Microalgae: a renewable source for second generation biofuels

Energy security has become a national issue and solemn attempts are being made to seek viable alternatives in the form of renewable energy to meet the futuristic needs. In fact 85% of the energy, which we use is obtained from the fossil fuels as energy source in the form of oil, coal and natural gas. This situation makes us to solely depend on fossil fuels to sustain the energy requirements. But the alarming common concern of today's world is that the natural source of fossil fuel is finite and is depleting rapidly due to uncontrolled consumption. This indicates the non-renewable nature of fossil fuels and it is important to have a renewable source as an alternate energy source. Renewable energy is any natural source that can replenish itself naturally over a short span of time. Renewable energy obtained from commonly known sources such as solar power, wind, running water, geothermal energy, etc.

In order to have energy security, India is committed to use renewable bio-energy sources to supplement its energy requirements. The Planning Commission of India[1] brought out a report on the development of biofuels in 2003. The National Biodiesel Commission was set up to look exclusively into issues pertaining to biodiesel and development of *Jatropha curcas* as feedstock for biodiesel production. The National Policy on Biofuel[2] has been proposed a blending target of ethanol (10%) and bio-diesel (20%) with petro-diesel for 2011–2012.

To achieve the national target of 20% blending, the country requires about 13.4 million tonnes of biodiesel. Although *J. curcas*, a non-edible oil-producing crop, has great potential for biodiesel production, it will take sometime to achieve the target depending on just one source. Therefore, in addition to Jatropha, other non-edible sources, as feedstock for biodiesel is necessary to achieve the target well in time. In this regard, algae-based biodiesel has gained a lot of potential in the current scenario. Microalgae are photosynthetic organisms, which convert sunlight, water and CO_2 to sugars; from these macromolecules, lipids and triacylglycerols can be obtained. Microalgae are widespread in diverse environmental niches which include fresh water, brackish, marine and hypersaline with a range of temperature and pH (ref. 3). Microalgae provide an excellent source of lipids for two major reasons. First, microalgae productivity can be an order of magnitude greater than the terrestrial vegetation used for biofuel feedstock. Second, the lipid content of microalgae can exceed 70% of their dry mass, although algae with lipid content of around 30% is more common[4]. Chemically algal biomass constitutes approximately about 60% natural stored lipids; rest is protein, carbohydrate and other nutrients. Thus, algae species can produce up to 60% of their body weight as stored lipids in the form of triacylglycerol[4]. Because of its unique ability to multiply fast with higher lipid content, it is highly significant to exploit these tiny microalgae for the production of biodiesel. High productivity combined with high lipid content results in a large amount of lipid that can be harvested periodically for biodiesel production. A wide range of oil content (4–80%) has been reported in different algal species, for example *Spirulina maxima* having 4–9%, whereas *Botryococcus braunii* have 25–80%[4]. Microalga has shown more than 250-fold higher biodiesel production when compared to first generation biofuel crops like corn and canola[5]. Recent and earlier reports reveal that oil content in microalgae can exceed up to 80% by weight of dry biomass[6,7]. By considering the above facts, microalgae appear to be the only source of biodiesel that has the potential to substitute the depleting fossil fuel sources[5]. Moreover algae do not compete with the inputs and natural resources, which are meant for production of food and oil seed crops. However, research dealing with improving production systems, identifying ideal microalgae for biofuel production and studying the sustainability of microalgal biofuel is necessary before large-scale microalgal biofuel operations are established.

Microalgae are one of the potential sources of biofuel feedstock due to several unique properties such as synthesis and accumulate large quantities of neutral lipids (20–50% of dry cell weight), renewable with higher multiplication rate (cell doubling time varies from 8 to 24 h), thrive in saline/brackish water/coastal seawater[3], utilize nutrients from a variety of agricultural, industrial and municipal wastewater with an additional benefit of wastewater bioremediation[8], sequester CO_2 from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing emission of major greenhouse gas (CO_2), produces valuable by-products (proteins, polysaccharides, pigments, animal feed, manure, hydrogen and biopolymers) and grows in culture vessels successfully throughout the year with high biomass productivity.

The Defence Institute of Bio-Energy Research (DIBER) along with other participating DRDO labs has undertaken a project 'DRDO – Army Biodiesel Programme' to fulfil the energy security mainly focusing on non-edible oil-bearing feedstock. DIBER actively engaged in research for developing technologies for mass-scale cultivation of non-edible oil-bearing bio-energy crops such as Jatropha (*J. curcas*), Cheura (*Diploknema butterissia*), Camelina (*Camelina sativa*), etc. along with research for enhancing seed yield and oil content by conventional breeding and genetic engineering approach. In this regard, R&D on algal biofuel has also been initiated with the prime objective of identifying an ideal locally adapted strain, which can withstand the varying geo-physical environmental conditions, i.e. temperature (day/night) and higher altitude (5500′) in order to produce biodiesel on a large scale to meet future demand. Therefore, work has been taken up to collect the locally adapted algal strain, culture them in open pond condition and evaluate oil productivity with less energy consuming harvesting techniques.

All the algal strains have been collected from Pithoragarh (Uttrakhand), situated in Central Himalayas, which extends from 29°29′–30°49′N lat. to 85°05′–81°31′E long. The annual rainfall is around 1250 mm, out of which 70–75% is received during rainy season. The temperature of Pithoragarh ranges from 35°C in summer to a level of –2°C in winter[9]. In general, algal strains were collected from 1400 to 1990 m msl, sunny and freshwater sources with their native pH ranging from 6.07 to 7.31. Of the collected algal samples (46), two locally adapted algal strains *Chlorococcum humicola* and *Scenedesmus bijugatus*

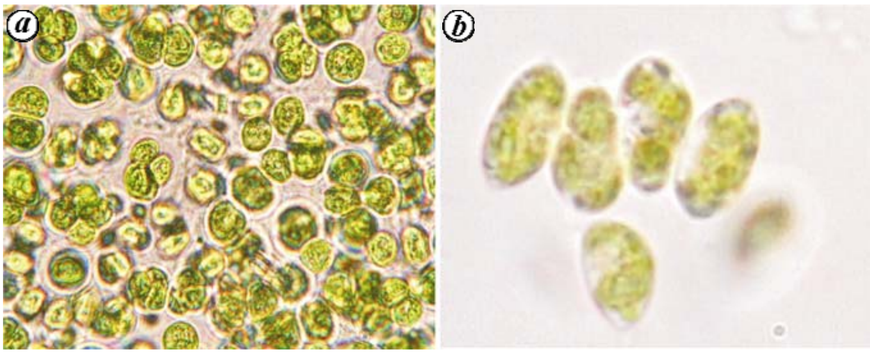

Figure 1. ***a***, *Chlorococcum humicola* and ***b***, *Scenedesmus bijugatus*.

Table 1. Biomass yield and productivity of locally adopted algal strains

Algal strain	Biomass productivity as dry weight (mg/l/day)	Total lipid productivity (mg/l/day)	Biomass yield as dry weight (kg/ha/day)
Scenedesmus bijugatus	150	17.7	450
Chlorococcum humicola	70	7.0	210

(Figure 1) were grown in open pond condition utilizing the naturally prevailing environmental conditions. These algal samples were grown for 15 days, biomass harvested by natural settling method and biomass was dried in sunlight till a crisp dry stage. The biomass was quantified gravimetrically and multiplication rate expressed as biomass productivity. Total lipids were extracted using chloroform : methanol (2 : 1 v/v) by soxhlet apparatus and the total lipid content was calculated gravimetrically and was expressed as mg/l/day. The biomass productivity of 150 mg/l/day and total lipid productivity of 17.7 mg/l/day were observed for *S. bijugatus* whereas for *C. humicola*, the values were 70 mg/l/day and 7.0 mg/l/day respectively (Table 1). The results are in conformity with Yoo *et al.*[10] who reported *Scenedesmus* sp. as a potential algal strain with the biomass productivity of 217 mg/l/day and total lipid productivity of 20.65 mg/l/day. Rajan *et al.*[11] also reported the potentiality of *C. humicola* for biodiesel production.

The potential benefits of large-scale production of microalgae for biofuels are more promising than the concerns raised for utilization of resources and freshwater with capital investment. At the moment, microalgae appear to be one of the best viable options for biofuel feedstock because of their tremendous productivity, ability to use wastewater in their production and the valuable by-products that can be produced. Despite having tremendous potential, technology for large-scale cultivation is still in R&D phase. Efforts are being made at DIBER, DRDO to evolve a low cost, easily available growth media to overcome the major limitation of algal biomass production in large scale. The selection of region-specific ideal algal strain along with low-cost nutrient medium will definitely boost the adoption of this technology for biofuel production provided that the other associated technology for processing of algal biomass is being standardized, economized and simplified.

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