



# CYANOBACTERIA AS A NOVEL SOURCE OF RENEWABLE ENERGY

**Garvita Singh**

Department of Botany, Gargi College, Sirifort Road, New Delhi 110049

## Abstract

Exploring cyanobacteria as a potent source of biofuel production is first step towards development of an ecofriendly sustainable society. Burning environmental issues, intensifying costs and limited availability of fossil fuels have generated the need of alternative energy sources. Nowadays several plants, microbes are being studied for their potential role in production of biofuel such as bio hydrogen, bioethanol and biodiesel. Cyanobacteria provide a quite promising platform due to their simpler organization and easy genome modification capability. This review put forward an insight on the vital issues of alternative energy where cyanobacteria prove to be one of the best choices as a source of renewable energy.

**Key words:** Biofuel, Hydrogen, carbon, Lipids, methane, ethanol biodiesel, cyanobacteria and renewable energy

## Introduction

World around is looking forward to reduce greenhouse gas emissions via alternative resources to depleting fossil fuels in order to go green and ecofriendly. The use of cyanobacteria as a renewable energy source is highly emerging because cyanobacteria promises as a model organism for the production of biofuels utilizing simplified source as carbon dioxide(CO<sub>2</sub>) and sunlight as their main sources of carbon and energy (Ducat *et al.*, 2011; Machado and Atsumi, 2012). Cyanobacteria are one of the oldest photosynthetic organisms on Earth that existed about 2.5-2.6 billion years ago (Schopf and Packer, 1987; Olson and Blankenship, 2004). Phylogenetic studies group them as Gram-negative prokaryotes having cosmopolitan distribution ranging from hot springs to the Arctic and Antarctic regions (Stanier and Cohen-Bazire, 1977). Complete transition from a reducing environment to oxidizing atmosphere paved way for the evolution of O<sub>2</sub> producing cyanobacteria and turned Earth as dwelling place for various life forms (Dismukes *et al.*, 2001). Thus, cyanobacteria were responsible for a major global evolutionary transformation leading to the development of aerobic metabolism (Pace, 1997). Because of their comparative cellular simplicity and ease of genetic manipulation, cyanobacteria are the object of numerous

biotechnological efforts for metabolic engineering for the renewable production of biofuels and high-value products. Compared with algae and plants, cyanobacteria are easier to genetically modify and are amenable to organism-wide metabolic modeling, which are attributes that lend themselves to synthetic biology approaches (Berla *et al.*, 2013). Cyanobacteria have thus become the targets of biofuel production that includes the engineered production of ethanol (Deng and Coleman, 1999), butanol, isoprene (Lindberg *et al.*, 2010), ethylene (Ungerer *et al.*, 2012), sugars (Ducat *et al.*, 2012), and lactate (Angermayr *et al.*, 2012).

The development and use of renewable energies provide a considerable number of benefits to nations around the world including an increment of the energy production, environmental protection, reduction in pollution and jobcreation. Solar, wind, hydroelectric, biomass and geothermal energy currently constitute the most common sustainable sources of energy. Each one of these sources has particular properties that determine their usefulness and application in our society (Afgan and Carvalho 2002). Sustainable energies represented about 18% of the global total energy consumption and are able to substitute traditional fuels at different levels including power generation, heating, transport fuel and rural energy. Because of its common use in developing countries for local energysupply, biomass represents the major source

of renewable energy (constituting up to a 75% of the renewable energy sources) (Hall and Moss 1983). Bioenergy is fuel derived from biological sources (biomass) and is also referred to as biofuel. Biomass is defined as any organic material coming from any form of life or its derived metabolic products. Biofuel (either biodiesel or bioethanol) is currently the only alternative energy source able to replace transport fuel in today's vehicles without involving major modifications to vehicle engines (Kaygusuz 2009). Biomass possesses important advantages if compared to other sustainable sources, for instance, it is available throughout the world, its processing is relatively simple without involving expensive equipment and it can be stored over long periods of time. This review highlights about utility of cyanobacteria as a source of energy by utilizing its organic and inorganic constituents.

#### **Why cyanobacteria as source of energy? Promises**

Cyanobacteria possess certain properties which have made them to be one of the most promising organisms for bioenergy generation (Deng and Coleman 1999; Dexter and Fu 2009):

1. Presence of considerable amounts of lipids in the thylakoid membranes.
2. Higher level of photosynthesis and growth rates as compared to other algae and higher plants.
3. They could be easily grown with basic nutritional requirements and are able to survive if supplied with air, water and mineral salts (especially phosphorous-containing salts) with light as the only energy source. These simplified needs make them best choice for their selection and are often termed as green *E. coli*.
4. Their cultivation is relatively simple and inexpensive.
5. Cyanobacteria possess a relatively small genome and many of them have already been completely sequenced, thus it is comparatively easier to perform system biology approaches in these organisms as compared to eukaryotic algae (Rittmann 2008).

#### **Types of fuels obtained from cyanobacterial biomass**

Cyanobacteria being photosynthetic, utilize the sun's energy, water and carbon dioxide to synthesize their energy storage components, *i.e.* carbohydrates, lipids and proteins. These energy storage components can be further converted into bioenergy. Cyanobacteria make up a promising model via its unique metabolism to transform all the C sources into valuable sustainable fuels. The following section details about the type of fuels obtained from cyanobacteria as source of renewable energy.

#### **Diesel**

Cyanobacterial biomass has traditionally been associated with the production of ethanol (Deng and Coleman 1999; Dexter and Fu 2009) or hydrogen (Hall *et al.* 1995) as an energy source. Around 2,000 species of cyanobacteria have been identified (Sheehan *et al.* 1998), but information regarding the production of biodiesel from these species or related parameters such as the biochemical profile, growth rate and energy content of the different species are scarce (Miao and Wu 2006). For the large-scale production, a wide range of variables are important of which (Griffiths and Harrison 2009; Grobbelaar 2000) lipid content (percent dry weight), productivity (milligrams per litre per day) and growth rates (doubling time) are keys for the production of biodiesel. Griffiths and Harrison (2009) collected data on the biodiesel production of 55 microalgae species. *Synechococcus* with a production of 75 mg/L of lipids per day was among the highest yielding strains of cyanobacteria. Vermaas and colleagues have genetically engineered a single gene mutant of *Synechocystis* able to accumulate up to 50% of dry weight in lipids (Rittmann 2008). A bioscience firm in the USA, Targeted Growth, has recently claimed to have developed a new technology to increase the lipid content of cyanobacteria by approximately 400% (Timmerman 2009). Biodiesel quality is also an important factor as it should meet various specifications before commercialization according to the European or American standards. 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. Generally, unicellular types of cyanobacteria lack PUFA (poly unsaturated fatty acid), while most of the filamentous species contain high levels of di- and trienoic fatty acids (Kenyon 1972; Kenyon *et al.* 1972). Thus, unicellular strains represent the most suitable choice for high quality biodiesel production because they have a larger MUFA (mono unsaturated fatty acids) amount.

#### **Ethanol**

Ethanol is formed by converting the starch (the storage component) and cellulose (the cell wall component) in the organism. As lipids can be made into biodiesel, the carbohydrates can be converted to ethanol. Cyanobacteria are the optimal source for bioethanol production due to the fact that they are high source of carbohydrates/polysaccharides and have thin cellulose walls. The advantage that cyanobacteria have over the traditional energy crops in producing ethanol is that they ferment naturally without the need to add yeast cultures as is the

case with fermentation of traditional energy crops (Atsumi *et al.*, 2008). This characteristic makes cyanobacteria a promising candidate for the production of ethanol. The first cyanobacterial species to be genetically modified in order to produce ethanol was *Synechococcus* sp. PCC 7942. The noncrystalline nature of cellulose makes it ideal source for ethanol production facilitating its hydrolysis (Nobles and Brown 2008). Ethanol is today the most common biofuel worldwide.

### Methane

Cellular biomass can be transformed to methane, under anoxic conditions, through a process known as anaerobic digestion. After lipid extraction from cyanobacterial biomass, the remaining material can be converted into methane by this process raising the total energy recovery. This will lead to a more favourable or positive energetic balance of the overall biofuels production by cyanobacteria, which could also decrease the total costs of the process for bioenergy production. *Spirulina maxima* is one of the species studied in terms of the methane production (Samson and Leduy 1982, 1986; Vareletal. 1988). The nitrogen-fixing cyanobacterium, *Anabaena* sp., was shown to be able to biodegrade cyanides and thereby producing methane in batch reactors (Gantzer and Maier 1990).

### Hydrogen

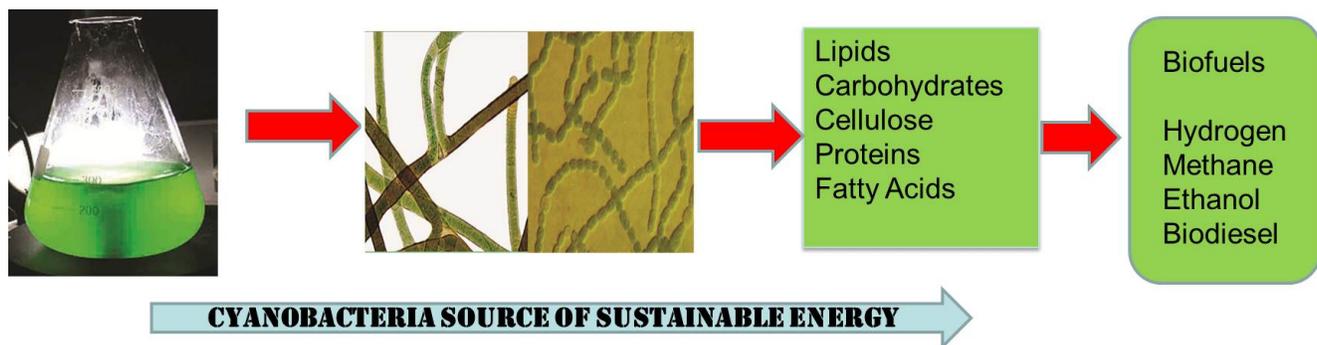
Biological hydrogen production as a source of renewable energy has been receiving considerable attention quite late. Active research into the production of hydrogen in cyanobacteria is at the moment focusing on the identification of new strains with specific hydrogen metabolism, optimising cultivation conditions in bioreactors and genetically modifying specific strains to enhance H<sub>2</sub> production (McKinlay and Harwood 2010; Schütz *et al.* 2004; McNeely *et al.* 2010). Hydrogen can be produced by many strains of cyanobacteria by the reversible activity of hydrogenase. When cyanobacteria

are grown under nitrogen-limiting conditions, hydrogen is formed as a byproduct of nitrogen fixation by nitrogenase (EC 1.7.99.2). It was also shown that non-heterocystous cyanobacteria are less efficient in hydrogen production than the heterocystous organisms. Several reports have reviewed cyanobacterial species capable of producing hydrogen (Abed *et al.* 2009; Das and Veziroglu 2001; Dutta *et al.* 2005) including at least 14 genera cultivated under different growth conditions. Several genera *Anabaena*, *Oscillatoria*, *Cyanothece*, *Nostoc*, *Synechococcus*, *Mycrocystis* etc. (Dutta *et al.* 2005). Among these genera it was reported that *Anabaena* spp. was able to produce the highest amount of hydrogen as a biofuel. Moreover, the emergence of synthetic biology approaches will facilitate the future development of more specialised and energy efficient strains for biofuel production Fig 1 (Huang *et al.* 2010).

Cyanobacteria have received significant consideration and furthermore offer a promising biomass feedstock for various organic (ethanol, methane and biodiesel) and inorganic (hydrogen and electricity) biofuels. They are a potential source of a wide range of valuable biofuels using different substrates for their production. Many of the cited strategies are still under development and their energy yield may not be economically feasible yet at industrial production levels. Therefore, the metabolic network needs to be optimized and extrapolated to generate an efficient and economic biofuel production system to a commercial scale.

### References

- Abed, R.M.M., S. Dobretsov and K. Sudesh (2009). Applications of Cyanobacteria in Biotechnology. *J. App. I. Microbiol.*, **106**:1–12.
- Afgan, N.H. and M.G. Carvalho (2002). Multi-criteria Assessment of new and Renewable Energy Power Plants. *Energy*, **27**: 739–755.
- Angermayr, S.A., K.J. Hellingwerf, P. Lindblad and M.J.T.



**Fig.1:** Utilization of organic and inorganic components of cyanobacteria for sustainable energy.

- de Mattos (2009). Energy Biotechnology with Cyanobacteria. *Curr. Opin. Biotechnol.*, **20**:257–263.
- Atsumi, S., T. Hanai and J.C. Liao (2008). Non-Fermentative Pathways for Synthesis of Branched-chain higher Alcohols as Biofuels. *Nature*, **451**:86–89.
- Berla, B.M., R. Saha, C.M. Immethun, C.D. Maranas, T.S. Moon and H.B. Pakrasi (2013). Synthetic Biology of Cyanobacteria: Unique Challenges and Opportunities. *Front. Microbiol.*, **4**: 246. doi:10.3389/fmicb.2013.00246
- Das, D. and T.N. Veziroglu (2001). Hydrogen Production by Biological Processes: A Survey of Literature. *Int. J. Hydrogen Energ.*, **26**:13–28.
- Deng, M.D. and J.R. Coleman (1999). Ethanol Synthesis by Genetic Engineering in Cyanobacteria. *Appl. Environ. Microbiol.*, **65**: 523–528
- Deng, M.D. and J.R. Coleman (1999). Ethanol Synthesis by Genetic Engineering in Cyanobacteria. *Appl. Environ. Microbiol.*, **65**:523–528
- Dexter, J. and P.C. Fu (2009). Metabolic Engineering of Cyanobacteria for Ethanol Production. *Energ. Environ. Sci.*, **2**: 857–864.
- Dismukes, G.C., V.V. Klimov, S.V. Baranov, N. Kozlov Yu, J. Das Gupta and A. Tyryshkin (2001). The origin of atmospheric oxygen on Earth In: *The Innovation of Oxygenic Photosynthesis. Proc. Nat. Acad. Sci., USA* **98**: 2170–2175.
- Ducat, D.C., J.A. Avelar-Rivas, J.C. Way and P.A. Silver (2012). Rerouting Carbon Flux to Enhance Photosynthetic Productivity. *Appl. Environ. Microbiol.*, **78**, 2660–2668. doi:10.1128/aem.07901-11.
- Ducat, D.C., J.C. Way and P.A. Silver (2011). Engineering Cyanobacteria to Generate High-Value Products. *Trends Biotechnol.* **29**: 95–103. doi:10.1016/j.tibtech.2010.12.003.
- Dutta, D., D. De, S. Chaudhuri and S.K. Bhattacharya (2005). Hydrogen Production by Cyanobacteria. *Microb Cell Fact.*, **4**:1–11
- Gantzer, C.J. and W.J. Maier (1990). Biological Degradation of Cyanide by Nitrogen-Fixing Cyanobacteria. *National Service Center for Environmental Publications, Cincinnati*.
- Griffiths, M.J. and S.T.L. Harrison (2009). Lipid Productivity as a Key Characteristic for Choosing Algal Species for Biodiesel Production. *J. App. l. Physiol.*, **21**:493–507.
- Grobbelaar, J.U. (2000). Physiological and technological considerations for optimising mass algal cultures. *J. App. l. Physiol.*, **12**:201–206.
- Hall, D.O., S.A. Markov, Y. Watanabe and K.K. Rao (1995). The Potential Applications of Cyanobacterial Photosynthesis for Clean Technologies. *Photosynth Res.*, **46**:159–167.
- Hall, D.O. and P.A. Moss (1983). Biomass for Energy in Developing Countries. *Geojournal*, **7**(1):5–14.
- Huang, H.H., D. Camsund, P. Lindblad and T. Heidorn (2010). Design and Characterization of Molecular Tools for a Synthetic Biology Approach Towards Developing Cyanobacterial Biotechnology. *Nucleic Acids Res.*, **38**:2577–2593.
- Kaygusuz, K. (2009). Bioenergy as a Clean and Sustainable Fuel. Energy Sources, part A: Recovery, Utilization and Environmental Effects. *Energ. Sourc.*, **31**:1069–1080.
- Kenyon, C.N. (1972), Fatty acid composition of Unicellular Strains of Blue-Green Algae. *J. Bacteriol.*, **109**:827–834.
- Kenyon, C.N., R.Y. Stanier and R. Rippka (1972). Fatty Acid Comopostion and Physiological Properties of some Filamentous Blue-Green Algae. *Arch. Mikrobiol.*, **83**: 216–236,
- Lindberg, P., S. Park and A. Melis (2010). Engineering a Platform for Photosynthetic Isoprene Production in Cyanobacteria, using. *Synechocystis* as the model organism. *Metab. Eng.*, **12**:70–79.
- Machado, I.M.P. and S. Atsumi (2012). Cyanobacterial Biofuel Production. *J. Biotechnol.* **162**: 50–56. doi:10.1016/j.jbiotec.2012.03.005.
- McKinlay, J.B. and C.S. Harwood (2010). *Curr. Opin. Biotechnol.*, **21**:244–251.
- McNeely, K., Y. Xu, N. Bennette, D.A. Bryant and G.C. Dismukes (2010). Redirecting Reductant Flux Into Hydrogen Production via Metabolic Engineering of Fermentative Carbon Metabolism in a Cyanobacterium. *Appl. Environ. Microbiol.*, **76**:5032–5038.
- Miao, X.L. and Q.Y. Wu (2006). Biodiesel Production from Heterotrophic Microalgal Oil. *Bioresour Technol.*, **97**: 841–846.
- Nobles, D.R. and R.M. Brown (2008). Transgenic Expression of *Gluconacetobacterxylinus* Strain ATCC 53582 cellulose synthase genes in the cyanobacterium. *Synechococcusleopoliensis* strain UTCC 100. *Cellulose*, **15**:691–701.
- Olson, J.M. and R.E. Blankenship (2004). Thinking about the Evolution of Photosynthesis. *Photosynth Res.*, **80**: 373–386.
- Pace, N.R. (1997). A Molecular view of Microbial Diversity and the Biosphere. *Science*, **276**: 734–740.
- Rittmann, B.E. (2008). Opportunities for Renewable Bioenergy using Microorganisms. *Biotechnol Bioeng.* **100**(2): 203–212.
- Samson, R. and A. Leduy (1982). Biogas Production from Anaerobic-digestion of *Spirulina* algal Biomass. *Biotechnol Bioeng.*, **24**:1919–1924.
- Samson, R. and A. Leduy (1986). Detailed study of Anaerobic Digestion of *Spirulina maxima* Algal Biomass. *Biotechnol Bioeng.*, **28**:1014–1023.
- Schopf, J.W. and B.M. Packer (1987). Early Archean (3.3-

- Billion to 3.5-billion-year-old) Microfossils from Warrawoona Group, Australia. *Science*, **237**: 70-73.
- Schütz, K., T. Happe, O. Troshina and P. Lindblad (2004). Cyanobacterial H<sub>2</sub> Production—A Comparative Analysis. *Planta*, **218**:350–359.
- Sheehan, J., T. Dunahay, J. Benemann and P. Roessler (1998). A look back at the U.S. Department of Energy's. *Aquatic Species Program-Biodiesel from Algae Report*
- Stanier, R.Y. and G. Cohen-Bazire (1977). Phototrophic Prokaryotes: the Cyanobacteria. *Ann. Rev. Microbiol.* **31**: 255-274.
- Timmerman, L. (2009). Targeted Growth Plots future as Agricultural Biotech, *Cleantech Pioneer*. Xconomy.<http://www.xconomy.com/seattle/2009/07/24/targeted-growth-plots-future-as-agriculturalbiotech-cleantech-pioneer/>
- Ungerer, J., L. Tao, M. Davis, M. Ghirardi, P.C. Maness , and J. Yu (2012). Sustained Photosynthetic Conversion of CO<sub>2</sub> to Ethylene in recombinant Cyanobacterium. *Synechocystis* 6803. *Energy Environ. Sci.*, **5**: 8998–9006. doi:10. 1039/c2ee22555g
- Varel, V.H., T.H. Chen and A.G. Hashimoto (1988). Thermophilic and Mesophilic Methane Production from Anaerobic Degradation of the Cyanobacterium *Spirulina maxima*. *Resour Conservat Recycl*, **1**:19–26
- Vermaas, W.F.J. (2001). Photosynthesis and Respiration in Cyanobacteria. *Encyclopedia of life Sciences*. Wiley, New York 1–7