

Chapter 6

Microalgae for Industrial Purposes

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Abstract The use of microalgae for the production of compounds of commercial relevance has received substantial interest in recent years, mostly because these organisms contain a plethora of valuable compounds and their high turnover rate and functional plasticity make them relatively easy to cultivate for the production of biomass and added-value molecules. The metabolic flexibility of algae allows using them for many commercial applications, but it also makes it easy for cultures to diverge from the intended biomass quality. A thorough comprehension of the principles that control growth and carbon allocation is therefore of paramount importance for effective production of algal biomass and derived chemicals. In this review, we intend to provide basic but exhaustive information on how algae grow and on their biotechnological potential. In addition to this primary goal, we also give the reader a succinct panorama of culturing systems and possible applications.

Keywords Algae • Carbon allocation • Genetics • Culture • Cell composition • Stoichiometry • Biofuel • Food • Feed

6.1 Introduction

The use of microalgal biomass for capturing CO₂, for the production of biofuel and pharmaceutical and nutraceutical products, as food and animal feed, and for wastewater treatment has recently become a focal point for research and the object of public interest (e.g., Rasala et al. 2014). Part of this growing popularity is due to the

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fact that microalgae are very versatile in their utilization. Furthermore, they represent an excellent alternative to land crops: with their fast turnover rates, microalgae can produce large amounts of biomass in a relatively small volume and within a relatively short time. Furthermore, microalgae cultivation can be carried out on land of low agricultural value, thus avoiding competition with traditional crops. Because of the unique metabolic plasticity of microalgae, it is relatively easy (and certainly easier than for most terrestrial embryophytes) to optimize resource allocation in the cell to favor the production of selected compounds, by controlling culture conditions. Their high photosynthetic yield and efficiency make microalgae potentially capable of capturing substantial amounts of CO₂, thereby contributing to the mitigation of global climate change, especially if microalgae cultures can be fed with flue gases from fossil fuel combustion.

In spite of the foregoing considerations and although the commercial production of microalgae has a rather long history (e.g., Ben-Amotz 2004), progress in the design of production plants and, in general, in the technologies (both biological and engineering) used to commercially exploit algae (Table 6.1) has been rather slow. The causes of this are various, with a great contribution attributable to the fact that players on the market tend not to share information (Grobelaar 2009). Furthermore, algal cultivation is often conducted by operators who lack a thorough understanding of the physiology of these organisms, thus having limited ability to identify and solve the problems that may arise from their functional complexity. This chapter intends to highlight the crucial aspects of algal physiology and to suggest ways to avoid difficulties and facilitate the attainment of biomass of a defined quality or of specific compounds. To do so, we provide some basic information on how algae fix and allocate carbon and on how culture conditions can influence the organic composition of cells. We also discuss how the chemical composition of culture media affects algal growth and biomass quality. Finally, we provide examples of applications in which physiological knowledge may bring obvious advantages.

6.2 What Are Algae?

In the words of Raven and Giordano (2014), “algae frequently get a bad press. Pond slime is a problem in garden pools, algal blooms can produce toxins that incapacitate or kill animals and humans and even the term seaweed is pejorative, a weed being a plant growing in what humans consider to be the wrong place. Positive aspects of algae are generally less newsworthy.” Giving a scientific sound definition of “algae” is not an easy task: algae are organisms that produce O₂ as a waste product of their photosynthesis, but are not “higher plants” (embryophytes) (Raven and Giordano 2014). According to this definition, prokaryotic (cyanobacteria) and eukaryotic photosynthetic organisms are algae. In the group of organisms included in this definition, we find organisms in an approximate size range from 1 μm to 1 mm,

Table 6.1 Main algal species used in biotechnological applications (the information reported in this table was mainly obtained from Enzing et al. 2014 and Borowitzka 2016)

Species	Phylum and class	Product	Production status
<i>Artospira platensis</i>	Cyanophyta, Cyanophyceae	Biomass as dietary supplement	Mass production
<i>Chaetoceros muellerii</i>	Bacillariophyta, Bacillariophyceae	Biomass as dietary supplement	Small scale
<i>Chlorella vulgaris</i>	Chlorophyta, Chlorophyceae	Canthaxanthin, astaxanthin, β -carotene, biomass as dietary supplement	Mass production
<i>Cryptocodinium cohnii</i>	Miozoa, Dinophyceae	Docosahexanoic acid	Mass production
<i>Dunaliella salina</i>	Chlorophyta, Chlorophyceae	β -Carotene, glycerol	Mass production
<i>Hematococcus pluvialis</i>	Chlorophyta, Chlorophyceae	Astaxanthin, cantaxanthin, lutein	Mass production
<i>Isochrysis</i> spp.	Chlorophyta, Chrysophyceae	Biomass as dietary supplement	Mass production
<i>Nannochloropsis</i> spp.	Ochrophyta, Eustigmatophyceae	Eicosapentanoic acid	Small scale
<i>Nitzschia closterium</i>	Bacillariophyta, Bacillariophyceae	Eicosapentanoic acid	Research
<i>Nostoc commune</i>	Cyanophyta, Cyanophyceae	Biomass as dietary supplement	Collected, not cultivated
<i>Nostoc flagelliforme</i>	Cyanophyta, Cyanophyceae	Biomass as dietary supplement	Collected, not cultivated
<i>Nostoc sphaeroids</i>	Cyanophyta, Cyanophyceae	Biomass as dietary supplement	Collected, not cultivated
<i>Odontella</i>	Bacillariophyta, Mediophyceae	Eicosapentanoic acid, docosahexanoic acid	Small scale
<i>Pavlova lutherii</i>	Haptophyta, Pavlovophyceae	Biomass as dietary supplement	Research
<i>Phaeodactylum tricoratum</i>	Bacillariophyta, Bacillariophyta incertae sedis	Eicosapentanoic acid	Small scale
<i>Porphyridium cruentum</i>	Rhodophyta, Protofloridae	Biomass as dietary supplement, arachidonic acid, triacylglycerols	Small scale
<i>Scenedesmus almeriensis</i>	Chlorophyta, Chlorophyceae	Lutein, β -carotene	Research
<i>Skeletonema</i> spp.	Bacillariophyta, Bacillariophyceae	Biomass as dietary supplement	Small scale
<i>Tetraselmis</i> spp.	Chlorophyta, Prasinophyceae	Biomass as dietary supplement	Research

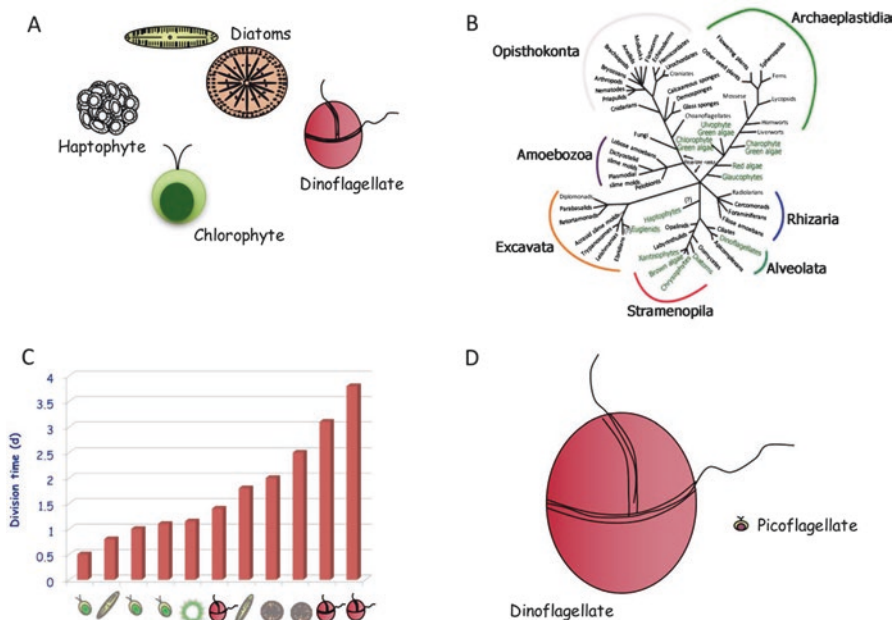


Fig. 6.1 The algae are extremely diverse in morphology (a), phylogeny (b), growth potential (c), and size (d). The information in b refers to eukaryotic algae and was derived from Knoll (2003) and Brodie et al. (2017). The growth rates in c are unpublished data; the species are not identified because of non-disclosure agreements

highly phylogenetically and morphologically diverse (and this diversity has only been minimally explored; de Vargas et al. 2015), with an extremely broad range of growth rates (Fig. 6.1).

6.3 Algae Produce Biomass Through Photolithotrophy, Heterotrophy, or Mixotrophy

Although the ability to carry out photosynthesis is certainly a key characteristic of algae, they are also capable of various degrees and various modes of heterotrophy. For some application, the ability of algae to combine autotrophy and heterotrophy can be advantageous, offering the ability to stimulate growth by supplying organic nutrients when light is not present or insufficient and when CO_2 is subsaturating. The ability to grow on organic substrates can also be exploited in phytodepuration processes that require the treatment of dissolved or particulate organic matter, in addition to that of inorganic nutrients. A brief description of algal trophisms may help the comprehension of the ensuing topics.

6.3.1 *Photolithotrophy*

Photosynthesis is a mode of nutrition that uses inorganic carbon in a light-dependent process to generate organic compounds (photolithotrophy). In photosynthesis the generation of energy in the form of reductants and ATP is conducted on the photosynthetic membranes, which in eukaryotes are located in the chloroplast (Chen et al. 2015a). The photosynthetic generation of reductants is the consequence of the excitation of specialized chlorophyll *a* molecules, which initiate an electron transfer along a redox gradient. This redox energy is employed chemo-osmotically (Mitchell 1961) to produce ATP, whereas the electrons are finally allocated onto soluble molecules (e.g., ferredoxins and NADPH) that can then be employed in metabolism (Schmollinger and Merchant 2014). The production of ATP and reductants is thus a function of the amount of photons captured by the so-called antenna systems, partially modulable ensembles of pigment–protein complexes. The energy made available by the processes occurring on photosynthetic membranes is then employed for the assimilation of primarily inorganic carbon. Appreciable proportions of reducing power and ATP are also used to acquire and assimilate other nutrients (N, P, S, etc.) and for various metabolic processes. CO₂ assimilation is carried out through the carboxylation of pentose ribulose biphosphate (RuBP) with the catalysis of the enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco) (Marcus et al. 2011). The enzyme rubisco can also catalyze the oxygenation of RuBP, and O₂ and CO₂ compete with each other at the active sites of rubisco (Bowes et al. 1971). The presence of O₂ in the gas phase decreases the efficiency and yield of photosynthesis and leads to a conspicuous increase in the energetic cost of CO₂ fixation. Although in the course of evolution the ability of rubisco to favor carboxylation versus oxygenation has increased, no rubisco present today on the planet is capable of conducting carboxylation in the absence of oxygenation (Giordano et al. 2005). Most algae (Raven et al. 2005, 2008, 2012) overcome the difficulties associated to the double activity of rubisco (carboxylation and oxygenation) and the low CO₂:O₂ ratio in the extant atmosphere by expressing CO₂-concentrating mechanisms (CCMs) that pump CO₂ toward rubisco in an energy-dependent matter (Giordano et al. 2005; Raven et al. 2014). The CCMs are of various sorts and their modulation is strongly responsive to the CO₂:O₂ ratio in the environment. The activity of CCMs may also depend on factors that are distinct from the availability of CO₂ and O₂: the presence of CCMs allows algae to decrease the amount of rubisco in the cell and this leads to substantial savings in N, S, and also to an enhanced Fe and light use efficiency (Beardall and Giordano 2002; Raven et al. 2014); the availability of these resources can therefore have a role in the modulation of CCMs (Beardall and Giordano 2002; Raven et al. 2008, 2012, 2014). Elevated CO₂ leads to the downregulation of CCM (Giordano et al. 2005). CCM downregulation allows cells to save the energy they would otherwise have invested into pumping inorganic carbon toward rubisco. However, this does not necessarily create a higher growth rate, because the energy saved is not always invested in growth, or growth limitation (before the CO₂ increase) may not reside in CO₂ (Giordano and Ratti 2013).

6.3.2 *Mixotrophy*

Mixotrophy can be defined as the concomitant occurrence of photolithotrophy and chemo-organotrophy (use of exogenous organic C as the source of both energy and C for metabolism; also see Raven and Beardall 2016). The uptake of organic C can be carried out on a molecule-by-molecule basis (osmotrophy) or through the acquisition of particles (phagotrophy) (Flynn et al. 2010; Raven et al. 2013). Algae are, for the most part, primarily photolithotrophs and recur to mixotrophy only when photolithotrophy is hindered by the scarcity of inorganic nutrients or light. There are, however, protists that, although they fall within the definition of “algae” sensu Raven and Giordano (2014), are primarily phagotrophic, but can photosynthesize under prey limitation. Finally, some organisms are obviously derived from photosynthetic organisms but are obligate chemo-organotrophs (Mitra et al. 2016). The ancestral condition in algae is probably that of obligate photolithotrophs (Beardall and Raven 2016), which is confirmed by the fact that basal cyanobacteria such as *Glaeobacter violaceus* (Blank and Sanchez-Baracaldo 2010) are incapable of chemo-organotrophy (Beardall and Raven 2016, and references therein). On the other hand, Raven et al. (2009) wrote that “without phagotrophy at the cell level there would be no photosynthesis in eukaryotes”; in other words, as the acquisition of the chloroplast is the consequence of a phagotrophic event, phagotrophy/mixotrophy appears to be an inherent property of eukaryotic photosynthetic organisms. It must be considered that primary endosymbiotic events, that is, those in which a heterotrophic prokaryote engulfed a photosynthetic prokaryote, have most likely been relatively rare, whereas subsequent secondary and tertiary endosymbiotic events, in which an eukaryote engulfed a photosynthetic eukaryote, may have occurred more readily (McFadden 2001; Gentil et al. 2017; Lane 2017). Among extant algae, clades deriving from primary endosymbiosis are rarely mixotrophic, whereas mixotrophy is much more frequent in algae originated from secondary and tertiary endosymbiotic events (Beardall and Raven 2016). According to Raven (1995, 1997), the cost of the photosynthetic apparatus and the uptake systems for nutrients different from C sums up to about 50% of C, N, P, Fe, and of the energy cost to make a cell in a photolithotroph. The corresponding cost for the heterotrophic (phagotrophic) apparatus is less than 10% (also see Jones 2000 for further discussion on these matters). The advantage of mixotrophy over obligate photolithotrophy and obligate photo-organotrophy emerges especially in the light (Jones 2000), although the large number of possible nutritional conditions makes it hard to provide an univocal outcome of competition between organisms with different trophisms. It should also be considered that chemo-organotrophy leads to greater C loss through respiration and thus can lead to an elemental stoichiometry with lower C relative to N, P, and Fe. The concomitant use of photosynthesis can compensate for such unbalance, to a variable degree (Beardall and Raven 2016). Osmomixotrophy may also be important to recapture leaked dissolved organic carbon (Raven and Beardall 2016).

6.4 Cell Composition Results from a Combination of Genotypic and Environmental Constraints

The composition of cells is the result of the interaction of the genome with the environment leading to the best suited structural and functional cell organization. The composition of the cell is thus strongly dependent on the condition in which cells live or are cultured. At the same time, because it depends on the genotype, cell composition is strongly species specific. Different genotypes will respond differently to the same kind of environmental perturbation, with different degrees of compositional and functional homeostasis (Giordano 2013). The attitude to homeostatically retain cell composition is possibly also a function of growth rate: Fanesi et al. (2014) showed that, other things being equal, fast-growing microalgae have a lower tendency to compositional homeostasis than slow-growing ones, because they have a higher probability of competitively taking advantage of the investment in reproduction, regardless of the duration of the perturbation (Giordano 2013). Depending on both the species and the type of environmental perturbations, cells can adjust their growth performance through (a) regulatory processes that do not require changes in the expressed proteome (Giordano 2013; Raven and Geider 2003); (b) the production of new protein and the degradation of protein present before the perturbation was applied (acclimation); and (c) by changes in the genotype (adaptation) (Giordano 2013; Raven and Geider 2003). Most species, if maintained for a prolonged time in the same conditions, will tend to change their expressed proteome and acclimate to the environmental condition (Giordano 2013), and if a sufficient genetic heterogeneity is present in the population, genotype selection is also likely (Venuleo et al. 2017).

The consequences of these considerations for the commercial cultivation of microalgae are that (a) a tight match between genotype and cultural conditions must be ensured to obtain the desired end product; (b) stability of culture conditions is required to ensure constancy in the quality of the product and in productivity; and (c) a control of the genetic stability of the population is important to prevent a shift in dominance that may lead to the prevalence of a strain with non optimal characteristics (from the commercial perspective).

6.4.1 *Elemental Stoichiometry and Organic Cell Composition*

Cell composition results from the availability of the various elements and their metabolic and structural requirements. Quigg et al. (2003, 2011) and Ho et al. (2010) reported the elemental composition for a large number of species cultured under presumably resource-replete conditions. The species-specificity of these cell stoichiometries emerges clearly; macronutrients (i.e., C, N, P, S) generally show a higher degree of similarity than micronutrients across species (Giordano 2013 and references therein). An obvious reciprocal relationship exists between cell

stoichiometry and environmental chemistry; the oceanic “Redfield ratio” (Redfield 1934) is a typical example of this. A mechanistic basis for the Redfield ratio was proposed by Loladze and Elser (2011), who suggested that the fairly constant elemental stoichiometry of phytoplankton in the ocean, especially with respect to the N:P ratio, is imposed by the fact that rapid growth constrains the ratio between cell protein (main sink for N) and RNA (main sink for P) (e.g., Norici et al. 2011; Raven et al. 2012; Geider and La Roche 2002). The relative content of N:P can be used as a proxy for the protein to RNA ratio and this can be related to growth through the capacity for protein synthesis (growth rate hypothesis: Elser et al. 2011; Flynn et al. 2010; Loladze and Elser 2011; Giordano et al. 2015b) It is worthwhile noticing, however, that the growth rate hypothesis does not always apply to microalgae (Flynn et al. 2010; Nicklisch and Steinberg 2009; but also see Giordano et al. (2015b).

In a commercial cultivation system, most likely, growth conditions are resource replete, which may lead to the fact that cell elemental composition does not reflect the requirement to achieve the maximum possible growth rate but is influenced by luxury accumulation of some elements (Giordano 2013).

Elemental composition is connected to the organic cell composition also because an imbalance between C and N (or C and P, or C and S) can lead to different C allocation patterns. When, for instance, the C:N ratio is higher than the C:N ratio in protein and nucleic acids and the excess of C surpasses the requirement for structural non-N/P-containing pools, two options exist: the C in excess (relative to other nutrients) is not acquired (Kaffes et al. 2010) or C is assimilated beyond strict growth requirements with a consequent increase in the size of pools that do not contain N and P, such as carbohydrates and lipids (Giordano et al. 2015a; Giordano and Ratti 2013; Montechiaro and Giordano 2010; Palmucci et al. 2011). Whether the excess C is allocated to carbohydrates or lipids depends on genotypic, energetic, and size constraints (Palmucci et al. 2011). The genotypic constraints are associated with the preference for some metabolic pathways by a species/strain (Palmucci and Giordano 2012). The energetic constraints are associated with the different cost of allocating C to carbohydrate or lipid and mobilizing it (Montechiaro and Giordano 2010; Palmucci et al. 2011); this of course becomes relevant only when energy availability (i.e., light; as is often the case in dense commercial cultures) limits growth (Ruan and Giordano 2017; Ruan et al. 2017). The spatial constraints occur because the lower hydration of lipids makes it easier for them to accumulate when space is limited (Palmucci et al. 2011). The outcome of an imbalance between C and N is thus not easily forecasted and should be assessed case by case.

6.5 Genetic Modification of Algae: Tools and Aims

The enormous pool of metabolic possibilities constituted by microalgae translates into a very large and mostly unexplored potential for applications. It also minimizes the need for genetic manipulations, because many functional variants are present in nature (although they may not all have been discovered yet). Many applicative

problems may be solved through the search of “natural” species/strains with the required metabolic capabilities. This notwithstanding, genetic manipulation is possible and, where allowed, it may offer the best solutions for specific problems (Gressel 2008). The generation of mutants is important for strain improvement for biotechnological applications, but it can also be used, in the secure space of a laboratory, for functional analyses of genes and proteins. Few genetically modified strains of microalgae are used commercially nowadays, partially because molecular tools (e.g., efficient nuclear transformation, availability of promoter and selectable marker genes, and stable expression of transgenes) are not available for some commercially important species (Amaro et al. 2011). However, recent developments of high-throughput technologies have enabled the profiling of mRNA, proteins, and metabolites, giving rise to the fields of transcriptomics, proteomics, and metabolomics, respectively (Lee et al. 2010); these methodologies allow the comprehension of the consequences of genetic manipulation at the whole cell level, thus facilitating their application in a productive context.

Despite the increasing number of sequenced microalgae genomes, precise and programmable genome editing has been reported for only a few eukaryotic microalgae, such as the eustigmatophyte *Nannochloropsis* sp. (Kilian et al. 2011), the green alga *Chlamydomonas reinhardtii* (Sizova et al. 2013), the diatoms *Phaeodactylum tricornutum* (Nymark et al. 2016) and *Thalassiosira pseudonana* (Poulsen et al. 2006). The genome editing methods used for these studies (Daboussi et al. 2014; Hopes et al. 2016; Nymark et al. 2016; Shin et al. 2016; Wang et al. 2016) (see following), together with the ever-growing number of tools for transgene expression, cloning, and transformation (e.g., Rasala et al. 2014; Scaife et al. 2015), open up very promising perspectives for the future of algal genetic manipulation.

The methods for targeted gene knockout and gene replacement based on homologous recombination (HR) have driven rapid progress in understanding many of the complex metabolic and regulatory networks in eukaryotic cells (Weeks 2011). The main obstacle for direct gene targeting is the low frequency of HR between nuclear genes and donor DNA. Recombination efficiency may be increased by the use of zinc-finger nucleases (ZFNs), which cut the genome at specific sites to facilitate HR. Sizova et al. (2013) published a nuclear gene targeting strategy for the green alga *Chlamydomonas reinhardtii* that is based on the application of ZFNs. In the case of *C. reinhardtii*, insertional mutagenesis to disrupt a gene of interest is commonly employed. For instance, by exploiting a collection of *C. reinhardtii* insertional mutants originally isolated for their insensitivity to ammonium, Emanuel et al. (2016) found a strain that, in addition to its ammonium-insensitive (AI) phenotype, was unable to correctly express nitrogen assimilation genes in response to NO signals. The difficulty of extending this approach to more species resides in the fact that it cannot prescind from the existence of large collections of mutants and from the screening of many thousands of clones.

MicroRNAs (miRNA) are 21- to 24-nucleotide RNAs present in many eukaryotes that guide the silencing effector Argonaute (AGO) protein to target mRNAs via a base-pairing process (Bartel 2009). Chung et al. (2016) showed that miRNAs in

C. reinhardtii regulate gene expression primarily by destabilizing mRNAs, using target sites that lie predominantly within coding regions.

Protein-based systems involving mega nucleases and “transcription activator-like effector nucleases” (TALENs) allow precisely targeted genome editing of eukaryotic microalgae genomes (Daboussi et al. 2014; Weyman et al. 2015). These systems bear great potential for research and generation of tailored strains, although they are labor intensive and rather costly. Recently, a much simpler and inexpensive method for genome editing in algae, CRISPR/Cas9, came about (Nymark et al. 2016). It was developed to generate stable targeted gene knockouts in the marine diatom *Phaeodactylum tricorutum*, but it should be easily adaptable for use in other microalgae. Shin et al. (2016) applied this system to *C. reinhardtii*; they directly delivered the Cas9 protein and the “single-chain guide RNAs” (sgRNAs) to three different genes, obtaining mutations at the Cas9 cut sites with a significantly improved targeted mutagenic efficiency. Wang et al. (2016) established a precise CRISPR/Cas9-based genome editing approach for the industrial oleaginous microalga *Nannochloropsis oceanica*, using the gene encoding nitrate reductase (NR; g7988) as an example. The isolated mutants, in which precise deletion of five bases caused a frameshift in NR translation, grew normally in the presence of NH_4^+ but failed to grow when N was supplied as NO_3^- . This demonstration of CRISPR/Cas9-based genome editing in industrial microalgae is very promising for microalgae-based biotechnological applications. Also, editing of the chloroplast genome is of interest for biotechnological applications, because it may allow transgene insertion via HR with expression that is not subject to nuclear gene-silencing mechanisms; furthermore, plastidial transformation may take advantage of the prokaryotic organization of chloroplast genomes to co-express multiple genes in operons (Wannathong et al. 2016). New simple and inexpensive protocols have recently been developed to this end (Wannathong et al. 2016), but their effectiveness for species different from *C. reinhardtii* is still to be demonstrated as is their applicability for biotechnological purposes.

Many cyanobacterial strains are amenable to transformation and homologous recombination. Cis genetic modification (through genome editing) is the most common approach for engineering cyanobacteria (Berla et al. 2013). Typically, chromosomal mutations are generated through the insertion of a plasmid that contains the gene(s) of interest, a selectable marker gene, and flanking sequences homologous to the targeted chromosomal sequence (homology arms). Numerous heterologous genes have been inserted through these methods in the model cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 (Savakis and Hellingwerf 2015). However, genome editing of cyanobacteria is more challenging than in model heterotrophic prokaryotes such as *Escherichia coli*, primarily because cyanobacteria often contain multiple genome copies per cell and long-term instability of the genes introduced (Kusakabe et al. 2013; Ramey et al. 2015). CRISPR interference is emerging as a promising method to repress expression of specific genes, with no need for gene knockout also for prokaryotes (Huang et al. 2016).

6.6 How Are Algae Cultured?

Large-scale microalgal cultivation can be attained by a number of culturing systems, the choice of which depends on cost, available technology, and desired quality of the biomass or added-value product. Consequently, no set recipe for a successful cultivation exists and physiological, engineering, and economic analyses must be conducted to ensure good results.

Algal commercial cultivation is often conducted empirically without a full understanding of the physiology behind it, which can lead to unsatisfactory results or to products that are highly variable in quality because of the lack of control on the biological processes controlling biomass production.

From a trophic point of view, microalgae can be grown photolithotrophically (phototrophy), chemo-organotrophically (heterotrophy), or mixotrophically (Chojnacka and Marquez-Rocha 2004; see above). Photolithotrophic growth is advantageous because it can use natural sunlight as the energy source and mineral media, which are relatively inexpensive; it is unavoidable when obligate photolithotrophs are used. Light must be in large supply to sustain photolithotrophic growth (Perez-Garcia et al. 2011), which makes this mode of mass cultivation economically convenient in area with high insolation. In intensive cultures, light penetration can be substantially attenuated and light can become the limiting factor for growth. This problem is usually addressed through careful design of culturing systems. In recent years, a molecular approach to the problem of light availability in intensive culture systems has also been taken, through the production of genetically modified strains that have antennae of smaller size and thus a decreased light attenuation across the culture (Mooij et al. 2015). These strains, however, have not yet found commercial application and still need to be tested for productivity at a usefully large scale.

In some cases, higher productivity can be attained through heterotrophic or mixotrophic cultivation methods (Chen et al. 2016). Heterotrophy can be maintained in total darkness by supplying organic compounds (e.g., glucose, acetate, glycerol) as both energy and carbon source, eliminating the need for illumination but adding a cost for the organic substrates. The cultivation of *Chlorella protothecoides* (now *Auxenochlorella protothecoides*) under mixotrophic condition was reported to increase the yield of biomass and lipid (Wang et al. 2013). The mixotrophic cultivation of the green alga *Chlorella* sp. C2 in a 5-L bioreactor resulted in a maximum biomass productivity of 9.87 g L⁻¹ day⁻¹; this productivity declined to 7.93 g L⁻¹ day⁻¹ when the culture size was scaled up to 50 L (Chen et al. 2016), which is still a very small volume for commercial application. The change in productivity with increasing cultural volume warns us about the extrapolation of data obtained from small-scale tests to larger-scale cultivation. Cultivation on organic substrates currently is rarely utilized in the commercial production of algal biomass because the number of heterotrophic or mixotrophic algal strains that can be used is limited, the presence of organic carbon makes it very difficult to control bacterial proliferation, cases of growth inhibition by soluble organic compounds were reported, and because of the higher cost of growth media (Zhang et al. 2014a). There are however

applications for which mixotrophy can be useful and almost unavoidable. One such application, for instance, is the use of algae in wastewater treatment, where the water affluent cannot be fully deprived of organic components and a concomitant utilization of the inorganic and organic components is desirable or necessary (Cai et al. 2013).

In terms of the engineering of the cultivation systems, both open ponds and photobioreactors are used for algal cultivation. When very large quantities of product must be generated at low cost, open ponds are the most common solution (Borowitzka 1999). However, not all species are amenable to effective cultivation in open ponds, and the susceptibility to weather (especially rain and temperature variations) and light availability makes an open pond suited mostly for tropical and subtropical regimes with low precipitation and cloud cover (Richmond 1986). Algae cultivation in open-pond production systems has been used since the 1950s (Chojnacka et al. 2004), in both natural (lakes, lagoons, ponds) and artificial basins. Shallow raceway ponds, in which the algal suspension is mixed with paddle wheels, are the most widely used systems because they are relatively easy and cheap to construct and operate (Doucha and Lívanský 2006). Currently, more than 90% of world microalgae biomass production is obtained in raceway ponds. Some ponds are built on non-arable lands adjacent to power plants to have access to CO₂ from flue gases or near wastewater treatment plants to easily access nutrient supplies. Although widespread, open ponds have their drawbacks such as relatively low culture density and biomass productivity, high evaporative losses, and susceptibility to weather and to contamination by bacteria or undesired algal strains (Chen et al. 2013; Richardson et al. 2012).

When the environmental conditions are not suitable for open ponds, or a high and verifiable quality of biomass is required or biomass is used for the production of added-value compounds, algae can be cultured in closed or nearly closed systems, the photobioreactors (PBR). The engineering of PBRs is very diverse (Behrebs 2005 and references therein), and there are various designs of PBRs for different uses and of different cost. We refer to the numerous reviews and research papers on these topics published in recent years for more details (e.g., Zittelli et al. 2013; Pires et al. 2017).

In a continuous culture system, such as most PBRs, the carrying capacity (i.e., maximum biomass that can be obtained) is determined by the concentration of the factor(s) that limit growth. If light is sufficient, the composition of the medium is therefore a crucial aspect in the planning of a successful production system. In most cases, care is taken to provide an excess of macronutrients but little attention is given to micronutrients and elemental stoichiometry. The consequence often is an imbalance in nutrient availability, resulting in unnecessary costs and lack of control on limiting factors (Giordano 2013). The rate of biomass production, instead, is a function of the rate by which limiting nutrients are supplied to the culture (dilution rate). A dilution rate that surpasses the genotypically fixed maximum growth potential of a strain will lead to the decrease of the cell number per unit of volume of culture. Dilution rates that are lower than this higher limit are sustainable. It should

be considered that a suboptimal growth rate may impact cell metabolism and thus affect biomass quality (Fanesi et al. 2014).

6.6.1 *Harvesting and Dehydration of Algal Biomass*

After cultivation, biomass has to be separated from the growth medium and recovered for downstream processing. Harvesting usually involves two steps: (1) bulk harvesting (or primary harvesting), to separate the microalgae from their growth medium, usually done by sedimentation, flocculation, or flotation; (2) thickening (or secondary dewatering), to concentrate the microalgal slurry after bulk harvesting, typically by centrifugation or filtration (Lam and Lee 2012; Zhang et al. 2014b). Thickening by centrifugation and filtration use up considerable energy and, although often employed, represents one of the main costs for commercial algae (and algal products) production. Flocculation is used to increase the efficiency of gravity sedimentation (Brennan and Owende 2010). However, conventional flocculants are often toxic, whereas non toxic flocculants (e.g., organic polymers) are presently too expensive for large-scale applications (Lee et al. 2013). Autoflocculation, which can be induced by increasing the H^+ concentration in the medium, and electrolytic flocculation may be used to separate algae from the medium without the addition of chemicals; estimates suggest that these methods would be significantly more economical than other harvesting techniques (Beuckels et al. 2013; Coons et al. 2014; Lee et al. 2013). Bioflocculation is the process of flocculation induced by microorganisms or by compounds they produce; it is possibly the most environmentally friendly among the flocculation methods (Wan et al. 2015). In a study by Wang et al. (2015), co-culturing of *Chlorella* and bioflocculant-producing bacteria was optimized to decrease adverse effect of co-culturing and proved to be effective in facilitating harvesting; such an approach may be a good option for the collection of algal biomass in wastewater treatment plants, where the bacterial component is unavoidable. Electroflocculation is another option: Coons et al. (2014) reported that, in the production of algal lipids, the cost of electroflocculation with inert electrodes was appreciably lower than that of membrane filtration, which, in turn, was less costly than centrifugation. The same authors suggested that ultrasonic harvesting, which operates through a standing wave created by forward and reverse propagating pressure waves in the water, could afford substantial economic advantages in comparison to other harvesting methods.

Drying of biomass is among the most expensive steps in microalgal production, because the evaporation of large volumes of water drains large amounts of energy; yet, it is usually a necessary step, because the presence of water interferes with transport and processing (Kumar et al. 2010). Spray, drum, freeze, and solar drying are commonly applied methods. Solar drying is economical, but it requires large extensions of land and is not feasible in temperate climates, where sunlight is not always sufficient (Zhang et al. 2014a).

Another common problem in algal commercial cultures is the identification of the appropriate time for harvesting. A method that appears especially suited for such task is Fourier-transform infrared spectroscopy (FTIR) (Domenighini and Giordano 2009; Giordano et al. 2001). A number of papers have proved the reliability, rapidity, and low cost of this methodology, which affords a snapshot of cell composition (Jebsen et al. 2012; Montechiaro and Giordano 2010; Palmucci and Giordano 2012; Palmucci et al. 2011) and allows reliably following changes in biomass quality over time (Giordano et al. 2017; Giordano and Ratti 2013; Memmola et al. 2014). The advantage of FTIR is that various organic pools can be determined concomitantly, with no need for extractive procedures and in quasi-real time. The disadvantage is that, in complex whole-cell spectra, the identification of specific compounds or small pools may not be easy (and sometimes is not possible). In these cases, other methodologies may be better suited. For lipids, fluorescent probes such as the lipophilic Nile red and BODIPY 505/515 can be used; these compounds can detect neutral lipids in intact cells (Cooper et al. 2010). However, the relatively long time required for staining and detecting the fluorescent probe, the relatively high cost of the probe, and the potential errors caused by the different permeability of the probes into different microalgae cells represent drawbacks of the use of these molecular probes. Qiao et al. (2015) developed a method to determine the optimal harvest time in oil-producing microalgal cultivations by measuring maximal photosystem II quantum yield (F_v/F_m); although this method afforded good results, it must be considered that F_v/F_m is associated with a number of events occurring within the cells and is not highly specific.

6.7 Products and Applications

6.7.1 Biofuels from Algae

Microalgae can, in principle, be used for the production of several different types of biofuels: biodiesel can be obtained from algal oil (Ahmad et al. 2011), biomethane, also known as biogas, can be produced through anaerobic digestion of algal biomass (Frigon et al. 2013), hydrogen can be generated photobiologically (Zhang et al. 2012), and bioethanol can be produced in the dark by anaerobic fermentation (Bigelow et al. 2014). The production of these biofuels can be combined in the same process, because the residue of the oil extraction for biodiesel production can be further processed into ethanol, methane, or H_2 (Mata et al. 2010; Singh and Cu 2010) (Fig. 6.2). The literature on algal biofuels is vast (e.g., Demirbas 2009; Kapdan and Kargi 2006; Mata et al. 2010; Spolaore et al. 2006). We shall therefore not linger on details in this section of our review. In spite of the broad interest in algal biofuels, the actual commercial production of such forms of renewable energy is, to say the least, limited: the very large volumes of cultures required to obtain a meaningful quantity of biofuels, together with the still relatively low price of fuels

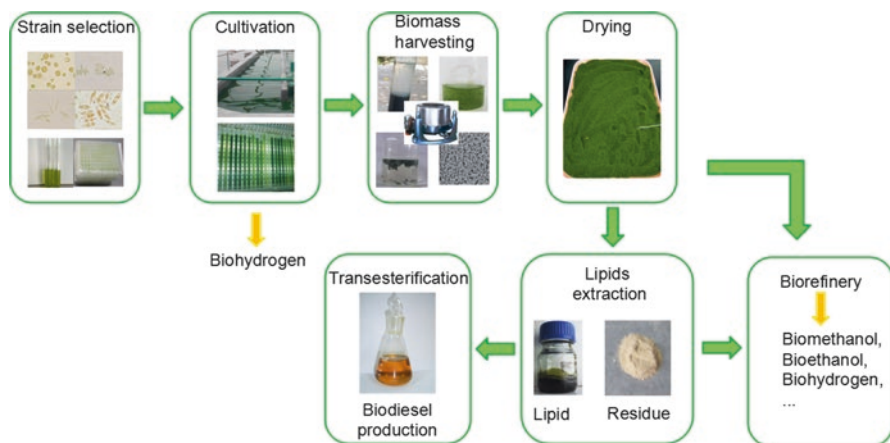


Fig. 6.2 The overall process flow for microalgal biofuel production

derived from fossil oil and other sources, is still in the way of a significant exploitation of algal fuels (Borowitzka 2016 and references therein).

6.7.1.1 Biodiesel

Biodiesel production comprises six steps: (i) strain selection; (ii) cultivation; (iii) biomass harvesting; (iv) biomass drying; (v) lipids extraction; and (vi) transesterification (Zhang et al. 2014b). We have already provided information on the first four steps; thus, the ensuing paragraphs focus on lipid extraction and transesterification.

Before lipid extraction, cells are usually lysed to facilitate access of the solvents to lipids within the cells. Various lysis procedures can be used: high pressure homogenization (HPH) (Samarasinghe et al. 2012), bead mills (Doucha and Lívanský 2008), ultrasonic disruption (Adam et al. 2012; Bigelow et al. 2014), and electroporation (Sheng et al. 2011). Ultrasonic disruption is possibly the procedure with the lowest energy requirement (Coons et al. 2014). Subsequently, lipids and fatty acids are extracted from the microalgal slurry mainly by two methods: the hexane Soxhlet method and the Bligh–Dyer method (Demirbas 2009; Kanda et al. 2013). Hexane-based oil extraction is more energy efficient and is therefore preferred for scaling-up efforts (Peralta-Ruiz et al. 2013). However, the use of chemical solvents has intrinsic problems associated with the toxicity of these compounds to humans and environment. Several supercritical fluids, especially supercritical CO₂, have been used for microalgal lipid extraction for the production of biodiesel. Although supercritical extraction is nontoxic and provides a nonoxidizing environment that avoids degradation of the extracts (Mouahid et al. 2013), it is expensive.

After lipid extraction, fatty acids transesterification is generally used to produce biodiesel (Lam et al. 2010). Lipid extraction and transesterification can be carried out simultaneously, simplifying the process and reducing the overall cost of

microalgal biodiesel production (Lam and Lee 2012). Biodiesel recovery in *in situ* transesterification is negatively affected by excessive biomass moisture (>20% m/m) (Sathish et al. 2014). Given the aforementioned high cost of biomass dehydration, this excessive moisture has a nontrivial effect on the economic performance of the production system. An improved *in situ* transesterification process that directly converts wet oil-bearing microalgal biomass into biodiesel was recently proposed (Dang-Thuan et al. 2013).

6.7.1.2 Other Microalgal Biofuels

Bioethanol

Most microalgae do not contain lignin; this property of algal biomass facilitates the enzymatic hydrolysis necessary for bioethanol production (Sun and Cheng 2002). Furthermore, in appropriate culture conditions, many algal species can accumulate high amounts of ethanol if substrates can be fermented (Bibia et al. 2017; Farias Silva and Bertucco 2016). Green algae of the genera *Scenedesmus*, *Chlorella*, *Chlorococcum*, and *Tetraselmis* and cyanobacteria of the genus *Synechococcus* have been reported to be potentially good sources of bioethanol (Farias Silva and Bertucco 2016). Typically, bioethanol is produced by the hydrolysis of sugars and their subsequent fermentation in microaerobic or anaerobic conditions using yeasts (Farias Silva and Bertucco 2016). Algae can generate ethanol directly in the dark, by fermentative metabolism (Ueno et al. 1998); however, this process does not seem to be sufficiently efficient for commercial exploitation. Algae can also produce ethanol directly via photofermentation (photanol) (Hellingwerf and Mattos 2009). In photofermentation, the glyceraldehyde-3-phosphate generated in the Calvin cycle is converted to phosphoenolpyruvate and then to pyruvate; pyruvate is decarboxylated to acetaldehyde (by pyruvate decarboxylase), which is finally converted to ethanol by alcohol dehydrogenase. Some engineered cyanobacteria have been made able to directly produce ethanol (and other compounds) through photofermentation in amounts and rates that appear to be compatible with their commercial exploitation (Farias Silva and Bertucco 2016 and references therein).

Molecular Hydrogen

Some green microalgae are capable of H₂ generation, a clean fuel with H₂O as the only major by-product. As opposed to non biological production processes, bio-H₂ can be produced at ambient temperature and pressure and has no demand for metal catalysts. The matter has been excellently summarized by Eroglu and Melis (2016). We shall therefore not overly linger on this theme and simply mention that two light-dependent electron transport pathways leading to H₂ production have been identified in *Chlamydomonas reinhardtii*: one draws electrons from water lysis at photosystem II, and the other uses the reducing power allocated on quinones through their reduction by hydrogenase. Also, a light-independent fermentative pathway leading to H₂ production has been identified in *C. reinhardtii* (Eroglu and Melis 2016). Incompatibility of simultaneous O₂ and H₂ evolution from microalgae has so

far hindered the development of large-scale bio-H₂ production (Rajvanshi and Sharma 2012). By using artificial miRNA (amiRNA) technology, a transgenic knockdown *C. reinhardtii* strain for the oxygen-evolving center (*OEE2* gene) was obtained; in this strain, O₂ is not released and the H₂ yield is about twofold higher than that of the wild type under similar growth conditions (Ngan et al. 2015).

Biogas

Microalgal biomass represents a potential alternative to biogas production from terrestrial crops (Dębowski et al. 2013). Photoautotrophically grown *Scenedesmus obliquus*, when used as biogas substrate, proved to produce more methane than maize silage (Wirth et al. 2015). However, a number of difficulties are associated with the use of algae for biogas production. For instance, the cell walls of some algae are resistant to anaerobic digestion and some algal strains generate compounds that are toxic to the bacteria that carry out anaerobic digestion; furthermore, in some cases, the C:N ratio of algae is unfavorable to anaerobic digestion (Dębowski et al. 2013 and references therein). This notwithstanding, the high turnover rate of algae and the possibility of selecting strains with suitable elemental stoichiometry make algae very interesting candidates for biogas production (Mussnug et al. 2010). Some authors have also reported that when algae are mixed with traditional feedstocks they improve the efficiency of biogas production (Mussnug et al. 2010; Zhong et al. 2012). Miao et al. (2014) showed that co-digestion of cyanobacteria with swine manure leads to an improved efficiency of both biodegradation and methane production as compared to the same processes without the addition of the algae. Zhao and Ruan (2013) also demonstrated the feasibility of adjusting the C/N ratio to increase biogas production by the addition of algae (mostly *Microcystis*) to kitchen wastes.

6.7.1.3 Challenges and Solutions for Algal Biofuel Production

Microalgal biofuel production is presently not conducted on a large scale because overwhelming investments in capital and operation are required (Chen et al. 2015b; Zhu 2015). In the case of biodiesel, for instance, the species that are known to be highly oleaginous often grow slowly. In such case, genetic manipulation may be advantageous and possibly necessary to obtain strains that can ensure sufficiently high productivity to make biodiesel production economically viable (Anandarajah et al. 2012; Iwai et al. 2014). It should also be considered that monocultures are susceptible to contamination, especially in conditions that intrinsically do not allow a tight control of the microbiota (e.g., wastewater); strains that grow slowly, such as the oleaginous ones, are especially likely to be outperformed by faster-growing competitors (Chen et al. 2015b). Mixed cultures of algae have been reported to persist in wastewater treatment systems and to be more stable and more resistant to exogenous invasion than monocultures (Chen et al. 2015b). This report would however need to be confirmed under a wider range of conditions; also, in-culture evolution (Borowitzka 2016) may have a stronger role in mixed cultures than in

monospecific cultures because of the selective pressure exerted by interspecific interaction (Venuleo et al. 2017). Some algal species used in large-scale open-pond commercial production are restricted to geographic locations with warm climates and would be unable to grow at acceptable rates during the hot or cold seasons of certain geographic regions (Holbrook et al. 2014). One solution to this problem is to identify indigenous algae that are adapted to the local environment (Holbrook et al. 2014).

Lipid accumulation occurs within microalgal cells according to the general principles outlined in previous publications (Giordano 2013; Palmucci et al. 2011; Raven and Giordano 2016). Zhang et al. (2013) suggested a possible connection between the oxidative stress induced by N-shortage and neutral lipid accumulation; applications of N-limitation or starvation, however, are inefficient methods to increase lipid accumulation because they can also significantly lower biomass and lipid productivity (Borowitzka 2016). A two-stage cultivation strategy has often been proposed for the production of stress-induced algal compounds (Borowitzka (2016) and references therein), in which a full-strength medium is used to promote biomass buildup, followed by a stress treatment (e.g., N-starvation; Zhu et al. 2014) to trigger the accumulation of the target compound. Also, “mid-point” approaches (i.e., compromises between best condition for growth and production of the compound of interest) have been suggested to simplify processes and decrease production costs (Borowitzka 2016). Zhu et al. (2016) proposed a single-step approach for boosting lipid production: these authors showed that the addition of trace amounts of urea to the growth medium significantly stimulated the accumulation of neutral lipids without affecting growth rates.

6.8 Microalgae for Bioremediation

6.8.1 *CO₂ Fixation and Flue Gas Treatment*

Carbon is the main nutrient in microalgal cells (36–65% of dry matter). It is therefore extremely alluring to use algae to sequester CO₂, at least temporarily (Singh and Ahluwalia 2013). The frequent suggestions to utilize algae for this purpose have rarely considered the physiological nuances of the responses of algal cells to elevated CO₂. As explained earlier, the excess CO₂ may be not taken up by the cells (thus conferring no advantage and only increasing the cost of new biomass production) or it is assimilated and subsequently elicits a nutritional unbalance leading to a change in biomass quality (Beardall and Giordano 2002; Giordano and Ratti 2013; Lynn et al. 2010; Palmucci et al. 2011; Raven et al. 2011, 2012). Also, the impact of elevated CO₂ on growth rates is variable, mostly species specific, and depending on energy availability (Beardall and Giordano 2002; Raven et al. 2011, 2012; Wu et al. 2012). Nevertheless, the utilization of algal biomass for the mitigation of CO₂ emission has its merits, provided that appropriate strains and suitable culture conditions

are selected and consideration is given to the fact that biomass will in the end release the CO₂ that it fixed. Liu et al. (2013) described a high-throughput screening method to rapidly identify microalgae strains that can tolerate high CO₂ condition or flue gases. Microalgae reported to tolerate high levels of CO₂ include *Chlorella* sp. (Qi et al. 2016), *Scenedesmus* sp. (Liu et al. 2013), and *Dunaliella tertiolecta* (Farrelly et al. 2013). Jacob et al. (2015) estimated that algal cultivation systems, whether they are tubular or flat photobioreactors or open ponds, can allow an effective and significant conversion of the CO₂ emitted by coal power plants into biomass. Once again, this work does not take into account the complex physiology associated with CO₂ fixation, but it does show that, at least in principle, algal cultivation can be coupled to industrial activity to minimize environmental impact. Experimental evidence (although in small-scale experiments) showed that the addition of flue gases to cultures of *Scenedesmus quadricauda* afforded a decrease by 85% v/v of CO₂, (and also 62% v/v of NO_x and 45% v/v of SO_x) in the flue gas; somewhat lower fixation capacities were obtained using *Botryococcus braunii* and *Chlorella vulgaris* (Kandimalla et al. 2016). Interestingly, the amount of fixed gases increased if the algae were cultured in mixotrophic conditions.

Flue gases contain different NO_x species, most of which are restricted by legislation and therefore must be removed (Van Den Hende et al. 2012). NO_x may serve as a nitrogen source for microalgae cultivation (Chen et al. 2016; Raven and Giordano 2016; Zhang et al. 2014a). Thus, “denoxification” (DeNO_x) by microalgae (bio-denox) may be a worthy contribution to flue gas treatment. As the efficiency of NO_x removal by microalgae varies dramatically among species, it is necessary to select or genetically modify suitable algal candidates for this purpose. Some strains of the genera *Chlorella*, *Scenedesmus*, and *Dunaliella* have been reported to significantly remove NO_x (Jin et al. 2008; Nagase et al. 2001; Santiago et al. 2010; Kandimalla et al. 2016), although high levels of NO_x tend to depress photosynthesis. In a typical flue gas from incineration processes, about 90–95% of the NO_x is given by NO (Fritz and Pitchon 1997). When NO dissolves in water, it is oxidized to nitrite and nitrate (Niu and Leung 2010). Nitrite has an inhibitory effect on algal growth, which is exerted through a retardation of electron transfer from Q_A to Q_B (Q_A is a bound quinone; Q_B is a quinone that binds and unbinds to photosystem II), and by interference with the donor side of PSII (Zhang et al. 2017). The screening of nitrite-tolerant microalgae species is therefore crucial for the use of algae in DeNO_x approaches. Li et al. (2016) analyzed numerous *Chlorella* strains in this respect and found that the degree of nitrite tolerance was a strain-specific feature, although most *Chlorella* strains showed the ability to withstand high concentrations of nitrite. The nitrate and nitrite generated by the dissolution of NO in water can be directly assimilated by algae (Giordano and Raven 2014; Raven and Giordano 2016); NO dissolution is however rather slow and often limits the rate of combined nitrogen assimilation. Zhang et al. (2014a) reported on a two-step microalgal bio-DeNO_x roadmap, in which NO_x-rich flue gases were first fixed, mostly as nitrite, to flue gas fixed salts (FGFS), and then used as nitrogen source for *Chlorella* sp. cultures. By using FGFS with NO₂⁻ equivalent to 5-fold that in the common culture medium BG11 (Stanier et al. 1971), up to 60% v/v of the NO_x was removed from the medium with an

inoculated cell density of 0.07 g DW L^{-1} , together with the production of 33% algae lipids (Zhang et al. 2014a). The mixotrophic cultivation of *Chlorella* sp. with FGFSS and glucose achieved an overall DeNO_x efficiency of 96%, demonstrating the feasibility and practicality of efficient biological DeNO_x by microalgae (Chen et al. 2016).

In most incineration flue gases, SO_x are also present; they mainly consist of SO₂, with a minor contribution (2–4% v/v) by SO₃; both SO₂ and SO₃ are highly soluble in water; SO₂ tends to hydrate to H₂SO₃, which dissociates in protons and sulfite (at pH >6) and bisulfite (prominent between pH 2 and 6); SO₃ hydrates to H₂SO₄, which typically dissociates in protons and sulfate (SO₄²⁻); SO₄²⁻ tends to prevail at pH >1.9; also the oxidation of H₂SO₃ can generate H₂SO₄ and SO₄²⁻ (Stumm and Morgan 1981; Van Den Hende et al. 2012 and references therein). The dissolution of SO_x, therefore, causes acidification of the medium, the extent of which depends on the SO_x content of the flue gas, which is a function of the combustion substrates from which it was generated. The consequence of SO_x dissolution in the growth medium can be such to limit the choice of algae to acidophilic and/or bisulfite-tolerant strains (see Van Den Hende et al. 2012 and references therein for details); in some cases, scrubbing SO_x from the flue gas may be a precondition for any microalgal treatment. If acidity and toxicity of SO_x-derived solutes do not prevent algal survival, algae can assimilate substantial amounts of SO₄²⁻ (Norici et al. 2005; Ratti et al. 2011; Giordano and Raven 2014; Prioretti and Giordano 2016), compatibly with elemental stoichiometry in the growth medium and stoichiometric constraints of cell growth (Giordano 2013).

6.8.2 Wastewater Treatment by Microalgae Cultivation

Large-scale microalgae culture may compete with crops and human activities with respect to water usage. Large amounts of nitrogen and phosphorus are also required, and their cost is high (Lardon et al. 2009). Both water and nutrients can be obtained from wastewaters; culturing algae in wastewaters also affords obvious environmental benefits. Microalgae are very effective at removing nitrogen, phosphorus, and toxic metals from wastewaters, producing cleaner effluents with high concentrations of dissolved oxygen (Gomez et al. 2013). Cabanelas et al. (2013) used *Chlorella vulgaris* for nitrogen and phosphorus removal from municipal wastewater with the highest removal rates of 9.8 (N) and 3.0 (P) mg l⁻¹ days⁻¹. Some studies also reported on the cultivation of microalgae in sewage under mixotrophic conditions. Cheng et al. (2013) found that mixotrophic microalga–bacteria systems significantly promoted algal growth and nutrient removal efficiency; maximal biomass and lipid productivity was attained when the alga *Desmodesmus* sp. CHX1 was used to treat piggy wastewater. Moreover, the co-culture of microalgae and bacteria in wastewater was reported to obtain 50–60% and 68–81% dissolved organic carbon (DOC) removal efficiency from municipal and industrial wastewater mixtures,

respectively (Nielsen 2015). Zhou et al. (2012) developed an effective organo-photolithotrophic system for improved wastewater nutrient removal, wastewater recycling, and enhanced algal lipid accumulation with *Auxenochlorella protothecoides* UMN280. Carbohydrate-rich and nitrogen-deficient solid wastes and some food industry wastewaters, such as olive mill wastewater, can be also used for hydrogen production (Keskin et al. 2011). It was reported that photosynthetic H₂ evolution from *C. reinhardtii* grown in advanced solid-state fermentation wastewater was increased by more than 700% compared to the cells grown in TAP medium (Chen et al. 2014). A study was also carried out to evaluate the potential of the green alga *Scenedesmus obliquus* grown in different concentrations of wastewater to produce biomass rich in sugar to produce bioethanol by fermentation processes; it was found that the highest removal efficiency of biological oxygen demand (BOD) and chemical oxygen demand (COD) were 18% for *S. obliquus* grown under aeration conditions and that the highest ethanol efficiency of biomass hydrolysate was 20.33% (Hamouda et al. 2016). Also, biomethane production in digesters could be improved by the addition of microalgae biomass harvested from algae-based swine wastewater digestate (Perazzoli et al. 2016). Because of the complex nature of wastewaters, issues such as contamination, inconsistent wastewater components, and unstable biomass production hinder efforts to use wastewater for large-scale algal cultivation (Cai et al. 2013).

The combination of CO₂ and/or NO_x fixation from flue gases and nutrient removal from wastewaters may provide a very promising alternative to current bioremediation strategies; the concomitant supply of nutrients from the gas and the liquid phase synergistically increases the effectiveness of depuration by algae (Chen et al. 2015b) and also stimulates algal growth and accumulation of added-value metabolic products (e.g., lipids) within the cells (Devi and Mohan 2012). Chinnasamy et al. (2010) cultured *Chlamydomonas globosa*, *Chlorella minutissima*, and *Scenedesmus bijuga* in untreated wastewater from the carpet industry to which a gas stream containing 5–6% v/v CO₂ was added; biomass productivity reached 5.9–21.1 g m⁻² day⁻¹. The cyanobacterium *Aphanothece microscopica* Nägeli cultivated in a photobioreactor using supplemented wastewater from an oil refinery was found to assimilate CO₂ when light was present; the capacity for CO₂ sequestration was lowered by one fourth when the algae were cultured in a light/dark photoperiod rather than under continuous light (Jacob-Lopes et al. 2010). The other important finding of this study was that only a small portion (about 3% v/v) of the CO₂ sequestered during cultivation was in the end effectively fixed in algal biomass, whereas the rest was probably released as biopolymers or volatile organic compounds. This finding is a warning about the direct extrapolation to commercial application of physiological studies that do not include a thorough analysis of biomass.

Recently, the use of microalgae for the concomitant remediation of environmental pollution and biofuel production has also been proposed; this would allow decreasing energy, nutrients, water cost, and also CO₂ emissions (Chen et al. 2015b; Sun et al. 2013), making biofuel production from microalgae more environmentally sustainable, cost-effective, and profitable (Chen et al. 2015b; Nayak et al. 2016) (Fig. 6.3).

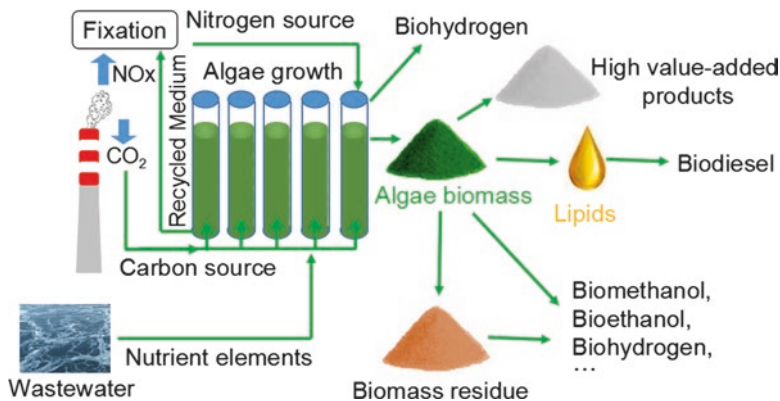


Fig. 6.3 Flowchart of the combination of environmental pollution control and biofuel production

Chlorogonium sp. showed good potential in the simultaneous purification of saline sewage effluent and CO₂ sequestration while delivering feedstock for potential biofuel production in a waste-recycling manner, achieving high removal efficiencies of NH₃-N, NO₃⁻-N, TN, and PO₄³⁻-P, at a CO₂ consumption rate of 58.96 mg l⁻¹ day⁻¹, and lipid content of 24.26% m/m of the algal biomass (Lee et al. 2015). An economically viable algal biofuel-based DeNO_x process using *Chlorella* was evaluated and verified in actual industrial flue gas condition by Zhang et al. (2014a). To reduce the mismatch between the large amount of NO_x contained in flue gases and the relatively low capacity for its assimilation in photolithotrophic algal growth, the possibility of managing NO_x by culturing oil-producing *Chlorella* strains mixotrophically was tested (Chen et al. 2016). After a stepwise optimization of mixotrophic cultivation of *Chlorella* using FGFS, an impressive DeNO_x efficiency of more than 96%, with a biomass productivity of 9.87 g l⁻¹ day⁻¹ and a high lipid productivity of 1.83 g l⁻¹ day⁻¹, were obtained.

6.9 Microalgal Cultivation for Food or Feed Production

The large and increasing demand for animal feed exerts a tremendous pressure on food crops, because, on a purely economic basis, the conversion of land use from crops (for humans) to animal feed production is more profitable; this trend is, however, in obvious conflict with the need to support the increasing human population on our planet. Microalgae can be effectively and conveniently used as animal feed (Norambuena et al. 2015; Packer et al. 2016; Tibbetts et al. 2017; Vidyashankar et al. 2015); furthermore, their cultivation poses minimal or no threat to crop production (Vidyashankar et al. 2015). The nutritional and bioactive effects of microalgal biomass have been assessed in a variety of studies (Benemann 2013; Wells et al. 2017). The composition of algae, whose cells are rich in carotenoids and other

antioxidants, essential polyunsaturated fatty acids, minerals, and protein with a balanced amino acid profile, makes them an excellent alternative to conventional feed-stocks such as corn, soya, barley, and skimmed milk (Shields and Lupatsch 2012). There are several examples of the utilization of algae for animal feed: *Chlorella vulgaris* has been used for the development of pet and fish feed (Groza et al. 1966; Li et al. 2015); *Dunaliella* can be used directly as a nutritional additive for fish or for secondary biological baits (such as rotifers and *Artemia*) and other aquacultured animals (Del Campo et al. 2007; Elbermawi 2009); *Spirulina maxima* has been used in swine feed (Saeid et al. 2013). Several studies have suggested that small amounts (2.5–10% of the diet) of algae in fish diets result in higher growth rates, feed utilization efficiency, carcass quality, physiological activity, intestinal microbiota, disease resistance, stress response, modulation of lipid metabolism, and protein retention during periods of reduced feed intake, and also lead to a higher palatability in sea urchin formulated feed (Cyrus et al. 2015; Valente et al. 2006; Nakagawa 1997; Norambuena et al. 2015; Wassef et al. 2005).

The widespread and growing interest in algae as food and food complements for humans emerges clearly in the recent literature (Cottin et al. 2011; Hafting et al. 2015; Harnedy and Fitzgerald 2011; Knies 2017; Packer et al. 2016; Pangestuti and Kim 2011; Sinéad et al. 2011; Wells et al. 2017). Limiting our *excursus* to microalgae (see Packer et al. 2016 for a panorama on macroalgae used for food), numerous species have been traditionally grown or have been collected as food: *Nostoc sphaeroides*, for instance, is an edible cyanobacteria widely cultivated in Hubei Province, China (Yi et al. 2016); there is evidence that in Central America the Aztecs were already eating cyanobacteria (*Spirulina*) collected from lakes in the fifteenth century; populations inhabiting the banks of Chad Lake, in Africa, have also traditionally used the cyanobacterium *Arthrospira* (formerly *Spirulina*) (Reed et al. 1985). *Arthrospira* was possibly the first microalga that spread widely across the shelves of supermarkets and “natural food” shops; this species encountered the favor of consumers for its rich protein, linolenic acid, and phycocyanin content. Nowadays, China is the main producer of *Spirulina* in the world (Lu et al. 2011). Also, *Dunaliella salina* encountered substantial success by its high content of β -carotene, an antioxidant in its own right and a precursor of vitamin A, with the first large production plants becoming operative in the 1980s in Israel, Australia, and the USA (Borowitzka 2016 and references therein). In more recent years, the fad of natural nutritional complements has facilitated the expansion of the market for nutritional products from algae, to which, with variably sound scientific bases, antioxidant, antibacterial, antiinflammatory, antiviral, and anti-cancer functions, for example, have been attributed (Wells et al. 2017). The consumption of microalgae as food has also, to some extent, been driven by the producers, who with the decline of profitability in algal biofuels have looked for alternative uses of their biomass (Packer et al. 2016). More species are now cultured in large-scale plants for the production and commercialization of β -carotene, astaxanthin, phycocyanin, some fatty acids (including Ω -3 and Ω -6), and other bioactive substances (Borowitzka 2016). *Chlorella* has been marketed as a health food because of its alleged ability to stimulate the human immune system; and its production is mainly distributed in

Japan, China, France, Portugal, and South Korea (An et al. 2008; Liu and Hu 2013; Saad et al. 2006). The green alga *Haematococcus pluvialis* can accumulate carotenoids, mostly astaxanthin and its ester derivatives, when subject to nutrient limitation, high temperature, or excessive light (Borowitzka 2016); these compounds have a high value on the market as antioxidants (Bagchi et al. 2001; Hagen and Grunewald 2001). The mass culture of *H. pluvialis* is mainly concentrated in Japan, Israel, and the USA (Gómez et al. 2013). Also, some heterotrophic species, such as the dinoflagellate *Cryptocodinium cohnii* and the labyrinthulid *Ulkenia*, have been used for production of docosahexaenoic acid (DHA), which has been proposed as a baby food additive (Ganuza et al. 2008; Lee Chang et al. 2014).

6.10 Conclusions

In the light of all these facts, it seems fair to conclude that, although some applications of microalgal cultivation appear not to be economically sustainable, at this point in time (e.g., the still fashionable use of these organisms for the sole production of biofuels), the use of microalgae in large-scale multifunctional plants is feasible and promising. Also, the direct use of algal biomass for human and animal nutrition or for the production of nutritional complements appears to have a positive outlook in terms of market demand and economic sustainability. However, further studies must be conducted, both on the engineering aspects of large-scale algal culturing systems and, possibly more importantly, on the specific challenges that industrial applications pose to algal physiology (e.g., responses to high CO₂, NO_x, and SO_x concentration, temperature, and low light penetration; C allocation under different growth regimes) and on the functional diversity of algae, which has been only marginally explored.

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References

- Adam F, Abert-Vian M, Peltier G, Chemat F (2012) “Solvent-free” ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. *Bioresour Technol* 114:457–465
- Ahmad AL, Yasin NHM, Derek CJC, Lim JK (2011) Microalgae as a sustainable energy source for biodiesel production: a review. *Renew Sust Energy Rev* 15(1):584–593. <https://doi.org/10.1016/j.rser.2010.09.018>
- Amaro HM, Guedes AC, Malcata FX (2011) Advances and perspectives in using microalgae to produce biodiesel. *Appl Energy* 88(10):3402–3410. <https://doi.org/10.1016/j.apenergy.2010.12.014>

- An HJ, Rim HK, Lee JH, Seo MJ, Hong JW, Kim NH, Myung NY, Moon PD, Choi IY, Na HJ (2008) Effect of *Chlorella vulgaris* on immune-enhancement and cytokine production in vivo and in vitro. *Food Sci Biotechnol* 17(5):953–958
- Anandarajah K, Mahendrapurumal G, Sommerfeld M, Hu Q (2012) Characterization of microalga *Nannochloropsis* sp. mutants for improved production of biofuels. *Appl Energy* 96(0):371–377. <https://doi.org/10.1016/j.apenergy.2012.02.057>
- Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ (2001) Oxygen free radical scavenging abilities of vitamins C, E, β -carotene, pycnogenol, grape seed proanthocyanidin extract and astaxanthins in vitro. *Res Commun Mol Pathol Pharmacol* 95(2):179–189
- Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136(2):215
- Beardall J, Giordano M (2002) Ecological implications of microalgal and cyanobacterial CO₂ concentrating mechanisms, and their regulation. *Funct Plant Biol* 29(2-3):335–347
- Beardall J, Raven JA (2016) Carbon acquisition by microalgae. In: *The physiology of microalgae*. Springer, Cham
- Behrebs P (2005) Photobioreactors and fermenters: the light and dark sides of growing algae. In: Andersen RA (ed) *Algal culturing techniques*. Academic Press, Burlington
- Ben-Amotz A (2004) Industrial production of microalgal cell-mass and secondary products major industrial species. In: Richmond A (ed) *Handbook of microalgal culture biotechnology and applied phycology*. Blackwell, Oxford, pp 273–280
- Benemann JR (2013) Microalgae for biofuels and animal feeds. *Biotechnol Bioeng* 110(9):2319–2321
- Berla BM, Rajib S, Immethun CM, Maranas CD, Seok MT, Pakrasi HB (2013) Synthetic biology of cyanobacteria: unique challenges and opportunities. *Front Microbiol* 4:246
- Beuckels A, Depraetere O, Vandamme D, Foubert I, Smolders E, Muylaert K (2013) Influence of organic matter on flocculation of *Chlorella vulgaris* by calcium phosphate precipitation. *Biomass Bioenergy* 54:107–114
- Bigelow TA, Xu J, Stessman DJ, Yao L, Spalding MH, Wang T (2014) Lysis of *Chlamydomonas reinhardtii* by high-intensity focused ultrasound as a function of exposure time. *Ultrason Sonochem* 21(3):1258–1264
- Bibia R, Ahmad Z, Imran M, Hussain S, Ditta A, Mahmood S, Khalid A (2017) Algal bioethanol production technology: a trend towards sustainable development. *Renew Sustain Energy Rev* 7:976–985
- Blank CE, Sanchez-Baracaldo P (2010) Timing of morphological and ecological innovations in the cyanobacteria—a key to understanding the rise in atmospheric oxygen. *Geobiology* 8(1):1–23
- Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J Biotechnol* 70(1-3):313–321
- Borowitzka MA (2016) Algal physiology and large-scale outdoor cultures of microalgae. In: *The physiology of microalgae*. Springer, Cham
- Bowes G, Ogren WL, Hageman RH (1971) Phosphoglycolate production catalyzed by ribulose diphosphate carboxylase. *Biochem Biophys Res Commun* 45(3):716–722
- Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sust Energy Rev* 14(2):557–577
- Brodie J, Chan CX, De Clerck O, Cock JM, Coelho SM, Gachon C, Grossman AR, Mock T, Raven JA, Smith AG, Yoon HS, Bhattacharya D (2017) The algal revolution. *Trends Plant Sci*. <https://doi.org/10.1016/j.tplants.2017.05.005>
- Cabanelas ITD, Ruiz J, Arbib Z, Chinalia FA, Garrido-Perez C, Rogalla F, Nascimento IA, Perales JA (2013) Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. *Bioresour Technol* 131:429–436. <https://doi.org/10.1016/j.biortech.2012.12.152>
- Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: status and prospects. *Renew Sust Energy Rev* 19(0):360–369. <https://doi.org/10.1016/j.rser.2012.11.030>
- Chen Y, Wang J, Zhang W, Chen L, Gao L, Liu T (2013) Forced light/dark circulation operation of open pond for microalgae cultivation. *Biomass Bioenergy* 56(56):464–470

- Chen M, Zhang L, Li S, Chang S, Wang W, Zhang Z, Wang J, Gang Z, Qi L, Xu W (2014) Characterization of cell growth and photobiological H₂ production of *Chlamydomonas reinhardtii* in ASSF industry wastewater. *Int J Hydrog Energy* 39(25):13462–13467
- Chen H, Hu J, Qiao Y, Chen W, Rong J, Zhang Y, He C, Wang Q (2015a) Ca²⁺-regulated cyclic electron flow supplies ATP for nitrogen starvation-induced lipid biosynthesis in green alga. *Sci Rep* 5:15117. <https://doi.org/10.1038/srep15117>. <http://www.nature.com/articles/srep15117#supplementary-information>
- Chen H, Qiu T, Rong J, He C, Wang Q (2015b) Microalgal biofuel revisited: an informatics-based analysis of developments to date and future prospects. *Appl Energy* 155:585–598. <https://doi.org/10.1016/j.apenergy.2015.06.055>
- Chen WX, Zhang SS, Rong JF, Li X, Chen H, He CL, Wang Q (2016) Effective biological DeNO_x of industrial flue gas by the mixotrophic cultivation of an oil-producing green alga *Chlorella* sp. C2. *Environ Sci Technol* 50(3):1620–1627. <https://doi.org/10.1021/acs.est.5b04696>
- Cheng HX, Tian GM, Liu JZ (2013) Enhancement of biomass productivity and nutrients removal from pretreated piggery wastewater by mixotrophic cultivation of *Desmodesmus* sp. CHX1. *Desalin Water Treat* 51(37-39):7004–7011. <https://doi.org/10.1080/19443994.2013.769917>
- Chinnasamy S, Bhatnagar A, Hunt RW, Das KC (2010) Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour Technol* 101(9):3097–3105
- Chojnacka K, Marquez-Rocha FJ (2004) Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology* 3:21–34
- Chojnacka K, Chojnacki A, Górecka H (2004) Trace element removal by *Spirulina* sp. from copper smelter and refinery effluents. *Hydrometallurgy* 73(1):147–153
- Chung BY, Deery MJ, Groen AJ, Howard J, Baulcombe DC (2016) mRNA turnover through CDS-targeting is the primary role of miRNA in the green alga *Chlamydomonas*. *bioRxiv*. <https://doi.org/10.1101/088807>
- Coons JE, Kalb DM, Dale T, Marrone BL (2014) Getting to low-cost algal biofuels: a monograph on conventional and cutting-edge harvesting and extraction technologies. *Algal Res* 6:250–270
- Cooper MS, Hardin WR, Petersen TW, Cattolico RA (2010) Visualizing “green oil” in live algal cells. *J Biosci Bioeng* 109(2):198–201. <https://doi.org/10.1016/j.jbiosc.2009.08.004>
- Cottin SC, Sanders TA, Hall WL (2011) The differential effects of EPA and DHA on cardiovascular risk factors. *Proc Nutr Soc* 70(2):215
- Cyrus MD, Bolton JJ, Scholtz R, Macey BM (2015) The advantages of *Ulva* (Chlorophyta) as an additive in sea urchin formulated feeds: effects on palatability, consumption and digestibility. *Aquacult Nutr* 21(5):578–591
- Daboussi F, Leduc S, Marechal A, Dubois G, Guyot V, Perez-Michaut C, Amato A, Falcitore A, Juillerat A, Beurdeley M, Voytas DF, Cavarec L, Duchateau P (2014) Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat Commun* 5:3831
- Dang-Thuan T, Chen CL, Chang JS (2013) Effect of solvents and oil content on direct transesterification of wet oil-bearing microalgal biomass of *Chlorella vulgaris* ESP-31 for biodiesel synthesis using immobilized lipase as the biocatalyst. *Bioresour Technol* 135:213–221. <https://doi.org/10.1016/j.biortech.2012.09.101>
- de Vargas C, Audic S, Henry N, Decelle J, Mahe F, Logares R, Lara E, Berney C, Le Bescot N, Probert I, Carmichael M, Poulain J, Romac S, Colin S, Aury JM, Bittner L, Chaffron S, Dunthorn M, Engelen S, Flegontova O, Guidi L, Horak A, Jaillon O, Lima-Mendez G, Lukes J, Malviya S, Morard R, Mulot M, Scalco E, Siano R, Vincent F, Zingone A, Dimier C, Picheral M, Searson S, Kandels-Lewis S, Acinas SG, Bork P, Bowler C, Gorsky G, Grimsley N, Hingamp P, Iudicone D, Not F, Ogata H, Pesant S, Raes J, Sieracki ME, Speich S, Stemann L, Sunagawa S, Weissenbach J, Wincker P, Karsenti E, Coordinators TO (2015) Eukaryotic plankton diversity in the sunlit ocean. *Science* 348(6237). doi:<https://doi.org/10.1126/science.1261605>
- Dębowski M, Zieliński M, Grala A, Dudek M (2013) Algae biomass as an alternative substrate in biogas production technologies: review. *Renew Sust Energy Rev* 27:596–604
- Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl Microbiol Biotechnol* 74(6):1163–1174

- Demirbas A (2009) Progress and recent trends in biodiesel fuels. *Energy Convers Manag* 50(1):14–34. <https://doi.org/10.1016/j.enconman.2008.09.001>
- Devi MP, Mohan SV (2012) CO₂ supplementation to domestic wastewater enhances microalgae lipid accumulation under mixotrophic microenvironment: effect of sparging period and interval. *Bioresour Technol* 112:116–123. <https://doi.org/10.1016/j.biortech.2012.02.095>
- Domenighini A, Giordano M (2009) Fourier transform infrared spectroscopy of microalgae as a novel tool for biodiversity studies, species identification, and the assessment of water quality. *J Phycol* 45(2):522–531
- Doucha J, Lívanský K (2006) Productivity, CO₂/O₂ exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a middle and southern European climate. *J Appl Phycol* 18(6):811–826
- Doucha J, Lívanský K (2008) Influence of processing parameters on disintegration of *Chlorella* cells in various types of homogenizers. *Appl Microbiol Biotechnol* 81(3):431–440
- Elbermawi NM (2009) Using *Dunaliella salina* extract to improve survival, stress tolerance and growth performance of freshwater prawn *Macrobrachium rosenbergii*. *Egypt J Nutr Feeds* 12(1):113–125
- Elser JJ, Acquisti C, Kumar S (2011) Stoichiogenomics: the evolutionary ecology of macromolecular elemental composition. *Trends Ecol Evol* 26(1):38–44
- Emanuel SL, Francisco OC, Aurora G, Emilio F, Amaury DM (2016) Characterization of a mutant deficient for ammonium and nitric oxide signalling in the model system *Chlamydomonas reinhardtii*. *PLoS One* 11(5):e0155128
- Enzing C, Ploeg M, Barbosa M, Sijtsma L (2014) Microalgae-based products for the food and feed sector: an outlook for Europe. JRC Scientific and Policy Reports. Report EUR 26255 EN. European Union 2014. doi:<https://doi.org/10.2791/3339>
- Eroglu E, Melis A (2016) Microalgal hydrogen production research. *Int J Hydrog Energy* 41(30):12772–12798. <https://doi.org/10.1016/j.ijhydene.2016.05.115>
- Fanesi A, Raven JA, Giordano M (2014) Growth rate affects the responses of the green alga *Tetraselmis suecica* to external perturbations. *Plant Cell Environ* 37(2):512–519
- Farrelly DJ, Brennan L, Everard CD, McDonnell KP (2014) Carbon dioxide utilisation of *Dunaliella tertiolecta* for carbon bio-mitigation in a semicontinuous photobioreactor. *Appl Microbiol Biotechnol*. <https://doi.org/10.1007/s00253-013-5322-y>
- Farias Silva CE, Bertuccio A (2016) Bioethanol from microalgae and cyanobacteria: a review and technological outlook. *Process Biochem* 51:1833–1842
- Flynn KJ, Raven JA, Rees TAV, Finkel Z, Quigg A, Beardall J (2010) Is the growth rate hypothesis applicable to microalgae? *J Phycol* 46(1):1–12
- Frigon J-C, Matteau-Lebrun F, Hamani Abdou R, McGinn PJ, O’Leary SJB, Guiot SR (2013) Screening microalgae strains for their productivity in methane following anaerobic digestion. *Appl Energy* 108(0):100–107. <https://doi.org/10.1016/j.apenergy.2013.02.051>
- Fritz A, Pitchon V (1997) The current state of research on automotive lean NO_x catalysis. *Appl Catal B Environ* 13(1):1–25
- Ganuza E, Benítez-Santana T, Atalah E, Vega-Orellana O, Ganga R, Izquierdo MS (2008) *Cryptocodinium cohnii* and *Schizochytrium* sp. as potential substitutes to fisheries-derived oils from seabream (*Sparus aurata*) microdiets. *Aquaculture* 277(1-2):109–116
- Geider RJ, La Roche J (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur J Phycol* 37(1):1–17
- Gentil J, Hempel F, Moog D, Zauner S, Maier UG (2017) Origin of complex algae by secondary endosymbiosis: a journey through time. *Protoplasma* 254:1835–1843
- Giordano M (2013) Homeostasis: an underestimated focal point of ecology and evolution. *Plant Sci* 211:92–101
- Giordano M, Ratti S (2013) The biomass quality of algae used for CO₂ sequestration is highly species-specific and may vary over time. *J Appl Phycol* 25(5):1431–1434
- Giordano M, Kansiz M, Heraud P, Beardall J, Wood B, McNaughton D (2001) Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga *Chaetoceros muellerii* (Bacillariophyceae). *J Phycol* 37(2):271–279

- Giordano M, Beardall J, Raven JA (2005) CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annu Rev Plant Biol* 56:99–131
- Giordano M, Palmucci M, Norici A (2015a) Taxonomy and growth conditions concur to determine the energetic suitability of algal fatty acid complements. *J Appl Phycol* 27(4):1401–1413
- Giordano M, Palmucci M, Raven JA (2015b) Growth rate hypothesis and efficiency of protein synthesis under different sulphate concentrations in two green algae. *Plant Cell Environ* 38(11):2313–2317
- Giordano M, Raven JA (2014) Nitrogen and sulfur assimilation in plants and algae. *Aquat Bot* 118:45–61. <https://doi.org/10.1016/j.aquabot.2014.06.012>
- Giordano M, Norici A, Beardall J (2017) Impact of inhibitors of amino acid, protein, and RNA synthesis on C allocation in the diatom *Chaetoceros muellerii*: a FTIR approach. *Algae* 32(2):161–170. <https://doi.org/10.4490/algae.2017.32.6.6>
- Gómez PI, Inostroza I, Pizarro M, Pérez J (2013) From genetic improvement to commercial-scale mass culture of a Chilean strain of the green microalga *Haematococcus pluvialis* with enhanced productivity of the red ketocarotenoid astaxanthin. *AoB Plants* 5(1):pt026
- Gomez C, Escudero R, Morales MM, Figueroa FL, Fernandez-Sevilla JM, Acien FG (2013) Use of secondary-treated wastewater for the production of *Muriellopsis* sp. *Appl Microbiol Biotechnol* 97(5):2239–2249. <https://doi.org/10.1007/s00253-012-4634-7>
- Gressel J (2008) Transgenics are imperative for biofuel crops. *Plant Sci* 174(3):246–263. <https://doi.org/10.1016/j.plantsci.2007.11.009>
- Grobbelaar JU (2009) From laboratory to commercial production: a case study of a *Spirulina* (*Arthrospira*) facility in Musina, South Africa. *J Appl Phycol* 21(5):523–527
- Groza I, Boldizar A, Vlad G (1966) *Chlorella vulgaris* an important source of protein and vitamins in animal feeding. *Revista Zooteh Med Vet* 7:24–26
- Hafting JT, Craigie JS, Stengel DB, Loureiro RR, Buschmann AH, Yarish C, Edwards MD, Critchley AT (2015) Prospects and challenges for industrial production of seaweed bioactives. *J Phycol* 51(5):821
- Hagen C, Grunewald K (2001) Compartmentation of astaxanthin biosynthesis in *Haematococcus pluvialis*. *Sci Access* 3(1). <https://doi.org/10.1071/SA0403037>
- Hamouda RA, Yeheia DS, Hussein MH, Hamzah HA (2016) Removal of heavy metals and production of bioethanol by green alga *Scenedesmus obliquus* grown in different concentrations of wastewater. *Sains Malays* 45(3):467–476
- Harnedy PA, Fitzgerald RJ (2011) Bioactive proteins, peptides, and amino acids from macroalgae. *J Phycol* 47(2):218
- Hellingwerf KJ, De Mattos MJT (2009) Alternative routes to biofuels: light-driven biofuel formation from CO₂ and water based on the ‘photanol’ approach. *J Biotechnol* 142:87–90
- Ho TY, Quigg A, Finkel ZV, Milligan AJ, Wyman K, Falkowski PG, Morel FMM (2010) The elemental composition of some marine phytoplankton. *J Phycol* 39(6):1145–1159
- Holbrook GP, Davidson Z, Tataru RA, Ziemer NL, Rosentrater KA, Scott Grayburn W (2014) Use of the microalga *Monoraphidium* sp. grown in wastewater as a feedstock for biodiesel: cultivation and fuel characteristics. *Appl Energy* 131(0):386–393. <https://doi.org/10.1016/j.apenergy.2014.06.043>
- Hopes A, Nekrasov V, Kamoun S, Mock T (2016) Editing of the urease gene by CRISPR-Cas in the diatom *Thalassiosira pseudonana*. *Plant Methods* 12:49
- Huang CH, Shen CR, Li H, Sung LY, Wu MY, Hu YC (2016) CRISPR interference (CRISPRi) for gene regulation and succinate production in cyanobacterium *S. elongatus* PCC 7942. *Microb Cell Factories* 15(1):196
- Iwai M, Ikeda K, Shimojima M, Ohta H (2014) Enhancement of extraplastidic oil synthesis in *Chlamydomonas reinhardtii* using a type-2 diacylglycerol acyltransferase with a phosphorus starvation-inducible promoter. *Plant Biotechnol J* 12(6):808–819. <https://doi.org/10.1111/Pbi.12210>
- Jacob A, Xia A, Murphy JD (2015) ChemInform abstract: a perspective on gaseous biofuel production from micro-algae generated from CO₂ from a coal-fired power plant. *ChemInform* 148(51):396–402

- Jacob-Lopes E, Scoparo CHG, Queiroz MI, Franco TT (2010) Biotransformations of carbon dioxide in photobioreactors. *Energy Convers Manag* 51(5):894–900
- Jebsen C, Norici A, Wagner H, Palmucci M, Giordano M, Wilhelm C (2012) FTIR spectra of algal species can be used as physiological fingerprints to assess their actual growth potential. *Physiol Plant* 146(4):427–438
- Jin H-F, Santiago DEO, Park J, Lee K (2008) Enhancement of nitric oxide solubility using Fe(II)-EDTA and its removal by green algae *Scenedesmus* sp. *Biotechnol Bioprocess Eng* 13(1):48–52. <https://doi.org/10.1007/s12257-007-0164-z>
- Jones RI (2000) Mixotrophy in planktonic protists: an overview. *Freshw Biol* 45(2):219–226
- Kaffes N, Thoms S, Trimbom S, Rost B, Richter K-U, Köhler A, Norici A, Giordano M (2010) Carbon and nitrogen fluxes in the marine coccolithophore *Emiliana huxleyi* grown under different nitrate concentrations. *J Exp Mar Biol Ecol* 393:1–8. <https://doi.org/10.1016/j.jembe.2010.06.004>
- Kanda H, Li P, Yoshimura T, Okada S (2013) Wet extraction of hydrocarbons from *Botryococcus braunii* by dimethyl ether as compared with dry extraction by hexane. *Fuel* 105:535–539. <https://doi.org/10.1016/j.fuel.2012.08.032>
- Kandimalla P, Desi S, Vurimindi H (2016) Mixotrophic cultivation of microalgae using industrial flue gases for biodiesel production. *Environ Sci Pollut Res* 23(10):9345–9354
- Kapdan IK, Kargi F (2006) Bio-hydrogen production from waste materials. *Enzyme Microb Technol* 38(5):569–582. <https://doi.org/10.1016/j.enzmictec.2005.09.015>
- Keskin T, Abo-Hashesh M, Hallenbeck PC (2011) Photofermentative hydrogen production from wastes. *Bioresour Technol* 102(18):8557–8568. <https://doi.org/10.1016/j.biortech.2011.04.004>
- Kilian O, Benemann CSE, Niyogi KK, Vick B (2011) High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. *Proc Natl Acad Sci USA* 108(52):21265–21269
- Knies JM (2017) Algae and algal products as novel foods. *Ernahr Umsch* 64(2):M84–M93
- Knoll AH (2003) Biomineralization and evolutionary history. *Rev Mineral Geochem* 54(1):329–356. <https://doi.org/10.2113/054032>
- Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J, Malcata FX, van Langenhove H (2010) Enhanced CO₂ fixation and biofuel production via microalgae: recent developments and future directions. *Trends Biotechnol* 28(7):371–380. <https://doi.org/10.1016/j.tibtech.2010.04.004>
- Kusakabe T, Tatsuke T, Tsuruno K, Hirokawa Y, Atsumi S, Liao JC, Hanai T (2013) Engineering a synthetic pathway in cyanobacteria for isopropanol production directly from carbon dioxide and light. *Metab Eng* 20(5):101
- Lam MK, Lee KT (2012) Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnol Adv* 30(3):673–690
- Lam MK, Lee KT, Mohamed AR (2010) Homogeneous, heterogeneous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: a review. *Biotechnol Adv* 28(4):500–518
- Lane N (2017) Serial endosymbiosis or singular event at the origin of eukaryotes? *J Theor Biol* 434:58–67
- Lardon L, Hélias A, Sialve B, Steyer J-P, Bernard O (2009) Life-cycle assessment of biodiesel production from microalgae. *Environ Sci Technol* 43(17):6475–6481
- Lee Chang KJ, Nichols CM, Blackburn SI, Dunstan GA, Koutoulis A, Nichols PD (2014) Comparison of thraustochytrids *Aurantiochytrium* sp., *Schizochytrium* sp., *Thraustochytrium* sp., and *Ulkenia* sp. for production of biodiesel, long-chain omega-3 oils, and exopolysaccharide. *Mar Biotechnol* 16(4):396–411
- Lee WNP, Wahjudi PN, Xu J, Go VL (2010) Tracer-based metabolomics: concepts and practices. *Clin Biochem* 43(16-17):1269–1277
- Lee YC, Kim B, Farooq W, Chung J, Han JI, Shin HJ, Jeong SH, Park JY, Lee JS, YK O (2013) Harvesting of oleaginous *Chlorella* sp. by organoclays. *Bioresour Technol* 132:440–445. <https://doi.org/10.1016/j.biortech.2013.01.102>

- Lee KY, Ng TW, Li GY, An TC, Kwan KK, Chan KM, Huang GC, Yip HY, Wong PK (2015) Simultaneous nutrient removal, optimised CO₂ mitigation and biofuel feedstock production by *Chlorogonium* sp. grown in secondary treated non-sterile saline sewage effluent. *J Hazard Mater* 297:241–250
- Li J, Liu Y, Cheng JJ, Mos M, Daroch M (2015) Biological potential of microalgae in China for biorefinery-based production of biofuels and high value compounds. *New Biotechnol* 32(6):588
- Li T, Xu G, Rong J, Chen H, He C, Giordano M, Wang Q (2016) The acclimation of *Chlorella* to high-level nitrite for potential application in biological NO_x removal from industrial flue gases. *J Plant Physiol* 195:73–79
- Liu J, Hu Q (2013) *Chlorella*: industrial production of cell mass and chemicals. In: Handbook of microalgal culture: applied phycology and biotechnology. Wiley, Chichester
- Liu Z, Zhang F, Chen F (2013) High throughput screening of CO₂-tolerating microalgae using GasPak bags. *Aquat Biosyst* 9(1):23. <https://doi.org/10.1186/2046-9063-9-23>
- Loladze I, Elser JJ (2011) The origins of the Redfield nitrogen-to-phosphorus ratio are in a homeostatic protein-to-rRNA ratio. *Ecol Lett* 14(3):244–250
- Lu YM, Xiang WZ, Wen YH (2011) Spirulina (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. *J Appl Phycol* 23(2):265
- Lynn SG, Kilham SS, Kreeger DA, Interlandi SJ (2010) Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *J Phycol* 36(3):510–522
- Marcus Y, Altmangueta H, Wolff Y, Gurevitz M (2011) Rubisco mutagenesis provides new insight into limitations on photosynthesis and growth in *Synechocystis* PCC6803. *J Exp Bot* 62(12):4173–4182
- Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. *Renew Sust Energy Rev* 14(1):217–232. <https://doi.org/10.1016/j.rser.2009.07.020>
- McFadden GI (2001) Primary and secondary endosymbiosis and the origin of plastids. *J Phycol* 37:951–959
- Memola F, Mukherjee B, Moroney JV, Giordano M (2014) Carbon allocation and metal use in four *Chlamydomonas* mutants defective in CCM-related genes. *Photosynth Res* 121:111–124
- Miao HF, Wang SQ, Zhao MX, Huang ZX, Ren HY, Yan Q, Ruan WQ (2014) Codigestion of Taihu blue algae with swine manure for biogas production. *Energy Convers Manag* 77:643–649
- Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature (Lond)* 191(478):144–148
- Mitra A, Flynn KJ, Tillmann U, Raven JA, Caron D, Stoecker DK, Not F, Hansen PJ, Hallegraef G, Sanders R, Wilken S, McManus G, Johnson M, Pitta P, Vage S, Berge T, Calbet A, Thingstad F, Jeong HJ, Burkholder J, Glibert PM, Graneli E, Lundgren V (2016) Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition: incorporation of diverse mixotrophic strategies. *Protist* 167(2):106–120
- Montecchiaro F, Giordano M (2010) Compositional homeostasis of the dinoflagellate *Protoceratium reticulatum* grown at three different pCO₂. *J Plant Physiol* 167(2):110–113
- Mooij TD, Janssen M, Cerezo-Chinarro O, Mussgnug JH, Kruse O, Ballottari M, Bassi R, Bujaldon S, Wollman FA, Wijffels RH (2015) Antenna size reduction as a strategy to increase biomass productivity: a great potential not yet realized. *J Appl Phycol* 27(3):1063–1077
- Mouahid A, Crampon C, Toudji SAA, Badens E (2013) Supercritical CO₂ extraction of neutral lipids from microalgae: experiments and modelling. *J Supercrit Fluids* 77:7–16. <https://doi.org/10.1016/j.supflu.2013.01.024>
- Mussgnug JH, Klassen V, Schluter A, Kruse O (2010) Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J Biotechnol* 150(1):51–56
- Nagase H, Yoshihara K, Eguchi K, Okamoto Y, Murasaki S, Yamashita R, Hirata K, Miyamoto K (2001) Uptake pathway and continuous removal of nitric oxide from flue gas using microalgae. *Biochem Eng J* 7(3):241–246. [https://doi.org/10.1016/S1369-703x\(00\)00122-4](https://doi.org/10.1016/S1369-703x(00)00122-4)

- Nakagawa H (1997) Effect of dietary algae on improvement of lipid metabolism in fish. *Biomed Pharmacother* 51(8):345–348
- Nayak M, Karemore A, Sen R (2016) Sustainable valorization of flue gas CO₂ and wastewater for the production of microalgal biomass as a biofuel feedstock in closed and open reactor systems. *RSC Adv* 6(94):91111–91120
- Ngan CY, Wong CH, Choi C, Yoshinaga Y, Louie K, Jia J, Chen C, Bowen B, Cheng HY, Leonelli L, Kuo R, Baran R, Garcia-Cerdan JG, Pratap A, Wang M, Lim J, Tice H, Daum C, Xu J, Northen T, Visel A, Bristow J, Niyogi KK, Wei CL (2015) Lineage-specific chromatin signatures reveal a regulator of lipid metabolism in microalgae. *Nat Plants* 1(8):15107
- Nicklisch A, Steinberg CEW (2009) RNA/protein and RNA/DNA ratios determined by flow cytometry and their relationship to growth limitation of selected planktonic algae in culture. *Eur J Phycol* 44:297–308. <https://doi.org/10.1080/09670260802578518>
- Nielsen A (2015) Treatment of wastewater with microalgae under mixotrophic growth: a focus on removal of DOC from municipal and industrial wastewater. Degree thesis in Geocology, Umeå University
- Niu HJY, Leung DY C (2010) A review on the removal of nitrogen oxides from polluted flow by bioreactors. *Environ Rev* 18:175–189. <https://doi.org/10.1139/A10-007>
- Norambuena F, Hermon K, Skrzypczyk V, Emery JA, Sharon Y, Beard A, Turchini GM (2015) Algae in fish feed: performances and fatty acid metabolism in juvenile Atlantic salmon. *PLoS One* 10(4):e0124042
- Norici A, Bazzoni AM, Pugnetti A, Raven JA, Giordano M (2011) Impact of irradiance on the C allocation in the coastal marine diatom *Skeletonema marinoi* Sarno and Zingone. *Plant Cell Environ* 34(10):1666–1677
- Norici A, Hell R, Giordano M (2005) Sulfur and primary production in aquatic environments: an ecophysiological perspective. *Photosynth Res* 85:409–417. <https://doi.org/10.1007/s11120-005-3250-0>
- Nymark M, Sharma AK, Sparstad T, Bones AM, Winge P (2016) A CRISPR/Cas9 system adapted for gene editing in marine algae. *Sci Rep* 6:24951
- Packer MA, Harris GC, Adams SL (2016) Food and feed applications of algae. In: Bux F, Chisti Y (eds) *Algae biotechnology, Green Energy and Technology*. Springer, Cham
- Palmucci M, Giordano M (2012) Is cell composition related to the phylogenesis of microalgae? An investigation using hierarchical cluster analysis of Fourier transform infrared spectra of whole cells. *Environ Exp Bot* 75:220–224
- Palmucci M, Ratti S, Giordano M (2011) Ecological and evolutionary implications of carbon allocation in marine phytoplankton as a function of nitrogen availability: a Fourier transform infrared spectroscopy approach. *J Phycol* 47(2):313–323
- Pangestuti R, Kim SK (2011) Biological activities and health benefit effects of natural pigments derived from marine algae. *J Funct Foods* 3(4):255–266
- Peralta-Ruiz Y, González-Delgado AD, Kafarov V (2013) Evaluation of alternatives for microalgae oil extraction based on exergy analysis. *Appl Energy* 101(0):226–236. <https://doi.org/10.1016/j.apenergy.2012.06.065>
- Perazzoli S, Bruchez BM, Michelon W, Steinmetz RLR, Mezzari MP, Nunes EO, da Silva MLB (2016) Optimizing biomethane production from anaerobic degradation of *Scenedesmus* spp. biomass harvested from algae-based swine digestate treatment. *Int Biodeter Biodegr* 109:23–28
- Perez-García O, Escalante FM, de-Bashan LE, Bashan Y (2011) Heterotrophic cultures of microalgae: metabolism and potential products. *Water Res* 45(1):11–36
- Pires JCM, Alvim-Ferraz MCM, Martins FG (2017) Photobioreactor design for microalgae production through computational fluid dynamics: a review. *Renew Sust Energy Rev* 79:248–254
- Poulsen N, Chesley PM, Kröger N (2006) Molecular genetic manipulation of the diatom *Thalassiosira pseudonana* (Bacillariophyceae). *J Phycol* 42(5):1059–1065
- Prioretti L, Giordano M (2016) Direct and indirect influence of sulfur availability on phytoplankton evolutionary trajectories. *J Phycol* 52:1094–1102

- Qi F, Pei HY, Hu WR, Mu RM, Zhang S (2016) Characterization of a microalgal mutant for CO₂ biofixation and biofuel production. *Energy Convers Manag* 122:344–349
- Qiao Y, Rong J, Chen H, He C, Wang Q (2015) Non-invasive rapid harvest time determination of oil-producing microalgae cultivations for biodiesel production by using chlorophyll fluorescence. *Front Energy Res* 3:44
- Quigg A, Finkel ZV, Irwin AJ, Rosenthal Y, Ho TY, Reinfelder JR, Schofield O, Morel FMM, Falkowski PG (2003) The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature (Lond)* 425(6955):291–294
- Quigg A, Irwin AJ, Finkel ZV (2011) Evolutionary inheritance of elemental stoichiometry in phytoplankton. *Proc R Soc Lond B Biol Sci* 278(1705):526–534
- Rajvanshi S, Sharma MP (2012) Micro algae: a potential source of biodiesel. *J Sustain Bioenergy Syst* 2(3):49–59
- Ramey CJ, Barón-Sola Á, Aucoin HR, Boyle NR (2015) Genome engineering in cyanobacteria: where we are and where we need to go. *ACS Synth Biol* 4(11):1186
- Rasala BA, Chao SS, Pier M, Barrera DJ, Mayfield SP (2014) Enhanced genetic tools for engineering multigene traits into green algae. *PLoS One* 9(4):e94028
- Ratti S, Knoll AH, Giordano M (2011) Did sulfate availability facilitate the evolutionary expansion of chlorophyll a+c phytoplankton in the oceans? *Geobiology* 9(4):301–312. <https://doi.org/10.1111/j.1472-4669.2011.00284.x>
- Raven JA (1995) Costs and benefits of low intracellular osmolarity in cells of fresh-water algae. *Funct Ecol* 9(5):701–707
- Raven JA (1997) Inorganic carbon acquisition by marine autotrophs. *Adv Bot Res* 27:85–209
- Raven JA, Beardall J (2016) Dark respiration and organic carbon loss. In: *The physiology of microalgae*. Springer, Cham
- Raven JA, Geider RJ (2003) Adaptation, acclimation and regulation in algal photosynthesis. In: Larkum AWD, Douglas SE, Raven JA (eds) *Photosynthesis in algae, Advances in photosynthesis and respiration*, vol 14. Springer, Dordrecht
- Raven JA, Giordano M (2014) Algae. *Curr Biol* 24(13):R590–R595. <https://doi.org/10.1016/j.cub.2014.05.039>
- Raven JA, Giordano M (2016) Combined nitrogen. In: Borowitzka M, Beardall J, Raven JA (eds) *The physiology of microalgae*. Springer, Cham, pp 143–154
- Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC (2005) Algae lacking carbon-concentrating mechanisms. *Can J Bot* 83(7):879–890
- Raven JA, Giordano M, Beardall J (2008) Insights into the evolution of CCMs from comparisons with other resource acquisition and assimilation processes. *Physiol Plant* 133(1):4–14
- Raven JA, Beardall J, Flynn KJ, Maberly SC (2009) Phagotrophy in the origins of photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs: relation to Darwin's insectivorous plants. *J Exp Bot* 60(14):3975–3987
- Raven JA, Giordano M, Beardall J, Maberly SC (2011) Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynth Res* 109(1-3):281–296
- Raven JA, Giordano M, Beardall J, Maberly SC (2012) Algal evolution in relation to atmospheric CO₂: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Philos Trans R Soc Lond B Biol Sci* 367(1588):493–507
- Raven JA, Beardall J, Larkum AWD, Sanchez-Baracaldo P (2013) Interactions of photosynthesis with genome size and function. *Philos Trans R Soc Lond B Biol Sci* 368(1622):20120264
- Raven JA, Beardall J, Giordano M (2014) Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms. *Photosynth Res* 121(2-3):111–124
- Redfield AC (1934) The haemocyanins. *Biol Rev Camb Philos Soc* 9(2):175–212
- Reed RH, Warr SRC, Richardson DL, Moore DJ, Stewart WDP (1985) Blue-green algae (cyanobacteria): prospects and perspectives. *Plant Soil* 89(1):97–106
- Richardson JW, Johnson MD, Outlaw JL (2012) Economic comparison of open pond raceways to photo bio-reactors for profitable production of algae for transportation fuels in the southwest. *Algal Res* 1(1):93–100

- Richmond AE (1986) Microalgaculture. *Crit Rev Biotechnol* 4:369–438. <https://doi.org/10.3109/07388558609150800>
- Ruan Z, Giordano M (2017) The use of NH_4^+ rather than NO_3^- affects cell stoichiometry, C allocation, photosynthesis and growth in the cyanobacterium *Synechococcus* sp. UTEX LB 2380, only when energy is limiting. *Plant Cell Environ* 40:227–236
- Ruan Z, Raven JA, Giordano M (2017) In *Synechococcus* sp. competition for energy between assimilation and acquisition of C and those of N only occurs when growth is light limited. *J Exp Bot* 68(14):3829–3839
- Saad SM, Yusof YAM, Ngah WZW (2006) Comparison between locally produced *Chlorella vulgaris* and *Chlorella vulgaris* from Japan on proliferation and apoptosis of liver cancer cell line, HepG2. *Malays J Biochem Mol Biol* 13:32–36
- Saeid A, Chojnacka K, Korczyński M, Korniewicz D, Dobrzański Z (2013) Biomass of *Spirulina maxima* enriched by biosorption process as a new feed supplement for swine. *J Appl Phycol* 25(2):667–675
- Samarasinghe N, Fernando S, Lacey R, Faulkner WB (2012) Algal cell rupture using high pressure homogenization as a prelude to oil extraction. *Renew Energy* 48:300–308
- Santiago DE, Jin H-F, Lee K (2010) The influence of ferrous-complexed EDTA as a solubilization agent and its auto-regeneration on the removal of nitric oxide gas through the culture of green alga *Scenedesmus* sp. *Process Biochem* 45(12):1949–1953
- Sathish A, Smith BR, Sims RC (2014) Effect of moisture on in situ transesterification of microalgae for biodiesel production. *J Chem Technol Biotechnol* 89(1):137–142. <https://doi.org/10.1002/jctb.4125>
- Savakis P, Hellingwerf KJ (2015) Engineering cyanobacteria for direct biofuel production from CO_2 . *Curr Opin Biotechnol* 33(33):8–14
- Scaife MA, Nguyen GTDT, Rico J, Lambert D, Helliwell KE, Smith AG (2015) Establishing *Chlamydomonas reinhardtii* as an industrial biotechnology host. *Plant J* 82:532–546
- Schmollinger S, Merchant SS (2014) Nitrogen-sparing mechanisms in *Chlamydomonas* affect the transcriptome, the proteome, and photosynthetic metabolism. *Plant Cell* 26(4):1410–1435
- Sheng J, Vannela R, Rittmann BE (2011) Evaluation of cell-disruption effects of pulsed-electric-field treatment of *Synechocystis* PCC 6803. *Environ Sci Technol* 45(8):3795–3802
- Shields RJ, Lupatsch I (2012) Algae for aquaculture and animal feeds. *Technikfolgenabschätzung – Theorie Und Praxis* 21(1):23–37
- Shin SE, Lim JM, Koh HG, Kim EK, Kang NK, Jeon S, Kwon S, Shin WS, Lee B, Hwangbo K, Kim J, Ye SH, Yun JY, Seo H, Oh HM, Kim KJ, Kim JS, Jeong WJ, Chang YK, Jeong BR (2016) CRISPR/Cas9-induced knockout and knock-in mutations in *Chlamydomonas reinhardtii*. *Sci Rep* 6:27810
- Sinéad L, Paul RR, Catherine S (2011) Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Mar Drugs* 9(6):1056–1100
- Singh J, Cu S (2010) Commercialization potential of microalgae for biofuels production. *Renew Sust Energy Rev* 14(9):2596–2610
- Singh UB, Ahluwalia AS (2013) Microalgae: a promising tool for carbon sequestration. *Mitig Adapt Strat Glob Chang* 18:73–79
- Sizova I, Greiner A, Awasthi M, Kateriya S, Hegemann P (2013) Nuclear gene targeting in *Chlamydomonas* using engineered zinc-finger nucleases. *Plant J* 73(5):873–882
- Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. *J Biosci Bioeng* 101(2):87–96. <https://doi.org/10.1263/Jbb.101.87>
- Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol Rev* 35:171–205
- Stumm W, Morgan JJ (1981) Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters, 2nd edn. Wiley-Interscience, New York. 780 pp
- Sun T, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour Technol* 83:1–11

- Sun X, Wang C, Li Z, Wang W, Tong Y, Wei J (2013) Microalgal cultivation in wastewater from the fermentation effluent in riboflavin (B2) manufacturing for biodiesel production. *Bioresour Technol* 143:499–504
- Tibbetts SM, Yasumaru F, Lemos D (2017) In vitro prediction of digestible protein content of marine microalgae (*Nannochloropsis granulata*) meals for Pacific white shrimp (*Litopenaeus vannamei*) and rainbow trout (*Oncorhynchus mykiss*). *Algal Res* 21:76–80
- Ueno Y, Kurano K, Miyachi S (1998) Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. *J Ferment Bioeng* 86:38–43
- Valente LMP, Gouveia A, Rema P, Matos J, Gomes FF, Pinto IS (2006) Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* 252(1):85–91
- Van Den Hende S, Vervaeren H, Boon N (2012) Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. *Biotechnol Adv* 30:1405–1424. <https://doi.org/10.1016/j.biotechadv.2012.02.015>
- Venuleo M, Raven JA, Giordano M (2017) Intraspecific chemical communication in microalgae. *New Phytol* 215:516–530
- Vidyashankar S, VenuGopal KS, Chauhan VS, Muthukumar SP, Sarada R (2015) Characterisation of defatted *Scenedesmus dimorphus* algal biomass as animal feed. *J Appl Phycol* 27(5):1871–1879
- Wan C, Alam MA, Zhao XQ, Zhang XY, Guo SL, Ho SH, Chang JS, Bai FW (2015) Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. *Bioresour Technol* 184:251–257. <https://doi.org/10.1016/j.biortech.2014.11.081>
- Wang Y, Rischer H, Eriksen NT, Wiebe MG (2013) Mixotrophic continuous flow cultivation of *Chlorella protothecoides* for lipids. *Bioresour Technol* 144:608–614. <https://doi.org/10.1016/j.biortech.2013.07.027>
- Wang Y, Yang Y, Ma F, Xuan L, Xu Y, Huo H, Zhou D, Dong S (2015) Optimization of *Chlorella vulgaris* and bioflocculant-producing bacteria co-culture: enhancing microalgae harvesting and lipid content. *Lett Appl Microbiol* 60(5):497–503. <https://doi.org/10.1111/Lam.12403>
- Wang Q, Lu Y, Xin Y, Wei L, Huang S, Xu J (2016) Genome editing of model oleaginous microalgae *Nannochloropsis* spp. by CRISPR/Cas9. *Plant J* 88(6):1071
- Wannathong T, Waterhouse JC, Young REB, Economou CK, Purton S (2016) New tools for chloroplast genetic engineering allow the synthesis of human growth hormone in the green alga *Chlamydomonas reinhardtii*. *Appl Microbiol Biotechnol* 100(12):5467–5477
- Wassef EA, El-Sayed AFM, Kandeel KM, Sakr EM (2005) Evaluation of *Pterocladia* and *Ulva* meals as additives to gilthead seabream *Sparus aurata* diets. *Egypt J Aquat Res* 31:321–332
- Weeks DP (2011) Homologous recombination in *Nannochloropsis*: a powerful tool in an industrially relevant alga. *Proc Natl Acad Sci USA* 108(52):20859–20860
- Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME, Brawley SH (2017) Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol* 29(2):949–982
- Weyman PD, Beeri K, Lefebvre SC, Rivera J, McCarthy JK, Heuberger AL, Peers G, Allen AE, Dupont CL (2015) Inactivation of *Phaeodactylum tricornerutum* urease gene using transcription activator-like effector nuclease-based targeted mutagenesis. *Plant Biotechnol J* 13(4):460
- Wirth R, Lakatos G, Bojti T, Maroti G, Bagi Z, Kis M, Kovacs A, Acs N, Rakhely G, Kovacs KL (2015) Metagenome changes in the mesophilic biogas-producing community during fermentation of the green alga *Scenedesmus obliquus*. *J Biotechnol* 215:52–61
- Wu XJ, Gao G, Giordano M, Gao KS (2012) Growth and photosynthesis of a diatom grown under elevated CO₂ in the presence of solar UV radiation. *Fundam Appl Limnol* 180(4):279–290
- Yi YU, Wang H, Yang Y, Pei G, Qin Z, Jiujun JI (2016) Study on preparation of nutritional *Nostoc sphaeroides* jelly. *Amino Acids Biot Resour* 03:34–39
- Zhang YM, Chen H, He CL, Wang Q (2013) Nitrogen starvation induced oxidative stress in an oil-producing green alga *Chlorella sorokiniana* C3. *PLoS One* 8(7):e69225. <https://doi.org/10.1371/journal.pone.0069225>

- Zhang Y, Fan X, Yang Z, Wang H, Yang D, Guo R (2012) Characterization of H₂ photoproduction by a new marine green alga, *Platymonas helgolandica* var. *tsingtaoensis*. *Appl Energy* 92:38–43
- Zhang X, Chen H, Chen W, Qiao Y, He C, Wang Q (2014a) Evaluation of an oil-producing green alga *Chlorella* sp. C2 for biological DeNO_x of industrial flue gases. *Environ Sci Technol* 48(17):10497–10504. <https://doi.org/10.1021/es5013824>
- Zhang X, Rong J, Chen H, He C, Wang Q (2014b) Current status and outlook in the application of microalgae in biodiesel production and environmental protection. *Energy Res.* <https://doi.org/10.3389/fenrg.2014.00032>
- Zhang X, Ma F, Zhu X, Zhu J, Rong J, Zhan J, Chen H, He C, Wang Q (2017) The acceptor side of photosystem II is the initial target of nitrite stress in *Synechocystis* sp. strain PCC 6803. *Appl Environ Microbiol* 83(3):e02952–e03016
- Zhao MX, Ruan WQ (2013) Biogas performance from co-digestion of Taihu algae and kitchen wastes. *Energy Convers Manag* 75:21–24
- Zhong W, Zhang Z, Luo Y, Qiao M, Xiao W (2012) Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. *Bioresour* 114:281–286
- Zhou W, Min M, Li Y, Bing H, Ma X, Cheng Y, Liu Y, Chen P, Ruan R (2012) A hetero-photoautotrophic two-stage cultivation process to improve wastewater nutrient removal and enhance algal lipid accumulation. *Bioresour Technol* 110(1):448
- Zhu LD (2015) Biorefinery as a promising approach to promote microalgae industry: an innovative framework. *Renew Sust Energy Rev* 41:1376–1384. <https://doi.org/10.1016/j.rser.2014.09.040>
- Zhu S, Wang Y, Huang W, Xu J, Wang Z, Xu J, Yuan Z (2014) Enhanced accumulation of carbohydrate and starch in *Chlorella zofingiensis* induced by nitrogen starvation. *Appl Biochem Biotechnol* 174(7):2435–2445
- Zhu J, Chen W, Chen H, Zhang X, He C, Rong J, Wang Q (2016) Improved productivity of neutral lipids in *Chlorella* sp. A2 by minimal nitrogen supply. *Front Microbiol* 7(557). <https://doi.org/10.3389/fmicb.2016.00557>
- Zittelli GC, Rodolfi L, Bassi N, Biondi N, Tredici MR (2013) Photobioreactors for microalgal biofuel production. In: *Algae for biofuels and energy*. Springer, Dordrecht