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Original Article

Effect of light intensity on carbohydrates, lipids contents, and bioethanol production in two algal species of *Coelastrella saipanensis* and *Oscillatoria duplisecta*

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Abstract: This study aimed to examine the feasibility of two algal species of *Coelastrella saipanensis* (Chlorophyceae) and *Oscillatoria duplisecta* (Cyanophyceae) to produce bioethanol production at different light intensities. In the present study, light-intensity treatments at 27, 36, and 67 µmol m^{-2} s^{-1} were used to stimulate bioethanol production from microalga. The effects of these treatments on *C. saipanensis* and *O. duplisecta* were investigated on their growth, carbohydrate and lipids contents. The results showed that the stationary phase of *C. saipanensis* started on the sixth day under light intensities of 27 and 36 µmol m^{-2} s^{-1} and on the eighth day under light intensity of 67 µmol m^{-2} s^{-1}. The stationary stage of blue-green algae *O. duplisecta* started on day eight, sixth, and seventh under light intensities of 27, 36, and 67 µmol m^{-2} s^{-}1, respectively. The highest amount of carbohydrate content was 0.182, and 0.310 mg/l for *C. saipanensis* and *O. duplisecta* under light intensity of 36 µmol m^{-2} s^{-1}. The highest amount of lipid was 0.95 g/l for *C. saipanensis* under a light intensity of 36 µmol m^{-2} s^{-1}, while 0.74 g/L was the highest amount of lipid for *O. duplisecta* under 67 µmol m^{-2} s^{-1} at a light intensity of 36 µmol m^{-2} s^{-1}. The highest percentage of bioethanol in *C. saipanensis* and *O. duplisecta* were 11.35 and 10.23%, respectively. The 18S rRNA and 16S rRNA genes were used for the identification, and the sequences of algae matched those registered in the GenBank (MT375484.1 for *C. saipanensis* and MW405018.1 for O. *duplisecta*). The phylogenetic tree of the ITS area was analyzed inside the 18S rRNA and 16S rRNA and the sequences showed a strong resemblance to those species registered in the Genebank.

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Introduction

The world is facing a serious problem represented by the high consumption of all kinds of energy, and this is due to the steady increase in human population as well as the development of civilization which depends heavily on civilian life. This prompted scientists to search for alternative sources of energy that are low-cost and usable in several areas (Sims et al., 2010). From this purpose, attempts emerged to use algae as a successful alternative to biofuel production. It has met with acceptable success because algae have great potential for biofuel production being a diverse group of organisms that are rich in lipids, making them a valuable raw material for biofuels. They can provide a range of solutions to energy requirements in several ways (Skrede et al., 2011). It also offers some of the best potential for sustainable supplies of renewable biofuels that come from the energy of sunlight entering the atmosphere that is used through the photosynthesis process that occurs in plants and algae (Saifuddin and Priatharsini, 2016).

Algae are efficient at using CO_2 from the atmosphere and are responsible for more than 50% of the total global carbon fixation (Feng et al., 2011). Algae are grown commercially to produce several different materials, including nutritional supplements and biochemicals. In addition, algae biomass can provide a wide range of products such as bioenergy, biofuel, and Bio-based products such as bio-plastics, bio-cosmetics, and bio-solvents (Ometto, 2014). Algal fuel or algal biofuel is one of their products and an alternative to liquid fossil fuel (Darzins et al., 2010). The mechanisms of photosynthesis in algae are similar to those in higher plants that are often more efficient

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at converting the sun energy into beneficial biochemical products such as lipids because of their simple cellular structure making cells grow in different aqueous solutions, and their high efficiency in accessing water, carbon dioxide and other nutrients. Moreover, many algae strains *in vitro* can produce more than 50% of their mass from lipids, and sometimes up to 80% (Metting, 1996). For these reasons, algae can produce a much higher amount of lipids per unit area of land than many terrestrial oilseed crops such as soybean and coconut. Therefore, algae is a promising new source of raw materials for biofuel production (Mata et al., 2010). It is expected that the use of algae to produce biofuels will play an important role in securing an alternative source of energy soon, especially in the depleted fossil fuel reserves and their environmental impacts (Pereira, 2017).

It requires studies regarding the growth rates of different algal species and their response to different environmental conditions, such as physicochemical for evaluation of their effect on the chemical composition of algal cells and the extent to which the living components that make up these cells are affected, especially fatty acids and total lipids, proteins, and other carbohydrates (Nzayisenga et al., 2018). The biochemical composition of microalgae is affected by the change in the environmental factors of the medium in which it lives, as there are many stimulating physical factors such as light, which is a limiting environmental factor for growth. Light's importance is due to its regulation and stimulation effects on plants and algal growth (Barsanti and Gualtieri, 2006). Light is an essential factor in the photosynthesis process for all photosynthetic organisms which take it from the sun with water and nutrients from their environment to synthesize and store organic matter (Chen et al., 2009).

The response to changes in light intensity is due to the algae that can initiate the process of Photo acclimation or Photo adaptation. In the process of photo accumulation, the level of synthesis and decomposition of the complex products in the photosynthesis mechanism is controlled and under sub-saturating light intensity. The total light-quotient complexes in the algae are stimulated, with an increased ability to determine it in pigments such as chlorophyll a and proteins of the photosynthesis reaction center such as D1 and D2 (Hegemann, 2008). Autotrophic organisms, including microalgae, need light which is the main energy source, and both light quality and intensity affect cell growth and biomass composition (Bialevich et al., 2022).

Several studies have indicated the possibility of producing biofuels from algae e.g. Minyuk et al. (2017) indicated that *Coelastrella* can produce amounts of lipids, which is a promising indicator of biotechnology. Metsoviti et al. (2019) in a study of the effect of solar irradiance on *Chlorella vulgaris* cultivated in open bioreactors under greenhouse conditions, showed that the light intensity and wavelength strongly affected the growth of *C. vulgaris*, higher lipid content (up to 22.2%) and its biomass nutrient composition. Nzayisenga et al. (2020) indicated the effects of light intensity on lipid production in *Desmodesmus* sp. and *Scenedesmus obliquus* that are grown in wastewater, and their results showed the importance of optimizing light intensities to improve lipid production by algae and their quality as sources of biodiesel. Bialevich et al. (2022) studied the effects of different light intensities and light spectra on the growth of three algal species, *Chlamydomonas reinhardtii*, *D. quadricauda*, and *Parachlorella kessleri*, showing increasing the light intensity can increase in maximum cell size and division into more daughter cells and improved the growth rates of all studied species. Therefore, this study aimed to isolate and purify *Coelastrella saipanensis* and *Oscillatoria duplisecta* from local habitats in Iraq, examine their ability for ethanol production under different light intensities, and their taxonomic statute using molecular phylogenetic and classical taxonomy of morphological characters.

Materials and Methods

Culture medium preparation and sterilization: Chu10 medium (Chu, 1942) modified by Kassim et al. (1999) was used to grow green *C. saipanensis*. The

medium BG-11 (Rippka et al., 1979) modified by Andersen (2005) was used for the culture and propagation of Cyanophyta *O. duplisecta*. The media were prepared from the stock solution and kept in the fridge at 4℃ without sterilizing until use.

Sampling of algae, isolation, and purification: The green algae of *C. saipanensis* samples were collected from open wastewater from the Al-Jameaa quarter in Diwaniyah City, Iraq, and Cyanophyta was isolated from the soil in Hayy Al-Hakim in the same city. Algae samples were isolated using the serial dilution method and streaking on plate agar techniques (Patterson, 1983; Mohammady et al., 2014). To obtain a pure axenic culture for *C. saipanensis* and *O. duplisecta*, the density gradient centrifugation method was used (Wiedeman et al., 1964). To make sure the culture of algae is empty of bacteria and fungi, the method of Anderson (2005) was used.

Morphological identification of algae: The morphological identification of *Coelastrella saipanensis* and *Oscillatoria duplisecta* was performed using a compound optical microscope using the taxonomic key (Martins et al., 2010; Buch et al., 2017; Guiry and Guiry, 2020).

Molecular study

Genetic diagnosis of algae: To confirm morphological identification, 18S rRNA and 16S rRNA gene-based genetic identification were used. The genes were used to determine the final diagnosis for green algae *C. saipanensis* and blue-green algae *O. duplisecta* using 18S rRNA and 16S rRNA, respectively, and comparing them with the GenBank database (NCBI), the primers were obtained from Geneaid Company (USA).

DNA extraction and sequencing: DNA was isolated using pre-equipped protocol steps Geneaid Company, and this kit provides an easy and quick way to obtain pure DNA from algae species. DNA was extracted from microalgae using the Genomic DNA extraction kit (Bacteria) from Geneaid Biotech Ltd., and the extraction was carried out according to the company's instructions. Samples from algae *C. saipanensis* and *O. duplisecta* are disrupted by both grinding in lysis buffer incubation and liquid nitrogen. The cell wall was disrupted with liquid nitrogen, before using genomic DNA in the polymerase chain reaction (PCR), which was stored at -20℃.

Two primers were used to conduct the PCR, the first primer was for the 18S rRNA gene (Moro et al., 2009) viz. forward primer (5′-TGG CCT ATC TTG TTG GT CTG T-3′) and reverse primer (5′-GAA TCA ACC TGA CAA GGC AAC-3) for the green algae identification. To amplify the partial 16S rRNA gene, two sets of cyanobacteria-specific primers, including forward (5′-CGG ACG GGT GAG TAA CGC GTGA-3′), and reverse (5′-GAC TAC TGG GGT ATC TAA TCC CATT-3′) primers were used (Nübel et al., 1997).

DNA profile: To confirm the concentration and purity of isolated DNA, a nanodrop spectrophotometer (Thermo Fisher / USA) was used. DNA is detected by determining the DNA conc. (ng / µl) and measure its purity by reading the absorbance at wavelengths of 260 and 280 nm. The extracted DNA is considered pure when the absorbance ratio is 2.1-1.8 ng / µl.

***DNA amplification program*:** The program of PCR reaction for gene amplifying ITS2 rRNA was as follows: 5 min at 95°C, followed by a cycle of 95°C (30s), 59°C (45s), and 72°C (90s) and a final extension for 10 min at 72°C were applied using a thermal cycler (Biorad, USA).

Gel electrophoresis: Agarose gel electrophoresis was carried out according to Russel (2001). The PCR products were electrophoresed on 1% agarose gel prepared in 1X Tris-acetate-EDTA buffer. Then, the electrophoresis gels were stained with ethidium bromide and visualized by the Scie-Plas gel documentation, UK. The gel containing the PCR product was checked using a UV source after the migration process was completed, and then photographed by a digital camera.

DNA sequencing: PCR products were sent for Sanger sequencing of 18S rRNA and 16S rRNA genes which was performed by the National Instrumentation Center for Environmental Management (NICEM), biotechnology lab, using DNA sequencer 3730XL (Applied Biosystem). Using Basic Local Alignment Search Tool (BLAST) program which is available at

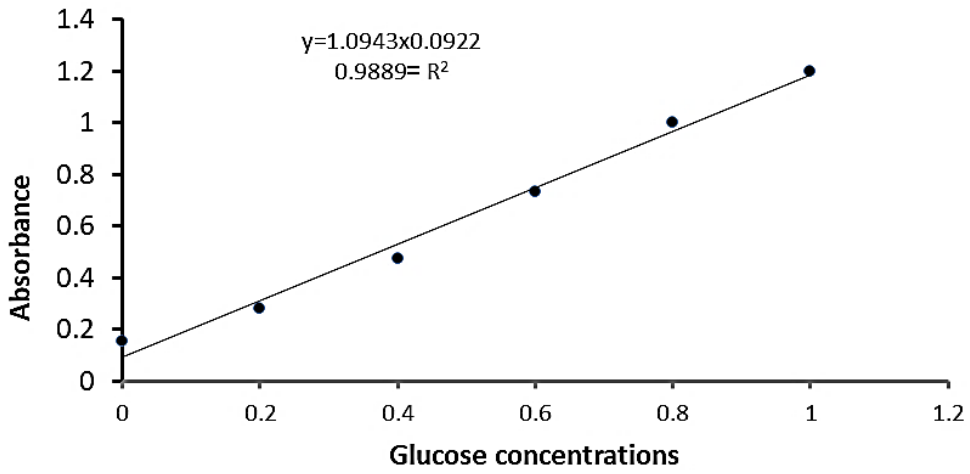

Figure 1. Standard curve of glucose sugar.

the National Center Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), the homology search was conducted. For the construction of the molecular phylogenetic tree, the Molecular Evolutionary Genetics Analysis program (MEGA 10.1) was used to reveal the relationship between the under-study algae and other species retrieved from NCBI.

Algal cultivation and biomass production: Algae are cultured for biomass production through batch culture. 10 ml of pure algal isolates were taken, and added to culture media in a 100 ml conical flask and then incubated in the incubator. Then the growths were transferred to a flask containing 1000 ml medium and incubated for 14 days. Finally, the growths were transferred to 5-liter glass for mass culture with ventilation and lighting system 8: 16h., light: dark, and with different light intensities of 27, 36, and 67 µmol m^{-2} s^{-1} to select the optimal light intensity for growth at a temperature 25±2℃ (Kawaguchi, 1980; Tredici, 2004). The cultures were shaken and changed at least twice a day to reduce the lighting difference and to prevent the accumulation of algae on the walls of the incubators.

Growth curve: The growth of alga was estimated using the standard methods (Tam and Wong, 1989) using the spectrophotometer (UV-Visible Apple, Japan) measurement at a wavelength of 540 and 750 nm for *C. saipanensis* and *O. duplisecta*, respectively, daily for 18 days in all treatments to identify the density of the studied algae. The readings were done daily to follow the growth curve**.**

Research experiments

Effect of light: The algae under study were grown under three light intensities of 27, 36, and 6)µmol m^{-2} s^{-1} to choose the best lighting. Light intensity was measured by Lux meter. The light system setup was 8:16 Light: Dark. After experiments, measuring the amount of carbohydrates, lipids, and bioethanol was done based on Jiminez et al. (2003).

Determination of chemical components of algae

Total carbohydrate: The total carbohydrate content in the filtrate residue was estimated after the centrifugation according to Dubois et al. (1956). The optical density was measured using a spectrophotometer and the standard curve for glucose (Fig. 1).

Total lipids content: The lipid content of algae was determined by the method of Bligh and Dyer (1959), where the extraction process was carried out using polar solvent methanol and non-polar chloroform. The lipid was extracted from the algae by adding 5 and 10 ml of chloroform and methanol to 8 ml of algae (the ratio of chloroform/methanol/algae was 1: 8: 2: 2). They mixed for 4 minutes with a rotary mixer and then adding 5 ml of chloroform again. Afterward, the solution was transferred to the centrifuge tubes, and the supernatant was discarded and centrifuged at 1000 rpm for 7 min, and the process was repeated 5 times. Three layers were observed, the lower layer was

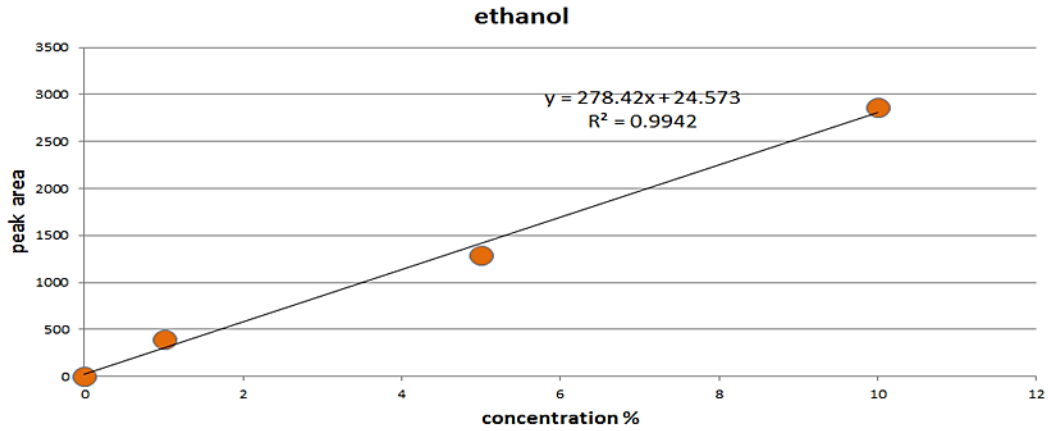

Figure 2. Standard curve of bioethanol analysis.

chloroform with lipid, which was transferred to a vacuum desiccator to evaporate the chloroform. The algal samples were dried, and the lipids were weighted and calculated using the following formula:

Total lipid =weight of lipid in aliquot ×volume of chloroform layer/ volume of aliquot.

Estimation of bioethanol production:

Hydrolysis process: 50 ml of each algae treatment was taken and placed in 500 ml glass flasks on a heating plate at a temperature of 100℃ for 2-4 hours. Then it cooled to 45±2℃ (Zhang and Feng, 2010). The α-amylase enzyme was added to each flask at a concentration of 0.06 g/50 mL and the flasks were incubated for 80 minutes at room temperature. Afterward, it was filtered using filter paper, the supernatant was taken and centrifuged at 9000 rpm for 15 minutes (Sulfahri et al., 2011a).

***Process of fermentation*:** 2.5 ml of Saccharomyces cerevisiae was added at a concentration of 10% (Light density = 0.5 at a wavelength of 600 nm) into the bottle fermenter of 100 ml containing 50 ml of substrate algae sample. Then it was incubated at room temperature 30±2℃ for 120 hrs and ethanol levels were observed. If ethanol levels showed an increasing trend, the fermentation process was continued until stopping the fermentation process when the levels of ethanol started to be reduced (Zhang and Feng, 2010).

***Bioethanol analysis process*:** The samples resulting from the fermentation process were filtered using a Millipore filter of 0.45 µm. Then, concentrations were determined by High-Performance Liquid Chromatography (HPLC) (Chen et al., 2014) by

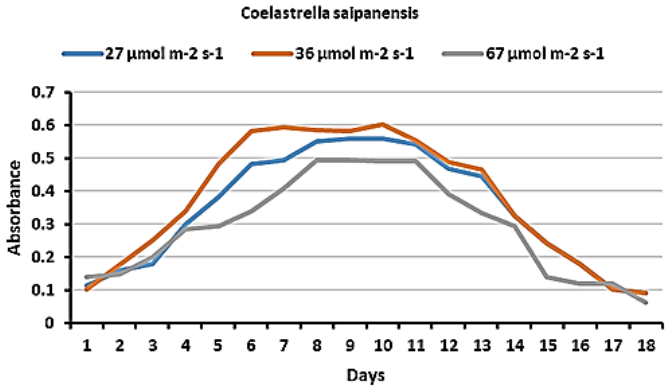

Figure 3. Growth curve of *Coelastrella saipanensis* under different light intensities.

determining the height of the curves and their relative area and comparing them with standard curves (Fig. 2).

Data analysis: The results were analyzed by SPSS software (version 25) using the One-Way ANOVA table, and the least significant difference (LSD) to compare the significant difference between means at the level of $P \leq 0.05$.

Results and Discussions

Algal used in the current study: The green algae *C. saipanensis* and cyanophyceae *O. duplisecta* were isolated successfully.

Effect of different light intensities on algal growth rate: According to the results of the present study, the two types of algae responded differently to light intensity during their cultivation and growth. The results showed that the stationary phase of *C. saipanensis* started on the sixth day under a light intensity of 27.36 µmolm^{-2} s^{-1}, and on the eighth day

Table 1. The effect of light intensity on carbohydrates, total lipids), and bioethanol for studied algae.

Algae	Light intensity (µmol m^{-2} s^{-1})	Biochemical Content		
		Carbohydrates (mg/l) Mean±SE	Total lipids (g/l) Mean±SE	Bioethanol% Mean±SE
C. saipanensis	27	0.140±0.04	0.44±0.041	3.64±0.351
	36	0.182±0.05	0.95±0.02	11.35±1.21
	67	0.168±0.05	0.61±0.04	5.62±0.42
O. duplisecta	27	0.180±0.02	0.74±0.041	1.11±0.02
	36	0.310±0.034	0.156±0.004	10.23±1.21
	67	0.225±0.004	0.79±0.04	4.59±0.35
LSD		0.039	0.065	1.33

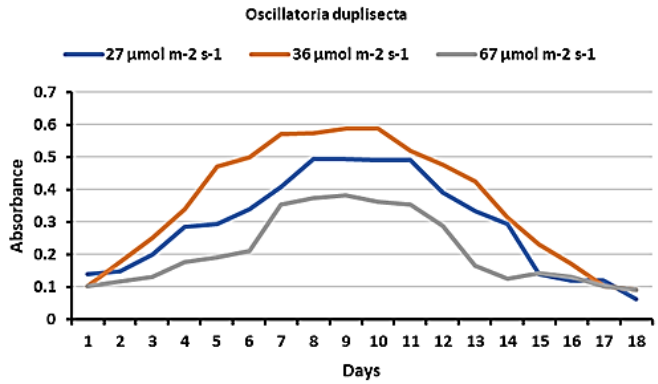

Figure 4. Growth curve of *Oscillatoria duplisecta* under different light intensities.

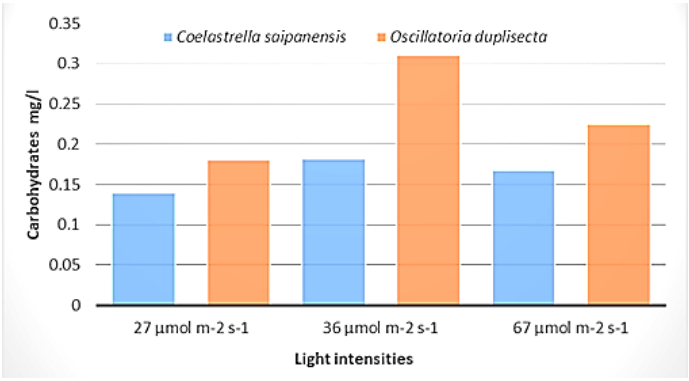

Figure 5. The effect of light intensity(µmol$m^{-2}s^{-1}$) on Carbohydrates (mg/l) for studied algae.

under a light intensity of 67 µmol m^{-2} s^{-1} (Fig. 3). While the stationary stage for blue-green algae of *O. duplisecta* started on eighth day under light intensity of 27 µmol m^{-2} s^{-1} and on day seven under light intensity of 36 and 67 µmol m^{-2} s^{-1}(Fig. 4). Based on the results (Figs. 3, 4) of the growth rate at different light intensities, the significant results of the 36 µmol m^{-2} s^{-1} lightening had the highest growth rate of 0.601 and 0.589 for *C. saipanensis* and *O. duplisecta*, respectively, compared to the other two intensities of 27 and 67 µmol m^{-2} s^{-1}. The lighting intensities of 67 µmol m^{-2} s^{-1} showed the lowest growth rate in the two algae compared to the intensities of 27 and 36 µmol m^{-2} s^{-1}, respectively. The reason for the decreased growth rate at higher light intensities is that excessive exposure to light can severely damage the mechanism of algal photosynthesis. Light is one of the factors that have a significant impact on microalgae growth affecting the growth and biochemical composition of microalgae (Zhou and Guo, 2020). our results agreed with the findings of Bialevich et al. (2022) on the effects of different light intensities and light spectra on the growth of three green algae *C. reinhardtii*, *D. quadricauda*, and *P. kessleri*. They also found the growth rate is limited at very low light intensities below the saturation point and the growth rate increases with light intensity up to a certain level but very high light intensities lead to photoinhibition and do not improve their growth. Similarly, Minhas et al. (2019) indicated that low light intensities have better the growth rate and production of biomass with the increment of light intensity showed a decrease in chlorophyll-a synthesis.

Effects of light intensities on the carbohydrate content: The results showed the highest carbohydrate (0.182 and 0.310 mg/l) in *C. saipanensis*, and *O. duplisecta,* respectively, at light intensity of 36 µmol m^{-2} s^{-1}. The lowest amount of carbohydrate (0.140 and 0.180 mg/l) was recorded under 27 µmol$m^{-2}s^{-1}$ light intensity (Fig. 5). There were significant differences between treatments. The increase in the content of carbohydrates in algal cells may be due to environmental conditions such as ventilation and

lighting, which contribute to the availability and abundance of carbon for the photosynthesis process (Sandens et al., 2005). Choixa et al. (2012) pointed out that the net accumulation of carbohydrates and starch is affected by several physical and chemical factors such as light-intensity, growth conditions. Adri et al. (2003) found that the second photosystem is responsible for the water molecule splits under high-intensity light effects.

The results of the present work revealed significant differences in carbohydrate content at light intensity of 36 and 67 µmol m^{-2} s^{-1} for *C. saipanensis* and 36 µmol m^{-2} s^{-1} for *O. duplisecta* (Table 1). The results of the current study agreed with the findings of Mardan (2020) in his study on four types of algae, including two Chlorophyceae (*Chlorella sorokiniana* and *Tetradesmus obliquus*) and two cyanophyceae (*Ancylothrix terrestris* and *Koinonema pervagatum*) which recorded the highest carbohydrate content when the light intensity increased.

Effect of light intensity on total lipid content: Microalgal biomass is mostly composed of lipids, carbohydrates, and proteins (Choudhary et al., 2015). The results revealed that the highest lipid was found as 0.95 g/L in *C. saipanensis* under a light intensity of 36 µmol m^{2} s^{-1} and the lowest was 0.44 under a light intensity of 27 µmol m^{-2} s^{-1}; and 0.74 g/L was the highest lipid content in *O. duplisecta* under 67 µmol m^{-2} s^{-1} of light intensity, while the lowest content was 0.156 g/L under light intensity of 27 µmol m^{2} s^{-1} (Fig. 6). The results revealed significant differences at 27 µmol m^{-2} s^{-1} of light intensity for both algal species in the current study (Table 1). The quality and quantity of fatty acid content vary as well as the entire biochemical composition in different environmental factors (Juneja et al., 2013).

The reason for the increase in lipid content may be due to light stimulating the process of lipid production through the effect of lighting on the enzymes responsible for producing these lipids (Yu Wang et al., 2007). An increase in the percentage of lipids in algal cells is directly proportional to the provision of optimum light density and the provision of nutrients that increase the accumulation of lipids in algal cells

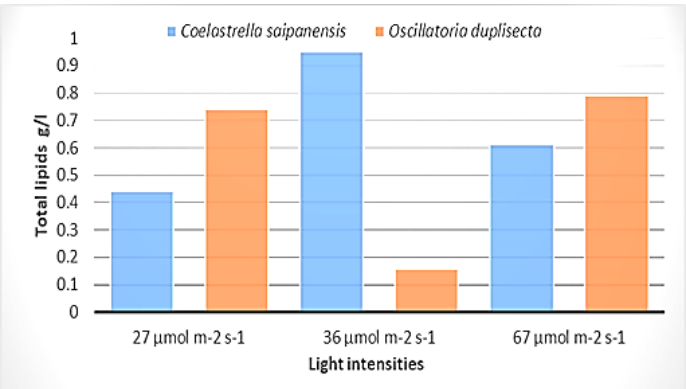

Figure 6. The effect of light intensity (µmol m^{-2} s^{-1}) on lipid (g/l) for studied algae.

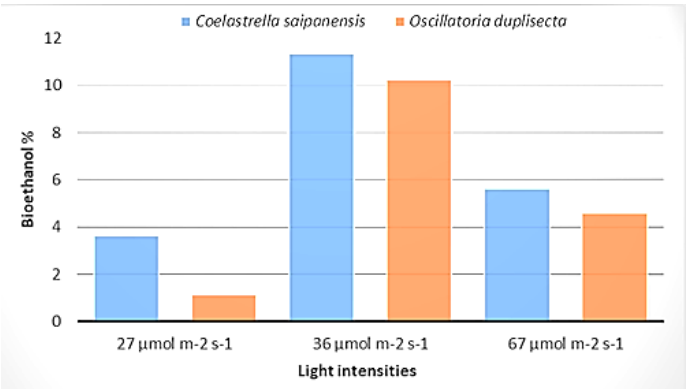

Figure 7. The effect of light intensity (µmol m-2 s-1) on bioethanol % for studied algae.

(Huang et al., 2016). The results of the current study are in agreement with the findings of Metsoviti et al. (2019) in the study of light intensity and quality on the growth rate and composition of *C. vulgaris* which indicated that an increase in light intensity at a certain level with red and white LED resulted in faster growth rates and higher lipid content.

Effects of light intensities on bioethanol production: The highest amount of bioethanol in *C. saipanensis* and *O. duplisecta* were recorded as 11.35 and 10.2%, respectively under light intensity of 36 µmol m^{2} s^{-1}. The lowest bioethanol production in *C. saipanensis* and *O. duplisecta* were 3.64 and 1.11%, respectively, in light intensity 27 of µmol m^{-2} s^{-1}. (Fig. 7). The results also revealed significant differences at 36 µmol m^{2} s^{-1} of light intensity on algal species' bioethanol production in the current study (Table 1). Similar findings were found in the study of Gupta et al. (2012) which reported a higher ethanol (85 ml) production by *Saccharomyces*

Table 2. Sequence homology between the green algae *Coelastrella saipanensis* isolated under study and algae found on the NCBI website.

Description	Scientific name	Max score	Query cover	Per. ident	Accession
Coelastrella saipanensis isolate CUKAN01 small subunit ribosomal RNA gene	*C. saipanensis*	813	100%	100	MT375484.1
C. astroideum strain UPMC-A0060 small subunit ribosomal RNA gene	*C. astroideum*	813	100%	100	MK834569.1
Tetradesmus acuminatus isolate Ta.TH03 small subunit ribosomal RNA gene	*T. acuminatus*	813	100%	100	MN886519.1
Scenedesmus sp. MF2 small subunit ribosomal RNA gene	*Scenedesmus* sp. MF2	813	100%	100	MN850521.1
Coelastrella sp. LQQ-1 small subunit ribosomal RNA gene, partial sequence	*Coelastrella* sp. LQQ-1	813	100%	100	MN688878.1
Scenedesmus sp. SWJ-2019 isolate Chil-gok small subunit ribosomal RNA gene	*Scenedesmus* sp. SWJ-2019	813	100%	100	MN604371.1
Asterarcys quadricellulare isolate FACHB-2316 18S ribosomal RNA gene	*A. quadricellulare*	813	100%	100	MH176109.1
Coelastrella tenuitheca isolate FACHB-2314 18S ribosomal RNA gene, partial sequence	*C. tenuitheca*	813	100%	100	MH176108.1
C. tenuitheca isolate FACHB-2313 18S ribosomal RNA gene	*C. tenuitheca*	813	100%	100	MH176107.1
C. tenuitheca isolate FACHB-2315 18S ribosomal RNA gene, partial sequence	*C. tenuitheca*	813	100%	100	MH176106.1
Coelastrella yingshanensis isolate FACHB-2311 18S ribosomal RNA gene	*Coelastrella yingshanensis*	813	100%	100	MH176103.1
C. thermophila var. globulina isolate FACHB-2308 18S ribosomal RNA gene	*C. thermophila* var. *globulina*	813	100%	100	MH176102.1
Coelastrella thermophila isolate FACHB-2305 18S ribosomal RNA gene	*C. thermophila*	813	100%	100	MH176100.1
C. thermophila var. globulina isolate FACHB-2309 18S ribosomal RNA gene	*C. thermophila* var. *globulina*	813	100%	100	MH176099.1

cerevisiae from extracted carbohydrate of *Chaetomorpha* sp. in the fermentation process. The current study agrees with the results of Bojorquez et al. (2016) that showed ethanol has been produced by microalgae *Dunaliella tertiolectafrom* extracted carbohydrate from hydrolyzed biomass fermented by *S. cerevisiae*. Similar to our findings, the results of Nzayisenga et al. (2020) showed the importance of optimizing light intensities to improve fatty acid production by microalgae and their quality as sources of biodiesel.

Genetic diagnosis and phylogenetic tree: Two types of algae viz. *C. saipanensis* and *O. duplisecta* were identified based on taxonomic keys (Prescott, 1973; Guiry and Guiry, 2019). These diagnosis were confirmed using the DNA sequencing of their 18S rRNA and 16S rRNA genes. Comparing the sequence of green algae *C. saipanensis* obtained in the present study with the same algae recorded in NCBI, showed an exact match (100%) between the isolated samples of *Coelastrella* registered in Genebank under accession number MT375484.1 (Table 2). The results confirmed that the green algae belong to the species *Saipanensis*. This study was in agreement with several studies that were adopted using the 18S rRNA gene for algae identification (Ancona-Canche, 2017; Abed, 2018).

The green algae *O. duplisecta* in the present study

Table 3. Sequence homology between the green alga *Oscillatoria duplisecta* isolated under study and algae found on the NCBI website.

Description	Scientific Name	Max Score	Query Cover	Per. Ident	Accession
Oscillatoria duplisecta PMC 1203.20 16S ribosomal RNA gene	*O. duplisecta* PMC 1203.20	1046	100%	98.81	MW405018.1
O. duplisecta PMC 1218.20 16S ribosomal RNA gene, partial sequence	*O. duplisecta* PMC 1218.20	1040	100%	98.64	MW405017.1
O. duplisecta PMC 1209.20 16S ribosomal RNA gene	*O. duplisecta* PMC 1209.20	1040	100%	98.64	MW405016.1
O. duplisecta PMC 1205.20 16S ribosomal RNA gene	*Oscillatoria duplisecta* PMC 1205.20	1040	100%	98.64	MW405015.1
O. duplisecta ETS-06 partial 16S rRNA gene, type strain ETS-06T	*Oscillatoria duplisecta* ETS-06	1029	100%	98.3	AM398647.1
Oscillatoria kawamurae gene for 16S ribosomal RNA, partial sequence, strain: Inba3	*Oscillatoria kawamurae*	1024	100%	98.13	AB741575.1
O. kawamurae gene for 16S ribosomal RNA, partial sequence, strain: Biwa6	*Oscillatoria kawamurae*	1024	100%	98.13	AB741574.1
O. kawamurae gene for 16S ribosomal RNA, partial sequence, strain: Lao7	*Oscillatoria kawamurae*	1024	100%	98.13	AB741573.1
O. kawamurae gene for 16S ribosomal RNA, partial sequence, strain: Inle1	*Oscillatoria kawamurae*	1024	100%	98.13	AB741572.1
Oscillatoria princeps PMC 960.16 16S ribosomal RNA gene, partial sequence	*Oscillatoria princeps* PMC 960.16	1024	100%	98.13	MW405021.1
Oscillatoria princeps PMC 959.16 16S ribosomal RNA gene	*Oscillatoria princeps* PMC 959.16	1024	100%	98.13	MW405020.1
O. *kawamurae* gene for 16S ribosomal RNA	*O. kawamurae*	1024	100%	98.13	AB298443.1
Uncultured *Oscillatoria* sp. clone P18cl8 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer	uncultured *Oscillatoria* sp.	1018	100%	97.96	MG250701.1
Uncultured *Oscillatoria* sp. clone 16cl10 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer	uncultured *Oscillatoria* sp.	1018	100%	97.96	MG250690.1
Uncultured *Oscillatoria* sp. clone 2cl4 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer	uncultured *Oscillatoria* sp.	1018	100%	97.96	MG250687.1
Uncultured *Oscillatoria* sp. clone l5 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer	uncultured *Oscillatoria* sp.	1018	100%	97.96	MG250682.1

with the same algae recorded at the Genebank National Center Biotechnology Information (NCBI), had an exact match (98.81%) with the recorded species of *Oscillatory* registered in (NCBI) with an accession number of MW405018.1 (Table 3). This is consistent with Ozturk et al. (2018) who compare Cyanobacteria and Chlorophyta species under the culture conditions using classical classification and molecular identification. Similarly, Al-Khazali (2019) identified two species of microalgae, *C. terrestris* (green microalgae) and *Wollea salina* (blue-green algae) using DNA sequences of ITS2 rRNA and ITS1 rRNA genes, respectively.

Genetic tree: After sequencing 18S rRNA for

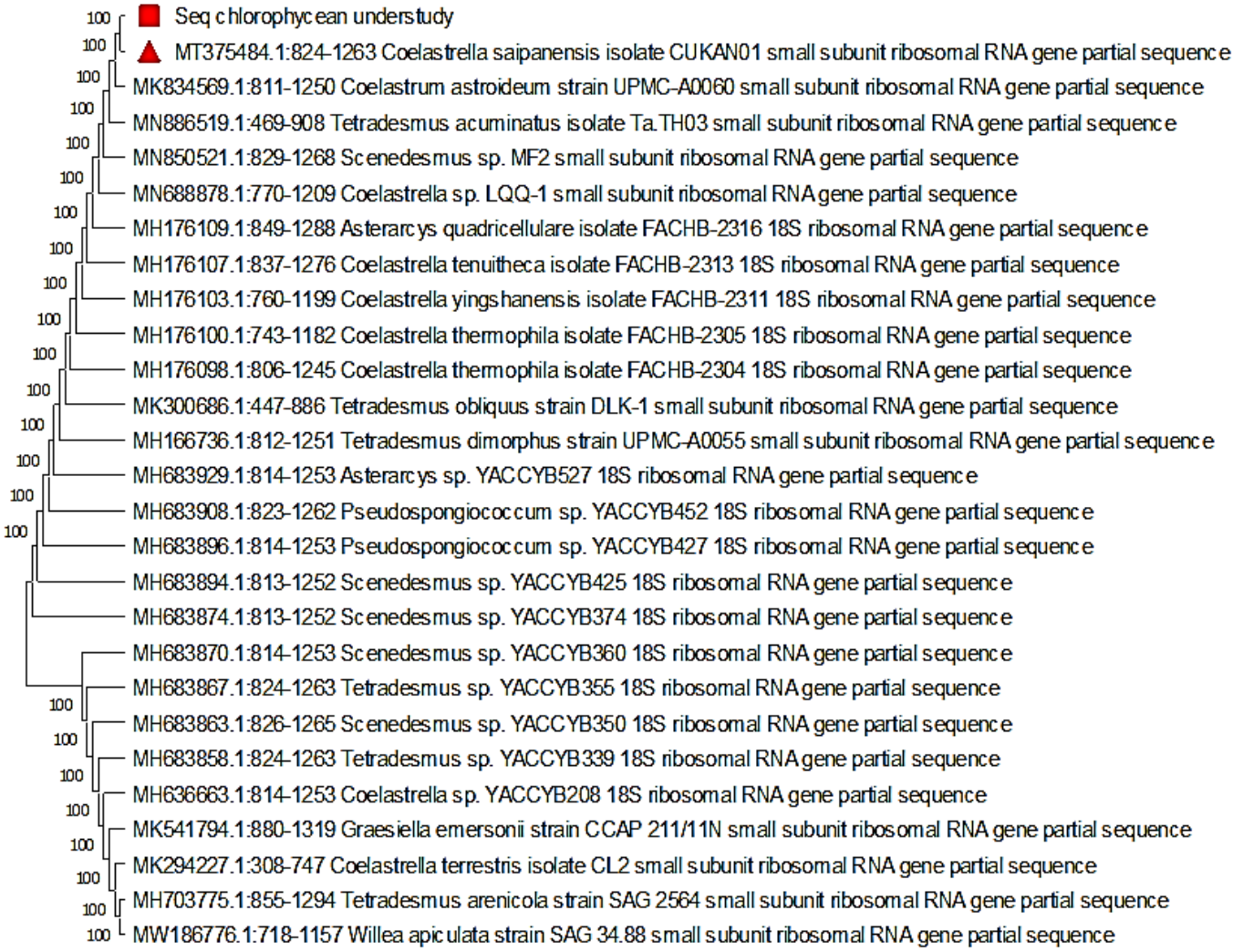

Figure 8. Maximum likelihood phylogenetic tree based on the 18S rRNA gene of *Coelastrella saipanensis* and other species of Chlorophyta from the NCBI site at indicated bootstrap values equal to 100%.

C. saipanensis isolated in the current study, the sequences of the same gene of *C. saipanensis* recorded at NCBI were retrieved (Table 2) and a phylogenetic tree was reconstructed based on UPGMA (Fig. 8). Also sequences of 16 rRNA for *O. duplisecta* isolated in the current study along with the sequences of the same gene of *O. duplisecta* recorded at NCBI were retrieved (Table 3), and a phylogenetic tree of UPGMA was drawn reconstructed (Fig. 9). Thus, the algae were diagnosed in the study based on the genes of 18S rRNA and 16S rRNA sequences.

Conclusions

The present study focuses on biodiesel production from green algae (*C. saipanensis*) and blue-green algae (*O. duplisecta*). These microalgae were identified and characterized morphologically and molecularly. The algae green of *C. saipanensis* and blue-green algae of *O. duplisecta* are sources of ethanol production. The different light intensities influenced the carbohydrate content, and total lipids and increased the bioethanol production. The high carbohydrate content and bioethanol yield were obtained when the light intensity was 36 µmol m^{-2} s^{-1}.

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Figure 9. Maximum likelihood phylogenetic tree based on the 16S rRNA gene between sequences of cyanobacterium *Oscillatoria duplisecta* and other species of Cyanophyta from the NCBI site at indicated bootstrap values equal to 100%.

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