Third generation biofuels from microalgae

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Biofuel production from renewable sources is widely considered to be one of the most sustainable alternatives to petroleum sourced fuels and a viable means for environmental and economic sustainability. Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO_2 fixation ability and high production capacity of lipids; they also do not compete with food or feed crops, and can be produced on non-arable land. Microalgae have broad bioenergy potential as they can be used to produce liquid transportation and heating fuels, such as biodiesel and bioethanol. In this review we present an overview about microalgae use for biodiesel and bioethanol production, including their cultivation, harvesting, and processing. The most used microalgal species for these purposes as well as the main microalgal cultivation systems (photobioreactors and open ponds) will also be discussed.

Keywords Microalgae; Biofuels; Biodiesel; Bioethanol; Global warming

1. Introduction

Concerns about shortage of fossil fuels, increasing crude oil price, energy security and accelerated global warming have led to growing worldwide interests in renewable energy sources such as biofuels. An increasing number of developed and rapidly developing nations see biofuels as a key to reducing reliance on foreign oil, lowering emissions of greenhouse gases (GHG), mainly carbon dioxide (CO_2) and methane (CH_4), and meeting rural development goals [1].

Biofuels are referred to solid, liquid or gaseous fuels derived from organic matter. They are generally divided into primary and secondary biofuels (Fig. 1). While primary biofuels such as fuelwood are used in an unprocessed form primarily for heating, cooking or electricity production, secondary biofuels such as bioethanol and biodiesel are produced by processing biomass and are able to be used in vehicles and various industrial processes. The secondary biofuels can be categorized into three generations: first, second and third generation biofuels on the basis of different parameters, such as the type of processing technology, type of feedstock or their level of development [2].

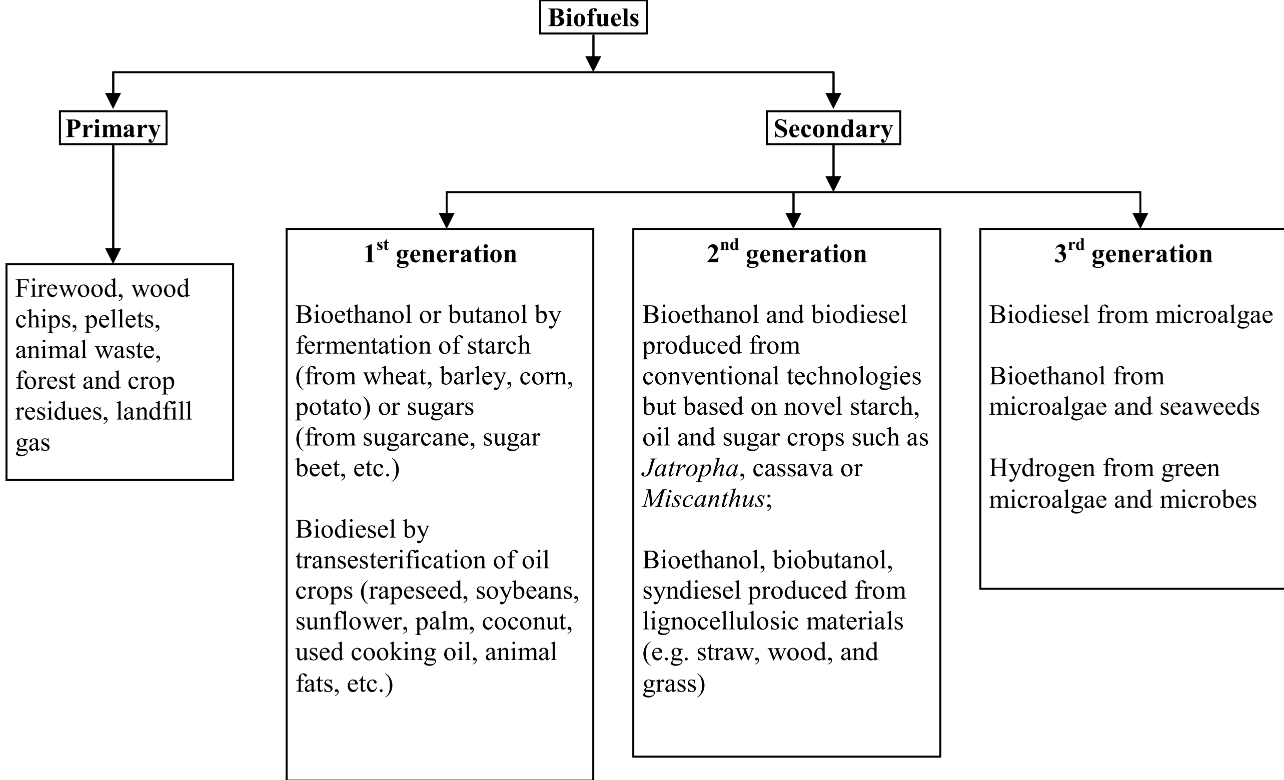

Fig. 1 Classification of biofuels (modified from [2]).

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Although biofuel processes have a great potential to provide a carbon-neutral route to fuel production, first generation production systems have considerable economic and environmental limitations. The most common concern related to the current first generation biofuels is that as production capacities increase, so does their competition with agriculture for arable land used for food production. The increased pressure on arable land currently used for food production can lead to severe food shortages, in particular for the developing world where already more than 800 million people suffer from hunger and malnutrition. In addition, the intensive use of land with high fertilizer and pesticide applications and water use can cause significant environmental problems [3].

The advent of second generation biofuels is intended to produce fuels from lignocellulosic biomass, the woody part of plants that do not compete with food production. Sources include agricultural residues, forest harvesting residues or wood processing waste such as leaves, straw or wood chips as well as the non-edible components of corn or sugarcane. However, converting the woody biomass into fermentable sugars requires costly technologies involving pre-treatment with special enzymes, meaning that second generation biofuels cannot yet be produced economically on a large scale [4].

Therefore, third generation biofuels derived from microalgae are considered to be a viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels [2, 5, 6]. Microalgae are able to produce 15–300 times more oil for biodiesel production than traditional crops on an area basis. Furthermore compared with conventional crop plants which are usually harvested once or twice a year, microalgae have a very short harvesting cycle (≈1– 10 days depending on the process), allowing multiple or continuous harvests with significantly increased yields [3].

2. Characteristics of microalgae

Microlgae, recognised as one of the oldest living organisms, are thallophytes (plants lacking roots, stems, and leaves) that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells [4]. While the mechanism of photosynthesis in these microorganisms is similar to that of higher plants, they are generally more efficient converters of solar energy because of their simple cellular structure. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, CO_2, and other nutrients [5].

Traditionally microalgae have been classified according to their colour and this characteristic continues to be of a certain importance. The current systems of classification of microalgae are based on the following main criteria: kinds of pigments, chemical nature of storage products and cell wall constituents. Additional criteria take into consideration the following cytological and morphological characters: occurrence of flagellate cells, structure of the flagella, scheme and path of nuclear and cell division, presence of an envelope of endoplasmic reticulum around the chloroplast, and possible connection between the endoplasmic reticulum and the nuclear membrane [7]. There are two basic types of cells in the algae, prokaryotic and eukaryotic. Prokaryotic cells lack membrane-bounded organelles (plastids, mitochondria, nuclei, Golgi bodies, and flagella) and occur in the cyanobacteria. The remainder of the algae are eukaryotic and have organelles [8].

Microalgae can be either autotrophic or heterotrophic. If they are autotrophic, they use inorganic compounds as a source of carbon. Autotrophs can be photoautotrophic, using light as a source of energy, or chemoautotrophic, oxidizing inorganic compounds for energy. If they are heterotrophic, microalgae use organic compounds for growth. Heterotrophs can be photoheterotrophs, using light as a source of energy, or chemoheterotrophs, oxidizing organic compounds for energy. Some photosynthetic microalgae are mixotrophic, combining heterotrophy and autotrophy by photosynthesis [8]. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2, the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth [4].

Microalgae are able to fix CO_2 efficiently from different sources, including the atmosphere, industrial exhaust gases, and soluble carbonate salts. Fixation of CO_2 from atmosphere is probably the most basic method to sink carbon, and relies on the mass transfer from the air to the microalgae in their aquatic growth environments during photosynthesis. However, because of the relatively small percentage of CO_2 in the atmosphere (approximately 0.036 %), the use of terrestrial plants is not an economically feasible option [4]. On the other hand, industrial exhaust gases such as flue gas contains up to 15 % CO_2, providing a CO_2-rich source for microalgal cultivation and a potentially more efficient route for CO_2 bio-fixation. Many microalgal species have also been able to utilize carbonates such as Na_2CO_3 and $NaHCO_3$ for cell growth. Some of these species typically have high extracellular carboanhydrase activities, which is responsible for the conversion of carbonate to free CO_2 to facilitate CO_2 assimilation. In addition, the direct uptake of bicarbonate by an active transport system has also been found in several species [9].

Growth medium must provide the inorganic elements that constitute the algal cell. Essential elements include nitrogen (N) and phosphorus (P). Minimal nutritional requirements can be estimated using the approximate molecular formula of the microalgal biomass, which is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ [5]. Nitrogen is mostly supplied as nitrate (NO_3^-), but often ammonia (NH_4^+) and urea are also used. Urea is most favourable as the nitrogen source because, for an equivalent nitrogen concentration, it gives higher yields and causes smaller pH fluctuations in the medium during algal growth

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[10]. On the other hand, nutrients such as P must be supplied in significant excess because the phosphates added complex with metal ions, therefore, not all the added P is bio-available [5]. Furthermore, microalgae growth depends not only on an adequate supply of essential macronutrient elements (carbon, nitrogen, phosphorus, silicon) and major ions (Mg_2^+, Ca_2^+, Cl^-, and SO_4^{2-}) but also on a number of micronutrient metals such as iron, manganese, zinc, cobalt, copper, and molybdenum [11].

3. Microalgae as a potential source of biofuel

There are several ways to convert microalgal biomass to energy sources, which can be classified into biochemical conversion, chemical reaction, direct combustion, and thermochemical conversion (Fig. 2). Thus, microalgae can provide feedstock for renewable liquid fuels such as biodiesel and bioethanol [12].

The idea of using microalgae as a source of biofuel is not new, but it is now being taken seriously because of the rising price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning of fossil fuels [5]. The utilization of microalgae for biofuels production offers the following advantages over higher plants: (1) microalgae synthesize and accumulate large quantities of neutral lipids (20–50 % dry weight of biomass) and grow at high rates; (2) microalgae are capable of all year round production, therefore, oil yield per area of microalgae cultures could greatly exceed the yield of best oilseed crops; (3) microalgae need less water than terrestrial crops therefore reducing the load on freshwater sources; (4) microalgae cultivation does not require herbicides or pesticides application; (5) microalgae sequester CO_2 from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing emissions of a major greenhouse gas (1 kg of dry algal biomass utilise about 1.83 kg of CO2); (6) wastewater bioremediation by removal of NH_4^+, NO_3^-, PO_4^{3-} from a variety of wastewater sources (e.g. agricultural run-off, concentrated animal feed operations, and industrial and municipal wastewaters); (7) combined with their ability to grow under harsher conditions and their reduced needs for nutrients, microalgae can be cultivated in saline/brackish water/coastal seawater on non-arable land, and do not compete for resources with conventional agriculture; (8) depending on the microalgae species other compounds may also be extracted, with valuable applications in different industrial sectors, including a large range of fine chemicals and bulk products, such as polyunsaturated fatty acids, natural dyes, polysaccharides, pigments, antioxidants, high-value bioactive compounds, and proteins [4, 12, 13].

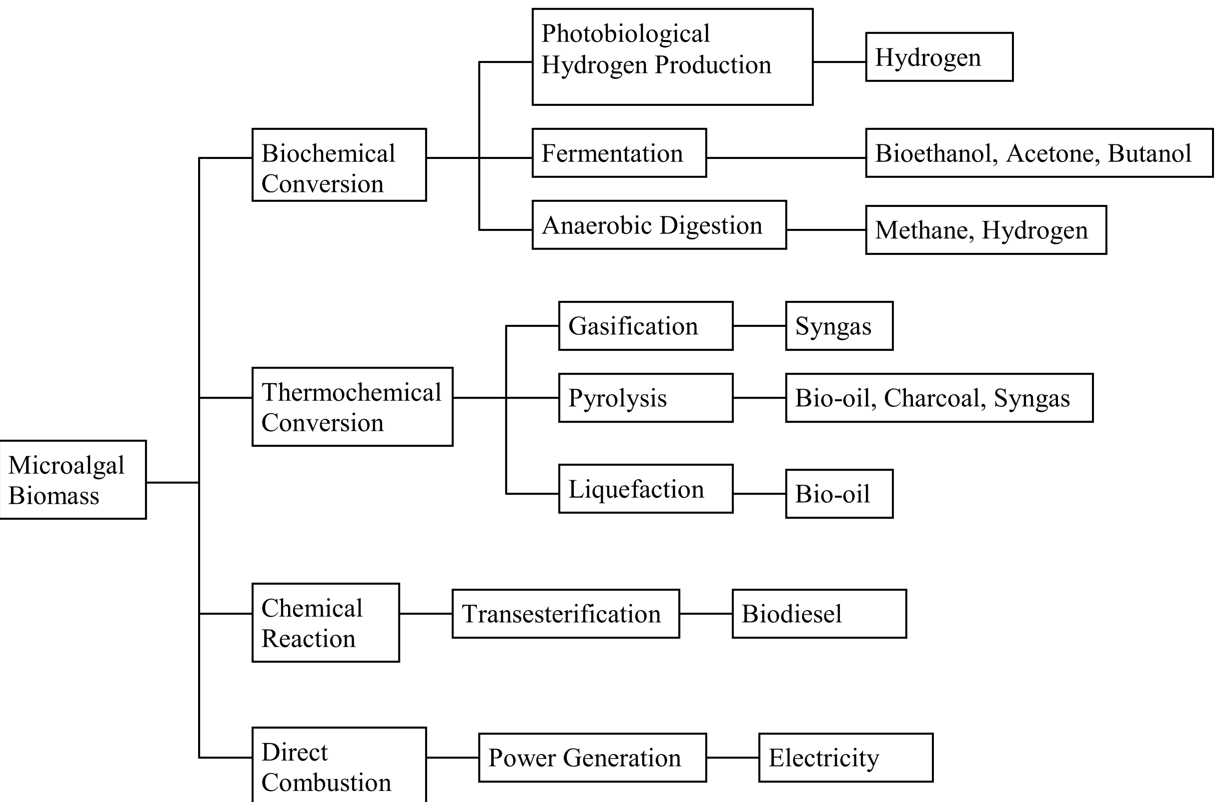

Fig. 2 Conversion processes for biofuel production from microalgal biomass (modified from [9]).

4. Biodiesel and bioethanol production from microalgae

Recent studies have shown that microalgal biomass is one of the most promising sources of renewable biodiesel that is capable of meeting the global demand for transport fuels. Biodiesel production by microalgae will not compromise production of food, fodder and other products derived from crops [5].

Microalgal biomass contains three main components: proteins, carbohydrates, and lipids (oil) [13]. The biomass composition of various microalgae in terms of those main components is shown in Table 1.

Table 1 Biomass composition of microalgae expressed on a dry matter basis ([13, 14]).

Strain	Protein	Carbohydrates	Lipid
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	40	2	33
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	41–58	12–17	10–22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Dunaliella tertiolecta	29	14	11
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus dimorphus	8–18	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	42–63	8–14	4–11
Synechoccus sp.	63	15	11
Tetraselmis maculata	52	15	3

Much of the on-going research work is focused on a small number of fast-growing microalgal species which have been found to accumulate substantial quantities of lipids, though under specific conditions. Within the green algae, typical species include *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species, as well as *Botryococcus braunii*, which although slow growing can accumulate large quantities of lipids [15]. While many microalgae strains naturally have high lipid content, it is possible to increase that concentration by optimising growth-determining factors such as the control of nitrogen level, light intensity, temperature, salinity, CO_2 concentration and harvesting procedure.

However, increasing lipid accumulation will not result in increased lipid productivity as biomass productivity and lipid accumulation are not necessarily correlated. Lipid accumulation refers to increased concentration of lipids within the microalgae cells without consideration of the overall biomass production. Lipid productivity takes into account both the lipid concentration within cells and the biomass produced by these cells and is therefore a more useful indicator of the potential costs of liquid biofuel production [4].

An integrated production of biofuels from microalgae (Fig. 3) includes a microalgal cultivation step, followed by the separation of the cells from the growth medium and subsequent lipid extraction for biodiesel production through transesterification.

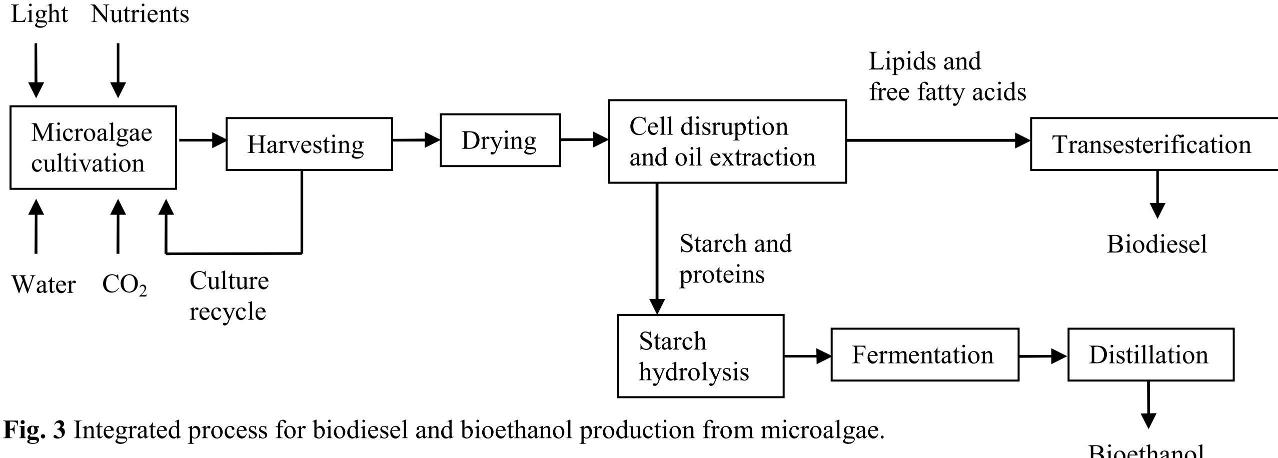

Fig. 3 Integrated process for biodiesel and bioethanol production from microalgae.

Following oil extraction, amylolytic enzymes are used to promote starch hydrolysis and formation of fermentable sugars. These sugars are fermented and distilled into bioethanol using conventional ethanol distillation technology.

4.1 Cultivation systems

After selecting the microalgae strain to obtain the product of interest, it becomes necessary to develop a whole range of bioprocesses that make viable its commercialization. Thus, the design and optimization of adequate bioreactors to cultivate these microorganisms is a major step in the strategy that aims at transforming scientific findings into a marketable product. Despite of many possible applications, only a few species of algae are cultured commercially because of poorly developed microalgal bioreactor technology.

From a commercial point of view, a microalgae culture system must have as many of the following characteristics as possible: high area productivity; high volumetric productivity; inexpensiveness (both in terms of investment and maintenance costs); easiness of control of the culture parameters (temperature, pH, O_2, turbulence); and reliability [16]. Cultivation systems of different designs attempt to achieve these characteristics differently. Although the term "photobioreactor" (PBR) has been applied to open ponds and channels, applied phycologists have generally distinguished between open-air systems and PBRs (devices that allow monoseptic culture). Thus in this chapter the term PBR is used only for closed systems.

4.1.1 Open-air systems

Open-air systems were extensively studied in the past few years [17-19], but these algae cultivation systems have been used since the 1950s. The classical open-air cultivation systems comprise lakes and natural ponds, circular ponds, raceway ponds and inclined systems. Open-air systems are the most widespread growth systems and all very large commercial systems used today are of this type. The reasons for this relate to economic and operational issues, since these systems are easier and less expensive to build, operate more durably and have a larger production capacity than most closed systems; further, they can utilize sunlight and the nutrients can be provided through runoff water from nearby land areas or by channeling the water from sewage/water treatment plants [20] making it the cheapest method of large-scale algal biomass production.

Although these systems are the most widely used at industrial level, open-air systems still present significant technical challenges. Generally ponds are susceptive to weather conditions, not allowing control of water temperature, evaporation and lighting, which make these systems dependent on the prevailing regional climate conditions (daily and annual temperature range, annual rainfall and rainfall pattern, number of sunny days, and degree of cloud cover). Furthermore, contamination by predators and other fast growing heterotrophs have restricted the commercial production of algae in open culture systems to fast growing, naturally occurring or extremophilic species. Consequently, this strictly limits the species of algae that can be grown in such systems. As a result, only *Dunaliella* (adaptable to very high salinity), *Spirulina* (adaptable to high alkalinity) and *Chlorella* (adaptable to nutrient-rich media) have been successfully grown in commercial open pond systems [20].

Natural and artificial ponds are only viable when a series of conditions are met. The existence of favorable climatic conditions and sufficient nutrients in order to the microalgae grow is profusely unavoidable and it also requires that the water presents selective characteristics (*e.g.* high salinity, high pH, high nutrients concentration) to ensure the existence of a monoculture. Successful examples of this type of cultivation are the *Arthrospira* production in Lake Kossorom (soda lake at the irregular northeast fringe of Lake Chad) where the Kanembu people harvest about 40 t/year of *Arthrospira* (*Spirulina*), to use it as food [21] and in Myanmar, where four old volcanic craters, full of alkaline water are used as cultivation system for the production of around 30 t/year of *Arthrospira* that are sold on the local market [22]. The Australian producer of *D. salina* (extremely halophilic and highly light-tolerant green alga) Betatene Ltd, uses very large ponds (up to 250 ha with an average depth of 0.2 to 0.3 m) at the extremely halophilic waters of Hutt-Lagoon, Western Australia which are unmixed other than by wind and convection [24].

The inclined system (cascade system) is the only open-air system which achieves high sustainable cell densities (up to 10 g l^{-1}). This system is very well suited for algae such as *Chlorella* and *Scenedesmus*, which can tolerate repeated pumping [23]. In inclined systems turbulence is created by gravity, the culture suspension flowing from the top to the bottom of a sloping surface, thus achieving highly turbulent flow and allowing the adoption of very thin culture layers (< 2 cm), facilitating higher cell concentrations and a higher surface-to-volume ratio (s/v) compared to raceway ponds.
Circular ponds with a centrally pivoted rotating agitator are widely used in Indonesia, Japan and Taiwan for the production of *Chlorella*. Depth is about 0.3 m. The design of these systems, however, limits pond size to about 10,000 m^2, because mixing by the rotating arm is no longer possible in larger ponds. Circular ponds are not favored in commercial plants since they require expensive concrete construction and high energy input for mixing [24].

Raceway ponds are the most commonly used artificial system. They are typically made of a closed loop, oval shaped recirculation channels, generally between 0.2 and 0.5 m deep, with mixing and circulation required to stabilize algae growth and productivity (Table 2). In a continuous production cycle, algae broth and nutrients are introduced in front of the paddlewheel and circulated through the loop to the harvest extraction point. The paddlewheel is in continuous operation to prevent sedimentation. At water depths of 0.15-0.20 m, biomass concentrations of up to 1 g l^{-1} and

productivities of 10-25 g m^{-2} d^{-1}, are possible [25]. The largest raceway-based biomass production facility located in Calipatria, CA (USA) occupies an area of 440,000 m^2 to grow *Spirulina* [26].

4.1.2 Photobioreactors

Photobioreactors (PBRs) are characterized by the regulation and control of nearly all the biotechnologically important parameters as well as by a reduced contamination risk, no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature, and flexible technical design [25]. These systems receive sunlight either directly through the transparent container walls or via light fibres or tubes that channel it from sunlight collectors.

Despite the relative success of open systems, recent advances in microalgal mass culture require closed systems, as many of the new algae and algal high-value products for use in the pharmaceutical and cosmetics industry must be grown free of pollution and potential contaminants such as heavy metals and microorganisms.

Many different designs have been developed, but the main categories include: (1) tubular (*e.g.* helical, manifold, serpentine, and α-shaped); (2) flat (*e.g.* alveolar panels and glass plates); and (3) column (*e.g.* bubble columns and airlift). A great amount of developmental work has been carried out in order to optimize different PBR systems for microalgae cultivation [17, 19, 27, 28].

4.1.2.1 Tubular photobioreactors

Tubular PBRs can be horizontal/serpentine- [29], near horizontal- [30], vertical- [31], inclined- [32] and conical-shaped [33]. Microalgae are circulated through the tubes by a pump, or preferably with airlift technology. Generally these PBR systems are relatively cheap, have a large illumination surface area and have fairly good biomass productivities.

Disadvantages include fouling, some degree of wall growth, dissolved oxygen and CO_2 along the tubes, and the pH gradients that lead to frequent re-carbonation of the cultures, which would consequently increase the cost of algal production (Table 2). The largest closed PBRs are tubular, e.g. the 25 m^3 plant at Mera Pharmaceuticals, Hawaii, and the 700 m^3 plant in Klötze, Germany. A maximum productivity of 25 g m^{-2} d^{-1} (*Spirulina*) has been achieved in a 10 m^3 serpentine bioreactor with intermitted culture circulation [34]. Further improvements were obtained by constructing a two-plane tubular photobioreactor with mean daylight productivities of about 30 g m^{-2} d^{-1} [35]. Helical tubular PBRs are a suitable alternative to straight tubular PBRs. The most frequently used layout is the Biocoil, currently traded by Biotechna (Melbourne, Australia). This reactor is composed of a set of polyethylene tubes (3.0 cm of inner diameter) coiled in an open circular framework, coupled with a gas exchange tower and a heat exchange system; a centrifugal pump drives the culture broth through the long tube to the gas exchange tower [28]. A 300 l α-shaped tubular PBR has been used for the cultivation of *Chlorella pyrenoidosa* [36]. That system comprises of an airlift pump to promote an ascending/descending trajectory, with several CO_2 injection points along its path.

4.1.2.2 Flat photobioreactors

Some of the earliest forms of closed systems are flat PBRs which have received much research attention due to the large surface area exposed to illumination and high densities (>80 g l^{-1}) of photoautotrophic cells observed [4].

In these PBR a thin layer of very dense culture is mixed or flown across a flat transparent panel, which allows radiation absorbance in the first few millimetres thickness. Flat PBRs are suitable for mass cultures of microalgae due to the low accumulation of dissolved oxygen and the high photosynthetic efficiency achieved when compared to tubular designs [4]. Usually, the panels are illuminated mainly on one side by direct sunlight and have the added advantage that they can be positioned vertically or inclined at an optimum angle facing the sun permitting a better efficiency in terms of energy absorbed from incident sunlight. Packed flat panels mixed by air bubbling can potentially achieve very high overall ground-areal productivities through lamination of solar light. Limitations include difficulty in controlling culture temperature, some degree of wall growth, scale-up requires many compartments and support materials, and possibility of hydrodynamic stress to some algal strains [12] (Table 2).

4.1.2.3 Column photobioreactors

Column PBRs are occasionally stirred tank reactors [37], but more often bubble columns [38] or airlifts [39]. The columns are placed vertically, aerated from the bottom, and illuminated through transparent walls or internally. Column bioreactors offer the most efficient mixing, the highest volumetric gas transfer rates, and the best controllable growth conditions. They are low-cost, compact and easy to operate. Their performance (*i.e.* final biomass concentration and specific growth rate) compares favorably with the values typically reported for tubular PBRs.

Vertical bubble columns and airlift cylinders can attain substantially increased radial movement of fluid that is necessary for improved light–dark cycling. These reactor designs have a low surface/volume, but substantially greater gas hold-ups than horizontal reactors and a much more chaotic gas–liquid flow. Consequently, cultures suffer less from photo-inhibition and photo-oxidation, and experience a more adequate light–dark cycle [12].

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4.1.3 Photobioreactor design and scale-up considerations

Despite various configurations, several essential issues need addressing when building a PBR: effective and efficient provision of light; supply of CO_2 while minimizing desorption; efficient mixing and circulation of the culture; scalable PBR technology and the material used in the construction of the PBR.

Light as the energy source for photoautotrophic life is the principal limiting factor in photobiotechnology. The light regimen inside the PBR is influenced by incident light intensity, reactor design and dimension, cell density, pigmentation of the cells, mixing pattern, etc. In outdoor PBRs the light regimen is also influenced by geographical location, time of the day, and weather conditions. Due to the light gradient inside the reactor and depending on the mixing properties, microalgae are subjected to light-dark cycles where the light period is characterized by a light gradient. These light-dark cycles will determine productivity and biomass yield on light energy [40]. Information about quantitative (photosynthetic photon flux density) and qualitative (spectral intensity distribution) aspects of light patterns in different points of a PBR can be obtained by using optical fiber technology [40].

The supply of CO_2 to microalgal mass culture systems is one of the principal difficulties that must be solved [41]. The principal point of all considerations relating to the CO_2 budget is that, on the one hand, CO_2 must not reach the upper concentration that produces inhibition and, on the other hand, must never fall below the minimum concentration that limits growth. These maximum (inhibition) and minimum (limitation) concentrations varies from one species to another and are not yet adequately known, ranging from 2.3×10^{-2} M to 2.3×10^{-4} M. Gas injection as minute bubbles into a column of a downcoming culture in which the culture velocity is adjusted to that of the rising CO_2 bubbles may increase the efficiency of absorption of CO_2 and thus the utilisation efficiency can be increased up to 70 % [42]. In a dual sparging bubble column PBR, the CO_2 transfer rate was increased 5 times compared to a similar reactor where the CO_2 was blended into the aeration air [43], while another study showed that, in the same PBR configuration, CO_2 transfer efficiencies were 100 % at certain conditions [44].

The level of mixing in a PBR strongly contributes to the growth of microalgae. Mixing is necessary to prevent cells from settling, to avoid thermal stratification, to distribute nutrients and break down diffusion gradients at the cell surface, to remove photosynthetically generated oxygen and to ensure that cells experience alternating periods of light and darkness of adequate length [19]. The fluid dynamics of the culture medium and the type of mixing influence average irradiance and the light regimen to which the cells are exposed, which in turns determine productivity. Fluctuations in light intensity faster than 1 s^{-1} enhance specific growth rates and productivities of microalgal cultures. In outdoor cultures exposed to photosynthetic photon flux densities above 1 000 μmol m^{-2} s light exposure times should be as short as 10 ms to maintain high photosynthetic efficiency [45]. The choice of the mixing device and the intensity of mixing should be dictated by the characteristics of the organism to be cultivated.

Tubular PBRs and raceway ponds are suitable for large-scale production [5]. The scalability of vertical air-lift PBR and bubble columns was considered an advantage of these systems [46]. Scale-up of closed systems is only possible by increasing the number of units in a production scheme. This method becomes extremely expensive, since each unit requires a variety of devices that control the wide range of growth factors (e.g. pH, temperature, aeration, CO_2 supply, nutrients supply). In addition, maintaining a monoculture in all of the units becomes challenging as the number of units to monitor grows [45]. Other than scale-up by multiplication of identical modules, the only way to increase volume is by increasing length or/and diameter or/and the light path of the PBR; however, this strategy is limited by the existence of changes in the performance of the PBR. Commercial-scale closed PBR have not been widely reported in scientific literature.

The type of material used is of fundamental importance for a suitable PBR construction. Materials such as plastic or glass sheets, collapsible or rigid tubes should have high transparency, high mechanical strength, high durability, chemical stability, low cost, must lack toxicity and be ease to clean [19].

Advantages and drawbacks of the most common materials used for building PBR have been reported in the literature [47].

4.1.4 Photobioreactors versus open-air systems

Table 2 shows a comparison between PBR (tubular, flat and column) and open systems for several culture conditions and growth parameters.

Table 2 Advantages and limitations of various microalgae culture systems

Culture Systems	**Advantages**	**Limitations**
Open systems	Relatively economical Easy to clean up Easy maintenance Utilization of non-agricultural land Low energy inputs	Little control of culture conditions Poor mixing, light and CO_2 utilization Difficult to grow algal cultures for long periods Poor productivity Limited to few strains Cultures are easily contaminated
Tubular PBR	Relatively cheap Large illumination surface area Suitable for outdoor cultures Good biomass productivities	Gradients of pH, dissolved oxygen and CO_2 along the tubes Fouling Some degree of wall growth Requires large land space Photoinhibition
Flat PBR	Relatively cheap Easy to clean up Large illumination surface area Suitable for outdoor cultures Low power consumption Good biomass productivities Good light path Readily tempered Low oxygen build-up Shortest oxygen path	Difficult scale-up Difficult temperature control Some degree of wall growth Hydrodynamic stress to some algal strains Low photosynthetic efficiency
Column PBR	Low energy consumption Readily tempered High mass transfer Good mixing Best exposure to light-dark cycles Low shear stress Easy to sterilize Reduced photoinhibition Reduced photo-oxidation High photosynthetic efficiency	Small illumination surface area Sophisticated construction materials Shear stress to algal cultures Decrease of illumination surface area upon scale-up Expensive compared to open ponds Support costs Modest scalability

Selection of a suitable production system clearly depends on the purpose of the production facility, microalgae strain and product of interest. In conclusion, PBR and open ponds should not be viewed as competing technologies.

4.2 Harvesting methods

Given the relatively low biomass concentration obtainable in microalgal cultivation systems due to the limit of light penetration (typically in the range of 1-5 g l^{-1}) and the small size of microalgal cells (typically in the range of 2-20 µm in diameter), costs and energy consumption for biomass harvesting are a significant concern that needs to be addressed

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properly [6]. In this sense, harvesting of microalgal cultures has been considered as a major bottleneck towards the industrial-scale processing of microalgae for biofuel production. The cost of biomass recovery from the broth can make up to 20–30% of the total cost of producing the biomass [48]. Microalgal biomass harvesting can be achieved in several physical, chemical or biological ways: flocculation, centrifugation, filtration, ultrafiltration, air-flotation, autoflotation, etc. Generally, microalgae harvesting is a two stage process, involving: (1) Bulk harvesting: aimed at separation of biomass from the bulk suspension. The concentration factors for this operation are generally 100–800 times to reach 2–7 % total solid matter. This will depend on the initial biomass concentration and technologies employed, including flocculation, flotation or gravity sedimentation; (2) Thickening: the aim is to concentrate the slurry through techniques such as centrifugation, filtration and ultrasonic aggregation, hence, it is generally a more energy intensive step than bulk harvesting .

4.2.1 Flocculation

Flocculation can be used as an initial dewatering step in the bulk harvesting process that will significantly enhance the ease of further processing. This stage is intended to aggregate microalgal cells from the broth in order to increase the effective ''particle'' size [49]. Since microalgae cells carry a negative charge that prevents them from self-aggregation in suspension, addition of chemicals known as flocculants neutralises or reduces the negative surface charge. These chemicals coagulate the algae without affecting the composition and toxicity of the product [48]. Multivalent metal salts like ferric chloride ($FeCl_3$), aluminium sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$) are commonly used [4].

4.2.2 Flotation

Some strains naturally float at the surface of the water as the microalgal lipid content increase. Although flotation has been mentioned as a potential harvesting method, there is very limited evidence of its technical or economic viability [4].

4.2.3 Centrifugation

Centrifugation involves the application of centrifugal forces to separate microalgal biomass from growth medium. Once separated, microalgae can be removed from the culture by simply draining the excess medium [49]. Centrifugal recovery is a rapid method of recovering algal cells, especially for producing extended shelf-life concentrates for aquaculture hatcheries and nurseries [48]. However, high gravitational and shear forces during the centrifugation process can damage cell structure. Additionally, it is not cost effective due to high power consumption especially when considering large volumes [49].

4.2.4 Filtration

Filtration is the method of harvesting that has proved to be the most competitive compared to other harvesting options. There are many different forms of filtration, such as dead end filtration, microfiltration, ultra filtration, pressure filtration, vacuum filtration and tangential flow filtration (TFF). Generally, filtration involves running the broth with algae through filters on which the algae accumulate and allow the medium to pass through the filter. The broth continually run through the microfilters until the filter contains a thick algae paste. Although filtration methods appear to be an attractive dewatering option, they are associated with extensive running costs and hidden pre-concentration requirements [49].

4.3 Extraction of microalgal lipids

4.3.1 Drying processes

Biomass drying before further lipid extraction and/or thermochemical processing is another step that needs to be taken into consideration. Sun drying is probably the cheapest drying method that has been employed for the processing of microalgal biomass. However, this method takes long drying time, requires large drying surface, and risks the loss of some bioreactive products [6]. More efficient but more costly drying technologies having been investigated for drying microalgae include drum drying, spray drying, fluidized bed drying, freeze drying and refractance window dehydration technology [4].

4.3.2 Cell disruption

The majority of biodiesel today is produced from animal or plant oils through a transesterification process following oil extraction with or without cell disruption [3]. Most cell disruption methods applicable to microalgae have been adapted from applications on intracellular non-photosynthetic bioproducts [4]. Cell disruption methods that have been used

successfully include high-pressure homogenisers, autoclaving, and addition of hydrochloric acid, sodium hydroxide, or alkaline lysis [50].

4.3.3 Methods for extraction of lipids

Numerous methods for extraction of lipids from microalgae have been applied; but most common methods are expeller/oil press, liquid–liquid extraction (solvent extraction), supercritical fluid extraction (SFE) and ultrasound techniques [49].

Expeller/oil pressing is a mechanical method for extracting oil from raw materials such as nuts and seeds. Press uses high pressure to squeeze and break cells. In order for this process to be effective, algae must first need to be dried. Although this method can recover 75% of oil and no special skills is required, it was reported less effective due to comparatively longer extraction time [49].

Solvent extraction proved to be successful in order to extract lipids from microalgae. In this approach, organic solvents, such as benzene, cyclo-hexane, hexane, acetone, chloroform are added to algae paste. Solvent destroy algal cell wall, and extract oil from aqueous medium because of their higher solubility in organic solvents than water. Solvent extract can then be subjected to distillation process to separate oil from solvent. Latter can be reclaimed for further use. Hexane is reported to be the most efficient solvent in extraction based on its highest extraction capability and low cost [49].

Supercritical extraction makes use of high pressures and temperatures to rupture the cells. This particular method of extraction has proved to be extremely time-efficient and is commonly employed [49].

Another promising method to be used in extraction of microalgae is the application of ultrasounds. This method exposes algae to a high intensity ultrasonic wave, which creates tiny cavitation bubbles around cells. Collapse of bubbles emits shockwaves, shattering the cell wall and releasing the desired compounds into solution. Although extraction of oil from microalgae using ultrasound is already in extensive use at laboratory scale, sufficient information on feasibility or cost for a commercial-scale operation is unavailable. This approach seems to have a high potential, but more research is needed [49].

4.4 Biodiesel production

After the extraction processes, the resulting microalgal oil can be converted into biodiesel through a process called transesterification. The transesterification reaction consists of transforming triglycerides into fatty acid alkyl esters, in the presence of an alcohol, such as methanol or ethanol, and a catalyst, such as an alkali or acid, with glycerol as a by-product [51].

For user acceptance, microalgal biodiesel needs to comply with existing standards, such as ASTM Biodiesel Standard D 6751 (United States) or Standard EN 14214 (European Union). Microalgal oil contains a high degree of polyunsaturated fatty acids (with four or more double bonds) when compared to vegetable oils, which makes it susceptible to oxidation in storage and therefore reduces its acceptability for use in biodiesel. However, the extent of unsaturation of microalgal oil and its content of fatty acids with more than four double bonds can be reduced easily by partial catalytic hydrogenation of the oil, the same technology that is commonly used in making margarine from vegetable oils [5]. Nevertheless, microalgal biodiesel has similar physical and chemical properties to petroleum diesel, first generation biodiesel from oil crops and compares favourably with the international standard EN14214 [4].

4.5 Bioethanol production

The current interests in producing bioethanol are focusing on microalgae as a feedstock for fermentation process. Microalgae provide carbohydrates (in the form of glucose, starch and other polysaccharides) and proteins that can be used as carbon sources for fermentation by bacteria, yeast or fungi [49]. For instance, *Chlorella vulgaris* has been considered as a potential raw material for bioethanol production because it can accumulate high levels of starch [52]. *Chlorococum* sp. was also used as a substrate for bioethanol production under different fermentation conditions. Results showed a maximum bioethanol concentration of 3.83 g l^{-1} obtained from 10 g l^{-1} of lipid-extracted microalgae debris [53].

Production of bioethanol by using microalgae can also be performed via self-fermentation. Previous studies reported that dark fermentation in the marine green algae *Chlorococcum littorale* was able to produce 450 µmol ethanol g^{-1} at 30 ºC [54].

Even though limited reports on microalgal fermentation were observed, a number of advantages were observed in order to produce bioethanol from microalgae. Fermentation process requires less consumption of energy and simplified process compared to biodiesel production system. Besides, CO_2 produced as by-product from fermentation process can be recycled as carbon sources to microalgae in cultivation process thus reduce the greenhouse gases emissions. However, the production of bioethanol from microalgae is still under investigation and this technology has not yet been commercialized [49].

5. Concluding remarks

Microalgae offer great potential as a sustainable feedstock for the production of third generation biofuels, such as biodiesel and bioethanol. However, several important scientific and technical barriers remain to be overcome before the large-scale production of microalgae derived biofuels can become a commercial reality. Technological developments, including advances in photobioreactor design, microalgal biomass harvesting, drying, and processing are important areas that may lead to enhanced cost-effectiveness and therefore, effective commercial implementation of the biofuel from microalgae strategy.

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