Effects of glycerol and glucose on the enhancement of biomass, lipid and soluble carbohydrate production by *Chlorella vulgaris* in mixotrophic culture

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Summary

Biodiesel-derived glycerol is a promising substrate for mixotrophic cultivation of oleaginous microalgae for the purpose of glycerol cyclic utilization and reduction of the cost of microalgae biodiesel. The objective of this study was to investigate the potential of using glycerol and glucose as the complex carbon substrate to produce microalgal biomass and biochemical components, such as photosynthetic pigments, lipids, soluble carbohydrates and proteins by *Chlorella vulgaris*. The results showed that *C. vulgaris* could utilize glycerol as a sole carbon substrate but was inferior to the mixed of glycerol and glucose for producing biomass and biochemical components. The effects of glycerol and glucose could enhance the algal cell growth rate, biomass content and volumetric productivity, and overcome the lower biomass caused by glycerol as the sole organic carbon source in mixotrophic culture medium. The utilization of complex organic carbon substrate could stimulate the biosynthesis of lipids and soluble carbohydrates as the raw materials for biodiesel and bioethanol production, while reduce the anabolism of photosynthetic pigments and proteins. This study provides a
promising niche in reducing the overall cost of biodiesel and bioethanol production from microalgae. This is because the raw materials (lipids and carbohydrates) and organic carbon substrates (soluble carbohydrates and glycerol) for mixotrophic cultivation of microalgae can be obtained from byproducts of algae biodiesel production and algal cell hydrolysis.

**Key words:** *Chlorella vulgaris*, glycerol, glucose, biomass production, biochemical components, mixotrophic cultivation

**Introduction**

As a promising source for production of biodiesel and other active ingredients (such as pigments, protein and unsaturated fatty acid), microalgae have drawn more and more attention of researchers because they possess high growth rate and provide lipids for biofuel production, while it becomes essential to increase biomass production and the productivity of lipid and other cellular composition rapidly and to decrease the cost of biodiesel production (1). The price of algal biofuel ultimately depends on the substrate cost, lipid yield, and the quality of the products formed by the downstream process (2). The cost of carbon source represents 50% of the cost of medium in algal cultivation (3). With the aim of commercializing biodiesel from algae, a substantial effort has been devoted to the development of improved algal strains and more efficient cultivation process. Recent studies have found that the biomass and lipid content of algae can be increased through changing cultivation conditions, such as CO₂ aeration fixation, temperature, salinity and nutrient concentration (4, 5, 6). Particularly, the effects of nitrogen sources and concentrations on lipid accumulation of microalgae have been examined widely (7).

The ability of growth transition from photoautotrophic to mixotrophic mode in microalgae is a phenomenon which appears to exist in a number of genera and species distributed throughout the major taxonomic divisions (8). Many algal organisms are capable of using either metabolic process (autotrophic or heterotrophic) for growth, meaning that they are able to photosynthesize as well as ingest prey or organic materials (9). The ability of
mixotrophism to process organic substrates means that cell growth is not strictly dependent on photosynthesis. Therefore, light energy is not an absolutely limiting factor for growth and light or organic carbon substrates can support the alga growth, hence, there is less biomass loss during the dark phase (10). Hayward (11) studied the effect of a range of externally supplied carbon compounds on the growth of *Phaeodactylum tricornutum* in the dark and in the light. *P. tricornutum* is able to respire glucose, mannitol and lactate without the energy released being apparently available for growth. Bouarab et al. reported that *Micractinium pusillum* grew in the presence of organic substrates, i.e., glucose and acetate, under mixotrophic condition as well as in the heterotrophic growth (12). The growth of *M. pusillum* was much more eugonic in the light than in the dark and more in the presence of glucose than of acetate. It can be implied from these features that mixotrophism would be an ideal nutritional mode for production of biofuels and functional components biosynthesis, with high density cultivation of microalgae.

During the biodiesel manufacturing process, one of the major byproducts is crude glycerol (13). With the rapid growth of biodiesel production, the market is flooded with crude glycerol. As a result, biodiesel producers must seek new uses for this waste stream. Recently, a process using crude glycerol as a substrate for the fermentation of the microalgae *Schizochytrium limacinum* has been developed (14). The oleaginous *S. limacinum* has capability of producing significant amounts of total lipid and docosahexaenoic acid (DHA, C22:6 n-3), especially, when growing in a variety of carbon sources such as glucose, glycerol or fructose (15). Above results suggested that biodiesel-derived glycerol is a potential substrate for mixotrophic cultivation of oleaginous microalgae with the purpose of cyclic utilization of glycerol and reducing the production cost of microalgae biodiesel.

However, there are few reports on the effects of carbon resources, especially, the effects of carbon sources on the biomass production and cellular components biosynthesis of algae under mixotrophic cultivation (10, 12). In this paper, the effects of glycerol and glucose on enhancement of the biomass, lipid and soluble carbohydrates production of *C. vulgaris* under mixotrophic conditions were investigated.
Materials and Methods

Microalgae and growth conditions

*C. vulgaris* was purchased from the Culture Collection of Algae, Institute of Hydrobiology, Chinese Academy of Sciences, and was grown on modified soil extract medium (SEM) which consisted of (per liter): 0.25 g NaNO₃; 0.175 g KH₂PO₄; 0.075 g K₂HPO₄; 0.075 g MgSO₄·7H₂O; 0.025 g NaCl; 0.025 g CaCl₂·2H₂O; 5 mg FeCl₃; 0.287 mg ZnSO₄·7H₂O; 0.169 mg MnSO₄·H₂O; 0.061 mg H₃BO₃; 2.5 µg CuSO₄·5H₂O; and 1.24 µg (Na)₆Mo₇O₂₄·7H₂O. The pH was adjusted to 7.2 prior to autoclaving at 120 °C for 20 min. In the tests, different contents of glycerol (1, 5, 10 g/L) and 2 g/L glucose were supplied in medium and cultured under illuminated condition. The autotrophic control group was cultivated in SEM with illumination. All cultures were maintained at 30 ± 1 °C in 250 ml flasks containing 100 ml culture under illuminated at 2500 Lux with 12 h light, 12 h dark and shaken at 120 rpm on an orbital shaker. Cultures were harvested on day 4 (96 h).

Determination of biomass content and productivity

Algal growth curves and biomass concentrations were determined by measuring the absorbance at 660 nm and dry cell weight, respectively. Cells were centrifuged at 2000×g for 10 min, rinsed twice with distilled water and dried at 70 °C for 24 h to give the dry cell weight (g/L).

The specific growth rate (µ, day⁻¹) of *C. vulgaris* at the exponential phase was calculated according to the equation $\mu = (\ln X_2 - \ln X_1) / (t_2 - t_1)$, where $X_2$ and $X_1$ are the dry cell weight concentration (g/L) at time $t_2$ and $t_1$, respectively. The biomass concentration (g/L) was recorded and the productivity ($P$, g/L/day) was from the equation $P=(X_2-X_1) / (t_2-t_1)$, where $X_2$ and $X_1$ are the dry cell weight concentration (g/L) at time $t_2$ and $t_1$, respectively (10).

Photosynthetic pigments extraction and determination

4 mL algal cultures were centrifuged at 2000×g for 10 min, rinsed twice with distilled water. The pellet was extracted with eight milliliters 80% (v/v) ethanol two times until the algal faded in 4°C refrigerator, followed by centrifugation at 2000×g for 10 min. The contents
of Chl a, Chl b, total Chl a+b and carotenoids in supernatant were determined by UV-VIS spectroscopy (16).

**Lipid extraction and determination**

Cells were harvested by centrifugation, washed with distilled water two times, and then dried by a freeze dryer. The dry biomass (100 mg) was homogenized in mortar and extracted with \( n \)-hexane (20 mL) for 30 minutes and centrifuged. The extraction process was repeated three times and supernatant was transferred to a pre-weighed glass vial and evaporated on rotary evaporator. The algae lipid was recovered after solvent evaporation and dried at 70 °C completely. The weight of glass vial containing oil was measured gravimetrically and the lipid concentration expressed as dry weight percentage (%) (17, 18). Meanwhile, the productivity of lipid (\( P \), mg/L/day) was calculated (10).

**Soluble carbohydrates extraction and determination**

Cells were harvested by centrifugation, washed with distilled water two times, and then dried by a freeze dryer. The dry sample was homogenized in a mortar and extracted by boiled water for 1 h and centrifuged at 2000× \( g \) for 10 min. The extraction process was repeated two times and the soluble carbohydrate content in the mixed supernatant was estimated. Anthrone - sulfuric acid method was adopted to determine the content of soluble carbohydrate (19). Briefly, 0.5 mL sample mixed with 0.5 mL distilled water and added in 5.0 mL anthrone agent. Homogeneous mixture was incubated in a boiling water bath for 10 min. After chromogenic reaction and cooling, the absorbance at 620 nm was measured with a spectrophotometer. Glucose was used as a carbohydrate standard. The soluble carbohydrate concentration was expressed as dry weight percentage (%).

**Soluble protein extraction and determination**

*C. vulgaris* cells were homogenized and extracted in 10 mL of 90 % ethanol 2 h, and the homogenate was centrifuged at 2000× \( g \) for 10 min. The pellet obtained was mixed with 5 mL of 10 % trichloroacetic acid (w/v) and recentrifuged. The pellet was repeatedly washed with ethanol (95%) for complete removal of trichloroacetic acid and dissolved in 0.1 M NaOH and
extracted 1 h at 60 °C two times in a waterbath and maintained the volume to 50 mL. Soluble protein content was quantified using Coomassie Brilliant blue with bovine serum albumin as a protein standard (20) and the concentration was expressed as dry weight percentage (%).

Statistical analysis

The data are presented in the figures and tables as the average of at least four replicates per treatment and means ± standard deviation. Each experiment was conducted in duplicate. The differences between means were calculated using a Tukey test at the 0.05 level by Origin 7.5 software.

Results and Discussion

Growth curves, kinetics and biomass production of C. vulgaris

Results presented in Fig. 1 and Table 1 demonstrate the effects of glycerol and glucose on the growth curves, kinetics and biomass production of C. vulgaris under mixotrophic cultivation (96h). With the cultivation of C. vulgaris under 30 °C and 2500 Lux, all of the cultures had an obvious growth except the control group. The samples supplied organic carbon sources (glycerol and glucose) displayed the superiority in their growth compared with the autotrophic control. Especially, the mixes of glycerol and glucose were better than the sole glycerol. After lag phase (about 12 hours), the algal cells got into their logarithmic growth phases. Till to 84 h the cell growth tended to stationary phases. Early in culture, the algal cells growth was inhibited by high concentrations of glycerol (10 g/L). Fortunately, the phenomenon vanished during the exponential phase. In comparison with autotrophic control, the specific growth rates of C. vulgaris were promoted by glycerol and glucose in medium under illumination and the maximal values of 0.99 day⁻¹ were obtained in the samples supplied 5 g/L glycerol and 2 g/L glucose and 10 g/L glycerol and 2 g/L glucose.

After 96 h cultivation, the maximal biomass content of 2.62 g/L was obtained in the culture added 10 g/L glycerol and 2 g/L glucose, which higher than the control group 7.71 times, however, it had no significant difference (p<0.05) with the culture medium supplied 5 g/L glycerol and 2 g/L glucose (2.60 g/L). Meanwhile, the results of biomass productivity for
C. vulgaris were similar to the biomass content. The biomass productivity increased with the supplement of glycerol and glucose, as well as the glycerol concentration. The maximal biomass productivity was 654.17 mg/L/day in the culture added 10 g/L glycerol and 2 g/L glucose, which had a significant difference (p<0.05) with control sample (85.42 mg/L/day), but the group supplied 5 g/L glycerol and 2 g/L glucose (650.00 mg/L/day).

For biomass production of microalgae, many cultivation modes, such as open pond, raceway and heterotrophic fermenter, have been established (21, 12). Some studies have focused on the methods to make biodiesel from microalgae more economical. One of them is to decrease substrate costs by using alternative carbon sources, such as corn powder hydrolysate (22), sweet sorghum juice (23). Alternatively, studies have sought to find marketable uses for the byproducts of algal biodiesel production, such as glycerol.

It is found that in the growth of Spirulina sp. there are three metabolic possibilities of culture: autotrophic, heterotrophic and mixotrophic. In mixotrophic growth there are two distinctive processes, photosynthesis and aerobic respiration. The former is influenced by light intensity and the latter is related to the organic substrate concentration (glucose) (24). C. vulgaris can grow with the similar rate on carbon sources and/or illumination. The high cells density of mixotrophic cultures demonstrates that the growth-stimulating effects of light and CO2 utilization in mixotrophic cultures were as strong as the effects of glucose (25). Early report showed that mixotrophic growth offered a possibility to increase microalgal cell concentration greatly and volumetric productivity in batch system. Meanwhile the ATP formed in the photochemical reactions accelerated the anabolism from glucose in the mixotrophic culture of Euglena gracilis. This should be a reason for the growth increase in the mixotrophic culture (26). The results of our study suggests that C. vulgaris has the potential to be a excellent biofuel producer due to the growth rate of the strains can be stimulated by organic materials.

Recent work showed that C. protothecoides showed slight inhibition from glycerol, and still could grow even the salinity reached to 35 g/L in the culture medium. C. vulgaris, however, had much smaller tolerance to glycerol and its growth was inhibited when the glycerol concentration reached to 15 g/L (25). In our study, slight inhibition from glycerol was observed during initial cultivation when the glycerol concentration reached to 10 g/L.
However, the growth dynamics parameters (specific growth rates, biomass content and productivity) of the alga had no significant differences between 5 g/L and 10 g/L glycerol added in media. Furthermore, this inhibition decreased by the mixture of glycerol and glucose. The results advised that using glycerol and glucose as complex carbon sources for mixotrophic cultivation of *C. vulgaris* is a feasible way to solve the problem of low algal cell density if glycerol as the sole carbon source in medium and stimulate the utilization of glycerol by *C. vulgaris*.

*Photosynthetic pigments content and productivity*

Chlorophyll is one of the cellular compounds that is used for estimating biomass of microalgae in culture and can be used to measure growth. As shown in Table 2, the effects of glycerol and glucose on the photosynthetic pigments contents and productivities of mixotrophic *C. vulgaris* were varied and significant. After 96 hours cultivation, the autotrophic control culture was obtained the maximum concentrations of chlorophyll a, b, total chlorophyll and carotenoids were obtained based on the dry cell weight. They were 23.53 mg/g, 12.55 mg/g, 36.08 mg/g and 4.88 mg/g, respectively. All the above values were higher than the samples when added 1 g/L, 5 g/L, 10 g/L glycerol or 2 g/L glucose (p<0.05). However, with the increase of glycerol (from 1 to 10 g/L) and the supplement of 2 g/L glucose in mixotrophic cultures, the photosynthetic pigments contents decreased obviously. In the groups supplied 1 g/L glycerol, 1 g/L glycerol and 2 g/L glucose, the decrease was more significant compared with the autotrophic control sample (p<0.05). The effects of glycerol concentrations at 5 g/L and 10 g/L on the photosynthetic pigments contents were not significant (p<0.05).

At the end of culture (96 h), the maximum photosynthetic pigments productivities were obtained in the group of 1 g/L glycerol and 2 g/L glucose, and the values of total chlorophyll and carotenoids productivities are 5.74 mg/L/day and 0.75 mg/L/day, which higher the autotrophic control 1.87 times and 1.83 times, respectively. However, the differences of chlorophyll and carotenoids productivities between the samples supplied 5 g/L and 10 g/L glycerol were not significant (p<0.05). According to previous reports, the utilization of an external organic carbon source may effect on the photoautotrophic growth processes in many
aspects, such as photosynthesis and respiration (27). Glucose inhibited the photosynthetic CO$_2$ fixation ten-fold and modified the pigmentary system. Light inhibited glucose uptake and assimilation, but under mixotrophic conditions maximal utilization of glucose was obtained. However, for *C. vulgaris* UAM 101, both light and dark respiration rates were enhanced by the addition of glucose, though the net photosynthetic rate was not influenced (28). The study of *Spirulina platensis* showed that both the photosynthetic and respiration rate were unchanged upon addition of glucose (29). Organic carbon assimilation under mixotrophic conditions induces changed in both respiratory and photosynthetic metabolism in cyanobacteria (30). Marquez et al. suggested that the photosynthetic activity and the organic carbon-dependent respiratory activity operated separately in those cells, because the total growth yields of *S. platensis* in mixotrophic cultures equal the yield in photoautotrophic growth in the light plus heterotrophic growth in the dark (29). However, Nieva and Valiente (31) observed a decreased rate of CO$_2$ fixation under mixotrophic growth, which suggested that some aspects of CO$_2$ metabolism may be modulated by organic compounds. That may be the reason for the inhibition of photosynthetic pigments synthesis under mixotrophic growth. Yamane et al. indicated that a good production of chlorophyll (39.4 mg/L) and carotenoids (13.8 mg/L) were attained in the mixotrophic culture of *E. gracilis*, due to the highest fermenter productivity with respect to biomass as well as chlorophyll and carotenoids (26). Our results showed that the mixotrophic cultures experience an increase in photosynthetic pigments productivities that were dependent on the increase of microalgae biomass content.

**Lipid content and productivity**

As summarized in Fig. 2, the effects of glycerol and glucose on the lipid content and productivity of *C. vulgaris* under mixotrophic conditions were markedly. For the lipid contents based on the dry cell weight, the lowest content of 7.48 % was obtained in the autotrophic control culture, and the highest value of 10.64 % was obtained in the group with 10 g/L glycerol and 2 g/L glucose supplied in SEM. With the increase of glycerol concentration, the lipid content increased slightly. The SEM samples supplied 10 g/L glycerol (and 2 g/L glucose) had significant difference compared with the control (p<0.05). But the addition of glucose did not promote the lipid accumulation (p<0.05). While the differences of
lipid volumetric productivities caused by effects of glycerol and glucose were more than the lipid contents. Lipid volumetric productivities of *C. vulgaris* stimulated by the glycerol and glucose since the organic carbons promoted the algal biomass content notably. The maximal lipid productivity (69.68 mg/L/day) was achieved in the group added 10 g/L glycerol and 2 g/L glucose, which higher than the control 10.96 times. In the mixotrophic samples, the lipid productivities in the groups using complex carbon sources were higher than the groups using different contents of glycerol as the sole organic carbon sources in SEM.

Recent work revealed that *C. vulgaris* could grow on autotrophic, mixotrophic and heterotrophic modes, and the mixotrophic cultivation could especially produce more cell biomass than the autotrophic, heterotrophic cultures individually or combined (25). Furthermore, the substrate concentration significantly influenced the final cell yield of the mixotrophic cultivations while the cell lipid content remained relatively constant. The sensitivity analysis results showed that the initial glycerin concentration was the most significant factor for algae growth and lipid production (32). The new study has demonstrated the promise of simultaneous high growth rates and lipid yields of *C. protothecoides* heterotrophically grown on mixtures of glycerol and glucose (33). In their work, the growth and lipid production of *C. protothecoides* using glycerol as the carbon source were performed to demonstrate the utility of recycling glycerol created during biodiesel production. Glycerol was examined as both the sole carbon source and combination with glucose. Algae cultures grown on sole glycerol in shake flasks showed a specific growth rate of 0.1/h and final lipid yield of 0.31 g lipid/g substrate, respectively. The values were similar to those observed on pure glucose, 0.096/h and 0.24 g lipid/g substrate. When the media contained a mixture of glycerol and glucose, simultaneous uptake of the two substrates was observed. Due to the difference in rates of lipid storage, lipid productivity was 0.077 g lipid / (l h) during growth on glycerol, while growth on glucose had a productivity of 0.096 g lipid / (l h). During growth on the 9:1 mixture of both glycerol and glucose, lipid productivity was 0.098 g lipid / (l h). Another report indicated that in batch mode, the biomass and lipid concentration of *C. protothecoides* cultivated in a crude glycerol medium were 23.5 g/L and 14.6 g/L respectively in a 6-day cultivation (34). In the fed-batch mode, the biomass and lipid concentration improved to 45.2 g/L and 24.6 g/L respectively after 8.2 days of cultivation. This work
demonstrates the feasibility of crude biodiesel glycerol as an alternative carbon substrate to glucose for microalgal cultivation and a cost reduction of carbon substrate feed in microalgal lipid production may be expected. Similar results were observed in our work. In mixotrophic cultures, the lipid contents were higher than (1.08-1.42 times) in the autotrophic culture. However, the mixotrophic cultures supplied glycerol and/or glucose experience an increase in lipid productivities that dependent on the increase in biomass content.

**Soluble carbohydrates and protein content and productivity**

From Fig. 3, it can be seen that the effects of glycerol and glucose on the soluble carbohydrate content and productivity of *C. vulgaris* under mixotrophic cultivation. The lowest soluble carbohydrates content and productivity of 6.04 % and 5.13 mg/L/day were obtained in the control sample. Meanwhile, the maximal values of 8.74 % and 57.26 mg/L/day were achieved in the group of 10 g/L glycerol and 2 g/L glucose added in SEM. In comparison of autotrophic control sample, the cultures supplied glycerol and glucose had higher soluble carbohydrates contents and productivities. At the 1 g/L glycerol level, the differences of soluble carbohydrates contents were not significant compared to the control. But those differences became significantly with the increase of glycerol contents (5 g/L and 10 g/L). Moreover, each culture supplied glycerol and glucose had higher carbohydrates content than only supplied glycerol. The differences of soluble carbohydrates productivities among the experimental groups were significant and enhanced by the glycerol concentration and glucose feed.

Fig. 4 indicates that the effects of the supplement of glycerol and glucose on the soluble protein content and productivity of *C. vulgaris*. The effects of mixotrophic (illumination and organic carbon stimulation) mode on the algal soluble protein anabolism were different from lipid and soluble carbohydrate, but similar to the photosynthetic pigments biosynthesis. The minimum soluble protein content (1.58 %) and productivity (10.37 mg/L/day) were obtained in the group with 10 g/L glycerol and 2 g/L glucose added in SEM, and the maximal values of 18.13 %, 15.41 mg/L/day were achieved in the autotrophic control group, respectively.

Carbohydrates have been found as the intermediary reserves in some algae, due to the fact that they are required when the nitrogen becomes limited in the lipid synthesis. In the present
study when protein content in *C. vulgaris* decreases, both lipid and carbohydrate were increased (Fig. 2-4). These changes in the constituents agreed with reports of others authors who mentioned that there was a reduction in the protein content of mixotrophic *C. vulgaris* UAM 101 cells, which was compensated by an increase in lipids and carbohydrates (28). Ogbonna and Tanaka reported that during the night, decreases were observed in the biomass concentration and carbohydrate contents of *C. pyrenoidosa* cells while their protein content increased (35). These changes implied that in the absence of light energy, intracellularly stored carbohydrate was metabolized as an energy source in part for cell maintenance and protein synthesis. Similar results were obtained in our study. With 2 g/L glucose and the more the glycerol concentrations feed, the higher the soluble carbohydrate content lower the protein of *C. vulgaris* were obtained based on the dry cell weight. The mixotrophic conditions and supplement of organic carbon resources promoted the biomass, lipid and carbohydrate production while reduce the pigments and protein biosynthesis, which implied the mixotrophic mode changed the metabolic pathways of nitrogen and carbon.

Previous work reported that nitrogen (nitrate) was essential for astaxanthin accumulation in *Haematococcus pluvialis*. They suggested that nitrogen was required for continuous synthesis of protein responsible for supporting the pigment formation (36). Another study concluded that higher chlorophyll and protein contents were found in *C. vulgaris* cultures with higher ammonia concentrations. The algal growth was accompanied by a decrease in nitrogen content in the medium, indicating that nitrogen removal was due to algal uptake and assimilation (37). Our results suggested that the supplement of organic carbon and energy (light and glucose) might convert the algal cell metabolic pathway. Those results suggested that changes in the cellular biochemical composition were influenced by the trophic mode and nutrient concentration in the medium.

**Conclusions**

In summary, the results from this paper showed that *C. vulgaris* can utilize glycerol as a sole carbon substrate. Yet, glycerol was inferior to the complex of glycerol and glucose for production of biomass and biochemical components. The effects of glycerol and glucose as
mixed substrates could enhance the *C. vulgaris* growth, biomass content and volumetric productivity, and overcome the lower biomass caused by glycerol as the sole organic carbon source. Besides, the utilization of mixed organic carbon substrates could stimulate the accumulation of lipids and soluble carbohydrates as the raw materials for biodiesel and bioethanol production, respectively, while reduce the anabolism of photosynthetic pigments and protein based on the dry cell weight. This provides a feasible way to reduce the cost of bioenergy production from microalgae by cyclic utilization of glycerol and hydrolysis of algal cell carbohydrate.

**Acknowledgments**

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**References**


30. A. Vonshak, S.M. Cheung, F. Chen, Mixotrophic growth modifies the response of


Table 1

Effects of glycerol and glucose on the biomass content, specific growth rate and productivity of *C. vulgaris*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 g/L Gly</th>
<th>1 g/L Gly +2 g/L Glu</th>
<th>5 g/L Gly</th>
<th>5 g/L Gly +2 g/L Glu</th>
<th>10 g/L Gly</th>
<th>10 g/L Gly +2 g/L Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass content, g/L</td>
<td>(0.34±0.06)a</td>
<td>(0.62±0.03)a</td>
<td>(1.48±0.19)b</td>
<td>(2.13±0.34)c</td>
<td>(2.60±0.18)d</td>
<td>(2.16±0.04)cd</td>
<td>(2.62±0.10)d</td>
</tr>
<tr>
<td>Specific growth rate -</td>
<td>(0.48±0.05)a</td>
<td>(0.63±0.01)b</td>
<td>(0.84±0.03)c</td>
<td>(0.94±0.04)d</td>
<td>(0.99±0.02)d</td>
<td>(0.94±0.02)d</td>
<td>(0.99±0.01)d</td>
</tr>
<tr>
<td>Biomass productivity, mg/L/day</td>
<td>(85.42±15.73)a</td>
<td>(154.17±7.22)a</td>
<td>(368.75±47.19)b</td>
<td>(533.33±83.93)c</td>
<td>(650.00±43.75)d</td>
<td>(539.58±9.55)cd</td>
<td>(654.17±25.26)d</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., *N*=4; mean values in the same line with different letters in the superscript are significantly different (p<0.05).
Table 2

Effects of glycerol and glucose on the photosynthetic pigments content and productivity of *C. vulgaris*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 g/L Gly</th>
<th>1 g/L Gly + 2 g/L Glu</th>
<th>5 g/L Gly</th>
<th>5 g/L Gly + 2 g/L Glu</th>
<th>10 g/L Gly</th>
<th>10 g/L Gly + 2 g/L Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a content, mg/g</td>
<td>(23.53±1.32)^a^</td>
<td>(17.62±1.30)^b^</td>
<td>(11.78±0.48)^c^</td>
<td>(6.51±0.10)^d^</td>
<td>(5.64±0.27)^de^</td>
<td>(5.94±0.26)^de^</td>
<td>(4.46±0.31)^e^</td>
</tr>
<tr>
<td>Chlorophyll a productivity, mg/L/day</td>
<td>(2.00±0.11)^a^</td>
<td>(2.73±0.20)^b^</td>
<td>(4.36±0.18)^c^</td>
<td>(3.47±0.05)^d^</td>
<td>(3.67±0.18)^d^</td>
<td>(3.21±0.14)^de^</td>
<td>(2.92±0.20)^be^</td>
</tr>
<tr>
<td>Chlorophyll b content, mg/g</td>
<td>(12.55±0.76)^a^</td>
<td>(8.30±0.45)^b^</td>
<td>(3.72±0.35)^c^</td>
<td>(1.49±0.06)^d^</td>
<td>(1.25±0.10)^d^</td>
<td>(1.60±0.13)^d^</td>
<td>(1.28±0.14)^d^</td>
</tr>
<tr>
<td>Chlorophyll b productivity, mg/L/day</td>
<td>(1.07±0.06)^a^</td>
<td>(1.29±0.07)^b^</td>
<td>(1.38±0.13)^b^</td>
<td>(0.79±0.03)^c^</td>
<td>(0.81±0.07)^c^</td>
<td>(0.86±0.07)^c^</td>
<td>(0.84±0.09)^c^</td>
</tr>
<tr>
<td>Total chlorophyll content, mg/g</td>
<td>(36.08±1.21)^a^</td>
<td>(25.93±1.72)^b^</td>
<td>(15.50±0.83)^c^</td>
<td>(8.00±0.16)^d^</td>
<td>(6.89±0.38)^de^</td>
<td>(7.55±0.39)^de^</td>
<td>(5.74±0.45)^e^</td>
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<tr>
<td>Total chlorophyll productivity, mg/L/day</td>
<td>(3.07±0.10)^a^</td>
<td>(4.02±0.27)^b^</td>
<td>(5.74±0.31)^c^</td>
<td>(4.26±0.08)^b^</td>
<td>(4.48±0.24)^b^</td>
<td>(4.07±0.21)^b^</td>
<td>(3.76±0.29)^bd^</td>
</tr>
<tr>
<td>Carotenoids content, mg/g</td>
<td>(4.88±0.44)^a^</td>
<td>(4.23±0.06)^b^</td>
<td>(2.03±0.15)^c^</td>
<td>(1.16±0.02)^d^</td>
<td>(1.03±0.05)^d^</td>
<td>(1.14±0.05)^d^</td>
<td>(0.79±0.01)^d^</td>
</tr>
<tr>
<td>Carotenoids productivity, mg/L/day</td>
<td>(0.41±0.04)^a^</td>
<td>(0.66±0.01)^b^</td>
<td>(0.75±0.05)^c^</td>
<td>(0.62±0.01)^b^</td>
<td>(0.67±0.03)^b^</td>
<td>(0.62±0.03)^ b^</td>
<td>(0.52±0.01)^d^</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., *N*=4; mean values in the same line with different letters in the superscript are significantly different (p<0.05).
Figure Captions

**Fig. 1.** Effects of glycerol and glucose on the growth curve of *C. vulgaris*.
Values are mean ± S.D., *N*=4.

**Fig. 2.** Effects of glycerol and glucose on the lipid content and productivity of *C. vulgaris*.
Values are mean ± S.D., *N*=4; the significance of differences were noted by different letters (p<0.05).

**Fig. 3.** Effects of glycerol and glucose on the soluble carbohydrates content and productivity of *C. vulgaris*.
Values are mean ± S.D., *N*=4; the significance of differences were noted by different letters (p<0.05).

**Fig. 4.** Effects of glycerol and glucose on the soluble protein content and productivity of *C. vulgaris*.
Values are mean ± S.D., *N*=4; the significance of differences were noted by different letters (p<0.05).
Fig. 1

Fig. 2
Soluble carbohydrate content (%)
Soluble carbohydrate productivity (mg/l/day)

Fig. 3

Soluble protein content (%)
Soluble protein productivity (mg/l/day)

Fig. 4