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Potential of Microalgae for Sustainable Biofuel Production

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Abstract

Bio-fuels have received prime focus of these efforts. First and second generation biofuels were unsuccessful due to extensive cropping land and water requirements. Presently, microalgae has been considered as the source of third generation biofuels as it can be produced on large scale without any detrimental impact on environment and arrest carbon emissions as well. For ensuring cost effective and sustainable production of microalgae, wastewater generated from municipal, agricultural and industrial activities may be considered as a potential medium for algal growth. The present study focuses on the simultaneous wastewater treatment and biofuel generating potentials of microalgae as well as advantages and disadvantages of the same. This review also discusses possibilities of rendering microalgal biodiesel production economically more beneficial than petro diesel and highlights the future perspectives by compiling initiatives taken so far.

Keywords: Bio-fuels; Microalgal; Biodiesel; Fuel reserves

Introduction

First- and second-generation biofuels like ethanol and biodiesel have a number of inherent limitations that make them less than ideal as a long-term replacement for petroleum. The primary feed-stocks for first-gen ethanol (corn and sugarcane) and biodiesel (rapeseed, soybeans, and palm) are all food-based crops that compete for scarce cropland, fresh water, and fertilizers. These fuels cannot be used in unmodified engines above small blends and are not applicable to the jet fuel market.

While first and second-generation bio-fuels account for more than 99% of current global biofuel production - and the U.S. already appropriates 30% of its corn supply to displace about 6% of its gasoline consumption - a number of important technologies are on the brink of commercialization that produce "drop-in" fuels with the same chemical characteristics of petroleum.

In recent times, global rise in energy demand by domestic and industrial sectors is being paralleled by a simultaneous increase in fossil fuel consumption. A considerable quantity of fossil fuel is still available at considerable cost but gradually inching towards depletion due to overexploitation of fuel reserves. Besides, excessive burning of fossil fuel has resulted in increased greenhouse gas emissions (GHG) and thereby contributed to global warming [1]. Excessive burning of fossil fuel may also result in generation of particulate matter and volatile organic compounds as well as other air contaminants like NOx, SOx, CO_2, CO, etc [2].

Biomass may be considered as a suitable alternative of fossil fuels as it is renewable and it may also significantly reduce GHG emissions through photosynthesis [3]. Large-scale production of biomass energy could result in sustainable benefits in environmental, social as well as economic sectors [2]. Presently, commercially available biofuels include bioethanol and biodiesel that are derived from starch and oil yielding crops respectively [1]. Although these biofuels are cleaner substitutes of fossil fuels, there still exists considerable doubt on whether they are economically competitive with fossil fuels. Moreover, wide scale cultivation of crops yielding biofuel might also trigger food scarcity [1]. Hence, in recent researches, algae have been gaining considerable attention as an alternative source of biofuel production.

For a long time now, unicellular microalgae have been considered as a significant source of renewable biofuel as it has appropriate amount of biomass as well as oil for biodiesel yield1. In comparison to plant crops, microalgae have been found to possess greater biomass productivity estimated in terms of cultivation land, incur lower cultivation cost and sequester GHG emissions as well. Like feedstocks obtained from plants, those derived from algae may also be processed directly or through different biochemical or thermochemical processes for yielding biofuel and gas [4-7]. Biochemical processes consist of fermentation and anaerobic degradation of biomass for production of bioethanol or methane [8]. Thermochemical processes for biofuel generation yield methods like pyrolysis, hydrogenation, liquefaction, etc. of algal biomass [7,8]. Besides, algae may produce hydrogen as well through bio-photolysis [9]. Transesterification is another process whereby triacylglycerol lipids extracted from microalgae are used for biodiesel production [9-11].

For the advantages offered by microalgae over other crop plants, the former has gained significant attention where production of biodiesel is concerned. Recent research has primarily reported identification of microalgal strains possessing high lipid synthesizing potentials and determination of suitable growth conditions which promote best lipid productivities [12,13].

Previous studies have reported higher neutral lipid (particularly triacylglycerol) accumulation in microalgal cells resulting from nutrient (nitrogen or phosphorous) [14,15]. However, it was often observed that nutrient stress induced rise in lipid productivity was paralled by a corresponding decrease in biomass productivity which in turn suppressed lipid productivity [13] rendering this process highly unsuitable for biodiesel production on a wide scale. Instead, determination of cultivation conditions that favored high biomass productivity were considered as more efficient method of ensuring elevation of total lipid productivity [13]. In this context it may be mentioned that large scale production of microalgal biofuel is economically more viable in comparison to other sources of biofuel and might be considered as a suitable substitute of fossil fuel if it is made available in sustainable quantities.

Background

Biodiesel, a combination of fatty acid alkyl esters, is mostly obtained from transesterification of plant lipids composed primarily of triglycerides (90–98 weight %), free fatty acids (1–5%) along with minute quantities of mono and diglycerides, phospholipids, carotenes, tocopherols, traces of water, etc. [16]. Transesterification is a series of reversible reactions, whereby, triglycerides are transformed to diglycerides, to monoglycerides and finally yield biodiesel (alkyl esters) with byproducts (glycerol) [17].

In a transesterification reaction, oil/fat is reacted with alcohol (preferably methanol) in a molar ratio of 6:1 and the reaction occurs in presence of a catalyst (generally NaOH) [17]. Usually this reaction is carried out in a stirred reactor on batch scale. In recent research, some improvisations like microwave assistance [18,19] cavitation stimulator [20,21] or ultrasound assistance [22,23] were introduced to facilitate the reaction in continuous mode with lower reaction time and improved mixing. In all current biodiesel production methods, the microalgae are grown in separate units prior to being extracted from growth medium for lipid extraction. Recently pyrolysis of triglycerides and organic compounds like small chained aliphatics, aromatics and carboxylic acids have been considered as an alternative to trans esterification [24,25].

Various recent studies have reported the benefits of obtaining biodiesel from microalgae in comparison to other feedstocks used previously [26-29]. Many microalgal strains have been reported to provide high oil yield on being induced for lipid accumulation as well [17]. Microalgae complete an entire life cycle in few days and can grow almost in any conditions in presence of sun light and other basic nutrients. Their growth rates may be hastened by providing specific nutrients and adequate aeration [30]. However, efficient yield of biodiesel from microalgal strains require appropriate optimization of cultivation, harvest and processing conditions. Different microalgal strains may adapt to a wide range of environmental conditions making it possible to obtain a best acclimatized strain for any experimental environment which is impossible for any other biofuel feedstock like sunflower, soyabean, etc. [17]. Microalgae pose no competition for cultivable land with other food crops and can provide feedstock for several fuels like biodiesel, ethanol, methane, hydrogen, etc. [17]. Algal biodiesel is much cleaner than petroleum diesel in emissions but was found to be similar in performance with the latter [17]. As microalgal strains provide benefits like wastewater treatment and sequestration of carbon emissions in addition to fuel supply, microalgae have been aptly considered as source of third generation biofuels [11].

Nevertheless, wide scale biodiesel production is still economically unstable due to innumerable technical obstacles faced by it. Besides, cost effective technologies for biomass harvesting as well as oil extraction are yet to be designed. Current efforts of deriving biofuel from microalgae should therefore primarily focus on cost reduction of small and large scale operations. This may be achieved by enriching microalgal growth with cost effective supply of CO_2 (from recycling flue gas, GHG, etc.) or application of nutrient enriched effluents. This reuse of waste products will not only reduce cost but also help lower GHG emissions and waste production and enhance microalgae productivity and applicability for other purposes like food, medicine, agriculture, etc. thus recycling of wastes will make microalgae cultivation sustainable and render microalgal biodiesel production as economically viable.

Algal Growth and Biodiesel Production

Algae cultivation

Microalgae have the potentials of growing in extreme environmental conditions by increasing their efficiency of resource utilization. The primarily require sufficient supply of carbon and light for performing photosynthesis. They can adapt to stressed conditions both biochemically and physiologically and can excrete compounds to promote or inhibit competitors [31]. Microalgae can execute any of the following metabolic pathways and also shift from one pathway to another in response to environmental adversities [32]:

Photoautotrophically, when food is primarily produced through photosynthesis;

Heterotrophically, when algae only depend upon organic compounds as growth factors;

Mixotrophically, where organic compounds and carbon dioxide are required besides other essentials for photosynthesis;

Amphitrophically, where algae switch between autotrophy and heterotrophy depending upon the availability of organic compounds and light;

Photoheterotrophically, where plants rely on light energy for utilization of organic compounds;

Previous studies have reported photoautotrophic, heterotrophic and mixotrophic behaviour in *Chlorella vulgaris*, *Selenastrum capricornutum, Haematococcus pluvialis, Scenedesmus acutus* and *Arthrospira platensis* [32].

Besides, carbohydrates, proteins and fats, algal strains may also require vitamins, salts and elements like nitrogen and phosphorous for algal growth when operational parameters (O_2-CO_2 balance, pH, temperature, light intensity, product removal, etc.) are maintained at equilibrium [17]. It is highly essential to optimize these parameters and investigate their interactions in order to monitor the quality of the biodiesel produced in large scale. Unmonitored culture conditions may result in infection of concerned algal strains [17].

The inability to achieve extensive growth of selected algal strains is a major drawback in large scale biodiesel production. However, Chlorella and Spirullina are two exceptions to this difficulty [17]. A drop in growth rates may be recorded when algal strains adapted to natural conditions are artificially cultured under semi-sterile monitored experimental conditions. Several abiotic (O_2-CO_2 balance, pH, temperature, light intensity, salinity, toxic chemicals, etc.) and biotic (pathogenic microorganisms) factors affect algal growth. Besides competition with other algal strains other operational factors like depth of cultivation site, harvest frequency, and bicarbonate addition may also influence algal growth rates [17]. Biological contaminants like redundant algal species, moulds, protozoa, fungi and bacterial strains may also contribute in collapse of large-scale microalgal cultures. Hence, often, closed culture setups prove to be more convenient over open culture setups for prevention of contamination of selected microalgal strains [17].

Effect of different environmental factors on algal growth as reported in previous studies has been given in table.

Microalgal strain	Factors Analyzed	Effects Observed	References
Pleurochrysis carterae	pH	Maximum productivities using plate photobioreactor at pH 7.7-8.0 Maximum productivity in outdoor raceway pond at pH 9.1-9.6. Best operational depth for outdoor raceway pond between 16 and 21 cm.	[17]
Not specified	Interaction between supplied CO_2 and pH	CO_2 supplied at near neutral pH resulted in huge CO_2 release into the atmosphere.	[31]
Not specified	Interaction between supplied CO_2 and pH	The hydration of CO_2 and subsequent formation of bicarbonate ion is faster at pH values below 8 Direct reaction of CO_2 with the hydroxyl ion to form bicarbonate occurred best at above pH 10.	[17]
Nannochloropsis oculata	CO_2 concentration	Biomass production and lipid accumulation increase with rise in CO_2 concentration	[33]
Scenedesmus obliquus *Chlorella kessleri* *Chlorella vulgaris* *Spirulina sp.*	CO_2 concentration	*Scenedesmus obliquus* and *Chlorella kessleri* were reported to possess potentials of CO_2 bio-fixation.	[34]
Haematococcus pluvialis	Aeration and light intensity	Better growth reported in aerated column as aeration prevented sedimentation and improved contact between cells and nutrients; Increase in light intensity facilitated higher cell density and specific growth rate up to a certain limit beyond which it inhibited growth.	[17]
Botryococcus *Nitzschia* sp. *Isochrysis* *Dunaliella* sp.	Nitrogen and salt stress	Under non-stressed conditions, *Botryococcus* supported highest lipid concentration; Lower levels of lipids were observed in *Dunaliella* sp. (23%), *Nitzschia* sp. (12%) and *Isochrysis* (7%).	[17]
Chlorella vulgaris	Iron concentration	High iron concentrations induced lipid accumulation	[35]
Chlorella emersonii *Chlorella minutissima* *Chlorella vulgaris*	Nitrogen stress	63%, 56% and 40% rise in lipid content was observed in *C. emersonii*, *C. minutissima* and *C. vulgaris* respectively	[36]
Botryococcus *Isochrysis* *Dunaliella bardawil* *Dunaliella salina*	Nitrogen stress	*Botryococcus* lipids increased (10%) with N2 increase *Dunaliella bardawil* and *Dunaliella salina* lipids decreased (10%) with N2 increase *Isochrysis* lipids and carbohydrates increased with N2 decrease. Protein and chlorophyll content decreased while carbohydrate and lipids demonstrated a species-specific trend.	[17]
Spirulina maxima	Nitrogen stress and Temperature	*Spirulina* lipids content increase approximately 3 times with the decrease of nitrogen content and temperature decrease, being the nitrogen concentration decrease more effective	[37]

Table 1: Effect of different environmental factors observed on algal growth.

Cultivation of third generation biofuels

Another favorable property of algae is the diversity of ways in which it can be cultivated. Algae can be grown in any of the following ways.

Open ponds: These are the simplest systems in which algae is grown in a pond in the open air. They are simple and have low capital costs, but are less efficient than other systems. They are also of concern because other organisms can contaminate the pond and potentially damage or kill the algae

Closed-loop systems: These are similar to open ponds, but they are not exposed to the atmosphere and use a sterile source of carbon dioxide. Such systems have potential because they may be able to be directly connected to carbon dioxide sources (such as smokestacks) and thus use the gas before it is every released into the atmosphere.

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Comparative Analysis of Open and Closed Systems

Productivity in algae cultivation units are evaluated on the basis of the three parameters given as follows [31]:

Volumetric productivity (VP) estimated as productivity/unit reactor volume (g L^{-1} d).

Areal productivity (AP) calculated as productivity/unit of ground area of reactor (g m^{-2} d).

Illuminated surface productivity (ISP) determined as productivity/unit illuminated surface area of reactor (g m^{-2} d).

Though closed systems do not ensure significant areal productivity, but it largely exceeds open systems in volumetric productivity (eight times) and biomass concentration (sixteen times) [31].

Harvesting

Algal harvesting is performed for biomass recovery from growth medium and accounts to nearly 20-30% of total cultivation cost38. In order to carry out solid liquid separation, various physico-chemical as well as biological methods have been employed for removal of large volumes of algae from water. Yet there still exists a need to design a suitable and cost effective harvesting system for microalgae.

Conventional methods of separation included sedimentation, centrifugation, filtration, combined of flocculation– flotation, etc. Flocculation usually facilitates aggregation of microalgal cells for other methods of separation like sedimentation, filtration, etc. [38]. Microstrainers have been widely utilised for algal harvest owing to its simple mechanism and wide availability. Many studies have concluded that it is essential to flocculate aglal cells before microstraining [17]. Simple filtration was found effective for large microalgae like *Coelastrum proboscideum* and *S. Platensis* but ineffective for algal strains with smaller cell dimensions like *Scenedesmus*, *Dunaliella* and *Chlorella* [17,38].

Of all existing methods, membrane filtration appeared to be the most promising for separation of fragile cells. Membrane filtrations applied so far are of two types namely microfiltration (pore diameter ranging from 100 nm to 10000 nm) and ultrafiltration (pore diameter ranging from 1 nm to 100 nm). These membranes are produced from different materials and may be of diverse geometry (compressed, tubular, multi-channeled, hollow, capillary or spiral) depending on its areas of application. With progress in preparation of cost effective and persistent membranes and wide applications, membrane technology may be successfully implemented for harvesting microalgae from growth medium. Membrane filtration is also capable of removing protozoans and viruses from the growth medium thereby rendering it recyclable. Additionally, absence of coagulants simplifies oil refining and promotes further use of biomass. Separated biomass requires immediate processing as it is vulnerable to quick damage from hot climate [39].

Lipid Extraction for Biodiesel Production

Harvested biomass is processed according to the desired products. Dehydrating increases the shelf life of bothe algal; biomass and its product. The most prevalent methods of dehydration include spray, drum, freeze and sun drying [31]. However, high water content of algae and cost incurred makes sun drying and spray drying respectively economically inapplicable for dehydrating algal biomass. Cell disruption of dried biomass releases desired metabolites. Metabolite extraction may be mechanical (homogenization, ultrasound irradiation, autoclaving, etc.) or non-mechanical (freezing, application of organic solvents, alteration of pH, osmotic shock, enzyme reactions, etc.) in nature [17]. A previous study reported that astaxanthin extraction was three times higher from autoclaved and homogenised biomass in comparison to other methods [31]. However, lyophilisation may be considered as a more suitable process as it disrupts cells into fine powder without any other treatment [17].

Biodiesel production requires lipid and fatty acid extraction from the microalgal biomass. Lipids are usually extracted (up to 98% purified) in solvents like hexane, ethanol, or a mixture of hexane and ethanol from lyophilised algal biomass [31]. Ethanol is avoided in case of only lipid extraction as it may often extract extracellular contaminants like carbohydrates, protein and its precursors, pigments and salts as well. Previous studies also reported improved extraction yield with ultrasound and microwave assistance in comparison to other conventional methods. Ultrasound oil extraction from marine microalgae *Crypthecodinium cohnii* increased to 25.9% from 4.8% as obtained in soxhlet method.

Prospects of Microalgal Biodiesel

About 0.53 billion m3 annual biodiesel production can meet half the present requirement of transport fuel in the US11, 40 show that depending on oil crops to suffice 50% of transport fuel need in US would require 24% of the total existing cropland which is reduced to 1-3% requirement for microalgae cultivation.

Comparative analysis of different sources of biodiesel yield. [m]: Required for meeting half of total transport fuel utilized in the United States; [n]: microalgae with high (70% by weight of biomass) oil yield; [o] microalgae with high (30% by weight of biomass) low oil yield

The microalgal biodiesel yields depicted and have been experimentally derived from microalgae cultivated in photobioreactors [11]. It is also evident from that microalgal strains of high oil content yield biodiesel which is twenty five times more in comparison to the traditionally used palm oil (*Elaeis guineensis*). Microalgae require 0.1 m^2 of land per kg biodiesel produced to provide an annual yield of 121,104 kg of biodiesel. For such voluminous yield of biofuel, microalgae have been considered to possess immense potentials of biodiesel production [40].

Oils, lipids and hydrocarbons present in microalgae are species specific [11]. Only a few of all oils present in microalgae are suitable for biodiesel production. For global acceptance, quality of biodiesel needs to be in accordance with existing standards. In the United States, ASTM Biodiesel Standard D6751 defines the biodiesel standards [11]. In the European Union, Standard EN 14214 and Standard EN 14213 define standards for transport oil and heating oil respectively [11].

Microalgal oils are rich in polyunsaturated fatty acids like eicosapentaenoic acid (EPA, C20) and docosahexaenoic acid (DHA, C22). However, presence of fatty acids and its esters render it susceptible to oxidation under storage conditions and impede its acceptability as biodiesel [11]. Though linoleic acid (C18) and linolenic acid (C18) present in vegetable oils show higher oxidative stability in comparison to EPA and DHA, the European Standard (EN 14214) restricts its presence in biodiesel for transport fuel to 12% (mol) [11]. Nevertheless, no such limitations have been prescribed for biodiesel used as heating oil, though acceptability of biodiesel requires the same to meet other criteria (iodine value) of unsaturation of oil. According to both EN standards (14214 and 14213) the iodine values of ideal biodiesel should be lower than 120 and 130 g iodine/100 g biodiesel correspondingly. Additionally both standards restrict the percentage of fatty acid methyl esters with four or higher unsaturated bonds to 1% mol by far [11]. Unsaturation in microalgal oils may be significantly reduced by subjecting the same to partial catalytic hydrogenation that is usually applied for preparing margarine from vegetable oils [11].

Microalgal biofuel may bring complete substitution of petroleum fuels if it is made available at a price roughly calculated from the equation given as follows [11]:

Cbiodiesel = 6.9 × 10−3C *petroleum*

Whereby, C*biodiesel* and C*petroleum* denote the price of microalgal biodiesel ($/L) and crude oil ($/Barrel) respectively.

This equation was designed with an assumption that algal oil possesses approximately 80% of the energy present in crude oil. Significant cost reduction of microalgal biodiesel production can be achieved by implementing a biorefinery based production approach with improved genetic engineering of microalgae and more advanced photobioreactors [11].

Enrichment of Microalgae Using Wastewater

Wastewaters from various sources contain high concentrations of organic (carbon) and inorganic (nitrogen and phosphorous) nutrients which may lead to eutrophication of water bodies that such effluents are discharged into 1. Phosphorous (P) requires special attention for removal from treated water. In commercial wastewater treatment, P is either precipitated out by chemical processes of converted to activated sludge by biological activity [1]. This isolated P is not fully recyclable and is hence either disposed as landfill or used as sludge fertilizer [1].

Microalgae are known to remove N, P and other toxic components from effluent streams [41] and are therefore capable of tertiary wastewater treatment [1]. Although use of microalgae is limited, it is being applied for small scale wastewater treatment across the world. Conventional oxidation ponds and shallow raceway type oxidation ponds aided with mechanical mixing have been reported as efficient for microalgal treatment of wastewater [1].

Cost effective and low energy requiring technologies of algal wastewater treatment has made the same more attractive to developing countries over conventional chemical treatments. Oxygenation of cultivation units is highly essential for efficient bioactivity. Significant O_2 production by the microalgae itself saves operational cost for mechanical aeration rendering the process more cost effective. Moreover, algal remediation is environmentally benign and highly sustainable as it does not produce any additional byproducts or sludge provides efficient treatment with nutrient recycling [1,42].

Most of the existing studies report application of laboratory and pilot scale cultures and performance of algal ponds under diverse effluent conditions. These studies have primarily focused on nitrogen and phosphorous removal besides metals from effluent streams. These results are of significant benefit for designing biofuel extraction from microalgae grown in wastewater.

Potential of Wastewater for Microalgal Cultivation

Microalgal growth is highly dependent upon ambient temperature, pH of the growth medium, concentration and ratio of nitrogen, phosphorous and organic carbon, intensity of light and availability of O_2 and CO_2 [1]. According to a previous study, microalgal growth in sewage water was found to increase with prolonged photoperiod and CO_2 supply while increase in temperature resulted in decrease in biomass [1]. Wastewaters are rich in nutrients like nitrogen and phosphorous and hence are highly suitable for the growth of microalgae. However, ammonia and other toxins like metals, dyes, etc. present in industrial wastewaters may inhibit algal growth [1]. Biological contaminants present in effluents include pathogens and zooplanktons. Besides, microalgae may face completion for essential nutrients from other microorganisms present in wastewaters as well. The initial biomass density of microalgae in wastewaters is also a deciding factor for thriving of the entire population [1].

Chemical and biological parameters of wastewaters depend on its source and are different at each treatment site. Ability of adapting to a specific wastewater condition also varies from one algal strain to another. Unicellular chlorophyll bearing microalgae have been reported to be more tolerant to wastewater due to their high nutrient accumulating potentials [30,43-49]. However, wastewater utilization efficiencies may differ in different chlorophyte species [1].

Potentials of dairy wastes for cultivation of microalgae were also investigated. Lipid accumulation in mixed algae cultures grown in anaerobically digested dairy manure was found to be significantly elevated after a six day treatment resulting in higher lipid productivity [49]. Similar observations were noted in Chlorella sp. [50,51]. In a separate study, pond-scale cultures of Rhizoclonium hieroglyphicum was carried out in combined swine and dairy effluent both in presence and absence of CO_2. Results revealed that total lipid content and algal productivity were high in microalgae grown in swine effluent and dairy effluent respectively. In algae grown in dairy effluent, fatty acid productivity was found to be higher in presence of added CO_2 [52]. These studies indicate the strong potential of wastewater utilization for cultivation of biodiesel yielding microalgae.

Limitations and Future Perspectives

Inspite of the huge biodiesel yielding potential of microalgae the following limitations need to be address for wide scale production and utilization of algal biodiesel.

Necessity of Efficient Microalgae Harvesting Processes

The small size and density as well as large handling volumes of cultivated microalgae necessitate efficient and cost effective methods of harvesting and processing. Absence of efficient algal harvesting technologies is a primary factor hindering large scale application of microalgae for industrial wastewater treatment. Contemporary methods of algal harvest include centrifugation, gravity sedimentation and filtration all of which may be made more convenient if preceded by flocculation as flocculation aggregates the algal cells increasing their size. Flocculation may be achieved by metal salts (FeCl3, Al2(SO4)3, etc.), cationic polymers or addition of alkali [4].

However, chemically induced flocculation may incur cost and require further purification of water prior to discharge or reuse. Auto flocculation or bio-flocculation may be considered as alternatives where algal cells spontaneously aggregate and are easily removed. Bio flocculation may also be induced artificially by limiting carbon resources or creating an oxygen deficient environment. Bio flocculation has been carried out on algae growing in synthetic medium but its feasibility in wastewater medium is yet to be investigated [44].

Centrifugation is often preferred over gravity sedimentation as it is applicable over diverse algal strains and provides highly efficient (>95%) cell harvesting. Nevertheless, filtration may be considered as another alternative method of algal cell harvesting. Membrane (micro and ultra) filtrations are advanced processes for separation of smaller algal cells like Scenedesmus and Chlorella which escape simple filtration [4]. However, cost of pumping and maintenance of membranes makes membrane filtration an expensive process [1].

A more recent attempt has been immobilization of microalgal cells for convenient removal of the same after growth period. Popular techniques of immobilization include artificial attachment and polymer encapsulation of algal cells. An effective immobilization should sustain live cells for maximum survival and permit high effluent flow rates for high nutrient availability. Immobilized microalgae (like Scenedesmus and Chlorella) were found capable of similar Nitrogen and Phosphorous accumulation as obtained with unattached algal cells [53]. However the possibility of lipid isolation from algal–polymer matrix for biodiesel production is yet to be achieved.

Appropriate Life Cycle Analysis of Produced Biofuel

Possibility of positive energy output and economic feasibility of a process determines its true potentials [1]. Besides it is essential to determine whether the process involves sustainable consumption of natural resources and is carbon neutral [1]. Analysis of recent attempts at using algal feedstocks for biofuel synthesis has revealed mixed conclusions [53-55]. Studies performing life cycle assessments have concluded that plant-based biofuels are more advantageous over algal biofuel as they have lower energy and water need and have lower GHG emissions as well. A major detrimental impact of using algal biofuels on environment is the requirement of CO_2 as fertilizer and nutrient source. Utilization of wastewater and flue gas may help meet the nutrient and CO_2 demands respectively. Anaerobic bacterial populations in wastewater will also generate CO_2 through respiration which in turn will be utilized by microalgae. A perious study reported that biodiesel obtained fromalgae grown on source-separated urine was more environmentally advantageous in comparison to plant based biofuel production [54]. A more detailed life cycle assessment of algal biodiesel production is required for making the process applicable on a large scale.

Achieving High Biomass and Lipid Productivity on a Large Scale

Biofuel extraction from microalgal biomass may be considered as an alluring option if residual biomass could be used for generating biogas [1]. Although establishes low total lipid content in microalgae grown in wastewater, the available high biomass will incur significantly high lipid productivity. However future attempts for sustainable algal biodiesel production should aim for large scale open cultivation of microalgae that can be maintained for extensive cultivation periods. Genetic alterations in microalgal cells may also be carried out for improving the algal growth and lipid content [56]. A recent study has proposed a photosynthesis–fermentation model (PFM) as an alternative approach for improving microalgal lipid productivity using Chlorella protothecoides [57]. In this model an initial autotrophic growth phase generated high algal biomass and was subjected to a heterotrophic fermentation phase in order to obtain maximum algal cell density as well as lipid accumulation. Results indicated that lipid yield of heterotrophic Chlorella protothecoides was significantly higher (~70%) when preceded by an autotrophic phase in the PFM model. This model is capable of giving rise to enhanced lipid productivity in algal cells cultivated using nutrient rich effluents [1].

Added Benefits of Microalgae Cultivation

Microalgae are unicellular organisms with short growth periods that render them highly advantageous over crop plants. Algae can be grown on any land without compromising on land required for cultivation of food crops. It can also utilize nutrients and CO_2 from wastewaters and flue gas respectively [58-60]. Nevertheless, cultivation of microalgae may cater to other purposes as well. Some other aspects of microalgal cultures are enlisted as follows:

Separation of CO_2 from industrial flue gases and subsequent reduction of GHGs emitted during biodiesel production;

Removal of NH4 +, NO3-, PO43- from wastewater [61];

The residual algal biomass from oil extraction has high nitrogen and phosphorous content and may be further burned for energy, used as organic fertilizer or processed to yield alcohol and livestock feed [61];

Cultivation of microalgae does not require arable land, are not influenced by weather changes and can utilize waste water and gas for nutrients

Different microalgal species may be utilized for other industrial purposes like synthesis of fine chemicals and bulk products including fatty acids, oil, dyes, sugars, pigments, antioxidants, and biomass [62-64];

Microalgal strains yield innumerable high value biological derivatives and therefore possess the potential of utilization in a number of biotechnological fields like biofuels, cosmetics, pharmaceuticals, nutrition and food additives, aquaculture, and pollution prevention [64,65].

Challenges for Algal Fuel Commercialization

Wide scale cost effective cultivation of microalgae for simultaneous biodiesel production and waste remediation is faced by many hurdles including optimization of algal growth, improved oil extraction and efficient fuel processing. A few major challenges faced in this concern have been detailed as follows:

Increasing the Efficiency of Algal Growth and Harvesting Processes

Appropriate engineering can improve nutrient circulation and monitor light exposure thereby ensuring proper growth conditions of microalgal strains. These challenges can be addressed either by designing cost effective photobioreactors (PBRs) for large scale implementation or by engineering microalgal species that can grow conveniently in inexpensive open setups [66]. However, PBRs support controlled algal growth thereby resulting in increased productivity and are hence considered more advantageous than open systems. Nevertheless PBRs are yet to be perfected for efficient gas exchange and system cooling [67,68]. Irrespective of the growth strategies employed, substantial improvement is required in present technologies of producing algal biodiesel which may be achieved from enhanced engineering or biotechnological advances in production strains.

Improvisation of Oil Extraction

Microalgal oil extraction is presently performed by either of oil press, hexane extraction, or supercritical CO_2 fluid extraction processes [68]. Though successfully established, these processes have expensive equipment and energy requirements. Since post extraction processing of fossil fuel and biodiesel are similar, it may be assumed that major oil companies are capable of efficient conversion of algal oil to liquid fuel with their processing efficiencies, albeit with improved catalysts [66].

Land Use

Above growth strategies employed and efficiency of oil withdrawal, the challenge of significant concern is substantial biodiesel production for significant substitution of fossil fuel in different applications. In 2008, the daily oil consumption for the USA alone was 19,497,950 barrels [66]. For any biofuel to meet this demand, the land requirement was estimated to be 30 million acres [66]. Of both terrestrial and marine strategies are required to attain large scale aquaculture of algae, marine strategies require enhanced investigations. The terrestrial strategies however can be implemented on non-arable land causing minimal environmental or economic stress [66].

Water Consumption

Water is a crucial commodity controlling algal growth. Non-arable land considered for algal cultivation is often accompanied by considerable non potable water reservoirs (highly alkaline or saline in nature) that meet the requirements of algal species. Microalgal water consumption in open systems is often equal to that of crop plants in order to reinstate water evaporated from surface [66]. Hence it is highly essential to determine alternative sources of water for wide scale implementation of microalgal cultivation. Domestic and industrial wastewaters may be considered as an alternative in this context, but it is essential to assess any wastewater induced toxicity in microalgal cultures prior to wide scale application.

Nutrient Balance

The cumulative presence of nutrients, illumination, water and a carbon source is highly essential for algal growth. The most vital nutrients include phosphorous, nitrogen, iron and sulfur. As algae can easily adapt to stress conditions, the nutrient availability for microalgae is often neglected. Effect of variations in nutrient load on algal growth needs more detailed investigation. It is highly inconvenient to achieve efficient algal growth by following a model based of terrestrial aquaculture. Nutrient balance is often maintained by addition of macro/micro nutrient supplements or fertilizers. Fertilizers generally applied are mostly prepared from fossil fuels and hence their extensive use is not advisable [69].

Fertilizers provide phosphorus, nitrogen and potassium, besides which algal strains often require chelated iron and sulfur as well. Additional nutrient supplements and fertilizers account for higher costs in cultivation of microalgae. Due to unavailability of adequate phosphate sources, it is almost imperative to recycle phosphate back into open ponds which may serve as only a temporary solution to the crisis. Unlike phosphorus, nitrogen is more widely available in bulk quantities but is a limiting macronutrient as it is only utilized by algae as ammonia and nitrates [66]. Almost all algal strains identified till date require an exogenous source of nitrogen, preferably ammonia [66]. Utilization of nitrogen-fixing cyanobacteria may help minimize the recurring cost of nitrogen supply [66].

Iron also plays an important role in inducing algal bloom and thereby promoting CO_2 sequestration [66]. In algal cells, iron is usually present as iron-sulfur clusters in different photosynthetic proteins [70]. Usually most algae utilize iron in chelated forms. However iron is more widely available in comparison to all other required nutrients.

Sulfur plays significant roles in electron transport chain, protein production and lipid metabolism. Inadequate sulfur results in reduced algal density and stunted growth [66]. Hence it is essential to determine the optimum quantity of sulfur for desired algal growth and best economic benefits.

The aforementioned elements along with potassium and other basic nutrients are highly essential for promoting algal biofuel production to acceptable levels [71]. Most of the required nutrients may be replenished in the algal growth medium by utilizing nutrient loaded wastewater and agricultural runoffs. Reuse of wastewater will encourage both water remediation and cost effective fuel production. These strategies require detailed investigation and a combined approach of different possibilities for nutrient recycling in algal cultivation thereby making algal fuel a feasible alternative to fossil fuels. However, anaerobic digestion may be considered as the most appropriate process of nutrient recycling in algal ponds [72]. This bacterial digestion yields a sludge rich in most of the nutrients that can be killed and reused as algal fertilizers. Bacterial treatment also liberates methane which may be utilized as energy source for farm operations. Hence it is essential to maintain a balance between efficiency of bacterial activity and production of beneficial byproducts.

Biological Contamination

Protection from biological pathogens and pests is a major challenge to biodiesel production using microalgae. Identification of strains with pathogen resistance or inclusion or engineered resistance in strains needs further investigations. An alternative approach may be utilization of mixed cultures which may reduce rate of host-specific pest infection of algal strains thereby preventing crop loss [66].

Pests may include nutrition competitors (other undesirable algal specie or bacteria), parasites like virus, fungus, etc. as well as predators such as protozoans, fungus or aquatic invertebrates [66]. These biological contaminations mostly occur in open culture setups. Closed systems however experience minimum contamination but at the expense of high capital. Conventional open pond systems have always utilized axenic (or nearly axenic) starter cultures to avoid contamination, but this method is unsuitable for continuous harvesting of wide scale cultures. Contamination may also be avoided by selecting highly resistant species like Dunaliella salina and Arthrospira that can tolerate extreme conditions of salinity (35%) and pH (10) [66]. Nevertheless, majority of algal strains cannot survive in adverse environmental conditions. Besides, there still remains a possibility of extremophile contamination [66].

However, many algal strains have inbuilt morphological, behavioral and chemical defenses against pathogens and predators. Chemical defense mechanisms in algal strains are active against competitors, parasites as well as protozoans [66]. Most antibiotics extracted from microalgae have been yielded by cyanobacteria, haptophytes, chrysophytes, diatoms, dinoflagellates and chlorophytes [66]. These antibiotics widely differ in their chemical properties. Some antibiotics accumulate in algal cells and act only when the cell is damaged or ingested. In other algal species, toxins are secreted in the surrounding media to ward of negative interactions [66]. Antibiotics can cause acute toxicity, reduced growth, feeding inhibition and evasion to algal grazers [66].

These natural defense mechanisms should be properly exploited in order to ascertain crop protection for biodiesel generation. Ideal selection criteria of microalgae should include sustenance in extreme conditions and possession of broad spectrum antibiotics. Since, these properties may not be present simultaneously in a single algal strain; crop protection may be enhanced by co-culture of algae that will synergistically provide crop defense. Crop defense may also be obtained by engineering a single algal species to yield higher number of antibiotics or to carry out specific crop protection [66]. A fundamental understanding of algal-pathogen interaction is required for effective crop management. In presence of limited fundamental data, it is important to balance cost of the solutions with increase in productivity and benefits on investment.

Bioprospecting

Different species of microalgae should be tested for biodiesel production. Most of the reported species belong to diatoms (Thalassiosira pseudonana and Phaeodactylum tricornutum), Chlorophyceae & trebouxiophyceae (Dunaliella salina, Chlamydomonas reinhardtii, Scenedesmus sp., Botryococcus sp., etc.), and Cyanobacteria. Further detailed study of these diverse classes may lead to identification of new species with better growth characteristics, higher fuel production and valuable co-products that contribute for improved characteristics of algal biofuels. Besides, knowledge of genomic data of these species can be combined with the existing for engineering other species with higher potential for algal biodiesel production [73].

Potential Benefits of Microalgal Oil Production

Microalgae include a wide variety of photosynthetic microorganisms capable of fixing CO_2 from the atmosphere and water to produce biomass more efficiently and rapidly than terrestrial plants. Numerous algal strains have been shown in the laboratory to produce more than 50 percent of their biomass as lipid with much of this as triacylglycerides (TAGs), also called triglycerides, the anticipated starting material for biodiesel fuels. Most of the observations of high lipid content come from algal cultures grown under nutrient (especially nitrogen, phosphorous, or silicon) limitation. Lipid content varies in both quantity and quality with varied growth conditions. While high lipid yields can be obtained under nutrient limitation, this is generally at the expense of reduced biomass yields. Nevertheless, the possibility that microalgae could generate considerably more oil than typical oilseed crops is an exciting opportunity. An additional benefit of growing algae as a biofuels feedstock is that they can be cultivated on otherwise non-productive (i.e., non-arable) land that is unsuitable for agriculture or in brackish, saline, and waste water that has little competing demand, offering the prospect of a biofuel that does not further tax already limited resources.

Future Research Directions

Different aspects of biodiesel synthesis from microalgal strains including detailed descriptions of steps in biodiesel production, its cost effectiveness in comparison to petroleum fuel and other valuable benefits from microalgal cultivation. This study has also discussed the potentials and challenges of algal biofuel production. Improper investigation in any process will lead to long term consequences overshadowing its short term benefits. Addressing the challenges highlighted in this study will help in rendering the described process cost effective and deployable on wide scale in a sustainable way. Advanced biotechnological and engineering endeavors are required to exploit all advantages of microalgal biodiesel and development of a green alternative energy for industrial and domestic use.

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