Marine Micro

5. Marine Microalgae

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Marine microalgae, the largest primary producer in the marine system, have been attracting wide attention as potential resources of new metabolites and biofuels. Whole genome sequencing and genetic modifications of microalgae have been rapidly advancing during the last few decades. In this chapter, the diversity of marine microalgae, the microalgal natural components, and the biotechnologies associated with microalgae are reviewed.

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5.1 Overview

Algae are the primary producers of oxygen in the aquatic environment. These microorganisms are widely distributed in marine systems and have a great diversity with respect to size, morphology, life cycle, pigments, and metabolism. About half of the global oxygen production is accomplished by marine microalgae. They play an important role in CO_2 recycling through photosynthesis, which is similar to higher plants in O_2-evolved systems (PSI (photosystem I) and PSII (photosystem II)). In addition to having a long history of use as food and as live feed in aquaculture, microalgae have also been considered as a promising source for high-value added products for pharmaceuticals, cosmetics, and other industrial applications, such as β-carotene, astaxanthin, polyunsaturated fatty acids (PUFAs). The utilization of microalgae as a suitable feedstock for sustainable biofuel production has gained worldwide attention over the last 20 years. In general, microalgal triacylglycerols, hydrocarbons, and polysaccharides are considered as biofuel precursors. Alkanes and short chain fatty acid methyl esters (FAMEs), ranging from C9 to C17, have been considered as the potential alternative jet fuel, while those ranging from C9 to C23 have been mainly recognized as biodiesel. FAMEs are normally generated from the methyl esterification of triacylglycerols. On the other hand, with the degradation and fermentation of microalgal polysaccharides, the generated ethanol can be used as an alternative fuel to gasoline. Compared with higher plants, biofuel from microalgae has two advantages: 1) a relatively higher productivity, and 2) no competition to agriculture.

On the other hand, increased reports of the microalgal whole genome sequence data have been significantly facilitating the better understanding of their evolutionary lineage and the species specificity of the microalgal metabolic pathway. In addition, gene transformation has been achieved in 18 microalgal genera.

In this chapter, we reviewed the fundamental characteristics of marine microalgae, the useful microalgal natural products, as well as the biotechnological aspects of marine microalgae.

5.2 Marine Microalgae

Algae are a very diverse group of photosynthetic organisms other than land plants, which have been classified into many classes, such as *Cyanophyceae*, *Chlorophyceae*, *Rhodophyceae*, *Cryptophyceae*, *Dinophyceae*, *Bacillariophyceae*, *Haptophyceae*, *Euglenophyceae* or *Prasinophyceae*. For convenience, they are referred to as blue-green algae, green algae, brown algae, or red algae due to the difference in composition of photosynthetic pigments. However, it is difficult to make a clear definition of algae because even multicellular eukaryotic microalgae (what is called seaweed) are also included. In this section, some representative marine microalgae are summarized to introduce their biotechnological applications.

Cyanophyceae (cyanobacteria, blue-green algae) are oxygenic photosynthetic prokaryotes that comprise a single taxonomic and phylogenetic group. Chloroplasts in eukaryotes evolved from endosymbiotic cyanobacteria. They show a large diversity in their morphology, physiology, ecology, biochemistry, and other characteristics. Typically, cyanobacteria contain chlorophyll a and phycocyanin. Three genera, i. e., *Prochlorococcus*, *Prochloron*, and *Prochlorothrix*, lack phycocyanin and possess chlorophyll a and b [5.1]. A unicellular cyanobacterium that synthesizes chlorophyll d has also been discovered [5.2]. Marine *Synechococcus* and *Prochlorococcus* contribute largely to global oxygen production. Cyanobacteria have gained attention as a source of bioactive compounds and biopolymers (polyhydroxyalkanoates (PHA)s) [5.3]. Bioactive compounds isolated from marine cyanobacteria were summarized by *Burja* et al. [5.4] and *Takeyama* and *Matsunaga* [5.5]. Several strains of cyanobacteria (*Synechococcus elongates* and *Anabaena variabilis*) have been reported to produce long-chain alkanes and alkenes [5.6]. These findings make cyanobacterial alkane and alkenes a promising source of biofuels [5.7]. Two enzyme families that are responsible for straight-chain hydrocarbon production in cyanobacteria have recently been identified as an acyl–acyl carrier protein reductase (AAR) and an aldehyde-deformylating oxygenase (ADO). These enzymes convert fatty acid intermediates to alkanes and alkenes. This discovery of the cyanobacterial alkane biosynthesis indicates possibilities for optimizing the biodiesel production in cyanobacterial strains with modest gains in alkanes [5.8].

Chlorophyceae, which are one of the classes of green algae, possess chlorophyll a and b, the same predominant photosynthetic pigments as those of land plants. *Chlorophyceae* form starch in the chloroplast as a storage product of photosynthesis. Especially, *Chlamydomonas reinhardtii* has been used as a representative eukaryotic microalgae for biology and molecular biology studies. Chloroplast transformation was firstly achieved in *C. reinhardtii* [5.9]. Some species of *Chlorophyceae* are found in the marine environment. A marine species of *Chlorophyceae*, *Dunaliella* has been cultivated commercially for food supplements and β-carotene production [5.10]. *Chlorella*, which is a genus of single-cell and chlorophyll a/b-containing algae, belongs to the phylum *Chlorophyta*. It has been known as a potential food resource because of its high content of protein and other nutrients. Miura and others [5.11] reported that *Chlorella* sp. NKG 042 401 contains 10% γ-linolenic acid (C18:3), which is present in the cells mainly in the form of galactolipids. In *Euglenophyceae*, the genus *Euglena* is well known. The chloroplast of *Euglena* originated from the eukaryotic green algae and contains chlorophyll a and b. Although most species are found in freshwater environments, some species also occur in marine environments.

Bacillariophyceae (diatoms) possess chlorophyll a and c, and fucoxanthin as the major carotenoid. Diatoms are widely used as feed in mariculture/aquaculture [5.12, 13]. *Chaetoceros calcitrans*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Skeletonema costatum*, and *Thalassiosira pseudonana* are commonly used as live feed for all growth stages of bivalve molluscs (e.g., oysters, scallops, clams, and mussels), for crustacean larvae, and for zooplankton used as feed for larvae. The genera *Navicula*, *Nitzschia*, *Cocconeis*, and *Amphora* also are used to feed juvenile abalone. They store energy either as lipids or as chrysolaminarin. Most diatoms have a high content of eicosapentaenoic acid (EPA) 20 : 5 (n-3). *Phaeodactylum tricornutum* and *Nitzschia laevis* have been especially investigated for EPA production. In addition, EPA production by diatoms was reviewed recently by *Lebeau* and *Robert* [5.14, 15]. Recent advances in heterotrophic production of EPA by microalgae were also reviewed by *Wen* and *Chen* [5.16].

The cells of *Haptophyceae* are brownish or yellowish-green and contain chlorophylls a/c and carotenoids such as β-carotene, fucoxanthin, diadinoxanthin, and diatoxanthin. The cells are commonly covered with scales made mainly by carbohydrates or calcium bicarbonate. Many species known as coccol-

ithophorids produce calcified scales called coccoliths. Most are primarily marine species inhabiting tropical seawater. Microalgal biomass of *Haptophyceae* is commonly used as living feed in aquaculture [5.17]. *Isochrysis galbana* and *Pavlova lutheri*, especially, are used as living feed for bivalve molluscs, crustacean larvae, and zooplanktons that in turn are used for crustacean and fish larvae. Some cells can produce PUFAs such as docosahexaenoic acid (DHA), or EPA. In addition, the DHA content in *I. galbana* has been shown to be enhanced by low temperature or incubation of the culture in the dark after reaching the plateau phase growth [5.18]. Furthermore, it was shown that these algae are useful for DHA enrichment of feed such as rotifers for the larvae of several marine fish species [5.19].

In *Dinophyceae*, a genus *Symbiodinium* (dinoflagellate) has been well described. Various marine invertebrates, such as reef-building corals, jellyfish, sea anemones, and bivalves form symbiotic associations with *Symbiodinium*, commonly known as zooxanthellae. *Symbiodinium* strains have been classified into more than three clades using restriction fragment length polymorphism based on 18S rRNA sequence analysis [5.20]. The composition of *Symbiodinium* populations may also play an important role in the tolerance or sensitivity of corals towards bleaching.

5.3 Microalgal Genomes

Sequencing of microbial genomes has become a routine procedure for gene discovery and genetic engineering of microalgae. *Synechocystis* sp. PCC 6803 was the first photosynthetic organism for which the entire genome sequence was determined. Currently, 72 finished cyanobacterial genome sequences are listed in GenBank, and many additional genome analyses are currently in progress. Most cyanobacteria possess a circular chromosome and a small number of additional plasmids. Genome sizes range from a minimum of 1.44 Mb for the marine cyanobacterium UCYN-A [5.21] to a maximum of 11.58 Mb for the *Calothrix* sp. PCC7103 [5.22]. Prokaryotes typically contain a single copy of their chromosome such as *Escherichia coli*, while large differences between cyanobacteria and other prokaryotes have been reported for chromosomal copy numbers. Some cyanobacteria are oligoploid, for example, *Synechocystis* sp. PCC 6803 are highly polyploid, and the motile wild-type strain contains 218 genome copies in exponential phase and 58 genome copies in linear and stationary phases [5.23].

Recently, a comparative genomics-based approach was used to screen cyanobacteria for the direct production of alkanes, the primary hydrocarbon components of gasoline, diesel, and jet fuel [5.8]. Eleven different cyanobacteria with available genome sequences were grown, and their culture extracts were evaluated for hydrocarbon production. Indeed, ten of these strains produced alkanes. The comparison of predicted proteins from these ten genomes against the eleventh finally led to the discovery of two hypothetical proteins as candidates for alkane biosynthesis. This discovery is the first description of genes responsible for alkane biosynthesis and the first example of a single-step conversion of sugar to fuel-grade alkanes by an engineered microorganism. A comparison of the genome sequences of producing and non-producing organisms led to the identification of the responsible genes.

In eukaryotic microalgae genomics, large-scale sequencing has been demonstrated by next-generation sequencing technologies. These have drastically increased the number of bases obtained per sequencing run while at the same time decreasing the costs per base. The first whole genome sequence of *C. merolae* was determined in 2004 [5.26]; this was the first identi-

Table 5.1 Sequenced whole genomes of microalgal strains

Microalgae species	Genome length (Mbp)	References
Ochrophyta		
Phaeodactylum tricornutum	27.4	[5.24]
Thalassiosira pseudonana	32.4	[5.25]
Rhodophyta		
Cyanidioschyzon merolae	16.5	[5.26]
Chlorophyta		
Chlamydomonas reinhardtii	121	[5.27]
Chlorella variabilis	46.2	[5.28]
Micromonas pusilla	21.9	[5.29]
Micromonas sp.	20.9	[5.29]
Volvox carteri	138	[5.30]
Ostreococcus lucimarinus	13.2	[5.31]
Ostreococcus tauri	12.6	[5.32]
Coccomyxa subellipsoidea	48.8	[5.33]

fied eukaryotic microalgal genome. Up until November 2012, the whole genome sequence of 11 strains of microalgae had been sequenced, including 2 diatoms (*Thalassiosira pseudonana* [5.24] and *Phaeodactylum tricornutum* [5.25]), a red alga (*Cyanidioschyzon merolae* [5.26]), and 8 green algae (*Chlamydomonas reinhardtii* [5.27], *Ostreococcus lucimarinus* [5.31], *Ostreococcus tauri* [5.32], *Chlorella variabilis* [5.28], *Volvox carteri* [5.30], *Coccomyxa subellipsoidea* [5.33], *Micromonas pusilla* [5.29], and *Micromonas* sp. [5.29]), see Table 5.1. In addition, the draft genome sequences of 17 strains of microalgae can been found in the NCBI GenBank databases [5.34]. With next generation technology, the draft genome sequence of the biodiesel producing microalga *Nannochloropsis gaditana* strain CCMP526 were also identified recently [5.35]. The identified microalgal whole genome sequences provide a powerful tool for the discovery of genes and metabolic pathways. Even though most of the predicted microalgal pathways have been proved to be similar to corresponding pathways in higher plants, the urea cycle identified from genomes of diatoms is absent in higher plants but present in animals [5.24]. The existence of an animal metabolic pathway in microalgal cells further highlights the importance of genome analysis for microalgae.

5.4 Genetic Engineering of Microalgae

5.4.1 Genetic Transformation Methods

Genetic studies on microalgae have been redirected mainly toward analysis of photosynthesis and metabolic pathways. A limited number of microalgae such as cyanobacteria have been used in biotechnological applications. The development of molecular techniques for physiological analysis and enhancement of biotechnological applications is necessary. Many attempts at gene transfer have been made in eukaryotic and prokaryotic microalgae. Genetic manipulation in prokaryotic microalgae cyanobacteria was studied extensively after several transformable unicellular strains were discovered. First, the freshwater cyanobacterium *Synechococcus* PCC7942 was reported to have the ability to take up DNA. Subsequently, several other naturally transformable freshwater strains were found. Gene transfer has been developed mainly in the freshwater strains *Synechococcus, Synechocystis, Anabaeba*, and *Nostoc* [5.69]. Several marine cyanobacterial strains of the genus *Synechococcus* have been also used for heterogeneous gene expression and other genetic applications [5.70, 71]. There are two commonly used gene transfer procedures: transformation using naturally occurring or artificially competent cells, e.g., conjugation with *Escherichia coli*, or physical methods for gene introduction, e.g., electroporation and particle bombardment. Natural transformation has been reported for *Synechococcus* sp. PCC7002 [5.72]. Other strains have been transformed successfully by electroporation or conjugation. Further, plasmids harvested from several marine microalgal species have been used as vector DNA for gene transfer. Marine plasmids have been found in *Synechococcus* sp. NKBG042902, which has a high phycocyanin content and a rapid growth rate. This strain contains more than three cryptic endogenous plasmids, and one of these, the plasmid pSY10 has the unique replication characteristic that its copy number increases under high salinity conditions [5.73]. Plasmids are maintained at a high copy number in cyanobacteria, which suggests the possibility that they act as a shuttle vector between cyanobacteria and *E. coli*. In fact, a shuttle vector with *E. coli* has been constructed using pSY10. Conjugative gene transfer using a broad-host range vector pKT230 was successful for the marine cyanobacterium *Synechococcus* sp. NKBG 15041C [5.74]. It has been demonstrated that this plasmid is stably maintained in cyanobacterial cells [5.75]. In marine cyanobacteria, in addition to the plasmid vector system, the construction of a phage vector system is also required to enable the cloning of large DNA fragments in specific cyanobacterial hosts. Since cyanophages were first reported by *Safferman* and *Morris* [5.76], various types of cyanophages have been found in seawater [5.77, 78] and characterized according to their genetic diversity and phylogenetic affiliations [5.79].

Due to the advance of genome, proteome, and metabolome analyses of microalgae, many attempts at gene transfer to eukaryotic microalgae have been made to enhance the production of useful compounds and biomass. However, because of the stiff cell wall of microalgae, the introduction of exogenous genes into microalgal cells could be challenging. The additional frustules and coccoliths surrounding some species of microalgae cells further increases the difficulty. Thus,

Table 5.2 Microalgal strains achieved for the stable transformation

Phylum species	Organelle	Transformation methods	Gene knock-down	References
Diatom				
Cyclotella cryptica	Nucleus	Biolistic		[5.36]
Cylindrotheca fusiformis	Nucleus	Biolistic		[5.37]
Chaetoceros sp.	Nucleus	Biolistic		[5.38]
Navicula saprophila	Nucleus	Biolistic		[5.36]
Phaeodactylum tricornutum	Nucleus	Biolistic	✓	[5.39, 40]
Thalassiosira pseudonana	Nucleus	Biolistic		[5.41]
Fistulifera sp.	Nucleus	Biolistic	✓	[5.42]
Chlorophyta				
Chlamydomonas reinhardtii	Nucleus	Biolistic, Electroporation, Glass beads, Agrobacterium	✓	[5.43–48]
	Chloroplast	Biolistic		[5.9]
	Mitochondoria	Biolistic		[5.33]
Chlorella spp.	Nucleus	Biolistic, Electroporation, Agrobacterium		[5.49–53]
Dunaliella spp.	Nucleus	Biolistic, Electroporation , Glass beads	✓	[5.54–58]
Haematococcus pluvialis	Nucleus	Biolistic, Agrobacterium		[5.59, 60]
Volvox carteri	Nucleus	Biolistic		[5.61]
Dinoflagellate				
Amphidinium sp.	Nucleus	Glass beads		[5.62]
Symbiodinium microadriaticum	Nucleus	Glass beads		[5.62]
Rhodophyta				
Cyanidioschyzon merolae	Nucleus	Glass beads	✓	[5.63, 64]
Porphyridium spp.	Chloroplast	Biolistic, Agrobacterium		[5.65, 66]
Euglenophyta				
Euglena gracilis	Chloroplast	Biolistic		[5.67]
Eustigmatophyte				
Nannochloropsis spp.	Nucleus	Electroporation		[5.35, 68]

the optimization of the gene transformation method for each specific species turns out to be important. Depending on the physiological characteristics of microalgal cells, electroporation, glass beads-mediated transformation, agrobacterium-mediated transformation, and biolistics have frequently been used. Moreover, the level of target protein varied due to multiple insertion, random integration, and (or) gene silencing [5.80]. Stable transformants that have already been reported are summarized in Table 5.2.

Biolistics, also referred to as a gene gun that was originally designed for the delivery of nucleic acid through the cell wall of intact plant cells, has been mostly applied for microalgae gene transformation. The payload in this system is a plasmid DNA-coated tungsten particle (particle size: $0.6-1.6\,\mu m$), which can be shot with helium gas. After bombardment, the tungsten particles were shot down to the plant organism or the cell culture on the petri dish. Some cells that are not disrupted by the firing may envelope the DNA-coated tungsten particles and the DNA can then migrate to and integrate into the plant chromosome [5.81]. The transformation efficiency of this methodology is not related to the physical property of the host cell but is highly controlled by the gas pressure at the point of firing. Therefore, theoretically, despite the hard cell wall and frustules, gene transformation can be achieved when the gas pressure is high enough.

Electroporation is a phenomenon when the electrical conductivity and permeability of the cell membrane increase by the externally applied electrical field. If the host cells and plasmids are mixed together, the plasmids can be transferred into the host cells through the transient holes in the cell membrane generated by the electronic shock. Electroporation-based gene transformation methodology has been commonly used to

transform mammalian cells with plasmids. The transformation efficiency was significantly decreased for plant cells due to their thick cell walls. Electroporation-based gene transformation has only been achieved with the *Chlamydomonas reinhardtii* cell wall-deficient mutant and *Dunaliella salina* cells, which have no cell wall [5.46, 57, 82]. However, in the studies mentioned above, the transformation efficiency was tenfold higher than the gene gun method applied to the corresponding strains [5.83].

The glass beads method is a relatively simple transformation procedure that has a higher transformation efficiency than biolistics but it is only capable of transforming cells without cell walls. Both the cell-wall deficient *C. reinhardtii* mutant and *D. salina* have been reported to have been successfully transformed by the glass bead method with a higher efficiency than with the gene gun method [5.56].

Agrobacterium-mediated transformation is based on the characteristic of the soil bacterium *Agrobacterium tumefaciens* that it naturally transfers and inserts its genes into plant chromosomes. Exogenous genes can be transferred into plant cells through *Agrobacterium* transformation using target gene inserted agrobacterium transfer DNA (T-DNA). Although reports of microalgae transferred by agrobacterium transformation are few, *Kathiresan* et al. achieved a twofold transformation efficiency with *Haematococcus pluvialis* over the gene gun method [5.60].

In the transformants generated by the methods mentioned above, it is not rare to find the continuous expression of the target genes in the chloroplast and (or) mitochondria due to the insertion of the target genes into their organelle genome. By using a specific vector containing a homologous sequence in the organelle genome, stable chloroplast and (or) mitochondria transformation can be expected. On the other hand, the target genes are usually found to be randomly inserted into the nucleic genome and even homologous recombination occurs. Thus, it is hardly possible to control the insertion site and the number of the target genes inserted into the nucleic genome, which has made gene functional analysis via gene knock-out difficult. With further consideration of the dual nature of the microalgal life cycle as either haploid or diploid, the possibility of complete knock-out dwindled significantly in diploid cells. The homologous recombination has been applied to the transformation of *C. reinhardtii* and *Volvox carteri*, which maintain an asexual haploid zoospore during the life cycle; their recombination efficiency, however, was inferior [5.84, 85]. Recently, highly efficient homologous recombination was reported in *Nannochloropsis* sp., which suggested the possible use in microalgal gene functional analysis [5.68]. For those diploid microalgae, the knockdown of the target gene via RNAi has been reported and considered as the substitute for knock-out [5.40, 44, 55, 64].

So far, six microalgae including *Phaeodactylum tricornutum*, *Thalassiosira pseudonana*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Volvox carteri*, and *Cyanidioschyzon merolae* have not only obtained stable transformants but also the whole genome sequence. *P. tricornutum* has been widely used for the studies of metabolic engineering towards enhanced lipid production. Yet, most research in this field has focused on the established stable transformant rather than on high oil-producing strains whose transformation method have not been determined.

5.4.2 Metabolic Engineering

Enhanced production of valuable primary or secondary metabolites in microalgae can be rendered possible by high density cultivation and/or application of genetic manipulation. Recent pharmaceutical interest in unsaturated fatty acids has triggered the search for sources of these valuable compounds. Several eukaryotic microalgae are known to produce highly unsaturated fatty acids such as EPA and DHA, which are valuable dietary components [5.16, 19]. Genetic engineering has been applied to produce EPA in the marine cyanobacterium *Synechococcus* sp. [5.71]. Cyanobacteria do not have the biosynthetic pathway to produce them. The EPA synthesis gene cluster (ca. 38 kbp) isolated from a marine bacterium *Shewanella putrefaciens* SCRC-2738 was cloned to the marine cyanobacterium using a broad-host cosmid vector. The content of EPA grown at 2 °C increased to $0.64\,mg\,g^{-1}$ dry cells after 24 h incubation at 17 °C. Furthermore, EPA production was improved by partial deletion of the EPA gene cluster to stabilize its expression and maintenance in host cyanobacterial cells [5.86].

Genetic engineering of microalgae for industrial purposes has also been performed in freshwater cyanobacteria where the ketocarotenoid astaxanthin, an extremely efficient antioxidant, was synthesized by the introduction of the β-c-4-oxygenase gene (*crt*O) from the green alga *Haematococcus* [5.87]. Ethylene production was also demonstrated in the cyanobacterium *Synechococcus elongates* PCC7942 by chromosomal insertion of an ethylene forming enzyme [5.88]. How-

ever, the reaction catalyzed by the ethylene forming enzyme induced metabolic stress, which was detrimental to the host cell.

Microalgal biodiesel production is expected to be improved though metabolic engineering. Several transformants have been established for the increased oil content of the microalgal cell, enhanced biomass productivity, and improved quality of the lipids. Acetyl CoA carboxylase (ACCase), which catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, the primary substrate of fatty acids synthesis, has been overexpressed in diatom cells to elevate the cell lipid content [5.36]. The vector containing the ACCase gene and its 5′ UTR (untranslated region) from the diatom *Cyclotella cryptica* was constructed and introduced into the diatoms *C. cryptica* and *Navicula saprophila*. Stable high ACCase expression transformants were obtained successfully, yet the predicted increase of neutral lipid content was not achieved, which indicates that TAG (triacylglycerol) accumulation in the microalgal cell is much more complex than previously assumed. With the expression of the hexose transporter that transfers the monohexose from the culture medium into the cell, the transformants of green algae *C. reinhardtii* and *V. carteri* as well as diatom *P. tricornutum* have been demonstrated to be capable to grow in the dark in a medium containing glucose [5.89–91]. Especially, the glucose transporter (Glut1) gene transformant of *P. tricornutum* cultured in dark conditions showed an almost threefold higher biomass production than in light conditions [5.91]. On the other hand, the de-regulation of the light-harvesting proteins in *C. reinhardtii* has been demonstrated to be able to elevate the solar energy conversion efficiencies in photosynthesis when the light-harvesting chlorophyll antenna size is minimized [5.92]. This permits a greater photosynthetic productivity under high cell density conditions as well as the possibility of culturing cells under high sunlight conditions. The transformation of *Cinnamomum camphora* (C12-TE) and *Umbellularia californica* (C14-TE) Acyl-ACP thioesterases genes into diatom *P. tricornutum* resulted in an increased lauric (C12:0) and myristic acid (C14:0) accumulation mutant [5.93]. Levels of lauric acid of up to 6.2% of total fatty acids and myristic acid of up to 15% by weight were achieved. Moreover, 75–90% of the shorter chain length fatty acids produced were demonstrated to be incorporated into triacylglycerols.

5.5 Photobioreactors for Marine Microalgae

Microalgae mass cultivation for the production of useful compounds has been widely discussed since the 1950s. Even though large-scale production of astaxanthin, DHA, and EPA from microalgae have been achieved, the industrial production of microalgal biofuel is still under development. Lower cost and higher productivity and efficiency than current bioreactors are necessary due to the extremely low final price of biodiesel (1 dollar L^{-1}) compared with those high value-added microalgal products.

Both the biology and the economics of microalgae mass cultivation are strongly influenced by photobioreactor design. Photosynthetic microalgae can be cultured in photobioreactors as either an open culture system or a closed system. Based on their localization, these photobioreactors can be divided into outdoor culture systems or indoor culture systems.

Outdoor open culture systems are the simplest method of algal cultivation due to the low construction cost and effortless operation. However, the productivity of these systems can be easily affected by several environmental factors such as contamination of other microorganisms, changes of weather conditions, and the disability of transgenic microalgae cultivation. The need to achieve higher productivity and to maintain monoculture of algae led to the development of closed photobioreactors. Despite higher biomass concentration and better control of culture parameters, CO_2 recycling efficiency, energy profit ratio, energy payback time, and cost of production in these enclosed photobioreactors are not better than those achievable in open systems.

The growth rate and maximum biomass yield of microalgal strains are affected by culture parameters (light, temperature, and pH) and nutritional status (CO_2, nitrogen, and phosphate concentration). On the other hand, increasing the density of cultures decreases photon availability to individual cells. Light penetration of microalgal cultures is poor, especially at high cell densities, and such poor photon availability decreases specific growth rates. Higher biomass yields can be expected if sufficient photons are provided in high density cultures of microalgae. Two major types of bioreactors (tubular [5.94, 95] or flat plate [5.96, 97]) are generally applied for the enclosed system (Fig. 5.1).

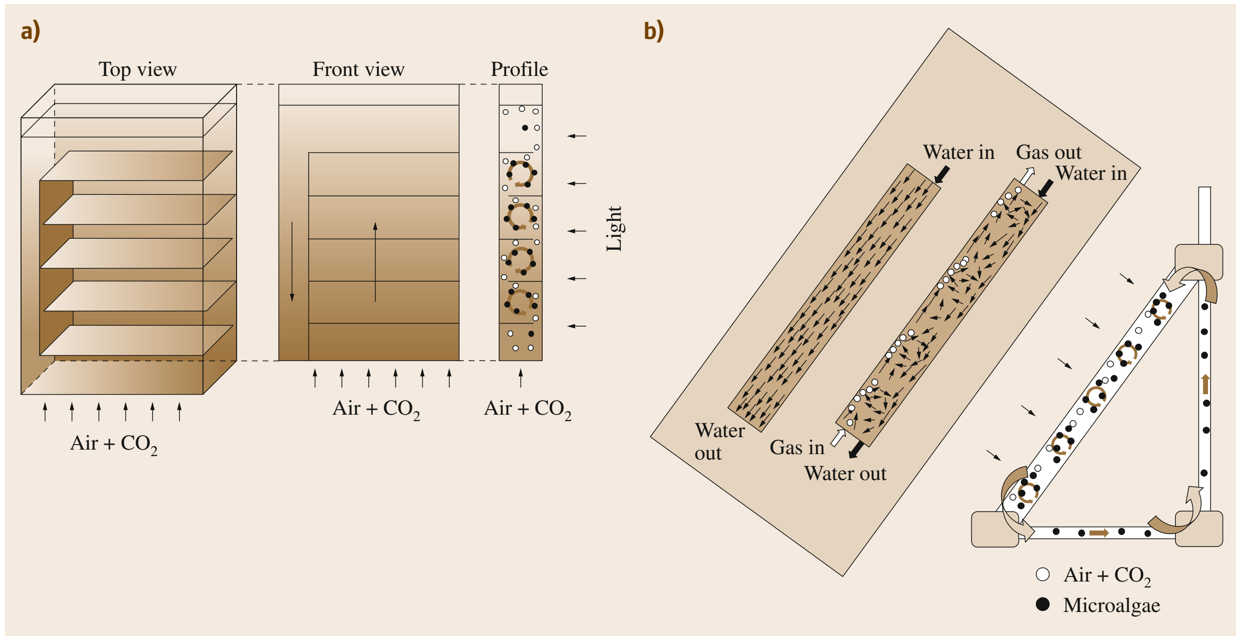

Fig. 5.1a,b Schematics of various photobioreactors. **(a)** After [5.95], **(b)** after [5.96]

Currently these enclosed photobioreactors are industrially applied for biodiesel production, for instance, the outdoor enclosed culture system used in Solix Biofuels Inc. and the indoor enclosed culture system used in Solazyme Inc. In addition, the use of intermittent light instead of continuous illumination can reduce the photoinhibition effect and improve the efficiency of light utilization through the flashing-light effect. On the other hand, the culture medium inside the photobioreactors needs to be stay flowing for mixing the algae and other nutrients. A newly developed flat plate photobioreactor achieved a 1.7-fold increased biomass production for *Chlorella vulgaris* [5.96] by applying the intermittent light from one side of the bioreactor, and the introduction of the air bubbling from the bottom of the bioreactor which generates turbulence inside the container. Recently, based on the flashing-light effect, a triangle bioreactor constructed by three tubular bioreactors with the air bubbling from the hypotenuse has achieved the currently highest volumetric productivity ($365\,\mathrm{t\,ha^{-1}\,a^{-1}}$) [5.95, 98].

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