Microalgae as Alternative Fuel for Compression Ignition (CI) Engines

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Abstract— **Microalgal biodiesel is a promising alternative fuel because of the following: 1) its high productivity in comparison with crops oil 2) it does not affect food production, and 3) its lower emissions and potential to reduce environmental pollution. The aims of this work are firstly to produce and extract lipid from a fresh water microalgae *Chlorella vulgaris* using iron as a stress treatment to achieve high lipid content. Secondly, the physical and chemical properties of *Chlorella vulgaris* and *Chlorella protothecoides* oil will be compared with diesel and biodiesel from other sources.**

Keywords-Biodiesel, microalgae, Chlorella vulgaris, Chlorella protothecoides, lipid analysis

I. Introduction

The diesel engine is a key component for the transportation and agricultural sectors. The dependence on fossil fuel in transport has been reported as one of the main factors causing a high CO_2 percentage in the atmosphere, leading to global warming. The use of biodiesel in conventional diesel engines has increased due to the depletion of fossil fuel, high fuel prices and health concerns and pollution associated with exhaust emissions. Biodiesel from crops such as oil crops, waste cooking oil and animal fat is a natural alternative fuel, which unfortunately cannot provide even a small fraction of the fuel required for transportation. Therefore, non-edible sources of biodiesel are considered a promising alternative for use in diesel engines [1]. Microalgae can produce oil which can be used as biodiesel and its properties are very close to those of the oil extracted from vegetables or fish. Microalgae are unicellular photosynthetic organisms that use light energy and carbon dioxide, with higher photosynthetic efficiency in comparison to plants grown for biomass production [2]. It appears to be the only renewable source of fuel that has the capacity to meet the global demand for transportation [3]. There are many reasons why microalgae has received a lot of attention recently. Firstly, growing microalgae does not require arable land or fresh water only; some species can grow in ponds in deserts using salt water or even in waste water. Secondly, microalgae oil productivity is many times higher than crops oil. The same amount of biodiesel from microalgae (for 30% w/w oil content) compared to rapeseed and soybean crops require much less land – up to 49 or 132 times respectively. Currently microalgal biomass production throughout the world is about 10,000 tons per year [4].

Biodiesel can be produced from storage lipids extracted from the biomass harvested from liquid cultures of the microalgae *Chlorella vulgaris*. This species has relatively acceptable biomass productivity and low lipid content. Therefore, increasing the lipid content in this species to increase the lipid productivity will be valuable. The dual requirements of maximizing biomass and lipid production are difficult to achieve, as lipid storage production in microalgae is enhanced only under conditions of environmental stress. Increasing the lipid content under stress conditions means the biomass productivity could be affected due to this stressor. The productivity of biomass and the productivity of lipid content of *Chlorella vulgaris* can both be enhanced if specific culture conditions are applied [5]. The lipid content in *Chlorella vulgaris* increased up to 56.6% of biomass by dry weight by adding 1.2×10^{-5} mol L^{-1} $FeCl_3$ to stress actively growing cells [6]. Alternatively, *Chlorella vulgaris* was cultured in a tubular photobioreactor to increase the calorific value of the lipids through nitrogen deficiency. The highest calorific value obtained for lipids extracted from a nitrogen-deficient *Chlorella vulgaris* culture was 28 kJ g^{-1} [7]. The lipid content of microalgae was significantly affected by the variation of parameters such as the temperature condition for *Chlorella vulgaris*. The lipid content decreased from 14.71% to 5.90% when the temperature increased from 25°C to 30°C, while the oil contained a high value of the preferred lipid palmitic acid, and the concentration of linoleic acid met the requirement of the European legislation for biodiesel [8]. In comparison with conventional diesel fuel, *Chlorella protothecoides* biodiesel met the terms of the American Society for Testing of Materials standard for biodiesel (ASTM 6751) and it is feasible for biodiesel production in industrial level [9]. Therefore, it is feasible to produce microalgae biodiesel and its characteristics are close to those of crops biodiesel. Until now, there has been no study testing microalgae in diesel engines to determine the engine performance and emissions. However, an unmodified single cylinder diesel engine was used to test the performance and emissions of a fuel consisting of rapeseed biodiesel, a surfactant and slurry of *Chlorella vulgaris* [10]. While the CO_2 levels were higher, the nitrous oxide (NOx) emissions were lower than that of diesel. Experimental evaluation of diesel engine performance and emissions of diesel fuel and biodiesel in different blends have been studied by many researchers. The experimental tests require time, effort and money. Therefore, an assimilation program to predict the engine performance and emissions using biodiesel and biodiesel blended with diesel will be valuable. A single-zone combustion model based on thermodynamic principles has been developed to predict the diesel engine performance under different blend ratios (20%, 40% and 60%) for diesel and Karanja biodiesel. This simulation predicted the engine performance data with 20% and 40% diesel blend well [11]. Mathematical modeling simulations of diesel engine processes have been widely used, but very few studies with biodiesel have been done and it is still a new area of research [11].

The objectives of this paper are:

- To culture the fresh water microalgae *Chlorella vulgaris* in a complete nutrient medium to compare the biomass productivity and lipid productivity with that published in the literature.
- To add iron to the microalgae culture at the late exponential growth phase to stimulate lipid production.
- To evaluate and compare some of the chemical and physical properties of microalgal oil and biodiesel extracted from *Chlorella vulgaris* and *Chlorella protothecoides* with diesel and biodiesel standards.

II. METHODS

A. *Microalgae cultivation*

A culture of the fresh water microalgae *Chlorella vulgaris* (CCAP 211/11) was obtained from the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia. The microalgae cells were grown in the complete nutrient medium MBL in sterile 5-liter flasks illuminated by 2000-lux fluorescent lights on a 16 to 8 hour light and dark cycle. Sterilized micro-irrigation risers attached to an air pump were inserted into the liquid medium to generate large, slow bubbles to aerate and circulate the medium.

B. *Growth rate of microalgae*

The cell density of microalgae was quantified using a haemocytometer and spectrophotometer with a 515 nanometer wave length. The cells' growth curve produced from the data was used to assess when the culture reached the late exponential growth phase.

C. *lipid content*

To stimulate lipid content the culture was divided into three parts. The first one was left as a control. The second consisted of microalgae cultured in MBL and 1.2×10^{-5} $FeCl_3$/EDTA per liter added at the late exponential growth phase (after 38days). The last one was created by adding new medium with the ratio of 3:1 (v/v) and after three days 1.2×10^{-5} $FeCl_3$/EDTA per liter was added to stress the culture.

D. *Microalgae harvesting and oil extraction*

The microalgae were harvested using a centrifuge at 8000 rpm for 10 minutes. In order to determine the dry weight of the microalgae, the resulting biomass was dried using an oven at 80^oC for 12 hours until the weight of the sample was constant. The oil was extracted as per Folch's method [12] using chloroform/methanol (2/1). The lipid was gravimetrically measured using an electronic scale.

E. *Transesterfication*

Microalgal oil extracted from *Chlorella protothecoides* obtained from the Soley Institute, Turkey, was converted to biodiesel using transesterification. The transesterification procedure was conducted by heating 50mL of oil to 48^oC. At the same time, 0.45g of NaOH was added to 11mL of methanol and mixed. The mixture was added to the oil and mixed for 40 minutes. After 10 hours, the oil phase was separated to another flask and centrifuged to remove the glycerin. The biodiesel was then washed with 25 mL of water. After 1 hour, another washing with 25mL of water was done. The mixture of biodiesel and water was centrifuged to remove all the water from the biodiesel. Lipids from *Chlorella vulgaris* oil was transesterified using a similar method on a smaller scale.

F. *Oil characterisation*

The fatty acid methyl esters (FAME) produced after transesterification from *Chlorella vulgaris* and from *Chlorella protothecoides* were compared with the chemical standard fatty acid methyl ester mixture 1892-1AMP (SIGMA-ALDRICH) using gas chromatography.

III. RESULTS AND DISCUSSION

A. *Biomass and lipid productivity*

Mass production of *Chlorella vulgaris* and increasing the lipid content are in progress. The first batch cultures before adding iron have been harvested. This results of biomass and lipid productivity were compared with others results in Table I for *Chlorella vulgaris.* The biomass productivity was 12.16 mg L^{-1} day^{-1} which is lower than that for *Chlorella vulgaris* in Lee et al. [13], which is 74.2 mg $L^{-1}day^{-1}$, and in [14]. At the same time the lipid productivity of 1.224 mg L^{-1} day^{-1} was very low in comparison with [14]. A possible reason might be that the inoculation ratio in this case was low. The medium was inoculated from fresh microalgae in the ratio of 1:10 (v/v). Therefore a long incubation time is required (40 days) to reach the stationary growth rate. If the inoculation ratio was increased to reach a high concentration of microalgae per liter in a short time, the productivity of the biomass in the last 7 days would be 69.5 mg L^{-1} day^{-1} which is close to the biomass productivity in [13]. In this case (last 7 days) the lipid productivity would be 6.99 mg L^{-1} day^{-1} which is still lower than that in the literature and that could be due to the fact that MBL is a minimal medium which is relatively poor in nutrients.

B. *Physical properties of microalgae oil and biodiesel*

The physical properties of microalgal oil extracted from *Chlorella protothecoides*, biodiesel from microalgae, diesel fuel, and biodiesel from ASTM standard in different methods are illustrated in Table II. In comparison with diesel, the microalgae oil produced from *Chlorella protothecoides* has a viscosity of 3.6 mm^2s^{-1} at 25°C which is at the limit of the ASTM specification for biodiesel (between 3.5 and 5.0 mm^2s^{-1} at 40°C). The oil is also at the limit of diesel (1.9 - 4.1 mm^2s^{-1}). It is lower than the viscosity of the biodiesel derived from microalgae. Viscosity is decreased in two ways, through the transesterification process and blending with conventional diesel. The density of *Chlorella Protothecoides* oil is 0.912 kg L^{-1} which is higher than the density of microalgae oil, diesel fuel and ASTM biodiesel in Table II. The density of *Chlorella protothecoides* biodiesel is 0.87. Residual carbon is 0.1% (m/m) which is higher than the limits in Table II (0.050 max wt%). *Chlorella protothecoides* oil has a calorific value of 35.800 MJ kg^{-1} which is lower than the microalgal biodiesel calorific value and diesel fuel. An Acid Number of 0.2 mg KOH/g, water 140mg/kg and phosphor 0.0008mg/kg were found to be lower than others results of microalgae biodiesel, biodiesel standers and diesel fuel in Table II. The percentage of biodiesel extracted from *Chlorella protothecoides* oil is 91.3 %.

TABLE I. MICROALGAE *CHLORELLA VULGARIS* PRODUCTIVITY

Properties	*Chlorella vulgaris*	*Chlorella vulgaris* [13]	*Chlorella vulgaris* [14]
Growing time (days)	40	7	-
Dry weight (g L^{-1})	0.487	0.5	-
Biomass productivity (mg L^{-1} d^{-1})	12.16	74.2	20–200
Average lipid content (mg L^{-1})	48.95	77.9	-
Lipid productivity (mg L^{-1} d^{-1})	1.224	11.1 – 6.91 **	11.2–40.0
Lipid %	10.06	14.96-15.58*	5.0–58.0

* Dividing Average lipid content (mg L^{-1}) by Dry weight (g L^{-1}) and dividing Lipid productivity (mg L^{-1} d^{-1}) by Biomass productivity (mg L^{-1} d^{-1})

TABLE II. THE PHYSICAL PROPERTIES OF MICROALGAE *CHLORELLA PROTOTHECOIDES* OIL, BIODIESEL FROM MICROALGAE, DIESEL FUEL AND FROM ASTM STANDARD OF BIODIESEL IN DIFFERENT METHODS.

Properties	***Chlorella protothecoides* oil (Soley Institute)**	[2]			[15]	
		Microalgal biodiesel	*Diesel fuel*	*ASTM biodiesel standard*	*ASTM D 6751 – 02 Limits*	*Method*
Appearance	Clear	-	-	-	-	-
Colour	Yellow/greenish	-	-	-	-	-
Viscosity (mm2s^{-1})	3.6 mm2/s	5.2	1.9–4.1	3.5–5.0	1.9–6.0	D 445
Tot. Contamination (mg/kg)	2	-	-	-	-	-
Carbon Residual% (m/m)	0,1	-	-	-	-	-
Water consentration	140 mg/kg	-	-	-	max 0.050 volume%	D 2709
Sulfur	2 mg/kg	-	-	-	max 0.05 wt%	D 5453
Iodine mg. iodine/100g	67	-	-	-	-	-
Specific Gravity at 25°C	0.91 – 0.92	-	-	-	-	-
Peroxide value max.	0.5	-	-	-	-	-
Density (kg/L)	0.912	0.864	0.838	0.86–0.9	-	-
Acid Number (mg KOH/g)	0.2	0.374	max 0.5	max 0.5	max 0.80	D 664
Phosphor	0.0008 mg/kg%	-	-	-	0.0010 wt%	D 4951
Calorific value (MJ/kg)	35.8	41	40–45	-	-	-
Oxidation Stability (C): 5h	110		-	-	-	-
Flash Point (°C)	220	115	75	min 100	min 130	D 93

C. Chemical properties

The main components in the microalgal oil are palmitic, oleic and linoleic acids at 51%, 39% and 7% respectively. These three components comprise 97% of the total fatty acid profile of microalgae *Chlorella protothecoides* oil in Table III.

The vegetable oil that most closely matches *Chlorella protothecoides* oil in terms of chemical composition is palm oil, which consists of 42.6% C16:0, 40.5% C18:1 and 10.1% C18:2 [16] see Table IV. Table V shows that *Chlorella vulgaris* contains of 56% of C 18 unsaturated fatty acid in which 2% of C18:1, 34% of C18:2 and 20% of C18:3. The main fatty acid methyl esters (FAME) in the *Chlorella protothecoides* biodiesel after transesterification are illustrated in Table VI. The ratios of the predominant fatty acids are very similar to the results of Xu et al. [17]. Biodiesel from *Chlorella protothecoides* analysed using gas chromatography using the 1892-1AMP standard gave a slightly different percentage of chemical components: $C_{19}H_{36}O_2$ and $C_{17}H_{34}O_2$ in *Chlorella protothecoides* were found to be higher than the respective components in Table VI with values of 70.48% and 13.64% respectively, while the amount of $C_{19}H_{32}O_2$ in the sample tested in Table VI was more than the percentage of this component in Table VII.

TABLE III. FATTY ACID PROFILE (%) (SOLEY INSTITUTE)

Fatty Acid	Carbon	Chemical formula	%
Palmitic	C 16:0	$C_{16}H_{32}O_2$	51
Stearic	C 18:0	$C_{18}H_{36}O_2$	2
Oleic	C 18:1	$C_{18}H_{34}O_2$	39
Linoleic	C 18:2	$C_{18}H_{32}O_2$	7
Others			1

TABLE IV. FATTY ACID PROFILES OF SOME COMMON VEGETABLE OILS USED OR SUGGESTED AS BIODIESEL FEEDSTOCK [16]

Vegetable oil	C16: 0	C18: 0	C18:1	C18:2	C18:3	Other
Castor	1.1	3.1	4.9	1.3		Ricionic acid 89.6
Coconut	7.8	3.0	4.4	0.8		(12:0= 48.8), (14:0= 19.9), (C18:4 65.7)
Palm	42.6	4.4	40.5	10.1	0.2	
Peanut	11	2	48	32	1.0	
Rapeseed	3.5	0.9	64.1	22.3	8.2	
Soybean	13.9	2.1	23.2	56.2	4.3	

TABLE V. FATTY ACID COMPOSITION (% TOTAL) [18]

Freshwater spp.	16:0	16:1	16:2	16:3	16:4	18:1	18:2	18:3	18:4	20:5	20:6
Scenedesmus obliquus	35	2	-	-	15	9	6	30	2	-	-
Chlorella vulgaris	26	8	7	2	-	2	34	20	-	-	-
Chamydomonas reinhardtii	20	4	1	4	22	7	6	30	3	-	-
Salt- tolerant spp.											
Ankisterodesmus spp.	13	3	1	1	14	25	2	29	2	1	-
Isochrysis spp	12	6	-	-	15	4	6	17	-	2	13
Nannochloris spp.	9	20	7	9	-	4	1	1	-	27	-

TABLE VI. THE CHEMICAL ANALYSES OF THE FAME OF *CHLORELLA PROTOTHECOIDES* MICROALGAE OIL (SOLEY INSTITUTE)

Formula	FAME	Relative Content (%)
$C_{15}H_{30}O_2$	Methyl tetradecanoate	1.3
$C_{17}H_{34}O_2$	Hexadecanoic acid methyl ester	12.8
$C_{18}H_{36}O_2$	Heptadecanoic acid methyl ester	0.9
$C_{19}H_{34}O_2$	9.12-Octadecadienoic acid methyl ester	17.4
$C_{19}H_{36}O_2$	9-Octadecenoic acid methyl ester	60.8
$C_{19}H_{38}O_2$	Octadecanoic acid methyl ester	2.8
$C_{20}H_{38}O_2$	10-Nonadecenoic acid methyl ester	0.3
$C_{21}H_{40}O_2$	11-Eicosenioc acid methyl ester	0.4
$C_{21}H_{42}O_2$	Eicosanoic acid methyl esteracid ester	0.4

TABLE VII. BIODIESEL COMPONENTS IN *CHLORELLA VULGARIS* AND *CHLORELLA PROTOTHECOIDES*

Formula	***Chlorella vulgaris* FAME Area %**	***Chlorella protothecoides* FAME Area %**
- N/A	6.71	-
- N/A	-	3.10
- N/A	1.80	-
$C_{17}H_{34}O_2$	1.99	13.64
$C_{19}H_{38}O_2$	0.0	0.0
$C_{19}H_{36}O_2$	3.77	70.48
$C_{19}H_{34}O_2$	59.08	4.45
$C_{19}H_{32}O_2$	2.76	-
$C_{21}H_{42}O_2$	0.0	0.0
- N/A	23.89	-
- N/A	-	2.14
- N/A	-	2.23
- N/A	-	3.96

IV. CONCLUSION

Microalgae biodiesel is a promising alternative fuel for the future. Different microalgae species produce different biodiesel components with different oil productivity. Although fresh water *Chlorella vulgaris* is a robust species, it has low lipid content and low lipid productivity. Therefore, increasing the lipid content and the lipid productivity is important. *Chlorella protothecoides* oil can meet the ASTM standards for biodiesel for most of the characteristics. A single cylinder four stroke CI diesel engine will be used to test different blend ratios of microalgae biodiesel and diesel fuel. The engine performance and the emission results will be evaluated using diesel fuel and microalgae fuel (different blend ratios) with the aid of a numerical model.

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