Topic 8: Algal Culture and Biotechnology

Michael Borowitzka

Background

Microalgae are not only important as major primary producers in all aquatic systems and, at times because of the detrimental effects of algal blooms, but also in aquaculture and industry as sources of nutrition, high value chemicals, in wastewater treatment and potentially as sources of renewable fuels and in CO_2 bioremediation. The microalgal industry is one of the largest biotechnology industries in Australia producing β-carotene from the green alga *Dunaliella salina* for the pharmaceutical and nutraceutical industry.

Concepts Covered

Microalgae growth requirements and limits to growth, growth measurement, microalgae isolation and purification, microalgae culture systems, algal products.

Learning Objectives

1. To understand the nutritional and environmental requirements of microalgae

2. To understand the principles of algal culture and learn some of its applications in research and industry

3. To develop the basic knowledge and tools for isolating and purifying microalgae and establishing laboratory and larger-scale culture systems.

4. To understand commercial-scale algae production systems.

What are Algae ? (singular *alga*)

The algae are an extremely diverse group of plant-like organisms for which it is quite difficult to find a clear definition.

Probably the best way of differentiating algae from chlorophyllous plants is to use their method of sexual reproduction. This differs from green plants as follows:

In unicellular algae, the organisms themselves may function as gametes;

In multicellular algae, the gametes may be produced in special *unicellular* containers or gametangia, or, in others, the gametangia are *multicellular* and every gametangial cell is fertile, i.e. produces a gamete.

These characteristics are not found in liverworts, mosses, ferns or angiosperms.

Algal Taxonomy

The algal Divisions recognised are:

Prokaryotic algae:

Cyanophyta	(blue-green algae, cyanobacteria)

Eukaryotic algae:

Chlorophyta	(the green algae)
Chlorophyceae*	
Charophyceae	(the charophytes)
Euglenophyta*	(the euglenoids)
Phaeophyta	(the brown algae)
Chrysophyta	(the golden-brown algae)
Chrysophyceae*	(the chrysophytes)
Prymnesiophyceae*	(the prymnesiophytes incl. the coccolithophorids)
Xanthophyceae	(the xanthophytes)
Eustigmatophyceae*	(the eustigmatophytes)
Bacillariophyceae*	(the diatoms)
Pyrrhophyta*	(the dinoflagellates)
Rhodophyta*	(the red algae)
Cryptophyta*	(the cryptophytes)

*These Divisions and Classes have unicellular representatives

Recent revisions to algal taxonomy have combined the Phaeophyta and Chrysophyta into a single Division, the Heterokontophyta.

Algae may be either unicellular and (usually) microscopic, or they may be multicellular and often quite large such as the brown kelps which are up to several meters (> 10 m) long. In these notes we will mainly consider the 'micro-algae', i.e. the microscopic unicellular and multicellular species.

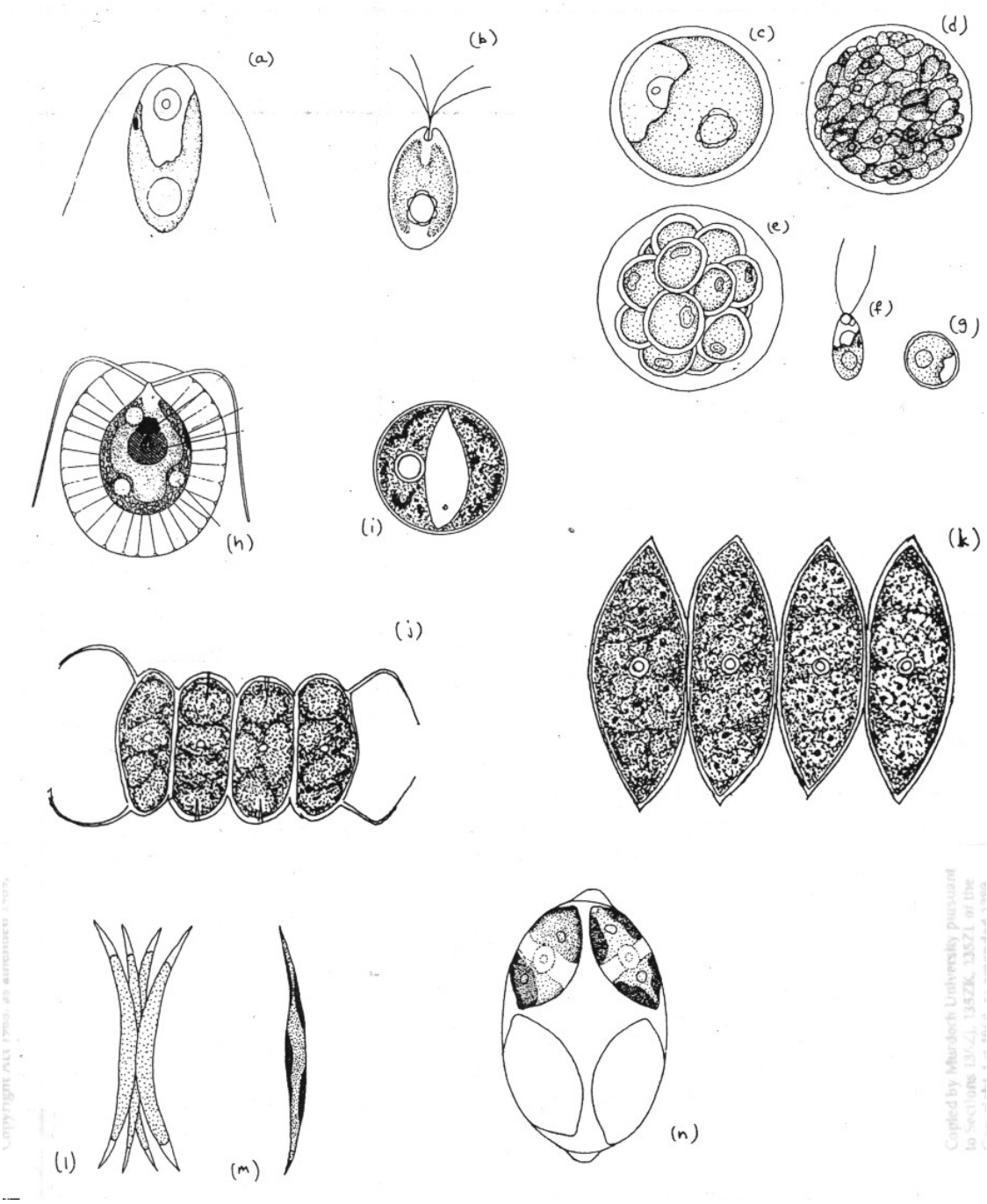


Figure 1. Illustration of several algae mentioned in the lecture course **N.B. Magnifications are approximate. (a) *Dunaliella salina* (x 1300); *Tetraselmis marina* (x 700); (c-g) *Nannochloris coherens* (x 900); (c) vegetative cell; (d) zoosporogenesis; (e) aplanospore formation; (f) zoospore; (g) young vegetative cell; (h) *Haematococcus pluvialis* (x 1200); (i) *Chlorella pyrenoidosa* (x 1500); (j) *Scenedesmus quadricauda* (x 900); (k) *Scenedesmus obliquus*; (1 & m) *Ankistrodesmus falcatus* (x 900); (n) *Oocystis marsonii* (x 790).**

Culture methods for the large algae (seaweeds) are available. For example, they may be cultivated in the laboratory in Petri dishes or small aquaria (West, 2005; Kawai et al.,

2005; Sahoo & Yarish, 2005) for a very good compilation of the methods and media used). They may also be farmed at sea attached to ropes, nets or rafts (Tseng, 1981; Sahoo & Yarish, 2005). These methods are used in China, Japan, Taiwan, Indonesia, the Philippines, Canada, USA, France and more recently in the UK, to farm algae such as *Porphyra, Gracilaria, Eucheuma, Kappaphyus* (Rhodophyta) and *Laminaria* and *Undaria* (Phaeophyta) as sources of hydrocolloids such as agar, alginates and carrageenan, and also for food. The world production of agar is in excess of 10 000 t per annum, and that of carrageenan is equally large. *Porphyra* biomass fetches more than \$19 kg^{-1} dry weight for good quality, and the world demand exceeds supply despite new farms being opened up in North America.

Figure 2. ***Dunaliella salina***

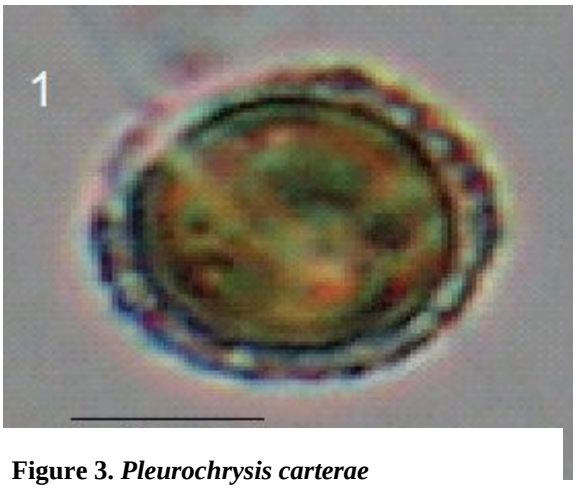


Figure 3. ***Pleurochrysis carterae***

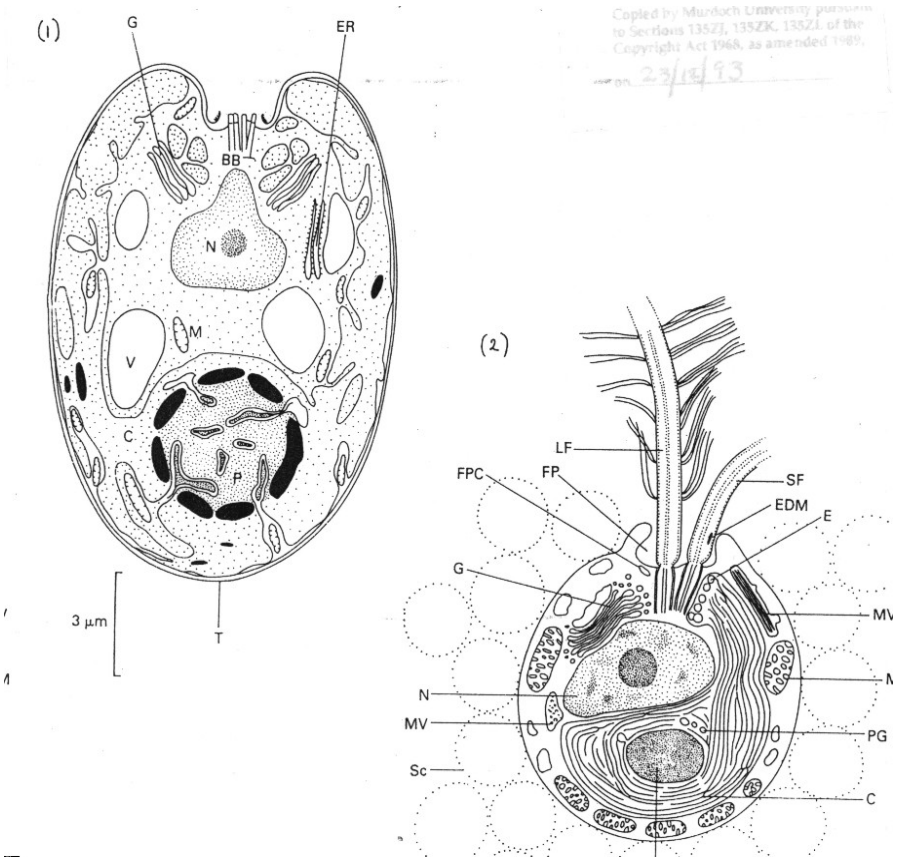


Figure 4. Sections through two unicellular algae showing organisation. **(1) *Tetraselmis astigmatica*, and (2) *Syncrypta glomifera*, showing the internal anatomy. Abbreviations: BB = basal body; C = chloroplast; E = eyespot; EDM = electron dense material; ER = endoplasmic reticulum; FP = flagellar pit; FPC = flagellar pit cisternum; G = Golgi body; LF = long flagellum; M = mitochondrion; MV = mastigoneme (flagellar hair) vesicle; N = nucleus; P = pyrenoid; PG = pigment granules; Sc – scales; SF = short flagellum; T = theca (or cell envelope).**

Macroalgae culture has become quite sophisticated and can involve breeding the algae in onshore facilities before transfer to the ocean for growth, as well as selection of faster growing strains, disease resistant strains, strains with a better quality product etc. Hybridisation and other plant breeding methods have also been employed to produce new strains.

In recent years research has also begun on tissue culture methods for macroalgae (see guierre-Lipperheide et al., 1995 for an excellent review). The methods used are similar to those you are using for the tissue culture of higher plants, except that much work remains to be done to optimise the media and growth conditions, on sterilisation of the explants and on algal hormones to regulate growth and development.

Structure and life histories

Micro-algae may be unicellular of multicellular, they may be non-motile or have one to several flagella. They may be naked (i.e. have no cell wall) (e.g. *Dunaliella*), have a cell wall, a cell covering of silica (e.g. diatoms), of proteinaceous plates (e.g. *Euglena*), be covered in organic scales (e.g. *Pyramimonas*), siliceous scales (e.g. *Mallomonas*) or calcareous scales (e.g. *Emilianea*). Figure 1 illustrates some of the variation in morphology found in the algae. Figure 2 and Figure 3 show micrographs of twp of these. Figure 4 shows a section through two microalgae, *Tetraselmis* (Chlorophyta) and *Syncrypta* (Chrysophyta) showing some of the internal organisation.

One potential problem in algal culture is the fact that some algae have complex life histories. The different stages of the life history may have different growth and nutrition requirements, and produce different compounds. An appreciation of algal life histories and an understanding of them is therefore of great importance. An understanding of the life histories also opens up the potential for genetic manipulation by selective mating and breeding experiments, or by more direct genetic manipulation such as genetic engineering.

We shall only briefly consider a few examples of algal life histories here. Figure 5 shows the life history of *Dunaliella salina* (see Borowitzka & Siva, 2007 for a more detailed description), and Figure 6 shows the life history of *Haematococcus pluvialis*, two green algae of great commercial importance with very different life histories.

More details on algal taxonomy, structure and life histories can be found in the books listed in the bibliography at the end of this guide. Particularly good and quite readable introductions are Bold & Wynne (1978) and several chapters in Clayton & King (1990).

Habitats

Algae are found in all water bodies (i.e. fresh-water, sea-water and hypersaline lakes). They are also found in soils, on plants (terrestrial and aquatic) and form symbiotic associations with a very wide range of plants and animals. Some algae have even been found growing in the human eye and in the intestine!

Nutrition

Algae have two major modes of nutrition: **Photoautotrophy** (lithotrophy) and **heterotrophy**. These can be further subdivided into sub-groups (see below).

Photoautotrophy

Photoautotrophs obtain all the elements they need from inorganic compounds and the energy for their metabolism from light.

Heterotrophy

Heterotrophs obtain their material and energy needs from organic compounds synthesized by other organisms. Few algae are obligate heterotrophs.

Auxotrophy

This is a form of heterotrophy in which the organisms require only small amounts of organic compounds (i.e. vitamins). Many algae are auxotrophs.

Mixotrophy

These algae use photosynthesis as the major source of energy, but may also use organic compounds i.e. it is *Photolithotrophic heterotrophy* (the best of both worlds?). This form of nutrition is quite common in the algae.

Photoheterotrophy

This is a form of nutrition where light is required to use organic carbon sources for growth. It is relatively rare in the algae.

Phagotrophy

Phagotrophic algae can take up solid food particles and digest these in food vacuoles. It occurs in some groups of the algae, especially the dinoflagellates.

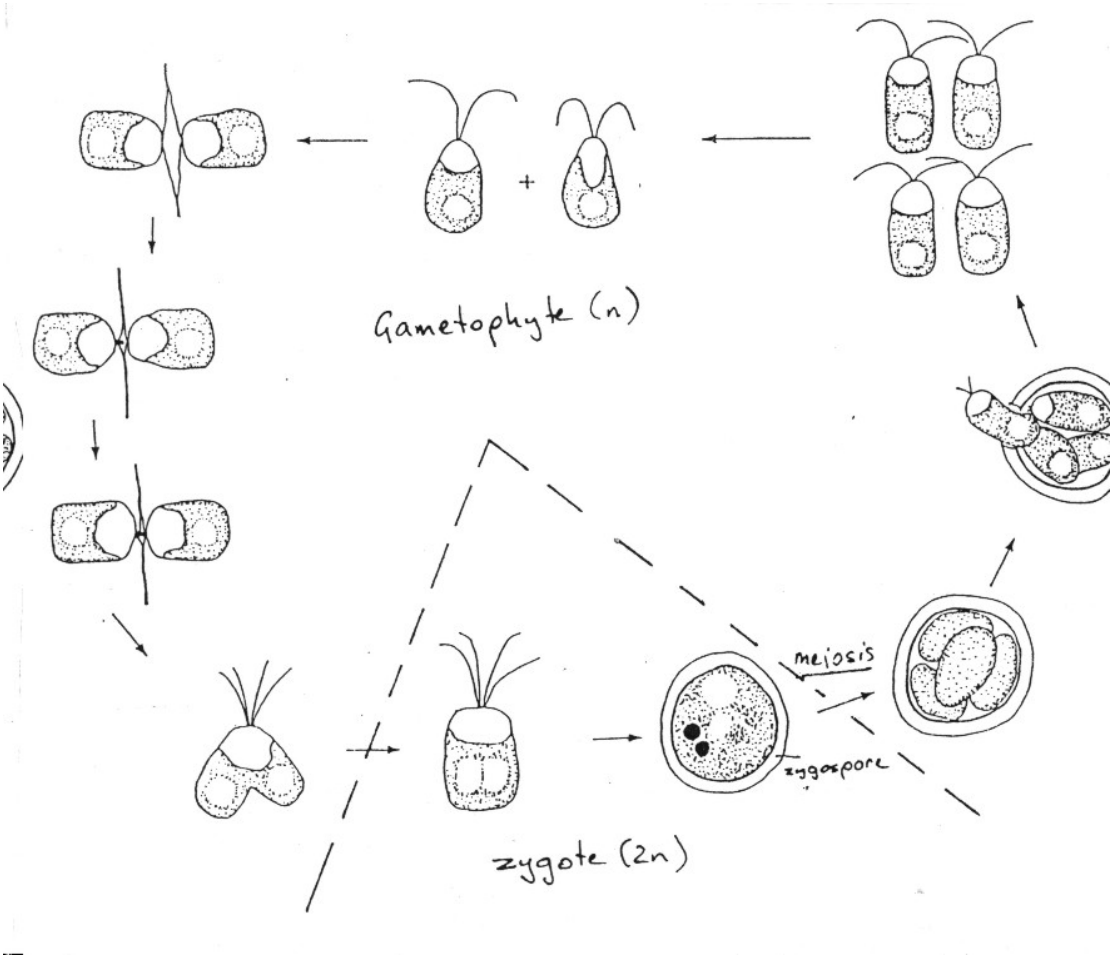


Figure 5. Life history of *Dunaliella salina* (sexual cycle only). The alga may also produce aplanospores (resting cysts) or form a non-motile palmella stage under certain conditions.

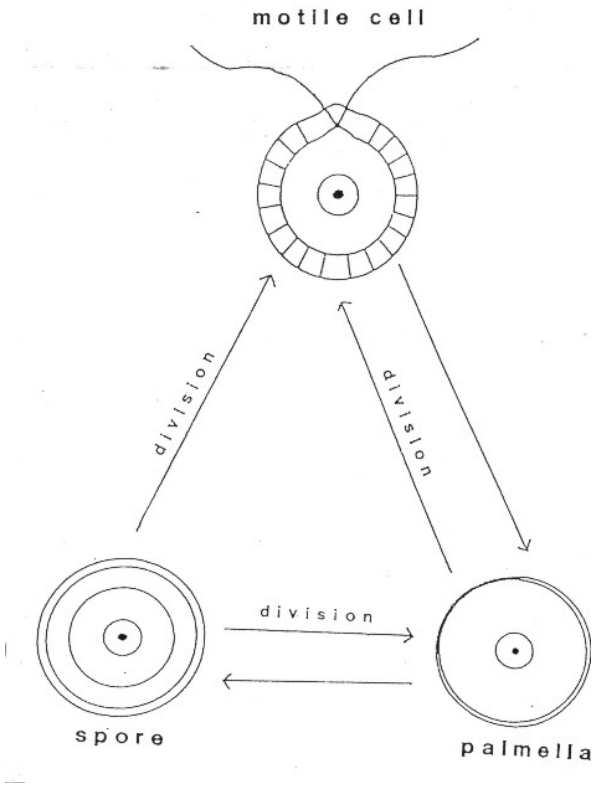


Figure 6. Life history of *Haematococcus* as usually observed in cultures. Sexual reproduction has also been reported, but is not

Inorganic nutrients

The great taxonomic diversity of the algae is also reflected in their nutritional requirements, and for most algal species we do not yet know what their exact nutrient requirements are. This state of ignorance is well reflected in the wide range of nutrient media used (see later). Nutrients can, however, be classified into **macronutrients** (i.e. those required at $g.L^{-1}$ concentrations) and **micronutrients** (i.e. those required at $mg.L^{-1}$ or $\mu g.L^{-1}$ concentrations). These are summarised in Table I. The role of these nutrients in the algae is summarised in Table II. The nutritional requirements of algae are well discussed in the article by Kaplan *et al.* (1986) and in many algal text books and will not be described here further in any detail.

Nitrogen

The usual nitrogen sources in algal media are (1) nitrate; (2) ammonium; or (3) urea. Some of the prokaryotic algae, the heterocystous blue-green algae (cyanobacteria), can also fix atmospheric N_2. Most algae can use nitrate (NO_3^-), nitrite (NO_2^-) or ammonium (NH_4^+) as an N source, with similar growth rates achieved irrespective of the nitrogen source.

When ammonium is used as the sole N source the pH of the medium may fall sharply, especially in dense cultures at high temperatures, often leading to a rapid decline in

growth and even death of the culture (Figure 7). High concentrations of >1 mM ammonium may inhibit growth, especially at high temperatures.

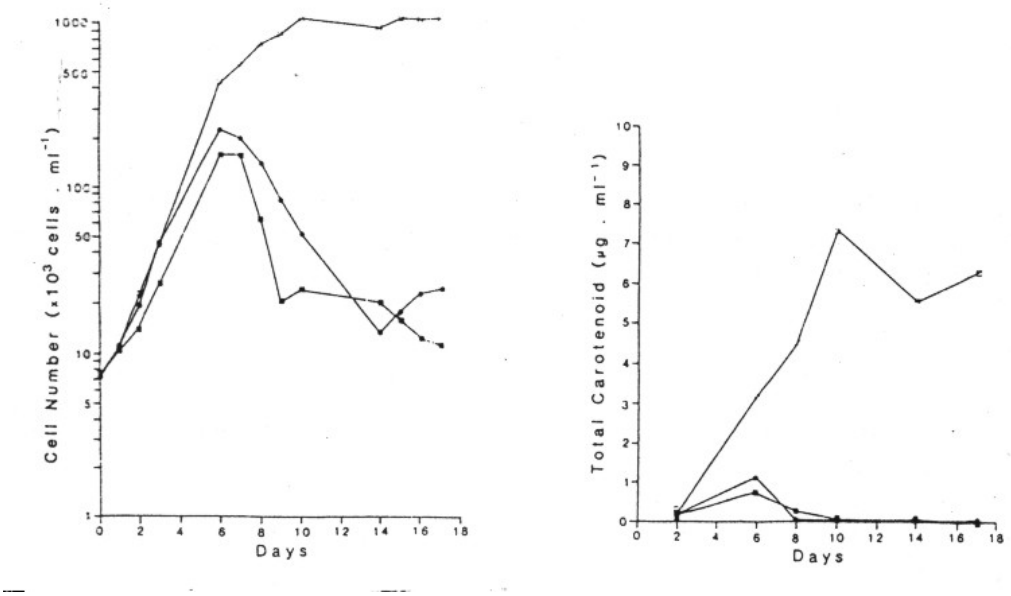


Figure 7. Effect of nitrogen source on (a) growth, and (b) carotenoid content of *Dunaliella salina* cultures. (□ = 1 g.l^{-1} KNO_3; ● = 1 g.l^{-1} NH_4NO_3; O = 0.5 g.l^{-1}

If both ammonium and nitrate are supplied the cultures generally do not take up the nitrate until the ammonium has been used up. This is because ammonium is the end product of nitrate reduction and therefore causes feedback-inhibition and repression of the nitrate uptake and reduction system.

Urea is also potentially a good nitrogen source for almost all algal species. Urea is usually hydrolysed before its N is incorporated into the algal cells. This occurs by the action of either the enzyme urease, or the enzyme urea amidolyase (UALase). Algae that metabolise urea have either urease or UALase, but not both.

Phosphorus

Phosphorous is another major nutrient for algae. The major form in which algae take up phosphorous is as inorganic phosphate ($H_2PO_4^-$ and HPO_4^{2-}). Those algae which can utilise organic phosphate compounds hydrolyse these extracellularly by the action of phosphoesterase or phosphatase enzymes and then take up the inorganic P produced. High concentrations of phosphorous may actually inhibit the growth of some algae.

Sulphur

Sulphur is required to form the sulphur-containing amino acids methionine, cystine, cysteine as well as biotin, pantothenic acid, thiamine, lipoic acid, sulphur lipids and sulphated polysaccharides etc. Most algae obtain the sulphur from inorganic sulphate, although a few species have also been shown to be able to take up organic sulphur compounds.

Calcium and Magnesium

Calcium is required for maximum growth by many algae although its exact function is unknown. Calcium does play a role in membrane stability and is incorporated into scales in some species.

Magnesium is essential for all algae. It is required for ribosome stability and for the function of chlorophyll.

Sodium, Potassium and Chlorine

Sodium is required by some algae, but apparently not by others. Marine species generally require some sodium.

Potassium is a cofactor for a variety of enzymes and is probably required by all algae.

Chloride ions also appear to be universally required, in part because of the role they play in potassium and sodium uptake.

Silicon

Most algae have a very low requirement for silicon which is probably met by silicon contamination of other media components, especially in seawater-based media. Diatoms, however, have a larger silicon requirement to form their siliceous valves. Lack of silicon can reduce growth, and in some species totally inhibit DNA replication. Silicon is only taken up as silicic acid (H_4SiO_4).

Germanium is taken up by diatoms in place of silicon but cannot be incorporated into the valve. If germanium is added at a molar ratio of Ge/Si of 0.1 to 0.2, the growth of almost all diatoms is inhibited. This can be used to remove diatom contamination from cultures during isolation.

Iron

Iron is essential for all algae. It is required for nitrogen assimilation, in photosynthesis and for the synthesis of cytochromes. In order to be taken up by algae it usually has to be available in a chelated form, usually with EDTA or with citrate. High concentrations of iron are inhibitory to growth.

Trace Elements

A range of trace elements are added to algal cultures. Specific requirements for some of these have been demonstrated in some algae, but much more work needs to be done on their functions and on the actual requirements. Trace elements usually added are boron (especially important to diatoms and blue-green algae), manganese, copper, zinc, molybdenum, vanadium, cobalt, nickel and selenium (especially important for some dinoflagellates).

Organic nutrients

Heterotrophic algae (i.e. auxotrophs, mixotrophs etc) can utilise a range of organic carbon sources, with acetate being most widely used. Other sources include glucose, galactose etc.

Organic nitrogen sources used by some algae include glycine, glutamine, aspartate, arginine and, in particular, urea.

Many algae also require vitamins, especially vitamin B_{12} and other B series vitamins.

Table 1. List of inorganic nutrients required by algae and their approximate concentration (NB. This list is not complete).

Element	Compounds	Conc range/L medium
C	CO_2, HCO_3^-, CO_3^{2-}, organic molecules	g
O	O_2, H_2O, organic molecules	g
H	H_2O, organic molecules, H_2S	g
N	N_2, NH_4^+, NO_3^-, NO_2^-, amino acids, purines, pyrimidines, urea, etc	g
Na	Several inorganic salts, i.e. NaCl, Na_2SO_4	g
K	Several inorganic salts, i.e. KC1, K_2SO_4, K_3PO_4	g
Ca	Several inorganic salts, i.e. $CaCO_3$, Ca^{2+} (as chloride)	g
P	Several inorganic salts, Na or K phosphates, Na_2 glycerophosphate	g
S	Several inorganic salts, $MgSO_4$, amino acids	g
Mg	Several inorganic salts, CO_3^{2-}, SO_4^{2-} or Cl^- salts	g
Cl	As Na^+ , K^+ , Ca^{2+}, or NH_4^- salts	g
Fe	$FeCl_3$, $Fe(NH_4)_2SO_4$, ferric citrate	mg
Zn	SO_4^{2-} or Cl^- salts	mg
Mn	SO_4^{2-} or Cl^- salts	mg
Br	As Na^+, K^+, Ca^{2+}, or NH_4^- salts	mg
Si	$Na_5SiO_59H_2O$	mg
B	H_5BO_5	mg
Mo	Na^+ or NH_4^+ molybdate salts	µg
V	$Na_5VO_4.16H_2O$	µg
Sr	SO_4^{2-}or Cl^- salts	µg
Al	SO_4^{2-}or Cl^- salts	µg
Rb	SO_4^{2-} or Cl^- salts	µg
Li	SO_4^{2-} or Cl^- salts	µg
Cu	SO_4^{2-} or Cl^- salts	µg
Co	Vitamin B_{12}, SO_4^{2-} or Cl^- salts	µg
I	As Na^+, K^+, Ca^{2+}, or NH_4- salts	µg
Se	Na_2SeO_3	ng (µg)

Table 2 The roles of the various inorganic nutrients in algal metabolism

ELEMENT	PROBABLE FUNCTION	EXAMPLES OF COMPOUNDS
Nitrogen	Major metabolic importance as compounds	Amino acids, purines, pyrimidines, porphyrins, amino sugars, amines
Phosphorous	Structural, energy transfer	ATP, GTP, nucleic acids, phospholipids, coezymes, coenzyme A, phosphoenol pyruvate
Potassium	Osmotic regulation, pH control, protein conformation and stability	Probably occurs predominantly in the ionic form
Calcium	Structural, enzyme activation, ion transport	Calcium pectate, $CaCO_3$
Magnesium	Photosynthetic pigments, enzyme activation, ion transport, ribosomal stability	Chlorophyll
Sulphour	Active groups in enzymes and coenzymes, structural	Methionine, cystine, cysteine, glutathione, agar, carrageenan, sulpholipids, coenzyme A
Iron	Active groups of porphyrin molecules and enzymes	Ferredoxin, cytochromes, nitrate reductase, ferretin, catalase
Manganese	Electron transport in Photosystem II, maintenance of chloroplast membrane structure	Manganin
Copper	Electron transport (photosynthesis), enzymes	Plastocyanin, amine oxide
Zinc	Enzymes, auxin metabolism (?), ribosome structure (?)	Carbonic anhydrase
Molybdenum	Nitrate reduction, ion absorption	Nitrate reductase
Sodium	Enzyme activation, water balance, enzymes	Nitrate reductase
Chlorine	Photosystem II	Terpenes
Boron	Regulation of carbon utilisation (?), RNA metabolism (?).required by heterocystous cyanobacteria through via its role in nitrogenase activity in N_2 fixation	
Cobalt	Component of vitamin B_{12}, C_4 photosynthetic pathway	Vitamin B_{12}
Bromine	?	Wide range of halogenated compounds in red and blue-green algae
Iodine	?	Wide range of halogenated compounds in red and blue-green algae

**Possibly an essential element in some algae*

Algal Culture Media

A very wide range of algal culture media are available and the best medium for a particular alga will have to be determined by experiment. A listing of various media can be found in Borowitzka (1988) and Anderson (2005)

A range of the major laboratory media is given in Tables A1-A3. These are a good place to start if no medium is known for an alga. Thus, for freshwater blue-green algae try BG-11, for other freshwater algae try Bold's Basal Medium (BB) or PHM-1. Zarrouck Medium (Z) has been optimised for the alkaliphilic blue-green alga *Spirulina*, and the modified Johnson's Medium (J/1) for *Dunaliella salina* and other halophilic *Dunaliella* species (the salinity is adjusted by altering the NaCl concentration in the medium). For marine algae, Guillard's f/2 medium has proven to be suitable for a large range of species (for some species the nutrient concentration, i.e. N and P is reduced further, and the vitamins may be omitted for some). The ASW medium is excellent for many marine diatoms and the MN medium for marine blue-green algae. The *Porphyridium* (Porph.) medium has been devised for the culture of the red alga *Porphyridium cruentum*.

For large-scale culture many of these 'laboratory' media are too complex and too expensive. A number of simplified media have, therefore, been devised including a range of media for *Spirulina* (Becker & Venkataraman, 1984) and a simplified medium for *Scenedesmus* (Becker, 1984). Commercial fertilisers may also be used, rather than more expensive laboratory chemicals.

Some general pointers on medium preparation

For all media, and especially seawater media, it is advisable to autoclave the phosphate and iron separately and to add it to the medium after autoclaving to avoid the precipitation of insoluble salts. If possible Teflon vessels rather than glass vessels should be used for autoclaving seawater to avoid precipitation.

Where vitamins are required, these should be filter-sterilised separately and added to the medium just before inoculation.

For the culture of diatoms, media which do not contain Si should be supplemented with 0.05 $g.L^{-1}$ $Na_2SiO_3.9H_2O$. Some diatoms, as for example *Skeletonema costatum*, have a very high Si requirement and will require Si supplementation to maintain good growth.

If required, media may be solidified with 1-1.5% agar.

If seawater is used as a base for the medium (i.e. f/2 medium) this should be collected as far off-shore as possible to reduce the possible effects of contaminants (organic substances, pesticides etc.) due to land-runoff or rivers. For best results the seawater should be 'aged' by keeping in a cold room (approx. 3^oC) in the dark for at least one week, and then stirring with about $2g.L^{-1}$ activated charcoal powder for 30 min before filtering through Whatman No. 1 filter paper. Small volumes of seawater media should be filter-sterilised rather than autoclaved to avoid possible changes in salinity. Larger volumes can be sterilised by autoclaving, pasteurisation[1] or with UV light. Detailed description of sterilization methods can be found in Kawachi and Noél (2005) If a fully

[1] Heat to 90 – 95^0C for 24 h.

defined medium is required artificial seawater mixes such as Artificial Pacific Seawater (APSW) (Borowitzka, 1988) may be used instead of natural seawater.

Table A1. Basic media for algal cultures. All weights are $g.L^{-1}$ deionised or distilled water, except for f/2 medium where the nutrients are added to seawater (see text), and MN medium where the nutrients are added to 750 mL filtered seawater and 250 mL deionised water. Composition of trace element solutions are given in Table A2 and for Fe-solutions in Table A3. pH is adjusted with HCl or NaOH.

	BG-11[1]	**BB**[2]	**PHM-1**[3]	**Z**[4]	**J/1**[3]	**F/2**[5]	**ASW**[6]	**MN**[8]	**Porph**[8]
KNO_3			1.0	3.0	1.0		0.303		1.0
$NaNO_3$	1.5	0.25				0.075		0.75	
K_2HPO_4	0.04	0.075	0.2		0.035			0.02	
KH_2PO_4		0.175					0.0456		0.07
$NaH_2PO_4.H_2O$						0.005			
$MgSO_4.7H_2O$	0.075	0.075	0.2		0.5		4.9	0.04	6.6
$MgCl_2.2H_2O$					1.5		4.1		5.6
$CaCl_2$							1.1		
$CaCl_2.2H_2O$	0.036	0.025			0.2			0.02	1.5
$CaSO_4$(sat.soln.)			20 mL						
NaCl		0.025		1.0	as reqd.		23.6		27.0
KCl					0.2		0.075		
K_2SO_4				1.0					
Na_2CO_3	0.02				0.043			0.02	
$NaHCO_3$				16.8					
Na-acetate			(0.1)						
$Na_2SiO_3.9H_2O$			(0.05)			0.03	0.04		
Citric Acid	0.006							0.003	
Na_2-EDTA	0.001			0.08			0.012	0.0005	
Fe-Ammonium Citrate	0.006							0.003	
$FeSO_4.7H_2O$				0.01					
Glycylglycine							0.66		
Tris HCl (1M)									20 mL
Thiamine HC1						100 µg			
Biotin						0.5 µg			
B_{12}						0.5 µg			

Fe-soln (1 mL)		BB	PHM-1		PHM-1	f/2			Porph.
Trace elements (1 mL)	A5	BB	J/1	Z_A+Z_B	J/1	f/2	ASW	A5	Porph.
pH	7.1	7.0	7.2	9.0	7.5	8.0	8.0	8.5	8.0

[1]Allen (1968); [2]Bischoff & Bold (1963); [3]Borowitzka (1988); [4]Vonshak *et al.* (1982); [5]Guillard & Ryther (1962); [6]Darley & Volcani (1969) ; [7]Waterbury & Stanier (1978); [8]Vonshak (1997)

Table A2. Trace element stock solutions for algal culture media (All weights in g.l.-1).

	A5	**BB**	**Z_A**	**Z_B**	**J/1**	**f/2**	**ASW**	**Porph.**
H_3BO_3	2.86	11.42	2.86		0.061		0.568	11.42
$MnCl_2.4H_2O$	1.81	1.44	1.81		0.0041	0.18	0.36	0.4
$ZnSO_4.7H_2O$	0.222	8.82	0.22			0.222		
$ZnCl_2$					0.0041		0.624	0.04
$Na_2MoO_4.2H_2O$	0.39					0.006	0.252	
MoO_3		0.71	0.015					
$(NH_4)_6Mo_7O_{24}.4H_2O$					0.038			0.0377
$CuSO_4.5H_2O$	0.079	1.57	0.079		0.006	0.01		
$CuCl_2.2H_2O$							0.268	0.4
$Co(NO_3)_2.6H_2O$	0.0494	0.49		0.044	0.0051			
$CoCl_2.6H_2O$						0.01	0.42	0.5
NH_4VO_3				0.023				
$K_2Ca(SO_4)_4.24H_2O$				0.096				
$NiSO_4.7H_2O$				0.048				
$Na_2WO_4.2H_2O$				0.018				
$Te_2(SO_4)_3$				0.04				
$FeSO_4$							1.36	
Na-tartrate							1.77	

Table A3. Fe stock solutions.

Bold Basal Medium (BB)

Add 1 mL each of the following stock solutions:

50 g EDTA and 31 g KOH (or 50 g Na_2 EDTA) in 1 L distilled water,

4.98 g $FeSO_4 7H_2O$ in 1 L acidified water (i.e. 1 mL H_2SO_4 in 1 L distilled water)

PHM-1

189 mg Na_2 EDTA and 244 mg $FeCl_3.6H_2O$ in 1L distilled water (autoclave to dissolve)

f/2

4.36 g Na_2 EDTA and 3.15 g $FeCl_3.6H_2O$ in 1 L distilled water (autoclave to dissolve)

Porph

240 mg $FeCl_3.4H_2O$ in 100 mL 0.05 M Na_2 EDTA (pH 7.6)

After autoclaving it is best to leave the medium to stand for about 24 h so that CO_2 can diffuse back into the medium.

At all times it is essential to ensure that all glassware etc. used for the culture of algae is clean and free of contaminants such as detergents. Glassware preparation involves a preparatory rinse in hot tap water, soaking overnight in a detergent bath (e.g. Pyroneg), scrubbing, washing in detergent, followed by at least 3 rinses in distilled water and drying. Similarly, the chemicals used for preparing the media should be of good quality and the water source as pure as possible.

Laboratory cultures are usually uni-algal, but quite often not axenic (i.e. bacteria and fungi free). For some purposes it is essential to have axenic cultures. To obtain these, one usually needs to use several different methods and have much patience. Methods to 'clean-up' cultures include the use of antibiotics, repeated streaking of cultures on agar, washing the cultures repeatedly with sterile medium and then isolating single cells etc. These methods are described in detail in Guillard (2005).

Limits to growth

(see also Borowitzka (1998))

Algal growth is influenced by a number of factors. In order to optimise growth one must understand what is limiting growth. If one is growing the algae as a source of a particular product one also has to understand what limits the formation of that product. Growth and product formation are often limited by different factors, and the final culture regime used will be a compromise.

Factor limiting growth include:

1. light
2. temperature
3. inorganic carbon supply
4. oxygen
5. Nutrients (e.g. N, P, Fe, etc)

Algae have two general kinds of response to changes in the concentration of limiting factors: (a) a change in the final yield (type I response – Figure 8), or (b) a change in the growth rate (type II response – Figure 8).

A Type II response is typical of phytoplankton in nature. It is often quite difficult to determine experimentally which type of response is occurring for several reasons. For example, another factor may also be limiting, or in batch cultures the concentration of the factor being studied changes as the algae grow. As soon as the net uptake of a nutrient is zero, growth should be completely inhibited. Many nutrients (especially P and N) are, however, stored by the algae in excess of their actual requirements. This means that normal growth may continue for some time after the concentration of the nutrient has

reached its lowest possible value. Growth at the expense of stored nutrients is especially well known for phosphorous, but also occurs with iron.

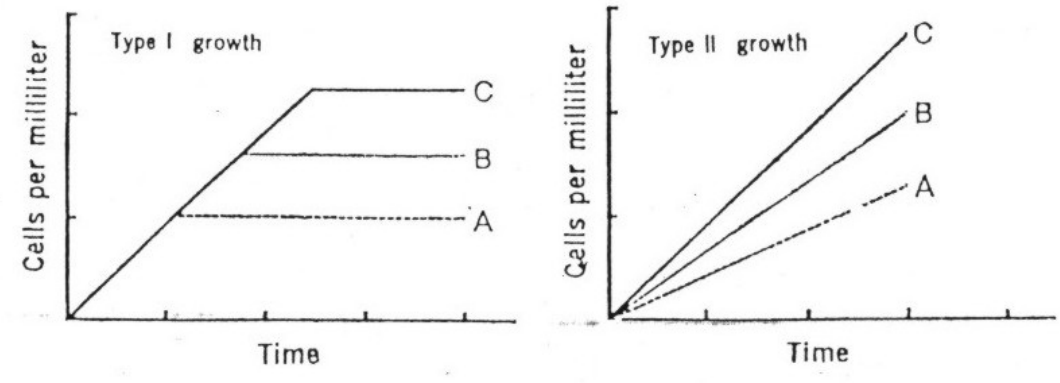


Figure 8. Type I and II growth patterns. The ordinate is a logarithmic scale. Increasing concentrations of the limiting factor are represented by A, B and C.

1.1 Light

Light is a complex factor affecting algal growth and metabolism. Not only is the energy supplied by light (i.e. the photon flux density - often erroneously called the light intensity) important, but also the spectral distribution of the light has to be considered. In the laboratory we usually use fluorescent lights, but these are deficient in the red part of the spectrum and this may have to be supplied by using incandescent bulbs or 'Grow-Lux' tubes. The maximum photon flux density which can be achieved using these is about 200 – 250 µmol photons.m^{-2}.sec^{-1}, while noon daylight is about 2000 µmol photons.m^{-2}.sec^{-1}.

In order to optimise productivity in large-scale cultures, pond depth (culture thickness) should also be optimal. V. Bush derived a theoretical equation to estimate the fraction, f_m , of the maximum possible photosynthetic efficiency that can be attained by a culture. Bush's equation states:

$$F_m = (S_s/S_o) [\ln (S_o/S_s) + 1] \quad (1)$$

where S_s is the photon flux density at which photosynthesis is saturated and S_o is the photon flux density at any instant. This predicted efficiency cannot, however, be attained in real cultures since they are also limited by the quantum efficiency of various wavelengths. Furthermore, one must also take account of night-time respiration before a net efficiency is obtained. The actual light utilisation efficiency of mixed cultures of algae ranges from less than 1% to a (rarely achieved) maximum of 5%.

Assuming that the major light absorbing components in a shallow algal culture are the algae, Oswald (1988) has empirically determined the following relationship (for green algae) between the concentration of algae, C_c ($mg.L^{-1}$) and the light penetration depth d_p (cm):

$$d_p = 6000/C_c \quad (2)$$

Field observations of large-scale cultures further indicate that the culture concentration in light-limited, continuously mixed culture approaches that which permits light to penetrate two-thirds of the actual culture depth, i.e.:

$$d_p = (2/3)d \quad (3)$$

and

$$C_c = 9000d \quad (4)$$

(Oswald 1988b)

A continuously mixed outdoor culture of green algae at 30 cm depth will therefore achieve an average maximum light-limited algal concentration of about 300 $mg.L^{-1}$. Higher photon flux densities will increase this only slightly, since the penetration of light is proportional to the log of its intensity. High concentrations of algae can therefore only be attained at shallow culture depth. Since shallow cultures are more prone to major temperature fluctuations, greater changes in medium composition due to evaporation and are more difficult and expensive to mix, the actual culture depth used must be a compromise.

Studies by Laws and coworkers (1986) using a system that introduces ordered vertical mixing into the culture by suspending small foils, similar to aeroplane wings, at regular intervals in the flowstream at a relatively high angle of attack and so generating regular vortices, have shown that culture yield can be increased by 50 to 100% for *Phaeodactylum tricornutum*. Whether this approach will be cost effective has yet to be determined.

Too high light can also lead to photoinhibition, especially in combination with high oxygen concentrations (Moheimani & Borowitzka, 2007). Most outdoor cultures of microalgae are photoinhibited at noon.

1.2 Temperature

Temperature affects metabolic rates (Figure 9.), and thus growth. Different species and strains have different temperature optima (e.g. Figure 11) and depending on other growth conditions different temperature extremes can be tolerated (eg. Figure 10).

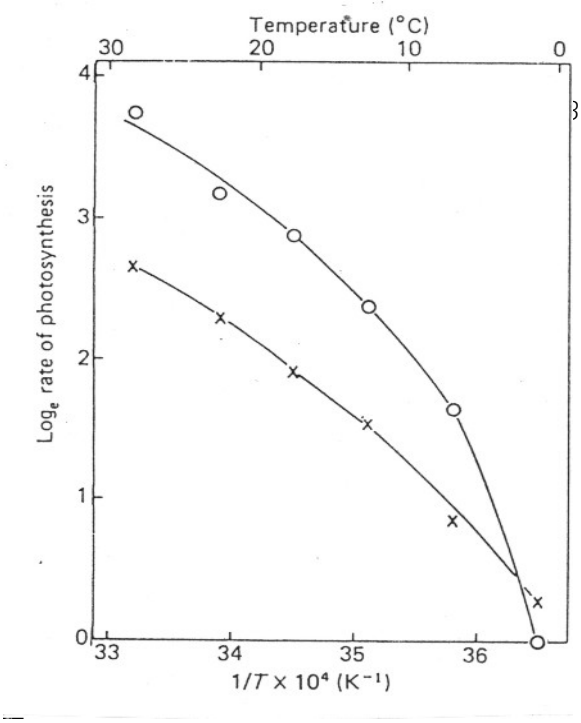


Figure 9. Effects of temperature on the photosynthetic rate of two species of *Chlorella*. (o) *Chlorella vulgaris*; (x) *Chlorella pyrenoidosa*.

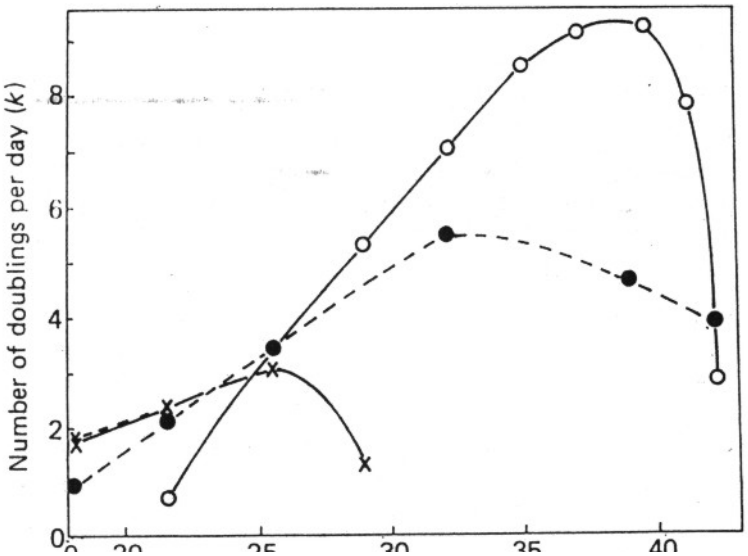


Figure 11. Growth rates of two strains of *Chlorella pyrenoidosa* at different temperatures. (●,O) Strain 7-11-05 at high (O) and low (●) light; (x) Emerson strain.

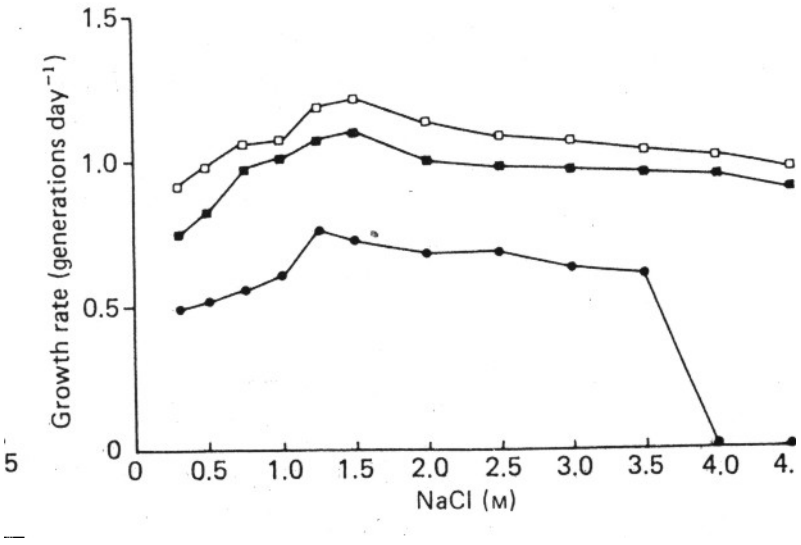


Figure 10. Growth rate of *Dunaliella viridis* over a range of NaCl concentrations at three different temperatures. (□) 30°C; (O) 26°C; (●) 14°C.

Inorganic carbon

Algae are aquatic organisms and in water inorganic carbon exists at CO_2, HCO_3^- and CO_3^{2-} i.e. the equilibrium can be represented as:

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + H^+$$

The relative proportion of these three species depends on the pH (Figure 12). All algae can take up CO_2 by diffusion, and many have active carbon uptake systems which can take up HCO_3^-. No algae can, however, take up CO_3^{2-} ions. The pH of the medium therefore has a profound effect on the effective concentration of inorganic carbon available to the algae to be taken up for photosynthesis. The total solubility of inorganic carbon is also dependent upon the ionic strength (salinity) of the medium. Halophilic

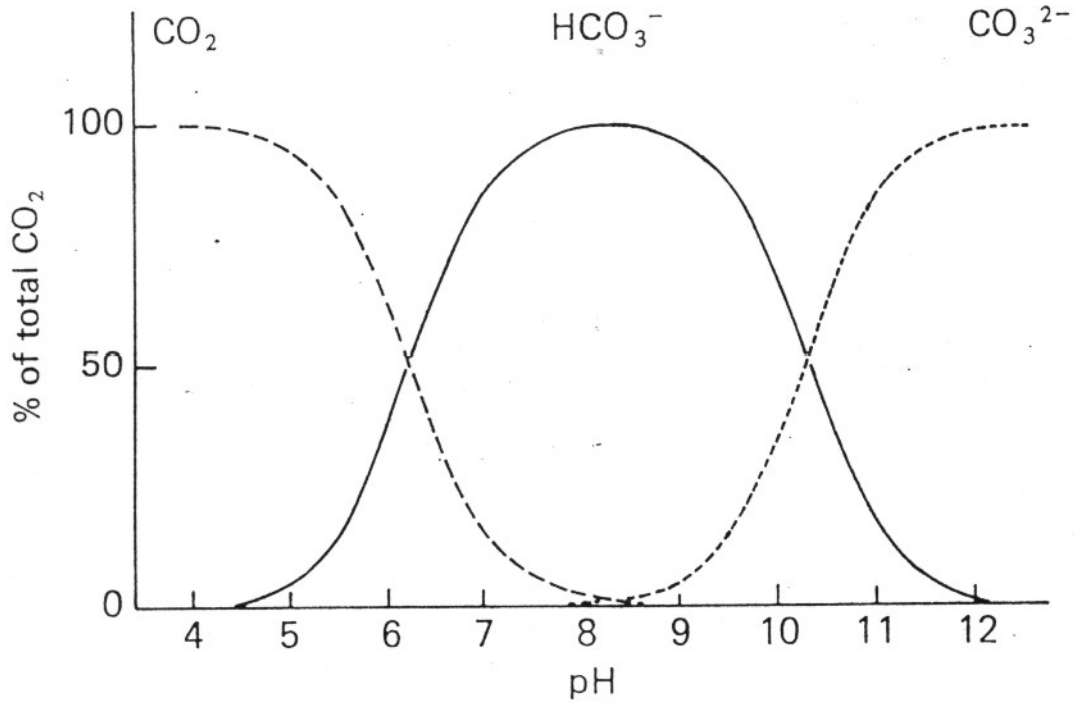


Figure 12. Relative proportions of CO_2, HCO_3^- and CO_3^{2-} in water with changing pH.

algae such as *Dunaliella salina* growing at extremely high ionic strengths are more carbon limited than marine species due to the reduced solubility of inorganic carbon in the highly saline brines.

The addition of CO_2 to cultures can improve growth and yields. However, CO_2 addition leads to acidification of the medium and must therefore be carried out in a controlled manner.

Oxygen

High concentrations of oxygen in the medium, as can occur in dense cultures under high light (i.e. at noon in outdoor cultures) results in photoinhibition and photorespiration and a reduction in photosynthesis and growth (for an example see Moheimani & Borowitzka, 2007).

Anoxia, which may occur in dense cultures, especially mixed outdoor cultures, at night can be tolerated by most algae for short periods but may have a growth-reducing effect. Oxygen supersaturation or anoxia can be alleviated, in part, by good mixing and aeration of the culture.

Nutrient limitation

The role of nitrogen, phosphorus, iron as well as cation such as Na^+, Ca^{2+}, Mg^{2+} and anions such as Cl^- and SO_4^- on growth and metabolism has already been considered. Some of these effects of limitation in these nutrients on growth and the formation of specific metabolites will be discussed.

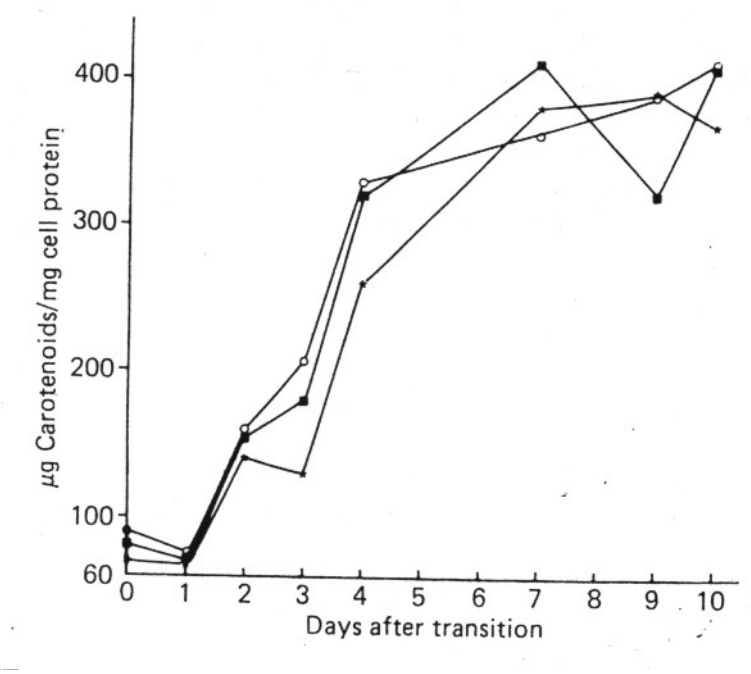


Figure 13. Increase in total carotenoid content of three strains of *Dunaliella salina* following an increase in the salinity of the medium form 15% (*w/v*) NaCl to 30%

Measuring growth

(see also Wood et al., 2005)

There are several ways to measure growth in algal cultures. These include:

1. direct counting of cells,
2. measurement of algal biomass by weighing,
3. measurement of the turbidity of the culture using a spectrophotometer
4. measurement of chlorophyll content or protein content of culture.

The latter two methods only work if the pigmentation of the algae does not change drastically during the growth cycle and, in the case of (3) if the medium does not contain other particulate matter as occurs in open, unlined ponds. Measurement of algal biomass by weighing is quick and easy, but requires clean cultures and a large sample volume. The most accurate, but generally most time consuming method is direct cell counting, either under a microscope or with the aid of an electronic particle counter. The latter will not work for fragile or colonial or filamentous species.

In order to calculate the actual growth rate growth curves are usually plotted (using a semi-log plot - Figure 14) and the growth rate determined.

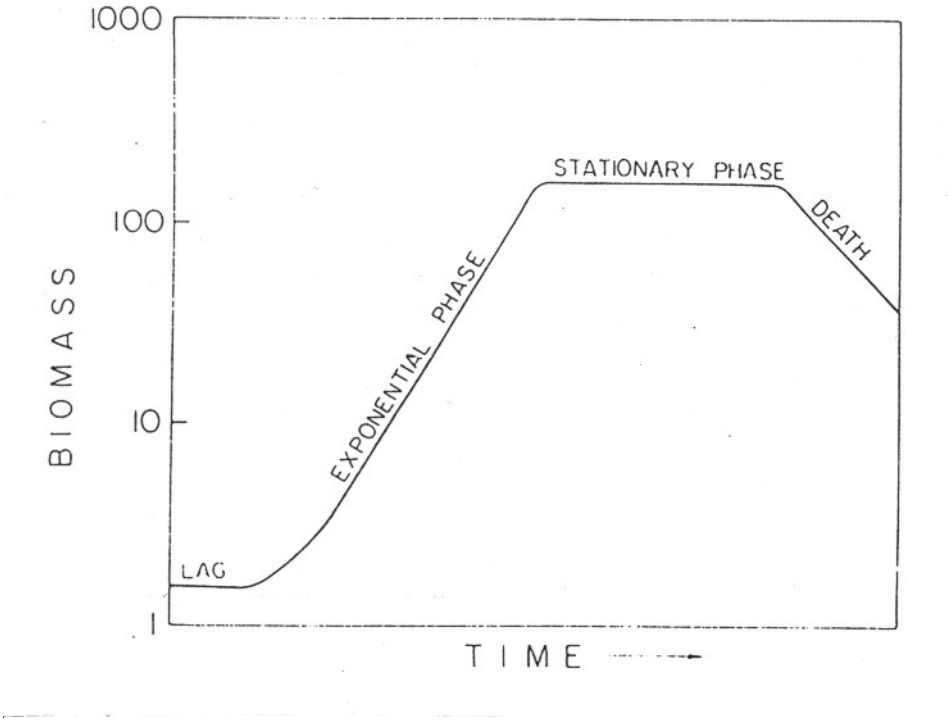


Figure 14. Diagram of idealised growth curve. Not actual growth curves may vary a little from this.

Algal growth curves

In an exponentially growing culture the increase in cell number (dx) will be proportional to the initial number of cells present (x) and the time interval dt.i.e.

$$dx = \mu dt \qquad (1)$$

where dx/dt represents the actual population growth rate and μ, having the dimension of 1/t, is termed the specific growth rate and represents the growth per unit time. Equation (1) can be integrated ($x=x_0$ at $t=0$) to:

$$x = e_0 e^{\mu t} \quad (2)$$

where *e* is the base of natural logarithms. Growth fits this relation (called the logistic growth curve model) during its exponential or logarithmic phase. Equation (2) can be further solved to:

$$\ln x/x_0 = \mu t \quad (3)$$

When $x = 2x_0$

$$\ln 2 = \mu t_2 \quad (4)$$

and

$$t_2 = \ln 2/\mu = 0.693/\mu \quad (5)$$

where t_2 is the doubling time of the algae and Equation (5) relates the specific growth rate to the doubling time.

By plotting the cell numbers vs time on semi-log graph paper one can easily determine the doubling time graphically and then calculate the specific growth rate, μ, using Equation 5.

It should be noted that one could use other measures of the growth rate such as, for example, dry or wet weight, chlorophyll *a* concentration, protein content or cell volume to calculate the growth rate.

Culture systems

Batch Cultures

Batch cultures are closed systems and nothing is added or removed from the liquid phase after inoculating the culture. Batch systems will support growth only until one or more nutrients become limiting, when growth stops. The type of growth curve observed is shown in Figure 14. Since cells in the stationary phase of growth still require energy to maintain viability some decline in cell numbers may occur. This decline is often quite dramatic; i.e. a 'crash'.

Continuous Cultures

Continuous cultures are open systems and differ from batch cultures in that a fresh supply of nutrients is continuously added at the same rate as medium is withdrawn from the culture so that the culture volume remains constant with time. This theoretically permits continuous exponential growth of the culture. Steady-state (exponential phase) growth is possible when all factors promoting cell growth are balanced by those factors contributing to the loss of cells, so that the cell concentration (biomass) is maintained as constant. This occurs when the flow rate of fresh medium into the culture (f = dv/dt) equals the flow rate of culture through the overflow. If V is the culture volume, then

$$(dv/dt)\ (1/V) = \mu$$

If we define D as the dilution rate, which also equals (dv/dt)/V, then $D = \mu$

There are basically three ways to cultivate algae in a continuous system, and these are outlined below.

Chemostat

Chemostat cultivation is based on the assumption that the algal growth rate is limited by the slowest step of nutrient metabolism under particular conditions and thus, by the concentration of a particular nutrient. The chemostat system consists of a reservoir containing the culture medium, a constant flow pump, and a culture vessel with a constant volume. Culture liquid leaves the vessel at the same rate as new liquid is added. The culture has to be homogeneously mixed.

Semi-continuous culture

Semi-continuous cultures are a type of batch culture which is diluted at frequent intervals. The concentration of biomass is monitored to estimate the proper frequency of dilution and the dilution ratio.

Turbidostat Culture

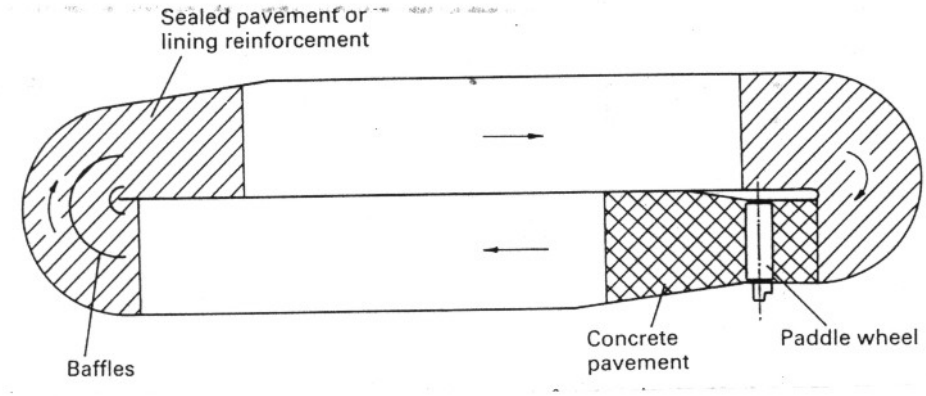
This type of culture depends on a continuous monitoring of the biomass, as, for example, by an optical device that measures turbidity. The dilution rate is controlled so that the culture biomass (turbidity) remains constant. Nutrients are always non-limiting. Light, on the other hand, may be a limiting factor unless the culture is very dilute.

Large-scale cultivation

Industrial cultivation of micro-algae may be either extensive or intensive, and may be either in open systems or in closed, non-axenic or axenic, systems (Borowitzka, 1999; 2005). No one culture method can be said to be better than another and the choice of culture system will depend, in large part, on the alga to be used and the product. Much of the technology of micro-algal mass-culture has been reviewed by Terry and Raymond (1985) who also give a good historical overview, and by Borowitzka (Borowitzka, 2005).

Extensive cultivation in large outdoor open ponds is the oldest of the industrial systems for algal cultivation and is used for the cultivation of algae such as *Chlorella, Scenedesmus, Spirulina* and *Dunaliella salina.* This cultivation method is inherently the simplest and cheapest method, but is generally only applicable if the alga to be cultured grows in a relatively selective and specialised environment. Thus, for example, *D. salina* grows in brines with NaCl concentrations > 20% w/v (seawater is about 3% w/v NaCl), and *Spirulina platensis* grows in alkaline waters with a pH > 9.2. That is not to say, however, that monocultures of algae growing in less selective environments (i.e. seawater or freshwater) cannot be maintained for long periods with judicious pond management (see Moheimani & Borowitzka, 2006).

The simplest type of extensive cultivation is used by Cognis in their *Dunaliella* plant at Hutt Lagoon, W.A. where each of the unlined 5 to 250 ha area production ponds is constructed on the lake bed of Hutt Lagoon with earthen berms (Figure 16). The cultures are approximately 20-40 cm deep and are not mixed other than by wind and convection (Borowitzka & Borowitzka, 1989).



The most common extensive open-air pond design, however, is the 'raceway' design which consists of long channels arranged in a single or in multiple loops and mixed by a paddlewheel. These shallow mixed ponds were originally introduced in the 1950s and 1960s by Oswald and co-workers. Many of the early designs used a configuration consisting of relatively narrow channels with many 180° bends and using propeller pumps to produce a channel velocity of about 30 $cm.sec^{-1}$. In the 1970s paddlewheel mixers of various designs were introduced and found to be more effective, with less energy requirements and reduced stress forces on the algal cells. Aside from propeller pumps and paddlewheels, airlifts have also been proposed for the mixing of 'raceway' ponds. These have, however, not been tested at a large scale to any extent and at this stage paddlewheels are the preferred mixing device.

The numerous bends in the channels of the older designs also led to hydraulic losses and problems with solids deposition. These were minimised by using a single-loop (racetrack) configuration, with suitable baffles being incorporated. **Error! Reference source not found.** shows the design of what is presently considered the optimal configuration (see also Figure 18 and Figure 20 for illustrations of raceway production ponds). The optimal pond size is a compromise between the hydraulics of the pond and the ability to actually construct the pond. Simple geometric optimisation shows that a large pond with a low length to width (L/W) ratio gives the largest pond area for the least wall length, and is therefore cheaper to construct. However, there are limits to the size of paddle wheels and therefore the widest ponds are about 6 m wide. The actual pond length needs thus to be evaluated to take account of head losses (which depend on velocity and roughness) relative to the mixing system to be used (Borowitzka, 2005).

Several factors need to be taken into account when designing the optimally sized pond. These include:

1. Optimal pond depth, taking into account the degree of light penetration.
2. Mixing velocity. This relation to the need to keep the algae in suspension, avoiding any dead spaces and the effects of turbulence on the pond materials.
3. Energy requirements for mixing.
4. Materials from which pond is constructed.

Experience, has shown that velocities generally greater that 10 $cm.sec^{-1}$ are necessary to avoid settling of cells. However, in order to cope with unavoidable variations in velocity, especially near the pond ends, a minimum design velocity of 20 $cm.sec^{-1}$ appears to be necessary (Dodd, 1986). In order to maximise productivity by ensuring the maximum exposure to light of each algal cell, velocities of up to 50 $cm.sec^{-1}$ have been suggested (e.g. Richmond & Vonshak, 1978), however such velocities are generally uneconomical because of the power requirement for mixing (Oswald, 1988; Borowitzka, 2005).

So far we have only considered open-air systems for large-scale algal culture. Such systems are, however, only suited to algal species which grow in a relatively extreme environment (i.e. *Dunaliell salina*, high salinity; *Spirulina*, high pH) where competition by other species and problems with predators and pathogens are reduced. Open systems may also work with fast-growing algae under optimum conditions (i.e. *Chlorella, Scenedesmus, Phaeodactylum*) which can outgrow most of their competitors.

In order to overcome these problems a large number of closed bioreactor systems have been and continue to be developed, mainly based on earlier designs (e.g. Jüttner, 1977; Pirt et al., 1983; Pulz et al., 1995; Hu et al., 1996; Tredici & Zitelli, 1997), and some of these are at a pilot stage of development. The most successful of these to date are the tubular photobioreactors (Borowitzka, 1996) (Figure 18 and Figure 21). Basically, these systems consist of a tubular solar receptor, a carbonation tower or similar system for CO_2 supply and a pump to circulate the culture between these two parts. For some algae and at some sites, these reactors appear to be superior (at least in biomass production for some species) to open systems (for example Torzillo et al., 1986).

Pilot 'Biocoil' facilities at Luton in the UK and in Perth, Australia have solar receptors arranged as a coil of approximately 30 mm diameter low density polyethylene tubing arranged around an open circular framework (Figure 15 and Figure 21). Temperature control is achieved by a heat exchange unit installed between the reactor and the pump or by evaporative cooling. The latter cooling system is used in the 1000L pilot-scale Biocoils at Murdoch University. A 1000 L unit would have an approximate photosynthetic surface area of 100 m^2.

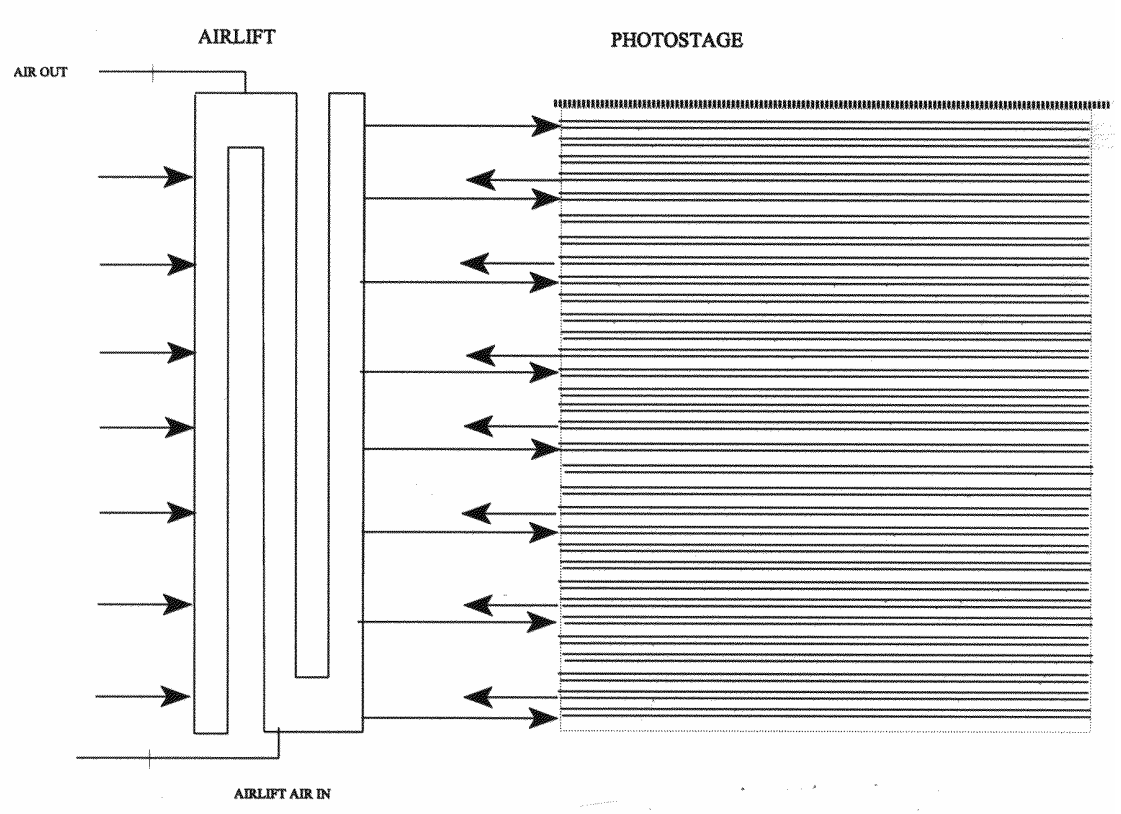


Figure 15. Schematic diagram of pilot-scale BIOCOIL at Murdoch University. The arrows indicate flow into or out of the photostage (for clarity connections are not shown). On the top of the photostage tower there is a perforated pipe (dark dotted line) which flows water over the photostage for cooling.

Although these systems are uni-algal, they are, at this stage at least, not axenic. For some algal species and/or products some means of sterilising the system and maintaining sterility may be required and this has not yet been developed. In fact it is probably impossible to maintain sterility for longer that about 2 weeks because of the large amount of air which has to be pumped through the units to provide CO_2 and remove O_2. The choice of pump used for circulating the culture is also critical to avoid damage to the algal cells.

Another potential problem which has appeared during scale-up is the build–up of O_2 in the system as a result of photosynthesis by the algae in the long tubes. Since high levels of O_2 inhibit algal photosynthesis this may limit the productivity of the system. CO_2 addition is also more of a problem at the larger scale, as it is for all bioreactors. CO_2 is quite expensive and more efficient carbonation towers are required to reduce operating costs.

Irrespective of the problems, the tubular photobioreactors represent an exciting new way to culture algae on a large scale and the pilot reactor of Cadarache has already achieved a steady state of operation using *Porphyridium cruentum* for periods in excess of 50 days, with a maximum production of 20 $g.m^{-2}.\ day^{-1}$ (Chaumont et al., 1988). Similarly we

have operated the Biocoils at Murdoch University growing *Tetraselmis* spp. for over 150 days with average productivity of 30 g.m $^{-2}$.day^{-1}.

A related development to the tubular photobioreactors are immobilised cell bioreactors. Such systems are being tested and developed for various processes such as ammonia production using blue-green algae (cyanobacteria) and polysaccharide production (Gudin & Thepenier, 1986; Robinson et al., 1986; Wilkinson et al., 1990). At this stage these systems must be considered experimental only and much work needs to be done before such systems become a commercial reality.

Figure 16. *Dunaliella salina* production plant at Hutt Lagoon, WA now operated by Cognis. Total pond area > 700 ha. This is the largest algae production plant in the world.

Figure 17 *Haematococcus* plant using tubular photobioreactors operated by Algatech Ltd, Kibbuz Ketura, Israel

Figure 18. Cyanotech production plant at Kona, Hawaii. Growing both *Haematococcus* and *Spirulina*

Figure 19. central pivot ponds used for the cultivation of *Chlorella* in Indonesia. Identical technology is used in Taiwan for *Chlorella* production.

Figure 20. *Spirulina* raceway ponds, used by in India by Parry Nutraceuticals to grow *Spirulina* or *Dunaliella*.

Figure 21. 1000L Pilot-scale tubular photobioreactor (BIOCOIL) at Murdoch University

Commercial Uses of Algae

Algae are being used to produce a wide range of compounds and in a wide range of applications. With continued research, many new applications for algae and new algal products are being discovered and developed, and new species with commercial potential are being isolated. The onset of the application of genetic technology to microalgae also has great potential.

Chemicals

Algae are being used to produce (cf. Figure 16):

polysaccharides (mainly from the brown and red seaweeds used to produce agar, alginate, agarose, carragheenan etc);
carotenoids (especially ß-carotene and astaxanthin);
biliproteins (phycocyanin and phycoerythrin);
lipids and fatty acids;
sterols;
vitamins;
amino acids (especially proline);
sugars (especially glycerol);
antibiotics and pharmaceuticals;
NH_4 (from N_2-fixing blue-green algae).

Processes

Waste water treatment in high-rate oxidation ponds;
Mineral extraction (i.e. Cu and Au);
Soil conditioners;
Animal and human food.

Algae of commercial importance

Every alga, and there are some 20,000+ species (!), may have commercial potential, however, we know very little about most of them. The list below gives some details of those microalgae in which there is some present interest or which are actually being used commercially.

CHLOROPHYTA

Dunaliella salina

This is the star of algal biotechnology. It is being grown commercially in Australia, Israel and India (and possibly in the future in China) as a source of natural ß-carotene. *Dunaliella salina* (also called *D. bardawil* in some papers) is the most halotolerant eukaryotic organism known and grows at salinities up to about 33 % (w/v) NaCl (= approx 5.5 M NaCl) i.e. up to NaCl saturation. It is this feature, as well as the very

high cell content of ß-carotene of up to 14% of dry weight which have made this alga so attractive. (Borowitzka & Borowitzka, 1988; Ben-Amotz, 1999; Borowitzka & Hallegraeff, 2007)

Chlorella spp.

Freshwater *Chlorella* species have been grown for over 30 years for the health food market in Japan and Taiwan and is also being used as carotenoid-rich (lutein) animal feed. It is also an important alga in some aquaculture feeds and for wastewater treatment. The mucilaginous species *Chlorella sajao* and *C. mexicana* are being grown in the USA for use as soil conditioners in agriculture. Marine *Chlorella* species are also of great interest as feed for larval fish, crustaceans and molluscs in aquaculture. (Oh-hama & Miyachi, 1988; Lee, 1997)

Scenedesmus spp.

This green alga had great popularity in the 1960's and 1970's as a possible form of single cell protein for human and animal consumption. Commercial realities have however meant that none of these projects have reached fruition where it is grown for the human and animal health market. See Soeder and Hegewald (1988) for details on this alga.

Nannochloris spp. (esp. *Nannochloris oleoabundans*)

This minute green alga is a contender as a future source of renewable liquid fuels and diesel oil substitutes. Research and development is under way in the USA and in France. Some species may also be excellent sources of fatty acids.

Botryococcus braunii

This is the original "oil alga". This green alga was first recognised in Australia as being the source of some fossil hydrocarbons. Under certain conditions it produces large quantities of long-chain hydrocarbons suitable for refining into transport fuels. Research and development is under way in Europe, the USA and elsewhere. Several faster growing strains have recently been isolated, however growth is still to slow for commercial use..

Tetraselmis spp.

This is also a very important genus in aquaculture .

Haematococcus pluvialis

This green alga has many animal-like features and is a natural source of the carotenoid astaxanthin. It is being grown commercially in Hawaii and Israel (Olaizola, 2000). The initial production of *Haematococcus* in Hawaii at Cyanotech takes place in closed culture systems, some as large as 40,000 L. This is followed by a short, 5 to 7 day, "reddening" cycle in 500,000 L open raceway ponds. After the reddening cycle, *Haematococcus* cultures are harvested, washed and dried and the *Haematococcus* biomass is extracted using supercritical carbon dioxide to produce a purified oleoresin.

<u>Other genera of interest:</u>

Chlamydomonas
Oocystis
Euglena
Asteromonas

RHODOPHYTA

Porphyridium cruentum

This unicellular red alga has attracted great interest over the years, firstly as a source of sulphated polysaccharides for use in industry, and lately as a source of the essential fatty acid arachidonic acid and of phycoerythrin. Research and development projects are under way in various parts of the world. See (Vonshak, 1988; Cohen, 1999) for details on this alga.

Other genus of interest:

Rhodella

CHRYSOPHYTA

Isochrysis galbana

important food source in aquaculture.

Other genera of interest:

Nitzschia
Cylindrotheca
Amphora
Chaetoceros

CYANOPHYTA

Spirulina spp.

Several *Spirulina* species (i.e. *S. platensis*, *S. mexicana*, *S. geitleri* etc.) are being farmed in the USA, China, India, Thailand, Japan, Taiwan and elsewhere for the health food market. The alga is also used as a source of the phycobilin pigment phycocyanin, and has been proposed as a potential source of several essential fatty acids. It has a long history as a human food dating back to the Aztecs in Mexico. It is easily cultured due to the high pH (pH > 9.0) required and is also easy to harvest since it is filamentous. See (Belay, 1997) for details on this alga.

Other genera of interest:

Anabaena
Aphanothece
Nostoc
Oscillatoria

Commercial Micro-algal Producers

This is a partial listing of commercial producers and their products

Australia:

Cognis Nutrition & Health	ß-carotene & *Dunaliella*
Aquacarotene Ltd.	ß-carotene

U.S.A.:

Cyanotech Inc.	ß-carotene
Spirulina	
phycobilins	
Haematococcus	astaxanthin
Earthrise Farms	*Spirulina*
Green Gold Corp.	*Spirulina*
R+A Plant/Soil Inc.	soil conditioners & fertilisers
Bio/Technical Res.	R+D (several products)
Martek Biosciences	heterotrophic culture of dinoflagellates to produce eicosapentaenoic acid.

Europe:

French Petroleum Inst.	R+D (*Spirulina*, *Porphyridium* etc.)
Montedison Co.	*Spirulina* + others

Israel:

NatureBeta	*Dunaliella*
Algatech	*haematococcus (in closed photobioreactors)*

Japan & Taiwan & India:

Dai Nippon Ink.	*Spirulina*
phycocyanin	
Taiwan Chlorella Corp. + others	*Chlorella*
Parry Nutraceuticals	*Spirulina, Dunaliella, Haematococcus*

Many other algal plants are in operation in Thailand, Indonesia and China.

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This lengthy bibliography is meant to serve as a reference guide both for this unit and in the future.

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