Scienza e società 2

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Sustainability, Technology and Selected Case Studies

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Alvise Perosa, Guido Bordignon,
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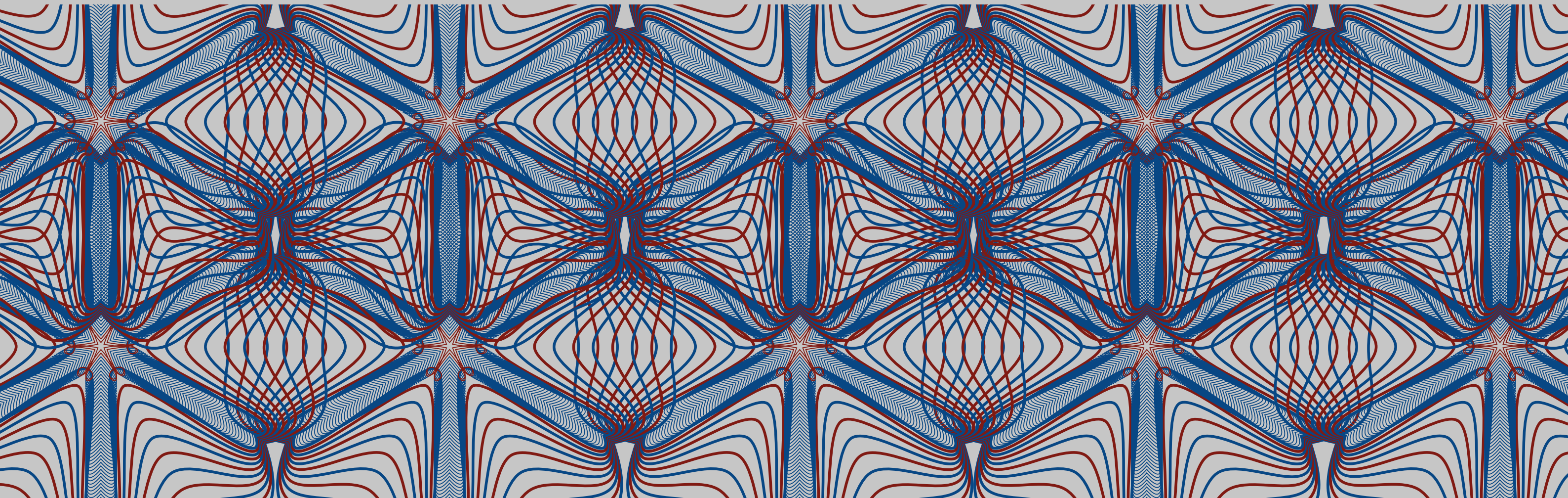

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2

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Summary

Foreword

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Using plant biomass for production of energy and added value products not only helps protecting the planet through the reduction of GHG emissions and explores alternatives to the alarmingly depleting fossil resources. It also means energy security and social benefits for many countries where the establishment of the new green industrial segment translates to new jobs and markets. Especially in developing countries and countries with economies in transition, the spin-off of the so-called bio-based industry to produce fuels, chemicals and materials from biomass is believed to stimulate both agricultural and industrial development resulting in the sustainable economic growth.

Many such countries historically rely on biomass to meet their energy needs. For some countries biomass has been the key to industrial development, like palm oil in Malaysia or sugar cane in Brazil, while for others their green potential still remains largely unexploited. The recent spread of edible oil and sugar based biofuels in industrialized countries was driven by the incentives policy and was mainly dependent on the provision of feedstock from less developed regions. Notwithstanding the new markets built in the supplier countries, neither proper industrial capacity nor capital was created locally. Moreover, these first generation biofuels were then seriously criticized for their competition with food production, as well as for their not always favourable greenhouse gas (GHG) balances.

It is now a commonly accepted vision that the sustainable biomass exploitation shall 1) valorise waste biomass, such as agricultural residues and food waste, and 2) look into alternative forms of biomass that do not compete with food production, do not cause environmental issues and are economically viable. The so-called next generation biofuels are often viewed in the context of biorefinery where not only energy but also other products and waste add value to the feedstock. The transition ton a sustainable bio-based industry requires in first place new feedstocks and new production processes.

It is worth mentioning that an essential obstacle to developing countries' taking lead in the emerging bio-industry is often the lack of S&T capacity. The latter is the main prerequisite for the in-house technology development and deployment that would rely on and benefit from the know-hows and knowledge of specific conditions and resources available locally. This knowledge together with other conditions stimulating and enabling investments could shift the paradigm from the developing countries' serving the industrialized economies as resource suppliers to their becoming end-product exporters.

In this edition we try to depict the emerging microalgae sector from the prospective of its potential use in the growing economies. In the recent years, microalgae (often simply called algae) have become a high topic with significant R&D and commercial implications of global dimension. In warmer climatic regions, microalgae have received increased attention because of the favourable conditions for their cultivation and not not very high investments needed to setup small-scale production plants. In fact, in some applications, such as food and chemicals production, microalgae projects have been commercially proven in the last thirty years. On the other hand, a vast variety of valorisation options and the overall complexity of production chains leave a number of possibilities for technology improvement. Together with the search for new and the genetic improvement of microalgae it explains the big number of R&D projects in microalgae.

The new green feedstock is seen as an alternative to the classical plant biomass for production of food, energy, drugs and chemicals and has a number of advantages like high growth rates and productivity or no land requirements. However, many issues also remain unsolved about microalgae, such as high technology cost for certain applications, high energy requirements for their processing and their sensitivity to various growth conditions affecting productivity. Most important applications of microalgae in the fields ranging from food and nutraceuticals to biodiesel, chemicals and environmental safeguard are highlighted. Various parts of the value chain are discussed from the cultivation and extraction to the chemical processing to end-products, considering both technical and economic implications.

The publication gives a general outlook on the developments in the field rather than a technical insight in specific issues or a theoretical background. It features some well-known applications and important developments while outlining trends and discussing potentials and risks. The underlying objective is to approach the question of whether microalgae can become an industrial opportunity for developing countries and where and how the progress more likely would take place.

Recognized experts in the field coming from both industrialized and emerging economies and representing different disciplines - from biology and chemistry to engineering and marketing - contributed to this task. Authors shared their vision on the potentials and challenges in their fields of competence, trying to identify promising niches for developing economies. Several case studies report on the on-going initiatives in research and commercial algae production from China, Malaysia and South Africa. In conclusion, a general analysis of challenges and opportunities of the whole algae industry for developing and transition economies is given in a separate chapter.

We believe that this publication will raise awareness in the developing nations' communities on the significant applications of microalgae, such as production of energy, food and chemicals, as well as their environmental use for water treatment and CO_2 sequestration. A multidisciplinary and generalization approach of this edition shall outreach to a wide range of specialists, from scientists and technologists to investors and decision makers. In the long term, sharing experiences on the state of the art of microalgae shall help countries establishing collaborations and partnerships in R&D and technology transfer projects, stimulate South-South cooperation and help adopting relevant policy instruments in order to promote the growth and dissemination of this innovative and green industry.

1 Algae: an Introduction

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1 Bases of Phycology, History and Classification of Algae

1.1 Bases

Microalgae are a group of photosynthetic organisms characterized by very simple structural organization, which use light energy, carbon dioxide (CO_2) and ions dissolved in the water for the synthesis of complex molecules and for the production of biomass. Microalgae are components of plankton and benthic communities and can live anywhere, in marine, fresh or transitional waters and with a very thin water film, they can also colonize rocks, soil, sand, polar and mountain ice. Only a certain number of species live in symbiotic association with different organisms while microalgae are usually free living as single cells or in colonies. The term microalgae, in applied phycology, include the microscopic algae *Sensu stricto* and the photosynthetic bacteria (i.e. cyanobacteria), formerly known as Cyanophyceae [1]. The cell structure is eukariotic in microalgae and prokariotic in cyanobacteria, but in terms of biomass, they are both considered as a potential source of energy, fuel, food and other interesting commercial products (lipids, proteins, carbohydrates). In ecology, these two phototrophic groups represent the first level of the trophic chain.

Microalgae, including cyanobacteria, share bioenergetic metabolism (oxygenic photosynthesis) and are directly responsible for just under 50% of photosynthesis on Earth; even if in terms of biomass, the aquatic organisms represent less than 1% respect the total plant biomass [2]. Therefore, the algae play a key role in regulating atmospheric CO_2 concentrations. In seawater rarely free CO_2 is found, while the inorganic carbon is present in the form of bicarbonate. However, the inorganic carbon equilibrium is the major determinant of pH, for this reason the pH cannot be considered as an independent variable. In the presence of light, only the obligate photoautotrophs are generally able to acquire and assimilate CO_2 as carbon sources for growth.

The first research on photosynthetic processes were largely based on data obtained using experimental designs with microalgae such as *Chlamidomonas* and *Chlorella* (i.e.) that have been chosen essentially for their easy growth or genetic manipulations. The photosynthetic processes are differently regulated in terrestrial and aquatic environments as consequence of many evolution and physiological adaptions occurred in the organisms. Up to now, we have sufficient knowledge regarding the basic mechanisms of the microalgae behaviour to some changes in their environment. Many works have suggested to use flue gas as carbon origin for microalgal cultures and to combine contemporarly both CO_2 mitigation strategies and biofuel production. The influence of high CO_2 concentration on microalgal growth must be investigated, particularly its effect can be examined on growth of a natural phytoplankton assemblage in controlled mesocosm experiments. In phytoplankton communities, during and after a bloom, diatom species responded differently when were exposed to varying CO_2 levels; only *Skeletonema costatum* showed an increase in growth rate with increasing seawater CO_2 while *Nitzschia spp.* mantained a constant growth rate [3]. These different responses of two diatoms to seawater CO_2 manipulation suggest that, for the future, in the surface ocean the structure of the diatom groups could potentially change at CO_2 concentration increase. Considering the growth characteristcs of *Botryococcus braunii*, a green colonial microalga with high hydrocarbon content, develops blooms in a variety waters, including freshwater, brackish and saline lakes [4,5,6,7]. In nature, *B. braunii* shows a potential competitive advantage and in photobioreactor experiments increased with increase of CO_2 concentration and in nitrogen deprivation in the culture [8]. In this study, the author reported that the maximum algal biomass of 2.31 gL-1 was obtained with 20% CO_2 on day 25. Under these experimental conditions, the lipid content was lower than others reported for the same species; this could be a consequence due to different algal strains. In fact, the response is species- and strain-specific. Interesting observations in this study were that the cells under variable CO_2 concentrations formed colonies of different size, color and compact structure. Green compact structure of the colonies was mantained at 2% CO_2, while became yellow at increasing of CO_2. To capture solar energy as CO_2 to generate metabolic flux. Chlorophyll is essential component and responsible for the photosynthetic mechanism. So a good cholorophyll content inside the cell can guarantee the cell growth and the lipid accumulation. The chlorophyll content increases in the beginning of the culture as the algal cells increase with the species growth. The cholophyll content in *B. braunii* under 2% CO_2 was generally higher than other concentrations and the cell growth did not cease after the decreased chlorophyll content indicating that photosynthetic pathways were active with other pigments and not with chlorophyll [8].

The first use of microalgae and cyanobacteria was historically in food (the case of *Spirulina spp.* in Chad) due to its high content of protein and other nutrients that make it an excellent food supplement, especially suited for cases of malnutrition.

High added value molecules such as fatty acids (omega 3, DHA, EPA), pigments (carotenoids, antioxidants), and biochemical stable isotopes can be extracted from algae. Furthermore, some metabolites seem to possess pharmacological activities: anti-cholesterol, anti-tumor, immunomodulatory, antibacterial and antifungal. Such fine-chemicals are used as food supplements, cosmetics and general treatments for the care and welfare of the person.

They are also used as feed for aquaculture; in fact microalgae constitute the main food of rotifers, in turn important live food for larvae of marine fish, seahorses, invertebrates, filter feeders, and many more. The use in animal feed for domestic animals and farmed species in poultry (broilers and laying hens) is yielding interesting results regarding the vitality and well-being of the animal: the color of meat, eggs, feathers are benefited.

The microalgal biomass has also given interesting results in using renewable energy to produce oil, ethanol or biogas. In the latter case, particularly, using wastewater to grow, one gets the double benefit of “phytoremediation” for wastewater and of energy from algal biomass. There are also species that, with proper cultivation systems, are able to produce molecular hydrogen. Integrated with other agricultural waste, algae can be treated by pyrolysis to obtain biochar. Biochar is a natural fertilizer showing results in the cultivation, in particular, restoring and maintaining the organic matter in the soil.

A recent technological application involves the use of microalgae for the capture of CO_2 from the exhaust gases, in particular from plants for the production of energy, heat or electricity. For every kg of biomass produced 2 kg of CO_2 are captured by photosynthetic microalgae [9]. Recent studies have demonstrated the effectiveness of using microalgae, cyanobacteria in particular, for the capture of heavy metals from water.

From an environmental point of view, microalgae are essential for the equilibrium of ecosystems. Algae can survive in hostile and extreme habitat, natural as well as those with strong human impact. In both marine or fresh waters, algae can be found at different temperature, salinity, concentration of main nutrients (nitrates, nitrites and phosphates), and in variable availability of light from the surface to the benthos as in coastal or offshore ecosystems. Also, the variability of pH due to different dissolution of CO_2 in seawater can represent another potential threat for marine calcifying organisms such as coral reefs, clams, oysters and mussels and, at the basis of the food chain, foraminifera and the coccolitophores that are protected by a theca built by calcium carbonate ($CaCO_3$) plates.

1.2 History

The term algae is pointed commonly to a body of simple structures, autotrophic, unicellular or multicellular organism, which produce oxygen and which do not have a differentiation in the tissues themselves. Not all have the common type of chlorophyll for photosynthesis or chlorophyll a. Simple forms of algae were already thriving 1.5 billion years ago.

In the past, single-celled organisms (which can unite in colonies) and autotrophic prokaryotes, were considered algae, and also called “cyanobacteria” or “blue algae”, now more properly included in the taxon of cyanobacteria belonging to the kingdom of Monera.

The real reason the eukaryotic algae, which traditionally belong to the domain of Protists, which can be either unicellular or multicellular. This grouping, however, is certainly paraphyletic or polyphyletic: the algae have recently been separated into different groups of eukaryotes, although the taxonomy is still very uncertain and varies considerably from author to author.

Algae are generally photosynthetic, although there are forms mixotrophic or facultative photoautotrophic such as *Chlamydomonas spp.* belonging to Chlorophyceae, that can use organic compounds to supplement its photosynthetic nutrition [2]. Other species such as *Noctiluca scintillans,* a dinoflagellate without chloroplasts, can be also included among the Protozoa for the presence of photosynthetic symbionts that determine a green coloration of its cytoplasm. Some unicellular algae depend solely on external energy sources and have limited or no photosynthetic apparatus.

The algae are structurally simple organisms. In most cases algae have a haploid life cycle, others are haplo-diploid - with alternating generations haploid (sporophyte) and diploid (gametophyte). Only the most advanced algae (such as the type Bacillariophyta) have a diploid cycle. The algal embryo is not protected by maternal cells, although during the life cycle, some dinoflagellates can produce temporary cysts and resting cysts that can survive in harsh environmental conditions in short or long periods until germination in response to better environmental change.

Generally microalgae live in the euphotic zone, preferably in the surface layer where light penetration is adapt for growth. They are single cells, completely self-sufficient. They are a component of phytoplankton and play a vital role as primary producers in the food chain from which the life of aquatic consumers are dependent. In aquatic ecosystems the process of primary production is related to the availability of external energy, which contributes to the injection of nutrients into the euphotic zone [10]. The mechanisms of photosynthetic processes capture and store CO_2 in organic carbon compounds and also supply the atmosphere with large amounts of oxygen.

1.3 Classification

1.3.1 Green Algae

Green algae are the large group of algae from which the embryophytes (higher plants) emerged. As such, they form a paraphyletic group, although the group including both green algae and embryophytes is monophyletic (and often just known as kingdom Plantae). The green algae include unicellular and colonial flagellates, most with two flagella per cell, as well as various colonial, coccoid, and filamentous forms, and macroscopic seaweeds. In the order Charales, the closest relatives of higher plants, full differentiation of tissues occurs. There are about 6,000 species of green algae. Many species live most of their lives as single cells, while other species form colonies, coenobia, long filaments, or highly differentiated macroscopic seaweeds. Almost all forms have chloroplasts. These contain chlorophylls a and b (as well as the accessory pigments beta-carotene and xanthophylls), giving them a bright green color, and have stacked thylakoids.

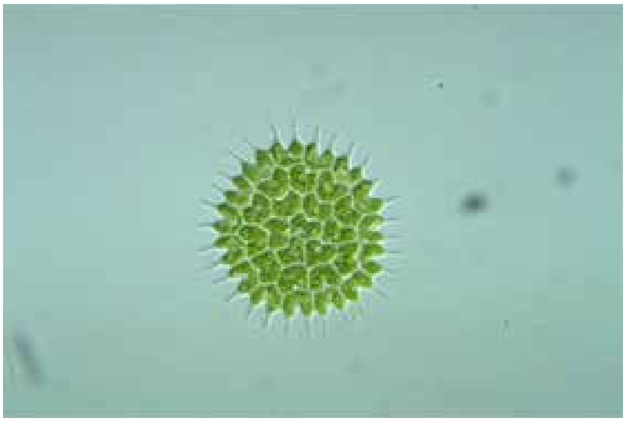

Figure 1. Green alga *Pediastrum boryanum.*

1.3.2 Blue-green Algae

Cyanobacteria also known as blue-green algae or blue-green bacteria, are of a bacteriaphylum Cyanophya that obtain their energy through photosynthesis. The ability of cyanobacteria to perform oxygenic photosynthesis is thought to have converted the early reducing atmosphere into an oxidizing one, which dramatically changed the composition of life forms on Earth by stimulating biodiversity and leading to the near-extinction of oxygen-intolerant organisms. According to endosymbiotic theory, chloroplasts in plants and eukaryotic algae have evolved from cyanobacterial ancestors via endosymbiosis. Cyanobacteria can be found in almost every terrestrial and aquatic habitat, from oceans to fresh water, and from bare rock to soil. They can occur as planktonic cells or form phototrophic biofilms in fresh water and marine environments, they occur in damp soil, or even on temporarily moistened rocks in the desert. A few are endosymbionts in lichens, plants, various protists or sponges and provide energy for the host. Some live in the fur of sloths, providing a form of camouflage. Aquatic cyanobacteria are probably best known for the extensive and highly visible blooms that can form in both freshwater and the marine environment and can have the appearance of blue-green paint or scum. The association of toxicity with such blooms has frequently led to the closure of recreational waters when blooms are observed. Marine bacteriophages are a significant parasite of unicellular marine cyanobacteria. When they infect cells, they lyse, releasing more phages into the water.

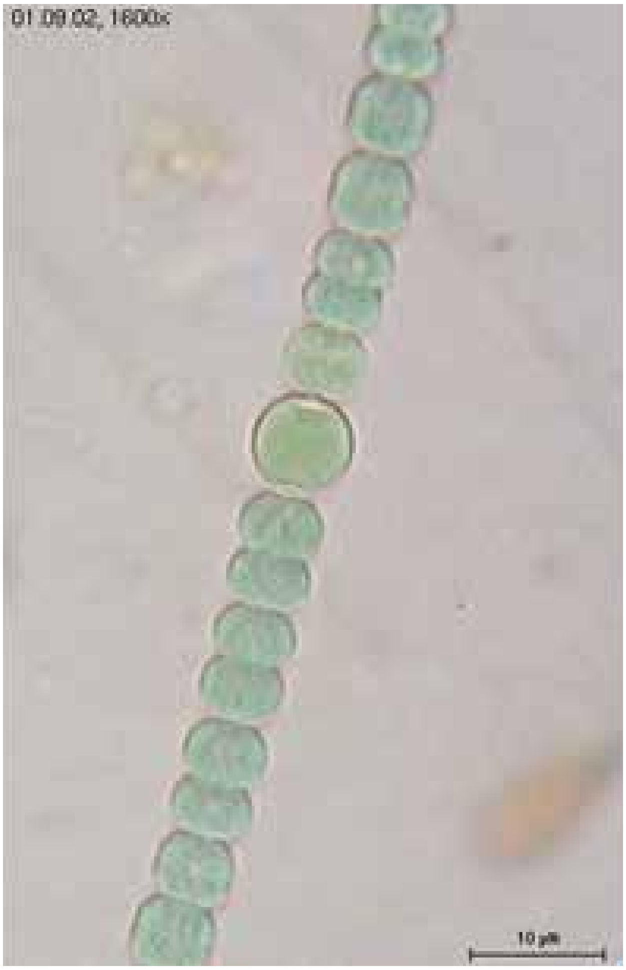

Figure 2. Cyanobacteria (blue-green alga) *Anabaena spherica.*

1.3.3 Red Algae

Red algae, or phylum Rhodophyta are one of the oldest groups of eukaryotic algae, and also one of the largest, with about 5,000–6,000 species of mostly multicellular, marine algae, including many notable seaweeds. Other references indicate as many as 10,000 species; more detailed counts indicate ~4,000 in ~600 genera (3,738 marine species in 546 genera and 10 orders [plus the unclassifiable]; 164 freshwater species in 30 genera in 8 orders). The red algae form a distinct group characterized by the following attributes: eukaryotic cells without flagella and centrioles, using floridean starch as food reserve, with phycobiliproteins as acessory pigments (giving them their red color), and with chloroplasts lacking external endoplasmic reticulum and containing unstacked thylakoids. Most red algae are also multicellular, macroscopic, marine, and reproduce sexually. Many of the coralline algae, which secrete $CaCO_3$ and play a major role in building coral reefs, are red algae. Red algae such as Dulse *(Palmaria palmata)* and Laver (Nori or Gim) are parts of European and Asian dishes and are used to make products such as agar carrageenans and other food additives.

Figure 3. Red alga *Polysiphonia spp.*

1.3.4 Brown Algae

The Phaeophyceae or brown algae, is a large group of mostly marine multicellular algae, including many seaweeds of colder Northern Hemisphere waters. They play an important role in marine environments, both as food and for the habitats they form. For instance *Macrocystis spp.*, a kelp of the order Laminariales, may reach 60m in length, and forms prominent underwater forests. Another example is *Sargassum spp.*, which creates unique habitats in the tropical waters of the Sargasso Sea. Many brown algae, such as members of the order Fucales, commonly grow along rocky seashores. Some members of the class are used as food for humans. Worldwide there are about 1,500–2,000 species of brown algae. Some species are of sufficient commercial importance, such as *Ascophyllum spp.* Brown algae belong to a very large group, the Heterokontophyta, a eukaryotic group of organisms distinguished most prominently by having chloroplasts surrounded by four membranes, suggesting an origin from a symbiotic relationship between a basal eukaryote and another eukaryotic organism. Most brown algae contain the pigment fucoxanthin, which is responsible for the distinctive brownish-green color that gives them their name. Brown algae are unique among heterokonts as they develop into multicellular forms with differentiated tissues, but they reproduce by means of flagellated spores and gametes that closely resemble cells of other heterokonts. Genetic studies show their closest relatives to be the yellow-green algae.

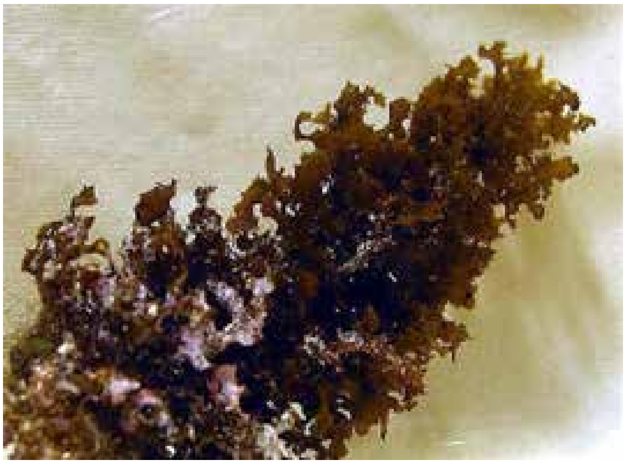

Figure 4. Brown alga *Sargassum polyphyllum.*

1.3.5 Yellow Algae

Yellow-green algae or xanthophytes are an important group of heterokont algae. Most live in freshwater, but some are found in marine and soil habitats. They vary from single-celled flagellates to simple colonial and filamentous forms. Xanthophyte chloroplasts contain the photosynthetic pigments chlorophyll a and b, beta-carotene and carotenoids i.e. diadinoxanthin. Unlike other heterokonts, their chloroplasts do not contain fucoxanthin, which accounts for their lighter color. Its storage polysaccharide is chrysolaminarin. Yellow algal cell walls are produced of cellulose and hemicellulose.

Figure 5. Yellow alga *Vaucheria sessilis*.

2 Characteristics of Selected Algal Strains of Potential Industrial Interests

Microalgae are unicellular organisms that can live alone or in colonies. Depending on the species they belong to, their dimensions may vary from a few to 100 micrometers (m). They are a very heterogeneous group of photosynthetic microorganisms and heterotrophic and, with extraordinary potential in the production of energy. They can be grown in aqueous media more variably, from fresh water to the extreme conditions of salinity and are able to produce a wide variety of products of commercial interest, such as oils, proteins, carbohydrates and bioactive compounds. One of the most interesting aspects of microalgae is that they can contain significant amounts of lipids (fats and oils) whose composition is similar to that of traditional vegetable oils.

What distinguishes them is that microalgae respond to fluctuating growth conditions or with different types of stress variations in lipid composition. The accumulation of lipids is linked to photosynthesis, and then the whole process that goes by the assimilation of CO_2 to the synthesis of triacylglycerols can be modulated. Some algae are able to produce oils in greater quantities compared to the best oil crops and for this reason can be a renewable source of oil for the synthesis of biodiesel. The use of microalgae for the production of biodiesel does not affect the production of food, feed and other products derived from crops and their cultivation can take place in areas not used in agriculture.

Today we are witnessing a resurgence of interest in microalgae, particularly in relation to their huge potential in renewable energy, although other applications such as purification of wastewater and the production of food supplements, animal feed, chemicals and drugs are just as promising, as shown in the scheme of Table 1.

Table 1. State of Production and products [12].

Products/ process	Species/production (t/y)	Culture
Food integrator	Arthospira (3000), Clorella (2000), Aphanizomenon (500), Dunaliella (1200), Haematococcus (300)	Lagoons, raceway track ponds photobioreactors
Pigments	Dunaliella, Arthospira, Haematococcus	Lagoons, raceway track ponds photobioreactors
Fatty acid 3	Schyzochitrium, Cryptecodinium	Fermenters
Marker, Enzyme	Arthospira, Anabaena, Anacystis	Fermenters, photobioreactors
Bioremediation	Scenedesmus and mixed cultures	Lagoons, pools, raceway track ponds
Acquaculture biomass	Various species	Pools, bags, photobioreactors

It is academically and practically relevant to study the effects of main nutrients in cultivation conditions on the microalgal cell growth. There are systematic studies focussed on the nutritional effects of algae utilized as feed for aquaculture species, less studies have observed methods to better the algal culture for lipid production for biodiesel production. A few microalgal species such as *Chlorella spp., Dunaliella spp., Nannochloris spp., Parietochloris incisa, Neochloris oleoabundans* and *B. braunii* have the capacity to accumulate large quantities of lipids [11]. In this context, the effect of nitrogen as sources on cell growth to accumulate lipid is significant. Usually high lipid cell contents are produced by cells under stress in nutrient limitation, often associated with low biomass productivity and consequently low overall lipid productivity. Among three tested nitrogen compounds, i.e. sodium nitrate, urea and ammonium bicarbonate, sodium nitrate was the most favorable nitrogen source for the cell growth and lipid production. In addition, the sodium nitrate is less expensive than potassium nitrate as recommended by the algal collection University of Texas, Austin and this represents an advantage for industrial processes. The results showed that at 10 mM of sodium nitrate concentration both the maximum biomass concentration and the biomass productivity reached the highest values and suggests that nitrogen was limiting at an initial nitrate concentration. Considering the high cell density obtained in the experiments, the light limitation was clearly one of the factors that caused the decreased cell growth to enter in the stationary phase at high nitrate concentration, therefore the sodium nitrate might be inhibitive [11].

In water bodies, when light and temperature are adequate and nutrients, especially nitrogen and phosphorus, not limiting, microalgae can grow to reach concentrations of hundreds of millions of cells per milliliter. This condition is sought in artificial culture (including commercial) where the primary objective is to maintain a single dominant species and a cell concentration that can intercept all the incident light and maximize productivity. In nature the accumulation of lipids in microalgae increases under certain conditions that should be considered when selecting the species for the production of biodiesel.

The large-scale cultivation of microalgae started in the 1980's, with experience on the cyanobacterium *Spirulina* (or *Arthrospira*) and marine microalgae that form the staple food for filter feeders and shellfish larvae of fish species in aquaculture. Culture systems have also been developed to achieve massive food supplements for humans and for animals (*Spirulina*, *Chlorella*, *Dunaliella*) or for the extraction of biomolecules such as astaxanthin, pro-vitamin A, vitamin B12, gamma linolenic acid (GLA), polyunsaturated fatty acids, phycocyanins, intended for various applications (nutraceuticals, cosmetics, dyes, drugs, reagents, etc.). The main production facilities are currently in the tropical belt in southern California, China, Taiwan, India, Cuba and Hawaii, as favored by high average temperatures that allow productions throughout the year. The most common cropping system is based on circuits Raceways pool with shallow water circulation and paddle stirrers ensured by energy electricity. The contents of the tanks matured is filtered, dried and reduced to powder or tablets, or subjected to chemical-physical treatments to extract substances of interest.

The monospecific outdoor crops are now used only for some species adapted to extreme environments (very salty water, alkaline water) and in certain geographical locations. In fact, the contamination by other micro-organisms plants and animals, in the absence of physico-chemical factors is limiting, as in the case of *Spirulina* or *Dunaliella* where outdoor growth lead to the disappearance of crop coming to maturity in a very short time. The use of greenhouses and crops indoors reduces the risk of contamination, but it increases production costs significantly. In plants indoors efforts to intensify production of microalgae and the density have been made with the use of photobioreactors.

2.1 Growth

2.1.1 The Growth of Algae Occurs in Three Main Phases:

Exponential phase stationary is the phase in which growth rate of the population is always positive in time and dependent on nutrients, temperature and lighting available in the environment (initially the low number of cells in culture minimizes the mutual shading and each cell is in light saturation);

Linear phase or stationary is the phase in which of population growth rate has slowed down or canceled and algal concentration reaches a high value. For extensive crops it is convenient to maintain the growth curve in this phase, ensuring a proper intake of nutrients, adjusting the concentration algal and ensuring sufficient light to the metabolically active cells;

Descending phase is the phase in which the cells tend to die, being suspended both the step of dividing both the metabolic phase. Generally coincides with excessive algal concentration, depletion of nutrients in the culture medium or with the onset of adverse growth conditions (temperatures not suitable, the presence of toxic substances, inadequate lighting).

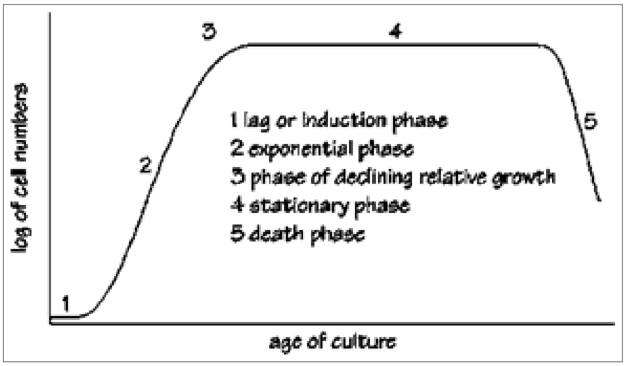

Figure 6. Algal growth curve.

The microalgae cultures are divided in laboratory cultures and cultures massive, the first to preserve purity in the different strains and arrive at volumes of inoculum for crops massive, the second to achieve adequate quantity and quality to the production needs.

2.1.2 Crops In The Laboratory

In addition to the maintenance and characterization of the algal species, the cultures in the laboratory allow basic research on microalgal species. Monospecific crops are started in small volumes of 10-50 ml (tube) under controlled environmental conditions, in liquid media or on solid medium (typically agar Petri dishes). In the first phase the main goal is to maintain quality of the strains that will be used for inocula in larger volumes. It is not required for rapid growth which, even if in liquid media, it is not necessary to blow air and CO_2. Microalgal populations are kept in clean room (to avoid contamination), at constant temperatures (typically 18-20°C) and permanent lighting with fluorescent cold low intensity (1,000 lux). The strains are replicated on a monthly basis to avoid that the crops exceed the linear phase of growth and then decay. The replicas are made by inoculating about 1/5-1/10 of the previous crops into new tubes previously filled with water salinity and nutrients desired, after chemical sterilization (hypochlorite and sodium thiosulfate) or in an autoclave at a temperature of 120°C; exceptions are the solutions that are vitamin degraded due to high temperatures. For strains on solid media, replication occurs by taking a small quantity of the previous crop and spread on Petri dish containing growth medium. The operations are performed under a laminar flow hood to avoid contamination. Periodic counts by microscope counting chamber (Neubauer hemocytometer) or by readings from spectrophotometers calibrated with cell density readings of the optical microscope, provide information on the frequency of algal cultures.

Figure 7. Algal growth on solid media.

2.1.3 Crops Massive

Crops massive are generally characterized by high concentrations of algal populations, so the farm conditions must allow a good development of phytoplankton. The techniques vary depending on the end use of the biomass. Vary substantially the volume of livestock and methods of cultivation systems (indoors or outdoors). It initiates the crops in small containers from a few liters to get the volume of use (from 20-30 the real growth basins) through passages in increasing volumes. It begins by maintaining strains that serve as 'inoculum' for 0.5 to 1 liter flasks. It then goes on then to the flasks to 3-10 volumes of 25-30 liters. This technique allows the reduction of time (crops find themselves always in the exponential growth phase), more accurate control and ability to develop a program.

Figures 8-9. Algal growth in liquid media flasks (8) and containers (9).

Once achieved the volumes of use, the cultures may be maintained in a semi-continuous, continuous or discontinuous.

The semi-continuous way is to keep the culture for long periods of time, by taking a sample at intervals of 20-30% and bringing to volume with half enriched vapor. The drawings begin when the concentration is of the order of millions of algal cell per ml with large variations depending on the size of the microalgae. When removal and replacement are continuous, it is called continuously. These techniques, however, expose crops to higher risks of pollution.

The discontinuous way consists in bringing the culture to the maximum possible concentration (which varies with the farmed species), and in the complete use at one time of the biomass obtained. Compared to the previous one, this technique is simpler and guarantees a greater purity of the population phytoplankton but requires a rigorous schedule of crops. In aquaculture systems for the reproduction of fish species is usually an entire industry devoted to the cultivation of plankton (phyto-zooplankton cells) in aseptic environments and maintained under controlled environmental conditions. These cells are equipped with lighting systems to sunlight or fluorescent cold (6,000-10,000 lux intensity between each), thermoregulation (constant temperature, usually 18-22°C, but variable in relation to the species) and of the distribution network air, CO_2 and water treated. Particular attention is drawn on the type of water used, which at this stage of the crops, must be of the highest purity to ensure sterility. The incoming water is decanted, filtered (mechanical filters in decreasing scale with a mesh from 50 to 1 micron) and sterilized chemically, or by autoclave or with ultraviolet rays. The cultures are kept in polyethylene bags with 20 to 400 liters, placed in front of neon lamps. In these microalgal population is inoculated from lower volumes after filling the bags with seawater filtered and enriched with nutrients. The insufflation of air and CO_2 at the bottom of the culture also determines the agitation.

In outdoor ponds, particular attention is paid to the design and construction of livestock facilities of phytoplancktonic populations; in order to achieve optimal conditions for algal growth factors to control such as nutrient availability, depth and concentration of the culture, turbulence of the medium, water filtration and sterilization. The major factors are not controllable: temperature and brightness, as they depend on season, weather events and their geographical location. Under optimal conditions, these populations can achieve average production of 15-30 g/m/2 days of dry biomass. The depth of the crops is inversely related to the cell concentration and the amount of available light. Depth of 15-40 cm represent a compromise between conflicting requirements such as power consumption for agitation and harvesting (rotor des, water pumps, air-lift) and the daily temperature variation. The size of the rearing ponds range from tens of square meters to 0.4-0.5ha with rounded shapes (thin partition walls and rounded corners prevent clumps of cells and impart rotary motion) type Raceways. In the basins on earth is often used a plastic sheet coated for better control of biotic parameters and to avoid seepage. In addition, limited to the cultivation of cyanobacteria (*Spirulina*) for industrial purposes, have been tested basins with inclined surface, small and shallow depths, when the crop is fed to the upper end and slides on the culture surface towards the collecting vessel placed the lower end. In the path, the culture encounters obstacles consist of corrugations of the bottom of the basin, in order to brake the rectilinear motion of fall of the liquid and increase the vertical motion of enhancing the favorable effect of flash illumination (flashing light). The major disadvantages of algal cultures open basins are represented by evaporation of the water especially in case of high temperatures, losses of CO_2, the risks of pollution from other undesirable algal species (which in the presence of favorable conditions can replace the initial culture) or by animals such as insects and aquatic fitoplanctofagi (rotifers, copepods, etc.) and protozoa whose development is almost always overwhelming. Open systems, however, can reach larger costs associated with much lower than those related to technical indoors.

Algae lipids that accumulate in large quantities were often found in specific environments and microclimates where microalgal growth is favored by environmental constraints. In these microenvironments the cells are still able to fix CO_2 in the form of photosynthesis and accumulate lipids and starch that perform the function of reserve to survive in environmental non-favorable conditions. In normal growing conditions many species of microalgae have a fat content between 10-30% on dry weight, but under special conditions, the fat content can be doubled or tripled. The algal biomass contains approximately 50% carbon on the dry weight. This carbon comes from CO_2 with which the algal cultures must be constantly fed. In the production of 100 tons of algal biomass 183 tons of CO_2 are fixed [9].

Graph 1. Algal biomass: Oil, Protein, Carbohydrates fractions [13].

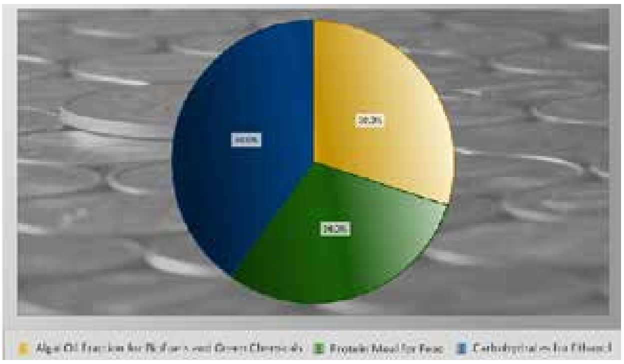

Table 2. Composition (%) of selected algae [14]

Strain	Protein	Carbo-hydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50-56	10-17	12-14	3-6
Scenedesmus quadricauda	47	-	1.9	-
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra spp.	6-20	33-64	11-21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39-61	14-18	14-20	-
Prymnesium parvum	28-45	25-33	22-38	1-2
Tetraselmis maculata	52	15	3	-
Porphyridium cruentum	28-39	40-57	9-14	-
Spirulina platensis	46-63	8-14	4--9	2-5
Spirulina maxima	60-71	13-16	6-7	3-4.5
Synechoccus spp.	63	15	11	5
Anabaena cylindrica	43-56	25-30	4-7	-

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2 Value-added Products from Algal Biomass

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Abstract Microalgae represent powerful unicellular factories with enormous potential to eliminate pollutants from effluent gas and sewage water. Mainly the exceptional capacity of microalgae for CO_2 fixation underlines their significance for diminishing current ecological problems. Together with environmental benefit, microalgae exhibit high nutritional value and synthesize prized marketable products as their cell constituents. Pigments, lipids, and certain polysaccharides are of major economic significance. Microalgae contain three weighty pigment groups: carotenoids, phycobillins, and chlorophylls. These pigments are responsible for light harvesting, CO_2 fixation, and algal coloration. They constitute the most promising microalgal candidates for quick commercial success and are applied in 'functional food', for therapeutic purposes, in cosmetics, aquaculture, or as food colorants. Microalgal oils can be commercialized as health food and for pharmaceutical and therapeutic applications at much better prices than after converting them to biodiesel. This is due to often high contents of polyunsaturated fatty acids that display crucial tasks in the human metabolism. After product isolation, the value-added conversion of remaining biomass for generation of green energy carriers and biofertilizers shall close the cycle of complete utilization of algal biomass. This strategy corresponds to a "biorefinery" concept as the final aim of microalgal-based technology.

Keywords Bioethanol; Biofertilizer; Biogas; Green energy carriers; Lipids; Microalgae; Pigments; PUFAs; Single-cell protein; Value-added products

1 Introduction

1.1 General Aspects

Microalgae can be considered as promising candidates for a broad range of applications in "White Biotechnology"; this goes as well for the production of food, feed, fine chemicals as for creation of different green energy carriers [1].

These unicellular microbes constitute a versatile polyphyletic group of organisms with the common capability of photosynthetic fixation of CO_2 for generation of various algal cell components, energy and molecular oxygen [2]. From the microbiological point of view, microalgae encompass eukaryotic and, if also including the cyanobacterial representatives (Cyanophyta; formerly also known as "blue-green algae"), prokaryotic microbial species [3, 4]. To underline the significance of these powerful microbes for the entire ecosphere, one should consider that the global fixation of CO_2 by algae amounts to about the same quantity as the photosynthetic performance accomplished by terrestrial green plants [5].

1.2 The Broad Range of Microalgal Products

1.2.1 General

Microalgal biomass itself is of interest for human nutrition due to its high protein content, as dietary or "health food", as protein and polyunsaturated fatty acids (PUFAs) source for aquaculture and feeding of cattle, pigs and poultry, for cosmetic purposes (coloration of pigments and especially anti-aging skin formulations; extracts from *Chlorella vulgaris* support collagen repair mechanisms), pharmaceutical purposes (immune response, weight control) [6] and for production of green energy carriers like biogas [3], biohydrogen, or bioethanol (see later).

1.2.2 Nutrition

Due to their high nutritional value, algae play a role for human food since thousands of years. This is documented in ancient Chinese literature since about two and a half millennia [7]. Especially in East-Asian regions and some other global areas like South Africa or Mexico, protein-rich algae like e.g. the microalgae *Spirulina* or *Nostoc*, or the macroalga *Porphyra* were applied for nutritional purposes. It has to be underlined that for example *Spirulina platensis* and *Arthrospira maxima* biomass contain about 60% of crude protein. Also *Chlorella vulgaris*, maybe the best studied green alga, harbors more than 50% of proteins in its cell mass [8].

In the mid of the 20th century, the growing world population and the resulting shortage in protein supply for mankind lead to increased activities for exploring novel and alternative protein sources, such as single cell proteins; this gave rise to develop enhanced cultivation strategies for large-scale production of algal biomass. The first examples for commercial scale production were realized in Taiwan (based on the green alga *Chlorella*) and the USA, China and India (using the blue-green algae *Arthrospira* and *Spirulina*). Additional genera that are predominantly cultivated for their high protein content are *Scenedesmus*, *Anabaena*, or *Synechococcus* [9].

1.2.3 High-prized Products

In addition to the utilization of proteinaceous microalgal biomass for food and feed purposes, one can take profit of additional products produced by these organisms, such as pigments, enzymes, sugars, fatty acids, vitamins (B-vitamins like B1, B2, B3, B5, B6, B7, B9, B12; vitamins A, C, and E) and other unusual bioactive compounds, in particular compounds displaying anti-cancer, anti-inflammatory and antibiotic (Chlorellin) activity, [9-11]. The exploitation of microalgae for such metabolites is highly attractive because those compounds very often display exceptionally high market values. As the best known and most powerful strains for production of high-value microalgal products, *Haematococcus* and *Dunaliella* have to be highlighted. In any case, the microalgal cell components discussed before render these organisms to perfect candidates for more and more fashionable “health food” or “functional food”. Indeed, the market for “functional food” surpasses by far all other applications of microalgae. This can be visualized considering the fact that only for Japan, 2400 tons of microalgal biomass is commercialized by year for “health food” purposes [12].

Apart from microalgal lipids and pigments, some carbohydrates produced by these organisms are quickening interest due to their potential for therapeutic application. Here, ß-1,3-glucan, a carbohydrate active as immunostimulator, antioxidant and reducer of blood lipids has to be mentioned; it is accessible from the cultivation of *Chlorella* strains. Further, sulfated polysaccharides produced by microalgae can be applied in anti-adhesive therapies against bacterial infections both in cold- and warm-blooded animals [13]. It has to be emphasized that ß-1,3-glucan displays a considerable higher market value if compared with other algal carbohydrates that are of importance for technical applications, such as the gelling or thickening compound agar (produced by macroalgae belonging to the *Rhodophyta*), alginates, or carrageenan that is used as emulsifier and stabilizer in various food products. Carrageenan, also known as food-additive E407, displays also potential pharmaceutical activity.

1.2.4 Dependence of Product Formation on Cultivation Conditions

It must be emphasized that many microalgal species are able to adapt to a broad range of process conditions during biosynthesis, such as salinity, temperature, pH-value and illumination (light intensity, dark-light cycles and spectral range). In this case, the extents of production of biomass, lipids, pigments and carbohydrates can vary considerably depending on the conditions the organisms are exposed to [14]. Therefore, the applied cultivation parameters have a major impact on the productivity of a desired end-product. For the selection of the appropriate production parameters, the decision has to be made as the case arises to which final product the nutrient flux should be directed. These facts are decisive for the design of an adequate photobioreactor system for high-performance cultivation of microalgae. Such systems should be flexible concerning different microalgal species to be cultivated therein, and concerning different final products.

1.3 Combining the Removal of Eco-Pollutants to Microalgal Product formation

Caused by prevailing ecological concerns affecting our planet, the biological sequestering of CO_2 by living algal cells for abatement of greenhouse gases has become a research field of increasing global significance [3, 15].

Also in the case of environmental pollution by precarious heavy metals like Ni(II), Fe(II/III), Hg(II), Cd(II), Cr(IV), Zn(II) or Au(II), microalgae were identified as potential candidates to remove them from various aquatic environments by “bioleaching”, especially from industrial waste water [16].

Formaldehyde, a compound severely toxic for skin, eyes and the respiratory system, is often released to marine environments *via* waste water from different industrial branches like paper, resin, and glue producing companies. Its elimination *via* biodegradation was demonstrated by the marine microalga *Nannochloropsis oculata* ST-3, a representative of the *Chlorophyta* [17].

If waste water is used for nutrient supply, naturally occurring bacteria accomplish the breakdown of the organic waste materials to such nitrogen and phosphate sources that can subsequently easily be converted by microalgae. Hence, symbiotic interactions exist between the metabolism of the bacteria and the algae. Diverse waste waters from agriculture, municipal origin or different industrial branches provide the ingredients required for algal nutrient supply. Additionally, the carbon present in waste water can also be converted by the algae during heterotrophic phases of cultivation.

By combining the application of available waste water bodies or solutions of hydrolysis products of organic rejects with the utilization of CO_2 stemming from industrial effluent gases, suitable raw materials are available for microalgal growth and formation of high value products. Using mixotrophic algal species, different waste streams can be used for different phases of cultivation. During the heterotrophic or mixotrophic cultivation, characterized by the formation of high densities of catalytically active algal biomass, carbon-, nitrogen-, and phosphate-rich waste streams of various origins can be supplied. The second phase of the cultivation, characterized by the generation of vendible products like pigments or special lipids provides a possibility of CO_2 mitigation for numerous involved industrial branches. This abating of CO_2 might contribute globally to make the agreed global goals for climate protection as nowadays frequently discussed come true.

Fig. 1 provides a schematic illustrating the potential microalgal products like lipids, pigments, biomass, carbohydrates, molecular oxygen or biohydrogen that can be obtained by converting the huge variety of discussed pollution streams of different origin like sewage water or industrial effluent gases. In addition, the areas of final application of the algal products, such as agriculture, aquaculture, generation of green energy carriers, and pharmaceutical and nutritional purposes are indicated.

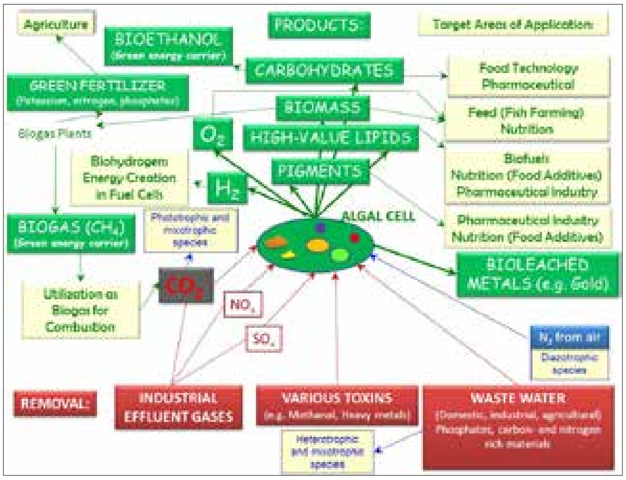


Figure 1. Combining the removal of pollutants with the production of high-value products by algal strains.

2 Market Potential Of Microalgal Products – Some Figures For Illustration

Algal biomass applied for human nutrition nowadays is sold at a market price of about 40 to 50 US-$ per kg, considering production strains like *Spirulina* or *Chlorella*. It is of essential importance to take into account that those algal products featuring the highest market values are present in the entire algal biomass in a quantity not exceeding a few percent per mass. This means that high efforts are needed for isolation und purification of the products; this contributes considerably to the final manufacturing prices.

Considering algal products that are marketed as nutraceuticals (“functional food”) for human consumption, one comes to a price per kg of about 120 US-$, corresponding to a global market volume of more than $7 * 10^7$ US-$. In the case of nutraceuticals for animal feeding and aquaculture, the price per kg of the final product is estimated with about 10 US-$, corresponding to a global market volume of more than $4 * 10^9$ US-$.

These figures are in clear contrast to the prizes that can be obtained for pure high-value algal products. In the pigment sector, the market value for ß-carotene, in dependence on the product purity, can be estimated with 300 to 3,000 US-$ per kg, with a global market size of $2 * 10^8$ US-$ per year. For astaxanthin, one can estimate, dependent on the source, about 2,500 to even more than 7,000 US-$ per kg. For the high-prized phycobiliproteins from *Spirulina* (phycoerythrin and phycocyanin), 3,000 to up to 25,000 US-$ per kg can be expected. In the case of astaxanthin, the global market potential of this pigment is estimated with 200 million US-$.

The market penetration of microalgal pigments is still in a very early state; this can be visualized by their shares on the entire volume of the pigments. In the case of ß-carotene, the portion produced by microalgae amounts to about a quarter of the entire ß-carotene market. The situation is even more tremendous in the case of astaxanthin, were less than 1% of the commercialized quantity are allotted to the algal-derived pigment.

Reliable market prices for polyunsaturated fatty acids accessible from microalgal cultivations can be estimated with about 50 US-$ per kg of docosa-hexaenoic acid (DHA), if production strains like *Crypthecodinium cohnii* or *Shizochytrium* are considered [18]. It has to be emphasized that the market price per kg of a microalgal products is classically inverse to its market volume. Considering biodiesel from algal oils, the market price per kg amounts to less than 0,5 US-$, whereas the market volume can be estimated in a magnitude of 10^9 US-$. This is clearly in contrast to the situation regarding microalgal neutraceuticals for human nutrition, where the market price per kg of about 120 US-$ corresponds to a market volume of more than $7 * 10^7$ US-$ [19].

3 Microalgal Pigments

3.1 General

According to their high market values, pigments are considered as those algal products of highest potential for commercial success [13, 20-22]. Algal pigments are responsible for light harvesting, CO_2 fixation, protection of the algal cells against excessive illumination, and, macroscopically, the appearance of the algal culture by its coloration.

Three major groups of pigments are found in microalgae, namely carotenoids (here, carotenes giving an orange coloration, and xanthophylls, responsible for yellowish coloration are distinguished), phycobillins (red or blue coloration), and chlorophylls (green coloration).

The pigment fraction of algae can be applied as nutrient supply due to their high contents of pro-vitamin A (E160a), vitamin E (E306, E307, E308) [19], other pharmaceutical, veterinary and medical purposes (anti-inflammatory effects, antioxidative effect, cancer prevention [23], in cosmetic industry and also for food industry. Additionally, ß-carotene and lutein are needed for poultry feeding because of its important for the yellow-orange coloration of egg yolk. These general facts are collected in Table 1, whereas Fig. 2 presents the chemical structures of some of the most important algal pigments.

3.2 Chlorophylls

As the most prominent algal pigments, chlorophylls absorb light mainly in the blue zone of the electromagnetic spectrum, and, to a minor extent, in the red zone. However, chlorophylls are just poor absorbers of green and near-green portions of the spectrum; this causes the typical and well-known green coloration of the green algae *(Chlorophyta)* that harbor chlorophylls as the predominant pigments. As the best-known representative, chlorophyll a is found in all microalgal species.

Applications of chlorophylls are found in the kitchen: This pigment is approved as a food additive (E140) for coloration. Famous "Chefs de cuisine" use chlorophyll to provide a green coloring to a variety of foods and beverages, such as pasta, pesto and absinthe. In addition, wasabi surrogates are commercialized that just constitute "simple" horseradish colorized with *Spirulina* chlorophyll. Also in the case of chlorophylls, anticancer activity is evidenced: A study reports the benefit of chlorophyll derivatives (chlorophyllin) against colon cancer cells [24]. Due to its deodorant nature, chlorophyll a is an ingredient in items of personal hygiene, such as deodorants, or, as pastilles, it is commercialized against bad breath.

3.3 Carotenoids

Carotenoids display so called "secondary pigments" because they support the "primary pigment" chlorophyll a in capturing light energy. These pigments absorb wavelengths of light mainly in the range between 400 nm and 500 nm of the light spectrum where chlorophylls are not able to absorb light energy; hence, carotenoids transfer this energy to chlorophyll a to be finally used for photosynthesis. In addition, they protect the algal cells against negative effects of solar radiation by their high potential to act as antioxidants by "catching" free radicals. Due to the fact that antioxidants are also required by the human metabolism to prevent the negative impacts caused by free radicals, this outstanding property explains the significance of carotenoids in "functional food" products.

Concerning microalgal carotenoid production, the saline organism *Dunaliella salina* is mainly cultivated in Israel and Australia for ß-carotene

Table 1. Microalgal pigments and potential fields of application: an overview

Pigment Group	**Predominant Colour of the Algal Culture**	**Examples of the Pigments**	**Microalgal Representatives**	**Colour of Pigment**	**Application of the Pigment**
Carotenoids (Carotenes)	brown- orange	β-carotene	*Dunaliella salina* (up to 14%!), *Botryococcus braunii*	yellow	Pro-vitamin A; antioxidant; Food additive E160a
(Tocopherol)		α-Tocopherol	*Nannochloropsis oculata, Stichococcus bacillaris, Euglena gracilis, Chlorella*	brown	Vitamin E; Food Additive E306, E307, E308 antioxidant in cosmetics and foods
Carotenoids (Carotenes)		Bixin	*Dunaliella salina*	yellowish to peach-color	Food additive E160b (colorant); cosmetics
Carotenoids (Xanthophylls)		Violaxanthin	*Botryococcus braunii*, *Dunaliella tertiolecta*	orange	Food additive E161e (approved in Australia and New Zealand); anti-cancer
Carotenoids (Xanthophylls)		Astaxanthin	*Haematococcus pluvialis, Botryococcus braunii*	reddish-salmon	Food additive E161j; antioxidant; farming of salmon and trout (colour, immuno-response)
Carotenoids (Xanthophylls)		Fucoxanthin	Brown algae (*Phaeophyceae*)	brown to olive	promotes fat burning (anti-adipositas)
Carotenoids (Xanthophylls)		Lutein	*Chlorella protothecoides, Botryococcus braunii, Chlorococcum citriforme, Muriellopsis* sp., *Neospongiococcum gelatinosum, Chlorella zofingiensis*	yellow-orange	Food additive E161b; yellow coloration of egg york (feed additive), pigmentation of animal tissues . Pharmaceutical: anti-macular degeneration, anti-colon cancer. Cosmetics: coloration
Carotenoids (Xanthophylls)		Zeaxanthin	*Botryococcus braunii*, *Dunaliella salina, Nannochloropsis oculata*	orange-yellow	Food additive E 161h, animal feed Pharmaceutical: anti-colon cancer
Phycobillins	red	Phycocyanin	*Spirulina*, *Arthrospira* (cyanobacteria)	blue-green („cyano“)	Food colorant (beverages, ice cream, sweets); cosmetics; Immunofluorescence techniques; antibody labels, receptors and other biological molecules
Phycobillins		Phycoerythrin	Cyanobacteria, *Porphyridium*	red	Immunofluorescence techniques; labels for antibodies, receptors and other biological molecules
Chlorophylls	green	Chlorophyll a	all phototrophic oxygenic algae	green	Pharmaceutical and cosmetical (deoderant)

biosynthesis. ß-carotene, also known as pro-vitamin A, is mainly applied for nutritional purposes as a vitamin supplement. Beside this, it plays a certain role in chicken nutrition to provide a typical orange color to egg yolk as desired by the customer. As mentioned before, algal pigments, especially carotenoids, possess high importance as colorants in food industry. An important example is the production of astaxanthin (E161j), the most powerful antioxidant, hence free radical scavenger that occurs in nature. Astaxanthin is used to give salmon or trout the typical reddish color, if the farming of these fishes is supported by artificial nutrient supplements. In addition to the coloration of salmonids as desired by the customer, astaxanthin, also a representative of the carotenoid pigments, displays a central role for the immune-system of these fishes. Further, astaxanthin is responsible for the pigmentation of various aquatic organisms such as shrimps, lobster and other crustaceans. For their coloration, all of these organisms are dependent on the supply with astaxanthin by the microalgal phytoplankton. Astaxanthin is also of importance for therapeutic applications due to its benefit in antibody production and its reported antitumor activity [25]. Nowadays, the most important microalgal production strain for astaxanthin is

Haematococcus pluvialis [11, 26]. Cultivation methods are described in literature to produce *Haematococcus* with exceptionally high astaxanthin contents of up to 3.0 % by dry mass [27].

For many dairy products such as cheese, butter or margarine, the food additive bixin (E160b), an additional example of carotenes, provides a yellowish to peach-color shade. Additionally, bixin is used as colorant in cosmetics. Lutein and zeaxanthin, two representatives of the xanthophyll group of carotenoids, are applied technically for coloration of chicken skin.

Violaxanthin, another member of the xantophylls, has an orange coloration and can technically be applied as a food colorant (E161e). Here, it has to be emphasized that violaxanthin is not approved for use as food additive in the EU or USA; this is in contrast to its approval in Australia and New Zealand. Potential producers of this pigment are *Dunaliella tertiolecta* and *Botryococcus braunii*. Recent studies indicate strong anti-proliferative activity of violaxanthin on human mammary cancer cells. The authors therefore suggest the detailed studying of the pharmacology of violaxanthin and its derivatives on cancer cells [28].

3.4 Phycobilins

Phycobilins (Phycocyanin and Phycoerythrin) are mainly found in the stroma of chloroplasts of cyanobacteria and red algae, glaucophytes and some cryptomonads; green algae are no natural producers of these chromophores. Phycocyanin is a blue pigment primarily found in cyanobacteria, while phycoerythrin is a pigment occuring in red algae and responsible for its characteristic red coloration. Phycobilins are unique among all known photosynthetic pigments because they are bonded to certain water-soluble proteins, building so called phycobiliproteins. Phycobiliproteins further pass the energy of the harvested light to chlorophylls for photosynthesis; hence, similar to carotenoids, the serve as “secondary pigments”.

Phycobilins fluoresce at a particular wavelength, and are, therefore, often used in research as chemical tags. Here, they act by binding phycobiliproteins to antibodies in immunofluorescence techniques. Such phycobiliproteins constitute those algal-derived products revealing the by far highest market values. Beside this highly sophisticated application as chemical tags, phycobilins are also used as food colorants and in cosmetics due to their high coloration effects.

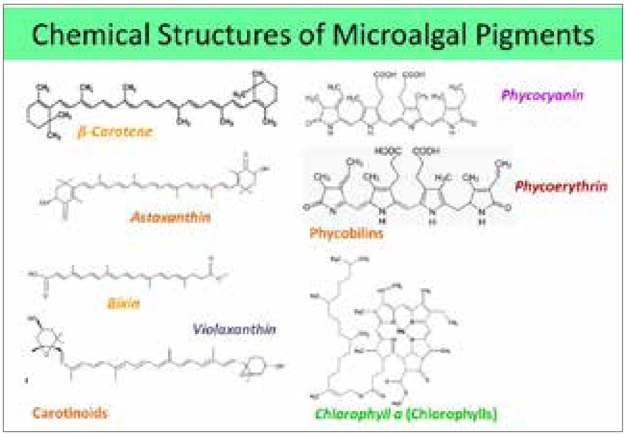


Figure 2. Chemical structures of selected microalgal pigments (*italic*; pigment groups: not italic).

4 Microalgal lipids

4.1 General

Different strains are discussed in literature as potential candidates for microalgal biofuel production. Especially among the genera *Botryococcus*, *Chlorella*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Scenedesmus*, *Dunaliella* and *Schizochtyrium*, several species are described to show exceptionally high amounts of lipids in their cell mass under optimal cultivation conditions [14, 16, 29, 30]. In the case of *Botryococcus braunii*, one of the best scrutinized microalgal lipid producers, a total of 75% (w/w) of hydrocarbons in cell mass were reached. The type of hydrocarbons produced by *Botryococcus braunii* depends on the race of this species (race A, B, L). A large number of monounsaturated, polyunsaturated, and even branched hydrocarbons is produced by *Botryococcus braunii*; these compounds can be converted by cracking them to fuels with properties similar to those of gasoline [14]. Other species produce high amounts of typical “vegetable oils” with high contents of monounsaturated, di-unsaturated, threefold and poly-unsaturated fatty acids. These oils can be classically converted to biodiesel by the well-known alkaline transesterification with alcohols like methanol [31, 32]. As the main by-product of this transesterification process, the glycerol phase can be digested anaerobically in biogas plants, can be thermally converted, or can be applied as an efficient carbon source for numerous biotechnological applications [30]. In addition, glycerol can be commercialized for manufacturing of e.g.

cosmetic products or be applied in food industry as humectant (E422).

In any case, biodiesel to be used as engine fuel is not the only conceivable product stemming from algal lipids. It has to be emphasized that many algal fats contain special, often polyunsaturated, fatty acids (PUFAs) with high market values, such as the ω−3 fatty acids EPA and DHA, and the ω−6 fatty acids GLA and AA (see Table 2). These acids are the components of microalgal cell membranes which serve as an energy reserve for unfavorable environmental conditions. In addition to their high energy content, ω−3 fatty acids are accumulated due to their excellent flow properties that are important for various cell functions.

These PUFAs can be commercialized for pharmaceutical and therapeutic applications yielding much higher prices than after converting the lipids to biofuels for combustion. PUFAs encompass ω−3 fatty acids and ω−6 fatty acids that are well-known to occur in fish oil. However, fish do not produce PUFAs themselves; they accumulate them by eating phytoplankton, hence microalgae or other algae-eating organisms. Regarding the application of microalgae in the entire field of aquaculture, the share for different aquatic animals to be cultivated can be estimated as 62% for mollusks, 21% for shrimps, and 16% for fish. Therefore, algae are the primary source of these essential nutritional components that display important functions in the human metabolism. Due to their manifold physiological roles, similar to microalgal carotenoids, PUFAs are important components of "functional food". Ω−3 fatty acids are known since a long time to be important components of human cell membranes, particularly of neuronal cells. Especially, they are frequently discussed due to their manifold role in cancer prevention [33] and cardiac protection [34].

The subsequent paragraphs, together with Table 2, provide an insight into potential applications of high-value microalgal fatty acids.

4.2 EPA

Eicosapentenoic acid (EPA), a ω-3 fatty acid, plays an important role as nutrient supplement. Essential biological functions of this acid are displayed by its significance for biosynthesis of eicosanoids which in succession are responsible

Table 2. Microalgal fatty acids and potential fields of application: an overview

Fatty Acid (Lipid)	**Microalgal Representatives**	**Application of Fatty Acid (Lipids)**
(5Z,8Z,11Z,14Z,17Z)-eicosa-5,8,11,14,17-pentenoic acid (EPA) ω−3 fatty acid	*Nannochloropsis oculata; Porphyridium purpureum, Porphyridium cruentum, Phaeodactylum tricornutum, Phaeodactylum cornutum, Isochrysis galbana, Nitzschia laevis, Chlorella minutissima, Pavlova viridis, Pinguiococcus Pyrenidosus, Traustochytrium* sp., *Dunaliella salina, Chaetoceros calcitrans*	Nutritional supplements: heart diseases, blood clotting, blood pressure, anti-thrombosis and anti-artherosklerosis; anti-inflammation Psychtherapeutic medication Aquaculture
(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA) ω−3 fatty acid	*Crypthecodinium cohnii, Shizochytrium limacinum, Pavlova lutheri, Pavlova viridis, Pinguiococcus Pyrenidosus, Isochrysis galbana, Traustochytrium* sp., *Chaetoceros calcitrans*	Nutritional supplements: important for brain and eye development at fetus and children adult dietary supplement in food and beverages significance for cardiovascular health Anti-colon cancer; anti-breast cancer; anti-inflammation Aquaculture
(5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraenoic acid (arachidonic acid, ARA) ω−6 fatty acid	*Porphyridium*	Nutritional supplements; anti-inflammatory; muscle anabolic formulations (body builder) Aquaculture
all-cis-6,9,12-octadecatrienoic acid (γ-linolenic acid, GLA) ω−6 fatty acid	*Arthrospira*	Nutritional supplements anti-inflammation and auto-immune diseases; potential to suppress tumor growth and metastasis (anti-cancer)
in addition:		
Mono- and polyunsaturated and even branched hydrocarbons (up to 75%!) n-alkadienes and trienes; triterpenoid botryococcenes and methylated squalenes, lycopadiene (a tetraterpenoid)	*Botryococcus braunii*	Biofuel production

for various functions of the immune system, the blood clotting, and the regulation of the blood pressure. In addition, EPA is reported to act beneficially for coronal heart diseases [34]. Further, this acid was investigated already a long time ago for its activity in prevention of thrombosis and atherosclerosis [35]. More recently, therapeutic applications of EPA are discussed for alleviating psychological problems like anxiety, depression, or even schizophrenia [36]. In addition, EPA is used in aquaculture for fish-farming; salmons and herring are known to harbor high amounts of this acid in their lipids based on the fact that EPA-producing algae play a crucial role in their nutrition. As the most prominent microalgal EPA-producer, the chlorophytal strain *Nannochloropsis oculata* has to be mentioned, whereas *Phaeodactylum tricornutum*, *Pavlova viridis* and *Pavlova lutheri* are reported to produce the highest amounts of EPA (and DHA) regarding the entire fatty acid pattern of their lipids [37].

4.3 DHA

Docosahexaenoic acid (DHA), another ω−3 fatty acid, is produced by microalgae such as *Crypthecodinium cohnii*, *Thraustochytrium*, *Shizochytrium limacinum*, or *Pavlova lutheri*. A recent study reports the heterotrophic utilization of by-products from the biodiesel production (crude glycerol phase) as raw material for DHA-biosynthesis by the alga *Schizochytrium limacinum*. The authors report high lipid contents (up to 50%) obtained by the cultivation and, besides palmitic acid, DHA as the predominant fatty acid. Further, the study investigates in details the inhibiting effects of additional compounds in crude glycerol phase, such as methanol and soaps [38].

DHA features a primary structural component of the human brain cerebral cortex, sperm, testicles and retina. It is especially important for infantile brain and eye development. Anti-inflammatory effects of DHA [39] and its importance for the developing human foetus and healthy breast milk [40] is also evidenced by recent scientific studies. Further, it is applied as dietary supplement in food and beverages due to its significance for the nervous system, cardiovascular health and other organs. In addition, activity of DHA against colon cancer [41], and breast cancer [42] is evidenced. Since more than two decades, its inclusion in infant formula is recommended by various health and nutrition organizations [18].

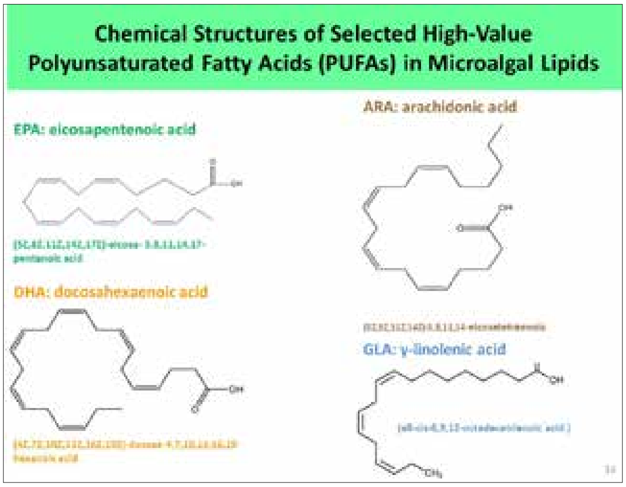
Also in the field of fish farming, this acid is applied. Especially cold-water oceanic fish oils are rich in DHA they get from the microalgal components of their nutrition. In contrast to the well-known fish-oil preparations like train oil or cod liver oil that are commercialized since a long time to provide supplementation of DHA and also EPA, algal-based formulations that are rich of these fatty acids become more and more important on the market.

4.4 ARA

Arachidonic acid (ARA), a four-fold unsaturated ω-6 fatty acid, is an essential component of membrane phospholipids. It acts as a vasodilator, shows anti-inflammatory effects [43] and is therefore used for nutrient supplements. ARA is necessary for the repair and growth of skeletal muscle tissue. This role makes ARA an important dietary component in support of the muscle anabolic formulations [44]. In addition, also ARA is applied in aquaculture [7].

4.5 GLA

γ-linolenic acid (GLA), an ω-6 unsaturated fatty acid, is mainly present in cyanobacterial representatives like *Arthrospira* [45]. Studies indicate that some people are not sufficiently supplied with GLA by their diet and need additional dosages of it by food additives. It is used by the human organism to make prostaglandins; further uses encompass therapeutic applications for its anti-inflammatory effects and for auto-immune diseases. More recently, its potential to suppress tumor growth and metastasis was recognized; hence, this compound plays an increasing role for anti-cancer therapy. In addition, GLA and GLA-rich supplements are promoted to help people with breast pain, skin allergies, diabetes, obesity, rheumatoid arthritis, heart disease, high blood pressure, premenstrual syndrome, multiple sclerosis, attention deficit hyperactivity disorder (ADHD), and neurological problems related to diabetes [46].



Chemical Structures of Selected High-Value Polyunsaturated Fatty Acids (PUFAs) in Microalgal Lipids

Figure 3. Chemical structures of selected high-value microalgal fatty acids.

5 Value-added application of residual algal biomass

5.1 Bioethanol

After cell harvest and isolation of lipids and high-value pigments from the microalgal cells by extraction or mechanical disruption methods, residual biomass is generated that can be converted in different directions. Attempts have been made to generate bioethanol from algal biomass. This can be accomplished by the fermentation of starch-rich algal biomass by the anaerobic action of yeasts [47]. Starch and starch-like polysaccharides constitute algal reserve materials typically produced by several species among the genera of *Chlorophyta*, *Rhodophyta*, *Cryptophyta*, and *Pyrrophyta*. Due to the low yields that characterize the anaerobic ethanol production by yeasts, the large-scale application of this strategy appears rather doubtful. In addition, a biotechnological production strategy using two types of microorganisms in two separated processes (starch accumulation by microalgae followed by the anaerobic conversion of starch to ethanol) is rather complex to install and demands a rather big number of intermediary process steps. Nevertheless, after the distillation process for generation of bioethanol, the so-called distiller soluble remains as a residue. These residues contain valuable compounds like minerals that render the material of interest for utilization as agricultural fertilizers ("biofertilizer").

5.2 Biogas

By anaerobic degradation in biogas plants, the residual biomass can be used for the generation of biogas, a more or less carbon neutral energy carrier. For this conversion, algal biomass does not have to be dried before; it can directly be subjected to the anaerobic break-down in biogas plants. The generated biogas typically contains comparable amounts of the energy carrier methane and CO_2. If compared to the production of hydrocarbons or biodiesel by algae, biogas generation from algal biomass is technically simple to realize. The so called "digestate" remains as residue from the biogas production. This material is rich in nutrients such as potassium, phosphates, and minor mineral components, and constitutes a precious green fertilizer in agriculture. In addition, it appears reasonable to apply the digestate as additional nutrient supply in subsequent algal cultivations. This recycling strategy should allow additional production of algal biomass and, in case that waste water is used as nutrient source for algal farming, act as a supplement to the nutrient supply obtained from the waste water input. In addition, the digestate that is rich in minerals like potassium, phosphates and many others, can be, similar to the distiller soluble discussed above, used as a valuable "green fertilizer" in agriculture.

Recent studies report that the potential for production of biogas is strongly dependent on the microalgal species and on the pre-treatment of the algal biomass. Here the application of the green alga *Chlamydomonas reinhardtii* is more effective in terms of biogas yield in comparison to e.g. *Scenedesmus obliquus* [48].

Recently, an integrated process for biogas production from and purification on cassava starch effluent was developed, where microalgae act as so called "bio-stabiliser agent". The main problem of the biogas production from cassava starch effluent is the rapid decline of the pH-value due to the action of acid forming-bacteria; this high acidity antagonizes the growth of methanogenic bacteria that are responsible for biogas generation. The study demonstrates that this problem can be overcome by adding microalgae as bio-stabiliser of pH-value. At the same time, the microalgae act as purifier agent by absorbing CO_2 that accrues as by-product of the biogas production process, resulting in an increased quality of the obtained energy carrier biogas by microalgal conditioning [49]. The utilization of the green alga *Chlorella vulgaris* SAG 211-11b for conditioning of biogas is also reported in literature [50]. In this study, the biogas components CO_2 and H_2S could be eliminated nearby completely, yielding high-quality biogas. Assessing these experiments, also an increase of microalgal biomass was observed, indicating the conversion of the unwanted biogas components CO_2 and H_2S as carbon and sulphur substrates by the microalgal cells.

A life-cycle assessment (LCA) of biogas production from the microalga *Chlorella vulgaris* was performed. It highlights the main bottlenecks in this production, and compares them with the advantages and the drawbacks of mature and other immature technologies like e.g. algal biodiesel production. The authors focused on a simplified process where methane was the only recovered product; based on the results, they concluded that the optimum outcome from both the environmental and the economical point of view is a process combining lipid recovery for a fraction of the algal biomass and methane production from both raw biomass and remaining biomass after lipid extraction [51].

In a recent study, the efficiency of abating CO_2 by using biogas stemming from algal biomass is compared to the utilization of natural gas. It was calculated that the production of 1 ton of algal biomass results in avoiding 0,5 tons of CO_2. It can be estimated that this value can be doubled if natural gas was replaced by coal fired energy generation, saving energy of conventional waste water treatment and replacing the energy demanding production of fertilizers by digestate [14].

Alternatively, residual algal biomass can be thermally converted *via* incineration to generate energy and ash. This can be regarded as the technologically simplest method for energy recovery from algal biomass. Similar to the digestate residues from biogas production, ash remaining from incineration can further act as a valuable agricultural biofertilizer or as mineral nutrient supply for subsequent algal cultivations.

6 Conclusion

Developing countries are most susceptible to the expected and already occurring negative impacts of climate change, and also to the proceeding shortage of resources for food, feed, and energy production. While these countries are often not the powerful decision-makers with sufficient possibilities to fight climate change, their health and security concerning food, water and energy supply is highly endangered. If done in a proper and effective way, concepts for efficient cultivation of microalgae for products needed by mankind can combat climate change, while, at the same time, *via* CO_2 sequestering, even diminish it to a certain extent. Especially in the case of developing and emerging countries, the industrial production of various algal products might be a viable strategy to create a broad range of differently qualified jobs, to enter global markets where such prized products can be commercialized, and, considering green energy carriers accessible from microalgae, a higher degree of independence in the energy sector.

Uniting the possible enhancements of each process step in microalgal cultivation, one can definitely make substantial progress towards a cost-efficient algal-based technology. In any case, the development of really efficient processes for manufacturing of algal products needs the narrow cooperation of experts from different scientific fields. Chemical engineers, microbiologists, genetic engineers and experts in the fields of Life Cycle Assessment and Cleaner Production Studies have to concentrate their special expertise and know-how in order to close the existing gaps between promising data from the laboratory scale *via* pilot plants to industrial realization. Until today, especially the lacking synopsis of skills in process design and well-grounded understanding of intracellular on-goings hampers the broad industrial implementation of these powerful phototrophic cell-factories.

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3 Nutritional Aspects of Algae: Food, Feed, Nutraceuticals

Microalgae as Novel Food Products and Ingredients

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Abstract It became evident that algae are able to enhance the nutritional content of conventional food and feed preparation and can to positively affect humans and animal health due to their original chemical composition of high protein content, with balanced essential amino acids pattern, carotenoids, fatty acids, vitamins, polysaccharides, sterols, phycobilins and other biologically active compounds [1, 2].

1 Introduction

Modern food industry leads to an increase of cheaper, healthier and more convenient products. The use of natural ingredients, like polyunsaturated fatty acids (PUFA's) and antioxidant pigments, exhibiting high impact on functional properties is important to reduce chronic diseases incidence, which are strongly considered of capital importance in Europe, where aging population and welfare costs are fatal for public resources management. The impact of natural substances introduced in the diet via 'usual' foods is proved to be efficient long term and does not present the drawbacks of traditional therapeutic actions based on medicines of short term impact.

Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications [3]. They can be used to enhance the nutritional value of food and animal feed, due to their well-balanced chemical composition. Moreover, they are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids, pigments, antioxidants, pharmaceuticals and other biologically active compounds. The application of microalgal biomass and/or metabolites is an interesting and innovative approach for the development of healthier food products. Microalgal biotechnology is similar to conventional agriculture, but has received quite a lot of attention over the last decades because they can reach substantially higher productivity than traditional crops and can be extended into areas and climates unsuitable for agricultural purposes (e.g. desert and seashore lands). Microalgal production is an important natural mechanism to reduce the excess of atmospheric CO_2 by biofixation and recycling of fixed C in products, ensuring a lower greenhouse effect, reducing the global environmental heating and climate changes. Microalgae are important as feed to aquaculture and life-support systems, and can effectively remove nutrients (or pollutants) (e.g. nitrogen and phosphorus) from water. Microalgal driven sunlight systems for environmental and production applications can clearly contribute to sustainable development and improved management of natural resources. Lately, microalgae have been seen with a great potential as a sustainable feedstock for biodiesel production, in substitution for oil from vegetable crops, and also for hydrogen production [4, 5]. This chapter reviews the main applications of microalgae in feed and food products.

2 Microalgae as Food

Microalgae use by indigenous populations has occurred for centuries. However, the cultivation of microalgae is only a few decades old and among the 30,000 species that are believed to exist, only a few thousands strains are kept in collections, a few hundred are investigated for chemical content and just a handful are cultivated in industrial quantities [6, 7, 8, 9]

Some of the most biotechnologically relevant microalgae are the green algae (Chlorophycea) *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* and the Cyanobacteria *Spirulina maxima* which are already widely commercialized and used, mainly as nutritional supplements for humans and as animal feed additives.

Chlorella vulgaris has been used as an alternative medicine in the Far East since ancient times and it is known as a traditional food in the Orient. It is widely produced and marketed as a food supplement in many countries, including China, Japan, Europe and the US, despite not possessing GRAS status. *Chlorella spp.* is being considered as a potential source of a wide spectrum of nutrients (e.g. carotenoids, vitamins, minerals) being widely used in the healthy food market as well as for animal feed and aquaculture. *Chlorella spp.* is important as a health-promoting factor on many kinds of disorders such as gastric ulcers, wounds, constipation, anemia, hypertension, diabetes infant malnutrition and neurosis [10]. It is also attributed a preventive action against atherosclerosis and hypercholesterolemia by glycolipids and phospholipids, and antitumor actions by glicoproteins, peptides and nucleotides [10]. However, the most important substance in *Chlorella spp.* seems to be a beta-1,3-glucan, which is an active immunostimulator, a free-radical scavenger and a reducer of blood lipids [1].

Haematococcus pluvialis has been identified as an organism in which can accumulate the highest level of astaxanthin in nature (1.5-3.0% dry weight). This carotenoid pigment is a potent radical scavenger and singlet oxygen quencher, with increasing amount of evidence suggesting that surpasses the antioxidant benefits of eta-carotene, vitamin C and vitamin E.

Haematococcus spp. is currently the prime natural source of this pigment for commercial exploitation, particularly in aquaculture salmon and trout farming [11]. Another natural source, *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*) yeast requires a large amount of feed for sufficient pigmentation [12].

Dunaliella salina is an halotolerant microalga, naturally occurring in salted lakes, that is able to accumulate very large amounts of beta-carotene, a valuable chemical mainly used as natural food colouring and provitamin A (retinol). The *D. salina* community in Pink Lake, Victoria (Australia) was estimated to contain up to 14% of this carotenoid in their dry weight, and in culture some *Dunaliella* strains may also contain up to 10% and more beta-carotene, under nutrient-stressed, high salt and high light conditions [13, 14, 15]. Apart from beta-carotene *Dunaliella* produces another valuable chemical, glycerol.

Arthrosphira (*Spirulina*) *spp.* grows profusely in certain alkaline lakes in Mexico and Africa and has been used as food by local populations since ancient times [10]. It is extensively produced around the world (3,000 tons/year) and broadly used in food and feed supplements, due of its high protein content and its excellent nutritive value, such as high alpha-linolenic acid level [16, 17]. In addition, this microalga has various possible health promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus spp.*, suppression of elevated serum glucose level anticarcinogenic effect and have hypocholesterolemic properties [1, 18]. *Spirulina spp.* is also the main source of natural phycocyanin, used as a natural food and cosmetic colouring (blue colour extract) and as biochemical tracer in immunoassays, among other uses [16, 17, 19].

Recently, attention has been drawn on the marine microalgae *Isochrysis galbana* and *Diacronema vlkianum* (Haptophyceae) due to their ability to produce long chain polyunsaturated fatty acids (LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:53) and also docosahexaenoic acid (DHA, 22:63), that are accumulated as oil droplets in prominent lipid bodies in the cell [20]. These microalgae have been used as a feed species for commercial rearing of many aquatic animals, particularly larval and juvenile molluscs, crustacean and fish species [21]. For example, in a relative ranking of microalgal diets for clam *Mercenaria mercenaria*, the microalga *I. galbana* was shown as the most suitable source of nutrition for rapid growth, while *D. vlkianum* resulted in high growth rates and low mortality for the Pacific oyster *Crassostrea gigas* larvae [22, 23].

These microalgae are also potentially promising for the food industry as a valuable source of LC-PUFA's, in alternative to fish oils, supplying also sterols, tocopherols, colouring pigments and other nutraceuticals [24].

As with any higher plant, the chemical composition of algae is not an intrinsic constant factor but varies over a wide range. Environmental factors, such as temperature, illumination, pH-value, mineral contents, CO_2 supply, or population density, growth phase and algae physiology, can greatly modified chemical composition.

Microalgae can biosynthesize, metabolize, accumulate and secrete a great diversity of primary and secondary metabolites, many of which are valuable substances with potential applications in the food, pharmaceutical and cosmetics industries [10]. One of the most obvious and arresting characteristics of the algae is their color. In general, each phylum has its own particular combination of pigments and an individual color. Aside chlorophylls as the primary photosynthetic pigment, microalgae also form various accessory or secondary pigments, such as phycobiliproteins and a wide range of carotenoids. These natural pigments are able to improve the efficiency of light energy utilization of the algae and protect them against solar radiation and related effects. Their function as antioxidants in the plant shows interesting parallels with their potential role as antioxidants in foods and humans [25]. Therefore, microalgae are recognized as an excellent source of natural colorants and nutraceuticals and it is expected they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature [12].

3 Algae as Animal Feed

The main applications of microalgae for aquaculture are associated with nutrition, being used fresh (as sole component or as food additive to basic nutrients) for coloring the flesh of salmonids and for inducing other biological activities [26]. Several investigations have been carried out on the use of algae as additives in fish feed. Feeding trials were carried out with many fish species, most commonly red sea bream (*Pagrus major*), ayu (*Plecoglossus altivelis*), nibbler (*Girella punctata*), striped jack (*Pseudoceranx dentex*), cherry salmon (*Oncorhynchus masou*), yellowtail (Seriola *quinqueradiata*), black sea bream (*Acanthopagrus schlegeli*), rainbow trout (*Oncorhynchus mykiss*), rockfish (*Sebastes schlegeli*) and Japanese flounder (*Paralichthys olivaceus*). Various types of algae were used; the most extensively studied ones have been the blue-green algae *Spirulina spp.* and *Chlorella spp.*; the brown algae *Ascophyllum spp., Laminaria spp.* and *Undaria spp.*; the red alga *Porphyra spp.*; and the green alga *Ulva spp.*

A summary of the results of selected feeding trials with algae as feed additives are presented in Table 1.3. Most of these research studies were conducted in Japan with Japanese fish species, although the results may well be applicable to other species and in other countries. The responses of test fish fed algae supplemented diets were compared with fish fed standard control diets. Although various types of algae and fish species were used in these evaluations, not all algae were evaluated as feed additives for every different species. As the main biochemical constituents and digestibility are different among algae, the effect of dietary algae varies with the algae and fish species [27]. While studying the effect of two seaweeds, *Undaria pinnatifida* and *Ascophyllum nodosum*, at different supplementation levels for red sea bream, Yone, Furuichi and Urano observed best growth and feed efficiency from a diet containing 5 percent *U. pinnatifida* followed by a diet containing 5 percent *A. nodosum* [28]. Similarly, Mustafa et al. observed more pronounced effects on growth and feed utilization of red sea bream by feeding a diet containing Spirulina compared to one containing *A. nodosum* [29]. In another study, Mustafa et al. studied the comparative efficacy of three different algae (*A. nodosum*, *Porphyra yezoensis* and *Ulva pertusa*) for red sea bream and noted that feeding *P. yezoensis* showed the most pronounced effects on growth and energy accumulation, followed by *A. nodosum* and *U. pertusa* [27]. However, research results obtained so far do not specifically identify any one specific alga as the most suitable for feed additives for any particular fish species.

Nevertheless, the results of various research studies show that algae as dietary additives contribute to an increase in growth and feed utilization of cultured fish due to efficacious assimilation of dietary protein, improvement in physiological activity, stress response, starvation tolerance, disease resistance and carcass quality. In fish fed algae-supplemented diets, accumulation of lipid reserves was generally well controlled and the reserved lipids were mobilized to energy prior to muscle protein degradation in response to energy requirements. In complete pelleted diets, algal supplementation of 5 percent or less was found to be adequate. *Spirulina spp.* are widely used as feed additives in the Japanese fish farming industry. Henson reported that *Spirulina spp.* improved the performances of ayu, cherry salmon, sea bream, mackerel, yellowtail and koi carp [30]. The levels of supplementation used by Japanese farmers are 0.5-2.5 percent. Henson further reported that Japanese fish farmers used about US$ 2.5 million worth of Spirulina in 1989 [30]. Five important benefits reported by using a feed containing this alga were improved growth rates; improved carcass quality and coloration; higher survival rates; reduced requirement for medication; and reduced wastes in effluents. However, the high cost of most of these algae may limit their use to the commercial production of high value fish only. Other example of algae for animal feed: Kalla et al. indicated that the addition of *Porphyra sp.* spheroplasts to a semi-purified red seabream diet improved specific growth rate (SGR) [31]. In addition, Valente et al. recorded improvements in SGR when dried *Gracilaria sp.* replaced 5 or 10 percent of a fish protein hydrolysate diet for European seabass [32].

Appler and Jauncey recorded a SGR of 58 percent of control diet when the filamentous green alga *Cladophora glomerata* meal was used as the sole source of protein for Nile tilapia (*Oreochromis niloticus*) [33]. Similarly, Appler recorded SGRs of 44 percent and 56 percent of control diets when the filamentous green alga *Hydrodictyon reticu-*

latum meal was used as the sole source of protein for *O. niloticus* and *Tilapia zillii* [34].

Ayyappan et al. conducted a *Spirulina* feeding experiment with carp species [35]. The fry stage of catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), silver carp (*Hypophthalmicthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) were fed with an experimental diet in which 10 percent dried *Spirulina* powder was added to a 45:45 mixture of rice bran and groundnut oil cake. A 50:50 bran-groundnut oil cake control diet was used. The experimental results clearly demonstrated the beneficial effect of the *Spirulina spp.* diet on the yield and quality of carp fry.

Dietary supplementation of *Chlorella ellipsoidea* powder at 2 percent on a dry weight basis showed higher weight gain and improved feed efficiency and protein efficiency ratios in juvenile Japanese flounders (*Paralichthys olivaceus*); the addition of *Chlorella sp.* had positive effects as it significantly reduced serum cholesterol and body fat levels and also led to improved lipid metabolism [36].

Based on the report of Hasan and Chakrabarti, moderate growth responses and good food utilization (Feed Conversion Ratio 1.5–2.0) were generally recorded when dried algal meal were used as a partial replacement of fishmeal protein [37]. However, the collection, drying and pelletization of algae require considerable time and effort. Furthermore, cultivation costs would have to be taken into consideration. Therefore, further cost-benefit on-farm trials that take these costs into consideration are needed before any definite conclusions on the future application of algae as fish feed can be drawn [37].

4 Nutraceuticals from Algae

Nutraceutical is a term combining the words nutrition and pharmaceutical. It is a food or food product that provides health and medical benefits, including the prevention and treatment of disease. A nutraceutical has beneficial effects because it possesses many compounds with antioxidant and intracellular signalling-pathway modulator effects. In recent years, it has been demonstrated that microalgae of the Chlorophyceae class could be excellent nutraceuticals because they contain polyphenols, chlorophyll, beta-carotene, ascorbic acid, lycopene, alpha-tocopherol, xanthophylls, and PUFAs. For this reason, research groups have studied the nutraceutical properties of the genera *Dunalliela spp.*, *Haematococcus spp.*, and *Chlorella spp*. Among the different compounds with health functional properties, antioxidants are the most widely studied [2, 3]. These compounds can play an important role in food technology because of their usefulness against lipid peroxidation. Usually, food production process and storage can generate important losses of endogenous antioxidants that limit their own protection against lipid oxidation. Moreover, the important role of antioxidants in human health has been demonstrated, thus increasing the interest in such products and their demand by consumers. Accumulating evidence from the literature indicates that algae can be a promising source of antioxidants including different phenolic compounds, carotenoids and tocopherols. In the search for feasible new sources of natural antioxidants that can be used in the food industry, algae have been suggested as possible raw materials. These organisms are widely known and consumed in certain countries, and numerous health benefits have been associated with their use. Therefore, algae are potentially a great source of natural compounds that could be used as ingredients for preparing functional foods [67].

Table 1. nutraceutical use of *Chlorella spp.*

Study	**Evidence**
The administration of *Chlorella sp.* reduces endotoxemia, intestinal oxidative stress and bacterial traslocation in experimental biliary obstruction [38].	*Chlorella spp.* administration inhibits bacterial culture and it avoids oxidative stress.
Hot water extract of *Chlorella vulgaris* induced DNA damage and apoptosis [39].	The extract of *Chlorella vulgaris* inhibited DNA synthesis, causing apoptosis and it increases p53, caspase-3, and Bax expression in hepatoma cells (HEpG2)
Attenuating effect of *Chlorella sp.* supplementation on oxidative stress and NFκB. Activation in peritoneal macrophages and liver of C57BL/6 mice fed on atherogenic diet [40].	*Chlorella spp.* supplementation decreases the NFκB activation and superoxide anion production and because it increases SOD and catalase activity.
Chlorella sp. accelerates dioxin excretion in rats [41].	*Chlorella spp.* enhanced dioxin metabolism and excretion by feces.
Effect of *Chlorella spp.* and its fractions on blood pressure, cerebral stroke lesions, and life-span in stroke-prone spontaneously hypertensive rats [42].	A *Chlorella spp.* supplemented diet decreases blood pressure and the incidence rate of Cerebral stroke in SHRSP.
Hypocholesterolemic mechanism of *Chlorella*: *Chlorella sp.* and its indigestible fraction enhance hepatic cholesterol 7α-hydroxylase in rats [43].	*Chlorella spp.* powder increases the expression of CYP7A1, a limiting enzyme of the main pathway of the cholesterol catabolism, lowering the concentration of LDL in plasma.

C. vulgaris triggers apoptosis in hepatocarcinogenesis-induced rats [44].	*C. vulgaris* inhibits the anti-apoptotic protein Bcl-2.
Effect of *C. vulgaris* on lipid metabolism in Wistar rats fed high fat diet [45].	*C. vulgaris* decreases HDL cholesterol concentration by a reduction in the intestinal absorption.
Antioxidant effect of the marine algae *C. vulgaris* against aphthaleneinduced oxidative stress in the albino rats [46].	*C. vulgaris* inhibits production of free radicals, decreasing lipoperoxidation, and increasing the activity of antioxidant enzymes as SOD, catalase, GPX and reduced glutathione, preventing from the toxicity of Naftalene.
Six-week supplementation with *Chlorella sp.* has favorable impact on antioxidant status in Korean male smokers [47].	*Chlorella spp.* supplement exhibits antioxidant activity decreasing ROS and increasing the activity of SOD and catalase
C. pyrenoidosa supplementation reduces the risk of anemia, proteinuria and edema in pregnant women [48].	*C. pyrenoidosa* exhibits an antiinflammatory activity regulated by cytokine. It increased the production of IL-10.
Effect of *Chlorella sp.* intake on cadmium metabolism in rats [49].	*Chlorella spp.* inhibits cadmium absorption and it promotes the excretion through the feces. Also, it stimulates the production of metallothionein in the small intestine.
Isolation of phosphorylated polysaccharides from algae: the inmmunostimulatory principle of *C. pyrenoidosa* [50].	The *Chlorella spp.* polysaccharides increases the production of NO in macrophages enhancing the innate immune response, mediated by Toll-like receptors (TLR-4).
Influence of *Chlorella* powder intake during swimming stress in mice [51].	*C. vulgaris* exhibits an antioxidant activity, reducing the lipoperoxidation, avoiding the DNA damage. However it does not show hypoglycemic activity.

Table 2. *Haematococcus spp.* and its astaxanthin as nutr aceutics.

Study	**Evidence**
Haematococcus sp. astaxanthin: applications for human health and nutrition [52].	This is a review about the uses of astaxantin from *Haematococcus spp.* in health.
Optimization of microwave-assisted extraction of astaxanthin from *Haematococcus pluvialis* by response surface methodology and antioxidant activities of the extracts [53].	The extracts have a high antioxidant capacity, inhibit peroxidation of linoleic acid, and neutralize free radicals.
Cardioprotection and myocardial salvage by a disodium disuccinate astaxanthin derivative (Cardax™) [54].	The astaxanthin is an antioxidant, antiinflammatory, and cardioprotective. reducer of levels of nitric oxide, tumor necrosis factor alpha, and prostaglandin E2.
Ulcer preventive and antioxidative properties of astaxanthin from *H. pluvialis* [55].	The astaxanthin exerts its gastroprotection of gastric ulceration by activation of antioxidant enzyme such as catalase, superoxide dismutase, and glutathione peroxidase. It inhibits the activity pump Na-K ATPase.
Safety assessment of astaxanthin-rich microalgae biomass: acute and subchronic toxicity studies in rats [56].	The administration of astaxanthin has no adverse effects.
Astaxanthin, a carotenoid with potential in human health and nutrition [57].	The antihypertensive and neuroprotective potentials of the compound.
Protective effects of *Haematococcus sp.* astaxanthin on oxidative stress in healthy smokers [58].	The results suggest that ASX supplementation might prevent oxidative damage in smokers by suppressing lipid peroxidation and stimulating the activity of the antioxidant system in smokers.
Astaxanthin-rich extract from the green alga *H. pluvialis* lowers plasma lipid concentrations and enhances antioxidant defense in apolipoprotein E knockout mice [59].	It results suggest that supplementation of astaxanthin-rich *Haematococcus spp.* extract improves cholesterol and lipid metabolism as well as antioxidant defense mechanisms, all of which could help mitigate the progression of atherosclerosis.

Table 3. Nutraceutical evidence of *Dunaliella spp.*

Study	**Conclusion**
In vivo antioxidant activity of carotenoids from *Dunaliella salina* a green microalga [60].	Carotenoids provide protection against CCI4-caused hepatic damage by restoring the activity of hepatic enzymes like peroxidase, super oxide dismutase, and catalase, which reduce ROS and lipid peroxidation.
9-*cis* beta-carotene-rich powder of the alga *Dunaliella bardawil* increases plasma HDLcholesterol in fibrate-treated patients [61].	*Dunaliella spp.* treatment increases plasma HDL-cholesterol and lower plasma triglyceride levels.
Ethanol extract of *D. salina* induces cell cycle arrest and apoptosis in A545 human non-small cell lung cancer cells [62].	Ethanol extract of *D. salina* inhibits cell proliferation and causes apoptosis possibly via p53 and p21 promoting the protein expression of Fas and FasL.
Protective effects of *D. salina* against experimental induced fibrosarcoma on Wistar rats [63].	The Chlorophyta has a protective effect against experimentally caused fibrosarcoma.
Hypercholesterolemia induced oxidative stress is reduced in rats with diets enriched with supplement from *D. salina* algae [64].	*D. salina* components inhibit lipid peroxidation and also increases Type1 5´-iodothyronine deiodinase (5´-DI) expression, which leads to a T3 level increase.
Evaluation of carotenoid extract from *D. salina* against cadmium-induced cytotoxicity and transforming growth factor 1 induced expression of smooth muscle lpha-actin with rat liver cell lines [65].	Carotenoid extract of *D. salina* contains abundant *cis* and *trans* beta-carotenes. These antioxidants decrease the lipid peroxidation and also inhibit activation of hepatic stellate cells (HSCs).
Protective effects of *D. salina,* a carotenoid-rich alga, against carbon tetrachloride-induced hepatotoxicity in mice [66].	Carotenoids of *D. salina* inhibit the lipid peroxidation and increases the antioxidant enzyme activity.

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4 Algal Biofuels and Bioenergy
Conversion Technology, Problems, Opportunities

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Abstract Algal biomass has large potential for the production of fuels and of value added chemical products. A brief survey of methods for the recovery of the biomass, for its successive transformation, and of the potential targets is here provided.

Keywords Algal biomass, extraction, purification, fuels, chemicals, upgrading conversion

1 Introduction

Microalgae can produce from 15 to 300 times more oil for biodiesel compared to conventional terrestrial crops per unit area [1]. Furthermore, compared with terrestrial plants that are harvested once or twice a year, microalgae are generally collected at much shorter time intervals (about 3-10 days) or even continuous, ensuring continuous production without the need to store large quantities of raw material. Algae capture more light with higher conversion efficiency leading to lower use of fertilizers and nutrients, thereby reducing waste and pollution potential. However the state of research does not yet provide precise estimates about the productivity of algal crops for biodiesel. Performance will vary considerably depending on the type of microalgal species, on the geographic location of the plant, on cultivation techniques, and on processing technologies.

For example, experimental production (not commercial) of marine microalgae Nanochloropsis in temperate latitudes can provide about 20 t/year of oil per acre, with 220-250 days per year production. For the purpose of fixing CO_2, Benemann [2] quotes a weight ratio of dry microalgae biomass and CO_2 fixed equal to about 1:1.6. Other authors [3], taking into account the energy input necessary for production processes, propose a 1:1 ratio.

2 Recovery of Algal Biomass

The recovery of microalgal biomass generally requires one or more solid–liquid separation steps and is a challenging phase of the algal biomass production process, [4] that accounts for up to 20–30% of the total costs. The processes involved include flocculation, filtration, flotation, and centrifugal sedimentation; some of which are highly energy intensive, [5] therefore the selection of harvesting technology is crucial and depends on a number of factors, including strain selection.

Extraction and purification of microalgal biomass: dehydration. The harvested biomass slurry (typical 5–15% dry solid content) is perishable and must be processed rapidly after harvest; drying is commonly used to extend the viability depending on the final product required. Methods that have been used include sun drying, low-pressure shelf drying, spray drying, drum drying, fluidised bed drying, freeze drying, and Refractance Window™ technology drying. All these technologies are summarized by Brennan et al..[5]

Sun drying is the cheapest dehydration method; but main disadvantages include long drying times, the requirement for large drying surfaces, and the risk of material loss. Spray drying is commonly used for extraction of high value products, but it is relatively expensive and can cause significant deterioration of some algal pigments. Freeze drying is equally expensive, especially for large scale operations, but it eases extraction of oils. Intracellular elements such as oils are difficult to extract from wet biomass with solvents without cell disruption, but are extracted more easily from freeze dried biomass.

Extraction and purification of biofuels. For the extraction of biofuels, it is important to establish a balance between the drying efficiency and cost-effectiveness in order to maximise the net energy output of the fuels. Drying temperature during lipid extraction affects both the lipid composition and the lipid yield from the algal biomass. [6] For example, drying at 60 °C still retains a high concentration of TAG in the lipids and only decreases slightly the lipid yield, with higher temperatures decreasing both the concentration of TAG and lipid yield. OriginOil (a biofuel company based in Los Angeles) developed a wet extraction process that combines ultrasound and electromagnetic pulse induction to break the algae cell walls. Carbon dioxide is added to the algae solution, which lowers the pH, and separates the biomass from the oil. [7]

Extraction and purification for algal metabolites. Cell disruption is often required to recover intracellular products from microalgae. Most cell disruption methods applicable to microalgae have been adapted from applications on intracellular non-photosynthetic bioproducts. Cell disruption methods that have been used successfully include high-pressure homogenisers, autoclaving, and addition of hydrochloric acid, sodium hydroxide, or alkaline lysis. [8]

Solvents are widely used to extract metabolites such as astaxanthin, β-carotene and fatty acids from algal biomass. [9] Conventional lipid extraction techniques rely on the use of organic solvents (hexanes, light chlorinated hydrocarbons, methanol) and require rinsing steps that generate waste and environmental and health risks. In addition these solvent extractions are often poorly selective for the lipid fraction, and the phospholipids as well as the pigments are extracted as well. Additionally, solvent extraction requires dry algal material. Properties of the cell membrane play an important part in solvent extraction process as well. For example, the presence of a cell wall may prevent direct contact between the solvent and the cell membrane and hinder extraction. More on this subject can be found in a dedicated chapter.

The greatest short-term potential for development is related to the production of biofuels, in particular biodiesel from the oil content in microalgae, which would allow a net reduction in CO_2 emissions compared to fossil fuels without competing for land used for food crops. What is still lacking is to define manufacturing processes that can provide adequate quantities of biodiesel with a positive economic and energy balance. The processes for the separation of biomass from the microalgae cultivation broth and to extract the lipid content are still critical issues to be solved using efficient, low cost solutions for large quantities. In particular, algae are cultivated and obtained as dilute aqueous suspensions that make lipid recovery complicated because dewatering and drying remain energy- and cost-intensive processes. [9] It also remains to be determined whether the quality of oil obtained from the particular species of microalgae is more or less suitable for processing into biodiesel (transesterification). After the extraction of oil for biofuel, the microalgal biomass remaining may still be used for the extraction of biomolecules of commercial interest or for the production of biogas.

Conversion technologies for utilising microalgae biomass can be separated into two basic categories: thermochemical and biochemical conversion (Fig. 1). Factors that influence choice of conversion process include: the type and quantity of biomass feedstock; the desired form of the energy; economic consideration; project specific; and the desired end form of the product.

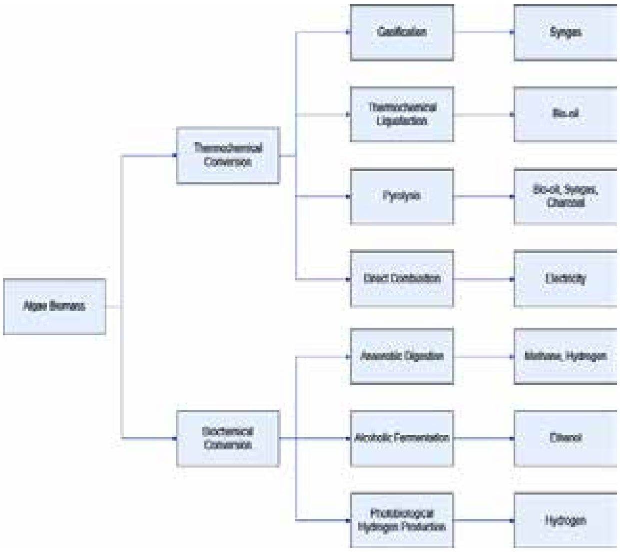


Figure 1. Algal biomass conversion processes (from references [5], [10]).

3 Biodiesel

A biodiesel production process that avoids biomass drying and organic solvents for oil extraction could lead to significant energy and cost savings. [11] Attempts to combine extraction with acid catalyzed transesterification in one step have been successful with dry algal biomass, but the reaction is severely inhibited by water. [12] This study investigated the influence of economic reaction factors on the conversion of microalgae oil to biodiesel using the acid-catalysed *in situ* transesterification process. These variables include the influence of reacting alcohol volumes, temperature, reaction time, biomass moisture content and stirring. Biomass drying was observed to play an important role, with an increase in the moisture content of the biomass resulting in significant reductions of the equilibrium FAME conversion yields. Therefore extensive biomass drying may be needed prior to biodiesel production by the in situ process. Although not covered in this study, continuous removal of the water product from the reactor, as well as an increase in the acidic catalyst concentration, are schemes which may be utilised to drive the forward reaction when microalgae biomass with high moisture content is used in the in situ transesterification process. In another study, direct transesterification of the oleaginous biomass resulted in a higher biodiesel yield and fatty acid methyl ester (FAME) content than the extraction-transesterification method. [13]

Levine et al. [14] have developed a two-step, catalyst-free biodiesel production process involving intracellular lipid hydrolysis coupled with supercritical in situ transesterification (SC-IST/E). Because of the low permeability and resistance of the cell membrane of the algae, the efficacy of the sc-CO_2 extraction depends on the pre-treatment of the algae. In the first step, wet algal biomass (80% moisture) reacts at subcritical water conditions to hydrolyze intracellular lipids, conglomerate cells into an easily filterable solid that retains the lipids, and produce a sterile, potentially nutrient-rich aqueous phase. In the second step, the wet fatty acid (FA) rich solids are subjected to SC-IST/E with ethanol to produce biodiesel in the form of fatty acid ethyl esters (FAEEs). This process eliminates both biomass drying and triglyceride (TG) extraction (e.g., with an organic solvent such as n-hexane). They also identified several factors that motivate this approach, e.g. oil hydrolysis, supercritical esterification, etc. Amassing cells into a filterable solid accomplishes additional dewatering prior to transesterification with minimal costs; retention of FAs and remaining lipids in a solid matrix obviates difficulties with lipid recovery from aqueous systems. Nutrients (N and P) and glycerol from processed biomass can be captured and reused in a sterile aqueous phase free of catalyst. Finally, a well-engineered process to produce biodiesel through supercritical alcohol transesterification may reduce costs and energy expenditure relative to those of conventional catalytic methods. [15] The same authors have demonstrated the feasibility of a two-step hydrolysis–solvolysis process to produce biodiesel from lipid-rich, wet algal biomass. The hydrolysis–solvolysis process obviates biomass drying, organic solvent extraction, and catalysts, while providing a mechanism for nutrient recycling. A cursory experimental investigation of the influence of some key process variables led to crude biodiesel and FAEE yields as high as 100% and 66%, respectively, on the basis of lipids within the hydrolysis solids. Considering that about 80–90% of lipids in the original algal biomass were retained in the solids recovered after hydrolysis, the total process yield was somewhat lower. The optimal time and temperature for hydrolysis must appropriately balance the desire for increased lipid hydrolysis with the likelihood of reduced lipid retention and solids yields at more severe conditions. In addition, it is imperative to improve the ester yield from SC-IST/E, which may have been limited by incomplete transesterification, decomposition/polymerization of unsaturated FA, hydrolysis of FAEE, or incomplete lipid extraction from the solid. A considerable benefit of the process described is the ability of its first step (hydrolysis) to create two sterile products: a relatively low moisture (<50% water), FA-rich solid and a nutrient-rich aqueous phase. Both are potentially amenable to a variety of downstream processes. Considering that the N and P required for producing algal biomass are nonrenewable resources, the ability to recycle these nutrients, together with a useful carbon source like glycerol, presents unique opportunities to further reduce the impact of algal biodiesel production. This approach might be attractive if algal biomass is grown in wastewater. (Significant significant part of this section has been directly quoted from Levine et al. [14]).

Another approach involves extracting the lipid content from wet biomass with supercritical carbon dioxide scCO_2. [16] There have been many studies on lipid extraction by scCO_2 in the food industry, particularly related to nutrient isolation from plant and algae products. These studies suggest that scCO_2 is efficient at extracting triacylglycerols as well as other lipid components from algal cells. However scCO_2 extraction research has not been thoroughly assessed or optimized for application to the extraction of algal lipids that are desired for use in biodiesel production. The quoted study assessed the viability of using scCO_2 for wet algal lipid extraction , in particular, by evaluating the scCO_2 extraction efficiency compared with conventional methods. The potential for scCO_2 extraction of algal lipids indicated that its efficiency, sustainability, safety and selectivity as compared to conventional extraction may contribute to the viability of algal biodiesel. The results indicate that optimized scCO_2 extraction yields are comparable to those resulting from conventional solvent extraction, and that scCO_2 produces a fatty acid methyl ester mass yield that represents 98% of the total collected lipid mass extracted. In addition, unlike conventional solvent extraction that indiscriminately extracts phospholipids (1% of total lipid fraction) and pigments, their results suggest that $scCO_2$ can selectivity extract triacylglycerols, significantly decreasing the extracted pigments and eliminating phospholipids that are undesirable for transesterification. The results show that water content does not change the quantity and profile of biodiesel produced, and thus $scCO_2$ extraction can use the more convenient and economical starting material of wet algae. Supercritical CO_2 extraction appears therefore as a promising means for lipid extraction from algae as it results in comparable efficiencies, fewer contaminants (*i.e.* nitrogen, phospholipids and pigments) and demonstrates solvent tuneability for increased selectivity. The absence of phospholipids from the lipid extract is important as phospholipids are deleterious to the transesterification process. In addition, during combustion , a decreased nitrogen content leads to decreased NO_x production. Not only is the selectivity and tuneability of $scCO_2$ extraction valuable for the production of biodiesel, but also for the viability of algae to be an economical starting material for other value-added materials, such as nutraceuticals , cosmetics, animal feeds, polymer development, and novel products and chemicals. With regard to the energy use of $scCO_2$ compared to conventional solvents , LCA of lipid extraction using conventional press and hexane extraction *vs.* $scCO_2$ extraction at 100 °C and 30 MPa shows that $scCO_2$ extraction would require about 38% of the energy needed for conventional extraction.

If algal biofuels are to be viable, an efficient refining process needs to be developed where all possible valuable materials and chemicals are harvested from the algal mass, much like the nearly complete utilization of distillation fractions associated with petroleum refining. The viability of this ideal goal may be in closer reach by using SFE in a streamlined process, where an algal bio-refinery would extract TAG, and multiple value-added chemical and material products at a single facility. Ideally sequential extraction would be executed for each desired product. For example, pure $scCO_2$ could be used to extract lipids. At high pressures, (*i.e.* above 7,000 psi) and moderate temperatures (*i.e.*70–80°C), the solubility of triglycerides in $scCO_2$ is very high. By dropping the pressure, a significant portion of the triglycerides can be recovered, or a small reduction of temperature at high pressure allows for the significant recovery of triglycerides. If a co-solvent is then introduced into the system, phospholipids can be extracted. Multiple extraction condition steps can be implemented to take advantage of both the changing solvent properties at different pressures and temperatures, and increasing polarities as co-solvents are added to the mix. Downstream CO_2 can be recycled or even fed into bioreactors for heightened algal growth.

SFE using $scCO_2$ has been shown to be a viable means for extracting algal lipids for biodiesel production, and the further development of this green solvent technology may contribute to the viability of the field of algal biofuel and the realization of a biorefinery approach analogous to petroleum refining. (Significant part of this section has been directly quoted from Zimmerman and Soh [16]).

4 Biogas

Algal biomass is rich in nutrients especially nitrogen and phosphorus, for which the use and potential loss may not be environmentally and economically sustainable. [17] A process to recycle nitrogen and phosphorus contained in algal waste after lipid extraction is therefore required in order to recover the nutrients that can be further utilized as fertilizers. Anaerobic digestion (AD) can be an answer to this problem, since this biotechnological process can mineralise algal waste containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can be used as a substrate for the microalgae. The AD of algal waste not only recycles the nutrients but also provide biomethane, a renewable energy. AD involves the breakdown of organic matter to produce biogas. [5] AD process is appropriate for high moisture content (80–90% moisture) organic wastes, [18] and can be useful for wet algal biomass. The AD process occurs in three sequential stages of hydrolysis, fermentation and methanogenesis. In hydrolysis the complex compounds are broken down into soluble sugars. Then, fermentative bacteria convert these into alcohols, acetic acid, volatile fatty acids (VFAs), and a gas containing H_2 and CO_2, which is metabolised into primarily CH_4 (60–70%) and CO_2 (30–40%) by methanogens.

Sialve et al. [17] calculated the methane potential and ammonia released during the anaerobic digestion of total biomass on the basis of composition of different algal species earlier given by Becker (Table 1). [19]

The biomass composition, pH, temperature, hydraulic and solid retention time (HRT and

Table 1. Composition of different algal species and their theoretical methane potential and ammonia release during anaerobic digestion of the total biomass (adopted from Sialve et al. [17]).

Algal species	**Protein (%)**	**Lipid (%)**	**Carbohydrate (%)**	**CH_4 (L g^{-1} VS)**	**N–NH_3 (mg g^{-1} VS)**
Euglena gracilis	39–61	14–20	14–18	0.53–0.8	54.3–84.9
Chlamydomonas Reinhardtii	48	21	17	0.69	44.7
Chlorella Pyrenoidosa	57	2	26	0.8	53.1
Chlorella vulgaris	51–58	14–22	12–17	0.63–0.79	47.5–54.0
Dunaliella salina	57	6	32	0.68	53.1
Spirulina maxima	60–71	6–7	13–16	0.63–0.74	55.9–66.1
Spirulina platensis	46–63	4–9	8–14	0.47–0.69	42.8–58.7
Scenedesmus obliquus	50–56	12–14	10–17	0.59–0.69	46.6–42.2

SRT) and loading rate determine the quantity and quality of biogas production during anaerobic digestion. The increase in temperature from 15 to 52°C improves the methane conversion of *Spirulina maxima*, and the productivity together with the volatile solids reduction is enhanced up to 35°C. [20] HRT and SRT should be high enough to allow the active microbial populations to remain in the reactor, especially methanogens, and not to limit hydrolysis which is generally the limiting-step of the overall conversion of complex substrates to methane. The optimal loading rates and HRT must be chosen depending on the type and composition of the algal biomass for maximum production of biomethane. When the cells are directly injected into the anaerobic process, accessibility of the intracellular content to the anaerobic microorganisms is limited by the resistance of the algal cell wall to hydrolysis. Thus, characteristics of algal species makes the difference for a given loading rate or HRT. The most important factor impacting CH_4 proportion in the biogas is the pH, which controls the speciation of the carbonate system and the release of CO_2. At high pH, due to high alkalinity from NH_3 release the gas content will shift more to CH_4, resulting higher content of CH_4 in the produced biogas.

Anaerobic digestion of the protein rich (60%) cyanobacteria *S. maxima* releases an extremely high concentration of ammonia (up to 7,000 mg L^{-1}). [20] Sánchez Hernández and Travieso Córdoba [21] observed a strong concentration of volatile fatty acids as a consequence of the toxic effect of ammonia on the anaerobic flora. Inhibiting concentrations vary in a wide range from 1.7 to 14 g L^{-1} and depend on several factors such as the acclimation period, the nature of substrate and inoculum together with operating conditions. [22] Thermophilic conditions enhance the inhibition effect. [17] High concentrations of ions such as Na^+, Ca^{2+} and Mg^{2+}, which increase alkalinity and decrease the fraction of unionized NH_3, can lower the inhibition effects. [23] Sodium ions are required by the anaerobic microorganisms for its metabolism in a range from 0.002 to 0.004 M, but above 0.14 M, they become strongly inhibitory, while marine microalgae require a culture medium with high sodium chloride content (0.5–1 M). However, it has been proved feasible to use salt-adapted microorganisms capable of withstanding high salinities. The selection of salt-tolerant microorganisms involves an adaptation of the sludge to high salt concentrations. As for NH_3 high temperature enhance the inhibition effect. The presence of other ions (Ca^{2+}, K^+, Mg^{2+}) can also play a significant, antagonistic or synergistic role on the potential toxicity of sodium [23].

Pre-treatment of a substrate prior to anaerobic digestion allows to significantly improve its biodegradability. [17] Separation techniques, concentration or dehydration, mobilize and maximize the proportion of organic matter in the fraction to be digested. Chemical treatments (acids, bases, ozonation), thermal treatment and ultrasonic lysis improve the disintegration of the most refractory organic fractions. [24] These operations increase kinetics of production and/or methane yield. Co-digestion is a strategy to increase the performance of a digester by ensuring an optimal influent composition. Yen and Brune [25] reported a significant enhancement of the methane production with an addition of waste paper to algal sludge feedstock, the optimum C/N was observed to be between 20 and 25 (this section has been quoted from Singh and Olsen [11]).

5 Hydrothermal Upgrading

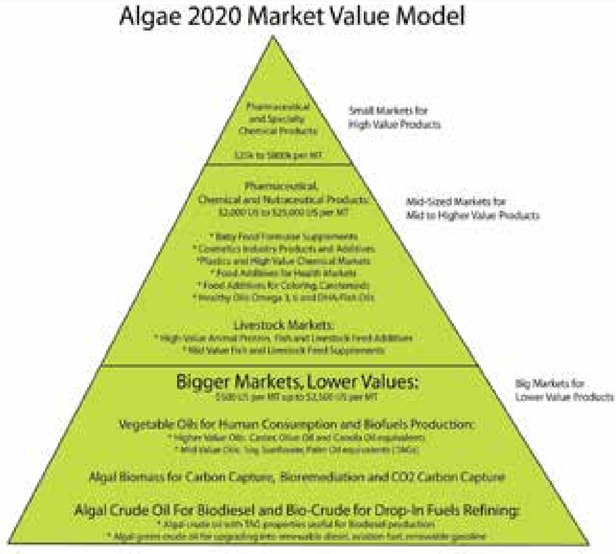
Thermochemical liquefaction is a process that can be employed to convert wet algal biomass material into liquid fuel. [26] Thermochemical liquefaction is a low-temperature (300–350°C), high pressure (5–20 MPa) process aided by a catalyst in the presence of hydrogen to yield bio-oil. [27] Reactors for thermochemical liquefaction and fuel-feed systems are somewhat complex, but have advantages in their ability to convert wet biomass into energy. The process utilises water in sub-critical conditions to decompose biomass materials down to shorter and smaller molecular materials with a higher energy density. [26] The microalgal biomass has relatively high water content (80-90%) that makes it difficult to use for heat and power generation as it requires pre-treatments to reduce the water content and increase the energy density. Direct hydrothermal liquefaction in sub-critical water conditions overcomes this drawback as it can be employed to convert wet biomass into liquid fuel. Goudriaan *et al*. [28] claim the thermal efficiency (defined as the ratio of heating values of bio-crude products and feedstock plus external heat input) for the hydrothermal upgrading process (HTU®) of biomass of a 10 kg dry weight h^{-1} pilot plant is as high as 75%. The main product of the process is bio-crude accounting for 45% wt. of the feedstock on dry ash free basis, with a lower heating value of 30-35 MJ kg^{-1}, which is compatible with fossil diesel and can be upgraded further. As moist biomass can be easily heated by microwave power, a process similar to the HTU® process using a novel microwave high-pressure (MHP) reactor has been developed in order to further minimize the energy consumption of the process. [29] Past research on hydrothermal technology for direct liquefaction of biomass rarely reported the use of algal biomass as feedstock. Minowa *et al*. [30] reported an oil yield of about 37% (organic basis) by direct hydrothermal liquefaction at around 300 °C and 10 MPa from *Dunaliella tertiolecta* with a moisture content of 78.4 wt%. The oil obtained at a reaction temperature of 340 °C and holding time of 60 min had a viscosity of 150–330 mPas and a calorific value of 36 kJ g−1, comparable to those of fuel oil. The liquefaction technique was concluded to be a net energy producer from the energy balance. In a similar study on oil recovery from *Botryococcus braunii*, a maximum yield 64% dry wt. basis of oil was obtained by liquefaction at 300 °C catalyzed by sodium carbonate. [31] Aresta *et al*. [32] have compared different conversion techniques *viz*., supercritical CO_2, organic solvent extraction, pyrolysis, and hydrothermal technology for production of microalgal biodiesel. The hydrothermal liquefaction technique was more effective for extraction of microalgal biodiesel than using the supercritical carbon dioxide, [32a] because liquefaction has lower energy input requirements (T= 200–300 °C versus 500 °C required for pyrolysis and 2,000 °C for gasification). Another advantage of liquefaction is the possibility of using algal biomass with high moisture content (>80–90 wt%). By increasing the temperature the total amount of oil extracted from the algae reaches a plateau at 350–395°C. From these two studies, it is reasonable to believe that, among the selected techniques, the hydrothermal liquefaction is the most effective technological option for production of bio-diesel from algae. Nevertheless, due to the level of limited information in the hydrothermal liquefaction of algae, more research in this area would be needed.

Several studies have investigated the characteristics of algal biomass as a feedstock. Dote et al. [33] successfully used thermochemical liquefaction at 300 °C on *B. braunii* to achieve a maximum yield of 64% dry wt. basis of oil with HHV of 45.9 MJ kg^{-1} and also declared a positive energy balance for the process (output/input ratio of 6.67:1). In a similar study, an oil yield of 42% dry wt. was obtained from *Dunaliella tertiolecta* giving a HHV of 34.9 MJ kg^{-1} and positive energy balance of 2.94:1. [30] These results indicate that thermochemical liquefaction is a viable option for the conversion of algal biomass-to-liquid fuel.

Hydrothermal upgrading of biomass to yield liquid fuels is currently being implemented well over the pilot plant scale, and in some cases actual production facilities are starting up. For example, a wide range of biomass feedstock is being converted into bio-crude oil by this kind of catalytic technology. [34] The missing link, constituted by employing algae as feedstock, is probably only a short step away.

6 Conclusions

According to Thurmond, [35] products derived from microalgae have a huge potential market in the world (Fig. 2), dominated by the production of biodiesel (50%), the absorption of CO_2 and production of food supplements, [36] and high-value pharmaceutical and specialty chemicals.



source: Emerging Markets Online Consulting Services: Algae 2020 study

Figure 2. Algae 2020 Market Value Model.

To balance economic, energy and emissions, and to achieve an advantage particularly in the production of biodiesel, it is necessary that the entire production cycle is based on processes with reduced energy consumption, moderate cost of installation, that uses wet algal biomass as feedstock. It would be desirable to build systems with renewable energy based systems (wind, solar, geothermal, etc.). As regards the absorption of CO_2, careful consideration should be given to the option of ad-hoc systems of gas insufflation, *e.g.* from power plants, which have a cost in the face of a possible small increase in productivity, rather than relying on the natural absorption of atmospheric CO_2 by microalgae, at no cost.

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5 Micro-algae: the Rise of Next Generation Biofuels

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Abstract The search for a suitable replacement to conventionally used fossil fuels as a feedstock for diesel production, has been gaining momentum over the recent years. The use of first generation feed-stocks such as edible and non-edible plant oils have been rendered non-feasible for large scale production, due to the food versus fuel dilemma. Research into the use of microalgae for the production of biodiesel has gained significant interest due to the ability of microalgal cultures to be grown to significant cell titres. They have an inherently faster growth rate in comparison to plant sources and have an ability to accumulate algal lipids up to 70% of its dry cell weight. Research into microalgae as a biodiesel feedstock is being conducted globally; these include many lavishly funded multidisciplinary international teams aiming to reduce the world's dependence fossil fuels.

Keywords Biodiesel, microalgal, lipids

1 Introduction

Energy is indispensable for economic growth and quality of life. The global demand for energy is constantly on the increase, particularly in the transportation sector resulting in a worldwide energy crisis [1]. The majority of the world's energy supply is derived from fossil fuels, which has been recognised as unsustainable due to depleting resources. This as a result increases petroleum prices and also contributes to the accumulation of greenhouse gases in the atmosphere [1]. Global warming as a result of increasing greenhouse gas emissions, of which most are a consequence of fossil fuel combustion, is a great concern. The implementation of alternative sources of energy, such as biofuels is imperative and has gained enormous interest in several countries.

Biofuels are defined as solid, liquid or gaseous fuels obtained from biological material and are an attractive alternative source of renewable energy [2]. The production of biofuels presents the added advantages of reducing greenhouse gas emissions and opportunities to sustain economic growth [3]. Common biofuels include biodiesel and bio-ethanol and these can be used as substitutes for diesel and gasoline respectively [3]. Biodiesel is biodegradable and has low toxicity which makes it a particularly interesting and beneficial alternative for diesel fuel [4]. Biodiesel has been recognised as the best replacement for diesel fuel as it can be used in a conventional compression ignition engine without requiring large modifications [5].

Chemically, biodiesel is a mix of methyl or ethyl esters of long chain fatty acids [6]. It is commonly produced in the transesterification of mono-, di- and triacylglycerides (TAGs) or in the esterification of free fatty acids (FFAs) with alcohols (methanol or ethanol) [7]. Transesterification is a multistep reaction where the addition of an alcohol such as methanol to a parent oil, for example a triglyceride, in the presence of a catalyst, forms methyl esters (biodiesel) and glycerol [3, 8]. The glycerol formed during the transesterification reaction has many applications – for instance as a strong detergent – and is a valuable by-product. Other biodiesel fuels include the original purified oils to be used directly in diesel engines, their blends with fossil diesel, micro-emulsions, and hydrocarbons obtained by thermal cracking or pyrolysis of oils [5]. Thermal cracking and transesterification are preferred, as no known problems occur when using the produced biodiesel in engines, but thermal cracking is very energy intensive and thus contributes to a much higher cost of production [5].

Feedstocks for biodiesel include vegetable oils (non-edible and edible), animal fats and recycled or waste oil [4]. At present, biodiesel is mostly produced from edible oils such as rapeseed, soybean, palm or sunflower oil. The use of vegetable oils to run diesel engines dates back to 1893, wherein diesel engine inventor, Rudolf Diesel, ran his engine model on peanut oil [9]. Producing biodiesel from food crops requires a large amount of arable land and increases competition with the edible oil market. This in turn increases the cost of edible oils and biodiesel [5, 6]. It is for these reasons that the focus of research has been diverted towards biodiesel production from non-edible sources, such as microalgae. If microalgae can be produced on large-scale industrially, approximately 0.4% of arable land would be required worldwide to meet the current fuel demands [10, 11]. This figure is significantly lower than what would be required from terrestrial oil seed crops, as microalgae can produce approximately 30 times more oil per unit area of land [11].

Microalgae are simple unicellular organisms that use photosynthesis to convert solar energy into chemical energy [12]. These organisms can utilise sunlight and carbon dioxide (CO_2) to produce biomass that can be utilised as a feedstock for biofuels, high-value bioactive compounds and food and feed products [8]. The four main classes of microalgae are *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including diatoms) and *Chrysophyceae* (including golden algae) [13]. Microalgae are known to have fast growth rates and they can grow in both freshwater and saltwater environments, requiring only sunlight and simple nutrients [3]. The basic requirements for growth media used to cultivate microalgae include CO_2, light and essential elements such as nitrogen, phosphate, iron and depending on the species, silicon [3, 8]. Microalgae are known to have higher growth rates and lipid contents in comparison to terrestrial plants-with biomass productivities up to 50 times higher [1, 6, 8, 7]. Other advantages of using microalgae include its low water demand and high efficiency of CO_2 mitigation [1].

Currently the only practical methods of large-scale microalgal cultivation are open raceway pond systems and closed system tubular or flat plate photobioreactors (PBRs) [8]. The raceway pond is a simple method of algal cultivation and it has a common technical design [14]. There are advantages and disadvantages of using both methods of large-scale cultivation. In general, the open pond system is less favourable due to the increased risk of contamination and algal biomass productivity is reported to be lower than with PBRs [12, 8]. PBRs are however more costly, are very specific to the microalgal specie being cultivated. They also have several operating challenges, such as overheating and are difficult to scale up to large volumes [12, 8]. A raceway pond being rather simple in design and easy to maintain makes the process a lot simpler and cost effective and as such is the preferred method for commercial production. The risk of contamination can be significantly lowered by selecting a microalgal specie with a very high growth rate as this decreases the chance of another algal specie out-competing the selected organism for nutrients, in addition to increasing the algal biomass productivity [6].

Regardless of the method of cultivation, harvesting of algal biomass is a process that forms a major part of microalgal biodiesel production. This process consists of concentrating algal biomass from the culture media and can occur via biological, physical or chemical means [3]. Methods of harvesting include centrifugation, filtration, ultra-filtration or a combination of flocculation-flotation [3]. The harvesting process can contribute to almost a third of the cost of biodiesel production and is an area with large scope for further research [3].

Despite the many advantages, production of biodiesel using microalgae is not yet economically feasible [6]. The cost of producing microalgal biodiesel is very high as a result of low lipid yield and the cost of the harvesting process [6]. However, with the enormous potential as an alternative energy source and the positive environmental benefits, cost should not be a discouraging factor. There is still a great lack of understanding around microalgae and further research needs to be undertaken in order to find solutions to the technological challenges that increase costs of biodiesel production [15]. The potential to find an alternate source of energy, to protect the environment and increase our quality of life substantiates the need for increased investigation into microalgae for biodiesel production.

2 Challenges

The current advances in the use of microalgae for biodiesel production has showed much promise in a field where ground breaking research is a necessity. Algae have many advantages over the use of first and second generation feed-stocks. Microalgal biomass with an oil content of 55% will need to be produced at a cost lower $340 per tonne for it to be deemed as a competitor to petroleum diesel [8]. The current costs of production will have to be decreased significantly for this technology to be taken up internationally.

There are many factors that hinder the algal biodiesel production process. Due to this area being a “new” area of research, many bottlenecks need to be streamlined in order to obtain a feasible production process. The highest realistic/obtainable threshold of biodiesel production using microalgae is in the region of 10 000 gallons per acre per year [16]. This figure could improve as a result of improved cultivation methods, genetic engineering, and species selection. However the laws of thermodynamics and photosynthetic efficiencies will not allow higher estimated values [16]. In addition to these basic laws of physics, there are additional problem areas that many researchers/start-up companies are faced with.

Selecting a production organism is a vital step in technology development. Many labs are involved in actively screening indigenous water bodies to isolate algae that could be used for biodiesel production. Previously, the purification and assessment of the ability of each isolate to be classified as a high lipid producing organism and for it to be used in biodiesel production was a tedious and time consuming task. More recently, high throughput screening methodologies and robotics techniques have been implemented to speed up the process. The selection of the production organism also has an impact on the choice of appropriate harvesting technologies and down streaming process technologies. This organism must have the ability to produce a lot of oil, whilst still remaining competitive in an open pond system [16].

Some organisms are capable of doubling their population within a day, however, if and when the microalgae diverts energy towards making and accumulating oil within the cell, they do not display a fast growth rate [17-19]. When a high growth rate is achieved, oil accumulation rarely does occur. This trade off will have to be well understood to ensure oil production whilst maintaining a decent growth rate. This switch of growth modes has stumped researchers for decades [16]. One could assume that the advent of recombinant DNA technology would solve problem, however, the resultant transgenic algal isolate may have a reduced fitness in an open pond system due to its commercial tailoring [16].

In addition to the challenge of selecting a suitable production isolate, both methods currently used for the cultivation of microalgae have their challenges and limitations. The main obstacle for the commercialization of algae-based biodiesel is its high production costs from requiring high-oil-yielding algae strains and effective large-scale bioreactors [20]. Open ponds require a strain that will perform optimally in a highly selective environment. In a production system using an open pond system; there is an inherent threat of contamination and pollution from other algal species, bacteria and protozoa [6, 12, 8]. An algal isolate that is capable of growing in an extreme environment such as high salinity, or high alkalinity would facilitate the growth of an algal monoculture by making the environment uninhabitable for other contaminating organisms [17]. These systems regardless of their lower cost of operability results in the lower production of biomass in comparison to closed systems. Many factors contribute to this lower biomass production. These include evaporation losses, fluctuation of the temperature of the growth medium, CO_2 deficiency, inefficient mixing and most importantly, light limitation [21, 22]. These factors influence the resultant low cell density and low productivity of open systems. This also results in poor batch to batch consistency and unpredictable culture crashes [8, 23, 24]. A major factor in the mass production of microalgae for biodiesel is the use of sunlight as a free natural resource. Sunlight however is limited due to diurnal cycles and seasonal variations. This leads to limited options for the commercial production areas to areas with high solar radiation. This factor could be overcome by utilising artificial lighting which would allow for continuous production but at a cost of significantly higher energy input. Many current systems use artificial lighting with is usually powered by electricity derived from fossil fuels. This set-up negates the aim of developing a competitive technology that reduces the carbon footprint [21]. Studies into the development of alternative renewable sources of electricity such as solar wind or hydro power; to suitably power up these artificial light sources need to be developed. Raceways are perceived to be less expensive than PBRs because they cost significantly less to build and operate [25]. However, they have a much lower productivity in comparison to PBRs.

Closed systems, such as the use of PBRs on the other hand, are more expensive and more complex to operate than open pond systems [12, 8]. These reactors were designed to overcome many of the shortcomings experienced during the cultivation of algae in open pond systems [17, 21]. Cell cultures obtained using this method of cultivation, reach higher densities in this closed environment. This higher cell titre also facilitates easier cell harvesting. As a result, the cost of harvesting, which is a major contributing cost to the process technology, is significantly decreased. It is however rather unfortunate that the capital and operating costs of a closed system still remains high and is therefore very difficult to render the process as being economically viable [21]. Some researchers say that by working in a closed bioreactor, some companies double the cost of a gallon of algal oil [16].

The successful harvesting of algae from a culture system presents itself as one of the major challenges experienced. Apart from the numerous challenges of algal cell cultivation, the harvesting of algal cells from the culture systems presents itself as another major bottleneck in biodiesel production [19, 21]. To ensure the effective removal of cells, this process step generally requires one or more solid-liquid separation step. Due to the various steps involved in a typical DSP process, cell harvesting techniques contributes to approximately 20-30% of the total production cost [21]. Many options such as filtration, flocculation, aggregation, flotation and centrifugation have all been tested for use in the selected cell production system. Most algal isolates can be recovered via centrifugation; however, this has been reported as probably the most expensive form of cell separation. Some researchers have reported that cells when exposed to high gravitational forces and shear forces they could become damaged [26, 19, 27, 28]. Studies have also proved that this method of separation is time consuming and very costly as a consequence [17]. When selecting a suitable cell separation step, it is imperative to understand and take into consideration the morphological characteristics of an organism. Algal cells, apart from being difficult to suitably remove from the broth culture, also have a negative charge, which hinders their stability in a dispersed state and makes the process of aggregating cells more problematic [21].

Once the first stage of the down-stream process viz., cell separation has been carefully selected, an efficient method of oil extraction needs to be implemented. Three main methods used in oil extraction have been described in detail. Of the three commonly used options, solvent extraction using n-hexane methods result in the highest oil yield. For algal oil to be suitably substituted as an alternative for petro-diesel it needs to be transesterified. This transesterification cost, along with the cost of recovering glycerol as a by-product; contributes to the major cost associated with this technology. One method of reducing the production cost is to have a continuous transesterification process. In addition, the recovery of a high quality glycerol will help to reduce the cost of biodiesel. When designing a biodiesel production plant, it would be beneficial and also to an extent imperative for all biodiesel plants to have a glycerol recovery facility [9, 25, 29-31]. The development of value added products from glycerol would significantly improve the overall economics of an algal biodiesel venture, as currently the crude glycerol market is saturated.

There are various methods available which can be applied to diverse production systems to effectively remove algal cells from the culture broth. Sometimes, as a result of cell separation, a large amount of water is also removed from the culture system. The most commonly used method of extracting this excess water is most probably performed by drying. The drying of the algal biomass would result in the use of either a convection dryer or similar option which ultimately results in a high energy consuming process [32]. More efficient and economic harvesting techniques need to be employed to enhance the commercial viability of microalgae biofuel industry [19]. Due to the numerous challenges experienced in the selection of a feasible harvesting process step, the resultant consequence is an abandonment of various up and coming technologies using algal biomass.

Currently, biodiesel costs 1.5-3 times higher than the cost of fossil diesel in developed countries. All governments need to place microalgal biodiesel on the biofuels priority list and also introduce incentives to promote biodiesel research. This will facilitate the lowering of the cost algal biodiesel and make prices more competitive with other conventionally used energy sources [4, 29-33]. In addition to costs of biodiesel production described in this chapter, such as, biomass growth, harvesting, oil extraction and transesterification; other costs such as; engineering, licensing, infrastructure and plant build-up, equipment purchase and integration also need to be considered and factored in the total cost of production [17].

The industrial viability of microalgal based biofuels hinges upon the economics underlying the process. Regardless of the fact of numerous advances are emerging in terms of technological innovations, they have not yet received enough excitement from the market to fund projects that are capital-intensive unless the risk-return ratio is acceptable [34]. The current reality is that the market has been over-saturated with overly optimistic claims that pressure the rest of the industry to match and create expectations. It has also been observed that venture capitalists fuel the resulting problem as they do not consider challenges and barriers that could arise during technology development.

It is imperative to ensure that all researchers, consultants and venture capitalists, work together in order to ensure that algal biodiesel, forms a suitable and effective alternative to non-renewable petro-diesel. The resulting fuel or fuel additive must be produced via a process that is technically feasible, economically competitive, environmentally acceptable, and easily available [35]. Process engineering provides a vibrant emerging area for industrial practice for the production of algal biodiesel with great promise for partially replacing the use of petro-diesel and biodiesel from oil crops [7].

3 Opportunities for Biodiesel

The two most important factors contributing to increased interest in and incentives for biodiesel production are the security of energy supply and the environmental effects [11]. Fossil fuel resources supply most of the world's energy and these reserves have been identified as unsustainable [1, 8]. Therefore, it is unquestionable that a transition from fossil fuel derived energy toward biofuels is an unavoidable and impending reality for the future [36].

Biodiesel fuel has the potential to play a significant part in strengthening a nation's energy security, as a renewable and biodegradable energy. Its production could also address the issue of the detrimental effects of increasing greenhouse gas emissions in the atmosphere [1, 9]. Europe has aimed to have cut greenhouse gas emissions by 20% in 2020 and included a demand for an increased proportion of biodiesel involvement in the transport sector [9]. The United States of America have also set targets of decreasing greenhouse gas pollution by 28% by 2020 and the Chinese have also set targets to decrease carbon dioxide emissions and also aid African countries in their development of clean energy sources [9]. Other countries that have also announced their involvement in promoting a reduction in greenhouse gases and development of alternative energy sources include Japan, India, Brazil and South Africa, among others [9]. The involvement of governments to set targets for gradual substitution of conventional petroleum fuels with biodiesel provides exceptional opportunities for the development of the biodiesel industry.

Biodiesel is one of the most common biofuels worldwide, due to it being compatible with conventional biodiesel fuel and it can be used without requiring major modifications to the current compression ignition engine design [5, 9]. First generation biodiesel is mostly produced from food crops. The production of these sources has the opportunity to increase net farm income and reduce government subsidies to farmers by increasing the oil crop market price [9]. However, the production of biodiesel from food crops requires a large amount of arable land and therefore competes with the edible oil market, thus increasing the cost of both edible oils and biodiesel [5, 6]. First generation biodiesel is therefore not completely sustainable as there is a resource limitation of arable land [37].

Focus of research has shifted towards production of biodiesel from microalgae – a non-edible source. Microalgae uses photosynthesis to convert sunlight and CO_2 into biomass from which non edible vegetable oils can be extracted [8]. These non edible sources can be converted into biodiesel via a transesterification reaction. The major reasons for the increased interest in microalgae over terrestrial plants are: higher growth rate (and thus biomass productivity), lower water demand, higher photosynthetic efficiency (and CO_2 mitigation efficiency) and non-competition with food crops for arable land [1, 8]. Significant challenges do exist, most of which are a result of the high cost of production and due to algae-based biofuel initiatives, presently surrounding research and development, being primarily based in developed countries [38]. However, successful implementation will result in a sustainable source of alternate energy that is not resource limited for arable land [37].

Microalgae are organisms with the potential to produce a variety of products, including nutraceuticals for human consumption, nutraceuticals for animal or fish feed produce, bulk chemicals and biofuels [38]. A main benefit of microalgae is their ability to co-produce products. This property can be exploited for a more economically feasible system of microalgal production. The production of for instance high-value products such as omega-3-fatty-acids and other polyunsaturated fatty acids (which consist of approximately 10% of algal biomass) can be co-produced with biodiesel (able to utilise a majority remaining biomass) [38]. By developing a system whereby co-production is possible, some of the economical barriers that are a current concern for biodiesel production from microalgae, might be overcome. Presently co-production is not yet a suitable method of biofuel production, as productivity at the current available scale is not yet on target. As a result, there is much room for implementing research and development incentives for large-scale microalgae cultivation for biodiesel production and co-production of high-value products.

Another very exciting opportunity exists to utilise microalgae for carbon dioxide sequestration. Biological mitigation of CO_2 (via photosynthesis) has been suggested as the only truly feasible method of reducing CO_2 emissions in the atmosphere [39]. As photosynthetic organisms, with much higher photosynthetic efficiencies than terrestrial plants, microalgae have major potential to be exploited for the purposes of CO_2 sequestration. Sources of CO_2 for microalgae are typically atmospheric CO_2, CO_2 from industrial exhaust gases (e.g. flue gas) and CO_2 in the form of soluble carbonates (e.g. $NaHCO_3$ and Na_2CO_3) [39]. The possibility of using waste CO_2 from fossil-fuelled power plants and other carbon emitting facilities offer exciting opportunities for meeting targeted reduction in greenhouse gas incentives. Prospects of coupling biodiesel production from microalgae to this process and co-production of high-value products, intensifies the idea of large-scale microalgal cultivation for biodiesel production.

The other possible application of microalgae cultivation, is additionally coupling wastewater treatment to biodiesel production [39]. Microalgae do not require water of high quality and wastewater from agricultural, municipal or industrial facilities can therefore be used for cultivation and as a minor source of nitrogen, phosphorous and other nutrients [39]. Currently, microalgae are being used for wastewater treatment as a tool to remove and recover nutrients, and under heterotrophic conditions, for the removal of heavy metals and organic substances [39]. This opportunity further substantiates economic and environmental incentives for microalgal biodiesel production [39].

Currently there are no commercial large-scale production facilities for microalgal biodiesel production. This is because an algal strain with a sufficient level of lipid productivity has not been grown on large scale to achieve yields comparable to C_4 plants such as sugarcane [38]. In addition, there is uncertainty surrounding the capital and operation costs and volatile market prices of biodiesel [40]. Studies have shown that the economic viability of large-scale microalgal biodiesel production is not yet attainable [40]. Major opportunities exist for large-scale production in the event that funded, comprehensive research and development programs are implemented. Fossil fuel reserves are, however, declining and this is a reality for the future – therefore as conventional energy sources are exhausting, the market value of alternate sources of energy, such as biodiesel, will increase [37].

The gaps in current research into microalgal biotechnology offer opportunities for further research. For effective CO_2 mitigation assessment via microalgae, large-scale facilities need to be employed. Currently, mostly bench-scale experiments have been done to confirm that a possibility for waste CO_2 utilisation from power stations exists [39]. As concentration of atmospheric CO_2 is not enough for efficient microalgal growth, additional CO_2 is required for cultivation and is currently contributing to the cost of production [39]. Costs can be decreased severely if appropriate research is funded to actualise waste CO_2 usage from high carbon dioxide emitting facilities. Additional research is also required for wastewater treatment via algae in conjunction with biofuel production in terms of large-scale feasibility as well as assessing the quality of the biodiesel produced by such means [39]. The biggest issues for large-scale production of biodiesel with microalgae as a feedstock include that methods of harvesting, dewatering and lipid extraction have not been developed and refined in term of efficient use of energy and low cost [6, 39]. Opportunities for increased bio-prospecting initiatives worldwide exist whereby microalgal strains can be evaluated for lipid content, growth rate (biomass productivity) and the combined lipid productivity – key criteria for strain selection [6]. Other criteria of an ideal strain include photosynthetic efficiency for CO_2 mitigation. There is a gap in research whereby microalgal strains are investigated and cultivated to maximize photosynthetic efficiency [11].

In order for the long term goal of commercial production of biodiesel to be achieved, the opportunities for microalgal biodiesel development need to be seized. This can only be done with adequate research and the availability of financial resources for that research.

4 Conclusions and Recommendations

Humanity is confronted by an energy crisis as fossil fuel reserves have been recognised to be on the decline. Fossil fuel combustion contributes to a majority of the greenhouse gas emissions in the atmosphere and the emerging threat of Global Warming caused a worldwide concern. Policies have been governed in major countries in order to protect the environment and find alternate sources of energy to sustain the quality of human life. Biodiesel is an attractive alternative source of fuel as it is environmentally friendly and has the potential to minimise the threat to the security of energy supply. Although biodiesel is mostly produced from edible vegetable feedstocks, interest has been shifted towards producing biodiesel from non-edible sources such as microalgae. The major reasons being that microalgae does not require the use of arable land and hence does not compete with the edible oil market, studies have reported higher oil yields and growth rates for microalgae than food crops and the photosynthetic efficiency of microalgae is much higher than terrestrial plants. Current technology is feasible for lab-scale and pilot scale production of microalgal biodiesel. Flow cytometry has been identified as a valuable tool for rapid screening of microalgal strains for lipid content and growth rate. Various culture methods can be applied when cultivating microalgae and these methods depend on the specific requirements of the specie being cultivated. The only practical methods of large scale cultivation at present are through closed system PBRs or open system raceway ponds. Both methods of cultivation have their advantages and disadvantages, but commercial production would favour open raceway pond systems due to lower cost and ease of maintenance. Several methods of cell separation and harvesting exists: gravity sedimentation, flocculation, floatation and centrifugation and filtration, however these methods still have significant challenges to overcome and comparative studies need to be done to assess the which method would be the most practical and of lowest cost. Microalgal lipid extraction can be done via various methods, but solvent extraction is the most common method and enzymatic extraction is the most environmentally friendly. Significant challenges exist for biodiesel production from microalgae – the biggest obstacle remaining the high cost of production. Microalgal biodiesel production is still far from being commercially feasible. The other challenges that contribute to the major problem concerning cost include shortcomings with screening systems for ideal strains, technological design of the culture vessels and the cell separation and harvesting techniques. There are still many opportunities for refining current technology for commercial production of biodiesel via microalgae. The scope for further research is very wide and incentives and financial resources need to be applied towards to bridge the gaps in research and development of biodiesel production from microalgae.

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6 Wastewater Treatment Using Algae Technology
An Emerging Environmental Innovation

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Abstract Treatment of contaminated waters is a crucial global issue. In particular, contaminated freshwater resources cause significant environmental problems and public health concerns. Algae are recognised to play a central role in the natural self-purification of contaminated waters, and algae-based wastewater treatment processes have been gaining tremendous attention following the pioneering work of Oswald. Here we present a review on the mechanism of action, capabilities and benefits of algae based bioremediation.

Keywords Contaminated water, pollution, algae self-purification wastewater treatment, phycoremediation

1 Wastewater Problems in the World

> There is a water crisis today. But the crisis is not about having too little water to satisfy our needs. It is a crisis of managing water so badly that billions of people - and the environment - suffer badly. [1]

The importance of water as a global resource for human life is irrefutable. A prominent threat to global water quality, in general, is its contamination with human- derived wastes of residential, industrial and commercial origins. This is particularly the case for freshwater resources, where human-derived wastewaters are one of the major sources of contamination and pollution [2]. The decline in water quality has given rise to significant environmental problems and public health concerns [3].

Municipal and industrial wastewater treatment is a key infrastructural process that preserves desirable water quality for multipurpose uses. In general, the treatment of domestic and agro-industrial wastewater still releases large amounts of phosphorus and nitrogen. These nutrients are directly responsible for eutrophication of rivers, lakes, and seas [4, 5].

Prior to discharging wastewater into water bodies, removing most nutrients is usually obligatory, even though it is not performed in many cases, especially in developing countries. The wastewater treatment industry presently uses several methods to remove phosphorus and nitrogen [6] and other pollutants. Some are used in large-scale treatment facilities and a few are experimental projects and used on a small-scale basis (from a process-engineering viewpoint [7, 8]).

Algae are recognised to play a central role in the natural self-purification of contaminated waters [9-11]. Algae based wastewater treatment processes have been gaining tremendous attention since 1960s [12-14]. The basics to purposely use Microalgae for wastewater treatment have been laid by multiple researchers [15-18]. Following the pioneering work of Oswald et al. [19], and the potential for microalgal wastewater treatment and nutrient removal has shown a great promise [20-23]. Oswald et al. [19] coined the term photosynthetic oxygenation to describe the treatment of wastewater by algal cells and heterotrophic bacteria. In this process the organic waste is decomposed by the bacteria to inorganic nutrients, and these are incorporated into algal biomass, which may then be separated from the effluent. Thus the waste is treated and nutrients recycled into algal biomass.

2 Algae in Wastewater Treatment: A Concise Literature Survey

Aziz and Ng et al. [24] studied the feasibility of using an activated-algal process to treat wastewater and found that it was able to reduce by 80-88% the biochemical oxygen demand (BOD), and by 70-82% the chemical oxygen demand (COD), as well as to remove 60-70% of nitrogen and 50-60% of phosphorus, with a retention period of 15 days.

Sawayama et al. [25] used *Botrycoccus braunii* to remove nitrate and phosphate from sewage after primary treatment along with the production of hydrocarbon-rich biomass.

Martinez et al. [26] achieved a significant removal of phosphorus and nitrogen from urban wastewater using the microalgal *Scenedesmus obliquus*. They were able to achieve 98% elimination of phosphorus and a complete removal (100%) of ammonium nitrogen in a stirred culture at 25 C° over 94 and 183 h retention time, respectively.

Gomez Villa et al. [27] experimented with outdoor cultivation of microalgal *Scenedesmus obliquus* in artificial wastewater, and achieved the reduction of dissolved nitrogen concentrations of 53% and 21% of initial values in winter and summer, respectively. Phosphorus, which was only removed during the day, showed a total reduction of 45% in the winter and 73% in the summer.

Hodaifa et al. [28] recorded 67.4% reduction in BOD with *Scenedesmus obliquus* cultured in diluted (25%) industrial wastewater from olive-oil extraction. The percentage of elimination reduced to 35.5% with undiluted wastewater because of low nitrogen contents, which inhibited the microalgae growth during the exponential phase.

Shen et al. [29] investigated treatment of livestock wastewater and production of biomass and lipid of *Botrycoccus braunii*. This alga was able to remove 88% of total nitorgen and 98% of total phosphorus over the course of two weeks while producing 19.8% (weight percent of the dry weight) crude oil content. The authors also found that under ideal laboratory conditions such as autoclaved wastewater and optimal lighting with additional nutrient supplies, *B. braunii could* achieve a biomass production of up to 2.5 g/L, which is a very high value compared to most batch systems.

Hu et al. [30] used the freshwater microalga *Scenedesmus* sp. LX1 that showed the best ability to adapt to grow in secondary effluent with the highest microalgal biomass (dry weight) of 0.11 g/L and the highest lipid content at 31–33%. Lipid content of *Scenedesmus* sp. LX1 changed with growth, and increased from 14% at the log phase to 31% at the stationary phase and reached its maximum after 10 days of growth.

Chinnasamy et al. [31] established the proof of concept for production of biodiesel from a consortium of native algae cultivated in carpet wastewater. They concluded that the recovery of energy from the consortium through anaerobic digestion or thermochemical liquefaction appears promising.

3 Mechanism of Action, Capabilities and Benefits of Algae Based Bioremediation

The algal cell maintains peculiar biological characteristics that vary from one species to another. Under light limiting conditions the algal cells can change their nutrient regime and assimilate organic carbon (heterotrophic growth) [32] as well as inorganic nutrients such as nitrogen and phosphorus (mixotrophic) [13] from the wastewater for their growth without an aerobic environment being created and maintained [33, 34].

Algae produce oxygen during photosynthesis and thereby provide a cheap and safe alternative to mechanical aeration while also contribute to CO_2 mitigation [35-37] (see Fig. 1).

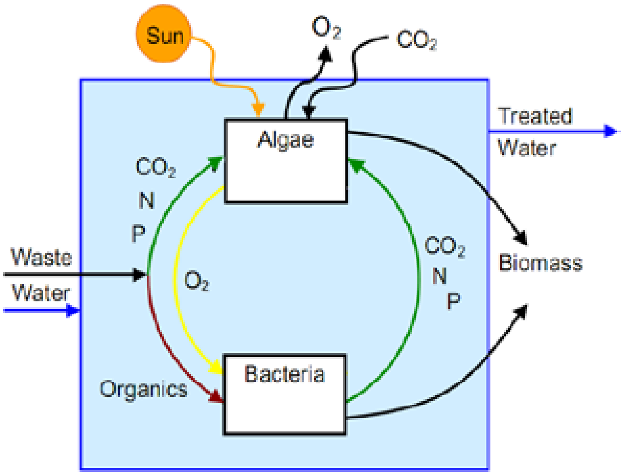

Figure 1. Algae-bacteria symbiosis in wastewater treatment [38].

Microalgae assimilate significant amounts of nitrogen and phosphorous for nucleic acids, phospholipids and proteins synthesis, which account for 45-60% of microalgae dry weight [35]. The N composition of different algae species ranges from 1% to 14% of algae dry weight and P ranges from 0.05% to 3.3% [38]. Microalgae are capable of taking up nitrogen and phosphorus in excess (luxury metabolism) [39, 40]. Microalgae intervene in the reduction of nutrients by inducing precipitation of phosphates and stripping of ammonia through photosynthetically created high pH values [41].

Wastewater is a good source of free nutrients for algae cultivation that can significantly reduce the operation cost of algal production systems [42, 43]. All of the essential macronutrients (e.g. N and P) and micronutrients (e.g. Fe, S etc.) are available in domestic and agricultural wastewater [44]. Major nutrients such as N and P alone contribute to ~10–20% of production cost of algae biomass [45]. Certain microalgae species can efficiently grow and build up biomass in brackish and saline wastewater [46].

Traditional wastewater treatment processes involve the high energy costs of mechanical aeration to provide oxygen to aerobic bacteria to consume the organic compounds of wastewater and high cost for subsequent sludge processing. Roughly one kg of BOD removed in an activated sludge process requires one kWh of electricity for aeration, which produces one kg of fossil CO_2 from power generation [47]. By contrast, one kg of BOD removed by photosynthetic oxygenation requires no energy inputs and produces enough algal biomass to generate methane that can produce one kWh of electric power [47].

The use of algae can also offer an increased benefit during treatment of hazardous pollutants that must be biodegraded aerobically but might volatize under mechanical aeration. Bioaccumulation of heavy metals and toxic compounds is a potential additional advantage of an algal wastewater treatment system [48] but contaminating the algal biomass produced and excluding the algae for certain uses. Some microalgae species maintain bactericidal capabilities that can reduce the propagation of pathogenic bacteria [49, 50]. Microalgae are active in reducing wastewater malodors by creating alkaline conditions in the upper part of the pond and chemically trapping H_2S, mercaptans and volatile fatty acids [51].

4 Potential Phycoremediation Processes Using Microalgae

4.1 Phycoremediation of Nutrients

Nitrogen is regarded as an essential macronutrient for the growth of microalgae which is needed for various biochemical processes, mainly for protein synthesis [52]. Microalgae showed their ability to increase growth by uptaking various forms of inorganic nitrogen mostly ammonium (NH^{4+}) and nitrate (No_3) with stripping off the toxic ammonia gas through the elevated pH [3, 53-57]. Kim et al. [58] reported 95.3% and 96% removal of nitrogen and phosphorus, respectively, by *Chlorella vulgaris* in 25% secondary treated swine wastewater after four days of incubation. Travieso et al. [59] treated distillery wastewater from an anaerobic fixed-bed reactor in a microalgae pond and obtained 90.2%, 84.1%, and 85.5% organic nitrogen, ammonia, and total phosphorus removal, respectively.

Phosphorus is a macronutrient that plays an important role in growth and metabolism of algae. It is required for most cellular processes, that involving energy transfer and nucleic acid synthesis. The two most important phosphorus forms used by algae are $(H_2PO_4)^-$ and $(HPO_4)^{2-}$ [60]. Organic phosphates are also found in larger concentrations in water than inorganic phosphates. They must be hydrolyzed by extracellular enzymes such as phosphoesterases or phosphatases. Algae stores phosphorus mainly in the form of polyphosphates and metaphosphates. These compounds are present in granular form in algae under excess phosphorus conditions and disappear in limiting conditions [61].

During photosynthesis, microalgae assimilate inorganic carbon from wastewater, mostly carbondioxide (CO_2) and bicarbonate anion (HCO_3-) [62]. Their consumption resulted in significant increase in medium pH to level that can strongly affect the wastewater chemistry. As a result, phosphorus can be readily precipitated with available cations to form metal phosphates, where calcium phosphates are the most common [14].

Chemical precipitation of phosphorus may comprise a significant part of the total phosphorus removal in algal wastewater treatment [63-65]. Particularly in areas with hard water, i.e., water with high concentrations of calcium and magnesium. The chemical stripping of phosphorus may be regarded as advantageous by-effect of the algal growth, with enhanced phosphorus removal as a result. Another advantage is that the chemical sludge which is produced is easier to harvest than free-floating cells due to the tendency of the precipitate to sink.

4.2 Phycoremediation of Heavy Metals

The intensive use of metals and chemicals in industrial processes has resulted in generation of large quantities of effluent that contain high level of toxic heavy metals and their presence creates environmental problems due to their non-degradable and persistence nature. In addition, mining, mineral processing and extractive-metallurgical operations also generate toxic liquid wastes rich in heavy metals content [66, 67].

Microalgae require different metals for biological functions. In this respect many studies have indicated that the certain species of microalgae are successful in removal of several heavy metals [68-71]. Omar et al. [72] reported that certain green microalgae by physical adsorption process can adsorb metals ion very quickly just in a few seconds or minutes. Then, these ions are transported slowly into the cytoplasm in a process called chemisorption. The author also indicated that polyphosphate bodies in algae enable storing of certain metals including Ti, Pb, Mg, Zn, Cd, Sr, Co, Hg, Ni and Cu. In this context, the polyphosphate bodies stored in algal cells can perform two different functions, namely provide a “storage pool” for metals and act as a “detoxification mechanism”.

The metal bioremoval process mainly combines two mechanism types, namely: passive uptake (metabolism-independent) and active uptake (metabolism dependent). The passive uptake (biosorption) occurs when the metal ion binds to the cell wall according to two different ways: a) ion exchange where monovalent and divalent ions in the cell wall are displaced by heavy metal ions; and b) the complex formation between metal ions and functional groups present at the cell wall. Biosorption is reversible and rapid (it is completed in 5-10 min). The amount of metal accumulated per unit of biomass is proportional to the concentration of metal ion in the solution [73] and largely be affected by pH and the presence of other ions in the medium. The active uptake (metabolism-dependent). may simultaneously involve metal ion consumption for the microalgae growth and/or intracellular accumulation of metal ions. Active uptake is more effective than biosorption for low metal ion concentrations. Both mechanisms can work simultaneously in microalgae and their relative importance may depend on the algal species, culture conditions and the metal chemical properties.

It is perhaps relevant to mention that the most common microalgae useful for metal removal from wastewater include: *Scenedesmus* obliquus [74, 75]; *Chlorella vulgaris* [76, 77] and *Synechococcus* [78].

4.3 Phycoremediation of Organic Pollutants

Several studies [79, 80] indicated that certain microalgae are capable of growing on wastewater rich in organic compounds. Walker et al. [81] performed experiments with the achlorophyllous alga *Prototheca zopfii* which was able to degrade petroleum hydrocarbons found in Louisiana crude and motor oils. Interestingly, in the crude oil, 38-60% of the saturated aliphatic hydrocarbons and 12-41% of the aromatic compounds were degraded, whereas in the motor oil, 10-23% of the saturated aliphatic hydrocarbons and 10-26% of the aromatic compounds were degraded. This suggested that the alga *Prototheca zopfii* was capable of degrading different oils to varying levels.

Jinqi and Houtian [82] investigated the degradation of azo-dyes by *Chlorella vulgaris* and *Chlorella pyrenoidosa* and found that certain dyes, such as Eriochrome blue SE and black T, could be decolorized and actually used as carbon and nitrogen sources. The degradation was found to be an inducible catabolic process. They also found that the algae degraded aniline, a potential degradation product of the azo dye breakdown. In another study, *Ochromonas danica*, a nutritionally versatile chrysophyte, grew heterotrophically on phenol or p-cresol, as the sole source of carbon, up to concentrations of 4 mM [83].

In their studies on bioremediation of industrial wastewater from ethanol and citric acid production, Valderrama et al. [84] indicated that the green microalga *Chlorella vulgaris*, reduced ammonium nitrogen by 72%, phosphorus by 28%, and COD by 61%. Lima et al. [85] reported p-nitrophenol removal of 50 mg/L/Day by a consortium of *Chlorella vulgaris* and *Chlorella pyrenoidosa*.

4.4 Phycoremediation of Animal Manures

A considerable number of earlier publications have demonstrated the ability of various algae to utilize animal waste as a growth medium [86-90]. Recently, Kebede-Westhead et al. [91] reported the use of an algal turf scrubber colonized with freshwater, filamentous algae for the treatment of raw swine manure. Filamentous algae have been used in combination with algae turf scrubbers to treat dairy and swine effluent [92-94]. The authors found that the algae colonies were able to remove between 42-100% of the initial ammonium nitrogen, and 58-100% of the total initial phosphorus in the wastewater. As ammonium was the only indicator of nitrogen that was measured, the algae were proven to remove between 33 and 42% of the total nitrogen.

Another study utilizing benthic algae for dairy manure nutrient recovery found that a consortium of benthic algae could contain large quantities of protein and would be an ideal animal or fish feed [95]. Fedler et al. [96] proposed a multi-step process where wastewater was introduced into an anaerobic system such as an integrated facultative pond. The resulting effluent was high in ammonia-nitrogen and also generated methane which could be used as a fuel. Ammonia which is toxic to many fish was consumed by algae, so the latter could be used as a feed.

5 Advantages and Drawbacks of Algae Phycoremediation

Becker et al. [97] pointed out some major advantageous of using algae for wastewater treatment. These advantages include, but not limited to, the following:

1) using of municipal wastewater to grow algae might obviate the need for freshwater;
2) providing of reusable water free from pollutants, pathogenic bacteria through bactericide action and bad odour;
3) recycling nutrients into algae biomass to be used as a fertilizer;
4) offsetting treatment cost by lowering cost of operation;
5) generating an oxygen rich water effluent after wastewater treatment using algae.

However, there are some technical problems of combining wastewater treatment with algae production. Sheehan et al. [98] concluded that the combination of high-lipid algal biomass production and wastewater treatment is problematic due to nutrient-rich condition that promotes a high productivity without inducing lipid accumulation, while the latter is a nutrient starvation phenomenon. In addition, the need for selective enrichment of high-oil algae species are hampered by contamination with native algae species and bacteria that are abundant in untreated wastewater and are more competitive than the target algae.

The major drawbacks of wastewater purification using microalgae is the harvesting of biomass from the treated effluent [99, 100]. A technical separation unit consisting of filtration or centrifugation has to be applied [101], but this will raise the operation costs. Adding chemicals such as slaked lime will result in secondary pollutants [102, 103]. The use of immobilization system is another possible solution [99, 104], but the known supports are costly and inefficient over a long operation time. Therefore, a more effective biomass harvesting strategy such as a settleable algae-bacteria system is required [105].

6 Types of Microalgae Wastewater Treatment Systems

Depending on objectives, the microalgae wastewater treatment systems can be employed in primary, secondary and/or tertiary (advanced) treatment and can be used solely or in combination with conventional treatment systems. Wastewater treatment systems provide primary treatment (removal of settleable and floatable solids), secondary treatment (removal of dissolved organics and reduction in BOD), as well as partial tertiary treatment (removal of nutrients) and disinfection [106, 107].

6.1 Oxidation Ponds (Facultative Ponds)

The oxidation ponds are usually of the facultative type which means that, in the bottom layer the anaerobic conditions prevail (breakdown of organic material) while in the upper layer the aerobic conditions (photosynthesis) predominate. Different processes, such as grease removal, primary sedimentation, methane fermentation of settled organics, biological oxidation of soluble organics, photosynthetic oxygen production, nutrient and toxicant removal, separation of algae and disinfection can be selectively enhanced through phase isolation in specially designed ponds placed in series [108, 109].

The major characteristics of oxidation ponds are the long detention time (weeks or months), a relatively long depth (1-2 m) and the absence of artificial mixing. The design of oxidation ponds is optimal for wastewater treatment but less for algal growth. Most algae in oxidation ponds are planktonic, especially the coccoid (green) and green flagellates [110]. These algae occur where there is sufficient light for photosynthesis. In a shallow pond they may be dispersed throughout, whereas in a deeper pond they will grow in the upper layer only. Floating mates of blue green algae (e.g. *Oscillatoria*) may also occur and may impair the efficiency of the pond by preventing light to reach the algae in the lower strata and thereby reducing the oxygen production [33]. In shallow ponds benthic algae like diatoms and filamentous blue green algae may also be important.

Oswald and Gotass et al. [111] considered that in large oxidation ponds with a retention time of several weeks up to six months, surface aeration is the most important source of oxygen, while smaller ponds with a retention time of less than a week are highly dependent on oxygen produced by algae.

Recirculating a part of the final treated effluent considerably increases the wastewater treatment efficiency, allowing increased wastewater loads and limiting the hindering anaerobic conditions (malodorous) through inoculation of the incoming wastewater with algae, and thus accelerating the purification process. According to Shelef at al. [112] a recirculation ratio (recirculated effluent: settled sewage) between 1.8 and 2.5 was found to give the best results as far as hydrogen sulphite control and BOD removal is concerned. During critical periods when increased loads and anaerobic conditions tend to dominate in these ponds, supplementary oxygenation with surface aerators may be necessary [113].

Disadvantages of oxidation ponds are the lack of control of algal biomass production (mainly due to predation of zooplankton) and the difficulty of algal biomass recovery as algae are, in the first place, used for their oxygenating capacity. An advantage of oxidation ponds, as an alternative for conventional wastewater treatment, is the relatively low cost of operation [115].

Algal communities play a number of roles in the biological processes involved in stabilization ponds: (1) they provide conditions to reduce BOD and COD, inorganic nutrients (N and P) and pathogens (coliform bacteria) [116, 117], and (2) at certain densities, they keep the aerobic phase of facultative ponds functioning. The capability of microalgae in reducing high wastewater organic loads has been investigated in tropical and temperate countries[118-122].

Species diversity, seasonal variations and plankton succession in wastewater stabilization ponds have been associated with organic load, day length and zooplankton grazing intensity [118, 123, 124]. Microalgae density and composition are useful indicators of pond status and effluent quality [118, 125-127]. A stabilization pond malfunction may occur if algal density is insufficient to treat water, which in turn may favour the predominance of the anaerobic phase and, consequently, lead to a reduction in the removal efficiency of organic matter from the system [117].

6.2 High-rate Algal Ponds

HRAP offer a great potential for wastewater treatment using algae. The mechanism of work of HRAP [128] involves feeding wastewater after primary or secondary treatment in a race track reactor of 0.3 to 0.4 m where algae and bacteria are cultured. Algae are continuously mixed to keep the cells in suspension and expose them periodically to light. Algae and bacteria remove organic matter by a “mutual relationship”: algae provide the dissolved oxygen required for bacterial decomposition of organic matter and bacteria provide carbon, nitrogen, and phosphorus essential for algal growth by degrading wastewater components. Algal biomass is then harvested.

In HRAPs, algae remove the nutrients directly through uptake and harvesting of the biomass. Nitrogen and phosphorus are removed indirectly by ammonia-nitrogen volatilization and orthophosphate precipitation, respectively. Directly and indirectly, the growth rate of algae controls the efficiency of nitrogen and phosphorus removal. The efficiency of nutrient removal in a HRAP is determined by cellular retention time, solar radiation, and temperature. These ponds have been successfully used for the treatment of anaerobic effluents from pig waste [55].

6.3 Conventional Ponds

Conventional ponds (Fig. 2) often stratify and can be divided into 3 zones: aerobic, facultative and anaerobic [106, 128, 129]. The anaerobic zone is at the bottom of the pond, where settled wastewater solids are degraded anaerobically. The aerobic zone is at the surface of the pond where algae grow. Conventional ponds are usually dominated by motile algae species such as *Euglena* sp. and *Chlamydomonas* sp. [130]. Algal photosynthesis and some diffusion from the atmosphere provide oxygen (O_2) for aerobic breakdown of organic matter to water (H_2O), carbon dioxide (CO_2) and nutrients; ammoniacal-nitrogen (NH_3-N) and phosphate (PO_4-P). Between these two zones is the facultative zone which is inhabited by microorganisms which can live in both anaerobic and aerobic conditions [129].

The depths of these 3 zones within a conventional pond vary considerably depending on factors such as local climate and season. Algal photosynthesis often leads to elevated daytime DO (dissolved oxygen) and pH levels in the surface layers of conventional ponds [106, 132]. Under such conditions some nutrients like NH_4-N and phosphates are removed by volatilisation and precipitation respectively, in addition, solar UV radiation augmented by the elevated DO and pH levels reduces pathogen levels [132-135]. The algae biomass production in conventional ponds is low and can change considerably with season, retention time, loading rate and algal species.

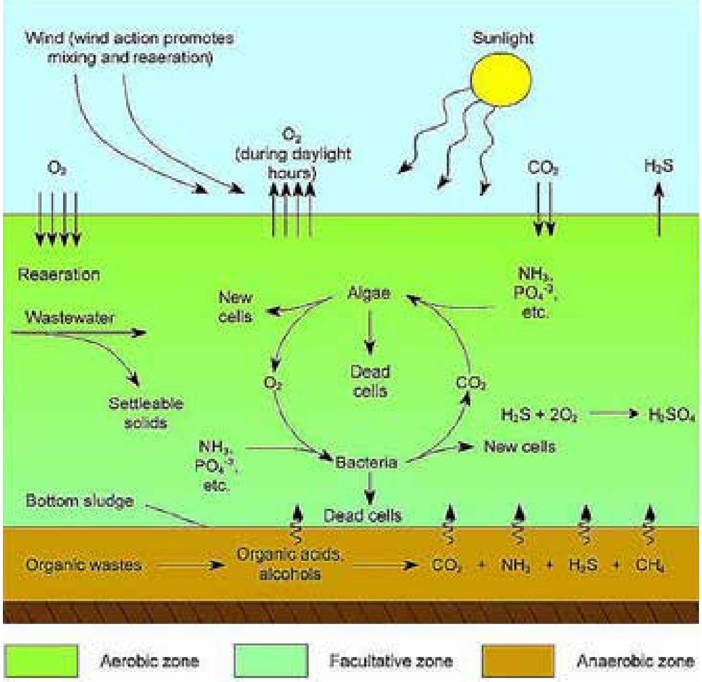

Figure 2. Schematic representation of aerobic - anoxic - anaerobic processes in facultative ponds.

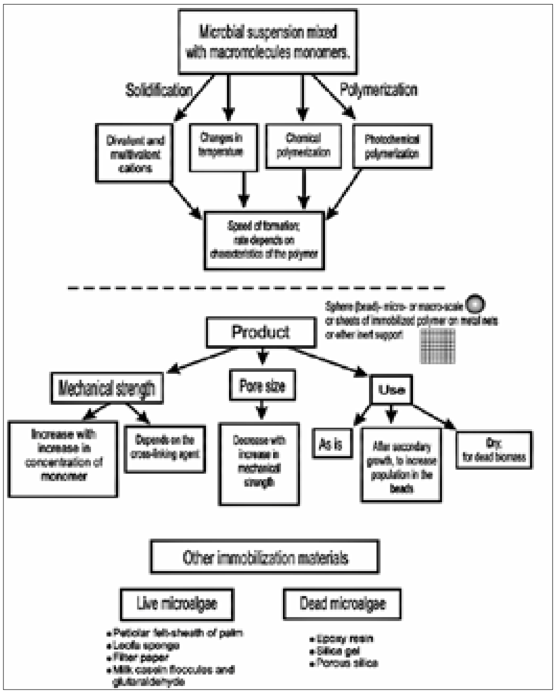

Figure 3. Flow chart for procedures for immobilization of microalgae in polymers.

7 Immobilization of Microalgae

An immobilized cell is defined as a living cell that, by natural or artificial means, is prevented from moving independently from its original location to all parts of an aqueous phase of a system [99]. Immobilized cells can increase the efficiency of microalgae-based wastewater treatment because of higher cell density. Microalgae can be entrapped in gel beads (Fig. 3) to remove nitrogen and phosphorus from wastewater. Studies have consistently indicated efficient and rapid removal of nitrogen and phosphorus from wastewater by immobilized algae. Carrageenan, chitosan and alginate are the polymers often used in these algal systems [104, 137-139], with alginate beads being used most frequently [13, 140, 141].

8 Algae Biomass to Biofuel

The integration of biofuels production and wastewater treatment with CO_2 supplementation, was first suggested by Oswald et al. [143]. Although the production of biodiesel from microalgae is technically possible, it still has a long way before it can be economically competitive with petroleum derived fuel [144]. The paramount economic hurdle in production of microalgal biodiesel is the biomass productivity [145].

Microalgae-based biofuel production in conjunction with wastewater treatment is the area with the most plausible commercial application [146]. It became evident that microalgae can assimilate nutrient and remove inorganic and organic contaminants from wastewater while producing biomass for biofuel production [35, 147].

Algae grown on wastewater media represent a potential source of low-cost lipids for production of liquid biofuel. Considerable attention has been given to biomass production stage, as it is the bottleneck in economic production of microalgal-derived biodiesel [145]. One of the ways to reduce biomass production costs and at the same time obtain environmental benefit is to couple emissions control and wastewater management with biomass cultivation [148].

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7 Carbon Fixation Potential

Are Algae an Opportunity to Mitigate GHG Emissions?

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1 Introduction

Since the industrial revolution our society has relied on petroleum-based products for fuels, materials, and specialty chemicals. For the last hundred years, the use of petroleum has been possible due to its relative low price and a balance between supply and demand. However, the increased consumption of energy has created an environment with high and variable prices and where demands could begin to exceed supplies [1]. An important aspect that became probably even more serious lately is the environmental impacts of petroleum consumption in terms of global warming due to the greenhouse gas (GHG) emissions. In terms of carbon, the fossil fuels consumed in one year release 44 × 1,018 g of carbon, and this is 400-fold the amount of annual carbon fixed during net primary productivity by the global biota [2], this is a massive influx of carbon into the atmosphere mediated through the burning and consumption of petroleum-based products. Fundamental and applied research now-a-days focus on feasibility and utility of the renewable energy sources that use direct phototrophic CO_2-fixation like microalgal biomass [3].

2 Mechanisms of CO_2 Sequestration by Microalgae

Microalgae, like every other photosynthetic organism, absorb carbon dioxide, a GHG, and combined with hydrogen, nutrients and sun energy form glucose and release oxygen. Microalgae are also at the base of the food chain, therefore, the carbon intake entering the food chain becomes suitable for other organisms. The sequestration of CO_2 operated by this system is compelling in terms of the potential for mitigating GHG emissions. The total volume of CO_2 intake by microalgae is the most promising since phytoplankton is the primary actor in the atmospheric carbon cycle; today microalgae produce over half of the oxygen we breathe.

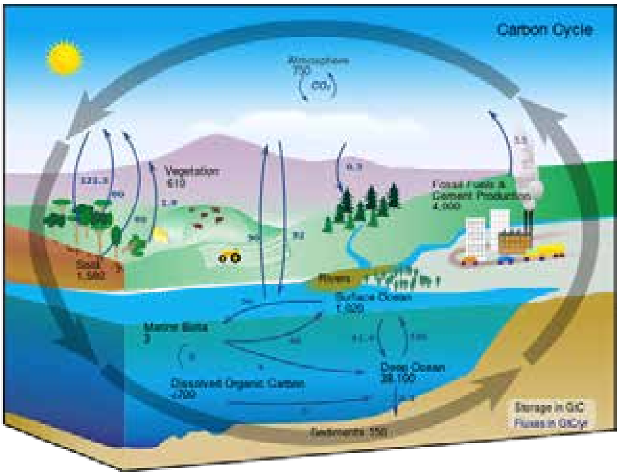

Figure 1. Carbon cycle. Source NOAA.

Phytoplankton have evolved carbon (CO_2)-concentration mechanisms (CCMs) to maintain photosynthetic carboxylation and reduce photorespiration via ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). Recently it is speculated that marine diatoms have two possible types of CCMs, a biophysical and a biochemical mechanism [4]. A biophysical CCM is the direct transport of inorganic carbon across multiple membranes using transporters and carbonic anhydrases. The other mechanism for concentrating inorganic carbon around the plastid is the use of a C_4- (four carbon intermediate) CCM. In a biochemical C_4-CCM, relatively high affinity carboxylases fix inorganic carbon to a C_3 intermediate and form C_4 compounds that can be transported to the chloroplast. The C_4 intermediates are then decarboxylated to deliver the inorganic carbon to the relatively low-affinity RubisCO [5], and the C_3 intermediate is recycled for another round of carbon fixation. In this sense, the CCM takes advantage of the C_4 carboxylases low Km for inorganic carbon and affords the cell an additional mechanism to concentrate carbon. CO_2 fixation by photoautotrophic algal cultures has the potential to diminish the release of CO_2 into the atmosphere, helping alleviate the trend toward global warming. To realize usable biological CO_2 fixation systems, selection of optimal microalgae species is vital. The selection of optimal microalgae species depends on specific strategies employed for CO_2 sequestration. A second key point is to use microalgae in the food chain, especially with organisms that can operate carbon sequestration. Filter-feeding bivalves such as mussels and clams can use the carbon from their microalgal based diet to build their shells, storing carbon and sequestering it from the cycle.

3 Potential Algal Species

Some microalgae species, such as *Chlorella, Spirulina and Dunaliella spp.* have commercial values. It is expected that commercial profit from biomass production will offset overall operational costs for CO_2 sequestration. *Chlorella spp.* has been studied for use in CO_2 sequestration. For example, Hanagata et al. reported that *Chlorella spp.* can grow under 20% CO_2 conditions [6]. It has been used as a health food as well [7]. CO_2 tolerance of *Dunaliella spp.* also has been examined and the species has been used in the industrial production of alpha-carotene [8]. Further potential applications of microalgal products are the utilization of secondary metabolites for fertilizer and biofuel production. In addition to CO_2 sequestration, another potential strategy to offset operational costs is to develop multifunctional systems such as waste treatment and aquaculture farms [9]. Since economic feasibility is one of the major issues to realize biological mitigation systems, seeking additional value for the system is an important criterion [10].

4 High CO_2 Tolerance

Direct utilization of power plant flue gas has been considered for CO_2 sequestration systems [11]. The advantage of utilizing flue gas directly is the reduction of the cost of separating CO_2 gas. Since power plant flue gas contains a higher concentration of CO_2, identifying high CO_2 tolerant species is important. Although CO_2 concentrations vary depending on the flue gas source, 15- 20% v/v is typically assumed. Several species have been tested under CO_2 concentrations of over 15%. For example, *Chlorococcum littorale* could grow under 60% CO_2 using the stepwise adaptation technique [12]. Another high CO_2 tolerant species is *Euglena gracilis*, the best growth was observed with 5% CO_2 concentration, however, the species will grow until greater than 45% CO_2 [13]. *Chlorella sp.* UK001 could grow successfully under 10% CO_2 conditions [14], 40% CO_2 conditions and *Chlorella sp.* T-1 can even grow under 100% CO_2 though maximum growth rate occurred under a 10% concentration [6,15]. *Scenedesmus sp.* could grow under 80% CO_2 conditions but the maximum cell mass was observed in 10-20% CO_2 concentrations [6]. *Cyanidium caldarium* and some other species of *Cyanidium sp.* can grow in pure CO_2 [8, 16]. As seen the candidates for industrial capture of emitted CO_2, microalgae are vastly promising and in particular the use of marine microalgae. Many CO_2 sources, such as power plants, are located along the coastal area. A number of marine algae species have been tested for CO_2 sequestration applications such as *Tetraselmis sp.* [17, 18], *Synechococcus sp.* [19], Chlorococcum littorale [20], *Chlamydomonas sp.* [21], *Nannochloropsis salina* [18, 22], and *Phaeodactylum tricornutum* [18]. Seawater could be used directly as a growing media so that maintenance costs of microalgae culture could be reduced.

The CO_2 in the atmosphere is in equilibrium with CO_2 in the ocean. During photosynthesis, phytoplankton removes CO_2 from sea water and releases oxygen as a by-product. This allows the oceans to absorb additional CO_2 from the atmosphere. If phytoplankton populations decrease, atmospheric CO_2 would increase. Phytoplankton also affect CO_2 levels when consumed by other organisms; in the case of filter feeding bivalves using their gills to capture phytoplankton from the water, the carbon from microalgae is transformed into live biomass and shell carbonates. Carbon intake that is used for shell carbonates is not only carbon captured but also carbon sequestered. Carbon sequestration also occurs when dead phytoplankton sink to the ocean floor. In this way, the oceans act as a sink, a place to dispose of global carbon, which otherwise would accumulate in the atmosphere as CO_2. Other global sinks include land vegetation and soil, however, these sinks frequently return carbon to the atmosphere as CO_2 by burning or decomposition.

5 Conclusions

For the purpose of CO_2 sequestration, the use of microalgae is a unique technology. For example, microalgae can assimilate CO_2 within various ranges of concentration from ambient (0.04%)

to 100% v/v CO_2 by selecting adequate species. The process works under a wide range of thermal conditions, ranging from 25 to 100°C. Using microalgae for the use of CO_2 sequestration also has the potential to produce useful byproducts in a bio-refinery integrated system, which is considered an environmentally friendly technology [10]. There are a variety of technological solutions possible for microalgal-based CO_2 sequestration systems, and thus optimal microalgae employed would differ from system to system. While efforts to find the “ideal” microalgae species will continue, strategic engineering decisions and engineering modifications will be taken into great consideration to realize effective microalgal CO_2 sequestration systems. Geological sequestration faces limited storage capacity and the non-trivial scenario of an accidental high-pressure leak or rock penetration. Chemical sequestration is also problematic as it is expensive and the energy requirement to complete this process is enormous. Biological sequestration, which includes the use of photosynthetic organisms such as algae, offers the unique potential to overcome many of the hurdles faced by other sequestration methodologies and has emerged simultaneously as a significant commercial opportunity with the production of renewable algal oil and residual biomass.

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8 Towards a Breakthrough in Algae Technology

Bioreactors Engineering and Selected Technical Aspects of Efficiency/ Productivity Issues of Algae Production Systems

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Abstract Photobioreactors (PBRs) are the key devices for industrializing microalgal biotechnology. The lack of large-scale photobioreactors with low cost and high efficiency is one of the bottlenecks for the commercialization of microalgal technology, especially for microalgal energy and CO_2 fixation. Hence, the design and development of photobioreactors is very important for the maximum production of algae. This review outlines various photobioreactors and their current status, and points out their advantages and disadvantages. Additionally, Computational Fluid Dynamics (CFD) has become a powerful tool for the study of photobioreactor. And the current situation of CFD method applied in the investigation of the PBR is also commented. Furthermore, the important design aspect and essential factors affecting performance of photobioreactor such as light and mixing are analyzed in detail. Finally, the outlooks and strategies for developing photobioreactors are proposed. It is expected that advanced photobioreactor obtained by further research and development will enable the commercialization of microalgal application such as microalgal biofuels within the next decade.

Keywords Microalgae, Photobioreactor, Computational fluid dynamics, Mixture, Optimization, Scale up.

1 Introduction

Microalgae are rich in proteins, lipids, pigments, vitamins and a variety of mineral elements, thus they have important values in the yields of food, feed, environmental protection (in particular, CO_2 bio-fixation and wastewater treatment) and the energy. Especially in recent years, it is clearly recognized and believed that microalgae have tremendous application potentials in the fields of bio-energy and environmental protection, thus the studies of microalgae applications on biofuel and carbon sequestration are becoming the worldwide hot spot of research.

However, the efficient cultivation of microalgae is the firstly critical step for applications of microalgae. The devices for microalgae photoautotrophic cultivation are defined as photobioreactor. Generally, the photobioreactor could be divided into two major types: the open culture systems and the enclosed photobioreactor. The open culture systems are mainly artificial open ponds such as raceway ponds, circular ponds and other any open containers for microalgae photoautotrophic cultivation (such as pots, slots and tanks, etc.). Broadly speaking, the open culture systems also include natural waters such as lakes and lagoons. Open ponds are the earliest systems for microalgal mass cultivation. After World War II, people began to use the artificial raceway ponds and circular ponds for mass culturing microalgae. The most typical open pond is the raceway pond proposed first by Oswald. And raceway pond is also the most widely used culture system in the commercial large-scale cultivation of microalgae. Due to the disadvantages of large power consumption and poor mixing near the centre of pond, circular ponds are applied less in mass cultivation of microalgae outdoor, and most are distributed only in Asia, such as Japan, Taiwan and India.

Because microalgal culture in open ponds is uncovered for air, it is difficult to control effectively the culture conditions which are appropriate for microalgae growth. Furthermore, the mixing is relatively poor in the ponds. These factors would result in low microalgal cell density and low productivity. In addition, the open ponds have high risk of microalgal culture contamination with protozoan, miscellaneous algae and bacteria, often causing culture failure or crashes. The significant drawbacks of the open culture systems have promoted the development of closed photobioreactors. The closed photobioreactor mainly have three types: the tubular reactor, the flat plate reactor and the vertical column reactor. The tubular photobioreactors have been proposed and developed, began from the pioneering works of Tamiya and Pirt. There are many various forms of tubular photobioreactors at present, however they have the similar main structures, which consist of the transparent pipes made of glass or plastic materials, gas exchange vessels and recirculation systems of the culture with the pump or airlift device. The prototype of the flat-plate photobioreactor was first proposed by Milner, whose work laid the foundation for the development and application of the flat plate reactor. Subsequently, the flat plate reactor with fluorescents was developed by Samson and Leduy in 1985. Roughly a year later, Ramos and Roux firstly employed transparent PVC material to develop the plate reactor for microalgae culture outdoor. After then, abundant forms of flat-pate reactors have been proposed and a lot of research work related to the flat plate reactor were emerged gradually. The third major type of enclosed photobioreactor is vertical column photobioreactor which has two forms including bubble column reactor and airlift reactor. Due to the simple structure, easy operation and low cost, the vertical column reactor have been widely used in chemical industry, biological chemical process and wastewater treatment for a long time. In recent years, many closed photobioreactors with different structures and forms are proposed and designed which bring a trend of diverse and innovative development for closed photobioreactor.

With the ceaseless development of microalgal biotechnology, considerable progress for photobioreactors has been achieved. Especially in recent years, the rapid development of the microalgae technology for bio-energy and carbon sequestration is bringing urgent demand for the large-scale photobioreactors with low cost and high efficiency.

2 The Status of Photobioreactors

2.1 The Status of Open Ponds

Open Raceway Ponds

The raceway pond is the most typical and widely used culture systems, and also is one of the oldest systems for microalgae mass culture. The raceway pond is usually a ring structure with two or more circular flow channels, and the depth of microalgal culture is usual 15~30 cm. The raceway pond is generally driven by paddle-wheels to promote the culture mixed (Fig. 1-a). At present, to culture microalgae on a large scale usually uses raceway ponds systems. For example, raceway ponds are used for production of Spirulina in a culture base with a total area reached 40,000 m^2 in California, United State. Furthermore, the main structure and form of raceway pond proposed in the 1960s is almost unchanged. Since the past 40 years, abundant researches on photoautotrophic cultivation of microalgae with raceway ponds have been reported. However, the studies on structure design, transformation, optimization and up-scale of raceway ponds are rare, and had been reported only many years ago. From 1980 to 1996, the U.S. Department of Energy conducted a research effort within a biofuels program, known as the Aquatic Species Program (ASP). The ASP research focused on using open pond raceway systems to culture microalgae to produce biodiesel. In this period, Law et al designed a “foil array” in the pond, which would generate a vortex that would create organized mixing in the ponds, expected to result in exposure of the cells to regular dark-light cycles. The

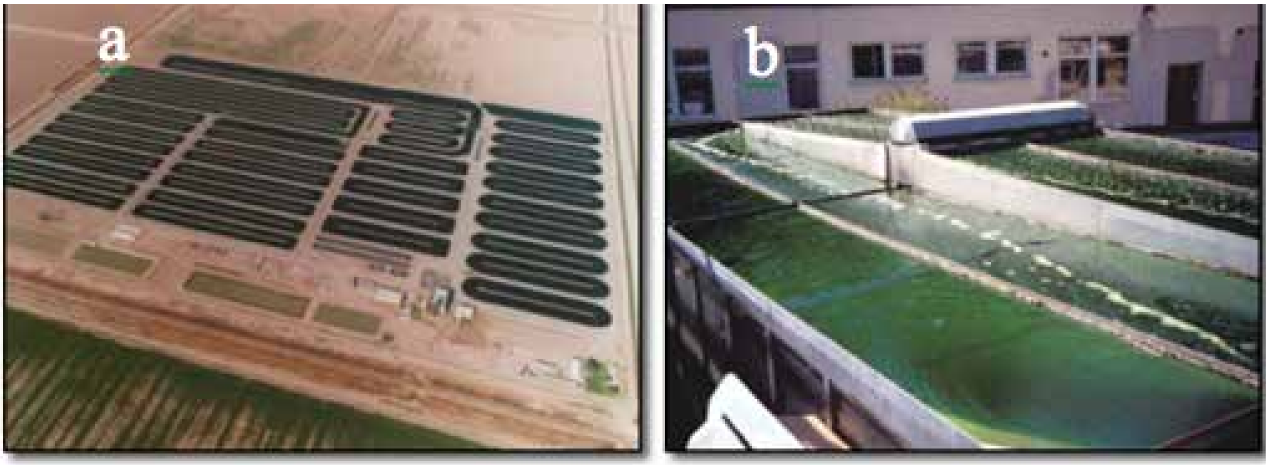

Figure 1. Open raceway ponds..

Table 1. DO distribution of the raceway pond at different point (NOs of sites correspond to Fig. 2-a).

Position	8	7	6	5	4	3	2	1
Dissolved oxygen Value (mg/L)	16.42	16.65	17.01	17.22	17.30	17.80	18.48	18.84

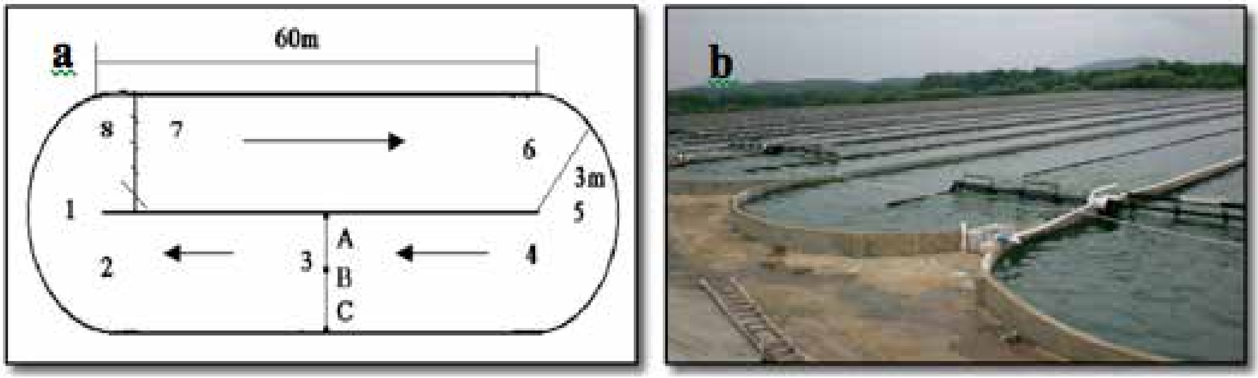

Figure 2. the DO measurement position and the photograph of the open raceways pond.

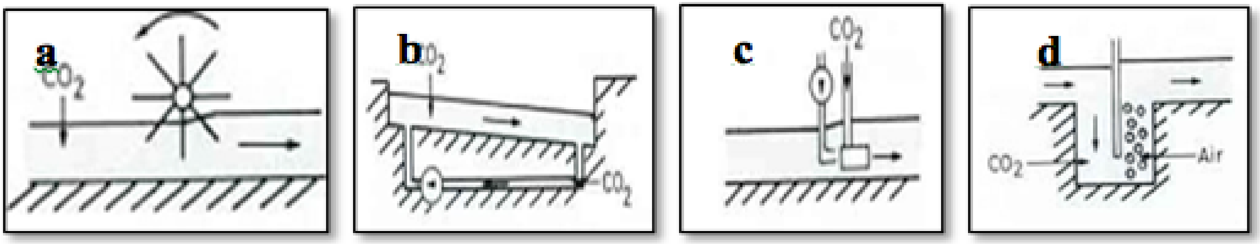

Figure 3. The approach of fluid hybrid in raceway ponds.

statistics of the average growth rate of microalgae showed the foils may bring a certain effect to promote the growth of microalgae.

The open raceway ponds are usually held horizontally and directly built on the flat ground, and however some ponds are inclined to be the inclined ponds (Fig. 1-b). In inclined raceway ponds, the culture suspension flows down along the sloping plane, and then would be pumped to the top of the slope, and continued such cycle. A higher cell density and productivity can be obtained in the inclined raceway pond. Setlik et al reported the cell density and the areal productivity of Chlorella reached respectively 10 g/L and 25 g m^{-2} d^{-1} in the photoautotrophic culture using the inclined raceway pond.

The mixing of microalgal culture in raceway ponds are driven by paddle wheels for almost raceway pond systems (Fig. 2-b and Fig. 3-a). Our studied showed that dissolved oxygen concentration was high at points which are far away the paddle wheel along the direction of fluid flow (Table 1). Specifically, mixing and gas-liquid mass transfer are better near the paddle wheel than that in the other places.

In addition, there are some other mixing methods. In inclined raceway ponds, microalgae fluids are generally transported from the low of the pond to the top by the pump and then flow to the bottom of pond through the gravity (Fig. 3-b). Another mixing way is using the injecting gas to promote the fluid flow. The gas mixture of air and CO_2 are injected horizontally into the pond to create mixing (Fig. 3-c), which can enhance the fluid turbulence, and can also promote the gas liquid mass transfer and CO_2 absorption for the microalgae culture. Additionally, the air-lift devices which can make the culture fluid to flow are installed for some ponds (Fig. 3-d). Usually, the devices can combine with the absorption of CO_2 by changed injected air into a gas mixture of air and CO_2. This gas lift pumps have both functions of the creation of mixing and the absorption of CO_2, thus generally have high CO_2 absorption efficiency.

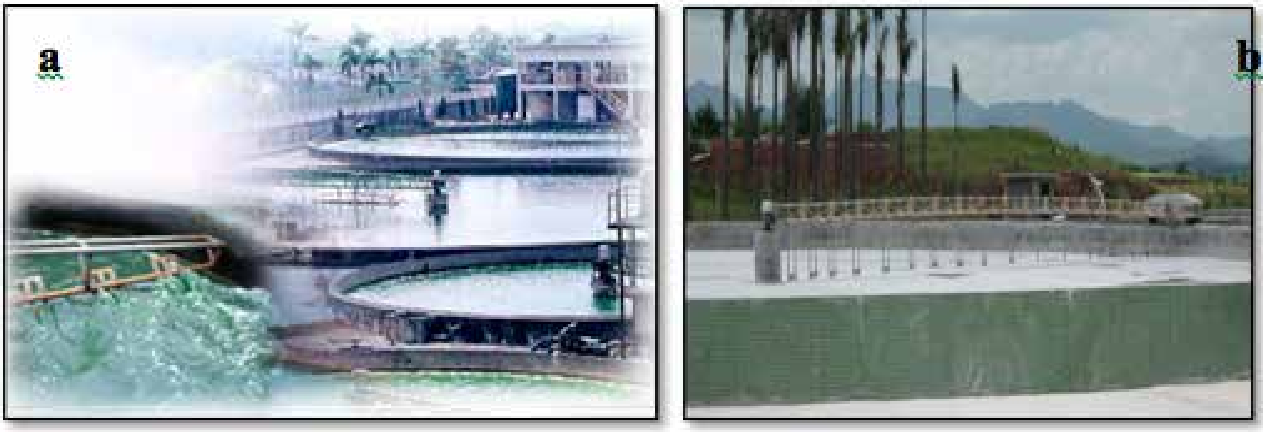

Figure 4. Mixing systems in circular ponds.

The raceway pond with gas lift pumps could also reduce the energy consumption. Ketheesan et al reported an airlift raceway pond, which is a semi-circular structure with the width of 14.5 cm and formed by the annular flow path, two vertical cylindrical air-lift devices installed in the middle of the reactor. Under the same conditions of the general flow rate of 14 cm s^{-1}, there is lower energy consumption and 80% of energy saved in the airlift raceway pond compared to that in the traditional raceway pond with the paddle wheel.

Open Circular Ponds

There is usually a rotary arm agitator to mix microalgal culture in the circular ponds. However, to scale up circular pond is generally difficult. The diameter of circular pond is larger, the mixing of pond is poorer, especially in the centre of pond. Moreover, the energy consumption increases with the increase of the diameter of pond. As reported, the largest diameter of the round pond is only 50 m.

At present, the circular pond is applied on outdoor large-scale culture commonly using single-arm rotating agitator whose form is usually like scraper at the top (Fig. 4-a) or bottom (Fig. 4-b) of the pond. The rotations of agitator rely on the trolley device moved around the edge of the pond. Compared to conventional rotation method for agitator in the fermenters, this approach can reduce efficiently the energy consumption.

Open ponds have a great deal of advantages, such as, easy to construct and operate, simply to scale up, low costs for construction and operation. These advantages make open ponds suitable for mass culture of microalgae with low cost, such as the culture of oilgae. However, the drawbacks of open pond are also very evident, and are mainly as follows:

Low culture efficiency. In open ponds, the culture of microalgae needs a long time, and the concentration of cell is usually low which is approximately 0.5~1.0 g/L. Meanwhile, the low cell density raises the difficulty for cell harvest, thus increase the cost;

Difficult to control culture conditions. Microalgae culture in open ponds utilizes mainly sunlight, and therefore it is fairly depend on environmental factors such as weather and climate;

Easy to be contaminated. The process of microalgae cultivation is vulnerable to the pollution of miscellaneous algae and protozoa as well as other organisms, which seriously affects the productivity of microalgae culture, or even cause complete failure;

Large amount of material are exchanged with the external environment. Rain will enter the culture medium to dilute the medium. The huge amount of evaporation for open pond at summer will increase the ion concentration in the medium, leading to the culture to be unstable;

To need a large area of land. Microalgal cells need adequate light to grow. Therefor the depth of the open pond is usual 15~30 cm, thus the large-scale cultivation of microalgae require a large area of land;

Applicable species are limited. Nowadays, microalgae which mass culture in open ponds are more demanding on growth conditions compared to other microalgae (such as Spirulina is resistant to high pH, Dunaliella is tolerance to high salinity, et al.). Therefore, most of microal-

Table 2. The status of tubular photobioreactors.

Placement	**Volume/diameter**	**Characteristics of reactors**	**Algal species**	**Yields**	**Authors and Reported time**
Horizontal	7 m^3/6 cm	Floating on or submerged in water to control the temperature	Outdoor/Porphyridium cruentum	20~25 g m^{-2} d^{-1}	Chaumount, 1993
Incline	300 L/3.2 cm	Be similar to the α and tilt	Outdoor/Chlorella pyrenoidosa	72 g m^{-2} d^{-1}	Lee et al., 1995
Horizontal	200 L/5.3 cm	Snake-like tube with degassing	Outdoor/Phaeodactylum tricornutum	1.90 g L^{-1} d^{-1}	Molina et al., 2001
Incline	6 L/3.8 cm	Slop to the ground with an angle and with the static mixer	Outdoor/Chlorella sorokiniana	0.43~1.47 g L^{-1} d^{-1}	Ugwu et al, 2002
Horizontal	75 L/3 cm	Spiral structure	Outdoor/Phaeodactylum tricornutum	1.4 g L^{-1} d^{-1}	Hall et al., 2003
Horizontal	11 L/1 cm	Wave structure, matrix-type arrangement	Outdoor/Spirulina	2.7ffl0.2 g $L^{-1}d^{-1}$	Carlozzi, 2003

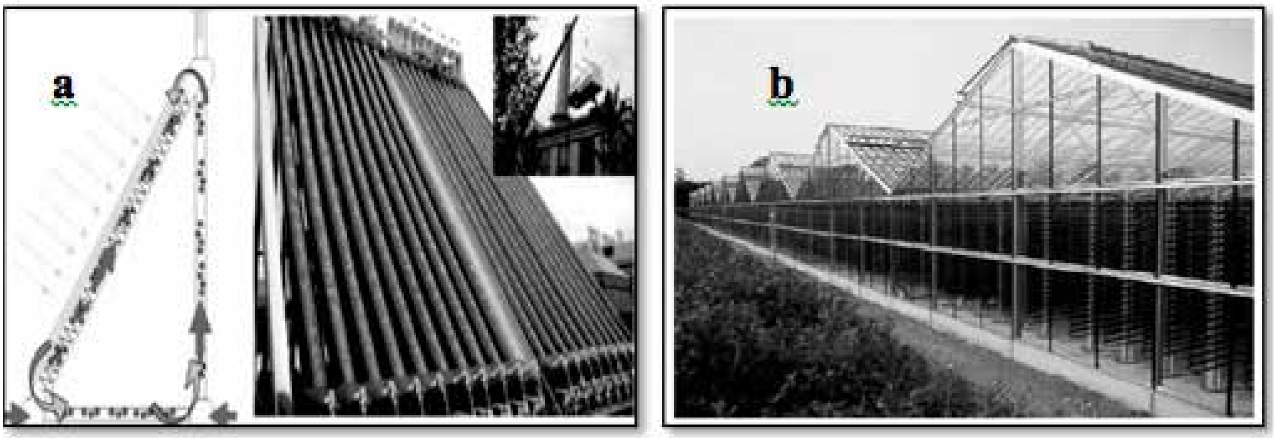

Figure 5. Tubular photobioreactors.

gae species, which need the moderate growth conditions, could not culture in open ponds.

Due to a number of major drawbacks of open raceway ponds, some researchers proposed an integrated culture system which is coupled the raceway pond with the flat-plate photobioreactor. The integrated culture system can control better temperature and improve the light availability of microalgae. Although this way can improve the cultivation efficiency of the raceway pond to some extent, it has not yet been effectively applied in the mass outdoor cultivation of microalgae.

Overall, the open pond is low-cost and easy to realize the mass culture of microalgae, but the low culture efficiency and limited microalgae species make people to focus their attentions on the closed photobioreactor.

2.2 The Status of the Closed Photobioreactor

Tubular Photobioreactors

Tubular photobioreactors are generally made of transparent glass or plastic material, relying on the pump or air-lift system to make the microalgae culture mixing and circulating in the reactor. Tubular photobioreactors are the most one widely used for mass culture in all the closed photobioreactors. They are mainly employed for production of high value substances in microalgae, such as Haematococcus pluvialis. Tubular photobioreactors have many forms, such as: parallel with the ground, placed perpendicular to the ground, tilted a certain angle with the ground, helical coiled and conical.

Furthermore, the green fuel technology company in U.S. has developed a triangle tubular photobioreactor (Fig. 5-a). The gas is injected respectively into two positions which are place at the lower vertices of triangle. The swirls or vortexes are produced as gas liquid counter-current flow in the inclined tubular of the reactor which can enhance the utilization efficiency of light for algae cells. Generally, scaling up a tubular photobioreactor is usually achieved by increasing the length of the tube. With increasing the tube diameter, the light zone in the reactor is narrowed. Therefore, the growth of algal cells would be limited since they are unable to obtain sufficient light for photosynthesis. It was reported the diameter of the tubular photobioreactor is best controlled in 10 cm. For example, it was confirmed that horizontal tubular photobioreactors, which were tightly arranged vertically were built in Wolfsburg, Germany (Fig. 5-b). The reactors were consisted of the diameter of 4 cm glass tubes with the length of 500,000 m and the total volume of 700 m^3. These reactors had a capability to produce 130-150 tons Chlorella annually.

The tubular photobioreactor has generally a great specific surface area. Therefore, the application of tubular photobioreactor for the microalgae culture can achieve a higher cell density and productivity (Table 2). However, there are still many disadvantages. In particular, engineering problems emerge when tubular photobioreactors are applied on large-scale production. The drawbacks of tubular reactor are embodied in the following aspects:

Poor mixing capabilities, studies have shown that the fluid within the tubular photobioreactor was basically in a laminar state, thus the overall mixing of the reactor was poor. At present, static mixer elements are usually used to improve the mixing in the tubular photobioreactors. Unfortunately, the static mixer could significantly increase the flow resistance along the tube, and it needs more energy to overcome this additional resistance from the mixers;

Dissolved oxygen accumulation, due to the poor mixing in the reactor, the gas liquid mass transfer coefficient is low, resulting in low oxygen release which have negative effect on microalgae growth and product;

Difficult to control temperature and pH, because of a large specific surface area, it is more easily to accumulate heat, resulting in difficulties on temperature control. In addition, pH and CO_2 in the tube will appear gradient distribution against microalgae growth if the tube is very long;

Difficult to clean, algal cells are easy to attach to the inner wall of the tubular reactor;

The costs of manufacturing, operation and maintenance are expensive.

Due to the above problems for practical applications of the tubular reactor, some plants using tubular reactors to culture microalgae are running hard, even close down. Therefore, the development of the tubular photobioreactor slows down in recent years, and is reported rarely.

Vertical Column Photobioreactors

Generally, vertical column photobioreactors can be divided into three forms, which are bubble column, draft tube airlift and spilt cylinder respectively. Vertical column photobioreactors have relative large diameter compared with tubular photobioreactors, and was made of glass or transparent materials.

Vertical column reactors have been used widely in bio-chemical, chemical and other fields for long time. Abundant studies related to mixing of column have been reported over the past years. Compared to the tubular photobioreactors, vertical column photobioreactors are mainly employed in the laboratories, and are applied rarely outdoor.

The optimization of the column photobioreactor is mainly carried out by installing or adjusting the internal structures and change the configuration. It is usual that to change the bubble column into a variety of air-lift reactors. Kaewpintong et al compared the performances of bubble column photobioreactor and draft tube airlift reactor of which volume are both 3.6r for culture of Haematococcus pluvialis. The results showed the highest cell density of 79.5 x 104 cells mL^{-1} was obtained in the draft tube airlift reactor, and was almost 2 fold than that in the bubble column.

Additionally, helical flow promoters were added in the downcomer of draft tube air-lift photo-

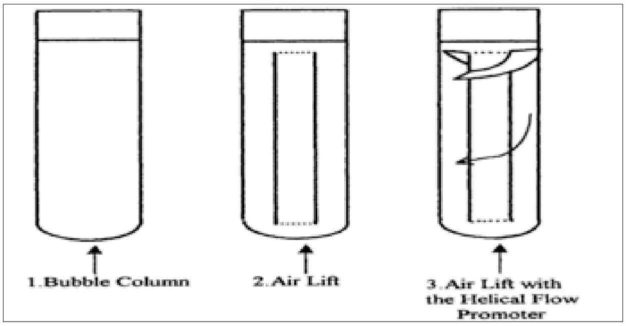

Figure 6. Vertical column photobioreactors.

Figure 7. the stand-up pouch or the punching bag systems of photobioreactors.

bioreactor by Merchuk et al. The flow promoter created the regular and orderly secondary circulation flow in the downcomer which could increase the mixing along the direction of the light attenuation. Meanwhile, it needed lower air flow to achieve sufficient mixing in the reactor with promoters, thus it can significantly reduce the energy consumption (Fig. 6).

As shown above, the vertical column photobioreactors are small and difficult to scale up. The amplification along the radial direction will increase the light path, so that algae cannot obtain adequate light to growth. And high demands for reactor materials and equipment are necessary for amplifying the column reactor along the vertical direction. Therefore scaling up the vertical column photobioreactor is often achieved by increasing the number of reactors, but the total scale of column reactors can still not reach the scales of the open raceway pond and the tubular photobioreactor.

At present, the vertical column photobioreactors for mass culture are the bag system proposed by Baynes for the commercial application (see Fig. 7-a and -b). Because the bag systems are built at low costan are easy to operate. Compare to the open culture system, the culture efficiency of the bag system has improved greatly. So far, the bags are applied in the culture of microalgae seed and the large-scale production of algae as aquaculture feeds. For example, the Sea salter Shellfish Company in United Kingdom built a relatively cheap culture system of bags for cultivation of microalgae for aquaculture using unit volume of 500 L vertical polyethylene plastic bags which supported by metal frame.

Flat-plate Photobioreactors

Compared to other types of photobioreactors, flat-plate photobioreactors have a large specific surface area to maintain short light paths, and they are relative easy to scale up and just need a little area of land. Therefor more attentions have been shifted to the flat plate photobioreactor in recent years. The flat-plate photobioreactor is usually laid up vertically, and it can also be tilted at an angle with the ground for receiving sunlight preferably. Hu et al. have studied on tilt angles of flat-panel photobioreactor for culture of Spirulina outdoor (southern Israel) under a variety of seasonal conditions. The results show that the best angle is 10~30° in summer and 60° in winter. Additionally, Degen et al reported a novel airlift photobioreactor with inner baffles. The fluid downward channel is divided into six connected parts by five horizontal baffles. Five baffles are alternately fixed on the front and rear panels of the reactor. Because of the specific arranges of the inner baffles, the microalgae culture suspension is circulated in every relative independent part of the reactor which increases the frequency of light and dark cycle of the algal cells. Li et al. invented a novel flat-panel photobioreactor with multistage separators. There are gaps between each separator that can make the fluid flow through the intervals. And artificial lights can be laid within the hollow between the separators. Due to the strong mixing of the micoalgal culture along the direction of light irradiance, the reactor improves significantly the light utilization efficiency of microalgae. And it has an advantage of avoiding the direct contact of lamps with media. At present, the photobioreactor is scaled up to 1 m^3 for the cultivation of algae (Fig. 8-a). Tredici et al designed a flat-plate

Figure 8. Flat-plate photobioreactors.

photobioreactor, known as the Green Wall Panel, is made of transparent plastic and supported by wires. (Fig. 8-b). Such design reduces significantly the manufacture cost of the photobioreactor, and is suitable for the mass culture with low cost. Subitec Company (Germany) developed a new type of flat panel airlift photob ioreactor. The volume of each unit is 180 L. Two synthetic film shells, structured by deep drawing, are welded together. Gas spargers lead to an upward movement of fluid in several parallel chambers, while baffles included in the panel wall induce defined vortices and improve light penetration of the fluid phase. Solix Biofuels Company (USA) has proposed a series of water-embedded plate photobioreactors systems which consist of film bags. External water cannot only support the bag reactor, but also played the role of regulating the temperature and the light intensity.

Using the flat-plate photobioreactors to culture microalgae can get a high cell density and high cell yield. Furthermore, the reactors are easy to scale up in length, and are convenient to operate and clean. Therefore, the flat plate photobioreactor are considered to be the most suitable one of closed culture systems for mass culture of microalgae, which could proved by the a great number of studied on flat plate photobioreactors in recent years.

People are trying to reduce costs of manufacturing and maintenance as much as possible by the exploration of the reactor materials and external support facilities, in order to make the closed reactor suitable for low-cost microalgae cultivation. The other approaches are the adjustments of reactor external shape and adjunctions of the internal constructional elements, which would make vortices in the reactor to increase the light-dark cycle frequency of algal cells, thereby increase the growth rate of the algae.

Figure 9. Dome shaped photobioreactors.

In addition to the above three kinds of relatively common closed photobioreactors, some photobioreactors with relatively special shapes or structures appear. Sato et al designed a dome shaped photobioreactor. The volume of the reactor with a hemisphere shape is 130 L, in which six gas inlets are evenly distributed along the bottom of the circumference. Meanwhile, there is a small device known as the “Train” which is moved around the bottom of the reactor by the recoil effect of the gas, thereby enhancing the overall mixing in the reactor. The reactors have been applied in mass culturing Haematococcus pluvialis outdoors (Fig. 9).

Besides, Hsieh et al reported a special trough photobioreactor. The reactor has a length of 43 cm, a width of 30.5 cm and a high of 24 cm, in which an open rectangular groove is set up for the entrance of light. The rectangular groove increases the light-receiving area of the reactor. The researchers investigated a variety of different size grooves, and ultimately obtained the optimal structure. For the culture of Chlorella using the reactor, the highest cell concentration and the yield can reach 3.75g L^{-1} and 0.34g L^{-1} d^{-1}, respectively. This reactor can increase the specific surface area of illumination, and is still being studied for further optimization and amplification in the future.

3 Computational Fluid Dynamics Applied in Photobioreactors

The fluid dynamics within the photobioreactor has already been studied, but the hydrodynamics in bioreactor could not completely be known due to the limitation of the measurement means. The development of computational fluid dynamics makes the detailed understanding of internal mixing conditions and hydrodynamics in the photobioreactor to be possible. CFD is an advance subject based on the basis of hydrodynamic theory and numerical calculation. CFD can simulate the mixing within the reactor to obtain the local mixing information and overall mixing parameters, which have been widely used to direct the design, the optimization and the scale up of the bioreactors and the chemical reactors. Compared to the traditional method of design and optimization with the empirical and tedious experiments, CFD method can save a lot of manual labour, material and financial capabilities as well as precious time.

In recent years, CFD has gradually become a strong tool for the research of photobioreactor. Perner et al used CFD method to optimize the structure of the flat-plate photobioreactor. The analysis of the influence from internal structure to fluid pressure loss and the dead zone based on CFD calculated data of is basically same with actual measurement results. Pruvost et al. used CFD to investigate the hydrodynamic in a torus photobioreactor with impeller, and the results were validated by the technology of particle image velocimetry. Sato et al. used CFD method to select better shapes of the chamber of photobioreactor from four different shapes of dome, parabola, pipe and diamond by studying the global mixing and the light reception capacity. Perner et al. used the particle tracking model in the CFD combined with signal analysis to calculate the light-dark cycle frequency of microalgae in the tubular photobioreactor with and without the static mixer.

Sato et al. combined the growth model of microalgae with light dark cycles of microalgae obtained by the CFD to simulate the algal cells growth in a virtual tube photobioreactor, but the results have not been practically tested by microalgae culture experiments. Wu et al. calculated the light history of algal cells in a series of spiral tube photobioreactors, indicating that the swirl motions formed the spiral tubular photobioreactor makes the algae cells to generate the rapid cycle between light and dark which is benefit for microalgae growth. Using CFD technology, Su et al. studied the mixing of flat plate photobioreactor with destabilizing structures, and optimized the structural parameters of reactor. Li et al. investigated flow characteristics in a wave-baffled panel photobioreactor using CFD, showed the CFD calculation and measured values are almost the same. Moberg et al. calculated the trajectories and the light-dark cycle frequencies of algal cells under the different fluid rates in different diameter tubular photobioreactors using CFD technology, the results showed that the cycle frequencies increase with the decrease of the reactor diameter and the increases of flow rate. Luo et al. measured flow field data to validate the calculation model of CFD. Further, the trajectories of the cell particles in the reactor were calculated and finally were compared with the actual tracking trajectories of particle.

However, there are following problems in the present literatures on CFD applied in the photobioreactor: 1) Most of the studies focused on closed photobioreactors, but less focused on open ponds. There are few research on open ponds with CFD on internet; 2) It relates only with the optimization of photobioreactor but not the scale up; 3) The results obtained from CFD almost have not been inspected realistically by the microalgae culture experiments. Thus it lack the analysis of the relationship among the structural parameters of reactors, the parameters of fluid dynamics, the light regime parameters of algal cells between the microalgae culture efficiency.

In allusion to above issues, our laboratory have carried out the study of photobioreactor based on CFD since 2004. The main work is the optimization and scale up of the flat-plate photobioreactor. Not only the results obtained by CFD calculation were validated by microalgae culture experiments, but also the photobioreactors are scaled up using CFD method.

Therefore, the CFD technology can get more information on the hydrodynamics and mixing in the photobioreactor, giving an important reference for the design and amplification of photobioreactor. According to the recent progress of photobioreactor, CFD has become an indispensable tool for the research of photobioreactors. Furthermore, a method of the design and optimization of photobioreactor is expected to be established based on the association of the CFD technology with the experiment of microalgae cultivation, and will lay the foundation for the rapid development of the photobioreactor, and can be used to develop the large-scale photobioreactors with low cost and high performance.

4 Analysis of Key Factors for PBR Design

Only if the excellent PBRs could have a high productivity of microalgae, low cost and the characteristic to scale up easily, the large scale microalgae cultivation with low cost and high productivity would be achieved. Light and mixing are the key factors to affect the performances of PBRs. The in-deep analysis for these two factors is as follows.

4.1 Light

The photoautotrophic growth of microalgae relays on photosynthesis. Any factors which are relative to photosynthesis influence the microalgae growth. The availability and intensity of light in PBRs are the major factors controlling the biomass productivity of microalgae culture.

Generally, the specific growth rate of microalgae increases as the light intensity raise when it below the saturation light intensity. If the light intensity continue to enhance and exceed the light inhibition point, the microalgae growth rate decrease sharply which called photo-inhibition phenomenon. Thus, the maximum growth rate could only be obtained when the microalgal cells reach the condition of saturation light intensity which should be homogeneously distributed in the entire PBR. However, this is impossible in practical cultivation systems, because the light attenuation is occurred in the microalgae cultures. The local light intensity of PBR decreases exponentially at the same cell concentration condition when the light path length is raised. And at the some light path length, the light intensity decrease gradually when the microalgal cell densities are enhanced (Fig. 10). Therefore, the light intensity in the PBR changes spatially and temporally. Obviously, this changing is nonlinearity.

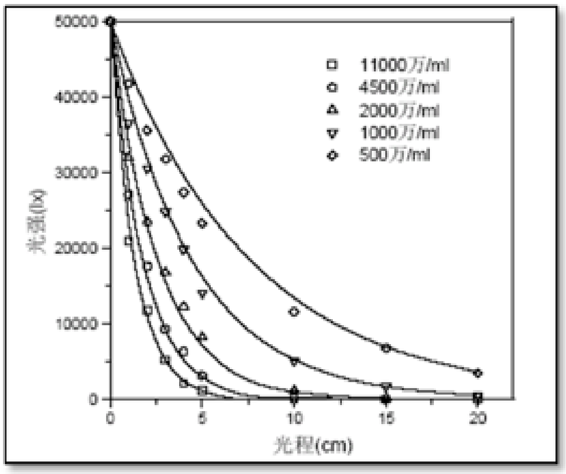

Figure 10. The light attenuation of microalgae at condition of different cell densities.

The phenomenon of light intensity changed constantly in PBR would affect markedly the growth rate of microalgae. The microalgae can achieve high growth rate at the initial stage of cultivation, because at this time the light is relatively well-distributed and sufficient for low cell concentration. With the extension of culture time, the concentration of microalgae increase gradually, and the gradient distribution of light is occurred, and cause that the microalgae in central zone of photobioreactor cannot acquire enough light and the growth rate decrease. The photobioreactor is divided into obvious light zone and dark zone when the cell density is high. And the photobioreactor is almost captured by dark zone which make the microalgae cannot exposure to sufficient illumination to grow.

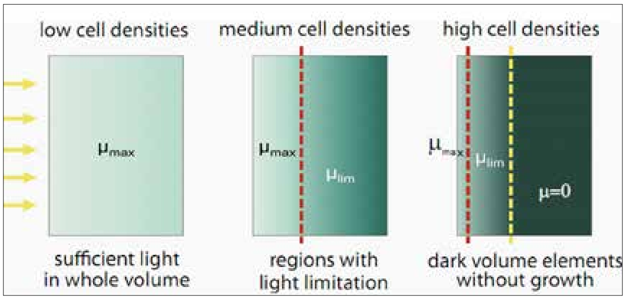

Figure 11. the change of light distribution and specific growth rate of microalgae under different cell densities conditions.

Therefore, it is difficult to achieve a high density of microalgae in photoautotrophic culture especially for photobioreactor scaled up by increasing the light path. The proportion of the light zone decrease with the light path increase, the algal cell cannot obtain enough light and the algal growth is significantly limited. According to the report, in flat-panel reactor, the maximum cell density can be up to 80g/L, but the light path of the photobioreactor is only a few centimetres. At present, due to the light path factor, various types of closed photo bioreactor are difficulty in scaling-up. Therefore, strengthening the mixing in the reactor, especially the mixing in light direction is conducive for algal cells to get more chance to get into the light zone and take light.

In order to increase the availability of light for the algal cells within the reactor, some researchers are trying to use built-in artificial light or introduce natural light into the reactor. Suh et al. reported a small photobioreactor with internal light source, in which six fluorescent tubes are installed, and the light intensity within the reactor can be adjusted by controlling the number of lamp on. Besides, many researchers have reported that the natural light is collected and then delivered into the photobioreactor through some certain medium such as fiber optic cables. Although the internal light sources can effectively improve the illumination area, shorten the light path and eliminate the heterogeneity distribution of light, some problems also exist, such as the high cost of manufacture and operation, difficulty in maintenance and running as well as scaling-up. Therefore, the internal-light source models can only be used in experimental research instead of industrial application. With the continuous development of related technologies, the internal light source should be possible to applied in the practice over coming years.

In addition, the light near the light source can be delivered to the dark zones far away from light source by setting transparent material that can be used to transfer the in the reactor. By this way, the transfer efficiency of the light in the reactor can be improved. At the same time, the distribution of light can be more heterogeneity. Meanwhile, the quality of the light also affects the growth of algae cells and intracellular components. Microalgae have different growth rate under various light quality. Thus, some related conversion membrane technology can be applied in filtering the sunlight to obtain the optimum light which has narrow light frequency for microalgae mass culture to improve the culture efficiency outdoor.

4.2 Mixing

Perner et al. proposed the relationship among the culture mixing and light utilization as well as algal cell biochemical metabolic in the microalgae cultivation process. As shown in Fig. 12, the mixing of the culture affects the pH、O_2 and CO_2 distribution in the photobioreactor, thus further influencts the biochemical metabolic reaction of the algal cells. Mixing also have an effect on the light/dark cycles frequencies by influencing the motion of the algal in the reactors. Consequently, mixing affects the light utilization efficiency and the growth of algal cells. Meanwhile, the growth of the algal cells changes the light distribution within the photobioreactor, and then affecting the light regime of algal cells. Therefore, the mixing, light and algal cell growth in the photobioreactor is closely related and influences each other.

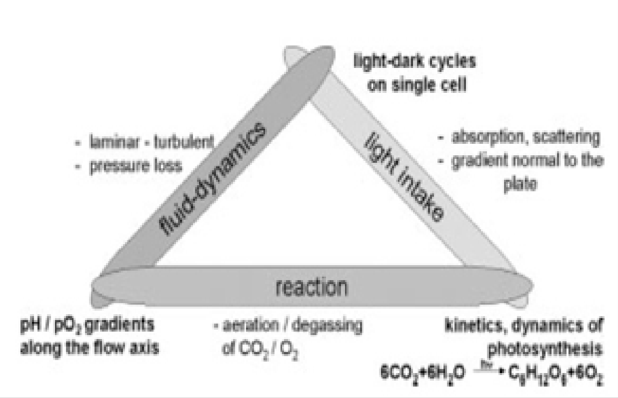

Figure 12. The relationship among the mixing and light utilization as well as microalgae growth.

The researchers believe that when nutrients are sufficient and environmental conditions is not limiting factor in the microalgae culture, mixing is the most important factor to obtain high microalgae yield. Mixing can affect the microalgae productivity greatly. When the flow rate inside the photo bioreactor is much slow, that may lead to algal cell sedimentation, then the algal cells deteriorate, decompose gradually and die finally. Besides, mixing could be beneficial to the gas-liquid mass transfer in the microalgal culture and accelerate the absorption of CO_2 as well as the release of O_2. O_2 will be produce with the microalgae photosynthesis and may be accumulate in the culture process. When the dissolved oxygen reaches a certain level, this preferred to inhibit the photosynthesis and growth of algal cells. Babcock et al. Studied the mixing time, gas holdup, velocity, Reynolds number and gas-liquid mass transfer coefficient within the tubular reactor, and the results show that oxidative stress caused by the difficulties in releasing oxygen which is the result of lower mass transfer coefficient that also is the main reason to limit the microalgae yield. On the other hand, the intense mixing will have negative effects on the algal cells, such as the velocity difference caused by agitation and aeration and the energy released when the bubble break could produce a greater shear force on the algal cells, resulting in algal cells damage and even death. Merchuk have obtained a result that in the optimal culture conditions, with the further increase of the superficial gas velocity, the Phorphyridium sp growth rate decline rapidly.

Light and mixing not only are the primary factors for algae cells culture productivity, but also have an important influence on the design of photobioreactor. Due to spatiotemporal nonlinear variety of local light intensity and the obvious gradient distribution of light in the photobioreactor, the light intensity that the single algal cell receives is not only related to the algal cell concentration, the external light intensity asl well as light path but also related with the mixing. The light intensity received by the single algal may be represented by the following formula: $I_x = f(I_0, C_x, L, Mixing)$, where I_x is the light intensity received by the single algal; I_0 is the external light intensity; C_x is the algal cells concentration, and L is the light path. The mixing in photobioreactor, especially the mixing of the fluid in the light attenuation direction will significantly affect the light regime and probability of expose to light for algal cells. Therefore, enhancing the mixing of algal cells in the light attenuation direction is significant to the growth of the algae cells. Ant the mixing of the fluid in the light attenuation direction in the photobioreactor is also one of the most crucial factor for photobioreactor design, optimization, and scaling-up.

In addition, the mixing will make the cells move frequently between dark and light zones, and form a light and dark cycles. This phenomenon is known as light flash effect, which can improve the efficiency of light energy utilization and photosynthetic of algal cells, and then increase the growth rate and productivity of algal cells. When the algal cells move into the dark zone, they can continue to use the light energy got from the light region. Hence, under the high frequency of light and dark cycles, the algae cells are able to produce high light yield (light yield of algal $Y_{X/E}$). However, when the mixing in the PBRs is poor, and the frequency of light/dark cycles is low, a negative effect for the regulation at the enzyme level and the gene level of the algal cells will exert, and reduce the microalgae growth rate and light energy utilization.

In the PBRs, the frequency of light/dark cycles is determined by a variety of factors, such as light path, cell density, turbulence intensity and external light intensity. The frequency of light/dark cycles of algal cells can strongly influence the productivity and light yield of algal. The study showed that under the condition of high frequency of light/dark cycles (cycle time ≤ 100ms), the growth rate and photosynthetic rate of algal cells is higher than that on the condition of continuous illumination. However, when the cycle time is 13~87 s, the growth rate and light yield of algal cells is similar or little lower than which under continuous illumination condition.

The mixing and the light regime characteristics of microalgae, such as the light/dark cycles formed by coupling the mixing and light distribution, have important effects on the growth of algal cells, especially when the PBRs is scaled-up, the influences will be more significant. Therefore, the frequency of the light/dark cycles is an important parameter for PBRs design. In general, there are two ways to increase the frequency of light/dark cycles: 1) to improve the mixing intensity in the PBRs, to make the cells spent a short time to move from the dark zone to the light zone; 2) to set some internal structures such as static mixers or baffles etc. in the PBRs, thus the reactor will be divided into several small parts, and relying on the fluid swirl generated caused by the specific internal structures, the cells will periodically move in the units, and then the frequency of light/dark cycles will be increased.

5 Summary and Outlook

The mass cultivation of algae for produce biodiesel mainly depends on the development of open photobioreactor systems such as raceway ponds, however the cultivation of photoautotrophic microalgae seed and high value-added microalgae majorly relay on the development of closed photobioreactor.

To date, a large number of studies on the closed culture system have been reported, but there are little of researches on the open culture system, making the culture efficiency of microalgae in the open pond is still low. The optimization and amplification of the open culture system, mainly raceway pond and a circular pond, can reduce the operating costs and increase the productivity, and are also important to accelerate the industrialization of the microalgal biotechnology, especially for the industrialization of energy microalgae. In order to reduce the manufacturing costs of the closed photobioreactors, the transparent thin film used to produce a variety of closed culture systems is an important trend in the development of the closed reactor.

At present, the optimization and scaling up of the photobioreactor are conducted according to the experience and experiments of microalgae culture, thus the large-scale reactor cannot achieve the most excellent performance due to different uniform criteria and parameters. Meanwhile, the design and optimization of photobioreactor lack the systematic theory and method. Hence, the effect of the optimization is not obvious, and the performance of photobioreactor is degraded after it is scaled up. Eventually, it leads to the low efficiency and high cost in the large-scale cultivation of microalgae. In recent years, it becomes a major trend to employ the CFD method for the research and development of photobioreactor, but the results derived by CFD are not almost tested by microalgae experimental in the current literatures. Therefore, it should be emphasized to obtain reliable CFD simulation method, which should combine with the practical experiments of microalgae culture to achieve the sensitivity parameters or parameter groups for the microalgae photoautotrophic culture. They will be helpful to establish the systematic and reliable theory and methods for the optimization and scaling up of photobioreactor. The research to establish methods of optimization and scaling up of photobioreactor is a significant orientation for the development of photobioreactor.

With the rapid development of microalgal biotechnology, there will be a major breakthrough of the development of photobioreactor for microalgae industry in the near future. In particular, it will provide a strong support of the equipment for the industry of microalgae especial for the applications in biofuel and carbon bio-fixation.

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9 Overview of Microalgae Production Technologies

Biomass Cultivation, Harvesting and Processing

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Abstract Microalgae are fast becoming a favoured feedstock for the production of advanced biofuels including biodiesel, biocrude, bioethanol, biomethane, green diesel and biojet fuels. Algae for their growth require sunlight, CO_2 from waste streams, unproductive lands and poor quality waters which include seawater, brackish water and agricultural, municipal and industrial wastewaters. Algae biomass is rich in lipids, carbohydrates and proteins. Currently more emphasis is given to extract lipids from the algal biomass to produce drop-in fuels. Algae can be cultivated through photoautotrophic and mixotrophic mode of nutrition using open raceway ponds and photobioreactors (PBRs). Heterotrophic mode of nutrition requires closed bioreactors and needs organic carbon sources which may prove to be costly for biofuels production. Harvesting of algae is a major energy consuming process in the fuel production path. Techniques such as centrifugation, filtration, flocculation, flotation and electrocoagulation-flocculation are assessed for their energy efficiency and cost effectiveness for commercial-scale operations. Downstream processing of algal biomass including cell disruption and extraction requires innovative technologies. This book chapter discusses algae biomass cultivation, harvesting, cell disruption (techniques such as bead beating, sonication, and pulsed electric field) and extraction technologies (using solvents, ionic liquids, subcritical and supercritical fluids) in great detail for biofuel production.

Keywords biocrude, biofuel, bio-oil, hydrothermal liquefaction, ionic liquids, microalgae, milking, oil, subcritical water extraction, supercritical fluid extraction.

1 Introduction

Algae are considered as potential biomass feedstocks for the production of food, feed, fuel and nutraceutical, cosmeceutical and pharmaceutical products. Micro and macroalgae are the two broad categories currently being exploited for the above applications. Various co-products derived from algal proteins, carbohydrates and lipids are given in Fig. 1. Microalgae can be cultivated in non-arable lands and poor quality waters including sewage, industrial wastewaters and seawater. In view of their superlative biomass production potential, microalgae are being promoted as a future source of transportation fuels. Recently, the major focus of biofuel research is mainly on developing viable technologies for commercial-scale production of biofuels from microalgae. However, the progress in the development of large-scale microalgae farms has been slow owing to major limitations in the mass cultivation, harvesting and downstream processing technologies [1]. Majority of R&D work carried out all over the world, focuses mainly on cultivation aspects of microalgae. Researchers give more emphasis to strain development/improvement and reactor design for algae cultivation. However, harvesting, dewatering, cell disruption, extraction and conversion into fuels remain the most critical steps which require more attention in the biofuel production path from microalgae. For biofuel production, the energy requirement and cost involved in cultivation, harvesting, cell disruption and extraction processes of biomass should be very attractive. This book chapter provides an overview of mass cultivation of microalgae, harvesting and extraction technologies currently used for algal biomass and analyses their sustainability for large-scale biofuel production in future.

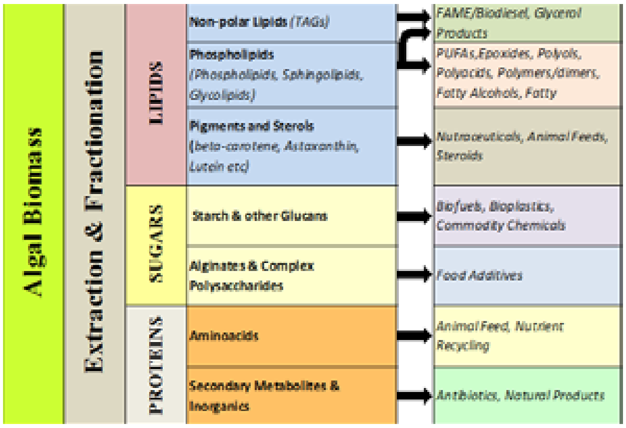

Figure 1. General schematic for algal biomass fractionation and-co-product generation (Foley et al. [2]).

2 Algae Production Technologies

2.1 Cultivation Systems

The type of system used for cultivating algae depends largely on the requirements of the organism being cultivated and on the nature of the product being harvested [3]. Commercial production of microalgae requires large amounts of biomass through mass culture [4]. Artificial production, therefore attempts to mimic and improve natural growth conditions [5]. Solar energy is one of the major natural resources required for the commercial production of microalgae. Algal production methods consist of photoautotrophic production by photosynthesis, heterotrophic growth using added organic carbon sources and mixotrophic growth combining photoautotrophic and heterotrophic growth [6-8]. All these cultivation methods have their own merits and demerits which are shown in Table 1.

Table 1: Comparison of various cultivation methods.

Cultivation Method	Advantages	Disadvantages
Photoautotrophic	Open sunlight	Light limitation in dense cultures
Heterotrophic	High cell densities and cell productivities	Few species capable of growth on organic carbon substrates
Mixotrophic	Either light or carbon supports cell growth	Expensive

2.1.1 Photoautotrophic Production

Phototrophic algae absorb sunlight, atmospheric carbon dioxide and nutrients from aquatic environments [5]. Borowitzka stated that there are several considerations when choosing a culture system to use i.e. biology of the alga, cost of land, labour, energy, water, nutrients, climate and the type of final product [9]. Many systems have been designed to maximize the surface to volume ratio of the culture [10]. Photoautotrophic production of microalgae can be achieved in open systems such as raceway ponds or closed systems using photobioreactors (PBRs).

2.1.1.1. Raceway Ponds

Raceway pond is a shallow artificial pond in which water is circulated with the help of a paddle wheel (Fig. 2). Water depths in the pond range between 10 and 20 cm [11]. Outdoor ponds of various sizes, shapes, mixing devices and construction materials have been designed and experimented for microalgal cultivation. Large ponds can be unlined (with a natural bottom), or lined with inexpensive materials (clay, brick or cement) or lined with expensive materials (PVC or fibreglass) [12, 13]. Open raceway ponds are the oldest, simplest and preferred systems for mass cultivation of microalgae, with about 98% of commercial algae biomass being produced in open ponds [14]. Mixing of the culture medium and circulation are by a paddle wheel and flow is guided around bends by baffles placed in the flow channel [15]. Large-scale production of microalgal biomass generally uses continuous culture, in which fresh culture medium is fed at a constant rate and microalgal culture medium is withdrawn continuously. Nutrients are provided during daylight hours when the algae are reproducing, whilst mixing remains uninterrupted to prevent settling of the biomass [15]. In raceways, culture conditions may be extremely variable due to exposure to the open environment, seasonal changes and diurnal cycles. For outdoor cultures, light is generally the limiting factor, therefore commercial production is limited to areas with high solar radiation [5].

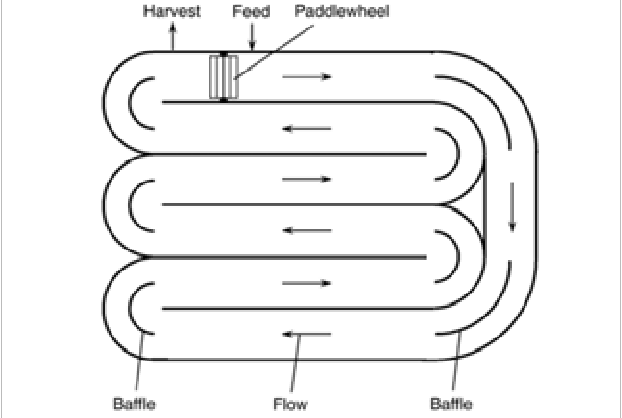

Figure 2. Aerial view of a raceway pond (Chisti [15]).

2.1.1.2. Photobioreactors (PBRs)

Closed system PBRs are an alternative method of cultivating microalgae designed to overcome the problems associated with conventional open raceway ponds [5]. PBRs are relatively safe from invading microorganisms and enable monoculture of microalgae for prolonged durations, with high algal concentrations and productivities and better control of culture conditions [16]. Maximal volumetric productivities are possible in PBRs because of higher surface-to-volume ratio. PBRs are similar to conventional fermenters, however they utilize light rather than an organic carbon source [10, 17]. To address the limitations with sunlight, the use of artificial lighting by fluorescent lamps allows for continuous production of algal culture. Typically, algal culture is circulated in glass or plastic tubes, generally 0.1 m or less in diameter, by a centrifugal pump with the culture medium intermittently passing through a degasser [18]. The most common types of closed PBRs reported in literature are tubular (Fig. 3) and flat panel types [3, 19-21]. Other designs reported include the helical-tubular photobioreactor, bubble column and airlift reactors [22-25].

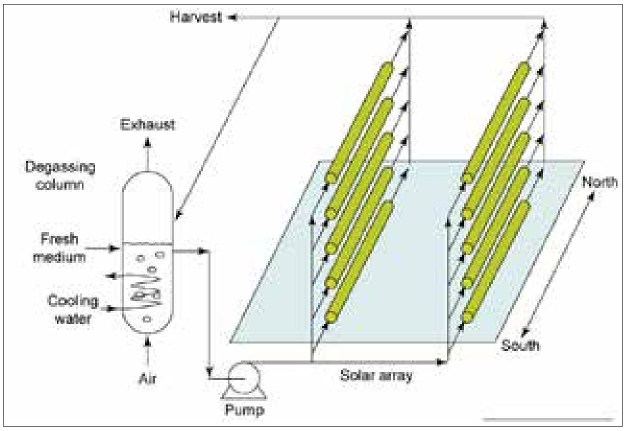

Figure 3. Schematic of a tubular photobioreactor with horizontal solar collectors (Chisti [26]).

Thin channel flat plate bioreactors are known to be highly efficient, because they have a large surface area exposed to illumination and high densities of photoautotrophic cells [27]. The reactors are constructed of transparent materials for maximum solar energy penetration, and a thin layer of dense culture flows across the flat plate, which permits algae in the medium to efficiently absorb radiation. Flat-plate PBRs are suitable for mass cultures of algae due to low accumulation of dissolved oxygen and the high photosynthetic efficiency achieved when compared to tubular versions [5].

Tubular PBRs seem to be suitable for outdoor mass cultures since they expose a larger surface area to sunlight [21], and have provided high biomass productivities for industrial-scale production of *Haematococcus* spp., *Chlorella* spp. and *Nannochloropsis* spp. [18]. However tubular reactors require large areas of land which is a significant disadvantage and therefore, cannot be scaled up indefinitely. Tubular PBRs have design limitations owing to high dissolved oxygen levels, gradients of oxygen and CO_2 transfer and photo-inhibition in long tubes [21].

Bubble column and airlift reactors are low-cost, easy to operate and more compact than tubular reactors and offer important advantages for large scale culture if a compromise is made to loss of productivity [17]. The vertical columns are illuminated internally or through transparent walls and aerated from the bottom offering the most efficient mixing and highest volumetric mass transfer rates, with performance comparing favourably with tubular PBRs [5].

2.1.1.3. Attached Cultivation Systems

Attached algal cultivation systems such as Algal Turf Scrubber (ATS) was developed by Walter Aday in 1980s in which benthic algae grow on the surface of solid substrates to capture nutrients from agricultural, municipal and industrial wastewaters (Fig. 4). Cultivation of algae using ATS systems to treat and recover nutrients from dairy and swine manure effluents was extensively studied and documented by the US Department of Agriculture's research facility in Beltsville, Maryland [28]. HydroMentia, Inc – a Florida based company in the US – commercialized ATS technology for nutrient scrubbing of agricultural non-point-source wastewaters in South Florida which can treat 3-110 million lpd.

Figure 4. Aerial view of 10 MGD Egret Marsh Algal Turf Scrubber located in Indian River County, Florida. Photograph: Courtesy of HydroMentia, Inc.

2.2 Heterotrophic Production

Heterotrophic mode of nutrition is an alternative to photoautotrophic growth and it is limited to a few algal species. In this method, microalgae are grown on organic carbon substrates in the absence of light in fermenters or bioreactors [29]. Fermenters eliminate contamination by other organisms and facilitate precise control of various growth parameters. Since growth is independent of light, heterotrophic algae can be grown to higher cell densities resulting in enhanced biomass productivity and lower harvesting costs. In addition, fermenters are easily scalable to nearly any volume desired [10]. Heterotrophic production has also been successfully used for production of algal biomass and metabolites [30-32]. A study using *Chlorella protothecoides* demonstrated that the lipid content in heterotrophic cells was much higher compared to photoautotrophic cells grown under similar conditions [33]. Hence, it was concluded that heterotrophic cultivation could result in higher production of biomass and accumulation of high lipid content in cells.

2.3 Mixotrophic Production

Mixotrophic production is a modification of heterotrophic mode of nutrition, where CO_2 and organic carbon sources present in the culture medium are simultaneously assimilated in the presence of a light source which allows respiratory and photosynthetic metabolism to operate concurrently [8]. The limitations imposed by pure photoautotrophic growth are potentially overcome by mixotrophic production [34]. Either light or organic carbon substrate can support mixotrophic growth whereby photosynthetic metabolism utilises light for growth, whilst aerobic respiration uses an added organic carbon source in the growth medium. Growth rates of mixotrophic algae compare favourably with cultivation of photoautotrophic algae in closed PBRs. The growth rates of algae under mixotrophic mode of nutrition in open ponds are higher but are considerably lower than for heterotrophic mode of production.

3 Harvesting/Cell Separation

The first step after algae cultivation is harvesting. Microalgae harvesting is an energy intensive process. An efficient microalgal separation process should consume less energy to harvest both freshwater and marine algae and yield a product with less moisture content and impurities such as ash [35]. Harvesting of algal biomass is a challenging task as the biomass concentration in the algal culture is only about 0.1% w/v. Hence, harvesting is done at two steps; first stage harvesting concentrates the algal culture to 2-3% solids followed by second stage dewatering step which further concentrates the algal solids to 15-20% (w/v). Currently, there are several methods to harvest microalgae such as natural gravity sedimentation, flocculation, flotation, centrifugation and filtration [36].

Centrifugation and filtration methods are very efficient for separating algal cells from the growth medium. However, they are not cost-effective to harvest microalgae for the production of low value products such as biofuels [37]. Centrifugation involves high energy consumption, capital and maintenance costs, whilst filtration methods are best suited to filamentous or colony forming microalgae [38].

Various methods borrowed from wastewater treatment technologies have been tested in the past to induce flocculation in algal cultures. The most commonly used coagulants–flocculants in wastewater treatment are ferric chloride ($FeCl_3$), ferric sulphate ($Fe_2(SO_4)_3$), ferrous sulphate ($FeSO_4$), aluminium sulphate ($Al(SO_4)_3$) and calcium hydroxide ($Ca(OH)_2$). The choice of a specific coagulation reagent is governed by its effectiveness and the cost relative to alternative reagents [39].

Organic polymeric flocculants which are biodegradable and less toxic helps in separating algal cells in an eco-friendly way [36]. Wu *et al*, identified flocculation by increasing pH as an attractive alternative because it is low-cost, low on energy consumption, non-toxic to algal cells and eliminates the use of flocculants [40]. Bioflocculation is a dynamic process resulting from synthesis of extracellular polysaccharides (EPS) by living cells and this has emerged as a new research trend in flocculation technology [41].

Immobilization technologies such as algae biofilm or attached algae cultivation systems exploit the natural tendency of some algae to attach and grow on supporting substrates which can be easily harvested (Fig 4). At present, such systems are mainly used for treating agricultural and municipal wastewater streams [28]. However, harvesting studies related to attached cultivation systems are limited in terms of energy efficiency and cost saving evaluation [36].

Dissolved air flotation has effectively been used to separate algae in the presence of cationic surfactants. A study by Cheng *et al*, utilized dispersed ozone flotation for harvesting and cell lysis of *Scenedesmus obliquus* FSP-3, a species that has a high lipid production potential [42]. The findings of this study suggest that aeration with ozone efficiently induces flotation and cell lysis and produces negatively charged algal cells with increasingly hydrophobic surfaces compared with intact cells. Flotation has advantages of flexible operation and small footprint when compared with coagulation–flocculation and sedimentation techniques. Levin *et al*, developed a highly efficient froth flotation procedure for harvesting algae from dilute suspensions [43]. This method obviates the need for addition of chemicals. Cell harvesting is carried out in a long column containing the feed solution which is aerated from the bottom. A stable column of foam is produced and harvested from a side arm near the top of the column. The cell concentration of the harvest is a function of pH, aeration rate, aerator porosity, feed concentration and height of foam in the harvesting column. The economic aspects of this process seem favourable for mass harvesting of algae for food or other purposes.

Xu *et al*, studied the use of Fe_3O_4 nanoparticles for harvesting algae by magnetic separation [44]. Following the addition of the magnetic nano particles to the algal culture, electrostatic attraction bound the cells to the particles. Algal cells were then separated by an external magnetic field. The nanoparticle coated microalgal cells were concentrated and separated from the suspension medium by decantation, when a permanent magnet was placed at the bottom of the vessel. The maximal recovery efficiency reached more than 98%, at a stirring speed of 120 rpm within a minute. This technology is cost effective and energy efficient. However due to the problems in manufacturing of the nano magnetic particles, practical application of this harvesting system is currently limited.

Electro-coagulation-flocculation technology has significant advantages over other conventional harvesting technologies as it requires less energy to harvest microalgae [36, 45]. In this technology, iron or aluminum ions are released from a sacrificial anode through electrolytic oxidation. Compared to conventional chemical coagulation-flocculation techniques, no anions such as chlorine and sulphate are released in the process water. The naturally occurring negative charge on the surface of algal cell wall is neutralized by the electroflocculation treatment which results in the reduction of electrostatic force of repulsion between algal cells in suspension and formation of inter-particle bridging to cause flocculation of cells in the culture [46]. Energy consumption in the electroflocculation technology reported was about 2 kWh/kg for harvesting freshwater alga *Chlorella vulgaris* and 0.3 kWh/kg for the marine alga *Phaeodactylum tricornutum* [36]. The total cost of harvesting through electroflocculation was estimated about 0.11 US$ for separation of 1 m^3 of algal suspension which was less when compared to the other separation processes such as plate centrifuge (0.80 US$ m^{-3}), sedimentation with flocculants (0.21–0.34 US$ m^{-3}) and flotation with flocculants (0.71–0.84 US$ m^{-3}). However, electroflocculation may require a further centrifugation or filtration step to increase the solids concentration above 10% (w/v).

4 Downstream Processing

4.1 Cell Disruption

Extraction of lipids or biocrude from algal biomass is considered problematic as algal cells are protected by tough cell walls that require extreme conditions warranting the use of intensive and costly energy inputs to break the cells. Algal cell walls are composite structures of linear and branched polysaccharides, including cellulose. Current extraction strategies for algae are focused on extraction of lipids for biodiesel production [47, 48-52]. In microalgae, excess moisture in the harvested biomass, type of microalgal species, cell size and the strength, structure and composition of the cell wall will greatly impact the cost and efficiency of lipid extraction. Lipid extraction in algae is mostly done using completely dried algae biomass. However, wet biomass extraction is advocated by many researchers to improve the energy economics of the extraction process.

Since most lipids are intracellular, any lipid extraction process will be effective only when the native structure of biomass is mechanically disrupted. Techniques like mechanical pressing, lyophilisation followed by grinding algal cells while frozen in liquid nitrogen, homogenization, bead beating, use of microwave energy, osmotic shock, sonication and pulsed electric field are commonly used for algal cell lysis [53].

Cooney *et al*, evaluated various physical and chemical cell disruption techniques applied to wet and dried algal biomass [1]. Among all, grinding the cells in liquid nitrogen proved highly effective as it resulted in 90-100% cell disruption. However, industrial-scale application of this technique will be energetically and financially very costly. As a case in point, freezing of microalgae with 20% solids and 80% water consumes 13% of total energy provided by algae oil considering 40% lipid content in the biomass. Cooney *et al*, also reported that the extraction of lipids and oil yield from oven dried and ground algal biomass sample was much better than the oil recovery obtained using frozen and ground wet biomass [1].

Mechanical pressing is normally used for extracting oil from oilseeds like sunflower using screw press. However, this technique is ineffective for microalgae as the cells can pass through the press as the cell size is very small [54]. Homogenization forces the biomass through a small orifice which results in pressure change and high shearing action [55]. Bead mills can disrupt 99% of cells. However, the energy consumption of bead mills is high compared to other methods [56].

Cell disruption through osmotic shock is achieved by placing freshwater algae in highly saline solution [hypertonic shock] or marine algae in freshwater (hypotonic shock) [57]. However, this technique was relatively ineffective for cell disruption when compared to microwave and sonication methods.

High intensity pulsed electric field (PEF) processing helps in enlarging the pores of the cell membranes which kills the cells and results in the release of cell contents. This process is known as electrophoration [58]. Recently, the US based company Origin Oil has developed a single step extraction process for microalgae (Fig. 5). This process disrupts the microalgal cells by pulsed electromagnetic fields after injecting CO_2 into the medium to reduce the pH and creating mechanical stress to microalgal cells through fluid effect [59, 60]. However, the commercial viability of this disruption cum extraction technique is yet to be proven in large-scale.

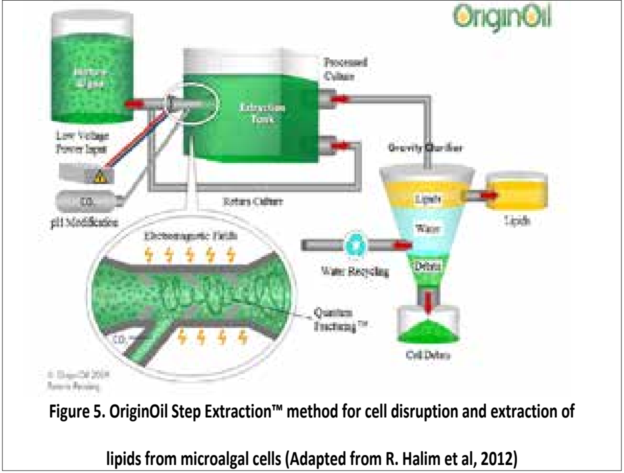

Figure 5. OriginOil Step Extraction™ method for cell disruption and extraction of lipids from microalgal cells (Halim et al., [59]).

In ultrasonication, sound waves having frequencies higher than 20 kHz are used to create mechanical vibrations in a solid, liquid and gas [61]. Low operational temperature and short duration of treatment are the major advantages of ultrasonication technique [47, 62]. Ultrasonic sound, triggers collisions among particles within the cell. This collision increases internal heat which helps in cell rupture and release of cellular contents [63].

Hydrodynamic cavitation is another novel technique which can effectively break the algal cells to facilitate extraction of oil [64]. However, this technology is currently not suitable for handling large volumes of algal cultures as the energy requirement is high.

Table 2 compares the energy requirements of various cell disruption processes and indicates the potential of methods like ultrasound and PEF which offer significant energy savings when compared to mechanical disruption processes [53].

Table 2. Comparison of cell disruption methods.

Details	**Homogeniser**	**Bead beater**	**Ultra-sound**	**PEF***
Algal biomass feed concentration (% dry weight)	15	15	15	2
Energy consumption/requirement:				
kWh/m³	38	1,500	10	15
kWh/kg	0.25	10	0.07	0.06

**pulsed electric field*

It was also reported that many of the lysing techniques were not effective at disrupting cell walls of marine microalga *Nannochloropsis* spp. due to the presence of insoluble and non-hydrolysable biopolymers in the outer cell walls which are termed as algaenans. Algaenans serve as protective coat surrounding the cells [65, 66]. *Haematococcus* spp. contains a thick sporopollenin wall which is highly resistant to cell wall disruption [67].

4.2 Extraction

4.2.1. Solvent Extraction

Organic solvents like chloroform, hexane, acetone, benzene and cyclohexane are very effective in extraction of oil/lipids from microalgae. However, a suitable solvent should be insoluble in water, should preferentially solubilize the compound of interest, have a low boiling point to facilitate its removal after extraction, and have a considerably different density than water. In addition the solvent system should be easily sourced, inexpensive and reusable. Currently hexane is considered a suitable solvent for large-scale extraction due to the qualities mentioned above [55].

The co-solvent approach described by Bligh and Dyer using a combination of chloroform, methanol and water has been used as a benchmark for comparison of solvent extraction methods [68]. However, use of chloroform and methanol for large-scale extraction may not be safe due to toxicity issues. Apart from saponifiable lipids, chloroform and methanol combination will extract pigments and other lipid and non-lipid contaminants. Hence, the oil extracted using these solvents require degumming and purification before further use.

In order to achieve better extraction efficiencies using solvents, pre-treatment is required to achieve complete cell disruption which will facilitate easy penetration of the solvent into the algal cell. Also the solvent used for extraction needs to match the polarity of the targeted compound. It has been reported that the sequence of solvent addition plays an important role in extraction and can also affect the extraction efficiency. Cooney *et al*, found that lipid extraction was more efficient when solvents were sequentially added to the dry algae biomass in the order of increasing polarity (i.e. chloroform, methanol and water) [1]. However, the presence of water in the wet algae biomass makes it more difficult for other less polar solvents such as chloroform to solubilise and extract the lipids because water molecules form a shell around the algal cells containing lipids.

Efficiency of solvent extraction can be enhanced by applying high pressure and temperature. This process is known as Accelerated Solvent Extraction (ASE). Effective percolation of solvent into the cells is facilitated by increased pressure (500-3,000 psi) and temperature (50-200^{o}C) when compared to atmospheric conditions. Though pressurized solvents at elevated temperature improves extraction efficiency, the energy and operating costs of this process can become highly prohibitive. Moreover, this extraction technique requires dried algal biomass, which requires more energy input for drying the algal biomass.

4.2.2. Ionic Liquids For Energy Efficient Extraction of Algal Biomass

To effectively fractionate the algal biomass components, an efficient cell disruption technique is needed. Binder and Raines, suggested the use of ionic liquids for complete lysis of cells for effective extraction of cell components [69].

Ionic liquids are green solvents which are organic salts, usually containing a quaternary ammonium cation, that are liquids at reasonable working temperatures (0°C to 140 °C). Ionic liquids possess relatively low vapour pressure and capacity to be tailored for a specific solubility, polarity, electrical conductivity, and relative hydrophobicity [70]. Ionic liquids are broadly categorised as hydrophilic (eg. *1-butyl-3-methylimidazolium chloride, 1-ethyl-3-methylimidazolium chloride, 1-allyl-3-methylimidazolium chloride and 1-(2-hydroxylethyl)-3-methylimidazolium chloride)* and hydrophobic (eg. *1-butyl-3-methylimidazolium hexafluorophosphate and 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide*).

Teixeira proposed an energy efficient approach to hydrolyze, solubilize and create cell free mixtures of algae biomass for production of biofuels and host of other green chemicals [71]. He conceptualized a simple ionic liquid based process scheme to use wet algae (10% solids w/w) and fractionate it into lipids, and ionic liquid solubles including sugars and proteins. The heated wet biomass is mixed with the ionic liquid in the reactor. Ionic liquid facilitates the hydrolysis process and helps in dissolving and releasing algal cell constituents. Lipids from ionic liquids can then be extracted using solvents.

Lardon *et al*, reported a Net Energy Gain (ratio of created energy vs spent energy) of 2-4 for the biodiesel production scenario where the alga was grown under low N conditions and the extraction was done with wet biomass (Table 3) [72]. Teixeira reported a Net Energy Gain (NEG) of ~10 for a processing path utilizing ionic liquids for algal hydrolysis [71].

This extraction technology looks attractive as it does not require acids, bases and other catalysts to create cell free mixtures from wet algal biomass and preserves the integrity of algal cell constituents. The cell free mixtures of algae can be used as starting material for the production of a wide range of products from lipids, carbohydrates and proteins in a biorefinery.

4.2.3. Extraction With Original Algae Oil

Benemann and Oswald suggested an approach for extraction of lipids using wet algae [73]. After initial dewatering step, the algal biomass with 1-3% (w/v) solid content is subjected to homogenization to facilitate cell disruption. The homogenized solution in the form of emulsion is extracted with hot solution of algal oil (previously extracted from algae) and the entire mixture is further separated into three harvestable product streams using a three phase centrifuge *viz*. a biomass cake, an organic and protein rich water stream and the lipid fraction. Though this concept is very attractive, the lipid concentration in the extraction mixture is very little compared to the volume of algal oil added to facilitate the extraction process.

Table 3. Net energy gain of algae extraction using ionic liquids in comparison with conventional extraction techniques (Teixeira [71])

Details	**Normal (Conventional Extraction)**		**Low N (Conventional Extraction)**		**Algae Hydrolysis Using Ionic Liquids**	
	Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Algae culture and harvesting						
Electricity (MJ)	1.3	1.3	1.5	1.5	1.3	1.5
Drying						
Heat (MJ)	14		14			
Electricity (MJ)	1.4		1.4			
Lipid extraction						
Heat (MJ)	1.2	2.7-6.6	1.2	2.7-6.6	0.3	0.4
Electricity (MJ)	0.3	1.0-2.4	0.3	1.0-2.4	0.1	0.1
Lipid transesterification						
Heat (MJ)	0.2	0.1	0.3	0.2	0.1	0.2
Electricity (MJ)						
Total Energy						
Consumption (MJ)	18	5-10	18	5-11	2	2
Production (MJ)	18	18	23	23	18	23
NET ENERGY GAIN	1.0	1.7-3.5	1.2	2.1-4.2	10	10

First four columns represent nutrient replete (Normal) and nitrogen-starved (Low N) algae processed via dry and wet solvent extraction. The last two columns represent algae hydrolysis using ionic liquids. Net Energy Gain is the ratio of energy production over consumption for each biodiesel path.

4.2.4. Milking

Milking for simultaneous production and selective extraction of target products in an aqueous –organic biphasic system was reported earlier for the production of pigments and hydrocarbons [74, 75]. Recently, the use of solvents like decane and dodecane to extract triglycerides and membrane bound free fatty acids from live microalgae cells without loss of algal cell viability was proposed for energy efficient extraction of oils. However, the effectiveness of this cell milking technique is largely limited to algal strains which lack cell wall (eg. *Dunaliella*) or algae known for the synthesis of extracellular oils (eg. *Botryococcus*). Though this technology appears promising, the following questions still need to be answered to adopt the process on commercial-scale. (1) How algal cells will cope with removal and regeneration of membrane lipids during the non destructive milking process using solvents? (2) What is the energy requirement for concentration and dewatering of algal cells to reduce the moisture level optimised for milking? and (3) While milking process may be economical for producing high value products from algae, will it be economical for biofuel production?

4.2.5. Subcritical Water Extraction/Hydrotehrmal Liquefaction (HTL)

Subcritical water extraction or hydrothermal liquefaction advocates the use of water at temperatures (100° to 374°C) below critical temperature and pressure high enough to maintain the liquid state of water. Subcritical or hot compressed water behaves differently from water at room temperature because of changes in properties such as solubility, density, dielectric constant and reactivity as water approaches its critical point (374°C, 22.1 MPa). This reactive water medium enhances depolymerization and repolymerization of lignins, celluloses, lipids, proteins and carbohydrates, transforming them into biocrude/bio-oil, gas and char [76]. Higher pressures are maintained in hydrothermal processing procedures to avoid energy losses due to phase change of water to steam [77].

Advantages of this process include shorter extraction times and lower cost of extracting agent. This process also eliminates the need for dewatering step in algae which consumes more energy. Toor *et al*, found that subcritical water was more effective than supercritical water for extracting biocrude from wet biomass of *Spirulina platensis* and *Nannochloropsis salina* [78]. However, large-scale unit operations using this approach can be energy intensive and costly due to high energy loads required to heat the system up to subcritical temperature and operate cooling systems [79]. However, development of commercial-scale continuous HTL reactor systems with efficient heat recovery arrangements can significantly enhance the energy economics of this extraction process.

This process has many advantages over conventional biomass extraction and conversion techniques as it can handle wet biomass. The biocrude or bio-oil yield in this process is not dependent on the lipid content in the biomass as the depolymerization and repolymerization reactions facilitate hydrocarbon production even from proteins and carbohydrates present in the biomass. Contaminated algal culture can also be converted into biocrude/bio-oil using this process. Though the process requires high temperature and pressure conditions, Jena and Das reported that hydrothermal liquefaction process can recover about 67.9% of the energy present in the original biomass [80]. They also reported that the Energy Consumption Ratio (Energy input ÷ Energy output) for hydrothermal liquefaction was 0.7 which indicates that the process is a net energy producer. Jena *et al*, proposed a scheme for HTL of algal biomass for the extraction of biocrude/bio-oil (Fig. 6) [81].

However, this process has a few drawbacks. The biocrude/bio-oil produced requires further upgrading for use in existing refineries and its recovery from the reaction mixture requires a solvent extraction step which adds to the cost. This process results in only one major product stream i.e. biocrude/bio-oil. The other HTL co-products such as aqueous fraction and solid residues are rich in nutrients where as the gas phase is rich in CO_2. HTL process seems very attractive for algae biofuel production as it does not require energy intensive drying and cell disruption steps.

4.2.6. Supercritical Fluid Extraction

Supercritical fluid extraction technique is based on the enhanced solvating power of fluids combined with gas-like mass transfer properties [59]. Most of the supercritical extraction applications use CO_2 because of its preferred critical properties (critical temperature of 31.1°C and pressure of 1,071 psi/72.9 atm), low toxicity and inertness. The major advantages of supercritical CO_2 are low processing temperature and selectivity to high value components and triglycerides. However, supercritical CO_2 extraction requires dry algal biomass and the algal oil yield observed in this extraction process is relatively poor when compared with organic solvents. In addition to CO_2, other fluids like water, methanol, ethane, nitrous oxide, sulphur hexafluoride, n-butane and pentane also can be used for supercritical extraction process [82]. One of the major advantages of this process is that the extracted material dissolved into the supercritical fluid, can be easily separated by depressurizing so the fluid returns to its original gaseous state while the extracted product remains as liquid or solid. Though lipids have been selectively extracted from microalgae at 40-50°C and pressures of 241-379 bar (3,495-4,596 psi), this process has a major limitation due to difficulties in scale-up and higher energy costs for biofuel applications.

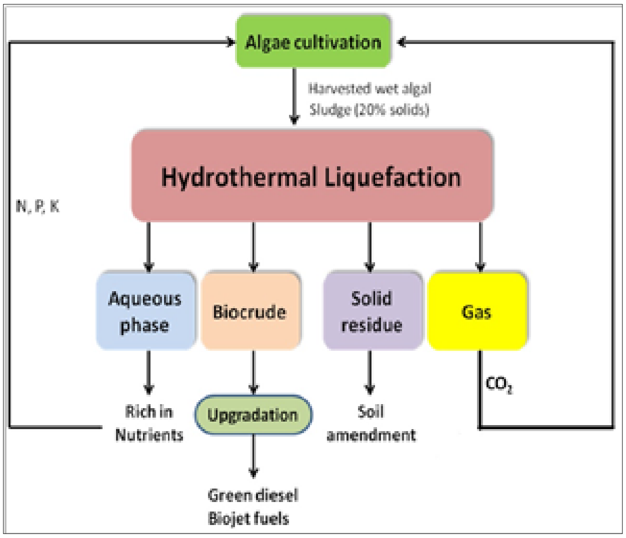

Figure 6. Proposed scheme for hydrothermal liquefaction (HTL) of algal biomass for extraction of biocrude/bio-oil (Jena et al, [81]).

Any viable extraction technology should consume less than 1/3rd of total energy content present in the algal biomass. Boer *et al*, reviewed several extraction technologies and concluded that hydrothermal liquefaction and pulsed electric field-assisted extraction followed by transesterification are energetically feasible methods over other extraction methods [53].

5 Conclusion

Commercial cultivation of algae for the production of feed and nutraceuticals is already proven and economically feasible as the selling cost of algal biomass is at least 4-5 times higher than the production cost. However, to produce biofuels from algae the production cost of algal biomass should be less than USD 0.15 per kg of dry biomass which is possible in very large-scale production facilities. There are a number of energy intensive processing steps involved in algae to fuels production which include harvesting, drying, cell disruption, extraction and conversion into fuels. To make algae fuels environmentally and economically sustainable the energy consumption and operating cost from cultivation to processing operations has to be significantly reduced. This can be achieved by enhancing algae biomass productivity in low-cost reactors for algae cultivation such as open raceway ponds and developing novel harvesting, dewatering, cell disruption and extraction techniques which require less energy. Currently biomass extraction using subcritical water or hydrothermal liquefaction appears promising as this technology does not require energy intensive drying of algae. The bio-oil or biocrude obtained through hydrothermal conversion technology can be processed in existing petrocrude refineries without creating any new infrastructure facilities for downstream processing. Currently, algae cultivation for the production of biofuel alone may not be economically sustainable as biofuels are low value products required in huge volumes. Hence, considerable research and development efforts are still required to develop commercial-scale technologies for the production high value co-products along with algae biofuels in an integrated biorefinery handling multiple biomass feedstocks.

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10 Algae Economics, Feasibility Studies and Financing Opportunities for Algae

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Different experts say that our planet will run out of oil resources within 30-100 years. In addition to this, water resources are also getting depleted. Finally, if we continue to emit CO_2 into the air at our current trend will the planet sustain the way we are polluting and destroying ecosystems?

Algae give us the opportunity to become more sustainable and to save resources. As larger corporations are beginning to realize the importance of algae and their economics, within the next few years we are also going to see a wave of change and awareness in the mindset of people.

Algae economics or 'algaenomics' has been around for some time, prior to the rise of the recent attention to algae, and have been generating revenue for many businesses. In fact, algae companies can generally be placed in two genres: 1) the traditional algae companies which have been producing algae and its products for decades, some for even hundreds of years, like the seaweed "nori" companies in Japan and Korea; 2) the new wave innovative algae companies which range from start-ups to large corporation relying on intensive technological research for innovations and breakthroughs, IP's including patents and discovering new ways of utilizing algae.

1 Algae Producers

In USA alone, there are more than 380 registered algae companies ranging from a one-man show to large corporations such as Sapphire Energy and Solazyme [1]. So, between the traditional players and the innovative newcomers, algaenomics will have a different prespective depending on these two sectors.

Companies such as Cyanotech started their operations in the early 80s [2], they have been producing

thousands of tones of *Spirulina* and other high value algae-derived products for the world market. The same goes for other traditional companies such as E.I.D. Parry's in India, whose history with algae can be traced back to 1977 [3]. These traditional companies must have a revenue generating strategy in order to have prospered till today. The algae pricing strategy is indeed a very interesting topic, it started at a very high premium price, and within the years it gradually dropped as more players and competition entered the market.

Spirulina used to be sold for USD 200-300 per kilogram in the about 2 or 3 decades ago [4, 5] and around early 2000 the Chinese started cultivating *Spirulina* on a larger scale. This entry by the Chinese producers resulted in enclosures of some of the smaller *Spirulina* producers and only the fittest survived, causing the market to sell *Spirulina* as low as USD 7.00 per kilogram However, the quality of spirulina from China was unable to maintain the high criteria required for nutraceutical graded *Spirulina*, therefore the market is slowly began demanding for high value premium *Spirulina* again. In such cases, long term experienced producers like Cyanotech and Parry's will benefit from this market shift, and are able to provide high quality at reasonable price.

Asides from the *Spirulina* players who are producing and selling, in some cases, up to 50 countries, there are also many other companies around the world who expanding their algae horizons and utilizing appropriate marketing strategy, some of which are selling thorough the MLM (multi-level marketing) strategy. Amway Corporation is one example of large corporation utilizing the MLM method. Beside from *Spirulina*, algae producers also benefit from other species such as chlorella, *Dunaliella* and *Haematococus* (Cyanotech and Eid-parry).

2 Traditional Producers vs. Innovative Algae Companies

Practically speaking, these are some of the general criteria's one can observed of the traditional algae industries which are mostly over 20 years old:

- They do not concentrate on R&D, but rather focus on specific production systems that have

been utilized throughout the years and maintaining traditional and well-proven culturing techniques.
- They tend to focus on production and generating revenue from their proven track records.
- They are secured and well known within their own market segment, limiting their exposure to emerging markets and emerging players.
- Some of the larger corporations have seen significant growth in their market share as people look for alternatives in high nutrients supplements. Based on these observations, it can be said that these well established companies are mostly contented with their annual growth and profit margins that they can isolate themselves from venturing into other aspects of the algae business. After all why does one need to change an already winning combination?

3 New Innovative Algae Companies – Come and Goes

Within less than 5 years, there has been more than a few hundred algae companies emerging like mushrooms all over the world, especially so in USA and Europe [1]. They are looking into many areas, but the most definitive one in economic terms are as follows:

a) New innovative companies, looking forward to sell and license their technology & services to customers [6]. Which can be further divided into categories; Such as:
   - Strain selections and screenings
   - Techniques of culturing
   - Special media and vitamins to accelerate algae growth
   - Machineries and equipment suppliers' dedicated only to algae

b) Machines and equipment sales for algae industry
c) Bio-remediation services for waste through algae
d) CO_2 sequestration projects consultants for algae projects

4 The Big Algae Craze

Based on the differences between the traditional algae producers and emerging algae companies and the traditional algae producers, the reality remains that most larger corporations are still skeptical about the potential of algae [7]. In 2006, there was a sudden craze in the algae industry as people started recognizing algae's incredible ability to grow in a comparatively short period of time than normal agriculture. The focus was completely on algae derived biofuels. Everyone was talking about algae becoming the new feedstock for biofuels. The whole industry was shaken with these fantastic new findings, universities and research centers around the world started applying for grants from governments and many succeeded in producing algae oil in their laboratories.

Thus alga being a single cell species become like a Holy Grail to biomass substitute. However the issues rise in the form of economics. Even if one can prove that such a concept on algae work, there is always a question of energy balance. When lot of energy is utilized to produce a little, the process requires serious optimization or reconsideration. The goal is to create a process that is highly energy efficient and economically viable. Shell in 2007 invested close to the USD 100 million in a facility in Kona, Hawaii, and a few years later pulled back from the joint venture [8]. Shell's reason for that is the algae biofuel is not yet economically viable and decided to streamline its alternative fuel portfolio. This scenario can be seen in a few other ventures which faced the same issues of economics [9].

While scientists around the world struggle to produce algae at USD 0.50 cents per kg, innovative algae companies are still in the process of optimizing their methods to meet the energetic and economic vision into reality [10]. However, if by any chance the price of oil would shoot up to USD 200.00 per barrel, it is inevitable that algae oil will be the next generation feedstock for the planet [11, 12]. There are many parameters and complex facets for the production of algae oil, and in order to make the process into become a reality, a larger sharing of knowledge and exchange of ideas are needed between all research institutes and companies around the world.

It is sure about algae that it is not a race that we can stop running. In the meantime, we can look into integrated algae projects where one can incorporate various aspects, such as:
- Sequestering of CO_2
- Cleaning of waste water
- Production of biomass feed for both human and animal
- Integration of oil production into the whole system to minimize cost and increase profitability

5 Potentials and Opportunities of Algae

The algae industry is a brand new industry that will change how people think about different traditional sectors, such as waste water remediation [13, 14]. To tap the vast opportunities algae can bring, one has to look into different aspects of the problems that algae can tackle. The vast potential and opportunities of algae has been generally categorized under these few topical points:

CO_2 sequestration is a huge problem faced by many different industrial sectors, from cement plants, power plants, fermentation plants and many other CO_2 emitters, including auto vehicles etc. The quest for clean and sustainable source is ongoing and the most promising so far is algae [15].

As all countries are segmented into their development and income status, the Clean Development Mechanism (CDM) has to face the reality that the GHG effect is not an individual country or regional problem, but rather a global issue. Developed nations that have been plundering the earth since almost 150 years ago with the industrial revolution, were not accountable for their pollution in the past, yet the newly emerging nations whose income per capita is still relatively low are held accountable and are facing harsh criticism for their effort to develop. USD 10.00 in Bangladesh will have a very different value to the same amount in the USA [16]. So it is unfair to ask such a poor nation pay for the pollution emitted by the developed nations a hundred years ago. It is therefore not a surprise that every climate meeting held for the purpose to realize the clean and sustainable future has never been successful in reaching an agreement. Algae can be a great ambassador in realizing a clean and sustainable future, as algae can virtually grow in all climates, and naturally, in all countries [11, 17].

Waste water has always been regarded as a problem faced by many industries and a natural price to be paid for development. There are many different types of waste ranging from animal waste (from cows, pigs, chicken, etc.), industrial waste, to aquaculture waste such as from fish farming and shrimp farming. There are also organic processing waste from sugar factory deriving from sugar cane, cassava and sago. These are all nutrient rich waste in which algae can actively thrive on [11]. These large amounts of waste are the usual suspects for polluting streams and rivers. The waste water steams, alongside with artificial man-made ponds and lakes are a huge source of energy that have largely been remained untapped for a very long time. Even though algae they are well known for their high resources in nutrients and energy, until recently, only a few studies have been done to look into these potentials [1]. In most cases, they are regarded as cost centres where huge investments are required to ensure that the final effluents conform to the requirements of each nation. Algae can harness these hidden sources and be processed to meet various requirements; however the economical means to this solution is yet to be realized.

Feasibility studies looking into these different potentials will be the keys to revolutionize the way how people think about solving waste water issues. Increasing amounts of industries are seriously looking into production of biomass from these waste streams. A very popular phrase now is waste to wealth [11]. Once again algae are the most flexible means to realize waste to wealth, and stands at the pinnacle of this revolution.

A regimented feasibility study should be carried out by different nations and focusing into converting these studies into real projects that will benefit the owners of the farms and various industries. Not only will algae purify their waste stream, but they will also provide a means to produce a certain extent of sustainable energy.

6 Financing Opportunities for Algae

Generally, banks are still skeptical about the algae business. Most traditional algae companies are very quiet on their activities and investments on algae business are not considered to be an important stream like farming, real estate developments, or manufacturing. In fact, algae culturing is yet to get its own category, it lies somewhere within farming, aquaculture and biotechnology. Algae business has yet to be crowned a proper name as an industry.

Traditionally, the algae windows of opportunities have been mostly in R&D. Not many SMEs have succeeded in raising a significant amount of money for algae projects. The larger algae companies, normally controlled by well-known businesses, have been known to raise significant amount of investments into energy projects which are mostly still in the R&D stage.

Algae are not only meant for energy sustainability, but also for food. As conventional agriculture faces more and more challenges in terms of soil and land area, water availability and the rising price of fossil oil for energy, alga culture shall merge as the new phenomena that will save the situation. Almost 80 % of all vitamins, antioxidants consumed today whether it is for animal feed or human are derived synthetic. The dramatic change on how human perceive synthetic foods is becoming clearer, as an increasing amount of individuals are demanding for pure organic foods [18, 19]. As this trend becomes a norm within the next decade, so will the future of the chemical and phyto-vitamins industry. Algae, the source of all other feed shall become the main source of all required organic ingredients, from nutraceutical to pharmaceutical and animal feed [9, 10]. The world will change and algae culturing shall play an important role in defining the future of agriculture [20].

There will be a wave of new investment opportunities for the algae industry, and a whole new algae economics that will churn out new companies dealing with the primary and secondary part of the algae business, from the design, construction, engineering and other parts of the entire algae chain. In a more sustainable future energy and food can be produced out of brackish and/or marine water, CO_2 and sunlight. This can change the way we look at traditional agriculture, where so much is wasted in every single kilogram of grain or rice produced. Algae can hold the key to unlocking a world were human population will strive without generating useless waste but rather waste which can be recycled for the benefit of all living creatures and ecosystems on Earth [21].

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11 Drivers, Potentials and Challenges for the Development of Microalgae Industry in Developing Countries

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Abstract The paper analyzes opportunities for microalgae applications in developing economies. Existing trends and barriers are presented including geographic factors and resource availability, technical issues and economic viability. Different applications, approaches and technologies for valorization of algae biomass are discussed, including bioreactors and open ponds, production of food and energy, waste treatment and integrated production. The latter is seen as a promising future prospective for developing countries while current applications mainly include nutrients. The role of the international community in supporting R&D in algae, applied technology initiatives, and fostering international cooperation in developing countries is also highlighted.

1 Introduction

Access to healthy food and clean water and affordable energy are the fundamental human rights. Coupled with the environmental safeguard they lay the basis of the sustainable growth. Closely related with most Millennium Development Goals (MDG), they are also the key for improvement of the quality of life in the underdeveloped regions. According to FAO, IFRI and IEA forecasts for 2030, the demands of food and energy will increase at 50% and 40%, respectively. Almost half the world's population will be living in areas of high water stress [1, 2].

The growing concern about the depleting fossil sources, the GHG effect and the relevant environmental and socio-economic consequences made us realize the need to diversify our energy sources. From prehistoric times, crop biomass has always given food and energy to people and till now remains to be the dominant energy source in the developing world, especially in the least developed regions. In Sub-Saharan Africa, biomass (mainly wood and charcoal) constitutes over a half of the total energy consumption of the continent (62% in 1995 and 49% in 2005 according to IEA) being a primary source of energy for over 600 million people. Nowadays a substantial part of renewable energy (biofuels) again comes from biomass, mainly crops. The unsustainable use of land has amplified food crisis in the poorest regions and, sometimes, has led to higher GHG emissions than those from production of fossil energy equivalents. Moreover, producing food and energy from crops can impact heavily on the shrinking resources of freshwater.

Seeking for more sustainable and affordable forms of renewable energy we now look into possibilities of using waste biomass and alternative biomass feedstocks. One of such feedstocks is microalgae or simply algae which apparently can solve the water, energy and food security nexus.

2 Algal Biofuels

In the last decade we have seen an explosion of interest in the topic of making energy from microalgae. Delighted by the promising figures of growth rate and oil content of algae against the background of the growing delusion in biofuels from energy crops, many wanted to see algae first of all as the long-awaited clue to a next generation of sustainable biofuels. Algal biofuels would in fact have many advantages before the traditional ones, namely:

- little or no competition for agricultural land;
- algae produce and not consume fertilizers;
- lower water requirements than traditional crops, tolerance to saline water,
- high biomass productivity;
- sequestration of CO_2 from production facilities;
- potential of improvement via biotech research.
- To cite some challenges or limitations:
- technologies are still in premature phase;
- need for nutrients/water;
- high investment costs;
- need for local research and country-region specific assessments, selection of native strains;
- other risks and issues include danger to ecosystems, waste water disposal and replenishment, sensibility of algae to toxicity, etc.

Microalgae indeed seem to be an ideal biofuels feedstock. However, approaching the practical systems and industrial scale many technical complexities of microalgae cultivation were revealed resulting in low productivity and too high energy requirements. So far, there is still no commercial production of bioenergy from algae, except few demonstration initiatives in industrialized countries, e.g. the Great Green Fleet initiative by US Navy [3]. In the industrialized world, many hundreds of millions dollars have been invested to support research and demonstrate viability of algae based alternatives to fossil fuels, including initiatives by US DOE and DOD. However, the results of these initiatives are far from optimistic. According to the report of the US National Research Council, algae biofuels are still underperforming in both economic and environmental terms, thus rendering unsustainable their substitution for at least 5% of transportation fuels in US [4]. In 2009, ExxonMobil partnered with Synthetic Genomics Inc. in an ambitious initiative to commercialize algal biofuels. According to the developers, new synthetic algae strains need to be discovered because the natural ones do not meet the requirements of economic sustainability [5].

Productivity forecast and economic analyses of algal biofuels vary significantly, where the productivity figures are often overestimated, e.g. some reporting over 60 t ha-1 yr-1 (corresponds to ca. 2% of solar energy conversion - the highest realistic value achievable in practice) or even exceeding the theoretical efficiency of photosynthesis of algae (12%). According to opinions of some experts, oil prices should rise above US $200 per barrel before the algal biofuels may become truly competitive, or even above US $800 (for cultivation in photo-bioreactors) [6, 7].

The net energy ratio (NER, produced vs. consumed energy) of algal biofuels produced in photo-bioreactors (PBR) are close to 1 at the farm gate which is the same as for the subsidized corn ethanol [4]. The law of the minimum NER says that either for fossil and bio-energy it should be at least 3. It is the excessive use of energy, needed for mixing, harvesting and oil extraction, production of nutrients and equipment that makes the algal biofuels so expensive. Forecasts report considerably higher production costs of algal biofuels than fossil fuels or traditional biofuels [8]. The price of algae oil is lower for the raceways technology compared to the expensive PBR, though it is still far above conventional vegetable oils. Among different technologies, only fermenters are approaching the oil costs of 1 US dollar which is far higher than conventional energy crops. Yet, significant research is to be done to make the fermenters technology mature. In general, research on strains, identification of locations, and optimization of energy inputs along the production chain are still main challenges for algal biofuels.

3 Algae for Food Production

A more realistic avenue for algae in the developing world can be seen from a food perspective. To add to the facts that algae do not compete with agricultural land and require less water than crops, it is worth noting that algae offer healthy food, as well as pharmaceuticals and high added value products.

The poor quality nutrients in developing countries are the real issue, causing obesity and various health problems [9]. Malnutrition is often more challenging than starvation. Lacking vitamins, minerals and essential amino acids in many developing regions poses significant risks especially on children. Many microalgae possess a very high nutritional value including the content of proteins and amino-acid profiles, carbohydrates and lipids. Algae are a source of many vitamins and antioxidants. One of the advantages of microalgae is the high content of rare polyunsaturated fatty acids, such as EPA and DHA which reduce cardiovascular risk by lowering plasma triglycerides and oxidative stress [10]. Historically the source of these acids was the oil of predatory fish species. However, the origin of EPA/DHA in aquatic ecosystems is algae. Now, organically produced DHA-rich microalgae oil is available.

Spirulina and Chlorella are the well-known microalgae for their wide use as dietary supplements for humans and livestock in dry form. The cyanobacterium Spirulina (recently reclassified as the Arthrospira species) has been rediscovered several decades ago. It was harvested from natural blooms by Aztecs in 16th century [11] and in Chad Africa in 9th Century [12]. Today, Spirulina is used in aquaculture and poultry industries [13], but its main potential is seen to be that of combating malnutrition.

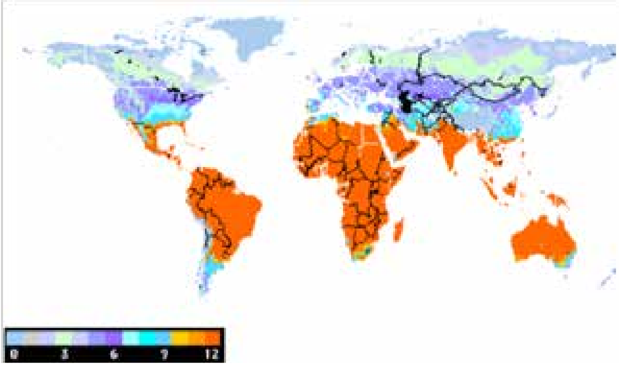
Spirulina contains 26 times more calcium than milk, 20 times protein more than milk and 6 times more protein than eggs [14]. It has all the essential amino acids with amounts of methionine, cysteine, and lysine lower than in meat, eggs, and milk but superior to their content in plants, including legumes [15]. It is a richest source of iron and beta carotene, as well as of vitamins and necessary enzymes and minerals. Spirulina extracts have been found to inhibit HIV [16].

Back to 1974, the UN recognized Spirulina potential to become a possible best food of the future. Today, Spirulina is grown in numerous countries in Africa (Mali, Burkina Faso, Niger, Mauritania, Senegal, Ivory Coast, Benin, Togo, Cameroon, CAR, DRC, Kenya, Malawi, South Africa, Madagascar, etc.), Asia (India, China, Thailand, Philippines, Cambodia, etc.), Europe (France, Spain, Italy, etc.) and America (United States, Mexico, Ecuador, Brazil, etc.). Spirulina has been rated G.R.A.S. (Generally Recognized As Safe) by the United States Food and Drug Administration and China has declared it a national food [14].

Today, nearly 9,000 tonnes of algal biomass is produced commercially for food supplements and nutraceuticals [17]. Food applications are the only widely explored commercial use of microalgae today. The market of nutraceuticals or dietary supplements is estimated to US $106 billion with the annual growth rate of 6.2%. Fatty acids are the fastest growing market, with the annually rate of 16%. Market demands for omega-3 fatty acids exceed current industry production capacity. Many current market suppliers of omega-3s are experiencing over 20 percent annual revenue growth for algae-based ingredients in food and nutritional products [12]. Applications of microalgae as feed for fish and animals are the other growing market opportunities.

4 Need for Resources: Land, Sunlight, Water, Nutrients

There is a very high potential of microalgae to create market in the countries with abundant sunlight, for example in Africa, South Asia and Latin America (see Fig. 1). Continuity of sun intensity throughout the year and constant temperatures are the favorable factors to reach high productivity of algae biomass.



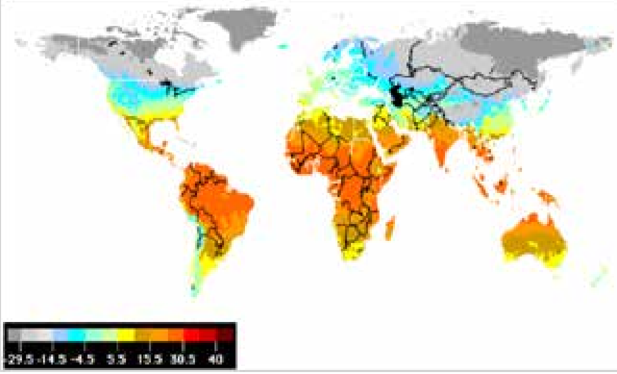


Figure 1. Number of months with average temperatures exceeding 10 °C (A) and average monthly temperature of coldest month in °C (B) [18].

As it was mentioned, an advantage of using microalgae to meet energy and food demands is that they do not require fertile land which can be an issue in some developing countries. In fact, algae could grow on arid lands or even in deserts, provided that ther is water and nutirents supply. Water is one of the principal concerns of the sustainable resource use by microalgae. In many developing regions water is given the same priority as energy and food. Especially freshwater resources are often limited. According to some forecasts, the race for traditional biofuels can result in the increase in global water consumption by agriculture by 20-100% [4], but would the next generation biofuels from algae help it?

A modeling study [19] compared two cultivation technologies (A) an autotrophic system with open pond raceways and seed cultivating PBR, and (B) a heterotrophic system with closed bioreactors for production of biodiesel. Table 1 reports the requirements of various resources per unit of energy produced. Data for the heterotrophic system, which requires organic carbon sources, include the upstream processes of sugar production from switchgrass. Compared to the real data for soy biodiesel and petroleum diesel, the calculations suggest that the only benefit appears to be a lower land use in the case of the autotrophic system, while significantly higher water resources are required for both.

Table 1. Environmental burdens to produce MJ of energy in the form of biodiesel per year using different algae systems, results of a modeling study [17].

	Process energy, MJ	**Water, L**	**Fertilizer, g**	**Land, m2**	**Net GHG, g CO_2 eq**	**Waste water, L**

Autotrophic system	0.5	160	5	0.02	27	120
Heterotrophic system + switchgrass	3	176	17	0.897	138	140
Soybean biodiesel	0.6	32	3	0.18	33	0.03
Petroleum diesel	0.2	0.01			94	0.007

Fortunately, not all microalgae require freshwater. Numerous species grow in saline water e.g. Dunaliella Salina (source of β-carotene), Nannochloropsis (biofuels and omega-3), etc. Open cultivation systems, which are the only economically viable option of cultivation of algae in developing countries, require large amounts of water because of evaporation and hence have to be built in the vicinity to water sources.

5 Environmental Applications of Microalgae

One of the key MDGs is to ensure access to drinking water. The lack in wastewater management practices is one of the factors causing the freshwater scarcity and poor quality of drinking water, especially in the highly populated regions of Africa and Asia. The use of microalgae for wastewater treatment is an example of innovative and holistic approach in wastewater management which was mentioned among the 19 solutions to Global Freshwater Crisis according to the Globescan/ Sustainability Survey Poll on Water [20].

Contaminated and wastewater from municipal, industrial and agricultural activities is the environmental issue of global dimension. Excessive nutrients are causing eutrophication and algal blooms that endanger natural ecosystems. The 3rd Global Biodiversity Outlook report published by UN in 2010 [21] notes the alarmingly growing number of 'dead zones', which are 'the coastal sea areas where water oxygen levels have dropped too low to support most marine life' (see Fig. 2). The phenomenon is caused by the fertilizers which are washed from inland agricultural areas and lead to the growth of algae in the sea. The latter 'die and decompose on the seabed, depleting the water of oxygen and threatening fisheries, livelihoods and tourism'.

Figure 2. Dead zones caused by the buildup of nutrients in the coastal zones [18].

Application of microalgae for wastewater treatment can be a relevant and short term option to produce value and to solve environmental problems related to wastewater management in developing countries. Cleaning wastewater microalgae time produce biomass at the same that can be used as fuel, feed or fertilizer. Because microalgae consume the ionic NH_4+, NO_3-, PO_{43}- species from contaminated water their biomass can offer a valuable source of nitrogen and phosphorus as organic fertilizers. Use of algae for bioremediation also solves the problems of the use of chemicals and of energy costs compared to traditional techniques.

Most microalgae currently applied or studied are the autotrophic cultures which build their biomass via the photosynthetic route. Because of the higher replication rate, algae are able to fix CO_2 several times faster than crops. The conventional facilities where algae are grown at atmospheric CO_2 concentration do not reach the maximum productivity. A number of algae species can assimilate the CO_2 concentrations up to 100% in a range of temperatures (see Fig. 3) which makes it possible to use algae facilities directly at CO_2 emission sources.

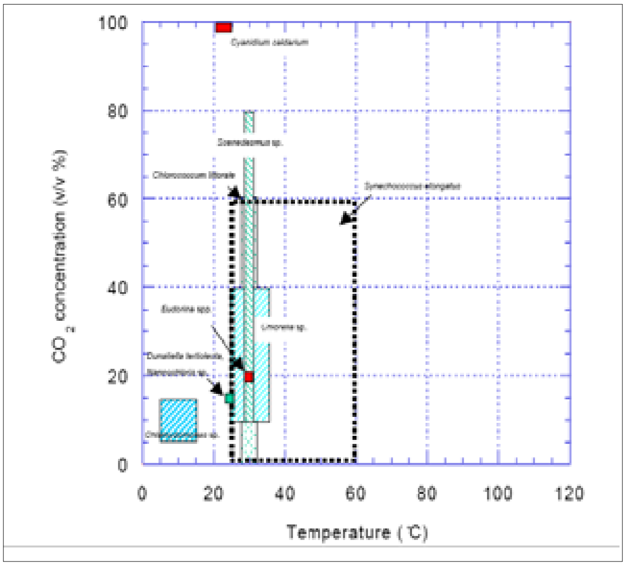


Figure 3. Habitable temperature-CO_2 concentration conditions of microalgae species [22].

Same as bioremediation, the environmental benefits of algal CO_2 sequestration facilities can become economically sustained given the possibility to use the biomass accumulated for energy purposes (due to the presence of contaminants it does not have a nutritional value). An economically viable solution in this case can be the anaerobic digestion process which produces bio-methane.

The added value through energy production in this case is complementary to the environmental financing, such as carbon credits and waste management services. For that reason, incentives in local policies can accelerate the development of environmental algae applications.

6 Technology Selection and Feasibility Assessment

Production of biofuels and other products from microalgae requires that realistic assessments be made for climatic conditions, water requirements, nutrients and sunlight availability together with any risk or potential social or environmental impacts. As there are so many variables and options there are no universal solutions but specific ones either for food, energy or wastewater treatment applications of algae. Especially in developing countries, considering the high degree of uncertainty of existing background data, poor infrastructure & supply and the lack of relevant experience the thorough analysis of risks is mandatory. As the starting point, the general assessment for countries regions on potential algae applications (a NREL study for India [23] is an example) would be very useful. New microalgae projects should obviously consider and benefit from former experiences and the necessary awareness and knowledge and technology transfer should be enacted through the international South-North and South-South cooperation, while local parameters have to be always given careful analysis.

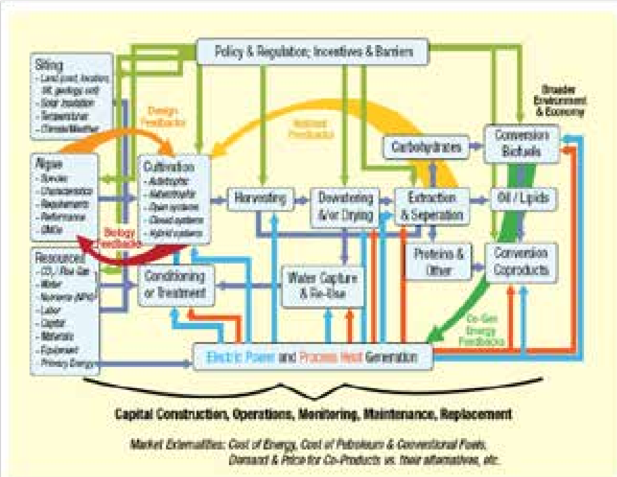


Figure 4. Coupling and interdependences across the algal biofuels supply chain [24].

For the case of microalgae energy applications, Fig. 4 outlines the complexity of production chain options and related factors that influence project performance. Sometimes general recommendations can be helpful for certain specific issues along the production chain. One example is the choice of the cultivation technology, i.e. closed or open system. There is no substantial difference in productivity, except at low temperatures, between ponds and PBR. The main advantages of PBR include lower culture contamination and better biomass concentration (see Table 2).

Table 2. Comparison of microalgae cultivation technologies, adapted from [21]

		Advantages	Disadvantages & challenges
Photo-autotrophic systems	PBR	Less loss of water Long term culture maintenance Higher cell concentration	Cost and scalability Temperature control and cleaning required Maximum light exposure
Open ponds	Evaporative cooling to maintain temperature Cost	Sensitive to climatic conditions Culture contamination	
Heterotrophic systems	Better control of conditions and cell purity High biomass concentrations	High GHG balance Cost and availability of feedstocks	

Open ponds is the main technology of commercial microalgae cultivation which is used for production of 95% algal biomass. The open ponds technology is also particularly suitable for projects in developing countries because of the labor costs constitute a bigger part of the cost breakdown. Much more expensive PBR have several advantages, but their applications are limited mainly to high added value products or in the locations where the open ponds are not efficient due to climatic conditions. A comparison of costs for raceways and PBR is presented in Table 3.

Table 3. Economic comparison of open ponds and photobioreactors [25]

	Open ponds and raceways	PBR
Start-up	6-8 weeks	2-4 weeks
Capital costs	High ~ US $100,000 per hectare	Very high ~ US $250,000 to 1,000,000 per hectare (PBR plus supporting systems)
Operating costs	Low (paddle wheel, CO_2 addition)	Higher (CO_2 addition, oxygen removal, cooling, cleaning, maintenance)
Harvesting cost	High, species dependent	Lower due to high biomass concentration and better control over species and conditions
Current commercial applications	5,000 (8 to 10,000) t of algal biomass per year	Limited to processes for high added value compounds or algae used in food and cosmetics

Given the complexity of the algae production systems and a number of factors which are locally specific or external to the technology, it can be recommended to carry out a detailed Techno Economic Analysis (TEA) for microalgae projects [21, 26, 27]. The TEA would be based on computer modeling of costs and technical details (e.g. energy yield) of the entire value chain of algae production and supported by other tools like LCA and GIS to provide decision support for the assessment of cost/benefits tradeoffs and of the environmental impact, as well as for the optimization of siting of new production facilities [21].

7 Role of International Organizations and Development Aid Community

It is important that all stakeholders including policy makers, investors, researchers and entrepreneurs are involved in the definition of efficient national strategies for the development of microalgae industry. Cooperation of parties locally and internationally should lead to mutual benefits and understanding and minimizing risks. The role of development aid community including international institutions NGO, national agencies can be instrumental in promoting education and knowledge transfer among different stakeholders, stimulating policy making, and supporting pilot production projects.

Because of their multidisciplinary and multipurpose nature, algae projects are potentially within the scopes of different organizations and programmes dealing with environmental protection, agricultural, industrial and food aspects. Below there are just several examples of international programmes and initiatives aimed at promoting algae in developing countries.

In 2001, several countries formed the Intergovernmental Institution for the Use of Micro-algae Spirulina Against Malnutrition (IIMSAM) [28]. IIMSAM aspires to build a consensus to make Spirulina a key driver to eradicate malnutrition, achieve food security and bridge the health divide throughout the world. CISRI-ISP is the Spirulina Programme of the Collaborative Inter-Governmental Scientific Research Institute [29]. The institute was established in 2000 under the agreement of several states registered with the UN Secretariat with the goal to produce microalgae biomass addressed to malnourished and victims of famine, draught and food emergencies. The global project aims at building ten productive centers for humanitarian purposes, where the algal biomass so obtained and mixed with cereals will be freely distributed by the Institution, to indigent people and to victims of humanitarian urgencies.

The FAO Inter-Departmental Working Group on Bioenergy reviewed the state-of-knowledge on algae-based biofuels to assess relevance and potential applications in developing countries and in 2009 published a review paper on challenges and opportunities of algae-based biofuels for developing countries [22]. The FAO paper recognizes the potential benefits of algal biofuels for developing countries in the long term while points out at significant challenges related to the attracting foreign investments, building capacity of institutions, gaining expertise in technologiy and assessing local potentials.

In 2012 the International Centre for Science and Technology of UNIDO (ICS-UNIDO) held a series of events [30] to survey the state of the art in microalgae R&D and commercial experience in developing countries and to identify strategies to promote cooperation and attract of SMEs in the algae sector. The following needs, recommendations and conclusions were drafted in an international forum of experts from R&D, academia and private sector:

- Studies on CO_2 balance acceptable for CDM revenues are needed.
- Nutrients and high added value products as well as application for remediation purposes in developing countries are to be given priority to biofuels/bioenergy production.
- Biorefinery concepts for algae should produce biofuels/energy as secondary products
- Open ponds are the most feasible technology for developing countries.
- Biogas can be considered as the most currently feasible technology for bioenergy from algae but needs to be linked to other primary application concepts.
- Emerging technologies, e.g. thermochemical, supercritical, ultrasound, pressure and other aimed at the pre-treatment/extraction/conversion of useful substances from algae need to be further improved and feasibility studies performed.
- Centrifugation to concentrate algae biomass must be avoided, especially for bioenergy applications.
- Developing countries have to look into local opportunities and needs (e.g. study and valorization of locally occurring cultures, wastewater treatment).
- The productivity of algae and the economic feasibility have to be carefully evaluated when planning commercial projects.
- Closer cooperation between R&D and industry is needed.
- Support to knowledge transfer, training and e-learning from community is needed.
- Support to local research and demonstration projects is needed.
- Investments and governmental support to commercial projects are needed.

8 Conclusions

According to the opinions of many experts, the most feasible scenarios for the development of microalgae industry in the short term future will be food and environmental applications rather than biofuels. However, the integrated production of energy from secondary streams of algae facilities is very attractive. Such algae biorefineries combining food/products, water remediation or CO_2 sequestration with added value through energy production would bring multiple benefits to the community at a higher economic sustainability.

The favorable climatic conditions and low labor costs are important factors that can help developing countries taking the lead in the emerging microalgae industry. The main challenges of the successful adoption of commercial algae projects are the accurate feasibility and techno-economic analysis studies, access to investments, cooperation and capacity building of local specialized institutions and R&D to enable smooth technology transfer and implementation. Last but not the least, the favorable policy should be put in place to stimulate investments and market development in microalgae products and applications.

There is a promising potential that the successful development of microalgae in developing countries can create a new independent niche in the agro-industrial sector which will rely on the market of specific products, specific equipment, specific knowledge and skills. This new sector is capable of generating new profits not only from the industrialization of developing economies but is also through the stimulation of agri and aquaculture. Algae industry will not compete with agriculture for the land and market, but instead will supply fertilizers for crops, feed for aquaculture and energy for rural areas. Environmental applications of microalgae are also closely related to the improvements and needs of local industrial and agro-practices. As a consequence, substantial benefit is seen from the social standpoint because new jobs will be created locally, including highly specialized jobs and R&D. Most algae technologies are scalable and algae products are high valued, so unlike other second generation biofuels and agro-food industries this new sector will be attractive to SMEs, given the necessary policy work and awareness building.

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Case Studies

Sequential Heterotrophy-Dilution-Photointroduction
A Novel Culture Model Achieving Microalgal High Biomass Productivity, High Quality and Rapid Lipid Accumulation

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Abstract Traditional algal industry for large-scale applications has still been limited by the low yield and high cost of biomass production. Our group has focused on this field for almost eighteen years and initiated a novel cultivation strategy called "Sequential Heterotrophy-Dilution-Photoinduction" (SHDP) as platform technology for efficient algal biomass production. SHDP has been confirmed as a cost-effective and scale-up feasible culture method for algal biomass mass production. Three Chlorella species were first cultivated heterotrophically to achieve high cell density, then the broth was diluted to suitable concentration (2~5 g/L) and transferred to light environment for photoinduction. With this strategy, the Chlorella intracellular protein, pigments and lipids increased rapidly within several hour, which make the quality close to the level of cells cultivated photoautotrophically. Therefore, the proposed strategy provided an effective approach for microalgal biomass production to meet the urgent need for both health food and biodiesel.

Keywords Microalgae, Sequential Heterotrophy – Dilution – Photoinduction, Chlorella, Biomass, Lipid.

1 Urgent Need for Novel Microalgale Culture Method

Microalgae have been extensively investigated and exploited in series of products owing to its high nutritive value. More recently, microalgae, which are able to grow fast and accumulate lipids either autotrophically or heterotrophically, have become one of the worldwide focuses as a potential biofuels feedstock. Although there are wide uses of microalgae, the large-scale applications have still been limited by the low yield and high cost of microalgal biomass production. Therefore, it is urgent that an efficient microalgae production strategy is needed to be developed.

Conventional commercial production of algal biomass, such as Spirulina spp., Chlorella spp., Dunaliella spp. and Haematococcus spp., are typically accomplished by the photoautotriphic cultures in open ponds. The option using photoautotrophic cultivation of microalgae for biofuels production also seems compelling and technically feasible. However, the low productivity and high production cost of this culture model seriously hinders the development of the microalgal industry. Furthermore, its large area coverage, uncontrollability of growing conditions, susceptibility to biological contamination and climatic condition are also the main limiting factors. Thus, several closed systems with better light supply and control of culture conditions have been investigated. However, compared with the open systems, the high cost for the suitable material and construction of the enclosed culture equipments are unnegligible problems. Therefore, researchers have focused on these problems and attempted to develop certain alternative culture systems to meet the urgent need for both algal health food and biodiesel feedstock.

Some algae species are able to grow heterotrophically where organic carbon serve as carbon source. Through heterotrophic cultivation, the cell density and productivity can achieve considerable high levels. However, under heterotrophic conditions, microalgae are lacking in photosynthetically derived compounds, leading the loss of its main advantage and practical application value. In addition, the source of organic carbons is an issue, as is the potential for contamination of cultures by heterotrophic fungi and bacteria. Thus, although a high biomass or lipid productivity could be achieved, the high cost of the organic carbon sources of heterotrophy and its poor biomass quality due to low photosynthetic product content probably do not meet the requirement of large-scale development and nutritional demand. In order to produce a high cell density with high cellular photosynthetic components, a few studies have been carried out. Ogbonna et al. heterotrophically cultivated C. pyrenoidosa in a 2.5 L fermenter to obtain the culture broth with high biomass concentration and then simply transfered it to an internally illuminated photobioreactor for autotrophic cultivation to improve chlorophyll and protein contents. Similar results were also achieved in a high cell density and a high level of valuable components in other alga species by a cyclic or successive heterotrophic/autotrophic cultivation. Moreover, Xiong et al adopted a photosynthesis-fermentation model to merge the positive aspects of autotrophs and heterotrophs, where C. protothecoides was firstly grown autotrophically for CO_2 fixation and then metabolized heterotrophically for oil accumulation. The above achievements demonstrated the notion that such two-step culture systems were desirable to obtain high biomass with high quality (high cellular photosynthetic components or high lipid content), yet it should be pointed out that the reported systems may be only applied to laboratory-scale research, but are not feasible in large-scale industrial production, as the scale-up of photobioreactors they used may have a lot of troubles. Photobioreactors are highly expensive to both construction and operation. In general, the photobioreactors are manufactured by glass and it is very difficult to sterilize the photobioreactors completely with steam. Besides, their operational difficulties may also include light limitation, high oxygen concentration inhibition and cleaning problems. Furthermore, it's difficult to maintain the cyclic culture process being stable for a long period due to the unavoidable contamination. The most serious problem with the systems reported is that, the light as the sole energy source would be insufficient for such high dense cells in photoautotrophic cultivation systems, resulting in death or extending the period of cultivation. Thus, the exploitation of a cost-effective and scale-up feasible culture method for high biomass and high quality microalgal production, especially for mass algal oil production, is particularly significant.

2 Chlorella and Sequential Heterotrophy – Dilution – Photoinduction

Green algae in the genus Chlorella is single-celled, microscopic plant that grows in fresh water and is believed to have been around for billions of years. It contains the highest amount of chlorophyll of any know plant and has been served as a popular food supplement and animal feed in Asia, U.S. and Europe for centuries. One specific niche of Chlorella spp. is the use of their heterotrophic growth capacity in the absence of light, replacing the fixation of CO_2 of autotrophic cultures with organic carbon sources dissolved in the medium. This unique ability of essentially photosynthetic microorganisms is restricted to a few microalgal species. Cost effectiveness and relative simplicity of operations and daily maintenance are the main attractions of the heterotrophic growth approach. For Chlorella, a side but significant benefit is that it is possible to obtain, heterotrophically, high biomass productivity that provides an economically feasible option for large scale cultivation. Heterotrophic Chlorella can remarkably increase its biomass yields, resulting powerful advantages of using heterotrophic cells as seeds for subsequent phototrophic growth for lipid production. Many recent studies showed that heterotrophic cultures of Chlorella were gaining increasing interest for producing a wide variety of microalgal metabolites at all scales, from bench experiments to industrial scale. However, traditional Chlorella industry for large-scale applications has still been limited by the low yield and high cost of biomass production by photoautotrophy.

Although the Chlorella cell density achieved by heterotrophic cultivation was much higher than those of other culture methods, previous studies revealed that the proportion of its intracellular photosynthetic components was low. In addition, higher cell density was easily obtained by increasing the glucose concentration when cultured heterotrophically, but it was difficult to efficiently supply light to such a dense culture solution (Fig. 1).

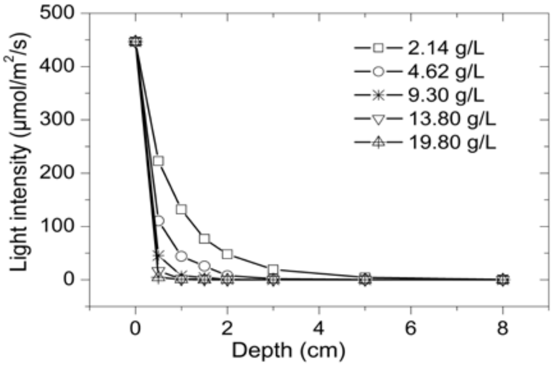

Figure 1. Light attenuation curve inside C. vulgaris suspensions with different cell concentration (incidence light intensity I0 = 447 μmol/m^2/s).

Our group has focused on this field for almost eighteen years and initiated a novel cultivation strategy called "Sequential Heterotrophy-Dilution-Photoinduction" (SHDP) as platform technology for efficient algal biomass production. SHDP

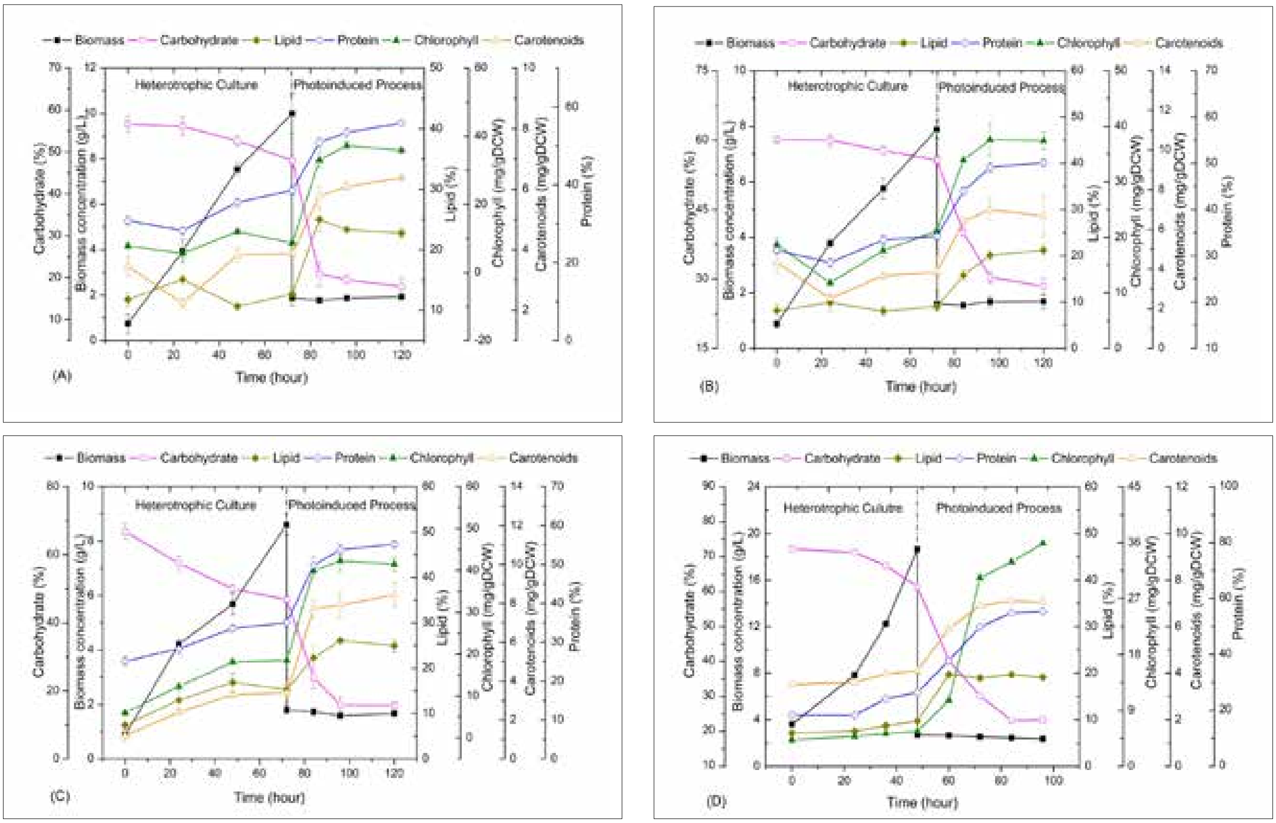

Figure 2. Alga growth and the cellular component temporal pattern characteristics during SHDP. (A) *C. vulgaris* in flasks; (B) *C. ellipsoidea* in flasks; (C) *C. pyrenoidosa* in flasks; (D) *C. pyrenoidosa* in 5-L fermenter/3-L flat panel photobioreactor. Error bars represent the mean ffl standard deviation (SD) of three independent biological replicates

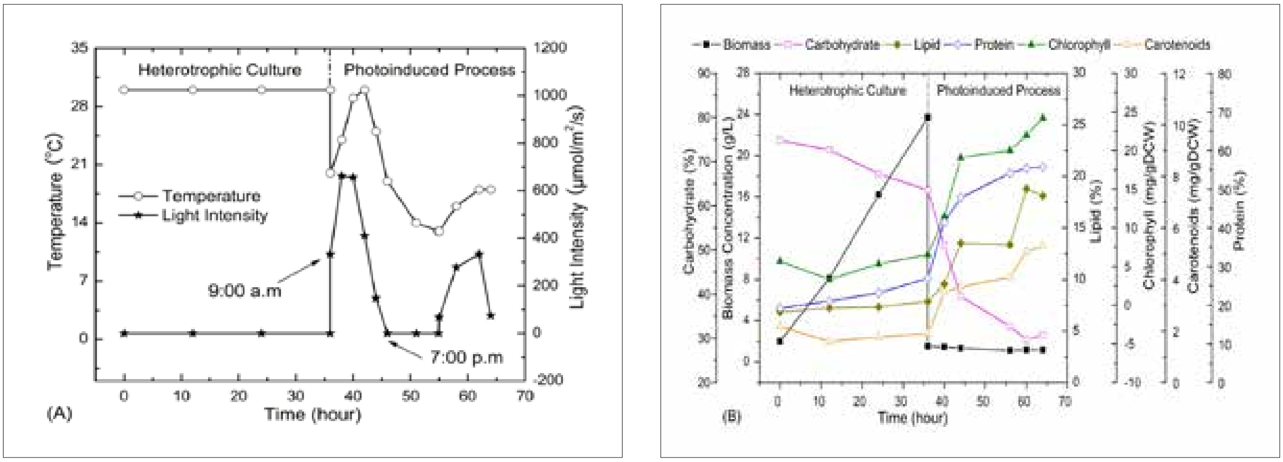

Figure 3. *C. vulgaris* growth and the cellular component temporal pattern characteristics during SHDP in 50-L fermenter/60-L outdoor plastic basin. (A) Natural tendency of day-to-day weather conditions; (B) Growth and the cellular component temporal pattern characteristics

has been confirmed as a cost-effective and scale-up feasible culture method for algal biomass mass production. Three Chlorella species including C. pyrenoidosa, C. vulgaris and C. ellipsoidea were adopted to confirm the feasibility of SHDP in a large scale approach for microalgal mass culture process (Fig. 2). High biomass productivities and high photosynthetic products were achieved, as well as intracellular lipids rapidly accumulated within several hours. Further outdoor studies were also carried out to confirm the feasibility of SHDP in a large scale approach for microalgal mass culture process (Fig. 3). Analysis of cell components and electron micrographs studies all suggested that shift from heterotrophy to photoautotrophy caused a rapid decrease in carbohydrate and corresponding increases in other intracellular components (protein, lipids, and pigments) within several hours (Fig 4).

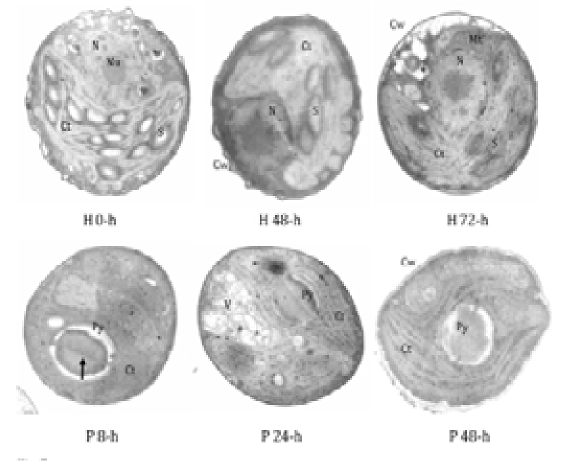

Figure 4. Changes in ultrastructure of C. pyrenoidosa during SHDP. N: Nucleus; Nu: Nucleolus; Ct: Chloroplast; Cw: Cell wall; S: Starch granule; Mt: Mitochondria; Py: Pyrenoid; V: Vacuole. Arrowhead indicates the thylakoid membranes penetrating the pyrenoid matrix. H means heterotrophic culture phase. P means photoinduction process.

It was important to ensure that the glucose was completely consumed by the end of heterotrophic culture process, otherwise the residuary glucose would cause serious contamination in the photoinduced process. Besides, the algal cells would also grow mixotrophically so as to influence the induction effect. It should be pointed out that dilution was critical before photoinduction, as the optimum duration of the photoinduced process would depend on the biomass concentration and the light supply coefficient of the illumination apparatus. It was found that dry cell weight decreased by 10~20% when the high-density heterotrophic cells were directly transferred into light environment without any dilution (Fig. 5), which was consistent with the results obtained in the literature, while further dilution caused a slight increase in the biomass concentration after light illumination (data not shown) and would also result in an increase in the harvesting costs. A putative reason for suitable dilution was that, in the photoinduced process, the cells with a high density (i.e., more than 5 g/L) might be lacking in metabolic energy, resulting in death, because the glucose had been depleted and light as the sole energy source was insufficient for such dense cells (Fig. 1). When the density decreased, the light energy supplied (300 μmol/m^2/s) could approximately meet the required metabolic energy balance, keeping the final density steady. The cells may also die due to the drastic change in cell metabolism from oxybiotic chemoorganotrophy to oxygen-evolving photolithotrophy, because we observed that the slight decrease in density mainly occurred in the early photoinduced period (0-12h, Fig. 2 and Fig. 3), and even if the broth was diluted to a very low density and cultivated with enough light, the density still decreased at the beginning and then reverted.

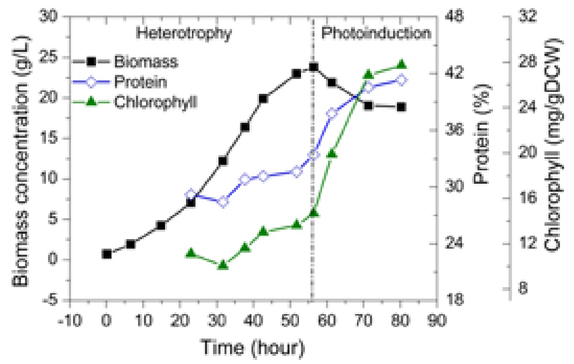

Figure 5. C. vulgaris growth and the cellular component temporal pattern characteristics when cultured in 5-L fermenter/3-L flat panel photobioreactor (without dilution).

As shown in Fig. 2 and Fig. 3, the total lipid could be accumulated to high levels by SHDP within several hours. In general, alga growth and lipid accumulation are inversely related, and the increment of lipid content usually occurs in many microalgae as a response to different stress conditions. In fact, sudden light irradiation for photoinduction is also an excess light stress to the heterotrophic cells. Under such photo-oxidative stress, the algal cell quickly stops division and accumulates TAG as the main energy storage. The fatty acid compositions of the three Chlorella cultured by SHDP are shown in Fig. 6. C14 to C18 are present, with the most dominating acids in three Chlorella being palmitic acid (16:0), hexadecatrienoic acid (16:3), linoleic acid (18:2) and linolenic acid (18:3), accounting for over 70% of the total fatty acids. No FAs with more than 20 carbon atoms were found in any sample. The result indicated that SHDP had the potential as a novel model for large scale microalgal biofuel production. SHDP could also help to meet an urgent need to provide a lot of feedstock for biofuels exploitation at this moment as the purely photoautotrophy's low efficiency now.

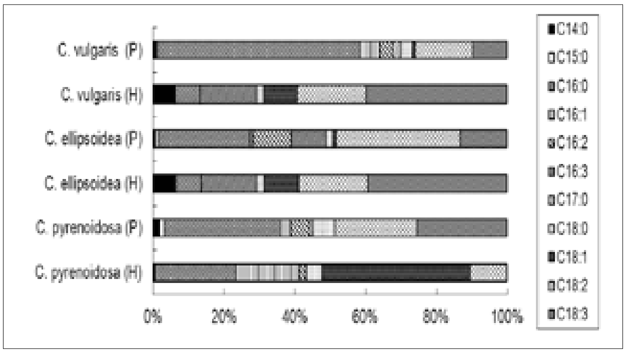

Figure 6. Fatty acid profiles of three Chlorella species during SHDP in flask. H means heterotrophic culture phase. P means photoinduction process.

3 Conclusion

The results in this report suggested that the three Chlorella species cultured by SHDP were very versatile on features, which allowed their broad uses in various applications. The main advantage of SHDP over the previous strategies was that heterotrophic culture and photoinduction were divided and could be optimized independently, which made SHDP easy to scale-up. In summary, the above SHDP approach, which could achieve high biomass productivity, high biomass quality and rapid lipid accumulation, is expected to have a great potential for the mass cultivation of heterotrophic microalgae to produce both algal health food and biofuels.

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Towards the Production of Epa: a Developing Country Perspective

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Abstract Omega-3 fatty acids have an attractive market potential as human nutraceutical supplements, as ingredients in functional foods and as animal feed supplements. The current market for these products is dominated by fish oils, which contain omega-3 fatty acids and are marketed in oil filled gel capsules or added to food or feed. Apart from an undesirable taste, the use of fish oils also results in the overexploitation of natural fish stocks. Many consumers are also seeking vegetarian alternatives to animal derived foods. Microalgae are considered alternate sources for omega-3 fatty acids. The two most common omega-3 fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Since microalgal oil rich in EPA is limited, efforts are focussed towards increased production of high purity EPA. This provides a window of opportunity for a supply of competing products that are rich in algal EPA. Recently the CSIR developed a technology for the production of EPA by an indigenous algal isolate designated Isolate A23.2. The process developed by the CSIR integrates multiple unit operations such as biomass and EPA production in a raceway pond system and various downstream processes which enabled production of product intermediates, which were used in the production of several end product prototypes such as functional foods, drinks and nutraceuticals. An intensive process development programme resulted in optimised growth and EPA production and the system was demonstrated to be operationally robust. The techno-economic assessment of the raceway system indicated an attractive business case especially when the business is operated at pilot or production scale. South Africa has significant climatic advantages, experience in algal production and a dire need for job creation and growth in the Bio-manufacturing Sector, therefore commercialization of this technology will provide opportunities for job creation of semi skilled people and further skills development to support a growing algal cluster.

Keywords Omega-3 fatty acids, EPA, DHA, isolate A23.2, raceway system

1 Introduction

Polyunsaturated fatty acids (PUFAs) have been regarded as essential components of human nutrition and valuable as animal feed supplements. They have been recognised as important dietary compounds for the prevention of a multitude of diseases such as cardiovascular and inflammatory conditions [1, 2, 3 and 4]. Omega-3 fatty acids are a group of PUFAs that have been attracting worldwide attention because they are essential for the proper functioning of the brain and for the development of the nervous system [5]. The two main essential omega-3 fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Over the years and even today, omega-3 EPA and DHA are regarded as common constituents of fish oil.

Higher plants, animals and human beings do not have the ability to synthesise these essential fatty acids. The main source has been fish and fish oil, however; there are challenges regarding the use of fish oil products. Fish oil fluctuates in cost and quality and there are other concerns regarding fish oil, such as contamination of fish oil with pesticides and heavy metals [3]. Furthermore, the taste and odour of fish oil is unpleasant, the sustainability of natural fish stocks is under threat and there is increasing consumer preference for non-animal derived supplements and foods. These challenges will limit the use of fish oil, as the demand for EPA and DHA increases [5, 4].

Microalgae have been considered potential alternate sources of DHA and EPA [3]. They are regarded as the best alternative source of EPA because their production technologies are relatively inexpensive and sustainable [6]. Of the two fatty acids, DHA is currently commercially produced [5, 4 and 7]. Although there are various microalgal species reported to produce EPA (*Monodus subterraneus* [8], *Navicula saprophila*, *Rhodomonas salina*, *Nitzschia* sp. [9], *Phaeodactylum tricornutum* [2], *Nannochloropsis* sp. [10] etc.), microalgal oil that is rich in EPA is still limited [4 and 7].

Currently the algal omega market is dominated by Martek Biosciences as the key supplier. Martek's technology has given rise to products such as algal-derived omega-3 DHA in infant formula, dietary supplements, functional foods and beverages, as well as animal feeds. Their products however lack high concentrations of EPA. Some argue that it is not imperative to consume both EPA and DHA as the human body efficiently converts EPA to DHA [11, 12]. Efforts are therefore focussed towards increased production of high purity EPA, thus satisfying the window of opportunity for a supply of competing products that are rich in algal EPA at affordable costs to developing nations. Algal omega-3 products that are currently on the market are not affordable to mass populations in developing countries, where perhaps the need is greatest to reduce disease burden on limited funds. There is also a need and gap in these markets for functional food products supplemented with algal omega-3 EPA to mitigate nutritional deficiencies that contribute to an array of socio-economic problems in developing countries. Incorporating EPA into preferred food products found in most South African households would raise awareness on functional foods as an alternative to supplements. Contrary to the rest of the world, the average South African consumer is unaware of functional foods as an alternative to supplements, and this is primarily due to the lack of marketing, education and awareness, in these poorer societies. The rise in consumer awareness globally has however resulted in an ever increasing market for omega-3 DHA/EPA functional food products. There exists currently an opportunity for product and ingredient manufacturers to fill that gap in the market place by providing consumers with added value. From a South African perspective as a developing country, local climatic advantages of high solar radiation and temperature, a diverse array of indigenous algae coupled to addressing socio-economic and health challenges are driving forces for the implementation of a technology that would give rise to algal EPA production.

The CSIR has recently developed a technology for the production of EPA rich oil using microalgae. This technology comprises a unique indigenous algal isolate, capable of producing high quantities of EPA. The process integrates multiple unit operations such as inoculum development, biomass production, EPA production and a downstream process that enables production of two product intermediates. The product intermediates can be used in the production of various end product forms such as functional foods, drinks and nutraceuticals, some of which have been demonstrated as prototypes by the CSIR. The products have multiple routes to market and satisfy a wide array of consumer needs. The unique production process contributes to the competitive edge of this technology. In comparison to the fish oil products, the CSIR's algal omega oil is sustainable and competitive, thereby offering many solutions to the future challenges of developing countries. The case study of the process and product development is described herein.

2 Screening for Potential Omega 3 Producing Algal Strains

The biodiversity of the South African coastline is remarkable; the western coast upwelling of the Benguela current supports an ecosystem of seaweed, fish, penguins and seals, with the warm east coast having even greater species diversity. The primary producers of both these rich marine ecosystems are algae [13]. Hence, the Microalgal Prospecting Program of the CSIR was launched to sample the natural algal biodiversity of South Africa (Fig. 1).

Figure 1. Environmental sampling across South Africa. Green dots represent sampling sites.

Samples were obtained from harbours, jetties, rock pools, inland dams and rivers. Upon sampling, isolates were kept in sample bottles preloaded with either Artificial Fresh water (AF6) or Artificial Sea Water (ASW) medium depending on the sample environment. During sampling, site specific information was noted and entered into a web based database that allowed a national network of partners to contribute to this program. The database is updated when ever new information pertaining to isolates becomes available.

The ability to screen for algal strains on a large-scale for commercial EPA production requires the identification of high-yielding algal strains, and identifying the most optimal methods/systems to cultivate them. For the commercial production of EPA or any other lipid based product from microalgae, the selected strain of algae must have suitable lipid productivity [14]. Other key factors include the extractability, yield, vulnerability, local conditions, as well as the capability for large-scale conversion of total algal oil into EPA.

Samples collected by the CSIR were isolated by mimicking the naturally occurring environmental conditions [15] in order to revive the organism after transportation from its natural habitat to the laboratory. After cultivation, samples were evaluated microscopically to confirm cell shape, size, colour (general morphology) and purity. There are two methods that were employed during the purification of the mixed algal cultures algal cultures once they were revived in the laboratory. The conventional method involved streak plating onto agar plates [16, 15] and a new automated method using flow cytometry to count and sort cells was also used, which results in the isolation of individual species from mixed samples with high purity and viability [17].

Single colonies obtained from either of the methods were passaged three times to obtain monoalgal isolates. The passaged colonies of isolates contained some bacterial contaminants, which were purified using kanamycin antibiotic and a dilution plate method. Single algal colonies without bacterial or fungal presence were picked and streaked onto new ASW/AF6 plates to obtain an axenic culture. Once monoculture status was reached, the isolate was grown in liquid ASW or AF6 media at ambient temperature and lightning.

In an effort to find an algal strain that produces omega 3, the 12 best high lipid producing monoalgal isolates (out of 500 isolates catalogued in the database at the time), were screened for EPA production. The isolates were screened qualitatively for lipid content using fluorescent microscopic analysis in conjunction with Nile Red dye. After lipid content was confirmed qualitatively, the lipid positive isolates were subjected to a quantitative lipid analysis by gas chromatography (GC). Total lipid was also determined by the Soxlett method [18, 19].

Table 1. Qualitative test for EPA, DHA and DPA in lipid positive isolates.

Isolates	EPA	DHA
A 4.1	-	-
A 15.1	-	-
A 15.2	-	-
A 23.1	-	-
A 23.2	+	+
A 26.1	+	-
A 26.2	-	-
A 41. 1	-	-
A 11.1	-	-
A 11.4	-	-
A 10.3	-	-
A 3.2	+	-
Phaeodactylum tricornutum (+ control)	+	+

Of the 12 organisms, isolate A23.2 was positively identified to contain EPA, using *Phaeodactylum tricornutum* as a positive control (table 1).

Based on the quantitative results obtained from the GC analyses, further studies on the process for production of EPA by isolate A23.2 were commenced.

The microalgal prospecting programme launched by the CSIR involved major research capabilities which contributed to the bioprocessing of algal based products. The aim of this programme was to screen the algal biodiversity of South Africa for indigenous microalgal strains capable of producing valuable products such as EPA. This would result in establishing algal technologies in South Africa as a developing country, using indigenous microalgal strains.

3 Biomass Production

Due to an increasing demand for algal EPA it is imperative that it is produced at a large scale where its productivity can be maximised. There are different systems designed for the production of microalgae at a large scale. Popular options include open ponds and photobioreactors [20, 3, 21], which are the most practical methods for the cultivation of microalgae. The cultivation of microalgae in open pond systems, in South Africa has potential as the climate and environment of the country are conducive to large scale cultivation of microalgae [22]. As funding for large capital projects is not easily available in developing countries, cheaper open pond raceway systems always remain a more attractive option. In South Africa, open ponds are viewed as an extension of agronomic type activities, requiring intermediate skills and potential to create much needed jobs. There is a fair amount of non-arable land, especially in the Northern Cape Region, which also has high solar radiation intensity. As food security is also a challenge in developing countries, the use of non-arable land becomes a key consideration. Open raceway ponds are however prone to the forces of nature and susceptible to contamination, therefore the strain for EPA production had to be carefully selected not only for EPA productivity but robustness against environmental fluctuations, process aberrations and contamination.

The steps towards the development of a high-yield EPA production process by microalgae included optimization of medium components and environmental factors [3]. These factors can significantly affect EPA yield and volumetric productivity. Biomass production by isolate A23.2 was conducted in a similar manner as suggested by [3] where the impact of environmental factors (pH, temperature, light and salinity) and medium composition on biomass were investigated. Results obtained confirmed the marine nature of isolate A23.2, which has process advantages in limiting potential contamination by freshwater microalgae. The nutrients impacting on growth and biomass production by isolate A23.2 were carbonate, nitrate, phosphate and silicate. The isolate assimilated carbonate at a faster rate than the other nutrients; possibly due the fact that carbonate is an essential source of carbon needed for the growth of cells [23, 3]. Following the identification of the key parameters influencing the growth of isolate A23.2, the culture was scaled up from a 2 L scale to a 200 L open raceway pond system where it was maintained in an actively growing state by the continuous feeding of the growth medium.

4 EPA Production

The actively growing culture of isolate A23.2 was thereafter transitioned to unfavourable growth conditions in a cascaded raceway configuration using a sequence of ponds. This was done to enhance EPA production by the isolate. The unfavourable growth conditions were created by limiting the supply of the key nutrients. It has been shown that cells undergo metabolic acclimatization when exposed to nutrient limited conditions; this usually results in changes of the cellular composition of macromolecules [22, 3, 23]. Carbonate was the only nutrient supplemented into the system as it is required for the normal functioning of the algal cells and for enhancing EPA production.

The cultivation of microalgae in open pond systems can be very challenging due to issues associated with contamination by unwanted algal species [3]. This has resulted in only a few species being successfully cultivated in open pond systems at commercial scale. The cascaded system designed by the CSIR showed robustness against contamination by other algal species over a period of ~6 months. The amount of EPA produced on a daily basis under stress conditions was calculated to be three fold higher than the EPA produced under optimal growth conditions. Although an open pond system usually results in relatively low biomass concentration, the construction, operating and maintenance costs of the ponds are very low, therefore rendering this system a competitive cultivation option [20]. The biomass produced from the cascaded system was used in the development of two product intermediates; algal powder and oil.

5 Biomass Recovery And Product Development

The first step towards product development is the separation of biomass from the medium. There are various methods available for the harvesting of biomass; these include centrifugation, flocculation, filtration etc. [20]. The harvest method chosen in our process was filtration, by passing biomass through a sieve. This method was chosen because isolate A23.2 has the tendency to form clumps as it grows. It therefore makes it easier to harvest biomass. Energy intensive separations such as centrifugation were avoided in this process because clumping of the cells facilitates settling and sieving. Although the clumping of cells is undesirable because it hinders the accurate enumeration of cells, it is advantageous in the downstream processing because it results in a cost effective process. It has been reported that about 60% of the costs arise from downstream processing of the biomass [1]. Biomass production only contributes 40% towards the cost of algal EPA. Therefore, reducing the costs of a downstream process can result in a cost effective process, which is an important consideration in developing countries. The cell recoveries and mass losses across the sieving step were negligible as there was little or no slippage of cells to the filtrate. The overall cell recoveries and mass balance closure across the sieving unit operation were 99%, thus implying that sieving is a suitable harvest method for isolate A23.2. Biomass from isolate A23.2 can be easily dried and milled into a fine powder which is a product intermediate that allows the design and production of various functional foods, feed and nutraceutical products. The overall EPA recovery after sieving and drying of biomass was higher than 80%.

The whole cell powder was used to produce whole cell tablets and it was also formulated into food prototypes. These included pasta, an energy drink, sports drink, orange juice, noodles, candy and flavoured yoghurt. Oil was extracted from the powder by using a solvent extraction method. The extracted oil was then used to make oil capsule prototypes. A cost comparison was conducted to compare the cost of EPA in fish oil products that are already in the market to the cost of EPA in isolate A23.2 algal oil (Fig. 2).

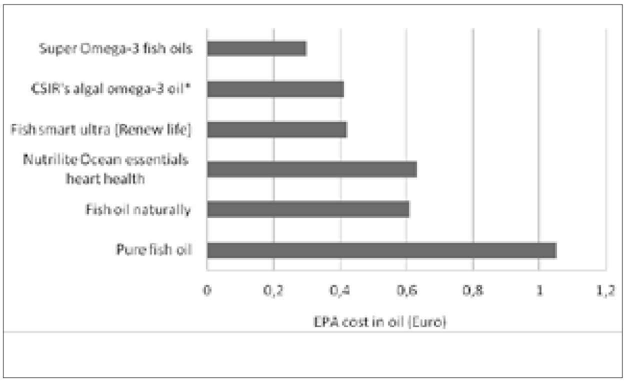

Figure 2. Cost comparison between fish oil product in the market and CSIR's product

*retail cost projection of CSIR EPA which includes 20% for packaging and 30% for margin (ex works) including a 4x multiple for distribution chain to estimate retail price

This comparison indicates that the cost of EPA in isolate A23.2 oil is comparable to fish oil products currently on the market. The CSIR's omega-3 oil would be an attractive product to the market due to its lower cost, yet algal EPA commands a price premium to fish oil. The fact that algal EPA is longer term more sustainable and also suitable for use by vegans are additional attractive attributes to this technology.

The lipid content of our algal oil was also compared to 5 other popular vegetarian and fish oil brands available on the shelves in South Africa (Fig. 3). This comparison indicates that the oil produced by the CSIR isolate A23.2 contains almost 20 times more EPA, yet very little DHA when compared to that of Solal; the only other algal and vegetarian alternative available on the market in South Africa.

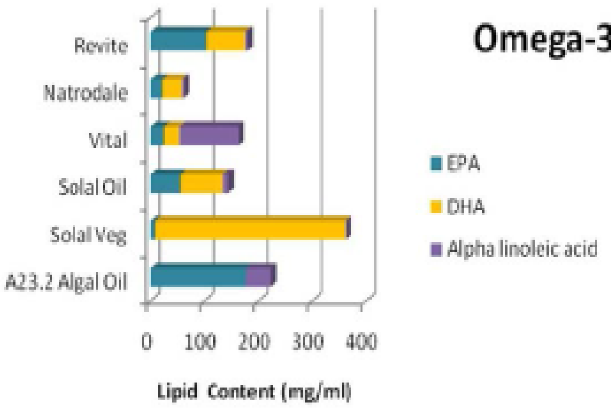

Figure 3. Popular nutraceuticals on the market and their respective Omega-3 fatty acid contents in comparison to A23.2 (mg. ml -1).

All other nutraceutical brands incorporate the traditional fish oil as a source of Omega. This comparison indicates that the isolate A23.2 algal oil is a potentially market competitive ingredient; not only in terms of nutraceuticals, but also as a functional ingredient in the food and beverage manufacturing sector.

The technology presented in this case study has immense potential, however it requires further development and pilot scale testing to further enhance the production of EPA by isolate A23.2 while reducing production costs. There are countless opportunities that could arise from this technology should it go to commercialization. Algal technology is an avenue worth pursuing for South Africa.

6 Commercial Foresight

Promoting the production of bio lipids from microalgae holds many advantages for South Africa. The production of lipids can yield a number of value added products – of which EPA is one. According to megadiverse.org, South Africa is one of the 17 countries considered to be rich in microalgal biodiversity. Microalgae are adapted to various niches and can be grown in wastewater and on non-arable land while utilising CO_2 as well as CO_2 rich flue gases. Hence, this ultimately results in the reduction of competition with food crops for agricultural land and fresh water sources, which are limited in South Africa. In addition, microalgae have the potential to aid in the rehabilitation of the environment [26, 16], to ensure longer term sustainability for less industrialized nations.

From a production point of view, algae can be grown in large scale race way ponds along the coast. Coastal cultivation is ideal for algal growth due to high levels of solar radiation. Algae can also be grown in industrial wastewaters as well as in water treated by sewage plants that are high in phosphates and nitrates. This would be beneficial in the sense that material cost of production can be reduced. The advantage to using local species for the production of EPA is that the species are already adapted to the South Africian climate and are therefore more likely to thrive.

An existing test facility in Upington, Western Cape, is the largest in South Africa and the local community comprises a population of 71 373 people [27], many living under impoverished circumstances, after the decline of the mining sector in that area. The technology developed by the CSIR has the potential for great socio-economic impact in terms of being a nucleus for resurrection of an algal technology hub in this area. Commercialization of this technology will provide opportunities for job creation for the local community surrounding the pilot plant and skills development to support a growing algal cluster. Although it is the technological advances that will set this project in motion, coupled to the social benefits, unique marketing strategies that embrace the challenges of developing countries are of paramount importance. It is the consumer who drives production. This means extending the product application profile; not only by producing novel products that are affordable and with indigenous appeal, but also products that can be freely distributed and are sufficiently stable to meet rural storage challenges.

In conclusion, there is massive scope for algal technology in South Africa. The key lies in developing the process and products to a higher stage of maturity to mitigate risk of commercial implementation, in a region where capital funding is scarce. Omega-3 EPA has shown to improve learning and cognitive behaviour in children, as well as benefits for pregnant and lactating women and infant development. The effect on new inflammatory diseases such as Rheumatoid arthritis and Systemic lupus are also seemingly positive. Aligning together with national and local government, education and awareness initiatives can be implemented and subsequently the potential products can be distributed at schools and clinics. This is one of many ways of how we can not only facilitate a healthy lifestyle but alleviate cognitive and behavioural problems in children, while enhancing their performance and interaction at school. The new generation African bio-economy must look at Africa's needs and emerging markets as a significant competitive advantage. There is also merit in adopting these types of technology implementations in other developing countries.

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