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Identification of Green Alga *Chlorella vulgaris* Isolated from Freshwater and Improvement Biodiesel Productivity via UV Irradiation

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Chlorella vulgaris **was isolated from the Nile River, Qalubia Governorate, Egypt, for possible use in biodiesel production. BG-II nutrient growth media was used for isolation and laboratory growth. Identification was performed via 18S rRNA gene amplification, followed by sequencing. The alga was exposed to UV-C (254 nm) for 15, 30, and 45 s to improve dry weight accumulation and to increase the oil production. Daily measurements of dry weight ($g \cdot l^{-1}$) were performed; oil content and volumetric lipid productivity were also determined. UV-C exposure led to an increase in the volumetric lipid productivity by 27, 27.3, and 32.4 $mg \cdot l^{-1} \cdot d^{-1}$ with 15, 30, and 45 s, respectively, as compared with the control, which resulted in 18 $mg \cdot l^{-1} \cdot d^{-1}$. Of the examined mutants, the one with the highest productivity was re-irradiated by UV-C (254 nm) for 15, 30, 45, and 60 s. For 15 s of exposure time, the oil content increased to 34%, while it was 31% at 30 s; further, it decreased to 22% at 45 and 60 s exposures. The fatty acid methyl ester profile was 82.22% in the first mutant at 45 s, compared with the wild strain that contained a total of 66.01% of FAs. Furthermore, the highest levels of polyunsaturated fatty acid methyl ester were observed in the mutant exposed for 45 s, and it reached 11.41%, which reduced the cetane number to 71.3.**

Keywords: ***Chlorella vulgaris*****, 18S rRNA gene, UV irradiation, fatty acid, fuel properties**

Introduction

All microalgae are capable to produce energy-rich oils since a lot of microalgae types accumulate naturally high levels of lipid in their biomass weight [1]. *Chlorella vulgaris*, as well as most algal categories, can enhance the potential use of microalgae feedstock in a variety of applications such as the production of biodiesel [2]. Algae have been reported to use in plant physiology and biochemical research as a model organism [3]. The generation of *Chlorella* bioenergy is a relatively new semblance of renewable energy research [4]. Actually, the phylogeny of microalgae deduces from 18S rRNA sequence comparisons including nine separate strains containing divisions *Chlorophyta, Heterokonta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta, Glaucocystophyta,* and *Rhodophyta* [5, 6]. Furthermore, more sequences of genes for algae ribosomal RNA in databases were deposited [7, 8]. The classification of microalgae, such as *Chlorella*, has been hindered by the absence of phenotypic traits suitable for border demarcation of species. For this reason, data of molecular phylogenetic, based primarily on the sequence of genes 18S rRNA presented the first real discernment into algae phylogenetic structure [9, 10]. The effects of ultraviolet (UV) radiation on biological materials have

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become the important issue over the past three decades since the first reports about the changes that man-made ozone layer in the atmosphere which covers and protects the Earth's surface from harmful UV irradiation. The term "UV" used to describe the ultraviolet irradiance from 280 to 400 nm, (UV-B) from 280 to 315 nm, (UV-A) 315–400 nm, taking into consideration that wavelengths of UV-B are less than 290 nm does not reach currently the plant cover [11, 12]. The "UV-C" definition of wavelength from 200 to 280 nm, but is completely absorbed from the atmosphere. Radiation may interact with the growing solar radiation via climate change factors [13]. The last group used was fresh water green algae; it is also used in another study on structural changes and adaptations in physiological systems under UV irradiation of different simulations [14]. UV irradiation are absorbed from nucleic acids and proteins which can cause photodamage and harmonic changes and then disrupt vital metabolic functions such as DNA transcription, replication, and translation [15, 16]. The detailed presentation of potential targets for ultraviolet damage in the photosynthesis of higher plants which can also be useful for Chlorophyta because the structure of plastid function is very similar. From this article, UV induced changes in plasmalemma and thylakoid structure can be expected as have been depicting also for microalgae [17]. Among the various physiological processes, it can be photosynthesis main objective of the UV, not only in higher plants but also because of microalgae in many of the potential impacts [18].

The present study aimed to develop total lipids and fatty acids component for microalgae *Chlorella vulgaris* strain isolated from Nile River in Egypt, to achieve this goal it was exposed to of UV-C irradiation for different times under laboratory conditions in order to increase biodiesel content and biodiesel quality.

Materials and Methods

Source of the isolation

Chlorella vulgaris was isolated from freshwater of Nile River in Qalubia Governorate, Egypt. Isolation and purification of alga were done based on dilution technique and microscopic examination. Sub-culturing and scaling up was employed using in BG-11 medium [19]. Samples were isolated and the cultures were maintained at 25℃ under illumination (120 μ·e). Illumination frequency was 16/8 h light-dark cycle in an orbital shaking incubator at 90 rpm at pH 7.1.

Sub-culture

Visually observed health colonies were transferred to the sub-culturing unit. For this purposes, a single colony was picked-up to the sterilized test tube containing 5 ml of growth medium, incubated at room temperature with about 80 μ·e. Once the green color appeared, a microscopical examination was performed. Re-isolation was repeated to obtain homo-culture.

Growth kinetics, biomass, and lipid productivity

1. Growth was monitored every 24 h, by spectrophotometer cell counting (cells per ml) and by optical density (OD). OD was determined at 650 nm using spectrophotometer SM1600Uv-VIS [20].

2. Biomass productivity (Pdwt), as the dry biomass produced (in gram per liter per day), during the exponential growth phase [21]. For Pdwt determination, samples were collected at the end of the exponential phase and cells were harvested by centrifugation for 10 min at 6000 rpm at 5℃. Supernatant discarded pellets were washed with distilled water and dried at 70℃ over night and the dry weight was determined [22].

3. Total lipids content (Lc), reported as percentage of the total biomass (in % dwt), determined by using the Petroleum ether (60–80℃) approach [23].

4. Volumetric lipid productivity (Lp), calculated according to the equation Lp = Pdwt × Lc and expressed as milligrams per liter per day [24].

Extraction and purification DNA

Total DNA was extracted from axenic cultures of *Chlorella vulgaris* according to the method outlined by [25]. Samples were suspended in the (CTAB) extraction buffer (3% CTAB, 0.1 mol Tris-HCl, 0.01 mol EDTA, 1.4 mol NaCl, 0.5% β-mercaptoethanol, 1% PVP) at pH 8.0. The mixture was incubated at 60℃ for one hour with shaking for every fifteen minutes, and was cooled down to room temperature. DNA was then extracted with an equal volume of chloroform: isoamyl alcohol (24:1) and precipitated from the supernatant by the addition of one volume isopropanol. DNA extract was re-suspended in TE buffer. DNA quality was controlled by

agarose gel electrophoresis.

18S rRNA gene identification

The genomic 18S ribosomal DNA region of microalgae was amplified by colony PCR as described previously by [26] using the C-2: 5'>ATTGGAGGGCAAGTCTGGT<3' forward and D-2: 5'>ACTAAGAACGGCCATGCAC<3' reverse primers. The PCR reaction were performed for the 18S rDNA gene in 25 μl volume by mixing 30 ng genomic DNA with 2 μl of primer (10 pmole/μl) and master max (Takara, Japan) and PCR water. The PCR reaction continued with denaturation for 5 min at 95℃, followed by 30 cycles of 30 s denaturation at 95℃, 1 min. annealing temperatures at 60℃, and 1 min extension at 72℃. The 30 thermal cycles were followed by a final extension of 5 min at 72℃. Following amplification, PCR products were electrophoresed on a 0.9% agarose gel and purified using a Thermo Scientific Gene Jet PCR purification Kit state.

18S rRNA gene sequence analysis

The current rDNA gene sequences were registered at DNA database under accession number. Sequences were compared with those available in the Gene Bank database using Blast. The Phylogenetic tree was constructed through two Bioinformatics Processes. In the first process, the nucleotide sequences of the recovered rDNA gene phenotypes and their homologs sequences, from the DNA database, were aligned using the online program "Clustal Omega". In the second process, the aligned sequences were submitted to the MEGA 7 software (http://www.megasoftware.net/) for drawing the phylogenetic tree. The Phylogenetic tree was constructed by applying the algorithms maximum likelihood in MEGA software.

Mutation by UV-C radiation

UV-C irradiation was done to log phase grown the culture. The Sample of algal culture (20 ml) was centrifuged at 6000 rpm for 10 min at 5℃. After washing the pellets with distilled water twice. The cells were re-suspended in 20 ml of physiological solution 0.9% NaCl are placed in sterile Petri dish with using magnetic stirrer. Petri dish on magnetic stirrer machine was placed inside the UV machine. and then exposed to UV irradiation using 2 lamps UV-C wavelength 254 with the intensity will be around 120 μW/cm^2 for 15, 30, 45 and 60 s at a distance of 40 cm. After Induced Radiation mutation by UV directly the treatment cells were kept in strongly darksome hours lest cause photoreactivation repair.

Fatty acids profiles of microalgae strains

Fatty acids profile was determined by the capillary column gas chromatographic method applied to the fatty acid methyl esters. Sample chromatograms were compared with standard FAME retention times in external standard [27]. Fatty acid methyl esters (FAME) were prepared by transesterification of lipid extracts.

a. Lipids extraction

For breaking cells of the microalgae, adding glass beads to 5 ml of culture followed by addition of 4.5 ml of chloroform:methanol (2:1) into the cells with 30 μl 1.0 M HCl. The samples were incubated at 4℃ for 2 h with continuous shaking. Then add 1.0 ml of 0.9% NaCl to the samples with the vortex. Samples then centrifuged at 200 g for 2 min and transferred the lower chloroform phase into glass tubes. Dry samples were re-suspended in 100 μl chloroform/methanol (1:1).

b. Transmethylation of esterified fatty acids

Dry sample then added to 333 μl MeOH:Toluene (1:1) with 167 μl of 0.5 M sodium methoxide ($NaOCH_3$). Samples were incubated at room temperature for 20 min followed by addition of 500 μl 1 M NaCl with 50 μl 37% HCl with 1.5 ml hexane followed by vortex. Then samples were centrifuged at 200 g for 2 min then transferring the upper hexane phase into 2 ml Eppendorf tube then dried and re-suspended in 40 μl acetonitrile to GC procedure.

Estimation of biodiesel fuel properties based on fame profiles

The cetane number (CN), saponification value (SV), iodine value (IV), The degree of unsaturation (DU), long-chain saturated factor (LCSF) and cold filter plugging point (CFPP) were determined by experimental equations according to [28]. The cetane number of the mixture was estimated by the experimental equations proposed by [29]. The cetane number, saponification value, and iodine value were calculated in accordance to the three following equations:

$$CN = 46.3 + (5.458/SV) - (0.225 \times IV) \quad (1)$$

$$SV = \Sigma\ (560 \times N)/M \quad (2)$$

$$IV = \Sigma\ (254 \times D\ N)/M \quad (3)$$

D is the number of double bonds in the fatty ester, M is the molecular mass of the fatty ester, and N is the percentage of the particular fatty ester in the oil sample.

The degree of unsaturation (DU) is calculated using the amounts of the monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) present in the oil; thus:

$$DU = MUFA + (2 \times PUFA) \quad (4)$$

The long-chain saturated factor (LCSF) and the cold filter plugging point (CFPP) are estimated:

$$LCSF = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (1.5 \times C22) + (2 \times C24) \quad (5)$$

$$CFPP = (3.1417 \times LCSF) - 16.477 \quad (6)$$

Statistical analysis

The statistical analyses were carried out using SPSS 16.0. The Data obtained were analyzed statistically to determine the standard deviation for first mutation, and re-mutation. Linear regression was used to determine the relation between the UV doses and mutant and determine the degree of significance by one and two-tailed t-test.

Results and Discussion

Total lipids

The potent algae species that isolate was characterized by 20% of total lipids, dry biomass 0.9 g $\cdot l^{-1}$ and volumetric lipid productivity (Lp) was 18 (mg $\cdot l^{-1} \cdot day^{-1}$). This green alga was tentatively identified as *Chlorella vulgaris* belonging to *Chlorophyta*. The isolate was selected due to its higher oil content. Oil determination was performed at the stationary growth phase that outset after the eleventh day to the eighteenth day. That is result accord with the result [30, 31].

18S rRNA sequences and phylogenetic tree

18S rRNA Sequences of the isolate the base size of *Chlorella vulgaris* was 744 bp. The DNA sequences of this isolate showed 100% identity to *Chlorella vulgaris*.

Although, morphological examination by light microscopy revealed that the strain had consistent morphology to *Chlorella vulgaris*.

After sequencing the current rRNA gene sequences was registered at DNA database under accession number LC333291 and named *Chlorella vulgaris* Eukalg-MG. The result obtained was compared in the gene bank (NCBI).

The Sequences of accession number LC333291 and eight accession numbers from gene bank were aligned using the online program "Clustal Omega" and phylogeny tree was submitted to the MEGA 7 software Analyses of Phylogenetic for the 18S rRNA sequences observed in (Table 1), the relationship between The Sequences of accession number LC333291 and eight accession numbers from National Center for Biotechnology Information (NCBI) database, USA (http://www.ncbi.nlm.nih.gov).

The result showed that the present specimens were

Table 1. Nucleotide sequence statistics for 18s rDNA sequences of the wild strain and 18s rDNA sequences for eight accession numbers from gene bank.

Name	Length	Adenine (A)	Cytosine (C)	Guanine (G)	Thymine (T)	SNPs	Gaps	% for Wild Strain
Wild Strain	744 bp	180	153	219	192	-	-	-
KX094755	744 bp	181	153	218	192	1	0	99.87%
JX097060	744 bp	180	154	219	191	1	0	99.87%
LC037427	744 bp	180	153	219	192	4	0	99.46%
KT250599	744 bp	180	153	219	192	1	1	99.19%
X73992	744 bp	176	156	221	190	6	0	99.19%
X56105	744 bp	177	155	222	189	8	0	98.92%
AB006046	744 bp	180	150	222	192	18	0	97.58%
X63505	743 bp	176	152	224	189	30	2	95.29%

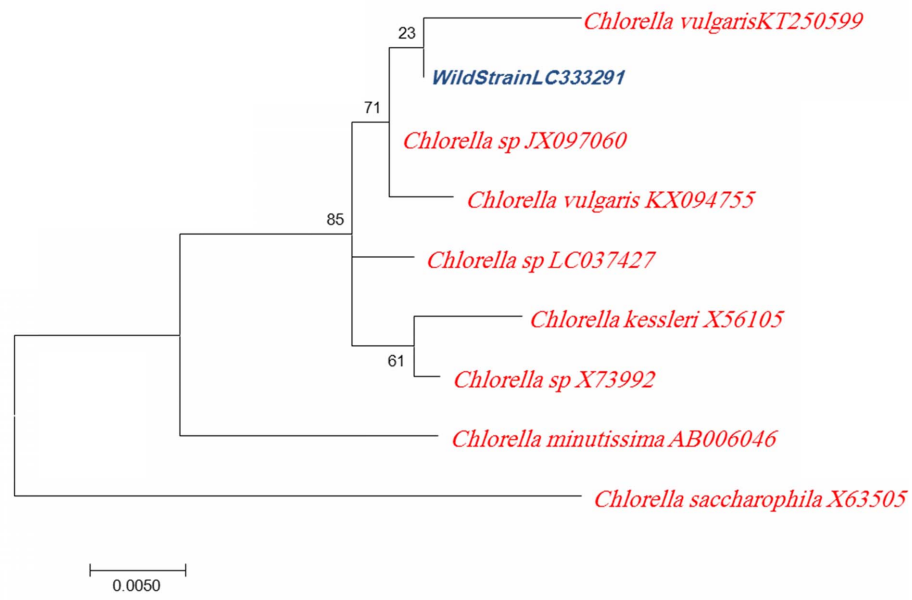

Fig. 1. The Relationship between The Sequences of accession number LC333291 and 8 Accession numbers from gene bank. The Phylogenetic tree was constructed by applying the algorithms maximum likelihood.

grouped in Phylum Chlorophyta (Fig. 1). The phylogenetic tree deducted from the sequence comparison of 18S rRNA region showed that the length of branch that represents an amount genetic change of 0.0050, and the 18S rRNA-based phylogeny tree included six clades. The clades grouping had low support (bootstrap value between 23–85%) and showed that the present specimen was placed in *Chlorella vulgaris* Sequence ID: LC333291 neighbor to *Chlorella vulgaris* Sequence ID: KX094755 that isolated from Germany isolation source is soil percentage of 99.87% and *Chlorella* sp. Sequence ID: - JX097060 that isolated from China percentage of 99.87% And showed Chlorella *vulgaris* Sequence ID: LC333291 distant on *Chlorella vulgaris* Sequence ID: KT250599 that isolated from Canada percentage of 99.19%, *Chlorella* sp Sequence ID: X73992 that isolated from Germany percentage of 99.19%, *Chlorella minutissima* Sequence ID: AB006046 that isolated from Japan percentage of 97.58%, *Chlorella saccharophila* Sequence ID: X63505 that isolated from Germany percentage of 95.29%, *Chlorella kessleri* Sequence ID: X56105 that isolated from Germany percentage of 98.92% and *Chlorella* sp Sequence ID: LC037427 that isolated from Japan percentage of 99.46%. That is mean that is the sequences of 18s rRNA common in Phylum Chlorophyta in the world in the different environment and under different conditions.

Radiation mutation by UV effecting on growth and oil content

Ultraviolet radiation is part of the electromagnetic spectrum, but with shorter, higher energy wavelengths than visible light. Prolonged exposure can be lethal to cells because when DNA absorbs UV radiation at 254 nm, the energy is used to form new covalent bonds between adjacent pyrimidines: cytosine-cytosine, cytosine-thymine, or thymine -thymine. Collectively, these are known as pyrimidine dimers, with thymine dimers being the most common. These dimers distort the DNA molecule and interfere with DNA replication and transcription [36].

Concerning radiation on algal biomass accumulation and oil productivity, radiation-induced mutation by UV-C (254 nm) for raising the production of biodiesel through increasing total lipid content and improving fatty acids and biomass dry cell weight as well. Here, UV radiation for 15 s increases totals lipids to reach 27% associated with about 1.0 $g \cdot l^{-1}$ of dry biomass. In addition, volumetric lipid productivity (Lp) was recorded as 27 ($mg \cdot l^{-1} \cdot d^{-1}$). By 30 s UV exposure, a total lipid

Table 2. The output of Radiation mutation.

Exposure time (s)	Total lipids (%)	Biomass (DCW g·l^{-1})	Lp (mg·l^{-1}·day^{-1})
Wild strain (0.0)	20	0.9	18
15 SA	27	1	27
30 SA	31	0.88	27.3
45 SA	32.4	1	32.4

std. deviation = 2.8, R = 0.963, Sig. (1-tailed = 0.003).
SA = Time per second in first exposure.

reached 31%, while a decrease in dry biomass was observed. As shown in (Table 2); the obtained biomass was found to be 0.88 g·l^{-1} and Lp was 27.3 mg·l^{-1}·d^{-1}, meaning that is almost a slight change in the total product with 15 s.

Results in the time 45 s showed an increase in total lipids to reach 32.4% with increasing of dry biomass to 1.0 g·l^{-1}. Also, Lp was raised to be 32.4 mg·l^{-1}·d^{-1} in comparison with the wild type strain that is containing total lipid of 20% and 0.9 g·l^{-1} of dry biomass with 18 mg·l^{-1}·d^{-1} of Lp. The wild type strain and UV mutants estimated total lipid many times and the results identical when decreasing total lipid in the wild type strain for any reason also decrease total lipid in UV mutants with keeping on total lipid ratio. The culture obtained for UV-C treatments during the logarithmic growth phase that beginning after the third day to the eleventh day for increase mutation rate because of increase division rate.

After Induced Radiation mutation by UV-C directly the treatment was put in strongly darksome hours lest cause photoreactivation repair. Was observed increase in mutation ratio with increasing radiation dose.

Effect of re-radiation mutation by UV-C

The best mutant resulted in 45 s (compared with the wild type strain) was re-exposed to UV radiation by the same aforementioned mutation technique. The results showed that the time 15 s increase total lipids to 34% compared with mutant 45SA that contain total lipid of 32.4%. By 30 s decreasing of total lipids to 26% was observed and UV-C radiation for 45 s decreases total lipids to 22% (Table 3). In the time 1.0 min, no change in total lipid compared with the time 45 s. That is mean that the percentage of total lipids was increased to the maximum extent possible to the cell then reveres mutation piecemeal when exposed to long dose from UV-C radiation was found.

Table 3. Re-radiation mutation of the mutant 45 s.

Exposure time (s)	15	30	45	60
Total lipids (%)	34	26	22	22

std.deviation = 5.66, R = 0.913, Sig. (1-tailed = 0.003).

Fatty acids profile for wild strain and mutant 45 s

As Shown in Table 3; the percentage of Caprylic acid C8:0 almost is equal between the wild type strain that contains 1.53% and the mutant 45SA is including 1.47% from C8:0. Pelargonic acid (C9:0) was observed in the wild type strain (3.91%); while disappeared in mutant 45 s. The wild type strain presented the highest percentage of behenic acid (20.16%) in comparison with mutant 45SA which contain from C22:0 (1.43%). And a remarkable not found significant change in the percentage of Lignoceric acid C24:0 among the wild type strain and mutant 45SA. The amounts of Palmitoleic acid (C16:1) in the mutant 45SA percentage was 1.39% was higher than those in the wild type strain (0.65%). An increase in Erucic acid (C22:1) was observed from 5.03% in wild type strain to be 9.32% in mutant 45SA. Mutant 45 s processes a high level of MUFA, where Nervonic acid C24:1 resulted in 53.37% and the wild type one content was 27.68%. Lower content of Linoleic acid (C18:2) in mutant 45SA was observed (3.14%) compared with 3.76% of wild type strain. With an absence of Docosadienoic acid C22:2 in wild type strain, the presence in the mutant was found (8.27%). Data was found in accordance with the result [30–33] concerning fatty acids include medium-chain (C10–C14), long-chain (C16–18) and very-long-chain (>C20) species and fatty acid derivatives.

As shown in Table 4; C8:0, C16:1, C18:2, C22:0, C22:1, C24:0 and C24:1 were the predominant fatty acid in the wild type strain and mutant 45 s lipid extracts. The percentage of saturated fatty acids in total fatty acids in wild type strain was 28.89% and mutant 45 s was 6.73%, The percentage of Monounsaturated fatty acids in total fatty acids in wild type strain was 33.36% and mutant 45 s was 64.08% and the percentage of polyunsaturated fatty acids in total fatty acids in wild type strain was 3.76% and mutant 45 s was 11.41%.

The FAME results the best prove on UV irradiation resulting convert in some fatty acids from wild type

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Table 4. Fatty acids profile percentage in wild strain and Mutant one after 45 s exposure to Ultraviolet.

Fatty acids	Name	Wild Strain FAs%	The Mutant 45SA FAs%
SFAs			
C8:0	Caprylic	1.53	1.47
C9:0	Pelargonic	3.91	ND
C22:0	Behenic	20.16	1.43
C24:0	Lignoceric	3.29	3.83
Total SFAs		28.89	6.73
MUFAs			
C16:1	Palmitoleic	0.65	1.39
C22:1	Erucic	5.03	9.32
C24:1	Nervonic	27.68	53.37
Total MUFAs		33.36	64.08
PUFAs			
C18:2	Linoleic	3.76	3.14
C22:2	Docosadienoic	ND	8.27
Total PUFAs		3.76	11.41
Total FAs		66.01	82.22

ND = None Detected.
R = .998, Sig. (2-tailed = 0.002).

strain to obtain other species from the green alga *Chlorella vulgaris* (45SA).

Estimation of biodiesel fuel properties based on FAME profiles

This appreciation has allowed enough specification characteristics. Cetane Number concludes for identity to the properties specifications. The estimated Cetane Number for the wild type strain showed CN 80, while the mutant one possessed 71.3 (Table 5). Saponification values were estimated for the wild type strain and mutant as 117.45 and 133.89, respectively. The Iodine values were appraised for the wild type strain and mutant as 56.68 and 69.82 by the same respect. Cold filter plugging point obtained for the wild type strain was CFPP -13.3 and the mutant one showed CFPP of -11.93.

The wild strain and the best mutant one will generate biodiesel of higher DU, higher CN, lower IV and higher oxidation stability.

The mutation results indicated that happening of many effects in the genes and pathways which in turn lead to more beneficial in dry cell weight and synthesis of total lipid and fatty acids components and Properties of biodiesel this is the target from this research.

Discussion

The microalgae simple identify extremely hard because of the size is small molecular techniques can be allowing an evaluation of the legitimacy of the morphological species idea for general microalgae [34]. Although, a lot of studies have observed that the 18S rRNA is in many cases also conserved to differentiate between closely linked genera and species [35].

Algae synthesize fatty acids as blocks to form different types of lipids. Fatty acids are synthesized by chain lengths ranging from C16 to C18 are similar to those of higher plants [36]. Monounsaturated and Polyunsaturated are the tasks of stabilizing biodiesel oxidation [37].

Erucic acid can use in biodiesel fuel [38]. Those strains contain oils with higher percentages of MUFA; generate biodiesel [37]. Linoleic acid discovered in the lipids of cell membranes [39]. In additions, PUFAs have been reported to be part of cell membrane components and to play a significant role in membrane development and cellular repair in microalgae [40]. In general, the longer the fatty acids chain, the higher the Cetane Number, combustion temperature and viscosity, so the preferable fatty acids for biodiesel fuel are long chain fatty acids (C16–18) [28–41]. Cetane number refers to the time delay in fuel combustion, for diesel cycle engines. The higher the Cetane Number, the shorter the ignition time. Increases of Cetane Number with unbranched length carbon chain FAME components [42]. The IV indicates to the tendency of biodiesel to interact with

Table 5. The estimated characteristics of biodiesel from micro-algae oils.

Micro Algae	CN	SVmg KOH g^{-1}	IVgI_2 100 g^{-1}	DU (Wt %)	LCSF (Wt %)	CFPP (°C)
Wild Strain	80	117.454	56.68	40.88	1.02	-13.3
Mutant 45SA	71.3	133.89	69.82	86.9	1.45	-11.93

DU = degree of unsaturation; CN = cetane number; SV = saponification value; IV = iodine value; LCFS = long-chain saturated factor; CFPP = cold filter plugging point.

oxygen at near ambient temperature. This property depends on the number and position of the double bonds in the carbon chains of alkyl esters. The higher the IV (the mass of iodine, in grams, that is exhaust by 100 g of a chemical substance), the higher the possibility of oxidation, deposits formation and deterioration of the biodiesel lubricity. The maximum IV accepted in Europe is 120 g I_2/100 g [29]. The CFPP are commonly used to predict the performance of biodiesel flow at low temperatures [42]. At low temperatures, crystallization of the FAME molecules grows and aggregates, blockage of fuel lines and filters. The larger the carbon chains or the higher the saturation of FAME molecules that make up biodiesel, the higher value and the worse the temperature properties [43].

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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