Fatty Acid Methyl Esters of the Aerophytic Cave Alga Coccomyxa subglobosa as a Source for Biodiesel Production

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Abstract: The microscopic alga Coccomyxa subglobosa, collected from the Głowoniowa Nyża Cave (Tatra Mountains, Poland), is a source of fatty acids (FAs) that could be used for biodiesel production. FAs from subaerial algae have unlimited availability because of the ubiquity of algae in nature. Algal culture was carried out under laboratory conditions and algal biomass was measured during growth phase, resulting in 5 g of dry weight (32% oil). The fatty acid methyl ester (FAME) profile was analyzed by means of gas chromatography–mass spectrometry (GC–MS). The presence of lipids and chloroplasts in C. subglobosa was demonstrated using GC–MS and confocal laser microscopy. Naturally occurring FAMEs contained C_{12}–C_{24} compounds, and methyl palmitate (28.5%) and methyl stearate (45%) were the predominant lipid species. Aerophytic algae could be an important component of biodiesel production, as they are omnipresent and environmentally friendly, contain more methyl esters than seaweed, and can be easily produced on a large scale.

Keywords: aerophytic algae; biodiesel; GC–MS

1. Introduction

Biofuels produced from plants and animal waste reduce the necessity for petroleum, coal, and natural gas use and they are also an environmentally sustainable alternative that allows for reductions in global pollutants. Microalgae can produce third-generation biodiesel, because of the high content of lipid reaching up to 40% lipid by weight [1]. Biodiesel is now typically commercially produced from vegetable and soybean oils. However, oilseed crops cannot displace conventional transport fuels such as gasoline and diesel. Fakhry and Maghraby noted that the main environmental benefits of biodiesel depend on its renewability, lack of toxicity, and biodegradability, as well as the fact that its combustion produces fewer harmful gaseous emissions, such as sulphur oxide [2]. Biodiesel is composed of long-chain alkyl (methyl, ethyl, or propyl) esters obtained from triglycerides by transesterification with alcohol [3,4]. Fatty acids (FAs) are composed of hydrocarbon and carboxylic acid groups, and are responsible for the hydrophobic properties exhibited by lipids. FAs differ in aliphatic chain length, degree of unsaturation, configuration, and position of double bonds, and also have various functions, and most of the commonly found FAs contain even numbers of carbon atoms (C_{4}–C_{28}) [5,6]. Algae are widely studied for FAs because of their chemotaxonomic, nutritional, and industrial importance. Lipids are defined as apolar natural compounds, which can be classified by their FA profiles [7]. FA profiles are used as chemotaxonomic markers to define groups of different
taxonomic levels for vascular plants and embryophytes [7]. Lipids consist of saturated and unsaturated FAs, and their ester attributes differ significantly as a function of available resources [8]. Carvalho et al. described the production of biofuel from algae, and noted that they are one of the most commonly occurring sources of lipids that is also renewable [9]. On the other hand, two studies found that algae have both high biomass and lipid content, which can be used to produce fuel [10,11]. Nevertheless, photosynthetic algae are useful in bioremediation and provide several types of renewable biofuels [12]. Chisti concluded that, although biodiesel is produced on an industrial scale from plant and animal oils, and not from algae, oil productivity from many microalgae greatly exceeds the production of energy derived both from plants and animal waste [10]. Biodiesel is made from diverse sources through a transesterification process; and, on the one hand, biofuel production from seaweed was increased because of the fact that algae are readily available and grow by photosynthesis; on the other hand, to our knowledge, subaerial algae have not yet been used for biofuel production [9].

Aerophytic algae are omnipresent and colonize epiphytic subaerial habitats, including exposed bedrock soil, terrestrial bryophytes, tree bark, rocks, and anthropogenic structures [13]. They are usually small in size, produce extracellular mucilage to improve water retention, are tolerant to desiccation and solar radiation, a broad pH range, can survive low nutrient levels, and are transported by wind, water, and birds [14]. The solar radiation in subaerial habitats are resulting in the production of internal or external protective pigments e.g., in aerophytic cyanobacteria found scytonemin (the UV-A absorbing pigment) and mycosporine amino acids (the UV-B absorbing pigment). For aerophytic algae adaptations to survival in environments are important such as tolerance to desiccation and to solar radiation, also dispersal and the colonization of new subaerial habitats. It is interesting that the majority of aerophytic species are soil algae having variability between different climates and their diversity in the atmosphere are changing to divers throughout year.

Gorbushina and Broughton suggest that aerophytic algae are exposed to harsher and more variable environmental conditions than freshwater algae, because water buffers abrupt changes in radiation and temperature [15]. Aerophytic algae can also be aerial, terrestrial, or airborne [16]. Algae use CO₂, and participate in more than 40% of global carbon fixation, mainly from seaweed, due to their rapid increase in biomass [17,18]. Aerophytic algae are usually cyanobacterial coccoid green algae, and because of their high lipid productivity can be used as an alternative feedstock for biodiesel. It is worth of noting that most algal species are composed of more than 50% triacylglycerols and alkanes by dry mass. Aerophytic algae with long-chain hydrocarbons from the terpenoid pathway are used in biodiesel production [19–21]. Oils, hydrocarbons, and algal biomass (containing lipids and FAs) can be used to produce animal feed, cosmetics, pharmaceuticals, and aerophytic algae constitute an important source of energy; furthermore, the overall energy and carbon balance would be favorable [10,22]. Biodiesel production from microalgae has attracted more attention because of its potential to reduce the use of agricultural land. However, the use of different habitats (water, land, and air) is characteristic of microalgae, and biofuel production from algae (50–60% oil content) is more efficient than that from agricultural oil plants (5–10% oil content). If production is economically profitable, microalgae will likely be used as effective biofuel producers in the future. The lack of non-renewable energy sources has inspired the development of alternative energy sources, e.g., biomass (from plants or plant-derived materials).

The aim of this study was to test whether FA methyl esters (FAMEs) of the aerophytic alga Coccomyxa subglobosa could be isolated as a method for a biodiesel production. FA profiles of certain FAMEs were estimated with gas chromatography–mass spectrometry (GS–MS).

2. Materials and Methods

2.1. Algal Isolation

Aerophytic algae were collected from the pseudokarstic Głowniowa Nyża Cave (Tatra Mountains, Poland). Green-colored patches of algae were scraped from granite cave walls, and from pine
twigs. Aerophytic algae were first cultivated in 1% Bold’s Basal Medium (BBM) with fresh agar. The cultures were maintained under controlled conditions (temperature, 20 °C; 12-h light/12-h dark cycle; 3000 μE m−2 s−1 lx (40-W cool fluorescent tubes). Approximately 2–3 months after the first cultivation of algae, a microscopy study was performed. All phototrophs were observed live and identified using a Jenamed 2 light microscope (Carl Zeiss, Jena, Germany).

2.2. Microscopy

Algae were observed and photographed with a Nikon Eclipse Ti inverted microscope, and with Nikon A1 confocal equipment (Nikon, Tokyo, Japan). The 405.488.561-nm laser heads and detection system comprised a PMT-DU4 detector in the range of 400–820 nm, a Plan Apo VC ×100 oil immersion lens, Nikon C-HgFiE mercury illumination, and a DS0Fi1C-U3 digital camera. Digital images were obtained with Nikon NIS-Elements software, and processed with Adobe Photoshop CS5 (Adobe, San Jose, CA, USA).

2.3. PCR

Total DNA was extracted from C. subglobosa dry cell mass using the DNeasy Plant Mini kit (Qiagen) according to the manufacturer’s instructions. This isolation kit provides silica-based plant DNA extraction in spin column format. For amplification, Coccomyxa-specific oligonucleotide primers were used (F, 5′-ATGCCTGAGATGAAGGACGTA-3′; R, 5′-TCTAGGTTAGGGTAGGTATTAA-3′). The PCR procedure consisted of an initial step for 3 min at 94 °C; followed by 35 cycles of 30 s at 94 °C, 1 min at 56 °C, 1 min 40 s at 72 °C; and a final extension of 5 min at 72 °C [23]. PCR products were subjected to electrophoresis in a 1% agarose gel. The gel was stained with ethidium bromide (0.5 µg/mL) and DNA was visualized under a UV light.

2.4. Lipid Extraction and Methyl Transesterification

C. subglobosa cells were cultivated under aerobic conditions in a fermenter (Biostat B, Sartorius Stedim Biotech, Gottingen, Germany) under the same conditions as the algal samples collected from the Glowoniowa Nysa Cave (cultured in the laboratory on Petri plates). Cells were collected (5000 g, 30 min) and lyophilized. Next, the dried cells (5 g) were suspended in 50 mL of a chloromethane–methanol 2:1 mixture for 60 min at room temperature with magnetic stirring. When the extraction was finished, the mixture was filtered with sintered glass in a Buchner funnel, and the solvent was evaporated. After filtration, water was added to create a biphasic system with a final ratio of 8:4:3 chloromethane:methanol:water. The organic phase containing lipids was isolated and vaporized under vacuum. The lipid fraction was suspended in 4.5 mL of a methanol–hydrochloric acid–chloromethane mixture for 4 h at 80 °C with magnetic stirring. The solution was cooled, mixed with 1.5 mL water, and 3 × 4 mL of a hexane–water mixture. The organic phases were collected and filtered with a funnel before GC–MS analysis [24].

2.5. GC–MS Analysis of FAMEs

Qualitative and quantitative analyses were conducted by the GC–MS method using the Clarus 600T instrument from PerkinElmer (Waltham, MA, USA) [24–27]. Compounds were separated on the Elite-5MS capillary column (5% phenyl methyl siloxane; length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm) at 60 °C for 1 min, and then the temperature was increased at a rate of 15 °C per min up to 250 °C, and maintained at 250 °C for 5 min. Total analysis time was 18.67 min. The injection port temperature was 250 °C, and the carrier gas helium was kept at a steady flow rate of 1 mL/min. The ion source and transfer line temperatures were set at 250 °C. The electron ionization energy in spectrometry was 70 eV. FAME identification was carried out with C8–C24 saturated and unsaturated external standards, a GC-grade n-hexane solution, and the NIST Mass Spectra Database.
3. Results

In this study, we present that the aerophytic alga *C. subglobosa* isolated from a cave, can be used as a potential source for biodiesel production. *Coccomyxa* cells were variable in shape, usually cylindrical, elongate–ovoid, or ellipsoid, sometimes asymmetrical, thin-walled, solitary or grouped within a mucilaginous envelope, and with or without concentric striations surrounding cell groups. Irregular, elliptical-to-globular, parietal chloroplasts were plate-like and lateral in position, without pyrenoid or flagellated stages. Each sporangium contained two to four autospores for asexual reproduction. The average cell size was 6–14 × 3–6 µm (Figure 1).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** (a,b) Confocal images of *C. subglobosa* chloroplasts. (c) 3D reconstruction image of *C. subglobosa* chloroplasts (scanner zoom, 1.773; calibration value, 0.02 µm/px). (d) Confocal image of intracellular lipid droplets (indicated by blue color).

Different life stages of *C. subglobosa* were found in all studied samples. Cells were 2–4 µm wide, 3–8 µm long, green-colored (indicating the presence of chlorophyll a and b), and broadly ellipsoid to subspherical to spherical, with mucilage surrounding the cells either homogeneously or with striations, as demonstrated by the Nikon Eclipse Ti inverted microscope with Nikon A1 confocal equipment. Figure 1 shows two parietal chloroplasts per cell, but pyrenoid and flagellated stages were not observed. The cell wall varied in thickness, from about 40 to 100 nm, and the cup-shaped chloroplast made up about half of the cell volume. Additionally, genetic analysis in this study confirmed *Coccomyxa* species (Figure 2).
In this work, FAs from *C. subglobosa* containing C12–C24 were isolated, and FA profiles revealed qualitative and quantitative differences. Figure 3 presents the GC–MS profile of methyl esters of free FAs from *C. subglobosa*.

Additionally, the content, density, and viscosity of FAMEs from *C. subglobosa* are presented in Table 1. This profile was consistent with the FA profile in seaweed [28]. Lang et al. identified 76 different FAs and ten other lipophilic substances from 2076 FAs profiles of microalgal strains [7]. However, only one genus, *Nostoc*, belongs to terrestrial habitats (aerophytic algae).

**Table 1.** FAME characteristics in *C. subglobosa* according to Figure 3.

| Retention Time (min) | FAME | Name    | Content (%) | Density * (kg/m³) | Viscosity ** (mPA s) |
|----------------------|------|---------|-------------|-------------------|-----------------------|
| 5.12                 | C6   | Caproate| 7.0         | 890.97            | -                     |
| 5.28                 | C8   | Caprylate| 9.5        | 883.04            | 0.99                  |
| 7.12                 | C10  | Caprate | 2.5         | 876.31            | 1.40                  |
| 9.27                 | C12  | Laurate | 1.5         | 873.28            | 1.95                  |
| 10.87                | C14  | Myristate| 1.5        | 868.18            | 2.69                  |
| 12.17                | C16  | Palmitate| 28.5       | 864.19            | 3.60                  |
| 13.45                | C18  | Stearate| 45.0        | 867.55            | 4.74                  |
| 13.63                | C20  | Arachidate| 2.0       | 866.28            | -                     |
| 15.67                | C22  | Docosanoic| 1.0       | -                 | -                     |
| 18.53                | C24  | Tetracosanoic| 1.5    | -                 | -                     |

* Density converted to 15 °C, estimated according to [29]; ** Viscosity measured at 40 °C, estimated according to [30].
In this study, C12:0, C14:0, C16:0, C18:0, C22:0, and C24:0, and other FAs were identified (Figure 3). C16 (methyl palmitate) and C18 (methyl stearate) were obtained. Figure 4 presents a portion of the chromatogram with dominant peaks of FAMEs: C16:0 (27.3%), C16:1 (3.1%), C16:2 (0.6%), C18:0 (47.3%), C18:1 (18.9%), and C18:2 (2.8%). Methyl palmitate (C16:0) and methyl stearate (C18:0) were the major saturated FAMEs. Methyl palmitoleate (C16:1) and methyl oleate (C18:1) were the major unsaturated FAMEs.

Figure 4. A portion of the chromatogram with dominant peaks of FAMEs. The remaining peaks are FAME isomers.

Additionally, the content, density, and viscosity of FAMEs from C. subglobosa are presented in Table 2.

Table 2. FAME characteristics of C16 and C18 FAs in C. subglobosa according to Figure 4.

| Retention Time (min) | FAME | Name         | Content (%) | Density * (kg/m³) | Viscosity ** (mPA s) |
|----------------------|------|--------------|-------------|-------------------|----------------------|
| 12.17                | C16:0| Palmitate    | 27.3        | 864.19            | 3.60                 |
| 12.24                | C16:1| Palmitoleate | 3.1         | 882.39            | -                    |
| 12.33                | C16:2| Hexadecadienoate | 0.6   | 903.85            | -                    |
| 13.22                | C18:2| Linoleate    | 2.8         | 893.18            | 3.05                 |
| 13.27                | C18:1| Oleate       | 18.9        | 877.46            | 3.73                 |
| 13.45                | C18:0| Stearate     | 47.3        | 867.55            | 4.74                 |

* Density converted to 15 °C; estimated according to [29]; ** Viscosity measured at 40 °C; estimated according to [30].

4. Discussion

Aerophytic algae offer a potential source (because of their small size and large biomass) for sufficient production of renewable fuels to impact the consumption of fossil fuels. Algae produce large amount of oil from oilseeds and this production is performed by different methods [21]. Furthermore, oil extracted from biomass of algae contains polar lipids, phospholipids and glycolipids, non-polar glycerides and free fatty acids. Marine, freshwater and aerophytic algae species exhibit different concentrations of total saturated and unsaturated fatty acids which a characteristic profile for each.

The aerophytic alga C. subglobosa isolated from a pseudokarstic cave was selected. Additionally, Coccomyxa simplex (SAG 216-9a), from the Culture Collection of Algae was used in many studies for
biofuel and polyunsaturated FA (PUFA) production [31]. Although seaweed has many PUFAAs and is a potential source of FAs, the possibility of using unprocessed glycerol, which can be a carbon source for green unicellular algae, such as *Chlorella pyrenoidosa* and *Coccomyxa subellipsoidea* [32], is of potential interest. Similar studies considering *C. subellipsoidea* for medical treatment made use of brewery effluent phytohormones to increase biomass and lipid accumulation in this species [7].

The FA composition of seaweed and aquatic and terrestrial algae differ significantly, but aerophytic algae are especially rich in long chain PUFAAs [33]. Table 3 presents chemical compositions of the high lipid content of various algae from different habitats: marine, freshwater, and terrestrial.

Table 3. FA content as FAMEs from FA fractions of algal lipid extracts.

| Fraction of Algal Lipid Extract (Free FAs) | FA Chain | FA Content in Algal Dry Mass (%) | References |
|-------------------------------------------|----------|----------------------------------|------------|
| *Scenedesmus dimorphus*                    | C16:0    | 0.685                            | [33]       |
|                                           | C16:1    | 0.327                            |            |
|                                           | C18:0    | 0.174                            |            |
|                                           | C18:1    | 0.804                            |            |
|                                           | C18:2    | 1.19                             |            |
|                                           | C18:3    | 1.26                             |            |
| *Chlorella vulgaris*                       | C8:0     | 0.25                             | [34]       |
|                                           | C14:0    | 0.95                             |            |
|                                           | C15:0    | 0.29                             |            |
|                                           | C16:0    | 15.58                            |            |
|                                           | C17:1    | 0.86                             |            |
|                                           | C18:2    | 0.15                             |            |
|                                           | C18:3    | 0.23                             |            |
|                                           | C20:4    | 0.17                             |            |
|                                           | C20:5    | 0.17                             |            |
| *Coccomyxa subglobosa*                     | C12:0    | 0.721                            | [35]       |
|                                           | C14:0    | 0.734                            |            |
|                                           | C16:1    | 1.05                             |            |
|                                           | C18:1    | 1.32                             |            |
|                                           | C20:0    | 0.54                             |            |
|                                           | C22:0    | 0.89                             |            |
|                                           | C24:0    | 0.654                            |            |

The genus *Coccomyxa* was described for the first time by Schmidle, and its classification as a close relative of *Dactylococcus* in the order Protococcoideae was finally included in the green algal class Trebouxiophyceae, which was separated by molecular approaches [36,37] into the *Chlorella*, *Oocystis* and *Trebouxia* genera [38], mostly consisting of terrestrial species. Darienko et al. stated that all species of *Coccomyxa* belong to the *Elliptochloris* clade [39]. The genus *Coccomyxa* is distributed worldwide in various ecosystems in diverse lifestyles (free-living, parasitic, or as photobionts). This cosmopolitan microscopic algal species forms macroscopic mucilaginous masses in terrestrial and aquatic habitats, rarely free-floating, but sometimes as a lichen phycobiont. Moreover, the *Coccomyxa* genus occurs as biofilms, in soil, on moss, as planktonic cells in limnetic ecosystems, as symbionts associated with fungi and vascular plants, and as parasites of marine mussels.

However, to date, only a limited number of algal species have been investigated for their FA composition. In this study, we evaluated *C. subglobosa* aerophytic algae as a potential source of oil products, by determining their total lipid content and FA profile. The total lipid content was different for species from the *Coccomyxa* genus, because it depends on the environmental conditions in which the algal isolates usually exist or survive. Generally, algae are very capable of surviving, reproducing, and growing in different environmental conditions, and lipid metabolism can be modified in response to habitat changes, which is reflected in the diversity and pattern of cellular lipids [40]. Juneja et al. determined the total lipid content and FA profiles in algae in natural habitats or under the influence of
environmental factors and nutrient availability [41]. Jensen showed that lipid content in seaweed is low (from 1% to 5% of dry matter), and is significantly different in various algal species (Table 3) [42]. The aerophytic alga C. subglobosa has higher total lipid content in comparison to the brown alga Padina pavonica and the red alga Jania rubens. Chao et al. investigated C. subellipsoidea as a model alga to augment lipid productivity in oleaginous vascular plants by manipulating nitrogen sources [43]. Seaweed has a low lipid content, and therefore is not very often used for biodiesel production (because of high cost), whereas aerophytic algae are more economical sources of biofuel products. Lang et al. examined 2076 strains from the algal culture collection (Göttingen University); among these were 76 different FAs and ten other lipophilic substances [7]. To define species or the algal taxonomic level, FA distribution patterns can be used as chemotaxonomic markers. Nevertheless, it is difficult to predict the FA profile of a new algal isolate, because of variation at the species level. Considering the diverse FA pattern distribution at the species level, it is difficult to define this profile in a new isolate. FA distribution shows phylogenetic relationships among different taxonomic levels in both genomic and molecular phylogenies.

Darki et al. suggested that amounts and types of fatty acids, along with biochemical composition, vary depending on environmental conditions [44]. Salinity, temperature, pH, and nutrients influence algal growth and biosynthetic lipid production, and promote the formation and accumulation of different lipids, which are indispensable for controlling intracellular stress. Additionally, technical aspects of biodiesel production are crucial, such as at the lipid-extraction stage. For example, ultrasonication is the best method to extract lipids from microalgae [45]. Biodiesel is important sources of renewable, biodegradable and environmentally fuel alternative and produces less harmful gas emissions. Biofuels produce from bio-sources reduce the need for petroleum oil and are favorable for sustainability and reduce pollutant and greenhouse gas emissions e.g., sulphur oxide [18]. Guzman et al. stated that, biodiesel reduces carbon dioxide emissions by 78% in comparison to conventional diesel fuel [27]. Knothe concluded that, for biodiesel production algae with a high proportion of saturated fatty acids are preferred because this leads to higher oxidative stability and higher ignition quality and produces an overall higher quality product [8]. Moreover, the fatty acid methyl ester profile is important such as a factor that determines the suitability of any feedstock using in biodiesel production.

Aerophytic algae synthesize and accumulate high amounts of lipids, proteins and carbohydrates in their cells and therefore they are good potentially sources of biodiesel and bioactive secondary metabolites. Algae can be used as renewable source for a biodiesel production because they are non-competitive and rapidly growing organisms, have capability to accumulate of lipids, metabolic flexibility and a biomass production occur during the whole year. Considering the climate and food problems, also energy crisis in the world, algae are now becoming the main source of biofuel producers. Moreover, algal biofuel does not contain harmful chemicals, so environment can be maintained clean after the combustion. For the clean and safe environment maintaining sustainability, renewable and environmentally friendly fuels are needed to be produce. Our C. subglobosa strain cultured in BBM medium could potentially be applied to biofuel production, by esterification of palmitic acid with methanol, ethanol, and isopropanol.

5. Conclusions

In this article, we report an aerophytic alga, C. subglobosa, isolated from a cave, which could potentially be used to produce biodiesel. Considering its FA profile, C. subglobosa was rated as a promising oil source. Cell capacity was $8.5 \times 10^6$ cells/mL culture, corresponding to a dry weight of 5.0 g/mL, producing 32% oil, and FA profiles were identified as C_{12}–C_{24}. According to chromatographic analysis (GC-MS) the dominant FAMEs were saturated compounds: methyl stearate (C_{18:0}) and methyl palmitate (C_{16:0}) and unsaturated compounds: methyl oleate (C_{18:1}) and methyl palmitoleate (C_{16:1}). Preparing cultures and further developing this algal project could be of great importance in producing biofuel on an industrial scale.
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References

1. Sobczuk, T.M.; Chisti, Y. Potential fuel oils from the microalga Choricystis minor. J. Chem. Technol. Biotechnol. 2010, 85, 100–108. [CrossRef]
2. Fakhry, E.M.; Maghraby, D.M. El Fatty Acids Composition and Biodiesel Characterization of Dunaliella salina. J. Water Resour. Prot. 2013, 05, 894–899. [CrossRef]
3. Van Gerpen, J. Biodiesel processing and production. Fuel Process. Technol. 2005, 86, 1097–1107. [CrossRef]
4. Rizwanul Fattah, I.M.; Ong, H.C.; Mahlia, T.M.I.; Mohijur, M.; Silitonga, A.S.; Ashrafur Rahman, S.M.; Ahmad, A. State of the Art of Catalysts for Biodiesel Production. Front. Energy Res. 2020, 8, 1–17. [CrossRef]
5. Gutnikov, G. Fatty acid profiles of lipid samples. J. Chromatogr. B Biomed. Sci. Appl. 1995, 671, 71–89. [CrossRef]
6. Orsavova, J.; Misurcova, L.; Vavra Ambrozova, J.; Vicha, R.; Mlcek, J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int. J. Mol. Sci. 2015, 16, 12871–12890. [CrossRef] [PubMed]
7. Lang, I.; Hodac, L.; Friedl, T.; Feussner, I. Fatty acid profiles and their distribution patterns in microalgae: A comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biol. 2011, 11, 124. [CrossRef]
8. Knothe, G. Dependence of biodiesel fuel properties on the structure of fatty acid alky esters. Fuel Process. Technol. 2005, 86, 1059–1070. [CrossRef]
9. de Carvalho, C.G.P.; Caldeira, A.; de Carvalho, L.M.; de Carvalho, H.W.L.; Ribeiro, J.L.; Mandarino, J.M.G.; de Resende, J.C.F.; dos Santos, A.R.; da Silva, M.R.; Arriel, N.H.C. Fatty Acid Profile of Sunflower Achene Oil From the Brazilian Semi-arid Region. J. Agric. Sci. 2018, 10, 144. [CrossRef]
10. Chisti, Y. Biodiesel from microalgae. Biotechnol. Adv. 2007, 25, 294–306. [CrossRef]
11. Resurreccion, E.P.; Colosi, L.M.; White, M.A.; Clarens, A.F. Comparison of algae cultivation methods for bioenergy production using a combined life cycle assessment and life cycle costing approach. Bioresour. Technol. 2012, 126, 298–306. [CrossRef] [PubMed]
12. Muñoz, R.; Guieysse, B. Algal-bacterial processes for the treatment of hazardous contaminants: A review. Water Res. 2006, 40, 2799–2815. [CrossRef] [PubMed]
13. Neustupa, J.; Štifterová, A. Distribution patterns of Subaerial corticolous microalgae in two European regions. Plant Ecol. Evol. 2013, 146, 279–289. [CrossRef]
14. Falasco, E.; Ector, L.; Isaa, M.; Wetzel, C.E.; Hoffmann, L.; Bona, F. Diatom flora in subterranean ecosystems: A review. Int. J. Speleol. 2014, 43, 231–251. [CrossRef]
15. Gorbushina, A.A.; Broughton, W.J. Microbiology of the atmosphere-rock interface: How biological interactions and physical stresses modulate a sophisticated microbial ecosystem. Annu. Rev. Microbiol. 2009, 63, 431–450. [CrossRef]
16. Czerwik-Marcinkowska, J.; Pusz, W.; Zagożdżon, P. Cyanobacteria and algae in an old mine adit (Marcinków, sudety mountains, southwestern Poland). J. Cave Karst Stud. 2017, 79, 122–130. [CrossRef]
17. Park, J.H.; Yoon, J.J.; Park, H.D.; Kim, Y.J.; Lim, D.J.; Kim, S.H. Feasibility of biohydrogen production from Gelidium amansii. Int. J. Hydrogen Energy 2011, 36, 13997–14003. [CrossRef]
18. Pittman, J.K.; Dean, A.P.; Osundeko, O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour. Technol. 2011, 102, 17–25. [CrossRef]
19. Scott, S.A.; Davey, M.P.; Dennis, J.S.; Horst, I.; Howe, C.J.; Lea-Smith, D.J.; Smith, A.G. Biodiesel from algae: Challenges and prospects. Curr. Opin. Biotechnol. 2010, 21, 277–286. [CrossRef]
20. Radakovits, R.; Jinkerson, R.E.; Darzins, A.; Posewitz, M.C. Genetic engineering of algae for enhanced biofuel production. *Eukaryot. Cell* **2010**, *9*, 486–501. [CrossRef]

21. Sivakumar, G.; Xu, J.; Thompson, R.W.; Yang, Y.; Randol-Smith, P.; Weathers, P.J. Integrated green algal technology for bioremediation and biofuel. *Bioresour. Technol.* **2012**, *107*, 1–9. [CrossRef] [PubMed]

22. Vlysidis, A.; Binns, M.; Webb, C.; Theodoropoulos, C. A techno-economic analysis of biodiesel biorefineries: Assessment of integrated designs for the co-production of fuels and chemicals. *Energy* **2011**, *36*, 4671–4683. [CrossRef]

23. Syasina, I.G.; Kukhllevsky, A.D.; Kovaleva, A.L.; Vaschenko, M.A. Phylogenetic and morphological characterization of the green alga infesting the horse mussel Modiolus modiolus from Vityaz Bay (Peter the Great Bay, Sea of Japan). *J. Invertebr. Pathol.* **2012**, *111*, 175–181. [CrossRef] [PubMed]

24. Bermúdez Menéndez, J.M.; Arenillas, A.; Menéndez Díaz, J.A.; Boffia, L.; Mantegna, S.; Binello, A.; Cravotto, G. Optimization of microalgae oil extraction under ultrasound and microwave irradiation. *J. Chem. Technol. Biotechnol.* **2014**, *89*, 1779–1784. [CrossRef]

25. Vlysidis, A.; Binns, M.; Webb, C.; Theodoropoulos, C. A techno-economic analysis of biodiesel biorefineries: Assessment of integrated designs for the co-production of fuels and chemicals. *Energy* **2011**, *36*, 4671–4683. [CrossRef]

26. Harwood, J.L.; Guschina, I.A. The versatility of algae and their lipid metabolism. *Biochimie* **2009**, *91*, 679–684. [CrossRef]

27. Guzmán, H.M.; de la Valido, A.J.; Duarte, L.C.; Presmanes, K.F. Analysis of interspecific variation in relative fatty acid composition: Use of flow cytometry to estimate unsaturation index and relative polyunsaturated fatty acid content in microalgae. *J. Appl. Physiol.* **2011**, *23*, 7–15. [CrossRef]

28. Silva, G.; Pereira, R.B.; Valentão, P.; Andrade, P.B.; Sousa, C. Distinct fatty acid profile of ten brown macroalgae. *Braz. J. Pharmacogn.* **2013**, *23*, 608–613. [CrossRef]

29. Lapuerta, M.; Rodriguez-Fernández, J.; Armaz, O. Correlation for the estimation of the density of fatty acid esters fuels and its implications. A proposed Biodiesel Cetane Index. *Chem. Phys. Lipids* **2010**, *163*, 720–727. [CrossRef]

30. Allen, C.A.W.; Watts, K.C.; Ackman, R.G.; Pegg, M.J. Predicting the viscosity of biodiesel fuels from their fatty acid ester composition. *Fuel* **1999**, *78*, 1319–1326. [CrossRef]

31. Ghosh, A.; Kharra, S.; Mondal, M.; Halder, G.; Tiwari, O.N.; Saini, S.; Bhowmick, T.K.; Gayen, K. Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: A review. *Energy Convers. Manag.* **2016**, *113*, 104–118. [CrossRef]

32. Khotimchenko, S.V.; Vaskovsky, V.E.; Titlyanova, T.V. Fatty acids of marine algae from the pacific coast of North California. *Bot. Mar.* **2002**, *45*, 17–22. [CrossRef]

33. Avula, S.G.C.; Belovich, J.M.; Xu, Y. Determination of fatty acid methyl esters derived from algae Scenedesmus dimorphus biomass by GC–MS with one-step esterification of free fatty acids and transesterification of glycerolipids. *J. Sep. Sci.* **2017**, *40*, 2214–2227. [CrossRef] [PubMed]

34. Sibi, G. Inhibition of lipase and inflammatory mediators by Chlorella lipid extracts for antiacne treatment. *J. Adv. Pharm. Technol. Res.* **2015**, *6*, 7–12. [CrossRef] [PubMed]

35. Maltsev, Y.; Maltseva, I.; Maltseva, S.; Kociolek, J.P.; Kulikovskiy, M. Fatty Acid Content and Profile of the Novel Strain of Coccomyxa elongata (Treouxibiophyceae, Chlorophyta) Cultivated at Reduced Nitrogen and Phosphorus Concentrations. *J. Phycol.* **2019**, *55*, 1154–1165. [CrossRef]

36. Lohtander, K.; Oksanen, I.; Rikkinen, J. Genetic diversity of green algal and cyanobacterial photobionts in Nephroma (Peltigerales). *Lichenologist* **2013**, *35*, 325–339. [CrossRef]

37. Zoller, S.; Lutzoni, F. Slow algae, fast fungi: Exceptionally high nucleotide substitution rate differences between lichenized fungi Omphalina and their symbiotic green algae Coccomyxa. *Mol. Phylogenet. Evol.* **2003**, *29*, 629–640. [CrossRef]

38. Darienko, T.; Gustavs, L.; Eggert, A.; Wolf, W.; Pröschold, T. Evaluating the species boundaries of green microalgae (Coccomyxa, Treouxibiophyceae, Chlorophyta) using integrative taxonomy and DNA barcoding with further implications for the species identification in environmental samples. *PLoS ONE* **2015**, *10*, e0127838. [CrossRef]

39. Darienko, T.; Gustavs, L.; Mudimu, O.; Menendez, C.R.; Schumann, R.; Karsten, U.; Friedl, T.; Prößchold, T. Chloroidium, a common terrestrial coccoid green alga previously assigned to Chlorella (Treouxibiophyceae, Chlorophyta). *Eur. J. Phycol.* **2010**, *45*, 79–95. [CrossRef]
40. Hwang, E.K.; Liu, F.; Lee, K.H.; Ha, D.S.; Park, C.S. Comparison of the cultivation performance between korean (Sugwawon no. 301) and Chinese strains (Huangguan no. 1) of kelp Saccharina japonica in an aquaculture farm in Korea. *Algae* 2018, 33, 101–108. [CrossRef]

41. Juneja, A.; Ceballos, R.M.; Murthy, G.S. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies* 2013, 6, 4607–4638. [CrossRef]

42. Jensen, M.D. Gender differences in regional fatty acid metabolism before and after meal ingestion. *J. Clin. Investig.* 1995, 96, 2297–2303. [CrossRef] [PubMed]

43. Chao, J.; Wolfaardt, G.M.; Arts, M.T. Characterization of pseudomonas aeruginosa fatty acid profiles in biofilms and batch planktonic cultures. *Can. J. Microbiol.* 2010, 56, 1028–1039. [CrossRef] [PubMed]

44. Darki, B.Z.; Seyfabadi, J.; Fayazi, S. Effect of nutrients on total lipid content and fatty acids profile of scenedesmus obliquus. *Braz. Arch. Biol. Technol.* 2017, 60, 1–12. [CrossRef]

45. Rizwanul Fattah, I.M.; Noraini, M.Y.; Mofijur, M.; Silitonga, A.S.; Badruddin, I.A.; Yunus Khan, T.M.; Ong, H.C.; Mahlia, T.M.I. Lipid extraction maximization and enzymatic synthesis of biodiesel from microalgae. *Appl. Sci.* 2020, 10, 6103. [CrossRef]

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