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Review

Types of microalgae cultivation photobioreactors and production process of microalgal biodiesel as alternative fuel

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Abstract: The main purpose of this review is to evaluate the design of several photobioreactors (PBR) systems with the microalgae cultures and the quality of the microalgae species related to the production of lipid for biodiesel. In general, microalgae cultivation is divided into two systems: open pond system (unstirred, circular, raceway) and closed system (flat-panel, horizontal tube, helical tube, vertical tube, stirred tank, big bag), made by transparent and waterproof materials, and able to provide an ideal cultivation environment for photosynthetic microalgae. There are some issues to be considered in microalgae cultivation systems such as modelling by simulation, data collecting, mixing, illumination, gas exchange, availability of the nutritions and the cost of the system. Most common microalgae for PBRs and their lipid percentages as follows: *Chaetoceros muelleri* (25.0-63.0%), *Chlorella emersonii* (5.0-58.0%), *Chlorella vulgaris* (18.0-57.0%), *Chlorococuum* sp. (20.0-51.1), *Dunaliella primolecta* (10-71%), *Dunaliella tertiolecta* (17.5-67.0), *Nannochloropsis* sp. (20.0-56.0), *Neochloris oleoabundans* (29.0-65.0%), *Phaeodactylum tricornutum* (18.0-57.0%), *Scenedesmus obliquus* (11.0-55.0%), *Skeletonema costatum* (13.5-51.3%) (based on dry mass). The high lipid content of the microalgae is not only sufficient parameter, but also they should be resistant to harsh conditions, capable of rapid growth and easy to culture. Ultimately, this article focuses on applications in PBRs and gives an outlook for this field, aiming at microalgae cultivation and biodiesel production from microalgal lipids.

Keywords: Photobioreactors, microalgae, biodiesel, transesterification

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Introduction

Nowadays, microalgal culture has been acquiring special interest, because microalgae can fix atmospheric CO_2 promoting the mitigation of the greenhouse effect. The studies on Photobioreactors (PBRs) have been popular for obtaining efficient and renewable energy, especially biodiesel (Breuer et al., 2015). Microalgae production in PBRs is a good system for fuel technology which allows major productivity and high quality of biofuels (Bouallagui et al., 2013).

PBR systems are designed in two different types, open ponds, and closed systems. Open ponds are generally designed as circular or raceway. Water is often kept in motion by a shovel. Although it is cheap and easy to build, poor light usage, pollution, water evaporation, low biomass output per area, and plenty of water requirements are among the challenges. Some difficulties can be overcome by building the roof; however, this increases costs. Open ponds are used for all commercial microalgae production, but it is difficult to achieve high yields as temperature and light intensity vary throughout the day

and the year. In addition to their low efficiency, the production and collection of gas products are technically more difficult in open ponds. As an alternative to open pools, different closed PBRs have been developed for microalgae cultivation. They consist of helical (Hoshino et al., 1991), vertical, horizontal tubular (Pirt et al., 1983), inclined and horizontal thin panel PBR (Tredici et al., 1991). Closed systems are mostly constructed as tubular or flat-plate reactors. PBRs, including flat-plate systems, are more preferred in the world because of their large illuminated surface areas, but their heating problems and their tendency to form biofilms in interior walls are also known as disadvantages. On the other hand, tubular systems reduce these undesirable disadvantages and increase optimum light penetration and high efficiency. For this reason, long-lasting and easy-to-clean closed tubular glasses are very suitable for highly reproducible microalgae cultivation, leading to the highest possible growth rates.

Microalgae are a group of photosynthetic microorganisms commonly found in nature that grows autotrophic, heterotrophic or mixotrophic. Microalgae cultivation has two main purposes. One goal is to produce high-value products by using sunlight, CO_2, and nutrients to form lipids, proteins, carbohydrates, fatty acids, natural pigments, pharmaceutics, and other biochemical products. Another goal is related to environmental purification. Microalgal photosynthesis provides CO_2 fixation and nutrient assimilation. This makes microalgae cultivation not only for biomass production but also for CO_2 fixation and removal of pollutants. For these two reasons, the production of microalgae for biomass is of great importance for the environment (Han et al., 2017; Bahadar and Han, 2013; Zeng et al., 2015). The growing and proliferation of microalgae are linked to the photosynthesis process. The gathering of light depends on the source and the way it penetrates the system, as well as CO_2 fixation, depends on a mass transfer from the gas phase to the medium and the aeration system. CO_2 fixation and illumination should be well known in PBR systems for efficiency (Janssen et al., 2003; Molina et al., 1999).

In the current paper, the microalgae cultivation PBR systems, containing open ponds and closed systems are detailed presented, as well as the production of microalgal biodiesel as an alternative fuel has been stated with most significant points and considerations.

A- Open Pond Photobioreactor Systems

Microalgae cultivation in open ponds is known as the oldest and simplest system and works under the same conditions as the outdoor environment. The idea of an open pond first emerged in the 1950s and is still widely used in large-scale outdoor microalgal farming. Many different designs have emerged for open pond systems (Shen et al., 2009). Large shallow pools, canal pools, tanks, and circular pools are the most commonly used open systems. A trough pool consists essentially of a rectangular channel having a flow of microalgae culture flowing from a supply portion to an outlet portion. The most important parameter is the length, depth and width ratio in an open pond system. Extremely large a width leads to unwanted poor flow for mixing and mass transfer. This is undesirable in open ponds. Length and depth ratio, light penetration and the amount of culture volume the unit can hold are determined by mathematical calculations (Chisti, 2007).

In open ponds, pollution from microbes and other fast-growing heterotrophs can cause degradation of the culture and reduce the production of the microalgae. Inadequate mixing can result in low mass transfer and low biomass efficiency. Such disadvantages pose a challenge to the proliferation of the culture. In recent years, improvements in open culture system technology have been made to improve the mixing systems to avoid sedimentation and to increase the efficiency of light use (Chisti, 2007).

a- Unstirred open pond system

Most of the natural open pond systems do not have a stirred unit. Most of the unstirred open ponds consist of lakes, lagoons, and natural pools. Such systems provide an economical, simple and convenient way to operate and monitor microalgal processing. The natural pond is generally no deeper than half meters to allow light to penetrate water and be absorbed by microalgae cells (Figure 1c). The low depth allows light to penetrate every region in unstirred ponds. Sometimes, plastic films can be used by coating the water surface for better temperature control. The depth of these pools is not more than 50 deep cm. *Dunaliella salina* can be produced very easily in unstirred open systems (Vonshak and Richmond 1988; Han et al., 2017). On the other hand, unmixed open ponds are highly susceptible to competitive organisms that contain contaminants such as protozoa, microalgae, viruses and bacteria that can grow under poor conditions.

b- Circular open pond system

The idea of using a circular pond with a long swivel arm had been designed from the circular reactor for wastewater treatment. Therefore, a circular pond is similar to a wastewater treatment pond. *Chlorella sp*. generally used in circular open pond systems. These types of ponds are usually built 30-70 cm deep and 40-50 m in diameter (Figure 1b). The long rotating arm (pivoted agitator) is located in the middle of the pond, which acts as a clock dial and functions as a paddle-wheel familiar to that of a raceway pond (Figure 1b). It is clear that the mixing of culture medium and microalgae cells is more efficient than in an unmixed pond, but it should be known that as microalgae are exposed to the external environment, contamination is inevitable (Han et al., 2017, Lee, 2001). Circular ponds can also be combined with wastewater treatment and used for water and environmental cleaning. *Oscillatoria* was cultured in circular ponds using diluted wastewater, and the resulting biomass efficiency was determined to be about 15 gm^{-2} d^{-1} with a reduction of more than 80% ammonia and 50% total organic carbon in the wastewater. This shows that this alga has a significant proportion of wastewater treatment (Shen et al., 2009).

c- Raceway open pond system

There are some points to be considered while building the raceway open pond. Otherwise, microalgae production yield decreases due to the variations. The raceway pond can be placed on the ground or dug into the ground and covered with a wall to prevent liquid from penetrating the ground. The configurations of the channel pond may vary, including a single channel and channel groups. The raceway pond usually ranges from 15 cm to 25 cm in depth (Figure 1a). In raceway ponds, the length/width ratio is an important parameter, larger widths can cause a poor flow rate, and larger length can cause larger land use. Most of the raceway ponds consist of paddle-wheel, baffles, and channels. The paddle-wheels stimulate the flow of liquid, allow the microalgae cell to be suspended in the culture medium and prevent sedimentation. The baffles govern the flow direction and avoid the dead flow zone where the cells will settle. As such, microalgae cells will be mixed sufficiently and provide a continuous flow of sunlight and CO_2 from the atmosphere. While the fresh culture medium is easily added, high-density microalgae can be collected at an outlet (Tredici, 2004; Han et al., 2017).

A velocity of 10 to 20 cm s-1 is effective and also higher speeds are preferred, but velocity greater than 30 cm s^{-1} can consume a lot of energy to be applicable (Shen et al., 2009). Raceway ponds are the most widely used open systems for commercial microalgae culture due to relatively low construction and maintenance costs (Shen et al., 2009). The entire production process of the raceway pond is very efficient and convenient, compared to other open systems. Therefore, for large-scale microalgae cultivation, such ponds are the most preferred. *Chlorella, Spirulina, Dunaliella* and *Haematococcus* have been reported to be the most common microalgae species that can be cultivated in raceway ponds (Tredici, 2004; Han et al., 2017).

As a result, contamination of other microorganisms (unwanted microalgae species, bacteria, fungi or viruses) is the most urgent issue to be seriously addressed in open pond systems. In the future, several tasks are worthy of further investigation by scientists and industrialists, such as how to increase light penetration, reduce evaporation of water, and improve mixing in an open pond (Han et al., 2017). The efficiency ratio in open pond systems is theoretically less than expected. Because external environmental factors are difficult to be controlled and small types of microalgae can be successfully developed in open systems. To be successful in open pond systems, the depth of the pool, providing sufficient light to the microalgae cells, forming a sufficient water depth for the mixture, preventing evaporation, controlling the major changes in the ionic composition are necessary. In addition, the diffusion of CO_2 is one of the most important challenges. Adding CO_2 to open ponds does not seem inefficient and economical. It is also known that such systems require a larger area than closed systems. Table 1 shows the differences between open ponds systems and closed systems in detail.

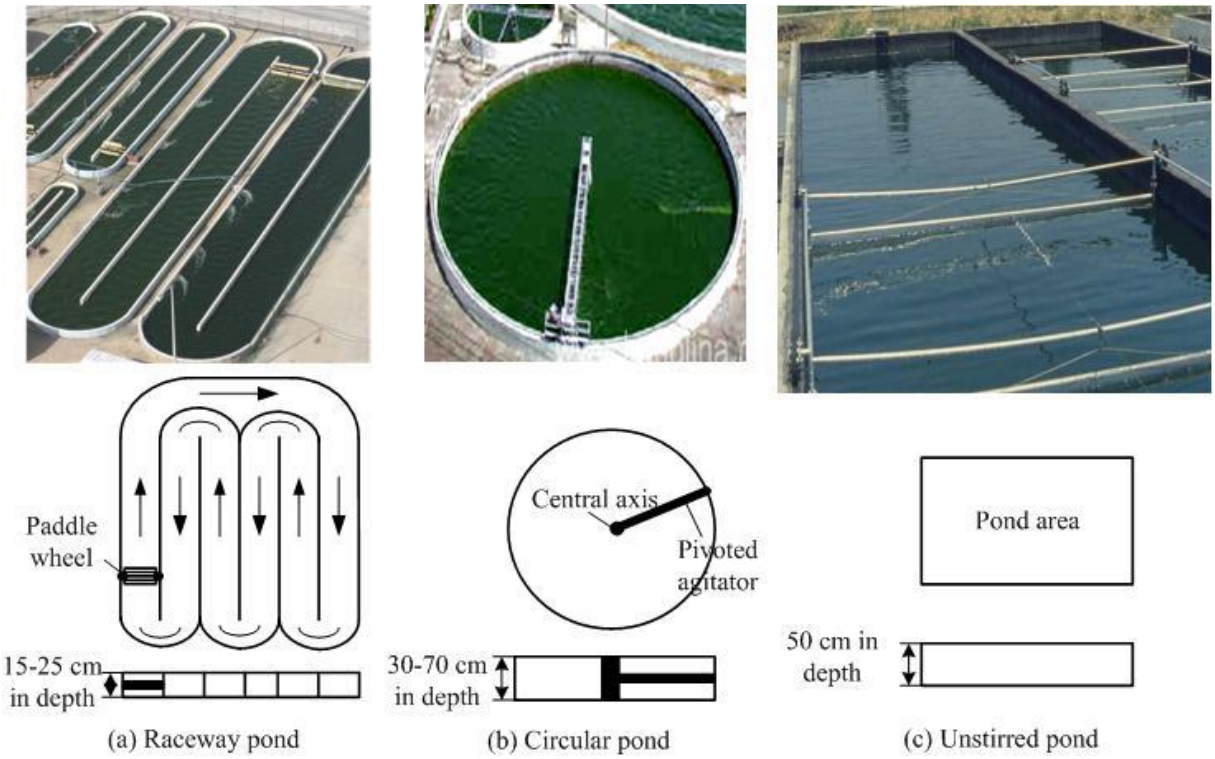

Figure 1. Three open pond systems (a and b: National Institute of Oceanography, Israel; c: University of Florence, Italy) (Shen et al., 2009).

B- Closed Photobioreactor Systems

Closed PBRs are mostly built indoors without direct exposure to the atmosphere. They are designed in a variety of types to maximize exposure of light, but the most preferred is the Flat-panel type (Figure 2). Closed PBRs are more suitable for large scale cultures that do not contain contaminants. The devices for closed systems are more expensive to build and maintain than open ponds and additional approaches such as airtightness, the system controlling, and mass transfer increase the cost; however, they may be the only option to obtain a large number of microalgal components. Furthermore, closed PBRs enable efficient control of culture variables such as pH, temperature, CO_2 concentration for fed of microalgae suspensions (Table 1) (Han et al., 2017; Xu et al., 2009; Behrens, 2005; Sanchez et al., 1999; Molina et al., 2001; Ugwu et al., 2002). No matter which photobioreactor is used, photosynthetic algae production is always accompanied by oxygen production and carbon dioxide intake. This fact causes changes in the culture medium and the pH is constantly changed. Oxygen levels above air saturation (0.225 mol O_2 m^{-3} at 20 °C, equivalent to 7.2 mg O_2 1^{-1}) can inhibit photosynthesis in many types of algae. In addition, high levels of oxygen combined with high levels of irradiation can lead to serious photo-oxidation, which reduces the yield of cultures. Therefore, an important aspect of the design of photobioreactors is to create combinations of mass transfer capacity and photosynthesis rate that do not allow oxygen to rise to inhibitory levels (Posten, 2009). The suitable Ph varies according to the type of algae used in the photobioreactor system because algae have different water environments and living conditions. Microalgae need CO_2 as a source of carbon; this should be at concentrations that do not limit their growth. Therefore, it has been suggested that partial CO_2 pressure should be higher than 0.2 kPa. Higher values might be necessary at high light intensities, or to support product formation (Yoo et al., 2010). The partial CO_2 pressure in the atmosphere is 0.04 kPa, which indicates that pure air is not sufficient for CO_2 supply. Therefore, an enriched gas mixture is required. Although flue gases can also be used, pure CO_2 is often used as the gas phase for carbon source and pH control. Pure carbon dioxide supply accounts for 30% of the total microalgae production cost (Acie´n et al., 2012).

Closed PBR is of great value in high value-added products such as biopharmaceuticals, cosmetics, human foods, and biofuels. Therefore, they have got many significant benefits for mankind. They can be in different designs. The closed PBRs generally include flat-panel, vertical tube, horizontal tube, stirred tank, big (plastic) bag and modified configurations (Han et al., 2017).

a- Flat-panel closed system

The flat panels are made of transparent or translucent materials such as glass, plexiglass or polycarbonate. They can be installed indoors exposed to artificial light sources or outdoors exposed to sunlight. The flat panels have a very short path of light which allows light to easily penetrate the culture fluid to increase the efficiency. Ordinary 16 mm thick plexiglass alveolar plates are used in the construction of flat-panel PBR due to their high surface/volume ratio (Figure 2f). The mixing is mainly driven by air bubbles produced from the air spreader. A pump is often used to circulate the microalgae cell suspension to use air bubbles from the air spreader. Furthermore, the movement of the culture, gas exchange and degassing is accomplished by passing air bubbles through the bottom of each channel (Han et al., 2017). It has been shown that high photosynthetic yields can be achieved with flat-plate PBR due to its large illumination surface area (Hu et al., 1998). The main advantages of flat-panels are that they are suitable for cleaning, having a high surface area to volume, not permitting dissolved oxygen accumulation, and flexibility (Han et al., 2017).

b- Horizontal tube closed system

Horizontal tubes PBRs are the reactors with the largest surface to volume ratio which are very useful for increasing the exposure of microalgae to light (Figure 2b, 3e). They consist essentially of tubes arranged in many possible orientations, such as horizontal, inclined, spiral, helicoid, and variations thereof (Figure 3). In addition to the arrangement of the tubes, tubular PBRs differ in tube length, flow rate, circulatory system, and a light receptor. The tubes can be designed in diameters from 10 mm to 60 mm and can even reach several hundred meters in length. Despite the largest surface-to-volume ratio and continuous culture treatment, the horizontal tube PBR has many limitations. One of these is the unbalanced mass transfer in the radial direction. This limitation is very easy to cause inhomogeneous temperature and CO_2 dispersions and leads to the accumulation of dissolved oxygen. Another limitation is that the system is difficult to scale outdoors because of the high land-use requirement and not economical in planting. Also, photo-inhibition caused by surface bioaccumulation can cause difficulty in cleaning the tubes. Nevertheless, the horizontal tube PBRs still have been chosen and have high performance (Sanchez Miron et al., 1999; Posten, 2009; Han et al., 2017).

c- Vertical tube closed system

Vertical tubular PBRs have large surface areas and are best suited for outdoor mass cultivation. These types of PBRs consist of transparent vertical tubes that allow light to enter the whole system (Figure 2c, 3a). Cultures are circulated in the system either with an air pump or with an airlift. There are two types of vertical tube PBR, known as bubble column and airlift. Both have an attached air spared at the bottom of the reactor. The reactor converts the spared gas into tiny bubbles to ensure microalgae cells suspension and to enhance the mass transfer. The bubble column reactor does not have an internal structure, so the fluid flow is driven by bubbles released at the bottom by the air spray. In a bubble column reactor, the gas flow rate, which is the only parameter to be considered during the process, can deeply affect the light and dark cycle. The main advantages of bubble column reactors are low cost, high surface area to volume ratio, simple configuration and satisfactory mass transfer (Sanchez Miron et al., 1999; Posten, 2009; Han et al., 2017).

d- Stirred tank closed system

Stirred tanks are conventional aerated PBRs (Figure 2e). Mixing is done by mechanical agitation. A mixed tank reactor inspired by the fermentation tank was invented for growing microalgae with an external light source such as fluorescent lamps or optical fibers. In a stirred tank reactor, agitation is carried out by the mechanical movement of the propeller-driven by the electric motor, so that the stirred tank reactor has the optimum heat and mass transfer and mixing. Stirred tanks PBRs contain a very effective mixing mechanism. This mixing allows the dark areas in the reactor to be reduced and to produce higher biomass. Therefore, in the system, mass transfer rates and light distribution are both higher and more efficient. The stirred tank has a really good performance in mixing, agitation, and indoor microalgae production, but it requires high energy consumption. However, the very low surface-area-to-volume ratio renders non-ideal light penetration, which significantly reduces the photosynthetic yield of the microalgae (Franco-Lara et al., 2006; Doran, 2013; Han et al., 2017).

e- Big (plastic) bag closed system

A bag closed system is carried out indoors or outdoors by a discrete method (Figure 2a). The large scale of the system limits the use of light. In this case, production may not be of the desired quality. The most widely used large-scale system is the big (plastic) bag system (nearly 1000 big bags). In these systems, large sterile plastic bags of approximately 0.5 m diameter are used. The main problem of the big bag system is the low photosynthesis due to the low light transmissions of artificial bags. Mostly the system preferred to be created indoors to keep the temperature under control. On the other hand, there is a greater need for labour in this system, as cultures are generally large-scale and there is an inadequate mix. All these factors increase the cost of microalgae production in bag systems (Yılmaz, 2006).

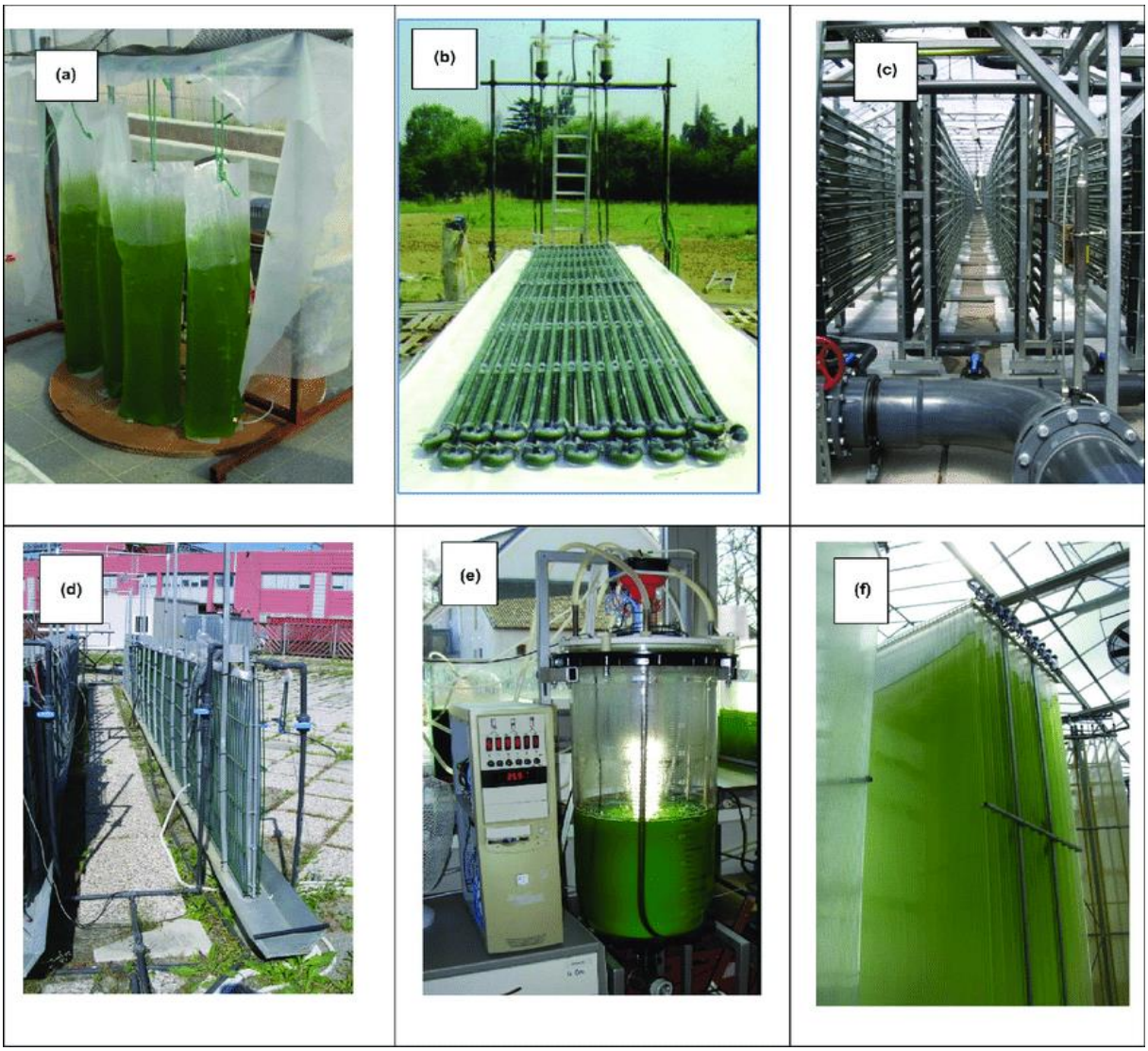

Figure 2. Design of closed PBRs (a) Big (Plastic) bags (Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Turkey); (b) Horizontal tubular (Institute for Ecosystem Study of the CNR, Florence, Italy); (c) Vertical tubular (Salata GmbH, Germany); (d) Vertical flat-panel (Institute for Ecosystem Study of the CNR, Florence, Italy); (e) Column photobioreactor (stirred tank) (Institute of Microbiology, Trebon, Czech Republic); (f) Flat-plate (Bruck a/L, Austria) (Masojidek and Torzillo, 2008).

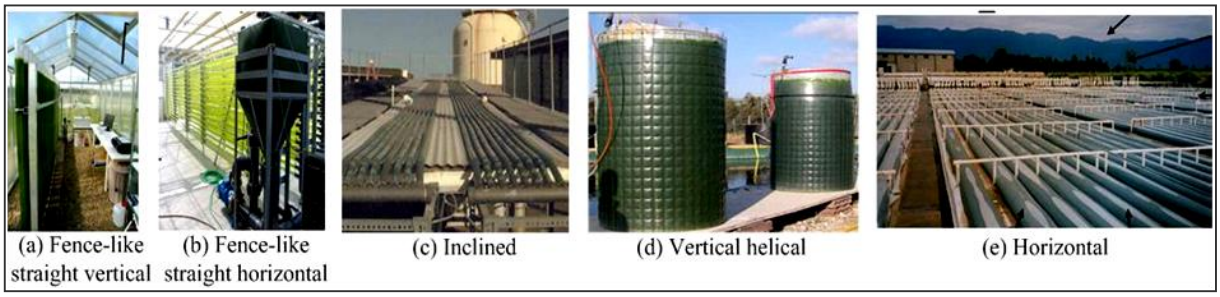

Figure 3. Different applications of tubular PBRs (a and b: Jacobs University in Germany; c, d, e: University of Florence in Italy) (Shen et al., 2009).

Table 1. Comparison of open pond system and closed systems (Adapted from Pulz, 2001).

Parameters	Open pond system	Closed system
Required space	High	Low
Water loss (evaporation)	Very high, may also cause salt precipitation	Low
CO_2-loss	High	Low
Oxygen concentration	Generally low enough due to continuous self-overflow	A build-up in the closed system requires gas exchange devices (excessive O_2 inhibit photosynthesis and cause photo-oxidative damage)
Temperature	Highly variable and hard to control	Cooling often required (by spraying water on PBR or immersing tubes in cooling baths)
Shear	Usually low (gentle mixing)	Usually high (fast and turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	Do not need much and easier	Required (wall-growth and dirt reduce light intensity), but causes abrasion, limiting PBR lifetime
Contamination risk	High (limiting the number of species that can be grown)	Low (Medium to Low)
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g/l	High, generally between 0.5 and 8 g/l
Production flexibility	Only a few species possible, difficult to switch	High, switching possible
Start-up	6 - 8 weeks	2 - 4 weeks
Operating costs	Low (for the paddle and CO_2 addition, biomass collection)	Higher (for CO_2 addition, oxygen removal, cooling, cleaning, maintenance)
Harvesting cost	High, species-dependent	Lower due to high biomass concentration and better control over species
Current commercial applications	5000 (8 to 10.000) t of algal biomass per year	Limited to processes for high added value compounds or algae used in food and cosmetics

C- Considerations for Photobioreactor Systems

There are some important points to consider for establishing effective and continuous PBR systems. The most desirable features of PBRs are economical, profitable, continuous and easy to control.

a- Collecting data and modelling of the system

Data measurement, monitoring, and designing models suitable for computer simulation are the most important factors for the installation of a PBR system. Simulated models and data can help to understand the real and expected microalgae growth with various parameters. Furthermore, on-site PBR design and optimization of operating conditions can be realized, which ensures the high efficiency of biomass production. For tubular reactors, installing high-precision sensors along the reactor axes can improve reactor performance by preventing restrictions or reducing energy demand by overfeeding (Fleck-Schneider et al., 2007; Zijffers et al., 2008; Matsudo et al., 2012).

b- Mixing and continuous mobility

Photosynthetic microalgae are very sensitive to mixing; some are movable or filamentous, which makes them very fragile and vulnerable to shear stresses. To make a good mixture, you need to get to know the features of microalgae. Mixing keeps the microalgae cells in suspension and ensures that the microalgae are distributed evenly in all regions and reduces thermal stratification, allows for even distribution of nutrients and prevents O_2 deposition. It is very important to completely mix the liquid to obtain a high cell concentration in PBR. Besides, the type of device used to mix and circulate the culture suspension is the key element in the design of successful PBR (Ugwu et al., 2008). Mixing reduces the degree of mutual shading and reduces the likelihood of photoinhibition. With proper mixing in the direction perpendicular to the flow (parallel to the path of light), mutual shading is significantly reduced, thereby increasing the space for cells exposed to light. Another important role of mixing is to position the microalgae to the light zone close to the lighting surfaces from the dark inner zones (Molina et al., 2001; Ugwu et al., 2008). It should be noted that over-mixing can damage microalgae cells and should be avoided (Lee and Palsson, 1994; Barbosa et al., 2003; Masojidek et al., 2003).

c- Light utilization and distribution

The use of light is a critical factor for the growth of microalgal cultures. Effective light supply to PBRs is the most important necessity to be considered in the design and construction of the reactor. Currently, the use of solar energy in open ponds seems to be the only way to commercially produce some cheap microalgae-derived products. Therefore, it is necessary to design a PBRs that can be easily illuminated by both solar and artificial light sources (Lee and Palsson, 1994). Generally, shallow or thinner culture media have higher cell density as the effects of self-shading are minimized, resulting in greater productivity. However, in dense cultures, the use of light is reduced as shading will be excessive. Because they have a larger surface area, flat-plate PBRs are generally more efficient than tubular PBRs in exploiting sunlight (Tredici and Zittelli, 1998). Therefore, the use of light can be effectively optimized by using flat transparent panel tubes in various configurations and by providing light with fiber optics and LEDs (Eriksen, 2008; Ugwu et al., 2008; Xue et al., 2013). As much as a light source, light distributions within the PBRs are significantly important in design. The light should be transmitted to the system to minimize photon loss, eliminate heat formation with the light source, and filter potentially harmful wavelengths. By using LEDs, all of these problems are bypassed. Internal lighting is the best way to minimize photon loss. Good light distribution maximizes systemic light using by minimizing mutual shading. It should not be ignored that any light that is not absorbed or used in photosynthesis will be converted into thermal energy (Lee and Palsson, 1994).

As it is known, the most important factor governing the efficiency of photosynthetic microorganism cultures is light availability, so photosynthesis ratio (PO_2) is a function of the irradiation to which algal cells are exposed. Irradiation is defined as the total amount of radiation that reaches a point in space from any direction, at any wavelength. Photosynthetic microorganisms can only benefit from photosynthetically active radiation in the 400-700 nm range. The rate of photosynthesis against irradiation response curves is measured under diluted conditions so that all cells are exposed to the irradiation provided. In addition, blocking of the light causes a great decrease in the rate of photosynthesis. The values of these irradiation constants are specific functions and can additionally change as a function of culture conditions. Compensation irradiance is in the range of 10-20 lE m^{-2} s^{-1} whereas the photosynthesis is saturated at irradiances from 100 to 500 lE m^{-2} s^{-1}. Photoinhibition is seen in rays above 1000 lE m^{-2} s^{-1} in most strains but may appear in low rays up to 300 lE m^{-2} s^{-1}. The effect of light on the rate of photosynthesis of a particular species should be examined in any case (Vejrazka et al., 2011). Whatever the model used to simulate the behaviour of the cells under the dynamic light in microalgae cultures, two scenarios are commonly considered. In full light integration, the photosynthesis rate is a function of time-averaged irradiance at which the cells are exposed to, according to the light regime defined by the optical conditions of the culture and cell movement. According to this, the frequency of light exposition must be high, up to values of 10-100 Hz (Brindley et al., 2011; Vejrazka et al., 2012). In local light use (usually open ponds), no integration of light is performed and photosynthesis rate is a function of local conditions at which the cells are exposed to in each time, overall photosynthesis rate being the results of integrating the photosynthesis rate in each position. The existence of dark zones in which the cells are performing respiration instead of photosynthesis reduces the yield of the cultures. This is relevant in the design of

photobioreactors for microalgae production because a photobioreactor necessarily contains an illuminated outer zone and a darker core. The movement of fluid between the illuminated zone and the dark interior unavoidably subject the cells to fluctuating illumination, but the higher the frequency of movement the higher the yield of the culture (Brindley et al., 2011).

d- Gas inflow and outflow from the system

Gas input and output is an important problem in PBR systems. CO_2 must be supplied and the produced O_2 must be released from the systems. CO_2 is used as a carbon source in photosynthesis by microalgae. Depending on the type of microalgae, CO_2 can be taken from the water as dissolved (HCO_3^- or CO_3^-) or undissolved (CO_2 or H_2CO_3). In both cases, the CO_2 should be dissolved in the culture broth. Oxygen is a product that inhibits the growth of microalgae and inhibits photosynthesis and causes photo-oxidative damage (Table 1). Therefore, to prevent oxygen from reaching the inhibitory level, the produced oxygen must be removed (Lee and Palsson, 1994).

e- Procurement of culture nutritions

The PBR systems should generally be saturated with fresh feeds such as nitrogen, phosphorus, and inorganic salts. Keeping all culture components in balance with growth and photosynthetic requirements is one of the important parameters in PBR systems. Online completion of media (supply of media components and removal of potentially harmful secondary metabolites) may be achieved by dialysis or ultrafiltration. Sometimes different sources of nutrients can be considered, for the easy and inexpensive supply of microalgae. For example, in open pond systems, wastewater sources can be used in PBR as a nutrition source for algae. Ultrafiltration is often preferred because it is both faster and has less impact on gas measurement (Lee and Palsson, 1994). It should be noted that microalgae should be reproduced in environments close to their natural habitats. Therefore, for some marine microalgae, seawater or high salinity water should be used.

f- Cost and economy of the system

The cost of PBR has a significant impact on the cost of production for large-scale biomass. The most important way to reduce cost is to determine the right microalgae strain, to establish an efficient PBR system and to determine the correct production technology of the biomass to be used. The major cost requirements for PBR include mixing system, culture medium detection, irradiation conditions, and photosynthetic efficiency of microalgae. The most effective effort in reducing PBR costs is to reduce the consumption of raw materials. CO_2 is the most expensive consumed product in biomass production. The utilizing of flue gases from industrial sources reduces CO_2 costs as close to zero (Acie et al., 2012). Wastewater can be used to reduce the price of mineral supply.

Recent research has focused on the production potential of microalgae and the amount of land needed to replace 50% of US transport fuels. Besides, various oil content levels of these species were examined by looking at microalgae strains production capacity. The scale of the area required to produce 100,000 kg of microalgal biomass was measured. Both efficient production paths and how to design the PBR production facility were discussed. The microalgal biomass is estimated to be US $ 2.95 per kg (PBR) and the US $ 3.80 per kg for the channel. These estimates are presumed to assume that CO_2 is free (Chisti, 2007).

g- Assessment of wastewater in cultivation

Industrial and agricultural wastewaters have a high concentration of nitrogen (N) and phosphorus (P) compared to natural water sources. In Europe, on average, 0.51 kg Phosphate and 2.52 kg Nitrogen per inhabitant and year are discharged in wastewater. P and N containing nutrients are valuable resources that can substitute expensive fertilizers for the production of crops or algae. Agricultural and livestock farms are using aquaculture systems (lagoons, aerobic, anaerobic, optional or mature ponds, constructed wetlands) designed to achieve specific wastewater treatment and to solve environmental and sanitary problems, while being economically efficient. Algal systems have been used traditionally as a potential secondary treatment system or as a tertiary process for removing all organic ions, biologically or chemically. A suitable algae presence in wastewater treatment systems is

determined by nutrients, temperatures, and sunlight. Contrary to standard biological treatments, algae improve the final effluent quality through natural disinfection and incorporation of contaminants like heavy metals, pharmaceuticals, and endocrine disrupters. Algae treatment systems are effective for removing coliform bacteria from wastewater, such as Salmonella, Shigella, viruses, and protozoa. Significant removal of coliforms can be achieved in stabilization ponds (a reduction of 88.8% in 11.4 days and even 99%). Chlorella vulgaris achieved a nutrient elimination efficiency of 86% for inorganic N and 70% for inorganic P. More than 1000 algae taxonomies have been reported in the literature as being tolerant of pollution, including 240 genres, 725 species, 125 varieties, and forms (Ungureanu et al., 2019; Abdel-Raouf et al., 2012).

D- Types of Microalgae Used in Photobioreactor Systems

Algae are organisms ranged from unicellular microalgae (3 -10μ) to multicellular forms, such as the giant kelp, large brown algae that may grow up to 50 m in length. Most are aquatic and autotrophic and lack many of the distinct cell and tissue types, such as stomata, xylem, and phloem, which are found in land plants. The largest and most complex marine algae are called seaweeds, while the most complex freshwater forms are the Charophyta. Microalgae are known as simple, chlorophyll-containing, single-celled, multi-celled or colony-forming prokaryotic or eukaryotic photosynthetic microorganisms. It is stated that there are more than 50,000 species in the world, but only about 30,000 microalgae have been studied (Mata et al., 2010).

There are different metabolic activities in microalgae; (1) mixotrophic, algae performing photosynthesis as the main energy source, but they need both organic compounds and CO_2; (2) autotrophic, algae using light as the sole energy source, converting light energy into chemical energy using CO_2 by photosynthesis; (3) heterotrophic, algae using only organic compounds as energy and carbon source; (4) photoheterotrophic, algae using light and using organic compounds as carbon source for photosynthesis (Chojnacka et al., 2004; El-Sheekh and Abomohra, 2016).

Features of the microalgae should be considered in the selection of the most suitable species or strain for biodiesel production. The proportions and contents of the fatty acids of the species have a significant effect on the quality of the biodiesel. Some microalgae species can be stimulated to accumulate a high amount of lipids (Molina et al., 2003). This contributes to a high lipid yield. The average lipid content of microalgae ranges from 1 to 70%, but under some conditions, some species contain up to 90% of their dry weight by lipid (Parker et al., 1967; Medina et al., 1998; Mansour et al., 1999). Lipid content in *Botryococcus braunii* can reach 75% by weight of dry biomass but has got low productivity. That is, besides the high accumulation of lipids, the rapid growth of the microalgae is also very important. The most common microalgae (*Isochrysis, Chlorella, Nannochloropsis, Crypthecodinium, Schizochytrium, Cylindrotheca, Nannochloris, Neochloris, Nitzschia, Phaeodactylum, Dunaliella, Chlorella* and *Porphyridium*) have lipid contents between 20 and 50% (Parker et al., 1967).

Unlike higher plants, microalgae show greater variation in fatty acid composition. Some microalgae are capable of synthesizing medium-chain fatty acids (e.g., C8, C10, C12, and C14), some others are capable of synthesizing long-chain fatty acids (C18, C20, C22). For example, the predominantly C10 fatty acid in the filamentous cyanobacteria *Trichodesmium erythraeum* accounts for 27-50% of the total fatty acids (Parker et al., 1967). In another example, the long-chain fatty acid docosahexaenoic acid (C22:6n-3) constitutes 24% of the total fatty acids of the microalgae of *Gymnodinium sanguineum* (Mansour et al., 1999; El-Sheekh and Abomohra, 2016). The lipid amounts of many other microalgae species are shown in Table 2. *Chlorella ellipsoidea* and *Botryococcus braunii* seem to be a suitable microalgal species for biodiesel production (Table 2). However, even if some other species are as suitable and productive as *Chlorella ellipsoidea* and *Botryococcus braunii*, in determining the most suitable species for biodiesel production, some factors such as identifying the most suitable environments in which microalgae can develop by using the right culture nutrients should be taken into consideration (Mata et al., 2010; El-Sheekh and Abomohra, 2016).

Table 2. Total Lipid content and productivities of different microalgal species cultivated in different cultivation conditions (based on % dry weight) (Xiao et al., 2013)

Types of algae	Lipid content (% dry cell weight)	Carbohydrate/ protein (wt%)	Lipid productivity (mg/l/day)[a]	Volumetric productivity of biomass (g/L/day)	Cultivation condition
Ankistrodesmus sp.	24.0-31.0	25/43	12.4	0.29	P
Botryococcus braunii	33.6	18.9/17.8	5.5	0.03	P
Chaetoceros muelleri	25.0-63.0	19.3/46.9	21.8	0.07	P
Chaetoceros calcitrans	19.0-22.0	-	17.6	0.04	P
Chlorella emersonii	5.0-58.0	26/44	10.3-50.0	0.036-0.041	P
Chlorlla protothecoides	10.0-48.0	8.70/41.60	0.2-5.4	2.00-7.70	P
Chlorlla protothecoides	43.0-46.0	-	1881.3-1840.0	0.01	H
Chlorella sorokinian	2.0	-	44.7	0.23-1.47	P
Chlorella vulgaris	18.0-57.0	18/22	11.2-66.3	0.02-0.20	P
Chlorella vulgaris	23.0-36.0	-	27.0-35.0	0.08-0.15	H
Chlorella vulgaris	21.0-34.0	-	22.0-54.0	0.09-0.25	M
Chlorela sp.	19.3	19/48	42.1	21.6-34.0	P
Chlorell pyrenoidosa	11.76	-	34.8	2.90-3.64	P
Chlorococuum sp.	20.0-51.1	22/41	53.7	0.28	P
Crythecodinium cohnii	6.0-45.0	-	28.0	0.20	P
Dunaliella salina	23.1	32/57	116.0	0.20-0.34	P
Dunaliella primolecta	10-71	19/48	36.4	0.09	P
Dunaliella tertiolecta	17.5-67.0	-	60.6-69.8	0.12	P
Dunaliella sp.	27.4	5/47	33.5	0.20	P
Elliposodiun sp.	14.0-20.0	20/46	47.3	0.17	P
Euglena gracilis	25.0	18/39	32.4	7.70	P
Haematcocuus pluvialis	13.58	18/34	-	0.05-0.06	P
Isochrysis galbana	7.0-40.0	26.8/47.9	-	0.32-1.60	P
Isochrysis sp.	7.1-33	12.9/50.8	37.8	0.08-0.17	P
Monodus subterraneus	16.0	22/33	30.4	0.19	P
Monalantus salina	20.0-22.0	17/49	-	0.08	P
Nannochloropsis sp.	20.0-56.0	19.81/32.82	84.0-142.0	0.37-0.48	P
Nannochloropsis oculata	22.7-29.7	23/44	37.6-90.0	0.17-1.43	P
Nannochloropsis sp.	12.0-53.0	28/48	90.0-134.0	0.18	P
Neochloris oleoabundans	29.0-65.0	9.2/16.8	-	0.20	P
Nitzschia sp.	16.0-47.0	26/48	-	0.24	P
Ocystis pusilla	10.5	19/44	49.4	0.16	P
Pavlova salina	30.9	28/42	31.2	0.14	P
Pavalova lutheri	35.5	28/42	40.2	0.18	P
Phaeodactylum tricornutum	18.0-57.0	28/42	29.2	0.14	P
Porphyridium cruentum	10.37	28/42	40.2	0.13	P
Scenedesmus obliquus	11.0-55.0	15/50	7.14	0.04-0.74	P
Scenedesmus obliquus	6.6-11.8	-	11.6-58.6	0.10-0.51	M
Scenedesmus quadricauda	1.9-18.4	14/47	35.1	0.19	P
Scenedesmus sp.	19.6-21.1	21/18	40.8-53.9	0.03-0.26	P
Skeletonema sp.	13.3-31.8	22/38	27.3	0.09	P
Skeletonema costatum	13.5-51.3	22/38	17.4	0.08	P
Spirulina platensis	4.0-16.6	14/26	14.2	0.06-4.3	P
Spirulina maxima	4.0-9.0	13/46	21.0	0.21-0.25	P
Tetraselmis suecica	12.78	33/57	27.0-36.4	0.12-0.32	P
Tetraselmis sp.	8.5-23.0	28/50	43.4	0.30	P
Thalassiosira pseudonana	12.6-14.7	12/44	17.4	0.08	P

The abbreviation: P is phototrophic, M is mixotrophic, and H is heterotrophic

There are several cultivation modes of microalgae, which include the photoautotrophic, photoheterotrophic, mixotrophic and heterotrophic modes. Photoautotrophic microalgae are the most commonly cultivated microalgae and utilize sunlight as their main energy source and atmospheric CO_2 as a carbon source. Nevertheless, other cultivation modes such as mixotrophic and heterotrophic microalgae are known to grow more rapidly with higher cellular oil content as compared to photoautotrophic cells, making them more promising as a biofuel feedstock. This benefit is compromised though with the higher requirement of organic carbon sources like glucose or acetate for their growth, which leads to an increase in the medium costs by up to 80%. Under growth-limiting conditions (such as nitrogen starvation), oleaginous algae switch their lipid synthesis pathway to accumulate TAGs (triacylglycerols). These TAGs cannot be used for synthesizing membranes; hence, they are stored as lipid molecules. In general terms, through the transesterification process, algal oil is converted into biodiesel. Extracted oil from the algae is mixed with alcohol and acid (or a base), which further produces the fatty acids methyl esters that makeup biodiesel, bioethanol, and biomethane (Mitra et al., 2012; Pal et al., 2019).

The cultivation of oleaginous microalgae species *Pseudochlorococcum* sp. in an attached biofilm favoured accumulating on the hydrophilic substrate than on hydrophobic substrate. In a study, it is reported, the use of chitosan and tannin as an algae flocculant that focuses on harvesting halophilic strains. The cost of the harvesting and dewatering process is significantly high for industrial-scale production. This calls for the need to develop energy-efficient techniques that will help to mitigate the energy- and cost-related problems. The flocculation of *Scenedesmus obtusiusculus* microalgae in a salt-based medium was investigated. Chitosan and tannin were added as algae flocculent and the process was induced by a pH shift. It was discovered that induction by pH shift is more cost-effective, but a large amount of the base is required to raise the pH due to the buffering effect of the saline cultivation medium. The tannin appears to be favourable for culturing the microalgae when compared to chitosan in the absence as well as in the presence of algae organic matter. The cultivation time did noticeably affect the flocculation efficiencies of tannin and other parameters like algae zeta potential and bacterial communities' presence remained stable when tannin was used as a flocculant. Possible efforts were studied for the production of cheap organic substrates for reducing the microalgae production cost without compromising the nutritional qualities of oleaginous microalgae. The use of cassava starch hydrolysate and corn powder hydrolysate has been reported to be useful as glucose substitutes for improving the lipid yields of microalgae *Chlorella protothecoides*. The heterotrophic growth of *C. vulgaris* was scaled up to a 6l stirred bioreactor and subjected to ultrasonication and solvent extraction treatments to recover intracellular oil from dried biomass. Depending on the growth media characteristics, the oil content from thin stillage was 43%; from soy whey, it was 11%; and from the modified basal medium, it was 27% (w/w). The oil produced from *C. vulgaris* grown on both thin stillage and MBM medium contains higher contents of linoleic and linolenic acids (Mitra et al., 2012; Pal et al., 2019).

Even though, the lipid composition of microalgae is roughly similar to the plants; there are significant differences in overall biomass productivity and biodiesel efficiency with a clear advantage for the resulting oil yield and biodiesel. Above all, in terms of less land use, high biomass productivity and high lipid production, microalgae production systems as PBRs are more advantageous than another oily plant harvesting (Table 3).

E- Microalgal filtration and harvesting systems

The high operational costs associated with microalgal harvesting are a major challenge due to the very dilute nature of the microalgal suspension and their small cell size. An optimal harvesting method for microalgae should be independent of the microalgal species being cultivated, and also should have a low chemical and energy demand. Centrifuge and belt filter are commonly used microalgal dewatering systems. The primary difference between a centrifuge and the belt filter system is the principle of separation. A centrifuge applies centrifugal forces to the solution to aid the separation of solid and liquid. For a belt filter system, the principle of separation is gravity drainage followed by compression shear.

Centrifugation is a highly effective method for harvesting microalgae but it has a high energy demand and is expensive. Compared to a centrifuge, belt filter system has lower energy consumption and operational costs have a continuous mode of operation and can be up-scaled. However, microalgal suspension with a concentration of 10-40 g dry wt/L is needed prior to dewatering on a belt filter (Sturm and Lamer, 2011). To further investigate this,

microalgal suspensions with feed concentrations of 4 g dry wt/L and 6 g dry wt/L were produced. A prototype belt filter dewatering system consisting of a filter section followed by two drying sections was designed and developed. A fine blade was installed at the end of the drying section to scrape off the dried algal cake. Air drying was the chosen drying method, due to its low energy and cost requirements. The design was based on filtration tests conducted on 50 g dry wt/L microalgal suspension. The prototype is a 1% scale of a system proposed to process 60.000 gallons of 50 g dry wt/L microalgal solution per day. The difference between a standard belt filter system and the prototype belt filter dewatering system developed is the dewatering mechanism. For a standard belt filter press, the principle dewatering mechanism is gravity drainage followed by compression shear. The principle dewatering mechanism of the prototype belt filter dewatering system is gravity drainage. Another system developed based on the belt filter gravity drainage dewatering mechanism is Salsnes Water to Algae Treatment (SWAT) technology. But, there are some differences between SWAT technology and the prototype belt filter dewatering system. Firstly, the filter section of the SWAT technology is enclosed in a chamber. Secondly, the belt movement in the filter sections of the prototype belt filter dewatering system and the SWAT technology are in opposite directions. Lastly, there is no drying unit in SWAT technology (Sandip et al., 2015).

Table 3. Conventional biodiesel sources and their capacities compared with oily microalgae (Mata et al., 2010).

Biodiesel sources	Oil content	Oil yield	Land need	Productivity
Microalgae (low lipid content)	30	58.700	0.2	51.927
Microalgae (medium lipid content)	50	97.800	0.1	86.515
Microalgae (high lipid content)	70	136.900	0.1	121.104
Corn/Maize (*Zea mays*)	44	172	66	152
Hemp (*Cannabis sativa*)	33	363	31	321
Soybean (*Glycine max*)	18	636	18	562
Jatropha (*Jatropha curcas*)	28	741	15	656
Camelina (*Camelina sativa*)	42	915	12	809
Canola/Rapeseed (*Brassica napus*)	41	974	12	862
Sunflower (*Helianthus annuus*)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747

F- Biodiesel Production from Microalgal Lipids

Although microalgae do not appear to be significantly different from other biodiesel raw materials, they are just small vulnerable photosynthetic microorganisms, for this reason, special harvesting, cultivation, and processing techniques are to be considered for the efficient production of biodiesel. The processes necessary for the production of biodiesel from microalgae consist of growing and removing microalgae from the culture and extracting of the lipids. Then, biodiesel is produced by using the same methods and technologies (Mata et al. 2010). Figure 4 shows a schematic representation of microalgae biodiesel production stages starting from the design and implementation of a cultivation system for microalgae growth and determining of microalgae species based on local specific conditions. It is then followed by biomass harvesting, processing, and lipid extraction to supply to the biodiesel production unit (Mata et al., 2010). Sometimes the harvesting method may involve several steps to separate large amounts of water from the biomass and obtain a high proportion of raw materials (Frac et al., 2010; Mata et al., 2010).

In general, conventional harvesting methods include centrifugation, flocculation, sedimentation, ultrafiltration,

and filtration. The selection of the appropriate harvesting method depends on the microalgae species, the growth medium of the desired end product and the production cost. Filtration by pressure or vacuum can be used to harvest large quantities of biomass, but for large scale production, filtration may be relatively slow and consequently insufficient (Mata et al., 2010). Furthermore, filtration is more suitable for large, filamentous microalgae such as *Arthrospira platensis* (*Spirulina platensis*); however, it is not very advantageous with smaller organisms such as *Dunaliella* and *Chlorella* (Molina et al., 2003). It is recommended that centrifugation be used for high-value products such as the recovery of high-quality microalgae for food or aquaculture. An important parameter for selecting an appropriate harvesting procedure is the potential of the harvesting method to adjust the density or acceptable moisture level in the resulting biomass (Mata et al., 2010). Besides, dewatering should be performed quickly after the separation of the microalgal biomass to prevent degradation of the biomass.

Extracted microalgal lipids mostly contain TAGs (Triacylglycerols), which are used extensively in the transesterification reaction for biodiesel. The essential condition during extraction is that lipids must be released and removed without significant contamination by other cellular components such as DNA or chlorophyll (Scott et al., 2010). The lipid extraction process should be more selective to biodiesel precursor acylglycerols than polar lipids and non-acylglycerol neutral lipids, including free fatty acids, hydrocarbons, sterols, ketones, carotenes, and chlorophylls that cannot be converted to biodiesel (Medina et al., 1998). Extraction can generally be divided into two methods: mechanical methods with expeller press and ultrasonic-assisted extraction, and chemical methods with organic solvent extraction and supercritical fluid extraction. There are drawbacks for each of these methods; the mechanical press requires drying of energy-consuming microalgae in general, the use of ultrasonic is an effective method for small-scale extraction, supercritical extraction requires high-pressure equipment that is energy-consuming and expensive. Although the use of chemical solvents is considered to be unsafe for safety and health issues, most microalgae oil manufacturers use chemical solvents to remove high levels of oil (Mata et al., 2010; El-Sheekh and Abomohra, 2016).

Transesterification is an esterification reaction used for biodiesel production. Biodiesel is a fatty acid methyl ester mixture obtained by the transesterification of lipids (Mata et al., 2010). Microalgal lipids consist of 90-98% by weight of triglycerides and small amounts of diglycerides, monoglycerides, free fatty acids and small amounts of phospholipids, tocopherols, sulfur compounds and water traces (Bozbas, 2008). Transesterification is a three-reversible step reaction in which triglycerides are first converted to diglycerides, then diglycerides to monoglycerides, and finally monoglycerides to esters (biodiesel) and glycerol (by-products) (Figure 5). Briefly, the reaction is also known as the glycerides present in the lipids reacting with an alcohol (usually methanol) in the presence of a catalyst (such as NaOH) to form methyl esters (Figure 5). To form ester and glycerol, the catalyst used in the reaction increases the speed and efficiency of the reaction. Because the transesterification reaction is reversible, excessive alcohol present in the medium can convert the reaction in favour of the forward direction. Theoretically, although the oil to alcohol ratio is known to be 3:1 mole, the 6:1 mole ratio is more preferred for the efficiency and cost of the reaction (Mata et al., 2010; El-Sheekh and Abomohra, 2016).

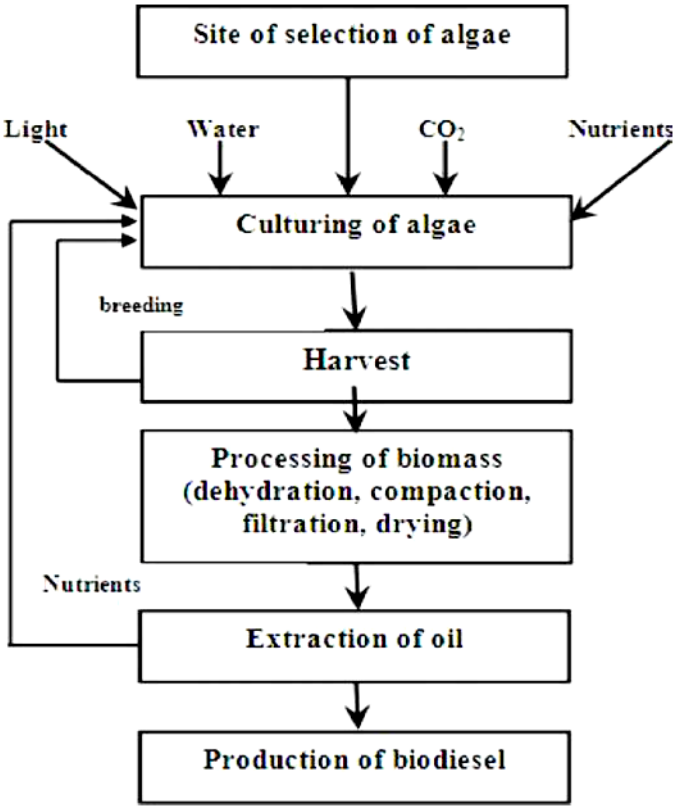

Figure 4. Schematic representation of biodiesel production steps for microalgal biodiesel (Frac et al., 2010).

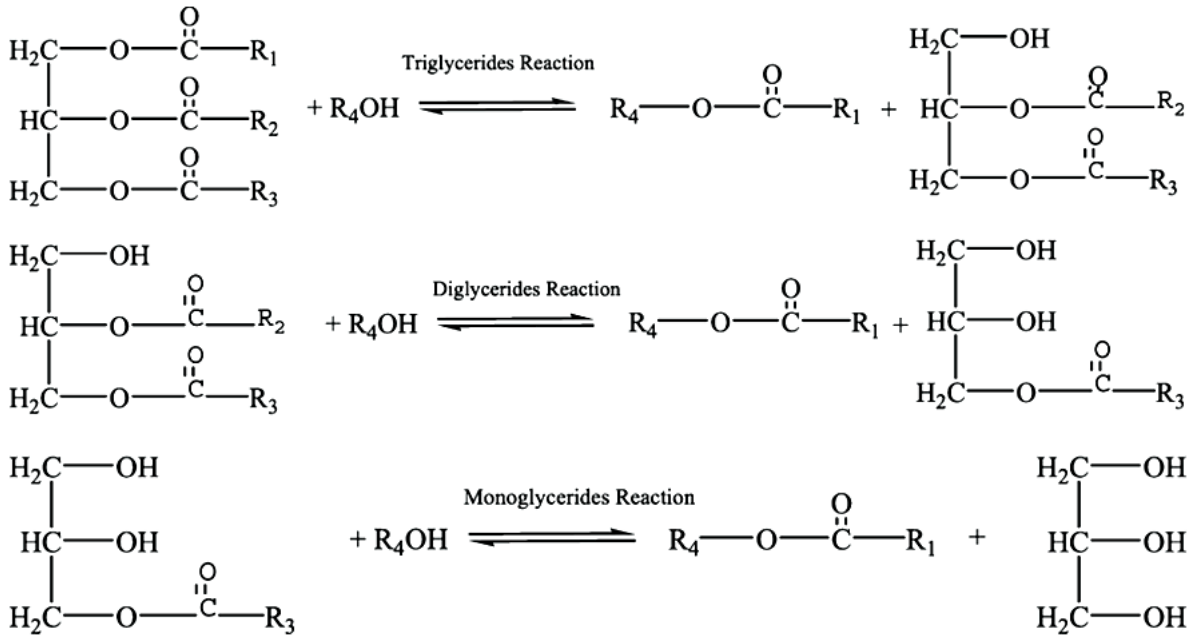

Figure 5. Transesterification reaction of biodiesel production from triglycerides, diglycerides and monoglycerides (The reaction consists of 3 stages) (Yin et al., 2012).

Discussion and Conclusion

For decades, oily seed crops such as canola, sunflower, soybean, palm and safflower have been used for biodiesel production. In recent years, however, biodiesel production from microalgae has become very popular due to the high amount of lipid content. As a result of detailed studies, it is determined that the growth rates of microalgae were higher compared to agricultural products and other aquatic plants and they are more resistant to changing environmental conditions. Many research reports mentioned the advantages of using microalgae for biodiesel production in comparison with other available feedstocks. It can be concluded as the following points:

- Microalgae complete the entire growth cycle every few days by converting solar energy into chemical energy in the photosynthesis process. Compared to other raw materials, microalgae have higher growth rates and productivity and requiring much less land area (Chisti, 2007).
- Microalgae are easy to grow, as they can grow using wastewater or seawater unsuitable for human use (Mata et al., 2010).
- Microalgae can produce different types of renewable fuels such as biodiesel, biomethane, hydrogen, and bioethanol (Pratoomyot et al., 2005).
- Because of their high-value biological derivatives, they can potentially revolutionize many biotechnology areas, including nutrition and food additives, pharmaceuticals, biofuels, cosmetics, and pollution prevention, with many possible commercial applications.
- Their growth rates and lipid content can be accelerated by adding or removing certain nutrients and can grow almost everywhere. Due to their low nutritional requirements, ability to grow under difficult conditions and not being overly affected by seasonal weather changes, they can be grown in areas unsuitable for agricultural purposes, so that they cannot compete for arable land use (Aslan and Kapdan, 2006; Mata et al., 2010).
- They remove CO_2 from industrial flue gases by bio-fixation, reducing the greenhouse gases (GHG) emissions (Wang et al., 2008).
- Microalgae can treat wastewater by removing NH_4, NO_3, PO_4 and can be grown using these water contaminants as nutrients (Wang et al., 2008).
- Some other valuable compounds such as oils, polyunsaturated fatty acids, natural dyes, sugars, pigments, carotenoids, antioxidants, high-value bioactive compounds, and chemicals may also be obtained by microalgae (Wang et al., 2008; Mata et al., 2010; El-Sheekh and Abomohra, 2016).

▪ Microalgae biomass may be processed as animal feed, or easily burned for energy cogeneration as well as used as organic fertilizer due to its high N:P ratio (Wang et al, 2008; Mata et al., 2010).

Conclusively, the new favourite of green capitalism seems to be microalgae that produce half of the oxygen in the atmosphere as well as to be the best way for our fuel crisis. Biodiesel production from microalgae is not technically very difficult. It is considered to be one of the main renewable sources and may completely displace oil-derived liquid fuels in the future. Since the serious negative consequences of the agricultural product-based biofuels debated, which has been propagated for years as an alternative to fossil fuels, microalgae studies have gained considerable importance.

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