viscosity (KV) are very important, as they have a direct impact on the injection of the fuel inside combustion chamber of the engine [44,45]. Moreover, these parameters affect the functioning of the diesel engine fuel feed system, especially the fuel filter at low temperatures, resulting in flow blockage, which is characterised by a cold filter plugging point (CFPP) [46]. During storage of biofuels, their stability is also very significant, as expressed by the parameters of oxidation stability (OS) and iodine number (IV) [47].

Our aim was to investigate the effect of stress induced by nutrient deprivation on microalgal biomass and lipid production and fatty acid profile (FA). This study was carried

out at technical scale. In addition, FAs were used to predict biodiesel properties and evaluate the suitability of microalgae for biofuel production.

2. Materials and Methods

2.1. Microalgae Strains

Microalgae strains of *Chlorella vulgaris*, *Chlorella fusca*, *Oocystis submarina* and *Monoraphidium* were obtained from the Culture Collection of Baltic Algae (University of Gdańsk, Gdańsk, Poland). The inocula were stored in liquid medium F/2 [48] containing [g·L^{-1}]: NaNO$_3$—0.075 g; NaH$_2$PO$_4$·2H$_2$O—0.00565 g; stock solution of trace elements—1 mL·L^{-1} (Na$_2$EDTA 4.16 g, FeCl$_3$ 6H$_2$O 3.15 g, CuSO$_4$ 5H$_2$O 0.01 g, ZnSO$_4$ 7H$_2$O 0.022 g, CoCl$_2$ 6H$_2$O 0.01 g, MnCl$_2$ 4H$_2$O 0.18 g and NaMoO$_4$ 2H$_2$O 0.18 g) and stock solution of vitamin mix—1 mL·L^{-1} (cyanocobalamin (vitamin B12) 0.0005 g, thiamine HCl (vitamin B1) 0.1 g, biotin 0.0005 g). Microalgae were stored at 4 °C, with lighting (12/12 h light/dark cycle).

2.2. Experimental Setup

Microalgae cultures were carried out in vertical tubular photobioreactors with a total volume of 100 L, with 70 L of growth medium. Microalgae were cultured in F/2 culture medium containing all required components at full dose (100%) or reduced by half (50%). A 20 L of 7-day microalgal inoculum was introduced into particular photobioreactors.

The culture was illuminated by light emitting diode (LED light with white, red and blue diodes) under 18/6 h light/dark cycle. Algae culture was mixed and aerated by 175 W membrane pump (HAILEA ACO-500A, Raoping, China) with a capacity of 275 L·min^{-1}. The experiment was carried out as a batch culture for 10 days.

2.3. Analytical Methods

2.3.1. Biomass Content Determination

Biomass content was determined by thermogravimetric measurement using a weigh-dryer (AXIS ATS60, Gdańsk, Poland) [49]. The results were recalculated and presented in mg·L^{-1}. Measurement was carried out on the last day of the experiment.

2.3.2. Lipid and Ash Content of Biomass

Lipid content in biomass was determined by the gravimetric method. To extract lipids from microalgal cell, a modified Bligh and Dyer method was applied [50]. Total lipids were determined after evaporation of the solvent. The lipid content was determined according to the following formula:

$$\text{LC} = \left(\frac{m_L}{m_{DAB}} \right) \cdot 100$$

where: m_L is the mass of lipids (g) and m_{DAB} is the dry microalgal biomass (g).

The ash content in biomass was determined gravimetrically according to the Standard PN-EN ISO 18122:2016-01 [51].

2.4. Fatty Acids Methyl Esters Analysis

Lipid extraction from microalgae biomass was performed using the method of Folch et al. [52]. For the determination of fatty acids, they were converted into fatty acid methyl esters according to the method of AOCS Ce 2-66 [53].

The fatty acid composition of the studied samples was estimated using gas chromatography (GC). Fatty acid methyl esters were separated using a Trace 1300 (Thermo Scientific, Waltham, MA, USA) gas chromatography system equipped with a SP TM-2560 capillary column (100 m × 0.25 mm × 0.2 μm) (Supelco, Bellefonte, PA, USA) and a flame-ionization detector (FID). Column temperature was held at 160 °C for 1 min, then the temperature was increased from 160 °C to 220 °C at 6 °C/min and held at 220 °C for an additional 35 min. The temperature of the injection port and the detector was set at 240 °C. Hydrogen was used as a carrier gas at a flow rate of 1.5 mL/min. The results were expressed as a percentage of the total peak area of all the fatty acids in microalgae lipid extract sample.

The peaks were identified by comparing the retention times with those of a mixture of external standard methyl esters (Supelco 37 FAME Mix C 4–C 24 Component, Sigma-Aldrich, St. Louis, MO, USA).

2.5. Biodiesel Properties According to Fatty Acid Profile

Analysis of selected biofuel properties was carried out using the BiodieselAnalyzer© software (version 2.2, BRTeam, Karaj, Iran), according to the methodology described in the works of Ramírez-Verduzco et al. [54] and Talebi et al. [55]. This software was designed to evaluate (predict) the biodiesel fuel properties based on the fatty acid profile determined by gas chromatography, therefore eliminate long and expensive laboratory tests.

Difficulties to determine the best feedstock for biodiesel production arise from the presence more than one evaluation criteria. Therefore, the multi-criteria decision problem required several mathematical programming models. The PROMETHEE method is one of such tools. According to the references [54–57], seven functional parameters were selected and associated with weight factors. The highest weight was assigned to the calorific value (HHV = 0.5), followed by the cetane number (CN = 0.2). The weighting coefficients for CFPP, D, KV, OS, and IV were the same and equals 0.06.

2.6. Statistical Analysis

All analyses were carried out in triplicate. Results were statistically analysed using Statistica software (version 13.3, 2016; Dell Inc., Tulsa, OK, USA). Two-factor analysis of variance was used. The significance of differences between means was assessed using Tukey's test at $p \leq 0.05$. The standard deviation (SD) was also determined.

3. Results and Discussion

3.1. Algal Biomass Concentration

The biomass content in the particular photobioreactors was presented in Figure 1. Higher values were observed at the 100% dose of nutrient solution. The biomass content ranged from 292 ± 14 mg·L^{-1} for *C. fusca* to 525 ± 29 mg·L^{-1} for *Monoraphidium*. These values correspond with results available in the literature [58]. Biomass production can be increased using nutrient-rich culture media [59] but the results are not always positive. In a study carried out by Sacristán et al. [60], increase in nutrient content in culture medium had a negative impact on the growth and development of *Scenedesmus acutus*. This could be associated with the high pH in the culture medium and the presence of toxins in the wastewater used in the cultivation.
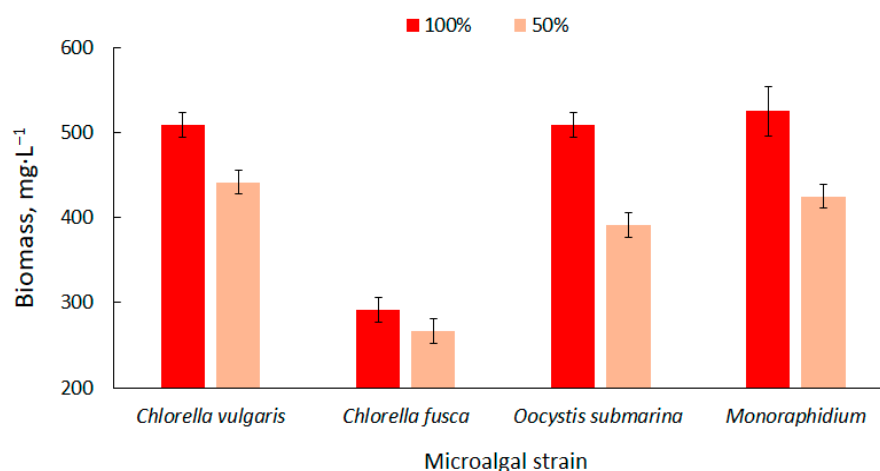

Figure 1. Dynamics of biomass content changes of four strains in relation to nutrient supply in the culture medium.

Nutrient limitation decreases the productivity of biomass [61] which is confirmed by the results of the present study. The amount of biomass decreased by more than 20% for *O. submarina*, 19% for *Monoraphidium*, 13% for *C. vulgaris* and 9% for *C. fusca*. Kim et al. [62] cultivated strains of *Chlorella* sp., *Dunaliella salina* and *Dunaliella* sp. and found that the appropriate amount of nutrients in the medium determines the effective production of algal biomass. They observed a higher increase in biomass in a culture medium with a higher nutrient content.

3.2. Lipid Accumulation under Nutrient Limitation Conditions

High lipid content in microalgae cells is one of the most important criteria that determines their potential use for biofuel production [63]. The lipid content for the tested microalgae strains is shown in Figure 2. The average amount of lipids in relation to dry matter, at the complete nutrient dose (100%), ranged from 12.33 ± 1.0 for *O. submarina* to $13.53 \pm 2.1\%$ for *C. fusca*. A higher lipid content *for O. submarina* was reported by Klin et al. [64], but increase in lipid accumulation could be associated with using a different culture medium and a significantly lower photobioreactor capacity.
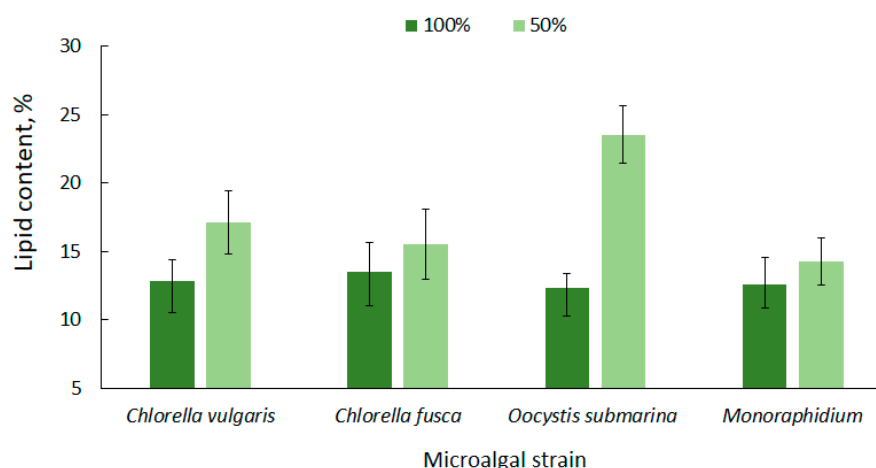

Figure 2. Lipid content in microalgal biomass under nutrient limitation.

Under nutrient stress treatment (50% F/2), there was a 14% increase in lipid accumulation for *Monoraphidium*, 15% for *C. fusca*, 31% for *C. vulgaris*, and over 90% dla *O. submarina*; however, only the *C. vulgaris* and *O. submarina* increase was statistically significant. Nutrient limitation to promote lipid accumulation in microalgal cells is a commonly used method [65,66]. Results presented by Converti et al. [67] confirmed the correlation between nitrogen depletion in the culture medium and an increase in lipid accumulation for *Nannochloropsis oculata* and *C. vulgaris*. A 75% nitrogen limitation resulted in a two-fold increase of lipids in the algal cells. In the present study, a slightly lower lipid content was observed for *C. fusca* (from $13.53 \pm 2.1\%$ to $15.53 \pm 2.5\%$) and for *Monoraphidium* (from $12.53 \pm 2.0\%$ to $14.27 \pm 1.7\%$), while for *O. submarina* the lipid content under stress conditions was $23.53\%. \pm 2.1$ A similar correlation was observed in a previous study [68] evaluating nutrient stress caused by nitrogen limitation in wastewater from salmon aquaculture on lipid content in *C. vulgaris* biomass. The highest value (11.81%) was determined at the lowest dose of aquaculture wastewater. In the present study, the lipid content in biomass of C. vulgaris, regardless of nutrient conditions, was in a range corresponding to values for this species reported by other authors [69,70].

3.3. Ash Content

Microalgal biomass has a high ash content compared to terrestrial plant biomass, and this is one of the major disadvantages in algae conversion to fuel [71]. The ash content in dry matter, depending on the microalgal strain and the culture conditions, is up to 43% [72].

This reduces the total volatile solids content of the feedstock, one of the most important parameters determining the quality of biofuel. A lot of final ash in algal biomass would require fuel purification [73,74].

According to the strain, the average ash content in biomass at 100% dose was in the range from 19.74 ± 1.70 for *O. submarina*) to 43.19 ± 0.24% for *Monoraphidium* (Figure 3). Similar results were presented by Bi and He [75] who found up to 19% ash content for green algae. In this study, the results show that nutrient stress not only promotes lipid accumulation, but generally causes a decrease in ash content in microalgal biomass. In the nutrient-limited medium (50%), ash content ranged from 11.16 ± 0.07% for *O. submarina* to 37.04 ± 0.38% for *Monoraphidium*. Increase in ash content was observed only for *C. fusca*, but it was statistically insignificant difference. The correlation between nutrient content and ash content in microalgal biomass was also reported by Roostaei et al. [76]. The limited availability of nutrients in *Chlorella vulgaris* culture significantly decreased ash content.
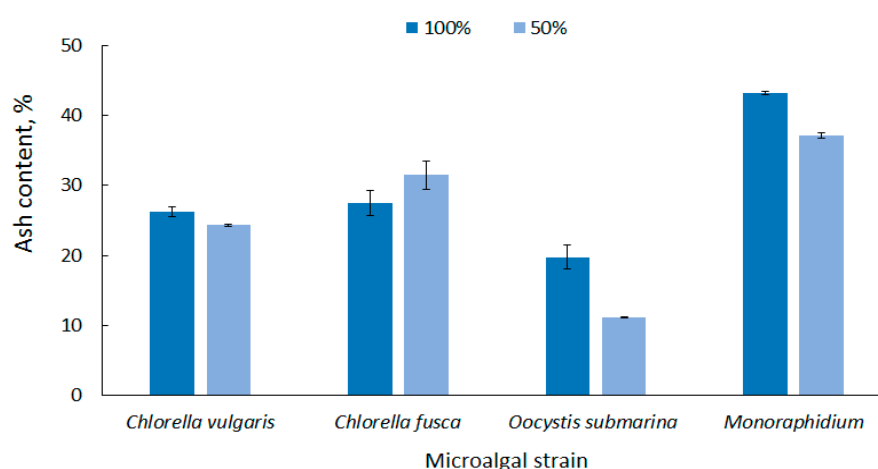

Figure 3. Ash content in microalgal biomass under nutrient limitation.

3.4. Fatty Acid Composition and Content

Microalgal oil is a source of saturated and unsaturated fatty acids [38]. In FAs profiles, groups with chain lengths of C16–C18 are most commonly determined [77], as also confirmed by the results of the presented study (Table 1, Figure 4A–D). The most important FAs that determine the quality of the biofuel are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) [78]. In microalgal biomass cultivated in culture medium with complete suggested nutrient rate (100% F/2), palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (C18:3 n-3) were dominant in the profile, but in different quantities for each strain. Due to environmental stress, fatty acid composition changes [79] which was also observed in this study. A 50% reduction of the dose for all medium components increased palmitic acid content for all strains, oleic acid content for *C. fusca* and *O. submarina*, and linoleic acid for *C. vulgaris* and *C. fusca*. Furthermore, acids that were not present in the 100% F/2 dose were determined, e.g., C16:1, C18:0, C18:3 n-6 and C18:4 for *Monoraphidium*. A higher content of saturated fatty acids (C16:0, C18:0) increases the cetane number (CN) [80]. FAs profiles change not only with species but also intraspecies [81]. Petkov and Garcia [82] analysed the fatty acid composition of three *Chlorella* strains, including freshwater and marine strains, and found that species belonging to the same genus contained the same acids but in different ratios. The presented study does not confirm these results. The C16:1 and C18:3 n-6 fatty acids determined in the FAs profile of *C. vulgaris* were not found in *C. fusca*. The change in FAs composition may also result from different cultivation methods (photoautotrophic, heterotrophic) [83] or from a change in cultivation conditions. In a study carried out by Yusof et al. [41], increasing amount of carbon dioxide in *C. vulgaris* culture medium from 1 to 10% increased concentrations of palmitic, oleic, linoleic, and linolenic acids. In the

present study, cultivations of all strains were carried out at one test site, at the same time, with uniformity of all growing parameters. This means that a biological factor, directly associated with the metabolic activity of particular microalgal strains, is essential for the observed changes. Analysis of FAs profiles in relation to growth stage and adjusting biomass harvesting to the most beneficial stage for optimal fatty acid composition may be important [81]. In a study carried out by da Costa et al. [84], growth phase induced more changes in the fatty acid profile of *Tisochrysis lutea* than stress associated with N limitation in culture medium.

Table 1. Fatty acid profile of microalgae biomass.

Fatty Acid (m:n *)	*Chlorella vulgaris*		*Chlorella fusca*		*Oocystis submarina*		*Monoraphidium*	
	100%	50%	100%	50%	100%	50%	100%	50%
C16:0	20.73	24.61	25.53	28.63	17.17	26.35	19.01	19.56
C16:1	1.46	1.46	ta **	ta **	ta **	ta **	ta **	2.52
C18:0	6.59	5.39	3.11	ta **	ta **	ta **	ta **	0.72
C18:1 cis	22.72	21.68	18.50	20.23	10.15	19.95	17.63	13.62
C18:2 cis	9.90	10.41	11.20	12.10	16.98	11.74	22.51	19.37
C18:3 n-6	1.98	2.37	ta **	ta **	ta **	ta **	ta **	1.09
C18:3 n-3	22.96	18.11	25.32	23.84	28.00	22.91	17.53	16.29
C18:4	3.87	3.68	3.76	ta **	2.77	2.99	ta **	7.91

* m:n ratio in FAs description denotes m carbon atoms and n double bonds between carbon atoms, ** ta—trace amounts.
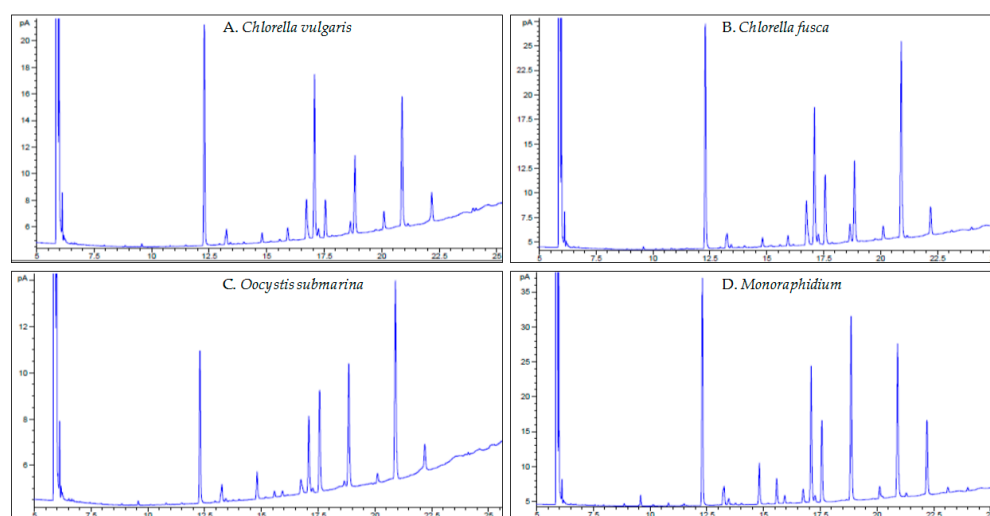

Figure 4. GC-FID chromatograms of fatty acids from particular microalgal strain.

The content of particular fatty acid categories is important for the quality of the biodiesel fuel. High concentration of saturated fatty acids (SFAs) ensures good oxidation resistance for biofuel [85]. In the presented study *C. vulgaris* has a total of 27.32% SFAs, *C. fusca* of 28.64%, *O. submarina* of 17.17%, and *Monoraphidium* of 19.01% (Figure 5). Taking into account not only oxidation stability, but also liquidity at low temperatures, monounsaturated fatty acids (MUFAs) are particularly important for biodiesel production [86]. The MUFAs content in the presented studies ranged from 10.15% to 24.18% of the total lipid composition. The lowest content was found for *O. submarina*, the highest for *C. vulgaris* (100%). Dahmen et al. [87] applied stress conditions (high salinity) to *Picochlorum* culture and observed lipid enrichment in saturated fatty acids. Nutrient stress from nutrient limitation (50% F/2) increased SFAs content to 30% for *C. vulgaris*, 26.35% for *O. submarina*, and slightly for *Monoraphidium* (to 20.28%). Arora and Philippidis [88] reported beneficial for biofuel production biosynthesis of SFAs and MUFAs in *C. vulgaris* culture using sweet sorghum pomace hydrolysate (mixotrophic conditions). Rohit and Mohan [89] under mixotrophic and heterotrophic conditions, found for *Chlorella* strain not only increase in

MUFAs but also PUFAs content. Polyunsaturated fatty acids are not good for biodiesel production. For different microalgae strains, the content of PUFAs ranges very widely from less than 3% to almost 69% [56]. In the present study, the lowest content of PUFAs in the profile was found for *C. vulgaris* (38.71%), the highest for *O. submarina* (47.75%). Under nutrient-limited conditions in the culture, beneficial changes, i.e., decrease in PUFA content, were observed for both species of *Chlorella* and for *O. submarina*. The high content of PUFAs may increase nitrogen oxide emissions [86] from the biofuel although according to a study carried out by Wahlen et al. [90] biodiesel from the microalgae *Chaetoceros gracilis* had lower NOx emissions compared to conventional diesel. Low amount of PUFAs have a positive effect on the biodiesel flow properties, especially at low temperatures [81].
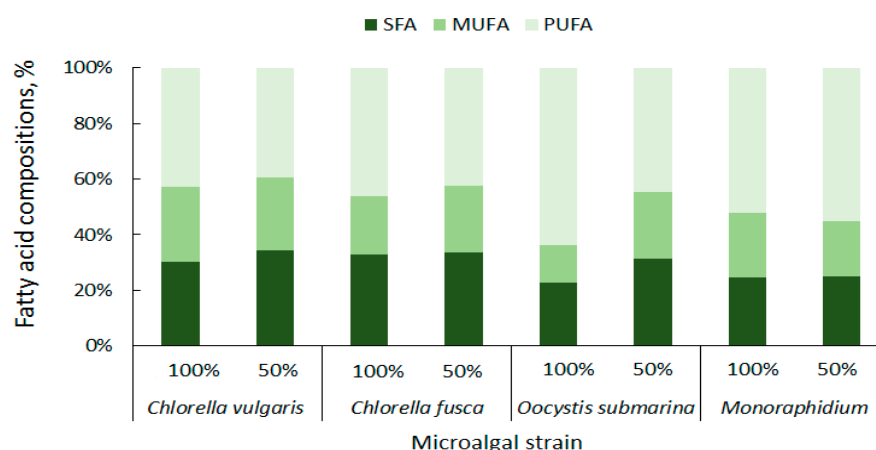

Figure 5. Summary composition of fatty acids in microalgal biomass.

3.5. Biodiesel Properties

Selected results from BiodieselAnalyzer© Version 2.2 are shown in Table 2. In terms of energy, nutrient limitation was found to increase only the HHV values only for *O. submarina* and *Monoraphidium*. Lower nutrient dose decreased HHV for both *Chlorella* species. The best feedstock for biodiesel production was oil from *C. vulgaris* 100%, HHV = 35.483 MJ·kg^{-1}. The high energetic potential of *Chlorella* was also confirmed in a study carried out by Sakarika et al. [91], who compared energy efficiency of biodiesel, biomethane, and solid biofuel produced from the biomass of this microalgae species. Among the high-energy microalgae, *Nannochloropsis* sp. may be interesting, especially in countries with high insolation, as highlighted in the paper of Pradana et al. [92].

Table 2. Biodiesel fuel properties calculated on fatty acid profile.

Parameter	*Chlorella vulgaris*		*Chlorella fusca*		*Oocystis submarina*		*Monoraphidium*	
	100%	50%	100%	50%	100%	50%	100%	50%
HHV	35.483	34.495	34.353	33.328	29.467	32.979	30.157	31.831
CN	48.401	52.013	49.704	53.83	53.361	52.507	57.601	50.651
D	0.795	0.772	0.771	0.747	0.665	0.74	0.676	0.717
KV	3.076	3.008	2.93	2.876	2.422	2.808	2.593	2.636
CFPP	0.388	−0.278	−3.571	−7.482	−11.083	−8.199	−10.505	−9.201
OS	5.975	6.408	5.82	5.872	5.212	5.994	5.536	5.799
IV	122.302	109.386	120.04	105.354	126.696	112.896	104.603	126.491

HHV: Higher Heating Value (MJ·kg^{-1}), CN: Cetane number, D: Density (g·cm^{-3}), KV: Kinematic Viscosity (mm^2·s^{-1}), CFPP: Cold Filter Plugging Point (°C), OS: Oxidation Stability (h), IV: Iodine Value.

Nutrient stress increased the cetane number for *Chlorella* strains, in contrast to *O. submarina* and *Monoraphidium*. The highest value of this parameter was obtained for *Monoraphidium* 100%, CN = 57.601. The CN value for oil of all tested microalgae species

was high compared to the results presented by Yaşar et al. [93] for oils from oil crops cultivated for biodiesel production. The rheological parameters of biodiesel produced from microalgal biomass, such as density and kinematic viscosity, presented in Table 2, suggest that nutrient stress increased their values for *O. submarina* and *Monoraphidium*. The opposite dependence was found for *Chlorella* strains. The highest values were observed for *C. vulgaris* cultivated with full dose of nutrients (C.v.100%). They were respectively $D = 0.795$ g·cm^{-3} and $KV = 3.076$ mm^2·s^{-1} and were most similar to the parameters of conventional diesel fuel ($D = 0.82$–0.845 g·cm^{-3} and $KV = 2$–4.5 mm^2·s^{-1}).

A significant parameter of biodiesel is the cold filter plugging point. The lowest and the most beneficial value of this parameter was obtained for *O. submarina* (O.100%), CFPP = -11.08 °C. Under nutrient stress conditions beneficial decrease in CFPP was observed only for *Chlorella* strains. The high oil content improves the CFPP index, therefore use of these microalgae is advantageous, especially when their cultivation is not competitive with food crops [94].

Oxidation stability, which indicates the formation of different types of sediments, resins, and acids in the fuel, increased for limited nutrient dose (50%) and had the most beneficial value for *C. vulgaris* (OS = 6.41 h). This is also confirmed by research carried out by Islam et al. [57].

Regarding iodine number IV, characterising the content of unsaturated compounds in biofuel, the 50% lower nutrient dose had a positive effect on this parameter except for *Monoraphidium*, which was characterised by the lowest value IV = 104.60 at complete nutrient dose (100%). In a study carried out by Islam et al. [57], the most beneficial value for this parameter was observed for *Calothrix* sp.

This study has shown that biodiesel produced from oil of different types of microalgae has some beneficial and some disadvantages. Other authors concluded the same [57]. The analysed parameters of biofuels produced from the investigated energy feedstocks do not provide their complete evaluation. According to a few studies carried out in this topic [95,96], a complete evaluation of such energy feedstocks can be obtained only during engine tests. In these tests, not only energy parameters determination, but also emission of toxic components of exhaust gases can be evaluated, thus also environmental assessment of such biofuels.

Figure 6 shows results of carried out analysis with highlighting those microalgae species whose position is most close to the so-called decision axis. It is important to note that all highlights were microalgal strains under nutritional stress. It is concluded that nutrient stress in three out of four possible cases (except *Monoraphidium*), had a beneficial effect on the functional properties of the newly produced biodiesel. The ranking of microalgae strains, considering the culture conditions, is shown in Table 3.

Table 3. Ranking list of microalgae according to their suitability for biodiesel production.

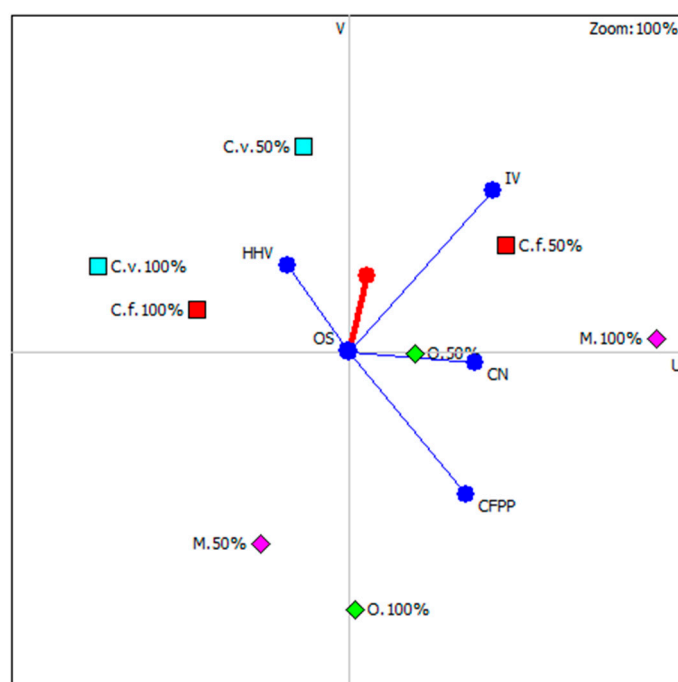| Rank	Microalgae Strains		F/2 Component Dose, %	Phi
	Description	Name		
1	C.f.50%	*C. fusca*	50	0.1586
2	C.v.50%	*C. vulgaris*	50	0.1097
3	O.50%	*O. submarina*	50	0.0664
4	M.100%	*Monoraphidium*	100	0.0294
5	C.v.100%	*C. vulgaris*	100	0.0150
6	C.f.100%	*C. fusca*	100	0.0067
7	M.50%	*Monoraphidium*	50	−0.1379
8	O.100%	*O. submarina*	100	−0.2479

Figure 6. Graphical multi-criteria analysis PROMETHEE-GAIA (C.f.—*C. fusca*, C.v.—*C. vulgaris*, O.—*O. submarina*, M.—*Monoraphidium*).

4. Conclusions

Nutrient limitation (50% F/2) decreased microalgal biomass production, while beneficial changes were observed in ash and lipid content. The fatty acid profile of tested green microalgae strains indicates that these algae can be a source of feedstock for advanced biofuel production. Under nutritional stress conditions lipid enrichment in saturated fatty acids for *C. vulgaris*, *O. submarina* and *Monoraphidium*, and monounsaturated fatty acids for *C. fusca* and *O. submarina* was noted, which improves the biodiesel fuel properties. The fatty acid profile gives some information about the quality of microalgal biomass as an energy feedstock, but without engine tests, this assessment is not sufficient. However, according to carried out analysis, it can be concluded that nutrient limitation has a beneficial effect on the functional properties of biodiesel produced from oil of the tested microalgae. Positive changes were found for *C. fusca*, *O. submarina* and *C. vulgaris.*

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