Effect of temperature and increased concentration of CO\textsubscript{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\textsubscript{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\textsubscript{2} and temperature 12°C. In this treatment, potential quantum yield of photochemical processes in photosystem II (F\textsubscript{V}/F\textsubscript{M}) was 0.44 and content of Chl \textsubscript{a} was 5.14 µg ml\textsuperscript{-1}. In contrast, the lowest efficiency of primary processes was found at the treatment with addition of CO\textsubscript{2} and temperature 8°C, where value of F\textsubscript{V}/F\textsubscript{M} reached 0.37 and content of Chl \textsubscript{a} was 3.71 µg ml\textsuperscript{-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\textsubscript{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, Treydt et al. 2006). Increasing of concentration of carbon dioxide in atmosphere also contributes to stratospheric ozone depletion, which leads to increased
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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>0.25 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.175 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.075 g</td>
</tr>
<tr>
<td>MgSO₄.7 H₂O</td>
<td>0.075 g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.025 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.025 g</td>
</tr>
</tbody>
</table>

Micronutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃BO₃</td>
<td>11.42 mg</td>
</tr>
<tr>
<td>KOH</td>
<td>31.0 mg</td>
</tr>
<tr>
<td>EDTA</td>
<td>50.0 mg</td>
</tr>
<tr>
<td>FeSO₄.7 H₂O</td>
<td>4.95 mg</td>
</tr>
<tr>
<td>ZnSO₄.7 H₂O</td>
<td>8.82 mg</td>
</tr>
<tr>
<td>CuSO₄.5 H₂O</td>
<td>1.57 mg</td>
</tr>
<tr>
<td>Co(NO₃)₂.6 H₂O</td>
<td>0.49 mg</td>
</tr>
<tr>
<td>MnCl₂.4 H₂O</td>
<td>1.44 mg</td>
</tr>
<tr>
<td>MoO₃</td>
<td>0.71 mg</td>
</tr>
</tbody>
</table>

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 CO₂ (850±50 ppm, checked by a EGM-2 gas monitoring system, PP Systems, UK) and two dishes served as control (ambient CO₂ 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.
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, samples were centrifuged (5 min. at 10 000 g). The pigment contents were determined spectrophotometrically (Specord 205, Analytic Jena, Germany) according to equations:

Chlorophyll-a
\[ Ca = 12.47 \times A_{665.1} - 3.62 \times A_{649.1} \]  
Eqn. 1

Chlorophyll-b
\[ Ch = 25.06 \times A_{649.1} - 6.5 \times A_{665.1} \]  
Eqn. 2

Total carotenoids
\[ Cx+c = \frac{(1000 \times A_{480} - 1.29 \times Ca - 53.78 \times Ch)}{220} \]  
Eqn. 3

Measurement of chlorophyll fluorescence

Chl fluorescence parameters were measured on control and samples cultivated in CO₂. Measurement of Chl fluorescence was carried out by the method of Chl fluorescence imaging by FluorCam (PSI, Czech Republic). Used fluorescence parameters (Fᵥ/Fₘ 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%).
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-
between the treatments at 8°C and 12°C with ambient CO₂ concentration. However, statistically significantly differences were not detected between the treatments at 8°C and 12°C with elevated CO₂ concentration. The highest amount of dry weight was observed in treatment at 12°C with ambient CO₂ 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 CO₂, namely 3.23 mg.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl a (µg ml⁻¹)</th>
<th>Chl b (µg ml⁻¹)</th>
<th>Carotenoids (µg ml⁻¹)</th>
<th>Biomass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂/T8</td>
<td>3.71±0.27</td>
<td>0.45±0.04</td>
<td>2.63±0.15</td>
<td>3.30±0.49</td>
</tr>
<tr>
<td>T8</td>
<td>3.87±0.25</td>
<td>0.43±0.05</td>
<td>2.70±0.15</td>
<td>3.23±0.50</td>
</tr>
<tr>
<td>CO₂/T12</td>
<td>5.14±0.40</td>
<td>0.55±0.08</td>
<td>3.36±0.09</td>
<td>3.73±0.45</td>
</tr>
<tr>
<td>T12</td>
<td>4.81±0.30</td>
<td>0.61±0.08</td>
<td>3.31±0.15</td>
<td>3.82±0.33</td>
</tr>
</tbody>
</table>

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 (LSD₀.₀⁵ tests).

![Graph](image)

**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 Fₖ/F₉, after the 12 days experiment were observed statistically significant differences between samples, which were cultivated at different temperatures, irrespective of the concentration of CO₂. The greatest capacity of the primary photosynthetic processes was observed at 12°C with increased concentration of CO₂ (Fₖ/F₉ = 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 analogous to changes in the parameter Fv/FM. In each of treatment, the higher was Fv/FM, the lower was Rfd. The lowest values of Rfd was recorded at 12°C with elevation of CO2 concentration (Rfd = 0.08).

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 (LSD0.05 tests).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO2/T8</th>
<th>T8</th>
<th>CO2/T12</th>
<th>T12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fv/FM</td>
<td>0.37±0.02</td>
<td>a</td>
<td>0.37±0.04</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>0.44±0.03</td>
<td>b</td>
<td>0.42±0.04</td>
<td>b</td>
</tr>
<tr>
<td>Rfd</td>
<td>0.19±0.03</td>
<td>ab</td>
<td>0.20±0.01</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>0.08±0.03</td>
<td>c</td>
<td>0.16±0.02</td>
<td>a</td>
</tr>
</tbody>
</table>

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

Discussion

Although many algae are able to utilise different sources of organic carbon, CO2 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 CO2 concentration. Statistically significant differences were observed between the treatments in different temperature, irrespective of the concentration of CO2. 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 CO₂ 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 (CO₂) 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 CO₂ were temperature inhibited at 8°C, where CO₂ 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 CO₂ had no statistically significant effect on biomass growth. This might be attributed to a CO₂ concentrating mechanism (CCM), i.e. ability of Trebouxia to the accumulate dissolved inorganic carbon internally and thus enhance their CO₂ 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 CO₂-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, CO₂-enriched world. We may, therefore, conclude that CCM may alter CO₂ 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 CO₂ 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 CO₂ enhances the photosynthetic activity, which in turn generates more reductant and energy donor (Chinnasamy et al. 2009). This phenomenon is confirmed by results of Fₐ/Fₘ, which expressed the capacity of photochemical processes. The highest value of this parameter is just in the treatment with increased level of CO₂ at 12°C. As reported by Miyachi et al. (2003), some microalgae can grow very
rapidly at elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂. A conclusion can be drawn that increased CO₂ 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


