Biodiversity and application of microalgae

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The algae are a polyphyletic, artificial assemblage of O_2-evolving, photosynthetic organisms (and secondarily non-photosynthetic evolutionary descendants) that includes seaweeds (macroalgae) and a highly diverse group of micro-organisms known as microalgae. Phycology, the study of algae, developed historically as a discipline focused on the morphological, physiological and ecological similarities of the subject organisms, including the prokaryotic blue-green algae (cyanobacteria) and prochlorophytes. Eukaryotic algal groups represent at least five distinct evolutionary lineages, some of which include protists traditionally recognized as fungi and protozoa. Ubiquitous in marine, freshwater and terrestrial habitats and possessing broad biochemical diversity, the number of algal species has been estimated at between one and ten million, most of which are microalgae. The implied biochemical diversity is the basis for many biotechnological and industrial applications.

Introduction

The phylogenetic diversity of the algae is very broad and is reflected in an equally wide range of metabolisms and biochemical properties. This paper considers the phylogeny (evolutionary relatedness), classification (taxonomy), ecology and distribution, biochemical diversity and industrial application of eukaryotic and prokaryotic microalgae in an attempt to illustrate the biodiversity of this assemblage of largely microscopic phototrophs. The volume of information on these subjects is more than sufficient for a number of textbooks and this article is by no means believed or intended to be authoritative or complete. For in depth information, interested readers are encouraged to consult the literature cited in the text and Bold and Wynne [10] and van den Hoek *et al* [93] on algal biology, systematics, and classification, Cox [26] on photosynthetic flagellates. Margulis *et al* [59] and O'Kelly [70] for perspectives on phylogeny and classification, Andersen [2], Norton *et al* [68], and Radmer [77] on biodiversity, Darley [28], Rogers and Gallon [85] and Stewart [91] on physiology and biochemistry, and Akatsuka [1], Borowitzka and Borowitzka [14], and Lembi and Waaland [55] on commercial applications. Much of our fundamental knowledge of microalgae is based on research with isolates cultured in the laboratory. For a discussion of culturing and a listing of culture collections throughout the world, see Andersen [3].

Phylogeny and classification

Our understanding and interpretation of the phylogeny of algae and related organisms has evolved dramatically in recent years. Molecular and ultrastructural evidence of evolutionarily conserved features (eg ribosomal RNA gene sequencing, flagellar hairs and roots, plastid and mitochondrial structure, the mitotic apparatus) has combined to create an exciting, dynamic field of inquiry. Biodiversity implies that groups of organisms are differentiated by some measure of the extent to which their gene pools are separated and how this is expressed phenotypically. For purposes of classification and for understanding biological and evolutionary relatedness (phylogeny), the species is the fundamental unit for classifying groups of organisms. Thus, in order to assess biodiversity, it is important to consider the species concept as it is applied to the algae. With the increasing availability of molecular information (ie protein and nucleic acid sequence data), there has been a movement toward reconciliation of taxonomic and phylogenetic approaches. This is leading to classification systems that reflect some biological reality, such as the degree to which groups of populations are genetically similar, with implications for evolutionary history and speciation. The results of ongoing molecular level interrogation of cultured and natural microbial populations is leading to completely new frameworks for categorization at the higher taxonomic levels. For an in depth treatment of the interrelationships among phylogeny and classification based on current debate on the species concept applied to algae, see Manhart and McCourt [58].

Historically, species of microalgae were recognized on the basis of phenotypic properties, such as whole organism morphology, cellular anatomy and ultrastructure, and metabolism and physiology and were described and categorized according to the International Code of Botanical Nomenclature. More recently, it has been recommended by some bacteriologists that the taxonomy of prokaryotic blue-green algae (cyanobacteria or cyanophytes*) and prochlorophytes be treated under the International Code of Nomenclature of Bacteria, with the botanical system serv-

*In this article, the terms blue-green algae, cyanophytes, and cyanobacteria are used interchangeably.

ing as the baseline [21]. However, very few species have been described under the bacteriological code and most researchers agree to the pressing needs for resolution of this issue. Geitler [32] continues to be used as the baseline authority for the identification and preliminary classification of genera and putative species of blue-green algae. Species are placed in Orders that are defined on the basis of morphology and contrasting modes of cell division.

Because of the dynamic nature of research and debate in the phylogeny of eukaryotic microorganisms and the current uncertainty about placement of a number of groups, and also for continuity with research in ecology and distribution, biochemical diversity and applied phycology, this article will rely on a classification scheme based on a traditional approach. Historically the major groups of algae are classified into divisions (equivalent to the zoological phylum) on the basis of pigmentation, the chemical nature of the photosynthetic storage product, thylakoid organization and other ultrastructural features of the chloroplast, the chemistry and structure of the cell wall, the number, arrangement and ultrastructure of flagella (if any), and the occurrence of any special features. Ten of the divisions recognized by van den Hoek *et al* [93], representing virtually all known algae, are listed in Table 1. It is now agreed that the algae are not a cohesive, natural assemblage of organisms. Depending on how the data for problematic groups are interpreted, algae are included in as many as seven [2], eight [70] or more eukaryotic and one or more eubacterial lines within the domains Eukarya and Bacteria (*sensu* Woese *et al* [99]). At this level, many people agree that eukaryotic algae for which sufficient data are available represent a minimum of four or five phyletic assemblages: green plants (including green algae and land plants), red algae, euglenoids, chromophyte algae, and dinoflagellates (which are either included with the chromophytes or considered to be an independent lineage). The diversity among these major lineages is illustrated in Figure 1, where it can be seen that algae are phylogenetically more diverse than either land plants or animals. Groups for which no clear consensus has yet emerged because of paucity of data or opposing interpretation of what evidence is available include the glaucophytes, prymnesiophytes, chloroacrachniophytes and cryptophytes (Table 1). Among the algae, fossil records show the blue-green lineage arising early in the Precambrian. The red and green lineages date from the mid- to late-Precambrian. Brown algae are first seen in the Paleozoic while most other lineages date from the early to late Mesozoic [3].

Blue-green algae and prochlorophytes

All blue-green algae and prochlorophytes are Gram-negative eubacteria classified by bacteriologists as the class Oxyphotobacteria in the eubacterial division Gracilicutes [22,67]. Species are described on the basis of morphology, although phylogenetic analysis based on molecular data has revealed that some morphologically cohesive assemblages, such as the non-heterocystous filamentous 'oscillatorian' algae, include phylogenetically distinct groups suggesting that some species and genera may require extensive taxonomic revision [33,97]. Among the approximately 2500 described species of blue-green algae are included unicellu-

lar coccoid, colonial, and filamentous forms with or without branching or differentiation of specialized cells, such as akinetes (spores) and heterocysts (anaerobic N_2-fixing cells). Blue-greens are thought to be the evolutionary origin of plastids within eukaryotic algae and land plants (an event that probably occurred more than once) [80] and responsible for oxygenation of the atmosphere early in our earth's history. Thylakoids (photosynthetic membranes) are not arranged in stacks, in contrast to prochlorophytes and most other algae. Pigmentation includes chlorophyll a and d, blue and red phycobilins, β-carotene, and several xanthophylls. The storage product, cyanophycean starch (an α-(1–4)-linked glucan), is formed as granules between the thylakoids [93]. Blue-green algae produce at least 50 compounds which are toxic to vertebrates in addition to antibiotics and other secondary metabolites [19].

Prochlorophytes are prokaryotic microalgae in the same bacterial division and class as the blue-green algae. They are distinct from the blue-greens by possessing both of the chlorophylls found in green algae, euglenoids and land plants (a and b or divinyl derivatives). Discovered less than 30 years ago as extracellular symbionts within subtropical ascidians (sea squirts), but now known to include free-living freshwater and marine plankton, the prochlorophytes may themselves be polyphyletic in origin [72].

Glaucophytes

The eukaryotic division Glaucophyta includes three genera of biflagellate unicellular microalgae whose chloroplasts strongly resemble coccoid blue-green algae in that the thylakoids are not stacked and are surrounded by a thin peptidoglycan layer (a wall typical of Gram-negative bacteria). Also like blue-greens, glaucophytes have chlorophyll a and phycobilins, although the range of xanthophylls present is smaller. They are rare freshwater inhabitants [93].

Red algae

Red algae (Rhodophyta) include seaweeds and microalgae distinct from other algae by having the following *combination* of properties: (1) the lack of any flagellate stages; (2) the presence of accessory phycobilin pigments (shared with cyanobacteria, glaucophytes, and cryptomonads); (3) chloroplasts with singly-occurring thylakoids; (4) floridean starch (an α-(1–4-glucan) as the photosynthate; and (5) oogamy in species with sexual reproduction [93]. Microalgal species have not been shown to reproduce sexually. Chlorophyll a is the only chlorophyll. In addition to phycobilins (phycoerythrin imparts the red color), rhodophytes have β-carotene, zeaxanthin and, as a group, five other known xanthophylls. Two classes are usually recognized on the basis of whether the plant body is relatively simple (Bangiophyceae, includes microalgae) or complex (Floridophyceae) together with a number of anatomical characteristics and attributes of the life history [93]. Red seaweeds are distributed worldwide along coastlines. In addition to marine habitats, red microalgae occur in freshwater, on soil, and as endosymbionts within tropical foraminifera (protozoa). One marine gastropod harbors a red algal chloroplast as a photosynthetic quasi-organelle. Nearly all of the approximately 5500 species are seaweeds.

Table 1 Biodiversity, distribution, and estimated numbers of algal species

Division[a] (common name)	Distinguishing features[b]	Major groups[a] (common name and/or features)	Marine	Fresh-water	Terrestrial	Symbiotic	Estimated number of species (microalgae and seaweeds)[c]
			Distribution of microscopic species				
Cyanophyta (blue-green algae)	Chlorophyll a,d: phycobilins: β-carotene; zeaxanthin, echinenone, canthaxanthin, myxoxanthophyll, oscillaxanthin, 3 minor xanthophylls; phycobilins; prokaryotic; Gram-negative cell walls	Chroococcales (Unicellular cocci and rods; binary fission and budding)	+	+	+	+	≈125 described
		Pleurocapsales (Unicellular and aggregate-forming; multiple fission)	+	+	–	–	≈35 described
		Oscillatoriales (Filamentous; binary fission; no specialized cells)	+	+	+	–	1000
		Nostocales (Filamentous; binary fission; specialized cells; heterocysts, akinetes)	+	+	+	+	1000
		Stigonematales (Filamentous; branched; cell division in 3 planes; specialized cells; heterocysts, akinetes and motile, gliding hormonogia)	–	+	+	–	≈35 genera
Prochlophyta (prochlorophytes)	Chlorophyll a,b; β-carotene; several xanthophylls; phycobilins, prokaryotic; Gram-negative cell walls	Prochlorales (Coccoid and filamentous; binary fission)	+	?	?	+	<10 described
Glaucophyta (glaucophytes)	Chlorophyll a,b; phycobilins; β-carotene; zeaxanthin, β-cryptoxanthin; starch; cellulose wall		–	+	–	–	3 described
Rhodophyta (red algae)	Chlorophyll a; α-, β-carotenes; phycobilins; zeaxanthin, 5 minor xanthophylls; floridean starch; cellulose and other cell wall materials	Bangiophycidae	+	+	+	+	5500–20 000 (both classes)
		Floridophycidae (seaweeds only)	+	+	–	–	
Cryptophyta (cryptophytes)	Chlorophyll a,c_2; α-, β-, ϵ-carotenes; phycobilins; alloxanthin, zeaxanthin, α-1,4 glucan starch; exterior organic periplast		+	+	?	?	1200
Chlorophyta (green algae)	Chlorophyll a,b; α-, β-, γ-carotenes; zeaxanthin, lutein, violaxanthin, neoxanthin; several minor xanthophylls; starch; diverse cell wall materials, incl cellulose	Micromonadophyceae (prasinophytes)	+	+	?	?	500
		Charophyceae (stoneworts, filamentous microalgae)	+	+	+	?	20 500
		Ulvophyceae (seaweeds, filamentous microalgae)	+	+	+	?	3000
		Pleurastrophyceae	+	+	+	+	6 genera
		Chlorophyceae	+	+	+	+	10 000–100 000

Table 1 Continued

Division[a] (common name)	Distinguishing features[b]	Major groups[a] (common name and/or features)	Distribution of microscopic species				Estimated number of species (microalgae and seaweeds)[c]
			Marine	Fresh-water	Terrestrial	Symbiotic	
Euglenophyta (euglenoids)	Chlorophyll a,b; β-,γ-carotenes; neoxanthin, diadinoxanthin, diatoxanthin; paramylon; wall-like organic pellicle internal to cell membrane		+	+	+	?	2000
Chlorarachnio-phytes	Chlorophyll a,b; unknown carotenoids; amoeboid with zoospores		+	–	–	+	2 genera, each with 1 species
Pyrrhophyta[d] (dinoflagellates)	Chlorophyll a,c; β-carotene; diadinoxanthin, diatoxanthin, fucoxanthin, peridinin, 5 minor xanthophylls; α-1,4 glucan starch; cellulose walls		+	+	?	+	3500–11000
Chromophyta (heterokont algae)[e]	Chlorophyll a,c_1,c_2,c_3; α-, β- and ϵ-carotenes; several major and minor xanthophylls; chrysolaminarin, other glucans, oils; variety of cell wall materials	Chrysophyceae (golden-brown algae)	+	+	?	?	3400
		Bacillariophyceae (diatoms)	+	+	+	?	100000–10000000
		Xanthophyceae (yellow-green algae)	+	+	+	?	2000
		Eustigmatophyceae (eustigmatophytes)	+	+	+	?	1000–10000
		Raphidophyceae (raphidophytes, chloromonads)	+	+	?	?	100
		Prymnesiophyceae (haptophytes or prymnesiophytes, including coccolithophorids)	+	+	?	?	2000
		Dictyochophyceae (silicoflagellates)	+	–	–	–	≈15
		Phaeophyceae (brown seaweeds, no microalgae)	+	+	+	?	2000

[a]*Sensu* Castenholtz and Waterbury [22] for cyanophyte orders, Lewin [56] for prochlorophyte orders, and van den Hoek *et al* [93] for all eukaryotic divisions and classes.
[b]Not all classes within a division include taxa displaying all the distinguishing features. In particular, classes of chromophyte algae vary considerably in pigmentation and nature of the photosynthate [9].
[c]From Andersen [2] and Norton *et al* [68].
[d]Some dinoflagellates include endosymbiotic brown algae so that while most species have peridinin as the principal xanthophyll, others have fucoxanthin. This may represent multiple endosymbiotic events in the evolutionary past [70].
[e]Some authors recognize additional classes of chromophyte algae, including the *Synurophyceae* and *Pelagophyceae* [2,88].

Cryptophytes

Cryptophytes (Cryptophyta) are a small group of mostly unicellular biflagellate microalgae with a broad collection of pigments, including chlorophylls a and c_2 and phycobilins, and a single chloroplast with a thylakoid structure intermediate between the red algae and other plants. The cell is enclosed by a stiff proteinaceous periplast made of rectangular or polygonal plates. Ultrastructural properties of the chloroplast and unique cellular inclusions place cryptomonads apart from other algae, although Cavalier-Smith [23] allies them with the chromophyte algae. Starch is stored as distinct granules and some species also store oils [93].

Green algae

The green algae (Chlorophyta) comprise one of the largest in terms of number of species, most widely distributed, and morphologically diverse groups of algae. Land plants probably evolved directly from green algae and share many biochemical, metabolic and ultrastructural features. Most chlo-
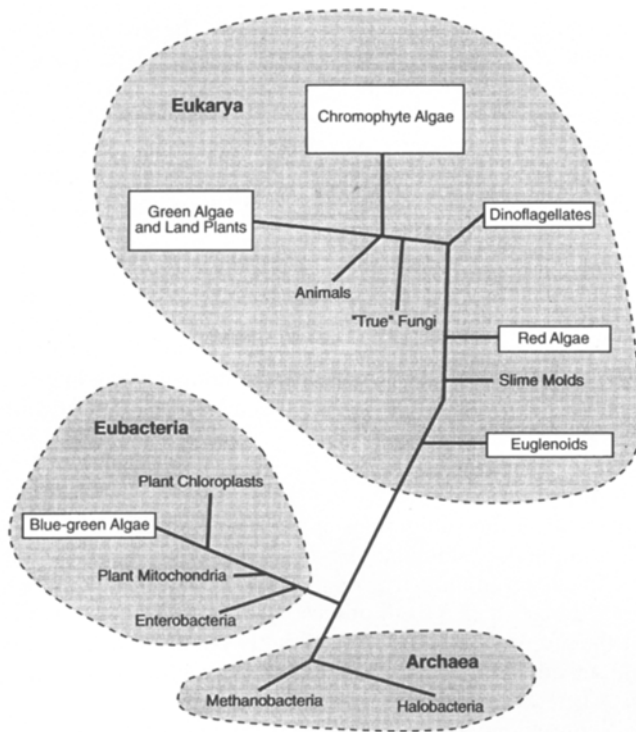
Figure 1 Depiction of the phylogenetic relatedness of some groups of algae. Lengths of line segments are proportional to evolutionary distance based on analysis of ribosomal RNA gene sequences (modified from Radmer and Parker [78]).

rophytes store starch and the group as a whole includes a wide diversity in the number and arrangements of chloroplasts and flagella associated with individual cells. Thylakoids are tightly stacked in bundles of from two to six lamellae within plastids bound by two membranes, in common with red algae and glaucophytes. Green algae include unicellular, colonial, filamentous and pseudoparenchymatous uni- or multinucleate forms and large numbers of both seaweeds and microscopic species have been described. Major taxonomic categories (Classes) listed in Table 1 are delineated largely on the basis of ultrastructural details of flagellate cells and mechanisms of cell division and so are thought to accurately reflect phylogeny within the division [60,93].

Chlorarachniophytes
This rare group includes species in two genera that appear to be some evolutionary combination of an amoeboid, plasmodial, phagotrophic host with an eukaryotic chlorophyll *a* and *b* containing endosymbiont derived from a euglenoid or chlorophyte [70]. They are described from association with marine siphonous green algae [93].

Euglenoids
Euglenoids (Euglenophyta) share pigmentation with the prochlorophytes, green algae, and land plants, but possess unique cellular and biochemical features that, together with ultrastructural and molecular evidence, places these microorganisms closer to trypanosomes than to any algal group [70]. The storage product is paramylon, a β-1,3 linked glucan, located freely in the cytoplasm that is also produced

by prymesiophytes, a class of chromophyte algae. Euglenoids do not have cell walls, but rather possess a proteinaceous pellicle internal to the cell membrane. Osmotrophic and phagotrophic facultative and obligate heterotrophs are common. All euglenoids are microscopic and include mostly uninucleate and unicellular flagellates, although colonial species are common [93].

Chromophyte algae
The chromophyte algae are a highly diverse group, most of which share a common storage product (chrysolaminarin, a β-linked glucan) and a preponderance of carotenoids over chlorophylls that results in all groups having golden, golden-brown, brown or yellow-green rather than the grass-green, blue-green or red coloration typical of other major algal divisions. Five of the many chromophyte classes (Table 1) feature chlorophylls a, c_1, and c_2. Eustigmatophytes have only chlorophyll a. Fats and oils are stored by many chromophyte algae and prymnesiophytes make paramylon, found elsewhere only in euglenoids. Ultrastructural and molecular sequence comparisons over the past decade have revealed that chromophytes may be closely related to certain fungi and protozoa as well and it was recently proposed to place them with these non-photosynthetic relatives into a single kingdom with the common name stramenopiles, reflecting the common presence of tripartite (bipartite for the prymnesiophytes) mastigonemes, or hairs on one flagellum [73]. This division includes ecologically and morphologically distinctive microalgal classes such as the diatoms, coccolithophorids, and silicoflagellates [93].

Dinoflagellates
The dinoflagellates (Pyrrhophyta) are a diverse assemblage of coccoid, filamentous, palmelloid, and amoeboid microalgae within which the evolutionary acquisition of plastids and phototrophy has occurred more than once [70]. Together with photosynthesis, a range of heterotrophic nutritional patterns is represented, with many saprophytic, phagotrophic, symbiotic and parasitic species having been described. Dinoflagellates share the presence of chlorophylls a, c_1, and c_2 and some carotenoids with chromophyte algae, but also have unique carotenoids and a cellulose wall. Most dinoflagellates have two uniquely positioned flagella. Ultrastructural aspects of flagellar and nuclear organization are also unique to this group. Dinoflagellates are important freshwater and marine inhabitants [93].

Ecology and distribution

As the predominant component of the marine and freshwater plankton, microalgae are primarily responsible for the 40–50% of total global photosynthetic primary production contributed by all algae [41]. Included in aquatic habitats are microplankton (20–200 μm), nanoplankton (2–20 μm) and picoplankton (0.2–2 μm) [90]. In recent years it has become evident that much or most primary production is contributed by cells <3–5 μm in diameter, known as ultraplankton, that together with similar-sized heterotrophic plankton constitute as much as 80% of the pelagic biomass [2,89]. While the contribution by pelagic, coastal, and freshwater communities is relatively well understood, the

significance of terrestrial microalgae is not known with any degree of precision, but they may be important on a local or regional scale because of their predominance on extensive areas of exposed land surface in arid and semi-arid steppes and deserts [63].

Phytoplankton reside throughout the photic zone from the surface layer (neuston) to 250 m or more in depth in some clear oligotrophic waters and depending on latitude. Andersen [2] calculated that as many as 3.6×10^{25} individual phytoplankters may inhabit the world's oceans at any given time and are responsible for an annual primary production of over 5×10^{13} kg, constituting the base of the marine food web. As a consequence of diurnal, seasonal, vertical, and geographic variation in nutrient availability, temperature, light and other factors, the distribution and metabolic activities of groups of microalgae may be considerably more heterogeneous than is currently understood. The interpretation of data from flow cytometry, extraction and analysis of community-level nucleic acids, phospholipid and other biochemical assays, remote sensing, and traditional microscopy has resulted in some generalizations regarding the distribution of phytoplankton. These are certain to be refined as more surveys across geographic and temporal gradients are reported and as our knowledge improves about the distribution and significance of viable but non-culturable species, believed by many people to include the bulk of microbial biodiversity. The discussion of the occurrence and general distribution patterns of the major groups of marine microalgae that follows is largely taken from Thomsen's 1986 survey [92] in which he indicated that the depth of information about the different algal groups is highly variable and that creation of a systematic framework for presenting or interpreting the current data is difficult at best. Thus, the following treatment is meant only to provide an appreciation for the ecological diversity of microalgae with the degree of attention paid to each group in no way reflecting its perceived or real importance in the biosphere.

Blue-green algae and prochlorophytes

Cyanobacteria are ubiquitous in marine, freshwater, and terrestrial environments as free-living populations and endosymbionts of marine animals, lichens, bryophytes, and cycad roots. In the past two decades, ultraplanktonic cyanobacteria (eg *Synechococcus*) have been shown to contribute as much or more to oceanic primary production as any other single group of microalgae [31,95]. In addition, filamentous cyanobacteria such as *Anabaena, Nostoc, Microcoleus, Oscillatoria* and *Mastigocladus* are commonly the dominant component of microbial mats in brackish waters, hot springs, on semi-arid and arid soils, and in rice paddies [20,62,84]. Prochlorophytes (eg *Prochloron*) were first described two decades ago from symbiotic association with marine didemnids and holothurians and are now known to also exist as free-living components of the pelagic nanoplankton where their distribution and importance in this habitat is becoming clearer [25].

Red algae

Consisting mostly of seaweeds categorized into two major botanical classes, the red algae include genera of free-living

microalgae in the order Porphyridiales. The genera *Rhodella* and *Rhodosorus* include marine phytoplankton species. They are regular inhabitants at some locations, such as Norwegian fjords. Under favorable circumstances, the release of male gametes by red seaweeds makes a significant ephemeral contribution to coastal benthic phytoplankton communities [92]. *Porphyridium* inhabits freshwater and terrestrial ecosystems [93].

Cryptophytes

Members of this group are sporadically present in freshwater and marine habitats. Some species of *Chroomonas* exhibit broad tolerance to salt concentrations, living in estuaries and salt marshes [92]. One species is symbiotic within the marine ciliate *Mesodinium*.

Green algae

Green algae are ubiquitous in freshwater habitats, where they commonly dominate phytoplankton assemblages, and are abundant in terrestrial environments as well. The green algae range in size from microscopic to macroscopic in all of these environments. Predominantly photosynthetic, there are nonetheless numerous examples of facultative and obligate heterotrophs [93].

Representative genera that are widely distributed in marine environments include mostly uniflagellate (eg *Pedinomonas, Micromonas*) and biflagellate prasinophytes (eg *Mamiella, Mantoniella*) and chlorophytes (eg *Pyramimonas, Tetraselmis*). Various coccoid chlorophytes less frequently documented (eg *Chlorella, Nanochloris, Halochlorococcum*) may also prove ultimately to have worldwide distribution and importance. *Dunaliella*, a unicellular biflagellate phytoplankter found in extremely saline habitats, such as seaside rock pools and saline lakes, including the Dead Sea and Great Salt Lake, is a focus of efforts for commercial production of β-carotene [15].

Green microalgae are abundant and diverse in freshwater habitats. For example, unicellular (eg *Chlamydomonas, Phacotus*) and colonial (eg *Volvox, Pyrobotrys*) flagellates, coccoid non-flagellate species (eg *Chlorella, Pediastrum, Ankistrodesmus, Scenedesmus*), and filamentous forms (eg *Chaetophora, Oedogonium*) are ubiquitous in ponds, lakes, and streams at most latitudes. Many green microalgae are important as 'weeds' in lakes, canals, and other waterways [54]. Most of these are mat-forming (eg *Spirogyra, Hydrodictyon*) or attached (eg *Cladophora, Ulothrix, Stigeoclonium*) filamentous species [10,93].

Green microalgae also occur in terrestrial habitats, including on and in soil and rocks. The most commonly encountered are coccoid (eg *Chlorococcum*), palmelloid—aggregates of cells invested by a common mucilage—(eg *Palmella, Gloeococcus*), sarconoid—packets of 4, 8, 16 or 32 cells—(eg *Tetracystis, Chlorosarcina*), and filamentous (eg *Stichococcus, Klebshormidium*) genera [62]. *Trentepohlia* inhabits tree trunks and other subaerial surfaces while other green microalgae are symbiotic, such as *Trebouxia*, a common lichen phycobiont.

Euglenoids

Euglena, Trachelomonas, Colacium and other euglenoid genera include many common freshwater and edaphic spec-

ies. This group is less common in marine environments, although species in the genus *Eutreptia* occasionally form very dense blooms in coastal waters. Most euglenoids are facultatively heterotrophic and/or phagotrophic. Colorless, phagotrophic species (eg *Peranema*) are also common [93].

Chromophyte algae

Chromophyte microalgae are highly diverse and important components of most marine and freshwater habitats. With the exception of *Pelagococus*, which has been cultured from across the north Pacific, golden-brown microalgae (Chrysophyceae: eg *Ochromonas, Chromulina*), although often encountered, are not often abundant in marine environments. Other golden-browns are, however, known to be important freshwater phytoplankton that commonly form blooms in lakes and ponds. *Synura*, for example, is a nuisance alga responsible for imparting undesirable odors and taste to drinking water from lakes and reservoirs. Golden-brown microalgae have not been reported from soil. In contrast, silicoflagellates (eg *Dichtyota*) are very common in the oceans, where they often form dense blooms. These microalgae are uncommon in freshwater and not known from terrestrial habitats. *Pedinella* is another pelagic genus whose abundance under ice can color the seawater yellow [92].

Diatoms (Bacillariophyceae) are ubiquitous in marine, freshwater and terrestrial environments and probably include the greatest number of extant species (up to ten million) of any group of microalgae [49,86]. Diatoms are categorized into three classes on the basis of having bilateral or radial symmetry. Bacillariophyceae include bilaterally symmetrical species with raphes (an opening or fissure in the valve). Common genera include *Navicula, Nitzschia*, and *Phaeodactylum*. Diatoms displaying bilateral symmetry but without raphes are in the class Fragilariophyceae (eg *Diatoma, Synedra, Fragilaria*). Radially symmetrical diatoms are in the class Coscinodiscophyceae (eg *Chaetoceros, Biddulphia, Thalassiosira*). Diatoms are mostly unicellular, although filamentous species are also abundant and diverse. These microalgae commonly dominate planktonic communities at all latitudes, particularly in and under ice, in temperate coastal and pelagic environments, on mud flats, in still or moving freshwater, and on some soils [86].

Yellow-green microalgae (Xanthophyceae: eg *Vaucheria, Bumilleria*) are primarily known from freshwater environments. Unicellular, aggregate and filamentous xanthophytes are also common on soil. The eustigmatophytes *Nanochloris* and *Nanochloropsis* (Eustigmatophyceae) have been discovered over the past two decades to be important contributors to primary production in estuaries, salt marshes, and brackish seas and are present, but less abundant, in the open ocean [3].

Prymnesiophytes, also known as haptophytes (Prymnesiophyceae or Haptophyceae) are microalgae that commonly dominate the marine phytoplankton in numbers and biomass (eg *Phaeocystis*). They are sometimes distinguished at the species level by the pattern of organic scales and, in the case of the coccolithophorids, $CaCO_3$ deposits on the cell surface. The biodiversity of prymnesiophytes is greatest in warm oligotrophic regions, such as the Red Sea and Gulf of California, although they are also known to bloom in eutrophic waters. Coccolithophores have been the subject of more investigations of geographic distribution in the oceans than most other major groups of algae. Subtropical and tropical marine waters support diverse communities that include *Emiliana, Discosphaera, Rhabdosphaera* and many other genera [98]. *Coccolithus* and *Emiliana* are two coccolithophore genera common in open oceans at arctic latitudes. Members of the genus *Chrysochromulina* are common in all marine environments where they often dominate and can form dense populations even in dilute brackish waters. Prymnesiophytes are uncommon in freshwater and unknown from terrestrial habitats.

Dinoflagellates

Dinoflagellates are often important components of the microplankton of freshwater and marine habitats in which they are found. *Dinophysis, Gymnodinium, Amphidinium, Peridinium, Ceratium* and *Prorocentrum* all include photosynthetic flagellate species found at most latitudes in fresh and marine bodies of water [92]. Dinoflagellates also include many specialized species, including endo- and ecto-parasites of marine animals (eg *Blastodinium, Haplozoon*) and the photosynthetic partners of many corals known as zooxanthellae [93]. Dinoflagellates include species capable of bioluminescence (eg *Gonyaulax, Pyrocystis*) and those responsible for 'red tides' (see section on Bioactive compounds, below).

Biochemical diversity

The phylogenetic breadth of microalgae is reflected in an equally broad biochemical diversity of pigments, photosynthetic storage products, cell walls and mucilages, fatty acids and lipids, oils, sterols and hydrocarbons, and bioactive compounds, including secondary metabolites. Much of the material to follow is from contributed chapters to volumes edited by Borowitzka and Borowitzka [14], Rogers and Gallon [85], and Stewart [91].

Pigments

Chlorophylls, carotenoids, and phycobilins comprise the range of pigments produced in various combinations by photosynthetic microalgae. Chlorophyll *a* is present in all algae and land plants, including the prokaryotic cyanobacteria and prochlorophytes. Other chlorophylls (b, c_1, c_2, d) are accessory light-harvesting molecules whose distribution among algal groups is used, in part, for taxonomic purposes at the division level (Table 1). Chlorophylls absorb blue and red light and can range up to 2% or more of cellular dry weight. Together with carotenoids and phycobilins, the synthesis, content, and abundance of chlorophylls is dependent on light, nutrition and other environmental factors [61,82].

Carotenoids are tetraterpenes comprised of eight branched 5-C isoprenoid units. The variety of carotenoids in algae is far greater than for land plants, with more than 40 carotenes and xanthophylls having been isolated and characterized [9]. Several algal groups derive their primary coloration from the mix of carotenoids they produce, for example the yellow-green, golden, golden-brown and

brown coloration found within the chromophyte algae. β carotene is chemically the most simple and is found in all algae and other plants. Hypersaline species of *Dunaliella* accumulate β-carotene up to 14% of total dry weight. Astaxanthin is another important carotenoid that is produced in large concentrations by green microalgae, including *Haematococcus* and others that inhabit snow and ice (eg *Chlamydomonas* spp). Many carotenes and xanthophylls are commonly restricted to one or a few algal groups. For example, lutein is shared by chlorophytes and land plants, myxoxanthin and myxoxanthophyll are characteristic of cyanobacteria, as are peridinin for dinoflagellates and fucoxanthin for brown algae and diatoms.

Phycobilins are water-soluble accessory pigments produced by cyanobacteria, glaucophytes, red algae, and cryptophytes. As many as three different phycobilins can be produced by an individual organism. In some algae, they modulate a metabolic process called chromatic adaption in which one form or another is preferentially synthesized according to the quality of available light [34]. Phycobilins are extracted from red and blue-green algae for use as fluorescent markers in cell biology and to impart color to food and cosmetic products [12].

Photosynthetic storage products

Microalgae synthesize a wide variety of photosynthetic storage products (photosynthates), the chemical nature of which is also used as a criterion for taxonomic treatment at the division level. Photosynthates include α-(1–4)-linked glucans (starches), β-(1–3)-glucans, fructans, low molecular weight carbohydrates, and fats and oils. Starches produced by various groups include floridean starch (amylopectin subunits) by red algae and myxophycean starch (amylopectin or glycogen-like subunits) by blue-green algae. Some green algae form a cross-linked amylose-amylopectin starch that is essentially similar to starch in land plants. Others store fructosans (inulin-like fructose oligosaccharides). Cryptophytes and dinoflagellates also store α-(1–4)-linked glucans. Chrysophytes store oils or chrysophycean starch, a water-soluble β-(1–3) glucopyranoside. Other chromophyte algae store various hydrocarbons and oils (see below). Paramylon, composed solely of β-(1–3)-linked D-glucose, is unique to euglenoids and prymnesiophytes [93].

Many microalgae in different divisions store monosaccharides (eg glucose, galactose, many others), disaccharides (ie sucrose, trehalose, maltose), glycerol glycosides, and polyols (eg mannitol). Low molecular weight compounds are also accumulated by microalgae for osmoregulation. Examples include sugars (eg *Ochromonas*), free glycerol (eg *Dunaliella* and various zooxanthellae) and proline (some *Chlorella* spp). Industrial processes for glycerol [7] and proline [53] production were developed, but not commercialized.

Cell walls and extracellular mucilages

Most microalgae form rigid cell walls. Exceptions include numerous 'naked' microalgae, such as *Dunaliella*, some dinoflagellates and chrysophytes, and the motile spores of various groups. Cryptomonads and euglenoids have distinctive proteinaceous pellicles, internal to the cell membrane

in euglenoids and to the outside in cryptophytes. Cyanobacteria and prochlorophytes have Gram-negative bacterial walls composed of α- and ϵ-diamino-pimelic acid and glucosamine (peptidoglycan). Many or most green algae and dinoflagellates have cellulose walls. Other chlorophyte walls are constructed from hydroxy-proline glucosides, xylans, or mannans; some are calicified [93].

Diatoms construct elaborately decorated walls of silicon (frustules) made of two identical halves (valves) that fit together like the lid and bottom of a petri dish. Devoid of walls, silicoflagellates instead produce siliceous internal and/or external skeletons, often with spiny projections, around and through which the protoplast moves. Prymnesiophytes, although without a rigid wall, are covered by superficial organic scales visible only with an electron microscope. These scales are calcified in the coccolithophorids [93].

Microalgal representatives in all of the divisions produce a variety of extracellular polysaccharide mucilages. Much smaller quantities are usually produced during active growth and cell division as compared to stationary phase. Mucilages can be responsible for aggregation of plankton, contribution to biofilm formation by attached species, and desiccation tolerance of terrestrial forms and can possess chelating and flocculant properties. The best-studied mucilages are produced by cyanobacteria [4], diatoms and other chromophyte algae [42], chlorophytes [79], and red microalgae [94]. Cell wall materials from brown and red seaweeds form the basis of a large marine hydrocolloid industry [77].

Lipids, oils, sterols and fatty acids

The total oil and fat content of microalgae ranges from 1% to 70% of the dry weight and tends to be inversely proportional to the rate of growth with greater accumulations during stationary phase [11]. The percent of total lipid as neutral lipid, glycolipid, and phospholipid also varies widely among and within groups of microalgae [11,50]. Some species manufacture hydrocarbons. An example is *Botryococcus*, a chlorophyte capable of accumulating up to 90% of its colonial dry weight as a mixture of ten hydrocarbon compounds in globules occluded among cells embedded in a colonial matrix. Hydrocarbons up to C-37 in size from different strains of *B. braunii* include straight chain alkadienes, branched triterpenoids (botryococcenes), and the tetraterpenoid lycopadiene [69].

A wide range of common and rare sterols are also synthesized by microalgae, including, for example, cholesterols (cyanobacteria, rhodophytes), chondrillasterol (chlorophytes, euglenoids), clinoasterol (xanthophytes), dinosterol (dinoflagellates), ergosterol (chlorophytes, rhodophytes, euglenoids), epibrassicasterol (diatoms), poriferasterol (chlorophytes, chrysophytes), and sitosterol (cyanobacteria, chlorophytes, xanthophytes) [37,75].

Because of increased awareness of the possible health aspects of including ω-3 fatty acids in the diet (see Applications, below), the manufacture and content of fatty acids by microalgae is the subject of a good deal of ongoing research and development [44]. Microalgal lipids are mostly esters of glycerol and fatty acids with a chain length of C_{14} to C_{22} and may be saturated or unsaturated. Cyano-

bacteria tend to have large amounts of polyunsaturated fatty acids while eukaryotic microalgae contain, in addition, a wide range of saturated and monosaturated fatty acids with fatty acid profiles widely variable among taxa. Specific fatty acid profiles vary widely among algal groups, with age and growth stage, and with environmental conditions. There are, however, some generalizations. For example, linolenic acid ($C_{18:3}$) is common in green algae, whereas diatoms contain palmitic ($C_{16:0}$), hexadecenoic ($C_{16:1}$) and C_{-20} polyenoic acids. Red microalgae have high contents of arachidonic acid ($C_{20:4}$) as well as palmitic, oleic ($C_{18:1}$) and linoleic acids. Chrysophytes contain significant quantities of highly unsaturated $C_{18:4}$ and $C_{22:6}$ acids in addition to unsaturated $C_{16:0}$ and $C_{20:0}$ compounds. Ω-3 fatty acids are produced by a variety of microalgae. Eicosapentaenoic ($C_{20:5}$) and docosahexaenoic ($C_{22:6}$) acids are produced by some species of green and red microalgae, cryptophytes, dinoflagellates, prymnesiophytes and diatoms. Certain species have also been shown to synthesize unusual fatty acids such as, for example myristic ($C_{14:0}$) in the diatom *Fragilaria*, lignoceric ($C_{24:0}$) in the premnesiophyte *Isochrysis*, gamma linolenic ($C_{18:3}$) in the golden-brown alga *Ochromonas*, and arachidonic ($C_{20:4}$) in the red microalga *Porphyridium* [24].

Bioactive compounds

Microalgae produce a wide array of compounds with biological activity including antibiotics, algicides, other toxins, pharmaceutically-active compounds, and plant growth regulators [13,65]. Antibiotic substances produced by microalgae include certain fatty acids, bromophenols, tannins, terpenoids, polysaccharides, alcohols, and other compounds [76]. Most antibiotics are known from chlorophytes and golden-browns, prymnesiophytes, diatoms, cyanobacteria, and dinoflagellates. Activity has been demonstrated against bacteria, fungi, protozoa and other microalgae. For example, the cyanobacterium *Scytonema hofmanii* is known to excrete a γ-lactone algicidal to a broad range of potentially competing microalgae in freshwater environments [36].

Dinoflagellates, cyanobacteria and other microalgae produce compounds toxic to invertebrate and vertebrate animals, including the neurotoxic agents of diarrhetic and paralytic shellfish poisoning (red tides) and ciguaterra fish poisoning, an endemic disease in human populations that consume reef-inhabiting fish in the South Pacific. A number of icthyotoxins are also produced by dinoflagellates. At least twelve toxins are produced by species of *Protogonyaulax* alone [47,65]. In very small quantities a saxitoxin has been used in microsurgical procedures and as an experimental treatment for near-sightedness. Neurotoxins (eg anatoxin, aphanatoxin) produced by the cyanobacteria *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, and hepatotoxins (eg microcystin) from *Microcystis aeruginosa* in temperate freshwater have been implicated in poisonings and occasional death of cattle and other livestock [19,38].

Applications

Microalgae have been the subject of applied research for their commercial and industrial potential since the early 1950s when productivity and yield were first studied in mass culture [18]. Early interest was focused on exploiting the fact that optimal culture conditions result in theoretical conversion of sunlight to biomass at yields of eight to ten times the most productive natural or agricultural plant communities. In practice, sustained commercial mass culture results in productivities of about 15 dry g cm^{-2} day $^{-1}$ (54 tons ha^{-1} $year^{-1}$). More recently, microalgae have been targeted as a source of bioactive compounds and pharmaceuticals, specialty chemicals, health foods, aquaculture feeds, and for waste treatment and agriculture [1,14,55,77].

Microalgal mass culture

Microalgal biomass is produced in engineered facilities whose fundamental design and infrastructure depend on the growth requirements of the microalgae of interest and the nature and value of the final product. Two principal production methods are the photobioreactor and the open pond. Photobioreactors are lit fermentors of variable sophistication that are closed to the environment in order to prevent chemical or biological contamination, including parasitism, predation, and the need to control competition from unwanted microalgae ('weeds') when the desired species is otherwise uncompetitive. Only products with high value such as reagent grade phycobilins or isotopically-labeled research compounds are produced in closed systems.

Industrial scale production of microalgal biomass for the health food market, for animal feed ingredients, and for wastewater treatment relies on engineered ponds open to the environment (Figure 2). Maintenance of single species (unialgal) cultures in open ponds relies on the competitive ability of the microalga of interest. Thus, *Spirulina* naturally predominates in highly alkaline water, as does *Dunaliella* in saline culture. Wastewater treatment systems that rely on microalgae for O_2 production are dominated by chlorophytes with high specific growth rates that tolerate environments with high concentrations of dissolved and particulate organic compounds (eg *Ankistrodesmus*, *Chlorella*, *Scenedesmus*). Engineering design considerations are considered in detail by Jassby [48], Oswald [71], and Richmond and Becker [83].

Pharmaceuticals

By virtue of their larger size and accessibility, seaweeds have until recently attracted more attention than microalgae as sources of natural products, including identification of novel compounds for pharmaceutical development [45,46]. Screening of marine algae for antitumor activity began in the 1970s. Early discoveries included tubercidin, a heterocyclic nitrogen compound from the cyanobacterium *Tolypothrix byssoidea*, shown to have *in vitro* activity against P-388 lymphocytic leukemia. Another compound, an L-aspinarigenase from the green microalga *Chlamydomonas*, inhibits growth of lymphosarcoma in mice. Marine blue-green algae have been one target of a more recent Natural Cancer Institute screening program aimed at anticancer and antiviral (anti-HIV) activity that has identified a number of compounds with potential for drug development, such as dibromoaplysiatoxin from *Lyngbya majuscula*. Approximately 10% of 600 cultures from numerous genera of marine blue-greens showed some activity in reducing cyto-
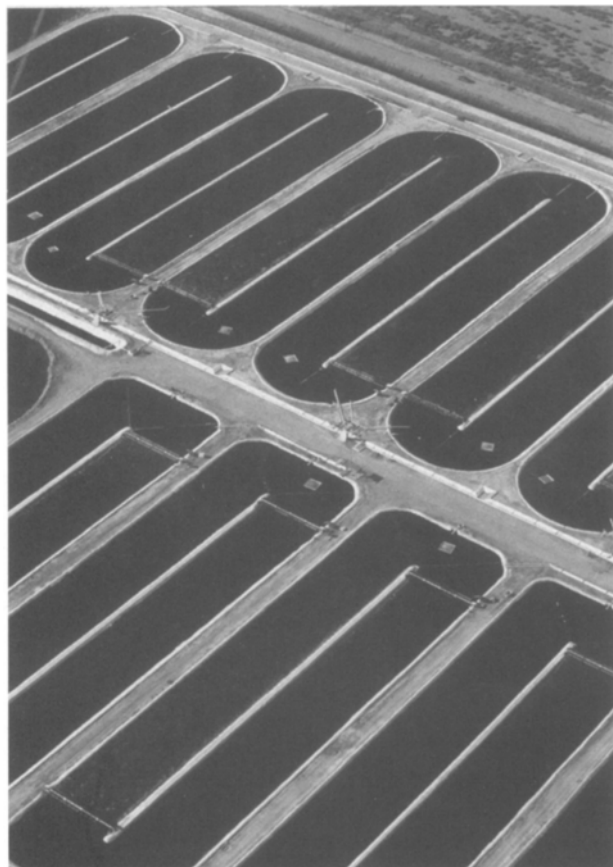
Figure 2 *Spirulina* biomass is produced in 5000 m² ponds at Earthrise Farms, Calipatria, CA for formulation and sale as health food supplements and poultry feed ingredients. Individual ponds are agitated by paddlewheels (courtesy of Earthrise Farms). The total area is 150 000 m².

pathic effects normally associated with cultured cell lines [74]. Microalgae, including cyanobacteria and colorless (apochlorotic) variants of diatoms, may also be potential sources of antiviral sulfolipids (eg sulfoquinosovyl diglyceride) [40].

Specialty chemicals

Markets for the production of high value chemicals and food supplements from microalgal biomass have grown considerably over the past decade. Microalgae have been investigated for the production of vitamins and vitamin precursors, including L-ascorbic acid (vitamin C), riboflavin, and α-, β-, and γ-tocopherol for food and cosmetic formulations and mariculture [16,17,29]. Commercially viable heterotrophic production of ascorbic acid by *Chlorella* was reported by Running *et al* [87]. Carotenoids, such as β-carotene from halophilic *Dunaliella* and astaxanthin from *Haematococcus* and other chlorophytes, euglenoids and red microalgae are also targets for commercialization. β-carotene (pro-vitamin A) is extracted on a commercial scale from *Dunaliella* mass-cultured in Israel and Australia for formulation into food supplements for human consumption [15]. Astaxanthin is responsible for the pink coloration of cultured salmonid fishes and other seafoods, but is not as yet commercially competitive with synthetic products in the aquaculture industry [8].

As the base of the marine food chain, microalgae are responsible for manufacture of the straight-chain polyunsaturated fatty acids associated with lessened incidence of heart disease in humans whose diets are rich in ω-3 fatty acids from consumption of cold water fishes. Prymnesiophytes, prasinophytes, diatoms, and golden-brown microalgae have been investigated as potential commercial sources of the fatty acids of interest. Eicosapentaenoic acid (EPA: $C_{20:5}$) and docosahexaenoic acid (DHA: $C_{22:6}$) from microalgae are available commercially from at least two companies who market them as additives to various products, including infant formulas [43].

Microalgae have been investigated as sources of polysaccharides, lipids, oils and hydrocarbons [11] and are used for production of high value research biochemicals such as phycobilins for fluorescent cell labeling [35] and stable isotopically-labeled fatty acids, proteins and other molecules, and deuterated lubricants [27,51]. Phycocyanin, a blue phycobilin from *Spirulina* is used to color food and cosmetic products in Japan [12].

Health foods

The market niche for microalgal products within the international market for 'health foods' is conservatively estimated at $20–25 million and is by far the largest commercial application of microalgal culture. Currently, the largest industrial operations product *Spirulina* biomass in outdoor ponds in Mexico, Hawaii, California, and Thailand for formulation into health food products and poultry feed supplements [6]. Worldwide annual *Spirulina* production may be as much as 1500 dry tons (Yoshmichi Ota, personal communication). On a smaller scale, *Chlorella* (Chlorophyceae) is also produced for consumption as a health food [81].

Nutritional research has focused on *Spirulina* as a source of protein based on standard measures of utilization, including protein content, digestibility, and biological value. As a source of useable protein, *Spirulina* is comparable to meat and dairy products, superior to plant materials, but inferior to poultry and fish. Its value as a source of protein quality is compromised by low levels of sulfur-containing essential amino acids, although it is comparable to other plant and single-cell protein sources and can be modified by strain selection and modification of culture conditions, such as providing high sulfate levels. *Spirulina* also contains unusually high concentrations of vitamins A and B_{12} [48]. Products sold as variously formulated tablets and powder in Europe, North America, and Asia also have putative therapeutic effects (eg antiviral, enhanced immune response, radioprotection) and other claims that lack rigorous clinical documentation [5]. Mention has already been made of commercial efforts to produce vitamins, carotenoids, and ω-3 fatty acids in various microalgae for formulation into traditional and health food products (see previous section).

Aquaculture feeds

Mass culture of microalgae as feed for molluscs (clams and oysters), crustaceans (shrimp), and fish is an important component of the mariculture industry [8,30]. Microalgal feeds are particularly valuable for seafood species with fas-

tidious dietary requirements that cannot be met by formulation with traditional agricultural commodity products, such as corn, soybeans, and fish and food processing by-products. In these cases, microalgae commonly provide essential amino acids, fatty acids or other unidentified growth factor requirements or are used to provide carotenoids for coloration of the final product. In nearly all cases, the microalgae are produced at the aquaculture facility, frequently by simple fertilization of incoming seawater, and fed directly to the animals as dilute, living cultures [8].

Waste treatment

Microalgae are principal or otherwise important biological components of various systems for treating municipal and industrial effluents. Oxygen from photosynthesis is the primary microalgal contribution to treatment of municipal wastewaters in open ponds or impoundments. The most common designs include facultative ponds, which are deeper and support surface growth of microalgae, and high-rate oxidation ponds which are specifically engineered to encourage maximum oxygenation by microalgae. High-rate ponds are shallow and depend on mechanical mixing to maximize algal production and removal of biochemical oxygen demand [71]. In both configurations, microalgal communities are commonly dominated by green microalgae (eg *Ankistrodesmus*, *Chlorella*, *Scenedesmus*). Biomass from high-rate oxidation ponds can be harvested for use as animal feed and the concept has been demonstrated as a component of integrated approaches to recycling of livestock wastes [57]. Non-living microalgal biomass has also been subject to investigation and early-stage commercialization for its potential in recovery of metals and radio-nuclides from aqueous wastestreams as an alternative to ion exchange resins [39]. Additionally, research has been targeted at the use of microalgal systems for mitigation of waste heat [96] and CO_2 [52] from industrial processes.

Biofertilizers

Microalgae are employed in agriculture as biofertilizers and soil conditioners [64]. Biofertilizers consist of N_2-fixing cyanobacteria in lowland tropical rice cultivation. Filamentous species of *Anabaena*, *Nostoc*, *Aulosira*, *Tolypothrix* and *Scytonema* are common in China, India and elsewhere in Asia, where they can provide 20 or more kg N ha^{-1} year^{-1}, sufficient for one third or more of the requirements of traditional rice cultivars [84]. In China, Vietnam and Africa, *Anabaena azollae*, symbiotic in leaf cavities of the water fern *Azolla* is used as a green manure or companion crop in rice production [66]. On a small scale, palmelloid (mucilage-producing) microalgae in the genus *Chlamydomonas* (Chlorophyceae) have been used as soil-conditioning agents for erosion control of pivot-irrigated soils in North America [64].

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