Potential of Microalgae for the Production of Different Biotechnological Products

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Microalgae have been recognized as powerful phototrophic cell-factories whose applications range from biomass production for food and feed purposes to the production of high-value products and biofuels. Microalgae have been considered a source of functional ingredients, such as polyunsaturated fatty acids, polysaccharides, essential minerals, vitamins and bioactive peptides that can have positive effects on human and animal health. Besides having high nutritional value due to the high percentage of proteins in their composition, microalgae generate high-value products, such as pigments, polysaccharides, bio-hydrogen, and even bio-polyesters with plastic-like properties. Algal biomass that remains after product recovery can be used as forage, biofertilizer or feedstock for biogas production. This step in overall algal production is important from an economic point of view due to the reduction in production costs. This paper presents the detailed study of the biotechnologically most important microalgae strains, and the design principles of photobioreactors for their cultivation. In addition, the main existing and potential high-value products derivable from microalgae, as well as utilization of microalgae for phytoremediation and biogas production, were reviewed.

Keywords:
microalgae, cultivation type, bioreactor systems, microalgal products

Introduction

Algae are generally defined as photosynthetic organisms. All microscopic algae, which are usually unicellular or filamentous, are called microalgae[1,2]. Microalgae are considered simple organisms, since they lack the complex cell structures and organs found in higher plants[1,3]. These unicellular microorganisms form a versatile polyphyletic group with the capability of photosynthetic fixation of CO_2 in order to generate different algal cell components, energy, and molecular oxygen[4,5]. Microalgae include both prokaryotic and eukaryotic photosynthetic microorganisms, including cyanobacteria, which are often referred to as blue-green algae[4,6]. These microorganisms can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure[6]. Cyanobacteria (formerly known as Cyanophyceae) are an example of prokaryotic microorganisms, while green algae (Chlorophyta) and diatoms (Bacillariophyta) are examples of eukaryotic microalgae[6,7]. Microalgae are present not only in aquatic but also in terrestrial ecosystems, representing a diverse collection of organisms whose diversity is not fully explored[6,8]. It has been estimated that there exist between 70 000 to one million species, but only about 44 000 have been described[9]. Algae are traditionally classified according to their colour (cyanobacteria, rhodophytes, chlorophytes, and chromophytes), and the current classification system is based on the kinds of pigments present in algae, chemical nature of storage products, and cell wall constituents[10,11]. Some additional criteria that are taken into consideration are the occurrence of flagellate cells, structure of the flagella, presence of an envelope of endoplasmic reticulum around the chloroplast, and path of nuclear and cell division[11]. Due to this evolutionary and phylogenetic diversity, the chemical composition of these microorganisms also varies greatly making them extremely attractive for bio-prospecting and exploitation of a wide range of bio-products[8]. Microalgae represent one of the most promising sources for new products (or applications), which can be potentially used as food, feed, fine chemicals or different green energy carriers[1,4,12,13]. Microalgae can be used as food supplements, but they are also an excellent source of vita-

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mins, proteins, pigments (*e.g.*, β-carotene and astaxanthin), lipids, bioactive compounds, polysaccharides (*e.g.*, β-1,3-glucans), bio-hydrogen, and even bio-polyesters[4,8,14]. Microalgae are of great significance to the sustainability of the Earth's ecosystem due to their exceptional capacity for CO_2 fixation. Global CO_2 fixation by algae amounts to about the same quantity as the photosynthetic performance accomplished by terrestrial green plants[13,15]. Besides mitigating CO_2 emission, they can eliminate contaminants from various environments, and therefore, microalgae can also be used in phytoremediation processes, particularly in tertiary wastewater treatment[14,16]. Finally, the residual algal biomass can be used as forage, biogas feedstock or biofertilizer to balance the material and energy cycles of entire bioprocess[4].

Cultivation of microalgae

The growth characteristics, microalgal composition, and product formation rates, significantly depend on the cultivation factors, such as quantity and quality of nutrients, light supply, and light intensity[13]. Microalgal biomass growth depends on the sufficient supply of carbon source and light to carry out photosynthesis[6]. Salinity, pH, temperature, and light intensity are decisive not only for biomass growth but also for product formation. Temperature range of 16–27 ºC, pH values of 4–11, salinities of 12–40 g L^{-1} and light intensity of 1000–10000 lux are typical values found in literature[17]. Microalgae adapt readily to strong fluctuating process conditions during biosynthesis[4]. They are able to assume many types of metabolisms, and therefore, four major types of cultivation regimes can be applied: (1) photoautotrophic, (2) heterotrophic, (3) mixotrophic and (4) photoheterotrophic[6,18]. Furthermore, microalgae are capable of a metabolic shift as a response to the changes in their environment. Photoautotrophic cultivation occurs when microalgae use light as a sole energy source and inorganic carbon as a carbon source to produce chemical energy through photosynthetic reaction[18,19]. These are the most commonly used cultivation conditions for microalgae growth[20,21]. In these conditions, light and CO_2 are usually growth-limiting substrates[22]. Some microalgae are able to grow under phototrophic conditions and use organic carbon in the dark. Heterotrophic cultivation is characterized by the utilization of only organic compounds as carbon and energy source[18]. In these conditions, the requirement for light is eliminated[22]. Glucose, acetate, fructose, sucrose, lactose, galactose, glycerol, and mannose can be used as an organic carbon source by microalgae[23]. Heterotrophic cultivation gives a possibility to increase biomass concentration, and consequently the productivity[22]. Although heterotrophic cultivation conditions result in much higher biomass and lipid production, this sugar-based system also often suffers from problems with contamination[18,24]. For strains capable of photosynthesis, such as *Chlorella vulgaris* Beijerinck*, C. sorokiniana* Shihira and R. W. Krauss*, C. regularis* Oltmanns, *C. pyrenoidosa* Chick*, Spirulina* sp.*, Haematococcus* sp.*, Scenedesmus* sp.*, Nostoc* sp. and *Synechocystis* sp., heterotrophic aerobic growth has been experimentally confirmed[2,4,8]. Another problem that might occur is a different quality of heterotrophically and photoautotrophically grown cells[22]. Microalgae grow mixotrophically when they perform photosynthesis, which is the main energy source, but both organic and inorganic (CO_2) carbon sources are essential for growth. One of the subtypes of mixotrophy is called amphitrophy, and it means that organisms are able to live autotrophically or heterotrophically depending on the concentration of organic compounds and available light intensity[6]. Mixotrophic organisms are characterized by the ability to assimilate organic compounds (*e.g.,* carbon source) while using inorganic compounds as electron donor[22]. It has been proved experimentally that in mixotrophic culture, the addition of organic substrate resulted in increased growth rate and final biomass concentration. Furthermore, in such culture, no photo-inhibition was observed, and photo-oxidative damage could be reduced because the cells were growing heterotrophically using dissolved oxygen produced by photoautotrophically growing cells, thus, lowering the oxygen concentration[22]. Photoheterotrophic cultivation requires sugar and light at the same time. This cultivation occurs when microalgae need light while using organic compounds as carbon source[18,22]. Photoheterotrophic metabolism is also known as photoganitrophy, photoassimilation and photometabolism[6]. Strains like *Chlorella vulgaris*, *Haematococcus pluvialis* Flotow, *Arthrospira* (*Spirulina*) *platensis* Gomont, can grow under photoautotrophic, heterotrophic, and mixotrophic conditions, while *Selenastrum capricornutum* Printz and *Scenedesmus acutus* Meyen are examples of strains that grow preferably photoautotrophically, heterotrophically or photoheterotrophically[22]. A general comparison between different microalgal cultivation regimes and systems is presented in Table 1.

Bioreactor systems for cultivation of microalgae

Microalgae can be grown in two types of bioreactor systems: open systems or closed systems – photobioreactors. In the cultivation of some algal species currently produced commercially, their common feature to grow in highly selective environments is an advantage that allows cultivation in

Table 1 – *Comparison of different cultivation regimes and systems*

Cultivation regime	Energy source	Carbon source	Light availability requirement	Metabolism variability	Bioreactor system	Cost	Challenges
Phototrophic	Light	Inorganic	Obligatory	No switch between sources	Open pond Closed photobioreactor	Low	Low cell density High condensation cost Scalability problems
Heterotrophic	Organic	Organic	No requirements	Switch between sources	Stirred tank bioreactors	Medium	Contamination High cost of substrate
Photoheterotrophic	Light	Organic	Obligatory	Switch between sources	Closed photobioreactor	High	Contamination High equipment cost High cost of substrate
Mixotrophic	Light and organic	Inorganic and organic	Not obligatory	Simultaneous utilization	Closed photobioreactor	High	Contamination High equipment cost High cost of substrate

open-air bioreactor systems without risking contamination by other algae and protozoa. For example, *Chlorella* sp. grows well in nutrient-rich media, while *Spirulina* sp. requires a high pH and bicarbonate concentration, and *Dunaliella salina* Teodoresco grows in very high salinity[25,26]. Most of the marine algae, like diatoms *Skeletonema*, *Chaetoceros*, *Thalassiosira*, chlorophyte *Tetraselmis,* and haptophyte *Isochrysis* and dinoflagellate *Crypthecodinium cohnii* Javornicky, have no environmental selective advantages and their cultivation requires closed systems[25,26]. In terms of cultivation mode, batch, fed-batch, repeated batch, repeated fed-batch or continuous setups can be applied in the design of the bioprocess[13].

Open bioreactor systems

Developing a cost-effective large-scale culture system is one among many factors that affect the success of commercial large-scale production of microalgae. Since closed systems photobioreactors have high capital and operating costs due to the need for artificial light sources causing relatively high electricity costs, most commercial cultivation of photosynthetic cells is done in open ponds utilizing solar light[27,28]. Open bioreactor systems can be divided into natural waters (lakes, lagoons, ponds), and artificial ponds or containers erected in different ways[29,30]. The most common types of culture systems are large shallow open ponds, circular ponds with rotating arms for mixing the culture, raceway ponds, and large bags and tanks also used in aquaculture[26,30]. In open bioreactor systems, light is supplied only through the exposed surface of ponds. Since optical absorption and self-shading by the algal cells limit light penetration through the broth, the light available per unit volume of culture declines with an increase in culture depth[3]. Therefore, ponds are kept shallow, and as such, they are more productive than deeper ponds providing the growth is exclusively photoautotrophic[31]. Raceway ponds usually operate at water depths between 10 and 50 cm[32]. They are generally designed to have a flat bottom and vertical walls[31]. Agitation is performed by a rotating arm in the simplest case of open spherical circulating ponds. The typical raceway pond is usually driven by low-energy-consuming paddle wheels for gas/liquid circulation, mixing, as well as prevention of sedimentation. A major advantage of open ponds is that they are less technically complex, and therefore, easier and cheaper to construct and operate than most closed systems[29,30,33]. They are good for the mass cultivation of algae and easy to clean after cultivation. In addition, the culture medium is exposed directly to the atmosphere, allowing liquid evaporation, which helps to regulate the bioprocess temperature[32]. These systems are typically used in commercial scale for the cultivation of cyanobacteria and microalgae, such as *Arthrospira platensis, Dunaliella salina, Phaeodactylum tricornutum* Bohlin*, Pleurochrysis carterae* Christensen, and species belonging to genera *Chlorella* sp*., Anabaena* sp. and *Nannochloropsis* sp*.,* among many others[32,34]. However, there are some major disadvantages of the open pond systems, such as little control of process parameters and limitations in controlling contamination[28]. Furthermore, limitations of open ponds include significant evaporative losses, diffusion of CO_2 into the atmosphere and requirement of large areas of land[29,30]. Mass transfer rates are very poor in such systems due to

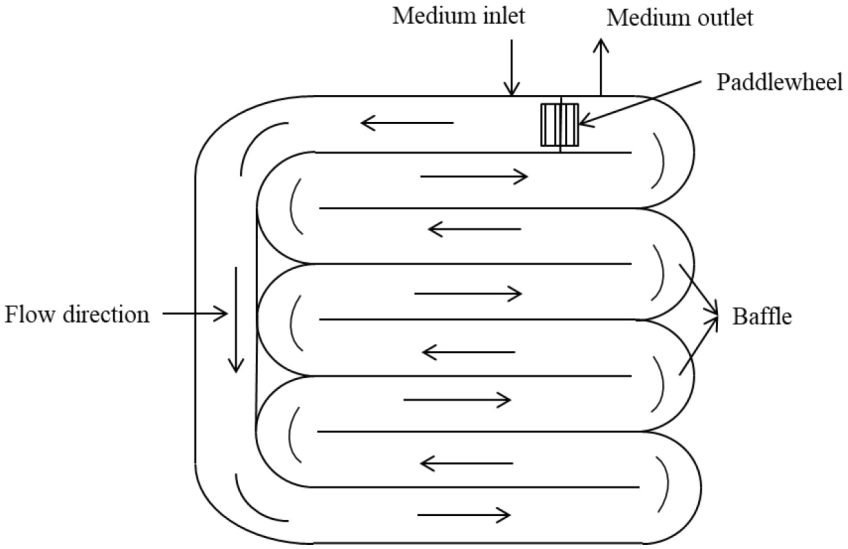

Fig. 1 – *Scheme of a raceway pond (adapted from Christi[35])*

inefficient mixing, which results in low concentration of algal biomass and intracellular accumulation products that can be reached[30]. This is also caused by light limitation due to the low penetration depth of the light necessary for the growth and biosynthesis of products[33]. By reducing the thickness of the algal layer to a few centimetres or even millimetres, it is possible to enhance light supply[29]. Due to direct contact with the atmosphere in such systems, the risk of contamination by predators and other fast-growing heterotrophs is high. That is why open culture systems are restricted for commercial production of extremophilic species only, and not suitable for the production of pharmaceutical or food ingredients[29,30,35] (Fig. 1).

Moreover, open cultivation systems are also subjected to the impact of weather conditions. Besides being excavated and lined with an impermeable material (*e.g.,* plastics), ponds can be constructed above ground with walls of brick or concrete blocks and floors. The choice of material for pond construction depends on many factors. For example, for the culture of algae such as *Chlorella,* concrete ponds are suitable, but for the culture of hypersaline *Dunaliella salina,* concrete is highly unsuitable because it gradually deteriorates in high salinities[36]. In terms of construction and operating costs, plastic-lined earthen raceways are the least expensive[31]. Plastic-lined concrete ponds are more expensive than plastic-lined compacted earth ponds[31,37]. For better control of the growth environment and protection from contamination, ponds can be enclosed in glasshouses or plastic-covered greenhouses[37]. Such ponds are even suitable for the production of high-value products, such as nutraceuticals[38]. One example of algal production in open ponds among many others is the production of *Dunaliella* in Israel, where paddle-wheeldriven raceway ponds are used[26]. Concerning the cultivation mode, batch or pseudo-steady state continuous culture can be applied in biomass production in raceways[31]. In the batch process, the nutrient medium is placed in the raceways and inoculated with chosen algal culture. In continuous mode, the culture generally begins as a batch, and once sufficient biomass concentration is achieved, the raceway is fed with fresh medium at the specific flow rate. During feeding, the algal broth is harvested from the raceway at a flow rate equal to feed rate. Feeding and harvesting occur only during daylight and must stop at night so the biomass is not washed out of the raceway overnight. In a well-operated raceway with a sunny locale and stable temperature of around 25 °C, annual dry biomass productivity of around 0.025 kg m^{-2} d^{-1} can be achieved[31].

Closed systems – photobioreactors

Although open pond cultivation is the most affordable option to overcome the problems with such systems, much attention is invested in the development of closed systems. Closed systems – photobioreactors, can be described as enclosed (or mostly closed), and illuminated vessels designed for controlled biomass production, where energy is supplied via electric lights[39,40]. These systems are required because many algal species of interest do not grow in highly selective environments. In addition, for production of some high-value algal products, a contamination free environment must be assured. For example, tubular photobioreactors can satisfy the Good Manufacturing Practice (GMP) requirements for pharmaceutical products, which enable their use in the production of biomass for food, feed, and additives[41]. Several designs of photobiore-

actors have been developed: flat-plate, tubular, vertical-column, internally-illuminated photobioreactors, airlift reactor, bubble column, stirred-tank, conical, torus, and seaweed-type photobioreactor[30,32,42]. The photobioreactors are categorized based on the illuminated surface as flat-plate, tubular and column, and based on their mode of liquid flow as stirred type, bubble column, and airlift reactor[43]. Although various types of photobioreactors have been developed, only a few can be used for industrial-scale cultivation. Besides open raceway ponds, tubular and flat panel photobioreactors are commercially applied today[44]. Compared with open-air systems, photobioreactors have several advantages, such as reduced risk of contamination, higher photosynthetic efficiency, higher concentrations, and areal productivities, ability to be used outdoors in natural daylight, and significantly smaller space requirements[42,45]. Higher operating cell densities reduce harvesting costs and land requirements. All these advantages enable the cultivation of a wider range of species by avoiding contamination, but also, bioreactors can be operated over a much wider climatic range than the open-air systems[26]. Closed systems also prevent water losses caused by evaporation and CO_2 losses[29,42]. There is also the greater ability to control culture conditions such as temperature, pH levels, mixing rates, efficient exposure to light, and to reproduce appropriate cultivation conditions. Therefore, the final product is of more consistent composition and quality, especially when operating in a continuous culture mode[26]. Some bioreactors, such as compact photobioreactors, can even be easily thermostated without high technical efforts by simply placing a reactor in a constant-temperature room[30]. Photobioreactors have been used in the cultivation of *Porphyridium cruentum* Nägeli, *Phaeodactylum tricornutum*, *Arthrospira platensis*, *Chlorella sarokiniana*, *Haematococcus pluvialis*, *Tetraselmis suecica* Butcher, *Chlorella vulgaris,* and *Nannochloropsis* sp.[32] A typical photobioreactor in autophototrophic cultivation is a three-phase system, where culture media represents the liquid phase, the cells compose the solid phase, and CO_2-enriched air the gas phase. Light is required in such systems and it can be referred to as the fourth phase[42]. Algae can be grown either photoautotrophically, mixotrophically or heterotrophically in closed systems. Heterotrophic culture has several advantages, as well as disadvantages. Cultivation systems are well understood and there is a wide experience in designing and operating such systems. In addition, high cell densities (from 20 to 200 g L^{-1}) can be achieved, thus reducing harvesting costs as well as capital costs of the cultivation vessels. However, the chemical composition of algae often changes under heterotrophic conditions, and not all microalgae are suitable for such cultivation conditions[26]. The most commonly used large-scale system for photoautotrophic cultivation, also commonly used in the aquaculture industry, is the "big bag" system, which uses large sterile plastic bags fitted with a system for aeration[26,42].

The main goal of photobioreactor construction is the reduction in biomass production costs by controlling environmental conditions, avoiding contamination, and improving the design of bioreactor. However, some major drawbacks make them uneconomical for low-cost end-products. Biomass production costs increase due to the high initial investment, operating and maintenance costs of photobioreactors[46]. Furthermore, light diffusion is limited at operating volumes above 100 L, and the microalgal biofilm on the surface limits light penetration, resulting in inefficient growth of microalgae[43]. All these challenges need to be overcome in order to design efficient photobioreactors. An efficient photobioreactor is characterized by minimal capital and operating costs, minimal energy consumption, minimal non-illuminated part, and highly transparent surface, and high mass transfer rates while avoiding damage to cultured cells and attaining high biomass growth[40,43,47]. In addition, such a photobioreactor should be suitable for the cultivation of various microalgal species universally. To that end, some important factors, such as light distribution, hydrodynamics, mass transfer, and growth kinetics must be considered while designing photobioreactors[42]. The advantages and disadvantages of different systems for microalgae cultivation are summarized in Table 2.

Design and characteristics of photobioreactors

The efficiency of photobioreactors can be determined by the integration of capturing, transportation, distribution, and utilization of light by microalgae through photosynthesis[48].

Light

Light supply plays a key role in photobioreactor design since it is essential for microalgal photosynthesis. The principle in designing photobioreactor is to maximize the surface area to volume ratio[49]. Another fundamental principle is to reduce the light path and by that to increase the amount of light available to each cell[26]. The level of light intensity is crucial, because if it is above a critical value, the growth will be inhibited by the light (photo-inhibition) and light will be wasted as fluorescence and heat.

On the other hand, if the light intensity is below the certain level necessary to balance the maintenance, photo-limitation occurs and the culture will

Table 2 – *Advantages and disadvantages of open and closed bioreactor systems for microalgae cultivation*

Parameter	Open system	Closed system
Space required	High	Low
Area/volume ratio	Low (5–10 m^{-1})	High (20–200 m^{-1})
Process control	Difficult	Easy
CO_2-losses	High	Low
Water losses	Very high	Low
Temperature	Highly variable	Cooling required
Gas transfer control	Low	High
Hydrodynamic stress on algae	Very low	Low-high
Weather dependence	High	Low
Biomass quality	Variable	Reproducible
Light utilization efficiency	Poor	Excellent
Contamination risk	High	Low
Productivity	Very low	Moderate to high*
Population density	Low	High
Cultivatable algal species	Restricted to a few algal varieties	High, nearly all microalgal varieties may be cultivated
Capital expenses	Low	High
Operating expenses	Low	High
Scale up	Easy	Difficult
Cleaning	None	Required
Harvesting efficiency	Low	High
Harvesting cost	High	Low
Most costly parameters	Mixing	Oxygen and temperature control
Maintenance	Simple	Complex

*(3–5 times more productive than open systems)

collapse due to growth limitation by light[42,47]. In addition, light energy that falls on the light-exposed surface is not always utilized efficiently. Most photosynthetic organisms will intercept too much light when close to the light-exposed surface even under low-intensity light[50]. Photobioreactor can be divided into three zones based on cell growth rate. First is the strong illumination zone, which has an inhibitory effect and extends from the illuminated wall to the point where the arriving light energy balances the level of light necessary for maximum growth rate. The next zone is the week illumination zone, which ends at the point where the light energy intake meets the energy requirement for maintenance, and it is followed by the final dark zone, where the cell growth rate is negative due to the limited availability of light[42]. Light spectral quality is also an important factor, because although sunlight covers a wide spectral range, only light within the range of 400 and 700 nm is photosynthetically active radiation[47]. Unfortunately, more than 50 % of incident solar radiation from natural light cannot be used by photosynthesis[49]. Materials that are used for photobioreactor construction should satisfy the transparency requirement and be mechanically sufficient for construction. The most commonly used materials are glass, plexiglas, poly(vinyl chloride) (PVC), acrylic-PVC, and polyethylene[47]. The material should also maintain the ability to prevent biofilm formation because it drastically reduces light transmission through photobioreactor and complicates cleaning. Besides biofilm on the surface, high cell density also affects light intensity and penetration, which is caused by mutual shading between different cells[47]. Therefore, critical cell density, which stands for a maximum cell concentration without mutual shading in algal cultures, can be used as a new operating parameter[51]. One of the most commonly adopted strategies to improve light distribution is limiting the length of the light path and improving mixing.

Mixing

Mixing of microalgal culture is necessary for several reasons: to prevent sedimentation of algal cells, to ensure uniform average exposure to light and nutrients, to improve gas exchange between the culture medium and the air phase, and to facilitate heat transfer to avoid thermal stratification[47]. Therefore, mixing in a reactor strongly contributes to the growth of algae and improves productivity[49]. Besides preventing cell sedimentation, mixing also prevents the emergence of dead zones and cell attachment to the walls of photobioreactor[42]. Settling and accumulation of cells in dead zones will cause deterioration, anaerobic decomposition and lowering of the quality of the product[52]. Mixing is usually induced by bubbling with CO_2-enriched gas bubbles, pumping, and mechanical agitation with rotation wheels or static mixer for instance, or by the combination of these methods[42,47]. However, mechanical agitation and bubble break-up often lead to hydrodynamic stress as well as shear stress that mechanically affects the cells most severely, resulting in restrictions to the algal growth and metabolic activity[52].

Temperature

Since microalgal production requires a lot of space and light, commercial cultivation is mostly located outdoors. These cultivation systems are exposed to a large range of day/night and seasonal temperature changes and light intensity. Although algae may be able to grow under a variety of tem-

peratures, each strain has a specific temperature required for optimal growth. The optimal temperature for microalgae cultures is usually between 20 °C and 24 °C, but most microalgae can tolerate water temperature between 16 °C and 35 °C[53]. For example, the optimum temperature ranges for *Nannochloropsis*, *Tetraselmis,* and *Isochrysis* were 19–21 °C, 19–21 °C, and 24–26 °C, respectively[49]. It has been reported that, without the temperature control equipment, the temperature in closed photobioreactors can reach from 10 to 30 °C higher values than the ambient temperature[52]. Some mechanisms that can be used to control temperature are submersion of the entire culture in a water pool, spraying the illuminated surface with water, shading or incorporation of a heat exchanger within photobioreactor for cooling[47,54,55]. Due to the expensiveness of cooling, the cultivation should be operated at the maximum possible temperature that still does not induce stress in the organism[52].

pH and pressure

Microalgae require CO_2 as a carbon source for growth, which contributes to control of the pH of the culture. Like temperature, each algal strain has a narrow optimal pH range. Most microalgae species have an optimal pH range between 8.2.–8.7, but they can be cultivated in the pH range between 7 and 9[42,47]. The pH of the culture medium affects both the liquid chemistry of polar compounds and the availability of many algal nutrients, such as iron, organic acids, and CO_2. Moreover, pH variation affects transport systems at the plasmalemma, the electrical charge of the cell wall surface and membrane potentials[52]. Therefore, it is crucial to maintain culture pH in the optimal range. When cultivating with high-density of microalgae and using CO_2-enriched air, CO_2 is consumed by microalgae during photosynthesis. Because of the carbon depletion through photosynthesis, pH in autotrophic algal cultures increases continuously[52]. The concentration of dissolved CO_2, which is the result of the balance between the mass transfer of CO_2 from the gas phase to the liquid phase and the consumption of CO_2 by cells, may be the dominant factor that determines the pH of culture[47]. The standard practice in conventional bioreactors is adding substances such as sodium bicarbonate to control the pH and keep it from rising too fast[42]. Along with temperature and pH, pressure also has a significant effect on the solubility of gases essential for algae, and hence, could have an indirect effect on growth[56].

CO_2 consumption and O_2 removal

One of the three competing cellular processes involved in microalgae cell growth is photosynthesis. The other two are photorespiration and dark respiration[42]. During photosynthesis, microalgae utilize light energy to fix CO_2 resulting in the release of O_2 as a by-product. Microalgae can use carbon dioxide as a carbon source only after it dissolves into the culture medium as bicarbonate. Maintaining the carbon dioxide level in the reasonable range is very important, since a too high concentration of CO_2 will inhibit growth, but it also must never fall below the minimum concentration that will limit growth[5,6]. Simple diffusion of CO_2 from the air (0.03 % CO_2) into water is too slow to replace the amount of CO_2 that is assimilated by rapidly growing algae, and thus, the algal cultures in photobioreactors are generally CO_2-limited[52]. Therefore, enclosed photobioreactors require a continuous supply of soluble inorganic carbon to provide satisfactory growth. Usually, that is achieved by introducing bubbles of CO_2-enriched gas mixture into photobioreactor[52]. An increase in CO_2 concentrations from 1 to 5 % could often lead to maximum growth, but on a laboratory scale, it is common to bubble algal cultures with 5–15 % CO_2 or even pure CO_2[42,49]. Oxygen is a product of photosynthesis with adverse effects on the growth of microalgae. High concentrations of dissolved oxygen are toxic to microalgae and in combination with intense sunlight can lead to photooxidative damage of algal cells[35,41]. For instance, oxygen radicals may develop upon exposure to strong sunlight, and they can cause damage to cytoplasmic membranes and other cellular components[47]. Maximal tolerable oxygen concentration has to be maintained below 400 % of air saturation[35,49]. Two main solutions for removal of excess O_2 are: (1) increasing turbulence by vigorous mixing and by that enhancing mass transfer between the gas and liquid phase, and (2) O_2 stripping with air[52].

Design of photobioreactors

Different types of photobioreactors have been designed and developed for the production of algae (Fig. 2).

Flat panel photobioreactor

Flat panel photobioreactor (Fig. 2a) consists of a frame covered by a transparent plate on both sides, and a pump for inducing the circulation of algal cell suspension[43]. This type of photobioreactor is characterized by a short light path, high illuminated surface to volume ratio, vertical or tilted inclination from the horizontal of the channels, and absence of mechanical devices for cell suspending[43,47]. Generally, it is made of transparent materials like glass, plexiglas, and polycarbonate for maximum utilization of solar light energy[40]. Wasanasathian and Peng report that typically 16 mm thick plexi-

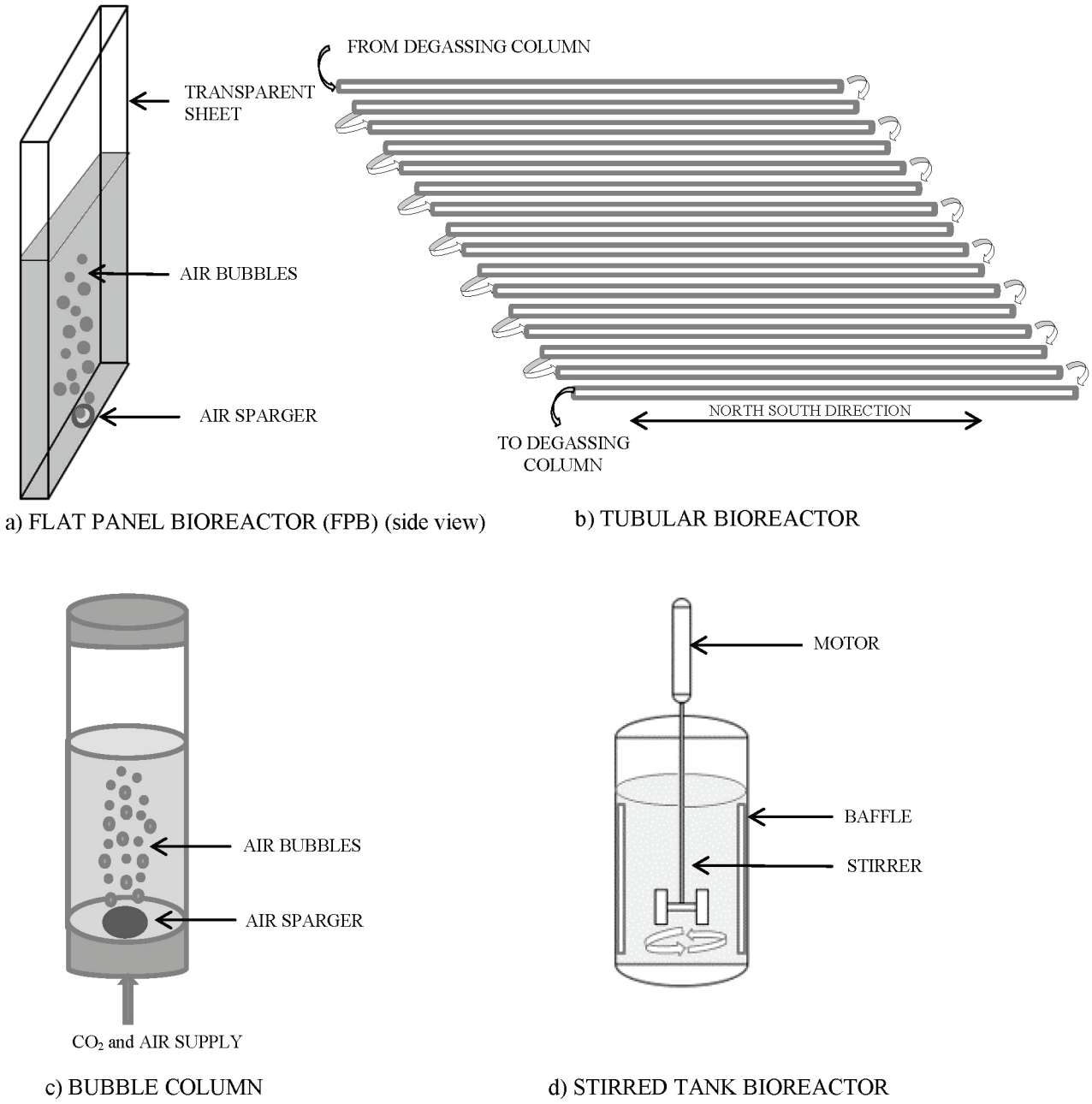

Fig. 2 – *The most common photobioreactor designs (adapted from Gupta et al.[43])*

glas alveolar plates are used in the construction[58]. The thickness of the plate is very important in the design because it determines the surface area/volume ratio and the length of the light path. Higher optimal cell density and biomass productivity are achieved with a shorter light path and smaller thickness[47]. High photosynthetic efficiencies can be achieved by using flat-plate photobioreactor[59]. When comparing to horizontal tubular photobioreactors, the accumulation of dissolved O_2 and its concentrations are relatively low in flat-plate photobioreactors[30,43]. Gas exchange, as well as movement, is performed by bubbling air from the base of each channel[43]. Scale-up can be done by arranging several plates over an area, increasing liquid height and elongating the light path, while enlargement of the bioreactor length is not recommended[40]. Some limitations of this type of bioreactor are the requirement of many compartments when scaling up, difficulty in controlling culture temperature, possible hydrodynamic stress to algae by aeration, and some degree of wall growth[30,43].

Tubular photobioreactors

Tubular photobioreactors are one of the most popular and suitable setups for outdoor biomass cultivations (Fig. 2b)[30,60]. They are comprised of the solar array for algae growth, the harvesting unit to

separate algae from suspension, a degassing column for gas exchange and cooling, and a circulation pump[47]. They can be in the form of a horizontal/serpentine, vertical, near horizontal, conical or inclined photobioreactor[30]. They are usually made of transparent glass or plastic tubes arranged in different patterns, such as the parallel set of tubes, loop shape, bent or spiral[40,45]. The diameter of the tubes is generally 10 mm to a maximum of 60 mm, while the length can be as long as several hundred kilometers[42,43]. In Germany (Wolfsburg), a tubular bioreactor for microalgae cultivation is 500 km long[29]. When compared with vertical bubble column, horizontal tubular photobioreactors have better characteristics, such as surface to volume ratio, amount of gas in dispersion, gas-liquid mass transfer characteristics, and nature of the fluid movement[43]. A greater surface to volume ratio can be provided more easily than with vertical column photobioreactor, because of the ability to decrease the diameter of the tubes without worrying about structural integrity[47]. The high surface to volume ratio, above 100 (m^2 m^{-3}) is one of the main advantages of this design[61]. In a tubular photobioreactor, air is usually introduced by an air-pump or airlift system which causes mixing of culture at the same time. Airlift system is especially attractive for a few reasons, such as (1) achievement of circulation without moving parts, thus providing robust system with a reduced contamination potential, (2) avoidance of the cell damage caused by mechanical pumping, and (3) dual functionality as a pump and gas exchanger for removing oxygen[62]. In an airlift-driven tubular photobioreactor, the culture circulates through a solar collector tubing where the photosynthesis occurs. Produced oxygen remains in the broth until the fluid returns in the airlift zone, where it is stripped by air. A gas-liquid separator in the airlift column prevents the recirculation of gas bubbles into the solar collector[62]. Tubular photobioreactor still has many disadvantages. When scaling up tubular photobioreactors, the poor mass transfer becomes a problem. The gradient of oxygen, CO_2, and pH along the tubes as well as a high level of dissolved oxygen can affect the growth of microalgae[62,63]. In addition, when scaling up by increasing the diameter of the tubes, photo-inhibition usually occurs, because the cells in the centre part of the tube do not receive enough light, and thus their growth is restricted[42]. Further disadvantages include difficult culture temperature control, land requirement, and power consumption[64]. To achieve turbulent conditions, the liquid velocities need to be around 20–50 m s^{-1} resulting in high energy consumption[42]. Energy consumption for tubular photobioreactors is about 2000 W m^{-3}, while for bubble column and flat-plate bioreactors[40] it is about 50 W m^{-3} .

Vertical column photobioreactors

Vertical-column photobioreactors are compact, low-cost, and easy to operate monoseptically[65]. Vertical column photobioreactors have some advantages for microalgal cultivation, such as no moving parts, low power consumption, high mass transfer rate, good solids suspension, homogeneous shear, rapid mixing, and less land requirement[66]. They are characterized by high volumetric gas transfer coefficients. The bubbling of gas from the bottom of the column enables efficient CO_2 utilization and optimal O_2 removal[47]. With a relatively low power input, a mass transfer coefficient of 0.006 s^{-1} can be achieved[66]. They are usually made of transparent materials constructed in a cylinder shape with a radius of up to 0.2 m and heights of up to 4 m [47]. The culture circulation is accomplished either with an air pump or by airlift system. Based on their mode of liquid flow, vertical photobioreactors can be divided into bubble column and airlift reactors. Their common feature is that mixing culture by gas bubbles is gentle and with very little shear stress[65]. Bubble column reactors are cylindrical vessels with a height greater than twice the diameter (Fig. 2c)[40,43]. In scale-up, perforated horizontal plates are used to break up and redistribute coalesced bubbles[43]. In order to improve the mass transfer of gases (CO_2 and O_2), some bubble columns can also be equipped with a rubber membrane diffuser or dual spargers[66]. Light is provided externally and its intensity decreases with distance from an irradiated surface due to self-shading and light absorption. Because of the light gradient across the reactor, algae are exposed to certain light/dark cycles[67]. Thus, as the liquid is circulated from the central dark zone to the external photic zone, the exposition to the light and dark cycle depends on the gas flow rate. Thus, the photosynthetic efficiency also greatly depends on gas flow rate[43]. Compared to the bubble column, airlift photobioreactors have shown superior growth of microalgae[66]. In the bubble column, cell flow patterns are more random, while the airlift system produces a more homogeneous flow pattern that moves cells from dark to light zones[68]. Thus, cells in a bubble column may reside in high or in low light intensities for a long time without circulation[66]. Increasing aeration rate increases mixing, liquid circulation, and gas-liquid mass transfer in both bubble column and airlift bioreactors. High superficial gas velocity also prevents oxygen accumulation and provides efficient use of CO_2, but some microalgal species also suffer from negative effects due to the high shear stress caused by high aeration rate[66]. To increase axial transport, the airlift principle has been employed, and a few different configurations of airlift vertical photobioreactors are possible. Since the airlift photobioreactor does not require a mechanical stirrer,

the risk of contamination and energy demand are reduced[69]. Conventional internal loop airlift photobioreactor is comprised of a transparent column, an internal column, and air sparger which introduces air or CO_2-enriched air in the internal column at the bottom[47]. In a split column airlift photobioreactor, a flat plate splits the diameter of the column and separates the column into two parts (1) the riser, in which liquid is carried upward by air introduced at the bottom, and (2) downcomer region (downer), in which the degassed liquid descends[47]. The next possible configuration is external loop airlift in which degassing occurs in a gas/liquid separation region on the top of the column, while the circulation of liquid is achieved through external circulation column[47]. Besides all the advantages, vertical column photobioreactors do have a few disadvantages as well. There are some restrictions regarding the height and diameter of vertical photobioreactors. The diameter should not exceed 0.2 m because the light would not be available in the centre of the column, which leads to considerable highly dark fraction in the middle of the cylinder that does not contribute to productivity[61]. The height is limited due to structural reasons, precisely the strength of the transparent materials used for construction, but also to reduce mutual shading in large commercial cultivations[42]. Furthermore, in tall columns, CO_2 gradient may be established, which can cause algal starvation and create pH gradients[66]. Moreover, in such columns, the residence time of photosynthetically generated O_2 is increased and it can reach an inhibitory level[47].

Stirred tank photobioreactor

Stirred tank bioreactor is a conventional aerated bioreactor where the mixing is achieved by mechanical agitation (Fig. 2d). This type of bioreactor has been turned into a photobioreactor by illuminating it externally with fluorescent lamps or optical fibers[40]. Stirred tank bioreactors have a very effective stirring mechanism, and hence, mass transfer rates and light dispersion are very high. In addition, a lower incidence of dark zones inside the bioreactor leads to higher biomass productivity[43]. The disadvantage of this type of bioreactor is the small surface to volume ratio, which results in a decrease in light harvesting efficiency[70].

Internally illuminated photobioreactors

One of the most important parameters is the ratio of illuminated surface to the volume of the bioreactor. The bioreactor can operate in higher cell concentration when the illuminated surface is higher. The main disadvantage of external illumination is that the specific volume of the bioreactor decreases when the surface to volume ratio is increased[71]. The purpose of internal illumination is to supply light energy efficiently and economically, to minimize the variation in light regimes in time and space, thus giving a possibility to adapt the light intensity to the growth of the microalgae. The lamp can be inserted in a transparent tube, and placed inside the bioreactor[69]. The other possibility is that the light is collected and concentrated outside the bioreactor and spread inside using glass or acrylic glass fiber optics[50]. The photobioreactor can be modified to utilize both solar and artificial light system, where the solar light intensity source is switched on whenever it decreases below a certain value (*e.g.*, during a cloudy day or at night)[30]. In that way, supply of light to the photobioreactor can be maintained continuously. An example of an internally illuminated photobioreactor is "annular photobioreactor", a special type designed by the Tredici group[72]. The annular bioreactor is constructed of two glass or Plexiglas cylinders of different diameters placed one inside the other to form the culture chamber 5–10 cm thick and 50–200 L in volume. Illumination can be provided by either natural or artificial light. This type of photobioreactor was used to cultivate bioactive *Nostoc* strains, *Nannochloropsis* sp., *Isochrysis* sp. and *Tetraselmis suecica* Butcher under artificial, natural, and combined illumination[72].

Microalgal products

Edible blue-green algae, including *Nostoc*, *Arthrospira* (*Spirulina*) and *Aphanizomenon* species, have been used as food for thousands of years[73]. Besides their use for food and feed purposes due to high protein and minerals content, these organisms are also sources of various commercially produced high-value chemicals, including carotenoids, long-chain polyunsaturated fatty acids, astaxanthin, β-carotene, pigments, and vitamins[8]. Therefore, algae can act as a chemical platform for cosmetic purposes, pharmaceutical, and therapeutic applications, food technology, and production of "green energy carriers" such as biogas, biodiesel, bio-hydrogen, and bioethanol (Fig. 3)[4]. Moreover, green microalgae are considered a good biomanufacturing system for the production of recombinant proteins because they are safe, easy to modify genetically, cheap to cultivate, and scalable[74].

Microalgae in human and animal nutrition

Microalgae can positively affect the health of humans and animals if used as an addition to conventional food preparations by enhancing their nutrient content[73]. Namely, microalgae are a rich source of carbohydrates, proteins, enzymes, and fibers[76]. As microalgae are capable of synthesizing all

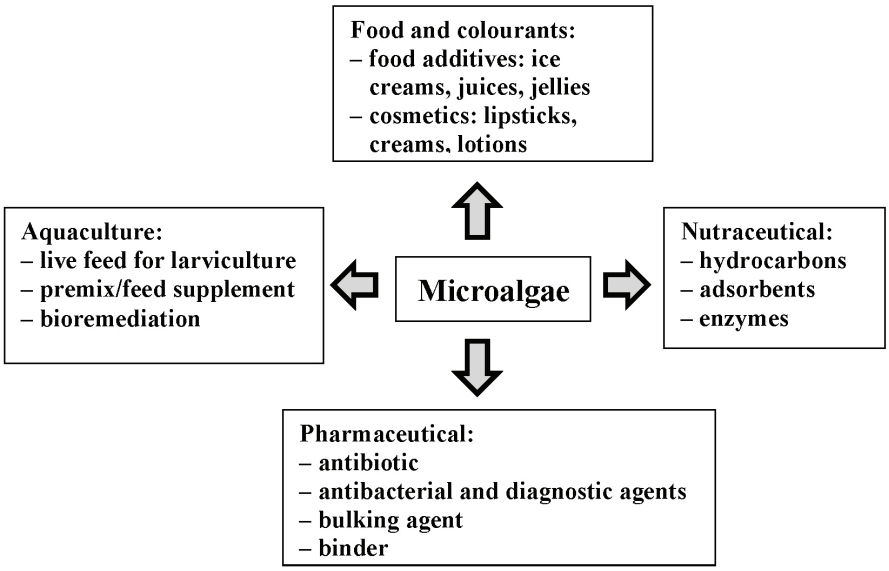

Fig. 3 – *Commercial applications of microalgae in different fields (adapted from Begum et al.[74])*

amino acids, they can provide the essential ones to humans and animals[77]. Carbohydrates in microalgae can be found in the form of starch, glucose, sugars among other polysaccharides. Their average lipid content varies between 1 % and 70 %, but it can reach even 90 % of dry weight under certain conditions[73]. Microalgae also represent a source of many vitamins and minerals, like vitamins A, C, B1, B2, B6, niacin, iodine, potassium, iron, magnesium and calcium[76]. Green algae *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* (Chlorophyceae), and *Spirulina maxima* Setchell and Gardner (Cyanobacteria) are some of the biotechnologically most interesting microalgae. They are mainly used as nutritional supplements for humans and as animal feed additives[76]. Commercial application is dominated by *Arthrospira platensis*, *Chlorella vulgaris*, *Dunaliella salina* and *Aphanizomenon flos-aquae,* Bornet and Flahault[2,73]. Some suggested health benefits of *Chlorella* are efficient on gastric ulcers, wounds and constipation together with preventive action against atherosclerosis, hyper-cholesterol, and anti-tumor activity[25]. *Chlorella* is one of the sources of β-1,3-glucan, which is an active immunostimulator, a free-radical scavenger and a reducer of blood lipids[73]. Another β-1,3-glucan source (paramylon) is *Euglena gracilis* Klebs, which is capable of growing as a strict photoautotroph, a photoheterotroph or a heterotroph. In heterotrophic conditions *Euglena gracilis* can efficiently produce paramylon on different complex media during the repeated batch cultivations, thus there is a high potential for industrial production of paramylon[78,79].

Spirulina is used in human nutrition because of its high protein content and excellent nutrient value[73]. It is also a valuable source of linolenic acid, an essential fatty acid that cannot be synthesized by humans[25]. *Spirulina* biomass used as an extract or processed in pasta, biscuits and other functional food products, supports the function of the digestive tract and helps maintain healthy intestinal bacteria[2]. Some more health-promoting effects are the alleviation of hyperlipidemia, suppression of intestinal *Lactobacillus* and suppression of elevated serum glucose level[80]. *Dunaliella salina* is grown for a source of photosynthetic pigment and beta-carotene which is used as orange dye and as a pro-vitamin A supplement[76]. The biomass of algae is marketed as tablets, capsules, and liquids, which are used as a nutritional supplement or added to pasta, snack foods or drinks as nutrition supplements or natural food colourants[76]. The market of functional foods is believed to be the most dynamic sector in the food industry, and could constitute up to 20 % of the whole food market within the next few years[2]. Besides in human nutrition, microalgae can be used in the feed for different animals, ranging from fish to pets and farm animals. In fact, 30 % of the current world algal production is used as feed supplement[81]. Microalgae have a significant role in aquaculture (mariculture) because they are food sources for larvae of different species of mollusks, crustaceans, and fish. Some of the most commonly used microalgae in aquaculture belong to genera: *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, and *Thalassiosira*[73]. They are utilized in aquaculture as live feeds for all growth stages of bivalve mollusks, for the juvenile stages of abalone, crustaceans and some fish species. Some species like *Dunaliella salina*, *Haematococcus pluvialis*, and *Arthrospira platensis* can be used as a source of natural pigments for the culture of prawns, salmonid fish, and

ornamental fish[81]. *Arthrospira* is also an example of microalgae used in animal feed for cats, dogs, birds, horses, cows, and breeding bulls[73].

Pigments

Synthetic colourants are often used in food products to make them more appealing. They are also commonly used in the nutraceutical and pharmaceutical industries. Their limited application due to regulatory practice for health reasons and their association with several health issues, such as attention deficit hyperactivity disorder, have resulted in rising interest for natural colourants[82,83]. Natural food colourants can be obtained from vegetables and fruits, but the exploitation of microalgal pigments as a source of natural colourants is a very attractive option. As microalgae culture is eco-friendly, renewable, growing rapidly, and can possess a wide range of pigments in higher concentration than those found in higher plants, there is a lot of interest in their use as a source of natural colourants[75,82]. Microalgal pigments find their application in food, nutraceutical, pharmaceutical, aquaculture, and cosmetic industry, but also clinical or research laboratories as a label for antibodies and receptors[75]. The pigments present in microalgae and cyanobacteria are grouped into three categories: chlorophylls, carotenoids, and phycobiliproteins. Astaxanthin, phycocyanin, and β-carotene are presently well-established microalgal pigments produced at large-scale in cultures of microalgae or cyanobacteria and used as natural colours in feed and foods, and as nutritional additives[84].

Chlorophyll is an essential compound used as an additive in pharmaceutical and cosmetic products. In microalgae, one or more types of chlorophyll can be present. Primary photosynthetic pigment chlorophyll *a* has been extensively used as a colouring agent because of its stability[83]. Chlorophyll derivatives can even exhibit health-promoting activities, have a medicinal application due to their wound healing and anti-inflammatory properties and even decrease the risk of colorectal cancer[85,86]. Due to its high deodorant capacity, chlorophyll is used as an ingredient in products for personal hygiene, such as deodorants, pastilles, and it is commercialized in formulations against bad breath[4]. *Spirulina* sp. is used as a colourant substitute for artificially synthesized chlorophyll[4,75].

There are over 400 known carotenoids, but only a few are used commercially, mainly β-carotene and astaxanthin, and of lesser importance, lutein, zeaxanthin, lycopene, and bixin[72]. They are used as food colourants (*e.g.* almond, chicken, egg yolk, butter) and as supplements for humans, and animal feeds[25]. β-carotene can be produced by *Dunaliella salina* which can accumulate β-carotene up to 14 % dry biomass weight when grown under conditions of high salinity and light intensity[10]. The advantage of natural β-carotene in comparison with the less expensive synthetic one is the provision of natural isomers in their natural ratio which are considered superior to the synthetic all-trans form[25,73,84]. The β-carotene is converted by the human body into vitamin A, which assists the body immune system, helps battle eye diseases, various skin ailments, acne, signs of aging and various forms of cancer[83]. It is also responsible for the prevention of toxin build-up in liver[87]. Since β-carotene is found to be negative in genotoxicity tests, it has been approved for use in the USA as a colour additive for foods, drugs, and cosmetics[75]. The second commercialized carotenoid from algae is astaxanthin, which is synthesized only by several green microalgae[73]. The richest source of natural astaxanthin is resting spores, haematocysts, of the freshwater microalga *Haematococcus pluvialis*[84]. *Haematococcus* can contain up to 3 % of dry weight biomass of astaxanthin when produced in two-stage bioprocess[25,73]. The first step is optimized for the production of green biomass of motile-stage algae, followed by an astaxanthin-accumulating stage under intense light conditions and preferably in a nutrient-poor medium[8,73]. The first stage must be performed in a closed photobioreactor, while the second stage can be either in open ponds or in closed photobioreactors[8]. Astaxanthin enhances the immunity of fish and shrimp for efficient growth and survival, but also has an efficient role in aquaculture production and livestock feed market[83]. Although aquaculture market is dominated by synthetic astaxanthin, natural astaxanthin is preferred for some applications (*e.g.* carp, chicken, and red sea bream breeding). Besides for aquaculture, astaxanthin can be used as a nutraceutical, antioxidant, and for health improvement. Astaxanthin is a powerful bioactive antioxidant that has demonstrated efficiency in animal or human models of muscular degeneration causing blindness and in the treatment of Alzheimer's and Parkinson's diseases[83]. This pigment is used in aquaculture especially to provide salmonids the typical ″salmon colouration″ as desired by the customer. It is approved as a food colourant for specific uses in animal and fish food by Food and Drug Administration (FDA), and as feed additive at EU level for salmon and trout at 100 mg kg^{-1} complete feed[75].

Some other carotenoids like lutein, zeaxanthin, and canthaxanthin are used for chicken skin coloration or pharmaceutical purposes[2]. Lutein and zeaxanthin are becoming important in the nutraceutical market since they play a significant role in eye health. Lutein, as a predominant pigment in macula, prevents cataract and macular degeneration[87].

Phycobiliproteins are water-soluble pigments that find their application in food and cosmetics as colours, as possible anti-oxidants in cosmetics, as photosensitizers in photodynamic therapy for the treatment of cancer, as a component of functional foods and as fluorescent tags for flow cytometry and immunology[4,8]. Red phycoerythrin (with phycoerythrobilin chromophores), blue phycocyanin and allophycocyanin (with phycocyanobilin chromophores) are the most often phycobiliproteins[84]. Phycocyanin cannot be made synthetically and is commercially produced using open pond raceway systems for cultivation of *Spirulina platensis,* which can contain phycocyanin in amounts of more than 15 % of dry weight biomass[8,84]. Another source of this pigment is also the red microalgae *Porphyridium aerugineum* Geitler, a red alga differing from other red algae in a lack of phycoerythrin. Its phycocyanin is C-phycocyanin rather than R-phycocyanin that accompanies phycoerythrin found in other *Porphyridium* species and red algae. The blue colour from *P. aerugineum* has yet to be approved for food use[83]. Phycocyanin has antioxidant, anti-inflammatory, neuroprotective and hepatoprotective properties, so it is also used as a pharmaceutical agent[75]. *Porphyridium* species are also a source of fluorescent pink colour, and the main phycobiliproteins are B-phycoerythrin and R-phycoerythrin. Phycoerythrin is also used in colour confectionary, gelatin desserts, fermented milk products, ice creams, sweet cake decoration, milk-shakes, and cosmetics[75]. Phycobiliproteins are widely used in clinical or research immunology[25]. Their properties, such as high molar absorbance coefficients, high fluorescence quantum yield, large Stokes shift, high oligomer stability, and high photo-stability, makes them very powerful and highly sensitive fluorescent reagents[73]. When used as chemical tags, they act by binding to antibodies in immunofluorescence techniques[4,75].

Polyunsaturated fatty acids

Humans and animals lack the requisite enzymes to synthesize polyunsaturated fatty acids (PUFAs) containing more than 18 carbon atoms. Humans and animals need to obtain these fatty acids from food, and therefore, PUFAs are known as essential fatty acids[25]. PUFAs encompass high market value ω-3 and ω-6 fatty acids, such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), g-linolenic acid (GLA), and arachidonic acid (ARA)[4,8]. Fish and fish oils are common sources of such long-chain PUFAs which they accumulate by consuming plankton, an important commercial source of PUFAs[25,88]. Although fish oils are still a less expensive natural source of PUFAs, they are associated with an unpleasant taste, typical fishy smell, poor oxidative stability, and potential danger of accumulated mercury and toxins[89,90]. Alpha-linolenic acid (ALA) is a short chain ω-3-PUFA found in vegetable oils (*e.g.*, flaxseed and rapeseed oil), which can be converted into eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid in the body. However, such conversion is insufficient for providing the amounts required for maintenance of good neural and cardiac tissue development[91]. EPA has been proven to prevent coronary heart disease and to lower blood cholesterol, while DHA also has an important role in the development of the central nervous system of infants[88]. Therefore, fortification of all infant formulas with EPA and DHA was recommended by the Food and Agriculture Organization and World Health Organization back in 1994, and in recent years many EPA/DHA fortified commercial infant formulas are seen on the market[91]. The most relevant microalgal EPA-producers are *Nannochloropsis* sp. and *Phaeodactylum tricornutum*[4]. Diatoms (Bacillariophyceae) can contain 15–30 % of total fatty acid content as EPA, and some representative examples are freshwater diatom *Navicula pelliculosa* Hilse and marine diatoms *Nitzschia frustulum* Cleve and Grunow, *Navicula incerta* Van Heurck, and *Biddulphia sinensis* Greville[91]. DHA is the only commercialized PUFA of algal origin, and it is produced by microalgae such as *Crypthecodinium cohnii*, *Pavlova lutheri* Green or *Schizochytriums limacinum* Honda[4]. Dinoflagellates represent a potential source for DHA production due to the high content of DHA ranging from 12–51% of total fatty acids content. Some representatives are *Crypthecodinium cohnii*, *Amphidinium carteri* Hulburt, *Gymnodinium simplex* Kofoid and *Gyrodinium cohnii* Schiller[91]. Important ω-6 unsaturated fatty acids are arachidonic acid and g-linolenic acid. Arachidonic acid (ARA) acts as a vasodilator and shows anti-inflammatory effects, so it is used for nutrient supplements. One of the potential sources of ARA is species *Porphyridium*[25]. GLA is mainly present in cyanobacterial representatives like *Arthrospira*[4]. GLA is used in the human organism to synthesize prostaglandins, but also can be used in therapeutic purposes for its anti-inflammatory effects and in battling auto-immune diseases[4].

Microalgae as feedstock for biofuels production

Microalgae can be used as feedstock for the production of several types of renewable biofuels. Methane is produced by anaerobic digestion of algal biomass, biodiesel is derived from microalgal lipids, while biohydrogen is produced photobiologically[35,92,93]. In addition, algae have drawn attention as an alternative renewable source of biomass for bioethanol production[94]. Another option is the direct combustion of algal biomass for the production of

steam or electricity[93,95]. Many advantages of using microalgae for biofuels production are described in research reports and articles. In comparison to other feedstocks, algae can provide a high-yield source of biofuels without compromising food supplies, rainforests or arable land[12,94]. It is estimated that algae have the potential to produce two to tenfold more biomass per unit land area than the best terrestrial systems, due to their higher photosynthetic efficiency and greater ability to capture light and convert it to usable chemical energy[96]. Algal cultivation requires less water than in cultivating crops, and moreover, they can grow in brackish water that contains high levels of salt, and in wastewater streams[6,7,97]. This means that algae technology greatly reduces the use of freshwater needed for domestic, industrial and agricultural use. Since microalgae can be cultivated on non-productive and non-arable land, the competition with the land used for the cultivation of crops is avoided[12]. Microalgae can tolerate and utilize high levels of CO_2, and by coupling the algae farm and CO_2 neutral fuel production with sequestration of CO_2 emitted from petroleum-based power stations or other industrial sources, can result in a reduction in greenhouse gas emission[94,95,97]. Moreover, microalgae produce both non-toxic, highly biodegradable biofuels and valuable co-products, such as previously mentioned polyunsaturated fats, pigments, antioxidants, and so on[6,12].

Production of bioethanol from microalgae can be accomplished through a few possible methods. Some microalgae species have high carbohydrate content in their cells making them excellent substrates for the production of bioethanol[98]. Microalgae accumulate carbohydrates mainly in the form of starch and cellulose that need to be hydrolyzed to fermentable sugars by chemical hydrolysis (acid or alkali) or enzymatic hydrolysis before ethanol fermentation with a suitable ethanol producer[94,98]. The cost of bioethanol from algae is increased because of this step due to the high cost of starch and cellulose depolymerizing enzymes. Employing genetic engineering, the production of all necessary enzymes (such as amylases and cellulases) could be triggered within the algae to reduce the production cost. After fermentation, the ethanol is purified by distillation to remove water and impurities, and concentrated ethanol is drawn off and condensed into a liquid form, which can be blended with other fossil fuels[94,99]. Microalgae grow faster and fix CO_2 at a higher rate than terrestrial plants, and do not contain structural biopolymers such as hemicellulose and lignin as higher plants. This, in turn, simplifies the process of bioethanol production by eliminating the chemical and enzymatic pre-treatment steps needed to breakdown these biopolymers into fermentable sugars[100,101]. Furthermore, algae can provide a huge amount of carbohydrate all year round, rather than seasonally[96]. It has been reported that microalgae like *Chlorella, Dunaliella, Chlamydomonas, Scenedesmus, Spirulina* can accumulate a large amount of starch and glycogen (>50 % of the biomass dry weight), making them good candidates for bioethanol production[94,98]. For example, genus *Chlorella* can accumulate high levels of starch, up to 37–55 % of its dry weight[98,102]. Some cyanobacteria and algae can even serve as self-biorefinery for ethanol production during anaerobic dark conditions by utilizing their photosynthates (glucose and sucrose)[12,94]. The cost of such a process could be reduced by the extraction of ethanol directly from the broth, which would eliminate the need to separate the biomass from water and extract[12]. Some microalgae perform so-called dark fermentation, where under dark and anaerobic conditions, polysaccharides in cells are catabolized to ethanol. These microalgae fall under classes Chlorophyceae (*Chlamydomonas, Chlorella*), Prasinophyceae, Cryptophyceae and Cyanophyceae (*Spirulina, Oscillatoria, Microcystis*)[94]. As a third option, there were attempts to produce genetically engineered microalgae for the direct production of ethanol[94]. Using such organisms to directly convert CO_2 to biofuel by photosynthesis would prevent unnecessary expenditure of energy needed to create and destroy biopolymers that are normally used for cell structure or energy storage[95].

Microalgae have a high potential for use in biodiesel production due to their ability to accumulate high amounts of lipids in their biomass. For example, species like *Botryococcus braunii* Kützing or *Schizochytrium* sp. can contain lipids up to 80 % of their biomass dry weight[103]. Lipids from microalgae *Botryococcus braunii* are of great interest because they are not in the form of triacylglycerides, but ″diesel-like″ hydrocarbons that can be branched or not branched, and saturated or unsaturated. They are not a source of biodiesel, and transesterification is not needed, but can be directly implemented after they are processed via hydrocracking[104]. Although microalgae have high potential in biodiesel production compared to other oil crops, biodiesel is currently produced from plant and animal oils. The most commonly used oleaginous crops for biodiesel production are rapeseed, soybean, sunflower, and palm[12,105]. However, to satisfy the needs for biodiesel, the area necessary for the cultivation of major oil crops is unsustainably large[35]. Microalgae are recognized as a suitable alternative feedstock, and some reports suggest that the average biodiesel production yield from microalgae could be 10 to 20 times higher than the yield obtained from oleaginous seeds and vegetable oils[105]. Furthermore, producing biodiesel from algae provides the highest net

energy, because converting oil into biodiesel is much less energy-intensive than methods for conversion to other fuels[12]. The oil content in microalgae is strain-dependent, but the average is from 20 to 50 % of biomass dry weight[19]. Although the oil content could reach up to 75 % of dry biomass weight, low productivity is often achieved[6]. Besides the strain, nutritional and environmental factors and cultivation conditions also affect the lipid content as well as fatty acid composition. When algae are grown under stressful conditions (nitrogen deficiency) or in the presence of supplemental reductants (sugar, glycerol), the metabolism of some species is redirected toward the production and accumulation of energy-dense storage compounds such as lipids[96]. Lipids are accumulated in so-called oil bodies at the expense of energy used for growth, leading to a decrease in growth rate and productivity[106]. The nitrogen starvation is the most efficient approach to induce the lipid storage as well as to control the lipid fractions ratio and the lipid biomass content (70–85 % of biomass dry weight)[19]. In these conditions, the change in biomass lipid composition can also be controlled. For example, it was reported that algae *Botryococcus braunii* had a higher content of oleic acid under nitrogen limitation, but the content of total lipids and triacylglycerols remained unchanged[19]. Production of biodiesel includes several phases. First is algal cultivation followed by harvesting of microalgal biomass. Harvesting of biomass is currently expensive due to high energy requirements and high capital costs[106]. The most common harvesting methods include centrifugation, flocculation, sedimentation, filtration, screening, and flotation[103]. This step of biomass recovery from the culture medium may contribute up to 20–30 % of the total biomass production cost[6]. In addition, algal biomass must be processed quickly after separation to avoid spoilage[6]. To extract oils from the algal biomass, the cells must firstly be disrupted[106]. However, before extraction, biomass must be dried, because the presence of water interferes with lipid extraction and biodiesel production[107]. However, drying microalgae can significantly increase the energy consumption (up to 69 % of total energy consumption), leading to the economic unsustainability of the entire bioprocess[108]. Lipid extraction can be accomplished using chemical solvents, supercritical CO_2, physicochemical, biochemical and direct transesterification[103]. Lowering the bioprocess cost could be accomplished by liquefaction, which has been developed to produce biofuel without the need of drying microalgae[19]. After extraction, the next step is transesterification. Oil characteristics like high viscosity and density could cause deposition in the combustion chamber in engines, and the transesterification process is an essential step, since it reduces the molecular weight and original viscosity and increases the fluidity[19,103]. In this process, a catalyst and an alcohol are added to a blend of microalgae lipids. Alkali, acid or enzyme catalyzed processes may be applied, and the most commonly used alcohols are methanol, ethanol, propanol, and butanol[19,93]. Methanol is the most commonly used due to its low price and physical advantages[103]. Since alkali-catalyzed transesterification is about 400 times faster than the acid catalyzed reaction, it is most frequently used commercially[19]. As the last step, biodiesel and by-products must be separated. For these purposes, hot water (50 °C), organic solvents such as hexane, and water-organic solvent for liquid-liquid separation are most commonly used[103]. Despite all the advantages of biodiesel production from microalgae, the costs of microalgae cultivation have to be drastically reduced to compete with traditional energy sources[12,35]. These bioprocesses are the most economic when they are combined with sequestration of CO_2 from flue gas emission, with wastewater remediation processes, and with the extraction of high-value compounds for application in other industries[6]. For example, one of the most interesting by-products is glycerol, and it can be transformed into value-added products for application in pharmaceutical, cosmetic, and soap industries[103]. Anaerobic digestion is applied to convert organic materials such as biomass into biogas[12]. As mentioned previously, large-scale production of biodiesel from microalgae is limited by the production and lipid downstream costs. The increasing profitability of such products could be achieved by the integration of algae cultivation with existing biogas plant[109]. Discharges of CO_2 and digestate can be used as nutrients for algae cultivation, and attained biomass can be converted to biogas via anaerobic digestion within existing infrastructure[109]. Anaerobic digestion process consists of biochemical degradation of complex organic matter resulting in biogas production. Biogas mainly constitutes methane (CH_4), carbon dioxide (CO_2), and trace amounts of hydrogen (H_2), nitrogen (N_2) and hydrogen sulfide (H_2S)[93,110]. Since a significant amount of biodegradable components is present in microalgae biomass, it is a favourable substrate for such bioprocesses. The advantage is that the algal biomass needs no drying before digestion, and can be directly subjected to the anaerobic break-down in biogas plant[4,111]. Residue from biogas production, so-called digestate, is rich in nutrients such as potassium, phosphates, and minor mineral components, making it a valuable green fertilizer for agriculture[4]. In addition, digestate could be used as an additional nutrient supply in subsequent cultivations[4].

It is expected that the importance of hydrogen (H_2) as a clean fuel in the future will increase. It is regarded a sustainable energy carrier for fuel cells[4]. It is the most advanced carbon-free and CO_2-neutral

fuel, which provides a common energy currency because it can be produced via a range of renewable technologies, including production with microalgae as a biological production system. Solar energy can be converted into chemical energy in the form of hydrogen gas using oxygenic and anoxygenic photosynthetic microbes such as green microalgae and cyanobacteria[112]. A selected group of unicellular algae and cyanobacteria evolved the ability to capture solar energy and use it to split water to produce molecular oxygen as well as H^+ and e^- that are combined to produce hydrogen by either hydrogenase or nitrogenase enzymes[113,114]. The water photolysis in the H_2 production process can be direct or indirect[112]. In the indirect process, solar energy is firstly converted into chemical energy in the form of carbohydrates, which are then used as substrates for H_2 production. This process occurs in cyanobacteria and microalgae, but cyanobacteria utilize both nitrogenases and hydrogenase, whereas microalgae rely on hydrogenases[114]. Due to the high O_2 sensitivity of these enzymes, the photosynthetic production of H_2 and O_2 must be separated either temporally or spatially[112,114]. Spatial separation refers to the production of H_2 in specialized cyanobacterial cells called heterocysts, which maintain low O_2 concentrations and CO_2 fixation in the vegetative cells[112]. However, the ratio of heterocysts to vegetative cells of about 1:10, limits H_2 production levels[114]. Temporal separation refers to the aerobic and anaerobic phase of bioprocess, where the photosynthetic storage compounds accumulate into H_2 either in the dark or in the light with the cells that have impaired O_2-evolving photosystem II activity[112]. In the indirect two-phased bioprocess, the oxygen and hydrogen production phases are successfully separated through sulphur depletion/repletion[113]. Water is oxidized to O_2 by photosystem II and H^+ and electrons are stored in the form of starch during photosynthesis. Under sulphur-deprived conditions, H^+ and e^- are extracted from starch and fed into the PQ pool and onto hydrogenase via photosystem I for H_2 production[113]. Direct photolysis has only been reported in microalgae, and it involves e^- derived from the light-driven water splitting reaction of photosystem II directly to H_2-producing hydrogenase[114]. Such bioprocesses can theoretically achieve 33 % higher efficiencies than the current two-phase process[113]. Although photo-H_2 production is attractive due to no greenhouse gases emission, consumption of CO_2 and advantages when compared to high energy demanding electrolysis of water, more efforts need to be put in technological improvements and genetic engineering of microalgae strains to improve the economic feasibility of bio-hydrogen production[4,112].

Production of polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) have received much attention as a replacement for well-established plastics of fossil origin. Their mechanical properties are similar to polypropylene but they also have attractive properties such as biocompatibility, thermoplasticity, hydrophobicity, piezoelectricity, and stereo-specificity[115,116]. Biodegradability of bio-plastic is an important property considering waste management, and degradation products are carbon dioxide and water. Currently, PHA is produced by heterotrophic bacteria such as recombinant *Escherichia coli* Migula and *Cupriavidus necator* Davis, and these bioprocesses require large amounts of organic carbon sources, which increases the costs of production[116]. Like many other prokaryotes, cyanobacteria can produce PHA as intracellular and carbon storage compounds, but in contrast to heterotrophic PHA producing bacteria, they require no organic carbon source[116]. Cyanobacteria are the only described group of PHA-accumulating oxygenic photoautotrophs[4,115]. The industrial utilization of cyanobacteria for production of PHA has the advantage of converting waste greenhouse gas carbon dioxide to environmentally friendly plastics using the energy of sunlight[117]. Poly(3-hydroxybutyrate) (PHB) is a common biopolymer, which is an attractive alternative to common plastics due to its advantageous properties such as hydrophobicity, complete biodegradability, biocompatibility, absolute resistance to water, and thermoplastic process ability[117,118]. PHB is also found frequently in cyanobacteria as an energy and carbon storage compound[116]. Since cyanobacteria have minimal nutrient requirements, due to their photoautotrophic nature, they are also considered as an alternative host system for PHB production[117]. Moreover, these organisms can be cultivated in wastewaters due to their ability to use inorganic nitrogen, phosphorous and wastewaters from farm-yards, fish farms, rubber industries, and sewage treatment plants, which are rich in nitrogen and phosphorous[117]. The PHB content of cyanobacteria is highly strain-specific but physiological stresses such as nutrient deficiencies are found to direct metabolic fluxes to provoke the PHB accumulation under photoautotrophic growth environments[115]. Nitrogen and phosphorus depletion are the most important factors to increase the PHB content, and often even necessary to produce any PHB at all[116]. Cyanobacteria can also accumulate PHAs under mixotrophic growth conditions with organic substrates such as acetate, glucose, propionate, valerate, and so on[115]. Although heterotrophic cultivation boosts the PHB content (over 30 % of biomass dry weight was reported when using acetate as carbon source), it impairs the attractive feature of cyanobacteria of converting CO_2 to PHB. Moreover, using organic sources of

carbon could easily lead to contamination and culture crashes[116]. Although this bioprocess is not economical today, cyanobacteria have the potential to produce biopolymers like PHB from CO_2 as the sole carbon source, but the yield of PHB could be increased by various means, such as nutrient limiting or stress conditions or different PHB enhancing precursors in vitro[117].

Wastewater treatment and phytoremediation processes using microalgae

Due to the ability of microalgae to remove nutrients, heavy metals, organic and inorganic toxic substances, and other impurities present in wastewater by using sunlight and CO_2, they can be used in phytoremediation and wastewater treatment processes[119]. Conventional wastewater treatment processes are simple and efficient but expensive bioprocesses that require high energy input, qualified personnel to manage bioprocess adequately, and they have a significant environmental impact due to the emission of greenhouse gases[120]. Because wastewater contains carbon, nitrogen, phosphorus, and other minor components, its composition is similar to the culture media usually used in the microalgae production, thus, it could be used for cultivating microalgae[120]. Algal wastewater treatment is effective in the removal of these nutrients (C, N, and P), coliform bacteria, heavy metals, reduction of chemical and biological oxygen demand, removal and degradation of xenobiotic compounds, and other contaminants[121]. The microalgae system can treat various types of wastewater such as domestic sewage, but also industrial wastewaters[119]. By metabolizing impurities from wastewater using sunlight and CO_2, microalgae synthesize their biomass and produce oxygen in amounts sufficient to meet the most aerobic bacterial requirements and release a large amount of simpler organic compounds that can be assimilated in an aqueous system. These heterotrophic bacteria, in turn, constitute an essential source of CO_2 for algal growth, stimulate the release of vitamins and organic growth factors, and change the pH of the supporting medium for algal growth, and further reduce nutrient concentrations[119,121]. Moreover, some algae show a high tolerance to heavy metals and high capacity for their accumulation. Combined with the ability to grow both autotrophically and heterotrophically, large area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation, some microalgae are ideal candidates for phytoremediation processes for selective removal and concentration of heavy metals[122]. These microalgae and cyanobacteria-based phytoremediation technologies have gained much attention recently for an eco-friendly approach to the cleaning of metal-contaminated wastewater, industrial effluents, and soil matrix[121].

Concluding remarks

Currently, there are several applications of microalgae, including human and animal nutrition, cosmetics and production of high-value products such as lipids, pigments, and vitamins. Although these microalgal products are well-established in the market, microalgae still represent an almost untapped resource. Considering their enormous biodiversity, the potential of microalgae for new products and applications is significant. The huge range of different products accessible from the primary and secondary metabolism of diverse microalgae species demonstrates their importance as cellular factories yet to be explored. Moreover, recent progress in the field of genetic engineering allows overcoming problems in cultivating microalgae, and thus making the production of these products economically cost-effective. To conclude, microalgae have huge potential, however, there are still many problems that need to be overcome to maximize utilization of these powerful phototrophic cell-factories.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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