CULTIVATION SYSTEM AND HARVESTING TECHNIQUES IN MICROALGAE BIOMASS PRODUCTION

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Abstract. Microalgae biomass is one of the very high potentials for the production of the third generation of biofuel. Public awareness of the importance of the environment and the lack of conventional fuel sources has been attracting the interest of researchers in exploring deeper on microalgae biomass production. Two major challenges need to be noted in respect of the microalgae industry is cultivation systems and effective harvesting techniques. Therefore, this review focuses on the main cultivation system and harvesting technique applied to microalgae. Besides that, the main advantages and disadvantages of each system and technique were also discussed further. The most suitable cultivation system is via closed photobioreactor while flocculation technique was preferred to harvest microalgae due to the low-cost method. But, selection of adequate cultivation and harvesting method depends on the characteristic of microalgae and also the value of the end product intends to be produced. Consequently, this review aims to provide information to enhance the development technology for microalgae biomass production in term of cultivation system and harvesting technique.
Keywords: *harvesting, microalgae cultivation, flocculation, coagulation, photobioreactor*

Introduction

Increasing demand for energy and awareness of environmental degradation has sparked initiatives to explore potential renewable energy sources that are environmentally friendly and sustainable. At present, most countries still rely heavily on conventional sources e.g. petroleum for power generation (Lam et al., 2019). Usage of non-renewable sources results in an increase in cost, as well as a continuous decrease in the supply of resources. About 57.7% of energy worldwide is consumed primarily by the transportation sector, where the consumption rate of fossil fuel was estimated to be 934 million tonnes per year (Lam et al., 2019). Uncontrolled utilisation of fossil fuel can lead to environmental problems too, such as global climate change, health problems, and environmental pollution (Chen et al., 2011). One of the most promising sources of renewable energy to address these problems is biomass, such as microalgae, which is presently considered as a promising source for biofuel production (Mata et al., 2010). Algae-based biofuel is not a new idea per se, as it has been discovered since 1940's as discussed by Borowitzka (2013). To date, production of fuel through algae biomass is still being studied for commercialization, particularly in developing countries (Rajkumar et al., 2014; Janta et al., 2013; Mahapatra and Ramachandra, 2013). The

allure of microalgae biomass as a source of biofuel is due to several special features including (Arnold, 2013): 1) Biofuel can be synthesized from a large variety of algae species; 2) Algae has a rapid growth rate compared to terrestrial plants; 3) Algae can be cultivated in brackish coastal water and seawater; 4) Some land areas that are unsuitable for agricultural can be used to cultivate algae; 5) Algae can take up high concentration of nutrients from municipal, agricultural, and industrial wastewater; and 6) Algae can utilize carbon dioxide from industrial sources. The only downside to this green source of energy is that the cultivation of algae is still considered relatively expensive, though this can be overcame by using wastewater as the growth media (Gani et al., 2016a; Razak et al., 2016). By treating wastewater through phycoremediation, the resulting production of biomass that can subsequently be used to produce biofuel will effectively reduce the cultivation costs, in addition to being environmentally friendly. Algae biomass production is also facing challenges in terms of the cultivation and harvesting techniques. As such, both stages of this biomass production must be taken seriously if one intends to produce biomass from microalgae commercially. Consequently, this review briefly discusses the cultivation and harvesting systems of microalgae for biomass production in term of application and selection of both techniques which use in current industry.

Cultivation of Microalgae

Growing microalgae either for commercial or research purposes can be done in both open and closed system. Their cultivation would require a huge volume of media containing necessary nutrients for their growth, though conditions may vary. Small-scale cultivations, for example, would utilise synthetic mediums, while large-scale cultivations normally utilise wastewater instead, especially from domestic sources, as the medium replacement. Domestic wastewater has been found to be a promising medium able to provide enough nutrients for their growth, while simultaneously allowing for the wastewater to be treated through phycoremediation (Gani et al., 2016b; Can et al., 2013; Teles et al., 2013; Devi et al., 2012). In spite of the nutrients provided, successful cultivation of microalgae still relies significantly on the culturing system used, which may be either through an open system, or a closed system, each of which is discussed further in the following sections.

Open Pond System

Open pond system is the most favourable method of microalgae biomass production, particularly in commercial projects to provide nutrition and produce biofuel. Several types of open pond system are available such as the raceway, shallow big and circular tank systems (Borowitzka, 2013). Raceway pond is the most famous cultivation system among these three, where typically, a fully closed loop and an oval shape circulation channel are constructed (Costa and de Morais, 2014). A raceway pond's shape resembles a race track and it is usually between 0.2 m – 0.5 m deep. The culture is stirred using paddlewheels for homogenisation, which would promote algal growth and biomass productivity. The pond can be made of concrete, glass fibre or membrane (Brennan and Owende, 2010). Compared to other open pond systems, the raceway is the most economic option for large-scale microalgae cultivation (Costa and de Morais, 2014; Chisti, 2007). Moreover, it is very easy to construct and operate, and has a very low power consumption (Ugwu et al., 2008). This cultivation technique that is exposed

to sunlight, however, is susceptible to water loss due to naturally high evaporation rate. In addition, the temperature and pH level are difficult to control, and the open system meant exposure to cross-contamination by undesired microorganisms. *Figure 1(a)* and *Table 1* show the schematic diagram and advantages/disadvantages of open raceway pond, respectively.

Table 1. *Advantages and disadvantages of open raceway pond and closed tubular photobioreactor.*

Raceway (Open Pond)	Column Heading
Advantages: 1. Low cost technique for large-scale cultivation. 2. Low power consumption/ requirement. 3. Easy to maintain and clean. 4. Capture atmosphere CO_2 effectively.	*Advantages:* 1. Large surface area that is exposed to natural light. 2. Higher productivity and easy to control. 3. Suitable for most microalgae species. 4. High mass-transfer rate with good mixing. 5. Compact, easy to operate, and low-cost, relatively. 6. Less water evaporation. 7. Low cross contamination risk.
Disadvantages: 1. Easily contaminated by other microorganisms. 2. Cell density is low due to shadowing of their cells. 3. Loss of water due to evaporation. 4. Uncontrolled temperature and pH level.	*Disadvantages*: 1. Small illumination surface area. 2. Cells sedimentation may occur during cultivation. 3. The growth of microalgae on the wall of photobioreactor due to inconsistent mixing. 4. Limit on the length of the tubes due to inefficient gaseous exchange.

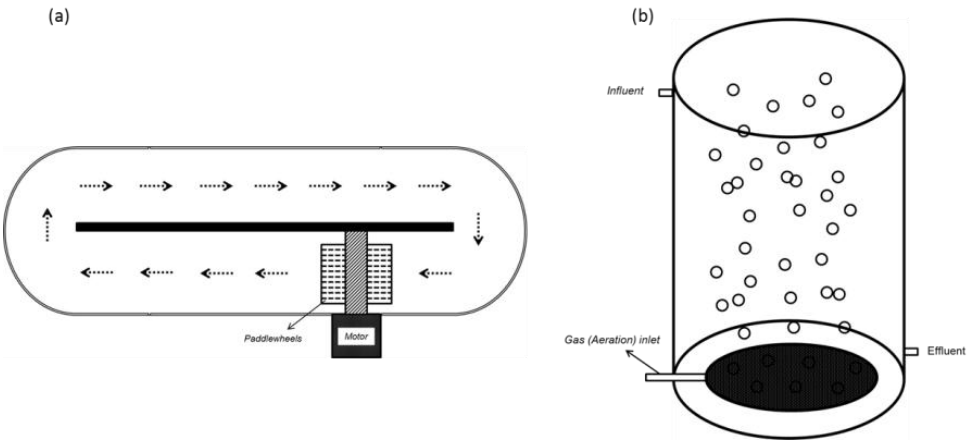

Figure 1. *Schematic diagram of (a) raceway open pond and (b) closed tubula photobioreactor.*

There are a few studies utilising open raceway pond to culture microalgae for many kinds of the application as stated in *Table 2*. Van Den Hende et al. (2014a), for example, used this technique to cultivate microalgae bacterial flocs in the outdoor

condition in Kortrijk, Belgium, for the purpose of aquaculture wastewater treatment and biomass production. They found that scaling up the culture decreases nutrients removal efficiencies and biomass productivity compared to a lab-scale system (Van Den Hende et al., 2014b). Another example of open raceway pond usage was by Ashokkumar & Rengasamy (Ashokkuar and Rengasamy, 2012) in Tamil Nadu, India, where *Botyococcus braunii* was grown for biofuel production.

A total 1800L of modified Chu 13 medium was prepared as the medium culture in the experiment, which was conducted in a concrete raceway pond lined with porcelain tiles having a total working volume of 2000L. After 15 days of cultivation, up to 1.8 g/L *Botryococcus braunii* biomass was able to be produced (Ashokkumar and Rengasamy, 2012). Yet another example of the open raceway pond usage was by Lim et al. (2010), in which wastewater from the textile industry was bio-remediated using *Chlorella vulgaris* cultivated in High Rate Algal Pond. The ponds were installed on the rooftop of the Institute of Postgraduate Studies, University of Malaya, with each single-loop raceway stirred using a paddlewheel at 15 rpm. A total 40L of textile wastewater was used for each pond and 10 days were allowed for treatment. There was a reduction of ammonium (44.4 – 45.1%), phosphate (33.1 – 33.3%), and COD (38.3 – 62.3%) while colour removal ranged from 41.8 to 50%. Biomass productivity ranged from 0.17 to 2.26 mg chlorophyll a/L in the textile wastewater (Lim et al., 2010).

Table 2. *Utilisation of raceway and photobioreactor system in previous studies*.

Type of cultivation system	Microalgae species	Location of study	Application	References
Raceway (Open Pond)	Microalgal bacterial flocs	Kortrijk, Belgium	Aquaculture wastewater treatment and biomass production.	Van Den Hende et al. (2014)
	Botryococcus braunii Kutz.	Tamilnadu, India.	Biofuel production	Ashokkumar and Rengasamy (2012)
	Chlorella vulgaris	Kuala Lumpur, Malaysia	Textile wastewater bioremediation	
	Spirulina platensis	Rio-Grande, Brazil	Biomass concentration and growth investigation.	Radmann et al. (2007)
	Species not specify	Madrid, Brazil	Investigation of effectiveness utilisation for flue gases.	Pawlowski et al. (2014)
Photobioreactor (Vertical)	*Chlorella vulgaris*, *Scenedesmus obliquus* and *Consortium C.*	Lisbon, Portugal	Wastewater treatment with Bioenergy potential for bio-hydrogen production.	Batista et al. (2015)
	Chlorella vulgaris	Kortrijk, Belgium	Biomass production and pre-harvesting of microalgae.	Bilad et al. (2014)
	Nannochloropsis oculata	Alicante, Spain	To obtained behaviour of microalgae/photobioreactor system related to the CO_2	Valdés et al. (2012)

		net balance.	
Oocystis sp.	Valladolid, Spain	Phycoremediation of fish processing wastewater.	Riaño et al. (2011)
Chlorella vulgaris	Nangang, China	Lipid production of algae cultivated in artificial wastewater.	Feng et al. (2011)

Over in Rio-Grande, Brazil, *Spirulina platensis* was cultivated using the same system by Radmann et al. (2007), for the purpose of investigating its growth and biomass concentration. Cultivation was carried out in a 6L acrylic open raceway pond containing 5L of Zarrouk culture medium, which was agitated using paddlewheels rotating at 18 revs/min and illuminated with 3000 Lux of light intensity. A Box-Behnken experimental design was used for the analysis, and the *Spirulina platensis* productivity was found to be at 0.028 to 0.046 g/L/day, with maximum specific growth rate being 0.038 to 0.138/day. Further in 2014, Pawlowski et al. (2014) demonstrated the cultivation of microalgae to address the effective utilisation of flue gases with the proper pH control in an open raceway pond. The raceway was operated at a constant depth of 0.2m, mixing was done using 1.2 m diameter paddlewheels with eight blades while flue gases were injected through membrane diffusers at the bottom of the sump. It was found that evaluated control algorithm significantly improves the pH control accuracy, which in turn has a direct influence on biomass productivity (Pawlowski et al., 2014).

Closed System

The drawbacks of the open pond application as stated in Table 1 have motivated intensive investigation into closed system especially for vertical photobioreactor (*Figure 1 (b*). This particular system is used for the cultivation of microalgae for high-quality products, such as pharmaceuticals and food supplement, where there is a strict requirement against contamination risk that is present in the exposed open system (Rawat et al., 2013). In a closed system's photobioreactor, there is no direct exchange of gases or contaminants between the cultivation system and the outside environment. Instead, gas exchange, which is essential for the mixing of algae in the culture, is provided using sterilised gas to avoid and minimise contamination inside the culture system. Another fundamental principle of photobioreactors' development is the total amount of illumination received by algae cells (Wang et al., 2012). Closed photobioreactors are more flexible than open systems on this as they can utilise artificial lighting to further increase the intensity given by natural sunlight, which enters the system and illuminates the microalgae culture inside through the transparent walls of the vertical photobioreactor tube. A gas inlet would be installed at the bottom of the reactor to supply CO_2 and to allow for mixing (*Figure 1 (b*). This naturally leads to a typical disadvantage of photobioreactors, which is the higher cost to build and operate. The advantages of a closed photobioreactor, as shown in *Table 1*, are the availability of larger surface area exposed to sunlight; higher biomass productivity, and higher mass-transfer rate with good mixing. Meanwhile, the drawbacks are the possibility of cell sedimentation during cultivation and the potential of microalgae growth on the photobioreactor's wall due to inconsistencies in mixing.

Several attempts have been made to cultivate microalgae in closed photobioreactors for various types of application as stated in *Table 2*. In 2014, Batista et al. (2015) have

demonstrated the use of photobioreactors for different microalgae species cultivated using urban wastewater collected from Aguas de Figueira, Portugal. A 150L tabular vertical photobioreactor was used to grow the microalgae under natural sunlight and outdoor temperature until nutrients depletion. This reduction in nutrients was done *Scenedesmus obliquus* for ammonium, phosphate and COD at 97.9%, 100%, and 54%, respectively. The microalgae species also produced the highest $BioH_2$ (56.8 mL H_2/gvs) compared to other species tested in the study (Batista t al., 2015). Bilad et al. (2014) meanwhile cultivated *Chlorella vulgaris* in a photobioreactor using Wright's cryptophytes medium to produce biomass, as well as to conduct a pre-harvesting investigation using different photobioreactors and membrane photobioreactors. The 25L cylinder photobioreactor operated continuously under different dilution rates for 45 days, after which a filtration system was added to the system, turning it into a membrane photobioreactor. It was found that the membrane photobioreactor was able to operate at higher dilution rate and thus increased growth rate compared to regular photobioreactors.

Another variation, the bubble column photobioreactor, was used by Valdes et al. (2012) to cultivate the *Nannochloropsis oculata* in modified seawater with the f/2 medium. The 1.7 m tall and 0.14 m wide photobioreactor was made of transparent PVC, with an estimated volume of about 25L, and it was placed outdoors in Alicante in September. The aim of their study was to investigate the behaviour of microalgae cultured in photobioreactor system in relation to CO_2 net balance using analysis of pH profiles. Mixing was done by injecting air using a microperforated circular pipe located at the bottom of the photobioreactor. A maximum photosynthesis active radiation (PAR) value of close to 55% was recorded at noon when the concentration of the culture was around 0.14 g/L. In an earlier study by Riaño et al. (2011), two photobioreactors were set up with a total working volume of 3L, where *Oocystis* sp. was cultivated using fish processing wastewater for phycoremediation potential. Each photobioreactor was exposed to light at 12,000 Lux for 24 hours and mixed using magnetic stirrers, though different temperatures were maintained for each reactor (23°C and 31°C). Similar TCOD and phosphate removal were achieved (about 70%) while ammonium concentration was completely exhausted in both photobioreactors. However, higher biomass productivity was recorded in the reactor with the higher temperature (about 55%) compared to the other one (Riaño et al., 2011).

Feng and Zhang (2011) investigated lipid production of *Chlorella vulgaris* cultured in sterilised artificial wastewater using four 2.2 L aerated column photobioreactors. The wastewater was inoculated with *Chlorella vulgaris* at 30°C and continuously illuminated at 3000 Lux intensity. The highest lipid productivity of *Chlorella vulgaris* was about 147 mg/L/day with nutrients removal of COD, ammonium and TP of 86%, 97%, and 96%, respectively. It was their conclusion from the research that the findings would lead to an economical technology of algal lipid production Feng and Zhang (2011).

Harvesting of Microalgae

Harvesting is another significant part of the biotechnology industry related to microalgae. In fact, the cost of harvesting is estimated to be up to 20% – 30% of the total cost of cultivation (Hattab et al., 2015). Many types of harvesting method are available and widely used presently, such as centrifugation, filtration, sedimentation,

and flocculation. The selection of harvesting method depends on several factors, including the application of the microalgae biomass, culture condition, and microalgae species. Each method has its advantages and disadvantages, as discussed further in the following section. Some previous studies in the literature reported that no single harvesting method is suitable for every microalga species since the particular strain and final product to be produced vary so widely (Granados et al., 2012). This makes the choice of harvesting technique to become more complex, particularly for large scale productions due to the need for optimisation, and the magnitude of laborious work to be carried out.

Centrifugation

Centrifugation is a common method used in lab-scale microalgae harvesting due to several advantages and accompanying disadvantages. While it is the fastest method to recover microalgae biomass, as well as the most efficient harvesting method compared to others due to its ability to recover the biomass of most microalgae species, it consumes very high amount of energy, leading to its higher cost (Barros et al., 2015; Raja et al., 2014). The process may also cause damage to microalgae cells due to its high spinning speed (Harun rt al., 2010). Due to this, the technique is neither recommended nor deemed feasible for large scale productions. Shah et al. (2014) reported that 80% - 90% of microalgae recovery can be achieved when laboratory centrifugation tests were conducted on pond effluent at 500-1000xg speed. Harun *et al.* (Harun et al., 2010) also recorded 88% - 100% cell viability and around 95% - 100% harvesting efficiency by centrifugation at 13000xg. *Table 3* shows the advantages and disadvantages of application of centrifugation in microalgae harvesting.

Table 3. *Advantages and disadvantages of microalgae harvesting technique [34, 54].*

Type of harvesting	Advantages	Disadvantages
Centrifugation	• High biomass recovery. • Quick method. • Suitable for most microalgae species.	• Needs more energy to operate. • Not cost effective. • Cell damage due to high speed.
Filtration	• Able to harvest microalgae cells of very low density. • High recovery efficiency.	• Filter medium easily clogged/fouled by algae cells. • The medium should be regularly cleaned. • Medium replacement and pumping are major contributors to its cost.
Sedimentation	• Very simple and low-cost method. • Requires less energy.	• Time-consuming due to slow sedimentation and possibly leads to deterioration. • Low algae paste concentration.
Flocculation	• Reliable and cost-effective. • Easy to conduct and very fast method.	• Chemical flocculants may be toxic to microalgae biomass.

	• Reusability of the media is limited.

Filtration

Filtration is a method of harvesting with the aid of porous media in which algae paste retains on the media while the water passes through. This conventional and competitive method of harvesting is sustainable for harvesting long length microalgae or those forming large colonies. It is also the preferable method to harvest algae cells of very low density (Show and Lee, 2014). The filter media can be categorised according to their pore size, viz. microfiltration (pore size of 0.1-10 μm), macrofiltration (pore size of >10 μm), ultrafiltration (pore size of 0.02-0.2 μm), and reverse osmosis (pore size of <0.001 μm) (Hattab et al., 2015). The main problem of this method, however, is the limitation on fluid flow volume, and clogging/fouling of the filter by deposited cells. The cost of filtration is mostly due to the frequent need to replace or clean the filter medium, leading to an increase in maintenance cost, and which may make it not cost-effective for small scale projects (Barros et al., 2015). Tabulation of these advantages and disadvantages are shown in *Table 3*.

Sedimentation

Sedimentation is a process of separating suspended solid, such as microalgae, from the liquid that is typically used in wastewater treatment plants (Shah et al., 2014; Show and Lee, 2014). In microalgae harvesting, suspended particles are separated from the culture gravitationally, the effectiveness of which depends on the density of the microalgae. Larger densities will result in faster sedimentation rate while the opposite would require a longer time to settle. Normally, flocculation is always used to increase the efficiency of gravity sedimentation (Hattab et al., 2015). As stated in *Table 3*, sedimentation is very straightforward, costs less, efficient and requires less energy. On the downside, time consumption due to slow sedimentation may lead to the deterioration of algae biomass.

Flocculation

Flocculation is done with the help of flocculants that cause the coagulation of microalgae cells into small clumps or formations, known as flocs (Shah et al., 2014; Zenouzi et al., 2013; Gramados et al., 2012; Harun et al., 2010). It serves as a preparatory step before other advanced harvesting methods such as sedimentation. As a harvesting method by itself, flocculation possesses certain advantages and disadvantages as well. It is easy to do and reliable in terms of cost. However, unsuitable chemical flocculants may be toxic to certain microalgae biomass, and reusability of the media is very limited as stated in *Table 3*. Two types of flocculation method are available, i.e. chemical flocculation and auto-flocculation. Auto-flocculation occurs due to the precipitation of algal cells, while chemical flocculation requires the addition of a chemical coagulant (inorganic or organic) to the microalgae culture (Hattab et al., 2015; Shah et al., 2014). Several studies have revealed that microalgae harvesting efficiency using chemical flocculants depend highly on three basic characteristics; pH, coagulant

dosage and coagulant type (Abdul Hamid et al., 2014; Huo et al., 2014; Liu et al., 2013; Surendhiran and Vijay, 2013; Udom et al., 2013; Granados et al., 2012; Marco et al., 2012; Harith et al., 2009). Since microalgae carry a negative surface charge, which prevents them from self-aggregation from the suspension, the coagulant is added to counter it. Flocculants normally used for harvesting include $A1_2(SO_4)_3$ (Aluminium sulphate), $FeC1_3$ (Ferric chloride) and $Fe_2(SO_4)_3$ (Ferric sulphate) (Harun et al., 2010). Organic chitosan flocculants have also been successfully applied in microalgae harvesting (Kurniawati et al., 2014; Rashid et al., 2013; Xu et al., 2013; Ahmad et al., 2011; Divakaran and Pillai, 2002).

pH sensitivity also affects the clumping ability of microalgae at the maximum efficiency. Flocculation of microalgae *Scenedesmus quadricauda* and *Chaetoceros muelleri* at pH 11.6 and 11.5 was observed to have the maximum flocculating activity of around 94.7% and 100%, respectively (Huo et al., 2014). Another microalgae species, *Nannochloropsis oculata* obtained from India was cultivated in Walnes's medium, and maximum flocculation was observed at 93.8% and 87.33% when $FeC1_3$ and $Fe_2(SO_4)_3$ were used at the concentration of 0.4 g/L and 0.6 g/L, respectively (Surendhiran and Vijay, 2013). In another study, an unspecified species of microalgae grown on wastewater was also harvested using flocculation (jar test) with the help of metal salts, a cationic polymer, anionic polymer and natural coagulant. Alum, ferric chloride, and cationic polymers were found to be able to achieve about 90% algal recovery at the optimal dosage (Udom et al., 2013). The green microalgae *Tetraselmis tetrahele* was also tested for use as a potential biodiesel feedstock. Two out of five flocculant types used, NOaH and $A_2(SO_4)_3$ showed the highest flocculating efficiencies at 96.15% and 98.65%, respectively, at the concentration of 200 mg/L (Marco et al., 2012).

Conclusion

This review presents existing cultivation systems and harvesting techniques for the development of biomass production from microalgae as a biofuel source, and for wastewater treatment. In terms of cultivation, both open and closed systems have their own pros and cons, though the selection of appropriate system depends on the application and end product desired. Though the closed system remains costly especially for large-scale projects, it is advantageous as the growth rate of algae can be fully controlled, and there is a lower risk of cross contamination from other microorganisms. For harvesting, it is apparent from past studies and industrial practices that flocculation is the preferred method due to its lower cost and higher harvesting efficiency. There is, however, still a need for better and more sustainable cultivation system and harvesting techniques to be developed, to further improve and enhance the productivity of microalgae biomass in the biotechnology industry.

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