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Study of the Fatty Acid Profiles at Different pH and Fatty Acid Methyl Ester Profiles of Cyanobacterial Strains

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Abstract: ***The search for development of new technologies related to use of cyanobacterial cells for production of renewable sources of energy gained momentum during present day's scientific world. Cyanobacterial lipids have potential commercial application as a source of renewable energy. The cellular fatty acids composition of cyanobacteria remain remarkable. Fatty acids are synthesized and then esterified to form complex lipid in cyanobacteria. The data observed during this study indicates that pH 6.5 remain suitable for palmitic acid, oleic acid and linoleic acid. pH 6.5 remain most suitable for saturated fatty acids and monounsaturated fatty acids. But pH 8.5 remain most suitable for polysaturated fatty acids. Data observed related to fatty acid methyl ester profile indicates that maximum amount of monosaturated and saturated type of fatty acids are produced by Synechococcus*** **sp. and polysaturated by** ***Synecocystis*** **sps. Fatty acid derived energy chemicals have many usages, thus active research attempts within the biofuel industry is required.**

Keywords: Fatty acids profiles, pH, Fatty acid methy ester profiles, Cyanobacteria.

1. Introduction

Fast growing and developing economics, increasing world population and changing climatic conditions demand significant consumption of energy and the limited, non - renewable fossil fuels as major energy resource, pose a world - wide challenge. These challenges have led mankind to search for alternative energy sources oth[illegible] fuels such as coal, petroleum, natural gas [illegible] respect, biofuels, especially those obtained from microalgae and cyanobacteria have emerged as a promising alternative to overcome the existing challenges.1 Cyanobacteria possess rapid growth rate in both fresh and waste water and their unique characteristic of glycogen, lipid and fuel molecules accumulation make them suitable for production of cost - effective biofuel.2

Cyanobacteria also called as Cyanophyta or blue green algae, belongs to a phylum of bacteria that is both old and well - diversified, however, modern cyanobacteria are distinguished by their diverse morphologies and distinct cellular differentiation. In food webs, they are essential as primary producers carrying out oxygenic photosynthesis. Additionally, cyanobacteria were important for the early biogeochemical fluxes, life, and the evolution of Earth.3 Since they are the only prokaryotic species capable of oxygenic photosynthesis, they are generally held responsible for the Great Oxidation Event (GOE), 2.4 billion years ago that led to an increase in oxygen levels in the atmosphere and oceans, with the help of geological processes.4 The development of complex life and the oxygenation of marine and terrestrial niches have been made possible by oxygenic photosynthesis.

During recent past photosynthetic production of fatty acids and fatty alcohols by genetically engineered cyanobacteria is studied by different researchers globally. The ability of cyanobacteria to trap and convert sunlight, water, CO_2 and nutrients into useful chemicals makes them ideal candidate for the bio industrial factories of the future.5

2. Methodology

[illegible]rial cultures were analysed by saponification, [illegible] and extraction process. Fatty acid profiles were examined at different pH. The identified triacyglycerol was converted into fatty acid methyl ester by the transesterification process. Then the volume of produced fatty acid methyl ester was noted. Fatty acid methyl ester profiles were analysed to determine the suitability of the cyanobacterial strains for the production of biofuels. The collected fatty acid methyl esters were processed with help of Gas Chromatography - Mass Spectrometry.

3. Result and Discussion

The data presented in Table - 1 indicates that pH 6.5 remain suitable for palmitic acid, oleic acid and linoleic acid. pH 8 remain most suitable for palmitoleic acid and pH 7.5 remain suitable for stearic acid. pH 8.5 remain suitable for hexadecadeinic acid, hexadecatrienoic acid and linolenic acid. pH 6.5 is observed most suitable for saturated fatty acids and monounsaturated fatty acids. pH 8.5 remain most suitable for polysaturated fatty acids.

Fatty acid methyl ester profile and relative abudance as percentage of total fatty acid was presented in Table - 2. Maximum amount of fatty acids produced by *Synechococcus* sps. remained as saturated and monosaturated types but in *Synechocystis* sps. maximum amount of fatty acids remain as saturated and poly - unsaturated fatty acids.

Table 1: Fatty acid profiles at different pH (% of total FAMEs)

Sl. No.	Fatty acid profiles	pH 6.5	pH 7	pH 7.5	pH 8	pH 8.5
1.	Palmitic	39.52+0.36	37.63+0.25	35.92+0.58	34.27+0.28	29.24+0.70
2.	Palmitoleic	3.46+0.26	2.55+0.10	2.87+0.44	3.72+0.08	2.12+0.05
3.	Hexadecadienoic	6.82+0.75	7.13+0.28	7.69+0.50	7.62+0.68	10.93+0.81
4.	Hexadecatrienoic	2.97+0.14	4.90+0.27	5.66+0.23	7.19+0.39	8.95+0.42
5.	Stearic	1.20+0.17	1.46+0.08	1.59+0.26	1.49+0.16	1.14+0.07
6.	Oleic	13.06+0.20	12.72+0.54	11.93+0.42	9.16+0.23	8.11+0.06
7.	Linoleic	24.98+0.50	23.39+0.31	21.70+0.75	22.41+0.64	21.85+0.25
8.	Linolenic	5.16+0.17	7.55+0.03	9.62+0.08	11.28+0.39	15.25+0.33
9.	NI	2.84+0.19	2.69+0.27	3.04+0.44	2.88+0.37	2.44+0.97
10.	SFA	40.72+0.19	39.09+0.17	37.50+0.33	35.75+0.14	30.37+0.76
11.	MUFA	16.51+0.07	15.27+0.64	14.80+0.05	12.88+0.16	10.22+0.10
12.	PUFA	39.93+0.30	72.97+0.03	44.67+0.13	48.50+0.10	56.97+1.81

Note: 1. Data are shown as means + SD, n=4
2. NI: Not identified.

Table 2: Fatty acid methyl ester (FAME) profiles of Cyonobacterial strains and Relative abundance of FAMEs (% of total)

S. No.	Taxonomy Fatty acid (%)	*Synechococcus* sp. UHCC0374	*Synechocystis sp.* PCC6803
A.	**Saturated**	51.84	45.25
1.	C14: 0	21.05	-
2.	C15: 0	0.44	-
3.	C16: 0	29.91	45.13
4.	C17: 0	0.44	0.12
5.	C18: 0	46.11	9.95
B.	**Monouunsaturated**	3.35	-
1.	C14: 1	0.79	-
2.	C15: 1	35.79	7.10
3.	C16: 1	1.22	2.84
4.	C17: 1	4.19	-
5.	C18: 1 n9	0.78	-
6.	C22: 1 n9	2.05	44.81
C.	**Polyunsaturated**	2.05	-
1.	C16: 4	-	9.02
2.	C18: 3 n3 (ALA)	-	9.09
3.	C18: 3 n6 (GLA)	-	18.38
4.	C18: 4	-	5.22
5.	C20: 3 n6	-	3.10

Synechocystis, a non - nitrogen fixing cyanobacteria is a single celled microorganism. This microorganism is often explored as a nodel system for biofuel production. The genome of *Synechocystis* is easy to manipulate due to absence of organellar compartmentation leading to simplification of metabolic engineering efforts. Cyanobacteria now have identified as novel microorganism for the producton of biofuels since first decade of twenty first century. Genetically engineered cyanobacteria remain capable to produce different types of biofuels.

Synechococcus strains also remain specific in demostrating fatty acid profiles and lack polyunsaturated fatty acids. Triacylglycerol, an excess carbon storage compound has been also reported in *Synechocystis* sp. Presence of this compound shows new opportunities for lipid production in these organisms. *Synenchococcus* is now - a - days extensively used in the researches related to biofuel production. This cyanobacteria is found in both fresh water and marine water bodies. This mocro - organism bears potential for biofuel production through its metabolic pathways. They can be easily manipulated due to diversity in its metabolism, through genome engineering. In *Synenchococcus*, a gap in DNA synthesis is observed between the cells exposed to light and exposed to dark. Presence of nitrogen in growth conditions can affect the doubling time of the colonies of this cyanobacteria.

Cyanobacteria converts solar energy into chemical energy in the form of ATP (Adenosine triphosphate) and reduced form of NADPH (Nicotinamide adenine dinucleotide phosphate). ATP and NADPH are produced by use of a linear and a cyclic pathway with the PSI (Photosystem I), a pigment protein complex. The genetically modified strain of cyanobacteria uses the cyclic pathway in suitable mode to raise APT: NADPH ratio. This process requires low temperature for suitable action. This process balance reductant requirements and maintain the redox poise of the electron transport chain. Thus development of cyanobacteria as photobiocatalysts for CO_2 reduction by sun light and biofuel production represents a critical step forward in the efficient production of biofuel by photosynthetic microorganisms.

The results of present research work are summarized as follows: -

1) The pH value at 39^{th} day of culture for all samples of *S. elongatus sp.* PCC 7942 varied between 9.01 to 10.17 and *Synechocystis sp.* PCC 6803 between 9.61 to 10.32.
2) The floculating pH for both the cyanobacterial species remain as 12.
3) *Synechococcus elongatus* PCC 7942 produces 0.01 g/L fatty acid ethyl esters and *Synechocystis sps.* PCC 6803 produces 0.197 g/L fatty acids. On the basis of this observation it became evident that *Synechocystis* sp. PCC 6803 remain more suitable for fatty acid derived biofuel production at commercial level as compared to *S. elongatus sps.* PCC 7942.
4) pH 6.5 was observed most suitable for saturated fatty acids as well as monosaturated fatty acids and pH 8.5 was observed most suitable for polyunsaturated fatty acids.
5) *Synechoccus* sps. produced maximum amount of saturated and monounsaturated fatty acids as 51.84% and 46.11% respectively. Thus this cyanobacterial species remain more suitable for biodiesel production.
6) *Synechocystis* sps. produces maximum amount of saturated and polyunsaturated fatty acids as 45.25% and 44.81% respectively.

It became evident from the interpretation and analysis of data mentioned in Table - 1 that the fatty acid profiles be influenced by culture pH. During increasing cultivation pH 6.5 to 8.5, the fractions of saturated fatty acids and monounsaturated fatty acid decreased. The monounsaturated fatty acids and polyunsaturated fatty acids have lower melting points than saturated fatty acids. They remain suitable for cold weather biodiesel production. But they require an oxidative stabilizer to be used safely. It is also a notable fact that monounsaturated fatty acids remain most favourable for biodiesel production.

Synechococcus produces more amount of saturated fatty acids (46.11%) than *Synechocystis* (9.95%). Thus *Synechococcus* remain most suitable for biofuel production at industrial level.

The FAME profiles observed for *Synechococcus* sps. and *Syechocystis* sps. was presented in table - 2. Both strains of cyanobacteria showed the presence of saturated fatty acids and monounsaturated fatty acids, but polyunsaturated fatty acids were present in moderate compositions in *Synechococcus* sps. Free fatty acid C16: 1 was most abundant in *Synechococcus* sps. as about 37% composition, for greater than *Synechocystis* sps. (7.10%). Thus this is the distinctive feature of *Synechococcus* sps.

Fatty acids are large block of carbon atoms and have hydrogen atoms that have a carboxyl group at one end. Fatty acids are found in a combined form with glycerol in the form of triglyceride. In present study, we used fatty acids for the conversion of cyanobacteria to fuels. The conversion metabolic pathway of cyanobacteria to fatty acids helps in producing biofuels that are less expensive than the other biomass recovery. Singh *et al*. (2017) stated that modification of cyanobacteria helps in producing and secreting fatty acids.6 Fuel must be a substance that provides power or heat for burning. Wood, oil, coal and petrol are the various kinds of fuel which are used to burn substances. In present study, we used cyanobacteria to produce fuel as cyanobacteria can grow faster grow and have the potential to produce renewable fuel with less emission of carbon dioxide. Biofuels reduce the emission of carbon dioxide from nature.

Zhou and Li (2010) stated that demand for energy will increase globally at tremendous rate in future.7 At present approximately 80% of the energy is generated by burning fossil fuel globally. The causes such as rapidly depleting fossul fuels, surge in crude oil prices and environmental pollution necessitate development of sustainable and environmentally suitable fuel substitutes. Biofuels are pospective renewable energy which depends upon incorporation of freely available solar energy in carbon - carbon bonds of photosynthesizing organisms inlcuding cyanobacteria. The energy stored in carbon - carbon bonds can be re - arranged by metabolic pathways of genetically engineered cyanobacteria to produce biofuels. Biofuel production methodology includes catalytic conversion of lipid into biodiesel or bio - conversion of sugars into ethanol, diesels and jet fuels.

The *Synechococcus* sp. demonstrates the best biodiesel qualities due to lack of polyunsaturated fatty acid content. *Synechocystis* sp. contains large abundance of polyunsaturated fatty acids (Table - 2). Linolenic acid (C18: 3) was observed as main contribution to polyunsaturated fatty acid in this strain of cyanobacteria. The majority of linolenic acid present in *Synechocystis* sps. was omega - 6 - gamma - linolenis acid.

Selection of a suitable microorganism for yield of biofuel plays a key role in success of this type of research. Finding of a microorganism with desirable properties is the pre - requisite of any research study aimed for metabolic biofuel production. The desired microorganisms should be amenable to genetic manipulation. High growth rate, tolerance to high temperature, pH, product toxicity and end products are considerable factors in this direction. The use of photosynthetic cyanobacteria remain suitable for the production of fuel compounds, on the basis of above mentioned parameters. These microorganisms capture solar visible light energy and convert that into high energy organic compouns using water. Many cyanobacterial strains are tested by serveral researchers for the production of valuable metabolities including fuels.

The manipulation of cellular metabolism for increased fatty acid production became possible by the tools of metabolic engineering and synthetic biology makes this method more efficient for fuel development. There is a rapid progress in fatty acid derived fuels. Now researches are focussed on the reduction of production cost of biofuel as well as increasing the efficiency of processes related to high productivity.

The outer membrane of cyanobacteria such as plasma membrane, outer membrane and thylakoidal membrane are three distrinct types of membranes which, however, share a few common aspects showing that the major functional components such as PSI, PS II, cytochromes and ATP synthase occur as multi sub unit complexes within the membrane.

4. Conclusion

It became evident from the results that pH 6.5 and 8.5 remain suitable for different types of fatty acids. The fatty acid secreting cyanobacteria are a promising biological material for renewable biofuel production. The *Synechococcus* and *Synechocystis*, both remain suitable for production of different fatty acids. The facts and findings of this research work demonstrated the potential of cyanobacterial lipids based biofuel in the blue bioeconomy related to lipid based biofuel industry. More genetic engineering work is needed to improve the cyanobacterial fatty alcohol production by introduction of novel gene for this purpose.

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