Biodiesel Properties of Two Strains of Cyanobacteria

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Abstract: ***Vast potential of Cyanobacteria as the most efficient primary producers makes them to become one of the most important alternative energy sources. Properties of the biodiesel obtained from Synechococcus sp and Synechocytis sp were observed during present research work. A comparative analysis of the Physical and Chemical properties of biodiesel obtained from Cyanobacteria and diesel fossil fuel were also conducted during present research study. The Synechoccus strain of Cyanobacteria had exceptionally high CN with values over 60. The lack of polyunsaturated fatty acids in the Synechococcus strain OS values of zero is surprising. It is also observed that the Synechococuus is most suited with cold weather with lower CFPA values. The values for density, viscosity and flash point remained higher in biodiesel as compared to diesel fuel. But heating value and HC ration remain almost similar in both biodiesel and diesel fuel. Thus, it became clear that biodiesel have properties superior than diesel fuel. The results of this study indicates that biodiesel have prospect to be used in place of fossil fuel.***

Keywords: Biodiesel, Cyanobacteria, Diesel fuel, Synechococcus sp., Synechocystis sp.

1. Introduction

Globally, energy security is one of the major problems. Therefore, researchers and policy makers are looking on non - conventional and renewable energy sources. Biofuel is an alternative for fossil fuel because biofuel may be renewable energy source and fossil fuels are non - renewable source of energy. The investigations related to development of renewable energy sources is necessary to combat global energy crisis. So, introduction of biofuel in energy market is necessary. Biofuels are novel solutions to replace the use of fossil fuels. Studies about cyanobacterial primary metabolism, viability and growth pathways should be extensively studied in direction of biofuel production.1 These steps could subsequently replace fossil fuels by renewable production of useful chemicals with help of cyanobacteria. Cyanobacteria are oxygenic photosynthetic microorganisms which adapt and flourish in a diversity of environmental conditions. These organisms utilize mechanism of lipid unsaturation to adjust to changing environmental conditions. Lipids remain major component of their membranes. Cyanobacteria have ability to change their features on prolonged exposure to a stable surrounding condition. The need for fermentable sugars, a carbon feedstock is needed for the production of fuels, however, cyanobacteria can directly fix carbon dioxide as its primary carbon source which reduces the emission of carbon.2 It is known that more than 14 genera of cyanobacteria, including *Anabaena, Aphanocapsa, Calothrix, Microcystis, Nostoc, Oscillatoria and Synechococcus*, etc. may create hydrogen in variety of growth conditions.3 *Synechococcus elongates* sp. PCC 794 strain is the first example of biofuel production in cyanobacteria. In addition to a pyruvate decarboxylase and an alcohol dehydrogenase, *Synechococcus elongates* produces ethanol, redirecting carbon from pyruvate.4 The improvement of ethanol for the production of fuel is a specific field of research.

2. Methodology

Cyanobacterial samples from 100 water bodies were carried to the laboratory adopting conventional method and precautions. Cyanobacterial strains were identified by staining, microscopy and with help of standard literature. Two cynobacterial strains such as *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 were isolated from the collected samples by serial dilution in specific broth. Biomass harvesting was conducted by centrifugation and flocculation techniques. Biodiesel properties were studies with help of standard methods such as thin layer chromatography.

3. Result and Discussion

Properties of the biodiesel obtained from *Synechococcus* sps. and *Synechocystis* sps. are presented in Table - 1. The physical and chemical properties of biodiesel and diesel fuel are presented in Table - 2.

Table 1: Biodiesel properties of the cyanobacterial strains

Normal Properties	Biodiesel properties	*Synechococcus* sp. PCC 7942	*Synechocystis* sp. PCC 6803
Combustion quality and oxidatve stability	SV	212.8	198.27
	IV (<120)	40.50+	117.79+
	CN (>120)	65.84+	47.33
	DU	41.41	90.52
	APE	8.51	83.42
	BAPE	0	63.96
	OS (>8h)	0	5.82
Cold flow properties	LCSF	3.37	4.51

	CFPP (< - 5ºC) +	- 5.89+	- 2.31
	CP	9.5	18.75
Physical properties	HHV	37.12	36.82
	v (3.5 - 5)	1.16	1.12
	p (0.86 - 0.9)	0.83	0.82

Table 2: Comparison of biodiesel and diesel fuel

S. No.	Properties	Biodiesel	Diesel Fuel
1.	Density Kg 1 - 1	0.864	0.838
2.	Viscosity Pa s	5.2×10 - 4 (40ºC)	1.9 - 4.1×10 - 4 (40ºC)
3.	Flash point ºC	65 - 115*	75
4.	Solidifying point ºC	- 12	- 50 - 10
5.	Cold filter plugging point ºC	- 11	- 3.0 (- 6.7 max)
6.	Acid value mg KOH g - 1	0.374	0.5 max
7.	Heating value MJ kg - 1	41	40 - 45
8.	HC ration	1.18	1.18

Based on data from multiple sources

It became evident from the data mentioned in Table - 1 that *Synechococcus* strain of cyanobacteria demonstrates the best overall potential by meeting iodine value (IV) and cetane number (CN). This strain also have lowest cold filter plug point. This cyanobacterial strain also shows lower oxidation stability (OS) value inspite of the lack of unsaturation and BAPE values. This feature indicates about high resistance to oxidation.

Medium chain saturated and unsaturated fatty acids of C12 to C - 18 have been found as most ideal for biodiesel production by cyanobacteria. Sarsekeyra *et al.* (2015) [5] also supported this fact on the basis of CN and IV values which are indicators of ignition and combustion quality. This resulted in high enough CN and low enough IV (Table - 1) values. The *Synechococcus* strain had exceptionally high CNs with values over 60. The lack of polyunsaturated fatty acids in the *Synechococcus* strain, OS values of zero (Table - 1) is surprising. It is also evident from the data mentioned in this table that the *Synechococcus* is most suited to cold weather with lower CFPP values.

During recent years, cyanobacteria have received a considerable attention among researchers as excellent organisms for renewable biofuel production.[6] Cyanobacteria commonly contain significant amount of lipids. Some species of cyanobacteria remain rich in essential fatty acids such as linoleic acids and gamma linoleic acids. Cyanobacteria have oxygenic photosynthesis as similar to higher green plants.[7] The modern technique available in modern age allow the biotechnological application of cyanobacteria to produce specific biofuels. Some cyanobacteria have large quantities of unsaturated fatty acid and few other strains lack poly unsaturated fatty acids in their lipids. Specific features of cyanobacteria entitled them to be most suitable for bioenergy generation. Lipids remain as most effective source of storage energy and also plays an important role as the structural component of cellular membranes of cyanobacterial cells.[8]

Researches around the globe proved that photosynthesis of cyanobacteria can be redirected to produce lipids and organic acids.[9] These lipids can potentially extracted from the cyanobacteria and converted into biodiesel, a new source of energy.

By introduction of fatty - alcohol producing pathway in cyanobacteria by metabolic engineering, the direct recycling of carbon dioxide to fatty alcohol can be produced for biofuels.[10]

Knowledge about natural function of alkanes in cyanobacteria is necessary for research regarding production of biofuels. Lipids obtained from cyanobacteria is converted into biodiesel (fuel) through process of transesterification. Biodiesel consits of triglycerides in which three fatty acid molecules were esterified with a molecule of glycorol. Triglycerides were reacted with methanol in a reaction known as transesterification or alcoholysis. Methyl esters of fatty acids were produced by this reaction. These are biodiesel and glycorol. This reaction takes place in three steps. Firstly triglycerides were converted to diglycerides. Then diglycerides were converted into monoglycerides and glyceral was finally obtained, from chemical conversion of monoglycerides. The transesterification reactions were catalyzed by acids, alkalis and lipase enzymes.[11] Then biodiesel is recovered after repeatedly washing with water. This process is conducted to remove methanol and glycerol. Biodiesel is methyl esters of fatty acid and produced by transesterification from triglycerides.

An emerging demand is moving around the world towards biofuel production to replace the fossil fuels. Cyanobacteria are identified as unique photosynthetic microorganism having features of accumulating glycogen, lipid and fuel molecules through natural metabolic pathways. Side - by - side cyanobacterial cells can be genetically engineered easily for systhesis of a large range of fuel molecules from CO_2.

Cyanobacteria have a specific pathway for conversion of fatty acids to a promising biofuel material, alkanes. Alkanes produced by cyanobacteria have high energy content. Cyanobacteria is a group of oxygenic photocutotrophs which remain capable of capturing CO_2 gas via the Calvin - Benson cycle and produces several organic compounds. According to Sharma *et al.* (2010), cyanobacteria contribute to a large share of the total photosynthetic fixation of solar energy and assimilation of CO_2 gas of the earth as compared to green plants and algae.[12] They also pointed out that these microorganisms accounts for 30% of the oxygen production annually on Earth. Genetically engineered cyanobacteria such as *Synechococcus* can directly convert CO_2 into alcohol

- based biofuels leading to the production of lipid. Biofuels metabolic engineering will likely promote the development of more efficient cyanobacterial cell factories for biofuel production.

Application of advanced techniques be efficiently used to fatty acids transformation into economical and environmentally responsible transportation biofuels. The findings of present research work paves the path for the future of energy with comparatively lesser bad - effects on our global environment. Cyanobacteria can act as renewable source containg rich lipids in their cells and they have potential to refill the energy demands in an eco - friendly way.

Different strains of cyanobacteria acts as excellent biological material for the production of carbon neutal and sustainbale biofuel. Cyanobacteria remain efficient for conversion of visible light energy into lipids. They grows on non - arable land. Cyanobacteria are a group of photosynthetic microorganisms. The production pathways regarding high concentration of diesel range hydrocarbons are exclusively remain present in cyanobacteria. The exclusive feature of cyanobacterial membranes is presence of hydrocarbons in large amount. Cyanobacteria remaim efficient in converting CO_2 gas and solar energy into fuels. The membranes of cyanobacteria remain made up of diacylglyerol lipids and they also have lipid biosynthetic metabolism (Wada and Murata, 1990).[13]

Cyanobacteria are more genetically maniputable than other photosynthetic microogranisms. Cyanobacteria remain suitable for metabolic engineering processes for the production of biofuels such as hydrocarbons, alkylesters, butanol and ethanol. Thus cyanobacteria remain suitable for photosynthetic biosynthesis of high density liquid biofuels. Alkanes are synthesized by many cyanobacteria but in minute quautities. Alkanes of defined chain lengths can be used as jet fuel. Notable amount of alkanes can be obtained by optimization of the expression of the alkane biosynthesis genes of cyanobacteria. Thus use of cyanobacteria for biofuel production offers great potential.

Fatty acid derived biofuels is the promising technology in the biofuel industry. For this purpose fatty acid biosynthetic pathway is utilized. Fatty acids and their derivatives are valuable biofuel molecules. Several researches are going on globally focused on development of new fuel - generating microorganisms as well as characterization of desired genes for production of desired fuel molecules with high yield using different biochemical tools. This may make bioguels cost competitive with petroleum products. The success of research in concerned field may maximize the yield of fatty acids by micro organisms. Fatty acid pathway has the potential to serve as an important fuel platform.

Biodiesel is one of the renewable biofuel. Which is a liquid fuel. Biodiesel is a form of fatty acid methyl ester (FAME) or methyl ester compound with long fatty acid chain. Fatty acid found in the entire cell of cyanobacteria remain composed of SFA (saturated fatty acids), MUFA (monounsaturated faty acid), PUFA (Poly unsaturated fatty acid), branched fatty acids and hydroxyl - substituted fatty acids. Palmitric acid is a type of saturated fatty acid which commonly found in cyanobacteria. Polyenoic fatty acid commonly found in filamentous cyanobacteria. Membrance lipids of cyanobacteria play an active role in the acclimation to different environmental conditions, such as high and low temperatures.

Synechococcus can be utilized in biodiesel production as the most promising biofuel alternative. The cyanobacterial cell remains capable of producing precursors for biodiesel production in form of fatty acids. They can also convert carbon dioxide gas into energy dense fuel molecules. *Synechococcus* does not contain a polyhydroxy butarate pathway thus has a district advantage to other cyanobacteria for fatty acid production. This cyanobacteria had the highest yield of lipid components.

4. Conclusion

The facts and findings of this research work indicates that two cyanobacterial strains such as *Synechococcus* sp. PCC 7942 and *Synechocystics* sp. PCC 6803 can be used for creating renewable transportation fuel without depleting agricultural land. The biodiesels obtained from cyanobacteria can minimize foreign dependence for petroleum and limit harmful emissions to our environment. Further research is needed for identification and testing of efficient cyanobacterial strains to produce a wide variety of biofuels.

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