# Effect of Increased $CO_2$ And Temperature on Growth, Photosynthesis And Lipid Content of Tropical Algae

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**ABSTRACT** Increasing temperatures causally linked with anthropogenically elevated atmospheric carbon dioxide ($CO_2$) levels characterise current 'global warming'. Very little is known about how these factors will affect tropical microalgae. The aim of this study was to investigate the $CO_2$ and temperature sensitivity of the photosynthetic process of two tropical *Chlorella* strains (*Chlorella vulgaris* UMACC 001 and *Chlorella vulgaris* UMACC 014). We grew cultures of the two strains under four conditions: (1) 28°C and 375 ppm $CO_2$ (control), (2) 28°C and 750 ppm $CO_2$ (high $CO_2$), (3) 32°C and 375 ppm $CO_2$ (high temperature), and (4) 32°C and 750 ppm $CO_2$ (combined factors). Based on specific growth rate (μ), both *Chlorella* strains grew best under the combined factors of high $CO_2$ level (750 ppm) and high temperature (32°C). Elevated $CO_2$ stimulated the production of lipid in both strains.

(**Keywords:** *Chlorella*, temperature, $CO_2$, photosynthesis )

## INTRODUCTION

Anthropogenic changes in the global environment, such as global warming and ozone depletion, have been particularly striking during the last two decades [1]. Average global temperatures have been and will continue to increase under the influence of greenhouse gases such as carbon dioxide ($CO_2$), nitrous oxide ($N_2O$), ozone ($O_3$), methane ($CH_4$) and chlorofluorocarbons (CFCs) in the atmosphere. Atmospheric $CO_2$ levels have increased from around 270 ppm before the start of the Industrial Revolution in the early Nineteenth Century, to over 391 ppm at present [2]. If no effective action is taken to suppress the emission of atmospheric $CO_2$, the most likely scenario is for a further two- to three-fold increase in atmospheric $CO_2$ concentration over the next century, attaining values between 750 and 1000 ppm by 2100 [1, 3, 4]. IPCC [1] concluded that, over the last 100 years (1906 – 2005), global mean surface temperatures (over land and sea surface temperature) have risen by 0.74°C ± 0.18°C as a result of the increasing $CO_2$ concentration. The widely used IPCC SRES B1 scenario predicts global temperatures to increase by 1.8°C over the next century, while even this scenario appears to be conservative in the face of accumulating evidence of more rapid changes [1]. According to Sarmiento *et al.* [5], global temperatures are expected to increase ~ 4°C over this period.

Microalgae play an important role in both terrestrial and marine ecosystems. They are primary producers accounting for more than half of the total primary production at the base of the food chain worldwide [6]. The cosmopolitan microalgal genus *Chlorella* includes small coccoid green algae and is one of the best studied photosynthetic eukaryotes. *Chlorella* species also have potential applications as dietary supplements in human nutrition [7] and have been widely used for a variety of applications in biotechnology such as waste water treatment [8], production of antioxidant compounds [9] and biofuel [10].

There is rapidly increasing interest in the responses of organisms exposed to a variety of stressors as a result of changes in environmental conditions, such as thermal stress, exposure to UVR, or elevated $CO_2$ [3, 11, 12]. However, although various impacts of climate change on microalgae have been widely studied, studies of stressors

acting in combination, such as $CO_2$ and temperature increase, are rare, especially in tropical microalgae. Therefore, the aim of this study was to investigate the physiological and biochemical responses of tropical *Chlorella* to the combination of $CO_2$ and temperature increase.

## MATERIALS AND METHODS

### Algae cultures

The microalgae used in the present study were obtained from the University of Malaya Algae Culture Collection (UMACC). *Chlorella vulgaris* UMACC 001 was isolated from a pond at the experimental farm of the University of Malaya, while *Chlorella vulgaris* UMACC 014 was isolated from Institute of Graduate Studies (IGS) farm of the University of Malaya. The cultures were grown in the laboratory as stock batch cultures in Bold's Basal Medium (BBM) and maintained in a controlled environment incubator at 28°C, with cool white fluorescent lamps (90 µmol $m^{-2}s^{-1}$) on a 12:12 h light-dark cycle.

### Experimental design

The temperatures used in this study were 28 and 32°C, simulating the typical current ambient temperature and the suggested increase of ~ 4°C over the next century. At each temperature, triplicate flasks were equilibrated with two different $CO_2$ concentrations: 375 ppm (close to current average atmospheric concentration) and 750 ppm (IPCC projected 2100 concentration). Continuous gentle bubbling of gas into the incubation system maintained the $CO_2$ concentration.

### Growth measurements

The inoculum (10%) used was obtained from exponential phase cultures standardised at an optical density at 620 nm ($OD_{620}$) of 0.2. The cultures were grown in 500 mL flasks and subjected to elevated $CO_2$ and temperature as described above. Growth was monitored based on two parameters: $OD_{620}$ and chlorophyll-*a* (chl-*a*) concentration. Chl-*a* concentration was determined by spectrophotometry after extraction of the filtered cells (glass-fibre filters, 0.45 µm) in acetone [13]. Specific growth rate (µ, $d^{-1}$) based on chl-*a* concentration was calculated using the following formula:

$$\mu\ (d^{-1}) = (\mathrm{Ln}\ N_2 - \mathrm{Ln}\ N_1)/(t_2 - t_1)$$

where $N_2$ is $OD_{620}$ at $t_2$, $N_1$ is $OD_{620}$ at $t_1$, and $t_2$ and $t_1$ are times within the exponential phase. The cells were harvested at the end of the experiment by filtration for determination of dry weight and the extraction of lipids.

### Dry weight determination

Blank glass-fibre filters (Whatman GF/C, 0.45 µm) were dried in a forced-air oven at 100°C for 24h. A known volume of the algal culture was filtered on a pre-weighed filter. The filter was placed in the oven and dried at 100°C for 24h. The algal dry weight (DW) was determined using the following equation:

$$\mathrm{DW\ (mg\ L^{-1})} = \frac{\text{[Weight of filter with algae (mg)]–[Weight of blank filter (mg)]}}{\text{Volume of algal culture (L)}}$$

### Lipid extraction

Lipids were extracted in methanol-chloroform-water (2:1:0.8) and the total content determined by gravimetry [14].

### Measurement of Photosynthetic Parameters

Chlorophyll fluorescence was measured using an Underwater Fluorometer Diving-PAM (Heinz Walz GmbH, Effeltrich, Germany). Before measurement, all the samples were dark-adapted for 15 min. The processing software generates values for minimum ($F_o$) and maximum ($F_m$) fluorescence, from which variable fluorescence ($F_v$) was calculated as $F_m - F_o$. The maximum effective quantum yield of PSII, $\Phi_{PSII}$, was then calculated as $F_v / F_m$. In addition, nine PAR irradiances, from dark to 1558 µmol photons $m^{-2}\ s^{-1}$ were applied sequentially with a duration of 60s at each level to produce a rapid light curve [15], and the fit parameters, α (the initial slop of the light curve proportional to the quantum yield, i.e. photosynthetic efficiency), $rETR_{max}$ (the maximum relative electron transport rate) and $E_k$ (the photoadaptive index) for each light curve were determined. All measurements were made in a darkened room.

### Data analysis

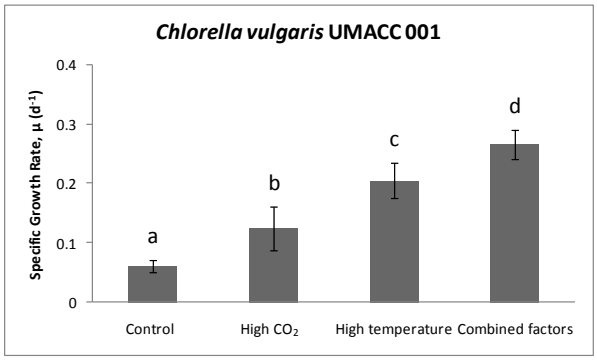
The data were analysed using one-way ANOVA followed by comparison of means using Newman-Keuls test (Statistica software, Version 5.0). Differences were considered significant at $p < 0.05$.

## RESULTS

### Growth

Specific growth rates (μ) of *Chlorella vulgaris* UMACC 001 and *Chlorella vulgaris* UMACC 014 under the four $CO_2$ and temperature conditions are shown in **Figure 1**. There was a significant increase in μ when *C. vulgaris* UMACC 001 was grown under elevated $CO_2$ or elevated temperature alone, or both variables were combined, as compare to μ measured under control conditions. However, no significant difference in the μ of *C. vulgaris* UMACC 014 was observed when the culture was grown under control and elevated temperature conditions. Both *Chlorella* strains showed similar growth trends when subjected to combined factors of elevated $CO_2$ and elevated temperature. The highest μ values were obtained when the two *Chlorella* strains were grown under these conditions.

(a)




(b)

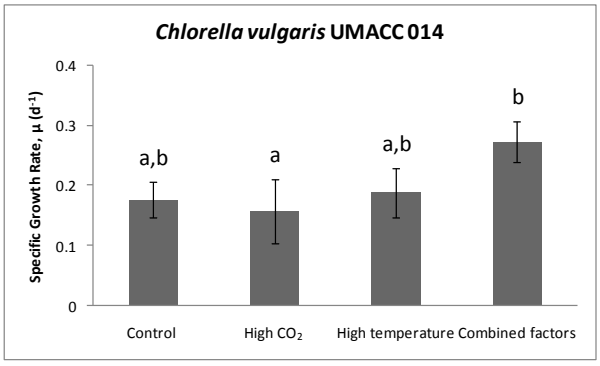
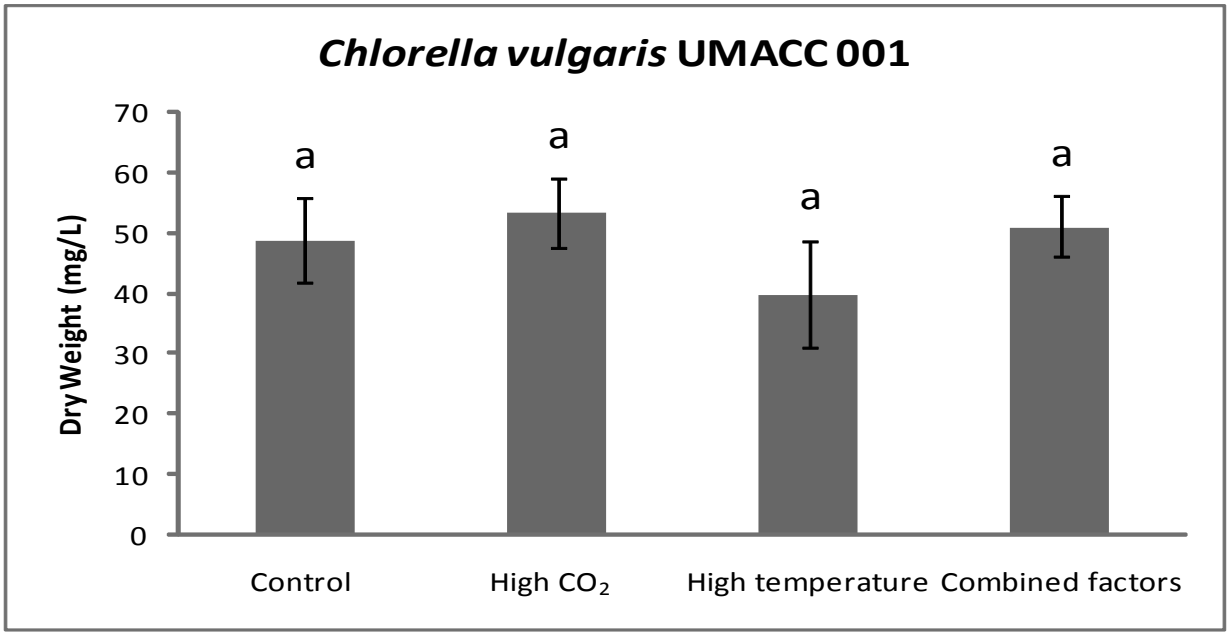



**Figure 1.** Specific growth rates (μ) based on chl-*a* concentration of a) *Chlorella vulgaris* UMACC 001, and b) *C. vulgaris* UMACC 014 grown under four different sets of experimental conditions. Data represent mean values of triplicate cultures and error bars are standard deviations. Different letters indicate significant differences at $p < 0.05$.

(a)



(b)

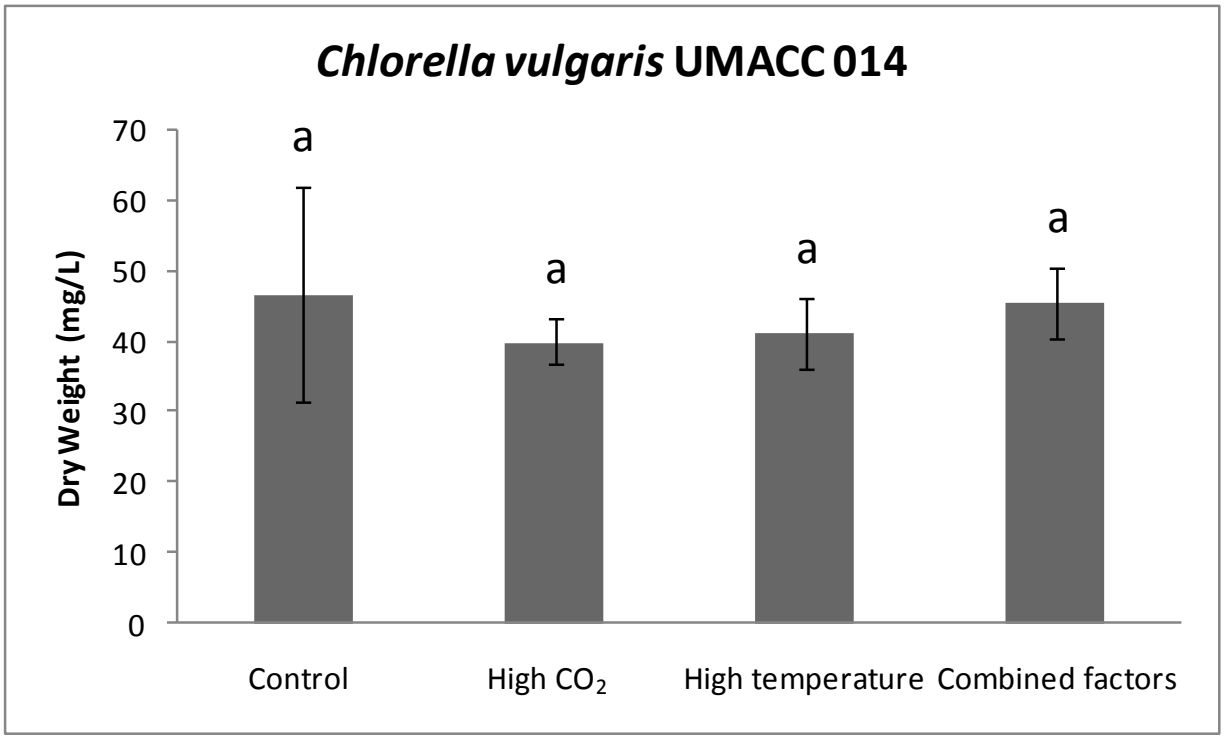
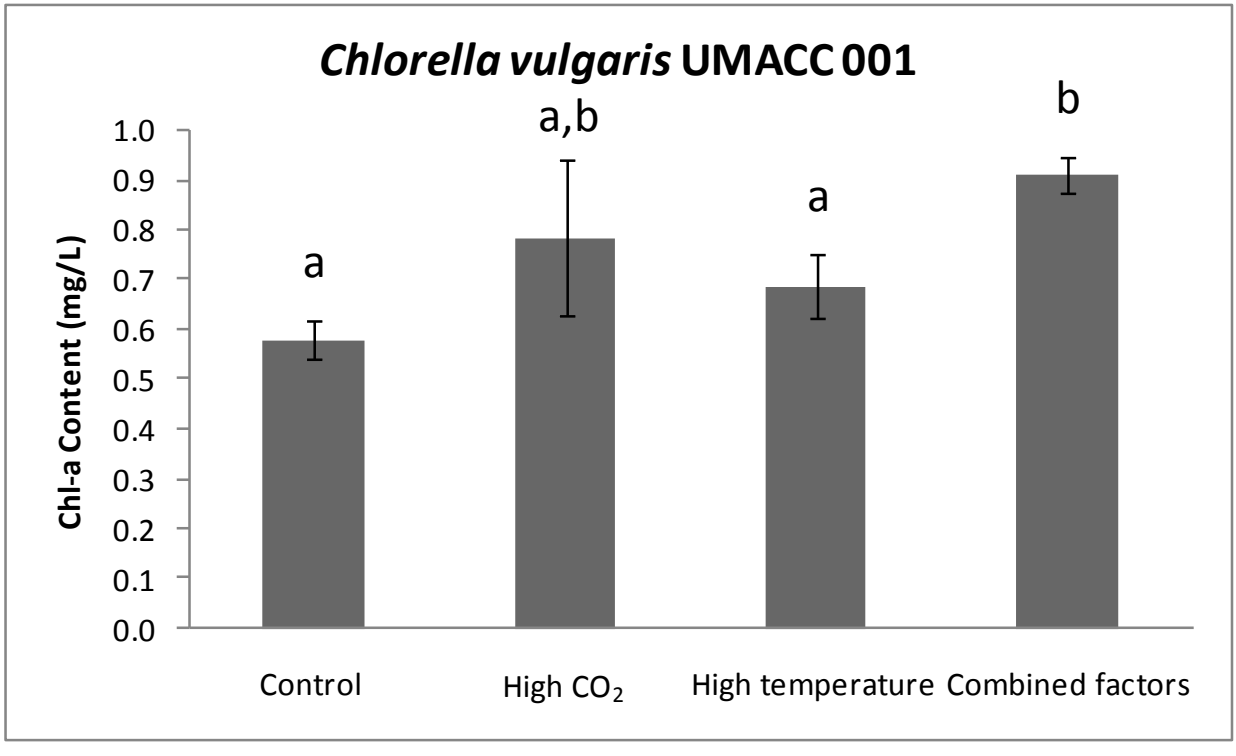



**Figure 2.** Final biomass (dry weight, mg/L) of a) *Chlorella vulgaris* UMACC 001, and b) *C. vulgaris* UMACC 014 grown under the four experimental conditions. Data represent mean values of triplicate cultures and error bars are standard deviations. Different letters indicate significant differences at $p < 0.05$.

(a)



(b)

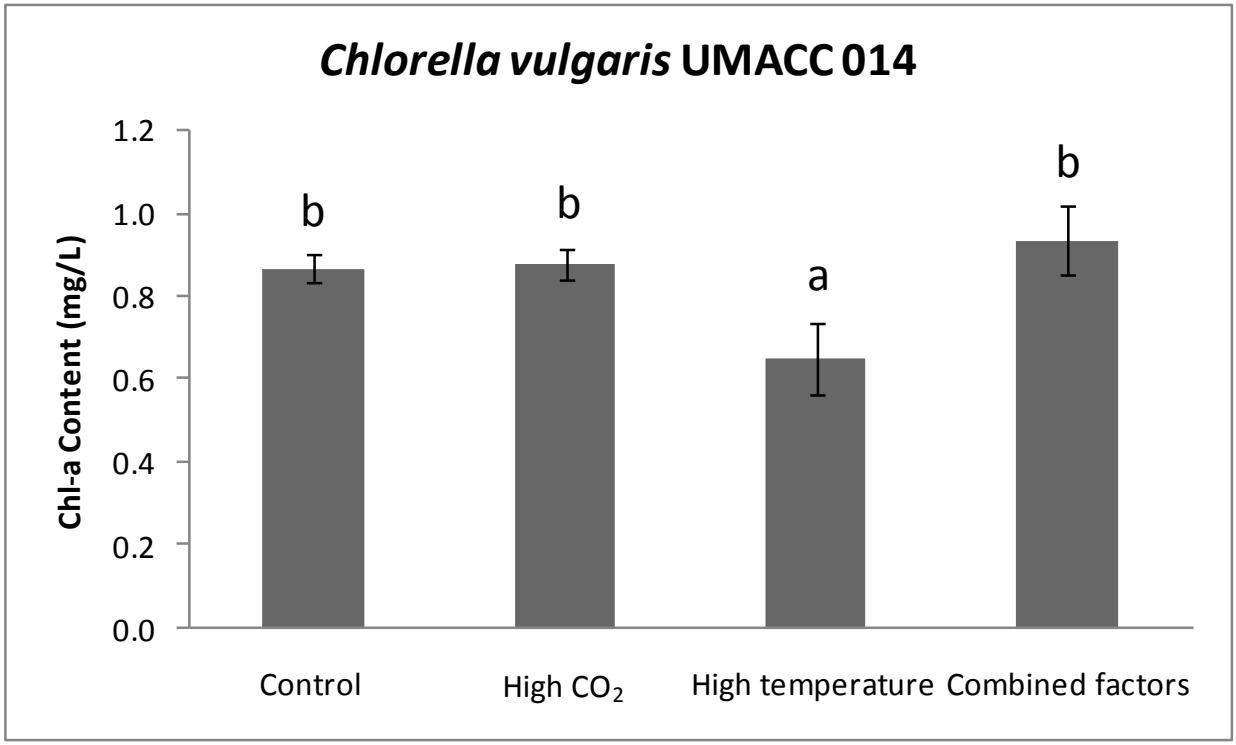



**Figure 3.** Final chl-*a* content (mg/L) of a) *Chlorella vulgaris* UMACC 001, and b) *C. vulgaris* UMACC 014 grown under the four experimental conditions. Data represent mean values of triplicate cultures and error bars are standard deviations. Different letters indicate significant differences at $p < 0.05$.

Overall, there was no significant difference in the final biomass attained by the microalgae when grown under the different conditions (**Figure 2**). Under elevated $CO_2$ and temperature conditions, there was a significant increase in the chl-*a* content of *C. vulgaris* UMACC 001 compared to those grown at other conditions (**Figure 3**). In contrast, the lowest chl-*a* content was observed in *C. vulgaris* UMACC 014 when the culture was grown under elevated temperature conditions.

## Lipid Content

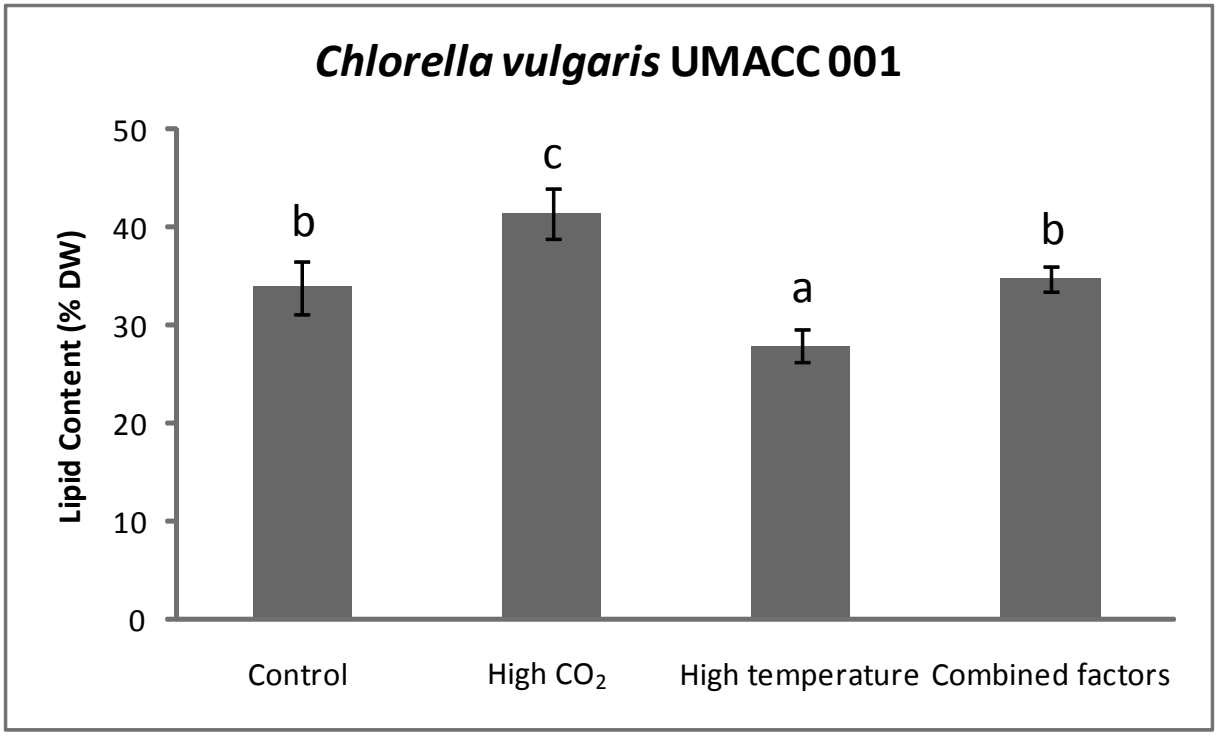
Effects of $CO_2$ and temperature on lipid content varied between the two strains (**Figure 4**). Elevated temperature caused a significant decrease in the lipid content of *C. vulgaris* UMACC 001 compared to those at other conditions, and a similar but non-significant trend was also observed in *C. vulgaris* UMACC 014. However, no significant difference in lipid content was found for the algae grown under control and high temperature conditions. In comparison, elevated $CO_2$ stimulated the production of the highest lipid content for *C. vulgaris* UMACC 001 and *C. vulgaris* UMACC 014 (41.48 % DW and 38.83 % DW, respectively).

## Photosynthesis

Maximum quantum yield ($F_v/F_m$) of *C. vulgaris* UMACC 001 ranged between 0.60 and 0.66. $F_v/F_m$ was significantly lower under the high temperature treatment compared to values obtained under other conditions (**Table 1**). In comparison to *C. vulg*aris UMACC 001, higher $F_v/F_m$ was obtained by *C. vulgaris* UMACC 014 when grown under each of the four different conditions. Increases in either $CO_2$ or temperature alone, or both factors in combination, increased $F_v/F_m$ in comparison with the control. No significant difference in photosynthetic efficiency (α) was observed in *C. vulgaris* UMACC 001 grown under the four different conditions (Table 1). Elevated $CO_2$, elevated temperature or the factors in combination led to significantly higher α in *C. vulga*ris UMACC 014.

Elevated $CO_2$ and temperature led to significantly higher $rETR_{max}$ in *C. vulgaris* UMACC 001 (Table 1). However, there was no overall difference in $rETR_{max}$ between samples of *C. vulgaris* UMACC 014 exposed to the four different conditions. There were no significant differences in the photoadaptive index ($E_k$) of either strain when exposed to the four different conditions (Table 1).

(a)




(b)

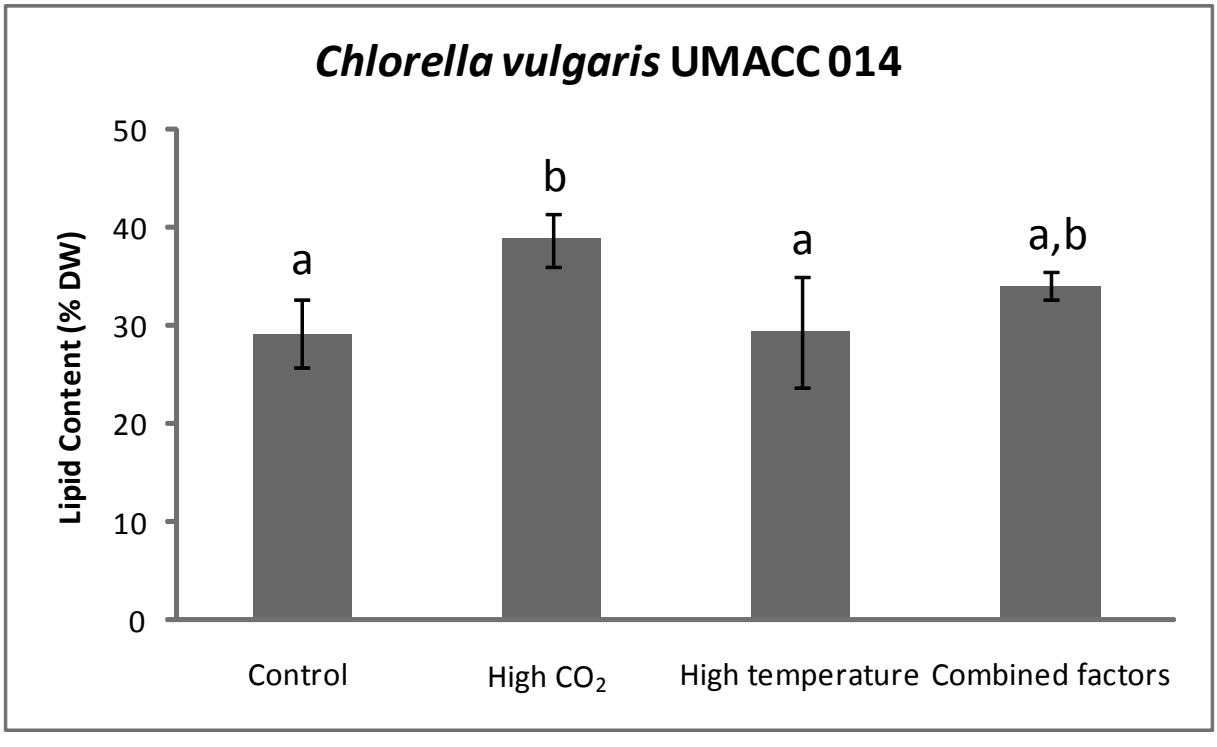



**Figure 4.** Lipid content (% DW) of a) *Chlorella vulgaris* UMACC 001, and b) *C. vulgaris* UMACC 014 grown under the four experimental conditions. Data represent mean values of triplicate cultures and error bars are standard deviations. Different letters indicate significant differences at $p < 0.05$.

**Table 1.** Statistical comparison of functional photosynthetic performance parameters of *Chlorella vulgaris* UMACC 001 and *C. vulgaris* UMACC 014 grown under the four experimental conditions. Data represent mean values and standard deviation of triplicate cultures. Different letters indicate significant differences at $p < 0.05$.

| | $F_v/F_m$ | $rETR_{max}$ (μmol electrons $m^{-2}s^{-1}$) | α | $E_k$ (μmol photons $m^{-2}s^{-1}$) |
|---|---|---|---|---|
| *C. vulgaris* UMACC 001 | | | | |
| Control | $0.659 \pm 0.034^{b}$ | $274.845 \pm 12.575^{a}$ | $0.584 \pm 0.018^{a}$ | $470.750 \pm 7.966^{a}$ |
| High $CO_2$ | $0.656 \pm 0.011^{b}$ | $300.198 \pm 14.980^{a,b}$ | $0.548 \pm 0.044^{a}$ | $550.507 \pm 50.817^{a}$ |
| High temperature | $0.599 \pm 0.025^{a}$ | $331.482 \pm 42.750^{a,b}$ | $0.555 \pm 0.048^{a}$ | $605.187 \pm 132.852^{a}$ |
| Combined factors | $0.653 \pm 0.035^{b}$ | $346.795 \pm 16.631^{b}$ | $0.579 \pm 0.030^{a}$ | $601.098 \pm 54.926^{a}$ |
| *C. vulgaris* UMACC 014 | | | | |
| Control | $0.625 \pm 0.020^{a}$ | $266.304 \pm 9.255^{a}$ | $0.568 \pm 0.031^{a}$ | $469.396 \pm 28.073^{a}$ |
| High $CO_2$ | $0.668 \pm 0.017^{b}$ | $271.908 \pm 14.530^{a}$ | $0.613 \pm 0.015^{b}$ | $444.110 \pm 34.347^{a}$ |
| High temperature | $0.690 \pm 0.010^{b,c}$ | $263.587 \pm 10.245^{a}$ | $0.626 \pm 0.008^{b}$ | $420.947 \pm 19.439^{a}$ |
| Combined factors | $0.703 \pm 0.003^{c}$ | $275.551 \pm 16.054^{a}$ | $0.633 \pm 0.021^{b}$ | $436.375 \pm 39.640^{a}$ |

## DISCUSSION

In this study two *Chlorella* strains were exposed to current and predicted future concentrations of $CO_2$ (375 and 750 ppm, respectively) in a two-way combination with ambient and elevated temperatures (28 and 32ºC) for 48 h. Although the two strains had the same tropical region origin, subtly different responses were observed to the experimental manipulations. Elevated $CO_2$ or elevated temperature alone, or the two factors combined, caused a marked increased in μ of *C. vulgaris* UMACC 001. This is consistent with previous findings on the algal genus *Synechococcus* when exposed to similar experimental conditions [16]. Conversely, the μ of *C. vulgaris* UMACC 014 only increased under the combined factors of elevated $CO_2$ and temperature. Our data and those of Fu *et al.* [16] demonstrate that the effects of predicted climatic changes in stimulating the growth of microalgae are likely to be both species-specific, and even strain-specific within species.

The highest values of μ were obtained in both *Chlorella* strains when grown under a combination of elevated $CO_2$ and temperature. This suggests that the predicted climate scenario modelled here will be beneficial to the growth of the two microalgae, as has also been reported in studies of *Synechococcus* [16] and *Heterosigma akashiwo* [17] grown under similar conditions. Sukenik *et al.* [18] also suggested that predicted further increases in atmospheric $CO_2$ and temperature would interact synergistically to increase algal production relative to current conditions. However, only two environmental factors were considered in this study and, in the natural environment, many others abiotic and biotic factors are involved and their interactions are complex [17, 19, 20]. Over the relatively short duration of this study the net production (final biomass) of the two microalgae was not significantly affected by either $CO_2$ concentration or temperature, again as reported in *Heterosigma akashiwo* and *Prorocentrum minimum* [17].

Increasing atmospheric $CO_2$ concentration is commonly predicted to lead to changes in biochemical composition of microalgae [21, 22]. Both studied *Chlorella* strains produced significantly higher amounts of lipid when exposed to elevated $CO_2$. This is consistent with the study of Widjaja [21], who reported that the lipid content of *C. vulgaris* increased with increasing $CO_2$ concentration. As microalgae form the basis of the food chain, changes in biochemical composition, including lipids, can alter their nutritional value, with consequences throughout the food chain.

Maximum quantum yield ($F_v/F_m$) is a parameter used to indicate photosynthetic stress in organisms such as plants and microalgae. Different classes of algae typically have different $F_v/F_m$ values ranging between 0.60 and 0.80. In the present study, the $F_v/F_m$ values measured in the two *Chlorella* strains were between 0.60 and 0.70, indicating that the cultures were healthy and not under stress when exposed to the four different experimental treatments. The increased photosynthetic efficiency (α) under increased $CO_2$ or temperature conditions in *C. vulgaris* UMACC 014 suggests that future predicted conditions will be favourable for this microalga. Increased maximum α and $rETR_{max}$ were noted in *C. vulgaris* UMACC 014 when grown under combined conditions, but neither $rETR_{max}$ nor photoadaptive index ($E_k$) were influenced by the $CO_2$ concentration or increased temperature when applied separately.

Based on the growth rates and photosynthetic parameters measured in this study, we conclude that the combined factors of elevated atmospheric $CO_2$ concentration and elevated temperature widely predicted under future climate scenarios will be favourable to both tropical *Chlorella* strains, and will enhance their growth. However, this is a relatively simple and short-term experimental approach. It is important also to consider both responses to a wider range of environmental variables acting in synergy, and the possible adaptive mechanisms and acclimatory processes available to the microalgae. Longer-term and multi-factorial experiments are required in order to better address these possibilities and improve predictions of the response of microalgae to future climate change.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding from the University of Malaya Research Grant No (UMRG, RG 098/10sus). We would like to thank Prof. Dr. Peter Convey for his comments which helped to strengthen the manuscript.

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