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REVIEW

# High-$CO_2$ tolerance in microalgae: possible mechanisms and implications for biotechnology and bioremediation

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**Abstract** Recent developments in the field of microalgal biotechnology, including $CO_2$ biomitigation and the discovery of new species of microalgae that are tolerant to extremely high $CO_2$ levels (40–100 vol%), have renewed interest in the physiological effects and mechanisms of high-$CO_2$ tolerance in photoautotrophs. Photosynthetic apparatus state transitions that increase ATP generation, upregulation of $H^+$-ATPases pumping protons out of the cell, rapid shutdown of $CO_2$-concentrating mechanisms, and adjustment of membranes' fatty acid composition are currently believed to be the key mechanisms governing cellular pH homeostasis and hence microalgae's tolerance to high $CO_2$ levels, which is especially characteristic of extremophile and symbiotic species. The mechanisms governing acclimation to high $CO_2$ comprise the subject of this review and are discussed in view of the use of $CO_2$ enrichment to increase the productivity of microalgal cultures, as well as the practice of carbon capture from flue gases.

**Keywords** Biomitigation · $CO_2$-concentrating mechanism · Microalga · pH homeostasis · Response of lipid metabolism

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## Introduction

The present-day atmospheric concentration of $CO_2$ (ca. 0.04 %[1]) is considered to be limiting for photosynthesis (Larkum 2010). In fact, the photosynthetic apparatus (PSA) and, in particular, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)—the stromal enzyme that catalyzes the entry of $CO_2$ into the Calvin–Benson–Bassham (CBB) cycle—seem to be adapted to the much higher $CO_2$ concentrations encountered by the ancestors of modern photoautotrophs (Kupriyanova and Pronina 2011). As the atmospheric $CO_2$ level gradually declined due to photosynthetic fixation and geochemical processes, photoautotrophic organisms evolved sophisticated $CO_2$-concentrating mechanisms (CCMs). These mechanisms facilitate $CO_2$ uptake and increase its

[1] Here and in the following, volumetric percentage of $CO_2$ in the gas mixture used for culture bubbling is specified as this parameter is more manageable and relevant to microalgal biotechnology than $CO_2$ partial pressure ($pCO_2$) in the medium.

concentration near Rubisco by one to three orders of magnitude relative to the atmospheric level, to maintain sufficient photosynthesis (Raven 2010). As a consequence, $CO_2$ levels as low as 2–5 % are saturating for the overwhelming majority of contemporary photoautotrophs, while higher $CO_2$ levels often exert deleterious effects on growth and photosynthesis.

The division of microalgae into $CO_2$-sensitive and $CO_2$-tolerant groups is, to a great extent, a matter of convention since $CO_2$ tolerance varies greatly not only between species, but within a single species as well, depending on growth conditions. In general, species inhibited by <2–5 % $CO_2$ are considered to be $CO_2$-sensitive (intolerant), those that can cope with up to 20 % $CO_2$ are referred to as $CO_2$-tolerant, and the species withstanding higher $CO_2$ concentrations are denoted as extremely $CO_2$-tolerant; accordingly, $CO_2$ concentrations of 2–5, 5–20, and 20–100 % are commonly referred to as high, very high, and extremely high, respectively (Miyachi et al. 2003).

Until recently, most studies in the field of microalgal physiology and biotechnology were dedicated to investigating the effects of relatively low (<5 %) $CO_2$ levels and CCM function, which is now quite well understood (Raven et al. 2008). In contrast, studies on the mechanisms governing tolerance to high $CO_2$ levels are scarce, even though this phenomenon has been known for over 40 years (Seckbach et al. 1970) and investigations into $CO_2$ acclimation of different microalgae, including species that are important for biotechnology, have been published (Table 1).

High-$CO_2$ acclimation is a complex process involving adjustment of numerous, if not all, functions of microalgal cells (Muradyan et al. 2004; Sergeenko et al. 2000). A thorough understanding of its underlying mechanisms is a prerequisite for employing $CO_2$ enrichment to increase productivity of mass-cultivated microalgae, as well as for efficient $CO_2$ fixation by microalgae for carbon-capture applications (Kumar et al. 2011; Wang et al. 2008). In particular, the proposed use of neutral lipids of microalgae as biofuel feedstock precursors recently inspired numerous attempts to examine the effect of sparging with gas mixtures having different $CO_2$ concentrations (from slightly above ambient to 100 %) on neutral lipid (triacylglycerol) production potential of microalgal cultures (Toledo-Cervantes et al. 2013; Yoo et al. 2013).

**Table 1** Examples of high-$CO_2$-tolerant species of microalgae

| Species | Max. $CO_2$ tolerated (% v/v) | Reference |
|---|---|---|
| *Cyanidium caldarium* | 100 | Seckbach et al. (1970) |
| *Scenedesmus* sp. | 80 | Hanagata et al. (1992) |
| *Chlorococcum littorale* | 60 | Kodama et al. (1993) |
| *Synechococcus elongatus* | 60 | Miyairi (1995) |
| *Euglena gracilis* | 45 | Nakano et al. (1996) |
| *Chlorella* sp. | 40 | Hanagata et al. (1992) |
| *Eudorina* spp. | 20 | Hanagata et al. (1992) |

Adapted from Raven et al. (2008)

The issue of microalgal tolerance to $CO_2$ is becoming increasingly important in view of the continuous rise in environmental risks imposed by anthropogenic $CO_2$ emission. Today, biological (photosynthetic) sequestration by microalgae is thought to be the most environmentally safe and sustainable way of mitigating $CO_2$ levels (Wang et al. 2008; Huntley and Redalje 2007; Kumar et al. 2011; Yoo et al. 2010; Borowitzka and Moheimani 2013). Indeed, there have been successful attempts at cultivating microalgae using $CO_2$ from the flue gas of a coal-combusting power plant (see e.g. Koberg et al. 2011). At the same time, considerable difficulties have been encountered in the development of efficient approaches for $CO_2$ biomitigation, due to insufficient understanding of the physiological effects of, and acclimation to high levels of $CO_2$. This situation, along with a considerable rise in the number of publications devoted to cultivation of microalgae under elevated $CO_2$ levels, raised the need for the present review.

## Acclimation of the photosynthetic apparatus (PSA)

The growth and photosynthesis of sensitive microalgae are largely inhibited by $CO_2$– at concentrations above 2–5 %. In tolerant species, growth and $CO_2$ fixation decline at considerably higher $CO_2$ levels; recovery often occurs after a lag period, depending on the species and the actual $CO_2$ concentration (Satoh et al. 2002). Such a lag is characteristic of *Chlamydomonas*, for example, but often lacking in *Chlorella* (Miyachi et al. 2003; Baba et al. 2011). However, $CO_2$

**Table 2** Growth and $CO_2$ fixation of high-$CO_2$-tolerant microalgae

| Species or strain | $CO_2$ (%) | $t$ (°C) | Growth (g $l^{-1}$ $day^{-1}$) | $CO_2$ fixation (g $l^{-1}$ $day^{-1}$) | Reference |
|---|---|---|---|---|---|
| *Chlorococcum littorale* | 40 | 30 | –[a] | 1.0 | Iwasaki et al. (1998) |
| *Chlorella kessleri* | 18 | 30 | 0.087 | 0.163 | de Morais and Costa (2007b) |
| *Chlorella* sp. UK001 | 15 | 35 | – | >1 | Murakami and Ikenouchi (1997) |
| *Chlorella vulgaris*[b] | 15 | | – | 0.624 | Yun et al. (1997) |
| *Chlorella* sp. | 40 | 42 | – | 1.0 | Sakai et al. (1995) |
| *Dunaliella* sp.[c] | 3 | 27 | 0.17 | 0.313 | Kishimoto et al. (1994) |
| *Haematococcus pluvialis*[d] | 16–34 | 20 | 0.076 | 0.143 | Huntley and Redalje (2007) |
| *Scenedesmus obliquus* | 18 | 30 | 0.14 | 0.26 | de Morais and Costa (2007a) |
| *Spirulina* sp. | 12 | 30 | 0.22 | 0.413a | de Morais and Costa (2007a) |

Adapted from Wang et al. (2008)

[a] Not recorded

[b] In artificial wastewater

[c] Accumulates β-carotene at high salinities

[d] In production-scale photobioreactor

tolerance of the same species may vary considerably in reports by different groups (Table 2).

The much-studied extremophile chlorophyte, *Chlorococcum littorale,* isolated from saline ponds, retains the capacity for rapid growth at $CO_2$ levels of up to 60 % (Miyachi et al. 2003). Studies of *C. littorale* have demonstrated that transfer to a high-$CO_2$ state brings about a rapid state transition of the PSA in this species (Demidov et al. 2000). A similar response was then found in other microalgae (Sergeenko et al. 2000). The high-$CO_2$-induced transition of the PSA from state I to state II was suggested to increase cyclic electron transport over photosystem (PS) I and to generate the added amounts of ATP necessary for support of pH homeostasis in the algal cell (Miyachi et al. 2003), and see the section on pH homeostasis further on). A common reason for the state transition in the PSA is reduction of the plastoquinone pool due to accumulation of NADPH (Antal et al. 2013). This situation usually arises when the dark reactions of photosynthesis are inhibited to a certain extent by a stressor (Strasser et al. 2004), such as elevated $CO_2$. Accordingly, the increase in the PSI/II ratio suggests that high-$CO_2$ tolerance requires, in particular, an increase in the size of the PSI light-harvesting antenna. Indeed, corresponding changes have been recorded in the PSA of $CO_2$-tolerant microalgae within several days of their transfer to high-$CO_2$ conditions (Satoh et al. 2002). Notably, the changes in the PSI/II ratio were reversible: the ratio could return to its initial level, probably due to a decrease in ATP demand as a result of overall acclimation of the microalgal cell to high $CO_2$_ and/or a decline in the CCM activity (Miyachi et al. 2003). In contrast, $CO_2$-intolerant species (such as *Dunaliella salina*) lack the state-transition response and display signs of PSI damage under high-$CO_2$ conditions (Muradyan et al. 2004).

Interestingly, elevated $CO_2$ promoted high-light-induced photoinhibition in sensitive species (e.g. in *Chlamydomonas reinhardtii*) but alleviated it in $CO_2$-tolerant species (*Chlorella pyrenoidosa* or *Scenedesmus obliquus*) in comparison with the same cultures grown under atmospheric $CO_2$ levels; at the same time, the high $CO_2$-adapted cultures recovered from the photoinhibition more rapidly (Yang and Gao 2003).

## Robustness of pH homeostasis in the cell

The rapid inhibition of photosynthesis observed under high-$CO_2$ conditions could be a consequence of inactivation of the key enzymes of the Calvin cycle due to acidification of the stromal compartment of the chloroplast (Krause and Weis 1991) under high-$CO_2$ stress. This suggestion was confirmed by Pronina et al. (1993) who demonstrated, using vital $^{31}$P-NMR, that the pH of the cytoplasm in the $CO_2$-tolerant microalga *C. littorale* does not drop below 7.0, even during

cultivation under extreme (40 %) $CO_2$ levels (Miyachi et al. 2003).

### Active transport of $H^+$

Taking into account these findings, it is obvious that efficient control of intracellular pH is essential for high-$CO_2$ tolerance. Such control might be achieved by pumping the protons from the cytoplasm into the vacuoles, for example, via consumption of the surplus ATP generated as a result of cyclic electron transport by the ATPase and $H^+$-pyrophosphatase associated with the tonoplast (Gogarten and Taiz 1992).

Normally, microalgal cells grown under low-$CO_2$ conditions are characterized by a certain level of CCM activity, including that of diverse carbonic anhydrase (CA) enzymes with different cellular localizations. Therefore, a considerable drop in pH is expected at the beginning of these cells' high-$CO_2$ acclimation, prior to CCM downregulation. This could explain the overlap of the peak demand for ATP with the acclimation period and the aforementioned reversal of the changes in PSI/II ratio thereafter. On the other hand, cultivation of microalgae at elevated $CO_2$ levels often leads to an increase in size and/or number of vacuoles in the cytoplasm (Pronina et al. 1993). Sasaki et al. (1999) showed that vacuolization of the cytoplasm in *C. littorale* is accompanied by significant induction of $H^+$-ATPase activity. The magnitude of this increase was strongly correlated with the rate of photosynthesis during acclimation to extremely high $CO_2$ levels.

An important factor of $CO_2$ tolerance is the ability of microalgal cells to increase the pH of the medium during active growth via nitrate uptake. Alkalization of the medium is believed to compensate, to a considerable extent, for the acidification effect of high $CO_2$ concentrations. This mechanism operates in *Emiliania huxleyi* (Fukuda et al. 2011) for example, as well as in a symbiotic *Desmodesmus* sp. (Solovchenko et al. unpublished). Obviously, a sufficient supply of $NO_3^-$ would be necessary for efficient acclimation to high $CO_2$ and carbon capture from flue gases by the microalgal cultures.

### Shutdown of the $CO_2$-concentrating mechanism (CCM)

Satoh et al. (2002) demonstrated a drop in intracellular pH within 1 h of transferring *C. littorale* cells growing in low $CO_2$ to 40 % $CO_2$. This effect was eliminated by treatment of the cells with ethoxzolamide, a membrane-permeable CA inhibitor. Cultivation of the low-$CO_2$-adapted microalgal cells at elevated $CO_2$ brought about a considerable decline in CCM activity. In *Chlamydomonas reinhardtii*, degradation of CA takes ca. 7 days (Baba and Shiraiwa 2012) and occurs in parallel with inhibition of active transport of bicarbonate (Bhatti and Colman 2008). This decline is often reversible: CCM activity returns to its initial level upon transfer of the cells to atmospheric $CO_2$. This process is thought to be regulated by CCM1/CIA5, the 290- to 580-kDa protein complex containing a zinc-finger domain (Fukuzawa et al. 2001). In contrast, cells of microalga adapted to 5 % $CO_2$ that possess no detectable intra- or extracellular CA activity do not display a growth lag upon transfer to extreme levels of (40 %) $CO_2$ (Satoh et al. 2002). Interestingly, the microalgae naturally adapted to growth under acidic conditions in the presence of elevated $CO_2$ concentrations such as *Synura petersenii* or *Tessellaria volvocina* apparently lack a CCM (Raven 2010; Bhatti and Colman 2008). In particular, cells of these species lack plasmalemma-bound CA, they are unable to take up bicarbonate, and their Rubisco is characterized by high affinity to $CO_2$ (Bhatti and Colman 2008). Interestingly, availability of alternative (organic) carbon sources leads to a decline in CA activity. It is thus apparent that the ability to swiftly downregulate CCM (or its effective lack) is an important prerequisite for attaining high $CO_2$ tolerance.

## Effects on lipid biosynthesis and fatty acid (fatty acid) composition

Many research groups are currently engaged in the isolation of novel $CO_2$-tolerant strains and investigations into their ability to accumulate storage lipids when sparged with high, or even pure, $CO_2$ (Kumar et al. 2011; Yoo et al. 2013; López et al. 2013; Solovchenko et al. 2013; Toledo-Cervantes et al. 2013). In general, a moderate (2–5 %) increase in $CO_2$ stimulates both growth and lipid accumulation by microalgal cells (Muradyan et al. 2004). For example, a green microalga isolated from a spring in Cuatro Cienegas, Mexico, and morphologically identified as *Scenedesmus obtusiusculus,* tolerated 10 % $CO_2$; its

neutral lipid content increased from 15 to 55 % of dry weight (DW) as the $CO_2$ level increased from 0.04 to 5 %, with a corresponding decrease in total carbohydrates (Toledo-Cervantes et al. 2013). In another study, a *Scenedesmus* sp. and *Chlorella* sp., both isolated from a Sonoran desert mineral spring, grew and tolerated exposures to up to 20 % $CO_2$ applied continuously in batch reactors (Westerhoff et al. 2010). Another chlorophyte, *Ettlia* sp., showed high growth rate and lipid content at 5–10 % $CO_2$; the highest lipid productivity (80 mg $l^{-1}$ $day^{-1}$) and total lipid content (up to 42 % of DW) was obtained with administration of 5 % $CO_2$ (Yoo et al. 2013). Increasing $CO_2$ from 1.5 to 5 % enhanced total lipid content in the biomass of the green alga *Parietochloris incisa* (Shuuluka et al. unpublished work). $CO_2$ levels higher than 20–30 % generally lead to a decline in reserve-lipid biosynthesis, often with overall inhibition of the culture's growth (Chiu et al. 2009). At the same time, $CO_2$ levels characteristic of flue gases (13–20 %) sometimes cause no inhibition, as in the case of *Botryococcus braunii* (Ge et al. 2011), or even promote lipid accumulation (Yoo et al. 2010). Thus, Tang et al. (2011) attempted to cultivate *S. obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2 with 0.03–50 % $CO_2$. Whereas both microalgae demonstrated optimal growth potential at 10 % $CO_2$, sustained, albeit slower, growth was recorded at 50 % as well. Total lipid content in the stationary cultures showed a moderate but gradual increase with the increase in $CO_2$ supply from ambient 0.03 to 50 % in *S. obliquus*, while increasing $CO_2$ from 5 to 50 %, did not exert any prominent effect on total lipid content in *Chlorella pyrenoidosa*. Notably, no significant differences were found between cultures of *Tetraselmis suecica* CS-187 and a *Chlorella* sp. supplemented with either pure $CO_2$ or untreated flue gas from coal-fired power plant, in biomass and lipid productivities at optimal pH (Moheimani 2013).

The availability of other carbon sources has a strong effect on lipid accumulation under elevated $CO_2$. In particular, the effects of high or low inorganic carbon concentrations ($CO_2$ and bicarbonate) on neutral lipid and starch accumulation were studied in the model alga *Chlamydomonas reinhardtii* during nitrogen depletion under photoautotrophic conditions (Gardner et al. 2013). Under low $CO_2$ (0.04 %), carbon limitation resulted in reduced accumulation of both storage products - triacylglycerol (TAG) and starch; at 5 % $CO_2$, the highest amount of TAG was produced, however under these conditions rapid TAG accumulation was followed by degradation (probably following acclimation to the elevated $CO_2$ level). Under high bicarbonate supplementation, the cell cycle was arrested and sustainable accumulation of both TAG and starch was recorded. In mixotrophic cultures (grown on an organic carbon source), raising $CO_2$ levels impaired the algae's capacity for import and assimilation of glycerol (Sforza et al. 2012). Obviously, lipid (TAG in particular) biosynthesis represents a sink for the excess products of carbon fixation, which is especially important under stressful conditions causing cessation of cell division (Solovchenko 2012; Hu et al. 2008). One could further speculate that the ability to channel the excess photosynthates to the biosynthesis of energy-rich compounds such as TAG may, in some situations, contribute to high-$CO_2$ tolerance of microalgae.

Apart from changes in the proportions of the major lipid classes, bubbling with gas mixtures enriched in $CO_2$ often leads to an increase in fatty acid percentages of DW, even in $CO_2$-sensitive species, e.g. by 30 % in *D. salina* at 10 % $CO_2$ (Muradyan et al. 2004); in $CO_2$-tolerant species, this effect is even stronger (Luo and Al-Dahhan 2011). One might assume that under high $CO_2$ conditions, the flux of de-novo-synthesized fatty acids toward assembly of acyl lipids is enhanced. An increased level of $C_{18}$ polyunsaturated fatty acids in total lipids was reported to occur with increasing $CO_2$ concentration, indicating increasing unsaturation of chloroplast lipids (Tang et al. 2011).

At the same time, $CO_2$-sensitive species respond to even short-term (1 day) exposure to elevated $CO_2$ level by a decline in the fatty acid unsaturation index, suggesting inhibition of fatty acid desaturation and $C_{16}$-to-$C_{18}$ elongation (Muradyan et al. 2004; Yoo et al. 2010). However, given the increased amount of fatty acid precursors that accumulated in the lipids of *D. salina* under elevated $CO_2$ conditions, the authors (Muradyan et al. 2004) suggested an alternative explanation: that fatty acid-modifying activities might not be affected and are not sufficient to convert the enhanced influx of de novo-produced fatty acids. As a result, an increase in the proportion of saturated fatty acids and monounsaturated oleic acid (18:1) occurred in *D. salina* along with a rise in the ω3/ω6 fatty acid ratio and in the proportion of the major chloroplast galactolipid, monogalactosyl diacylglycerol (MGDG),

while the second major galactolipid—digalactosyl diacylglycerol (DGDG)—decreased (Muradyan et al. 2004). Importantly, high amounts of saturated palmitic acid (16:0), a product of de-novo fatty acid synthesis, accumulated in MGDG under high $CO_2$. These results suggest possible rearrangements in chloroplast membranes in high-$CO_2$-sensitive microalgae. The non-bilayer-forming MGDG is typically characterized by a high abundance of polyunsaturated fatty acids which forces it to adopt a so-called hexagonal II structure; hence high levels of saturated fatty acids in MGDG are likely to affect phase behavior of the lipids in the thylakoid and envelope membranes.

## Implications for mass cultivation of microalgae

It is obvious from the above considerations that $CO_2$ enrichment is an efficient technique for enhancing the productivity of microalgal cultures, provided that a proper approach is taken. In particular, it is essential to establish an optimal $CO_2$ feeding rate to the culture. This rate is usually limited by $CO_2$ tolerance (especially by its low-pH-related component) as described above. Aside from other factors, the efficiency of $CO_2$ utilization by microalgal cells is limited by mass transfer between the gaseous phase and the liquid culture medium. On the other hand, one cannot increase the rate of sparging of the culture and/or the intensity of agitation above a certain limit imposed by shear stress without negatively influencing culture growth. Separate concerns exist regarding the capture of $CO_2$ directly from flue gases, which usually contain some nitrogen and sulfur oxides that form acids upon dissolution in the culture medium, as well as other toxic pollutants. Therefore, flue gases require special treatment, e.g. pre-filtering and neutralization, before they are fed to microalgae (López et al. 2013). Collectively, these circumstances call for specific features in the design of dedicated photobioreactors for $CO_2$ capture by microalgae. For example, an important aim is maximizing contact between the sparging gas and the culture to achieve the most efficient mass transfer. Taking into account the necessity to avoid shear stress, a closed photobioreactor system with mixing by airlift and recycling of the sparging gas seems to be the best choice, although efficient open systems (raceway-based) for carbon capture have been implemented as well (Koberg et al. 2011). In other respects (achieving high photic volume, absence of stagnation zones, removal of $O_2$ formed in the course of photosynthesis, etc.), the construction of these photobioreactors should be governed by the common design principles outlined in recent papers (Greenwald et al. 2012; Sieblist et al. 2011a, b; Carvalho et al. 2011).

## Conclusion and outlook

A considerable number of microalgal species appear to be tolerant to moderate levels of $CO_2$; at the same time, species which are able to withstand extreme $CO_2$ concentrations (20–40 % and higher) are quite rare. Nevertheless, high-$CO_2$-tolerant microalgae need to be isolated for the development of efficient $CO_2$-capture methods. Summarizing the experimental data obtained to date, one can conclude that $CO_2$ tolerance in microalgae is achieved via several mechanisms. These include the responses preventing acidification of the chloroplastic stromal compartment and cytoplasm to maintain sufficient Rubisco activity. First, state transition of the PSA increases ATP generation which is spent on maintaining a suitable pH by active transport. Second, the ability to rapidly and reversibly shut down the CCMs operating under atmospheric $CO_2$ levels but facilitating the pH drop in microalgal cells under elevated $CO_2$ seems to be of considerable importance. Third, various adjustments in lipid metabolism provide for optimal balance of source and sink under stressful conditions, as well as for swift rearrangements of PSA membranes.

Mechanisms of high-$CO_2$ tolerance have important implications for microalgal biotechnology. The insights obtained in the studies of these mechanisms reveal additional strain-selection criteria for the development of efficient microalgal cultivation methods based on high-$CO_2$ enrichment for diverse applications, especially in the case of mixotrophic cultures. In particular, one should look closely for the presence of the responses outlined above. Promising sources of high-$CO_2$-tolerant isolates include extremophiles from geysers and thermal springs (López et al. 2013). An additional benefit of exploiting thermophilic species is the reduced energy input required for cooling and hence more cost-efficient carbon capture, since flue gases are discharged at high temperature. Symbiotic microalgae may also have a great potential for carbon capture (Solovchenko et al.

2013). Another potential mechanisms (but, to the best of our knowledge, not yet explored) approach would involve artificial down-regulation of the CCM in well-known biotechnologically important species to improve their $CO_2$ tolerance without harming their ability to accumulate value-added compounds.

**In conclusion**, despite the considerable progress achieved to date, our understanding of the mechanisms of high-$CO_2$ tolerance in microalgae is far from complete and requires a considerable research effort. Application of the advanced 'omics' tools in this field seems to be promising for improving current microalgal biotechnologies for environmental protection and obtaining diverse bioproducts.

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