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Biomass from Microalgae: An Overview

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Abstract

Microalgal biomass offers a number of advantages over conventional biomass such as higher productivities, use of non-productive land, reuse and recovery of waste nutrients, use of saline or brackish waters, and reuse of CO_2 from power-plant flue-gas. The production of microalgal biomass reduces Greenhouse Gases (GHG) and provides biofuel as a replacement for fossil fuels. They are useful for production of food, health supplements, fodder, biofuel, aquaculture, fine chemicals and various biotechnological applications. The most commonly used marine algal cultures are *Botryococcus braunii*, *Chlorella vulgaris*, *Chaetoceros muelleri*, *Dunaliella salina*, *Nannochloropsis oculata, Arthrospira maxima*, *Scenedesmus quadricauda*. The current review provides details of the microalgal biomass with emphasis on strain selection, cultivation, strain improvement and biotechnological potentials.

Keywords: Microalgae; Biomass; Open raceway ponds; Photobioreactor; Nutrients

Introduction

Microalgae are most abundant primary unicellular producers found in all the aquatic systems such as, freshwater, seawater, hypersaline lakes and even in deserts and arctic ecosystems [1,2]. Sub-divided into eukaryotic and prokaryotic algae, eukaryotic possessing defined cell organelles such as nuclei, chloroplasts, mitochondria, prokaryotes (cyanobacteria or blue-green) are primitive, possessing the simpler cellular structure of bacteria. They convert light energy and carbon dioxide (CO_2) into biomass (e.g.) carbohydrates [3]. Their photosynthetic mechanism is similar to land-based plants, but due to a simple cellular structure, and submerged in an aqueous environment where they have efficient to access water, CO_2 and other nutrients, they are more efficient in converting solar energy into biomass [4]. Microalgae can also grow in extreme environments; it could be produced on agricultural and non-agricultural lands. It could also make use of fresh, brackish, saline, wastewater, municipal sewage and industrial effluents [5]. Many microalgae species are able to switch from phototrophic to heterotrophic growth conditions. As heterotrophs, the algae rely on glucose or other carbon sources for carbon metabolism and energy and some algae can grow mixotrophically. Some of the important updates on the biomass of algae have been elaborated in the following sections.

Microalgae are energy-rich feed stocks which have received so far more attention due to their facile adaptability to grow in the photobioreactors or open ponds for high yields and multiple applications. They have many inherent advantages, some of them are: higher productivity (biomass) in few days, easy adaptability to new environments and high-lipid content. Algae are cultivated not only for food source but also the cultivated biomass is used for feedstocks too [6] and scaling up algae farming could lead to yields of other commercially viable products [7,8]. Since two thirds of earth's surface is covered with ocean, algae would be the option with greatest potential for food including aquaculture industries, pharma products and global energy needs (Figure 1) [9].

Selection of new strains

Microalgae can be purified from a wide range of environments and they have to be screened for biofuel production, accumulation of desired storage compounds (high lipid strains), high-value products (unsaturated fatty acids), salt and temperature tolerance, resistance to predation and ease of harvesting. A variety of techniques can be used for isolation of microalgae, including physical extraction from crude water samples (micromanipulation), dilution to resolve individual cells, antibiotic selection and enrichment cultures using specific selection pressures (photoautotrophic conditions). Individual algal strains can be purified based on traditional method or high throughput Fluorescence-Activated Cell Sorting (FACS) approaches before the resulting axenic cultures are cryopreserved for storage to prevent genetic drift. Screening of algae is often based on optimal growth or the production of specific metabolites and the use of response surface modeling and principle component analysis to identify these conditions and the key

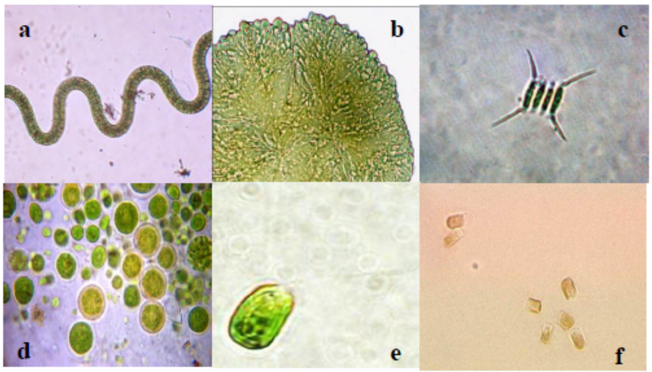

Figure 1: Few commercially important microalgal strains. (a) *Arthrospira maxima* (b) *Botryococcus brauanii* (c) *Scenedesmus quadricauda* (d) *Chlorella vulgaris* (e) *Dunaliella salina* (f) *Chaetoceros muelleri.*

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variables controlling those [10]. It is important to select the strain that grows well in the particular geographical location and environment, as it easier to manipulate acclimatized strains. Most companies have recognized the importance of utilizing the highest performing algal strains, whereas some favour screening locally harvested natural strains others prefer to genetically modify strains [11]. The selected marine microalgae strains would be suitable for maximum biomass production, where the total biomass composition includes, total caloric value of the biomass, % lipids and lipid composition (for biodiesel), % starch and carbohydrate composition (for bioethanol and to identify higher value byproducts, % protein and protein composition (soluble/insoluble for food/feed purposes). There are lots of important characters ideally be (quantitatively) measurable. For these criteria one should do an outdoor cultivation to get maximum as well optimum biomass in large scale systems to commercialize the product [2]. Productivity of specific biomass would help to increase production of components such as fine chemicals, nutraceutical and lipids.

Strain development and nutrients

For autotrophic growth, microalgae require many elements, which fall under two categories macronutrients (required in g/l concentrations) and micronutrients (required in mg/l concentrations). In addition to macronutrients such as inorganic elements (P, N, and C), microalgae require sufficient light, and favorable temperatures to grow. Hydrogen (H) and oxygen (O) are also essential for algal growth. Micronutrients are elements are needed for algal growth in trace amounts such as calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), manganese (Mn), sulfur (S), zinc (Zn), copper (Cu), and cobalt (Co) [5]. It is also important to understand that different algal species do not have identical nutritional needs, and it also depends on their source of environment such as freshwater, marine, halotolerant/halophilic species. Screening of algae is often based on optimal growth or the production of specific metabolites and the use of response surface modelling and principle component analysis to identify these conditions and the key variables controlling the production of microalgal biomass [10]. Alternatively, since microalgae represent a much simpler system for genetic manipulations compared to higher plants, they could be exploited for strain improvement. Additionally microalgae have significant advantages such as they have life cycles of hours or days instead of seasonal cycles, their unicellular nature and absence of cell differentiation helps in miniaturization of breeding systems and thereby the cost; they can replicate both sexually and asexually making the versatile tools for generation of higher genetic diversity compared to prokaryotic organisms and finally selection, screening and UV and chemical mutagenesis can be more easily applied [10]. But both genetically and non-Genetically Modified Organisms (GMOs and non-GMOs) can be screened for efficient phenotypes.

Genetic modifications in microalgae through a wide range of target mechanisms such as stress tolerance, product accumulation pathways, cellular chlorophyll contents, novel metabolic pathways, resistance to pathogens and competition, etc, make them better suitable for industrial applications. For instance, development of new genetic transformation tools for will provide metabolic engineering opportunities for overproduction of astaxanthin in microalgae. One possible application is to enhance astaxanthin production in *H. pluvialis* or *C. zofingiensis* by overexpressing PSY and CrtR-b genes, which previously have been shown to be possible rate-limiting steps for astaxanthin synthesis in *H. pluvialis* [12-14]. Additionally, an alternative strategy for enhancing astaxanthin production in microalgae is through random mutagenesis. Through chemical mutagenesis, several *Haematococcus* mutants with improved phenotypic traits have been generated, and one such example is *Haematococcus* astaxanthin-hyper-accumulating mutant strain MT 2877 [15].

There has been lot of advancements in algal genetic engineering, including genetic transformation, gene expression to use molecular genetics to advance algal biofuel production. An efficient transformation method was developed for *Nannochloropsis* sp., a fast-growing, unicellular alga capable of accumulating large amounts of oil by generated knockouts of the genes encoding nitrate reductase and nitrite reductase, resulting in strains that were unable to grow on nitrate and nitrate/ nitrite, respectively [16]. More recently, in a *Chlamydomonas reinhardtii* mutant with inactivated ADP-glucose pyrophosphorylase, an enzyme involved in starch synthesis, the triacylglycerol content exhibited a tenfold increase, from 2 to 20.5%, under nitrogen starvation [14]. Genetically modified microalgae can offer varied advantages, which is achieved by engineering the metabolic pathway to flux the metabolites towards the desired metabolite production or altering the regulatory factors such as transcription factors for over-expression of specific genes. On the whole collection, cultivation, selection and breeding of naturally occurring strains with high productivity or genetically modified microalgae with desired phenotype is required for both increase in biomass as well as high value product production.

Maintenance of axenic culture

Microlgae isolated from natural environments or genetically modified organisms are unlikely to be optimally adapted to the new environment, either in open pond cultivation or using photobioreactors. Contamination by predators and other fast growing heterotrophs have restricted the commercial production of algae. There are many factors that determine the species dominance such as resource-growth rate relationships for different algae, variable environmental conditions, inhibition of one organism by another through excrete dallelopathic substances, loss of growth due to predation or sinking. Free fatty acids, cell wall degradation products, exo-metabolites produced by algae and cyanobacteria, such as cyanobacterin [17] and fischerellin [18] could also have allelopathic potential.

The effect of single or specific variables or combination variables on the growth of organisms in a laboratory scale can be made. The key issue is difficulty in simulation of the outdoor environment in the laboratory conditions or extrapolation of data from lab to mass culture condition. The problem of species dominance and competition in outdoor mass cultures can be controlled by variations of biota, light, temperature, pH, oxygen and nutrient supplies. For example, high ammonia or pH will inhibit most zooplankton infestations, or selecting a growth environment for specific microalgae species such as a very high alkalinity selects for *Arthrospira* and high salinity for *Dunaliella*, the two major species. These techniques are expensive and result in severe reduction of algal productivities. High density inoculations and semi-batch operations could reduce contamination in *Chlorella* production, but this often resulted in reduced overall productivity. Both *Dunaliella* and *Chlorella* are dominant in their optimal environments. Contamination can be better managed in closed photobioreactors, but upon continuous cultivation both open and closed systems become more susceptible. Careful strain isolation and characterization, cultivation parameters of individual microalgal candidates and the expected contaminants in the region can reduce the contamination issues [19].

Biomass production

Microalgae is the best choice for recombinant protein productions, for production of fine chemicals, pharma products, poultry feeds, feed stock and the source of future biofuel (biodiesel, hydrogen, methane and bioethanol) because they have simple and inexpensive growth requirements (free seawater, inexpensive nitrogen, phosphorus, and carbon sources), rapid growth rates with sufficient light. The high density and large-scale cultivation of microalgae is the preferred way for the development and production of these high-value products. This could be achieved by either growing algae in closed photobioreactors or open-pond systems.

Photobioreactor: Basically, Photobioreactors (PBRs) are closed cultivation systems to grow microalgae under photo-autotrophic conditions [20]. Ideal growth conditions for microalgal cultures are strain specific and the biomass productivity depends upon many factors. These include abiotic factors like temperature, pH, water quality, minerals, carbon dioxide, light cycle and intensity. Water, nutrients and CO_2 are provided in a controlled way, while oxygen has to be removed. All parameters (nutrients, light regime, gas exchange) are maintained to realize optimal culture conditions. The contamination level is much lower compared to open systems.

Open raceway ponds: Open pond systems are shallow ponds (a maximum of 70 cm) in which algae are cultivated. Nutrients can be provided by mixing with few litres of water nearby paddle wheels. The water is typically kept in motion by paddle wheels and some mixing can be accomplished by appropriately designed guides. Raceway ponds are being in use in Israel, USA, China, India, Taiwan, Japan and other countries. Fertilizer is used for mass cultivation and the culture is agitated gently by paddle wheel (Figure 2d). The advantages of open ponds are low costs and that they are easy to operate. However, they are sensitive to contamination leading to introduction of unwanted fast growing organisms in the ponds. Especially heterotrophic organisms will graze on the autotrophic biomass and lead to loss of productivity. Therefore, the present commercial production of microalgae in open culture systems is restricted to only those organisms that can grow

Figure 2: (a) Culture scale-up for open raceway ponds (b) A typical lab-scale photobioreactor (c) French press (side view) (d) Open raceway pond (e) Flocculated culture shows algal clumping (f) CO_2 cylinder (g) Culture storage tank (h) French press (front view) (i) Wet algal biomass collected from French press (j) Hot air dryer (Courtesy: Aquatic Energy LLC, Louisiana).

under extreme conditions (i.e.) high pH or salinity. Thus, a limited range of microalgae can be maintained as monoculture in open ponds in long-term operation. Currently there is lot of algae mass cultured and marketed commercially they are *Skeletonema* sp, *Chaetoceros* sp, *Dunaliella* sp, *Arthrospira* in high alkalinity and *Chlorella* in high levels of nutrients have been successfully cultivated.

Biomass harvest

Biomass harvesting is a kind of technique and it accounts 15-20% of the production costs. The very small size of algae and their low concentration in the culture medium makes the cell recovery, harder one. The harvesting cannot be done by a single process because of the several species of algae with varying characteristics like shape, size and motility that influence to a big extent for their settling. Centrifugation is one of the most commonly used techniques to harvest microalgae in a lab scale as well in R&D laboratories. Algae are commonly used as a feed for aquaculture and they harvested by centrifuging to produce concentrates with longer shelf-life. Filter presses are used to recover fairly large microalgae like *Arthrospira* sp but are not suitable for smaller microalgae like *Scenedesmus*, *Dunaliella,* and *Chlorella* [19]. Using centrifuge in algal industries to harvest biomass is not a recommended and feasible one. Algae with autoflocculation characteristics simplify the harvesting step and minimize the energy and cost in the downstream processing of algae production [21]. The harvested algae biomass is dried so that the product can be stored without spoilage when algae are used as aquaculture feed (Figure 2).

Economic importance of algal biomass

Health supplements: Commercial use of microalgae as sources of specific chemicals began with *D. salina* for the production of β-carotene in the 1970's [1] followed by the use of *Haematococcus pluvialis* Flotow as a source of astaxanthin [22] and *Crypthecodinium cohnii* Seligo for long-chain Polyunsaturated Fatty Acid (PUFA) and Docosahexaenoic Acid (DHA) [23]. In the past few decades, microalgal biomass was directly utilized in the health food market with more than 75% of the annual microalgal biomass production being used for the manufacture of powders, tablets, capsules, or pastilles [2]. The two major species cultivated for this purpose are the unicellular green alga, *Chlorella* and more recently, filamentous blue-green alga (Cyanobacterium), *Spirulina*. A multitude of compounds are produced by microalgae which include Polyunsaturated Fatty Acids (PUFA), carotenoids, phycobiliproteins, polysaccharides and phycotoxins (Table 1).

Animal feed and aquaculture: Universally, more than 40 species of microalgae are used in aquaculture. Microalgae provide food for zooplankton they also help to stabilize and improve the quality of the culture medium. Indeed, for numerous freshwater and seawater animal species, the introduction of phytoplankton to rearing ponds leads to much better results in terms of survival, growth and transformation index [24]. The excreted biochemical compounds induce behavioural processes like initial prey catching and the regulation of bacterial population, probiotic effects and the stimulation of immunity [25,26]. Several factors can contribute to the nutritional value of a microalga (including its size and shape, digestibility, biochemical composition, enzymes, toxins and the requirements of animal feeding on the alga). Studies have attempted to correlate the nutritional value of microalgae with their biochemical profile [27]. However, results from feeding experiments that have tested microalgae differing in a specific nutrient are often difficult to interpret because of the confounding effects of other microalgal nutrients.

Microalgae grown to late logarithmic growth phase typically contain 30-40% protein, 10-20% lipid and 5-15% carbohydrate [28]. In the stationary phase, the proximate composition of microalgae can change significantly (e.g.) when nitrate is limiting, carbohydrate levels can double at the expense of protein [29]. There does not appear to be a strong correlation between the proximate composition of microalgae and nutritional value, though algal diets with high levels of carbohydrate are reported to produce the best growth for juvenile oysters, *Ostrea edulis* [30]. Larval scallops, *Patinopecten yessoensis* provided polyunsaturated fatty acids in adequate proportions. In contrast, high dietary protein provided best growth for juvenile mussels, *Mytilus trossulus* and Pacific oysters, *Crassostrea gigas* [31].

Microalgae are being in use for to improve the nutritional quality and gives coloration to the shrimps. Among these *Chlorella* grows well in nutrient-rich media, while *Arthrospira* sp requires a high pH of 9.5-11 with appropriate concentration of bicarbonate. Similarly, *Dunaliella salina* grows at the very high salinity of 0.5-6 M [32]. Several algae such as *Chaetoceros* sp, *Isochrysis* sp, *Skeletonema* sp, *Thalassiosira* sp, *Tetraselmis* sp and *Crypthecodinium cohnii* are being useful in the aquaculture industry which does not have these selective advantages, must be grown in closed systems. Table 2 summarizes the commercial algal culture and their uses. Factors to be considered for production of microalgae include: the biology of the alga, cost of land, labor, energy, water, nutrients (climate if the culture is outdoors) and the type of final product. Microalgae are necessary from the second stage of larval development (zoea) and in combination with zooplankton from the third stage (myses). Naturally occurring microalgal blooms are encouraged in large ponds with low water exchange where the larvae are introduced. Sometimes fertilizers and bacteria are added to induce more favorable conditions.

A sustainable and profitable biodiesel production from microalgae is possible. The biofuel can overcome the energy and environmental needs by integrating new technologies [33]. Large quantities of algal biomass needed for the production of biodiesel could be grown in

Microalgae	Products	References
Pavlova, Nannochloropsis, Phaeodactylum	Eicosapentaenoic acid (EPA)	Borowitzka and Borowitzka [1], Chisti [37] Raja, et al. [2]
Crypthecodiuimu and *Schizochytrium*	Docohexaenoic acid (DHA)	Borowitzka and Borowitzka [1], Chisti [37]
Spirulina	γ-linolenic acid (GLA)	
Porphyridium	Arachidonic acid (AA)	
Spirulina platensis	Phycocyanin	
Porphyridium cruentum	Phycoerythrin Polysaccharides	
Dunaliella salina	β-carotene	Borowitzka and Borowitzka [1], Chisti [37] Raja, et al. [2]
Haematococcus pluvialis	Astaxanthin	
Aphanizomenon flosaquae	Mycosporine-like amino acids (MAA)	

Table 1: High value products from microalgae.

Genus	Morphology	Purpose
Nannochloropsis sp	Small green algae	Growing rotifers and in fin fish hatcheries, used in reef tanks for feeding corals and other filter feeders, very high EPA level
Pavlova sp	Small golden-brown flagellate, very difficult to grow so it is not produced by many hatcheries	Used to increase the DHA/EPA levels in broodstock, oysters, clams, mussels and scallops, sterol composition so it is popular with cold water fish hatcheries (cod) for enriching rotifers
Isochrysis sp	Small golden-brown flagellate	Enrichment of zooplankton such as Artemia, used in shellfish hatcheries and used in some shrimp hatcheries, good size for feeding brine shrimp and copepods, oysters, clams, mussels, and scallops
Tetraselmis sp	Large green flagellate	Excellent feed for larval shrimps and contains natural amino acids that stimulate feeding in marine animals, used in conjunction with *Nannochloropsis* for producing rotifers, good size for feeding brine shrimp, standard feed for oysters, clams, mussels, and scallops, excellent feed for increasing growth rates and fighting zoea syndrome
Thalassiosira weissflogii	Large diatom	Used in the shrimp and shellfish larviculture, considered by several hatcheries to be the single best alga for larval shrimps, also good for feeding copepods and brine shrimps, post-set (200l and larger) oysters, clams, mussels, and scallops for brood stock conditioning
Dunaliella sp	Small green flagellate	Used to increase vitamin levels in some shrimp hatcheries and also for the coloration
Chaetoceros sp	Diatom	Used to increase vitamin levels in some shrimp hatcheries

Table 2: Commercial algal culture and its applications (Hemaiswarya et al.) [46].

photobioreactors combined with photonics and biotechnologies. However, more precise economic assessments of production are necessary to establish with petroleum derived fuels. The direct hydrothermal liquefaction is an energy-efficient technique for producing biodiesel from algae without the need to reduce the water content of the algal biomass [34].

Microalgae have high growth rates and photosynthetic efficiencies due to their simple structures. The efficiency is much higher (6-8%) than that of terrestrial plants (it is, 1.8-2.2%). The idea of using microalgae is not new, but it is now being taken seriously in several countries because of the emerging concern about global warming that is closely associated with burning of fossil fuels [35]. Microalgal biomass contains approximately 50% carbon by dry weight; therefore, it is also used to produce methane by anaerobic digestion. The process is technically feasible, but it cannot compete with many other low-cost organic substrates that are available for anaerobic digestion [36]. Depending on species, microalgae produce different kinds of lipids, hydrocarbons, and other complex oils [37,38]. The extent of unsaturation of microalgal oil and its content of fatty acids with more than four double bonds can be reduced easily by partial catalytic hydrogenation of the oil. The challenge lies in harvesting algal biomass and the extraction of biodiesel [39,40]. The heterogeneity of algal species and growth parameters makes this bio-inspired option a technical challenge for scale-up consideration.

Greenhouse gas reductions and global warming

The combustion of fossil fuel generates carbon dioxide, a major greenhouse gas that is considered as a huge threat because of its potential to cause severe global warming. Microalgae are particularly considered for bio-fixation because of their ability to grow fast and fix greater amounts of carbon dioxide. The bio-mitigation of carbon dioxide and other flue gases by microalgae have significantly gained interest in reducing the emissions from coal-fired power plants. The algae biomass thus produced by capturing carbon can be used in generating valuable products such as fuel, animal feed and fertilizer. Power plants are the major sources of CO_2 and release 5.7 giga tones of carbon dioxide per year [41]. CO_2 is produced by both stationary and mobile sources. Microalgae offer a natural way to recycle carbon dioxide from the flue gas and thus help in reducing the effects of global warming and climate change.

Microalgal biomass is composed of 45 to 50% carbon based on dry weight measurements [42]. The high carbon content of microalgae makes it suitable for storing carbon. CO_2 present is flue gas can significantly raise the growth rates of microalgae. Microalgae can be engineered in open ponds or photobioreactors to maximize CO_2 conversion to biomass thereby sequestering carbon and also producing a biofuel. The selection of microalgae is the most important factor in the bio-mitigation of carbon dioxide from flue gases generated by power plants. The algae should have high growth and CO_2 utilization rates. The other suitable characteristics for carbon dioxide bio-fixation are their ability to tolerate SOx and NOx, and thrive in mass cultures without contamination.

Prominent issues in commercializing microalgae

In the current situation, commercially biodiesel is not produced from microalgae [36]. One of the biggest challenges is to reproduce the closed laboratory conditions on a large scale. It is understood that in the lab, it is easier to produce oil [43] and several companies are attempting to commercialize microalgal biodiesel [43,44]. Oil productivity is directly related with algal growth rate and the oil content of the biomass. Hence, it is well known that the microalgae with high oil productivities are desired for producing biodiesel. However, producing biomass is generally more expensive than growing crops. Temperature must remain within 20 to 30°C. To minimize the expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels [38]. The crucial economic challenge for algae producers is to discover low cost oil extraction and harvesting methods [45]. Singh and Gu [45] suggested that the utilization of fatter algae with higher oil content (around 60%) in comparison to lower oil content algae can reduce up to half of the size and footprint of algae biofuels production systems and reduces the capital and operating costs. In addition, a cheaper and easier process can provided a better ground to commercialize. Using a non-lethal extraction called milking, can avoid a serious of processes such as harvesting, extraction etc. Carotenoids, high value lipids have also been selectively extracted from the green alga *Chlorella* sp. using decane [46]. Such methods have the capability to reduce the production cost significantly and simplify the biodiesel production process from algal biomass.

Conclusion

As far as the algal biomass production has concerned, currently there are lot of projects going on in many countries particularly in India, China, United States, European Union etc. and they are using these harvested biomass for multitude of applications. Algae also have the scope of growing in municipal sewages (or from effluents) containing heavy metals and unwanted chemicals which are bio-absorbed. This is the best biological method recommended by naturalists and scientists to protect the environment clean. At the same time, algae capturing atmospheric CO_2 as well as contribute to reduce global warming considerably. The greatest problem in algae biomass production development of superior strains of algae is with hurdles in collection from natural habitats, isolation, selection, maintenance, and genetic improvement.

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