# RESEARCH ON THE INFLUENCE OF GROWING FACTORS ON THE CYANOBACTERIA BIOMASS TO OBTAIN BIODIESEL

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**Abstract.** *Now days renewable biofuels are needed to displace petroleum derived transport fuels, which contribute to global warming and are of limited availability. Biodiesel is a potential renewable fuel that has attracted the most attention. Biodiesel from cyanobacteria seems to be a renewable biofuel that have the potential to displace petroleum-derived transport fuels, to accumulate significant amounts of lipids without affecting supply of food and also can use non-arable land. Cyanobacteria has attracted the attention of researchers because unlike crop plants their energy is converted to lipids and not fibers in the process of photosynthesis, so they are able to synthesize vegetable oils in large quantities, which can be converted into biofuels through transesterification, but there is necessary a rigorous evaluation to determine whether this type of fuel is competitive to the fossil fuels or not. This paper presents an overview of the possibility to obtaine biodiesel from cyanobacteria. The final aim of the study is to control growing factors, aiming to develop procedures to increase the biomass of cyanobacteria and fat content which could serve as a source of biodiesel production. A main objective is to develop techniques to achieve productivity closer to the photosynthesis maximum efficiency.*

**Keywords:** cyanobacteria, economical viability, biodiesel production, lipids, solar energy conversion.

## INTRODUCTION

In recent years, biomass-derived fuels have received increasing attention as one solution to our continued and growing dependence on fossil fuel, which exposes the world to the risk of critical disruptions in fuel supply, creates economic and social uncertainties for businesses and impacts our environmental security (Ferrell et al., 2010).

Traditional feedstock for nonpetroleum diesel includes vegetable oil and animal fat. Unless these materials come from food residuals, they divert materials from the food supply of human society and already have led to surges in food prices. In contrast, feedstock lipids generated from phototrophic microorganisms, have higher areal yields than plants and do not compete with the food supply or contaminate water resources (Chisti, 2007; Hu et al., 2008; Rittmann, 2008).

During the early years, the emphasis was on using cyanobacteria to produce hydrogen, but the focus changed to liquid fuels – biodiesel (Ferrell et al., 2010).

Photosynthesis is the key to making solar energy available in useable forms for all organic life in our environment. These organisms use energy from the sun to combine water with carbon dioxide (CO2) to create biomass. Photosynthetic bacteria, efficiently utilize the energy from the sun to combine water with carbon dioxide (CO2) to create biomass (www.nrel.gov).

The conversion of extracts derived from cyanobacteria sources is the typical mode of biofuel production from cyanobacteria. The most common type of cyanobacteria extracts under consideration are lipid-based, e.g., triacylglycerides, which can be converted into biodiesel. Biodiesel is defined as "mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats". Biodiesel has been demonstrated to be a viable fuel, both when used as a blend with petroleum-derived diesel and when used in its neat form (Ferrell et al., 2010).

Biodiesel fuel is a biodegradable, renewable and non-toxic fuel. It contributes no net carbon dioxide or sulfur to the atmosphere and emits less gaseous pollutants than normal diesel (Lang et al., 2001; Antolin et al., 2002; Vicente et al., 2004). The most common way to make biodiesel is transesterification as the biodiesel from transesterification can be used directly or as blends with diesel fuel in diesel engine (Peterson et al., 1991; Zhang et al., 2003). Exploring ways to reduce the high cost of biodiesel is of much interest in recent biodiesel research, especially for those methods concentrating on minimizing the raw material cost. Microorganisms have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops (Milne et al., 1990; Ginzburg, 1993; Dote et al., 1994; Minowa et al., 1995).

## MATERIAL AND METHOD

Cyanobacteria are photosynthetic prokaryotes possessing the ability to synthesize chlorophyll a. Typically water is the electron donor during photosynthesis, leading to the evolution of oxygen. Cyanobacteria have until recently also been characterized by their ability to form the phycobilin pigment, phycocyanin. It is the high concentration of this pigment occurring under some conditions which leads to the bluish colour of the organisms (Whitton and Potts, 2002).

Several names are currently used for these organisms, including 'cyanobacteria,' 'cyanophyceae,' 'cyanophytes' and 'blue_green algae'. Until electron microscopy and biochemical analyses could show convincingly that the cyanobacteria were procaryotes, these organisms were generally considered as algae (Annick Wilmotte, 2004).

Cyanobacteria can be cultivated via photoautotrophic methods (where cyanobacteria require light to grow and create new biomass) in open or closed ponds or via heterotrophic methods (where cyanobacteria are grown without light and are fed a carbon source, such as sugars, to generate new biomass). Choices made for the cultivation system are key to the affordability and sustainability of cyanobacteria to biodiesel systems. Three major components can be extracted from cyanobacteria biomass: lipids, carbohydrates, and proteins. While lipids and carbohydrates are fuel precursors, proteins can be used for co-products (Ferrell et al., 2010).

The lipid composition of cyanobacteria differs from that of bacteria, in which phospholipids are the major glycerolipids (Murata and Nishida, 1987; Lechevalier and Lechevalier, 1988). Cyanobacterial cells contain four glycerolipids, namely, MGDG, DGDG, SQDG and PG, and these glycerolipids are the major glycerolipids in the inner envelope and thylakoid membranes of the chloroplasts of higher plants (Block et al., 1983). The relative level of MGDG in cyanobacterial cells is slightly higher than 50% of the total glycerolipids, and relative levels of DGDG, SQDG and PG range from 5% to 25%. Cyanobacteria also contain a minor glycerolipid, GlcDG, which is absent from chloroplasts. There are several aspects of cyanobacteria biodiesel production, these include (Sato and Murata, 1982a): 1) high productivity; 2) non-food based feedstock resources; 3) use of non-arable land; 4) utilization of a wide variety of water sources; 5) production of both biofuels and valuable co-products; 6) potential recycling of CO2 and other nutrient waste streams.

## RESULTS AND DISCUSSION

Light is needed for adequate rates of lipid formation and, in addition, may be absolutely required for the production of certain molecules. For temperature, most attention has focused on low temperature and chilling stresses. However, high stress temperatures may also affect lipid metabolism and function. Low temperature exposure may have a number of effects of which increased unsaturation is the most common change (Siegenthaler and Murata, 2004)

Many organisms, including both prokaryotes and eukaryotes, respond to changes in environmental temperature by altering the fatty acid composition of their membrane lipids. When the growth temperature is decreased, the extent of unsaturation increases and the chain length of fatty acids decreases (Russell, 1984). Similar changes in fatty acid composition with growth temperature are observed in cyanobacteria.

The unsaturation of the fatty acids in the glycerolipids of biological membranes can be modified by various environmental factors. Growth temperature is the major factor that influences the unsaturation of fatty acids in thylakoid membranes. In cyanobacteria, a decrease in growth temperature induces the desaturation of the fatty acids of membrane lipids. Such temperature-induced changes in the extent of unsaturation can be explained in terms of the regulation of membrane fluidity, which is decreased at low temperatures and is increased by the desaturation of the fatty acids of the membrane lipids. It has been suggested that such a regulatory mechanism is necessary to maintain the optimal functioning of biological membranes (Cossins, 1994).

When the photosynthetic activities of *Synechocystis* sp. PCC 6803 that had been grown at various temperatures were compared under isothermal conditions, those of cells grown at lower temperatures were found to be higher than those of cells grown at elevated temperatures.Such changes in photosynthetic activity with growth temperature were regarded as a result of alterations in the extent of unsaturation of glycerolipids (Gombos et al., 1992).

High temperature acclimation is often accompanied by increased lipid saturation. The progressive disruption of the PS II complex by high temperature can be monitored by chlorophyll fluorescence and the threshold temperature at which Fo increase is initiated (Ti) has been widely used as a measure of thermostability of thylakoid function. The effects of unsaturation of fatty acids in membrane lipids on physiological characteristics, such as growth and chilling tolerance, have been studied in genetically manipulated strains of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942. It has been found that polyunsaturated fatty acids are important for growth and the ability to tolerate photoinhibition of photosynthesis at low temperature (Gombos et al., 1992).

Accelerated desaturation of fatty acid, upon a downward shift in temperature, results in the activation of desaturases by the changes in membrane fluidity, sow low temperature induced desaturation of fatty acids requires the reactions of the photosynthetic electron transport system in the thylakoid membranes. The primary signal in the biological perception of temperature was studied by catalytic hydrogenation of membrane lipids of *Synechocystis* sp. PCC 6803 (Vigh et al., 1993).

Gombos et al. (1987) examined the effects of nitrate starvation on the growth, lipid and fatty acid composition of cells of *Anacystis nidulans*. A remarkable decrease in the protein content and a disruption of endomembrane system in the cells were observed when the cells were transferred from a normal growth medium to a nitrate-free medium. However, the cells retain their ability even for days. An increase in the level of 18:0 with an accompanying decrease in the level of 16:1 in all lipid classes was also observed after the transfer of the cells from a normal growth medium to a nitrate-free medium. By contrast, the lipid composition did not change and no significant changes in the fluidity or in the phasetransition temperature of cytoplasmic membranes were observed during the nitrate starvation.

The effects of salinity on the lipid and fatty acid composition of membranes were studied in *Synechococcus* sp.PCC6311. Growth of *Synechococcus* sp. PCC 6311 in the presence of high concentration of NaCl was accompanied by significant changes in fatty acid and lipid composition. Upon transfer of cells from a growth medium that contained 15 mM NaCl to one that contained 500 mM NaCl. The level of MGDG decreased, whereas those of DGDG and PG increased after transfer of cells from low to high salinity. These changes in the lipid and fatty acid composition of membranes are observed in both the thylakoid and the cytoplasmic membranes. These findings suggest that might be important for acclimation of the cells to high salinity (Huflejt et al., 1990).

## CONCLUSIONS

Cyanobacteria constitute a vastly diverse group of oxygenic photosynthetic prokaryotes with metabolic versatility that allows them to adapt to a wide range of habitats. Cyanobacteria have attracted interest as catalysts for the conversion of solar energy into a variety of valuable products. Much exciting work has been done in the past 5 years demonstrating that cyanobacteria can produce desirable industrial platform chemicals like hydrogen, alkanes, fatty acids, butanol/iso-butyraldehyde, and isoprenes. Cyanobacteria offer great promise in contributing to the renewable fuels standard, for example the potential oil yields is significantly higher than the yields of oilseed crops. Because of the potential for photosynthetic microorganisms to produce more lipids per unit area for biofuel production than land plants, these microbes are in the forefront as future biodiesel producers.

## REFERENCES

1. Antolin G., F.V. Tinaut, Y. Briceno, V. Castano, C. Perez, A.I. Ramirez (2002). Optimisation of biodiesel production by sunflower oil transesterification. Bioresour. Technol. 83.

2. Annick Wilmotte (2004). The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers.

3. Block M.A., A.J. Dome, J. Joyard, and R. Douce (1983). Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. II. Biochemical characterization. J Biol Chem.

4. Briana A. Whitton, M. Potts (2002). The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic Publishers.

5. Chisti Y. (2007). Biodiesel from microalgae. Biotechnology Advances 25.

6. Cossins A.R. (1994). Homeoviscous adaptation of biological membranes and its functional significance. Temperature Adaptation of Biological Membranes. Portland Press, London.

7. Dote Y., S. Sawayama, S. Inoue, T. Minowa, S. Yokoyama (1994). Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. Fuel 73.

8. Ferrell J., Valerie Sarisky-Reed, D. Fishman, R. Majumdar, Joanne Morello, R. Pate, J. Yang (2010). National Algal Biofuels Technology Roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program.

9. Ginzburg B.Z. (1993). Liquid fuel (oil) from halophilic algae: a renewable source of non-polluting energy. Renew. Energy 3.

10. Gombos Z., M. Kis, T. Pali, and L. Vigh (1987). Nitrate starvation induces homeoviscous regulation of lipids in the cell envelope of the blue-green alga, *Anacystis nidulans.* European Journal of Biochemistry 165.

11. Gombos Z., H. Wada, and N. Murata (1992). Unsaturation of fatty acids in membrane lipids enhances tolerance of the cyanobacterium *Synechocystis* PCC 6803 to low-temperature photoinhibition. Proceedings of the National Academy of Sciences USA 89.

12. Hu Q., M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant Journal 54.

13. Huflejt M.E., A. Trémolières, B. Pineau, J.K. Lang, J. Hatheway, and L. Packer (1990). Changes in membrane lipid composition during saline growth of the freshwater cyanobacterium *Synechococcus* 6311. Plant Physiology 94.

14. Lang X., A.K. Dalai, N.N. Bakhshi, M.J. Reaney, P.B. Hertz (2001). Preparation and characterization of bio-diesels from various bio-oils. Bioresour. Technol. 80.

15. Lechevalier H., and M.P. Lechevalier (1988). Chemotaxonomic use of lipids – an overview. In: Ratledge C and Wilkinson SG Microbial Lipids, Vol 1, Academic Press, San Diego.

16. Milne T.A., R.J. Evans, N. Nagle (1990). Catalytic conversion of microalgae and vegetable oils to premium gasoline, with shape selective zeolites. Biomass 21.

17. Minowa T., S.Y. Yokoyama, M. Kishimoto, T. Okakurat (1995). Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction. Fuel 74.

18. Murata N., and I. Nishida (1987). Lipids of blue-green algae (cyanobacteria). In: The Biochemistry of Plants, Vol 9. Academic Press, Orlando.

19. Peterson C.L., M. Feldman, R. Korus, D.L. Auld (1991). Batch type transesterification process for winter rape oil. Appl. Eng. Agric. 7.

20. Rittmann B.E. (2008). Opportunities for renewable bioenergy using microorganisms. Biotechnology and Bioengineering 100.

21. Russell N.J. (1984). Mechanisms of thermal adaptation in bacteria: blueprints for survival. Trends Biochem Sci 9.

22. Sato N., and N. Murata (1982a). Lipid biosynthesis in the bluegreen alga, *Anabaena variabilis.* I. Lipid classes. Biochim Biophys Acta.

23. Siegenthaler P., N. Murata (2004). Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer Academic Publishers.

24. Vicente G., M. Martinez, J. Aracil (2004). Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Bioresour. Technol. 92.

25. Vigh L., D.A. Los, I. Horváth, and N. Murata (1993). The primary signal in the biological perception of temperature: Pd-catalyzed hydrogenation of membrane lipids stimulated the expression of the *desA* gene in *Synechocystis* PCC6803. Proceedings of the National Academy of Sciences USA 90.

26. Zhang Y., M.A. Dube, D.D. McLean, M. Kates (2003). Biodiesel production from waste cooking oil. Process design and technological assessment. Bioresour. Technol. 89.

27. *** www.nrel.gov