

Review

Extremophilic micro-algae and their potential contribution in biotechnology



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HIGHLIGHTS

- Extremophilic micro-algae have a potential role in the biotechnology industry.
- We consider extremes of temperature, light, CO₂, pH, salt and metal in this review.
- We present phylogenetic analysis of extremophilic microalgae.
- We discuss organisms' adaptive mechanisms to tackle these stresses.
- Physiology, metabolic engineering and molecular biology need further studies.

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ABSTRACT

Micro-algae have potential as sustainable sources of energy and products and alternative mode of agriculture. However, their mass cultivation is challenging due to low survival under harsh outdoor conditions and competition from other, undesired, species. Extremophilic micro-algae have a role to play by virtue of their ability to grow under acidic or alkaline pH, high temperature, light, CO₂ level and metal concentration. In this review, we provide several examples of potential biotechnological applications of extremophilic micro-algae and the ranges of tolerated extremes. We also discuss the adaptive mechanisms of tolerance to these extremes. Analysis of phylogenetic relationship of the reported extremophiles suggests certain groups of the Kingdom Protista to be more tolerant to extremophilic conditions than other taxa. While extremophilic microalgae are beginning to be explored, much needs to be done in terms of the physiology, molecular biology, metabolic engineering and outdoor cultivation trials before their true potential is realized.

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1. Introduction

Atmospheric carbon dioxide levels have been rising rapidly for the past 200 or so years (Falkowski et al., 2000). This post industrialization effect has been attributed primarily to anthropogenic CO₂ emissions caused by combustion of fossil fuels to meet our energy demands. These include direct energy consumption (such as electricity and transport) as well as indirect consumption for production and processing of various materials (such as steel, cement and plastic) used by mankind. Clearly, alternate sources of energy and products are needed that are carbon neutral and sustainable. Furthermore, with predictions that the world population will have

increased by another 2 billion by 2050, a major challenge for the planet is to provide enough food for its population. Current estimates indicate that sufficient water and arable land is not available to support such a demand (Fedoroff et al., 2010). Photosynthetic organisms such as cyanobacteria and eukaryotic algae have the potential to meet a significant fraction of the requirements of energy, products, food and animal feed. For ease of reference, while recognizing their phylogenetic diversity, we loosely refer to these organisms collectively as micro-algae in this review. Micro-algae grow much faster and show greater photosynthetic efficiency compared with land plants. Average areal biomass productivities of up to 20 kg/m²/year have been reported for micro-algal mass cultures, with a potential for further improvement with strain selection, strain improvement and process engineering (Williams and Laurens, 2010). Moreover, micro-algae have the potential to

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produce materials that are of commercial interest, such as astaxanthin (Fan et al., 1998) and long chain omega-3 polyunsaturated fatty acids (Khozin-Goldberg et al., 2011). Of equal importance is the possibility that micro-algal biomass offers an additional mode of agriculture that will provide food and animal feed produced on marginal land and using marginal water resources so as not to compete with resources utilized in conventional agriculture.

A key challenge in mass culturing of micro-algae is to find strains that not only produce marketable products or biomass for energy and alternative agriculture, but also grow well under industrially relevant outdoor conditions. These may include the need for growth under (i) high (or low) temperatures due to local climatic conditions or bubbling of hot flue gases, (ii) wide temperature variations over a diurnal cycle typically seen in desert conditions, (iii) high light and UV radiation when using solar radiation to drive photoautotrophic growth, (iv) high CO₂ while bubbling flue gases or limiting CO₂ while growing with ambient CO₂ if no source of CO₂ supplementation is available, (iv) local water conditions such as high salt content (e.g., seawater), alkaline or acidic pH and metal and organic carbon content originating either from local water bodies or from industrial wastewater that needs to be used for algal growth. Some of these can be considered extreme conditions, as most organisms will not survive in such environments. In view of this, extremophilic micro-algae have the potential to play an important role in the eventual commercial exploitation of micro-algae based biofuels, bioproducts and agriculture.

1.1. Definition of extremophiles

Most organisms have evolved under relatively benign climates and are not normally able to survive in extremes of environment such as temperature, pH or in the presence of xenobiotics. However, there are areas on Earth where environmental conditions are beyond the normal limits for growth and can thus be considered as extreme. Thus organisms that can cope with extremes of pH, temperature, pressure, and salinity have all been considered extremophiles. Sometimes these organisms possess additional qualities such as the ability to cope with very high levels of gases such as CO₂, or grow in the presence of high concentrations of metals and some can thrive in combinations of more than one stress (polyextremophiles). Some organisms even possess a remarkable ability to grow under very high ionizing radiation levels and to accumulate radionuclides (Rivasseau et al., 2013). It is our contention that some of these properties may be of use in biotechnological applications. While prokaryote extremophiles have been of undoubted value to biotechnology to date, we here concentrate on the potential application of phototrophic micro-algae in biotechnology.

2. Role of extremophilic micro-algae in biotechnology

Despite their commercial potential having been recognized for over 50 years, the number of micro-algal species that are currently produced on a large scale in a sustainable economic process is limited (Richmond, 2004). A major constraint in creating a new flourishing, micro-algae based, agro biotechnology lies in achieving large-scale under outdoor conditions (Torzillo et al., 2003). High light, temperature, seasonal and diurnal fluctuations in light and temperature and contamination by other organisms affect growth and productivity in outdoor algal ponds (Vonshak and Richmond, 1988). Of the few micro-algal strains that have reached a stage of being a commercially traded product, two are extremophiles. The first example is of *Dunaliella*, a green unicellular micro-algae isolated from high salinity water bodies with NaCl concentrations exceeding 3 M (Borowitzka and Huisman, 1993). The other one is

Spirulina, a filamentous cyanobacterium that blooms in alkaline lakes with high pH in the range of 9–11 (Silli et al., 2012). *Dunaliella* is used as a natural source of β-carotene while *Spirulina* has a market as a food and feed additive in human and animal nutrition. A key factor in the commercial success of these two species is their ability to grow under specific extreme conditions that help in reducing the contamination by other algal species (Avron and Ben-Amotz, 1992). Under mass culturing conditions, these species are reported to grow at 10–60 g m⁻² day⁻¹. In view of this, extremophilic micro-algae may offer the following advantages in biotechnological applications.

2.1. Ability to grow under local climatic conditions and exclude potential contaminants

This involves the selection of micro-algae for its ability to grow under extreme conditions. This will not only optimize biomass production but also minimize contamination by other algal species. These extreme conditions may include high daytime temperatures, bubbling with flue gases or using water of specific quality (e.g., high salinity). The extreme conditions will need to be in sync with the local climatic conditions and water quality to minimize costs involved in maintaining such conditions. For example maintaining high salinity will require a high cost in medium preparation unless seawater is used. Another challenge will be the need to maintain the level of salinity within acceptable bounds and avoid increases due to evaporation (or dilution by rainwater).

2.2. High value products from extremophilic micro-algae

Extremophiles have developed special mechanisms that allow the cell to grow and thrive under extreme conditions. The most common example is accumulation of glycerol as an osmo-regulant in *Dunaliella* or the accumulation of β-carotene as a protective agent against excess light. Such a phenomenon is the basis for the development of the mass culturing of *Haematococcus pluvialis* for the extraction of astaxanthin (Fan et al., 1998). Similar cases can be found in micro algae that grow in snow and as a result have had to develop a unique membrane structure to maintain their fluidity or protect the cell from freezing damage, or in the case of algal species that thrive in hot springs and represent a potential source of enzymes that are resistant to high temperatures. This approach requires the development of an intensive collecting and screening protocol that will identify the potential strains and then go into the process of developing the biotechnology for mass culturing of the selected strain. Representative examples of potential biotechnological applications of micro-algae from extreme temperatures are presented in Table 1.

2.3. Sources of genes that yield products of interest

The third approach, and one that is gaining more interest in recent years, is to view the extremophiles more as a source of genes that can be isolated and cloned into other organisms that are more easily mass cultured and in some cases even have the capacity to be grown heterotrophically.

The real challenge is to identify a product that will represent a unique advantage to be produced from the microbial biomass and represent a true economic advantage over the traditional sources of conventional agriculture, chemical synthesis or standard fermentation technology. This paper gives an overview of extremophilic micro-algae (cyanobacteria and eukaryotic microalgae) and asks the question as to whether adaptations to extreme environments in these organisms confers a particular ability to cope with the constraints of growing in mass cultures or induces production of unique compounds of potential use in biotechnology.

Table 1

Potential biotechnological applications of some psychrophilic and thermophilic algae and cyanobacteria. Representative applications are listed based on specific reports.

	Group	Product/application	References ¹
<i>Psychrophilic algae (snow algae)</i>			
<i>Chlamydomonas nivalis</i>	Green algae	Astaxanthin	(1)
<i>Raphidonema</i> sp.	Green algae	α -Tocopherol (vitamin E) and xanthophyll cycle pigments	(2)
<i>Mesotaenium berggrenii</i>	Green algae	Sucrose, Glucose, Glycerol	(3)
<i>Chloromonas</i> sp. (CCCr0 020–99)	Green algae	Glycerol	(4)
<i>Nostoc commune</i>	Cyanobacteria	Myxoxanthophylls and canthaxanthin	(5)
<i>Thermophilic cyanobacteria and algae</i>			
<i>Phormidium</i> sp.	Cyanobacteria	Thermostable restriction enzyme	(6)
<i>Thermosynechococcus elongatus</i> BP-1	Cyanobacteria	Thermostable phosphate kinase	(7)
<i>Desmodesmus</i> sp. F51	Green algae	Lutein (xanthophyll) [feed additive & natural colorant for pharmaceuticals]	(8)
<i>Galdieria sulphuraria</i> 074G	Red algae	Blue pigment phycocyanin (PC) used as a fluorescent marker in histochemistry	(9)
<i>Galdieria sulphuraria</i> CCME 5587.1	Red algae	Wastewater treatment	(10)
<i>Chlorella sorokiniana</i> UTEX 2805	Green algae	Wastewater treatment (Ammonium removal)	(11)
<i>Desmodesmus</i> sp. F2 and F18	Green algae	High lipid content (up to 58% of dry biomass)	(12)

¹ The references cited here have been listed in [Supplementary information](#).

3. Constraints in algal biotechnology – how can extremophiles fill the gap?

For biotechnological purposes, micro-algae are generally grown either in closed photobioreactors or in open raceways. Although phototrophic cultures are frequently light limited at depth, at the surface of cultures they can be exposed to the full force of the sun. In open raceways where there is no screening, this can also involve exposure to UV radiation. In closed systems, oxygen build-up in cultures can, in combination with other stressors lead to significant formation of damaging reactive oxygen species (ROS). Freshwater is often in scarce supply, especially in areas where solar radiation is high enough to minimize light limitation of algal mass cultures. Access to organisms that have a high tolerance to brines or hypersaline environments can get around constraints on freshwater supply. In dense cultures, inorganic carbon can be rapidly depleted, thus limiting photosynthesis and growth. This is overcome in most algae and cyanobacteria by operation of a CO₂ concentrating mechanism. In practice, CO₂ levels in raceways and PBRs can be maintained by sparging with CO₂ – this being especially important in the use of photoautotrophs to remediate CO₂ emissions. Given that CO₂ in waste gases from power-plants and other industrial applications can be very high (>12% for power-plant flue effluent) an ability to tolerate high CO₂ and the low pH values that result can be important.

4. Phylogeny of extremophilic micro-algae

Micro-algae belong to a number of evolutionarily distinct major groups within the Kingdom Protista (see e.g. Yoon et al., 2004). Cyanobacteria are an ancestral clade of prokaryotes originating 2700–3500 million years ago (MYA) (Falkowski et al., 2004) and are also the progenitors of plastids in photosynthetic eukaryotes. For the purpose of this review, it is apparent that in oxygenic phototrophs, extremophiles can be found among cyanobacteria, red algae (especially in the Cyanidiales) and green algae (chlorophytes) (Fig. 1). More recent, but ecologically important, taxa such as the diatoms and prymnesiophytes (members of the Heterokont algae) and the dinoflagellates do not have any reported extremophilic representatives with the exceptions of the diatom *Pinnularia* sp., found in some low pH freshwaters (Aguilera et al., 2006) and a reasonably extensive flora of psychrophilic species such as the polar/sea-ice diatoms *Entomoneis* and *Fragilariopsis* (Seckbach, 2007). Among the photosynthetic eukaryotes, the red algal Cyanidiales are an ancient lineage with the majority of high temperature and acid-tolerant eukaryotic alga belonging to this branch. The chlorophytes are not reported to tolerate temperatures above

55 °C while red algae, especially the Cyanidiales, tolerate up to 60 °C, though, as we will see, green algae exhibit tolerances to other extremes. Some cyanobacteria are reported to tolerate up to 74 °C. Higher plants are believed to have evolved ~ 470 MYA and are not known to tolerate temperatures above 50 °C, though some species have other extremophile characteristics such as desiccation tolerance or capacity for metal hyperaccumulation, that are outside the scope of this review.

5. Low and high temperature tolerant algae

Temperature is an important growth-determining factor for organisms. Extreme temperatures such as the extreme cold of the frozen deserts of Antarctica and temperatures above boiling in the hot springs of Yellowstone National Park present challenging growth environments to biota. Consequently, species diversity is quite low in these harsh environments. Yet there are organisms which thrive and complete their life cycle at such extreme temperatures.

Depending upon the optimal growth temperature, species can be broadly classified as (1) Psychrophiles growing optimally below a temperature of 15 °C, (2) Thermophiles growing at temperatures >50 °C and (3) Mesophiles growing best at intermediate temperature. A fourth class, Hyperthermophiles, have optimum temperatures of >80 °C. As observed above, most of the psychrophilic and hyperthermophilic organisms belong to archaeal or bacterial domains (D'Amico et al., 2006), Fig. 2 places some representative micro-algae on a temperature scale based on their optimal temperatures for growth. Although there are photosynthetic prokaryotes such as a few cyanobacteria and some purple and green sulfur bacteria which grow at temperatures up to 74–75 °C, the upper temperature limit for eukaryotic algae is 62 °C (Rothschild and Mancinelli, 2001) (Fig. 2). No photosynthetic organisms have been reported that grow beyond 75 °C, possibly due to the instability of chlorophylls beyond this threshold. At low extremes, some snow and ice algae grow even at 1 °C (Fujii et al., 2010).

5.1. Psychrophiles

Extremely cold regions of the Arctic and Antarctic, and moderately cold mountainous regions are dominated by psychrophiles and psychrotrophs. Psychrophiles grow in permanently cold environments whereas psychrotrophs are not completely adapted to cold and sometimes have upper temperature limits of >20 °C. The dominant phototrophic cyanobacteria in the Antarctic region include the genera *Oscillatoria*, *Phormidium*, and *Nostoc* (D'Amico et al., 2006) (Fig. 2), though there are a great many cold tolerant

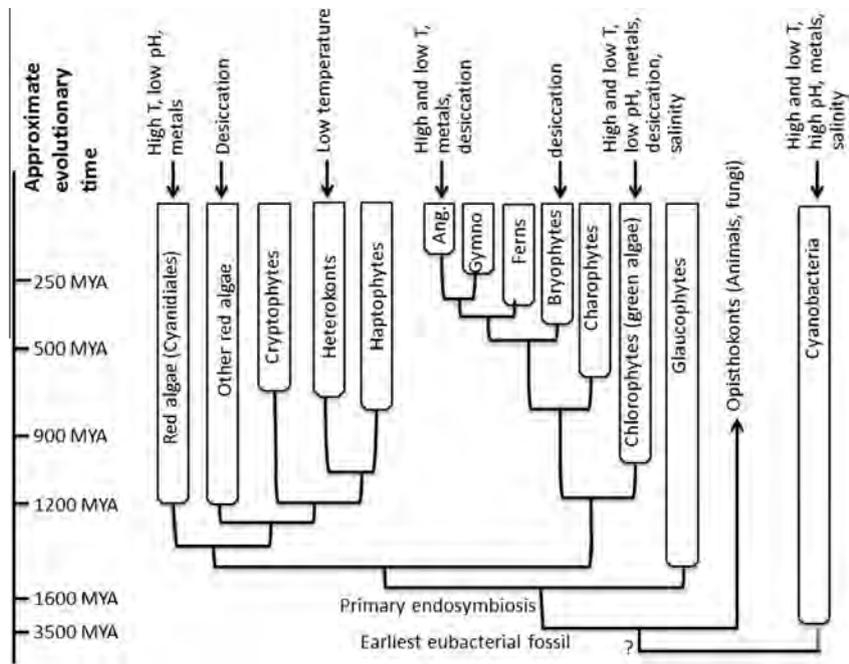


Fig. 1. Phylogeny of photosynthetic extremophiles. Extremophiles are found predominantly in the red algal (Cyanidiales) and chlorophyte lines of eukaryotes and the ancestral cyanobacterial line. A few angiosperms (Ang.) have extreme desiccation tolerance, as do some bryophytes. (Gymno = gymnosperms; Heterokonts include diatoms, chrysophytes and phaeophytes; haptophytes include the ecologically important coccolithophores). Not all algal lines are shown (dinoflagellates in the Alveolates are missing, but have no extremophile representatives) and timing of events is approximate. Redrawn after (Yoon et al., 2004).

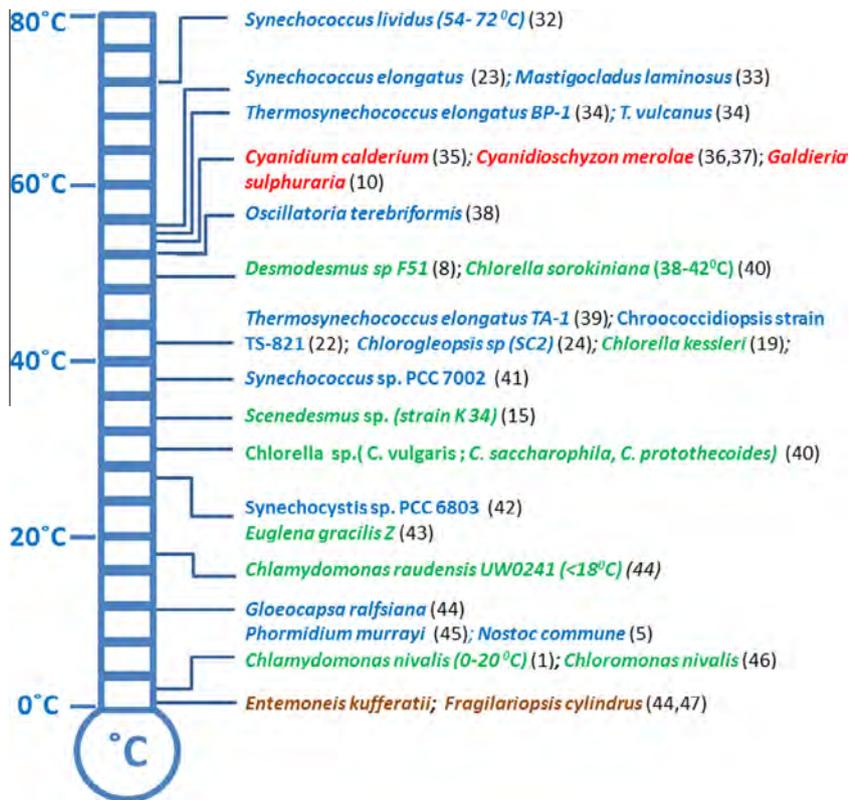


Fig. 2. Temperature limits for microalgal life. Optimal reported growth temperature for representative microalgae are shown. Red algae are indicated in red, green algae in green, blue-green algae (cyanobacteria) in blue, and diatoms in brown. The numbers in parenthesis indicate the reference number as listed in the [Supplementary information](#). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

diatoms and other psychrophilic eukaryotic algae that are the major primary producers in polar marine environments. Snow algae, which grow in cold regions, create huge blooms of

macroscopically visible pigmentation on the snow with different colors such as red, orange, pink and green. The green color is a result of actively dividing sexual and asexual stages of cell,

whereas the red color is due to carotenoids, produced especially in resting stages. Common eukaryotic snow algae genera are *Chlamydomonas*, *Chloromonas*, *Microglena*, *Chlorella* and *Scenedesmus*.

Psychrophiles have the ability to overcome adverse effects of low temperatures that cause an exponential drop in biochemical reaction rates. Thus, enzymes of these microorganisms are adapted to cold temperatures and have high catalytic efficiency at low temperature relative to their mesophilic and thermophilic counterparts. They are also capable of tolerating the increased water viscosity, which roughly doubles in going from 37 °C to 0 °C. Psychrophiles can potentially be used for the production of detergents, fine chemicals, in food industry and for the treatment of hydrocarbon contaminated soil in Antarctica (Hoover and Pikuta, 2010). Psychrophiles maintain their membrane fluidity at low temperature by incorporating a higher level of polyunsaturated fatty acids (PUFAs) in membrane lipids, thereby making them a potentially useful source of PUFAs for nutraceutical products such as eicosapentaenoic acid, arachidonic acid and docosahexaenoic acid.

5.2. Thermophiles

Though thermophilic life has been known for many years, reported studies were very limited until the thermophilic bacterium *Thermus aquaticus* was discovered in 1969 in the Mushroom Spring of Yellowstone National Park (Brock and Freeze, 1969). Since then, hundreds of thermophilic species have been identified in all the three domains of life, namely bacteria, archaea and eukarya. Thermophiles have thermostable enzymes with temperature optima for activity as high as 90 °C. Organisms that have adapted to extremes of temperature have been the subject of some interest for biotechnology (and it must be pointed out that thermo- and psychrophiles are often subject to additional stresses such as very high irradiance, and thus qualify as polyextremophiles). For instance, Leya et al., 2009 have indicated the psychrophile *Raphidoneima* may be a good source of α -tocopherol and various carotenoids, while the snow algae *Chloromonas nivalis* and *Chlamydomonas nivalis* synthesize high levels of astaxanthin (Remias et al., 2010, 2005). Further examples are given in Table 1. Biotechnological interest in thermophilic microalgae is more centered on their use as sources of thermostable enzymes. While thermostable restriction enzymes are usually sourced from the bacterium *T. aquaticus*, the cyanobacterium *Phormidium* has also been shown to produce a similar enzyme. Thermophilic alga *Galdieria sulphuraria* (a red alga) and *Desmodesmus* (a green alga) have both been investigated for useful pigment production. *G. sulphuraria* has also been tested for its ability to remove nutrients from primary wastewater effluent. (Table 1).

6. Ability to grow under low and high CO₂ levels

In dense mass cultures, intense photosynthetic activity decreases the dissolved inorganic carbon (DIC) concentration significantly. In poorly buffered systems, CO₂ uptake by the culture causes the pH to rise, and values of pH 9–10 are not uncommon. This rise in pH in turn leads to a decrease in the CO₂ to bicarbonate ratio as well as the decrease in absolute CO₂ concentration. Thus, DIC levels in cultures, even if quite well aerated, are frequently below atmospheric-equilibrium. For instance, Williams and Colman, 1996 showed that the DIC concentration in cultures of the acidophile *Chlorella saccharophila* dropped from 450 μ M (air equilibrium) to below 30 μ M over 3 days under low aeration rates. This decrease in DIC below air-equilibrium can impose limitations on diffusive supply of CO₂.

While it is possible in some instances to rectify DIC depletion by using sources of high CO₂, such as flue gases, mass algal cultures in

remote areas without a CO₂ source nearby will be severely carbon limited. In these circumstances, efficient use of the available inorganic carbon by the algal/cyanobacterial cells would be advantageous.

6.1. RuBisCO and CO₂ concentrating mechanisms (CCMs) under extreme conditions

All cyanobacteria and eukaryotic microalgae (and indeed almost all autotrophs) rely on the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) and the Photosynthetic Carbon Reduction Cycle (PCRC; the Calvin–Benson–Bassham Cycle) for net assimilation of inorganic carbon to organic matter. In addition to the fixation of CO₂ to the acceptor molecule ribulose-1,5-bisphosphate (RuBP), giving rise to 2 molecules of 3-phosphoglycerate, RuBisCO also catalyses an oxygenase reaction, the products here being one molecule each of phosphoglycerate and phosphoglycolate. One of the 2 carbons in phosphoglycolate can be recovered and converted to phosphoglycerate by the reactions of the photosynthetic carbon oxidation cycle (PCOC) in the process of photorespiration, but one carbon is lost as CO₂ and represents a potentially significant inefficiency in the carbon assimilatory process (Giordano et al., 2005). The carboxylase and oxygenase activities of RuBisCO are competitive and dependent on the ratio of oxygen and CO₂ at the enzyme active site, according to Eq. (1)

$$S_{rel} = \frac{\frac{k_{cat}(\text{CO}_2)}{K_{1/2}(\text{CO}_2)}}{\frac{k_{cat}(\text{O}_2)}{K_{1/2}(\text{O}_2)}} \quad (1)$$

where the selectivity factor S_{rel} defines the ratio of rates of carboxylase to oxygenase reactions and the k_{cat} and $K_{1/2}$ values correspond to the maximum specific reaction rates and half-saturation concentrations for the respective substrates. Different forms of RuBisCO that have evolved in autotrophic organisms. Most microalgae and cyanobacteria have forms of RuBisCO with 8 large and 8 small sub-units (L₈S₈) and are known as Form I (marine *Synechococcus* and all *Prochlorococcus* species possess Form IA RuBisCO while most freshwater cyanobacteria and some eukaryotic algae have Form IB and red algae, cryptophytes, haptophytes and diatoms all have Form ID). Form II RuBisCO comprises only 2 large subunits (L₂) and is found in dinoflagellates and *Chromera veria*. Form III is found only in archaea and will not be discussed further. The evolutionary origins of the different forms of RuBisCO are discussed in detail by (Raven et al., 2012).

These forms of RuBisCO possess different kinetic properties in relation to affinity for CO₂ ($K_{1/2}(\text{CO}_2)$) and specificity for CO₂ vs O₂ (S_{rel} , Eq. (1)) (Raven et al., 2012, 2011; Whitney et al., 2011). Values for these parameters are highly variable (Giordano et al., 2005); Miller et al. (2007) report the highest recorded value for $K_{0.5}(\text{CO}_2)$ for a Form I RuBisCO of 750 μ M for the marine cyanobacterium *Prochlorococcus marinus* whereas green algae have RuBisCOs with $K_{1/2}(\text{CO}_2) \sim 30$ μ M. Form II RuBisCOs have very low S_{rel} values and dinoflagellates might struggle to perform net C assimilation under air equilibrium CO₂ levels (Giordano et al., 2005). The general trend across all autotrophs is that a low $K_{1/2}(\text{CO}_2)$ and high S_{rel} are correlated with a low $k_{cat}(\text{CO}_2)$, and vice versa (Raven et al., 2012).

RuBisCO evolved at a geological time in which CO₂ levels were very much higher than at the present day. The subsequent long-term drop in CO₂ levels and rise in oxygen has led to a situation where competition between O₂ and CO₂ has become restrictive to net carbon fixation. Thus, in general (and there are exceptions – see below), at present-day dissolved CO₂ levels of ~ 15 μ mol L⁻¹ (the exact concentration depending on salinity and temperature), organisms will have RuBisCOs operating well below maximum capacity if the internal CO₂ is in equilibrium with (or lower than)

the external CO₂. However, cyanobacteria and algae have mechanisms to enhance CO₂ levels at the active site of RuBisCO (CCMs; see recent reviews by (Giordano et al., 2005; Raven et al., 2012). There is evidence that there is an inverse relationship between kinetic characteristics of RuBisCOs and the extent to which algae and cyanobacteria express CCMs, such that species with high S_{rel} RuBisCOs have lower levels of CCM expression than do species with low S_{rel} RuBisCO (Tortell, 2000). Thus there seem to be two approaches to the “low CO₂” problem, one involving evolution of active CCMs and the other involving the evolution of RuBisCOs with higher affinity for CO₂. CCM activity is strongly regulated by environmental factors (Raven et al., 2011). Foremost among these is the CO₂ level in solution (though cyanobacterial CCMs seem to be controlled directly by HCO₃⁻ concentrations instead (Mayo et al., 1986).

Thus high salinities, in which dissolved CO₂ at air equilibrium is lower, resulted in increased CCM activity in the halophile *Dunaliella salina* (Booth and Beardall, 1991). Low temperatures result in increased solubility of CO₂ and higher inorganic C concentration, and also influence the ratio of carboxylase to oxygenase activities in favor of carboxylation (Descolas-Gros and de Billy, 1987), but studies to date suggest that polar species adapted to very cold environments do not show diminished CCM activity compared to their temperate counterparts (Beardall and Roberts, 1999). On the other hand, some thermophilic (and acidophilic) extremophile algae have evolved RuBisCOs with very high affinity (low $K_{0.5}$) for CO₂; Uemura (1997) report $K_{0.5}$ CO₂ values of 6.6 and 6.7 μM for the red algae *Galdieria partita* and *Cyanidium caldarium*, respectively, with specificity factors over 220, compared to a value for 143 for RuBisCO from the temperate red alga *Porphyridium purpurum* ($K_{0.5}$ CO₂ = 22 μM). Other, non-thermophilic, acidophiles such as *C. saccharophila* and *Chlamydomonas acidophila* have retained CCMs (Spijkerman, 2005; Beardall 1981); and so has the acidophilic red alga *Cyanidioschyzon merolae* (Zenvirth et al., 1985), although there are no data on the kinetics of RuBisCO from these species. It is apparent from the above discussion that extremophiles may have representatives with especially good affinities for inorganic carbon which offer a high capacity for overcoming potential limitations of growth at high cell density in mass cultures.

6.2. Tolerance to high CO₂ levels

In order to (a) prevent CO₂ limitation and (b) to carry out remediation of CO₂ emissions from industry, there is considerable interest in using industrial flue gases to supplement CO₂ supplies to microalgal cultures. Since flue gases contain CO₂ at high levels (typically 10–12% compared to 0.04% in the present day atmosphere), using micro-algae tolerant to such concentrations (and the decrease in pH they engender) is of considerable importance. Flue gases also contain SO₂ which, despite efficient scrubbing technologies, may still reach 2400 ppm in the CO₂ stream (Lee et al., 2009). Species tolerant to high CO₂ and SO₂, together with low pH could thus be highly desirable. Many green algae and some cyanobacteria are tolerant to quite high CO₂ levels (>20%) and in the case of *Chlorella* T-1 and *Scenedesmus* strain K34 (see Table 2) will survive exposure to 100% CO₂ in a gas stream. However, few of these are also tolerant to the high temperatures such gases can potentially attain when they leave the flue – the cyanobacterium *Chroococcidiopsis* and the green alga *Chlorella* T1 are possible exceptions. On the other hand species such as *C. caldarium*, originating as it does from hot springs where high levels of subterranean gases bubble up from underground, have exceptional thermal and CO₂ tolerance. This suggests that species from hot springs and volcanic seeps may be good candidates for growth on flue gases.

Table 2

Representative examples of microalgae that can tolerate high CO₂ levels. Some strains also tolerate high temperatures. Note that the optimum growth temperatures and CO₂ levels may be different than those listed as the tolerated values.

Algal species	Temperature tolerance (°C)	Maximum CO ₂ tolerance (v/v) (%)	References ¹
<i>Red algae</i>			
<i>Cyanidium caldarium</i>	56	100	(13)
<i>Green algae</i>			
<i>Chlorella</i> sp. T-1	35–45	100	(14)
<i>Scenedesmus</i> sp. strain K34	40	100	(15)
<i>Chlorella</i> sp. K35	45	80	(15)
<i>Chlorella</i> ZY-1	40	70	(16)
<i>Chlorella</i> sp. UK001	30 (up to 45)	40	(17)
<i>Chlorella vulgaris</i> UTEX 259	20	30	(18)
<i>Chlorella kessleri</i>	50	18	(19)
<i>Scenedesmus obliquus</i>	50	18	(19)
<i>Nannochloris</i> sp. (NOA-113)	25	15	(20)
<i>Monoraphidium minutum</i> (NREL strain MONOR02)	25	13.6	(21)
<i>Cyanobacteria</i>			
<i>Chroococcidiopsis</i> strain TS 821	50	80	(22)
<i>Synechococcus elongates</i>	60	60	(23)
<i>Chlorogloopsis</i> sp. (SC2)	50	5	(24)

¹ The references cited here have been listed in [Supplementary information](#).

7. Tolerance to high levels of solar radiation

One of the major constraints in algal and cyanobacterial mass culture is the limited penetration of photosynthetically active radiation (PAR) through the cultures. Approximately 90% of the incident photons are absorbed by the uppermost 10% of the culture. The remaining culture volume (90%) is thus using photons very efficiently, but is severely light limited (Ritchie and Larkum, 2012). Changes in light availability over the seasons and with latitude may restrict sustainable, year-long, high yields to lower latitudes (<35°, Williams and Laurens, 2010). At these low latitudes, solar radiation is much higher than at high latitudes and can potentially cause severe photoinhibition in cells in the upper regions of cultures.

Similarly, UVB fluxes are considerably higher in the tropics than at high latitudes, though UVBR fluxes at high latitudes are more seasonally variable (Whitehead et al., 2000). UV radiation has a range of inhibitory effects on the physiological activities of algae and cyanobacteria (Beardall et al., 2009). These include inhibition of damage to DNA and to light transduction and carbon assimilation mechanisms as well as inhibition of nutrient uptake. Algae have a range of physiological strategies to cope with excess PAR and UVB. These include a range of antioxidants, mechanisms for scavenging reactive oxygen species (ROS), non-photochemical quenching for excess light dissipation, and mechanisms for repairing damage from excess PAR and UVB, though these can be energetically costly (Raven and Ralph, 2014). Algae and cyanobacteria also have a range of inducible UV-screening compounds, which can reduce UV-A and UV-B absorbance and minimize damage (Rozeema et al., 2002). Using cyanobacteria as sources for production of UV-sunscreens has been proposed by Browne et al. (2014).

Given that algae and cyanobacteria have evolved mechanisms to cope with high levels of PAR and UV radiation, it is likely that

organisms from environments with very high solar radiation (low latitude desert regions, with little screening from clouds) may have evolved exceptional capacities to cope with such stresses. There are certainly reports of positive effects of UV-B on recovery of algae from photoinhibition and stimulation of photoprotection mechanisms (Flores-Moya et al., 1999). Thus organisms that are habitually exposed to high UVB might be expected to show especially high capacity to withstand this stress. Not only would such algae and cyanobacteria be capable of growing well under extremes of PAR and UVB, they might also be excellent sources of antioxidants and protective compounds. Examples of the latter include β -carotene production by the green alga *Dunaliella tertiolecta* and astaxanthin formation by *Haematococcus*, as well as production of the antioxidant α -tocopherol by *Raphidoneima*, as mentioned above.

Formation of reactive oxygen species (ROS) is a common response to a range of stresses and can be a by-product of both respiration and photosynthesis. In photosynthetic metabolism ROS arise when there are insufficient electron sinks available so electrons from the light harvesting process are passed on to oxygen. Cells possess a range of antioxidative mechanisms, which ensures that ROS molecules are reduced before they can cause serious oxidative damage to cell components. When ROS levels exceed the antioxidative capacity of cells, oxidative damage will result in damage, causing alteration of cell structure and symptoms of oxidative stress.

Organisms commonly exposed to very high light, especially if combined with other stresses, are particularly likely to produce high levels of ROS. However, many extremophilic organisms possess remarkable abilities to withstand these stresses. Of particular note are those cyanobacteria and algae found in biological sand crusts (BSCs). For example the cyanobacterium *Microcoleus* up-regulates light energy quenching mechanisms, allowing it to continue to photosynthesise at very high light without photoinhibition (Ohad et al., 2010). Among eukaryotes, a newly described *Chlorella* species, *C. ohadii*, is well adapted to the harsh conditions of the BSCs and thrives at extremely high light (Treves et al., 2013). Organisms from environments such as BSCs may thus be ideal candidates for biotechnological exploitation as well as potentially acting as a source of genes to improve the performance of non-extremophiles under stress conditions.

8. Tolerance to high levels of metals and low pH

Naturally occurring acidic environments harbor a diverse range of acidophilic microorganisms, including algae, which tolerate, and in some cases thrive at pH values most microorganisms would not cope with (Souza-Egipsy et al., 2011). Such naturally occurring environments are a result of the leaching of substances, for example fuming and fulvic acids from podocarp rainforests, or volcanic activity (Collier et al., 1990). Highly acidic environments also result from anthropogenic activity. Highly acidic environments facilitate metal solubility, resulting in an environment in which metal-tolerant acidophiles reside (Novis and Harding, 2007). Rio Tinto, a river in southwestern Spain, is an example of a system with low pH and metal levels toxic to most aquatic organisms, yet it harbors a diverse range of eukaryotic microorganisms that are the main contributors to the biomass of the river (Souza-Egipsy et al., 2011). An acido-thermophile *Cyanidioschyzon* sp. that lives in algal mats in environments high in arsenic, in conjunction with *Cyanidium* and *Galdieria* (order Cyanidiales), plays a role in biotransforming arsenic present in their environment (Qin et al., 2009). Fig. 3 provides a glimpse of micro-algal representatives that grow optimally under acidic or alkaline pH.

Many microorganisms that grow in such environments grow as biofilms. It is thought that the formation of extracellular polymeric substances (EPS) is potentially responsible for the detoxification of the metal as well as an aid for the formation of the biofilm (García-Meza et al., 2005). Other methods of dealing with metal toxicity are exclusion from the cell, binding to the cell wall, sequestration within the cells either by incorporation into cellular processes, or by complexation with organic compounds and storage in the vacuole (Rai and Gaur, 2001).

Photosynthetic organisms are of great interest in toxicological studies, as they are the point of entry into the food chain (Sabatini et al., 2009), and have been used in developing toxicological bioassays (Stauber and Davies, 2000). This ability to accumulate metals, and other pollutants can be harnessed in creating remediation solutions using actively growing photosynthetic biofilms found in acidic environments with high levels of metals.

Systems using immobilized algae are being developed for treatment of wastewater, due to their photosynthetic capabilities resulting in the production of useful biomass, as well as the

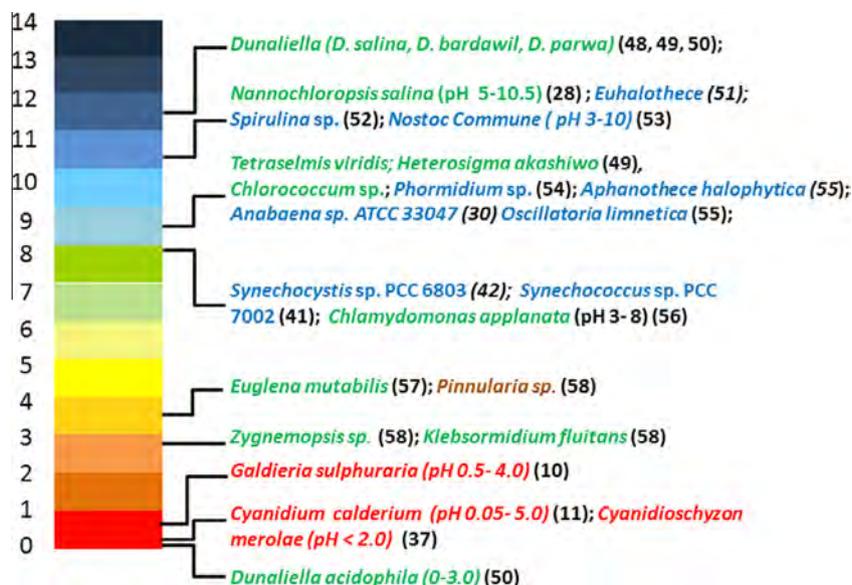


Fig. 3. pH limits for microalgal life. Examples of known pH limits for representative acidophilic, mesophilic and alkalophilic algae and cyanobacteria are shown with the same color scheme as in Fig. 2. The numbers in parenthesis indicate the reference number as listed in the Supplementary information. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

treatment of wastewater (Mallick, 2002). Membrane transport, ion-exchange columns, flocculation and other treatments can be expensive and also produce secondary pollution (Fu and Wang, 2011). Algae grow photosynthetically on limited substrates, and are therefore efficient and environmentally favorable for remediating point pollution, for example arsenic-contaminated drinking water in Bangladesh. An optimized and scaled-up phycoremediation system coupled with physico-chemical methods might offer solutions to larger scale remediation projects (Abdel-Raouf et al., 2012). An extreme form of metal tolerance is found in those organisms that can grow in the presence of radionuclides and which are therefore extremely resistant to ionizing radiation. For example Rivasseau et al. (2013) have isolated *Coccomyxa actinabiotis* from a pool used to store spent fuel elements and this is reported to withstand gamma ray doses of radiation up to 20 KGy and to accumulate, and remove from solution, a range of nuclides including ^{110m}Ag , ^{65}Zn and ^{137}Cs , ^{60}Co , and ^{238}U . Earlier work has also shown cyanobacteria to possess a degree of radiation resistance (Kraus, 1969). The possibility therefore exists to use phycoremediation of dealing with radiation contamination in water.

8.1. Adaptations to extremes: low pH and metals

Low pH increases the solubility of metals, and in turn, high metal levels cause toxicity (Novis and Harding, 2007), either through molecular mimicry, or competition with other nutrients within the growth media (Pinto et al., 2003). Often, at polluted sites where many metals are present, extremophiles have either a genetic or physiological adaptation that allows them to tolerate many metals at once, further increasing their applicability to remediation solutions (Rai and Gaur, 2001). Cellular response to a metal ion can be either exclusion of the metal from the cell, the uptake and modification of the metal to a less toxic form, followed by the metal's transportation out of the cell, or by internal sequestration (Hall, 2002). These processes are a result of a coordinated network of biochemical processes, which increase the cell's ability to maintain homeostasis, and minimize oxidative insult as a result of the production of ROS.

Metal intoxication increases ROS levels as they play a significant role in many ROS-producing mechanisms, including the Haber-Weiss cycle, Fenton's reactions, disruption of the photosynthetic electron chain leading to O_2^- , and reduction of the glutathione pool (Pinto et al., 2003). ROS also act as signalling molecules that induce the production of a network of antioxidants, antioxidant enzymes, and other stress related molecules (Panchuk et al., 2002). Components of the antioxidative response activated by metal stress include antioxidant enzymes and low molecular weight antioxidants, metal chelating molecules such as phytochelatin, metallothioneins, urate and glutathione.

A well-known limitation of biological-based remediation is the reliance on natural systems, which are not yet fully understood. Further elucidating oxidative and stress responses of algae would aid in optimizing productivity of algal bioprocess systems for metal remediation. Acidophilic extremophiles are a great tool for such studies, as they are known for their metal tolerance under already extreme environments (Whitton, 1970). They have the potential to provide much knowledge of cellular tolerance mechanisms to advance and optimize the bioremediation potential of extremophiles, as well as providing many potential species for the construction of bioremediation systems.

ROS signalling pathways and the antioxidative response may hold the key to understanding how extremophilic organisms tolerate metal oxidative stress. For example, in the extremophilic bacterium *Pseudomonas fluorescens*, it was shown that a ROS triggered cascade of biochemical reactions lead to an increased level of NADPH, therefore an increased reductive capacity and tolerance

Table 3

Examples of micro-algae that are tolerant to high salt levels and some of their potential products.

Species	Product/Application	Reference ¹
<i>Green algae</i>		
<i>Dunaliella salina</i>	β carotene (antioxidant)	(25, 26)
<i>Dunaliella bardawil</i>	β carotene (antioxidant)	(25)
<i>Dunaliella</i> sp.	Glycerol	(27)
<i>Nannochloropsis salina</i>	Higher level of lipids (up to 28%)	(28)
<i>Cyanobacteria</i>		
<i>Aphanocapsa</i> halophytica MN 11	EPS (Exopolysaccharides)/RPS (Released Polysaccharide)	(29)
<i>Anabaena</i> sp. ATCC 33047	EPS	(30)
<i>Cyanothece</i> sp. ATCC 51142	EPS	(31)

¹ The references cited here have been listed in [Supplementary information](#).

to the oxidative stress inducing Al (Singh et al., 2005). A comparative study of the neutrophilic alga *Chlamydomonas reinhardtii* and the acidophilic *C. acidophila* show the *C. acidophila* had an increased basal level of HSP, which is thought to be an adaptive mechanism to extreme acidic environment (Gerloff-Elias et al., 2006). Another comparative study of *C. reinhardtii* and *C. acidophila* showed that in face of metal stress, the acidophile produced higher levels of the antioxidative enzymes ascorbate peroxidase (APX) (Garbayo et al., 2007). Panchuk et al. (2002) found that in *Arabidopsis*, the production of heat shock transcription factors was closely tied with the production of not only HSP, but also APX. The acidophiles *Coccomyxa onubensis* and *C. acidophila* increased their production of the antioxidants lutein and β -carotene in the presence of copper at 0.2 mM and 4 mM, respectively (Garbayo et al., 2008; Vaquero et al., 2012).

There is still much to learn about genes and metabolic pathways involved in metal tolerance and sequestration (Hall, 2002). Understanding vacuolar transporters functioning in metal sequestration, membrane transporters that aid in phytochelatin and glutathione transport, and gene-expression induced by metal stress would greatly aid in identifying hyperaccumulators, and also aid the engineering of cells with enhanced metal tolerance. Further, multiple tolerance mechanisms can be introduced to cells to produce highly tolerant photosynthetic organisms to one or more abiotic stressors (Dobrota, 2006).

9. Tolerance to extreme salinity

As has already been flagged, growth under conditions that are unfavorable to grazers or competing microalgae can be a desired characteristic for large scale, particularly outdoor, cultures. A number of species of microalgae capable of growth under hypersaline conditions are well established in biotechnology. Thus the green alga *D. salina* (and *Dunaliella bardawil*, sometimes considered a strain of *D. salina*) grow well on very high salinity up to 3 M and large, open ponds of these algae are used for β -carotene production. *Nannochloropsis salina* has been grown for lipid production. A number of cyanobacteria are also capable of growth under high salinities and *Aphanocapsa halophytica* and strains of *Anabaena* and *Cyanothece* produce high levels of extracellular polysaccharides under those conditions (Table 3). While the microalgal flora of hypersaline environments is somewhat restricted, further investigations may uncover additional halophiles or new and desirable properties of known halophilic species.

10. Concluding remarks and future directions

Extremophilic microalgae have evolved mechanisms that allow them to tolerate conditions that would be toxic to other organisms.

Bioprospecting for new species/strains may identify yet more useful characteristics for biotechnology. We argue that extremophiles are currently an under-exploited resource and that further investigations on the following may be needed:

- (1) Tolerance mechanisms to environmental stresses.
- (2) Suitability of known extremophiles under outdoor conditions.
- (3) Metabolic engineering (Alagesan et al., 2013) of extremophiles; molecular biology tools.
- (4) Circadian rhythms in extremophiles, a concept that is widely researched in mesophilic cyanobacteria (Krishnakumar et al., 2013).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2014.11.040>.

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