gene activation libraries derived from transposon-tagging or CRISPRa, which will be useful in understanding gene function. Additionally, cell-type and tissue-specific promoters and reporter genes will be helpful for generating transgenic lines to characterize and engineer traits in a controlled manner. Finally, the community would benefit from the establishment of a standardized set of protocols for transformation, floral induction, and controlled pollinations for specific duckweed clones that have high-quality genome sequence information. This will facilitate a turn-key duckweed model for basic and applied plant research in the future.

Where can I find out more?

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Primer

Microalgae

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Microalgae, in the strictest definition, are eukaryotic, unicellular microorganisms that are photosynthetic and typically have an aquatic lifestyle. Despite the fact that cyanobacteria (or 'blue-green algae') are prokaryotic, and are therefore not true algae, we have included them in this overview because they have a similar physiology and ecology to eukaryotic microalgae, and share many biotechnological applications. In this Primer, we discuss the diversity of microalgae, their evolutionary origin and ecological importance, the role they have played in human affairs so far, and how they can help to accelerate the transition to a more sustainable society.

Photosynthesis originated around 3.5 billion years ago in cyanobacteria, most likely in the oceans. Eukaryotes emerged much later, following 1–1.5 billion years of prokaryotic evolution. One lineage of eukaryotes, the Archaeplastida, acquired photosynthesis through primary endosymbiosis (or internalisation) of a cyanobacterium (Figure 1) and gave rise to the red (rhodophytes) and green (chlorophytes) algae, alongside the glaucophytes. Other lineages of

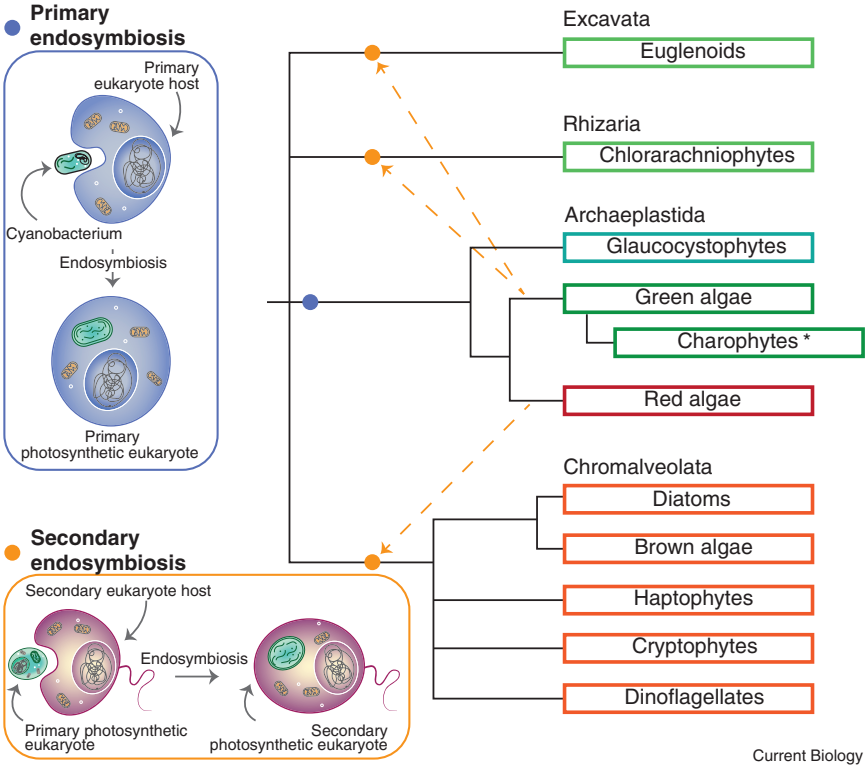

Figure 1. Broad overview of microalgal phylogeny.
Primary endosymbiosis of an ancestral, oxygenic cyanobacterium gave rise to the first lineage of eukaryotes that are capable of photosynthesis, the Archaeplastida. Three distinguishable groups evolved from the Archaeplastida: red algae, green algae, and glaucophytes. Subsequent endosymbiotic events of red and green algae led to further diversification of algal lineages, including, among others, dinoflagellates, cryptophytes, haptophytes, diatoms, and euglenoids. Green, blue, and red rectangles indicate the lineages of primary plastid-containing eukaryotes (Archaeplastida) and eukaryote lineages with secondary plastids of red (Chromalveolata) or green (Excavata, Rhizaria) algal origin. Branch lengths and placement of the secondary symbiosis arrows (orange and dashed) are arbitrary and do not reflect timing of diversification or endosymbiotic events. Note that microalgae are polyphyletic, and this schematic figure only intends to show a selected number of taxa. *One lineage of green algae, the charophytes, gave rise to the origin of all terrestrial plants.

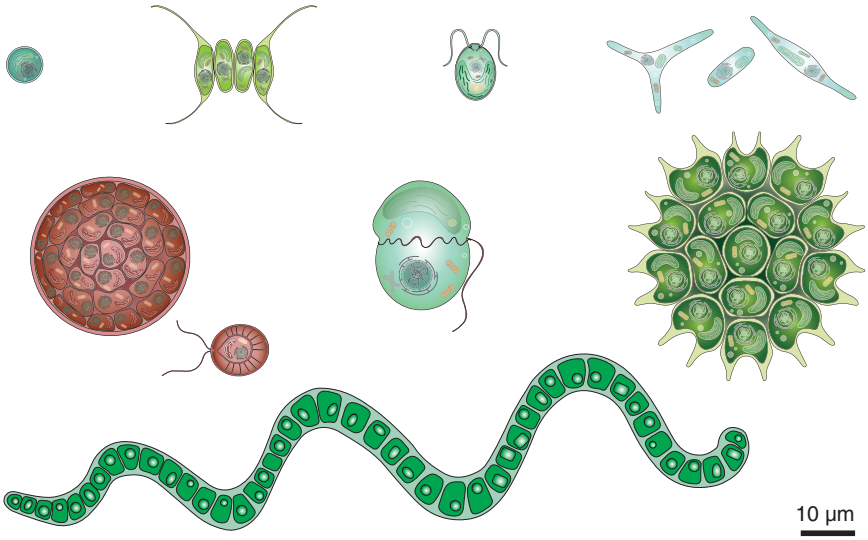

Figure 2. Phenotypic diversity of phytoplankton.
Microalgae live individually or in groups of individual cells, and can vary widely in shape and size, ranging from a few to several hundred micrometres. To date, over 30,000 microalgae species have been described, but the true diversity may well be orders of magnitude greater. Here, only a selection is shown: the green algae *Chlorella*, *Desmodesmus*, and *Chlamydomonas* (top row, in respective order); the diatom *Phaeodactylum tricornutum* (top right); two different life stages of the red algae *Haematococcus pluvialis* (middle left); a dinoflagellate (middle centre); the colony-forming green alga *Pediastrum* (middle right), and the colony-forming cyanobacterium *Arthrospira* (bottom).

eukaryotes acquired photosynthesis through secondary endosymbiosis of red (e.g., chromalveolates) or green (e.g., euglenoids) microalgae. A single lineage of the green algae (charophytes) would give rise to the origin of all terrestrial plants (embryophytes), around 500 million years ago. The process of endosymbiosis has resulted in remarkable phylogenetic diversity among microalgae that, as we will see, is reflected in a large metabolic diversity.

Even though oxygenic photosynthesis — by which algae convert inorganic carbon (CO_2) into biomass — is an important feature in the definition of microalgae, it is not necessarily a common denominator, as there have been multiple losses of photosynthetic capacity in several microalgal lineages. Such species rely on heterotrophic (i.e., using organic carbon as an energy source) rather than photoautotrophic growth, and are therefore able to grow in complete darkness. Other species, often referred to as mixotrophs, combine both strategies.

Ecological importance of microalgae

Microalgae and cyanobacteria that live in rivers, lakes, and oceans are collectively called phytoplankton (Figure 2), and are the main primary producers in these ecosystems, providing energy and organic matter for zooplankton and fish. Phytoplankton also provide essential nutrients to higher trophic levels (Figure 3A). For example, fatty fish species such as herring, sardines, and mackerel obtain essential fatty acids from lipid-rich phytoplankton.

Primary production of phytoplankton in aquatic ecosystems is driven mainly by the availability of inorganic nutrients rather than by temperature or irradiance. Hotspots of phytoplankton primary production in the oceans include coastal waters that receive nutrient-rich run-off from land, and areas where nutrient-rich deep oceanic waters reach the surface, such as upwelling zones along continental margins or the arctic oceans. In contrast, warm oceanic waters far from land are nutrient-poor and have a very low phytoplankton productivity.

The enrichment of rivers, lakes, and coastal waters with nutrients from agriculture, industry, or wastewater discharges often causes massive proliferation of phytoplankton through a process called eutrophication. This can result in discoloration of the water, also known as algal blooms (Figure 3B,C). Breakdown of organic matter produced by these algal blooms may result in depletion of the dissolved oxygen concentration in water, and a resulting loss of animal life, which leads to so-called ‘dead zones’. In addition, some bloom-forming phytoplankton species can produce potent toxins. In freshwater, some cyanobacteria produce toxins that may contaminate drinking water, while in coastal waters, several phytoplankton species produce toxins that can accumulate in the food chain and contaminate seafood.

As phytoplankton are responsible for almost half of global primary production, it is not surprising that they have a major impact on biogeochemical cycles. All phytoplankton mediate transfer of carbon from the atmosphere to ocean sediments, coccolithophores are responsible for the formation of calcium carbonate sediments, cyanobacteria transfer nitrogen from the atmosphere to the oceans, and diatoms play a major role in the global silica cycle. Microalgae are also important symbionts with other organisms, forming a close alliance with polyps in corals, and with fungi in lichens (Figure 3D).

Cultivation of microalgae

Historically, microalgae and cyanobacteria have rarely been exploited by humans. Only the cyanobacterium *Arthrospira platensis* (colloquially referred to as ‘spirulina’) has been traditionally consumed, and only by the Aztec people in central Mexico and the Kanembu people in Chad. Cultivation of microalgae started in 1890, when the green alga *Chlorella* was isolated by Dutch researchers and maintained as a pure culture in the lab. Cultivation for commercial purposes started in the 1970s with the production of *Arthrospira* and *Chlorella*. Today, most microalgae are cultivated in shallow ponds, also called raceway ponds (Figure 4A). These ponds are shallow, U-shaped basins in which the water is gently mixed and recirculated using a powered paddle wheel. These systems produce around 30 tons dry biomass per hectare per year. However, photosynthesis in raceway ponds is restricted due to self-shading of the suspended cells. Productivity can be more than doubled in so-called photobioreactors (Figure 4B). These are closed, transparent reactors

that are designed to optimise light penetration into the culture and facilitate photosynthesis by reducing self-shading. Photobioreactors exist in many designs, and can vary in shape from flat panels to vertical or horizontal tubular arrays. Alternatively, many microalgae are capable of growing mixotrophically, which means they can grow using a combination of light and simple organic carbon sources (e.g., glucose, acetic acid) as a source of energy. Some microalgae have lost their capacity for photosynthesis altogether, and grow completely heterotrophically. The production technology for heterotrophic microalgae is very different from that of auto- or mixotrophic species, instead resembling conventional fermentation (Figure 4C).

Because microalgae have a high exponential growth rate, they achieve higher areal biomass productivities compared with terrestrial crops. Moreover, whereas terrestrial crops produce a biomass that can only be partly used (e.g., the economic value of seeds and carbohydrate-rich tubers) and that has a high content of fibre (mainly cellulose and lignin), microalgae biomass can almost be used entirely, as it has a very low fibre content, and is rich in protein, lipids, and/or easily available carbohydrates like starch. Microalgae have a biomass composition that is roughly comparable to soybeans, yet can produce 10× more protein per hectare than soy. Microalgae could thus provide much more protein on the same area of land than conventional crops. Moreover, microalgae cultivation does not depend on fertile soils, and can be carried out on land that is unsuitable for agriculture. Many microalgae strains grow in seawater and therefore do not necessarily depend on our limited freshwater resources. Furthermore, while modern agriculture systems typically lose over half of the applied nitrogen to the environment due to inefficient nutrient uptake by crops, losses of nitrogen from microalgae cultivation systems are minimal.

The combination of high productivity with an attractive biomass composition initially attracted interest in the bulk production of microalgae. During World War II, lack of access to petroleum prompted researchers in Germany to evaluate the potential to produce oil from lipid-rich microalgae. In the decade after World War II, the use of *Chlorella* was investigated as a source of proteins for the rapidly growing global population. In response to the 1970 oil crisis, the United States launched a new research program to investigate microalgae cultivation for biofuel production. In the early 2000s, rising energy prices, in combination with an urgent need to wean the global economy from oil, initiated a new wave of research into the production of microalgae biofuels. Despite these repeated bouts of interest in large-scale production of microalgae for biofuels or bulk protein, microalgae are still not able to compete with agricultural commodity crops. There are several reasons for the high cost of production of microalgae compared with conventional crops.

Firstly, cultivation systems such as raceway ponds are costlier per unit of land area than conventional arable land. Closed photobioreactors are even more expensive, in particular when additional heating or cooling is needed to reach optimal growing conditions.

Secondly, harvesting and downstream processing of microalgal biomass (Figure 4D) is more energy-intensive compared with that of conventional crops. Microalgae biomass usually consists of small cells that form relatively dilute suspensions (0.5 to 5 g dry matter per litre). Harvesting, therefore, involves processing large volumes of culture broth. Commercial microalgae producers often use industrial centrifuges, but these are both energy-intensive and expensive. Lower-cost approaches such as membrane filtration, flocculation, or dissolved air flotation are being investigated, but are not yet proven on a large scale. Next, because freshly harvested microalgal biomass is a wet product, it needs to be dried to improve its shelf-life (e.g., through lyophilisation or spray-drying), which is also an energy-intensive process. Furthermore, cells of many microalgae are surrounded by a tough cell wall that needs to be disrupted in order to extract proteins or lipids. This can be achieved by high-pressure homogenisation, ultrasonication, or bead-milling but requires a lot of energy.

Finally, like terrestrial farming, microalgae cultivation can suffer large

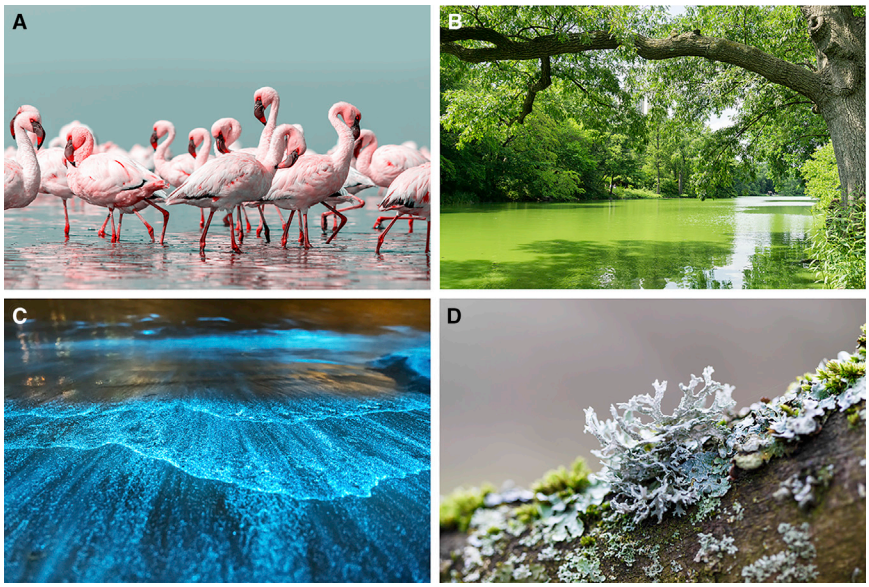

Figure 3. Microalgal signature in the environment.
(A) *Dunaliella salina* is a salt-tolerant microalga that can synthesise the red pigment β-carotene. This pigment can accumulate up the food chain and is the reason why flamingos turn pink. (B) Algal blooms occur when several environmental factors, including nutrient availability and temperature, create optimal conditions for excessive growth of microalgae. (C) Some blooming dinoflagellates are bioluminescent and emit a blue-green light when disturbed. (D) Although the majority of microalgae are aquatic and free-living, some species inhabit non-aquatic environments by virtue of specific adaptations such as desiccation tolerance or by living in symbioses with fungi, known as lichens. Photo credits: (A) iStock.com/Yulia Lakeienko; (B) iStock.com/UpdogDesigns; (C) iStock.com/RugliG; (D) iStock.com/mgfoto.

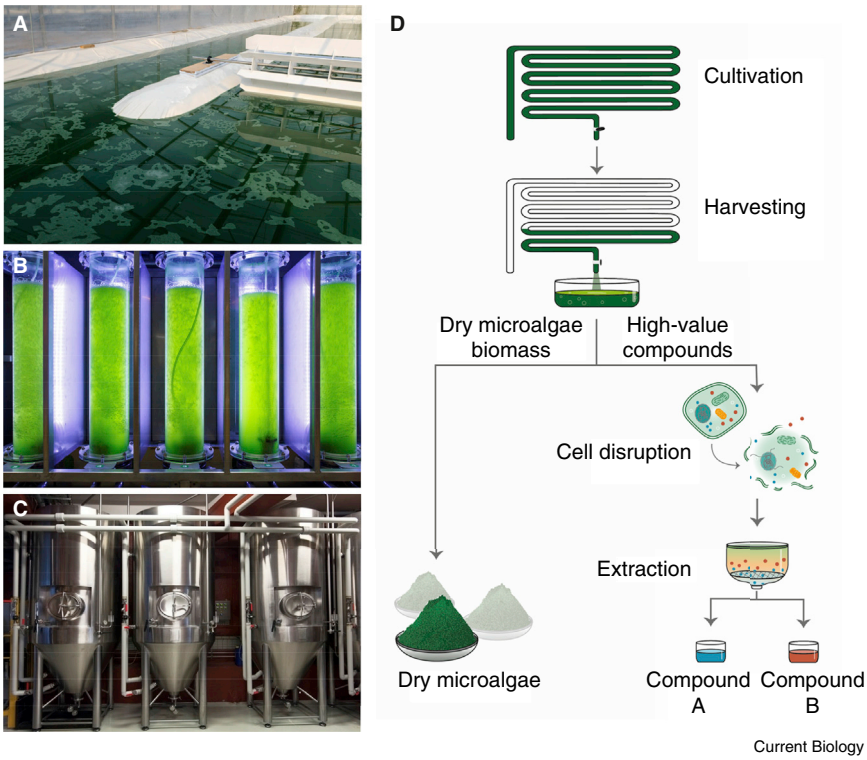

Figure 4. Microalgae cultivation and processing.
(A–C) Various technical systems exist to cultivate microalgae. For commercial purposes, microalgae can be grown photoautotrophically in open raceway ponds (A) or closed photobioreactors (B), or heterotrophically in fermenters (C). (D) Manufacture of microalgal products starts with cultivating microalgae for the production of biomass. Next, the culture broth is processed to harvest the wet biomass, typically as concentrated biomass with about 20% dry matter (called algae paste). This can be dried to algae powder or further processed (including cell wall disruption) for extraction of compounds. Photo credits: (A) iStock.com/corridor91; (B) iStock.com/Toa55; (C) iStock.com/ClarkandCompany.

losses in productivity due to pests that contaminate the culture. These include protozoans or small metazoans (e.g., rotifers and microcrustaceans) that feed on or parasitise microalgae, or unwanted microalgae that invade and take over the culture. These pests can cause large losses because they often have high growth rates, and can lead to a collapse of the microalgal culture within days. Currently, very little is known about these pest species, and research on prevention and control of contamination is still in its infancy. Risk of contamination is lower in closed photobioreactors than in open pond cultures, but even there it cannot be completely avoided.

Reducing the cost of microalgae production will require optimisation of the entire production process. Genetic engineering may play an important role in this. Specifically, it may help to increase biomass productivity by improving photosynthetic efficiency, or to enhance the yield of specific bioproducts by increasing their concentration in the biomass. It may even be possible to facilitate harvesting by creating auto-flocculating microalgae, and facilitate extraction of products by modifying the cell wall composition. Genetic engineering may also allow for the development of strains that are resistant to certain pests. In this regard, a challenge of genetic engineering of microalgae is that these species often belong to very different evolutionary lineages, and each lineage requires the optimisation of genetic toolboxes for manipulating the genome. Genetically modified microalgae are also faced with important regulatory hurdles.

High-value applications of microalgae and promising developments

Because (micro)algal lineages started diverging long before terrestrial plants, they have a deeper phylogenetic diversity, and this is reflected in a high metabolic diversity. Consequently, microalgae contain metabolites that are not found to the same extent, or at all, in terrestrial plants. In fact, since the 1970s, several companies worldwide have produced microalgae commercially (such as *Arthrospira* and *Chlorella*), not for production of biofuels or bulk protein but for high-value applications such as nutritional supplements (Figure 5A). For example, several microalgae accumulate high concentrations of carotenoids. *Dunaliella salina* grows in hypersaline water and is commercially produced as a source of β-carotene. This species accumulates up to 14% β-carotene, which is much higher than the concentrations found in terrestrial plants. Another example is *Haematococcus pluvialis*, which accumulates high concentrations of the carotenoid astaxanthin, a red pigment that is produced to cope with oxidative stress and is not synthesised by any terrestrial plant. It is used as an antioxidant (e.g., in sunscreens to protect against UV-induced oxidative damage) or as an ingredient of salmon feed to impart a pink colour to salmon meat (Figure 5B). Other pigments that are exploited by the food and pharmaceutical sector include fucoxanthin, an orange-brown pigment produced by several microalgae, including the diatom *Phaeodactylum tricornutum*, and the blue pigment phycocyanin, which is produced by *Arthrospira*.

Many microalgae contain high amounts of omega-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. These fatty acids are important for cardiovascular health and the development of the central nervous system. Today, our primary dietary source of omega-3 fatty acids is fish, which in turn obtain it through their diet. However, they can also be harvested directly from microalgae. The heterotrophic microalgae *Schizochytrium* and *Crypthecodinium cohnii* are commercially cultivated for this purpose. Also, autotrophic microalgae are currently being explored as a source of omega-3 fatty acids, such as *Nannochloropsis*, *Isochrysis*, and *Phaeodactylum*.

Figure 5. A selection of current and emerging applications of microalgae.
(A) *Arthrospira* (cyanobacteria) and *Chlorella* (green algae) are already cultivated worldwide for use in food. (B) Astaxanthin is a red pigment that is naturally produced by *Haematococcus pluvialis* and is used as an ingredient in salmon feed to impart the familiar pink colour of salmon fillets. (C, D) Researchers anticipate a wealth of sustainable applications of microalgae, including, but not limited to, biostimulants to increase crop vigour (C) and bioplastics (D). Photo credits: (A) iStock.com/pilipphoto; (B) iStock.com/Fudio; (C) iStock.com/Allexxandar (D) iStock.com/Whity2j.

The macroscopic counterparts of microalgae, called macroalgae or seaweeds, often contain complex polysaccharides with unique rheological properties. Examples of commercially important polysaccharides extracted from seaweeds include alginate, agar, and carrageenan, which are used as thickeners and gelling agents. Several microalgae, including the red alga *Porphyridium* and many species of cyanobacteria, also produce extracellular polysaccharides with promising rheological properties, but these bioproducts have so far not been exploited. Other examples of unique and promising metabolites that are currently left unexploited include toxins produced by bloom-forming phytoplankton, some of which are extremely potent and could be used to control tumour growth in targeted chemotherapy. Others, including diverse polysaccharides and phytohormones, could be used as plant biostimulants to optimise the nutrient-use efficiency of crops and increase their tolerance to environmental stress (Figure 5C). Microalgae are also an emerging feedstock for bioplastics production (Figure 5D).

We may also exploit the unique metabolic pathways of microalgae by incorporating them into terrestrial crops. For example, genes of microalgal origin can be expressed in terrestrial crops to produce omega-3-enriched oil. Other examples include carbon-concentrating mechanisms used by cyanobacteria to reduce photorespiration (i.e., light-dependent uptake of O_2) and boost primary production in crops. *Vice versa*, microalgae can be used as a platform to synthesise metabolites that are naturally found in other organisms. For example, microalgae are promising for the manufacture and (oral) delivery of various biopharmaceuticals.

One interesting development is to link supply chains in a circular economy framework by cultivating microalgae on waste- or side-streams of various industrial processes. Microalgae can be used to recover valuable resources, such as water and nutrients from wastewater, and to capture CO_2 from flue gases, in this way degrading or reducing waste products, while simultaneously producing marketable biomass.

Microalgae production is rapidly growing around the globe, and companies expand by addressing new markets with novel products. Still, the market success of new microalgal products not only depends on a cost-efficient production process, but also on consumer acceptance and demand (particularly for food applications). Furthermore, all products must strictly comply with the relevant regulations (e.g., food and feed regulations) which may be complex and ill-suited for new microalgal products. For example, in the European Union, only a handful of microalgae species is currently authorised for the food market, and achieving commercial authorisation is still a bottleneck that limits the full potential of microalgal products.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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