Review of the biological and engineering aspects of algae to fuels approach

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Abstract: Current biofuel production relies on limited arable lands on the earth, and is impossible to meet the biofuel demands. Oil producing algae are alternative biofuel feedstock with potential to meet the world's ambitious goal to replace fossil fuels. This review provides an overview of the biological and engineering aspects in the production and processing technologies and recent advances in research and development in the algae to fuels approach. The article covers biology, selection and genetic modification of algae species and strains, production systems design, culture media and light management, harvest and dewatering, downstream processing, and environment and economic assessment. Despite the many advances made over several decades, commercialization of algal fuels remains challenging chiefly because of the techno-economic constraints. Technological breakthroughs in all major aspects must take place before commercial production of algal fuels becomes economically viable.

Keywords: algae, microalgae, open pond, enclosed photobioreactor, light, harvest, dewatering, extraction, hydrothermal liquefaction, gasification, pyrolysis, fermentation

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1 Introduction

There has never been so much interest, effort, and investment in biofuels technology development as there is now. Bioethanol and biodiesel are the two most

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successful biofuels widely available in many parts of the world. However, the pursuit for biofuel alternatives does not stop there for a number of reasons. First, current production of biofuels cannot meet the existing and future demands. Many countries have set ambitious targets replacing fossil fuels with alternatives including biofuels within 15 to 20 years. Aside from the limited production capacity, production of grains and oil crops based biofuels is also limited by the available arable lands on the earth. The United States roughly uses 60 billion gallons (1gallon = 4.5461L) diesel and 120 billion gallons gasoline per year[1]. The 120 billion gallons of gasoline is equivalent to 78 billion gallons of diesel (Gasoline contains about 65% of the energy of diesel). Therefore the total transportation fuels need is translated to 138 billion gallons of diesel, equivalent to 140.8 billion gallons biodiesel (Biodiesel is 2% less than petrol diesel in terms of fuel efficiency). If the entire arable land of the United States (roughly 435 million acres, 1 acre = 4046.86 m^2= 4.047×10^{-3} km^2) were used to grow soybean for oil, it would produce about 21 billion gallons of biodiesel per year (based on 48 gallon/acre/yr), only about 15% of the total US biodiesel need. Secondly, current production of biofuels especially bioethanol and biodiesel displaces croplands currently for food and feed production, and has been blamed for food price hike, threatening food security and putting tremendous burden on the poor[2,3]. Thirdly, there are tremendous business opportunities in the biofuel sector, which attract interests and investments from large and small entrepreneurs and investors.

It is under this context that a renewed interest in algae has come into play in the biofuel sector. The April 2009 GreenTech Innovations report published by GreenTechMedia[4] listed more than 50 algae related companies in the United States alone, most of which are start-up companies formed in recent years. Hundreds of higher education and research institutes worldwide are conducting research on algae. The efforts span over many areas of "algae to fuels" technologies including production system development, algae harvest, algae strain development and genetic modification, algae products development, etc.

Algae offer many potential advantages:

- algae can potentially produce 1 000-4 000 gallon/ acre/yr significantly higher than soybeans and other oil crops (Table 1)
- they do not compete with traditional agriculture because they are not traditional foods and feeds and they can be cultivated in large open ponds or in closed photobioreactors located on non-arable land
- they can grow in a wide variety of climate and water conditions; they can utilize and sequester CO_2 from many sources
- finally, they can be processed into a broad spectrum of products including biodiesel via trans-esterification, green diesel and gasoline replacements via direct catalytic hydrothermal conversion, and catalytic upgrading, and bioethanol via fermentation, methane via anaerobic digestion, heat via combustion, bio-oil and biochar via thermochemical conversion, and high protein animal feed.

Table 1 Comparison of oil yields from biomass feedstocks (Modified from DOE[5])

Crop	Oil Yield (gallon/acre/yr)	Land needed to produce 140.8 gallons biodiesel (million acre)
Soybean	48	2 933
Camelina	62	2 270
Sunflower	102	1 380
Jatropha	202	697
Oil palm	635	2 217
Algae	1 000-4 000	140.8-35.5

Above all, the huge productivity potential of algae is the most important driving force behind the algae fever. To put it in perspective, to produce 140.9 billion gallons

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biodiesel from algae would require a total area of 35-140 million acres marginal or non-arable land, which is about 60%-250% of the size of the State of Minnesota, based the conservative oil yields of 1 000-4 000 gallon/acre/yr.

The concept of using algae as energy feedstock dates back to the late 1950s. However, this concept did not attract serious attentions until the oil price surge and the oil embargo of the early 1970s. In 1978, the US Department of Energy (DOE) started its 18-year Aquatic Species Program (ASP), which produced a report in 1998[6]. The program represents the most comprehensive research efforts on algae, and the report is the must-read document for any researchers who are interested in algae to fuels approach. The report identified many barriers chiefly economic barriers which could not be overcome without breakthrough technological innovations. Since then, interests and investments in algae were low, which could be attributed to the low oil price in that long period. Interests and investments in algae have been quickly picking up pace in recent years. Several US federal agencies have significantly increased their funding for algae research and development. Many new research and development efforts have advanced the algae to fuels technologies to a new high level, which deserves a comprehensive review.

The purpose of this review is to provide readers not only the important knowledge but also new advances in algae to fuels technologies. The review is structured in five sections covering such subjects as biology, production, harvest, processing, environmental and cost analysis, and future trends in R&D. Interested readers are encouraged to find further reading materials on the cited references list.

2 Biological aspects of algae

2.1 Species and strains

Algae are defined as any organisms which are plant-like and perform photosynthesis. Based on their morphology and size, algae are typically subdivided into two major categories—macroalgae and microalgae. Macroalgae, for example kelps, are composed of multiple cells which organize to structures resembling roots, stems, and leaves of higher plants. In contrast, microalgae are a large group of microscopic photosynthetic organisms, many of which are present in a unicellular manner and found in diverse environments. Microalgae are the subject of this review. The terms “microalgae” and “algae” will be used interchangeably throughout this article.

Microalgae could be further subdivided into prokaryotic cyanobacteria which clearly lack the nuclear structures and eukaryotic algae. Eukaryotic algae can be classified into at least 12 major divisions, in which microalgae find their locations. Among those divisions, some frequently mentioned classes include diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), red algae (*Rhodophyceae*), yellow-green algae (*Xanthophyceae*), golden algae (*Chrysophyceae*), brown algae (*Phaeophyceae*), and Euglenoids. Except *cyanobacteria* whose lipid contents are believed to be as low as less than 10% of dry algal weights, species capable of producing high levels of lipids could be identified from each of these eukaryotic algal classes[7].

Microalgae are believed to be one of the earliest life forms on the earth[8]. They thrive in diverse ecological habitats such as freshwater, brackish water, or seawater, and also adapt to various extreme temperatures and pH conditions. In addition, many microalgae show rapid growth under optimal conditions. For example the doubling time of some *Chlamydomanos* species is as short as six hours. Due to their strong ability in adaptation, microalgae therefore become dominating among the organisms on the earth, exemplified by their diverse unusual features such as being rich in starches, oils, and proteins, and able to accumulate important secondary metabolites (e.g., carotenoids and cancer chemopreventives). It was estimated the upper limit of algal species in the nature is about 10 million[9], only small portion of which is identified in taxonomy, including several thousand algal species collected and stored in institutions for research and only about 15 species used for industrial production of foods, feeds, drugs, and fine chemicals. Therefore, there is a huge potential to identify new algal species with economic values from natural environments.

In contrast to crops such as maize whose domestication began 6 000-10 000 years ago, the history of algal domestication for mass culture by human beings is quite short, less than 100 years. The major push for the algal domestication to overcome food and energy shortage occurred in the 1960-70's. Some algal species such as *Spirulina* containing abundant proteins and of high biomass production are cultivated in Asian countries and other regions of the world. It was hypothesized that microalgae like diatoms deposited millions years ago might be a source of petroleum and natural gas[10]. Mass cultivation of microalgae as the energy crop was triggered by the petroleum crisis starting from the early 1970s, marked by DOE's Aquatic Species Program. By then, the discovery that many algal species were able to accumulate high contents of lipids as storage oil droplets under certain growth conditions such as nitrogen deficiency suggested the promising use of algae as an energy crop. The recent rise in petroleum price has re-ignited research and investment into this hot field of algal biofuel production. It has been estimated that biodiesel productivity of some algae strains could be as high as 100-fold of that from traditional oil crops[11].

2.2 Photosynthesis and growth

Photosynthesis is the most basic and important way in which living organisms obtain their energy and nutrients, directly and indirectly. Algal photosynthesis is a unique process by which solar energy is converted into chemical energy that is stored in organic carbon matter through the cycling of atmospheric CO_2. Photosynthesis takes place in specialized organelles called chloroplasts of eukaryotic species, and also in a membrane-bound sac known as a thylakoid of the *cyanobacterium* due to lack of defined chloroplast structure in the prokaryotic organism. Photosynthesis is generally conducted in two separate steps—the light and dark reactions. In the light reaction, photons from sunlight are absorbed directly by chlorophylls and accessory pigments to excite electrons in water to a higher energy state. The latter is converted to ATP and NADPH, and molecular oxygen is released as a result of water splitting during the process. In the dark reaction, atmospheric CO_2 is converted to sugar with the energy in the form of ATP and NADPH generated during the light reactions.

There are generally two carboxylation pathways converting CO_2 to the organic carbon in algae—C3 and C4 carbon fixation pathways. In the C3 pathway, the enzyme Rubisco (ribulose-bisphosphate carboxylase) catalyzes the reaction of RuBP + CO_2 + HO_2 to 2 PGA (phosphoglyceric acid), a 3-carbon compound. PGA enters the Calvin cycle which results in sugar. It is assumed that most algae and higher plants employ the C3 pathway to fix the inorganic carbon. Some algae and plants evolved alternative C4 pathway—CO_2 is first converted into a four-carbon organic compound and then CO_2 released for fixation by Rubisco. The C4 pathway apparently only occurs in some eukaryotic algae since not a single case was found in any prokaryotic cyanobacteria so far. In all algal species tested there is a common carbon concentrating mechanism before the inorganic carbon moves into the catalytic sites of Rubisco for fixation. Under the aquatic environments, algae use carbonic anhydrase located on the surface of the cell to promote the conversion of dissolved CO_2 to HCO_3^-, the latter being transported into the cell and reversed to CO_2 by cytoplasmic carbonic anhydrase close to the Rubisco catalytic sites. Due to the higher dissolution of bicarbonate than carbon dioxide in solution, the carbon concentrating mechanism enhances the photosynthetic efficiency in algae.

Cell growth is another fundamental feature of algal cell biology, which, together with photosynthesis and nutrition supplements, determines the maximal potential of the algal biomass production. Cell growth probably consists of two phases—earlier cell proliferation and later enlargement in cell volume, both of which directly contribute to algal biomass accumulation. Therefore, it is expected that rapid division and growth of the cell after division are essential to the biomass production on a large scale. For some smaller and round microalgae whose cellular diameters are around several micrometers, the biomass production is determined mostly by the proliferation rate.

2.3 Metabolic pathways and their association with biomass and biofuel production

As mentioned earlier, most algal species are able to

synthesize organic macromolecules via photosynthesis through either C3 or C4 pathways under light. The process termed autotrophy represents the sole manner for organisms to convert inorganic carbon to organic matters which then provide energy and substances essential to survival and growth of all organisms on the planet. Many algal species are also able to take up small organic molecules in the environments and turn them into the building blocks of their own. This metabolic activity is called heterotrophy. If the external small organic molecules are considered as some sorts of intermediates of the whole metabolic system, it seems the efficiency of heterotrophy is higher than that of autotrophy. In addition, heterotrophic algae can grow well at extremely high cell density which is not possible for autotrophic algae. Therefore, the biomass production from heterotrophic algae is usually two-order higher than that from autotrophic algae, say 200 g (DW)/liter under heterotrophy vs. 2 g (DW)/liter under autotrophy. In addition, heterotrophic cells (*Chlorella protothecoides*) were found to accumulate higher levels of oils than the autotrophic ones[12]. Under light, some algal species can use either CO_2 or organic carbon through a so-called photoheterotrophic or mixotrophic pathway. In that situation, the biomass yield is probably between the autotrophic and heterotrophic biomass production. Apparently, heterotrophy with very high biomass production but also consuming sugar or other organic substrates at high costs is of economic importance only to those high value chemicals, which will compromise its significance in the biofuel production that must be built on low cost but large scale algae production. In the future, to improve the photosynthetic efficiency through genetic and cultivation approaches is still a major task for the biofuel community. As a short term strategy, given that photoheterotrophy in certain special situations (e.g., municipal wastewater) could achieve intermediate levels of biomass production, it might be practical to improve biomass yield through screening and selecting excellent algal species and strains from natural environments or commercial banks.

2.4 Components and structures of eukaryotic algal cells

The unique chemical composition and structure of algae give rise to several major issues associated with downstream processing. First, algal biomass contains not only C, H, O, but also N, P, S. The content of the nitrogen element is significantly high, such as 8% in the *Scenedesmus* species[13]. Besides, ash contents in algal biomass are usually higher than those in lignocellulosic feedstocks. These properties have significant impact on the products from thermochemical conversion of algal biomass. For example, de-nitrogenation could become a serious issue in algal biomass conversion and biorefinery. Second, eukaryotic microalgal cells possess highly differenciated organellar structures, typically cell walls, nuclei, mitochondria, chloroplasts, vacuoles, plasma membranes and endogenous membrane systems including the endoplasmic reticulum (ER) and the Golgi apparatus. TAGs are synthesized in the ER and stored in the cytoplasm. It is often necessary to break the cell walls and other cellular structures in order to release the lipids prior to physical and chemical extraction of TAGs from algal cells. Unfortunately, there is a lack of understanding of relationships among lipid extraction, the association of lipids with cellular structures, and disruption of the cellular structures.

Some species such as *Dunaliella* are able to move hydrocarbons from within the cells to the surrounding media via a secretory pathway involving transporters. If the lipid accumulation and secretion capabilities in these species could be enhanced through screening and genetic engineering techniques, there is a potential to develop techniques to collect lipids from living algal cells without harvest and extraction.

2.5 Lipid accumulation

Lipids are the most important targeted product of current mass algae production. Lipids in algae are present mostly as structural components of algae cells and organelles. Some of them are associated with proteins in chloroplasts. Lipids are traditionally thought to be energy reserves. However there are evidences to suggest that lipids also play a role in signal transduction.

Majority of the fatty acids are produced in chloroplasts for the construction of chloroplast membranes which may be attributed to the fact that

chloroplast metabolism is predominant in green algae. Some of the fatty acids produced in the chloroplasts are transported to elsewhere for construction of non-chloroplast membranes, which may play an important role in heterotrophic pathway (see below). In some rare cases, especially under nitrogen depletion conditions when lipid accumulation is enhanced, lipids may be present in free droplet form.

Triacylglycerols (TAGs) are one of the main storage compounds present in many algal species under stress conditions, such as nitrogen starvation. Some algal species are able to accumulate TAGs to high levels (e.g., 70% of dry weight in *Botryococcus braunii*). The major pathway for the formation of TAG involves *de novo* fatty acid synthesis in the stroma of plastids (or chloroplasts) and subsequent incorporation of the fatty acid into the glycerol backbone and acyl transfers from acyl CoA in the endoplasmic reticulum, eventually leading to TAG formation. The rate-limiting step in fatty acid synthesis is the conversion of acetyl CoA to malonyl CoA, catalyzed by acetyl CoA carboxylase (ACCase). The 16- or 18-carbon fatty acids (or both) produced through through this pathway are then used as precursors for the synthesis of cellular and organellar membranes as well as TAGs. TAGs are believed to be synthesized via a direct glycerol pathway. Positions 1 and 2 of glycerol-3-phosphate are transferred with fatty acids produced in chloroplasts. A third fatty acid is transferred to the position 3 of the diacylgylecrol to form triaceylglycerol (TAG), catalyzed by diacylglycerol acyltransferase. Unfortunately, at the biochemical and molecular levels, the TAG synthetic pathways and their regulation are still not fully understood, particularly in algae, which in turn limits the efforts to improve the TAG accumulation in algae through genetic and genomic engineering. Additional layers of difficulties in utilizing knowledge from other species than algae have been revealed by the comparative genomics between higher plants and *Chlamydomonas reinharttii*, a model green alga.

TAGs are the chemical stock for trans-esterification reaction for biodiesel production, and thus mass algae culture technology is tailored mostly for high TAG yield. Various stresses including nutrition starvation in N, P, and Si promote TAG accumulation in algal cells. However, this increase in TAG content is at the cost of decrease in algal cell growth, and ultimately the total TAG yield is not necessarily high. On the other hand, one may consider certain algal species with higher levels of TAGs under normal growth conditions. Unfortunately, at present these oilgae exhibit slow growth too in most situations—apparently, a conflict between rapid biomass growth and oil accumulation commonly occurrs in algae. To adress the conflict represents the great challenge in the basic research seeking solutions to the issue.

3 Mass culture

Algae must be grown on a large scale to have a substantial impact on biofuel production. Naturally occurring algae are very low in density. In order to significantly increase the productivity, it is necessary to find ways to increase the growth rate and density of algae in the culture media. It is also necessary to increase the lipid yield if biodiesel and other hydrocarbon fuels are the desirable products. This section will discuss screening of algae species and strains for specific growing conditions and purposes, production systems, culture media, and control of key growth parameters.

3.1 Screening and genetic manipulation of species and strains

Algae species and strains vary greatly in terms of growth rate and productivity, nutrient and light requirement, ability to accumulate lipids or other desirable compounds, ability to adapt to adverse conditions, etc. Therefore, the first step in mass cultivation of algae is to find or engineer right species and strains for specific purposes and cultivation systems.

There are tens of thousands of algae species and strains in the world. A small number of them grow well in the laboratories, but not all of them are suitable for mass cultivation for biofuel feedstock. Researchers have attempted to screen algae collected from fields. The criteria for algae screening may be formulated into three categories: growth physiology, metabolite production, and robustness[5]. The growth physiology is

evaluated based on maximum specific growth rate, maximum cell density, tolerance to environmental variables (temperature, pH, salinity, oxygen levels, CO_2 levels), and variability of *in situ* versus laboratory performance. Other ideal features are capabilities of heterotrophic or mixotrophic growth and growing to high cell density. Metabolite production is assessed for both the unit concentration as well as the total yield of the metabolites useful for biofuels production or other purposes (e.g., nutraceuticals). The ability of an algal species to secrete metabolites in liquid or volatile forms is another feature of potential significance for harvest. A robust algal strain should be characterized by parameters such as high culture consistency, reasonable resilience, high community stability, and low susceptibility to external predators.

A logical strategy is to select strains from environments where the strains are to be grown on a large scale. Another strategy is to acclimate or "train" the natural strains to adapt to certain environment in which these strains do not normally grow well. For example, to adapt an algal strain to concentrated wastewater environment, one may gradually increase the amount of wastewater added to an artificial medium and ultimately grow the strain in 100% wastewater. A third strategy is to use genetic approach to probe, understand, and modify regulation of key metabolisms pathways important to all performance parameters in the three screening criteria areas. Work in this area is still at its very early stage and slow in progress. An early research[14,15] was able to isolate Acetyl CoA Carboxylase (ACCase) from a diatom, an enzyme found to be responsible for catalyzing a key metabolic step in lipid synthesis in algae. However, over-expression of the ACCase gene did not demonstrate increased oil production in the cells[6]. Some basic researches such as genome sequence[16], insertional mutagenesis[17], RNA interference (RNAi) methods[18,19], molecular map[20], annotations of lipid genes[21,22]) are expected to speed up the genetic modification of algae.

Researchers are facing tremendous challenges with screening methodologies. There is not a reliable standard lipid analysis protocol. Assessment of most of the growth and metabolite parameters are time-consuming and labor-intensive. There is a strong demand for high throughput screening methods. Development of a "universal" culture system with a well balanced nutrition profile to support normal growth of most species with diverse growth properties would greatly reduce the work necessary to develop a specific culture medium for a given strain. Flow cytometry cell sorting may also play an important role in high throughput screening.

3.2 Production systems

Although some companies, especially nutraceutical companies, harvest algae from natural waters, most believe that algae should be grown in controlled environments for best productivity and quality. Outdoor open ponds and enclosed photobioreactors (PBR) are the most common production systems. Hybrid systems having some features of both open pond and enclosed PBR are emerging. Production systems vary in terms of growth parameters control, contamination, water evaporation, productivity, downstream processing characteristics, capital and operational costs, etc.

3.2.1 Open ponds

Open ponds are the most widely used system for large-scale outdoor microalgae cultivation in Southeast Asia, Australia, Middle East for food and medicine supplements during the last few decades[23]. Open pond systems are commercially economical, easy to build and operate. Depending on their size, shape, type of agitation and inclination, the open pond systems can be classified into (a) raceway pond, (b) circular pond, and (c) sloped pond (Figure 1)[24,25].

Raceway ponds are generally constructed either as a single unit or multiple joint units with agitation by means of a paddlewheel, propeller or air lift pumps. They are typically about 15-25 cm deep (Figure 1). Agitation and circulation are produced by a paddlewheel that operates all the time to prevent sedimentation[11]. Since 1950, raceway ponds become the most commonly used open systems for commercial algae culture because of their relatively low capital and maintenance costs[27].

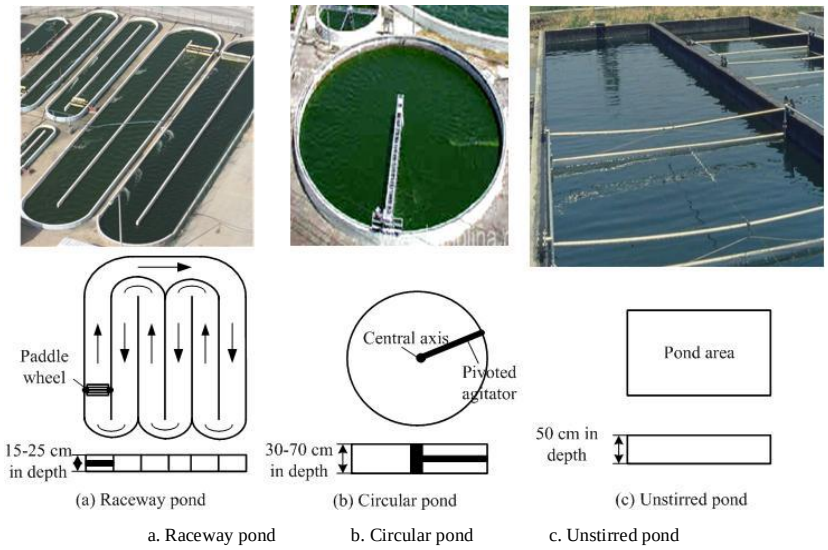


Figure 1 Three different designs of open pond systems (a and b: courtesy of A. Ben Amotz, National Institute of Oceanography, Israel; c: courtesy of M. R. Tredici[26], University of Florence, Italy; d: courtesy of M. A. Borowitaka[27])

Circular ponds use a rotation arm to provide agitation and were primarily operated in Japan and Taiwan during the early stages. The extended rotation arm can be as long as 45 m in diameter. Disadvantages of the circular ponds system, such as expensive concrete construction, high energy consumption of stirring, mechanical complexity of supplying CO_2, and inefficient land use, made them less popular than raceway ponds[25].

Sloped pond uses pumping and gravity flow to generate mixing of algal suspension. It was reported that higher yields can be obtained with inclined surfaces than raceway pond due to higher temperature that can be transferred from the circulation pump to the medium[25]. Productivities of all three types of open ponds are listed in Table 2.

Table 2 Productivities of open pond systems

Pond type	Total volume/L	Areal Productivity range /g dry weight · m^{-2} · d^{-1}	Volumetric Productivity range /g dry weight · L^{-1} · d^{-1}	Strain	Location	Ref.
Circular	1 960	1.61-16.47 2.43-13.52	0.02-0.16 0.03-0.13	*Chlorella sp.* *Scenedesmus sp.*	Japan	[28]
Circular		15	-	*Oscillatoria*	USA	[6]
Cascade (sloped)	1 970	25	10	*Chlorella sp.*	Czech Republic	[29]
Sloped	1 990	24.8	-	*Scenedesmus obliquus*	Peru	[25]
Raceway	-	9-13	-	*Spirulina sp.*	Mexico	[30]
Raceway	282	14.47	0.183	*Spirulina platensis*	Italy	[31]
Raceway	300	9.4-23.5	0.031-0.078	*Anabaena sp.*	Spain	[32]
Raceway	135 000	2-17	0.006-0.07	*Spirulina sp.*	Spain	[33]
Raceway	-	1.6-3.5	-	*Dunaliella salina*	Spain	[34]
Raceway	750	15-27	0.06-0.18	*Spirulina platensis*	Israel	[35]
Raceway	4 150	2.4-11.3	0.0028-0.13	*Phaeodactylum tricornutum*	Hawaii	[36]

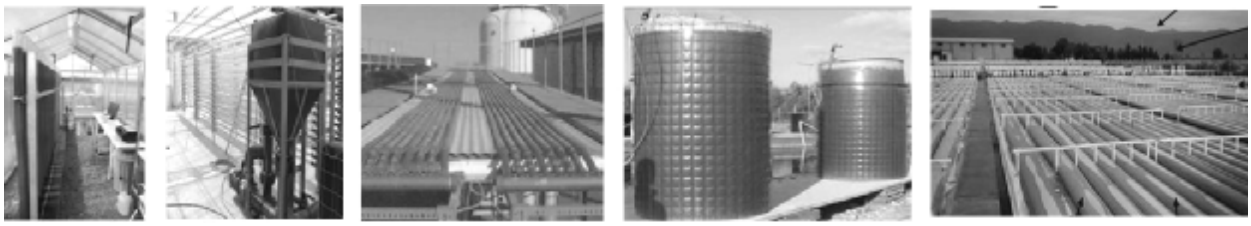
Although open pond systems have advantages of low construction and operation cost, there are many limitations that were widely discussed. They are (1) low productivity, (2) high harvesting cost, (3) water loss through evaporation. (4) temperature fluctuation, (5) contamination by predators, and (6) lower carbon dioxide use efficiency[11,24,25,29].

3.2.2 Enclosed PBR

Due to the limitation of open pond systems, enclosed photobioreactors (PBR) have evolved in the last 50 years. Two major types of enclosed PBR are tubular and plate types. Due to enclosed structure and relative controllable environment, enclosed PBR can reach high cell density and easy to maintain monoculture[29,37].

Figure 2 shows different types of tubular and plate PBRs[24,26].

Tubular PBR, constructed with transparent glass or plastic, is one of the popular outdoor systems for mass algae cultivations. By shape, it can be horizontal, vertical, conical, and inclined. By mixing, it can be airlift or pump system[37]. Plate type of PBR can be vertical, horizontal and inclined. The advantages of tubular and plate types of PBR are narrow light path (1.2-12.3 cm) that allows much higher cells concentration than open pond system, large illuminating area, and less contamination issues. The disadvantages are gradients of pH, dissolved oxygen and CO_2 along the tubes, wall growth, fouling, hydrodynamic stress, and expensive to scale up[29,37]. The productivities of major production systems were summarized in Table 3.



a. Fence-like straight vertical b. Fence-like straight horizontal c. Inclined d. Vertical helical e. Horizontal

f. Inclined plate PBR g. Vertical plate PBR

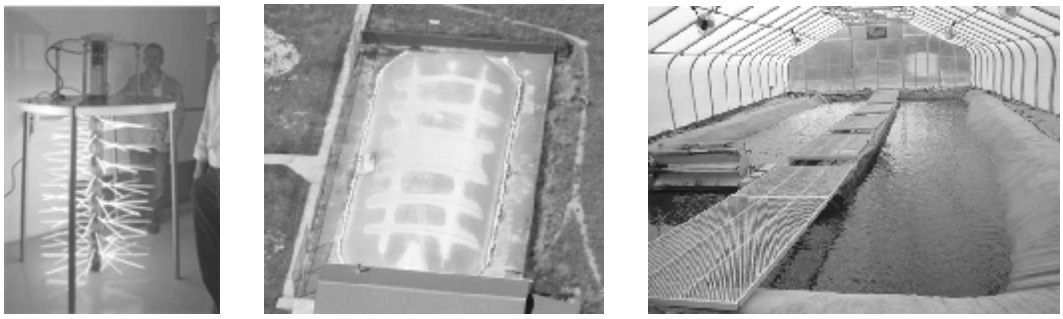
Figure 2 Different designs of tubular and plate PBRs (a and b: courtesy of L. Thomsen, Jacobs University, Germany; c, d, e: courtesy of M. R. Tredici[26], University of Florence, Italy; f: courtesy of A. Richmond[38], BenGurion University, Israel; g: courtesy of Q. Hu and M. Sommerfeld[7], Arizona State University)

Table 3 Productivities of enclosed PBR systems

Photobioreactors	ID/cm	Volume/L	Strain	Productivity/g · L^{-1} · d^{-1}	Ref.
Airlift tubular	-	200	*Porphyridium cruentum*	1.50	
Airlift tubular	-	200	*Phaeodactylum tricornutum*	1.20	
Airlift tubular	-	200	*Phaeodactylum tricornutum*	1.90	
Inclined tubular		6.0	*Chlorella sorokiniana*	1.47	
Undular row tubular	-	11	*Arthrospira platensis*	2.70	[37]
Outdoor helical tubular	-	75	*Phaeodactylum tricornutum*	1.40	
Parallel tubular	-	25,000	*Haematococcus pluvialis*	0.05	
Bubble-column	-	55	*Haematococcus pluvialis*	0.06	
Flat plate	-	440	*Nannochloropsis sp.*	0.27	
Horizontal Tubular	3.0	-	*Phaeodactylum*	2.76	
Inclined Tubular	2.5	-	*Chlorella pyrenoidosa*	2.90	
Vertical Coil	2.4	-	*Tetraselmis chuii*	1.20	
Vertical column	20.0	-	*Phaeodactylum*	0.69	[29]
Inclined plate	10.4	-	*Spirulina platensis*	0.30	
Inclined plate	1.3	-	*Spirulina platensis*	4.30	
Inclined plate	3.2	-	*Spirulina platensis*	0.80	
Vertical Coil	1.9	15	*Chlamydomonas reinhardtii*	2.0	[39]

3.2.3 Hybrid systems

Other types of systems are illustrated in Figure 3. Figure 3a is an internally-illuminated photobioreactor (Helix PBR) developed by Originoil company (Los Angeles, CA, www.originoil.com). The light array rotates vertically that allows algae growth in deep media and provides agitation. The light array consists of blue, red and white lights, which are the wavelengths the algae prefer. Green Star Products, a US company, has combined an enclosed bioreactor system with a low-cost open pond setup (Figures 3b & 3c) which is located in Montana (http://green.autoblog.com/2007/05/13/green-star-completes-first-phase-of-algae-biodiesel-demonstrati o/). The enclosed pond was able to maintain water temperatures of at least 18°C even when outside temperatures dipped as low as 1°C, which is warm enough to maintain optimum algae growth.



a. Helix photobioreactor b. Enclosed pond c. Greenhouse based open pond

Figure 3 Other types of PBRs (a: courtesy of Originoil company; b and c: courtesy of Green Star Products)

3.3 Culture media and nutrient management

3.3.1 Culture media

Algae can grow in various aquatic environments, such as fresh water[40], marine water[40], municipal wastewaters[41], and diluted animal manures[42] as long as there are certain amounts of carbon (organic or inorganic), nitrogen (ammonium or nitrate), and phosphorus present.

Growing algae on wastewater streams have a number of benefits. It will offset additional costs for nutrient removal from wastewater streams, and the costs associated with nutrient and water supplies for algae growth will be greatly reduced or eliminated. If it is grown on municipal wastewater, algae help remove nutrients particularly phosphorous and nitrogen (See 6. Environmental and cost analysis). Algae are able to use large quantities of organic carbon which would otherwise be emitted to the atmosphere.

Animal manure is another untapped resource for algae production. Take the state of Minnesota in the US for example, the state has a growing-finishing pig inventory for 2008 around 6.91 million, which produces about 30,000 metric tons of manure per day (solids amount at 11% = over 3,000 tons). This large amount of manure, not only stores more than 5×10^{10} BTU energy (equivalent to electrical power of 15.4 million kWh per day), but also contains all the necessary nutrients for algal growth. Co-locating an algal production facility with an animal farm will not only utilize the nutrients and water source but also cut the need and expenses to treat the manure wastewater.

However, there are a number of technical and economic issues which must be addressed in order to mass cultivate microalgae on wastewaters. These issues include finding suitable algae strains, transformation of the nutrients to readily available forms for algae to use, presence of solids and competing microflora, and turbidity.

3.3.2 Nutrient management strategy to maximize growth and lipid accumulation

Nutrients necessary to support algal growth and development usually include macronutrients (C, N, P, S, K, Na, Fe, Mg, and Ca) and micronutrients (B, Cu, Mn, Zn, Mo, Co, V, and Se). Some algae such as diatoms specifically require Si in fair amounts for cell wall components. Nutrition level in the aqueous habitats determines algal growth rate and potential of biomass

production. Some elements like zinc are critical to mitosis and photosynthesis of algae, and therefore even a small variation in zinc concentration will have significant impact on algal growth. In addition to the overall quantity of nutrients, the deficiency or excess of some particular elements may greatly alter some metabolic pathways such as TAG accumulation in algal cells as discussed above.

Ideal artificial culture medium should include all necessary elements to realize the maximal growth potential. However, in practice well balanced formulas of certain media are rarely available—not only due to the cost concern but also the difficulty in monitoring changes in the nutrition levels during cultures. Municipal wastewaters with most necessary nutrients offer a suitable alternative to artificial media. However, toxic and inhibitory substances in the wastewater suppress the growth of most algal species tested. Finding strains well adapted to hazardous conditions through screening and/or acclimation could provide a solution to this problem. This same strategy may be used to solve problems with other wastewaters such as animal manures and industrial wastewaters, the latter characterized by toxic heavy metals. In a broad sense, many kinds of natural water sources such as seawater and brackish water may be suitable media for algae production. Additional carbon source, for example CO_2 from flue gases, and other nutrients may be necessary for these low nutrition waters.

Synthesis and accumulation of large amounts of TAG occur in the cell when oleaginous algae are placed under stress conditions such as nitrogen deficiency, salinization, and very low or very high pH value of growth-medium. Growth phase and aging of the culture also affect TAG content and fatty acid composition[7]. Stress conditions, although boosting TAG accumulation in algal bodies, would compromise productivity to different extents. Up to now, there is no satisfactory nutrient management strategy to balance a good productivity and a high lipid content. However, some physical stimuli like higher temperature are reported to be beneficial for increased lipid generation. Tedesco and Duerr[43] found that growth and total lipid content of *S. platensis* increased when temperature was increased from 25 to 38°C. The increase in total lipid content was attributed to growth rate increase accompanied by the increase in storage of carbon in the cells.

3.4 Light administration

Light is critical to autotrophic growth of algae. Natural light fluctuates in either intensity or quality daily and seasonally. There are also significant differences in light resources between the south and the north of the United States. On a sunny day, the light intensity at noon over 1 500 mol/m^2/s is inhibitive to the surface layer of algae in the water column; however at the bottom of the water column (assuming of 30 cm height) the reduced light intensity might be fine or insufficient for photosynthesis due to, for example, blockage of upper layers of algae to light transmission. Both photoinhibition and low light stress of photosynthesis causes decrease in biomass production[44]. Furthermore, photosynthetic pigments (chlorophylls) exhibit best light absorption at around 440 and 680 nm wavelengths. White light with full spectral coverage cannot be fully absorbed. Part of the light will be reflected or transmitted as wasted energy. In theory, artificial light sources provided around these two wavelengths would result in best light efficiency for algae growth[45]. Sophisticated artificial light collection and distribution for algae growth are being studied.

3.4.1 Maximize natural light utilization

Using natural light is the first option. Photon absorption is affected by many factors, such as pigmentation in the algae cells, density of the culture, and the specific position of the cell[38]. Photoinhibition occurs during prolonged exposure to high irradiance[44]. Light is found to be a major limiting factor of productivity and growth when nutrition and temperature are satisfied[46]. Maximizing light utilization through design and operations is critical. Hu and Richmond[47] indicated a positive relationship between light intensity and productivity in which the maximal mixing-enhanced cell concentrations and productivity of biomass were obtained at the highest light intensity used. The rate of mixing required careful optimization: when too low, maximal productivity resulting from the most efficient utilization of light could not be obtained. Too high a rate of mixing resulted in cell damage and reduced output rate.

In open pond system, self-shading is affected by the cell density and depth of the pond. Due to irradiance variation throughout the year, the pond needs to be operated at different depths and cell densities in different seasons. For multi-stack and multi-row enclosed PBRs, the structure, orientation, and arrangement of the PBRs must be optimized to receive direct and diffuse light.

3.4.2 Artificial lighting

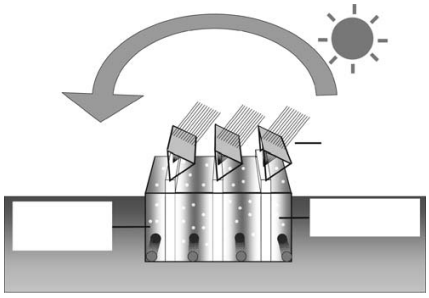
Artificial lighting may be necessary if algal growth 24-hour a day is desired and when natural lighting is inadequate. Fluorescent plant growth lights of full spectrum or specific spectrum are commonly used. LED is a very attractive alternative because of high energy efficiency although they are more expensive. Collecting and distributing solar lights for algae growth is another "artificial" lighting option.

3.4.2.1 LED

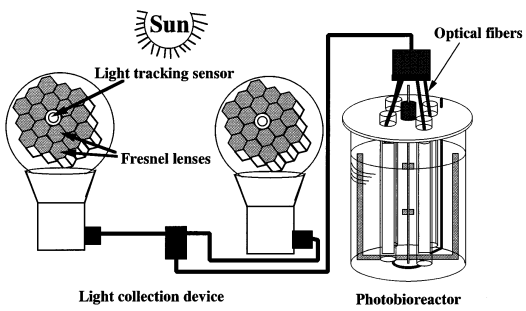
Light emitting diodes (LED) can convert electrical energy into radiation energy at up to 80%, which is the most energy efficient light source. Red LED is very attractive for photosynthesis because its emission spectrum fits with the photon energy needed to reach the first excited state of chlorophylls a and b. Blue light, of which photons contain about 40% more energy than the red light, can be absorbed by chlorophyll as well. Wang et al. [48] found red LED exhibited the highest specific growth rate under the condition of 3000 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} for *Spirulina platensis*. Blue LED showed the least efficiency in the conversion of photons to biomass. Light at other wavelengths is not suitable for photosynthesis but may play an important role in the regulation of cell growth and metabolism. Several studies utilized flashed LED light to simulate the light/dark cycle to prevent photoinhibition. Both the flux density and time frequency can affect algae growth rate. Terry[49] indicated that short duration flashed (<10 μs) with dark intervals of about 10 times longer duration (>100 μs) would be the optimal frequency. Nedbal et al.[50] obtained higher growth rates using the flashing light rather than the equivalent continuous light. Lee and Palsson[51] compared LED light (680 nm) with fluorescent light using *Chlorella vulgaris*. They found that the final cell mass and specific cellular growth rate under LEDs were comparable to those obtained under fluorescent light. However the narrow red light was found to reduce the average cell volume to half of cultivated under fluorescent light, but the total biomass production was not affected.

3.4.2.2 Solar light collectors

In mass algal production system, light can be the greatest limiting factor for scaling up. The efficiency of the photobioreactor is determined by the integration of: light capturing, light transportation, light distribution, and light usage. Zijffers et al. [52] has developed Green Solar Collector (Figure 4), an area-efficient photobioreactor for the outdoor cultivation of microalgae in which sunlight is captured into vertical plastic light guides. Sunlight reflects internally in the guide and eventually scatters out of the light guide into flat-panel photobioreactor compartments. Ogbonna et al.[53] used Fresnel lenses coupled with a light tracking sensor as light collector, and then distributing light inside the reactor through optical fibers. A light intensity sensor monitors the solar light intensity and the artificial light is automatically switched on or off, depending on the solar light intensity. In this way, continuous light supply to the reactor is achieved by using solar light during sunny period and artificial light at night and on cloudy days.



a. Green solar collector



b. Solar light collection device

Figure 4 Different solar collectors (a: courtesy of Zijffers et al.; b: courtesy of Ogbonna et al.[53])

3.4.3 Temperature control

Most commonly cultured species of algae are able to grow at temperatures between 16°C and 27°C. Optimal growth temperatures vary with culture medium, species, and strain cultured are usually in the range of 18–20°C. Temperatures beyond the optimal temperature range will slow down the growth or kill the algae. Temperature tolerance may be found in some species or strains through screening, or may be trained through acclimation. In regions where winter is extremely cold, the algae production facility may be enclosed in a greenhouse to maintain a suitable temperature.

3.4.4 Bacterial control

Although in laboratory research pure or unialgal species in the culture system are possible, in reality contamination of algae with other aquatic organisms and lower metazoan is unavoidable, and is sometimes beneficial but often times disastrous. Bacteria are one of the major sources of contamination. Laboratory work showed that many bacteria if co-cultured with algae inhibit growth of algae probably by secreting toxic factors and interfering with algal metabolisms. However, some bacteria when introduced into the algal culture system could promote algal growth[54]. They are thus called growth-promoting bacteria. It is believed that these growth promoting bacteria are capable of generating and releasing some beneficial biofactors, but the identities of these biofactors remain unknown in most situations.

4 Harvest, dewatering, and drying

4.1 Harvest and dewatering

Live microalgae are tiny particles (1 to 30 *m*m) suspended in the culture media. The dry weight of the culture media at the time of harvest is about 0.5-3.0 grams per L. Therefore separating and collecting these fine particles with low specific gravity from the bulk liquid is challenging and costly. A literature review provided for the US DOE is a good summary of algae harvest techniques[55]. Several physical, chemical, and mechanical harvest methods, individually or in combination, have been tested.

Membrane filtration with the aid of a suction or vacuum pump is usually the preferred method. This method is simple and simultaneously removes water and collects algae. Membranes are usually made of modified fibers or cellulose. A number of filtration systems such as drum filter with[56] or without scrubber[57], and disc filter, have been developed for algae harvest. However, membrane fouling and clogging are major problems associated with cell penetration into the membrane structures and cell packing. A reverse-flow vacuum filtration method, in which liquid moves upward across the membrane because of the vacuum above the membrane, was to avoid cell penetration and packing[58,59]. Stirring the media to avoid settling on the filter membrane during ultrafiltration of alage was also demonstrated[60]. Removing such large amounts of water through filtration can be very energy consuming. Innovation in new membrane materials that facilitate water removal and algae recovery could provide a solution to these problems. AlgaeVenture Systems Company developed a belt type harvest system based on advanced membrane (Figure 5). The system removes water and dries algae to 5% moisture content continuously. The company claims that its system reduces 95% energy compared with traditional centrifuge method.

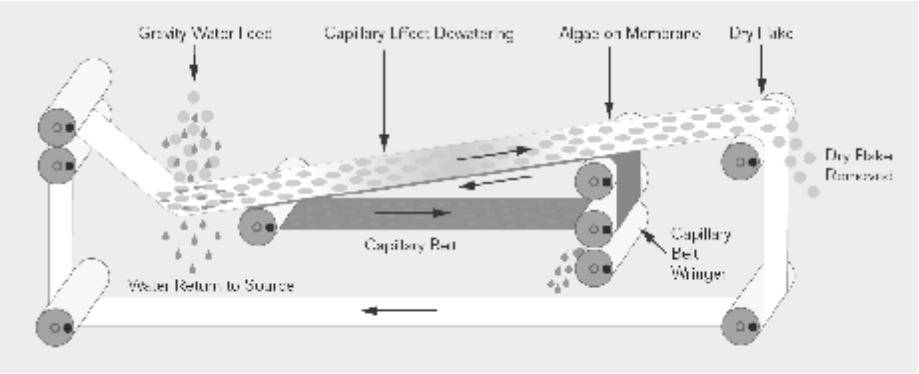


Figure 5 AlgaeVenture harvest, dewatering, and drying system

Chemical flocculation also appears to be a viable method. Microalgae have negative charges on their surfaces that keep individual cells separated in suspension. When adding coagulants (e.g., iron, alum, lime, cellulose, salts, polyacrylamide polymers, surfactants, chitosan, and other man-made fibers), the negative surface charges are disrupted, causing microalgae in suspensions to flocculate and settle. Researchers at the University of Minnesota have developed a method employing coagulants, equipment, and operational procedures currently used at wastewater plants. This method allows 95% recovery of algae from the culture media.

Air flotation, dissolved air flotation, and suspended air flotation[61-64] are methods in which fine air bubbles are generated through air injection and adhered to algal cells, causing algal cells to float as foam to the top of a treatment column. The foam with concentrated algae is removed from the top, or the water below the foam is drained or siphoned off. Flotation methods are commonly combined with chemical flocculation. Flotation methods can be expensive to operate because they involve energy-intensive air compression.

Centrifugation is another widely tested method[62,65]. It may be used alone or as a second step to further remove water from concentrated algae collected with other methods. Centrifugation of large volumes of algal culture may be carried out using large centrifuges such as a cream separator. Algal cells are deposited on the walls of the centrifuge head as a thick algal paste.

Ultrasound wave is a relatively new method in which algal cells experience low energy ultrasound waves and move to the low pressure nodes of ultrasound waves, causing the algal cells to agglomerate (Figure 6). The cell agglomeration is aided by the acoustic interaction forces and particle-particle interaction forces. Algae aggregates grow to such a size that they settle due to gravity when the ultrasonic field is turned off[66]. The advantages of this technique are that it is non-fouling, causes no shear, and is free of mechanical failures because it does not involve moving parts and offers the possibility of continuous operation. Its major disadvantages are high power consumption and low concentration factors compared with traditional centrifugation and flocculation methods.

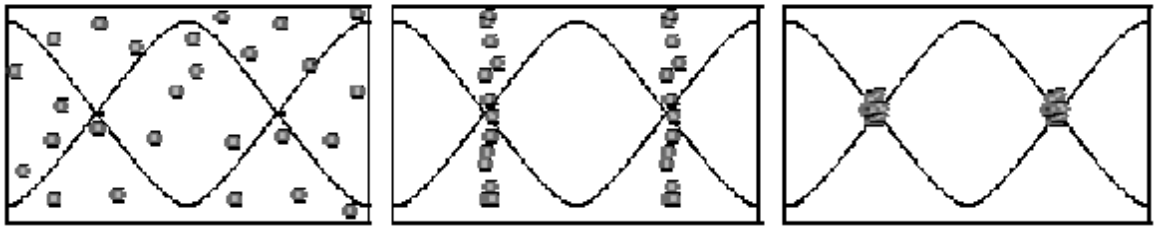


Figure 6 Principle of the ultrasonic harvesting process: When algal cells are exposed to an ultrasonic standing wave (a), they are driven to the planes of the low pressure nodes of the field (b). Subsequently, agglomeration of the cells occurs into the knots of the ultrasonic field (c). When the field is nullified, the large algae aggregates settle rapidly from the fluid due to gravity forces[66]

4.2 Drying

Harvested algae contain 97%-99% water. Removal of most of the water is necessary for long term storage of the algae feedstock and is required for many downstream processes. To keep algae from prolonged microbial growth, the moisture level of the harvested algae should be kept below 7%. Drying is an energy intensive process and can account for up to 30% of the total production costs. Natural drying (solar and wind) is the most economical way; however, its weather dependent nature could easily put the operation at risk of spoilage. It also requires a large space. AlgaeVenture's harvest, dewatering and drying system combines natural energy and artificial heat in addition to the strong water pulling power of the membrane. Many other artificial drying methods have been tested or proposed for algae drying[25,55]. Table 4 summarizes various drying methods. Selection of the dry methods should depend on

the algal species, the scale of operation, and final use of the dried products, costs and energy requirements[25]. Nevertheless, drying remains one of most costly operation in algae to fuels approach. Either new low cost drying methods must be developed, or conversion processes requiring no drying or minimal drying must be devised.

Table 4 Algae drying methods[25]

Method	Advantages	Limitations	Remarks
Drum-drying	Fast and efficient	Cost intensive	Ruptures cellulosic cell walls, sterilizes the product, not suitable for *Spirulina*
Spray-drying	Fast and efficient	Cost intensive	Sterilizes the product, breakage of cellulosic cell walls not always guaranteed
Sun-drying	Very low fixed capital and no running costs	Slow process, weather dependent	Biomass may ferment, sterilization not possible, does not break cellulosic cell walls
Solar-drying	Low capital costs	weather dependent	Does not break cellulosic cell walls, sterilization not possible
Cross-flow-drying	Faster than sun- and solar-drying, cheaper than drum-drying	Requires electricity	Does not break cellulosic cell walls, sterilization not possible
Vacuum-shelf-drying	Gentle process	Cost intensive	Does not break cellulosic cell walls, product becomes hygroscopic, sterilization not possible, preserves cell constituents
Freeze-drying	Gentle process	Slow process, Cost intensive	Does not break cellulosic cell walls, sterilization not possible, preserves cell constituents

5 Processing

Extracting the oil and converting the oil from algae to biodiesel are the primary driving force for algae to fuels technology development. The oil extracted can be converted to biodiesel via trans-esterification reaction. Nevertheless, the whole algae or the residues from oil extraction are excellent feedstock for making other fuels and products via different processes. For example, the starch and cellulose components are suitable for ethanol fermentation[67]. This section will discuss oil extraction, *in situ* trans-esterification, ethanol fermentation, thermochemical conversion, anaerobic digestion, fractionation,

5.1 Oil extraction

Extraction of oil from algal biomass has proven to be difficult and expensive. There is not a well-defined and ready-to-scale-up lipid extraction process currently available on the market. Most of current extraction methods are facing challenges with high costs associated with water removal and difficulties with disrupting the cellular structure to make lipids sufficiently accessible.

Organic solvent extraction is a widely used method for lipid extraction from traditional oilseed plants, and different extraction systems have also been tested with algae cultures[68-72]. The lipid classification of microalgae is shown in Table 5. In order to maximize the lipid extraction efficiency, the organic solvent used has to match the lipid polarity profile in the cells. For industrial applications, the extraction solvents should be cheap, easy for removal, free from toxic, insoluble in water, efficient in dissolving targeted components, and ideally recyclable .

Table 5 Microalgal lipids classification[71,73]

Neutral lipids	Polar lipids	
	Phospholipids	*Glycolipids*
Triglycerides	Phosphatidylcholine	Sulfoquinosyldiglyceride
Wax esters	Phosphatidylethanolamine	Monogalactosyldiglyceride
Hydrocarbons	Phosphatidylserine	Digalact osyldiglyceride
Free fatty acids	Phosphatidylglycerol	
Sterols	Phosphatidylinositol	

A lipid extraction method using a mixture of chloroform and methanol (2:1 (v/v))[74] has been widely used for a variety of materials including animal or plant tissue, and microorganisms. In microalgae, chloroform extractables include hydrocarbons, carotenoids, chlorophylls, sterols, triacylglycerols, wax esters, long-chain alcohols, aldehydes and free fatty acids, and methanol extractables include phospholipids and traces of glycolipids[71], many of which are non-lipid compounds. One drawback of this system is the flammability and

toxicity of the solvents used. Fajardo et al.[72] reported a two step extraction-purification method for lipid extraction from *Phaeodactylum tricornutum*, which included a first step of adding ethanol (96% V/V) to extract the lipids from the lyophilized biomass, and a second step of forming a biphasic system by adding water and hexane to the extracted crude oil. Cartens et al.[70] also suggested that ethanol (96%) is more efficient than hexane-ethanol (96%) (1:2.5, V/V) in terms of fatty acids extraction from *P. tricornutum*, since ethanol has higher polarity, which matches the polarity profile of the lipids in the cells. Nagle and Lemke[68] examined three solvents for their lipid extraction efficiencies from diatom *Chaetoceros muelleri*, and found that the most efficient solvent of the three was 1-butanol, followed by hexane/2-propanol and ethanol. Grima et al.[69] tested seven solvent systems and stated that ethanol (96%) and hexane/ethanol (96%), 1:2.5 V/V produced the best results when extracting lipid from *Isochrysis galbana*. Another low-toxicity solvent extraction system adopted by other researchers is hexane-isopropanol (3:2 V/V) system[75]. The purification is done by washing the extract with aqueous sodium sulfate. This system has the merit that the extract contains less non-lipid products. However, it gave significantly lower lipid yields when used with microalgae[68]. It is worth noting that in these alcohol-containing co-solvent systems, alcohol can add the benefit of inactivating many of the lipid-degrading phosphatidases and lipases[76,77], and also disrupting the lipid-protein complexes, and thus dissolve maximum amount of lipid. Among all the solvent systems examined for microalgal lipid extraction, chloroform-methanol system provided the highest extraction efficiency.

In order to promote better penetration of the solvent into the cells and increase the lipid yield, some techniques, such as autoclave, bead-beating, microwave, sonication, grinding and osmotic shock, to disrupt the cells before or during the extraction have been proposed for lipid extraction from microalgae.

Chisti and Moo-Young[78] reviewed a variety of mechanical cell disruption methods available for microorganisms, including bead milling, ultrasonication, high pressure homogenization. Other techniques of less industrial significance include freeze-press, osmotic shock, enzymatic and chemical lysis[78].

Lee et al.[79] examined the effect of different cell disruption procedures on three algae strains, and found that microwave oven and bead-beating methods were most effective for *Botrococcus sp.*, microwave and autoclaving methods were most effective for *Chlorella vulgaris*, and microwave method was most effective for *Scenedesmus sp*. They also suggested that microwave oven method was most applicable for scale-up. They found that bead-beating method extracted higher lipid content than sonication, homogenization, freeze-press and lyophilization when working with *Botrococcus braunii*. Pernet and Tremblay[80] tested the effects of grinding, ultrasonication and their combination on lipid extraction from *Chaetoceros gracilis*, and found that the effects varied with different storage time and sample amount. The combination of ultrasonication and grinding was more efficient in lipid extraction for large amount of samples and long storage time (more than 6 months) than ultrasonication or grinding alone. Dunstan et al.[81] applied ultrasonication between each operation when extracting lipids from green algae species *Chlorophyceae* and *Prasinophyceae* using chloroform-methanol-water (1:2:0.8 by vol).

Since cell wall structures vary a great deal with the algae species, one should be cautious when choosing the treatment method. Grima et al.[82] reported that direct extraction of fatty acids from wet *P. tricornuturn* biomass with 96% ethanol produced only slightly lower yields than those obtained from lyophilized biomass, in which case cost of extraction may be reduced by omitting lyophilization .

Supercritical fluid extraction (SFE) and subcritical water extraction (SWE) are relatively new techniques featuring high selectivity, short operation time, and toxic solvent-free[83]. When above their critical points, the supercritical fluids (SF) exhibit enhanced diffusivity and decreased viscosity, making SF diffuse more easily through solid materials than ordinary fluids, and thus provide higher extraction efficiency[84]. SWE is based on the fact that water becomes less polar under subcritical

conditions, and thus non-polar organic compounds have a higher solubility in supercritical conditions than that in non-supercritical conditions[85]. Chueng[86] used SFE to selectively extract lipid from macroalgae at the pressures of 241- 379 bar (1 bar=100 kPa) and temperatures between 40-50°C. Mendes et al.[87] used SFE to extract diolefines from *Botrycoccus braunii* cells and they found that 300 bar was the optimum pressure in terms of yield and extraction speed. Mentdes et al.[88] also used SFE to extract carotenoids from microalgae *Chlorella vulgaris* and the optimum conditions found were 55°C and 350 bar. A third microalga species studied by Mendes et al.[89] was *Arthospira (Spirulina) maxima*, which is able to produce large amount of γ-linolenic acid (GLA). CO_2 with 10% of ethanol as co-solvent at 350 bar and 60°C was optimal for this species. Herreo et al.[83] used SWE for extraction of antioxidant components from microalgae *S. platensis*. Denery et al.[90] conducted carotenoid extraction from the microalgae *Haematococcus pluvialis* and *Dunaliella salina*, using pressurized ethanol.

Some emerging techniques that have the potential to break the cell walls and extract the lipids are also at their early development stages. Nano-dispersion (NANO Dispersions Technology Inc., City of Knowledge, Clayton, Panama) is a method that uses a proprietary milling device, which operates on purely fluid mechanics principles, to disperse particle into nano size range. If this method can be combined with solvent extraction, it has the potential to mingle two processes into one. Electroporation was reported to have positive effect on lipid extraction when using Nile Red staining (Seattle University). Researchers from Hawaii Natural Energy Institute investigated a co-solvent system containing ionic liquid to co-extract bio-oil and protein from algae, and the extracted bio-oil can automatically separate into the upper layer. Other techniques like steam explosion, the combination of microwave and ultrasonication, and electro-magnetic field are also mentioned by the researchers in this field, but no scientific literature is available yet.

It is worth mentioning that although different extraction methods have been tested for microalgal lipid extraction on a laboratory scale, none of them is proved effective, economical and practical for commercial biodiesel production.

5.2 *In situ* transesterification

Conventional transesterification process requires costly oil extraction and separation. If fatty acid containing lipids are simultaneously extracted and transesterified, it would eliminate the need to extract and separate the lipids and fatty acids contained in the algae. Direct or *in situ* transesterification has been proven in a number of feedstocks including marine tissues[91], yeast and fungi[92], bacteria[93], microheterotrophs[94], algae fatty acid[95], and municipal primary and secondary sludge[96]. Johnson and Wen[97] compared *in situ* transesterification of dry and wet algae with conventional transesterification of extracted oil. *In situ* transesterification of dry algae with extraction solvent (chloroform, hexane, or petroleum ether) added resulted in higher crude biodiesel yield than the conventional transesterification process. *In situ* transesterification of wet algae compared unfavorably with conventional process. A very recent study by Ehimen et al.[98] examined the key variables such as alcohol volume, moisture content, temperature, reaction time, and mixing on an acid-catalyzed *in situ* transesterification process for production of biodiesel from microalgae lipids. They found that the *in situ* transesterification was inhibited when the biomass water content was greater than 115% w/w (based on oil weight). More efforts to evaluate and improve this method are worthwhile.

5.3 Ethanol fermentation of starch and cellulose in algae

Algae contain substantial amounts of starch and cellulose which, in theory, can be fermented to ethanol using existing technologies. The concept appears to be straightforward and has attracted business attention. There is no significant work on this in the scientific literature. Nakas et al.[99] studied several algal species for production of mixed solvent including ethanol through sequential bacterial fermentations. Bush and Hall[100] invented a process to produce ethanol from algae. In their process, harvested algae are first placed in a dark and anaerobic aqua environment to induce biomass degradation and then subjected to yeast fermentation to

produce ethanol. Algenol, a US company, is developing a DIRECT TO ETHANOL® process in which unique algal species are able to convert sugars to ethanol directly within the cells and allow the ethanol to diffuse out of the cells and evaporate quickly. The ethanol vapor can then be recovered and condensed. It is not clear when this technology will be commercially available.

Ethanol fermentation may use oil extraction residues as feedstock. On the other hand, fermentation of whole algae may breakdown the cell walls and release oil. More studies are certainly warranted in this area.

5.4 Thermochemical conversion

Algal biomass, either whole algal cells or extraction residues, are suitable feedstock for thermochemical conversion such as gasification, pyrolysis, and hydrothermal liquefaction and gasification to produce syngas, bio-oil, biopolyols, and biochar. Syngas can be combusted directly to produce heat or to generate electricity. Bio-oil can be used as heating oil or upgraded to liquid transportation fuels. Biopolyols are chemical stocks for material synthesis. Biochar can be used as active carbon, fertilizer, and soil amendment agent. Biochar is recently considered as one of the best ways for long term and low cost carbon sequestration[101,102]. One major advantage of thermochemical conversion of algae over other conversion technologies is its high efficiency due to short retention time, ranging from seconds to minutes. Scientific research on thermochemical conversion of algal biomass is very limited because most of the processing related research has been focused on lipid extraction and conversion.

5.4.1 Gasification

In gasification, biomass is converted to a combustible gas mixture called "synthesis gas (syngas)" or "producer gas" through partial oxidation reactions at high temperature typically ranging from 700 to 1 100°C. Syngas may vary in composition with type and moisture content of feedstock, type of gasifiers, gasification conditions, etc. Syngas can be burned to produce heat or used in gas engines or gas turbines to produce electricity. Gasification units are commercially available. Syngas clean-up and conditioning has been identified as a key technical barrier to the commercialization of biomass gasification technologies and has the greatest impact on the cost of clean syngas. Catalytic reforming and fermentation of syngas to other chemicals such as short chain fatty acids, methanol, ethanol, other mixed alcohols, hydrogen, aldehydes, olefins, and polyhydroxyalkanoates (PHA) are being investigated.

Gasification of algae biomass is largely unknown. Demirbas[103] studied the steam gasification of mosses, macroalgae (*Cladophora fracta*) and microalgae (*Chlorella protothecoid*). At temperatures ranging from 550 to 950 °C, the biomass was converted to CO_2, CO, H_2, and CH_4. The amount of target gas, H_2, varied with temperature and type of biomass. The microalgae resulted in highest H_2 yield. It is unclear whether the higher H_2 yield for the microalgae is due to its higher hydrocarbon content compared with the macroalgae.

5.4.2 Pyrolysis

Pyrolysis is another important thermochemical conversion process in which biomass is degraded to bio-oil, syngas, and biochars at medium high temperature (300-600°C) in the absence of oxygen[5,104]. Biomass is usually heated through heated surface or sands. The pyrolysis products generally include bio-oil, gas and char, and nowadays bio-oils are preferred because they have the potential to be upgraded to liquid transportation fuels. Pyrolysis yield and product compositions are a function of feedstock type, temperature and residence time[104,105]. Based on operating conditions, pyrolysis can be mainly categorized into two types: conventional pyrolysis and fast pyrolysis. Conventional pyrolysis operates at relatively low temperatures and produces mainly biochar. Fast pyrolysis is conducted at very high heating rate and short residence time with rapid cooling of gas products. It often takes places at temperatures in the range of 425-650°C[104].

A new type of pyrolysis process using microwave heating is being developed at the University of Minnesota. The technical advantages of microwave-assisted pyrolysis (MAP) over conventional pyrolysis include: (1) Microwave heating is uniform and easy to control for most of biomass with particle size of 1 inch or less; (2) It does not require a high degree of

feedstock grinding (e.g., even large chunks of wood logs can be used) and can handle mixed feedstock (e.g., municipal solid wastes); (3) The conversion products (pyrolytic gas and bio-oils) are cleaner than those from gasification and conventional pyrolysis because this process does not have to use biomass powder and does not require agitation and fluidization; (4) The syngas produced has a higher heating value since it is not diluted by the carrying gas for fluidizing the biomass materials; (5) Microwave heating is a mature technology, and development of microwave heating systems for biomass pyrolysis is of low cost.

During pyrolysis of algae, not only lipids but also other non-lipid components such as protein, starch, and cellulose are converted into bio-oil accompanied by combustible gas and biochar[106]. Higher yield and higher quality bio-oil was produced from algal biomass than mosses[103], which may be attributed to the higher hydrocarbon contents in the algal biomass. Miao and Wu[107] reported that bio-oil yields from fast pyrolysis of *Chlorella protothecoides* grown under autotrophic and heterotrophic conditions were very different. They found that the heterotrophic *Chlorella protothecoides* cells yielded 57.9% bio-oil, which was 3.4 times higher than autotrophic cells. The bio-oil from the heterotrophic *Chlorella protothecoides* cells was characterized by a much lower oxygen content, with a higher heating value (41 MJ/kg), a lower density (0.92 kg/L), and lower viscosity (0.02 Pa·s) compared with those from autotrophic cells and wood. These differences are believed to a result of chemical composition variations due to different metabolic pathways. Heterotrophic *Chlorella protothecoides* contained a higher crude lipid content (55.20%) compared with 14.57% in autotrophic cells, whereas crude protein in autotrophic cells (52.64%) was about five times that in heterotrophic cells (10.28%). The heterotrophic cells had a higher carbohydrate content and a lower moisture content than autotrophic cells.

The bottle-neck of pyrolysis of algae into bio-oil is the dewatering process prior to pyrolysis. This process requires high energy input. Pyrolysis technology is expected to become a cost-effective conversion method only if dehydration/drying becomes inexpensive. Another challenge of pyrolysis is that the components of bio-oil are very complex. Currently, much research has been focused on upgrading of bio-oil generated by reducing acidity and complexity as well as increasing stability. Wan and her co-workers' research demonstrated that some catalysts, for an example, $MgCl_2$, significantly improved bio-oil yield and the product selectivity through microwave-assisted pyrolysis of biomass[108]. Another study showed that the ester compounds in the upgraded bio-oil were increased significantly by the vacuum pyrolysis over the Mo-Ni/γ-Al_2O_3 catalyst[109].

5.4.3 Hydrothermal liquefaction and gasification

Hydrothermal liquefaction refers to decomposition reactions taking place in water media at high temperature and high pressure. The high temperature and high pressure create a unique condition termed "supercritical water" in which chemical reaction rates are significantly enhanced[110]. At lower temperature range (200–400°C) the reactions produce more liquid products (bio-oil or bio-crude), and therefore termed hydrothermal liquefaction. Hydrothermal gasification processes generally take place at higher temperatures (400–700°C) and produce methane or hydrogen gases.

Hydrothermal processes open tremendous opportunities for algae processing because they do not require drying, resulting in a huge cost saving in water removal operations. Therefore, hydrothermal processes deserve much more attention and investment. In addition to hydrothermal liquefaction and gasification, the possibility of using supercritical conditions to facilitate other reactions such as ***in situ*** conversion of lipids to biodiesel and other high grade fuels must be explored.

Very little work has been done on hydrothermal conversion of algae. Minowaa et al.[111] converted *Dunaliella tertiolecta* with a moisture content of 78.4wt% directly into about 37% oil (an organic basis) through hydrothermal liquefaction at around 300°C and 10 MPa. The oil had a viscosity of 150–330 mPa·s and a calorific value of 36 kJ/g, comparable to those of fuel oil. Dotea et al.[112] hydrothermally converted the artificially

cultivated *Botvyococcus braunii* Kiitzing Berkeley strain. This strain contained about 50% hexane extractables which were considered hydrocarbons by the authors. The hydrothermal liquefaction process with sodium carbonate as catalysts at 300°C resulted in 57% petroleum like bio-oil. Similar work was done on *Microcystis viridis* harvested from a lake[113]. Hydrothermal gasification of algae strains was done by Stucki et al.[114] on *Spirulina platensis* and Chakinala et al.[115] on *Chlorella vulgaris*. The syngas consists of CO_2, CO, CH_4, H_2, and varied with reaction temperature, residence time, algae content, and catalysts.

Hydrothermal processes are faced with several technical and engineering challenges. Heating rate and residence time are believed to be critical to the reactions and final products. The decomposition reactions under supercritical water conditions are supposed to complete in seconds or tens of seconds. Current research data for hydrothermal processes were obtained mostly with batch reactors. These reactors usually take a long time to reach the designed reaction temperatures and also a long time to cool down before the reactors can be opened, which may add up to a few hours. Under this situation, biomass may experience a thermal history with a wide range of temperatures over an extended period of time, and therefore the reaction kinetics may be complicated and the products may be very different from when reactions take place at the "ideal" heating rate and residence time. A better control of residence time at steady state is possible with a continuous reactor. However, fast heating rate, clogging-free flow, and high pressure pumping remain engineering challenges. Furthermore, incorporating catalyst bed into the reactor is difficult in terms of avoiding clogging and finding water-tolerant catalysts.

5.5 Anaerobic digestion

Marine algae consist of polysaccharides (alginate, laminaran and mannitol), with no or very low lignin and low cellulose content, making them a good material for methane fermentation[116]. The harvested algae biomass, under anaerobic condition and inoculation of certain groups of bacteria, i.e. hydrolytic and fermentative bacteria, acetogenic bacteria, methanogenic bacteria, can be fermented to methane, which is another way to produce renewable energy.

Vergara-Fernándeza et al.[116] evaluated the anaerobic digestion of *Macrocystis pyrifera*, *Durvillea antarctica* and their blend 1:1 (w/w) in a two-phase anaerobic digestion system consisting of an anaerobic sequencing batch reactor (ASBR) and an upflow anaerobic filter (UAF). The results show that 70% of the total biogas produced in the system was generated in the UAF, and both algae species have similar biogas yield of (180.4±1.5) mL g^{-1} dry algae d^{-1}, with a methane concentration around 65%. The same methane content was observed in biogas yield of algae blend; however, a lower biogas yield was obtained. In conclusion, either algae species or their blend can be utilized to produce methane gas in a two-phase digestion system.

Other algae species, such as *Tetraselmis*, *Gracilaria tikvahiae*, *Hypnea* and *Ulva*, could also be good feedstocks for the anaerobic digestion process, due to their high conversion rates and efficiencies obtained[117,118].

5.6 Fractionation

As summarized in Table 6, in addition to hydrocarbon fuels, microalgae can provide a large number of co-products ranging from food ingredients and feed to valuable products for nutraceutical, pharmaceutical and ecological applications[119].

Algal biomass has been commercially used as human food or animal (including fish, pet and farm animals) feed after harvesting and drying due to its high protein content and other health promoting ingredients[120-123]. The major food derived from algae include nori from red macroalgae, wakame from brown algae, kombu from brown macroalgae, alginates from brown macroalgae, carrageenans from red algae, agars, agarose derived from agar and seaweed meal[122]. The major strains for human nutrients supplement production include *Spirulina*, *Chlorella*, *Dunaliella salina and Aphanizomenon flos-aquae*[123], and that for the animal feed include *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira*[123-127].

Table 6 High value chemicals produced from algae with application areas[119]

Species/group	Product	Application areas
Spirulina platensis/Cyanobacteria	Phycocyanin, biomass	Health food, cosmetics
Chlorella vulgaris/Chlorophyta	Biomass	Health food, food supplement, feed surrogates
Dunaliella salina/Chlorophyta	Carotenoids, β-carotene	Health food, food supplement, feed
Haematococcus pluvialis/Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives
Odontella aurita/Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food
Porphyridium cruentum/Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition
Isochrysis galbana/Chlorophyta	Fatty acids	Animal nutrition
Phaedactylum tricornutum/Bacillariohyta	Lipids, fatty acids	Nutrition, fuel production
Lyngbya majuscule/Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition

Polyunsaturated fatty acids, which can be added to human food or animal feed, are a major group of high value chemicals produced by algae. Docosahexaenoic acid (DHA), which is helpful for brain and eye development in infants and cardiovascular health in adults[128], can be produced by *Crypthecodinium* and *Schizochytrium*[123]. Eicosapentaenoic acid (EPA), which has been shown to have positive effects on prevention and treatment of several human diseases and disorders[129], is produced by *Nannochloropsis, Phaeodactylum,* and *Nitzschia*[123]. Arachidonic acid (AA) and γ-Linolenic acid (GLA), which are used in infant formulas and nutrients supplements, are produced by *Arthrospira* and *Porphyridium*, respectively[123]. The technology for DHA production has already been commercialized, while that for EPA, AA, and GLA are still under vigorous investigation[119].

Another group of high value chemicals derived from algae is pigments including carotenoids (e.g. *β*-carotene, astaxanthin, lutein, zeaxanthin, lycopene and bixin) and Phycobiliproteins (e.g. phycoerythrin and phycocyanin)[119]. Carotenoids are used as natural food colorants, additive for animal feed, and also in cosmetics (Pulz and Gross, 2004[123]). *β*-Carotene is mainly produced by *Dunaliella.salina,* and used in health food as a vitamin A precursor. Astaxanthin is originated from *Haematococcus. Pluvialis,* and mainly consumed by salmon feed industry. Lutein, zeaxantin and canthaxantin are used for chicken skin coloration and pharmaceutical purposes (Pulz and Gross, 2004[123]).

Phycobiliproteins are mainly produced by cyanobacterium *Arthrospira* and the rhodophyte *Porphyridium*[130,131], and used in food and cosmetics, as well as in industry, clinical and research immunology laboratories as, for example, labels for antibodies, receptors and other biological molecules in a fluorescence-activated cell sorter[119,123]).

Antioxidant products derived from microalgae, including *β*-carotene, tocopherol, antioxidant extract, and PUFAs extracts are another commercial application of algal high value chemicals[119]. They are mainly used in face and skin care products, as well as sun protection and hair care products[123]. Their ability for functional food and therapy of oxidationassociated diseases is also of growing interest[119].

Some other valuable special products originated from algae such as toxins and isotopes are also under intensive research for their application and commercialization[119].

Pulz and Gross[119] reported that the size for microalgal biomass is about 5 000 t-dry-weight/year, which creates a profit of U.S. $ 1.25×10^9/year. Thus if the co-products generation can be integrated into the renewable fuel production, the economics of the whole process will be greatly improved.

5.7 Biorefinery approach

Algal biomass contains 20%-40% protein, 30%-50% lipid, 20% carbohydrate, and 10% other compounds. Depending on the conversion processes, a range of products can be obtained from algal biomass. If a system approach is taken towards the processing of algae biomass, it is possible to maximize the utilization of the biomass for maximum economic and environmental benefits. Biorefining is such a system approach. Biorefining is a concept derived from petroleum refining.

A biorefinery uses biomass as feedstock as opposed to fossil resources used in a petroleum biorefinery. The goal of biorefining is to produce a wide range of products such as fuels, materials, chemicals, etc., from one or more biological resources. Because biomass is not a heterogeneous feedstock, several biorefinery platforms such as biological platforms and thermochemical platforms have been proposed. A biorefinery uses a portfolio of conversion and refining technologies and may be integrated with biomass feedstock production. An integrated biorefinery is capable of producing multiple product streams and thus multiple income streams from a single biomass feedstock and, therefore, more economically viable than single product-based production schemes. Figure 7 shows a biorefining scheme for algal biomass utilization. The heat and energy generated in the scheme may be used within the scheme to make the system partially self sufficient in terms of energy.

Development of new processes, design of the system, and life cycle analysis are necessary for the development and implementation of algae based biorefineries.

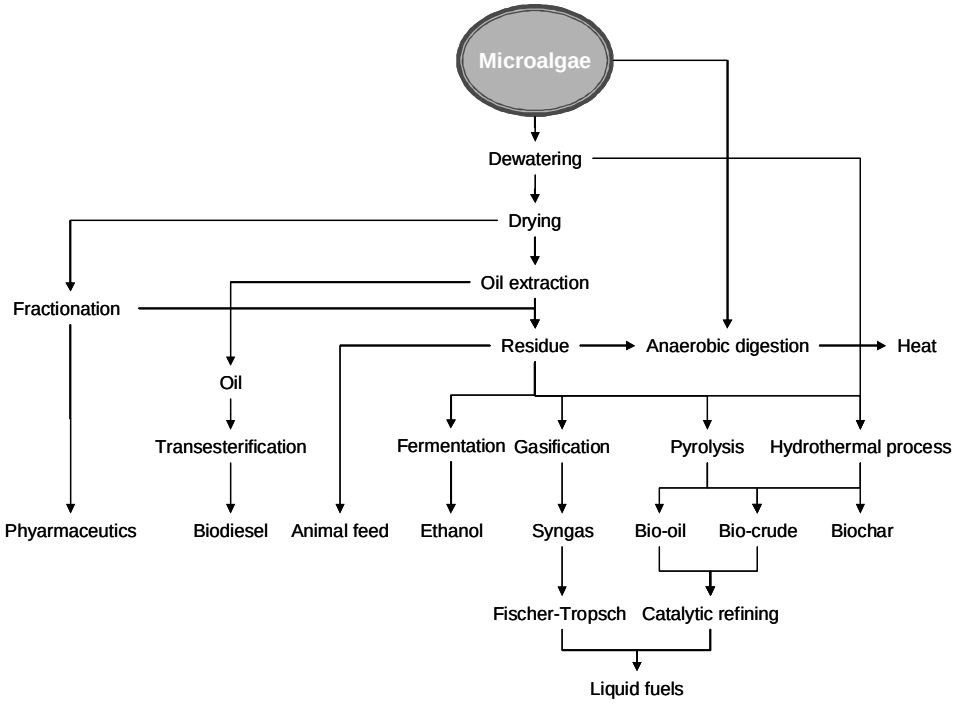


Figure 7 An integrated biorefining scheme for algal biomass utilization

6 Environmental and cost assessment

Algae based energy solutions share the same environmental benefits with other biofuels, i.e., most notably a reduction in greenhouse gas (GHG) emissions. Algae offer additional environmental benefits when they are grown on wastewaters and CO_2 containing flue gas.

6.1 Contaminant removal from wastewater

As the regulation of water discharge standards becomes more and more stringent, municipal wastewater treatment plants are facing a big challenge to optimize their secondary treatment process or consider introducing efficient tertiary treatment in order to further reduce nitrogen and phosphorus levels in the water to be discharged. Microalgae, a perfect candidate acting like activated sludge in aeration tanks, can take up and metabolize these nutrients even at very low levels[132,133]. Many studies demonstrated the success of using algae to clean wastewater rich in nitrogenous and phosphorus compounds[134]. Compared to the conventional wastewater treatment process, which introduces activated sludge, a biological floc, to degrade organic carbonaceous matter to CO_2, algae can assimilate organic pollutants into cellular constituents such as lipid and carbohydrate, thus achieving pollutant reduction in a more environment-friendly way.

Research on using algae cultivation as a tertiary wastewater treatment process started as early as 1970s[134-136]. While the initial purpose of introducing algae pond process was to further treat the secondary effluent in order to prevent from causing eutrophication[137,138], it was observed that the treatment removed nutrients from settled domestic sewage more efficiently than activated sewage process did, suggesting that it would be more economical and desirable to employ the algal system as the secondary rather than tertiary treatment process[138].

A wastewater stream generated from centrifuging of activated sludge, named centrate, contains highest amount of ammonia nitrogen and active phosphorus among several wastewaters at different stages in a municipal wastewater treatment plant, which could be a sutiable growth medium for microalgae for the dual purposes of removing nutrients and obtaining a feedstock for biofuel production.

From a study of an outdoor algal turf scrubber (ATS) raceway system, Mulbry et al.[139] concluded that projected annual operation costs were well below the costs cited for upgrading existing water treatment plants in sensitive watersheds, indicating that the algal technology for dairy manure treatment is very appealing from the environmental standpoint.

6.2 Carbon capture

For every ton of algal biomass produced, approximately one ton of CO_2 is fixed (assuming 40% of dry algae biomass is carbon) through autotrophic or heterotrophic metabolisms in algae. Most plants capture very dilute CO_2 from the atmosphere during photosynthesis but algae are able to use concentrated CO_2 sources such as flue gases. Flue gases are the major form of CO_2 emission from fossil fuel fired power plants, industrial processes, transportation, and residential and commercial buildings. The concentrations of CO_2 in flue gases vary from 12% to 20% with different sources. Flue gases from power plants and other fixed processing facilities can be transported to algae cultivation facilities co-located with power plants or fixed processing facilities. The algal utilization of the high doses of CO_2 in flue gases remains challenging. Although algal photosynthesis could be enhanced by an increase in atmospheric CO_2 up to 5%, beyond which growth of many algal species and strains is inhibited, meaning that the ability of algae to capture CO_2 from flue gas is limited. A few CO_2-tolerant algal species could survive in an environment with 40% CO_2[140]. Careful regulation of CO_2 input could maximize CO_2 utilization and minimize undesirable CO_2 inhibition. Additional issues such as NO_x and SO_x present in the flue gas inhibiting algal growth should be taken into account[141].

6.3 Cost analysis

Several key economic concerns of the mass algal production system considered are: (a) the cost of the resources such as nutrients needed for growing algae, CO_2 and water availability, (b) cost of construction and maintenances of the culture system, (c) the capital and operational costs of harvesting systems, and (d) downstream processing and refining cost. The cost of large scale cultivation varies with algal species, growth rate, lipid content, plant location, and type of culturing system. When algal cultivation is combined with municipal and animal wastewater treatment, CO_2 usage from ethanol plant or utilization of flu gas, the cost of resources can be reduced considerably. Figure 8 indicates that the costs for producing a gallon of algal oil differ greatly with different production systems and conditions. The average cost is US$109/gal with a wide variability (Std. Dev. = US$301/gal). The variability arises largely from the uncertainties in facility and operating costs while land cost is either not considered or small in most sources relative to total capital cost (Figure 9) [142]. This information suggests that facility and operation are where technological innovations have potential to reduce costs substantially.

General Atomics, a US company, estimated the costs for algal oil are in the range of $20.0 to $32.8 based on an open pond algae farming system[4]. The cost breakdown is shown in Table 7. The growth cost accounts for 60%-75% of the total costs.

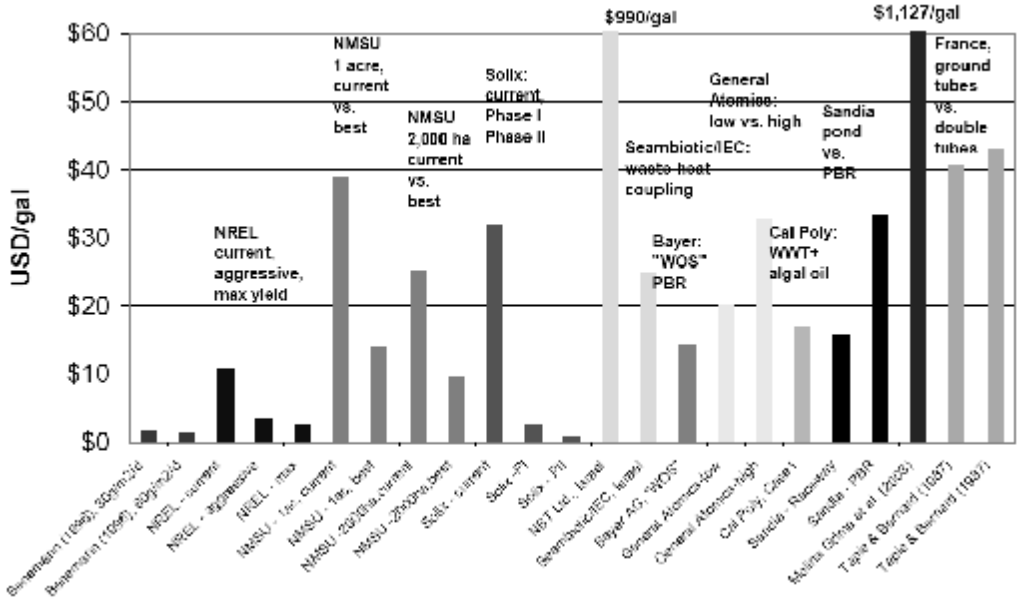


Figure 8 Standardized algal oil cost comparison[142]

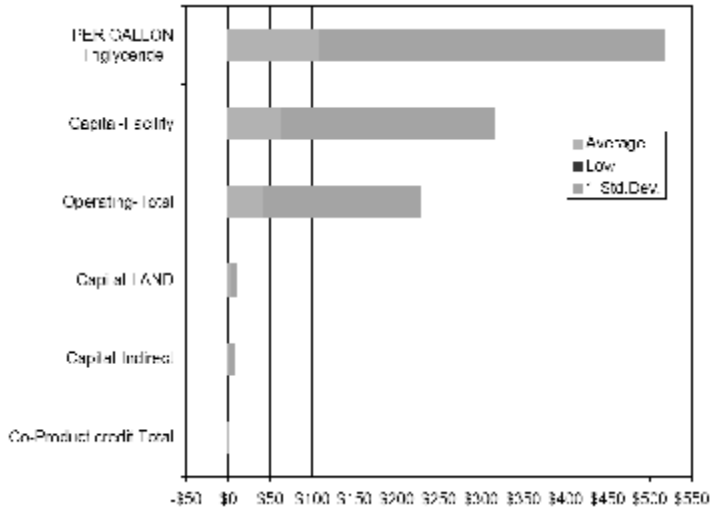


Figure 9 Cost uncertainties by categories[142]

Table 7 Cost breakdown for producing algal oil (open pond)[4]

Cost component	$/gallon
Algae growth	15.00-20.00
Water and nutrient supply	0.40-0.70
Carbon Dioxide supply	1.20-2.40
Harvesting	0.80-1.60
Oil extraction	1.50-2.60
Inoculation	1.10-5.50
Algae oil subtotal (General Atomics)	20.00-32.80

In a case study based on a 63-ha raceway located in west Australia, the total capital cost, including site preparation, culture system, engineering fee, contingency and land, was Aus$18.4M. The total annual productivity of *P. carterae* for the plant is about 1 170-1 480 tons per year in which the nutrient cost Aus$298K per year. The labor cost Aus$942 500 per year and power cost Aus$3.9M per year. By using 12 years return the calculated costs is about 5.3 Aus$ kg-1 *P. carterae* at unregulated pH[143]. Algae biomass production cost for raceway system and tubular PBR were summarized by Shen et al.[24] (Table 8). Downstream processing that include harvesting, drying and oil extraction accounts for 40% of the total cost, which is about the same as algae culture cost. It is estimated that 14%, 10%, and 16% of total production costs come from harvesting, drying, and

oil extraction, respectively[24]. Due to the high production cost, producing biodiesel from algae still not economically feasible today. Future research will have to significantly reduce production costs through innovations in areas such as metabolic and genetic engineering, algae biorefinery, production system engineering and downstream processing[24].

Table 8 Algae production costs of raceway and tubular PBR systems[24]

	Raceway	Tubular PBR
Algae strain	*Dunaliella*	*S. almeriensis*
Final product	β-carotene	biofuel
Scale	10 ha	650 m^2, 30 m^3
Biomass yield		
g $m^{-2}d^{-1}$	2	50
tons $ha^{-1}. yr^{-1}$	7	100
Capital cost ($)		
Major purchase equipment	4 300 000	290 720
Installation	--	29 070
Building	1 000 000	29 070
Infrastructure	1 000 000	264 140
other	300 000	--
Total capital costs	6 600 000	613 000
Depreciation (10 years, $.$yr^{-1}$)	660 000	61 300
Operating cost ($ .yr^{-1})		
Fertilizers	36 000	4 720
Labor	500 000	127 930
Electricity	180 000	18 130
Water	220 000	--
CO_2	150 000	8 810
Other	80 000	--
Total operation costs	1 166 000	159 590
Total production cost ($. yr^{-1})	1 826 000	220 890
Algae biomass production cost ($ kg^{-1})	26	34

7 Conclusions

Algae have a great potential for meeting the world's energy need. Many R&D efforts so far have advanced the technologies. However, the commercialization of algal fuels is very challenging chiefly because of the techno-economic constraints.

Facility and operation are areas with potential for substantial cost reduction through technological innovation. While open pond production systems may be practical in some areas, low cost enclosed photobioreactors with high photosynthesis efficiency must be developed and evaluated. Production systems which can be operated year round with good control of competitors, grazers, and pathogens are desirable. Wastewaters rich in nutrients are the preferred culture media for algae production because they offer many economic and environmental benefits. Screening and genetic modification of algae strains will play an increasingly important role. Genetic engineering has the potential to improve the overall algal biomass yield and lipid yield. Discovery of new strains and genetically modified strains capable of secreting hydrocarbons to extracellular spaces will open some new opportunities; however, challenges with recovering the secreted liquids or volatiles remain. There is a need to develop high throughput screening and analysis methods. Current harvest and dewatering are still too energy intensive. New techniques and strategies must be devised to lower the costs. Direct conversions such as *in situ* transesterification and hydrothermal liquefaction offer the possibility to process wet algae. Fractionation of algal biomass, before or after oil extraction, deserves a closer look because it may play an important role in offsetting the costs. New techniques to disrupt algae cellular structures to improve oil extraction efficiency are needed. A bioerfining scheme is believed to maximize the economic return of downstream processing. A systems approach, which minimizes production costs, maximizes product recovery and utilization, and provides environmental benefits, must be adopted in order to reduce the overall costs of algal fuels. Stable pilot to large scale operations must be established for meaningful life cycle analysis before commercialization can take place.

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