Chapter 15
Microalgae: Cultivation and Application

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15.1 Introduction

Algae are recognised as one of the oldest life form (Falkowski and Raven 1997) and include large group of organisms from different phylogenetic groups, representing many taxonomic divisions. In general they are primitive plants (thallophytes), i.e. lacking roots, stems and leaves, vascular tissues and a sterile covering of cells around the reproductive organ (Khan et al. 2009; Brennan and Owende 2010; Mutanda et al. 2011). Most of the algae contain chlorophyll by virtue of which they get green colour; however, some of the algae are not green but appears brown and red due to the presence of other pigments such as carotenoid (Wang and Chen 2009). Algae are oxygen evolving photosynthetic microorganisms containing chlorophyll 'a' as primary photosynthetic pigments and grow in various aquatic environments (fresh, marine and brackish water streams) including hot springs (Wang and Chen 2009). Some species can grow on rocks, soils, plants, etc., with minimum nutrient requirements (Zhou 2014). Algae are unicellular or colonial. When the cells are arranged end to end, the algae are said to be filamentous that may be unbranched filaments or more intricate branched filaments (Wang and Chen 2009).

Most of the algae are microscopic known as microalgae, whereas some are quite large and known as macroalgae. Microalgae are unicellular or simple multicellular organism with size ranges from 2 to 200 μm, whereas the macroalgae are completely multicellular organism, some growing to over 100 ft in length (Madigan et al. 1997; Wang and Chen 2009; Mutanda et al. 2011). Among the algae,

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microalgae have been extensively studied for not only due to their ubiquitous nature but also for their simple structure that renders them to grow and proliferate in a wide range of environmental condition (Vonshak 1990; Hu et al. 2007). This simple cellular structure provides a large surface to volume body ratio, which gives them the ability to uptake large amount of nutrients (Brennan and Owende 2010). The mechanism of photosynthesis in microalgae is similar to that of higher plants; however, they are generally considered as the more efficient converters of solar energy than higher plants because of their simple cellular structure (Walter et al. 2005; Spolaore et al. 2006; Khan et al. 2009; Kirrolia et al. 2013).

The present review aims to describe microalgae and its applications, with special emphasis on microalgal biodiesel production and CO_2 sequestration. This work starts by describing microalgae, its chemical composition, cultivation and harvesting system and various applications.

15.2 Microalgae

In applied phycology, the term microalgae covers all unicellular and simple multicellular oxygenic photosynthetic organism with chlorophyll 'a' as common photosynthetic pigment (Richmond 2008). The biodiversity of microalgae is outstanding, and it is estimated that from 200,000 species to several million species exist in nature; however, a very limited number have been studied and analysed (Norton et al. 1996; Mata et al. 2010). Among the microalgae, the most important groups of microalgae in terms of abundance are diatoms, green algae, blue-green algae and golden algae (Table 15.1).

Chlorophyta (green algae) includes a large number of microalgae with great morphological variability like coccoid, unicellular or colonial flagellates and multicellular or multinucleated filaments (Richmond 2008). They contain

Table 15.1 The four important group of microalgae (Khan et al. 2009)

Sl No	Algae	Known species (near about)	Morphology	Storage material	Habitat
1	Diatoms (Bacillariophyceae)	100,000	Unicellular	Chrysolaminarin (polymer of carbohydrates) and TAGs oceans	Fresh and brackish water
2	Green algae (Chlorophyceae)	8,000	Unicellular to leafy	Starch and TAGs	Freshwater, brackish water
3	Blue-green algae (Cyanophyceae)	2,000	Unicellular	Starch and TAGs	Different habitats
4	Golden algae (Chrysophyceae)	1,000	Unicellular	TAGs and carbohydrates	Freshwater

chlorophyll a, b which gives it a complete green colour. Along with chlorophylls, carotenoids are synthesised and accumulated in chloroplast.

15.2.1 Habitats of Microalgae

Microalgae are some of the most robust organisms on earth and are able to grow in almost every habitat in every part of the world. These are able to grow in a wide range of aquatic to terrestrial environment. The aquatic environment includes lacustrine, brackish, fresh water, higher saline water, hot springs, waste water maturation ponds, dams, rivers, marine and coastal areas. Besides all the above places, microalgae grow in rocks (internal and surface), mud, sand and terrestrial plants (tree trunks, branches, shady sides of trees). Interestingly some of the microalgae grow on and in other organism. Main habitats are fresh water, brackish and marine ecosystem. Among all, a number of microalgae are found in brackish water due to the nutritional composition and warmer temperature that results from the mixture of seawater and fresh water (Woelfel et al. 2007). It usually occurs at the river mouth on the coastline, and the microalgae always remain in suspended form due to the rapid water movement (Anandraj et al. 2007; Bhakta et al. 2011).

15.2.2 Microalgae Cell Components

Microalgae are simple eukaryotic plants lacking root, stem and leaves and possess all other common cell components that are present in higher plants, i.e. membrane bound organelles (Golgi body, endoplasmic reticulum, vacuoles, mitochondria, centrioles, plastids) with specialised function. The eukaryotic microalgal cell is surrounded by a thin, rigid cell wall. Some algae have an outer matrix lying outside the cell wall, similar to bacterial capsules. The cell wall provides a barrier between the environment outside the cell and that inside the cell. The cell wall is composed of a network of cellulose fibrils with the addition of polysaccharides such as pectin, xylans, mannans, alginic acids or fucinic acid whereas in some cases only with cellulose fibrils. But in case of diatoms, the cell wall is composed of silica along with protein and polysaccharides which gives a high rigidity to the cell. Cell wall of microalgae is more porous, containing small pores about 3–5 nm, that allows to pass only low molecular weight substances such as water, inorganic ions, gases and nutrients, however impermeable to macromolecules (Wang and Chen 2009).

The nucleus is bound with a nuclear envelope having pores (nuclear pores) and contains nucleolus, chromatin and karyolymph. The nucleus is larger in size, and within the nucleus, the DNA is kept organised. Photosynthesis is carried out in specialised organelles called chloroplast. The chloroplast contains a series of membrane bound sacs called thylakoids that hold the chlorophylls and surrounded by a matrix called stroma. In thylakoid the light reaction of photosynthesis takes

place, whereas in stroma the dark reaction of carbon dioxide fixation takes place. In some cases a pyrenoid, a dense proteinaceous area, is present in the chloroplast. This pyrenoid is associated with synthesis and storage of starch material. Mitochondrial structure varies greatly in the algae. Some algae (euglenoids) have discoid cristae (folds in the inner mitochondrial membrane); some have lamellar cristae (green and red algae); and the remaining (golden-brown and yellow-green, brown and diatoms) have tubular cristae (Prescott et al. 2002).

15.2.3 Chemical Composition of Microalgal Cell

The microalgae biomass is mainly composed of proteins, lipids, carbohydrates and nucleic acid. Protein is always the major organic constituent, followed by lipid and then by carbohydrate. Most of the microalgae possess high-protein content and is used as an unconventional source of protein and nutrition supplement (Pulz and Gross 2004; Soletto et al. 2005). The cells are capable of synthesising all essential amino acids which compares favourably with that of other food protein and can be used as an essential source for human and animal nutrition (Priyadarshani and Rath 2012). Microalgal cells contain all essential vitamins such as A, B1, B2, B6, C, E, nicotinate, biotin, folic acid and pantothenic acid (Spolaore et al. 2006; Kirrolia et al. 2013). Carbohydrates in the microalgae are mainly found in the form of starch, glucose, sugars and other polysaccharides (Wang and Chen 2009; Kirrolia et al. 2013). The lipid content of microalgae widely varies from species to species. Generally microalgae possess average lipid content of 5–20 % and can reach up to 80–90 % of dry weight under specific condition (Hu et al. 2008). Algal lipids are mainly composed of glycerol, sugars or bases esterified to saturated and unsaturated fatty acids (short to long chain). Among the fatty acids present in microalgae, polyunsaturated fatty acids such as omega 3, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and omega 6, γ-linolenic acid (GLA) and arachidonic acid (AA), are of particular interest for nutraceutical application (Spolaore et al. 2006; Bishop and Zubeck 2012). The fatty acid proportion and amount are dependent on the nutritional and environmental factors. Microalgae synthesise and accumulate a high amount of triacylglycerol (TAG) in the range of 20–80 % of dry weight under adverse condition. These TAGs are extracted and converted to biodiesel and used as an alternative renewable fuel (Hu et al. 2008; Chisti 2007).

Microalgae come in various colours due to the presence of different types of photosynthetic pigments (chlorophylls, carotenoids and phycobiliproteins). Different group of algae have different combination of chlorophyll molecules. These molecules have a wide range of commercial application. Among the pigments, carotenoid is a special class of natural fat-soluble pigment having high antioxidant property (Munir et al. 2013). Some of the microalgae (diatoms) contain pigment fucoxanthin that belongs to the group of xanthophyll having several biological

properties such as antioxidant, antimicrobial and anticancer activities (Rajauria and Abu-Ghannam 2013).

15.3 Microalgal Growth Requirement

Media used for cultivating microalgae must supply all the necessary components required for growth and maintenance of the organism. Optimal parameters as well as tolerated range are species specific, and each parameter must be determined individually (Lavens and Sorgeloos 1996). All these parameters not only affect photosynthesis and productivity of cell biomass but also influence the pattern, pathway and activity of cellular metabolism and thus change in cell composition (Richmond and Hu 2013). Hence while cultivating microalgae, several factors must be considered, and the most important parameters regulating algal growth include:

- Nutrients quantity and quality
- Light
- Carbon dioxide
- Temperature
- pH
- Turbulence and salinity

15.3.1 Nutrients

Nutrients are inorganic or organic compound other than CO_2 and water, used for growth (Neenan et al. 1986). The media used for culturing the microalgae should have sufficient amounts of nutrients in them in adequate proportion for their proper growth. Minimal nutritional requirements can be estimated using the approximate molecular formula of the microalgal biomass, that is, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. This formula is based on data presented by Grobbelaar (2004). Microalgal culture media that was first introduced by Pringsheim (1950) consists of a biphasic soil-water medium. However, the chemical composition of the media was not defined properly. Hence to overcome the problem, Vonshak (1986) established another microalgal culture media that contain carbon source (organic or inorganic), nitrogen source, trace elements and chelating agents, vitamins, salt content and other ionic components (potassium, magnesium, sodium, sulphate and phosphate) based on cellular composition. A vast number of culture media with various proportions of nutrient have been designed, while some media are derived from analysis of water in the native habitat and ecology of microalgae. Furthermore some media are species specific, while some are very general used for various microalgae. A detailed report on microalgal culture media was available in Culture Collection of

Algae and Protozoa (CCAP) (2013). Some of the commonly used media are described below:

- Bold basal media (Bold 1949; Bischoff and Bold 1963; Stein 1973).
- BG11 media-growth medium for blue-green algae and fresh water green algae Rippka et al. 1979).
- F/2 medium (Guillard and Ryther 1962; Guillard 1975) is a widely used media (generally enriched with sea water) for diatoms and marine microalgae.
- Walne medium (Walne 1970) and ASN-III (Rippka et al. 1979) used for culture of marine cyanobacteria and eukaryotic green algae.

However, due to the complexity and cost of the above culture media, it may not be feasible to use them for large-scale production of microalgal biomass. Alternative growth media comprised of commercially available agricultural-grade fertiliser (NPK, urea, potash, superphosphate) are suitable for large-scale microalgal cultivation. Among the nutrient, nitrogen accounts for about 7–10 % of cell dry weight (DCW) and is an essential constituent of all structural and functional proteins in algal cells. Microalgae have a limited ability to produce nitrogen-stored materials when growing under nitrogen-sufficient conditions. Discoloration of microalgal cell is common in nitrogen-deficient media due to the decrease in chlorophyll content and increase in carotenoids. Further nitrogen limitation shows active and specific degradation of phycobilisomes, and photosynthesis continues at a slow rate. As a result, photosynthetically fixed carbon is diverted from the protein synthesis into the pathways for carbohydrate and lipid synthesis. Further nitrogen deficiency may cause several changes in cell, where nitrogen limitation could activate diacylglycerol acyltransferase that converts acyl-CoA to triglyceride (TAG) (Xin et al. 2010). Nitrogen may be supplied in the form of urea, nitrate and ammonia. Among all the source of nitrogen, nitrate is mostly used; however, urea and ammonia also show similar growth of microalgae. A combination of urea and sodium nitrate for the organism *Scenedesmus* sp. showed highest ash-free dry biomass content with yield of 4.15 ± 0.38 g l^{-1} (Lin and Lin 2011). Further on consumption of urea by the microalgae, the carbon atom present in urea is released as CO_2 and used as carbon source in photosynthesis (Neenan et al. 1986).

Besides nitrogen, phosphorous is another important element to carry out many cellular processes such as energy transfer and biosynthesis of nucleic acid. Other than nitrogen and phosphorous, sulphur, potassium, sodium, iron, magnesium, calcium and trace elements like magnesium, zinc, molybdenum, cobalt and vanadium are also important for microalgal growth (Grobbelaar 2004). Further silicate is used as a major source for some microalgae culture (diatoms) for their cell wall synthesis. Apart from all these, some microalgae need some vitamins such as thiamin (B1), cyanocobalamin (B12) and sometimes biotin.

15.3.2 Light

Like plants, microalgae require light as the main source of energy to carry out fixation of CO_2 into organic matter in the process of photosynthesis. For proper photosynthesis, three variables of light are important such as intensity, spectral quality and photoperiod (light/dark period) (Lavens and Sorgeloos 1996). Usually most of the problem in cultivating microalgae is related to the light intensity as low intensity causes photo-limitation and higher intensity causes photo-inhibition. Light source for microalgae growth may be natural (sunlight) or artificial supplied by fluorescent tubes. The requirement of light varies greatly with culture growth (density) and culture system (depth). Generally, as the microalgae grow and reproduce, biomass density increases. As a result, microalgae distant from the surface are shaded by the microalgal culture present between it and the light source, thus receiving lesser amount of light. In this case the light intensity must be increased to penetrate through the culture (1,000 lux of light for Erlenmeyer flask and 5,000–10,000 is required for larger volume). Further too high light intensity (than needed) may result in photo-inhibition which causes decrease in photosynthetic pigments (Adir et al. 2003). Although the range of solar radiation is very broad, only radiation between 400 and 700 nm can be used by microalgae. This part of the solar spectrum is called "photosynthetic active radiation" (PARS) and accounts for 43 % of the solar radiation (Thimijan and Heins 1983). Microalgae cells cultivated under limited light conditions assimilate carbon towards the synthesis of amino acids and other essential cell constituents, but under saturated light conditions, sugars and starch are formed via the pentose phosphate-reducing pathway, suggesting the dependence of the biomass composition with the light availability (Harun et al. 2014). Fluorescent tubes emitting either in the blue or the red light spectrum may be preferred as these are most active portion of the light spectrum for photosynthesis. Further unlike light intensity, photoperiod (light/dark cycle) plays a great role for microalgae culture. This is because cell division occurs under dark conditions for many unicellular photosynthetic cultures, while for others, cell division occurs both in the dark and the illuminated phase (Harun et al. 2014). Although the photoperiod varies from organism to organism, for industrial applications relating the ratio between the cost of energy and the corresponding biomass production, 12–15 h of illumination is considered as the optimal period (Harun et al. 2014).

15.3.3 Temperature

The optimal temperature for phytoplankton culture is generally between 20 and 24 °C. Further the temperature range varies with culture medium composition and organism cultivated. Most commonly used microalgae tolerate temperatures between 16 and 27 °C. Temperature below the optimal temperature may not kill

the microalgae but reduce the growth rate, whereas high temperature will kill most of the microalgae.

15.3.4 Salinity

The total salt concentration mostly depends on the ecological origin of the organism. Salinity changes normally affect microalgae in three ways: osmotic stress, ion stress and changes of cellular ion concentration due to the selective permeability of ion through the membrane. Marine microalgae are extremely tolerant to changes in salinity. Most of the organisms grow best at a salinity that is slightly lower than that of their native habitat. Salinities of 2.0–2.5 % have been found to be optimal for microalgae (Lavens and Sorgeloos 1996).

15.3.5 pH

Most of the microalgae grow in the pH range of 7–9, while the optimum range is 8.2–8.7 (Lavens and Sorgeloos 1996). As the culture grows, pH of the culture medium increases with time as a result of continuous consumption of CO_2. If the pH is not maintained within the optimum pH range, it may result in disruption of many cellular processes, leading to the inhibition of biomass growth. The pH in the growing culture can be maintained either through simple aeration or through addition of extra CO_2.

15.3.6 Aeration and Mixing

Continuous mixing of the culture is essential for successful microalgal biomass production. Mixing provides appropriate distribution of nutrients, light, dissolved CO_2, O_2 elimination, maintenance of pH, temperature gradient and evade algal sediment formation (Lavens and Sorgeloos 1996). Further it improves the gas exchange between the culture medium and the air. Upon algae growth, the dissolved CO_2 in the culture become limited. In this case diffusion of culture with CO_2 as microbubbles may be carried out by enriching aeration (only air containing 0.03 % CO_2) or by mixing pure CO_2 gas with air in case of heavy biomass density (at a rate of 1 % of the volume of air) for proper microalgal growth. This process not only provides CO_2 for better growth but also maintains pH by CO_2/HCO_3^- balance and provides better mixing of the algal culture. Depending upon the culture volume, mixing is achieved by using stirrer in bioreactors or photosynthetic orbital shaker, but for large-scale operations like raceway ponds, the paddle

wheels are more suitable. Further the mixing of culture is species specific (Gouveia 2011).

15.4 Biomass Production

Under natural growth conditions, photoautotrophic algae absorb sunlight and assimilate CO_2 from air and nutrients from the aquatic habitats. Hence any artificial production should attempt to replicate and enhance the optimum natural conditions (Brennan and Owende 2009). Microalgae production can be done in a variety of systems either as pure culture or as consortium with minimal sophistication and equipments. Understanding and taking advantage of the biology of algal strains selected for the use in production systems is the foundation for processing feedstock into fuels and products (Chisti 2007).

15.4.1 Growth of Biomass

After selecting the microalgae strain, it is necessary to develop a range of bioprocesses that make its commercialisation viable. Thus, the design and optimisation of bioreactors to cultivate these microorganisms is a major step in transforming scientific findings into a marketable product. From a commercial point of view, a microalgae culture system must have as many of the following characteristics as possible: high area productivity, high volumetric productivity, inexpensiveness (both in terms of investment and maintenance costs), simple control of the culture parameters (temperature, pH, CO_2, turbulence), energy efficiency and reliability (Olaizola 2003). The cultivation methods adopted for microalgae are traditionally either in open ponds, known as high rate ponds (HRP) or raceway ponds (RP), or in enclosed systems known as photobioreactors. Macroalgae have been grown for some time in attached systems; the best examples of these are Algal Turf Scrubber (Craggs and Adey 1995). In order to minimise costs, algae is often grown using sunlight as a free source of light, even though it is variable with daily and seasonal changes in the amount of available light (Molina 1999; Molina and Fernandez 1999). Each system has its own advantages and disadvantages.

15.4.1.1 Open Pond Systems

The classical open-air cultivation systems comprise lakes and natural ponds, circular ponds, raceway ponds and inclined systems. Open-air systems are the most widespread growth systems for microalgae since these systems are easier and less expensive to build, operate more durable and have a larger production capacity than

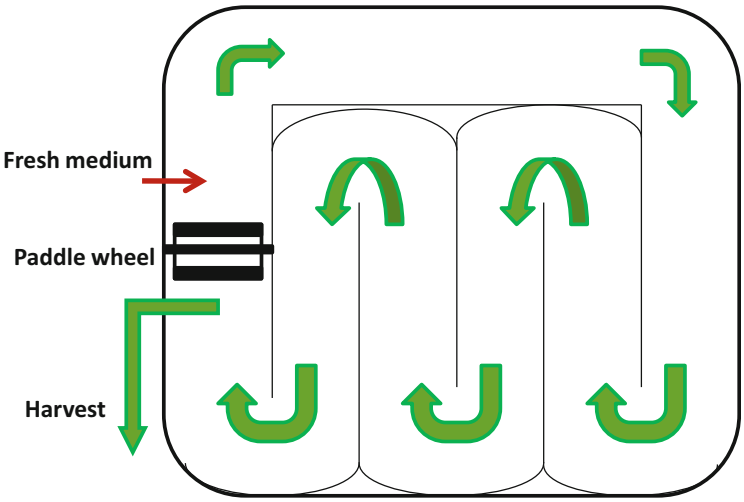

Fig. 15.1 Schematic of a raceway pond

most closed systems. They can utilise sunlight and the nutrients provided through runoff water from nearby land areas or by channelling the water from sewage/water treatment plants thus making it the cheapest method of large-scale algal biomass production (Carlsson et al. 2007).

Raceway ponds are the most commonly used artificial culture system (Fig. 15.1). The pond is usually designed in a 'raceway' or 'track' configuration generally between 0.2 and 0.5 m deep, in which a paddlewheel provides circulation and mixing of the algal cells and nutrients. Raceways are typically made from poured concrete or simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around bends. Pure carbon dioxide or air-CO_2 mixture is usually supplied through membrane diffusers or pipes along the channels to dispense tiny bubbles. Surface evaporation results in some loss of liquid, but it also helps in regulating the temperature of the medium. In a continuous production cycle, algae broth and nutrients are introduced in front of the paddlewheel and circulated through the loop. Growth period depends upon the species, inoculum density and other physicochemical parameters. Biomass concentrations of up to 1 g l^{-1} and productivities of 10–25 g m^{-2} day^{-1} are possible (Pulz 2001). The largest raceway-based biomass production facility located in Calipatria, CA (USA), occupies an area of 440,000 m^2 to grow *Spirulina* for producing biomass for food (Chisti 2007). Seaweed cultivation (macroalgae) for production of particular compound or product such as carrageenan, liquid fertilisers, agar, etc., has been demonstrated and licensed by CSIR-CSMCRI, Bhavnagar, India. Figure 15.2 shows large-scale microalgae cultivation facility available at CSIR-IMMT comprising of eight raceway ponds with 40,000 l capacity each.

Fig. 15.2 Large-scale microalgae cultivation facility at IMMT (CSIR), Bhubaneswar. Eight raceway ponds fitted with paddle wheels and CO_2 supply system

Although these systems are cost effective and easy to operate, open-air systems present significant technical challenges. Ponds are susceptible to weather conditions, with no control of physical parameters such as water temperature, evaporation and lighting (Molina 1999; Molina and Fernandez 1999). Furthermore, biomass productivity is also limited by contamination with unwanted algal species as well as organisms that feed on algae. Consequently, this strictly limits the species of algae that can be grown in such systems. As a result, very few species with high adaptability to salinity (*Dunaliella*), alkalinity (*Spirulina*) (Carlsson et al. 2007) and nutrient-rich conditions (*Chlorella*) have been successfully cultured so far.

15.4.1.2 Closed Systems: Photobioreactors

Photobioreactors (PBRs) give control on nearly all the biotechnologically important parameters. They present reduced contamination risk and CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature and flexible technical design (Pulz 2001). Recent advances in microalgae mass culture

require closed systems, as many of the new algae and algal high-value products for use in the pharmaceutical and cosmetics industry must be grown free of pollution and contaminants such as heavy metals and microorganisms (Janssen et al. 2003; Tredici 2004).

PBR are generally made of glass/fibre/plastic with sufficient strength against failure. They receive either direct sunlight through the transparent container walls or indirectly via light fibres or tubes that channel it from sunlight collectors. Some PBR have artificial light sources. The ground beneath the solar collector is often painted white or covered with white sheets of plastic to increase the light received by tubes. Mixing is achieved through circulation of culture or sparging gas through culture fluid.

There are several designs of PBR that have been reported, but the basic design in many of them is common. The main categories include (1) tubular (helical, manifold, serpentine and α shaped), (2) flat plate (alveolar panels and glass plates), (3) column (bubble columns and airlift) and (4) stirred tank reactor. A significant amount of work has been carried out in optimisation of different PBR systems for microalgae cultivation (Chaumont 1993; Janssen et al. 2003; Tredici 2004; Carvalho et al. 2006; Li et al. 2007; Aishvarya et al. 2012). A PBR has inlet for fresh feed, outlet for recirculation or harvest and a column for gas removal and settling purpose. Schematic for typical tubular PBR is given in Fig. 15.3. Tubular PBR have horizontal tubes transparent to sunlight enclosing the culture, and the tube diameter is often less than 0.1 m as light penetration is difficult in larger tubes (Chisti 2007). Bubble columns consist of a long column connected to a reservoir,

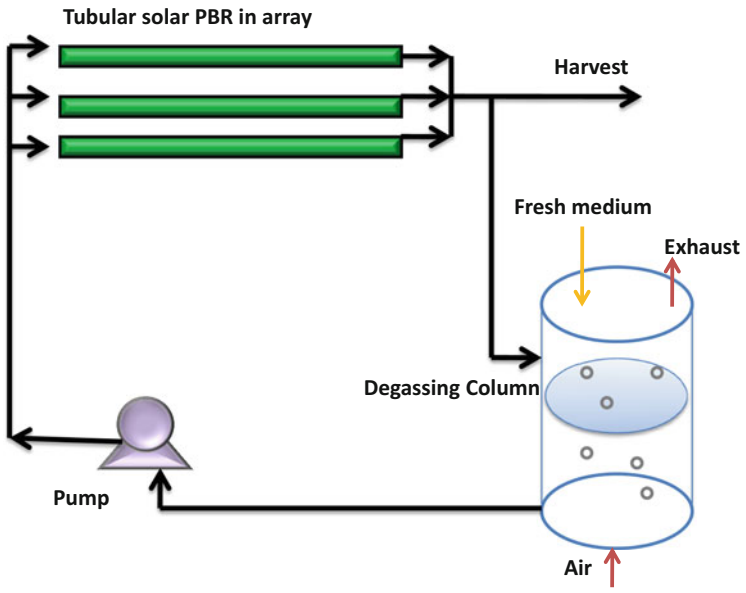

Fig. 15.3 Schematic of tubular PBR using solar light

Table 15.2 Comparison of properties of large-scale cultivation methods

Properties	Paddle wheel RWP	Stirred tank reactor	Tubular reactor	Column reactors
Light utilisation efficiency	Fairly good	Fairly good	Very good	Fairly good
Gas transfer	Moderate	Low-high	Low-high	High
Mixing	Fairly uniform	Almost uniform	Uniform, complete mixing	Almost uniform
Species control and sterility	Poor/none	Very good	Good	Good
Disadvantages	Large area of land required, low productivity	Difficult to scale up, high initial and operational costs	Fouling of tubes especially at bends, difficult to clean	Variable shear stress, high operational costs, difficult to scale up

and the liquid culture and gas are circulated either in same or opposite directions. As the effective surface area exposed to light is less compared to tubular reactors, bubble columns require artificial lighting along their length for good productivity. Due to design limitations, only tubular reactors (serpentine type) have been widely used in scale up yielding reasonable energy and cost efficiency.

A comparison between different modes of cultivation is given in Table 15.2 based on the vital parameters controlling the productivity of biomass (Borowitzka 1999; Brennan and Owende 2010).

15.5 Harvest of Biomass

The first step in the downstream processing of microalgae production is harvesting of cells. This is a major challenge in the microalgae cultivation as enormous amounts of liquid have to be processed to separate microalgae cells from the culture fluid. There are many techniques followed based on the species of algae, target products, the cell size and composition (high or low lipid content) as it decides the economy and efficiency of the process (Brennan and Owende 2010). Microalgae harvesting can be done in two stages involving (1) bulk harvesting and (2) thickening. Some commonly followed procedures are given in Table 15.3 with advantages and disadvantages.

Solid-liquid separation processes can be classified into two kinds. In the first, the liquid is constrained in a vessel and particles can move freely within the liquid (e.g. sedimentation and flotation). In the second kind, the particles are constrained by a permeable medium through which the liquid can flow (e.g. filtration and screening) (Shelef et al. 1984).

Table 15.3 Methods of harvesting microalgae

Stages of harvesting	Methods	Advantages	Disadvantages
Bulk harvesting	Sedimentation	Low cost, useful as first stage in separation to reduce energy input and cost	Settling rate specific to algae species; best for dense non-motile cells; can be slow
	Flotation	Uses air or gas bubbles; rapid than sedimentation	Species specific; oil-laden cells easily separated; air bubbling costs can be high
	Flocculation	Range of techniques available with low to high cost	Removal of flocculants and chemical contamination of harvested biomass
Thickening	Centrifugation	Can handle most algal cell types, efficient harvesting	High capital and operational costs
	Filtration	High concentrations can be achieved, efficient for large cells	Species dependent; clogging or fouling of filters; membrane costs can be high
	Ultrafiltration	Can handle delicate cells	High capital and operational costs

Sedimentation and flotation are gravity separation processes and depend on density of cells. Sedimentation occurs when cells settle due to gravity and can be increased by increasing cell dimension (i.e., coagulation) as sedimentation rate depends on particle size. Flotation is based on the attachment of air or gas bubbles to solid particles, which are then carried to the liquid surface and accumulate as float which can be skimmed off. The density difference between cell and culture medium decides which process is suitable. Oil-laden cells with low cell density can be separated by flotation.

Chemical flocculation is a well-known traditional method used in water treatment. Addition of chemicals to algal cultures to induce flocculation of cells is a routine procedure in various separation technologies. These chemicals can be inorganic such as Al^{3+} in alum, Fe^{3+} in ferric sulphate, $Ca(OH)_2$, etc., or polymeric (cationic, anionic, non-ionic) in nature (natural and synthetic polymers). During flocculation, single cells form larger aggregates that can be easily separated from the medium by simple gravity sedimentation. Flocculation is also performed as a first step before filtration in some cases as the cost and energy demand are significantly reduced if the cells are preconcentrated.

Flocculation can also be achieved by other methods which have been explored in the recent years such as electroflocculation, autoflocculation, flocculation using physical forces, bioflocculation, etc. (Vandamme et al. 2013).

Electroflocculation Flocculation is induced through electrolytic release of metal ions from a sacrificial anode. The efficiency of this method might be improved by changing the polarity of the electrodes. Similar to flocculation by metal salts, electroflocculation results in contamination of the biomass with metals, although to a lesser extent than when metal coagulants are directly used.

Autoflocculation Microalgal cultures spontaneously flocculate when pH increases beyond 9 due to photosynthetic CO_2 depletion. Autoflocculation is known to occur with formation of inorganic precipitates (calcium and magnesium) at high pH, and thus the harvested biomass contains high concentration of minerals.

Physical methods Forces such as ultrasound waves and magnetic nanoparticles have been studied to avoid contamination in the biomass. Magnetite (Fe_3O_4) nanoparticles adsorb directly on microalgae cells which are then separated by applying magnetic field, thus combining flocculation and separation in one step. The nanoparticles can be recovered after harvesting by desorption and reused.

Bioflocculation Certain extracellular polymer substances secreted by some species in the medium result in spontaneous flocculation. Some microalgae species flocculate more readily than others. Bacteria or fungi can also induce bioflocculation with their exopolysaccharides or positively charged hyphae, respectively. The mechanisms behind bioflocculation are poorly understood deserving further research.

15.6 Biotechnological Applications of Microalgae

Microalgae have a number of uses in industries. The algal biomass contains three main components: carbohydrates, protein and lipids/natural oil. High content of these commercially important molecules has seen escalation of research in areas from food industry, nutra- and pharmaceuticals to biofuels and phycoremediation (Fig. 15.4). Microalgal biotechnology has also gained importance due to diverse commercial application, such as value-added products for pharmaceutical purposes, human and animal nutrition, cosmetics, high-value molecules such as fatty acids, pigments and as energy sources (Spolaore et al. 2006; Mata et al. 2010). Recently, various nanoparticles and nanocomposites have also been developed using microalgae for environmental applications (Jena et al. 2013, 2014a). Some of the microalgal species in its raw or semi-decomposed form can be used as an organic biofertiliser. Further algae have seen applications in solving environmental issues as well by means of remediation, biosorption and latest for energy production.

Besides all the above potential application, the use of microalgae for biofuel production and carbon dioxide sequestration on an industrial scale is an important area of research owing to the increasing energy demands, predicted fossil fuels shortage in the near future and environmental concerns such as the production of greenhouse gas carbon dioxide.

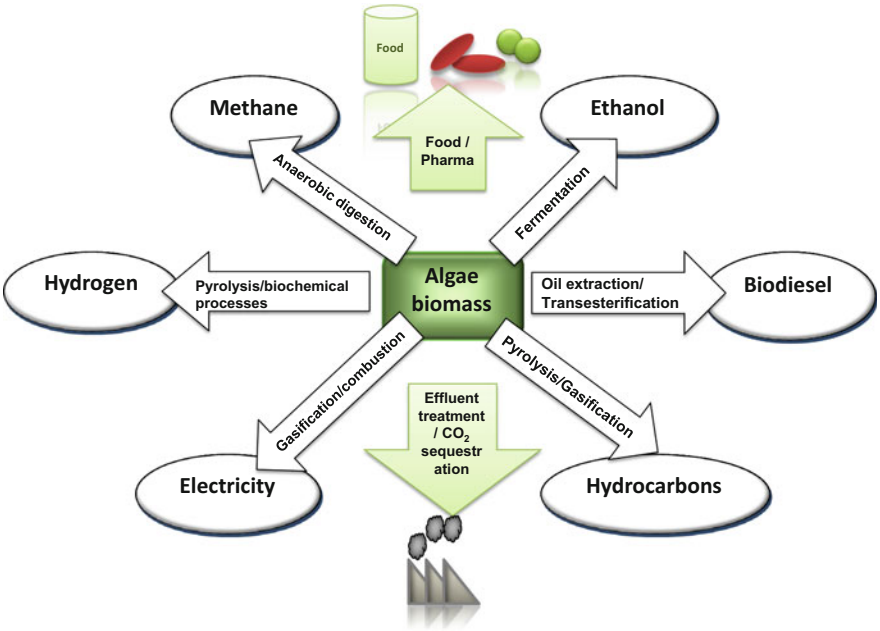

Fig. 15.4 Current industrial uses of algae

15.6.1 Food and Pharmaceuticals

Seaweeds are used as food for both humans and livestock. Due to the high-protein content, *Chlorella* sp. is widely used as health food for human beings and as animal nutritional supplements (Metting 1996). Some microalgae produce valuable by-products like proteins, pigments, biopolymers and fatty acids (docosahexaenoic acid) including antioxidant substances for commercial or pharmaceutical purpose (Priyadarshani and Rath 2012). Carbohydrate exists in several forms in the microalgae, such as starch, glucose, sugars and polysaccharides (Spolaore et al. 2006), and finds its potential applications in food, cosmetic and pharmaceutical industries (Banerjee et al. 2002). Seaweed is rich in many vitamins as A, B1, B2, B6, B12, C and niacin. Algae are also rich in iodine, potassium, iron, magnesium and calcium and are used in production of food supplements (Poulickova et al. 2008). The cyanobacterium *Arthrospira platensis* and *Arthrospira maxima*, more commonly known as '*Spirulina*', are widely consumed by humans as whole food or dietary supplements due to their rich protein and vitamin content. Also, algal hydrocolloids alginate, agar and carrageenan are produced from seaweeds (especially macroalgae) and largely used as viscosity-modifying agents in foods and pharmaceuticals (Mata et al. 2010). Many types of algae are also rich in omega-3 fatty acids and as such are used as diet supplements and as a component of livestock feed. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are

two relatively rare and valuable fatty acids found in microalgae, and these essential fatty acids are critical structural component of our cell membranes and play a vital role in brain function (Crawford 1990). Blue-green algae contain a wide range of antioxidants in the form of amino acids, vitamins and especially pigments like carotenes (astaxanthin) and potent green-blue pigments (phycocyanins, phycobiliproteins) (Gonzalez et al. 1999) that have opened a new direction in dermatological treatment for radiation damage.

15.6.2 CO_2 Sequestration

Algae being autotrophic in nature can use CO_2 as a nutrient through photosynthetic metabolism, the most highly polluting green house gas. Coupling of CO_2 scrubbing and algae production is widely practiced as it fixes carbon in algal biomass, a renewable energy source (Campbell et al. 2011). With respect to air quality maintenance and improvement, microalgae is being considered as a eminent CO_2 fixation system that can reduce the green house gas emission caused by the burning of fossil fuel for various purpose. As reported, a 1 kg of microalgal biomass can efficiently fix 1.83 kg of CO_2 (Chisti 2007). Flue gases from power plants are responsible for more than 7 % of the total world CO_2 emissions from energy use. Also, industrial exhaust gases contain up to 15 % CO_2, providing a CO_2-rich source for microalgae cultivation and a potentially more efficient route for CO_2 bio-fixation (Mata et al. 2010). Therefore, the use of flue gas emissions from an industrial process unit (e.g. from fuel-fired power plants) as a source of CO_2 for the microalgae growth is envisioned to have a great potential to diminish CO_2 and to provide a very promising alternative to current green house gas emissions mitigation strategies (Danielo 2005). Enhanced sequestration of CO_2 in the form of bicarbonate is possible in mildly alkaline medium (pH-10.0) and can be exploited industrially (Aishvarya et al. 2012). Some species with tolerance to high CO_2 levels have also been identified and studied in these environments. Various microalgal genera including *Chlorella* (Douskova et al. 2009; Ramanan et al. 2010; Borkenstein et al. 2011; Aishvarya et al. 2012), *Scenedesmus* (Morais and Costa 2007), *Chlorococcum*, *Synechococcus*, *Thermosynechococcus* (Hsueh et al. 2009), *Nannochloropsis* (Hsueh et al. 2009), and *Spirulina* (Ramanan et al. 2010) have been reported for CO_2 sequestration ability.

15.6.3 Effluent and Wastewater Treatment

Since most of the nutrients that algae needs are often found in wastes, such as nitrogen, phosphorous and ammonia, utility of algae in wastewater treatment has become a widespread notion. The exponential growth of algae under ideal nutrient loads has led to the idea of algae as a phycoremediation tool (Olguin 2003). Some

unicellular green microalgae species are particularly tolerant to sewage effluent conditions, most notably those of the *Chlorella* and *Scenedesmus* genus (Pittman et al. 2011). Growth of microalgae in primary settled sewage water was shown to increase significantly under long photoperiod conditions following addition of CO_2, while increased temperature decreased algal biomass concentration (Ip et al. 1982). Various species of *Chlorella* and *Scenedesmus* can provide very high (>80 %) and in many cases almost complete removal of ammonia, nitrate and phosphate from secondary treated wastewater (Martinez et al. 2000; Zhang et al. 2008; Ruiz-Marin et al. 2010). In small-scale treatments, algae can be used effectively in a pond or tank like setup. Since species control is not a priority in many cases of treatment, consortium can work better than single species. A consortium of algae has been used to treat carpet mill waste streams and simultaneously accumulate lipid for fuel purposes (Chinnasamy et al. 2010). Heavy metal in waste streams from electroplating, ceramics and plastics industries often polluting open water sources are toxic and accumulate in food chain. Biological treatment of such heavy metal containing waters is possible with microalgae by means of biosorption, bioaccumulation, retention and desorption (Chojnacka 2010). Biosorption of heavy metals has been studied on metal ions like Cr^{3+}, Cd^{2+}, Cu^{2+}, etc. (Chojnacka et al. 2005; Jena et al. 2014b).

15.6.4 Microalgae as a Source of Biodiesel

The possibility of using algae as a source of energy received widespread attention after the energy crisis of the 1970s. Algae has several advantages like higher photosynthetic efficiency, higher growth rates and biomass production compared to other energy crops, no competition with food crops for land, less water and nutrient requirements and their ability to accumulate lipids. Microalgal lipids are chemically similar to common vegetable oils (Dunahay et al. 1996; Chisti 2007). Many microalgae can accumulate lipids due to excess photosynthesis, and some species can accumulate high amount of lipids under heterotrophic or environmental stress, such as nutrient deficiency or salt stress (Takagi and Karseno 2006).

Biodiesel is defined as the mono-alkyl esters of fatty acids derived from vegetable oils, animal fats, waste cooking oil and jatropha oil (Felizardo et al. 2006; Chisti 2007). The oil of biodiesel consists of triglycerides in which three fatty acid molecules are esterified with a molecule of glycerol. The nature of fatty acids in biodiesel affects its properties. The percentage saturation and unsaturation of biodiesel determine its fluidity at room temperature, lubricity, viscosity, cetane number and its emission characteristics. This can be important when selecting the biodiesel for a particular application. Traditionally oil from algae is extracted by solvent extraction, Soxhlet extraction and recently by supercritical CO_2 extraction and pyrolysis (Miao and Wu 2004). Solvent extraction is widely done using chloroform and methanol (2:1, v/v) or hexane. The general procedure is to treat the biomass with solvent at 130 °C for 4 h, the extract is then passed through

anhydrous sodium sulphate to remove the moisture and the solvent is evaporated under vacuum to get the oil.

Biodiesel from each feedstock is different from the other in that they are made of different proportions of saturated, monounsaturated and polyunsaturated fatty acids. Microalgal oils differ from most vegetable oils in being quite rich in polyunsaturated fatty acids with four or more double bonds like EPA (C20:5n-3; five double bonds) and DHA (C22:6n-3; six double bonds) that occur commonly in algal oils. The stability can be improved by reducing the extent of unsaturation in microalgal oil with more than four double bonds, by partial catalytic hydrogenation of the oil (Jang et al. 2005), the same technology that is commonly used in making margarine from vegetable oils. Recent research aims to solve this by enhancing the transcription rate of MUFA genes and changing the metabolic pathway to improve the triglyceride conversion.

15.6.5 Energy Source

The concept of biodiesel from algae has accelerated the algae production manifold at different levels. But algal biomass can serve as energy source in more ways than just oil. The biomass left after the extraction of oil is rich with cellular storage products. Ethanol, a valuable fuel, can be produced from the leftover biomass by fermentation. Residual biomass may be used to produce methane by anaerobic digestion, for generating the electrical power necessary for running the microalgal biomass production facility (Spolaore et al. 2006). Although the microalgal biomass can be directly used to produce methane by anaerobic digestion, it cannot compete with the many other low-cost organic substrates that are available (Chisti 2007). After anaerobic treatment, the residue can be used as low-cost fertilisers as they are rich in N and P (Pittman et al. 2011). Research on hydrogen production from algae is another recent venture with algae. Algae biomass can be subjected to pyrolysis to extract hydrocarbons and synthetic gas from the biomass. Gasification of biomass can lead to electricity generation. All these potential utilities have led to integrated biorefinery using algae for multiple end products. A microalgal biorefinery can simultaneously produce biodiesel, animal feed, biogas and electrical power.

15.7 Concluding Remarks

The success of algae cultivation and its application have been one of the highly debated areas in biotechnology. But a strict control of desired conditions and judicious selection of appropriate microalgae species after considerations of its properties can result in predictable outcomes. With the wide range of applications

that they offer, microalgae can be considered one of the inevitable options of the future biotech industry.

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