Effect of temperature and increased concentration of CO_2 on growth and photosynthetic activity of polar alga *Trebouxia* sp.

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Abstract

Trebouxia sp., a lichen symbiotic alga, was isolated from lichen *Usnea antarctica* collected at James Ross Island, Antarctica. After isolation, the alga was cultivated on Bold's Basal Medium (BBM-agar) with addition of nitrogen for 12 days. Growth of alga and its photosynthetic properties were studied in relation to increased concentration of CO_2 (850±50 ppm) and two cultivation temperature (8 and 12°C). Physiological status of algae was evaluated by chlorophyll fluorescence parameters. Simultaneously, content of pigments and changes in biomass were evaluated during cultivation period. Evaluation of physiological state of *Trebouxia* sp. was carried out after the end of experiment. Results of chlorophyll fluorescence induction parameters and content of pigments showed that the highest efficiency of primary processes of photosynthesis was found at the treatment with elevated concentration of CO_2 and temperature 12°C. In this treatment, potential quantum yield of photochemical processes in photosystem II (F_V/F_M) was 0.44 and content of Chl *a* was 5.14 μg ml^{-1}. In contrast, the lowest efficiency of primary processes was found at the treatment with addition of CO_2 and temperature 8°C, where value of F_V/F_M reached 0.37 and content of Chl *a* was 3.71 μg ml^{-1}.

Key words: *Trebouxia* sp., carbon dioxide, fluorescence of chlorophyll, pigments, Antarctica, lichen

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Introduction

Due to anthropogenic activities occurs to increasing the concentration of atmospheric carbon dioxide and this elevation of concentration of CO_2 contributes significantly to the global warming. Warming of the Earth is causing regional climate changes and influencing many physical, biological and chemical processes (Walther et al. 2002, Treydte et al. 2006). Increasing of concentration of carbon dioxide in atmosphere also contributes to stratospheric ozone depletion, which leads to increased

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levels of biologically harmful UV-radiation. Moreover, elevation of CO_2 levels causes sea-level rise and significantly influences ocean chemistry (Hallock 2005).

Algae are distributed in a variety of environments and play an important role as primary producers in aquatic ecosystems (Yang et al. 2008). In polar regions, the algae occur either component of lichens or free living organisms in various types of ecosystems. Lichens are dominant inhabitant in cold environment and they have to possess unique properties to survive in such harsh condition as well as free living algae. Algae in lichen association are protected by symbiotic fungal partner which consumes a large amount of carbon. In contrast, free-living algae have not such protective partner and they have to cope with direct effect of unfavourable conditions. However, during the evolution they developed a lot of effective adaptations that help them survive or even thrive in the extreme environment.

Ecological effects of increasing atmospheric CO_2 concentration have been well studied in terrestrial plants (Bowes 1993, Ziska et Bunce 2007), however algae and their response to increased concentration CO_2 have been understood to much less extent (Badger et al. 2003). Recent studies showed that elevated CO_2 concentrations enhanced growth of some terrestrial cyanobacteria, freshwater green algae and some marine microalgae (Riebesell et al. 1993, Rost et al. 2003, Qiu et Gao 2002, Yang et Gao 2003, Schippers et al. 2004). However, enhancement of photosynthesis or growth under CO_2-enriched conditions can be species-specific, depending on their physiological characteristics (Yang et al. 2008). For example, *Chlamydomonas reinhardtii* concentrates CO_2 only when growing under low-CO_2 conditions. However, because more than 90% of the Rubisco is localized in pyrenoid, one question is whether pyrenoidal Rubisco is active in CO_2 fixation or whether pyrenoid serves as storage body (Moroney et Somanchi 1999).

In the present study, we have investigated effects of increased concentration of CO_2 and two levels of temperature on photosynthetic activity and physiological state of model alga, *Trebouxia* sp. Response was monitored as changes in biomass, content of pigment and also by induced fluorescence of chlorophyll.

Material and Methods

Cultivation of material

Stock cultures of the lichen photobiont *Trebouxia* sp. were maintained in axenic culture on Bold's Basal Medium (BBM-agar) with addition of nitrogen (*see* below), in Petri dishes at temperature 10°C and 16/8 photoperiod with irradiance of 10 μmol m^{-2} s^{-1}. The medium was prepared according to Ahmadjian (1993) – *see* Table 1. After 8 weeks cultivation on BBM agar medium, 207.6 mg of algal cultures were transferred into 150 ml of liquid 3N BBM medium.

Preparing of culture for experiment

From the liquid medium were transferred 5 ml on each of 29 glass microfiber filters and they were placed into 8 cultivation flasks with 3N BBM. Each of flasks contained three filters (Fig. 1). The five remaining filters were placed into Eppendorf tube as control samples.

Macronutrients					
$NaNO_3$	KH_2PO_4	K_2HPO_4	$MgSO_4 .7\ H_2O$	$CaCl_2$	NaCl
0.25 g	0.175 g	0.075 g	0.075 g	0.025 g	0.025 g

Micronutrients				
H_3BO_3	KOH	EDTA	$FeSO_4 . 7\ H_2O$	$ZnSO_4 . 7\ H_2O$
11.42 mg	31.0 mg	50.0 mg	4.95 mg	8.82 mg
$CuSO_4 . 5\ H_2O$	$Co(NO_3)_2 . 6\ H_2O$	$MnCl_2. 4\ H_2O$	MoO_3	
1.57 mg	0.49 mg	1.44 mg	0.71 mg	

Table 1. Nutrient composition of 1 litre of Bold Basal Medium - agar, according to Ahmadjian (1993) - used in this study.

Set-up of experiment

All of 8 cultivation dishes were placed to the thermostat, where were set two levels of temperature (8°C, 12°C). Four dishes were placed at 8°C and remaining dishes were placed at 12°C. In both temperatures, two dishes were affected by increased concentration of CO_2 (850±50 ppm, checked by a EGM-2 gas monitoring system, PP Systems, UK) and two dishes served as control (ambient CO_2 concentration). The technical solution of the experiment is shown on Fig. 3.

Fig. 1. Cultivation dishes with algal cells on glass microfiber filters. Three filters were placed in each of dishes.

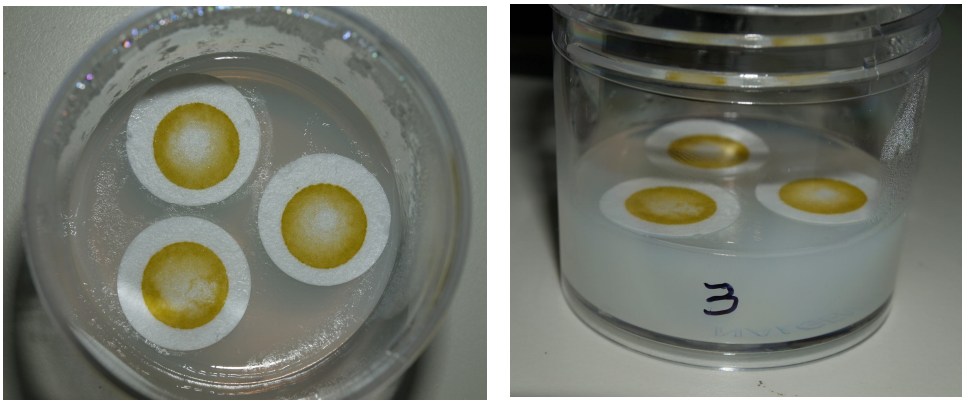

Fig. 2. Glass microfiber filters with colonies of *Trebouxia* sp. placed on top of agar in cultivation dishes as viewed from top (left) and side (right).

Extraction of pigments in algal cells

Extraction of algal pigments was carried out according to the method proposed by Wellburn (1994). As an organic solvent were used dimethyl sulfoxid (DMSO). After exposition, algal cells were freeze dried, DMSO was added in volume of 1.5 ml and suspenses were vortexed for 1 min. The pigments were extracted in thermostat at 70°C for 60 min. After extraction period, smaples were centrifuged (5 min. at 10 000 g). The pigment contents were determined spectrophotometrically (Specord 205, Analytic Jena, Germany) according to equations:

Chlorophyll-*a*	$Ca = 12.47 * A_{665.1} - 3.62 * A_{649.1}$	*Eqn. 1*
Chlorophyll-*b*	$Cb = 25.06 * A_{649.1} - 6.5 * A_{665.1}$	*Eqn. 2*
Total carotenoids	$Cx+c = (1000 * A_{480} - 1.29 * Ca - 53.78 * Cb) / 220$	*Eqn. 3*

Measurement of chlorophyll fluorescence

Chl fluorescence parameters were measured on control and samples cultivated in CO_2. Measurement of Chl fluorescence was carried out by the method of Chl fluorescence imaging by FluorCam (PSI, Czech Republic). Used fluorescence parameters (F_V/F_M and Rfd) were determined from analysis of the slow Kautsky kinetic (Roháček et al. 2008). After 10 minutes lasting dark-adaptation was run the kinetic.

Statistical processing

The experiment data were statistically processed by one-factor ANOVA. Data normality and homogeneity of variance were confirmed by Shapiro-Wilk's test and Cochran, Hartley, Bartlett's test. For assessment of statistically significant differences, Fischer LSD test was used (level of significance 95%).

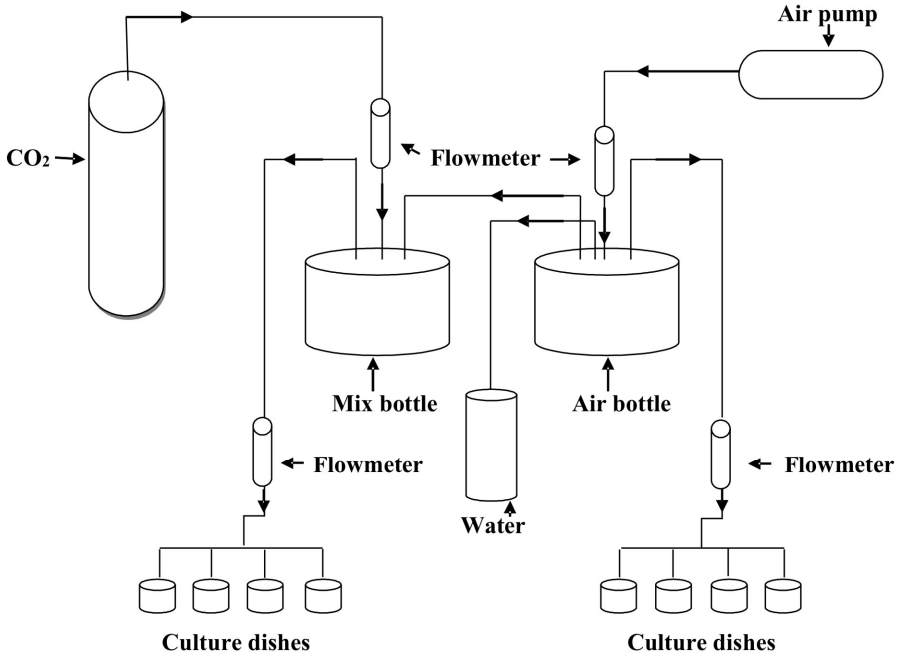

Fig. 3. The technical set up of the experiment. An apparatus shows a system for maintaining constantly elevated CO_2 concentration.

Results

Growth response to temperature and increased concentration of CO_2

As expected, statistically significant differences were observed between samples, which were cultivated at different temperatures, irrespective of the concentration of CO_2 after the 12 days experiment. The highest content of Chl *a* was found in the algae treated 12°C and supplied with elevated concentration of CO_2. In this treatment the average content of Chl *a* in cells of *Trebouxia* sp. was 5.14 μg ml^{-1}. On the contrary, the lowest mean content of Chl *a* was detected in algae exposed to 8°C with elevated concentration of CO_2, where the mean content of Chl *a* was 3.71 μg ml^{-1}. As regards the content of Chl *b* and the carotenoids, the highest content of Chl *b* in cells of model alga was observed at 12°C with ambient CO_2 concentration namely 0.61 μg ml^{-1}. While, the highest content of the carotenoids was found at 12°C with increased concentration of CO_2, where the average content was 3.36 μg ml^{-1}. The lowest content of Chl *b* was observed at 8°C with ambient CO_2 concentration, when the average content of Chl *b* was 0.43 μg ml^{-1}, but the lowest content of the carotenoids was detected at 8°C with elevated CO_2 concentration. The content was 2.63 μg ml^{-1}.

Also, changes were observed in the amount of biomass, where statistically significantly differences were observed be-

tween the treatments at 8°C and 12°C with ambient CO_2 concentration. However, statistically significantly differences were not detected between the treatments at 8°C and 12°C with elevated CO_2 concentration. The highest amount of dry weight was observed in treatment at 12°C with ambient CO_2 concentration, when amount of dry weight was 3.82 mg. In contrast, the lowest amount of dry weight were found at 8°C with ambient CO_2, namely 3.23 mg.

Treatment	Chl *a* (µg ml^{-1})		Chl *b* (µg ml^{-1})		Carotenoids (µg ml^{-1})		Biomass (mg)	
CO_2/T8	3.71±0.27	**a**	0.45±0.04	**a**	2.63±0.15	**a**	3.30±0.49	**ab**
T8	3.87±0.25	**a**	0.43±0.05	**a**	2.70±0.15	**a**	3.23±0.50	**a**
CO_2/T12	5.14±0.40	**b**	0.55±0.08	**b**	3.36±0.09	**b**	3.73±0.45	**ab**
T12	4.81±0.30	**b**	0.61±0.08	**b**	3.31±0.15	**b**	3.82±0.33	**b**

Table 2. A table of content of pigments in cells of model alga *Trebouxia* sp. The data represent means and standard deviation (6 reps). Averages within the each column of data, followed by the same letter code are statistically significantly different ($LSD_{0.05}$ tests).

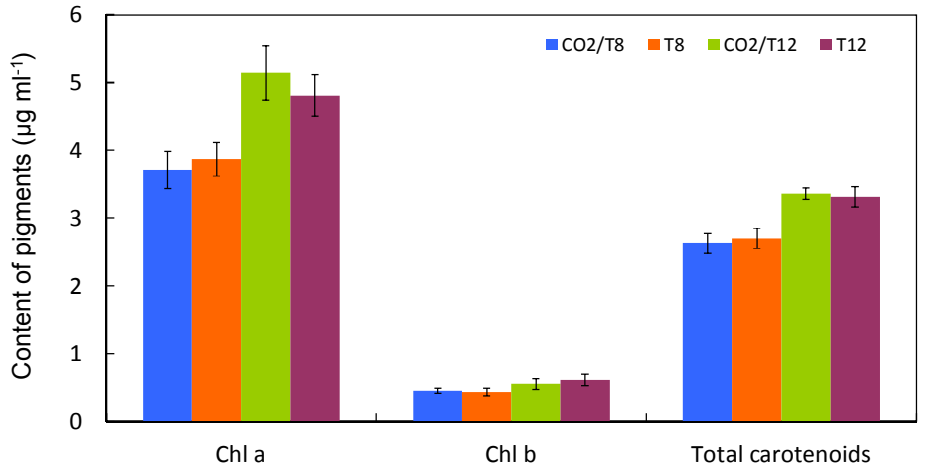

Fig. 4. Total content of pigments in cells of *Trebouxia* sp. after 12 days cultivation under specific conditions.

Evaluation of the physiological state by induced chlorophyll fluorescence

In the parameter F_V/F_M, after the 12 days experiment were observed statistically significant differences between samples, which were cultivated at different temperatures, irrespective of the concentration of CO_2. The greatest capacity of the primary photosynthetic processes was observed at 12°C with increased concentration of CO_2 (F_V/F_M = 0.44).

In the parameter Rfd, the statistically significant differences were detected between all of the tested treatment (Tab. 2). Changes in the parameter Rfd were analogical to changes in the parameter F_V/F_M. In each of treatment, the higher was F_V/F_M, the lower was Rfd. The lowest values of Rfd was recorded at 12°C with elevation of CO_2 concentration (Rfd = 0.08).

Treatment	**CO_2/T8**		**T8**		**CO_2/T12**		**T12**	
F_V/F_M	0.37±0.02	**a**	0.37±0.04	**a**	0.44±0.03	**b**	0.42±0.04	**b**
Rfd	0.19±0.03	**ab**	0.20±0.01	**b**	0.08±0.03	**c**	0.16±0.02	**a**

Table 3. A table of the fluorescence parameters, which characterized physiological state of model alga *Trebouxia* sp. The data represent means and standard deviation (6 reps). Averages within the each column of data, followed by the same letter code are statistically significantly different ($LSD_{0.05}$ tests).

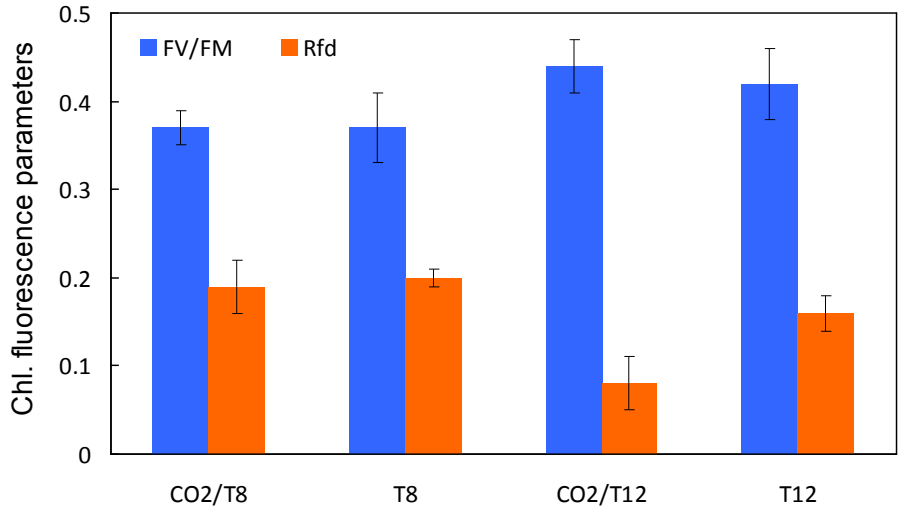

Fig. 5. Results of fluorescence analysis, which serves to characterization of the physiological state. For evaluation were used the parameters F_V/F_M and Rfd.

Discussion

Although many algae are able to utilise different sources of organic carbon, CO_2 is the main source of carbon for the majority of them under illuminated conditions (Chinnasamy et al. 2009). A model alga *Trebouxia* sp. used in the present study showed significant growth and physiological response to different temperature and elevated CO_2 concentration. Statistically significant differences were observed between the treatments in different temperature, irrespective of the concentration of CO_2. This fact indicates that as a more significant factor is reflected temperature. However, as reported Chinnasamy et al. (2009), temperature effectively regulates

various metabolic processes and the interaction between CO_2 concentration and temperature is bound to reflect in growth and physiology of algae.

The primary photochemical processes are temperature independent (Raven et Geider 1988), however the enzymatic reactions as part of the secondary processes of photosynthesis are temperature dependent. In spite of the fact that lichen symbiotic algae are adapted/acclimated to low temperature, they experience in the field during growth season, their optima of photosynthesis ranges 0.6-9.0 μmol (CO_2) m^{-2} s^{-1} as reported for a variety of species (reviewed by Palmqvist 2000). For primary photosynthetic processes, temperature optimum in lichens may reach 16-22°C as shown for *Cetraria islandica* (Hájek et al. 2001).

Therefore, it is likely, that the processes related to fixation of CO_2 were temperature inhibited at 8°C, where CO_2 had not positive effect on content of Chl *a*, carotenoids and physiological state. Moreover, although the primary photochemical processes are temperature independent, the impact of the thermal stress is thought to be primarily on the temperature-sensitive enzymes, which in turn cause a secondary photoinhibitory response in PS II (Koroleva et al. 1994). This support the opinion, that also primary photosynthetic processes were inhibited at 8°C. There is, however, a difficulty to evaluate true optimum for photosynthesis since the data from literature were gained from field, laboratory experiments as well as on a wide range of experimental set-up (lichen thalli, isolated algae, algae grown on agars, liquid growth media). All this together with species- and site-dependent differences makes it difficult to generalize temperature optima for photosynthesis. In spite of the fact that photosynthetic optimum for *Trebouxia* sp. are in the range of 12-20, the species show a resistance to shock freezing (-196°C) since 30-60% per cent of *Trebouxia* sp. may survive (Hájek et al. 2012).

Increasing concentration of CO_2 had no statistically significant effect on biomass growth. This might be attributed to a CO_2 concentrating mechanism (CCM), *i.e.* ability of *Trebouxia* to the accumulate dissolved inorganic carbon internally and thus enhance their CO_2 use efficiency (Palmqvist et al. 1997). Such mechanism, however, is much more effective in lichens that have cyanobacterial then algal partner (Badger et al. 1993). A contribution of CCM to total carbon gain in CO_2-treated *Trebouxia* is, however, unpredictable, at recent state of knowledge. Smith et Griffiths (1996), however, reported that CCM may contribute to a total carbon gain. Similarly, Raven et al. (2011) estimated an importance of CCM in algae and cyanobacteria in future, CO_2-enriched world. We may, therefore, conclude that CCM may alter CO_2 fixation efficiency in *Trebouxia* sp., the extent of such alteration is not easy to estimate. Contribution of carbon from cultivation agar to algal carbon gain can be omitted, since there was no carbon in medium in our experiment (*c.f.* Table 1). When carbon is present in cultivation medium, then it must be considered as a source for carbon metabolism of *Trebouxia* sp. due to switch from autotrophy to heterotrophy (Stocker-Worgotter et Elix 2006).

Elevated concentration of CO_2 had positive effect on physiology and content of pigments at 12°C. This facts suggest, that both photochemical and biochemical processes are more effective in such conditions. In addition, the presence of an efficient light harvesting system at higher level of CO_2 enhances the photosynthetic activity, which in turn generates more reductant and energy donor (Chinnasamy et al. 2009). This phenomenon is confirmed by results of F_V/F_M, which expressed the capacity of photochemical processes. The highest value of this parameter is just in the treatment with increased level of CO_2 at 12°C. As reported by Miyachi et al. (2003), some microalgae can grow very

rapidly at elevated CO_2 concentrations. This statement does not apply to model alga in present study. In our study, the highest biomass production of *Trebouxia* sp. was found at the treatment combining 12°C with ambient CO_2 concentration.

Our unpublished data (MS in prep.), *i.e.* simultaneously measured effective quantum yield of photosynthetic processes in PS II and photosynthetic oxygen evolution rate (OER) in response of photosynthetically active radiation, *i.e.* light-response curves, revealed that for *Trebouxia* sp. higher photosynthesis is reached in 10 than 20°C. Moreover, if unstressed, *Trebouxia* sp. showed linear Φ_{PSII} to OER relationship suggesting that under physiological conditions, primary and secondary photosynthetic processes are well coupled (Barták et al. 2013).

Conclusion

In the present study, a statistically significant difference was found in the cultivation of model species *Trebouxia* sp. at different temperature. A quality of growth and physiology were on high level at 12°C. Cultivation at elevated CO_2 concentration were a statistically insignificant, but had a positive effect at 12°C. The best growth and physiology response of all treatments were at 12°C with increased concentration of CO_2. A conclusion can be drawn that increased CO_2 in cultivation chamber does not bring any growth surplus as hypothesized. Cultivation temperature has more pronounced effect on growth characteristics of *Trebouxia* sp. The future of follow up studies will focus a detailed analysis of photosynthetic processes, *e.g.* relation of effective quantum yield of photosynthetic processes in PS II (Φ_{PSII}) to photosynthetic oxygen evolution rate (OER) under a variety of cultivation and experimental conditions.

References

AHMADJIAN, V. (1993): The lichen symbiosis. John Wiley & Sons, 264 p.

BADGER, M. R., PFANZ, H., BÜDEL, B., HEBER, U. and LANGE, O. L. (1993): Evidence for the functioning of photosynthetic CO_2-concentrating mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta*, 191: 57-70.

BADGER, M. R., PRICE, G. D. (2003): CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany*, 54: 609-622.

BARTÁK, M., VÁCZI, P., SKÁCELOVÁ, K., NÝVLT, D., MIGUEL VILUMBRALES, D., SEHNAL, L., TRNKOVÁ, K., HAZDROVÁ, J. (2013): Photosynthetic processes in two algal species from Antractic lakes in response to stress factors. Book of Abstracts. 54th Conference of the Czech Algological Society, Třeboň, Czech Republic, p. 18

BOWES, G. (1993): Facing the inevitable: plants and increasing atmospheric CO_2. *Annual Review of Plant Biology*, 44: 309-332.

CHINNASAMY, S., RAMAKRISHNAN, B., BHATNAGAR, A. and DAS, K. C. (2009): Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO_2 and temperature. *International Journal of Molecular Sciences*, 10: 518-532.

HÁJEK, J., BARTÁK, M. and GLOSER, J. (2001): Effects of thallus temperature and hydration on photosynthetic parameters of *Cetraria islandica* from contrasting habitats. *Photosynthetica*, 39: 427-435.

HÁJEK, J., VÁCZI, P., BARTÁK, M. and JAHNOVÁ, L. (2012): Interspecific differences in cryoresistance of lichen symbiotic algae of genus *Trebouxia* assessed by cell viability and chlorophyll fluorescence. *Cryobiology*, 64: 215-222.

HALLOCK, P. (2005): Global change and modern coral reefs: new opportunities to understand shallow-water carbonate depositional processes. *Sedimentary Geology*, 175: 19-33.

KOROLEVA, O. Y., BRÜGGEMANN, W. and KRAUSE, G. H. (1994): Photoinhibition, xanthophyll cycle and in vivo chlorophyll fluorescence quenching of chilling-tolerant *Oxyria digyna* and chilling-sensitive *Zea mays*. *Physiologia Plantarum*, 92: 577-584.

MIYACHI, S., IWASAKI, I. and SHIRAIWA, Y. (2003): Historical perspective on microalgal and cyanobacterial acclimation to low-and extremely high-CO_2 conditions. *Photosynthesis Research*, 77: 139-153.

MORONEY, J. V., SOMANCHI, A. (1999): How do algae concentrate CO_2 to increase the efficiency of photosynthetic carbon fixation? *Plant Physiology*, 119: 9-16.

PALMQVIST, K. (2000): Carbon economy in lichens. *New Phytologist,* 148: 11-36.

PALMQVIST, K., DE LOS RIOS, A., ASCASO, C., and SAMUELSSON, G. (1997): Photosynthetic carbon acquisition in the lichen photobionts *Coccomyxa* and *Trebouxia* (Chlorophyta). *Physiologia Plantarum*, 101: 67-76.

QIU, B., GAO, K. (2002): Effects of CO_2 enrichment on the bloom-forming cyanobacterium *Microcystis aeruginosa* (Cyanophyceae): Physiological responses and relationships with the availability of dissolved inorganic carbon. *Journal of Phycology*, 38: 721-729.

RAVEN, J. A., GEIDER, R. J. (1988): Temperature and algal growth. *New Phytologist*, 110: 441-461.

RAVEN, J. A., GIORDANO, M., BEARDALL, J. and MABERLY, S. C. (2011): Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynthesis Research*, 109: 281-296.

RIEBESELL, U., WOLF-GLADROW, D. A. and SMETACEK, V. (1993): Carbon dioxide limitation of marine phytoplankton growth rates. *Nature,* 361: 249-251.

ROHÁČEK K., SOUKUPOVÁ J. and BARTÁK M. (2008): Chlorophyll fluorescence: A wonderful tool to study plant physiology and plant stress. *In*: B. Schoefs (ed.): Plant Cell Compartments - Selected Topics. Research Signpost, Kerala, India, pp. 41-104.

ROST, B., RIEBESELL, U., BURKHARDT, S. and SÜLTEMEYER, D. (2003): Carbon acquisition of bloom-forming marine phytoplankton. *Limnology and Oceanography*, 48: 55-67.

SCHIPPERS, P., LÜRLING, M. and SCHEFFER, M. (2004): Increase of atmospheric CO_2 promotes phytoplankton productivity. *Ecology Letters*, 7: 446-451.

SMITH E. C., GRIFFITS, H. (1996): The occurrence of the chloroplast pyrenoid is correlated with the activity of a CO_2-concentrating mechanism and carbon isotope discrimination in lichens and bryophytes. *Planta*, 198: 6-16.

STOCKER-WORGOTTER, E., ELIX, J. A. (2006): Morphogenetic strategies and induction of secondary metabolite biosynthesis in cultured lichen-forming Ascomycota, as exemplified by *Cladia retipora* (Labill.) Nyl. and *Dactylina arctica* (Richards) Nyl. *Symbiosis*, 41: 9-20.

TREYDTE, K. S., SCHLESER, G. H., HELLE, G., FRANK, D. C., WINIGER, M., HAUG, G. H. and ESPER, J. (2006): The twentieth century was the wettest period in northern Pakistan over the past millennium. *Nature*, 440: 1179-1182.

WALTHER, G.-R., POST, E., CONVEY, P., MENZEL, A., PARMESAN, C., BEEBEE, T. J., FROMENTIN, J-M., HOEGH-GULDBERG, O. and BAIRLEIN, F. (2002): Ecological responses to recent climate change. *Nature*, 416: 389-395.

WELLBURN, A. R. (1994): The Spectral Determination of Chlorophylls *a* and *b* as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *Journal of Plant Physiology*, 144: 307-313.

YANG, H., XU, Z., FAN, M., GUPTA, R., SLIMANE, R. B., BLAND, A. E. and WRIGHT, I. (2008): Progress in carbon dioxide separation and capture: A review. *Journal of Environmental Sciences*, 20: 14-27.

YANG, Y., GAO, K. (2003): Effects of CO_2 concentrations on the freshwater microalgae, C*hlamydomonas reinhardtii, Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). *Journal of Applied Phycology*, 15: 379-389.

ZISKA, L. H., BUNCE, J. A. (2007): Predicting the impact of changing CO_2 on crop yields: some thoughts on food. *New Phytologist*, 175: 607-618.