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Direct transesterification to produce bio-diesel from *Oedogonium* algae and duckweed plant

Wed Al-Graiti[1*], Asaad H. Sayer[2], Saleem Ethaib[3], Sameerah Jameel Tarfa[1], Amina Sabah Hashem[1]

1. College of Dentistry, University of Thi-Qar, Thi-Qar, 64001, Iraq
2. Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq
3. College of Engineering, University of Thi-Qar, Thi-Qar, 64001, Iraq

** Corresponding author's Email: assa.sayar@sci.utq.edu.iq*

ABSTRACT

Biodiesel from renewable sources is one of the possible alternatives support the energy security. This study aimed to produce biodiesel from *Oedogonium* algae and duckweed plant by direct transesterification. An amount of 5 g of dry biomass was taken for the considered duckweed plant and the algae was immersed in 50 mL of methanol: hydrochloric acid: chloroform (10:1:1 v/v/v) solvent to extract fatty acid methyl esters (FAME). The extracted materials were taken for the characterisation using FT-IR and GC-Mass spectrometry. The prepared samples were specifically diagnosed for the identification of compounds particularly lipids. The results showed presence of saturated and unsaturated fatty acids in the formed ester (biodiesel). Saturated and unsaturated fatty acids identified in the biodiesel derived from duckweed plant were 24.19% and 20.34% respectively and from *Oedogonium* algae were 19.92% and 17.2% respectively. These results demonstrated a high potential to produce biodiesel from these types of biomass which could provide another route for energy supply.

Keywords: Biodiesel, *Oedogonium*, Duckweed, Direct transesterification, Fatty acid methyl esters.
Article type: Research Article.

INTRODUCTION

The continuous increase in energy demand and the increase in political conflicts, pollution damages, and global warming put pressure to find alternatives to the conventional energy resources represented by coal, oil, and oil derivatives. Moreover, depleting conventional fuels (fossil fuels) fasted and forced research to investigate alternative energy sources to save the global economy and environment (Ethaib *et al.* 2020a). Renewable sources could offer a viable route to tackle conventional energy defects (Sayer *et al.* 2020). Biofuels have emerged as promising alternative energy sources. For the first generation of biofuels, bioethanol and biodiesels are produced from food feedstock such as starch, sugar, and oil derived from agricultural crop plants such as corn, wheat, and soybeans (Neto *et al.* 2019). Using food crops feedstocks to generate biofuels triggers the debate to choose between fuel and food (Alaswad *et al.* 2015). Moreover, to create enough biomass, food crop feedstocks need huge agricultural areas which may lead to land destruction, biodiversity loss, habitat loss, water depletion, and air pollution (Neto *et al.* 2019). Therefore, the research is directed to produce biofuels from non-food crops utilizing grass, wood, lignocellulosic biomass and other organic wastes which is called second generation of biofuels. The complex structures of the lignocellulosic materials require a pre-treatment process for efficient conversion during hydrolysis (Ethaib *et al.* 2020b). A wide array of pre-treatment processes have been applied. However, most of these processes suffer from technical difficulties which ultimately reflects on the cost of final product (Ethaib *et al.* 2020c). In the search for viable and cost-effective alternatives, algae and algae-derived biomass gains considerable attention or the production of an improved version of biofuels (Gajraj *et al.* 2018). Employing alga

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and non-food feedstocks such as aquatic plants could be considered in the production of feasible biofuels and making a balance between biofuel production and food security. Moreover, Microalgae and macroalgae can fix 1.83 kg CO_2, while producing 1 kg biomass (Brennan & Owende 2010; Khaliullina 2021). Besides proteins and carbohydrates, microalgae also produce lipids for biofuels (Chew *et al.* 2017). Microalgal lipid output is 20,000-80,000 L/acre/year, which is 30 times that of seed crops fuel. Among biofuels, biodiesel can be an applicable alternative to the petroleum-based fuels. Recently, employing microalgae as a substrate for biodiesel production has gained widespread interest. Direct transesterification of microalgal lipids is an easy, straightforward, and quick method for measuring fatty acids by combining extraction and transesterification in a single step (Yousuf *et al.* 2017). Solvent-mediated extraction of microalgal lipids is followed by solvent evaporation and FAME synthesis (Sati *et al.* 2019). In this approach, wet or dry biomass is treated with methanol and an inorganic acid or base catalyst in a single reactor, resulting in the reactive extraction of lipids as fatty acid alkyl esters, usually fatty acid methyl ester. Methanol is a solvent and esterification reagent (Park *et al.* 2015). The simultaneous lipid extraction and transesterification to fatty acid methyl ester saves time and decreases organic solvent use. This technique reduces instrument installation, maintenance, and energy use (Yousuf *et al.* 2017; Sati *et al.* 2019). Duckweed plant also is a small floating aquatic plant that grabs the attention to be a substrate for biodiesel generation. Several studies extracted the lipids in this plant and converted into biodiesel via transesterification reaction. Besides the high potential of duckweed to convert into a biofuel, this plant has the ability of wastewater phytoremediation. It has a remarkable capability to absorb nitrogen from wastewater and release oxygen to water (Ge *et al.* 2012; Mohedano *et al.* 2012; Zhang *et al.* 2014; Rezania *et al.* 2016; Newete *et al.* 2016; Kamil & Taha 2022). Thus the current study aimed to evaluate the production of biodiesel from *Oedogonium* macro-algae and duckweed by direct transesterification.

MATERIALS AND METHODS

Materials

Oedogonium macro-algae and duckweed were cultivated inside wastewater ponds in Najaf and Nassiryai cities respectively. Then samples were dried in shadow. The dried samples of algae and duckweed were ground to prepare the powder required for the extraction process.

Methods

Direct transesterification: The methylation method suggested by Lewis *et al.* (2000) was followed in triplicate, an amount of 5 g of dry biomass was taken for the considered plant and algae. The suggested solvent by Lewis *et al.* (methanol: hydrochloric acid: chloroform, 10:1:1 v/v/v) was prepared for the extraction process. To the biomass sample (5 g) a quantity of 50 mL solvent was added. The samples were re-suspended by vortex for 30 seconds. After the resuspension, each sample was placed in a preheated ultra-sonic bath at a constant ultrasonic pulse (35 kHz) at 80 °C for 90 min to perform the required transesterification reaction (Ríos *et al.* 2013). After 90 min, the reaction was completed and the reaction mixtures were allowed to cool in room temperature. To each cold sample, a quantity of 15 mL distilled water was added with mixing. To extract the formed fatty acids (FA), a mixture of hexane-chloroform (4:1 v/v) was used (12 mL). The mixture of hexane-chloroform was added in batches and then vortexed for 20 second. Finally, two layers was observed, the organic layer contains the extracted fatty acid methyl esters (FAME). Samples were taken from the produced ester for the characterisation. FT-IR spectra of the products were performed by FT-IR device type BRUKER/FT-IR Affinity-1 spectrophotometer using KBr disks. The device was available at the Department of Chemistry, College of Sciences, University of Thi-Qar, Iraq. Samples of FAME were also diagnosed using GC/Mass device. GC/Mass spectrometer with the following specifications was used: Gas Chromotograph: Agilent (7820A) USA; GC/Mass spectrometer with analytical column Agilent HP-5MS Ultra Inert (30 m length × 250 µm inner diameter × o.25 µm film thickness); Injection volume was 1 µL.

RESULTS AND DISCUSSION

The prepared samples were diagnosed for the identification of compounds particularly lipids. The devices of FT-IR and GC-Mass spectrometry types were used for the identification of compounds formed after the direct esterification reaction. For duckweed plant, Figs. 1-2 show charts of FTIR spectrophotometry, while Fig. 3 illustrates the GC-Mass spectrum. Fig. 1 represents the FTIR spectrum of the upper layer of the solution which has fatty acids and other compounds.

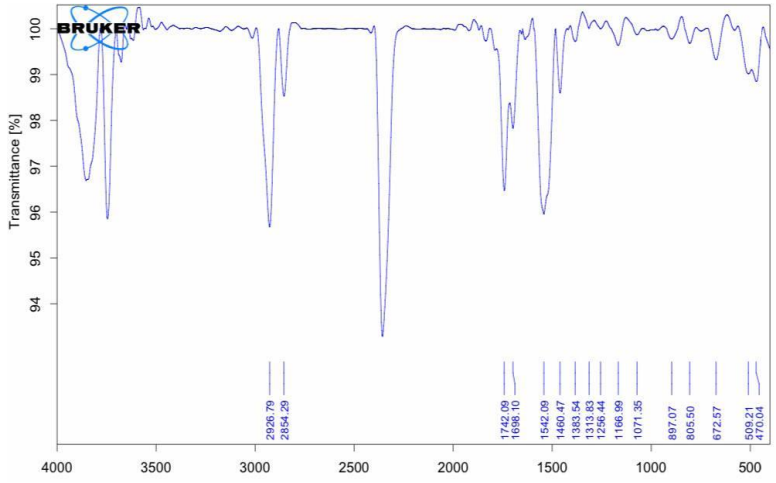


Fig. 1. The FTIR spectrum of the upper layer of duckweed plant after the rection.

It is clear from Fig. 1 that there is a apparent peaks at 2926 cm^{-1} and 2854 cm^{-1} respectively. These peaks refere to the presence of amide group resulting from NH stretching (Yu *et al.* 2011). There is also a sharp peak at 2250 cm^{-1}. Moreover, there are two clear peaks at 1742 and 1542 cm^{-1}. The latest two peaks are an indication of the presence of C=O at the stretching region of carboxylic acids (Lim *et al.* 2014; Merdas *et al.* 2022; Al-Graiti *et al.* 2019). Moreover, there are peaks between 1256 to 897 cm^{-1} which verfiy the presence of carbohydrates (Yu *et al.* 2011). Peaks at 1460 and 1542 cm^{-1} refer to amide derived from NH pending also from CN stretching. Fig. 2 demonstrates FTIR spectrum of the lower layer of the mixture which is anticipated to represent the glycerol layer.

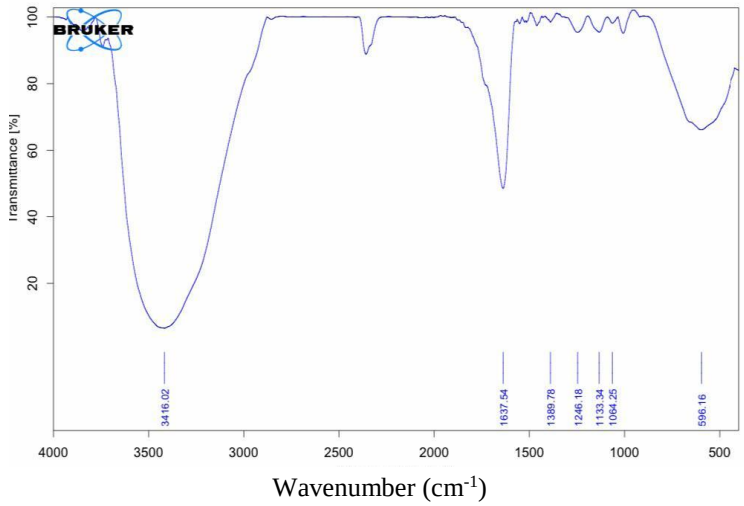
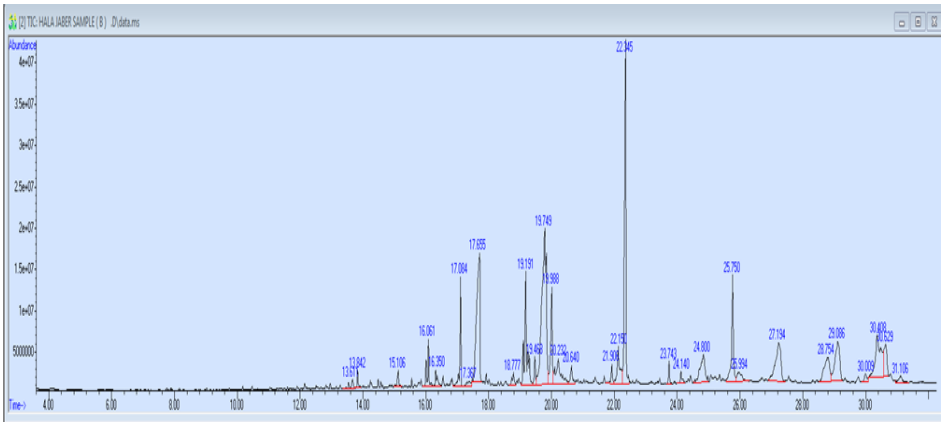


Fig. 2. The FTIR spectrum of the lower layer (glycerol layer) of duckweed plant.

As commonly known that glycerol is the main by-product of transesterfication, in the present study it was observed that glycerol represented the lower layer of the mixture in the separatory funel. As shown in Fig. 2 that the plotted chart by FTIR device is approximately similar to the available pure chart of glycerol, there is a broad peak at 3416 cm^{-1}. The broad peak refers to the presence of multiple OH functional group (Al-Graiti 2017). Furthermore, there are clear peaks at 1637 cm^{-1} belonging to hydrogen bonds formed by water molecules (Guimarães *et al.* 2016). For further identification to the available saturated and unsaturated fatty acids in the formed ester (biodiesel), the GC-mass test was perormed for duckweed plant and shown in Fig. 3.



Wavenumber (cm^{-1})

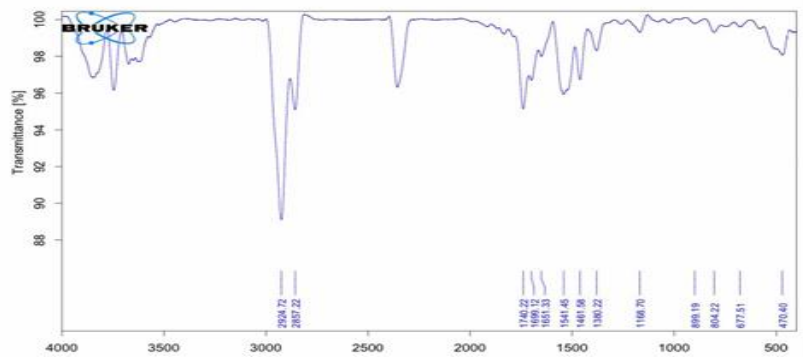
Fig. 3. The GC/mass spectra of biodiesel produced from duckweed plant.

According to information available in Fig. 3, Table 1 was derived, exhibiting the available saturated and unsaturated acids with their molecular formula for the plant of duckweed.

Table 1. Saturated and unsaturated fatty acids identified in the biodiesel derived from duckweed plant.

NO	Fatty acid Name	Chemical Formula	R. Time	Carbon Number	Saturated acids% Area	Unsaturated acids %Area
1	Tetradecanoic acid	$C_{14}H_{28}O_2$	15.109	C14:0	0.58	
2	Pentadecanoic acid	$C_{15}H_{30}O_2$	16.350	C15:0	0.71	
3	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	17.084	C17:0	2.79	
4	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	17.655	C16:0	10.26	
5	n-Decanoic acid	$C_{10}H_{20}O_2$	16.777	C10:0	0.66	
6	11-Octadecanoicacid, methyl ester	$C_{19}H_{36}O_2$	19.187	C19:1		6.28
7	Methyl stearate	$C_{19}H_{38}O_2$	19.467	C19:0	0.77	
8	Oleic acid	$C_{18}H_{34}O_2$	19.748	C18:1		16.98
9	Octadecanoic acid	$C_{18}H_{36}O_2$	19.986	C18:0	4.02	
10	Bihenic acid	$C_{22}H_{44}O_2$	24.139	C22:0	0.55	
11	9,12-Octadecadienoic acid(z,z)-	$C_{18}H_{32}O_2$	20.644	C18:2		0.93
					20.34	24.19

It is apparent from Table 1 that there are ranges of saturated and unsaturated fatty acids in the formed ester (biodiesel). This variation in acid availability is desirable in biodiesel. In the case of *Oedogonium*, the reaction of direct esterification was performed and the identification results are illustrated in Fig. 4-6. Fig. 4 explains FTIR spectrum of upper layer of *Oedogenium*. From this spectrum, multiple peaks are observed in the range between 1380 cm^{-1} and 1460 cm^{-1} belonging to C-N stretching bond of amino acids (Khilji *et al.* 2022). A peak observed at 1165 cm^{-1} may belongs to C-O-C of the polysaccharides. A clear peak seen at 1740 cm^{-1} attributes to the vibration bending of C=O of the proteins or the remaining acetate (Khilji *et al.* 2022). At 2924 cm^{-1} and 2857 cm^{-1} two main peaks were formed refer to C-H stretching (Saifuddin *et al.* 2014). Fig. 5 shows the FTIR spectrum of the glycerol layer.



Wavenumber (cm^{-1})

Fig. 4. FTIR of the upper layer of *Oedogonium*.

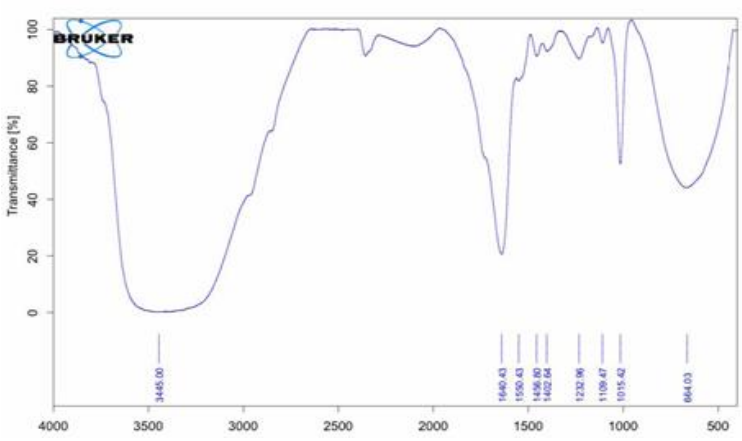


Fig. 5. FTIR of the lower layer (glycerol layer) of *Oedogonium*.

The FTIR technique was performed in order to investigate the lower layer of the *Oedogenium*. The glycerol layer at duckweed has revealed almost same spectrum when *Oedogenium* lower layer is examined by FTIR technique. A broad peak observed at 3445 cm^{-1} belongs to the existence of multiple OH peaks (Saifuddin *et al.* 2014; Al-Graiti *et al.* 2023). In addition, at 1640 cm^{-1}, a sharp peak observed, refers to the presence of hydrogen bond formed by water molecules. The GC-Mass spectra is illustrated in Fig. 6.

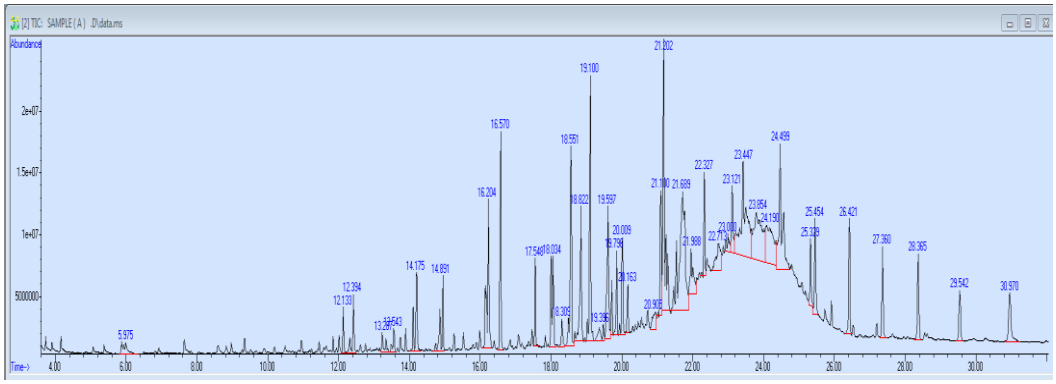


Fig. 6. GC-MS spectra of the *Oedogonium*.

Similar to the case when duckweed was used, there are many saturated and unsaturated acids shown in Fig. 6. This variation gives a prior indication that this biofuel can be suitable biodiesel. Table 2 was derived from Fig. 6 to show in more detail the formed acids.

Table 2. Saturated and unsaturated fatty acids identified in the biodiesel derived from *Oedogonium.*

No	Fatty acid Name	Chemical Formula	R. Time	Carbon Number	Saturated acids% Area	Unsaturated acids %Area
1	3-Hydroxy-2-methyl-octa-4,6- dienoic acid,methyl ester	$C_{10}H_{16}O_3$	13.545	C10:2		0.54
2	Nonanedioic acid,dimethyl ester	$C_{11}H_{20}O_4$	14.171	C11:0	2.43	
3	Tridecanoic acid,12-methyl-,methyl ester	$C_{15}H_{30}O_2$	16.566	C15:0	3.05	
4	Phytol, acetate	$C_{22}H_{42}O_2$	18.313	C22:1		0.52
5	Cis-5,8,11,17-Eicosapentaenoic acid, methyl ester	$C_{21}H_{32}O_2$	18.551	C21:5		4.07
6	9-Hexadecenoic acid,methyl est -er,(z)-	$C_{17}H_{32}O_2$	18.820	C17:1		2.93
7	Hexadecenoic acid,methyl ester	$C_{17}H_{34}O_2$	19.101	C17:0	4.58	
8	Methyl 5,12-octadecadienoate	$C_{19}H_{34}O_2$	21.097	C19:2		2.28
9	Nonahexacontanoic acid	$C_{69}H_{138}O_2$	21.205	C69:0	7.14	
10	Oleic acid	$C_{18}H_{34}O_2$	21.690	C18:1		9.58
					17.2	19.92

It is apparent from Table 2 that there are many saturated and unsaturated fatty acids in the formed ester (biodiesel) after the completion of the reaction. The table shows that saturated acids represent 17.2% while unsaturated acids 19.92%. According to Table 2, the produced biodiesel can be utilised as a biofuel, and this of course after measuring other physical and chemical properties of the fuel.

CONCLUSION

Two types of biomass including *Oedogonium* and duckweed were processed using direct transesterification to investigate their potential to produce biodiesel. The results of FTIR and GC-Mass spectrum showed that fatty acids and other compounds. Reasonable content of saturated and unsaturated acids were detected in the extracted lipids from the biomass substrates which refer to the high potential to produce biodiesel. Therefore, as a future study recommend to evaluate other physical and chemical properties of the produced biodiesel to assess the possibility of using it as fuel engine.

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