Review

Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions

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Unbalanced production of atmospheric CO_2 constitutes a major challenge to global sustainability. Technologies have thus been developed for enhanced biological carbon fixation (also referred to as CO_2 mitigation), and one of the most promising capitalizes on microalgae. However, the ''best bioreactor'', which would be able to achieve maximum productivity and maximum energy efficiency under a given set of operational costs, does not exist. This review briefly examines the current technologies available for enhanced microalgal CO_2 fixation, and specifically explores the possibility of coupling wastewater treatment with microalgal growth for eventual production of biofuels and/or added-value products, with an emphasis on productivity. In addition, an overview of reactor configurations for CO_2 fixation and bottlenecks associated with the underlying technology are provided. Finally, a review of life cycle analysis studies is presented, and routes for improvement of existing processes are suggested.

Introduction

An increase in atmospheric CO_2, derived from fossil fuel combustion, poses great challenges to worldwide sustainability [1]. Available technologies for CO_2 removal/capture include physicochemical absorbents, injection into deep oceans and geological formations, and enhanced biological fixation (or mitigation). Adsorbent materials (e.g. LiOH) are typically non-renewable and require significant space for storage. Other abiotic methods are based on direct injection of CO_2 into the deep ocean, geological strata, old coal mines, oil wells or saline aquifers, as well as mineral carbonation of CO_2. These methods present significant challenges, including high space requirements and potential CO_2 leakage over time [2]. Hence, biological mitigation is the only economically feasible and environmentally sustainable technology in the long term. Biological mitigation does not provide for permanent CO_2 sequestration because carbon taken up during photosynthesis is actively cycled (e.g. released during combustion of the biofuels produced). Instead, atmospheric carbon is conveyed into a cycle in which no additional CO_2 is created, while nutrient utilization and energy production can be achieved in a sustained fashion.

The urgent need for substantive net reductions in CO_2 emissions to the atmosphere can be addressed via biological CO_2 mitigation, coupled with transition to more extensive uses of biofuel, nuclear and renewable energy sources. Microalgae have attracted a great deal of attention for CO_2 fixation and biofuel production because they can convert CO_2 (and supplementary nutrients) into biomass via photosynthesis at much higher rates than conventional biofuel crops can. This biomass may then be transformed into methane [3,4] or hydrogen, using processes mediated by anaerobic bacteria; an integrated process for hydrothermal production of methane via microalgae has been discussed recently [5]. Of particular interest is the production of oils by microalgae because of the ease of their synthesis (a lack of a nitrogen source usually suffices to trigger this form of secondary metabolism). Lipid extraction and re-esterification are accomplished with short-chain alcohols and other by-products of secondary metabolism (i.e. polyunsaturated fatty acids, β-carotenes or polymers [3]). Upon extraction, such oils can be hydrolyzed and then re-esterified with methyl- or ethyl alcohol moieties to obtain biodiesel.

Microalga-mediated CO_2 fixation and biofuel production can be rendered more sustainable by coupling microalgal biomass production with existing power generation and wastewater treatment infrastructures (Figure 1). Microalgae can utilize low-quality water, such as agricultural runoff or municipal, industrial or agricultural wastewaters, as a source of water for the growth medium as well as a source of nitrogen, phosphorus and minor nutrients [6]. Hence, an additional economic and environmental incentive exists as a result of the decreased cost of water and chemicals required for the formulation of the growth medium, while providing a pathway for wastewater treatment [7,8]. Although the most common application of

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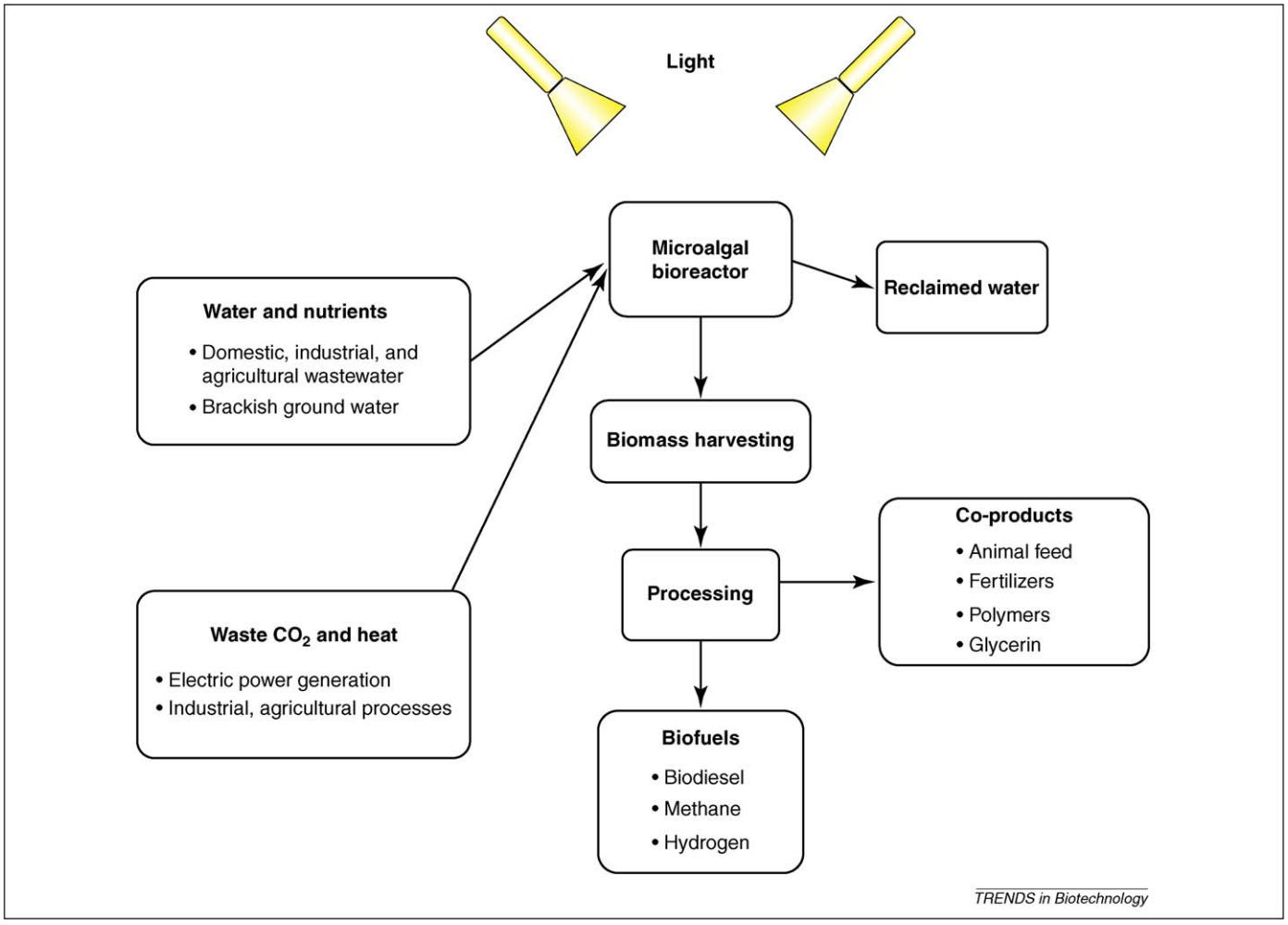

Figure 1. Integration of microalgal bioreactors into existing wastewater and power generation infrastructures. The overall process uses microalgae to capture industrially produced waste CO_2 in photobioreactors, coupled with treatment of nutrients in wastewater. CO_2 is converted into algal biomass by photosynthesis in the presence of light. After processing (biological, physical or thermochemical), the biomass generated can be used for production of biodiesel, methane or other fuels and co-products (e.g. animal feeds and polymers).

microalgae in wastewater treatment aims at nutrient removal/recovery, microalgae have also been utilized for removal of heavy metals and organic matter; the latter requires heterotrophic metabolism. A consortium of microalgae and bacteria has been used to biodegrade black oil and detoxify industrial wastewater [10]. Biogas production can be accomplished through digestion of microalgae or co-digestion of microalgae and sewage sludge [4]. Finally, secondary utilization of microalgae has been successful in toxicity monitoring [9].

The combination of the three roles of microalgae – CO_2 fixation, wastewater treatment and biofuel production – has the potential to maximize the impact of microalgal biofuel production systems, and has accordingly been investigated [11,12]. However, a number of crucial research gaps remain that must be overcome to achieve full-scale operation [13,14], including: (i) improved algal growth and nutrient uptake rates; (ii) integration of biosystems with waste gas, wastewater and water reclamation systems; (iii) improved gas transfer and mixing; (iv) improved algal harvesting and dewatering; and (v) life cycle analysis (LCA) and associated economic assessment. In addition, there is a lack of fundamental information needed to rationally optimize the performance of existing bioreactors. Novel bioreactor configurations and designs are also needed that promote microalgal growth, characterized by volumetric productivities at least one order of magnitude above those of conventional open pond facilities.

This article reviews the major issues associated with the dominant biotic and abiotic factors that affect microalgal growth and bioreactor systems, with an emphasis on reactor configurations and associated operational features. Downstream separation and production of valuable products, and how they might affect environmental sustainability and economic feasibility of the underlying microalga-based processes are also discussed.

Biocatalysts and biotic/abiotic factors

Microalgae are part of a large and diverse group of photosynthetic microorganisms, which can exist as individual cells, chains or groups, and are found in either freshwater or marine environments. Cyanobacteria – also known as blue-green algae – constitute a phylum of bacteria that also obtain their energy from photosynthesis; these are included in the general group of microalgae for the purpose of this review, even though they are rather distant taxonomically (e.g. the latter are prokaryotes, whereas the

former are eukaryotes) [6]. To date, over 40 000 species of microalgae and cyanobacteria have been catalogued. Among the largest groups of microalgae, *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including diatoms) and *Chrysophyceae* (including golden algae) are the most frequently cited as carrying one or more of the desirable features for efficient and economical combination of CO_2 fixation, wastewater treatment and lipid synthesis toward biofuel production.

The most relevant environmental factors that affect the growth of microalgae include light, temperature, pH, salinity, nutrient qualitative and quantitative profiles, and dissolved oxygen (DO), as well as levels of toxic elements/compounds, such as heavy metals or synthetic organics. Biological factors that might constrain microalgal growth rates include predation, viruses, competition and growth of epiphytes [15]. Finally, microalgal growth can be affected by such reactor operating conditions as hydraulic residence time, harvesting rates, gas transfer and mixing equipment, because they affect CO_2 availability, shear rates and light exposure.

Energy harvesting

Sunlight is the most common source of energy for microalgae, to an extent that is rather species-dependent. When light is the only limiting factor, microalgal productivity becomes proportional to the light conversion efficiency [16]. Although light intensity requirements of typical microalgae are relatively low compared with those of higher plants, microalgal activity usually rises, with increasing light intensity, up to 400 $\mu mol\ m^{-2}\ s^{-1}$ [9]; as an example, the saturating light intensity of *Chlorella* and *Scenedesmus* sp. is of the order of 200 $\mu mol\ m^{-2}\ s^{-1}$ [17]. Many microalgal species can switch from phototrophic to heterotrophic growth, and some can even grow mixotrophically (i.e. using photosynthesis for energy production, but organic carbon compounds for biosynthesis) [15].

Carbon uptake

Biological CO_2 fixation can be carried out by higher plants and microalgae, yet the latter possess a greater ability to fix CO_2 [3,18,19]. Usual sources of CO_2 for microalgae include: (i) atmospheric CO_2; (ii) CO_2 from industrial exhaust gases (e.g. flue gas and flaring gas); and (iii) CO_2 chemically fixed in the form of soluble carbonates (e.g. $NaHCO_3$ and Na_2CO_3). The tolerance of various microalgal species to the concentration of CO_2 is variable; however, the CO_2 concentration in the gaseous phase does not necessarily reflect the CO_2 concentration to which the microalga is exposed during dynamic liquid suspension, which depends on the pH and the CO_2 concentration gradient created by the resistance to mass transfer. Under heterotrophic or mixotrophic conditions, some microalgal species can metabolize a variety of organic compounds, including sugars, molasses and acetic acid, as well as compounds present in wastewater and petroleum [6].

Atmospheric CO_2 levels [~0.0387% (v/v)] are not sufficient to support the high microalgal growth rates and productivities needed for full-scale biofuel production. Waste gases from combustion processes, however, typically contain >15% (v/v) CO_2; this percentage indicates, in principle, that combustion processes will provide sufficient amounts of CO_2 for large-scale production of microalgae. Owing to the cost of upstream separation of CO_2 gas, direct utilization of power plant flue gas has been considered in microalgal biofuel production systems [2]. Flue gases that contain CO_2 at concentrations ranging from 5 to 15% (v/v) have indeed been introduced directly into ponds and bioreactors of various configurations that contain several microalgal species (Table 1).

Nutrient requirements

Apart from carbon, nitrogen is the most important element that is required for microalgal nutrition [6] and, as a constituent of both nucleic acids and proteins, nitrogen is directly associated with the primary metabolism of microalgae. Fast-growing microalgal species prefer ammonium rather than nitrate as a primary nitrogen source [21]; intermittent nitrate feeding, however, will enhance microalgal growth if a medium that lacks nitrate is used [22]. Under partial nitrogen deprivation, microalgae grow at lower rates (as expected), but produce significantly more lipids, which are reserve compounds synthesized under stress conditions, even at the expense of lower productivities [23].

Phosphorus is the third most important nutrient for microalgal growth, and should be supplied to significant excess as phosphates because not all phosphorus compounds are bioavailable (e.g. those combined with metal ions) [12]. In the case of marine microalgae, seawater supplemented with commercial nitrate and phosphate fertilizers is commonly used for production of microalgae [21]. Nevertheless, trace species, such as metals (Mg, Ca, Mn, Zn, Cu and Mb) and vitamins, are typically added for effective cultivation [6]. Note that phosphorus is among the crucial elements that limit human growth, so any form of bioenergy production should be efficient in cycling of phosphorus (unlike nitrogen, which can be fixed from atmospheric N_2).

Temperature effects

Temperature is one of the major factors that regulate cellular, morphological and physiological responses of microalgae: higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of microalgal growth [9]. The optimal temperature varies among microalgal species [24]; however, optimal temperatures are also influenced by other environmental parameters, such as light intensity. Optimal growth temperatures of 15–26 °C have been reported for some species, with maximum cell densities obtained at 23 °C. Only daytime higher temperatures were observed to have clearly favorable effects on microalgal growth rates due to photosynthesis, except when the night temperature was as low as 7 °C [25].

pH effects

Most microalgal species are favored by neutral pH, whereas some species are tolerant to higher pH (e.g. *Spirulina platensis* at pH 9 [26]) or lower pH (e.g. *Chlorococcum littorale* at pH 4 [27]). There is a complex relationship

Table 1. Performance of microalgal bioreactors with various configurations

Reactor type	Microalga species	CO_2 feed gas (%)	CO_2 fixation rate ($g/m^3/h$), or removal efficiency (%)	[d]Specific growth rate (/h), or [e]biomass productivity ($g/m^3/h$)	Refs.
Batch reactors	*Chlamydomonas reinhardtii*	30*	NA	0.08 ± 0.01[d]	[29]
	Chlorella pyrenoidosa	100*	NA	0.09 ± 0.09[d]	[29]
	Scenedesmus obliquus	60*	NA	0.06 ± 0.04[d]	[29]
	Chlorogleopsis sp.	5	0.8-1.9[a]	0.0007-0.0060[d]	[27]
	Spirulina platensis	0.03	NA	0.0082 ± 0.0020[d]	[12]
Open pond reactors	*Nannochloropsis salina*	15	NA	4.1[e]	[20]
	Chlorella sp.	6-8	10-50%	NA	[20]
	N. salina	5	NA	1.25[e]	[32]
Bioreactors	*Porphyridium* sp., *Botryococcus braunii*	2-3	3-18[a]	NA	[37]
	Euglena gracilis	11	3.1[a]	4.8[e]	[36]
	Chlorella vulgaris	1	128[b] and 141[b]	NA	[38]
Membrane bioreactors	*C. vulgaris*	1	NA	4[e]	[39]
	Nannochloropsis sp.	1	NA	4.2-5.8[e]	[14]
			NA	0.8-41.7[e]	[14]
	C. vulgaris	1	80-260[b]	NA	[35]
	C. vulgaris	1	43[b] and 275[b]	NA	[38]
	C. vulgaris	0.045	148[a]	NA	[40]
	S. platensis	2-15	38.3-60[c]	3-17.8[e]	[12]

*μM in medium.

Abbreviation: NA, not available.

CO_2 fixation rate calculated from: [a]biomass carbon content and growth rate ($g/m^3/h$); [b]$R_{CO2} = [(P_0 + \rho gh)y_{CO2,in} - P_0 y_{CO2,out}]F_{air}MW_{CO2}/8.314TV$, in which $y_{CO2,in}$ and $y_{CO2,out}$ are the CO_2 molar fractions in the inlet and outlet gas/phases, respectively, P_0 is the atmospheric pressure (Pa), ρ is the density of liquid (kg/m^3), h is the vertical height of the medium (m), F_{air} is the air volume flow rate in the gas phase (m^3/h), MW_{CO2} is the molecular weight of CO_2 (g/mol), T is the ambient absolute temperature (K) and V is the volume of the culture medium (m^3); [c]difference in inlet and outlet CO_2 concentrations, divided by the gas residence time in the reactor ($g/m^3/h$).

between CO_2 concentration and pH in microalgal bioreactor systems, owing to the underlying chemical equilibria among such chemical species as CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}. Increasing CO_2 concentrations can lead to higher biomass productivity, but will also decrease pH, which can have an adverse effect upon microalgal physiology. By contrast, microalgae have been shown to cause a rise in pH to 10–11 in open ponds because of CO_2 uptake [28]. This increase in pH can be beneficial for inactivation of pathogens in microalgal wastewater treatment, but can also inhibit microalgal growth. Similarly, the speciation of NH_3 and NH_4^+ in microalgal bioreactors is strongly dependent on pH – NH_3 uncouples electron transport in the microalgal photosystem and competes with water molecules in oxidation reactions, thus leading to release of O_2 [26].

Toxic compounds

Elements and compounds that may be toxic to microalgae include heavy metals and various gases, such as CO_2, NO_x, SO_x, O_2 and NH_3. Optimal CO_2 concentrations vary greatly among microalgal species, as discussed previously. Common freshwater microalgae exhibit changes in photosynthetic characteristics when grown under high CO_2 concentrations [i.e. above 5% (v/v)]. These changes include lower affinity to CO_2, higher photosynthetic sensitivity to O_2, higher CO_2 compensation points and lower activity of carbonic anhydrase [29]. The effect of trace acid gases (NO_x and SO_x) on microalgal growth has been determined using both model gases [31,39] and actual flue gases [32]. Maeda *et al.* [31] have examined the tolerance of a strain of *Chlorella* to NO_x and SO_x, and found that the strain can grow under trace element addition. Furthermore, Yoshihara *et al.* [30] have reported that *Nannochloropsis* sp. can grow with 100 ppm NO, whereas Matsumoto *et al.* [32] have observed that *Tetraselmis* sp. can withstand flue gases that contain up to 185 ppm SO_x and 125 ppm NO_x, as well as 14.1% (v/v) CO_2. When the concentration of SO_2 is high (>400 ppm), the pH of the medium will decrease, thus resulting in low productivity. By contrast, NO at ~300 ppm does not directly influence microalgal growth because NO absorbed by the cultivation medium is changed to NO_2^-, and thus can be further utilized as a nitrogen source.

Photosynthesis is a reversible set of reactions, and excessive dissolved oxygen, DO (i.e. >35 mg/l), can inhibit the metabolic processes [33]. DO supersaturation in enclosed bioreactors designed for mass microalgal cultivation can reach levels as high as 400%, thus severely inhibiting microalgal growth [34]. Furthermore, microalgae are negatively charged on their surface, so they can strongly adsorb polyvalent cations; this ion exchange capacity is the basis of the microalgal potential to remove heavy metals from wastewaters [9]. However, heavy metals are potent inhibitors of microalgal photosynthesis because they can replace or block the prosthetic metal atoms in the active site of relevant enzymes – or otherwise induce morphological changes in the microalgal cells that lead to physiological incompatibility.

Bioreactors and biotic/abiotic factors

Production of microalgae can be accomplished using either open pond processes or enclosed bioreactor systems; the latter generally offer better process control and higher biomass productivity. Additionally, enclosed bioreactors can be constructed vertically to minimize space requirements [13]. Nevertheless, enclosed bioreactors have higher construction costs and more complex operation

and maintenance, thus potentially leading to poorer overall energy efficiency and cost effectiveness [9]. In the case of systems that combine microalgal biomass production with wastewater treatment, additional advantages are gained by decreasing the discharge of pollutants into the environment, thus mitigating CO_2 and producing more high-added-value compounds – all of which will lead to different cost structures. A schematic description of the interrelationships between the various parameters that influence overall performance of microalgal bioreactors is shown in Figure 2. Several important considerations for bioreactor design, scale-up and operation are discussed below, including configuration, gas transfer, mixing rates, light provision and metabolite recovery. Although there are conflicting views as to whether the design of algal biofuel systems should be based on the overall goal of maximizing energy efficiency or biomass productivity, this review specifically focuses on the latter.

A summary of results from representative bioreactor configurations is presented in Table 1. A wide range of biomass production and CO_2 fixation rates can be found in the literature, thus reflecting the many factors discussed previously that influence biomass productivity and CO_2 uptake [35]. The extent of CO_2 fixation and the rate of biomass production tend to be higher in membrane bioreactors than in more conventional bubbling systems. However, several gaps still need to be filled regarding membrane bioreactors for microalgal production, including membrane durability and performance, and biofouling during long-term operation [35].

Reactor configuration

Bioreactors employed for cultivating microalgae include horizontal tubular reactors, vertical tubular reactors, helical tubular reactors, fermentor-type reactors, flat plate reactors and hollow fiber membrane reactors [33]. Photobioreactors can offer much higher areal production rates, provided that improvements are introduced regarding: (i) solar energy and CO_2 utilization; (ii) gas transfer and mixing; and (iii) harvesting. Unfortunately, none of the aforementioned bioreactor configurations is able to control effectively all process parameters that are required for maximum microalgal growth and metabolic rates.

Gas transfer

Gases introduced into bioreactors serve a number of purposes in microalgal cultivation, including: (i) supply of CO_2 and possibly other gases (e.g SO_x or NO_x) as sources of carbon (or sulfur and nitrogen, respectively) for biomass primary and secondary metabolism; (ii) provision of internal mixing, which avoids nutrient concentration gradients; (iii) promotion of exposure of all cells to light (especially in high density cultures), while minimizing self-shading and phototoxicity; (iv) control of pH by assuring dissolution of CO_2 and avoiding gradients thereof; and

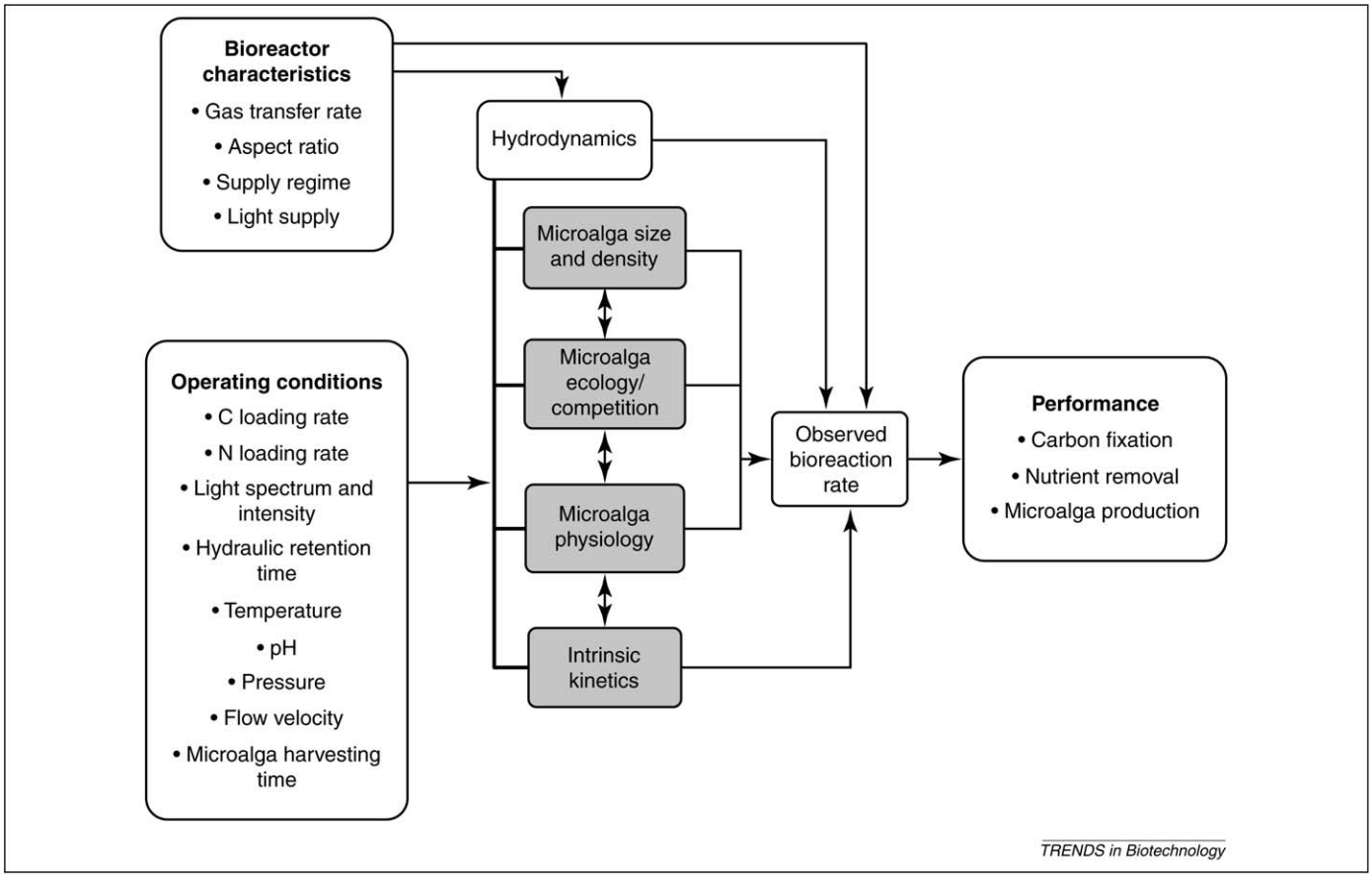

Figure 2. Impact of various factors upon performance of microalgal photobioreactor processes. Bioreactor characteristics and operating conditions affect physical hydrodynamics of the medium supporting system, growth kinetics and metabolism of the microalgae; these will lead to the observed bioreaction rate, which can be assessed in terms of several performance indicators, including carbon fixation, nutrient removal and microalgal production.

Table 2. Methods for CO_2 supply in microalgal bioreactors with various configurations

Process	Type of reactor	Gas transfer	Mixing	Hydrodynamic stress	Scale-up	Refs.
Surface aeration	Unstirred shallow ponds	Poor	Poor	Low	Difficult	[47]
Membrane transfer	Hollow-fiber membrane	Very good	Uniform	Medium	Medium	[33]
Gas injection	Airlift Bubble column Stirred-tank Flat-plate Tubular	Fair to good	Uniform	Low to high	Medium	[42]
Gas exchange	Tubular solar receptor	Good	Good (by pumping)	Medium	Difficult	[33]

(v) stripping of accumulated DO, hence reducing its toxicity to microalgae [13].

Available gas transfer equipment includes mechanical systems (e.g. propellers, blades and brushes), coarse and fine bubble diffusers (e.g. perforated piping, slotted tubes, discs or domes), jet aerators, aspirators, U-tubes and hollow-fiber membrane modules (Table 2). Among the various alternatives, bubbling CO_2-enriched air into the bottom of the bioreactor with bubble diffusers has been the most frequently used approach. Moderate overall transfer efficiencies (13–20%) can be achieved by this mode of gas delivery [33]; however, associated drawbacks are loss of CO_2 to the atmosphere, biofouling of diffusers, and poor mass transfer rates owing to a relatively low interfacial specific surface area. Better overall efficiencies are expected for hollow-fiber membrane bioreactors in which the slightly lower mass transfer coefficients that arise from a less turbulent local hydrodynamic pattern are compensated by the much larger area per unit volume available for mass transfer. In addition, the area of mass transfer is well defined, and the pressure on the gas side can be controlled so as to supply only the required amount of CO_2, hence permitting more accurate control of the transfer rate and a dramatic reduction in the amount of CO_2 lost to the atmosphere [14].

In addition to the physical transport of CO_2 required by microalgal photosynthesis, mixing also promotes chemical reactions between CO_2 and H^+, OH^-, H_2O and NH_3; all of these exert a great influence upon CO_2 uptake rates. These reactions are essentially reversible, so pH regulation is essential in microalgal bioreactors for control of CO_2 transfer. The most common system employed for pH control is the on–off type system in which CO_2 is injected into the culture when the pH exceeds a predefined set point. Hence, CO_2 is used not only as a critical nutrient, but also to buffer the medium; this adds to the complexity of microalgal bioreactors as compared to their bacterial, yeast or fungal counterparts.

Another specific issue of microalgal bioreactors is the accumulation of photosynthetically generated oxygen, especially when the rate of photosynthesis, which often correlates with the rate of CO_2 transfer, is high (as typical in horizontal tubular reactors). Most solutions to this problem rely on the use of a degasser (or gas exchange unit), where DO can be released [41]. However, to attain effective separation between the gas and liquid phases, the path through the degasser should be such that the smallest bubbles have sufficient time to disengage from the liquid. In solid tubular bioreactors, connections between tubes can incorporate a tube specifically for oxygen degassing, or a layer of parallel tubes connected by two manifolds: the lower manifold is used to inject air into the culture, and the higher one acts as the degasser. Nevertheless, microalgal productivities were lower than expected in these tubular systems, possibly because of build-up of DO during high light intensity periods and along the reactor path between manifolds. In systems with exhaust gas recirculation, DO accumulation can be avoided by bubbling exhaust gas through a sodium sulfite solution before its return to the bioreactor [42].

Unfortunately, the efficiencies of most techniques used to date for DO removal from microalgal cultures are still not satisfactory. As a result, the classical bubbling mode of operation has been employed to avoid the costlier need for degassing devices. The use of several small bioreactors instead of one larger unit also alleviates this problem. Scale-up is indeed easier for facilities that use many small reactors in parallel, even though investment costs might be higher than with fewer large equipment units. Continued research is needed to accurately match the amount of CO_2 supplied to the actual uptake requirement of the metabolizing microalgae, as well as the amount of O_2 removed to the actual amount of O_2 produced.

Mixing rates

Mixing is a key parameter for acceptable performance of microalgal bioreactors. Low mixing rates hamper gaseous mass transfer and might even permit biomass settling. In either case, poor mixing leads to emergence of stagnant zones, where light and nutrients are insufficiently available and anoxic/anaerobic conditions will thus prevail, which results in a decrease of productivity. Culture viability might also be compromised by production and accumulation of toxic compounds in stagnant zones [6]. Conversely, high mixing rates can cause shear damage to cells [15], besides requiring a large energy input.

The most common methods of mixing in microalgal bioreactors are pumping, mechanical stirring and gas injection. Pumping offers fair mixing efficiency, but low gas transfer rates; the associated hydrodynamic stress increases with the rotation speed of the pumps, or the number of passes of the microalgal suspension through the pump units [43]. Mechanical stirring has been reported to provide good mixing efficiency and gas transfer; however, it is likely to produce significant hydrodynamic stress [44], which can be managed via adequate use of baffles to create a controlled turbulence pattern. Gas injection (bubbling) produces lower hydrodynamic stress, while providing good gas transfer and reasonable mixing efficiency [45]; however, cell damage in sparged cultures increases as the

biomass concentration increases, because exponentially higher degrees of stirring are needed to maintain a high-density culture at a predefined level of mixing [13]. One approach to minimize this problem is to maintain a low gas input per nozzle, so as to reduce shear stress and consequent cell damage [46].

Light provision

Light is the basic energy source for phototrophic microorganisms. The intensity and utilization efficiency of the light supplied are thus of crucial importance in microalgal bioreactors. Light intensity decreases deeper within the culture medium, especially in high-density cultures; hence, the issue of optical depth, which measures the proportion of radiation absorbed or scattered along a path through a partially transparent medium, should be considered in microalgal bioreactor design.

Both sunlight and artificial light have been used via outer surface exposure as well as inner volume exposure, through the placement of lighting devices (e.g. LEDs or optical fibers) inside the reactor itself [48]. The photosynthetically active radiance is normally assumed to be 43–45% in the wavelength range of 400–700 nm [49]. The light intensity available to microalgae in high-density cultures is significantly attenuated by mutual shading; to maximize light absorbance and minimize light attenuation, bioreactors should be designed with a high surface area-to-volume ratio, coupled with a short light path [16]. Good microalgal growth rates have been reported [26] under a light intensity of 4000 μmol m^{-2} s^{-1}; this intensity is twice the solar flux in a medium latitude spot at midday during summer. However, a strong species-dependence exists that should be taken into account. By contrast, light above a saturation point causes light inhibition, which can be counterbalanced by exposing microalgal cells to very short cyclic periods of light and darkness [13].

The ratio of light to dark (or low-intensity light) periods in a cycle is crucial for microalgal productivity [9]. Similar overall numbers of moles of photons do not necessarily produce equal growth rates of (or CO_2 assimilation by) microalgae. When the light/dark cycle period approaches the photosynthetic unit turnover time (equal to the dark reaction time, estimated to lie within 1–15 ms), maximum photosynthetic efficiencies can be achieved [16]. Moreover, compared with periodic darkness, periods of low light intensity significantly increase growth, CO_2 assimilation and lipid productivity in microalgae for a given whole light level [50]. This type of lighting design can be achieved via artificial light, such as hybrid lighting systems [51]. Different lamps generate distinct spectra, and different microalgal species possess dissimilar absorption optima; therefore, each individual case should be studied before deciding on the set point of this important operational parameter. Variation of the exponential growth rates of *Phorphyridium cruentum* have been recorded [48] with variable radiation energies and light spectra, concluding that blue light (400–500 nm) increases cell growth and polysaccharide production.

Among the reactor designs available and tested to date, the maximum light-harvesting capacity is associated with alveolar panel [32] and tubular bioreactor configurations. Successful bioreactors (i.e. reactors that are able to achieve high productivity under moderate energy efficiency and cost effectiveness) combining natural and artificial light have also been described [52]. In terms of artificially illuminated bioreactors, the need for small reactor diameters to increase the illuminated surface area per unit volume of culture can be circumvented through provision of internal illumination. High biomass yields are more crucial in the case of artificially illuminated reactors, because the light provided adds to the overall operational cost of the underlying process. Such costs can be kept below acceptable thresholds via *in situ* growth-monitoring and associated online control of the intensity of light supplied – as is the case of flow injection analysis systems based on turbidimetric measurements [53] or light transmittance sensors [54].

Biomass and metabolite recovery

Biomass harvesting is necessary during continuous operation to secure high-quality effluents and to prevent cell washout [9,16]. The two main difficulties encountered in harvesting microalgae arise from the relatively low biomass concentration in conventional bioreactors, coupled with the small size of its constituent microalgal cells. Regardless of the interest in the microalgal biomass itself (e.g. for aquaculture feed) or in the feedstock for eventual extraction of bulk or fine chemicals (e.g. for biodiesel manufacture or production of anti-angiogenic active principles, respectively), development of economical harvesting methods is nuclear for the feasibility of the associated processes, because harvesting typically contributes to 20–30% of the total cost of microalgal biomass production [15]. Microalgal species, cell density and culture conditions greatly affect the application of a given harvesting method. Existing harvesting methods suitable for microalgae encompass coagulation/flocculation/sedimentation, centrifugation, foam fractionation, ultrasonic separation, flotation and membrane filtration [15]. Microalgal cell immobilization has been proposed to circumvent the harvesting issue, but large-scale applications are limited. Further investigation is clearly needed to optimize operating conditions and design new processes [7]. Recent developments in membrane filtration, specifically for wastewater treatment, could find a useful application in microalga harvesting [9,55].

Dewatering is another major challenge that affects microalga production. Following biomass harvest by centrifugation or filtration, microalgal paste traditionally consists of $\sim$ 90% (w/w) water, which meets the requirements for anaerobic digestion. However, it is necessary to reduce this value to a maximum of 50% (w/w) water for efficient oil extraction [56]. Despite its energy-intensive nature (and consequently high cost), drying has often been the dewatering process of choice. As a result of economic considerations, solar drying has been chiefly considered, yet it requires large land areas for industrial-scale operation. As a result, more efficient approaches, such as using waste heat from power plants, should be considered [56].

The final processing step in biodiesel production is recovery of specific endocellular metabolites from microalgal biomass. Several techniques have been assessed for lipid extraction, which usually requires $<$10% water [57],

including the use of supercritical CO_2 or organic solvents. Almost 90% of the energy required for biodiesel production is indeed accounted for by harvesting and dewatering of biomass, besides lipid extraction itself [23], thus providing an impetus for the design of more energy-efficient extraction technologies. In addition to lipid extraction for biodiesel production, a novel process that gasifies biomass to methane and concentrated CO_2 has recently been proposed [72] for improved overall energy efficiency.

Major products

Products that result from microalgal cultivation include animal and human food products and fertilizers, as well as industrial chemicals and bioenergy feedstocks. The use of microalgal biomass as healthy human food, or as an ingredient in the formulation of animal and aquaculture feed, is in fact a fast-growing market [58]. Presently, microalgal biomass is an essential source of nutrients in aquaculture [58], owing mainly to its high content of polyunsaturated fatty acids, which are well-established growth factors. It has also been used as a functional food in the form of tablets or powder, and as nutraceutical food additives [47,59]. Microalgae are used worldwide as agricultural fertilizers, which, besides their significant nutritional content, can improve the water-binding capacity and the mineral composition of the soil [60]. The major products are described in more detail in Box 1.

Life cycle analysis

LCA is a systems approach that is aimed at evaluating the environmental burden associated with the entire life cycle of the product of interest (microalgae, in our case), to avoid problem-shifting between life cycle stages and to identify technological innovation opportunities. A limited number of thorough LCA studies have been performed to assess the potential environmental impact of microalgal bioenergy systems [21,57,64–66]. Related studies [56,63,68–70] either have conducted a preliminary LCA or have only briefly discussed the sustainability issues of microalgal bioenergy production. The findings of LCA studies are briefly reviewed in Box 2.

Box 1. Major products from microalgae

Key issues

Industrial chemicals extracted from microalgae include: (i) glycerol, which is widely employed in food and personal care products [58]; (ii) astaxanthin and other carotenoids, used as antioxidants and coloring agents in food, cosmetics and aquaculture; (iii) fatty acids, used in cosmetics; (iv) poly-β-hydroxybutyrate, used in plastics; and (v) polysaccharides, such as agar, alginates and carrageenans, which are employed as thickening agents for foods [58]. Several recent reviews have focused on the isolation of health-promoting biomolecules from microalgae (e.g. ω-3 and other polyunsaturated fatty acids) [58]. Large bio-prospecting programs have meanwhile been created to encompass wild microalgae, in an attempt to identify compounds of potential therapeutic significance [61,62].

Bioenergy from microalgal products includes: (i) biogas produced via anaerobic digestion or co-digestion of microalgal biomass (e.g. with sewage sludge); (ii) electricity through direct biomass combustion or indirectly via combustion of the biogas derived from microalgae; (iii) biodiesel after oil extraction and re-esterification with small-chain monoalcohols; (iv) ethanol through fermentation; and (v) liquid fuels via thermochemical conversions, such as pyrolysis, gasification or liquefaction. Most bioenergy studies that pertain to microalgae have focused on biodiesel production [23,63] because this is generally perceived as the technology that is closest to commercial-scale viability, despite some overly optimistic views. Several other studies have investigated alternative energy products and are therefore worth highlighting: for example, the production of methanol from microalgae [64]; the production of electricity and biodiesel from microalgae grown in salt-water ponds [65]; co-firing of microalgae with coal [66]; extraction of oil from microalgae with CO_2 supplied from a power plant [57]; and anaerobic digestion of microalgae to produce biogas [4,67] (which possesses the major advantage that no dewatering is required).

Challenges and research needs

Enhanced biological fixation of CO_2 has attracted a great deal of attention because it leads to production of biofuels or other industrial products with a market value, together with a potential reduction in greenhouse gases (GHGs) and treatment of impaired waters. In addition, the high growth and CO_2-fixation rates of microalgae lead to a number of advantages as compared with conventional energy oil crops (e.g. palm oil), especially because the yields of oils, on a mass basis, are considerably higher. Finally, microalgal biomass can be used for animal and human food formulation or as land fertilizer. However, several challenges for microalgal-based CO_2 sequestration remain, some of which were addressed in this review and are further summarized below.

Most studies reported to date have been performed on the bench-scale, and were conducted under strictly controlled conditions. As a result, little is known about the feasibility of reactor scale-up, or the effects of competition by other microbial species inadvertently introduced into the bioreactor with the feed stream. Current investigations focus mainly on closed bioreactors, whereas future research should also consider open systems, owing to the possibility of more widespread use of biological CO_2 mitigation. Among these, technologies that support the supply of adequate amounts of CO_2, nutrients and light to microalgal cells, consume minimal energy, and release less CO_2 into the atmosphere are in special demand.

Enhanced CO_2 levels (typically well above its atmospheric level) are needed for efficient microalgal growth and metabolism, and currently are major contributors to the overall cost of microalgal cultivation. However, different sources of CO_2 and supplementary nutrients will greatly improve the overall environmental performance of microalga-based bioenergy production. Future research should explore existing sources, such as CO_2 from ammonia plants or flue gases from power stations. Furthermore, nutrients could easily be extracted from wastewater or agricultural wastes owing to their richness in nitrogen and phosphorus. Note, however, that the quality of the flue gas might hamper specific applications in the medical and food fields. Studies might also be required to discern whether microalgal biomass production using wastewater [7,12] produces sufficient-quality effluent for discharge into the environment, or even eventual reuse. Waste streams are, in general, small compared with energy demands; as a result, transportation costs to concentrated production sites should be assessed in advance. Co-digestion of microalgae with wastewater sludge for biogas production should also be considered, because this strategy could be integrated into the existing wastewater infrastructure.

Box 2. Life cycle analysis

System boundaries

The system boundary considered in the LCA studies conducted thus far has been limited to microalgal cultivation and harvesting and bioenergy production, such as oil extraction and biofuel production. By contrast, several groups [23,64,65] have extended the boundary to the consumption of bioenergy, such as combustion of biofuels (''cradle-to-grave''). The characteristics of biofuels derived from microalgal biomass through various conversion processes might differ from those of fossil fuels; therefore, the emission profile of the former is different during the combustion stage. As such, it is important to conduct a full LCA. Note, however, that this type of study is based on microalgae that are optimized for biomass production, rather than energy efficiency, which suggests that these assessments erroneously produce negative ratings. Maximizing biomass production is accordingly sought, while energy gains (relative to existing processes) also arise: directly, because no extra aeration and mixing are needed; and indirectly, because no extra supply of carbon is required.

Impact categories

In most LCA studies that encompass microalgal bioenergy, CO_2 emissions, GHG reduction potential and energy balance have been the only environmental impact categories evaluated. Additional impact categories have been specifically considered elsewhere [23,66]. The development of bio-based products will disrupt not only the carbon cycle, but also the nitrogen cycle [71], as the contribution of agriculture to reactive nitrogen flux in the biomass cultivation stage might produce deleterious environmental consequences. Therefore, the more impact categories considered, the broader the insight into environmental trade-offs of microalgal bioproducts.

Key findings

The results of the LCA studies applied to distinct microalgal systems are somewhat controversial. From an energy perspective, Campbell *et al.* [65] have concluded that microalgal biodiesel is very favorable when compared with ultra low sulphur (ULS) diesel: the total energy required is 0.206 MJ/ton/km for the scenario of 100% CO_2 truck delivered, compared with 1.074 MJ/ton/km for ULS diesel, because of less fossil fuel inputs and credits from electricity production. In the best case scenario entertained elsewhere [57], the authors have claimed generation of a net energy of the order of 11 000 MJ per dry ton of macroalgae, which compares with 9500 MJ/ton that is relevant to microalga gasification. However, other studies [22,64] have asserted that microalgal bioenergy is unfavorable when compared to energy from fossil fuels because of the significant amount of energy consumed in microalgal cultivation, harvesting [64] and oil extraction [23].

Consistent findings have been reported in the literature for GHG emissions, with microalgal biofuels favored over fossil fuels. Negative GHG emissions for microalgal biodiesel have been reported (−183 $kg_{eq.CO2}$/MJ) [69]. Total GHG emissions have been calculated to be 7.757 $g_{eq.CO2}$ for the scenario of 100% CO_2 truck delivered [65], whereas 75.040 $g_{eq.CO2}$ has been found for ULS diesel. GHG emissions of 302.1 $g_{eq.CO2}$/g have been indicated for microalga-based methanol, with 1204 $g_{eq.CO2}$/g for gasoline [64]. Finally, the overall GHG emissions for microalgal biodiesel have been reported at 25% less than that for regular diesel [23]. With respect to other impacts, the authors have noted that microalgal biodiesel is less favorable as compared with regular diesel in the categories of acidification, eutrophication, human toxicity, marine toxicity, land competition, radiation and photochemical oxidation [23]. Conversely, it has been concluded [64] that the environmental performance of microalga-based methanol is generally better than gasoline, with lower acidification, photochemical oxidation and eutrophication.

Harvesting, dewatering and lipid extraction from microalgal biomass are still challenging issues because they consume large amounts of energy – mainly because of the small cell size and relatively low biomass levels of microalgal cultures. Research efforts are therefore warranted for cultivation under higher cell densities, which pose engineering challenges with regard to cell accessibility to light and gas. In addition, the possibility for chemically induced or auto-flocculation of microalgal cells needs to be addressed.

Finally, a key challenge for microalgal biodiesel production is the use of microalgal species that can maintain a high growth rate in addition to a high metabolic rate, thus leading to significant lipid yields. This major challenge can be duly addressed via extensive bio-prospecting or target-oriented genetic engineering – both of which are now starting to appear as promising approaches.

Acknowledgements

Project OPTIC-ALGAE (ref. PTDC/BIO/71710/2006). from FCT (Portugal) and supervised by author F.X.M., for partial funding.

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