A Comparative Analysis of Emissions from a Compression–Ignition Engine Powered by Diesel, Rapeseed Biodiesel, and Biodiesel from Chlorella protothecoides Biomass Cultured under Different Conditions

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Abstract: The priority faced by energy systems in road transport is to develop and implement clean technologies. These actions are expected to reduce emissions and slow down climate changes. An alternative in this case may be the use of biodiesel produced from microalgae. However, its production and use need to be justified economically and technologically. The main objective of this study was to determine the emissions from an engine powered by biodiesel produced from the bio-oil of Chlorella protothecoides cultured with different methods, i.e., using a pure chemical medium (BD-ABM) and a medium based on the effluents from an anaerobic reactor (BD-AAR). The results obtained were compared to the emissions from engines powered by conventional biodiesel from rapeseed oil (BD-R) and diesel from crude oil (D-CO). The use of effluents as a medium in Chlorella protothecoides culture had no significant effect on the properties of bio-oil nor the composition of FAME. In both cases, octadecatrienoic acid proved to be the major FAME (50% wt/wt), followed by oleic acid (ca. 22%) and octadecadienoic acid (over 15%). The effluents from UASB were found to significantly reduce the biomass growth rate and lipid content of the biomass. The CO₂ emissions were comparable for all fuels tested and increased linearly along with an increasing engine load. The use of microalgae biodiesel resulted in a significantly lower CO emission compared to the rapeseed biofuel and contributed to lower NOₓ emission. Regardless of engine load tested, the HC emission was the highest in the engine powered by diesel. At low engine loads, it was significantly lower when the engine was powered by microalgae biodiesel than by rapeseed biodiesel.

Keywords: microalgae; algae cultivation; biodiesel; emission; exhaust gases; trans-esterification; compression–ignition diesel engine

1. Introduction

The emission of exhaust gases from various sources of fossil fuels poses one of the main threats to the environment; hence, its reduction is a serious challenge for fuel producers and engine manufacturers [1,2]. Reduced emissions can be achieved by increasing the use of energy from renewable sources [3,4]. One of the priorities for scientists, designers, and operators of energy systems is therefore to develop and effectively implement clean energy technologies, including liquid fuels, to be used in road transport [5].

The knowledge gained thus far allows considering microalgae as a competitive source of biomass for biofuel production [6]. Scientific papers provide detailed descriptions of biofuel production technologies with this taxonomic group [7,8]. Microalgae have been shown to be the most efficient, prospective, and environmentally friendly source of biomass
and bio-oil, which can reduce greenhouse gas emissions to the atmosphere [9]. They can compete with typical, terrestrial higher plants, such as rapeseed, soybean, or oil palm, in terms of the efficiency of biocomponent production [10,11], as they exhibit a very high photosynthetic efficiency, fast biomass growth, and resistance to various types of pollutants [12]. They can be cultured on managed lands unsuitable for agricultural purposes [13] and are usually grown without herbicides or pesticides [14,15]. Additionally, a broad range of their strains can be universally used for bioenergy purposes [16]. Finally, microalgae production systems are also seen as installations offering efficient bio-sequestration of carbon dioxide [17,18].

Despite many strengths of microalgae technologies, the previous attempts to implement them in the EU have proven technological complexity, operational difficulties, and high investment expenditures of these operations [19,20]. The investment and operating costs required for microalgae cultivation are estimated to be a few to several times higher than those incurred for the production of lignocellulosic biomass [21,22]. Therefore, it is necessary to search for new, alternative, and competitive solutions, the use of which will be economically and environmentally viable. This can be achieved through the implementation of technologies exploiting microalgal biomass in the broadly understood environmental engineering [23]. Thus far, investigations have suggested the feasibility of using microalgae in the processes of wastewater and effluent treatment, waste and sewage sludge management, carbon dioxide biosequestration, bio-gas enrichment, or exhaust gas purification [24,25]. It seems that the effluent from anaerobic reactors used for wastewater treatment might prove a viable component of culture media, improving the economy of the process and final technological effects [26,27]. This type of effluent represents a culture medium highly saturated with carbon dioxide, rich in nutrients, including mineral forms of nitrogen and phosphorus, and simultaneously devoid of factors hampering microalgal growth, namely, high concentrations of organic matter, turbidity, and suspended solids [28].

On the one hand, the replacement of expensive culture media with waste significantly increases the profitability of microalgae production technology, but on the other hand, it may have a negative impact on the composition and characteristics of the produced biofuels, including biodiesel [29]. It is therefore necessary to verify the impact of alternative culture methods on the final parameters of biodiesel, its energy performance, and pollutant emissions. The characteristics of biofuels determine the course of the combustion process, engine performance, emissions, and environmental impact [30]. Depending on the technological parameters of microalgal culture, biofuels produced from their biomass may affect many factors that define the combustion and emission characteristics [31]. One of the current research challenges is to verify and determine the importance of using culture media for the effectiveness of biomass growth, its composition, and characteristics, as well as the parameters of the recovered bio-oil, the characteristics of biodiesel, and the impact of these factors on the combustion process and pollutant emissions. On the other hand, the literature presents many studies on emissions from energy units powered by biodiesel obtained from microalgal biomass. However, their results are often inconclusive and lead to contradictory conclusions. Therefore, it seems necessary to extend the current knowledge in order to define and describe universal protocols for using microalgal biodiesel, covering both the emission of pollutants to the atmosphere and the projected engine performance. The research led to the verification of the following hypotheses: I. the use of effluent from an anaerobic reactor (UASB) treating dairy wastewater as a medium for Chlorella protothecoides production will not affect the quality of biodiesel, II. The use of biodiesel from microalgal biomass will reduce emissions compared to other tested fuels.

The main objective of this study was to present the characteristics of a compression-ignition engine powered by biodiesel produced from the Chlorella protothecoides bio-oil depending on the microalgae cultivation method. The microalgal biomass produced using a pure chemical medium (BD-ABM) and a medium based on the effluents from an anaerobic reactor (BD-AAR) was tested in the study. The results obtained were compared to the
emissions produced by conventional liquid fuels, i.e., commercial diesel oil and biodiesel from rapeseed oil.

2. Materials and Methods

2.1. Experimental Design

The study was divided into four stages differing in fuel type. Stage 1 (D-CO) was performed with commercial diesel oil, stage 2 (BD-R) with biodiesel produced from rapeseed oil, stage 3 (BD-ABM) with biodiesel produced from the biomass of *Chlorella protothecoides* microalgae cultured using a medium based on pure chemical reagents, and finally, stage 4 (BD-AAR) with biodiesel produced from the biomass of *Chlorella protothecoides* cultured using a medium based on effluents from an anaerobic reactor treating dairy wastewater. Each experimental stage was divided into five technological variants differing in the load of a compression-ignition engine. In variant 1 (0% EL), engine load was at 0% of the rate power; in variant 2 (25% EL)—at 25%, in variant 3 (50% EL)—at 50%, in variant 4 (75% EL)—at 75%, and in variant 5 (100% EL)—at 100%. Analyses performed included the evaluation of the effectiveness of microalgal biomass growth, characteristics of bio-oil and biodiesel, and characteristics of exhaust gases, including concentrations of CO, CO\textsubscript{2}, hydrocarbons (HC), and NO\textsubscript{x}, as well as smoke opacity.

2.2. Materials

Diesel oil (D-CO) used in the study was a commercial product derived from a widely available source of its distribution. Its basic physical and chemical properties were as follows: boiling point range: 170–400 °C, flash point: min. 56 °C (closed cup), upper explosion limit: 6.0% (V/V), lower explosion limit: 1.3% (V/V), vapor pressure at 40 °C: 0.4 kPa, relative density at 15 °C: 830 kg/m\textsuperscript{3}, self-ignition temperature: 255 °C, and kinematic viscosity at 40 °C: 2.0 ÷ 4.5 mm\textsuperscript{2}/s.

The biomass of winter rape (*Brassica napus*), used to produce biodiesel tested in the BD-R stage, derived from rape crops grown at the teaching—experimental station of the University of Warmia and Mazury in Olsztyn in Bałdy village (GPS N 53.599, S 20.608). Doses of fertilizers used in rape cultivation were as follows: 260 kg N/ha, 120 kg P\textsubscript{2}O\textsubscript{5}/ha, 330 kg K\textsubscript{2}O/ha, 200 kg Ca/ha, 55 kg MgO/ha, 70 kg S/ha, 1500 g Fe/ha, 600 g Mn/ha, 500 g Zn/ha, 100 g Cu/ha, 40 g Mo/ha, and 800 g B/ha.

The *Chlorella protothecoides* biomass originated from the Culture Collection of Algae at The University of Texas at Austin (UTEX). The biomass was cultured in photobioreactors (PBR) located at the Center of Aquaculture and Ecological Engineering (GPS N 53.753, S 20.461), at a temperature of 20 °C, with a mix of warm-white and cool-white light provided by fluorescent lamps with intensity of 3000 lux and periodicity of 12 h lightness/12 h darkness. In the BD-ABM stage of the study, the microalgae were cultured using a Bristol Medium, composed of: 2.94 mM NaNO\textsubscript{3} (Fisher BP360-500), 0.17 mM CaCl\textsubscript{2}·2H\textsubscript{2}O (Sigma C-3881), 0.3 mM MgSO\textsubscript{4}·7H\textsubscript{2}O (Sigma 230391), 0.43 mM K\textsubscript{2}HPO\textsubscript{4} (Sigma P 3786), 1.29 mM KH\textsubscript{2}PO\textsubscript{4} (Sigma P 0662), and 0.43 mM NaCl (Fisher S271-500). In the BD-AAR stage, *Chlorella protothecoides* microalgae were cultured with the effluent from an anaerobic reactor (UASB) treating dairy wastewater. The UASB reactor was exploited at the organic load rate (OLR) of 10 kg COD/m\textsuperscript{3}·d and hydraulic retention time (HRT) of 24 h. Before being used as the culture medium, the post-fermentation effluent was pasteurized (90 °C for 30 min) to ensure microalgal culture purity. The effluent was fed to the PBR at a dose of 40.0 dm\textsuperscript{3}/m\textsuperscript{3}·day. The characteristics of the post-fermentation effluent was as follows: BOD\textsubscript{5}—492.0 ± 99.4 mgO\textsubscript{2}/L, COD—891.2 ± 118.1 mgO\textsubscript{2}/L, BOD\textsubscript{5}/COD—0.6 ± 0.1, pH—7.2 ± 0.2, TS—98.2 ± 21.2 mg/L, TN—302.8 ± 99.4 mgN/L, AN—222.0 ± 70.7 mgN–NH\textsubscript{4}/L, TP—61.7 ± 11.9 mgP/L, P—PO\textsubscript{4}—46.5 ± 12.7 mg/L. The composition of rapeseed biomass and *Chlorella protothecoides* biomass is presented in Table 1.
Table 1. Composition of *Brassica napus* biomass (BD-R) and *Chlorella protothecoides* biomass (BD-ABM, BD-AAR).

| Parameter                  | Unit          | Brassica Napus | Chlorella Protothecoides |
|----------------------------|---------------|----------------|--------------------------|
|                            | Stage 2       | Stage 3        | Stage 4                  |
| Dry Mass                   | (% f.m.)      | 94.5 ± 13.22   | 78.43 ± 10.59            | 77.19 ± 12.95 |
| Organic dry matter         | (% d.m.)      | 95.14 ± 0.98   | 87.12 ± 0.97             | 91.47 ± 0.92 |
| Mineral dry matter         | (% d.m.)      | 4.46 ± 0.62    | 12.88 ± 0.97             | 8.53 ± 0.92  |
| $N_{\text{tot}}$           | (mg g d.m.$^{-1}$) | 37.5 ± 1.43    | 43.37 ± 1.75             | 58.07 ± 5.67 |
| $P_{\text{tot}}$           | (mg g d.m.$^{-1}$) | 7.9 ± 0.88     | 19.96 ± 1.32             | 10.31 ± 0.97 |
| $T_c$                      | (mg g d.m.$^{-1}$) | 512 ± 19.24    | 474.80 ± 11.50           | 493.40 ± 17.10 |
| $T_{oc}$                   | (mg g d.m.$^{-1}$) | 488 ± 31.64    | 439.40 ± 27.27           | 434.30 ± 12.74 |
| Protein                    | (% d.m.)      | 23.46 ± 1.97   | 27.11 ± 2.72             | 36.29 ± 8.92 |
| Lipids                     | (% d.m.)      | 42.69 ± 1.76   | 14.19 ± 0.65             | 7.38 ± 0.37  |
| Saccharides                | (% d.m.)      | 5.13 ± 0.45    | 39.77 ± 1.29             | 41.38 ± 0.36 |

2.3. Biodiesel Production

Bio-oils were produced from the biomass of *Brassica napus* and *Chlorella protothecoides* using an M222/15F screw press (Miramar Sp. z o.o.). Rape seeds used for pressing had an average temperature of 20 °C. Microalgal biomass was centrifuged (Z41 model CEPA flow centrifuge) and air-dried at a temperature of 20 ± 2 °C. Afterward, the dry biomass was cold-pressed in the screw press without electrical screw heating. The lipid fraction was extracted from the microalgal biomass acc. to Bligh and Dyer, using a chloroform: methanol solvent mixture [32]. The transesterification reaction was performed in a glass reactor with full mixing, exploited under atmospheric pressure, with a temperature of 65 °C, and an oil to methanol molar ratio of 1:9. NaOH was used as a homogeneous catalyst of the reaction, at 2% of oil weight (wt/wt). The exact transesterification reaction lasted 2 h. Glycerol was separated from biodiesel using a separator and then rinsed with water with 5% acid. Biodiesel was separated using a rotary evaporator at 80 °C and then dried at 100 °C.

2.4. Experimental Station

Experiments aimed to determine exhaust gas emissions were carried out using a vertical, single cylinder, water-cooled compression–ignition diesel engine (Kirloskar AV1). Its technical characteristic is presented in Table 2. Engine loads tested were as follows: 0%, 25%, 50%, 75%, and 100% of engine power. The scope of the study included analyses of the composition of exhaust gases, including CO, CO$_2$, HC, NO$_x$, and smoke opacity.

Table 2. Technical specification of the engine used in the study.

| Parameter                          | Unit            | Value                        |
|------------------------------------|-----------------|------------------------------|
| No. of cylinders                   | –               | 1                            |
| Bore × stroke                      | (mm)            | 80 × 110                     |
| Cubic capacity                     | (Ltr)           | 0.553                        |
| Compression ratio                  | –               | 16.51                        |
| Rated output                       | kW(hp)          | 3.7 (5)                      |
| Rated speed                        | rpm             | 1500                         |
| Torque at full load (crankshaft drive) | kN–m         | 0.024 (2.387)                |
| Crank shaft center height          | (mm)            | 203                          |
| Specific fuel consumption (sfc)    | (gm/hp–hr)      | 195 + 5%                     |
| Lube oil consumption               | –               | 0.8% of SFC max.             |
| Lube oil sump capacity             | (Ltr)           | 3.7 at higher level on dipstick |
| Fuel tank capacity                 | (Ltr)           | 6.5                          |
| Physical dimensions of bare engine (length × width × height) | (mm) | 617 × 504 × 843 |
Table 2. Cont.

| Parameter                                 | Unit     | Value       |
|-------------------------------------------|----------|-------------|
| Engine weight (dry)                       | (kg)     | 130         |
| Rotation while looking at the flywheel    | –        | Clockwise   |
| Power take-off                            | –        | Flywheel end|
| Starting                                  | –        | Hand start  |
| Governing                                 | –        | Class “B1”  |
| Type of fuel injection                    | –        | Direct injection |
| Overloading capacity of engine            | –        | 10% of rated output |

2.5. Analytical Methods

The qualitative analysis of *Chlorella protothecoides* biomass was conducted via microscope analysis using a BBE Algae OnLine Analyzer (Moldaenke). Contents of dry matter, organic dry matter, and mineral dry matter in the biomass were determined with the gravimetric method. Biomass samples dried at 105 °C were determined for contents of total carbon (TC), total organic carbon (TOC), and total nitrogen (N<sub>tot</sub>). The above analyses were performed using a Flesh 2000 elementary particle analyzer (Thermo). The content of total phosphorus (P<sub>tot</sub>) was determined with the colorimetric method with ammonium metavanadate (V) and ammonium molybdate after prior mineralization of the sample in a mixture of sulfuric (VI) and chloric (VII) acids, at a wavelength of 390 nm using a DR 2800 spectrophotometer (HACH Lange). The content of total protein was estimated by multiplying N<sub>tot</sub> content by a protein conversion factor of 6.25. The content of reducing sugars was determined by the colorimetric method with an anthrone reagent, at the wavelength of 600 nm using a DR 2800 spectrophotometer (HACH Lange). Lipid concentration was determined with the Soxhlet method using an extraction apparatus (Buchi).

Properties of bio-oils were determined using the following standards: density at 15 °C—ISO 3675, viscosity at 40 °C—ISO 3104, flash point—ISO 15267, carbon residue—EN ISO 10370, total contamination—EN 12662, oxidative stability at 110 °C—EN 14112, calorific value—DIN 51900, acid value—EN 14104, iodine value—EN 14111, water content—EN ISO 12937, sulfur content—EN 10540. The degree of transesterification of bio-oil triglycerides was determined with the HPLC technique (Shimadzu LC-10AT). The HPLC system consisted of a C-18 column and two DAD detectors (wavelength λ = 205 nm). The HPLC analysis was conducted at the following conditions: flow rate—0.9 cm<sup>2</sup>/min, injection volume—1.0 µL, and column temperature—25 °C. Solvent A—isopropanol-hexane (4/5) and solvent B—methanol were used as a mobile phase in the following gradient system: 0 min—solvent A 100%, 20 min—solvent A 100%, 45 min—solvent A 100%, 70 min—solvent A 100%, 71 min—solvent A 100%, and 75 min—solvent A 100%.

The products of algae oil transesterification were analyzed using the GC system consisting of a gas chromatograph (Shimadzu GC-15A) and an Rt-2560 column (110 m × 0.20 µm L.D. × 0.25 mm film thickness). The qualitative and quantitative analyses of fatty acid methyl esters (FAME) were carried out using the following analytical standards: Food Industry FAME Mix containing 37 methyl esters (C4:0–C24:1) and F.A.M.E. Mix C18:0–C20:0 (certified reference material containing: methyl arachidate 10% (w/w), methyl elaidate 20% (w/w), methyl linoleate 20% (w/w), methyl linolelaidate 20% (w/w), methyl oleate 20% (w/w), and methyl stearate 10% (w/w)). The GC analysis was conducted at the following conditions: column flow rate—0.80 mL/min, split ratio—10:1, make-up gas flow rate—40 mL/min, starting column temperature—100 °C, and temperature gradient: 4.0 °C/min—185 °C, 0.5 °C/min—220 °C, and 5.0 °C/min—240 °C.

The composition of exhaust gases was analyzed by means of an InfraLYT N-V101 mobile exhaust gas analyzer for compression—ignition engines (Test—Therm Ltd.). Analyses of CO, CO<sub>2</sub>, and HC were performed by their optical measurement using an infrared beam, whereas those of the other NO<sub>X</sub> were tested by the electrochemical method. The measuring ranges of the analyzer were as follows: 0–2000 ppm for CO, 0–20% for CO<sub>2</sub>, and...
0–2500 ppm for HC, 0–2500 ppm for NO, and 0–500 ppm for NO₂. Analyzer operating conditions were as follows: operating temperature—5–40 °C, measuring gas temperature at probe tip—5–500 °C, ambient pressure—860–1060 hPa, main voltage—AC 230 V ± 10 % (50 Hz 2 %), and power consumption—max. 60 VA. Smoke opacity was measured using an AVL 439 Opacimeter (AVL List GmbH).

2.6. Statistical Methods

The statistical analysis of the results was carried out with the Statistica 13.3 PL package (Statsoft, Inc., Tulsa, OK, USA). The hypothesis on the distribution of each analyzed variable was verified with a Shapiro-Wilk W-test. One-way analysis of variance (ANOVA) was applied to determine the significance of the differences between variables. Variance homogeneity in groups was checked with a Levene’s test, whereas the significance of the differences between the analyzed variables was determined with a Tukey HSD test. In all tests, the level of significance was adopted at α = 0.05.

3. Results and Discussion

3.1. Algae Growth and Bio-Oil Properties

The study proved that the culture medium used had a significant effect on the effectiveness of *Chlorella protothecoides* biomass growth and the content of lipid substances in the biomass produced. In the BD-ABM stage, the final biomass concentration in PBR reached 3570 ± 139 mg d.m./dm³, whereas in the BD-AAR stage, it was at 2850 ± 223 mg d.m./dm³ (Figure 1). The use of the pure culture medium enabled shortening the lag phase to 4 days and allowed the *Chlorella protothecoides* population to reach the exponential phase faster. In the BD–AAR stage, the lag phase spanned for 6 days, which indicates that the microalgae need to adapt to a less-beneficial culture medium. Yu et al. (2019) reported similar results from their study, where *Chlorella vulgaris* cultured on effluents from anaerobic reactors treating food waste was characterized by a lower biomass growth rate and lower sedimentation capability compared to the culture grown on the Bristol Medium [33].

![Figure 1. Changes in microalgal biomass concentration in particular study stages.](image)

The mean biomass growth rate was at 245 mg d.m./day in the BD-ABM stage and 186 mg d.m./day in the BD-AAR stage. In turn, lipid content in the biomass reached 36.3 ± 3.1% d.m. in stage 3 and 24.6 ± 4.7% d.m. in stage 4 (Figure 2). Additionally, other researchers pinpointed the significant effect of culture conditions on the concentration of lipid substances in microalgal biomass [34,35]. In the study conducted by Singh et al. (2011), the lipid content ranged from 3.9 to 10% in the biomass of *Scenedesmus bijuga* cultured on the effluent from poultry litter fermentation [34]. In turn, Xie et al. (2019) reported lipid
content of 44.72% in the biomass of Chlorella vulgaris grown with a mixture of wastewater from anaerobic fermentation and rainfall water [35]. Table 3 presents properties of tested bio-oils. Data presented therein indicate that culture conditions had no significant effect on bio-oil properties.

The use of effluents as a medium in Chlorella protethecoides culture did not significantly affect the bio-oil FAME characteristics. Octadecatrienoic acid (C18:3, all-cis —9,12,15) proved to be the major FAME in both these culture types. In BD-ABM stage, its content reached 50.14 ± 0.86% wt/wt, whereas in BD-AAR stage, it was at 51.43 ± 1.42% wt/wt. In turn, in BD-R stage, it was at 8.74 ± 0.11% wt/wt (Table 4). Other major FAMEs identified in microalgal bio-oils included oleic acid C18:1 (cis-9) with its concentration approximating 15.5% wt/wt (Table 4). The concentrations of palmitic and stearic acids were below 10% wt/wt (Table 4). In turn, oleic acid C18:1 (cis—9) was found to be the major FAME of BD-R, with its content at 51.43 ± 1.42% wt/wt (Table 4). It was followed by octadecadienoic acid at 19.84 ± 0.31% wt/wt (Table 4). The other components of the bio-oil tested were found in trace amounts. The above results are consistent with findings reported by Gölçüyurt et al. (2016) for the FAME profile of Chlorella protothecoides. They found oleic acid (C18:1) to be the major methyl ester, accounting for over 50% of...
total FAME content. In addition, they showed that the content of linolenic acid (C20:1), known to adversely affect the oxidative stability and cold flow properties of biodiesel, approximated 5% of total FAME [36].

Table 4. Content of n-paraffin hydrocarbons in diesel and characteristics of fatty acid methyl esters (FAME) in the tested bio-oils.

| Hydrocarbon/ Fatty Acid | Stage 1 D-CO | Stage 2 BD-R | Stage 3 BD-ABM | Stage 4 BD-AAR |
|-------------------------|-------------|--------------|----------------|----------------|
| C8:0                    | 0.15 ± 0.04 | 0.07 ± 0.03  |                |                |
| C9:0                    | 0.50 ± 0.08 |              |                |                |
| C10:0                   | 1.84 ± 0.11 | 0.05 ± 0.04  | 0.03 ± 0.01    |                |
| C11:0                   | 3.70 ± 0.23 | 0.06 ± 0.05  | 0.02 ± 0.01    |                |
| C12:0                   | 3.26 ± 0.27 |              | 0.03 ± 0.01    |                |
| C13:0                   | 2.94 ± 0.15 | 0.07 ± 0.01  | 0.04 ± 0.02    |                |
| C14:0                   | 2.62 ± 0.12 |              |                |                |
| C14:1 (cis–9)           | 1.85 ± 0.13 | 0.03 ± 0.02  | 0.02 ± 0.01    |                |
| C15:1 (cis–10)          | 0.50 ± 0.08 | 0.05 ± 0.02  | 0.04 ± 0.00    |                |
| C15:0                   | 3.26 ± 0.27 |              | 0.07 ± 0.01    |                |
| C16:0                   | 3.26 ± 0.27 | 4.78 ± 0.16  | 6.14 ± 0.19    | 5.52 ± 0.09    |
| C16:1 (cis–9)           | 3.26 ± 0.27 | 0.27 ± 0.02  | 0.09 ± 0.03    | 0.07 ± 0.02    |
| C17:0                   | 0.70 ± 0.04 | 0.07 ± 0.02  | 0.07 ± 0.01    |                |
| C17:1 (cis–10)          | 0.70 ± 0.04 | 0.05 ± 0.02  | 0.04 ± 0.00    |                |
| C18:0                   | 0.25 ± 0.03 | 2.38 ± 0.13  | 4.72 ± 0.01    | 4.56 ± 0.03    |
| C18:1 (trans–9)         | 0.25 ± 0.03 |              | 0.03 ± 0.02    | 0.03 ± 0.01    |
| C18:1 (cis–9)           | 59.68 ± 0.31| 21.88 ± 0.63 | 21.56 ± 0.45   |                |
| C18:2 (all–cis–9,12)    | 19.84 ± 0.31| 15.45 ± 0.57 | 15.43 ± 0.56   |                |
| C18:3 (all–cis–6,9,12)  | 0.55 ± 0.04 | 0.24 ± 0.01  | 0.27 ± 0.01    |                |
| C18:3 (all–cis–9,12,15) | 8.74 ± 0.11 | 50.14 ± 0.86 | 51.43 ± 1.42   |                |
| C19:0                   | 0.06 ± 0.01 | 0.11 ± 0.01  | 0.10 ± 0.03    |                |
| C20:0                   | 0.01 ± 0.01 | 0.07 ± 0.01  | 0.02 ± 0.01    |                |
| C20:1 (cis–11)          | 1.77 ± 0.15 | 0.20 ± 0.05  | 0.19 ± 0.01    |                |
| C20:2 (all–cis–11,14)   | 0.22 ± 0.01 | 0.07 ± 0.07  | 0.02 ± 0.01    |                |
| C22:0                   | 0.34 ± 0.03 | 0.09 ± 0.01  | 0.10 ± 0.03    |                |
| C22:1 (cis–13)          | 1.3 ± 0.12  | 0.14 ± 0.05  | 0.08 ± 0.02    |                |
| C20:4 (all–cis–5,8,11,14)| 0.04 ± 0.01 | 0.04 ± 0.01  |                |                |
| C22:2 (all–cis–13,16)   | 0.04 ± 0.01 | 0.09 ± 0.03  |                |                |
| C24:0                   | 0.34 ± 0.04 | 0.12 ± 0.02  | 0.14 ± 0.02    |                |
| C24:1 (cis–15)          | 0.06 ± 0.01 |              |                |                |
| C22:6                   |              |              |                | 0.05 ± 0.02    |

3.2. Engine Emission
3.2.1. Carbon Dioxide (CO₂)

The CO₂ emissions were comparable for all fuels tested and increased linearly along with an increasing engine load (Figure 3b). At 0% EL, CO₂ concentrations ranged from 1.6 ± 0.3% in the BD-AAR stage to 2.4 ± 0.1% in BD-R (Figure 3a). At 100% EL, the CO₂ emissions were the highest and similar for all fuel types. The concentrations of CO₂ ranged from 5.9 ± 0.2% in the DCO stage to 6.3% on average in experimental stages with BD-R and BD-AAR. A correlation between CO₂ emission increase and engine load was described with a linear function. The greatest fit of the model to empirical data, at R² = 0.9928, was observed in the D-CO stage (Figure 3b). This observation agrees with study results reported by other authors and is indirectly due to a higher fuel consumption at higher engine loads [37]. Additionally, Sanjid et al. (2014) confirmed this correlation [38]. In turn, other scientists proved that the use of biodiesel or diesel blends with bio-oils led to a decreased CO₂ concentration in exhaust gases [39]. The above observation was confirmed by Bazooyar et al. (2014), who investigated the effect of increasing input air on combustion.
performance and emissions from a boiler powered by biodiesel and diesel. They achieved similar results regarding thermal capacity and temperature of exhaust gases at lower CO\textsubscript{2} emission from the biodiesel [40]. Lower CO\textsubscript{2} emissions were reported for biodiesel produced from nettlespurge (Jatropha), oil palm, rape, and algae compared to diesel oil [41,42]. Another study demonstrated an increased CO\textsubscript{2} concentration in exhaust gases emitted during the combustion of biodiesel from microalgae [43]. Additionally, Namitha et al. showed higher CO\textsubscript{2} emissions from biodiesel synthesized from Monoraphidium sp. and Chlorella sorokiniana compared to conventional diesel oil at higher loads [44].

3.2.2. Carbon Monoxide (CO)

Regardless of engine load, the lowest CO emission was determined in the D-CO stage. The CO concentrations ranged from 360 ± 41 ppm at 100% EL to 840 ± 46 ppm at 25% EL (Figure 4a). The CO emissions determined upon the use of BD-ABM and BD-AAR biodiesel were lower compared to BD-R, with the greatest differences noted in variants 25% EL and 50% EL. In the case of using BD-ABM, the CO concentrations reached 1030 ± 40 ppm and 630 ± 42 ppm, whereas in the BD-AAR stage, they reached 1000 ± 59 ppm and 720 ± 39 ppm, respectively. In the BD-R stage, the CO emission was at 1200 ± 77 ppm in the 25% EL variant and at 930 ± 50 ppm in the 50% EL variant (Figure 4a). Analyses conducted over the study period revealed that CO emission was high at low engine loads (0% EL, 25% EL) and decreased in the subsequent engine load variants (Figure 4b). In the 0% EL variant, the CO emission was higher by nearly 50% compared to the 100% EL variant (Figure 4a). The CO emission is mainly due to a low combustion temperature and a rich fuel-air blend produced inside the engine [45]. It is believed that higher CO emissions are obtained at lower engine loads due to a low cylinder temperature, poor blend, and poor biodiesel atomization, as well as higher biodiesel viscosity and density [46]. At higher engine loads, the concentration of molecular oxygen improves and the temperature in the cylinder increases, which promotes oxidation and reduces viscosity. This leads to lower CO emissions noted for both diesel and biodiesel [47]. Some authors claim that increasing the engine load leads to a lower air demand coefficient, which results in a more efficient combustion and a reduced CO production [48]. Arunkumar et al. (2018) [49] and Rajak et al. (2018) [50] confirmed the above findings. However, some other studies reported an opposite phenomenon, namely, a significantly lower CO emission during the combustion of biodiesel than diesel oil. One of these is the research by Azad et al., who compared the performance, emissions, and characteristics of an engine powered by biodiesel from macadamia and grape seeds with that powered by diesel oil. They found that all biodiesel
blends reduced CO emissions compared to diesel [51]. The above finding can be explained, among others, by the fact that biodiesel contains more oxygen, which leads to more efficient carbon oxidation [41,52].

\[ y = -30x^2 + 42x + 930 \\
R^2 = 0.9182 \]

0
200
400
600
800
1000
1200
1400
0 25 50 75 100
CO (ppm)
Engine load (%)

This phenomenon is explained by the short combustion time, which directly shortens the emission increases with temperature and the excess air factor used [53,54]. What is puzzling and contrary to the above theory, however, is that the studies so far have proven that the use of algae biodiesel resulted in a high heat release and NO\textsubscript{x} reduction [55,56]. This phenomenon is explained by the short combustion time, which directly shortens the time available for N\textsubscript{2} conversion to NO\textsubscript{x}. In addition, this phenomenon also reduces the mean gas temperature and leads to reduced NO\textsubscript{x} emissions [53]. There are also some studies reporting increased NO\textsubscript{x} emissions upon the use of biofuels, including works by Miri et al. (2017) [57], Ozsezen et al. (2009) [58], and Özer et al. (2014) [59]. The NO\textsubscript{x} formation percentage in the exhaust gas emissions produced by biodiesel fuel is still unclear [60]. Although NO\textsubscript{x} emissions increase in some cases, these increases can be minimized by exhaust gas recirculation (EGR) or use of other additives [61,62].

Figure 4. The CO emission in particular study variants (a); correlation between engine load and CO emission depending on fuel tested (b).

3.2.3. Nitrogen Oxides (NO\textsubscript{x})

The study proved that using biodiesel produced from microalgal biomass (BD-ABM and BD-AAR) allowed reducing NO\textsubscript{x} emissions compared to D-CO in all engine load variants (Figure 5a). The noted emissions were lower by 11.4% to 34% depending on the variant. This tendency was also observed for BD-R, but only in engine load variants from 50% EL to 100% EL (Figure 5a). In the D-CO stage, the NO\textsubscript{x} emission increased from 111 ± 21 ppm at 0% EL to 437 ± 31 ppm at 100% EL. In stages 3 and 4, the NO\textsubscript{x} concentrations determined in exhaust gases were similar and ranged from 73 ± 12 ppm to 403 ± 27 ppm for BD-ABM and from 84 ± 16 ppm to 399 ± 20 ppm for BD-AAR, depending on the EL. The NO\textsubscript{x} emission was found to increase proportionally to engine load (Figure 5b). Depending on the study stage, the coefficient of determination (R\textsuperscript{2}) ranged from 0.9586 for BD-ABM to 0.9690 for BD-R. The formation of NO\textsubscript{x} mainly depended on combustion temperature and available oxygen concentration at the flash point. It is well known that NO\textsubscript{x} emission increases with temperature and the excess air factor used [53,54].
3.2.4. Unburned Hydrocarbons (HC)

The HC emissions are due to incomplete fuel combustion in the engine [63], which in turn may be caused by a too low combustion temperature, a too low oxygen concentration in the chamber, or too short fuel retention time at the flash point [64]. Considering biodiesel characteristics and, most of all, increased oxygen concentration in the fuel, the HC emission is expected to be lower compared to the use of diesel oil [65]. Such observations were made by Gharehghani et al. (2017) [47] and Palash et al. (2015) [66]. The results of our study also confirm this phenomenon. Regardless of engine load, the HC emission was the highest in the D-CO stage, with HC concentrations in exhaust gases fitting within a narrow range from 40 ± 4 ppm at 50% EL to 46 ± 6 ppm at 25% EL (Figure 6a). At low engine loads, the HC emissions determined for BD-ABM and BD-AAR were significantly lower than for BD-R (Figure 6a). No correlation was found between HC emission and engine load (Figure 6b). There are also available works that challenge the above theory. Some studies have proved higher HC emission during the combustion of bio-esters [45]. This may be due to the higher biodiesel viscosity which indirectly affects the droplet size of atomized fuel [67]. A greater diameter of aerosol particles may hamper complete fuel combustion, which consequently leads to increased HC emissions [68]. This phenomenon was proved by Nabi et al., who demonstrated increased HC emission (by max. 13%) in the case of using Licella biofuel blends compared to diesel oil [69]. This phenomenon is of significantly lesser importance at higher engine loads, because a richer fuel-air blend is then injected into the combustion chamber (higher air/fuel ratio). Higher concentrations of air and oxygen inbuilt into the fuel result in more efficient combustion and lower HC emission [70].

3.2.5. Smoke Opacity

The smoke opacity of exhaust gases develops during the combustion of a rich blend at a high temperature and pressure [71]. Differences in its concentrations depend on fuel type and engine load [72], as confirmed by multiple experiments, including the present study. Regardless of study stage, an increase in smoke opacity was linearly correlated with an increasing engine load (Figure 7b). In the variant with 100% EL, the smoke opacity of exhaust gases was significantly lower during engine feeding with biodiesel from microalgal biomass (stages BD-ABM and BD-AAR) compared to rapeseed biodiesel and petro-diesel (Figure 7a). In stages 3 and 4, the smoke opacity was at 47 ± 5% and 48 ± 6%, respectively, whereas in stages D-CO and BD-R, it reached 66 ± 1% and 59 ± 3%, respectively. The biofuel from microalgae is partly aerated, which promoted the oxidation of combustible
particles [73]. Biodiesel is also less calorific than diesel oil, which enables decreasing combustion temperature and pressure, thus contributing to smoke emission reduction [74]. In the remaining engine load variants, the differences in smoke opacity of exhaust gases were lesser, regardless of fuel type (Figure 7a).

**Figure 6.** The HC emission in particular study variants (a); correlation between engine load and HC emission depending on fuel tested (b).

**Figure 7.** Smoke opacity of exhaust gases in particular study variants (a); correlation between engine load and smoke opacity depending on fuel tested (b).

4. **Conclusions**

The culture medium used had a significant effect on the effectiveness of *Chlorella protothecoides* biomass growth and the content of lipid substances in the biomass produced. Higher technological effects were achieved with the medium composed of crude chemical reagents compared to the culture fed with effluents from an anaerobic reactor treating dairy wastewater. The *Chlorella protothecoides* culture conditions had no significant effect on the basic properties of bio-oil nor on FAME characteristics. In both culture variants, octadecatrienoic acid proved to be the major FAME (above 50% wt/wt), followed by oleic acid (ca. 22%) and octadecadienoic acid (over 15%).

The CO₂ emissions were comparable for all fuels tested and increased linearly along with an increasing engine load. At 100% EL the concentrations of CO₂ ranged from
5.9 ± 0.2% in D-CO stage to 6.3% on average in experimental stages with BD-R and BD-AAR. A linear correlation was found between CO₂ emission and engine load. Regardless of engine load, a lower CO emission was determined when the engine was powered by petro–diesel, ranging from 360 ± 41 ppm at 100% EL to 840 ± 46 ppm at 25% EL. The CO emissions determined upon the use of BDABM and BD-AAR biodiesel were lower compared to BD-R, with the greatest differences noted in variants 25% EL and 50% EL.

The study proved that using biodiesel produced from microalgal biomass (BD-ABM and BD-AAR) allowed reducing NOₓ emissions compared to D-CO in all engine load variants. The noted emissions were lower by 11.4–34% depending on EL. An increase in NOₓ emission was noticed proportional to the engine load. Regardless of engine load tested, the HC emission was the highest in the engine powered by diesel oil. At low engine loads, the HC emission was significantly reduced when the engine was powered by microalgae biodiesel than by rapeseed biodiesel.

Smoke opacity of exhaust gases increased proportionally to the engine load, and was significantly lower at the highest-tested engine load with biodiesel from microalgal biomass. Using algal biodiesel, smoke opacity was below 50%, whereas in stages D-CO and BD-R, it reached 66% ± 3% and 59 % ± 3%, respectively.

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