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Screening of Cyanobacterial Strains as a Smart Choice for Biodiesel

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ABSTRACT

Today, the prices rise and consumption of energy resources and creating more greenhouse gases have created incentives for use of biological resources in order to provide biofuels. The aim of this research was introducing selected cyanobacteria as potential candidates for biodiesel production. Samples were collected from Kharg Island. After purification, samples were kept in BG110 and BG11 media in 25 ºC under constant light of 40 to 60 µM $Em^{-2}s^{-1}$ and pH 7. Semi-permanent slides were prepared and identification was carried out by morphological keys and confirmed via molecular techniques. Growth rate, chlorophyll and phycobiliproteins were measured spectrophotometrically. The total amount of fatty acids was measured with FTIR instrument and profile of amino acids and fatty acids were determined with HPLC and GC-Mass respectively. Results showed the dominant genera as: *Synechococcus* sp. ISC 106*, Fischerella* sp. ISC 107*, Schizothrix* sp. ISC 108, *Nostoc* sp. ISC 101. Volumetric lipid productivity varied among strains from 22.61 to 204.91 mg L^{-1} day^{-1}. The highest lipid yields were observed for *Synechococcus* sp. ISC 106. There was no significant difference between the chlorophyll content of cyanobacteria but Allophycocyanin, Phycocyanin and Phycoerythrin of *Synechococcus* sp. ISC 106 was more than the others (12.02, 23.68 and 6.63 µg mg dw^{-1}, respectively). The maximum lipid content of cyanobacteria belonged to *Synechococcus* sp. ISC 106 and included Myrestic acid (41.44%), Palmitic acid (15.53%), Stearic acid (5.28%), Palmitoleic acid (30.74%), Oleic acid (5.13%), Linoleic acid (0.95%), α-Linolenic acid (0%). According to the importance of growth rate and ratio of C18 and C16 fatty acids in selecting candidate for biodiesel production, *Synechococcus* sp. ISC 106 can be considered as raw material for biodiesel production.

KEYWORDS: Amino acids, Biodiesel, Cyanobacteria, Fatty acid productivity, Species selection.

Abbreviation: APC: Allophycocyanin; PC: Phycocyanin; PE: Phycoerythrin; Chl: Chlorophyll

1- INTRODUCTION

Due to rising oil and gas prices and increase in greenhouse gases emissions, resulting in global warming and environmental concerns, researchers are looking for renewable resources of fuel. Recently, researchers focused on the development of different types of biofuels. Bioethanol and biodiesel are the dominate forms of biofuel use to solve the worldwide energy problem (Lu *et al*., 2011; Donmez and Karatay, 2011).

Biodiesel is typically a group of esters produced by a trans esterification reaction between fatty acids (come from animal fat or vegetable oil) and an alcohol in presence of catalyst. It is renewable resource, biodegradable, and nontoxic. Biodiesel in conventional diesel engines reduces emissions of unburned hydrocarbons, carbon monoxide, sulfates, polycyclic aromatic hydrocarbons and nitrated polycyclic aromatic hydrocarbons. One of the biggest challenges in biodiesel production is the availability of feed-stock (Chisti, 2007; Ghasemi *et al.*, 2012).

Biomass can be transformed into biofuel by chemical or biological conversion or a combination of both strategies. Yet, each type of biomass presents its own set challenges that must be overcome if biofuel is to be produced at low fiscal and environmental costs (Lu, 2010). Thus far, biodiesel in the world produce from vegetable oil extracted from food crops such as corn, canola, soybeans, and palm. The increasing criticism of the sustainability of many first-generation biofuels has stimulated the interest in developing second-generation biofuels produced from non-food feed stocks (Gong and Jiang, 2011). Microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Ryan, 2009).

As candidates for biofuel-producing microbial system, cyanobacteria have favorable characteristics. For example high lipid content, rapid growth rate compared to other energy crops, higher photosynthetic efficiency, higher biomass production simple input requirements, tolerance of marginal agricultural environments, more amenable to genetic manipulation for increasing biofuel, and carbon-neutral applications and biodiesel production

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its cheapness (Li *et al.*, 2008). They utilize solar energy as the energy source, carbon dioxide as the carbon source and cyanobacteria as the biological system (Lu *et al*., 2011), with a wide range of tolerance to different temperature, salinity, pH and nutrient availabilities (Hu *et al*., 2008; Brennan and Owende, 2010). For strain selection, some factors are: lipid content, more the distribution of free fatty acids and triglycerides not only the total lipids; resistance to environmental conditions changes, competition from other microalgae species and/or bacterial; nutrients availability; ease of biomass separation and processing; possibility of obtaining other valuable chemicals. Even when the species are not quite desirable for the purpose in commercial use, the utilization of genetic engineering may be a solution (Lu, 2010).

Although almost all types of cyanobacteria lipids can be extracted, only TAGs are easily trans esterified into biodiesel by traditional methods. Analysis of cyanobacteria species have shown tremendous difference in lipid content among different strains, ranging from 1% to approximately 85% of dry cell weight (DCW) (Spolaore *et al.,* 2006; Chisti, 2007; Li *et al.*, 2008). Cyanobacteria produce a wide variety of fatty acids with chain length from C10 to C24 (Hu *et al.*, 2008), depending on species or strains.

Biodiesel quality is also an important factor as it should meet various specifications before commercialization according to the European or American standards (UNE-EN 14214 and US ASTM D6751, respectively). Important parameters including oxidation stability, cetane number, iodine value and cold-flow properties are closely correlated to the fatty acid composition and are determined by the degree of saturation and the chain length of the fatty acids. Low cetane numbers are associated with shorter chain lengths and an increase in the level of unsaturation in the fatty acid. Moreover, a high content in unsaturated fatty acids is responsible for decreasing the oil oxidation stability while biodiesel consisting of saturated long chain fatty acids shows poor cold-flow properties (Knothe, 2008; Pinzi *et al.,* 2009). The fatty acids C10:0, C16:1 and C18:1, have the best combination of properties to produce high quality biodiesel. Investigation of the fatty acid profile of the raw material is thus important when selecting cyanobacteria species for biodiesel production.

For the production of lipid-based biofuels, cyanobacteria have received less attention than other feedstocks such as microalgae (Miao and Wu, 2006; Rodolfi *et al.,* 2009) or crops. For economic biodiesel production, residual algae biomass plays an important role. Lipidless biomass contains proteins, which can enhance the amount of nutritions of conventional food preparations (Spolaore *et al.*, 2006). Nagarkar *et al*. (2004) could correlate the high calorific values with high protein content. The utilization of amino acids of algae biomass alongside fatty acids increases the economic basis of the biodiesel production process.

Nostoc sp. and *Fischerella* sp. (from Nostocales) are filamentous and have been isolated from a wide range of habitats largely varying in their salinity (Vonshak and Tomaselli, 2000). *Synechococcus* sp. belongs to order Chroococcales and forms one of the most important components of the prokaryotic autotrophic picoplankton in the temperate to tropical oceans. *Schizothrix* sp. Belongs to order Oscillatoriales, grows in littoral of lakes joined to the stony or wooden substrates (usually in surf zone) or in metaphyton among water plants and in moors and swamps.

Choosing species for the large-scale production, a wide range of variables are important of which lipid content (percent dry weight), productivity (milligrams per liter per day) and growth rates (doubling time) are keys for the production of biodiesel (Griffiths and Harrison, 2009; Grobbelaar, 2000).

This work aimed to bioprospecting and screening cyanobacterial strains by applying, as selective criteria, the growth rate, volumetric lipid productivity and fatty acid profiles, used for estimating the biodiesel fuel properties.

2- MATERIALS & METHODS

Collection of the cyanobacteria was done from soils and waters of Kharg Island in Persian Gulf. Isolation, purification and liquid cultures were performed by solid agar and routine procedures with BG11 and $BG11_0$ media (Andersen, 2005). Each cyanobacterium was precultured in Erlenmeyer flasks in temperature 30±2 ºC and under continuous light arranged by three white fluorescent lamps emitting 60 µmol photon m^{-2} s^{-1} of light intensity.

Cell growth was checked by evaluation of algal dry weight (dw) every 2 days in duplicate, as defined by Leganés *et al.,* (1987).

Regarding morphological investigations, Semi-permanent slides were prepared. Preparation of samples for scanning electron microscopy (SEM) was completed as following: species were fixed in 2.5% glutaraldehyde for 4 hours and washed in buffer phosphate (PBS). Then, samples were centrifuged and dehydrated in consecutively increasing concentrations of alcohol (10%, 30%, 50%, 70%, 90% and 100%). Lastly, all samples were mounted on metal stubs and coated with a layer of gold (Diestra *et al.*, 2007).

To extract DNA, fresh biomass of cyanobacreria was taken by centrifuging at 12000 rpm and using Fermentas kit (k0512). The applied PCR condition has been defined by Nübel *et al.* (2000). PCR amplification, cloning and

sequence analysis of 16S DNA content was first extracted from the cyanobactrium, and subsequently, PCR was applied by use of two set of primers. Sequences were amplified by the primers PA 5′-AGA GTTTGATCCTGGCTCAG-3′ as forward and 5′-TTACCTTGTTACGACTT-3′ as reverse 1492R, which amplify a ~2000 –bp region of the 16Sr RNA gene. PCR products were gotten by electrophoresis in a 1% (w/v) agarose gel by means of TBE buffer comprising DNA set stain.

The sequence was evaluated by the Pishgam Company with the primers. The sequence data was examined via a likeness search through the BLAST via the website of the NCBI.

Chlorophyll *a* (Chl. *a*) concentration was evaluated performing overnight extractions with methanol. Centrifuged extracts were measured at 665 nm and calculated by the extinction coefficient of Marker (1972). Phycobiliproteins were extracted after osmotic shock and evaluated spectrophotometrically at 750, 652, 615 and 562 nm. O_2 evolution was measured with a Clark-type O_2 electrode from Hach Chemical Company (Soltani *et al*., 2012).

For FTIR spectroscopy, 0.5 ml of sample was taken from each sample, the supernatant was removed and the cells re-suspended in about 100 ml of distilled water. After centrifuge, oven-dried at 40 ºC for 30 min. The sample (10 mg) was mixed with 1000 mg potassium bromide (KBr) by grinding in a vessel. A portion (200 mg) of this mixture was compressed by an oil pressure machine. Spectra were collected over the wave number range 300–4000 cm^{-1}. Each sample was analyzed three times. Spectra were baseline corrected by the automatic baseline correction algorithm (Pittman *et al*., 2010).

In order to examine the fatty acid composition, 0.02 g sample lyophilized was added to 500 µl of methanolic H_2SO_4 (5%). Next, it was placed in 80ºC termocycler and was shaken 2 h (750 rpm). After cooling, 300 µl of NaCl (0.9%) and 150 µl n-hexane was added for the esterification process and the mixture was centrifuged. The hexane layer was collected and analyzed by GC. The analysis for fatty-acid-based biofuels was performed on a Varian 4000 gas chromatography with FID detector. A capillary column CBP1-M25-025 (Having highly pure silica inner surface) with dimension of 25 m × 0.22 mm, 0.25 mm film thickness was used for the separation of fatty-acid-based biofuels. The oven temperature was primarily kept at 180 ºC for 1 min, increased at 4 ◦C/min to 240 ◦C, (held for 15 min). The split ratio was 1:10, and helium was used as carrier gas at a flow rate of 1.0 mL/min in the constant flow mode. The injector and detector temperatures were both 250 °C. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–650 m/z. The injection sample volume was 2.0 µl (Lu *et al*., 2011). The components were identified by comparing their retention time and fragmentation patterns with those for standards seven fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3) were used as standard mate.

Amino acids were measured via HPLC system. 100 mg of freeze-dried biomass was resuspended in 1 mL ethanol 80%. Sampels shaked in 80 °C for 1 hour and Then was centrifuged (5 min, g 14000, 4 °C). Solutions were dried at freeze-drier and filtered. 250 µl filtered sample was added 200 µl borate buffer, 100 µl OPA and 50 µl of HCl 0.5 M. Probes were evaporated, dissolved in a sample solution buffer and injected on a cation separation column (4.6×150 mm, HALO C18; Knauer, Germany) and were identified at 330 nm and at 450 nm. The analyses were run under the following conditions: analysis cycle time 25 min; flow rates 1.1 ml min^{-1} for buffer. The amino acid content is given as the summed content of alanine, asparagine, aspartic acid, arginine, citrulline, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, taurine, methionine, phenylalanine, proline, serine, threonine, tyrosine, α-amino butiric acid, tryptophane and valine.

Data are the means and standard deviation of at least three replicates. Statistical differences were examined by ANOVA test by software SPSS ver. 19.

3- RESULTS & DISCUSSION

In the present study, four cyanobacteria species were separated from soils and waters of Kharg Island in Persian Gulf, included: *Nostoc* sp. ISC 101, *Synechococcus* sp. ISC 106, *Schizothrix* sp. ISC 108, *Fischerella* sp. ISC 107. The findings obtained from morphological observations with light and scanning electron microscope (SEM) are spresented in Table 1 and follows by their micrographs revealed in Figs. 1–2.

Hence, *Nostoc* sp. ISC 101 is a filamentous cyanobacterium in a condensed sheath. Likewise, all the trichomes accumulate in a thick sheath (Table 1, Figs. 1-2). Vegetative cells are more squared or cylindrical. Heterocysts are present apically. The terminal cells of strain are somewhat narrowed, and obviously differentiated by the absence of rounded terminal cells. There is no sign of akinetes. *Synechococcus* sp. ISC 106 has single cellular, coccoid cells less than 3 µm in diameter and non-heterocytous (Table 1, Fig. 1-2). *Schizothrix* sp. ISC 108 is filamentous; solitary or bundled (Table 1, Fig. 1-2). *Fischerella* sp. ISC 107 has heterocytous and akinetes. It has real branching (Table 1, Fig. 1-2).

Table 1. The morphological characteristics of four cyanobacteria grown under above conditions. Data shows X±SE.

Morphological characteristics	Cyanobacteria			
	***Nostoc* sp. ISC 101**	***Synechococcus* sp. ISC 106**	***Schizothrix* sp. ISC 108**	***Fischerella* sp. ISC 107**
Morphology of filaments	Solitary or bundled, curved	Single Cell	Solitary or bundled, curved or straight	Solitary or bundled, curved or straight
Mucilage sheath	+	-	+	+
Terminal cell	Semi-rounded	-	Semi-rounded	Semi-rounded or tapered
Vegetative cells shape	Squared-cylindrical	oval	Squared-cylindrical	Squared-cylindrical
Vegetative Width (µm)	16.32 ± 1.12^{d}	9.82 ± 1.01^{c}	11.86 ± 0.79^{b}	20.59 ± 0.56^{a}
Vegetative Length (µm)	16.80 ± 1.53^{b}	14.98 ± 1.70^{b}	14.16 ± 1.87^{b}	30.89 ± 0.83^{a}
Heterocyst Width (µm)	7.01 ± 0.13^{a}	-	-	7.06 ± 0.30^{a}
Heterocyst Length (µm)	6.52 ± 1.14^{a}	-	-	7.09 ± 1.94^{a}
Akinet shape	Oval	-	-	Oval

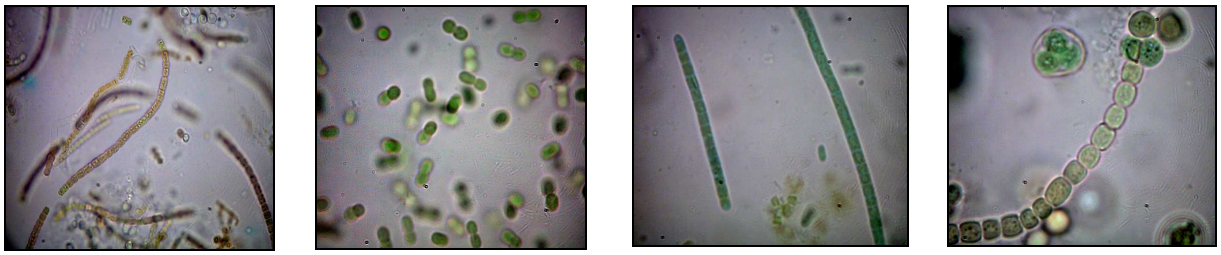

Fig. 1. Micrographs of strains in liquid medium *Nostoc* sp. ISC 101, *Synechococcus* sp. ISC 106, *Schizothrix* sp. ISC 108, *Fischerella* sp. ISC 107 from left to right respectively.

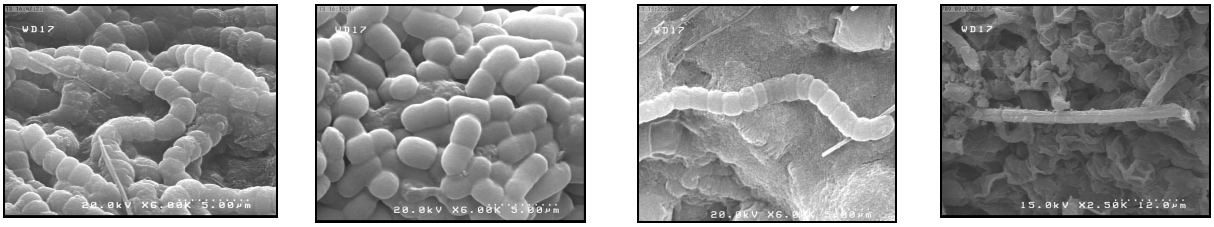

Fig. 2. The shape of cells *Nostoc* sp. ISC 101, *Synechococcus* sp. ISC 106, *Schizothrix* sp. ISC 108, *Fischerella* sp. ISC 107 by SEM from left to right.

Molecular techniques were adopted in order to identify strains. Phylogenetic analysis was done on the basis of the 16S rRNA gene partial sequence for strains *Nostoc* sp., *Synechococcus* sp., *Schizothrix* sp., *Fischerella* sp.. The sequences were compared with those of representative heterocystous cyanobacteria which are accessible in GenBank (NCBI). The 16S rRNA sequences were combined with other species available in the database (Casamatta and Johansen, 2003). The nucleotide sequences defined in this work have been submitted to the NCBI under the accession number NCBI: JX972170, KF443074.

Growth rate and oil content (% dwt) have been the two most evaluated factors in the pursuit of the accomplishment in large-scale cultivation of cyanobacteria for biofuels production (Griffith and Harrison, 2009). Nevertheless, rapid growth only seldom correlates with high lipid productivity. Lower growth rates and/or small cell size may explain lower the biomass productivity, even when the lipid content is high (Rodolfi *et al*., 2009). Consequently, biomass yields may be regarded as a sufficient principle for biodiesel production only when associated with lipid productivity (Lp) (Griffith and Harrison, 2009). That's why, the lipid volumetric productivity and the qualitative lipid composition should be regarded as the most suitable factors to enable decision making on species selection for biodiesel (Huerlimann et al., 2010).

In this investigation, four cyanobacteria strains were compared on the basis of their biomass and lipid productivities (Table 2). The highest biomass does not conform to the highest lipid producers. Relative lipid content was measured by calculating the ratio of the lipid (1705-1740 cm^{-1}) band with FTIR (Pittman *et al*., 2010). The total lipids ratios in dry biomass differed between 20.364 % (*Synechococcus* sp. ISC 106) to 6.384% (*Nostoc* sp. ISC

101). Lipid productivity was measured as the product of average lipid content and biomass productivity in milligrams per liter per day. The range of lipid productivity was from 22.61 to 204.91 mg L^{-1} day^{-1}.

Griffiths and Harrison (2009) gathered data on the biodiesel production of 55 microalgae species. They stated that the lipid content for cyanobacteria varies from 5% to 13% dw, with an average of 8% dw, whereas our findings exhibited noticeably higher lipid content. These values, along with those for the cyanobacterium *Synechococcus*, which, relatively amazingly given its low lipid content, has fairly high lipid productivity (75 mg L^{-1} day^{-1}).

Liu *et al.* (2013) specified that the lipids content in some strains varied between 8–12% dw, of which *Nodularia* and *Nostoc* had the highest amount.

In 1998, 3.000 species of microalgae were screened in the Aquatic Species Program so as to recognize species with high lipid content. They revealed that cyanobacteria have the fastest growth rates and that the lipid productivity was among the highest in exponentially growing cultures (Sheehan *et al*., 1998).

A high doubling time explains a low specific growth rate. The average doubling time for *Synechococcus* sp. ISC 106 is 13.60 h, which explains a μ of 0.051 day^{-1}. Growth data in this research is in agreement with already published data: Rodolfi *et al*. (2009) in screened 4 cyanobacteria strains, biomass productivity found between 0.04 and 0.37 gL^{-1} day^{-1}.

Table 2. Growth kinetics, lipid content, and lipid productivity of cyanobacteria strains

Local strains	Specific growth rate (d^{-1})	G (h)	Lipid content (% dw)
***Nostoc* sp. ISC 101**	0.025	28.47	6.384
***Synechococcus* sp. ISC 106**	0.051	13.60	20.364
***Schizothrix* sp. ISC 108**	0.024	29.54	13.995
***Fischerella* sp. ISC 107**	0.013	58.09	16.780

Nevertheless, the local strain *Synechococcus* sp. ISC 106 exhibited a higher growth significantly (Fig. 5). The *Synechococcus* sp. ISC 106 culture reached a maximum standing biomass of about 7.23 mg mL^{-1} at the 12 day, whereas *Nostoc* sp. ISC 101, *Fischerella* sp. ISC 107 and *Schizothrix* sp. ISC 108 showed algal biomass of 5.03, 4.37 and 3.79 mg mL^{-1} respectively. Earlier research showed that lower oil containing strains grow faster than higher one (Vasudevan and Briggs, 2008). However, our results in contrast with this report agree with Becker *et al*. (1994) that presented microalgae with 30% oil grow 30 times faster than those with 80% oil.

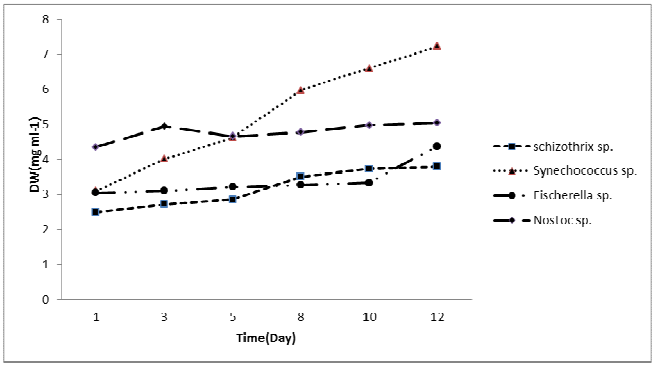

Fig. 5. Dry weight contents in four cyanobacteria.

Table 3 displays the photosynthetic pigments concentrations in four various species of cyanobacteria. About chlorophyll content, the most chlorophyll amount, 1.759 μg $mgdw^{-1}$ belonged to *Synechococcus* sp.. Regards to other pigments, the same results were found. The highest concentration of phycobiliproteins was distinguished in *Synechococcus* sp. ISC 106 with values 12.021, 23.684 and 6.631 μg $mgdw^{-1}$ for APC, PC and PE, respectively.

Table 3. Photosynthetic pigments amount in different cyanobacteria grown under the above conditions. Data shows X±SE.

Cyanobacteria	APC	PC	PE	Chl	PBP
	(μg $mgdw^{-1}$)				
Nostoc sp. ISC 101	2.384±0.28 ^{b}	5.370±0.55^{d}	5.050±0.50^{b}	1.071±0.01^{b}	12.804±0.64^{c}
Synechococcus sp. ISC 106	12.021±2.02^{a}	23.684±0.91^{a}	6.631±0.48^{a}	1.759±0.02^{a}	42.335±1.51^{a}
Schizothrix sp. ISC 108	9.974±1.48^{a}	13.084±0.25^{b}	4.708±0.15^{b}	1.225± 0.01^{b}	27.767±1.27^{b}
Fischerella sp. ISC 107	1.722± 0.37^{b}	10.456±0.36^{c}	0.388±0.25^{c}	1.165±0.20^{a,b}	12.566±0.57^{c}

The size of phycobilisomes can be frequently characterized by the ratio (PE+PC)/APC (Wyman and Fay, 1986). The ratio was higher at *Fischerella* sp. ISC 107 and significant differences between species were seen except *Synechococcus* sp. ISC 106 and *Schizothrix* sp. ISC 108 (Table 4).

The ratios of PBP/Chlorophyll are assumed to display the association between photosystem II and photosystem I (Yamaka and Glazer, 1981; Poza- Carrión *et al.*, 2001). *Synechococcus* sp. ISC 106 and *Schizothrix* sp. ISC 108 were higher (Table 4).

Table 4. (PE+PC)/APC, PBP/Chlorophyll ratios, of cyanobacteria grown under the above conditions. Data shows X±SE.

Cyanobacteria	(PC+PE)/APC	PBP/Chl *a*
***Nostoc* sp. ISC 101**	4.403±0.45^{b}	11.955±0.61^{b}
***Synechococcus* sp. ISC 106**	2.572±0.46^{c}	24.076±1.13^{a}
***Schizothrix* sp. ISC 108**	1.812±0.29^{c}	22.673±1.49^{a}
***Fischerella* sp. ISC 107**	6.493±1.37^{a}	10.790±0.60^{b}

Fig. 6 displays the highest photosynthesis rate for *Fischerella* sp. ISC 107 (13.498 µmol O_2 mg Chl^{-1} h^{-1}). Cyanobacteria gather lipids in thylakoid membranes. It means cyanobacteria gather lipids with high levels of photosynthesis and a rapid growth rate. Moreover, this data indicated the likelihood of the absence of phycoerythrin in *Synechococcus* sp. ISC 106.

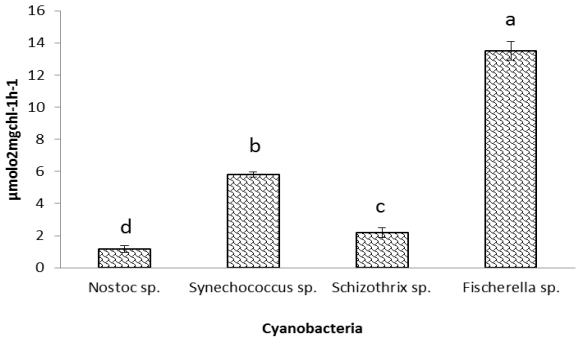

Fig. **6.** Photosynthesis values shows cyanobacteria.

Although there are several papers about eukaryotic microalgae, research on adoption of cyanobacterial lipids as a feedstock for biodiesel production is less. Chemical formula of biodiesel is C14–C24 methyl esters with boiling point of >475°K, and light to dark yellow. Cyanobacteria oils are frequently comprised of four unsaturated fatty acids, i.e. palmitolleic (16: 1), oleic (18: 1), linoleic (18: 2) and linolenic acid (18: 3). Saturated fatty acids like palmitic (16: 0) and stearic (18: 0) also present with a small proportion (Meng *et al*., 2009).

The profiles of fatty acids of our cyanobacteria are revealed in Table 5. Palmitic acid (C16:0) was the main fatty acid in most of the cyanobacteria lipid extracts. The highest percentage was gained with *Synechococcus* sp. ISC 106 about 10.53%. Maximum percentage of α-linolenic and oleic acid was for *Fischerella* sp. (6.43% and 66.33% respectively). The highest percentage palmitoleic was attained with *Synechococcus* sp. ISC 106 about 30.74%.

Extreme low content of linoleic acid (C18:2ω6) and α-Linolenic (C18:3ω3) was the reason for the difference in main range of fatty acids in *Synechococcus* sp. ISC 106 and in other species.

For some of the evaluated strains, the results are consistent with earlier findings. Differences in the overall ratios can be elucidated by the variety of strains and cultivation situations. However, the results of this study also display that *Synechococcus* sp. ISC 106 are frequently characterized by saturated, with chain lengths from C14 to C18. Unlike fuels that are composed typically by polyunsaturated FAME, the high quantity of saturated and MUFA in cyanobacteria oils of *Synechococcus* strains, may incur in less problems with fuel polymerization during combustion (Canakci and Sanli, 2008).

Table 5. Fatty acids profiles (%) in cyanobacteria oils.

Fatty acid	*Nostoc* sp. ISC 101	*Synechococcus* sp. ISC 106	*Schizothrix* sp. ISC 108	*Fischerella* sp. ISC 107
Myrestic (C14:0)	1.58	41.44	0.24	0.18
Palmitic (C16:0)	5.51	15.53	9.22	5.12
Stearic (C18:0)	2.3	5.28	7.37	2.47
Palmitoleic (C16:1)	n.d.†	30.74	7.37	0.24
Oleic (C18:1ω9)	53.78	5.13	50.35	60.33
Linoleic (C18:2ω6)	11.18	0.95	11.18	15.9
α-Linolenic (C18:3ω3)	5.58	n.d.	5.32	6.43

†Nd= Not determined

The entire lipids ratios (as percent of dry biomass) and the percentages of saturated, MUFA and PUFA in the dry biomass are revealed in Fig. 7. *Synechococcus* sp. ISC 106 created values 63.25 % of saturated FA, 35.87 of MUFA and the lowest percentages of PUFA (0.95%). The highest percentages of PUFA were detected for *Schizothrix* sp. ISC 108 (22.33%). If saturated and monoenoic FA are reflected in combination, the highest values for the sum of saturated and MUFA, though, were detected for *Synechococcus* sp. ISC 106 (99.12 %).

Numerous reports of the fatty acid composition of various cyanobacteria species developed under varying situations are accessible. Unicellular strains denote the most appropriate choice for high quality biodiesel production since they have a larger MUFA amount (Kenyon 1972; Kenyon *et al*., 1972). Our results approve earlier findings.

Donmez *et al*. (2011) indicated that *Synechococcus* sp. (at pH 7) exhibited 19.2% lipid content. The experiments were done the maximum lipid contents and C16 and C18 methyl ester yields were evaluated as 42.8% and 46.9% for *Synechococcus* sp., 45.0% and 90.6% for *Phormidium* sp. The saturated compounds were 74.5%, 84.7% for *Synechococcus* sp. and *Phormidium* sp., respectively.

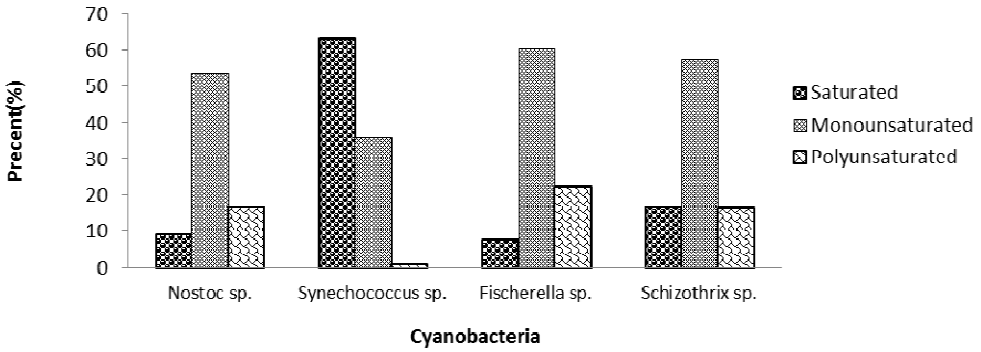

Fig. 7. Percentages of saturated, monounsaturated, and polyunsaturated FA in cyanobacteria dry biomass.

Proteins are main organic elements in algae biomass and are the chief reason for favorability of food preparations (Cornet, 1998; Soletto *et al*., 2005). Lipidless biomass comprises proteins, which can improve nutritional value of conventional food preparations (Hempel *et al*., 2012; Spolaore *et al*., 2006).

Results for efficiency of amino acids of all screened strains are provided in Fig. 8. Amino acids content of *Synechococcus* sp. ISC 106 were recognized as 15.26% fresh weight. Protein content of *Nostoc* sp. ISC 101 (0.73% fw), *Schizothrix* sp. ISC 108 (13.42% fw) and *Fischerella* sp. ISC 107 (3.12% fw) were detected. The chief fraction of amino acids for *Synechococcus* sp. ISC 106 contained alanine, glutamic acid, histidine, serine, tryptophan, valine and leucine.

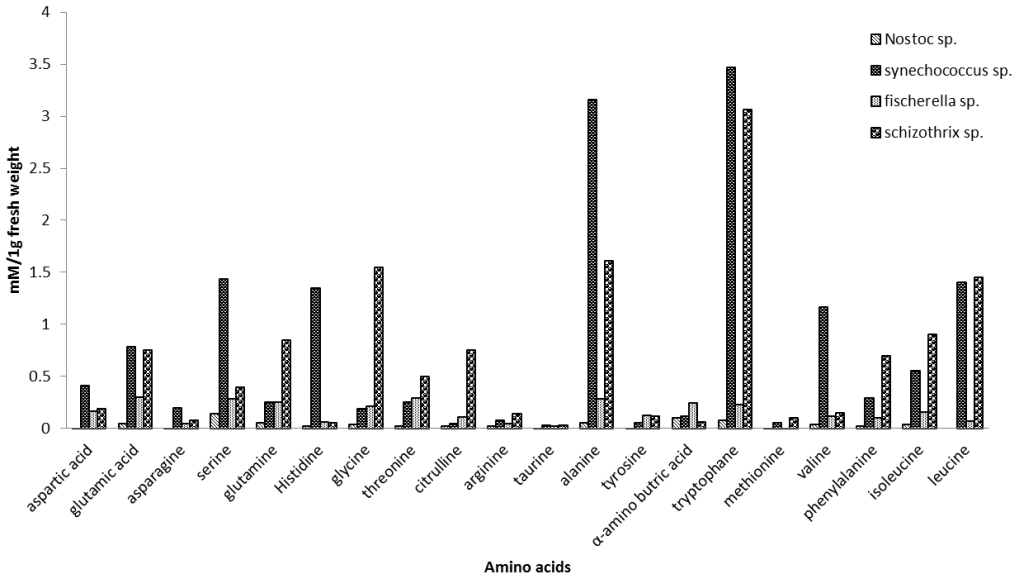

Fig. 8. Profile of amino acids of all screened strains

In literature, sometimes a robust negative correlation between lipid content and biomass productivity has been assumed according to the high metabolic cost of lipid biosynthesis (Rodolfi *et al*., 2009). Nevertheless, on the basis of screening results of the present investigation, this effect was not established.

4- Conclusion

In this work, high lipid accumulation characteristics were detected for the *Synechococcus* sp. ISC 106, albeit there are not any reports on the lipid accumulation properties of the cyanobacterial cells for biodiesel production. The highest lipid contents were detected as 20. 4% for *Synechococcus* sp. ISC 106 at optimal situations. The highest mono-saturated and saturated compounds were attained 99.12%.

Since the physicochemical characteristics of biodiesel are evaluated by the molecular constructions of the components FAME, this investigation proposes that the satisfactory fatty-acids composition of cyanobacterial oil and the volumetric lipid productivity must be the priority standards for strains choice, to make feasible the cyanobacteria-based biodiesel industry. In relation to their FA profiles, numerous cyanobacteria display the potential to yield biodiesel within most of the biodiesel standards. Nevertheless, none of the examined species would certainly yield a lipid capable of achieving all the necessities for a biodiesel of the chief quality grade. On the other hand, as most of them included one or more of the chief features describing such top quality, it is recommended that a good quality biodiesel may be attained by a blend of the different lipid extracts, gained from various species. With regard to the dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters and the rare evidence on the qualitative structure of cyanobacteria oil, this investigation offers a significant contribution for further bio prospection associated with cyanobacteria for biodiesel production. The results acquired from the investigation indicates that the crude lipids achieved from cyanobacteria could be the promising feedstock for biodiesel production.

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