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BIODIESEL PRODUCTION FROM *SCENEDESMUS ECORNIS* BY TRANSESTERIFICATION PROCESS

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Abstract

In this study *Scenedesmus ecornis* microalgae isolated from Chnarok-Koya city**.** After identification of genera their optimum growth condition studied by using the effecting of temperature, pH and light intensity to their fresh weight, dry weight, turbidity and chlorophyll content. In the result, the optimum growth rate showed at 28℃, pH 8 and 1800 lux. Species one genus that showed high growth rate selected and diagnosed by molecular identification as *Scenedesmus ecornis*, then the selected species used in biodiesel production.

The process of biodiesel production involved a chemical reaction between algal oil and methanol through transesterification. The result showed Production of biodiesel from *Scenedesmus ecornis* oil. The obtained biodiesel analyzed using ASTM methods to determine the characteristic fuel properties; kinematic viscosity (2.57 mm^2 sec^{-1}), density (0.8689 g cm^{-3}), total sulfur content (0.0116 %), flash point (153℃), water content (0.00 %) for *Scenedesmus ecornis*.

Keyword : Biodiesel, Microalgae, Dry biomass, transesterification, oil

Introduction

Biodiesel is an environmentally friendly energy source of renewable energy. Biodiesel produces less greenhouse gas, produces less carbon dioxide and low sulfur content, while also reducing the sulfur and carbon monoxide content. Technically speaking, 90% of air toxicity and 95 % of cancers can be reduced by biodiesel (Huang *et al.*, 2010; Ren, 2014).

Due to the elevated viscosity of the oil, diesel engines are not fitted to operate on pure vegetable oil (Carter *et al.*, 2005). The oil must, therefore, be modified to decrease its viscosity. The method used to chemically change the oil to decrease its viscosity is called transesterification (Carter *et al.*, 2005). Which is a multi-step response, composed of three reversible steps, where triglycerides are transformed to diglycerides, diglycerides to monoglycerides and monoglycerides to esters (biodiesel) and glycerol as a by-product. (Rajvanshi and Sharma, 2012).

However, biodiesel production techniques for vegetable oils can be applied to the manufacturing of biodiesel for microalgae oils due to comparable physical and chemical properties. Alcohols are important substrates for transesterification (Huang *et al.*, 2010). The addition of alcohol removes the structure of glycerol and replaces it with a lower methyl group. The large molecule is therefore divided into methyl esters and glycerol products during transesterification (Carter *et al.*, 2005). Transesterification is an equilibrium reaction that describes the alcoholism of carboxylic esters generally conducted in the presence of standard catalysts (e.g. NaOH and KOH) for a precious acceleration of the balance adjustment to obtain greater yields of esters (Mumtaz *et al.*, 2017).

Microalgae have fast development potential and many species have an oil content of 20-50 % of the dry weight of biomass, with an exponential growth rate that can double their biomass by as little as 3.5 hours (Haldar *et al.*, 2018). Biodiesel yields from microalgae maybe 10 to 20 times greater than those from oleaginous seeds and/or vegetable oils. Oil content in some microalgae may be relatively high and may be caused to generate even greater levels of lipids through the use of low nitrogen media, variable concentrations of Fe^{3+} and enhanced light intensity (Illman *et al.*, 2000; Liu *et al.*, 2008). Thus the purpose of this study was to investigate biofuel production from microalgae *Scenedesmus ecornis* by transersterification process.

Material and Methods

Isolation and identification of microalgae

Water samples diluted and plated on the BG11 medium. The algal isolates were then transferred to sterilized media for purification and identification. After day 15 purified single cells and filaments removed with pasture micropipettes placed on a glass slide covered with a cover slid and examined under light microscope for identification purposes based on their morphology as described by (Prescott, 1963; Desikachary, 1968; Rippka, 1988; Holt *et al.*, 1994). Microalgae strains have been recognized using conventional methods based on the morphological characteristics of the species. Classic morphological taxonomy involves the use of features that can be observed with light microscopies, such as Filament or unicellular, Akinet, Hormogonia, Heterocyst, color, chloroplast, and Cell shape (Prescott, 1963; Desikachary, 1968; Rippka, 1988; Holt *et al.*, 1994). Morphological characteristics of isolated microalgae were photographically registered at 10–40X magnification using Olympus BL51 microscope (Sarwa and Verma, 2017). After identification the pure colonies were gently blown into liquid medium then incubated at 30 °C and pH 8 to obtain biomass . After 14 days incubation microalgae were harvested by centrifugation at 4000 rpm for 10 min. To determine cell dry weight the collected sample dried in oven at 70 °C 4-6hrs. and weighted quickly after drying as described by (Becker, 1994).

Molecular identification of microalgae

The targeted region of 16S rRNA of microalgae was amplified by PCR using universal primers, forward primer A8F (5- AGAGTTTGATCCTGGCTCAG-3), and the reverse primer A1492R (5- TACGGCTACCTTGTTACGACTT-3),

were designed and selected by (Ponnuswamy *et al.*, 2008). A total of 50 µl volume of the reaction mixture was prepared to contain 40–55ng DNA, 100 mM of each primer, 0.05 U/µl Taq DNA polymerase, 4 mM MgCl2, and 0.4 mM of each dNTP in a thermocycler MJ Research, Applied Biosystem (AB). The cycling profile consists of an initial denaturation step of 2 min at 94˚C followed by 25 cycles of 60 sec. at 94˚C, 60 sec. at 52.3˚C, 60 sec. at 72˚C, and final extension 2 min at 72˚C.

A partial region of 16S rRNA was amplified by PCR using the universal primers. In the present study, ABI 3130XL genetic analyzer (USA) was employed to find the nucleotides order of 16S rRNA for different algae samples. The PCR fragments of the algae samples were cut from the agarose gel and utilized as a source of DNA template for sequence-specific PCR amplification and sent to the private medical genetic laboratory in Intergene Genetic Center, Ankara, Turkey.

Biodiesel production from microalgal isolates:

Oil extraction

The selected samples that showed a high rate of biomass were powdered after drying. A sample of 50 g of dried microalgae was put in the Soxhlet extractor. The Soxhlet extractor put on a flask including the extracting solvent (Hexane). Then the Soxhlet is fitted with a condenser.

The condenser ensures that any solvent vapor cooled into the chamber containing the solids is dripped. The chamber containing the solid material slowly filled with warm solvents (Figure 1). The cycle has been repeated for different periods. A part of the oil is dissolved in hexane during each cycle. After many such cycles, the required oil was focused on the distillation bottle (Topare *et al.*, 2011).

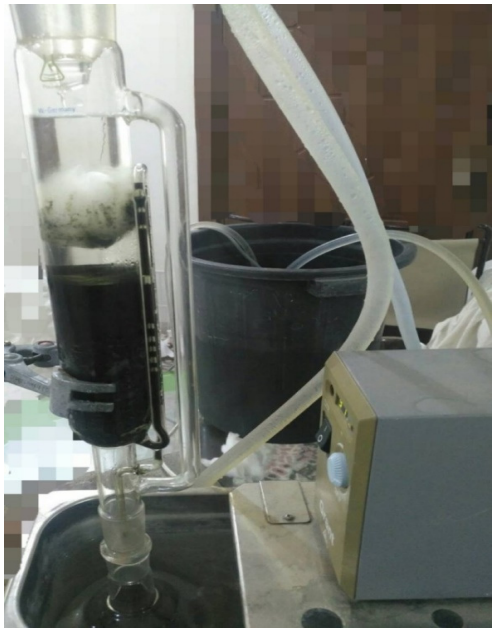

Fig. 1 : Oil extraction from algae using a Soxhlet extractor

Transesterification and biodiesel production

The extracted oil evaporated under a vacuum to release solutions of the solvent mixture using a rotary evaporator at temperature 40-45 ℃. Transesterification was done in a (1 L three-neck glass flask) connected to a reflux condenser using tap water to condense methanol vapor and thermometer. The mixture was stirred using a stainless steel stirrer. The reactor was placed in a hot water tank. KOH pellets were dissolved in methanol before being poured into the reactor containing approximately 200 ml of oil sample heated to the desired temperature of 60 ° C. The reaction was held at a temperature of 120 min. The molar ratio of the methanol and the oil sample was 6:1, while the KOH catalytic value was 1.5 wt per cent KOH of the oil sample. After some time, the mixture was poured into a separate funnel. The ester layer was separated by gravity and put in the upper layer. Glycerol, extra methanol and undesired materials were in the lower layer and decanted. The ester layer was washed several times, each with a small amount of hot water until the washes were neutral (Younis *et al.*, 2014).

The physical properties of the raw samples, biodiesel and its blends with petro-diesel were measured by using American Society for Testing and Materials "ASTM" standard methods, including kinematic viscosity (using U tube viscometer " SCOTT CT52", ASTM D 445), flash point (using Tanaka ACO-7, ASTM D 92), density (ASTM D1298), total sulfur content (using Tanaka RX-360SH, ASTM D4294) and water content (ASTM D6304).

Result and Discussion

Morphological and molecular identification of microalgae:

The microalgae identified on the base of morphological characteristics, after observation under microscope the microalgae was unicellular, cylinder shape with lamellate chloroplasts that identified as *Scenedesmus sp.*

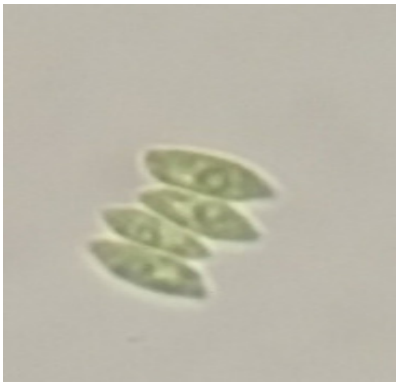

Fig. 2 : *Scenedesmus* sp. under microscope

The molecular identification showed that the sequence from 16S rDNA of algae specimens was made of 1200-1400 bp (amplified fragment was 1468 bp, while after sequencing some miss-nucleotides were excluded, related to quality of sequencing analysis) and put to BLAST then compared with other stored genus of algae sequences from GenBank database. The BLAST results was 99.20 % Identified as *Scenedesmus ecornis.*

The primary sequence analysis using universal primers of studied specimens revealed that the algae from northern Iraq belongs to genus *Scenedesmus ecornis.* Its rDNA conforms with the same rDNA sequence fragment marker, available at the Gene Bank in National Canter for Biotechnology Information (NCBI). Figures (3 and 4) showed pair wise analysis and partial sequence of the algae specimens. (Ponnuswamy *et al.*, 2008) isolated and identified

microorganism using genomic DNA and 16S rRNA gene amplification followed by sequencing. The resulting sequences were compared with those available through the BLAST Bioinformatics tool on the GSPI website database. The results showed a high 99 per cent correlation with known nucleotide sequence identities with *Chlorella vulgaris*. Moreno. (2012) conducted that the microorganisms have been isolated and identified using genomic DNA and 16S rRNA or 18S rRNA gene amplification followed by sequencing.

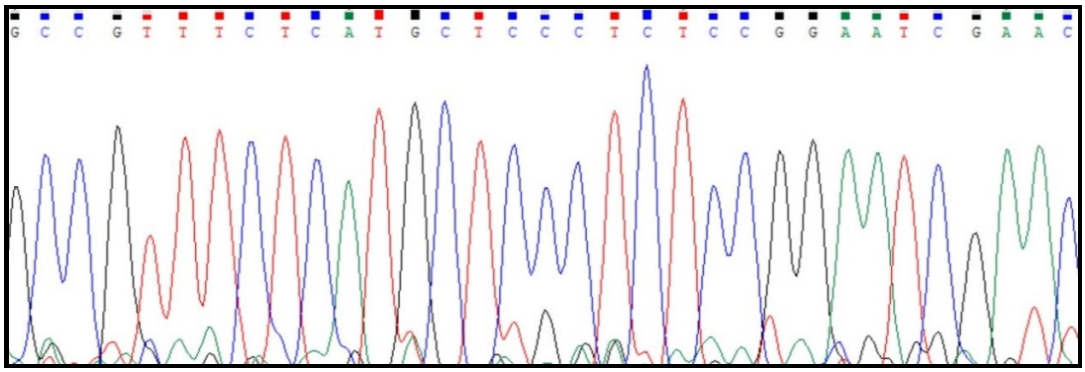

Fig. 3 : The partial sequencing result of 16S rDNA of *Scenedesmus ecornis.*

Score	Expect	Identities	Gaps	Strand
1583 bits(857)	0.0	873/880(99%)	3/880(0%)	Plus/Plus

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Query  1    AATATGATTACAGGTGCTGCGCAAATGGATGGTGCTATTTTAGTAGTATCAGGTGCTGAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AATATGATTACAGGTGCTGCGCAAATGGATGGTGCTATTTTAGTAGTATCAGGTGCTGAC  60

Query  61   GGTCCAATGCCACAAACAAAAGAACATATTTTATTAGCAAAACAAGTTGGTGTACCAAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GGTCCAATGCCACAAACAAAAGAACATATTTTATTAGCAAAACAAGTTGGTGTACCAAAT  120

Query  121  ATGGTTGttttttttAAACAAAGAAGATCAAGTTGATGATGCTGAATTATTAGAATTAGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ATGGTTGTTTTTTTTAAACAAAGAAGATCAAGTTGATGATGCTGAATTATTAGAATTAGTT  180

Query  181  GAATTAGAAGTTCGTGAAACATTAGATAAATATGAATTCCCAGGAGATGAAATTCCAGTT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GAATTAGAAGTTCGTGAAACATTAGATAAATATGAATTCCCAGGAGATGAAATTCCAGTT  240

Query  241  GTAAGTGGATCAGCATTATTAGCTTTAGAAGCTCTTGTTGCAAATCCTGCAATTAAAAAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GTAAGTGGATCAGCATTATTAGCTTTAGAAGCTCTTGTTGCAAATCCTGCAATTAAAAAA  300

Query  301  GGTGAAAACAAATGGGTCGATAAAATCTATGATTTAATGGATCAAGTTGATAAATATATT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GGTGAAAACAAATGGGTCGATAAAATCTATGATTTAATGGATCAAGTTGATAAATATATT  360

Query  361  CCAACACCAGATCGTGAAACAGATAAACCTTTCTTATTAGCCGTGGAAGATGTTTTATCA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CCAACACCAGATCGTGAAACAGATAAACCTTTCTTATTAGCCGTGGAAGATGTTTTATCA  420

Query  421  ATTACTGGTCGTGGTACAGTAGCAACTGGACGCGTTGAAAGAGGAACTTTAAAAGTAGGT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATTACTGGTCGTGGTACAGTAGCAACTGGACGCGTTGAAAGAGGAACTTTAAAAGTAGGT  480

Query  481  GAAAATATTGAATTAGTTGGATTAAAAGATACAAAATCAACAGTTGTAACAGGTCTTGAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  GAAAATATTGAATTAGTTGGATTAAAAGATACAAAATCAACAGTTGTAACAGGTCTTGAA  540

Query  541  ATGTTTaaaaaaaCATTAGATGAAACTATGGCTGGTGATAACGTTGGTGTACTTTTACGT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  ATGTTTAAAAAAACATTAGATGAAACTATGGCTGGTGATAACGTTGGTGTACTTTTACGT  600

Query  601  GGTATTCAGAAAAAAGACGTAGAACGTGGAATGGTTTTAGCAAAACCTGGTTCAATTACT  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  GGTATTCAGAAAAAAGACGTAGAACGTGGAATGGTTTTAGCAAAACCTGGTTCAATTACT  660

Query  661  CCACATACAAAATTTGAAGCACAAGTTTATGTATTAACAAATGAAGAAGGTGGTCGTCAC  720
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  661  CCACATACAAAATTTGAAGCACAAGTTTATGTATTAACAAAAGAAGAAGGTGGTCGTCAC  720

Query  721  TCACCTTTTTTAGTAGGATATCAACCACAATTCTTTATTCGT-CGACTGATGTTTCTGCA  779
            |||||||||||||||||||||||||||||||||||||||| |||||||||||| ||| |
Sbjct  721  TCACCTTTTTTAGTAGGATATCAACCACAATTCTTTATTCGTACGACTGATGTTACTGGA  780

Query  780  AAAATTG-AAGTTTTTCACATATTCAAATG-AAAATCCTTCTTCAGTTGCAGAAGAACAT  837
            |||||||| |||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  781  AAAATTGTAAGTTTTTCACATATTCAAATGAAAAATCCTTCTTCAGTTGCAGAAGAACAT  840

Query  838  TCATATAAAATGGCAATGCCAGGAGATCGTATTGAAGTAA  877
            ||| ||||||||||||||||||||||||||||||||||||
Sbjct  841  TCAAATAAAATGGCAATGCCAGGAGATCGTATTGAAGTAA  880
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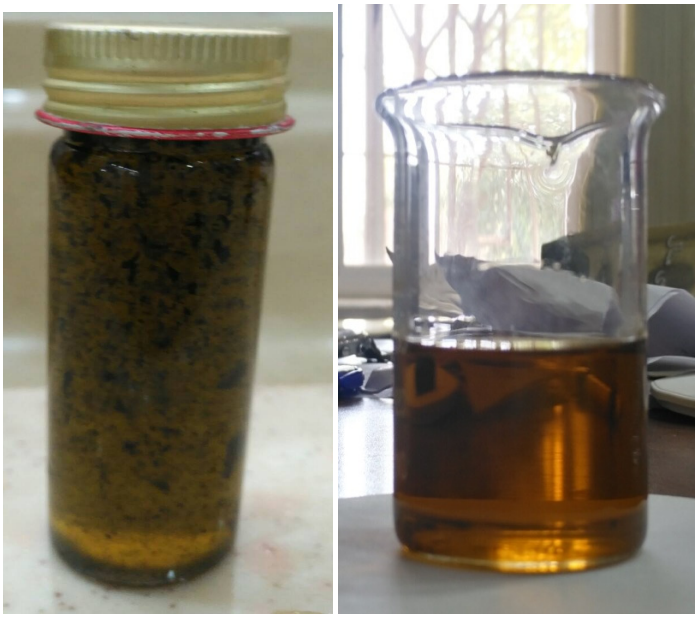
Fig. 4 : Pair wise alignment of 16S rDNA sequence of *Scenedesmus ecornis*, Query is the study or sample sequence and Sbjct is the GenBank sequence.

Microalgae oil extraction and characterization:

Table (1) showed oil extraction and characterization to determine the content of microalgae oil and compression of some characteristic of oil such as (viscosity, density, total sulfur content, flash point and water content) that extracted from *Scenedesmus ecornis.*

Table 1 : Physicochemical properties of Microalgae oils.

No.	Characteristics	Unit	Test method	*Scenedesmus ecornis* oil
1	Kinematic Viscosity @40℃	mm² sec -¹	ASTM D445	14.68
2	Density @15.5℃	g cm-³	ASTM D1298	0.9889
3	Total Sulfur Content	mass%	ASTM D4294	0.0119
4	Flash point	℃	ASTM D92	302
5	Water content	vol%	ASTM D6304	0.00



C- Microalgae oil before filtration D- Microalgae oil after filtration

Fig. 5 : Extracted oil from *Scenedesmus ecornis* before and after filtration

Biodiesel production and characterization by Transesterification process:

The process of transesterification from microalgae, was carried out under the temperature 60 °C, for 120 min. The molar ratio of the methanol and the oil sample was 6:1, while the KOH catalytic value was 1.5 wt per cent of the oil sample. The methanol to oil molar ratio was 6:1 and the quantity of catalyst was 1% by weight of microalgae. In this case, the reaction time was 4 h instead of 2 h, since a time of 4 h is necessary to extract all the oil.

Viscosity is an important parameter for biodiesel equipment and applied to determine the conversion of microalgae oil to methyl-esters. As shown in (Table 2. And Figure 6.) The viscosity of produced biodiesel from microalgae was 2.57 mm² sec^{-1} for *Scenedesmus ecornis* and this value is much lower than that of the crude microalgae oil which was 20.68 mm² sec^{-1} and the density in microalgae oil was more than the microalgae biodiesel. The result is agreed with (Rahman *et al.*, 2017) reported that the viscosity and density of produced biodiesel of crude *Spirulina maxima* oil was 0.864 kg/m^3 and 4.47 mm^2/s respectively. Saydut *et al.* (2010) reported that the viscosity values of methyl esters of vegetable oil decrease sharply after transesterification.

The flashpoint of produced microalgae oil was 302 while in microalgal biodiesel decreased to 153 respectively. (Younis *et al.*, 2014) conducted that the flashpoint for Sesame seed oil , oil Corn oil Sunflower, oil Hazelnut oil was 312, 330, 309, 301 and 325 respectively and reduced in vegetable biodiesels to 155, 150, 158, 154 and 165 respectively. These properties of biodiesel from microalgae oils compared with American Society for Testing and Materials "ASTM" standard methods, this is an agreement with (Atabani *et al.*, 2012; Zahan and Kano, 2018).

The obtained results (Table 2) show that the properties of biodiesel from microalgae oils is near to that obtained by (Abdulrahman *et al.*, 2015).

Table 2 : Physicochemical properties of biodiesel from microalgae oils

No.	Characteristics	unit	Test method	*Scenedesmus ecornis* biodiesel
1	Kinematic Viscosity @40℃	mm² sec -¹	ASTM D445	2.57
2	Density @15.5℃	g cm-³	ASTM D1298	0.8689
3	Total Sulfur Content	mass%	ASTM D4294	0.0116
4	Flash point	℃	ASTM D92	153
5	Water content	vol%	ASTM D6304	0.00

Fig. 6 : Biodiesel production from - *Scenedesmus ecornis* by transesterification process

Conclusion

The study is concluded that it is quite possible to produce biodiesel from *Scenedesmus ecornis* oil by using transesterification reaction method.

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