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Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review

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ABSTRACT

Microalgae have the ability to mitigate CO_2 emission and produce oil with a high productivity, thereby having the potential for applications in producing the third-generation of biofuels. The key technologies for producing microalgal biofuels include identification of preferable culture conditions for high oil productivity, development of effective and economical microalgae cultivation systems, as well as separation and harvesting of microalgal biomass and oil. This review presents recent advances in microalgal cultivation, photobioreactor design, and harvesting technologies with a focus on microalgal oil (mainly triglycerides) production. The effects of different microalgal metabolisms (i.e., phototrophic, heterotrophic, mixotrophic, and photoheterotrophic growth), cultivation systems (emphasizing the effect of light sources), and biomass harvesting methods (chemical/physical methods) on microalgal biomass and oil production are compared and critically discussed. This review aims to provide useful information to help future development of efficient and commercially viable technology for microalgae-based biodiesel production.

1. Introduction

Today about 80% of global energy demand is produced from fossil fuels. However, extensive utilization of fossil fuels has led to global climate change, environmental pollution, and health problems (Hallenbeck and Benemann, 2002). Many countries are thus turning their attention to the development of new, clean, and sustainable energy sources. Among the various potential sources of renewable energy, biofuels are of most interest and are expected to play a crucial role in the global energy infrastructure in the future. Biodiesel, one of the most commonly used biofuels, is recognized as an ideal recyclable energy carrier, and thus also as a possible primary energy source (Chisti, 2007). Commercial biodiesel is currently produced from animal fat, waste frying oil and vegetable oils (Barnwal and Sharma, 2005), whose competition with edible vegetable oil for agricultural land is still a controversial issue (Mata et al., 2010). Consequently, microalgae that can grow rapidly and convert solar energy to chemical energy via CO_2 fixation are now being considered a promising oil source for making biodiesel (Mata et al., 2010).

Under suitable culture conditions, some microalgal species are able to accumulate up to 50–70% of oil/lipid per dry weight (Chisti, 2007). The fatty acid profile of microalgal oil is suitable for the synthesis of biodiesel (Gouveia and Oliveira, 2009). The major attraction of using microalgal oil for biodiesel is the tremendous oil production capacity by microalgae, as they could produce up to 58,700 L oil per hectare, which is one or two magnitudes higher than that of any other energy crop (Chisti, 2007). However, mass production of microalgal oil faces a number of technical hurdles that render the current development of the algal industry economically unfit. In addition, it is also necessary, but very difficult, to develop cost-effective technologies that would permit efficient biomass harvesting and oil extraction. Nevertheless, since microalgae production is regarded a feasible approach to mitigate global warming, it is clear that producing oil from microalgal biomass would provide significant benefits, in addition to the fuel. Microalgae have thus been widely recognized as the feedstock for third-generation of biofuels (Chisti, 2007), and this review critically assesses the literature on the cultivation, photobioreactor design and harvesting of microalgae for biodiesel production.

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2. Effects of cultivation conditions on microalgal oil production

The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions (Chojnacka and Marquez-Rocha, 2004). There are four major types of cultivation conditions for microalgae: photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic cultivation (Chojnacka and Marquez-Rocha, 2004). Table 1 summarizes the lipid content, lipid productivity and the microalgal biomass production under different cultivation conditions for different microalgae species. Each type of cultivation is discussed in detail in the following sections.

2.1. Phototrophic cultivation

Phototrophic cultivation occurs when the microalgae use light, such as sunlight, as the energy source, and inorganic carbon (e.g., carbon dioxide) as the carbon source to form chemical energy through photosynthesis (Huang et al., 2010). This is the most commonly used cultivation condition for microalgae growth (Gouveia et al., 2009; Gouveia and Oliveira, 2009; Illman et al., 2000; Mandal and Mallick, 2009; Yoo et al., 2010). Table 1 shows that under phototrophic cultivation, there is a large variation in the lipid content of microalgae, ranging from 5% to 68%, depending on the type of microalgae species. Normally a nitrogen-limiting or nutrient-limiting condition was used to increase the lipid content in microalgae (Mata et al., 2010). As a result, achieving higher lipid content is usually at the expense of lower biomass productivity. Thus, lipid content is not the sole factor determining the oil-producing ability of microalgae. Instead, both lipid content and biomass production need to be considered simultaneously. Hence, lipid productivity, representing the combined effects of oil content and biomass production, is a more suitable performance index to indicate the ability of a microalga with regard to oil production. The highest lipid productivity reported in the literature is about 179 mg/L/d by *Chlorella* sp. under phototrophic cultivation using 2% CO_2 with 0.25 vvm aeration (Chiu et al., 2008) (Table 1). The major advantage of using autotrophic cultivation to produce microalgal oil is the consumption of CO_2 as carbon source for the cell growth and oil production. However, when CO_2 is the only carbon source, the microalgae cultivation site should be close to factories or power plants which can supply a large quantity of CO_2 for microalgal growth. Moreover, compared to other types of cultivation, the contamination problem is less severe when using autotrophic growth. Therefore, outdoor scale-up microalgae cultivation systems (such as open ponds and raceway ponds) are usually operated under phototrophic cultivation conditions (Mata et al., 2010).

2.2. Heterotrophic cultivation

Some microalgae species can not only grow under phototrophic conditions, but also use organic carbon under dark conditions, just like bacteria. The situation when microalgae use organic carbon as both the energy and carbon source is called heterotrophic cultivation (Chojnacka and Marquez-Rocha, 2004). This type of cultivation could avoid the problems associated with limited light that hinder high cell density in large scale photobioreactors during phototrophic cultivation (Huang et al., 2010). As indicated in Table 1, higher biomass production and productivity could be obtained from using heterotrophic cultivation. Some microalgae species show higher lipid content during heterotrophic growth, and a 40% increase in lipid content was obtained in *Chlorella protothecoides* by changing the cultivation condition from phototrophic to heterotrophic (Xu et al., 2006). Microalgae can assimilate a variety of organic carbon sources (such as glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose) for growth (Liang et al., 2009). Some studies have thus focused on finding cheaper organic carbon sources, such as corn powder hydrolysate (CPH) instead of sugars, resulting in high biomass (2 g/L/d) and lipid (932 mg/L/d) productivities (Table 1) (Xu et al., 2006). The highest lipid productivity (3700 mg/L/d) was reported by Xiong et al. (2008) using a 5-L fermentor operated with an improved fed-batch culture strategy. Using heterotrophic growth gives much higher lipid productivity, as the highest lipid productivity from heterotrophic cultivation is nearly 20 times higher than that obtained under phototrophic cultivation (Table 1). However, the sugar-based heterotrophic system frequently suffers from problems with contamination.

2.3. Mixotrophic cultivation

Mixotrophic cultivation is when microalgae undergo photosynthesis and use both organic compounds and inorganic carbon (CO_2) as a carbon source for growth. This means that the microalgae are able to live under either phototrophic or heterotrophic conditions, or both. Microalgae assimilate organic compounds and CO_2 as a carbon source, and the CO_2 released by microalgae via respiration will be trapped and reused under phototrophic cultivation (Mata et al., 2010). Compared with phototrophic and heterotrophic cultivation, mixotrophic cultivation is rarely used in microalgal oil production (Table 1).

2.4. Photoheterotrophic cultivation

Photoheterotrophic cultivation is when the microalgae require light when using organic compounds as the carbon source. The main difference between mixotrophic and photoheterotrophic cultivation is that the latter requires light as the energy source (Table 2), while mixotrophic cultivation can use organic compounds to serve this purpose. Hence, photoheterotrophic cultivation needs both sugars and light at the same time (Chojnacka and Marquez-Rocha, 2004). Although the production of some light-regulated useful metabolites can be enhanced by using photoheterotrophic cultivation (Ogbonna et al., 2002), using this approach to produce biodiesel is very rare, as is the case with mixotrophic cultivation.

2.5. Comparison of different cultivation conditions

Although oil production of microalgae is strain-dependent, Table 1 seems to indicate that heterotrophic growth could give much better oil productivity than other cultivation conditions, and this approach has thus attracted considerable interest. However, heterotrophic culture can get contaminated very easily, especially in open cultivation systems, causing problems in large-scale production (Table 2). In addition, the cost of an organic carbon source is also a major concern from the commercial aspect. Phototrophic cultivation is most frequently used, and easiest to scale up, as an open pond system, and is promising because microalgae could uptake CO_2 from the flue gas of factories and convert it to oil. However, the oil productivity of this approach is usually markedly lower than that of heterotrophic cultivation, due mainly to slow cell growth and low biomass productivity. On the other hand, the lower cost for scaling-up of phototrophic cultivation mean that this method is still very attractive (Table 2). To date, there is little information in the literature concerning using mixotrophic and photoheterotrophic cultivation for microalgal oil production, but those two cultivation conditions are also restricted by contamination risk and light requirements, and may require the design of a special photobioreactor for scaling-up, thereby increasing the operation cost (Table 2) .

Table 1
The lipid content and productivities of different microalgae species under different cultivation conditions.

Microalgae species	Cultivation condition	Biomass productivity (g/L/d)	Lipid content (% of DCW)	Lipid productivity (mg/L/d)	Reference
Botryococcus braunii UTEX 572	Phototrophic[a]	0.03	20.8	5.5	Yoo et al. (2010)
Chaetoceros calcitrans CS 178	Phototrophic[a]	0.04	39.8	17.6	Rodolfi et al. (2009)
Chaetoceros muelleri F&M-M43	Phototrophic[a]	0.07	33.6	21.8	Rodolfi et al. (2009)
Chlorella emersonii CCAP211/11N	Phototrophic[b]	0.04	25.0–34.0	10.3–12.2	Scragg et al. (2002)
Chlorella emersonii CCAP 211/11N	Phototrophic[a]	0.03–0.05	29.0–63.0	8.1–49.9	Illman et al. (2000)
Chlorella minutissima UTEX 2341	Phototrophic[a]	0.02–0.03	31.0–57.0	9.0–10.2	Illman et al. (2000)
Chlorella protothecoides CCAP 211/8D	Phototrophic[a]	0.002–0.02	11.0–23.0	0.2–5.4	Illman et al. (2000)
Chlorella protothecoides	Heterotrophic[f]	4.0–4.4	43.0–46.0	1881.3–1840.0	Cheng et al. (2009)
Chlorella protothecoides	Heterotrophic[c]	2.2–7.4	50.3–57.8	1209.6–3701.1	Xiong et al. (2008)
Chlorella protothecoides	Heterotrophic[c,g]	2.0	46.1	932.0	Xu et al. (2006)
Chlorella protothecoides	Heterotrophic[c]	1.7–2.0	43.0–48.7	732.7–932.0	Li et al. (2007)
Chlorella sorokiniana UTEX 1230	Phototrophic[a]	0.003–0.005	20.0–22.0	0.6–1.1	Illman et al. (2000)
Chlorella sorokiniana IAM-212	Phototrophic[a]	0.23	19.3	44.7	Rodolfi et al. (2009)
Chlorella sp. F&M-M48	Phototrophic[a]	0.23	18.7	42.1	Rodolfi et al. (2009)
Chlorella sp.	Phototrophic[a]	0.37–0.53	32.0–34.0	121.3–178.8	Chiu et al. (2008)
Chlorella vulgaris KCTC AG10032	Phototrophic[a]	0.10	6.6	6.9	Yoo et al. (2010)
Chlorella vulgaris #259	Phototrophic[a]	0.01	33.0–38.0	4.0	Liang et al. (2009)
Chlorella vulgaris #259	Heterotrophic[c,d]	0.08–0.15	23.0–36.0	27.0–35.0	Liang et al. (2009)
Chlorella vulgaris #259	Mixotrophic[c,e]	0.09–0.25	21.0–34.0	22.0–54.0	Liang et al. (2009)
Chlorella vulgaris INETI 58	Phototrophic[b]	0.18	5.1	7.4	Gouveia and Oliveira (2009)
Chlorella vulgaris Beijerinck CCAP 211/11B	Phototrophic[a]	0.03–0.04	18.0–40.0	5.4–14.9	Illman et al. (2000)
Chlorella vulgaris Beijerinck CCAP 211/11B	Phototrophic[b]	0.02–0.04	28.0–58.0	11.2–13.9	Scragg et al. (2002)
Chlorella vulgaris CCAP 211/11B	Phototrophic[a]	0.17	19.2	32.6	Rodolfi et al. (2009)
Chlorella vulgaris F&M-M49	Phototrophic[a]	0.20	18.4	36.9	Rodolfi et al. (2009)
Chlorococcum sp. UMACC 112	Phototrophic[a]	0.28	19.3	53.7	Rodolfi et al. (2009)
Dunaliella tertiolecta IPIMAR	Phototrophic[b]	0.12	16.7	20.0	Gouveia and Oliveira (2009)
Dunaliella tertiolecta ATCC 30929	Phototrophic[a]	0.10	60.6–67.8	60.6–69.8	Takagi et al. (2006)
Ellipsoidion sp. F&M-M31	Phototrophic[a]	0.17	27.4	47.3	Rodolfi et al. (2009)
Isochrysis sp. (T-ISO) CS 177	Phototrophic[a]	0.17	22.4	37.7	Rodolfi et al. (2009)
Isochrysis sp. F&M-M37	Phototrophic[a]	0.14	27.4	37.8	Rodolfi et al. (2009)
Monodus subterraneus UTEX 151	Phototrophic[a]	0.19	16.1	30.4	Rodolfi et al. (2009)
Nannochloris sp. UTEX LB1999	Phototrophic[a]	0.04–0.35	29.9–40.3	15.6–109.3	Takagi et al. (2000)
Nannochloropsis CS 246	Phototrophic[a]	0.17	29.2	49.7	Rodolfi et al. (2009)
Nannochloropsis oculata NCTU-3	Phototrophic[a]	0.37–0.48	22.7–29.7	84.0–142.0	Chiu et al. (2009)
Nannochloropsis sp.	Phototrophic[b]	0.09	28.7	25.8	Gouveia and Oliveira (2009)
Nannochloropsis sp. F&M-M24	Phototrophic[a]	0.18	30.9	54.8	Rodolfi et al. (2009)
Nannochloropsis sp. F&M-M26	Phototrophic[a]	0.21	29.6	61.0	Rodolfi et al. (2009)
Nannochloropsis sp. F&M-M27	Phototrophic[a]	0.20	24.4	48.2	Rodolfi et al. (2009)
Nannochloropsis sp.	Phototrophic[a]	0.17	35.7	60.9	Rodolfi et al. (2009)

(*continued on next page*)

Table 1 (*continued*)

Microalgae species	Cultivation condition	Biomass productivity (g/L/d)	Lipid content (% of DCW)	Lipid productivity (mg/L/d)	Reference
F&M-M28					
Nannochloropsis sp. F&M-M29	Phototrophic[a]	0.17	21.6	37.6	Rodolfi et al. (2009)
Neochloris oleabundans UTEX 1185	Phototrophic[a,b]	0.03–0.15	15.9–56.0	10.7–38.8	Gouveia et al. (2009)
Neochloris oleabundans UTEX 1185	Phototrophic[b]	0.09	29.0	26.1	Gouveia and Oliveira (2009)
Neochloris oleoabundans UTEX 1185	Phototrophic[a]	0.31–0.63	7.0–40.3	38.0–133.0	Li et al. (2008)
Pavlova lutheri CS 182	Phototrophic[a]	0.14	35.5	50.2	Rodolfi et al. (2009)
Pavlova salina CS 49	Phototrophic[a]	0.16	30.9	49.4	Rodolfi et al. (2009)
Phaeodactylum tricornutum F&M-M40	Phototrophic[a]	0.24	18.7	44.8	Rodolfi et al. (2009)
Porphyridium cruentum	Phototrophic[a]	0.37	9.5	34.8	Rodolfi et al. (2009)
Scenedesmus obliquus FCTU Coimbra	Phototrophic[b]	0.09	17.7	15.9	Gouveia and Oliveira (2009)
Scenedesmus obliquus	Phototrophic[b]	0.06	12.7	7.14	Mandal and Mallick (2009)
Scenedesmus obliquus	Mixotrophic[c]	0.10–0.51	6.6–11.8	11.6–58.6	Mandal and Mallick (2009)
Scenedesmus quadricauda	Phototrophic[a]	0.19	18.4	35.1	Rodolfi et al. (2009)
Scenedesmus sp. KCTC AG20831	Phototrophic[a]	0.22	9.5	20.7	Yoo et al. (2010)
Scenedesmus sp. DM	Phototrophic[a]	0.26	21.1	53.9	Rodolfi et al. (2009)
Scenedesmus sp. F&M-M19	Phototrophic[a]	0.21	19.6	40.8	Rodolfi et al. (2009)
Skeletonema costatum CS 181	Phototrophic[a]	0.08	21.1	17.4	Rodolfi et al. (2009)
Skeletonema sp. CS 252	Phototrophic[a]	0.09	31.8	27.3	Rodolfi et al. (2009)
Spirulina maxima LB 2342	Phototrophic[b]	0.21	4.1	8.6	Gouveia and Oliveira (2009)
Tetraselmis sp. F&M-M34	Phototrophic[a]	0.30	14.7	43.4	Rodolfi et al. (2009)
Tetraselmis suecica F&M-M33	Phototrophic[a]	0.32	8.5	27.0	Rodolfi et al. (2009)
Tetraselmis suecica F&M-M35	Phototrophic[a]	0.28	12.9	36.4	Rodolfi et al. (2009)
Thalassiosira pseudonana CS 173	Phototrophic[a]	0.08	20.6	17.4	Rodolfi et al. (2009)

[a] CO_2.
[b] Air.
[c] Glucose.
[d] Acetate.
[e] Glycerol.
[f] Jerusalem artichoke hydrolysate (JAH).
[g] Corn powder hydrolysate (CPH).

Table 2
Comparison of the characteristics of different cultivation conditions.

Cultivation condition	Energy source	Carbon source	Cell density	Reactor scale-up	Cost	Issues associated with scale-up
Phototrophic	Light	Inorganic	Low	Open pond or photobioreactor	Low	Low cell density High condensation cost
Heterotrophic	Organic	Organic	High	Conventional fermentor	Medium	Contamination High substrate cost
Mixotrophic	Light and organic	Inorganic and organic	Medium	Closed photobioreactor	High	Contamination High equipment cost High substrate cost
Photoheterotrophic	Light	Organic	Medium	Closed photobioreactor	High	Contamination High equipment cost High substrate cost

3. Microalgae culture system

In both indoor and outdoor microalgae cultivation systems, the light source and light intensity are critical factors affecting the performance of the phototrophic growth of microalgae (Mata et al., 2010). For outdoor cultivation, sunlight is the major light source, while some innovative artificial light sources (e.g., LED and optical fiber) are of interest for indoor cultivation systems. In addition, it is also possible to transmit solar energy from outside to illuminate indoor photobioreactors, such as solar-energy-excited optical fiber systems (OF-solar). Table 3 summarizes the features and electricity consumption of using different light sources for microalgae growth.

Table 3
Features and electricity consumption for different artificial light sources.

Light source	Feature	Operation stability	Electricity consumption of the light source[a] (kw-h)
Conventional artificial light sources	Higher biomass productivity, higher stability, large illumination area, low constructing cost	High	40.32
LED	Lower energy consumption, lower heat generation, longer life-expectancy, tolerate higher frequency of on–off switching, higher stability, low constructing cost	High	20.16
Optical fiber excited by metal–halide lamp (OF-MH)	Higher energy consumption, lower area of land required, good light path, uniform light distribution, lower space requirement, low contamination risk	Moderate	36.0
Optical fiber excited by solar energy (OF-solar)	Low electricity consumption, good light path, uniform light distribution, lower space requirement, low contamination risk, lower cost	Low	1.0
LED/OF-solar combined with wind power/solar panel	No electricity consumption, good light path, uniform light distribution, lower space requirement, low contamination risk	High	0

[a] The electricity consumption of light sources was based on a 40 L photobioreactor.

3.1. General light sources development for the microalgae culture systems

Although many efforts have been made to develop efficient and cost-effective photobioreactors, the high cost of installing and operating artificial light sources in conventional photobioreactors with artificial illumination systems remains a major problem. Moreover, according to Eq. (1), the light intensity decreases exponentially with distance from a reactor wall as the concentrations of both cell and product increase

$$I_L/I_o = \exp(-\gamma L) \quad (1)$$

where I_L is the light intensity at depth L, I_o is the original incident intensity, γ is the turbidity. Hence, the light intensity tends to decrease rapidly due to the light shading effects arising from increases in the concentrations of both cell and product or from formation of biofilm on the surface of reactor vessels (Chen et al., 2008). Furthermore, although a short light path is theoretically favorable for achieving high light efficiency, conventional light sources cannot be in close contact with the microalgae culture, because they usually generate a considerable amount of heat. Consequently, the light conversion efficiency of conventional photobioreactors has been limited. Due to the problems and limitations associated with conventional light sources, various photobioreactor designs with different illumination strategies have been developed to enhance the microalgae production rate and oil/lipid content (Ma and Hanna, 1999).

As seen above, microalgae biomass production performance is often limited by the light energy supplied and the cell concentration. Hence, without taking into account the issue of economic viability, different photobioreactors have been constructed and designed to improve light supply and microalgae biomass production performance. El-Shishtawy et al. (1997) proposed a photobioreactor which was designed by combining a light receiving face and reflection sheet to transfer light sources. Some researchers also developed a photobioreactor composed of three concentric glass cylinders with incandescent lamps placed directly into the photobioreactor (Tsygankov et al., 1994). Issarapayup et al. (2009) used a flat plate airlift photobioreactor to determine the optimal downcomer-to-riser area ratio for the cultivation of *Haematococcus pluvialis* NIES-144. Although many different photobioreactors have been designed and shown to be effective for microalgae growth, the major obstacle to their practical application is the high power consumption and operating cost due to the need for artificial light sources.

3.2. Innovative light sources for microalgae culture systems

To effectively exploit the commercial potential of algae, a cheap, durable, reliable and highly efficient light source is needed. If the light source has a narrow spectral output that overlaps the photosynthetic absorption spectrum, the emission of light at unusable frequencies would be eliminated, therefore improving the overall energy conversion. Among the light sources currently available, light-emitting diodes (LEDs) are the only ones that meet the foregoing criteria. LEDs are light and small enough to fit into virtually at any photobioreactor, and their other advantages include a longer life-expectancy, lower heat generation, higher conversion efficiency and a greater tolerance for switching on and off. In addition, LEDs have a narrow light emission spectra between 20 and 30 nm, which can be matched with photosynthetic needs. For instance, the adsorption wavelength of blue LED and red LED are around 450–470 nm and 645–665 nm, respectively (Yeh and Chung, 2009). Wang et al. (2007) found that the highest specific growth rate and biomass production were obtained by using red LED in the photoautotrophic cultivation of *Spirulina platensis* (Wang et al., 2007). Moreover, several studies reported that the optimal wavelength condition could vary from species to species. Katsuda et al. (2004) proposed that illumination with red LED is suitable for microalgal cell growth, while switching to illumination with blue LED could improve the astaxanthin production by *H. pluvialis*. Katsuda et al. (2006) also indicated that incident light intensity, duty cycle and flashing frequency have clear effects on the algal cultivation. Their results showed that using flashing light with blue LED is a promising illumination method for algae cell growth and astaxanthin production. Lee and Palsson (1994) applied LED as the sole light source for indoor cultivation of the green alga *Chlorella pyrenoidosa*. Their study tried to combine different LED with different absorption wavelengths to enhance the production of microalgae biomass and target products, as LED with a wide variety of absorption wavelengths are now available.

In addition to LED, optical fiber excited by artificial lights is another potential light source to improve microalgae culture systems. Original plastic-clad optical fiber is end-light illuminated, but if the fiber is polished mechanically to obtain a rough surface, then this allows light emission from the entire surface of the core, producing so-called side-light optical fibers. Such fibers are expected to markedly enhance the light conversion efficiency of the photobioreactors, because they can provide uniform light distribution (Lee and Kim, 1998) with a high surface-to-volume ratio, and can be directly immersed in the microalgae cultivation system to achieve efficient light energy transfer without heat generation. Side-light optical fibers can be excited by different artificial light engines to supply light with different wavelength distributions, and can be used as internal light sources for photobioreactors. Most optical-fiber-based photobioreactors are used for growing algae (Matsumaga et al., 1991). In addition, side-light optical-fibers have also been applied to design photobioreactors for microbial desulfurization (Henshaw and Zeu, 2001).

3.3. Sunlight as the light source for microalgae culture systems

As shown in Table 3, the major problems associated with microalgae culture systems are the high power consumption and high operating cost of the artificial light sources. To improve the light efficiency and increase the microalgae growth rate at a lower cost would thus be a substantial step towards the development of a successful microalgae production process. Among all the light sources available, that from the sun is clearly the most abundant, as its radiation provides the highest energy flow of ca. 5.7×10^{24} J year^{-1}, which is about 10,000 times more than the total energy consumed by human beings every year (Miyake et al., 1999). At mid-day, the sunlight provides the highest light intensity, at 1100 W/m^2 (Miyake et al., 1999), which exceeds the intensity required for efficient production of microalgae. Sunlight is often used as the energy source for microalgae cultivation (Chisti, 2007), and this approach has numerous advantages. First, sunlight is free, whereas artificial light sources are very expensive (Chen et al., 2008). Second, solar energy contains the full spectrum of light energy (Chen et al., 2008), and, through a specific UV filter, it can provide a suitable absorption wavelength for both microalgae cell growth and target product production (Chen et al., 2008). Efficient utilization of solar energy can simultaneously solve the problems of a high operating cost, electricity consumption and environmental pollution. Therefore, artificial light engines have been replaced by sunlight, which can excite side-light optical fibers. In this process, sunlight is directly introduced via optical fibers into the photobioreactors for illumination, which significantly reduces the electricity consumption down to 1.0 kW-h. However, the diurnal variation of light intensity is considered a major problem with using OF-solar as the light source.

Most of the commercial cultivation of microalgae is carried out in open pond systems, with solar light energy being directly utilized (Pulz, 2001). However, the performance of these outdoor open pond photobioreactors is usually poor, due to the problems of it being difficult to control the culture conditions, direct exposure to UV irradiation, contamination, low light intensity and uneven distribution (Chen et al., 2008), day–night cycles, diurnal variation and the need for a large area of land. Although all of these issues limit the light conversion efficiency and productivity of outdoor photobioreactors, it is the day–night cycles and diurnal variation in light intensity that are considered the major problems when using sunlight. Depending on the weather, season, solar spectrum and operation time, the length of time during which the light intensity is high enough to support microalgal growth can be very short. In the absence of light energy, the cellular metabolism mode will switch (Kitajima et al., 1998), and thus both the productivity and biochemical composition of the microalgal cells are affected by the availability of the light. It has also been discovered that biomass concentration and carbohydrate content decrease during cultivation of *C. pyrenoidosa* at night (Ogbonna and Tanaka, 1998). Ong et al. (2010) used an outdoor closed and vertical bubble column photobioreactor with 40 L culture volume. The CO_2 fixation rate reached 25.65 mg/min when using semi-continuous cultivation within a thermal-tolerant mutant *Chlorella* sp. In addition, Sato et al. (2006) used a new outdoor closed type photobioreactor, and found that the productivity of *Chaetoceros calcitrans* was 37.3 g/m^2/day with the maximum cell density of 2.5 g/L. (Carlozzi, 2003) also reported the maximum photoautotrophic cyanobacterium biomass productivity of 2.7 g/L/d with a maximum biomass concentration of 6.0 g/L by using an outdoor undulated tubular reactor. (Hall et al., 2003) obtained the microalga *Phaeodactylum tricornutum* productivity of 1.4 g/L/d with a maximum biomass concentration of 3.0 g/L with an outdoor cylindrical shaped helical tubular photobioreactor. Doucha and Livansky (2009) reported that biomass productivity of the microalga *Chlorella* sp. reached 4.3 g/L/d in an outdoor open thin-layer photobioreactor. In addition, Vonshak et al. (1996) successfully used outdoor tubular photobioreactors for the cultivation of *S. platensis*, while Ugwu et al. (2005) reported that the biomass productivity of *Synechocystis aquatilis* achieved 9 g/m^{-2}/d in an outdoor tubular photobioreactor equipped with static mixers. Finally, Li et al. (2007) demonstrated the feasibility of outdoor tubular cultivation of the marine microalga *Pavlova viridis* for photoautotrophic production of eicosapentaenoic acid (EPA).

3.4. Combining OF-solar/multi-LED with solar panel/wind power generator for microalgae culture systems

Since the light intensity of sunlight varies greatly with the weather, season, location and operating time, the light supply of OF-solar systems is unstable. To solve this problem, Chen et al. (2008) installed a light dependent resistor (LDR) for online monitoring of the irradiation intensity on the photobioreactor. In this new illumination system, the OF-solar or solar was used for daytime illumination. If the solar light intensity decreased to a set value (due to cloudy/rainy days and at night), the compensative artificial light source (multi-LED light sources) was automatically turned on, thus ensuring that a continuous, sufficient and stable light supply toward the inside and outside of the photobioreactor was maintained.

To further decrease electricity consumption, solar panels and a wind power generator were also introduced our work (Ramachandra et al., 2009). The solar panels were used to collect solar radiation from the sunlight, converting it into usable electricity. Because sunlight is not continuously supplied during the operating time, the wind power generator was also used as an additional complement to a solar panel system. This earlier study also used a wind power generator to blow the propeller round and then drive the generator to produce extra electricity. The solar panels and a wind power generator were together able to supply all of the energy required by the multi-LED light sources. The conceptual photobioreactor combining OF(sunlight) and multi-LED light sources with solar panels and a wind power generator, as shown in Fig. 1, has the potential to be developed into a commercially viable microalgae cultivation system with zero electricity consumption.

3.5. Comparison of different light sources for microalgae culture systems

A microalgae cultivation system can be illuminated by artificial light, solar light or combinations of different light sources. The oil yield in an outdoor microalgae cultivation system by using natural light sources (sunlight) is between 100 and 130 m^{-3} ha^{-1} (Chisti, 2007). In contrast, the oil yield can reach 172 m^{-3} ha^{-1} by using artificial general light sources in the laboratory-scale microalgae cultivation system. This is due primarily to the stability and continuous light energy provided by artificial light to enhance the microalgae growth and oil accumulation. Most of laboratory-scale photobioreactors are illuminated by using fluorescent lamps, which require high power consumption and high operating cost. At the same total light intensity, replacing fluorescent lamps by multi-LED light source resulted in a 50% decrease in electricity consumption (from 40.32 to 20.16 kW-h). Most commercial microalgae cultivation systems are carried out in open systems (such as open ponds) using solar energy as the light source, which is the cheapest light source available. However, the performance of outdoor systems is usually poor and requires a large area of land. Using optical fiber (OF) as the internal light sources to illuminate a microalgae cultivation system could increase the light efficiency and reduce electricity consumption. In particular, using OF excited by solar energy (OF-solar) requires only 1.0 kW-h of electricity (Table 3). However, the major drawback for OF-solar has been the instability of the

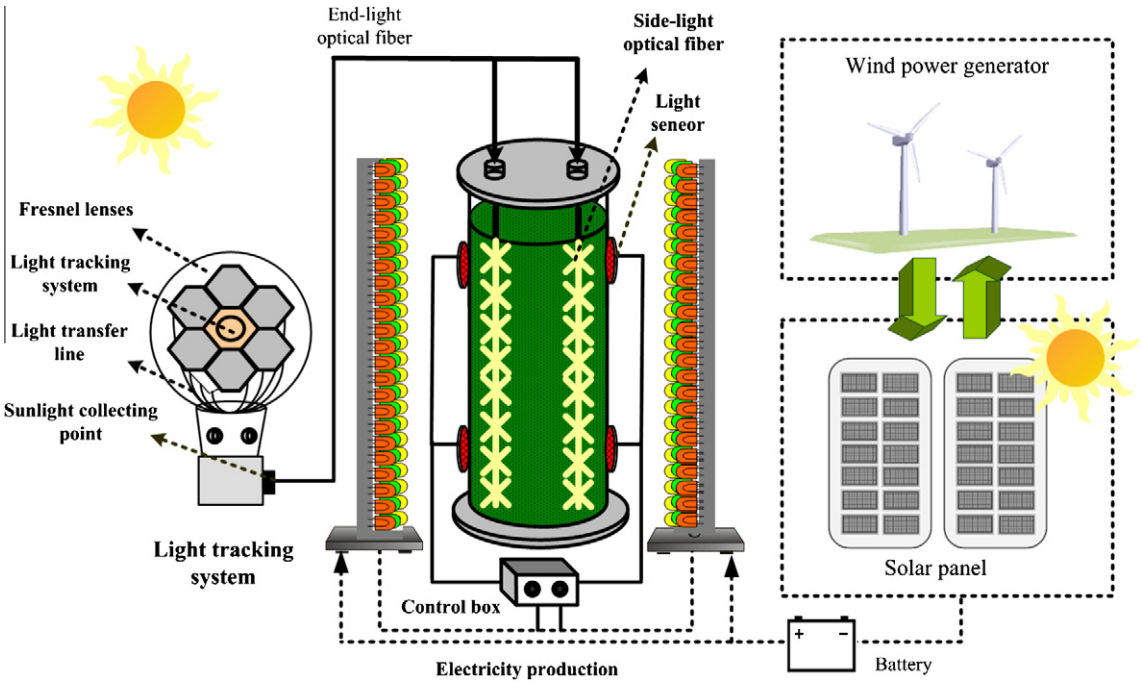

Fig. 1. Schematic description of the solar-energy-excited optical fiber photobioreactor system with an internal light source (optical fiber excited by sunlight collecting system) and a multi-LED light source using the electricity generated by a solar panel and wind power generator.

solar energy supply, which is a general problem limiting the applicability and productivity of outdoor photobioreactors. Combining OF-solar with solar panels and wind power generators was thus introduced to supply the power for multi-LED light source and other artificial light sources for the cultivation of microalgae. As shown in Table 3, the electricity consumption for different light sources decreased in the order of conventional artificial light (40.32 kW-h) > OF-artificial (36.0 kW-h) > LED (20.16 kW-h) > OF-solar (1.0 kW-h) > LED/OF-solar or solar/solar panel/wind power generator (0 kW-h). Although the foregoing results indicate that combining LED/OF-solar with solar panels and wind power generators could markedly decrease electricity consumption in all categories, further development of technologies that could enhance the microalgae growth rate, enable outdoor cultivation and avoid contamination problems is still needed.

4. Microalgal harvesting technologies

Efficient harvesting of biomass from cultivation froth is essential for mass production of biodiesel from microalgae. The major techniques presently applied in the harvesting of microalgae include centrifugation, flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques (Uduman et al., 2010). The cost of algae harvesting can be high, since the mass fractions in culture broth are generally low, while the cells normally carry negative charge and excess algogenic organic matters (AOM) to keep their stability in a dispersed state (Danquah et al., 2009).

The selection of harvesting technique is dependent on the properties of microalgae, such as density, size, the value of the desired products (Brennan and Owende, 2010). Microalgae harvesting can generally be divided into a two-step process, including:

1. *Bulk harvesting.* The purpose of this is to separate microalgal biomass from the bulk suspension. By this method, the total solid mater can reach 2–7% using flocculation, flotation, or gravity sedimentation (Brennan and Owende, 2010).
2. *Thickening.* The purpose of this harvesting is to concentrate the slurry, with filtration and centrifugation usually applied in this process. This step needs more energy than bulk harvesting (Brennan and Owende, 2010).

4.1. Centrifugation

Most microalgae can be recovered from the liquid broth using centrifugation. Laboratory centrifugation tests were conducted on pond effluent at 500–1000×*g* and showed that about 80–90% microalgae can be recovered within 2–5 min. Grima et al. (2003) concluded that centrifugation is a preferred method for harvesting of microalgal biomass, especially for producing extended shelf-life concentrates for aquaculture. Knuckey et al. (2006) states that exposure of microalgal cells to high gravitational and shear forces can damage cell structure. In addition, processing a large amount of culture using centrifugation is time consuming and costly (Grima et al., 2003).

4.2. Flocculation

Flocculation is a process in which dispersed particles are aggregated together to form large particles for settling.

4.2.1. Autoflocculation

Autoflocculation occurs as a result of precipitation of carbonate salts with algal cells in elevated pH, a consequence of photosynthetic CO_2 consumption with algae (Sukenik and Shelef, 1984). Hence, prolonged cultivation under sunlight with limited CO_2 supply assists autoflocculation of algal cells for harvesting. Laboratory experiments also revealed that autoflocculation can be simulated by adding NaOH to achieve certain pH values.

4.2.2. Chemical coagulation

Adding chemicals to microalgal culture to induce flocculation is a common practice in various solid–liquid separation processes as a pre-treatment stage, which is applicable to the treatment of large

quantities of numerous kinds of microalgal species (Lee et al., 1998). There are two main classifications of flocculants according to their chemical compositions: (1) inorganic flocculants and (2) organic flocculants/polyelectrolyte flocculants. The utilization of microorganisms to recover microalgae has also been investigated, with (Oh et al., 2001) studying the use of *Paenibacillys* sp. AM49 for effective harvesting of microalgae.

4.2.2.1. Inorganic coagulants. Microalgal cells are negatively charged, as a result of adsorption of ions originating from organic matter and dissociation or ionization of surface functional groups (Uduman et al., 2010). By disrupting the stability of the system, successful microalgal harvesting can be obtained. Addition of a coagulant, like iron-based or aluminum-based coagulants, will neutralize or reduce the surface charge (Grima et al., 2003). Alum was utilized for harvesting of *Scenedesmus* and *Chlorella* via charge neutralization (Grima et al., 2003). Microalgae can also be flocculated by inorganic flocculants at sufficiently low pH (Uduman et al., 2010). However, despite its advantages, coagulation using inorganic coagulants suffers from the following drawbacks:

1. A large concentration of inorganic flocculant is needed to cause solid–liquid separation of the microalgae, thereby producing a large quantity of sludge.
2. The process is highly sensitive to pH level.
3. Although some coagulants may work for some microalgal species, they do not work for others.
4. The end product is contaminated by the added aluminum or iron salts.

4.2.2.2. Organic flocculants. To achieve effective sedimentation, floc size should be more than 100 μm, with the addition of a high molecular weight bridging polymer increasing floc size and improving microalgal settling (Edzwald, 1993). Flocculation by aluminum sulfate followed by certain polyelectrolytes is effective in microalgal harvesting (Pushparaj et al., 1993). Biodegradable organic flocculants, such as chitosan, are produced from natural sources that do not contaminate the microalgal biomass (Divakaran and Pillai, 2002). The most effective flocculants for the recovery of microalgae are cationic flocculants (Bilanovic et al., 1988). Anionic and nonionic polyelectrolytes have been shown to fail to flocculate microalgae, which is explained by the repulsion existing between charges or the insufficient distance to bridge particles. Polymer molecular weight, charge density of molecules, dosage, concentration of microalgal biomass, ionic strength and pH of the broth, and the extent of mixing in the fluid have all been found to affect flocculation efficiency (Grima et al., 2003). Bilanovic et al. (1988) noted that flocculation by cationic polymers can be inhibited by the high salinity of a marine environment. High molecular weight polyelectrolytes are generally better bridging agents. A high biomass concentration in the broth also helps flocculation due to the frequent cell–cell encounters. Mixing at a low level is thus useful, as it helps bring the cells together, but excessive shear forces can disrupt flocs. In addition to all of the factors mentioned before, functional groups on microalgal cell walls are important, because they stimulate the formation of negative charge centers on the cell surfaces (Uduman et al., 2010).

4.2.2.3. Combined flocculation. A combined flocculation process is a multistep flocculation process using more than one type of flocculant. Sukenik et al. (1988) studied a combined flocculation process with marine microalgae. To induce flocculation in sea water, two methods were found. The first is combining polyelectrolytes with inorganic flocculants, such as ferric chloride or alum, and the second is ozone oxidation followed by flocculant addition. Muylaert et al. (2009) demostrated the feasibility of using cationic starch for flocculation of both freshwater microalgae and marine water microalgae.

4.2.3. Electrolytic process

Electrocoagulation mechanisms involve three consecutive stages: (1) generation of coagulants by electrolytic oxidation of the sacrificial electrode, (2) destabilization of particulate suspension and breaking of emulsion, and (3) aggregation of the destabilized phases to form flocs. Azarian et al. (2007) investigated the removal of microalgae from industrial waste-water using continuous flow electrocoagulation. Different from electrolytic coagulation, electrolytic flocculation does not requires the use of sacrificial electrodes. Electrolytic flocculation works based on the movement of microalgae to the anode in order to neutralize the carried charge and then form aggregates. Poelman et al. (1997) showed that the efficiency of algal removal is 80–95% when electrolytic flocculation is applied.

4.3. Gravity sedimentation

Gravity sedimentation is commonly applied for separating microalgae in water and waste-water treatment. Density and radius of algae cells and the induced sedimentation velocity influence the settling characteristic of suspended solids (Brennan and Owende, 2010). Enhanced microalgal harvesting by sedimentation can be achieved through lamella separators and sedimentation tanks (Uduman et al., 2010). Flocculation is frequently used to increase the efficiency of gravity sedimentation. The success of solids removal by gravity settling depends highly on the density of microalgal particles. Edzwald (1993) found that low density microalgal particle do not settle well and are unsuccessfully separated by settling.

4.4. Filtration and screening

Grima et al. (2003) reviewed harvesting process options to recover biomass and the related economic costs. Screening involves introducing the suspension through a screen with a particular pore size. Microstrainer and vibrating screen filters are two of the primary screening devices in microalgae harvesting. Microstrainers can be realized as rotating filters with fine mesh screens with frequent backwash. A high microalgal concentration can result in blocking the screen, whereas a low microalgal concentration can result in inefficient capture (Wilde et al., 1991). Microstrainers have several advantages, such as simplicity in function and construction, easy operation, low investment, negligible abrasion as a result of absence of quickly moving parts, being energy-intensive and having high filtration ratios. Grima et al. (2003) found that filters which operate under pressure or in a vacuum are able to recover relatively large microalgae, although they failed when applied to organisms approaching bacterial dimensions. Tangential flow filtration is a high rate method for microalgal harvesting, and (Petrusevski et al., 1995) recovered 70–89% freshwater algae using this approach. Additionally, tangential flow filtration retains the structure, properties and motility of the collected microalgae.

4.5. Flotation

Flotation is a gravity separation process in which air or gas bubbles attache to solid particles and then carry them to the liquid surface. Chen et al. (1998) noted that flotation is more beneficial and effective than sedimentation with regard to removing microalgae. Flotation can capture particles with a diameter of less than 500 μm by collision between a bubble and a particle and the subsequent adhesion of the bubble and the particle (Yoon and Luttrell, 1989).

Based on bubble sizes used in the flotation process, the applications can be divided into dissolved air flotation (DAF), dispersed flotation and electrolytic flotation.

4.5.1. Dissolved air flotation

The DAF entails the pressure reduction of a water stream that is presaturated with air at excess pressures to produce 10–100 μm bubbles (Uduman et al., 2010). Factors determining DAF harvesting of microalgae include the pressure of the tank, recycle rate, hydraulic retention time, and floating rate of particle. Chemical flocculation has been used with DAF to separate microalgae (Uduman et al., 2010). Microalgae autoflocculation using dissolved oxygen which is produced photosynthetically has also been studied after flocculation using alum or C-31 polymer (Koopman and Lincoln, 1983), and about 80–90% microalgal removal was obtained when about 16 mg/L microalgal float concentration was used. Edzwald (1993) found that DAF removed microalgae more effectively than settling, although flocculation pre-treatment was required in the former process.

4.5.2. Dispersed air flotation

Dispersed air flotation entails 700–1500 μm bubbles formed by a high speed mechanical agitator with an air injection system (Rubio et al., 2002). Chen et al. (1998) compared dispersed air flotation efficiencies for microalgae using three collectors, and noted that the cationic *N*-cetyl-*N*-*N*-*N*-trimethylammonium bromide (CTAB) effectively removed *Scenedesmus quadricauda*, while the nonionic X-100 and anionic sodium dodecylsulfate did not. They attributed these differences to changes in surface hydrophobicity with collector adsorption.

4.6. Electrophoresis techniques

The electrolytic method is a another potential approach to separate algae without the need to add any chemicals. In this method, an electric field drives charged algae to move out of the solution (Mollah et al., 2004). Water electrolysis generates hydrogen which adheres to the microalgal flocs and carries them to the surface. There are several benefits to using electrochemical methods, including environmental compatibility, versatility, energy efficiency, safety, selectivity, and cost effectiveness (Mollah et al., 2004). An investigation into the removal of microalgae electrolytically in batch and continuous reactors by flotation was conducted by Alfafara et al. (2002). The results for a batch system showed that by increasing the electrical power input the rate of chlorophyll removal increased and the electrolysis time decreased.

4.7. Harvesting techniques

Golueke and Oswald (1965) compared algae removal using filtration, flotation, centrifugation, precipitation, ion exchange, passage through a charged zone, and ultrasonic vibration. They concluded that only centrifugation and chemical precipitation are economically feasible options, with centrifugation being marginally better. An optimal harvest method of algae for biofuel production should be species independent, use minimal chemicals and energy, and, if possible, preferentially release intracellular materials for collection. Mass cultivation of algae needs a high overflow rate, which favors flotation in which algae are moving upwards instead of sedimentation in which algae are moving downward (Edzwald, 1993). Gravity sedimentation is only suitable for harvesting of large size microalgal, such as *Spirulina*. In order to enhance the separation of microalgae and the sedimentation rate, a flocculant can be added to the system. If land is available and product contamination by coagulants is not a concern, gravity sedimentation alone concentrates microalgal suspension to 1.5% w/w solids at minimal cost.

The cost of microstrainers may be low for large-sized microalgae. However, the problems encountered using microstrainers could be incomplete solids removal and difficulty in handling solids fluctuations (Middlebrooks et al., 1974). Another problem related to microstrainers is the increase in bacterial and algae slime on the microfabric (Middlebrooks et al., 1974), which thus require regular cleaning. Alternatively, the mesh surface may also be modified to reduce affinity between dissolved or colloidal algogenic organic matters (AOM) and the collector surface. The cost of applying tangential flow filtration relies on membrane replacement and pumping, and large scale harvesting using this method is limited by this. Rossignol et al. (1999) studied the use of polymer membranes for continuous harvesting of two marine microalgal species, *Haslea ostrearia* and *Skeletonema costatum*. However, to date the interactions between algal cells or AOM with the surfaces of membranes made of different materials remain unclear. Changes in the surface hydrophobicity of the applied membrane may be promising to reduce fouling during algal harvesting. The electrolytic method has the potential to separate algae without the addition of chemicals. Nevertheless, the high power that needs to be input results in a temperature increase that may damage the system, and fouling of the cathodes remains the major disadvantage of this method. Assisted flotation, such as the use of an oxidant to destabilize suspended algal cells (Betzer et al., 1980) presents a potential alternative for efficient mass harvesting of algal cells. Understanding the characteristics of AOM and suspended algal cells and associated changes subjected to coagulation or oxidation essentially determines the design and operation of algal harvesting processes.

5. Conclusions

Performance of microalgal oil production is strain- and metabolism-dependent. Heterotrophic cultivation usually exhibits much higher oil productivity due to higher growth rate and cell density. Phototrophic growth of microalgae yields lower oil productivity, but is able to fix CO_2 and suitable for outdoor cultivation (e.g., using open-pond). Photobioreactor could be effective to grow microalgae by using favorable light source and reactor configuration. Collection and concentration of microalgal biomass from cultivation systems contribute heavily to the operation cost of the overall process. Therefore, more efficient and economic harvesting technology should be developed to enhance the commercial viability of microalgae biofuels industry.

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