CrossMark

Changes in growth and photosynthesis of mixotrophic *Ochromonas* sp. in response to different concentrations of glucose

Lu Zhang[1] · Bangping Li[1] · Zhongqiu Wu[1] · Lei Gu[1] · Zhou Yang[1]

Abstract *Ochromonas* sp. (Chrysophyceae) occupies different trophic levels depending on its prevalent nutritional strategy in the field. *Ochromonas* sp. can utilize inorganic and organic carbon to grow photoautotrophically, mixotrophically, and heterotrophically. To examine how exogenous organic carbon influences population growth and photosynthesis in *Ochromonas* sp., we cultured *Ochromonas* sp. in media with different glucose concentrations (0, 50, 100, and 150 mg L^{-1}) and two treatment modes (single addition of glucose at the beginning and fed-batch mode with repeated addition every 12 h) for 15 days. Results showed that (1) compared to photoautotrophic growth, adding glucose significantly enhanced the growth rate and carrying capacity of *Ochromonas* sp. in a concentration-dependent manner; (2) photosynthesis also increased with glucose addition during the exponential growth stage; (3) repeated glucose addition caused higher biomass and photosynthetic performances than single addition, and (4) with depletion of glucose and the population reaching a stationary stage, photosynthetic parameters decreased slightly but remained at a relatively stable level, which was similar to that of photoautotrophic growth. We conclude that in comparison with pure photoautotrophy, mixotrophic growth can result in higher population growth rate and final biomass in a shorter time, especially in fed-batch cultures, indicating that this would be a highly efficient way for mass cultivation of the flagellate *Ochromonas*.

Introduction

Mixotrophic microorganisms, whose nutritional mode lies between photoautotrophy and heterotrophy, can utilize both light and organic sources for food. Therefore, mixotrophs occupy multiple trophic levels and confer ecological benefits to aquatic ecosystems (Sanders 1991; Jones 2000). Mixotrophs are very common among protists such as some flagellates and ciliates, as well as other mixotrophic organisms (Stoecker 1998). They can utilize inorganic carbon to grow photoautotrophically, take up dissolved organic carbon osmotrophically, or prey phagotrophically on small phytoplankton to grow heterotrophically, and grow as photoheterotrophs when provided with light and organic carbon (Boëchat et al. 2007). In nature, photoautotrophic growth is thought to facilitate survival under poor environment (Andersson et al. 1989), and mixotrophic mode is especially important under low-light conditions with added carbon source (Rothhaupt 1996). Under mixotrophic growth, *Ochromonas* sp. can usually ingest food more efficiently than under heterotrophic growth because it can derive more energy from the photosynthetic apparatus (Wilken et al. 2014). Boëchat et al. (2007) have proposed that the success of mixotrophic growth can be attributed to the combination of biochemical benefits of heterotrophic and photoautotrophic metabolic pathways.

Several methods of cultivating algae based on algal nutritional modes have been reported to date. To achieve greater algal production, dissolved organic carbon resources are used osmotrophically in mixotrophic or heterotrophic culture of

✉ Zhou Yang
yangzhou@njnu.edu.cn

[1] Jiangsu Key Laboratory for Biodiversity and Biotechnology, School of Biological Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210023, China

⨺ Springer

algae. Pålsson and Daniel (2004) reported that the growth rate and biomass of mixotrophic flagellate *Poterioochromonas malhamensis* increased in the presence of organic carbon compared with those in photoautotrophy. Higher biomass and lipid production of mixotrophic *Chlorella sorokiniana* have been achieved by the addition of glucose (Li et al. 2014). However, Girard et al. (2014) suggested that photoautotrophic culture accumulated more lipids than mixotrophic cultures with whey permeate as organic carbon for the green microalga *Scenedesmus obliquus*. Besides glucose, other commonly used organic carbon sources such as acetate, sucrose, and glycerol can increase the final cell abundances and final exopolysaccharide concentration in mixotrophic growth of the cyanobacterium *Nostoc flagelliforme* (Yu 2011). Also, molasses stimulated the biomass of *Arthrospira* (*Spirulina*) *platensis*, a microalga with rich proteins, vitamins, and polyunsaturated fatty acids (Andrade and Costa 2007). Wang et al. (2012) even reported that diluted primary piggery wastewater could contribute to lipid production of *Chlorella pyrenoidosa*, which also reduced the high organic content of piggery waste simultaneously. Taken together, these reports indicate that mixotrophic growth offers many advantages in the cultivation of algae.

Mixotrophic *Ochromonas* sp., an organism with multiple nutritional modes, has been reported to have a broad range of habitats in marine and fresh waters (Sanders 1991). Several studies have showed that *Ochromonas* sp. can strongly reduce the biomass of *Microcystis* and its toxin content (Yang et al. 2008; Van Donk et al. 2009; Zhang et al. 2010) and suggested that *Ochromonas* might serve as a possible biological control agent against *Microcystis* blooms. However, mass culturing *Ochromonas* sp. is necessary before using the mixotrophic organisms to control *Microcystis* in the field. Based on the above studies on the contribution of organic resources to rapid population growth of some mixotrophic organisms, glucose was used as a source of organic carbon in the current study to stimulate the growth of mixotrophic *Ochromonas* sp.

In this study, *Ochromonas* sp. was cultured in media with different glucose concentrations and two addition treatments (single addition of glucose at the beginning and repeated addition every 12 h) for 15 days to examine how exogenous organic carbon influences growth and photosynthesis. We hypothesized that (1) glucose addition will promote population growth of *Ochromonas* and that the carrying capacity improves with increasing glucose concentration and (2) the fed-batch mode with repeated addition can produce higher biomass of *Ochromonas* compared to single addition. The population growth rate and carrying capacity, specific growth rate, and photosynthetic performances were measured and analyzed to test the above hypotheses.

Materials and methods

The strain of mixotrophic chrysophyte *Ochromonas* sp. obtained from Meiliang Bay, Lake Taihu, China, was isolated under a microscope through stepwise dilution after being fed with autoclaved wheat seeds. Subsequently, a single cell was maintained in axenic mineral medium under photoautotrophic growth. The medium used was BG-11 (Stanier et al. 1971). Cultures of *Ochromonas* sp. were maintained at 25 ± 0.5 °C with constant light intensity of 40 μmol photons m^{-2} s^{-1} under a 12:12-h light-dark cycle.

Experimental design

The experiments were performed in a 250-mL flask containing 150 mL of sterilized BG-11 medium with pH adjusted to 7.3. *Ochromonas* sp. cultures were incubated at 25 ± 0.5 °C with constant light intensity of 40 μmol photons m^{-2} s^{-1}. *Ochromonas* sp. was grown osmotrophically by supplying glucose as organic carbon source. Three treatments were set up: (1) photoautotrophically grown *Ochromonas*, without glucose addition; (2) mixotrophically grown *Ochromonas* with a single glucose addition at the beginning; and (3) mixotrophically grown *Ochromonas* in fed-batch culture by repeated glucose additions at 12-h intervals for 9 days. The duration of all experiments was 15 days. Thus, mixotrophically grown *Ochromonas* with repeated glucose addition stopped receiving glucose after day 9. Four glucose concentrations (0, 50, 100, and 150 mg L^{-1}) were used in the experiments. In all treatment conditions, *Ochromonas* sp. was precultured at the described light intensity and glucose concentrations were monitored for 4 days prior to the start of the experiments. Each flask was inoculated with *Ochromonas* sp. to a final concentration of $\sim 1.0 \times 10^4$ cells mL^{-1}. To avoid bacterial growth, the whole experiments (including inoculating and sampling *Ochromonas*) were operated in sterile conditions and the glucose solution was filter-sterilized, but antibiotics were not added in the cultures. Samples (2 mL) for cell counts were taken every day, with 1 mL for determining glucose concentration every 12 h until glucose was used up. Photosynthetic parameters were measured every day until day 9, after which they were measured every 2 days. All treatments were performed in triplicate.

Ochromonas sp. growth

Samples for counting were fixed in Lugol's solution (2 %). Abundance was determined using a hemocytometer. Daily specific growth rates (μ) were calculated as $\mu = \ln N_{t+1} - \ln N_t$, where N_{t+1} and N_t are cell abundances (cells mL^{-1}) at times $t+1$ and t, respectively. The population dynamics were fitted using the logistic model

$f = \frac{K}{1+\left(\frac{K}{n}-1\right)\times e^{(-r\times x)}}$ to obtain the population growth rate (r) and carrying capacity (K).

Determination of glucose concentrations

To test the glucose concentration, samples (1 mL) were harvested by centrifugation at 8000 rpm for 10 min, and the supernatant was filtered through a 0.2-μm filter (Salus, USA). Glucose concentration was determined by the glucose oxidase-peroxidase method using a commercially available glucose assay kit (Jiancheng F006, Nanjing, China) every 12 h, and the appropriate amount of glucose was added to the constant addition groups until the final concentration in each group was reached. After that, the final concentration was measured. The dynamics of glucose concentrations are shown in Fig. 1.

Photosynthesis measurement

Photosynthetic activities of *Ochromonas* sp. during the experiments were measured using a Phyto-PAM (Walz, Germany). Photosynthetic parameters including maximal efficiency of PSII photochemistry (F_v/F_m), effective quantum yield of PSII photosynthetic efficiency (Φ_{PSII}), maximum relative electron rate ($rETR_{max}$), and initial slope α (α_{ETR}) were calculated from the fitting photosynthesis-irradiance (*P-E*) curve.

Statistical analyses

All data are presented as mean±SD and showed normal distribution and homogeneity of variance by the Shapiro-Wilk and equal variance tests. The growth rates and carrying capacity (K) among treatment groups were assessed by one-way ANOVA followed by the Tukey test ($\alpha = 0.05$). Photosynthetic performances and instantaneous growth rates among treatments were assessed by two-way ANOVA followed by the Holm-Sidak method ($\alpha = 0.05$), with time and glucose concentrations as the two factors. All statistical analyses were performed using Sigmaplot 11.0.

Results

Ochromonas sp. growth in response to different glucose concentrations

Cell abundances in treatments with glucose addition were significantly higher than those in photoautotrophic growth, i.e., adding glucose significantly promoted the carrying capacity of *Ochromonas* sp. (Figs. 2a–c and 3; $P < 0.05$). The *Ochromonas* sp. population in the photoautotrophic growth (no glucose added), single addition of glucose, and repeated addition of glucose (fed-batch culture) groups reached stationary phase on days 6, 4, and 7, respectively. The carrying capacity of the repeated-addition treatment group increased with rising glucose concentration and reached a significantly higher level than those of the single-addition treatments (Fig. 3; $P < 0.05$).

Adding glucose significantly enhanced the population growth rates of *Ochromonas* sp. in comparison with those in photoautotrophic growth (no glucose added) (Fig. 3; $P < 0.05$). In the single-addition treatment group, the population growth rate was the highest at 150 mg L^{-1} (2.66 ±0.36 days^{-1}), and no significant difference was found in

Fig. 1 Glucose concentrations in mixotrophic cultures. **a** Glucose was added once at the beginning of the experiment and determined every 12 h. **b–d** Glucose was added to the culture every 12 h before day 9, and glucose concentrations were measured before and after additions. *Error bars* indicate standard deviation
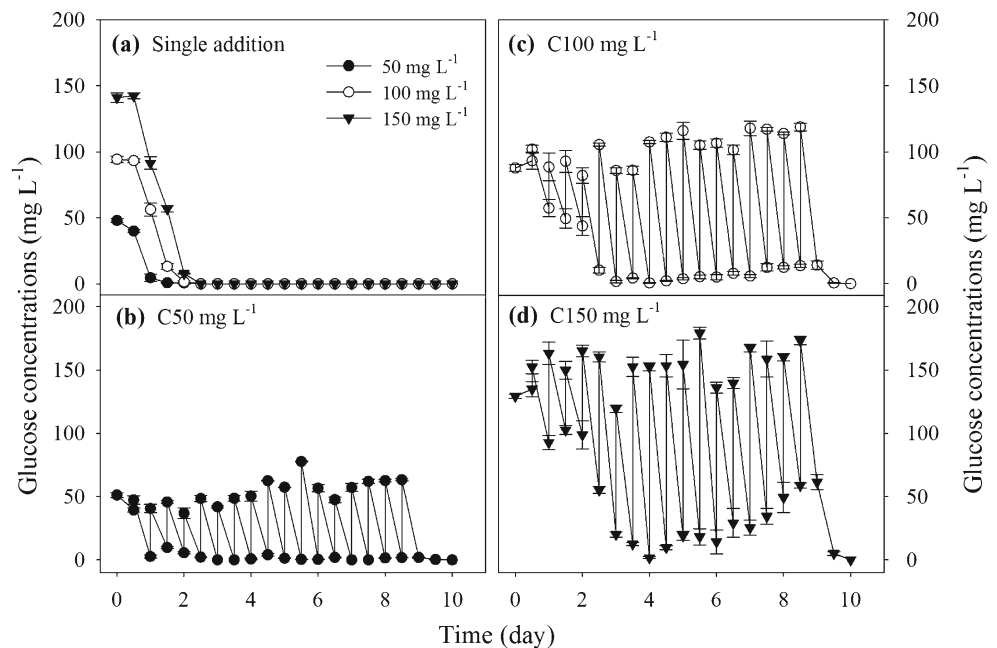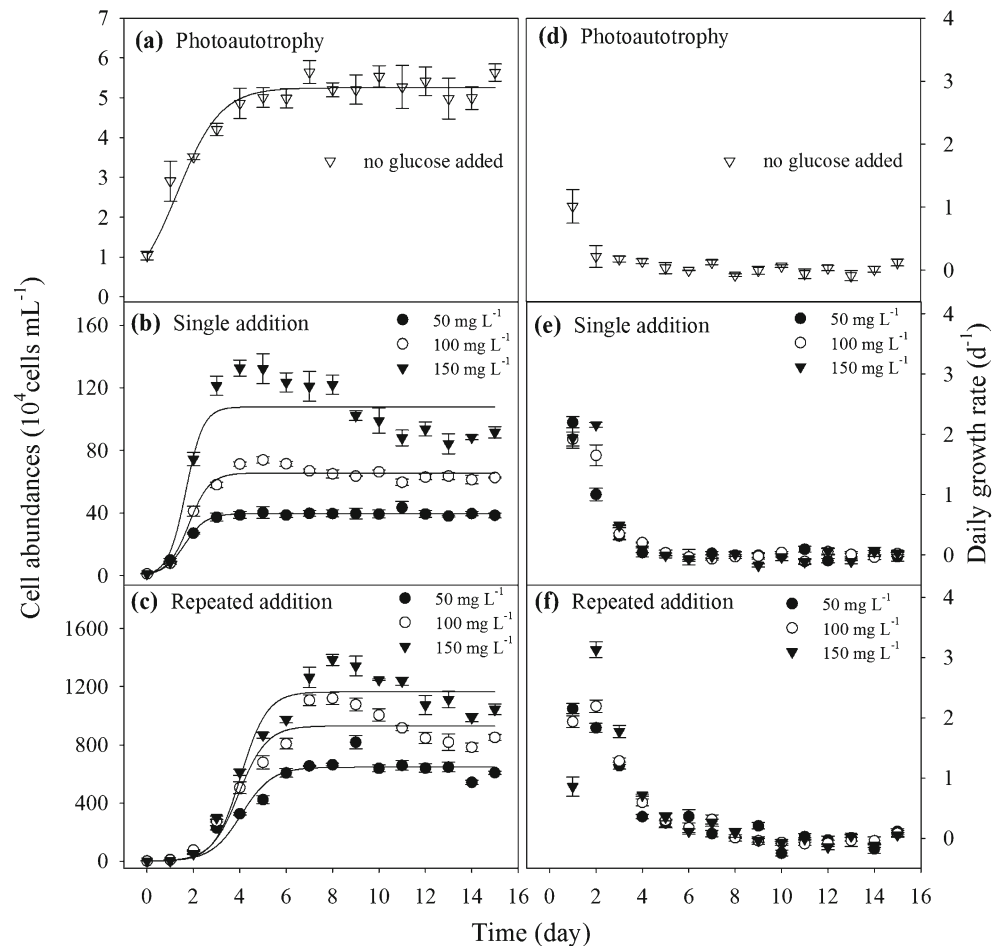
Fig. 2 **a–c** Growth curves and **d–f** daily specific growth rates (μ) of *Ochromonas* sp. at different concentrations of glucose cultures. *Lines* show time-response curves described in the logistic model $f = \frac{K}{1+\left(\frac{K}{n}-1\right)\times e^{(-r\times x)}}$ fitted to the points. Glucose addition ceased on day 9. *Error bars* indicate standard deviation

the population growth rates between 50 and 100 mg L^{-1} ($P = 0.738$). Besides, the population growth rate at 150 mg L^{-1} was significantly higher than that at 50 mg L^{-1} in the fed-batch treatment group ($P < 0.05$).

Daily specific growth rates in all treatment groups gradually decreased with time, until the populations reached their

Fig. 3 Population growth rate and *K* (carrying capacity) at different concentrations of glucose cultures. *C* indicates repeated-addition treatment. *Error bars* indicate standard deviation. Significant difference is indicated by *different lowercase letters* in the growth rate (one-way ANOVA, $P < 0.05$). Values marked with the *same uppercase letters* are significantly different in terms of carrying capacity ($P < 0.05$)

respective carrying capacities (Fig. 2). During the exponential phase, daily specific growth rates in the mixotrophic treatment increased with rising glucose concentration and became significantly higher than those in photoautotrophic growth (Fig. 2d–f; $P < 0.05$). These data indicated that adding glucose could efficiently promote the growth rate of *Ochromonas* sp., especially at higher glucose concentration.
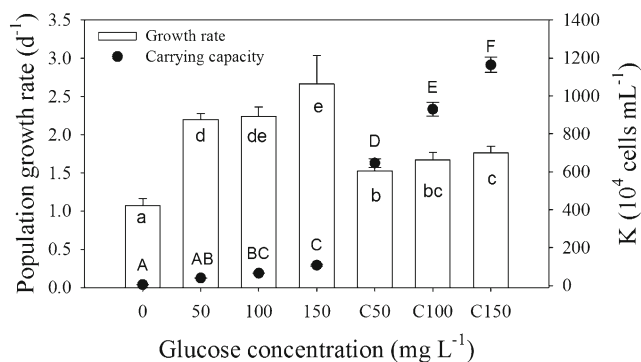
Changes in photosynthetic capacities

The photosynthetic activities in all treatment groups were higher at the beginning than at a later stage—it decreased with decreasing daily specific growth rate but finally stayed in stationary phase when population stability was reached (Figs. 4 and 5). Adding highly concentrated glucose (150 mg L^{-1}) caused significant enhancement of F_v/F_m and Φ_{PSII} on day 2 when compared with those in photoautotrophic growth (Fig. 4; $P < 0.001$). Meanwhile, rETR$_{max}$ at 100 and 150 mg L^{-1} in mixotrophic culture was especially higher than that of photoautotrophically growing microalgae on day 1 (Fig. 5a–c; $P < 0.001$). Significant difference in a_{ETR} was also observed between photoautotrophic and fed-batch treatments on day 2 (Fig. 5e–f; $P < 0.05$).

Fig. 4 a–c Maximal efficiency of PSII photochemistry (F_v/F_m) and **d–f** effective quantum yield of PSII photosynthetic efficiency (Φ_{PSII}) of *Ochromonas* sp. at different concentrations of glucose in cultures. *Error bars* indicate standard deviation. Glucose addition ceased on day 9
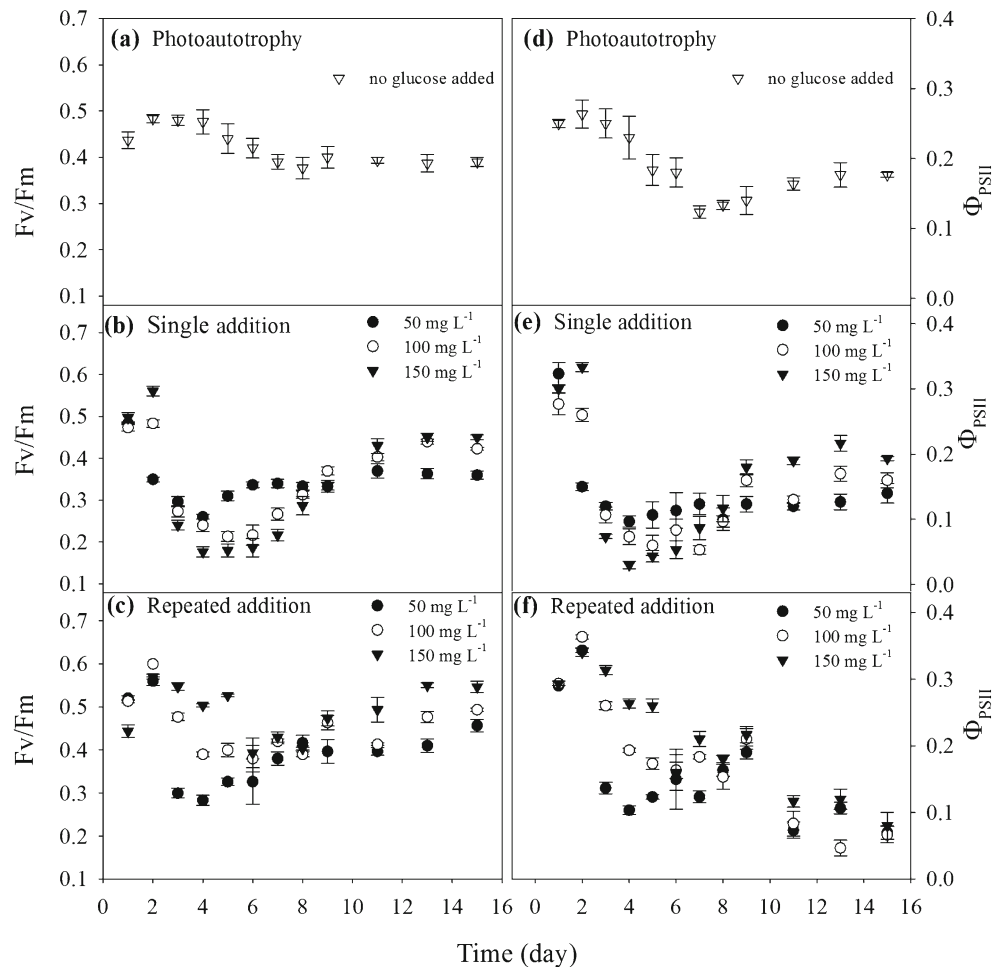
In the single-addition treatment group, photosynthetic activities of mixotrophic *Ochromonas* sp. on day 2 increased significantly with increase in glucose concentration (Fig. 4; $P < 0.05$), which was also observed on days 3 and 4 in the fed-batch treatment group (Fig. 5; $P < 0.001$). From day 3, photosynthetic performances began to decrease rapidly with decreasing glucose in all single-addition treatment and repeated 50-mg L^{-1} glucose addition treatment groups. However, it was observed that photosynthetic performances were always higher when high glucose was repeatedly added, compared to those receiving single addition of glucose. At the end of the experiment, the photosynthetic activities were stable across all groups. In particular, photosynthesis of *Ochromonas* in the groups receiving glucose addition was almost close to that of groups grown under photoautotrophic condition.

Discussion

In the presence of organic carbon, the growth rate and biomass of microalgae are commonly increased compared with those in photoautotrophy. The results of our study showed that glucose assimilation by mixotrophic *Ochromonas* resulted in enhancement of population growth. The carrying capacity and growth rate in mixotrophic cultures were at least 7 times and 1.4 times higher compared to those of cultures grown photoautotrophically. In particular, cell abundance reached 1×10^7 cells mL^{-1} in fed-batch treatment, which was more than 200 times that found in purely photoautotrophic culture. This finding is consistent with that of Li et al. (2014), who reported that the growth rate and biomass of *Chlorella sorokinian* fed with glucose were higher compared to those under photoautotrophic culture. As shown in Fig. 3, cell density increased with higher glucose concentration, i.e., it was concentration-dependent. This result was also observed in the species *Tetraselmis* (*Platymonas*) *subcordiformis* (Xie et al. 2001). In our study, a decline in cell abundance occurred at high glucose concentration during the stationary stage, which was possibly a result of limited organic resource (Simonds et al. 2010), metabolite accumulation, and expansion of living space.

In addition, we found that fed-batch mode by repeated addition of glucose resulted in higher biomass of *Ochromonas* than single addition of glucose. The fed-batch

Fig. 5 **a–c** Maximum relative electron rate (rETR$_{max}$) and **d–f** initial slope alpha (α_{ETR}) of *Ochromonas* sp. at different concentrations of glucose in cultures. *Error bars* indicate standard deviation. Glucose addition ceased on day 9
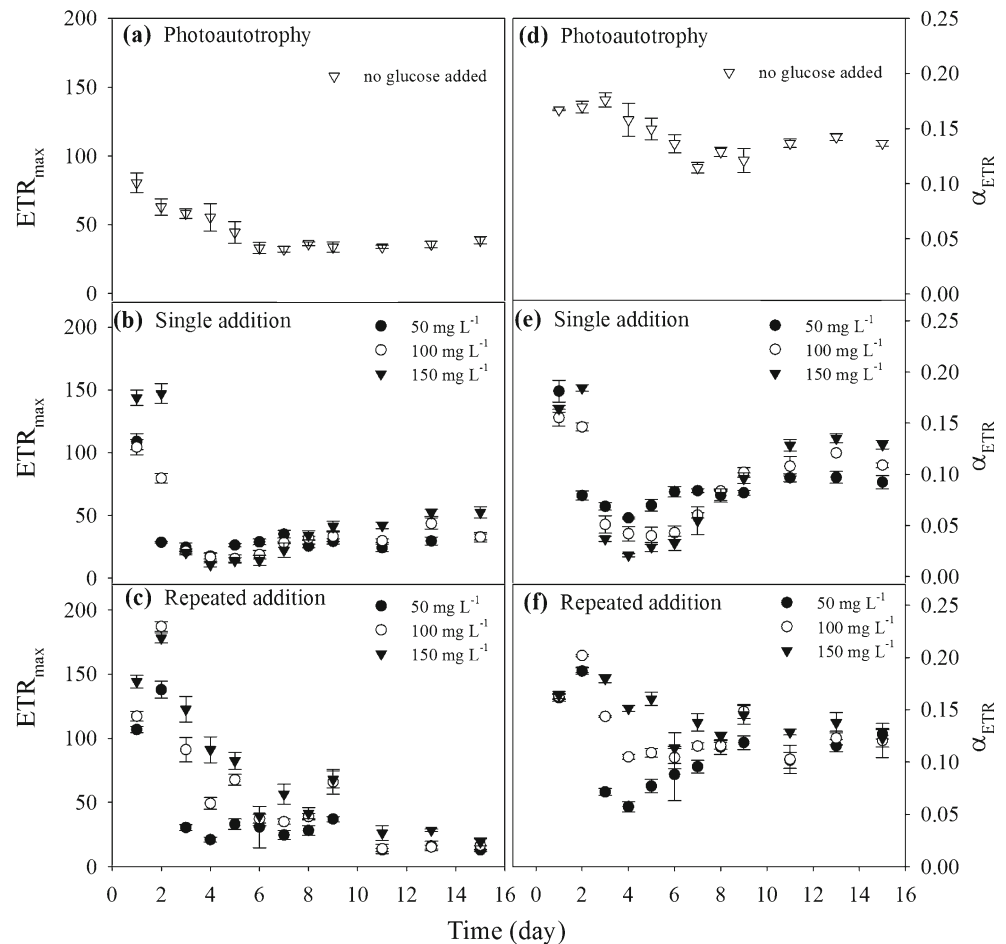
system is routinely used to improve biomass productivity in a mixotrophic process, such as glucose-grown *Nannochloropsis* sp. (Xu et al. 2004; Benjamas and Salwa 2012) and *A. (Spirulina) platensis* (Chen and Zhang 1997), acetate-grown *Haematococcus pluvialis* (Hata et al. 2001), and *A. platensis* (Matsudo et al. 2014). Cerón-García et al. (2013) suggested that semi-continuous mode could lead to much higher biomass productivities of *Phaeodactylum tricornutum* than fed-batch mode. Thus, mixotrophic growth, especially fed-batch mode, would be an ideal method of high-density cultivation of *Ochromonas* sp. in a short time. In this experiment, antibiotics were not added into the medium although we have adopted some measures to avoid bacterial growth, and thus, it was possible that a few bacteria grown in the medium could be ingested as carbon source by *Ochromonas* (Porter 1988; Sanders et al. 2001; Wilken et al. 2013). In this case, bacteria may be an additional carbon source for *Ochromonas* when glucose was used up.

Surprisingly, constant addition of glucose did not have a more positive effect on population growth rate compared to once addition (Fig. 3). This result is similar to those previously described by Rym et al. (2010), who showed a low growth rate at high glucose concentration (0.35 day^{-1} at 2500 mg L^{-1}

glucose). Benjamas and Salwa (2012) suggested that high organic nutrient concentration could inhibit cell growth and lipid accumulation in mixotrophic cultures. In some cases, less polyunsaturated fatty acids were produced compared to saturated fatty acids under mixotrophic and heterotrophic conditions (Boëchat et al. 2007). Also, the failure of consumer *Ochromonas* to feed on the cultures might be the cause of low population growth rate since high cell abundance produced by mixotrophic cultivation is known to reduce glucose availability (Chrzanowski and Foster 2014). Instead, the low population growth rate in photoautotrophic condition may be caused by increase in photorespiration (Wilken et al. 2013).

The work of Wilken et al. (2014) suggested that the photosynthetic machinery of mixotrophic *Ochromonas* sp. remains fully functional. Benefiting from the capacity for photosynthesis, mixotrophs can dominate in terms of biomass over heterotrophs (Pålsson and Daniel 2004). In addition, supplementing organic carbon contributes to higher cell abundance of mixotrophs than that of photoautotrophs (Cerón-Garcí et al. 2000). Thus, photosynthesis and intake of organic carbon are correlated and mutually dependent in influencing the growth of mixotrophic organisms. The present result showed that photosynthesis of

Ochromonas sp. changed along with population growth under mixotrophic culture. Maximal efficiency of PSII photochemistry (F_v/F_m), effective quantum yield of PSII photosynthetic efficiency (Φ_{PSII}), initial slope α (α_{ETR}), and maximum electron transport rate (rETR$_{max}$) are known as important photosynthetic indicators that show the integrity of the PSII reaction center and photosynthetic capacity. The photosynthetic performances (F_v/F_m, ΦPSII, α_{ETR} and rETRmax) of *Ochromonas* sp. increased at first, then decreased, and finally stayed constant regardless of glucose addition (Figs. 4 and 5).

On the one hand, the photosynthetic performances of *Ochromonas* sp. fed with glucose were more enhanced than those in photoautotrophic growth at the beginning. This result is consistent with that obtained by Vonshak et al. (2000) with *A. platensis*, and verified our starting hypothesis that glucose addition could enhance the growth of *Ochromonas*. Van Wagenen et al. (2015) reported that adding glucose in the dark could increase photobioreactor efficiency of mixotrophic *C. sorokiniana*. Higher photosynthetic parameters were also observed when high glucose was added continuously during the exponential phase (Figs. 4 and 5). On the other hand, the photosynthetic capacities decreased slightly with exhaustion of glucose after the exponential phase, which was possibly caused by enhancement of respiration (Orús et al. 1991). When glucose is added as an exogenous organic resource, some are converted to starch, whereas others normally remain to be oxidized through the glycolytic pathway or are directly oxidized into pentose phosphate and eruythrose-4-phosphate through the pentose phosphate pathway (Yang et al. 2000). The active glucose metabolism process may explain the decreases in photosynthetic capacities, i.e., heterotrophic metabolism dominated during this phase, bringing more positive effects on cell abundance of *Ochromonas* sp. In some instances, photoautotrophic microalgae switch to mixotrophic growth in the presence of organic carbon by decreasing the photosynthesis rate (Matantseva and Skarlato 2013). An early study by Liu et al. (2009) demonstrated that uptake of carbohydrates reduces F_v/F_m in *P. tricornutum*, a mixotrophic marine diatom. Similar reduction was observed in mixotrophic *Ochromonas* sp. when heterotrophic bacteria were added as prey, because adding glucose decreases the expression of the photosynthetic genes *PsbA* and *Rubisco*, which results in low capacity for carbon fixation (Wilken et al. 2014). Finally, photosynthesis rate recovered at the end of the experiment when population stability was reached. In particular, the photosynthetic performances of the single-addition treatment group were almost identical to that of photoautotrophic *Ochromonas* sp., which indicates that the former likely adapted to the environment and transitioned into photoautotrophic mode.

An interesting phenomenon observed in the experiment is that inhibition of photosynthetic performances occurred not only in mixotrophic cultures but also in photoautotrophic condition, which might be related to population growth. Thus, all of the above results confirmed that photosynthetic capacity could be enhanced in the early growth stage under mixotrophic condition, and then weakened with decreasing daily specific growth rate, and finally kept stable. Repeated addition of glucose at high concentration could make photosynthesis of mixotrophic *Ochromonas* sp. more active. However, the coincidence and independence between photoautotrophic and heterotrophic modes in mixotrophic culture (Martínez and Orús 1991; Marquez et al. 1993) should still be further discussed. Moreover, glucose used as an organic source in the present study is known to cause osmotic stress as well (Kanwal et al. 2014), so it should be investigated further to explain the observed inhibition of photosynthesis.

We conclude that heterotrophic growth can promote population growth and photosynthesis of mixotrophic *Ochromonas* sp., especially fed-batch culture, and can thus be a highly efficient way for mass culturing *Ochromonas* sp. to control *Microcystis* in the field.

References

Andersson A, Falk S, Samuelsson G, Hagstőm A (1989) Nutritional characteristics of a mixotrophic nanoflagellate, *Ochromonas* sp. Microb Ecol 17:251–262

Andrade MR, Costa JAV (2007) Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 264:130–134

Benjamas C, Salwa T (2012) Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. Bioresour Technol 110:510–516

Boëchat IG, Weithoff G, Krüger A, Gücker B, Adrian R (2007) A biochemical explanation for the success of mixotrophy in the flagellate *Ochromonas* sp. Limnol Oceanogr 52:1624–1632

Cerón-Garcí MC, Fernández-Sevilla JM, Acién-Fernández FG, Molina-Grima E, García-Camacho F (2000) Mixotrophic growth of *Phaeodactylum tricornutum* on glycerol: growth rate and fatty acid profile. J Appl Phycol 12:239–248

Cerón-García MC, Fernández-Sevilla JM, Sánchez-Mirón A, García-Camacho F, Contreras-Gómez A, Molina-Grima E (2013) Mixotrophic growth of *Phaeodactylum tricornutum* on fructose and glycerol in fed-batch and semi-continuous modes. Bioresour Technol 147:569–576

Chen F, Zhang Y (1997) High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzym Microb Technol 20:221–224

Chrzanowski TH, Foster BLL (2014) Prey element stoichiometry controls ecological fitness of the flagellate *Ochromonas danica*. Aquat Microb Ecol 71:257–269

Girard JM, Roy ML, Hafsa MB, Gagnon J, Nathalie F, Heitz M, Tremblay R, Deschênes JS (2014) Mixotrophic cultivation of green

microalgae *Scenedesmus obliquus* on cheese whey permeate for biodiesel production. Algal Res 5:241–248

Hata N, Ogbonna JC, Hasegawa Y, Taroda H, Tanaka H (2001) Production of astaxanthin by *Haematococcus pluvialis* in a sequential heterotrophic-photoautotrophic culture. J Appl Phycol 13:395–402

Jones RI (2000) Mixotrophy in planktonic protists: an overview. Freshw Biol 45:219–226

Kanwal S, Rastogi RP, Incharoensakdi A (2014) Glutamate decarboxylase activity and gamma-aminobutyric acid content in *Synechocystis* sp. PCC 6803 under osmotic stress and different carbon sources. J Appl Phycol 26:2327–2333

Li T, Zheng Y, Yu L, Chen S (2014) Mixotrophic cultivation of a *Chlorella sorokiniana* strain for enhanced biomass and lipid production. Biomass Bioenerg 66:204–213

Liu X, Duan S, Li A, Xu N, Cai Z, Hu Z (2009) Effects of organic carbon sources on growth, photosynthesis, and respiration of *Phaeodactylum tricornutum*. J Appl Phycol 21:239–246

Marquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S (1993) Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J Ferment Bioeng 76:408–410

Martínez F, Orús MI (1991) Interactions between glucose and inorganic carbon metabolism in *Chlorella vulgaris* strain UAM 101. Plant Physiol 95:1150–1155.

Matantseva OV, Skarlato SO (2013) Mixotrophy in microorganisms: ecological and cytophysiological aspects. J Evol Biochem Physiol 49:377–388

Matsudo MC, Moraes FA, Bezerra RP, Arashiro RE, Sato S, Carvalho JCM (2014) Use of acetate in fed-batch mixotrophic cultivation of *Arthrospira platensis*. Ann Microbiol 65:1721–1728

Orús MI, Marco E, Martínez F (1991) Suitability of *Chlorella vulgaris* UAM 101 for heterotrophic biomass production. Bioresour Technol 38:179–184

Pålsson C, Daniel C (2004) Effects of prey abundance and light intensity on nutrition of a mixotrophic flagellate and its competitive relationship with an obligate heterotroph. Aquat Microb Ecol 36:247–256

Porter KG (1988) Phagotrophic phytoflagellates in microbial food webs. Hydrobiologia 159:89–97

Rothhaupt KO (1996) Utilization of substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp. Ecology 77:706–715

Rym BD, Nejeh G, Lamia T, Ali Y, Rafika C, Khemissa G, Jihene A, Hela O, Hatem BO (2010) Modeling growth and photosynthetic response in *Arthrospira platensis* as function of light intensity and glucose concentration using factorial design. J Appl Phycol 22:745–752

Sanders RW (1991) Mixotrophic protists in marine and freshwater ecosystems. J Protozool 38:76–81

Sanders RW, Caron DA, Davidson JM, Dennett MR, Moran DM (2001) Nutrient acquisition and population growth of a mixotrophic alga in axenic and bacterized cultures. Microb Ecol 42:513–523

Simonds S, Grover JP, Chrzanowski TH (2010) Element content of *Ochromonas danica*: a replicated chemostat study controlling the growth rate and temperature. FEMS Microbiol Ecol 74:346–352

Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order Chroococcales). Bact Rev 35:171–205

Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. Eur J Protistol 34:281–290

Van Donk E, Cerbin S, Wilken S, Helmsing NR, Ptacnik R, Verschoor AM (2009) The effect of a mixotrophic chrysophyte on toxic and colony-forming cyanobacteria. Freshw Biol 54:1843–1855

Van Wagenen J, Francisci DD, Angelidaki I (2015) Comparison of mixotrophic to cyclic autotrophic/heterotrophic growth strategies to optimize productivity of *Chlorella sorokiniana*. J Appl Phycol 27:1775–1782

Vonshak A, Cheung SM, Chen F (2000) Mixotrophic growth modifies the response of *Spirulina* (*Arthrospira*) *platensis* (Cyanobacteria) cells to light. J Phycol 36:675–679

Wang H, Xiong H, Hui Z, Zeng X (2012) Mixotrophic cultivation of *Chlorella pyrenoidosa* with diluted primary piggery wastewater to produce lipids. Bioresour Technol 104:215–220.

Wilken S, Huisman J, Naus-Wiezer S, Van Donk E (2013) Mixotrophic organisms become more heterotrophic with rising temperature. Ecol Lett 16:225–233

Wilken S, Schuurmans JM, Matthijs HCP (2014) Do mixotrophs grow as photoheterotrophs? Photophysiological acclimation of the chrysophyte *Ochromonas danica* after feeding. New Phytol 204:882–889

Xie Y, Zhang Y, Li Y, Wang Y (2001) Mixotrophic cultivation of *Platymonas subcordiformis*. J Appl Phycol 13:343–347

Xu F, Cai ZW, Fan O (2004) Growth and fatty acid composition of *Nannochloropsis* sp. grown mixotrophically in fed-batch culture. Biotechnol Lett 26:1319–1322

Yang C, Hua Q, Shimizu K (2000) Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. Biochem Eng J 6:87–102

Yang Z, Kong FX, Shi XL, Zhang M, Xing P, Cao HS (2008) Changes in the morphology and polysaccharide content of *Microcystis aeruginosa* (Cyanobacteria) during flagellate grazing. J Phycol 44:716–720

Yu HF (2011) Effect of mixed carbon substrate on exopolysaccharide production of cyanobacterium *Nostoc flagelliforme* in mixotrophic cultures. J Appl Phycol 24:669–673

Zhang X, Hu H, Men Y, Christoffersen KS (2010) The effect of *Poterioochromonas* abundance on production of intra- and extracellular microcystin-LR concentration. Hydrobiologia 652:237–246