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Review

Cyanobacteria and microalgae: A positive prospect for biofuels

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ABSTRACT

Biofuel–bioenergy production has generated intensive interest due to increased concern regarding limited petroleum-based fuel supplies and their contribution to atmospheric CO_2 levels. Biofuel research is not just a matter of finding the right type of biomass and converting it to fuel, but it must also be economically sustainable on large-scale. Several aspects of cyanobacteria and microalgae such as oxygenic photosynthesis, high per-acre productivity, non-food based feedstock, growth on non-productive and non-arable land, utilization of wide variety of water sources (fresh, brackish, seawater and wastewater) and production of valuable co-products along with biofuels have combined to capture the interest of researchers and entrepreneurs. Currently, worldwide biofuels mainly in focus include biohydrogen, bioethanol, biodiesel and biogas. This review focuses on cultivation and harvesting of cyanobacteria and microalgae, possible biofuels and co-products, challenges for cyanobacterial and microalgal biofuels and the approaches of genetic engineering and modifications to increase biofuel production.

1. Introduction

Human society has an insatiable appetite for fuels and today's supply of liquid fuels worldwide is almost completely dependent on petroleum. Bioenergy production has recently become a topic of intense interest due to increased concern regarding limited petroleum-based fuel supplies and the contribution of the use of these fuels to atmospheric CO_2 levels. Finding sufficient supplies of clean energy for the future society is one of the most daunting challenges and is intimately linked with global stability, economic prosperity and quality of life. This leads to interesting questions and debate over the choice of new fuels, produced from new raw materials to complement or replace present petroleum-based fuels (Posten and Schaub, 2009).

Biofuel research is not just a matter of finding the right type of biomass and converting it to fuel, but it must also find environmentally and economically sound uses for the by-products of biofuel production. Biofuels target a much larger fuel market and so in the future will play an increasingly important role in maintaining energy security. Currently, fuels make up approximately 70% of the global final energy market. In contrast, global electricity demand accounts for only 30% (Hankamer et al., 2007). Yet, despite the importance of fuels, almost all CO_2 free energy production systems under development are designed to drive electricity generation (e.g., nuclear, photovoltaic, wind, geothermal, wave and hydroelectric). Given the above situation, there is presently a debate as to which fuels from biomass with their yield potentials appear most attractive. Several biofuel candidates were proposed to displace fossil fuels in order to eliminate the vulnerability of energy sector (Korres et al., 2010; Singh et al., 2011b). Much of the discussion over biofuels production has focused on higher plants such as corn, sugarcane, soyabean, algae, oil-palm and others (Pandey, 2008; Gnansounou et al., 2008) and the problems associated with their use, such as the loss of ecosystems or increase in the food prices. While most bioenergy options fail on both counts, several microorganism-based options have the potential to produce large amounts of renewable energy without disruptions. Cyanobacteria and their superior photosynthesis capabilities can convert up to 10% of the sun's energy into biomass, compared to the 1% recorded by conventional energy crops such as corn or sugarcane, or the 5% achieved by algae. Photosynthetic microorganisms like cyanobacteria and microalgae can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner and at rates high enough to replace a substantial fraction of our society's use of fossil fuels (Li et al., 2008).

There are several aspects of cyanobacterial and microalgal biofuel production that have combined to capture the interest of researchers and entrepreneurs around the world. These include: (1) They are able to perform oxygenic photosynthesis using water

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as an electron donor, (2) They grow to high densities and have high per-acre productivity compared to typical terrestrial oil-seed crops. Consequently, mass cultivation for commercial production of cyanobacteria can be performed efficiently, (3) They are non-food based feedstock resources, (4) They use otherwise non-productive, non-arable land, (5) They utilize wide variety of water sources (fresh, brackish, seawater and wastewater) (Tamagnini et al., 2007), and (6) They produce both biofuels and valuable co-products.

Cyanobacteria are oxygenic photosynthetic bacteria that have significant roles in global biological carbon sequestration, oxygen production and the nitrogen cycle. Cyanobacteria can be developed as an excellent microbial cell factory that can harvest solar energy and convert atmospheric CO_2 to useful products. Fossil traces of cyanobacteria are claimed to have been found from around 3.5 billion years ago, and most probably played a key role in the formation of atmospheric oxygen, and are thought to have evolved into present-day chloroplasts of algae and green plants (Tamagnini et al., 2007). Cyanobacteria, also known as blue-green algae, exhibit diversity in metabolism and structure also along with morphology and habitat. Moreover, cyanobacteria and microalgae have simple growth requirements, and use light, carbon dioxide and other inorganic nutrients efficiently. Cyanobacteria and microalgae are the only organisms known so far that are capable of both oxygenic photosynthesis and hydrogen production. Photobiological production of H_2 by microorganisms is of great public interest because it promises a renewable energy carrier from nature's most plentiful resources: solar energy and water. They have been investigated to produce different feed stocks for energy generation like hydrogen (by direct synthesis in cyanobacteria), lipids for biodi[illegible] and jet fuel production, hydrocarbons and isoprenoids for gaso[illegible] production and carbohydrates for ethanol production. Beyond th[illegible] the complete algal biomass can also be processed for syngas pro[illegible]duction followed or not by a fischer–tropsch process[illegible]hermal gasification for hydrogen or methane production[illegible]thane[illegible]oduction by anaerobic digestion, and co-combustio[illegible] electr[illegible] production. Hence, cyanobacterial and microalg[illegible] contribute to a sustainable bioenergy pr[illegible]ction. [illegible]ever different biotechnical, environmental and [illegible]omic challe[illegible] have to be overcome before energy product[illegible]ese systems [illegible] enter the market.

The objective of this review [illegible]ticle is to giv[illegible] overview of cyanobacteria and microalgae [illegible] prospective sour[illegible]or potential future biofuels (biohydrog[illegible]bioeth[illegible], biodiesel and biomethane), the brief outline of the p[illegible]sse[illegible]volved in biofuel production, i.e. cultivation, downstream p[illegible]sing, ex[illegible]tion and fractionation, and biofuels c[illegible]n tec[illegible]logi[illegible] genetic engineering and modification[illegible] cya[illegible]acteria/[illegible]algae for biofuel–bioenergy productio[illegible]d the c[illegible]enges of cyanobacteria and microalgal cultivation for [illegible]

2. The cyanobacter[illegible]icroalgae-to-biofuels opportunity

The schematic diagram for cyanobacteria/microalgae-to-biofuel opportunities has been shown in Fig 1. The overall system for producing biofuels includes growth of primary biomass and the processing of biomass. Research in the last six decades has demonstrated that cyanobacteria and algae produce a diverse array of chemical intermediates and hydrocarbons, precursors to biofuels. Hence, cyanobacteria-to-fuel offers promise as potential substitute for products currently derived from fossil fuels.

Cyanobacterial biomass can be directly used as food source or various feedstock. Various important biomolecules such as antioxidants, coloring agents, pharmaceuticals and bioactive compounds can be obtained. Biomass can be converted to biomethane (biogas) on anaerobic digestion. Cyanobacterial photosynthetic system is able to diverge the electrons emerging from two primary reactions, directly into the production of H_2. Calvin cycle leads to the production of carbohydrates, proteins, lipids and fatty acids. Carbohydrates can be converted into bio-ethanol by fermentation. Lipids can be converted into biodiesel. Fatty acids on fermentation form acetate, butyrate and propionate which on stabilization form CH_4, H_2 and e^-.

3. Cultivation and down-stream processing of cyanobacteria and microalgae

The process of cultivation, harvesti[illegible]and proces[illegible] of biomass has been described in details in other [illegible]ws (Singh [illegible]l., 2011a). In this review, we have present[illegible]a brie[illegible]ount of [illegible] processes in general.

Harvesting solar energ[illegible]a pho[illegible]ynthesi[illegible]one of the nature's remarkable achiev[illegible]nts[illegible]anobacteria and microalgae capture light energy[illegible]ough[illegible]osynthe[illegible]nd convert inorganic substances into si[illegible]le sugars[illegible]g c[illegible]ured energy. The prime factors that de[illegible] the grow[illegible] of cyanobacteria/microalgae are ligh[illegible]idea[illegible]mperature, [illegible]medium, aeration, pH, CO_2 requirements and ligh[illegible] dark periods. Some of the important nutrie[illegible] the growth[illegible]cyanobacteria/microalgae are NaCl, NaN[illegible], $MgSO_4$, $CaCl_2$, KH_2P[illegible]$_4$, citric acid and trace metals.

3.1. [illegible]tivation

Exte[illegible]dies have been carried out for the cultivation of [illegible]erent cyanobacteria and microalgae using a variety of cultivat[illegible]ms ranging from closely-controlled laboratory methods [illegible]less predictable methods in outdoor tanks.

The most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds (Oron et al., 1979; Seshadri and Thomas, 1979; Vonshak et al., 1985). One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems (Borowitzka, 1999). However, major limitations in open ponds include poor light utilization by the cells, evaporative losses, diffusion of CO_2 to the atmosphere and requirement of large amounts of water and land and low biomass productivity (Posten and Schaub, 2009). Furthermore the water medium has to provide extremophilic conditions to some extent, otherwise the cultivated species will be outcompeted by other algae or diminished by predator organisms.

An alternative to open ponds are closed ponds where the control over the environment is much better than that for the open ponds. Closed pond systems are more cost intensive than the open ponds, and considerably less than photobioreactors for similar areas of operation. It allows more species to be grown, it allows the species that are being grown to stay dominant, and it extends the growing season. Usually closed ponds are used in *Spirulina* cultivation (Santillan, 1982).

Closed bioreactors have some specific advantages (Pulz, 2001; Posten and Schaub, 2009). Firstly, they can distribute the sun light over a larger surface area, which can be up to 10 times higher than the footprint area of the reactor. Secondly, evaporation can be avoided. The only water loss is due to the water content in the wet cyanobacteria product. This allows for the cultivation of cyanobacteria also in arid areas, where classical terrestrial agriculture is not possible. Limiting factors are the high reactor costs and the need for auxiliary energy requirements. However, ongoing research in the reactor field is promising and will lead to cheaper and more energy-effective designs (Posten and Schaub, 2009).

The cultivation of cyanobacteria/microalgae in sewage and wastewater treatment plant is expected to bring double benefit

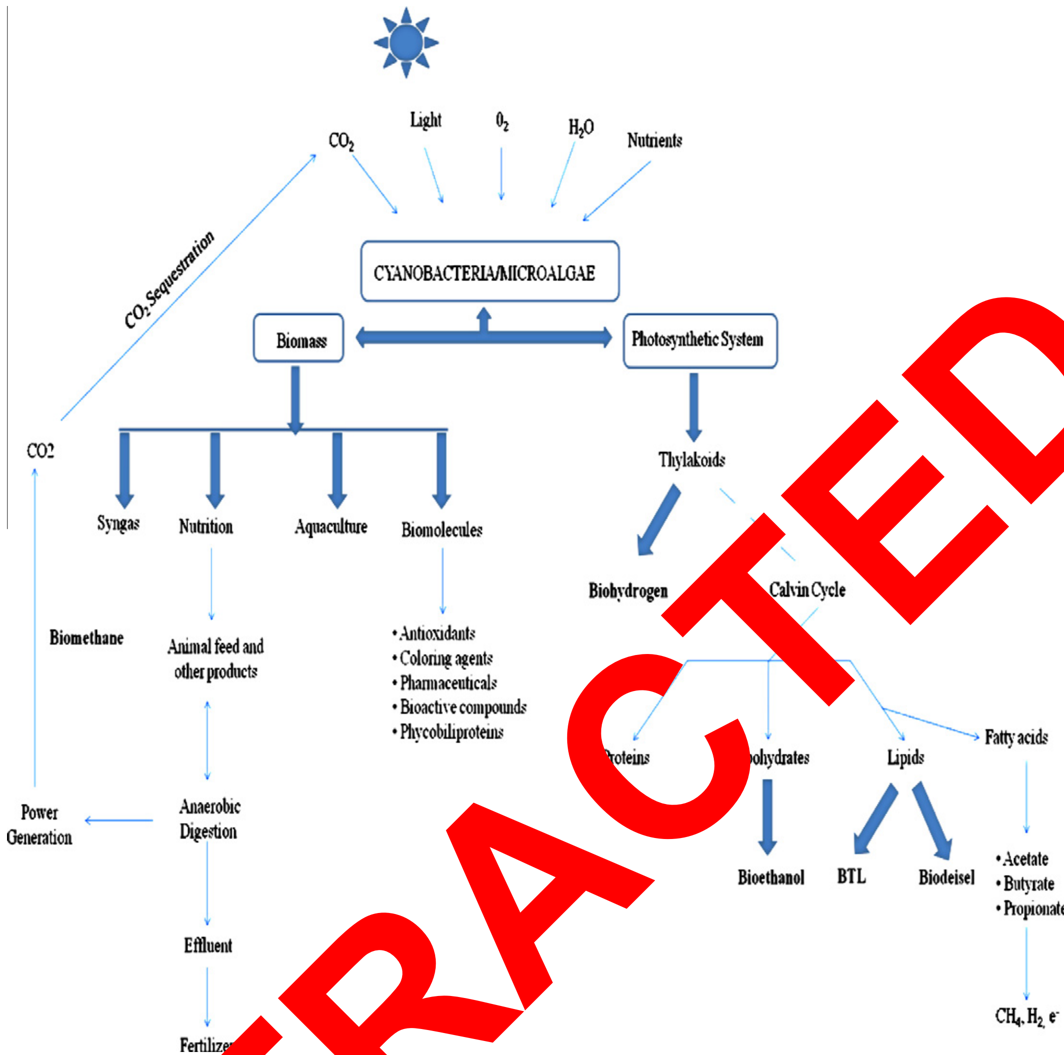

Fig. 1. Op[illegible]ities of cyanob[illegible] and microalgae for production of various biofuels and co-products.

to the environment since th[illegible] they can [illegible]ed to extract nutrients from waste water, [illegible] convert it to [illegible] for biodiesel production and reduces [illegible]llution from the [illegible]mosphere. Unlike other algal-biofuel t[illegible]ologi[illegible] this approach relies on 'wild algae' – i.e. algae that [illegible]y colo[illegible] sewage ponds already (Metcalf and E[illegible] 1980). [illegible]ther [illegible]nomical way of cultivating cyanobacte[illegible] [illegible]gae is [illegible]ater (salt water). The main nutrients [illegible]eded f[illegible] their grow[illegible] is already present in seawater. Seawate[illegible] a s[illegible] [illegible]alts of nearly constant composition, dissolved [illegible]ble amounts of water. There are over 70 elements dissol[illegible]n seawater with six of them make up >99% of all the dissolve[illegible]lts; all occur as ions – electrically charged atoms or groups o[illegible] atoms (Sodium (Na^+), Chlorine (Cl^-), Magnesium (Mg^{2+}), Potassium (K^+), Sulfate (SO_4^{2-}) and Calcium (Ca^{2+})) (Matsunaga et al., 2005).

3.2. Downstream processing of cultures

Obtaining fuels from cyanobacterial cultures requires processing steps such as harvesting, dewatering and extraction of fuel precursors. The selection of downstream processes depends on type of culture, feedstock and on desired product. High water content and high N and P content is the major limitation in downstream processing of cyanobacteria and microalgae. Besides these, other economical and practical issues such as energy costs, plant site, transportation, water quality and recycling issues have to be considered to make a feasible cyanobacteria-to-fuel strategy.

3.2.1. Harvesting of cyanobacteria and microalgae

The term harvesting refers to concentration of cyanobacterial/algal suspensions till a thick paste/dry mass is obtained depending on the need for the desired product. The main methods involve filtration, centrifugation, sedimentation and flotation.

Filtration, a conceptually simple process, is carried out commonly on membranes of various kinds with the aid of suction pump. The greatest advantage is that it is able to collect cells of very low density. However, various issues such as clogging of filter (Borchard and Omelia, 1961), appropriate pore size, recovery efficiency of cell mass and washing requirements, have been the biggest hindrances till now. Several methods such as reverse-flow vacuum, direct vacuum with a stirring blade above filter to prevent particles from settling and other changes in filtration design are making this process economically feasible (Danguah et al., 2008). Centrifugation is a method of settling the cells to the bottom by applying the centrifugal force. The biggest concern for centrifugation technologies is high throughput processing of large quantities of water and cultures. Centrifugation techniques are expensive initially but for commercial and industrial scale on long term basis they are economically feasible (Golueke and Oswald, 1965). Flocculation is a technique where in flocculants (chemical additives) are added to increase the size of the cell aggregates. Alum, lime, cellulose, salts, polyacrylamide polymers, surfactants, chitosan, etc. are some chemical additives that have been studied. Manipulating suspension pH (Sukenik et al., 1985) and bioflocculation (co-culturing with another organism) (Golueke and Oswald, 1965) are the other options to the chemical additives. Flocculation is always followed by either sedimentation or flotation. Naturally, flocculation leads to sedimentation in many older cultures, otherwise forced flocculation is required to promote sedimentation. To induce flotation, air is bubbled through the cell suspension causing cell clusters to float to the surface and top layer is removed as a scum (Parker, 1975).

3.2.2. Dewatering and drying

Dewatering and drying are used to achieve higher dry mass concentrations. Drum dryers (Prakash et al., 1997) and other oven-type dryers (Desmorieux and Decaen, 2006) are used to provide heat required for drying. However, the costs climb steeply with the increase in time and temperature. Air-drying is also possible in low humidity environment but this requires extra space and considerable time. Solutions involving either solar and wind energy are also possible.

3.2.3. Extraction and separation

Cyanobacteria and microalgae differ from traditional biomass feed stocks in several respects, such as cell wall chemistry, presence of large amounts of water and smaller cell size. These differences highlight the importance of the specific extraction techniques. Various methods like mechanical, chemical and enzymatic are applicable for extraction of biomass/biofuel.

Cell structure presents a formidable barrier for access to the molecules. This generally requires that the biomass must be mechanically disrupted prior to any further processing. The most common of these are (a) freezing and thawing ([illegible] et al., 2009; Parmar et al., 2010), (b) grinding cells while frozen in liquid nitrogen (Soni et al., 2008), (c) lyophilization followed by grinding, (d) pressing (with expeller), (e) ultrasonication, (f) bead mill and (g) homogenizers. Chemical methods include (a) hexane solvent method (Cartens et al., 1996), (b) soxhlet extraction (hexane/petroleum ether) (Park et al., [illegible]), (c) two solvent systems (Lewis et al., 2000), (d) supercritical fluid extraction (methanol or CO_2) (Herraro et al., 2006), (e) accelerated solvent extraction (high pressure) (Schafer, 1998), (f) subcritical water extraction (Metting et al., 1990; Ayala and Castro, 2001), (g) milking (two phase system of aqueous and organic phases) (Hejazi et al., 2002) and (h) transesterification (Carvalho and Malcata, [illegible]). Enzymatic extraction uses enzymes to degrade cell walls, making fractionation much easier. However, cost of this extraction process are as of now making this process not viable. Osmotic shock is a sudden change in osmotic pressure, which can cause cells in a solution to rupture. Osmotic shock leads to release of cellular components.

In the existing marketplace, the number of companies producing algal-based products is quite modest. Most of these companies focus on cultivating blue-green algae for food supplements, beta-carotene and related pigments for the nutraceuticals and food markets (Olaizola, 2003). The cyanobacteria are harvested, dried and formulated into pellets, pills, or powders for consumption. Pigments and other nutraceuticals can be further extracted by grinding or ball milling the dried cyanobacteria. Commercially cyanobacteria are grown at large scale and are harvested using the cell itself as the finished product.

4. Biofuels and co-products from cyanobacteria and microalgae

Cyanobacteria are a diverse group of prokaryotic photosynthetic microorganisms that can grow rapidly due to their simple structures. They have been investigated for the production of different biofuels including biohydrogen, biodiesel, bioethanol and biomethane. Cyanobacterial biofuel production is potentially sustainable. To make biofuel production economically viable we also need to use remaining algal biomass for co-products of commercial interests. It is possible to produce adequate cyanobacterial biofuels to satisfy the fast growing demand within the restraints of land and water resources. The flowchart representing the cultivation, downstream processing and production of biofuels along with co-products from cyanobacteria and microalgae has been shown in Fig. 2.

4.1. Biohydrogen

Hydrogen gas is seen as a future energy carrier by virtue of the fact that it is renewable, does not evolve the "greenhouse gas" CO_2 in combustion, liberates large amounts of energy per unit weight in combustion. Biological hydrogen production has several advantages over hydrogen production by photoelectrochemical or thermochemical processes. Biological hydrogen production by photosynthetic microorganisms for example requires the use of a simple solar reactor such as a transparent closed box, with low energy requirements. Electrochemical hydrogen production via solar battery-based water splitting on the other hand, requires the use of solar batteries with high energy requirements.

Cyanobacteria can be used for the production of molecular hydrogen (H_2), a possible future energy carrier, has been the subject of several recent reviews (Levin et al., 2004; Sakurai and Masukawa, 2007; Tamagnini et al., 2007). Cyanobacteria are able to divert the electrons emerging from the two primary reactions of oxygenic photosynthesis directly into the production of H_2, making them attractive for the production of renewable H_2 from solar energy and water. In cyanobacteria, two natural pathways for H_2 production can be used: first, H_2-production as a by-product during nitrogen fixation by nitrogenases; and second, H_2-production directly by bidirectional hydrogenase (Angermayr et al., 2009). Nitrogenases require ATP whereas bidirectional hydrogenases do not require ATP for H_2-production, hence making them more efficient and favorable for H_2-production with a much higher turnover.

The fundamental aspects of cyanobacterial hydrogenases, and their more applied potential use as future producers of renewable H_2 from sun and water, are receiving increased international attention. At the same time, significant progress is being made in the understanding of the molecular regulation of the genes encoding both the enzymes as well as the accessory proteins needed for the correct assembly of an active hydrogenase. With the increasing interest of both scientific and public community in clean and renewable energy sources, and consequent funding opportunities, rapid progress will be made in the fundamental understanding of the regulation of cyanobacterial hydrogenases at both genetic and proteomic levels.

Bandyopadhyay et al. (2010) have described *Cyanothece* sp. ATCC 51142, a unicellular, diazotrophic cyanobacterium with capacity to generate high levels of hydrogen under aerobic conditions. Wild-type *Cyanothece* sp. 51142 can produce hydrogen at rates as high as 465 μmol/mg of chlorophyll/h in the presence of glycerol. Authors also report that hydrogen production in this strain is mediated by an efficient nitrogenase system, which can be manipulated to convert solar energy into hydrogen at rates that are several fold higher, compared to other previously described wild-type hydrogen-producing photosynthetic microbes.

4.2. Bioethanol

Cyanobacteria and algae are capable of secreting glucose and sucrose. These simple sugars by anaerobic fermentation under dark

Fig. 2. The flowchart depicting the cultivation, downstream processing and production of biofuels and co-products from cyanobacteria and microalgae.

conditions to produce ethanol. If ethanol can be extracted directly from the culture media, the process may be drastically less capital- and energy-intensive than competitive biofuel processes. The process would potentially eliminate the need to separate the biomass from water and extract and process the oils. Professor R. Malcolm Brown Jr. and Dr. David Nobles Jr. said that 'The cyanobacterium is potentially a very inexpensive source for sugars to use for ethanol' and hypothesized that they could produce an equal amount of ethanol using an area half that size with the cyanobacteria based on current levels of productivity in the lab, but they caution that there is a lot of work ahead before cyanobacteria can provide such fuel in the field. Work with laboratory scale photobioreactors has shown the potential for a 17-fold increase in productivity. But this will be significant only if it can be achieved in the field and on a large scale.

Another approach, 'Photanol', employs nature's mechanisms of capturing solar energy to convert this energy into the reducing power of fermentation end products by highly efficient pathways of fermentative metabolism. Most importantly, this type of metabolism, which we refer to as 'photofermentation', involves a minimal number of steps in the conversion of CO_2 to biofuel, by bypassing the formation of the complex set of molecules of biomass. Therefore, the theoretical efficiency of biofuels production, expressed as liter of biofuel produced per unit of surface area per year can be significantly increased (Angermayr et al., 2009).

Bioethanol could be very important to foster energy independence and reduce greenhouse gas emissions. A very strong debate on gradual substitution of petroleum by use of renewable alternatives such as biofuels dominates the political and economic agenda worldwide (Demain, 2009). Alternative bioethanol production methods from cyanobacteria and microalgae need to be developed so that the costs associated with the land, labor and time of traditionally fermented crops can be circumvented.

Ueda et al. (1996) have patented a two-stage process for microalgae fermentation. In the first stage, microalgae undergo fermentation in anaerobic environment to produce ethanol. The CO_2 produced in the fermentation process can be recycled in algae cultivation as a nutrient. The second stage involves utilization of remaining algal biomass for production of methane, by anaerobic digestion process, which can further be converted to produce electricity. Bush and Hall (2006) pointed out that the patented process of Ueda et al. (1996) was not commercially scalable due to the limitations of single cell free floating algae. They patented a modified fermentation process wherein yeasts, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*, were added to algae fermentation broth for ethanol production.

Recently Harun et al. (2010) have studied the suitability of microalgae (*Chlorococum* sp.) as a substrate, using yeast for bioethanol production by fermentation. They achieved a productivity level of around 38% weight which supports the suitability of microalgae as a promising substrate for bioethanol production.

4.3. Biodiesel

Biodiesel is usually produced from oleaginous crops, such as rapeseed, soybean, sunflower and from palm, by a mono-alcoholic transesterification process, in which triglycerides reacts with a mono-alcohol (most commonly methanol or ethanol) with the catalysis of enzymes (Hankamer et al., 2007; Li et al., 2008). However, the use of microalgae and cyanobacteria can be a suitable alternative because algae are the most efficient biological producer of oil on the planet and a versatile biomass source and may soon be one of the Earth's most important renewable fuel crops (Li et al., 2008). Biodiesel from the photosynthetic algae which grow on CO_2 has great potential as a biofuel. These organisms are being seriously considered as a substitute for plant oils to make biodiesel. Producing biodiesel from algae provides the highest net energy because converting oil into biodiesel is much less energy-intensive than methods for conversion to other fuels. This characteristic has made biodiesel the favorite end-product from algae. Producing biodiesel from algae requires selecting high-oil content strains, and devising cost effective methods of harvesting, oil extraction and conversion of oil to biodiesel.

Singh and Gu (2010) in their review article have compared the biodiesel yields from microalgae with other best feedstocks [illegible] diesel yield is 58,700 l/ha from microalgae containing only 30% oil (w/w), compared to 1190 l/ha for rapeseed and canola (Schenk et al., 2008); 1892 l/ha for jatropha ([illegible] 2007); 2590 l/ha for karanj (*Pongamia pinnata*) (Lele, http://www.[illegible]le.com/karanj.htm); 172 l/ha for corn; 446 l/ha for Soybean; [illegible] l/ha for Peanut; 2689 l/ha for coconut; 595[illegible] l/ha for oil palm.

Chisti (2007) discussed the economics and quality constraints of biodiesel from microalgae in his review paper. He pointed out that the cost of growing microalgae for biofuel production must be drastically reduced to compete directly with traditional energy sources. It is essential to consider the other roles cyanobacterial cultures can play concurrently with biofuel production and the long term benefits [illegible] (Chisti, 2007).

The economics of biodiesel production could be improved by advances in the production technology. Specific outstanding technological issues are efficient methods for recovering the algal biomass from the dilute broths produced in photobioreactors. A different and complimentary approach to increase productivity of cyanobacteria is via genetic and metabolic engineering. This approach is likely to have the greatest impact on improving the economics of production of microalgal diesel (Hankamer et al., 2007). In Washington State, Targeted Growth announced it has developed a process to increase the lipid content of cyanobacteria by approximately 400%.

4.4. Biomethane

Organic material like biomass can be used to produce biogas via anaerobic digestion and fermentation (Hankamer et al., 2007). Organic biopolymers (i.e. carbohydrates, lipids and proteins) are hydrolyzed and broken down into monomers, which are then converted into a methane-rich gas via fermentation. Carbon dioxide is the second main component found in biogas (approximately 25–50%) and, like other interfering impurities, has to be removed before the methane is used (Hankamer et al., 2007). Methane in the form of compressed natural gas is used as a vehicle fuel, and is claimed to be more environmentally friendly than fossil fuels such as gasoline/petrol and diesel.

The research work of Converti et al. (2009) showed biogas production and purification by a two-step bench-scale biological system, consisting of fed-batch pulse-feeding anaerobic digestion of mixed sludge, followed by methane enrichment of biogas by the use of the cyanobacterium *Arthrospira platensis*. The composition of biogas was nearly constant, and methane and carbon dioxide percentages ranged between [illegible]–76.0% and 13.2–[illegible]%, respectively. The data of carbon dioxide removal from biogas revealed the existence of a linear relationship between the rates of *A. platensis* growth and carbon dioxide removal from biogas and allowed calculating carbon utilization efficiency for biomass production of almost 95% (Converti et al., 2009). [illegible]veth (2005) reported that *Laminaria* sp. produced methane yield of 0.26–0.28 $m^3\ kg^{-1}$. Otsuka and Yoshino (2004) used constant temperature (mesophilic) for anaerobic digestion of [illegible] sp. and found 180 ml/g of methane yield.

4.5. Co-products

To make biofuels economically viable, using appropriate technologies, the primary components of algal biomass – carbohydrates, lipids (oils), proteins and a variety of inorganic and complex organic molecules – must be converted into different products, either through chemical, enzymatic or microbial conversion means. The nature of the end products and of the technologies to be employed will be determined, primarily by the economics of the system, and they may vary from region to region according to the cost of the raw material (Willke and Vorlop, 2004).

A large number of different commercial products have been derived from cyanobacteria and microalgae. These include products for human and animal nutrition, poly-unsaturated fatty acids, anti-oxidants, coloring substances, fertilizers and soil conditioners, and a variety of specialty products such as bioflocculants, biodegradable polymers, cosmetics, pharmaceuticals, polysaccharides and stable isotopes for research purposes.

4.5.1. Nutrition

The consumption of cyanobacterial and microalgal biomass as a human health food supplement is currently restricted to only a few species, e.g., *Spirulina* (*Arthospira*), *Chlorella*, *Dunalliella*, and to a lesser extent, *Nostoc* and *Aphanizomenon* (Spolaore et al., 2006). However, the market is expected to grow in the future.

Microalgae and cyanobacteria are also used as feed in the aquaculture of mollusks, crustaceans (shrimp) and fish (Beneman, 1990). Most frequently used species are *Chaetoceros, Chlorella, Dunaliella, Isochrysis, Nannochloropsis, Nitzschia, Pavlova, Phaeodactylum, Scenedesmus, Skeletonema, Spirulina, Tetraselmis* and *Thalassiosira*. Both the protein content and the level of unsaturated fatty acids determine the nutritional value of microalgal aquaculture feeds.

Microalgal and cyanobacterial biomass have also been used with good results (i.e. better immune response, fertility, appearance, weight gain, etc.) as a feed additive for cows, horses, pigs, poultry, and even dogs and cats. In poultry rations, biomass up to a level of 5–10% (wt) can be safely used as a partial replacement for conventional proteins (Spolaore et al., 2006). The main species used in animal feed are *Spirulina, Chlorella* and *Scenesdesmus*.

4.5.2. Fertilizers

Cyanobacterial and microalgal biomass are used as a plant fertilizer and to improve the water-binding capacity and mineral composition of depleted soils (Metting et al., 1990). Moreover the effluent generated during anaerobic digestion for biomethane production can also be used as a fertilizer.

4.5.3. Biomolecules

Phycobiliproteins, phycoerythrin, phycocyanin and allophycocyanin produced by the cyanobacteria are used as food dyes, pigments in cosmetics, and as fluorescent reagents in clinical or research laboratories (Spolaore et al., 2006; Singh et al., 2009; Parmar et al., 2010, 2011). Microalgae-produced coloring agents are used as natural dyes for food, cosmetics and research, or as pigments in animal feed (Borowitzka, 1986). A number of anti-oxidants, sold for the health food market, have also been produced by microalgae (Borowitzka, 1986; Beneman, 1990). The most prominent is β-carotene from *Dunaliella salina*, which is sold either as an extract or as a whole cell powder. Moreover, bioflocculants (Borowitzka, 1986), biopolymers and biodegradable plastics (Wu et al., 2001; Philip et al., 2007), cosmetics (Spolaore et al., 2006), pharmaceuticals and bioactive compounds (Olaizola, 2003; Singh et al., 2005), polysaccharides (Beneman, 1990) and stable isotopes for research (Beneman, 1990; Radmer and Parker, 1994) are other important co-products obtained from cyanobacteria and microalgae.

4.5.4. Polyunsaturated fatty acids (PUFA)

Microalgae and cyanobacteria can also be cultured for their high content in PUFAs, which may be added to human food and a[illegible] feed for their health promoting properties (Beneman, 1990[illegible] mer and Parker, 1994). The most commonly considered P[illegible]s are arachidonic acid (AA), docohexaenoic acid (DHA), γ-linole[illegible] acid (GLA) and eicosapentaenoic acid (EPA). AA [illegible]n show[illegible] to be synthesized by *Porphyridium*, DHA by *C[illegible]theco[illegible]ium* an[illegible] *Schizochytrium*, GLA by *Arthrospira* and EP[illegible]y *Nan[illegible]hloropsis*, *Phaeodactylum* and *Nitzschia* (Spolaore et al., [illegible]6).

Worldwide industries have focuss[illegible]n eco[illegible]cally feasible processes. Many factors such as pr[illegible] of available [illegible] materials, land costs, water resources, tran[illegible]n costs and [illegible]ers influence the commercial price of t[illegible] prod[illegible] As a result a strategy successful at one location m[illegible]t not be suc[illegible]ful at other location or even vice versa. Con[illegible]ently, depending [illegible] the geographical and socio-political sc[illegible]rio co[illegible]nies develop their own strategies. Generally, comp[illegible] p[illegible] to have natural set-ups for cultivation like seawater, ope[illegible]ds or ot[illegible]s so as to reduce the costs of infrastruct[illegible]blishi[illegible] Pe[illegible]un situated in Arizona, USA use saltwa[illegible] pond[illegible]r cultiv[illegible] whereas Aquaflow Binomics is targeti[illegible] becor[illegible] the first company to produce biofuel from wild alga[illegible] Wh[illegible] not possible then they will decide upon closed [illegible]ems or bioreactors so as to minimize evaporation and other such [illegible]es. Solazyme Inc. situated in San Francisco, USA grows algae in da[illegible]where they are fed sugar for growth. To make the biofuel economical, companies focus on remaining algal biomass for co-products. Nearby industries and their raw material requirements, food sources, social acceptability and other such points can help in deciding on which biofuel along with co-products will be a good choice. Neptune industries situated in Boca Raton, USA has patented Aqua-Sphere system wherein fish waste is used to create additional revenue streams through the growth of algae for biofuel and methane. GreenFuel Technologies Cambridge situated in Massachusetts, USA have developed a system whereby they can capture up to 80% of the CO_2 emitted from a powerplant. The major research in companies is focussed on manipulations in cyanobacteria or microalgae by genetic engineering or other approaches so as to increase the productivity and make the recovery of desired products easy and less expensive. Aurora Biofuels use the genetically modified algae to efficiently create biodiesel using a patented technology, developed at University of California, Berklay and claim to create biofuel with yields 125 times higher and at costs 50% less than other production methods.

5. Challenges and hurdles in biofuel production from cyanobacteria and microalgae

Cyanobacterial and microalgal systems could contribute to a sustainable bioenergy production howev[illegible]different biotechnical, environmental and economic challen[illegible]hav[illegible]e overcome before energy products from these sy[illegible]ns can e[illegible]the market.

5.1. Biotechnical challenges

The main biotechni[illegible]hallen[illegible] addre[illegible]below are cultivation, harvesting and [illegible]tic [illegible]gineering of cyanobacteria and microalgae.

5.1.1. Large-[illegible]oduction

The ma[illegible]ity o[illegible]nmercial cyanobacteria and microalgae production occurs in u[illegible]histicated, low-productive artificial open po[illegible]isti, 2007). S[illegible]ined open pond production has been s[illegible]essful only for a limited number of cultures like *Spirulina* and *[illegible]naliella* with [illegible]xtreme conditions such as very high salinity or [illegible] pH. Despi[illegible]the success of open systems, future advances in cy[illegible]bacterial [illegible]d microalgal cultivation might require closed systems[illegible]algal species on interest do grow in highly selective environments. The concept of closed systems has been around for a [illegible]time. However, their high costs have largely precluded their com[illegible]ercial application until recently. Light is the source of energy for algal growth, but too high light intensity may result in photo-inhibition or overheating. That is why the physics of light distribution and its utilization inside photo-bioreactor is one of the major biotechnical challenges in bioreactor design.

5.1.2. Recovery and extraction

Cyanobacterial and microalgal cultures are usually very dilute suspensions. Several techniques like filtration, centrifugation, sedimentation and flocculation are used for their harvesting (Benemann and Oswald, 1996). However, the costs and energy demands for harvesting algal biomass by these methods are high. The present harvesting techniques are not applicable for large-scale and low-cost harvesting to produce low-value energy products. However, different approaches exist for a further development of harvesting techniques. A technique with low-energy demand is settling of algae by induced flocculation. However, flocculation of algal biomass is still poorly understood which makes it difficult to control this harvesting process.

Extracting lipids from microalgae is another biotechnical challenge due to the sturdy cell wall making oil hard to get out. Generally oil is expelled out from dried algae by using a press and the mashed up pulp is treated with solvent to get the remaining oil. Though the combination removes 95% of the oil, it is energy intensive. An alternative to this is the use of super-critical fluids but the process requires special machinery adding to the expense. In recent times a method called 'milking technique' has been described to harvest β-carotene from *D. salina* in a two-phase reactor and reuse of algae for continuous production (Hejazi and Wijffels, 2004).

5.1.3. Genetic engineering of cyanobacteria and microalgae

Among the around 10,000 algal species that are believed to exist, only a few thousand are kept in collections, a few hundred are investigated for chemical content and just a handful are cultivated in industrial quantities (Spolaore et al., 2006). Although some of these algae are commercially cultivated for a long period of time, metabolic engineering of these algae now seems to be necessary in order to enhance productivity, achieve their full processing capabilities and to optimize them for cultivation and harvesting.

Large-scale cultivation of genetically modified strains of algae compounds the risks of escape and contamination of the surrounding environment and of crossing with native strains. Moreover, modified strain could be transported in the air over long distances, and survive a variety of harsh conditions in a dormant stage. Thus, cultivation of genetically modified strains can have unintended consequences to public health and environment. These concerns have to be integrated in the design of large-scale production systems working with modified cultures. However the development of a number of transgenic algal strains boasting recombinant protein expression, engineered photosynthesis and enhanced metabolism encourage the prospects of engineered microalgae (Rosenberg et al., 2008).

5.2. Ecological challenges

A major advantage of cyanobacterial and microalgae is their ability to capture additional environmental benefits (CO_2 recycling and wastewater treatment). However, to realize these benefits some hurdles addressed below need to be overcome.

5.2.1. Recycling of CO_2

For photosynthetic organisms, water, nutrients and carbon dioxide are vital to growth. The atmospheric CO_2 concentration limits the growth of these organisms. Thus a cheap source of C to fuel their photosynthetic process is needed (Wang et 2008). If the purpose of algae cultivation is to sequester the indu trial CO_2 outputs of fossil-fueled power plants, it has to be take into account that during night time and during clou the algae slow down their reproduction rate and thus e up s CO_2. This would require the installation of gas stor acilitie o cope up with the influx of CO_2 during night. Before n deployment of microalgae systems becon feasib e challenge of limited availability of land for lar cale CO_2-ca ing from industrial or power plants by micr ga ve to be ov me by sophisticated area-efficient techniques to le CO_2 by microalgae (Sydney et al., 2010). Hov er it is wort ing that sequestering industrial CO_2 outpu hrough algae cult on is temporal storage as it is emitted ng th nversion of the algae and its use as energy.

5.2.2. Nutrient r nts

Cyanobac a and croalga high nutrient requirements especially conte of N and P. It may account to several-fold higher than he belaar, 2004). Thus their cultivation may invol ge quantities of N and P for which environmental and econom pact may not be sustainable. Therefore, strategies to reduce demand of fertilizers are required.

Microalgae ponds have been utilized for the treatment of sewage and wastewaters since they provide dissolved oxygen for bacterial composition of organic wastes. The major limitations in recycling nutrients from wastewater are relatively low loadings that can be applied per unit area-time, limited nitrogen and phosphorous removal, increasing land requirements and the high costs of removing the algal cells from the ponds effluent. Recycling nutrients via anaerobic digestion could be an answer to nutrients challenge, since this process can mineralize algal waste containing organic N and P, resulting in a flux of ammonium and phosphate that can be used for the cyanobacteria and microalgae. Another concept to minimize the demand of N fertilizer might be to engineer photosynthetic algae in a way that they are capable to fix nitrogen.

5.2.3. Availability and suitability of land

Cyanobacteria and microalgae produce much higher yields than traditional energy crops and thus need much less land. Nevertheless, it is unclear how much land is available and suitable to produce high yields and utilize waste CO_2 and nutrients.

5.3. Economic challenges

The development of cyanobacteria and microalgae for mass energy production is in its infancy. Because of that it seems critical to base the cost assumptions on state-of-the-a chniques used for small-scale production of high-value pr ts. ing and processing algae consumes energy, both nfrastruct and operation. Depending on the cultivation a e process harvesting and on yield, the energetic inpu of mi gae pro tion could exceed the energetic output (ten and S h, 2). However, ongoing research in the r or des is pro g and will lead to cheaper and more e effe e designs. Economics of biofuel production fro yan ia and croalgae can be improved by captur additio eve rom co-production of food, feed and value prod astewater treatment and net fertilizer ue e of nitrog fixing algae.

The capital costs fo ting a cyanobacterial/algal biofuel project m ude expen or land (if required), infrastructure esta ment, bioreactors, abor and many overhead expenses. Sig cant funding in research would be required to obtain maximu levels of pr ctivity for a successful commercial-scale produc The prod ion costs may include expenses for cultivation (exper ents); harvesting and dewatering; and extraction and separation. Besides these, costs for maintenance, compo replacement, transportation and overhead expenses. e a number of companies and government organizations have developed different methodologies as well as designs and prepared cost estimates for commercial-scale production. Many of these investigations recommend that algae to biofuels plants may be effectively developed on land adjacent to power stations (to convert CO_2 from exhausts into fuel); in wastewater treatment plants; or in seawater (to save land and fresh water) and many such useful suggestions (Singh and Gu, 2010).

Global warming will accelerate unless we take action to reduce the net addition of CO_2 to the atmosphere. The only hope for achieving a major slowing and ultimately a reversal in net CO_2 accumulation is greatly reducing the combustion of fossil fuels. Fossil-fuel use will decline only when society comes up with renewable, C-neutral alternatives in very large quantity. One of the best options in the long term is bioenergy, in which the sun's energy is captured as biomass and converted to useful energy forms. Successful bioenergy faces two serious challenges. The first is producing enough biomass-derived fuel to replace a significant fraction of the ~13 TW of energy generated today from fossil fuels. The second challenge is producing the bioenergy without incurring serious damage to the environment and to the food-supply system. Of the many bioenergy options on the table today, most fail on both counts. However, cyanobacteria and microalgal-based bioenergy options have the potential to produce renewable energy on a large scale, without disrupting the environment or human activities.

6. Genetic engineering and modifications in cyanobacteria/microalgae for biofuel–bioenergy production

With rising concerns of energy sustainability and climate change, genetic and metabolic engineering strategies must be applied to advent the development of biofuels. Photosynthetic microorganisms offer a promising solution to these challenges, while at the same time, addressing growing environmental concerns through CO_2 mitigation. Although the applications of genetic engineering to increase energy production in microalgae and cyanobacteria is in its infancy, significant advances in the development of genetic tools have recently been achieved with microalgal model systems and are being used to manipulate central carbon metabolism in these organisms. It is likely that many of these advances can be extended to industrially relevant organisms. This section is focused on potential avenues of genetic engineering that may be undertaken in order to improve cyanobacteria/microalgae as a biofuel platform for the production of bioenergy.

Sequencing the genome of cyanobacteria will examine for their potential as one of the next great sources of biofuel. Manipulation of metabolite pathways can redirect cellular functions towards synthesis of preferred products. Metabolic engineering allows direct control over the organism's cellular machinery through mutagenesis or the introduction of transgenes (Rosenberg et al., 2008). Many research works are focussed on altering the cyanobacterial cell wall properties (Lui and Curtiss, 2009; Leonard et al., 2010), transforming novel genes for hydrogen or other products (Brennan and Owende, 2010), increasing the lipid synthesis (Song et al., 2008), finding novel precursors and many more such interesting and useful areas. All these will make the biofuel generation economically viable and fruitful. Researchers from Arizona State believe that they have found a way to make biofuels cheaper and easier to produce by genetically programming microbes to self-destruct after photosynthesis, thus making the recovery of biofuel precursors easier and potentially less costly. The genes were taken from the bacteriophage (Lui and Curtiss, 2009).

In recent years, there have been attempts to overcome these barriers and problems related to hydrogen production, mainly by targeted genetic engineering of cyanobacterial strains; with reduced or deficient uptake hydrogenase activity; heterologous expression of an active ion hydrogenase; overexpression of H_2 evolving enzymes (nitrogenase(s) and/or bidirectional hydrogenase(s)); introducing less oxygen sensitive hydrogenases [illegible], introducing a synthetic, polypeptide based on proton channel into thylakoid membranes to dissipate the proton gradient across thylakoid membrane; increasing quantum efficiency of both PS I and PS II; directing the electron flow towards the H_2 producing enzymes and away from any other competing pathway (Tamagnini et al., 2007).

Some nitrogen-fixing cyanobacteria are potential candidates for practical hydrogen production. Hydrogen production by nitrogenase is, however, an energy consuming process due to hydrolysis of many ATP molecules. On the other hand, hydrogenase-dependent hydrogen production by cyanobacteria and green algae is economic in that there are no ATP requirements. This mechanism of hydrogen production is however sustainable under light conditions. Water splitting by hydrogenase is potentially an ideal hydrogen-producing system. Asada and co-workers attempted to overexpress hydrogenase from *Clostridium pasteurianum* in a cyanobacterium, *Synechococcus* PCC7942, by developing a genetic engineering system for cyanobacteria. These workers also demonstrated that clostridial hydrogenase protein, when electro-induced into cyanobacterial cells is active in producing hydrogen by receiving electrons produced by photosystems (Asada and Miyake, 1999).

Photosynthetic cyanobacteria can be redesigned for highly efficient ethanol production by the combination of gene transformation, strain/process development and metabolic modeling/profiling analysis. Dexter and Fu (2009) have transformed pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adh*) genes from *Zymomonas mobilis* into *Synechocystis* sp. PCC 6803. This strain can phototrophically convert CO_2 to ethanol. Earlier Deng and Coleman (1999) had also cloned the same set of genes in *Synechococcus* sp. PCC7942.

Algae, natural photosynthetic oil producers, are the focus of most of biodiesel research efforts, and little attention has been given to other photosynthetic microorganisms, particularly cyanobacteria. Cyanobacteria do not naturally produce oil like algae; however, there are other advantages of using cyanobacteria for biodiesel feedstock production. Unlike algae, cyanobacteria have well established methods for genetic engineering, as evidenced by genetic engineering of cyanobacteria for the production of first generation biofuels including ethanol and butanol. Furthermore, cyanobacteria will secrete free fatty acids (FAA), a biodiesel precursor, into extracellular media, simplifying downstream product isolation. These attributes motivate the investigation of cyanobacteria as a potential source for biodiesel feedstock. The cyanobacterium *Synechococcus elongatus* PCC7942 was engineered for the production of FAA. The metabolite engineering strategy involves the elimination of FAA metabolism, removal of feedback inhibition of the fatty acid synthesis pathway, improving carbon flux through the fatty acid and photosynthetic pathways, and elimination of competing pathways. Overexpression of acetyl-CoA carboxylase (ACCase) has been [illegible] for increasing the lipid biosynthesis. Certain obligate photoautotrophs, formerly unable to metabolize sugars, have been transformed with hexose transporters and thus making them suitable for heterotrophy. Higher light intensity can overwhelm the photosystems, hence using RNA interference technology, LHC proteins were down regulated and consequently strain exhibited higher resistance to photodamage (Rosenberg et al., 2008).

Advances in genetic manipulation of crucial metabolic networks will form an attractive platform for production of numerous high-value compounds (Rosenberg et al., 2008). The development of a number of transgenic strains boosting recombinant protein expression, engineered photosynthesis and enhanced metabolism encourage the prospects of modified cyanobacteria for biofuel generation.

7. Conclusion

Cyanobacterial and microalgal systems have many advantages over traditional energy crops however, its production could become economically feasible in the future when biotechnical, environmental and economic hurdles will be surmounted. Ultimately, cyanobacteria offer the potential to have a profound impact on the future welfare of the planet by addressing the pressing issues of alternative energy resources, global warming, human health and food security. Nonetheless, we believe the time is now to implement the advanced technologies, which are based on sustainable and renewable systems, to address current international issues.

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