Microalgae for Future Biotechnology Industries

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ABSTRACT

Algae are diverse group of photoautotrophic organisms and have chlorophyll and unicellular reproductive structures. They have 4 to 13 divisions with as many as 24 classes, and about 26,000 species. The size of algae varies from micrometer to several meters. The micrometer ranges algae abundant in nature are known as microalgae. The simple cellular structures of microalgae provide a large surface to volume ratio of their bodies facilitating large amount of nutrients uptake. They are more efficient in converting solar energy to chemical energy during the photosynthesis compared to the higher plants. The growth rate of the microalgae is much rapid to produce large biomass within few days. Worldwide, scientists from different domains are involved in the research relating microalgae and their applications. The applications of microalgae include food, fodder, medicine, bio-fuel, biofertilizer, environmental remediation, waste-to-wealth, and many more. With wide range of applications that microalgae can be considered one of the inevitable options of the future biotech industry. The aim of this paper is to show how microalgae can be used to solve the water and food shortage especially in the developing countries.

Keywords: Microalgae; Carbon Sequestration; Biodiesel; Nutraceuticals; Wastewater

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INTRODUCTION

In the technological revolution biotechnology emerges as a potential alternative to the chemical technology techno-economical and eco-friendly advantages. In the biotechnological process different indigenous microorganisms play important role in various applications including food, fodder, medicine, bio-fuel, biofertilizer, environmental remediation, waste-to-wealth and many more. Microalgae are microscopic photosynthetic organisms found in both marine and freshwater environments. Their photosynthetic mechanism is similar to land based plants, but due to a simple cellular structure and being submerged in an aqueous environment where they have an efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy to biomass than terrestrial plants and are efficient CO_2 fixers (1). Many algae can switch from phototrophic to heterotrophic growth and some can also grow mixotrophically. They have a high growth rate and can complete an entire growing cycle every few days. In fact, the biomass doubling time of some microalgae can be as short as 3.5 hours in the exponential growth phase (3). Thus combined with their high productivity, they are possibly a rich candidate for different applications, if the economics of the production process works out favorably.

IDENTIFICATION OF MICROROALGAE

The algae are photosynthetic plants that live in water. Most species are small to very tiny; many can only be seen with a microscope and often float or swim as plankton in fresh or salty water. Some algae, what we often call pond scum or seaweed, are conspicuous plants that can form dense mats of vegetation. The largest of these, the kelp, can be hundreds of feet in length and form impressive underwater forests. The biodiversity of Microalgae is outstanding, and it estimated that from 200,000 species to several million species exit in nature; however, a very limited number have been studied and analysed (1,2). Up to 90 percent have special chemical and nutritional compounds which are not yet fully known. Algae are chlorophyll-containing organisms that range from one-celled organisms to large, multi cellular seaweeds. Unlike plants, algae don't have true roots, stems and leaves. Algae contain high levels of protein, minerals, vitamins and trace elements. Algae are one of the few vegetable sources of vitamin B-12, one reason they are often included in vegetarian diets to avoid deficiencies. They have significant iodine content, and calcium and iron tend to accumulate at much higher levels in seaweed than in terrestrial plants. Algae are low in fat but high in fiber.

CARBON SEQUESTRATION BY MICROALGAE

In the Copenhagen conference on Climate change, India assured the World community that it would voluntarily cut down the rate of emission of Green House Gases (GHG) by 20% by the year 2020, while making it abundantly clear that India would not approve any external monitoring on its activities. We have taken significant initiatives for generation of renewable energy. The next step is to take forward a carbon sequestering master plan study. At the Paris climate conference (COP21) in December 2015, 195 countries adopted the first-ever universal, legally binding global climate deal. The agreement sets out a global action plan to put the world on track to avoid dangerous climate change by limiting global warming to well below 2 °C.The agreement is due to enter into force in 2020.

It is estimated that India and China, the two most rapidly developing countries, would account for 86% of incremental World coal demand between now and 2030. But the good news is that as per a study conducted in Massachusetts Institute of Technology coal still could be used as a sustainable source for power generation provided capture and sequestration (CCS) is used as the critical enabling technology. No doubt massive plantation programme could make some dent on reducing the net emission, but that can never be sufficient. Therefore, it is necessary to look for other sequestration methodologies. CO_2 emitted as flue gas or in coal gasification process plants could be captured through photosynthesis to generate biomass, which could be processed to generate bio-fuel and other valuable biomaterials. But the viability of such a system could be critically dependent upon the volume of biomass that would be generated. In this context growing algal biomass could be the right answer.

Integration of algae production to liquid or gaseous effluent treatment is a well known option. Production of microalgae can be simultaneously, absorb CO_2 emitted from the stacks, utilize wastewater effluents, and lead to production of renewable energy and biomaterials.

Algae, the most efficient photosynthesis and CO_2 sequestering organisms on earth, have great potential to reduce dependence on petrochemical resources. Unlike crop-based biological systems, their metabolism as biomass producers is linked only to the availability of CO_2 and light. Algae's productivity is directly proportional to the CO_2 supplement that is provided to it, a characteristics not shared by plants. Micro-algae could achieve growth rate that is ten times that of other land plants. Faster growth implies more photosynthesis and hence higher CO_2 consumption. In recent years, algal technologies have seen a surge of interest & investment around the world. Not with standing this, certain challenges prevent algae from becoming a large-scale commercial reality, which include insufficient algal strain improvement, lack of understanding of technological barriers, and inadequate demonstration of biomass production at commercial scale.

Certain microalgae strains are known to be accumulating significant amount of lipid or proteins and utilize CO_2 efficiently. These strains could be cultivated and grown to increase biomass content and then induced for the lipid accumulation pathways followed by biomass recovery and downstream processing to obtain bio fuel. The parameters of cultivation, growth cycle and field application could be precisely evaluated. Thus, appropriate assessment of environmental impact of algal biomass value chain systems, their sustainability and impacts could be specifically assessed for accumulation of lipids, bio-fuel and biomass for biomaterial application.The algal biomass generated by the sequestration process has immense sustainable and commercially viable end uses, and can be straight away used as industrial absorbent in coke processing plants for removal of cyanide and phenol from effluents.

India being in the temperate climate zone is ideally suited for algae cultivation , and CO_2 emitted from Thermal power Plants and coal gasifiers could be successfully utilized for testing, demonstration and development of the technology. Study can be conducted with the objective of developing an industry driven carbon sequestering master plan for effective and large-scale generation of green energy. A comprehensive approach is needed for tactical research that would improve system performance and demonstrate viability in places where biomass could be grown and “green” products could be subsequently utilized in energy, chemical and material industries. The long-term objective would be to support and develop a viable algal biorefinery system that utilizes a range of feed stocks to produce Biofuel and biomaterials. Nevertheless, with diverse applications, microalgae could be considered as one of the foremost players in the clean energy market and holds future promise for developing countries in creating more jobs and opportunities.

BIODIESEL PRODUCTION

Microalgae as the third generation bio feedstock possess inherent advantage such as they grow fast and have high biofuel yields. According to a report, algae yield 30 times more energy per acre than various oil plants and can produce up to 100,000 lit oil per hectare per year (which is higher than any other land crop). It has high adaptability, hence can be grown under any unsuitable conditions like marine water or wastewater ponds like industrial and municipal waste waters or from household toilets and sinks, agricultural run-off, it provides an economic and environment friendly remedy for sustainable and renewable algae based biofuel.)

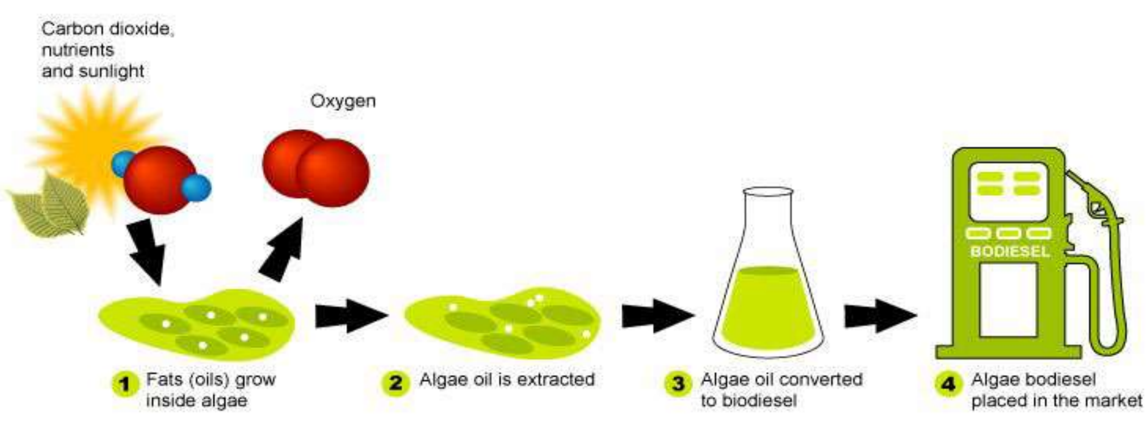

Figure 1. Biofuels production from microalgae (18).

Figure 2. Mass cultivation of microalgae using local microalgae strain at CSIR- IMMT Raceway pond (Capacity 30000 L) (15).

MICROALGAE DIVERSITY

Among the various potential sources of renewable energy, biofuels are of most interest. Marine microalgae are the most promising oil sources for making biofuels, which can grow very rapidly and convert solar energy to chemical energy via CO fixation. The fatty acid profile of almost all the microalgal oil is suitable for the synthesis of biofuel. Marine microalgae are microscopic plant organisms that are tiny, generally unicellular, autotrophic, and live within ocean and passively 'drift' along with water currents (3). Nano-phytoplankton (<20µm) are more productive in Oceanic region as compared to micro-phytoplankton (20-200µm) which are abundantly present in neritic regions (4). Generally, density of most phytoplankton is heavier than water and is constituted with skeletons made up of silica, calcium, carbonate and cellulose.

Freshwater has become relatively scarce and there are competing uses making freshwater species less attractive for biofuels applications. Marine microalgae do not require freshwater and can be grown in mass culture on land that is less suitable for other uses. It is also more favorable to isolate local marine species for any mass culture endeavor due to possible accidental release to the environment. Two groups of marine microalgae, diatoms and chlorophytes, have shown great potential for biofuels production. Diatoms often have greater rates of synthesis of biofuel lipids (5).

SEAWEED

Seaweeds are marine algae: saltwater-dwelling, simple organisms that fall into the somewhat outmoded, but still useful, category of "plants". Most of them are the green (more than1500 species), brown (about 2000 species) or red (over 7000 species) kinds, samples of which are each illustrated on this page, and most are attached by holdfasts, which generally just have an anchorage function, although a particularly efficient one (6).

NATURAL EXTRACTS

Marine microalgae and cyanobacteria are very rich in several chemical compounds and, therefore, they may be used in several biological applications related with health benefits, among others.The most studied applications of substances such as PUFA, sterols, proteins and enzymes, vitamins and pigments, in areas so diverse as human and animal nutrition, therapeutics, and aquaculture have been reported (7).

Polysaccharides are characteristic metabolites of many marine organisms, particularly of algae. Macrophytes such as brown, red, and green algae are known as traditional food ingredients for people populating seaboard geographic areas. Optimization of microalgae production is receiving a growing attention due to their potential to produce drugs, vitamins and energy. It is predicted that cancer will cause 12 million deaths worldwide in 2030, over 30% of the world’s population are anaemic due to iron deficiency and 250,000 children only in South East Asia become blind because of Vitamin A deficiency. These statistics demonstrate the importance of producing more drugs and nutrients with less cost. This concept can be an essential step in drug development using algae for more production of beta-carotene, Vitamin B-12, Vitamin E, antibiotics, probiotics, iron and other nutrients and vitamins (8, 9)

MICROALGAE STRAINS IN ODISHA COAST

Odisha state is located on the east coast region of India and has a large coastal plain called “Hexadeltaic region". It stretches along the coast of the Bay of Bengal. Odisha has a large brackish aquatic systems. A number of microalgal strains prefer brackish conditions because of its nutritional composition of the aquatic system and the warmer temperatures.If this long coast could be used for microalgal biodiesel production using the locally isolated strains then it may prove to be advantageous as they are already adapted and dominant strains (10). In the present field survey a total of 41 algal taxa have been identified from collection of 150 algal samples (11). The experimental result suggests that among the tested strains, *Scenedesmus* sp. was found to be the best candidate for biodiesel production due to high lipid content and high lipid productivity (12). Study of six different freshwater microalgae, collected from Odisha, eastern region of India, has been carried out to find out their potential for biodiesel production. The growth, total lipid, and fatty acid composition of six microalgal strains were determined. Chlorella sp. IMMTCC-2, which exhibited high lipid content with considerable amount of unsaturated fatty acids, was selected for culture in a self-designed photobioreactor in order to study the scale-up possibilities (13). The ability of OH^{-} ions in fixing dissolved CO in form of HCO − in algal growth medium was studied using a *Chlorella* sp. and scaled-up in a photobioreactor (14,15).

The study of a potential brackish water microalga *Scenedesmus* sp. has been carried out in outdoor raceway pond for biodiesel production. The designed raceway pond is a closed circular loop made up of concrete and consists of agitation system (paddle wheels), CO sparging system (CO diffuser) and flow mixing system (Baffles). The study deals with the culture of brackish water microalgal strain Scenedesmus sp. isolated from Odisha coast, eastern region of India in batch mode for mass cultivation, that are previously not been studied with respect to eastern region of India. The biomass yield and lipid yield during 18th day of cultivation time was found to be satisfactory. Again Fatty acid composition of the strain at different time interval at stationary phase of growth show high MUFA and PUFA content. The oil mainly comprised of palmitic acid, oleic acid as major fatty acid components, while linolenic acid was in considerable range, which show its suitability for biodiesel production in large-scale. Further process control and optimization studies are worth studying. Hence the result concludes that Orisha isolate microalga Scenedesmus sp. proves as one of the potential strain of eastern for commercial biodiesel production in pilot scale, due to high lipid content and high biomass yield.

MICROALGAE FOR WASTEWATER TREATMENT

Microalgae are oxygen-evolving photosynthetic microorganisms and commonly grow in various aquatic environments, such as fresh and marine water, wastewater streams from a variety of wastewater sources such as agricultural run-off, concentrated animal feed operations, industrial and municipal wastewaters, domestic water releases. The advantage of wastewaters for microalgae as they provide water medium and necessary nutrients suitable for cultivation of microalgae evidenced by high algae growth rate and productivity and nutrient removal efficiency. Therefore, coupling with wastewater treatment microalgae cultivation offer an economically viable and environmentally friendly way for sustainable renewable algae-based biofuels and chemicals production. Microalgae developed in wastewater retain large amounts of lipids, carbohydrates and proteins suitable for energy production, without a biomass limit or transformation. Scientists at the National University of Mexico (UNAM) can produce biofuels in three hours (16). Microalgae basically need nitrogen, phosphorous, water and light. The first three elements are present in urban wastewaters, but in order to obtain sufficient energy, the reactors must be exposed to sunlight like solar panels. Reactors must not be too deep because light would not reach the chlorophylls of the microalgae (17).

Microalgae are a proven low initial capital cost, low operational cost supplement or alternative to mechanically aerated wastewater treatment systems, explains Dr Brian H. Kiepper Assistant Professor & Extension Specialist at the College of Agricultural & Environmental Sciences, University of Georgia (18). Thermochemical conversion of whole microalgae into bio-crudes, which can be further upgraded into chemicals and fuels, offers a highly credible route to cost-effective fuel production. Here, oil is formed from the entire biomass, allowing use of non-lipid producing algae more suited to bioremediation. Additionally, a secondary concentrated waste stream containing heavy metals is also produced, suitable for reprocessing and additional are not only promising as waste converters and recyclers (19). The algal cell contains many useful substances and microalgae are cultivated increasingly for the production of valuable raw materials. For example, it is possible to produce oil, proteins, starch and pigments e.g., beta-carotene (20). Applications of these materials are numerous, ranging from biodiesel and bioplastics to colorants and hamburgers (21). Granular activated algae system proved to be a promising alternative for wastewater treatment which sustains cost-efficient microalgae harvesting, microalgae recovery efficiency ranging between 99.85 and 99.99% after granules settling with a velocity of 19 ± 3.6 m/h (22).While research and development of algal biofuels are currently receiving much interest and funding, they are still not commercially viable at today's fossil fuel prices. However, a niche opportunity may exist where algae are grown as a by-product of high rate algal ponds (HRAPs) operated for wastewater treatment (23).

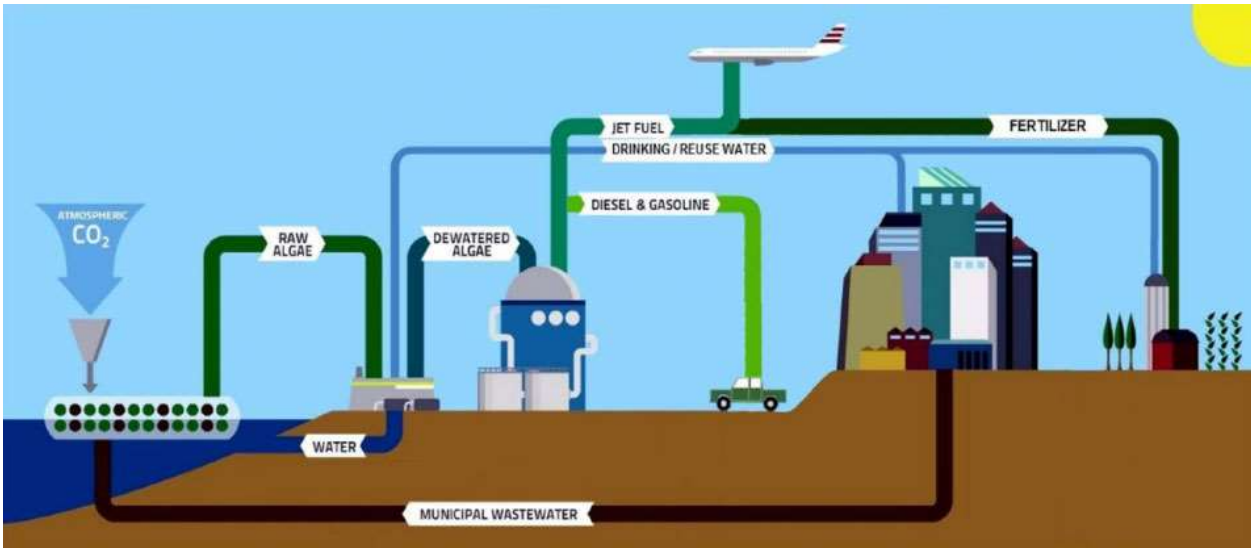

Figure 3. Wastewater process treatment using microalgae (18).

Pollution of surface water has become one of the most important environmental problems. Two types of large and long-lasting pollution threats can be recognized at the global level: on the one hand, organic pollution leading to high organic content in aquatic ecosystems and, in the long term, to eutrophication. It is a well-known fact that polluted water can reduce water quality thus restricting use of water bodies for many purposes.

Bioindicator organisms can be used to identify and qualify the effects of pollutants on the environment. Bioindicators tell about the cumulative effects of different pollutants in the ecosystem and about how long a problem may persist. Although indicator organisms can be any biological species that defines a trait or characteristics of the environment, algae are known to be good indicators of pollution of many types for the reasons like, (a) algae have wide temporal and spatial distribution, (b) many algal species are avaliable all the year, (c) response quickly to the charges in the environment due to pollution, (d) Algae are diverse group of organisms found in large quantities, and (e) easier to detect and sample. The presences of some algae are well correlated with particular type of pollution particularly to organic pollution (24). Analysis of water samples collected from lakes, streams and other bodies determines the diversity and density of algal species and provides potentially useful early warning signs of deteriorating conditions. The two Palmer Algae Pollution indices (one listed by genera, the other by species) were compiled from reports by 165 authors, and rank the genera/species most often encountered in waters with high rates of organic pollution. The algae are assigned a pollution index value of 1-6. When microscopic analysis shows that these alga genera are present at a density of 50 or more individuals in a 1ml sample, their index value is recorded. Following analysis, the values are totaled. A score of 20 or more is regarded as confirmation of high organic pollution in the water body. Scores from 15-19 indicate probable organic pollution. Some of the more common pollution-tolerant genera encountered in Washington water bodies including *Chlamydomonas*, *Desmodesmus (Scenedesmus withspines)*, *Euglena*, *Lepocinclis*, *Nitzschia, Oscillatoria, Pandorina,* and *Phacus*. Nutrient-related pollution significantly impacts drinking water supplies, aquatic life, and recreational water quality by supporting excessive algae growth. Nutrients reach water bodies through agricultural and urban runoff, sewage discharges and detergents containing phosphorus. Laboratory microscopic analysis, which reveals the composition and density of the algal flora present in a water body, is an important component of monitoring programs and is valuable in determining diverse trophic conditions. In addition to cyanobacteria, the populations and species diversity of green algae, flagellates and diatoms, which reflect different trophic conditions, are important indicators for evaluating water quality. The indices developed by Palmer are useful analytical tools for assessing non-eutrophic and eutrophic water conditions by categorizing algae that are present or absent in various aquatic environments (25).

Current standardized methodologies are based on microscopic determinations, which is time consuming and prone to identification uncertainties. The use of DNA-barcoding has been proposed as a way to avoid these flaws. Combining barcoding with next-generation sequencing enables collection of a large quantity of barcodes from natural samples. These barcodes are identified as certain diatom taxa by comparing the sequences to a reference barcoding library using algorithms. Proof of concept was recently demonstrated for synthetic and natural communities and underlined the importance of the quality of this reference library. We present an open-access and curated reference barcoding database for diatoms, called R-Syst-diatom, developed in the framework of R-Syst, the network of systematic supported by INRA (French National Institute for Agricultural Research) (26). Biological indicators like algae have only recently been included in water quality assessments in some areas of Malaysia. The use of algal parameters in identifying various types of water degradation is essential and complementary to other environmental indicators (27).

The world is witnessing an urgent need of shifting towards cleantech industry as the consequences of global warming are getting quite apparent. Significant support is being provided to startups in cleantech area. Microalgae technology has opened up an ocean of opportunities for the entrepreneurs in terms of research and development, biofuels production and refineries, and many more. The renewable energy market is currently valued at US $17 billion and growing annually at the rate of 15% which makes it a hot area for international ventures to support interesting technologies to get higher yields.Government funding and significant supports are available in India at all stages to boost this promising algal technology in the production of biofuels (28).

Conclusions

Microalgae are photosynthetic micro-planktons and commonly found in various nutrient rich aquatic ecosystems like municipal wastewater, industrial wastewater streams, agricultural run-off, shorelines, mine seepage, and concentrated animal feed operations. They are more efficient than terrestrial plants converting solar energy and CO_2, results huge biomass production ability. They utilize the dissolved nutrients like N and P in wastewater to convert carbohydrate to energy rich complex organic lipids and proteins. The complex organic compounds having large active sites both in- and out-side of the cells of microalgae transform the inorganic and organic pollutants present in the wastewater by the chemical reactions and adsorption process.

Wastewaters provide necessary nutrients in aqueous medium suitable for cultivation of microalgae evidenced by high algae growth rate and productivity, and a simultaneous removal of pollutants like heavy and toxic metals, TSS, TDS, FOG, BOD and COD from the wastewater. Another simulated technique of granular activated pellets of microalgae proved promising alternative way for efficient wastewater treatment. Enhanced cultivation of microalgae in wastewater retains large amounts of lipids, carbohydrates and proteins, suitable for energy production, without a biomass limit or transformation. This may be coupled with an economically viable and environment friendly way for sustainable renewable algae-based biofuel and biogas production. While research and development of algal biofuels receiving much interest and funding, they are still not commercially viable compared to the price of the conventional fossil fuel, however, a niche opportunity may exist where algae are harvested as a by-product of high rate algal ponds (HRAPs) or photobioreactors operated for wastewater treatment. Therefore designing of suitable HRAPS or photobioreactors for large cultivation and harvesting during the wastewater treatment vis-à-vis biofuels production in an integrated process for the commercial exploration of prospective algal energy.

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