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Response of Microalgae Growth and Cell Characteristics to Various Temperatures

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Microalgae are considered to be a promising alternative feedstock for next generation biofuels because of their rapid photosynthetic growth rates and less impact on land-use. In this study, we employed a series of laboratory microcosm experiments to explore effects of temperature on growth and cell characteristics of 12 species. The research results indicted a wide range of responses of population demographic rates and physiology of phytoplankton cells to temperature. Chlorophyta could grow at a wide range of temperature from 15 to 35 ºC, with the optimum temperature of 20 to 30 ºC, mainly around 25 ºC; whereas cyanobacteria had a higher tolerance to high temperature than chlorophyta, the optimum temperature for growth was between 30 and 35 ºC. At the optimum temperature, most species (mainly large-sized green algae) generally showed grow rapidly with slightly high lipid and chlorophyll a contents in algal cell, whereas chlorophyll a content in algal cell was not completely determined by algal size and volume. In conclusion the study identifies key tradeoffs among functional traits to determine the suitability of diverse algal species as biofuel feedstock, further to provide scientific basis on screening successfully the potential candidate species for use in bio-oil production under temperature stress.

Keywords: Microalgae, Temperature, Biofuel, Cell characteristics, Cyanobacteria, Chlorophyta.

INTRODUCTION

Population growth together with increased motorization has led to an overwhelming increase in the worldwide demand for fuel[1,2]. Petroleum reserves are globally finite and indeed peak oil production may already have occurred[3,4], so efforts to develop alternative sustainable energy sources are under aggressive global development. Recently, biodiesel has received more attention due to the fact that it is a renewable, biodegradable and environmentally friendly fuel. Current sources of commercial biodiesel are primarily soybean oil, rapeseed oil, palm oil, corn oil, waste cooking oil and animal fat. However, these sources of biodiesel cannot realistically meet even a small fraction of the existing demand for transportation fuels. Microalgae have become one of the most promising feedstocks for biodiesel production due to their widespread availability and high oil content which are two important characters of ideal candidate species of microalgae[5]. Moreover, microalgae, in contrast to traditional oilseed crops, can grow in ponds, wastewater or fomenters and thus avoid occupying land required for crops and forests[6].

As we know, the growth and production of lipids in cell of microalgae are affected by many environmental conditions such as light, temperature, salinity and pH[7,8], nutrients and CO_2 concentration[9,10]. The temperature of the culture could influence markedly the growth of the microorganisms not only by influencing the cell growth and death but also, indirectly, by affecting CO_2, O_2 and solubility within the medium[11]. Moreover, some studies found changes in cell characteristics including cell size, cell volume and cell density, *etc*. under different conditions would have a critical effect on cellular lipid yield and composition[8,12]. Our recent research which focused on the impact of light and nutrient treatments on the growth and lipid content of different algae also found large-celled species (primarily green algae) grew rapidly and reached high asymptotic biomass; and the most lipid rich species had low N and P but high minimum light requirements and were mostly small and slow growing. Whereas compared to light and nutrient condition, study reports about temperature effecting on the growth especially on the cell characteristics are still few.

One of the major difficulties with algae production in traditional raceways is the difficulty of maintaining a suitable

environment for the algal culture, particularly its temperature[13,14]. Open raceway algae production systems have diurnal and seasonal temperature variations, which may lead to undesirable conditions for algal growth[15]. Therefore, in this study, we try to employ laboratory microcosm experiments to mainly investigate the effects of temperature on the growth and cell characteristics of diverse microalgae by measuring biomass, special growth rate (r), algal cell density, cell size, cell volume, contents of chlorophyll-a and neutral lipids in algal cell of 12 species cultured across ranges of temperature and furthermore to explore the influence mechanism of thermal limits to microalgae growth and oil yield. Finally to infer the optimum temperature for diverse microalgae growth and screen the best energy microalgae under different temperature condition.

EXPERIMENTAL

Source and pre-culture of species: The microalgae used in this experiment were obtained from San Diego center for algae biotechnology in University of California at San Diego, USA. Experimental algae involves three cyanobacteria species including *Synechococcous elongatus* PCC 7942 (SYEL), *Synechocytis* sp. PCC 6803 (SYSP), *BLO 902* (B902) and nine chlorophyta species including *Scenedesmus obliquus* UTEX 393(SCOB), *Scenedesmus dimorphus* UTEX 1237(SCDI), *Chlorella minutissima* UTEX 2219(CHMI), *Chlorella minutissima* UTEX 2219(CHVU), *Chlamydomonas reinhardtii* (CHRE), *Chlorococcoum* oleabundans (CHOL), *Tetraselmis sp.* UTEX LB 2767(TESP), *Nannochloropsis oculata* UTEX LB 2164(NAOC), *BLO 910* (B910).

Pre-culture: Twelve species stock cultures were maintained in 250 mL Erlenmeyer flasks containing 100 mL WC medium[16] closed with a cellulose stopper. The flasks were placed at 20 ºC in 50 ± 5 µmol photons m^{-2} s^{-1} provided in a 12:12 h light-dark cycle. Prior to the experiment, the microalgae were acclimatized to the experimental conditions. Each culture was transferred to clean 50 mL Erlenmeyer flasks that contained 20 mL medium. The initial species concentration in each flask was 1 µg chlorophyll a L^{-1} for cyanobacteria and 3 µg chlorophyll a L^{-1} for eukaryotes, which was determined using *in vivo* fluorescence in a Turner Trilogy fluorometer. These flasks were placed in incubators for 15 days at the same light and temperature regimes that would be used during the experiment. Flasks were shaken manually twice every day.

Experimental design: Each per-cultured species was cultured in monoculture at six levels of temperature including 10, 15, 20, 25, 30 and 35 ºC with five replicates per treatment. Nutrient supply was always WC medium[27]; the light supply level was 50 ± 5 µmol photons m^{-2} s^{-1} PAR for eukaryotes and 15 ± 5 µmol photons m^{-2} s^{-1} µmol PAR for cyanobacteria. The experiment was performed in 50 mL Erlenmeyer flasks containing 20 mL using WC medium and a dilution rate of 20 %/day (renewed every two days). Growth kinetics was monitored every other day as chlorophyll-a concentration and the experiments lasted until the species reached steady state in a majority of treatments (15 to 21 days).

Samples were collected at the end of each growth experiment to analyze for neutral lipid content, cell number, cell size of the algal biomass. The Nile Red method was used to measure neutral lipid content according to the methods of Chen *et al.*[17]. Cells per replicate were counted in a hemacytometer and fifty cells for each alga were measured microscopically with an imaging system (Olympus CX51, Japan). The cell volumes were calculated using a set of geometric shapes and mathematical equations for biovolume calculations[18].

Data statistics and analysis: According to the chlorophyll-66 density measured, growth curves were gained which were used to exponential growth rate (r) and critical minimum limiting thresholds for temperature[19]. Statistical analyses were done with the software SPSS and R studio, mainly using one-way and two-way analysis of variances (ANOVA), the level of significance at $p < 0.05$ or 0.001. All data were reported as means.

RESULTS AND DISCUSSION

Effect of temperature on the growth of microalgae: There were positive correlations between growth rate and culture temperature in cyanobacteria B902, *Synechocytis* sp. and *S. elongatus* (r^2 = 0.897, 0.964, 0.817, respectively, $p <$ 0.05 in all cases) (Fig. 1), with the highest chlorophyll a content reaching 199.678 µg L^{-1} in *Synechocytis* sp. at 30 ºC (Fig.2); but differences were showed in nine chlorophyta species, there were non-linear relationships between temperature and growth rate, with maximum growth rate within temperature range of 20-30 ºC, highest chlorophyll density reaching 1985.892 µg L^{-1} in C. *reinhardtii* at 25 ºC. Also the optimum growth temperatures of cyanobacteria and chlorophyta were found in this study (Fig. 1). As for cyanobacteria, the optimum temperature for growth varied between 30 and 35 ºC, in contrast to low temperature, the species showed better growth advantage at high temperature (> 25 ºC) (NAOVA, $p < 0.05$). For chlorophyta, the optimum temperature range for growth was 20-30 ºC, mainly around 25 ºC. Mean time, it's observed that *C. oleabundans* showed the highest exponential growth rate among tested all species (r = 4.017 ± 0.373, t = 25 ºC). Compared to other species, chlorophyta *C. reinhardtii*, *S. obliquus*, *S. dimorphus* and *Tetraselmis* sp. always maintained better growth rates through the culture period at each of temperature.

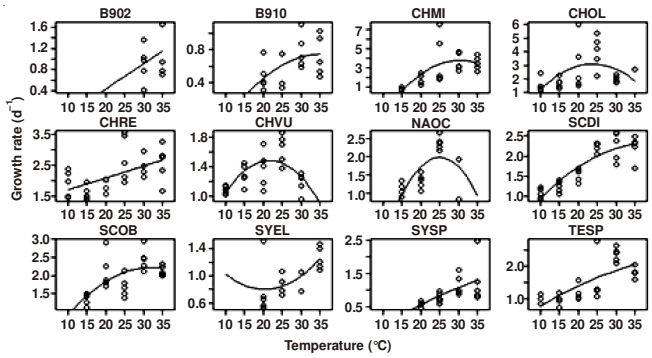

Fig. 1. Comparison of growth rate (r) in diverse species at different temperatures

Effect of temperature on cell characteristics of microalgae: Similarly to the growth trend, there were positive correlations between algal cell density and culture temperature in cyanobacteria B902, *Synechocytis* sp. and *S. elongatus* (r^2 = 0.53, 0.687, 0.657, respectively, $p < 0.05$ in all cases), the maximum cell density likewise was appeared in *Synechocytis*

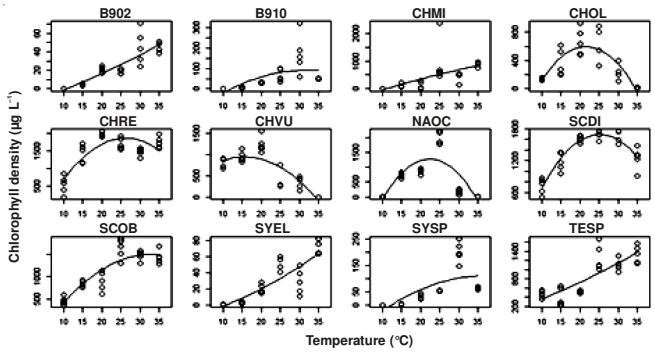

Fig. 2. Comparison of maximum chlorophyll density in diverse species at different temperatures

sp. with 3.51×10^6 cell L^{-1} at 30 ℃ (Fig. 3). On the contrary, cell size/volume of cyanobacteria generally showed a decreased trend expect for B902 with increasing temperature, with the range of 11.27-630.93 μm^3, whereas B902 showed went slightly up, with the maximum cell volume reaching 842.194 μm^3 (Fig. 4). There were no significant difference in all cyanobacteria (NAVOA, $p > 0.05$).

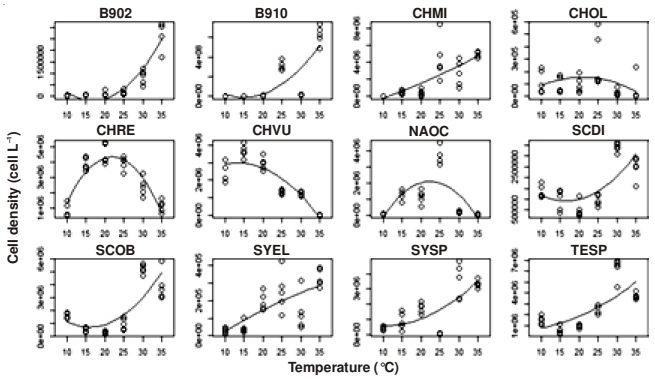

Fig. 3. Comparison of cell density of diverse species at different temperatures

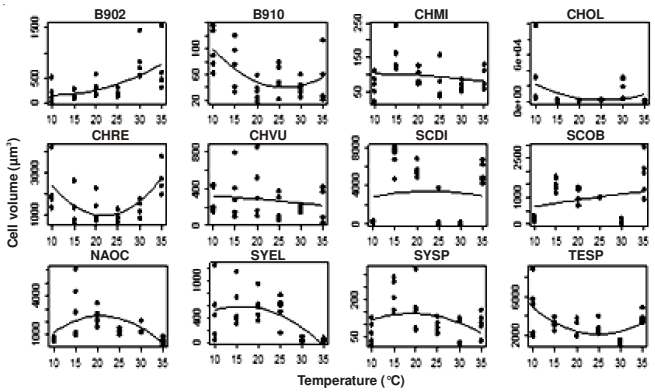

Fig. 4. Comparison of cell size/volume of diverse species at different temperatures

As for chlorophyta, similar trends were observed in Scenedesmus including *S. obliquus*, *S. dimorphus* and *Tetraselmis* sp. ($r^2 = 0.498, 0.419, 0.549$, respectively, $p < 0.05$ in all cases); whereas there were non-linear relationships between temperature and algal cell density in *C. oleabundans, C. reinhardtii*, *C. vulgaris*, *N. oculata*, with maximum value within temperature range of 20-25 ℃, maximum algal cell density reaching 4.5×10^6 cell L^{-1} in *C. reinhardtii* at 25 ℃. In general, similarly decreasing trends of cell size to cyanobacteria were observed in some chlorophyta species, but cell size of species such as *C. minutissima*, *C. oleabundans*, *C. vulgaris* showed no significant changes with temperature increasing.

Overall chlorophyta displayed cell size/volume larger than those of cyanobacteria at each of the temperatures, with the average cell volume (1875.04 μm^3) being 7.13 times as that of cyanobacteria (262.787 μm^3) (NAVOA, $p < 0.01$), in particular *C. oleabundans* and *C. reinhardtii*, with the maximum cell volume reaching 20638.46, 2864.32 μm^3, respectively.

Effect of temperature on chlorophyll a content in cell and lipid yield of diverse microalgae: There were non-relationship between chlorophyll a contents and temperature in all tested species only except for *C. reinhardtii* (Fig. 5). The optimum temperature range for chlorophyll a content in algal cell except for *C. reinhardtii* was 20-30 ℃, mainly around 25 ℃, the highest chlorophyll a content appeared in *C. oleabundans* at 30 ℃ with 1.41×10^{-5} μg $cell^{-1}$; generally, chlorophyll a contents in chlorophyta were higher than those in cyanobacteria, but no significantly different ($p > 0.05$).

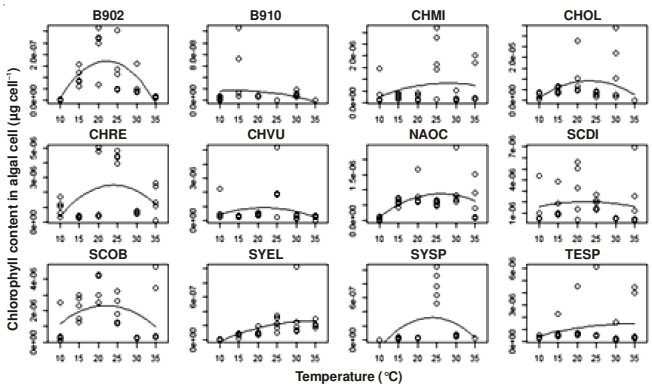

Fig. 5. Comparison of chlorophyll content in algal cell of diverse species at different temperatures

The culture temperature had a significant effect on the lipid yield of cyanobacteria (Fig. 6), obviously positive relationships between lipid percent and temperature were established in B902, *Synechocytis* sp. and *S. elongatus* ($r^2 = 0.535, 0.996, 0.67$, respectively, $p < 0.05$ in all cases), similar to the growth trends, the maximum lipid percents appeared at the same temperature of 35 ℃ with reaching 35.5, 63, 33.9 %, respectively, but no difference among them (NAOVA, $p > 0.05$). As for chlorophyta, there were non-liner correlations between lipid percent and culture temperature (Fig. 6), optimum temperature advantage for lipids accumulation in diverse species were evidently different, most chlorophyta were observed around 25 ℃ such as *C. vulgaris*, *Tetraselmis* sp., *C. oleabundans*, *S. obliquus*, *C. reinhardtii*, *S. dimorphus*. Whereas once temperature exceeded 35 ℃, lipid content decreased dramatically close to zero in some species such as *C. oleabundans and N. oculata.*

Also it was found from Figs. 3-6 that large-sized species (mainly green algae, *C. reinhardtii* and *S. dimorphus*, *S. obliquus*, *Tetraselmis* Sp., *N. oculata*) grew rapidly, with having relatively high lipid and chlorophyll a contents in their cells at optimum temperature.

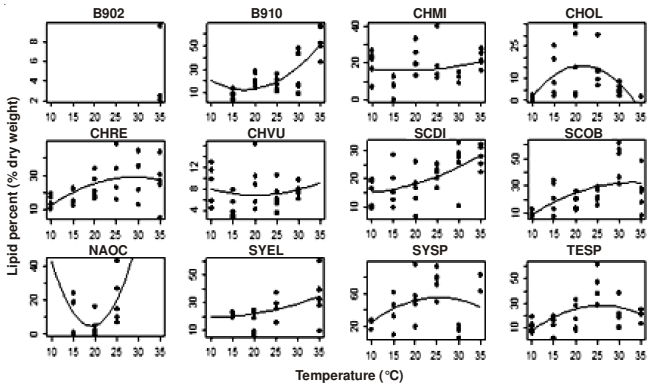

Fig. 6. Comparison of lipid contents in diverse species at different temperatures

The growth response of the algae could be linked to the variations in lipid contents and other biochemical composition with regards to changes in temperature. In the study, the optimum temperatures for cyanobacteria and chlorophyta growth were noted. Most chlorophyta growth was found to be around 25 ºC (Figs. 1-4), whereas over 30 ºC, particularly at 35 ºC, the taxa grew poorly and reached lower biomass with the sharp decline of cell density, indicating that the maximum tolerable temperature for growth occurred between 30 and 35 ºC. It's probably due to multiple mutations occurred affecting not only starch biosynthesis but also the flagella and the cell wall synthesis[20] under high temperature stress. In contrast, the optimum temperature for cyanobacteria growth found in our experiment was generally higher than chlorophyta and also higher than those retrieved from published data, which indicated the tested cyanobacteria species could thrive under warm condition. While the culture temperature was lower 15 ºC, practically at 10 ºC, the maximum growth rate of all species reduced remarkably.

Rhee and Gotham[21] found that the effects of temperature on cellular chlorophyll a content were species specific.

Previous studies were reported that the changes in algal size are mainly dependent on different environmental factors. For temperature effect, Jezberova and Komarkova[22] indicated the negative relationship between algal size and temperature. Rhee and Gotham[21] also reported the negative effect of temperature on cell volume under sufficient or limited nutrient treatments. The similar trends were also revealed in our study, *e.g*. cyanobacteria *Synechocytis* sp. and *S. elongatus* and some green algae *Tetraselmis* sp., *N. oculata*. The probable reason could be due to the accumulation of intracellular metabolites (as suggested by the granular appearance of cells observed at 10 or 15 ºC) or a decreased cell division. However, different results were observed that there was no significant changes in cell size/volume among B910, *C. minutissima*, *C. oleabundans*, *C. vulgaris* in response to increasing temperature, in particular B902 even showing a positive correlation between cell size and temperature, which was consistent with some studies on the effect of temperature (10-28 ºC) on cell morphometry[23] for which the highest cell size was found at temperatures of 28 ºC, hence prompting a species-specific response. It is also indicted that B902 has relatively high tolerance to thermal stress[24]. Numerous studies also showed that there seemed no single rule for the relationship between temperature and cellular chlorophyll a content of different algae. Lu *et al*.[25] showed that temperature had a positive effect on chlorophyll a concentration. In our experiment, only *C. reinhardtii* displayed the similar result with high chlorophyll a content in algal cell at high temperature, but other species didn't showed such same results, meantime we found that chlorophyll a content was not totally determined by algal size and volume, which was in agreement with the document[26]. In fact, many other environmental factors have important impacts on chlorophyll a content of algal cell.

As an important index of microalgae, lipid in algae includes extra- and intracellular composition. The studied reported previously show that high- and low-temperature stress could alter the composition of the lipids, particularly the degree of fatty acid saturation[27,28]. The present study showed that there was positive relationship between the lipid content and growth rate ($r^2 = 0.621$, $p < 0.05$). The maximum lipid productions of most species were observed at or close to optimum temperatures, for cyanobacteria at 35 ºC and for most chlorophyta at the range temperature from 20-30 ºC, which demonstrated growth and energy production in different species under thermal constraints were different evidently. The results could contribute to scale-cultivation of energy microalgae.

Meantime other valuable findings were presented in the study that large-sized species (mainly green algae) grew rapidly, with having relatively high lipid and chlorophyll a content in their cells, which was analogous to the documents reported previously. The possible reason was explained as Cermeno *et al*.[29] reported that large-sized phytoplankton could sustain a higher C-specific photosynthetic rate than do small sized cells and under conditions favorable for growth only large cells will be able to increase their intracellular quota of molecules involved in metabolic processes.

Conclusion

This study demonstrated that temperature had a significant impact on the growth and cell characteristics of diverse microalgae. Chlorophyta could grow at a wide range of temperature from 15-35 ºC, with the optimum temperature for growth of 20-30 ºC, mainly around 25 ºC; whereas cyanobacteria had a higher tolerance to high temperature than chlorophyta, the optimum temperature for growth was between 30 and 35 ºC. At the optimum temperature, most species generally showed grow rapidly with slightly high lipid and chlorophyll a contents in algal cell, especial in large-sized species (mainly green algae). In addition, we found chlorophyll a content in algal cell was not completely determined by algal size and volume. Overall this study identify key tradeoffs among functional traits to determine the suitability of diverse algal species as biofuel feedstock and screen successfully the potential candidate species for use in bio-oil production under different temperature constraints.

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