Chapter 4 Bioreactor for Microalgal Cultivation Systems: Strategy and Development

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Abstract Microalgae are important natural resources that can provide food, medicine, energy and various bioproducts for nutraceutical, cosmeceutical and aquaculture industries. Their production rates are superior compared to those of terrestrial crops. However, microalgae biomass production on a large scale is still a challenging problem in terms of economic and ecological viability. Microalgal cultivation system should be designed to maximize production with the least cost. Energy efficient approaches of using light, dynamic mixing to maximize use of carbon dioxide $(CO₂)$ and nutrients and selection of highly productive species are the main considerations in designing an efficient photobioreactor. In general, optimized culture conditions and biological responses are the two overarching attributes to be considered for photobioreactor design strategies. Thus, fundamental aspects of microalgae growth, such as availability of suitable light, $CO₂$ and nutrients to each growing cell, suitable environmental parameters (including temperature and pH) and efficient removal of oxygen which otherwise would negatively impact the algal growth, should be integrated into the photobioreactor design and function. Innovations should be strategized to fully exploit the wastewaters, flue-gas, waves or solar energy to drive large outdoor microalgae cultivation systems. Cultured species should be carefully selected to match the most suitable growth parameters in different reactor systems. Factors that would decrease production such as photoinhibition, self-shading and phosphate flocculation should be nullified using appropriate technical approaches such as flashing light innovation, selective light spectrum, light- $CO₂$ synergy and mixing dynamics. Use of predictive mathematical modelling and adoption of new technologies in novel photobioreactor design will not only increase the photosynthetic and growth rates but will also enhance the quality of microalgae composition. Optimizing the use of natural resources and industrial wastes that would otherwise harm the environment

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A. A. Rastegari et al. (eds.), *Prospects of Renewable Bioprocessing in Future Energy Systems*, Biofuel and Biorefinery Technologies 10, https://doi.org/10.1007/978-3-030-14463-0_4

should be given emphasis in strategizing the photobioreactor mass production. To date, more research and innovation are needed since scalability and economics of microalgae cultivation using photobioreactors remain the challenges to be overcome for large-scale microalgae production.

4.1 Introduction

Algae are ubiquitous microscopic and macroscopic plants in both marine and freshwater ecosystems, and their biomass production is known to exceed those of terrestrial plants (Schenk et al. [2008;](#page-40-0) Kraan [2013;](#page-37-0) Guyon et al. [2018\)](#page-36-0). Many microalgae species contain various high-value compounds with wide range of industrial applications. Thus, microalgae are important sources for various products including feedstocks of biofuels (Schenk et al. [2008;](#page-40-0) Pittman et al. [2011;](#page-39-0) Georgianna and Mayfield [2012;](#page-36-1) Medipally et al. [2015;](#page-38-0) Rastogi et al. [2018\)](#page-40-1), biomass and pigments for aquaculture industry (Angeles et al. [2009;](#page-34-0) Alishahi et al. [2015;](#page-34-1) Liu et al. [2017\)](#page-38-1), and commercially important compounds for food and health industries (Goh et al. [2014;](#page-36-2) Foo et al. [2015\)](#page-36-3). Studies on biofuel production indicated that microalgae are more superior and sustainable source compared to terrestrial crops such as corns, coconut, jatropha and oil palm (Chisti [2007;](#page-35-0) Rastogi et al. [2018\)](#page-40-1) due to their fast growth. In addition to biodiesel production, the use of wastewater and flue-gas for microalgae mass production helps to reduce water and air pollution, respectively (Cheah et al. [2015;](#page-35-1) Guldhe et al. [2017;](#page-36-4) Cao et al. [2017\)](#page-35-2).

Microalgae are natural sources of valuable fatty acids and amino acids that can be utilized in food, nutraceutical, pharmaceutical and cosmeceutical industries (Pennington et al. [1988;](#page-39-1) Jin et al. [2003;](#page-37-1) Xia et al. [2013\)](#page-41-0). Many species are capable of producing bioactives such as carotenoids, phenolic acids, flavonoids and highly unsaturated fatty acids (HUFAs) that can be used as additives and supplements for human health-promoting products and animal feeds (Natrah et al. [2007;](#page-39-2) Ebrahimi Nigjeh et al. [2013;](#page-35-3) Goh et al. [2014;](#page-36-2) Foo et al. [2017\)](#page-36-5). These secondary metabolites produced in microalgae cells have been proven effective as antioxidant, antimicrobial, anti-inflammatory, anticancer and many other ailments (Ryckebosch et al. [2014;](#page-40-2) Foo et al. [2015;](#page-36-3) Guyon et al. [2018\)](#page-36-0). In addition, they are useful as prebiotics and immunomodulatory agents. With valuable bioactive compounds in their cells, some microalgae commodities have been granted GRAS (generally regarded as safe) status as novel food products for health and medicines.

In aquaculture, microalgae have the potential to be used as colourants, prebiotics and enhancement of fish and invertebrate immunity (Peng et al. [2012;](#page-39-3) Liu et al. [2017\)](#page-38-1). As a colourant source, carotenoids in microalgae such as canthaxanthin, astaxanthin and lutein have been regularly used as feed ingredients to enhance colour of the fish. In fact, β-carotene has been effectively used as pro-vitamin A (retinol) in multivitamin preparation and is usually included in the formulation of healthy feeds (Begum et al. [2016\)](#page-34-2). Polyunsaturated fatty acids from microalgae, such as EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3), have been shown to

positively affect immune responses in cultured fish and invertebrates by modulating fish immunity through enhancement of lymphocyte proliferation, cytokine production and natural killer (NK) cells activity (Vallejos-Vidal et al. [2016;](#page-41-1) Gbadamosi and Lupatsch [2018\)](#page-36-6). Microalgae are also useful prebiotics that can act as stimulant for beneficial microbes (Panjiar et al. [2017\)](#page-39-4) and inhibitor for pathogenic bacteria (Natrah et al. [2014\)](#page-39-5). In addition, microalgae are an essential component of aquaculture system to ensure good water quality by efficient uptake of toxic compounds such as ammonia and nitrite (Mohamed Ramli et al. [2017\)](#page-38-2). In general, the use of microalgae in aquaculture will improve water quality and provide protection of the cultured animals against various diseases through improvement of their diets and enhancement of their immune system. In addition, the current research effort to utilize microalgae as a vaccine carrier will further enhance not only the fish health but contribute to the sustainability of aquaculture industry.

At present, the production of microalgae biomass is still low, and adequate production to satisfy the increasing demand from various industries remained a challenging bottleneck. One of the main strategies of microalgae production is the use of appropriate microalgae cultivation system using natural or cheap resources such as wastewaters for nutrients, solar energy for light, flue-gas for $CO₂$ and waves for mixing. There are many options for microalgal cultivation such as photobioreactors, raceways, tanks and ponds (Table [4.1\)](#page-3-0). Among many types of microalgae production system, photobioreactors are key devices for pure single species culture where contaminants that occur in pond or raceway cultures can be controlled. However, like other photosynthetic systems, the success of photobioreactors will depend on all factors that affect energy consumption and maintenance of optimum culture condition. In mass microalgae cultivation, availability of water, light, nutrients and energy would be the main items to be factored into the production cost. The production can be further improved by species or strain selection and optimization of all related culture conditions. The use of wastes and natural resources for the culture would make the microalgae production more economical, and to some extent improves the pollution pressure on the environment.

4.2 Photobioreactor Development—Strategies

Conventional microalgae culture is mainly carried out in open space cultivation, especially in ponds, tanks or raceways. With comparatively lower construction and operating cost compared to closed system, open space cultivation is relatively easy to operate and relatively cheap as most utilize natural sunlight and aeration (Table [4.1\)](#page-3-0). However, open system cultivation is prone to contamination which can affect the quality of the produced microalgae biomass and the extracted compounds such as astaxanthin and other carotenoids used in health and food industries. Thus, closed system cultivation is the better alternative for the production of high-value microalgae products (Table [4.1\)](#page-3-0).

Table 4.1 (continued)

Fig. 4.1 Schematics of an open raceway system (**a**) and a closed horizontal photobioreactor with shallow water depth and high S/V ratio (**b**)

Photobioreactors have been developed since 1950s for biomass production of a specific microalgae species in order to overcome food supply crisis. Several configurations such as raceway system (Fig. [4.1\)](#page-6-0), bubble column (Fig. [4.2\)](#page-7-0), flat plate (Fig. [4.3\)](#page-7-1) and tubular (Fig. [4.4\)](#page-8-0) have been used (Olivieri et al. [2014\)](#page-39-7). The early bioreactor design was very simple consisting of tubes and light sources. In the earlier years, bioreactors were relatively small, but the photobioreactor volume is getting bigger with more sophisticated design. Novoveská et al. [\(2016\)](#page-39-8) designed a large microalgae photobioreactor in the offshore area to treat municipal wastewater, up to 50,000 gallons/day, whereby 75% of total nitrogen, 93% of total phosphorus and 92% of biological oxygen demand (BOD) of the influent wastewater was removed, and 3.5–22.7 g m⁻² d⁻¹ of microalgae biomass was produced.

Photobioreactors are often categorized into (1) open and closed system, or (2) vertical and horizontal flow of culture media (Table [4.1\)](#page-3-0). Most bioreactors have different specifications in terms of materials, light pass length, working volume and volume/surface ratio. Common features in bioreactors include (1) light receiver to capture light energy effectively, (2) loading ports for the culture media, carbon dioxide and harvesting and (3) mixing function to remove produced oxygen and to increase mass transfer efficiency in the culture media. Open raceway system is the most popular microalgae production system. The basic design was derived from oxidation pond in wastewater treatment. In general, the open raceway system has one or multiple paddles for circulating the media in the trough that has 20–30 cm water depth (Fig. [4.1a](#page-6-0)). The paddle mixing has higher energy efficiency compared to aeration mixing used in other closed photobioreactors due to low energy loss in the former. However, the lower cell density was often reported in raceway system due to the longer light path length (\approx 30 cm) compared to other closed photobioreactors. However, only the species that has low contamination risk can be cultured in this system.

Fig. 4.2 Schematics of several types of column bioreactors; **a** normal column bioreactor, **b** column bioreactor with concentric airlift, **c** annular bioreactor

Fig. 4.3 Schematics of flat plate reactor (**a**) and flat panel airlift (FPA) photobioreactor (**b**)

Fig. 4.4 Schematics of tubular photobioreactor

To overcome the contamination issue, Dogaris et al. [\(2015\)](#page-35-8) modified the raceway system to develop a new horizontal photobioreactor (HBR) that has thin light pass length of 5 cm with airlift pumps (Fig. [4.1a](#page-6-0)). The HBR system achieved a maximum biomass concentration of 4.3 g L⁻¹ and average biomass productivity of 18.2 g m⁻² d^{-1} over the course of 165 days without any contamination problem (Dogaris et al. [2015\)](#page-35-8). Column and flat plate systems are categorized as vertical mixing photobioreactors, in which the agitation and mixing are accomplished by aeration. The main advantage of these bioreactors is the homogeneous and efficient mass transfer by entire mixing of the water column, while the raceway and tubular systems undergo partial mixing by paddle and airlift systems. To improve mixing efficiency, airlift column bioreactor was invented $(Fig. 4.2)$ $(Fig. 4.2)$. An airlift column bioreactor has a physical separation of the two interconnecting zones; the center column (dark zone) for upper flow and external side (light zone) for the downstream. The circulation of the dark and light cycles of overall media in the column provides constant light energy to all cells in the bioreactor.

To scale-up a column bioreactor, the reactor diameter increases and its surface/volume (S/V) ratio decreases, resulting in a decrease of cell density in the bioreactor. Lower biomass concentration in the harvested media requires higher cost and energy, when the harvested culture media is concentrated and dried. To avoid decreasing S/V ratio, the annular reactor was developed (Chini Zittelli et al. [2006;](#page-35-9) Posten [2009\)](#page-39-9). The structure of the annular bioreactor is actually wrapped flat plate bioreactor with the appearance of a column bioreactor (Fig. [4.2c](#page-7-0)). The flat plate photobioreactor uses simple geometry and it can be designed to reduce light path length and keep high S/V ratio (Fig. [4.3a](#page-7-1)). The reactor is placed in a vertical or tilted

inclination to receive sunlight energy effectively. The vertical mixing in column and flat plate bioreactors uses aeration which requires high energy consumption.

The performance of energy consumption in bioreactor is evaluated by net energy ratio (NER) that is the energy balance between total energy produced by the microalgae biomass (energy output) and energy requirement in the biomass production (energy input). Generally, the raceway system shows high NER ratio (>1.0) and high energy efficiency. On the other hand, vertical mixing reactor shows relatively low NER due to high energy consumption of aeration mixing (Burgess and Fernández-Velasco [2007;](#page-34-5) Huesemann and Benemann [2009;](#page-37-2) Jorquera et al. [2010\)](#page-37-3). In order to improve the energy efficiency, the flat panel airlift (FPA) bioreactor with rectangular channel airlift which improves the efficiency of light utilization was designed (Degen et al. [2001\)](#page-35-10) (Fig. [4.3b](#page-7-1)). Degen et al. [\(2001\)](#page-35-10) reported that the FPA bioreactor showed 1.7 times higher productivity than the conventional flat plate reactor in *Chlorella vulgaris* cultivation.

Tubular reactor is one of the typical closed photobioreactors consisting of a tube and pump system (generally airlift pump system) to circulate culture media and works as degasser to remove oxygen produced by photosynthesis (Fig. [4.4\)](#page-8-0). The advantage of the system is the high flexibility for the setting and it can be arranged horizontally, vertically and any other shape that is optimized to receive light source (Carlozzi [2003\)](#page-35-11). However, the oxygen resulting from photosynthesis often increases up to an inhibitory level since it is only partially removed in the airlift system (Sánchez Mirón et al. [1999\)](#page-40-6). In addition to the oxygen accumulation problem, the tubular system consumes high energy to circulate the culture media. Jorquera et al. [\(2010\)](#page-37-3) reported that the tubular system requires >2500 W/m³ (NER = 0.2) to generate turbulent for suitable gas/liquid mixing and mass transfer in the systems while the raceway and flat plate systems consume 3.72 W/m³ (NER = 8.34) and 53 W/m³ (NER = 4.51) for the mixing and/or aeration, respectively. However, these energy consumption values greatly vary with the culturing conditions and assumptions made during the calculation of NER.

4.3 Strategies to Increase Efficiency of Photobioreactor Systems

Microalgae are flagged as the next generation biomass feedstock for bioenergy and biochemical for the growing world population. Since its production is associated with reducing the impacts of climate change and enhancing of food security, microalgaebased industries have high potential to assist the socio-economic development of the global community. Thus, upscaling of microalgae products should be pursued by improving its production systems.

There is a great need to develop efficient photobioreactors to satisfy the high demand for microalgae biomass. The strategy to design a highly efficient bioreactor system is to focus on all factors that affect the microalgae physiological responses and biomass quality. Microalgae require light, carbon dioxide and nutrients to produce biomass and biocompounds, the rates of which are governed by the metabolic properties of the cultured species itself and the culture conditions (Lucker et al. [2014\)](#page-38-5). Optimizing the delivery of these factors to increase photosynthetic rates in photobioreactors would be the best strategy to obtain the maximum microalgae production. Thus, bioreactors have been designed to increase efficiencies in light, gas and nutrient utilization with increased outputs (Table [4.2\)](#page-11-0).

4.3.1 Selection of Microalgae Species

Many microalgal species have variable contents of high-value compounds such as fatty acids, amino acids and carotenoids. Thus, for photobioreactor production, microalgae species with high yield biomass and rapid growth rate should be carefully selected to suit targeted products. For example, *Haematococcus* spp. have high carotenoids contents, especially astaxanthin (Guyon et al. [2018;](#page-36-0) Lim et al. [2018\)](#page-37-4) and *Chlamydomonas* spp. are known sources for carbohydrates (Gifuni et al. [2017\)](#page-36-8). In fact, some species have compounds that cannot be found in other species. For example, fucoxanthin is only found in brown seaweeds and diatoms (Foo et al. [2015\)](#page-36-3). Molina-Miras et al. [\(2018\)](#page-38-6) reported the production of amphidinols, a group of polyketides with high bioactivities from a marine dinoflagellate, *Amphidinium carterae*. Thus, concentration of a target compound can also be an important criterion for selecting an algal species for mass production in a photobioreactor.

Physiological parameters and biochemical composition of microalgae biomass also determine the productivity and quality. The culture environment has a high influence on the species physiological response. Zhang et al. [\(2017a,](#page-41-2) [b\)](#page-41-4) manipulated the glucose, nitrogen and light levels to enhance astaxanthin production in *Chlorella zofingiensis*. In a study of tropical microalgae, Rocha et al. [\(2017\)](#page-40-7) reported that different chlorophyte strains of *Scenedesmus, Chlamydomonas, Chlorella, Monoraphidium, Scenedesmus* and *Selenastrum* have variable fatty acids, carbohydrate and protein contents and their metabolism and composition were closely related to the culture conditions. Guyon et al. [\(2018\)](#page-36-0) also suggested that microalgae productivity and carotenoid contents are species-specific and influenced by a wide range of environmental parameters.

Different species require different light intensity and spectra to maximize their growth and productivity. Vadiveloo et al. [\(2015\)](#page-41-5) showed that a green microalga, *Nannochloropsis* sp. produced the highest biomass when cultured under blue light (400–525 nm). Hidasi and Belay [\(2018\)](#page-36-7) reported that biomass composition of *Spirulina platensis* showed diurnal changes with lower photosynthetic pigments during the light hours, but recovered during the night. In fact, optimal growth factors (light, $CO₂$ and nutrients) are essential to achieve maximum production, but the exact requirements differ from one species to another. Mondal et al. [\(2017b\)](#page-38-7) reported that light intensity of 80 µmol m⁻² s⁻¹ and photoperiod of 12L:12D were the optimal conditions for *Chlorella sorokiniana* culture, whereas other species require higher

Type	Design and capacity	Special feature	Biomass production (g) L ⁻¹)/productivity (g m ⁻² d ⁻¹)	Microalgae species	References
Floating large modular offshore pho- tobioreactors	189.3 m^3 , 45.7 m long \times 1.83 m wide	Nutrient uptake-75% of total nitrogen, 93% of total phosphorus	3.5–22.7 $\rm g~m^{-2}$ d^{-1}	Mixed species, Scenedesmus, Chlorella and Cryptomonas	Novoveská et al. (2016)
Energy-free rotating floating pho- tobioreactor (RFP)	Outdoor rotating floating pho- tobioreactor powered by flowing water-with plexiglass serving as paddles and culture barrels in-between them	Two-step cultiva- tion-high biomass yield fermentation and outdoor culture induction	Biomass: 98.4 g L^{-1} Astaxanthin: 73.3 mgL^{-1}	Chlorella zofingiensis	Zhang et al. (2017b)
Vacuum airlift photo- bioreactor	An outdoor 500 L pilot plant	20 m high airlift system with 8 cm internal diameter using novel double- degaser that provided good gas-liquid separation	na	na	Marotta et al. (2017)
Flat plate gas-lift pho- tobioreactors	Scale-up of biomass production	300-L Pilot scale-opti- mization of gas, light and nutrients	Biomass: $14-19$ g m ⁻² d^{-1}	Scenedesmus spp.	Koller et al. (2018)
Twin-layer biofilm pho- tobioreactors (TL-PBRs)	Twin-layer sheet of 1 m^2	Use of high light $(1023 \,\mu \mathrm{mol m}^{-1})$ s^{-1}) and CO ₂ (3.0%) on immobilized microalgae	31-50 g m ^{-2} d^{-1}	Halochlorella rubescens	Schultze et al. (2015)

Table 4.2 Various microalgae photobioreactors and their production

(continued)

Type	Design and capacity	Special feature	Biomass production (g) L^{-1})/productivity $(g m^{-2} d^{-1})$	Microalgae species	References
Suspended- solid phase photobiore- actors (ssPBR)	Solid attachment carriers floating in the bioreactor by aeration	Attached microalgae cultivation on cotton carriers	70% higher than the conventional system	Scenedesmus. LX1	Zhuang et al. (2018)
Resonant ultrasound field incorporated dynamic pho- tobioreactor (RUF-DPBS)	Semi- automatic RUF-DPBS high-density microalgae culture in continuous mode	Use of acoustic radiation forces and gravity for cell retention and medium replacemen- t-reduced cost, labour and contami- nation	Biomass: 2.6 folds Total lipids: 2.1 folds	NannochloropsisLee and Li aculata	(2017)

Table 4.2 (continued)

light intensity (Schultze et al. [2015\)](#page-40-5). On the other hand, Holdmann et al. [\(2018\)](#page-37-7) reported that *Chlorella sorokiniana* produced the highest biomass under strong light intensity and shorter photoperiod, probably due to different strains and culture conditions. Some species such as *Chlorella sorokiniana* and *C. minutissima* are capable of using pentoses which otherwise do not have any significant industrial application as a carbon source (Freitas et al. [2017\)](#page-36-9). In fact, some species, such as *Scenedesmus obliquus*, was shown to sustain cell growth up to 2 h in the dark without affecting the photosynthetic rate (Maroneze et al. [2016\)](#page-38-9).

Thus, one of the strategies for optimized photobioreactor production is to explore the vast sources of microalgae diversity and select those strains with high potential for different biotechnological applications. Gonçalves et al. [\(2016\)](#page-36-10) showed that culture of mixed compatible species resulted not only in higher biomass production with higher nutrient removal, but also increased amount of lipids. Future research should focus on the selection and engineering of high-value species with robust characteristics and high growth rate. In addition, optimal culture conditions should be developed to enhance the microalgal biomass and high-value compounds production such as lipids, fatty acids, carotenoids and proteins (Rezvani et al. [2017;](#page-40-8) Zhuang et al. [2018\)](#page-41-6). Manirafasha et al. [\(2018\)](#page-38-10) demonstrated that supply of nitrogen source with metabolic stress resulted in high *Arthrospira platensis* growth with high accumulation of phycocyanin.

4.3.2 Aeration and Mixing

Aeration is important in providing adequate carbon dioxide and nutrients for microalgal cells to photosynthesize and synthesize organic compounds. In addition to delivering gas and nutrients, aeration also controls the mixing of the water column moving the algal cells to various parts of the reactors, from the light zone near the illumination surfaces to the darker-interior area. With mixing, algal cells are shuttled back and forth between the light and dark zone, enabling the microalgal cells to undergo short light–dark cycles that can promote faster growth and higher production of biomass compared to those bioreactors with limited optimized mixing. Ugwu et al. [\(2005,](#page-41-7) [2008\)](#page-41-8) reported that short light–dark cycles could promote growth of microalgal cells. In addition, with regulated mixing and proper supply of carbon dioxide and removal of oxygen, microalgal cells are kept in suspension in suitable zones to efficiently harvest the light and nutrients for their growth. In general, mixing is one of the important aspects in photobioreactor development. Thawechai et al. [\(2016\)](#page-40-9) optimized all interacting growth factors using Resonance Surface Methodology to enhance microalgae lipid and pigment production.

4.3.2.1 Carbon Dioxide

Carbon dioxide (CO_2) is readily available in the atmosphere with concentrations ranging from $0.03-0.06\%$ (v/v) depending on the area. There is a global trend of increasing $CO₂$ from anthropogenic activities especially in congested urban and industrial areas where flue-gas can contribute significantly to the $CO₂$ pool (Rahaman et al. [2011;](#page-40-1) Norhasyima and Mahlia [2018\)](#page-39-10). Microalgae, on the other hand, can efficiently sequester $CO₂$ at the rate of approximately 1.8 kg for every 1 kg of microalgae produced (Jiang et al. [2013\)](#page-37-8). In addition, flue-gas which can be obtained from various industries can be utilized to enhance microalgae productivity to new production level and contribute to the reduction of greenhouse gases. Carbon dioxide uptake by microalgae can be enhanced in tandem with other growth factors, such as light (Mondal et al. [2017b\)](#page-38-7) and nutrients (Yan et al. [2016\)](#page-41-9) to promote high growth rates in microalgae. Schultze et al. [\(2015\)](#page-40-5) reported that the increase of carbon dioxide together with light improved the production to 31–50 g m⁻² d⁻¹, using twin-layer biofilm photobioreactors (TL-PBRs), the highest microalgae dry biomass productivity reported to date (Table [4.2\)](#page-11-0). Cheah et al. [\(2015\)](#page-35-1) also reported the use of atmospheric CO₂ and flue-gas for microalgae biomass production.

4.3.2.2 Nutrients

Carbon, nitrogen and phosphorus are the three major nutrients that are essential for microalgae growth. Carbon dioxide can be obtained from the atmosphere by aeration, but reactive nitrogen and phosphorus have to be supplied to the culture media.

Microalgae are effective in consuming nutrients from wastewaters, such as domestic sewage, tannery wastewaters and aquaculture sludge which normally have organic contents (Table [4.3\)](#page-15-0). da Fontoura et al. [\(2017\)](#page-35-12) reported that *Scenedesmus* sp. showed a maximum biomass production of 210.5 mg L^{-1} d⁻¹ when cultured in tannery wastewater with high uptake rate of ammoniacal nitrogen (85.6%) and phosphorus (96.9%). Other industries with discharges of nutrients can also use microalgae culture to reduce their nutrient loadings into the ecosystem. Yan et al. [\(2016\)](#page-41-9) reported that removal efficiencies of total oxygen demand, total nitrogen and total phosphorus by *Chlorella* culture in a simultaneous biogas upgrading and nutrient reduction system were 93%, 81% and 80%, respectively, illustrating that microalgae can efficiently remove nutrients from wastewaters. Groundwater can also have high contents of nutrients. Rezvani et al. [\(2017\)](#page-40-8) used groundwater to cultivate *Ettlia* sp. with biomass productivity of 0.2 g L^{-1} d⁻¹.

Zhuang et al. (2018) reported that nitrogen and phosphorus were the two major determinants not only for microlagal biomass but also for improvement of protein synthesis. His idea was supported by many other studies that reported higher microalgae compounds are synthesized under adequate culture environment (Manirafasha et al. [2018\)](#page-38-10). In fact, a culture consisting of a consortia of species showed higher nutrient removal compared to a single species culture (Gonçalves et al. [2016\)](#page-36-10). Manipulations of major nutrients could enhance lipid production in marine microalgae (Adenan et al. [2016\)](#page-34-6). In addition, light can also influence the production of lipids. Using a chemostat culture system at 1500 μ mol m⁻² s⁻¹ light intensity, Seo et al. [\(2017\)](#page-40-10) showed that high lipid productivity of 291.4 mg L⁻¹ d⁻¹ could be obtained. Some minerals also show effects on microalgae production. In a phototrophic culture, addition of calcium ions (Ca^{2+}) would decrease the microalgae biomass production because the increase Ca^{2+} would increase the phosphate precipitation (Di Caprio et al. [2018\)](#page-35-13).

4.3.3 Light and Temperature

In addition to carbon dioxide and nutrients, light is a critical factor in promoting microalgal growth and biomass/biocompound accumulation. Light does not only affect microalgae but also microbes. Nitrite oxidizers are light sensitive, and nitrite accumulation may occur if light intensity is increased (Vergara et al. [2016\)](#page-41-10), and this might have some implication in photobioreactors using wastewater as the culture medium.

For photosynthetic-based industries, light is one of the main limiting factors for an efficient system. Thus, for the development of technological applications of producing energy from living biomass, the design of the culture vessels should ensure the availability of light to the producing cells both in terms of quantity and quality. Based on this premise, some models to predict the availability of light and its spectral distribution has been developed for microalgae bioreactors to increase biomass production and high-value compounds (Table [4.4\)](#page-17-0). Fuente et al. [\(2017\)](#page-36-11) developed a light

field model to predict light attenuation in bioreactors which can be easily modified to accommodate different microalgae species in different photobioreactor types. The ability to predict the light intensity and spectral distribution are fundamental for productivity enhancement of these photobiological processes, the microalgal biomass production. In temperate countries when the growing season is short, photobioreactor engineering would focus on lengthening the photoperiod and maintaining a suitable temperature for the microalgae optimum growth and biomass production (Saeid and Chojnacka [2015\)](#page-40-11).

Light distribution in a bioreactor depends on the incident light intensity, the configuration of the vessel and the algal biomass concentration (Zhang et al. [2017a,](#page-41-2) [b\)](#page-41-4). Naderi et al. [\(2017\)](#page-39-11) developed a model of light distribution in a bioreactor based on the Beer–Lambert model which could provide useful information on light distribution and predict light reduction in the culture vessel. In bioreactors, light intensity attenuates sharply with the distance from the irradiated surface due to self-shading in the inner areas and light absorption by the dense microalgae cells. However, Hu and Sato [\(2017\)](#page-37-9) proposed an internal light-limiting diode (LED) system that does not limit the volume of the reactor vessel, and light attenuation could be avoided by decreasing the light spacing (Table [4.4\)](#page-17-0). In a bioreactor, not all zones are well lighted. Thus, strategies should be made such that the distance between the light source to the algal cells be optimized. Sun et al. [\(2016\)](#page-40-12) illustrated the use of light guide to bring light close to the growing algal cells using hollow polymethyl methacrylate (PMMA) tubes embedded into a flat plate photobioreactor. In this way, the incident light can be transmitted and emitted to the interior of the PBR, providing a secondary light source for cells in light-deficient regions.

Different light spectrum has different effects on microalgae photosynthetic rates, which is further dependent on specific species (Vadiveloo et al. [2015\)](#page-41-5). Schulze et al. [\(2016\)](#page-40-13) suggested that LEDs emitting spectra between 390–450 (blue) and 630–690 nm (red) should be combined to increase high-quality microalgae biomass. Blue spectrum has been shown to be effective in increasing the microalgae productivity (Atta et al. [2013;](#page-34-7) Vadiveloo et al. [2015\)](#page-41-5), in addition to the red spectrum (Detweiler et al. [2015;](#page-35-4) Schulze et al. [2014,](#page-40-14) [2016;](#page-40-13) Gao et al. [2017;](#page-36-12) Yan et al. [2016\)](#page-41-9). Lima et al. [\(2018\)](#page-38-12) showed that using LEDs with 70% red and 30% blue spectra with light intensity of 100 μ mol m⁻² s⁻¹ provided relatively high biomass productivity of 0.145 g L−¹ d−¹ for *Athrospira platensis* cultured in modified Zarrouk's medium. Thus, both red and blue spectrum are needed to boost the microalgae production. Interestingly, Leonardi et al. [\(2018\)](#page-37-10) reported that it was not the blue or red spectrum individually that caused the increase in microalgal biomass (*Scenedesmus quadricauda*), but the interactions of all the photons in the absorption process. In addition to enhancing microalgae growth rates and biomass production, specific light spectrum can also influence the quantity and quality of biochemical compounds synthesized in microalgae cells. Vadiveloo et al. [\(2015\)](#page-41-5) reported that the lipid content in *Nannochloropsis* sp. was highest under the blue spectrum.

However, increasing light intensity is not necessarily good for all microalgae. Naderi et al. [\(2017\)](#page-39-11) demonstrated that increasing light intensity in dense cultures did not result in increased biomass due to light absorption and scattering. To accurately

Light system	Advantages	Strategies	References
Use of light-limiting diodes (LEDs)	Optimize biomass and high-value compounds (carotenoids and phycocyanin)	Suitable light spectra for the highest microalgae biomass productivity-0.15 g L^{-1} d ⁻¹	Yan et al. (2016), Lima et al. (2018)
Internal (light-limiting diode) LED illumination system-flashing light effects or dynamic light condition	Volume of reactor vessel is not limited; flashing lights decrease the occurrence of photoinhibition, more light absorption with less xanthophyll cycle and less thermal dissipation	Efficient use of light by the microalgae cells	Abu-Ghosh et al. (2016) , Hu and Sato (2017)
	A serial lantern shaped draft tube (LTD)	Increased mixing and enhanced flashing light effects	Ye et al. (2018)
Light and $CO2$ synergy	Synergistic action of light and $CO2$ -Enhanced biomass and lipid production	Efficient (regulated) supply of CO ₂ and nutrients. With light of 1500 μ mol m ⁻² s^{-1} , algal (<i>Ettlia</i> sp.) productivity (1.48 g L^{-1} d ⁻¹)	Seo et al. (2017)
		60 µmol m ⁻² s ⁻¹ , algal (Nannochloropsis sp.) productivity 0.73 $g L^{-1} d^{-1}$	Thawechai et al. (2016)
The green solar collector (GSC)—use lenses and light guides	Efficient capturing mechanism of solar energy, reduced operation cost	High light utilization efficiency with low cost	Zijffers et al. (2008)
Mechanically stirred bioreactor	The different zone in the reactor can be controlled by geometric configuration and impeller stirring mechanism	High light utilization efficiency and production of high-quality biomass	Zhang (2013)
Use of selected light spectrum for specific species: i. Photovoltaic panels ii. Use of blue and red spectra	Increase the photosynthetic efficiency of the algal cells and enhanced growth rates	The specific spectrum best match the physiological requirements of the species	Atta et al. (2013), Vadiveloo et al. (2015) , Detweiler et al. (2015), Schulze et al. (2016)

Table 4.4 Use of light in photobioreactor systems

(continued)

Light system	Advantages	Strategies	References
Use of light guide	Light can be transferred to the interior parts of the bioreactor where incident light cannot reach	Make light available to all cells in the bioreactor	Sun et al. (2016)
Light in immobilized cell cultures	Microalgae cell immobilized in agar gel to minimize contamination and easy metabolite recovery	Light can be supplied through immobilized biopolymer	Kandilian et al. (2017)
Central composite design (CCD) approach	Three main factors, light, temperature and $CO2$ were optimized using response surface methodology (RSM)	Chlorellla sp. BA9031-0.235 g L^{-1} d ⁻¹	Mondal et al. $(2017a)$

Table 4.4 (continued)

determine the light availability to microalgae cells, Kandilian et al. [\(2016\)](#page-37-12) proposed a simple method to measure microalgal spectral absorption cross-section that can be used to predict and control light transfer and biomass production in a photobioreactor. Too strong light can cause photoinhibition. In their study of cyanobacteria culture in raceways, Hidasi and Belay [\(2018\)](#page-36-7) reported that photosynthetic depression occurred at midday when the sunlight was highest. Aly et al. [\(2017\)](#page-34-9) estimated that photoinhibition could cause 30–40% reduction in net microalgae biomass in an outdoor bioreactor. Yan et al. [\(2016\)](#page-41-9), in their study of growing *Chlorella* sp. using biogas slurry nutrient, suggested that light intensity should be low (approximately 400 μ mol m⁻² s⁻¹) during the early phase of the culture to avoid photoinhibition, and increase accordingly (to approximately 1000 μ mol m⁻² s⁻¹) as the microalgae density increases. To prevent photoinhibition, Hidasi and Belay [\(2018\)](#page-36-7) used flashing light in his raceway culture and showed that the microalgae growth rates were significantly higher compared to those that received continuous light. Application of flashing light approach by using different technological devices and/or by optimizing the mixing velocity of the culture at a suitable microalgae density, can also be integrated into the photobioreactor design to decrease the effect of photoinhibition and increase the microalgae biomass production (Abu-Ghosh et al. [2016\)](#page-34-8).

4.3.3.1 Light Sources

Light can be obtained from the sun which is free but subjected to inconsistencies due to daily or seasonal, environmental and climate changes. In spite of the problems, solar energy should be fully utilized to decrease the cost of energy used. Zijffers et al. [\(2008\)](#page-42-0) used Fresnel lenses to guide solar energy to focus on the microalgae cells in the photobioreactor. Vadiveloo et al. [\(2015\)](#page-41-5) used blue photovoltaic filters to increase biomass production of *Nannochloropsis* sp. in large outdoor cultures as this species illustrated that blue light was the most efficient light to biomass conversion. In addition, trapped solar energy can be used as a source of electricity to run the microalgae cultivations system such as pumps and aerators (Parlevliet and Moheimani [2014\)](#page-39-12). Thus, photobioreactor innovations should be strategized to fully exploit the natural, free and clean solar energy to drive large outdoor microalgae cultivation system, not only to increase the productivity of the cultivated microalgae, but also for electricity production to drive the cultivations system. On the other hand, the artificial light from lamps such as fluorescent tube, high intensity discharge lamp (HID) and light-limiting diode (LED), is costly, but consistent (Blanken et al. [2013\)](#page-34-10). Thus, in designing an efficient microalgae production bioreactor, light factor, either from solar energy or artificial light, has to be optimized to ensure its availability to the photosynthesizing cells.

The effects of light of microalgae production also depend on other growth factors, such as the use of wastewater. Using a higher light intensity of 182.5 µmol m⁻² s⁻¹, da Fontoura et al. [\(2017\)](#page-35-12) reported *Scenedesmus* biomass productivity of 0.211 g L−¹ d−¹ cultured in tannery wastewater. Thus, optimization of light, both in terms of intensity and spectral distribution with respect to other growth factors such as temperature, pH, aeration, nutrients and cultured species is the most important strategy to be considered in designing a photobioreactor (Mondal et al. [2017b;](#page-38-7) Seo et al. [2017;](#page-40-10) Lima et al. [2018\)](#page-38-12). Willette et al. [\(2018\)](#page-41-13) demonstrated that microalgae growth and photosynthetic rates declined at extreme temperatures (<15 °C), but the cold stress could boost the lipid and fatty acids production. In addition to temperature, photoperiods also play an important role in microalgae biomass production. Maroneze et al. [\(2016\)](#page-38-9) showed that manipulations of photoperiod can reduce energy cost in *Scenedesmus obliquus* culture.

4.4 The Performances in Different Types of Photobioreactors

Upscaling of microalgae cultivation is crucial in the assessment of its economics and ecological viability. In assessing the performance of different types of photobioreactors, the cell density (g L^{-1}) and biomass production rate (g m⁻² d⁻¹) are the most important parameters in terms of bioprocess engineering, although construction and running costs and energy expenditure are also crucial for the actual industrial process. High cell density culture has the merits of (1) efficient light utilization, (2) low energy consumption for pumping and circulating of culture media and (3) saving energy in dewatering and biomass concentration for downstream use of the biomass. Thus, high cell density culture is one of the keys for improvement of mass production of microalgae. Based on 48 previous works on outdoor microalgae

Fig. 4.5 The relationship between cell density (g-dw L^{-1}) and light path length (m) of each reactor in outdoor culture. The data are collected from 48 previous studies on outdoor culture works listed in Table [4.1](#page-3-0)

culture in different countries, species and culture media (Table [4.5\)](#page-21-0), there is a negative between the cell density (g-dw L^{-1}) and light path length (m) in outdoor microalgae cultures (Fig. [4.5\)](#page-20-0). The cell density increased with decreasing light pass length or volume/surface ratio (m) of the bioreactor. Doucha and Lívanský [\(2006\)](#page-35-14) reported that high cell density of 43 g L^{-1} in the closed raceway system with 1 cm light path length. Ozkan et al. [\(2012\)](#page-39-13) achieved extremely high cell density of 96.4 g L−¹ in a biofilm reactor.

For higher production rate, the bioreactor requires higher light intensity, since the production of microalgae are the conversion process of light energy to biomass energy. The areal production rate $(g.m^{-2} day^{-1})$ seems to increase with daily solar radiation-PAR (MJ m⁻² day⁻¹) (Fig. [4.6\)](#page-31-0). The areal production is not much different among bioreactor types and the rate tends to increase with higher daily solar radiation-PAR until around 13 MJ m^{-2} day⁻¹ since the photosynthesis is the energy conversion process of light and biomass energy. However, lower production values were often reported even in the bioreactor that received higher solar radiations. These low values are causally related to (1) lack of nutrients and $CO₂$, (2) insufficient mass transfer efficiency to distribute nutrients and $CO₂$, (3) unsuitable environmental factor of pH and temperature, (4) non-optimal dilution rate and (5) variation of species-specific growth rate.

To increase the light energy received by a photobioractor, the second generation of internally irradiated photobioreactors using optical fibers (Javanmardian and Palsson [1991;](#page-37-13) Ogbonna et al. [1999\)](#page-39-14) and fresnel lenses (Ogbonna et al. [1999\)](#page-39-14) as light-concentrating devices, were developed. Masojídek et al. [\(2003\)](#page-38-14) used fresnel lenses to concentrate light energy on the surface of tubular reactor and achieved high light intensity of 7000 μ E m⁻² s⁻¹ and 31.5 MJ m⁻² day⁻¹ (Masojídek et al.

(continued)

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(continued)

Fig. 4.6 The relationship between areal production rate (g-dw m−² day−1) and daily solar radiation-PAR (MJ m−² day−1) in outdoor culture. The data are collected from 48 previous studies on outdoor culture works listed in Table [4.1](#page-3-0)

[2003\)](#page-38-14), although the areal production was not the highest. The idea of the internal irradiation by light-concentrating device is not only to concentrate light energy but also to diffuse strong light in order to avoid photoinhibition. However, this bioreactor structure becomes complex and its cost of construction also increases. The strategy of using light concentration technology may not be suitable for mass production of microalgae that requires low cost and low energy consumption.

4.4.1 Technology Improvements

There are technologies to improve microalgae biomass production using photobioreactors by strategizing the use of growth factors especially increasing the efficiencies of light, carbon dioxide and nutrient utilization by different species (Table [4.6\)](#page-32-0). Holdmann et al. [\(2018\)](#page-37-7) illustrated an extremely effective technology using an airlift reactor showing 300% of production compared to the conventional method. To address the major problems in microalgae biomass and biomolecule production, Lee and Li [\(2017\)](#page-37-6) proposed resonant ultrasound field incorporated dynamic photobioreactor (RUF-DPBS) that is labour-efficient, cost-effective and non-fouling. Huang et al. [\(2015\)](#page-37-20) developed a novel internal mixers optimized with computational fluid dynamics to improve the performance of their flat plate photobioreactors to about 32.8% higher than the conventional mixer. In general, innovative and cost-effective technologies for microalgae biomass production are still urgently required to satisfy the market demand for microalgae biomass by microalgae-based industries. Conventional technologies cannot keep up with the increasing demand for microalgae.

System	Percent improved production compared to conventional system	Technology	References
Flat plate Bioreactor-Archetype reactor	32.8% (Chlorella pyrenoidosa)	Optimized internal mixer using computational fluid dynamics	Huang et al. (2015)
Flat panel airlift (FPA)	300% (from $<1-4$ g L^{-1}) (Chlorella sorokiniana)	Airlift reactor mixed solely by aeration with sterile air	Holdmann (2018) —commercialized by Subitech GmbH
A serial lantern shaped draft tube in (LTD) Gas-lift circumflux column (GCC) photobioreactor	50% (Chlorella)	The serial lantern shaped draft tube (LDT improved $CO2$ fixation in a by generating vortices to increase radial velocity between dark and light region. Mass transfer coefficient increased by 26% and mixing time decreased by 21%	Ye et al. (2018)
Submerged-light photobioreactor $(SL-PBR)$	51% (Chlorella vulgaris)	Free floating wireless internal light source powered by near field resonant inductive coupling for Chlorellla vulgaris $(51\%$ increase) and Haematococcus pluvialis (53%)	Murray et al. (2017)
ePBR-novel environmental photobioreactor	Chlorella sorokiniana $(25-150 \text{ mg L}^{-1})$	Algal culturing platform for simulating dynamicsof natural environments	Lucker et al. (2014)
Predictive system, the laboratory environmental algae pond simulator (LEAPS) photobioreactor	88.7-109.2% (Chlorella sorokiniana and Nannochloropsis salina)	Screening of microalgae strains and photobioreactor operating conditions for high biomass and biocompound yields in outdoor systems	Huesemann et al. (2017)

Table 4.6 Improvements of microalgae biomass production using novel technologies

4.4.2 Mathematical Modelling

Due to many interacting factors influencing microalgae biomass production, mathematical modelling becomes a useful tool in predicting the behaviour and impacts of different factors, which in turn affect the design of suitable culture vessels and microalgae production systems. Thus, integrated modelling of an efficient and strategic photobioreactor for optimum and sustainable production of microalgae should encompass light intensity and spectral distribution, carbon dioxide and nutrient supply and uptake, optimization of environmental factors in culture vessels, dissolved oxygen removal and growth biokinetics with reference to selected species (Al Ketife et al. [2016\)](#page-34-14). Mondal et al. [\(2017a\)](#page-38-13) used response surface methodology (RSM)-central composite design approach to model three interacting factors (light intensity, $CO₂$) and temperature) to determine optimal culture conditions for *Chlorellla* sp. Gao et al. (2018) suggested a light distribution model to accurately predict the light intensity required for the fast growth of *Haematococcus pluvialis* culture under red LEDs. Aly et al. (2017) produced a mathematic model for the microalgae growth and $CO₂$ sequestration in outdoor photobioractors, whereas Al Ketife et al. [\(2016\)](#page-34-14) suggested a model that could permit optimization and scale-up of microalgae biomass production based on light, nutrients and carbon dioxide and their kinetics.

4.5 Conclusions and Future Perspectives

Microalgae are known to be sustainable feedstocks for biofuels and valuable compounds which are important in food, health and animal production industries. However, biomass production on a large scale is still an insurmountable challenge that need to be solved in terms of technological, economics and ecological viability. Photobioreactor is the best alternative to produce high-quality microalgae biomass but strategies are needed to build an economical, efficient and high-throughput microalgae production system. Efficient production of biomass through balancing the use of energy and reducing cost should be the focus in designing bioreactors. Microalgae growth factors including light, carbon dioxide and nutrients have to be technologically manipulated to develop a simple, efficient and cost-effective photobioreactor with high production rate but minimal construction and operation cost. Additional features to increase efficiency of the bioreactor such as efficient light harvesting with suitable light spectrum and adjustable photoperiod, suitable fluid dynamics to ensure optimised dispersion of microalgal cells, adjustable application of nutrient stress to trigger the production of high lipids contents in the algal cells, and automated oxygen discharge structure are necessary to overcome biomass production limitation. Natural light, gas and nutrient sources should be used to defray the operation cost. Strategic bioreactor should be flexible and adjustable to suit different species of microalgae and the target compounds and can be used in many areas with different climatic conditions. Large-scale photobioreactors should not be only technically improved

but should be made economically feasible. Once technologically and economically improvised, photobioreactors could generate all the resources that are valuable and useful to global communities.

Acknowledgements This study is partially supported by the SATREPS-COSMOS Malaysia-Japan collaborative project under the auspices of the Department of Higher Education, Ministry of Education Malaysia.

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