The effects of culture depth (2–10 cm) and cell density on the growth rate and biomass productivity of Chlorella sp. XQ-200419 were investigated through the use of a self-designed open circular pond photobioreactor–imitation system. With increases in culture depths from 2 to 10 cm, the growth rate decreased significantly from 1.08 /d to 0.39 /d. However, the biomass productivity only increased slightly from 8.41 to 11.22 g/m$^2$/d. The biomass productivity (11.08 g/m$^2$/d) achieved in 4 cm culture with an initial OD$_{540}$ of 0.95 was similar to that achieved in 10 cm culture with an initial OD$_{540}$ of 0.5. In addition, the duration of maximal areal productivity at a 4 cm depth was prolonged from 1 to 4 days, a finding that was also similar to that of the culture at a 10 cm depth. In both cases, the initial areal biomass densities were identical. Based on these results and previous studies, it can be concluded that the influence of culture depth and cell density on areal biomass productivity is actually due to different areal biomass densities. Under suitable conditions, there are a range of optimal biomass densities, and areal biomass productivity reaches its maximum when the biomass density is within these optimal ranges. Otherwise, biomass productivity will decrease. Therefore, a key factor for high biomass productivity is to maintain an optimal biomass density.

**Key words:** Chlorella, culture depth, cell density, growth rate, biomass productivity, shallow culture

Chlorella has been mass produced for use in health foods for years, and is also well known for its great potential as a biomass feedstock for biodiesel production. Many studies have been conducted on its nutritional physiology, and on the influence of light intensity and temperature on Chlorella growth [11, 14, 15, 18, 19]. With increased interest in oleaginous microalgae, researchers found that Chlorella could accumulate large amounts of lipids under certain conditions, such as nitrogen-deficiency stress [8]. Therefore, the potential for producing biofuel from Chlorella has received widespread attention [2, 4, 5, 10, 12, 20, 21, 23].

In every cultivation system, the culture depth and optical cross-section are basic factors to be considered, which affect productivity in mass microalgal cultures [13]. Commercial Chlorella production plants usually employ open ponds as photobioreactors, with the culture depth always within the range of 10–30 cm [9]. Previous work on other microalgae has indicated that cell density and light path were also key factors influencing algal growth and biomass productivity [6, 7, 17, 20]. However, to date, there has been no published research on the effects of these factors on the growth rate and productivity of Chlorella.

Mass cultivation of Chlorella at a deep culture depth requires large amounts of energy to circulate the algal suspension, thus making the establishment of a “green” culture mode of low energy consumption a priority.

Doucha and Livanský [2] used an inclined photobioreactor to culture Chlorella sp. at a depth of 6–7 mm, and achieved a biomass density of up to 40–50 g/l of dry weight. Their work indicates that a reduction in culture depth, without losing high biomass productivity, is possible by properly controlling the cell density of the algal suspension. Chlorella sp. XQ-200419 is a highly oleaginous strain screened by our laboratory [15], which grows rapidly, produces a large quantity of biomass, and can accumulate high amounts of lipids under suitable conditions. Therefore, it is an ideal strain for use as a resource for biodiesel production. The objective of this paper was to explore the feasibility of establishing a shallow (< 10 cm depth) culture
system with water and energy saving properties, while maintaining a high cell density of *Chlorella*.

**Materials and Methods**

**Strain and Growth Conditions**

The *Chlorella* sp. XQ-200419 used in this research was provided by the Algae Culture Collection of the Wuhan Botanical Garden, Chinese Academy of Sciences. *Chlorella* sp. XQ-200419 is an unicellular green microalga grown on a modified 2× BG-11 media [23].

In order to determine the effects of culture depth on the growth and biomass productivity of *Chlorella*, the culture depths were set at 2, 4, 6, 8, and 10 cm, respectively, and the initial optical density (OD<sub>540</sub>) was 0.5. To study the effect of cell density on biomass productivity, 4 and 10 cm depths, representing shallow and deep cultures, respectively, were selected. The initial OD<sub>540</sub> was 0.95 for the 4 cm culture and 0.5 for the 10 cm culture.

The culture conditions for all experiments in this study were 30°C, 200 µmol/m²/s, a 14/10 h light/dark cycle, and 50 rpm of mixing rate. The pH of the algal suspensions was adjusted to be within the range of 6.5–7.5. Pure CO₂ was bubbled into the algal suspensions through an aeration tube, which extended into the bottom of the reactor to provide a carbon source and to adjust the pH at intervals of 2–4 h during the light cycles. Sterile distilled water was added to the algal suspension to replenish water lost due to evaporation every 3 h. Each experiment was repeated at least three times.

**Culture System**

The culture system for this research was a self-designed photobioreactor that consisted of a double-layered circular tank, a light source, a temperature control system, a horizontal rotating stir system, and a CO₂ supply system [22]. The double-layered circular tank was made of a clear acrylic plastic, and had an outer diameter of 36 cm and an inner diameter of 10 cm. The solution depth ranges of 2 to 12 cm corresponded to volumes of 1–6 L. The main component of the automatic temperature control system was a thermostatic circulator, which was linked with the circular tank by outlet and inlet water pipes. Light was supplied by 12 fluorescent light tubes, and the light intensity was adjusted by altering the distance between the light tubes and the solution’s surface. The stir system was made up of a central vertical axis and a stir blade. The stir blade was 25 cm in length and fixed at the top of the central vertical axis. The CO₂ supply system was a manually controlled system, which provided a flow rate of 0–1.0 L/min.

**Light Transmittance of Algal Suspension**

The photobioreactor was made of transparent materials that hardly reduced light irradiation, and the side face of the photobioreactor was covered with light-proof material so that light could only vertically illuminate the upper algal suspension’s surface. Light intensity was measured by a TES-1332A irradiance meter (TES Electrical Electronic Corp., Taipei, China) at the surface of the algal suspension and at the bottom of the photobioreactor. The light transmittance (T) of the algal suspension was calculated as follows:

\[ T = \left( \frac{I}{I_0} \right) \times 100\% \]

in which I<sub>0</sub> is the incident light intensity in the vertical direction on the suspension surface, and I is the transmitted light intensity at the bottom of the reactor.

**Determination of Optical Density, Cell Density, and Biomass Dry Weight**

Cells of *Chlorella* in the exponentially growing stage were concentrated by centrifugation to reach an OD<sub>540</sub> of 2.0. The concentrated algal suspension was diluted to 1, 1/2, 1/4, 1/6, 1/8, 1/10, and 1/20 times by volume, and then the OD, dry cell weight, and cell numbers of each dilution were determined. The optical density of the algal suspension was measured by a UV752C spectrophotometer (Shanghai Lengguang Technology Corp., Shanghai, China) at a wavelength of 540 nm and a light path of 1 cm. Cell numbers were measured by counting cells with a hemocytometer under a microscope. To measure dry cell weight, a certain volume of algal suspension was centrifuged at 7,000 × g for 8 min, and then the cell pellets were rinsed twice with distilled water and finally dried in a vacuum dryer to a constant weight. The dry cell weight was then gravimetrically measured.

**Growth Measurements**

Algal growth was determined by measuring the OD<sub>540</sub> every 24 h, and then the biomass dry weight was calculated based on the obtained regression equation and the measured OD. The specific growth rate and areal biomass productivity of the algae were estimated by measuring changes in cell numbers and biomass dry weights. The specific growth rate (K) was calculated using the follow equation:

\[ K = \frac{(\ln N_2 - \ln N_1)/(T_2 - T_1)}{H} \]

in which N<sub>i</sub> and N<sub>j</sub> are the mean cell numbers of Chlorella sp. XQ-200419 at the times T<sub>i</sub> and T<sub>j</sub>, respectively.

Areal biomass productivity (P) was calculated by

\[ P (g/m²/d) = 10 \times H \times (B_2 - B_1)/(T_2 - T_1) \]

in which H is the culture depth (cm), and B<sub>i</sub> and B<sub>j</sub> are the mean biomass dry weights at the times T<sub>i</sub> and T<sub>j</sub>, respectively.

**Results**

**Changes of Light Transmittance of Algal Suspension During Culture**

In order to study the influence of effective light energy on algal growth, the light transmittance of different depths of cultures was investigated. The results showed that light transmittance of algal suspensions of >1% were only observed on the first 2 days in a 2 cm culture and on the first day in a 4 cm culture. Light transmittance of the remaining cultures was seen to be approximately zero. Thus, the loss of light energy during culture at different depths was negligible when light penetrated into the algal suspension (data not shown).

**Effects of Culture Depth on Algal Growth and Productivity**

Fig. 1 depicts the growth curves of *Chlorella* sp. XQ-200419 cultured at different depths. Shallower cultures
resulted in higher biomass dry weights. When the culture depth was 2 cm, the biomass dry weight reached a maximum value of 1.1 g/l after 5 days of culture (Fig. 1A). Although the general trend was that the highest growth rate occurred on day 2 and then the rate started to decline afterwards, culture depths had a remarkable influence on growth rates (Fig. 1B). During the first 2 days, the growth rates increased with increasing culture depths. After that, growth rate declined more rapidly in shallower cultures.

As shown in Fig. 2, the areal biomass productivity of all cultures increased rapidly during the first 2 days, and the maximum value was obtained on day 2. With increases in culture depths from 2 to 10 cm, the duration of maximum areal biomass productivity was gradually prolonged. Once maximum biomass productivity occurred, the value either immediately declined (e.g., in the 2 and 4 cm cultures), or remained more or less stable for 2–4 days and then declined (e.g., in the 6, 8, and 10 cm cultures).

The Fig. 3 shows the changes in maximum specific growth rates and areal biomass productivities of *Chlorella* sp. XQ-200419 during cultivation at different culture depths. The specific growth rate was greatly reduced with increasing culture depths. There was an essentially linear decline in the growth rate with increases in depths from 2
to 6 cm. When the culture depth was >6 cm, the growth rate decreased at a slightly slower rate. The specific growth rate for the 2 cm culture was 1.08/d, which was 3-fold higher than that of the 10 cm culture (0.39/d). Deeper cultures resulted in higher biomass productivities. When the culture depth was increased from 2 to 10 cm, the biomass productivity increased by about 33%, from 8.41 to 11.22 g/m²/d.

Effects of Cell Density on Biomass Productivity

Plotting the areal productivity as a function of biomass density revealed a clear relationship between culture depth and optimal biomass density (Fig. 4). For each culture depth, there is a range of optimal areal density, which is defined as the biomass density that yields the highest areal biomass productivity. Higher areal density is required for deeper cultures to achieve maximum productivity.

To explore the effects of cell density on the areal biomass productivity of *Chlorella* sp. XQ-200419, we carried out two group tests that had the same initial areal biomass density. The 4 cm culture with an OD₅₄₀ of 0.95 represented a shallow and high cell density culture; and the 10 cm culture with an OD₅₄₀ of 0.5 represented a deep and low cell density culture. The initial areal biomass densities of both were 17.6 g/m². When microalgae were cultured at a 4 cm depth with an OD₅₄₀ of 0.95, the medium was modified to 5× BG-11, so the quantity of each component in the culture medium was equal to that contained in the 10 cm culture medium with an OD₅₄₀ of 0.5.

By increasing the initial OD₅₄₀ from 0.5 to 0.95, the biomass density of the 4 cm culture was adjusted equally to that of the 10 cm culture with an initial OD₅₄₀ of 0.5. As a result, the highest biomass productivity was increased from 9.67 to 11.08 g/m²/d, which was approximately equal to the productivity achieved by the culture at a 10 cm depth (Table 1). Furthermore, the duration of the highest biomass productivity for the culture at a 4 cm depth was prolonged from 1 to 4 days, which was also a similar value achieved by the culture at a 10 cm depth.

**DISCUSSION**

Culture depth influenced the biomass productivity of *Chlorella* sp. XQ-200419 in two regards; namely, by influencing the maximal areal biomass productivity and the duration of maximal biomass productivity. Both these factors increased with increasing culture depths. Theoretically, deeper cultures can absorb and utilize more light energy. In our study, however, the light transmittance of shallower cultures was not significantly higher than that of deeper cultures. Hence, we conclude that the change in light transmittance at different culture depths is not the major reason for the concurrent increase in areal biomass productivity.

As low yields have been one of the major limiting factors in the mass production of algal biomass, the attainment of maximal productivity and its maintenance should be one of the most important operational objectives [4]. Hu *et al.* [7] studied the growth of *Spirulina platensis* using a flat plate photobioreactor with a light path of 7.5–200 mm. The results revealed an important rule for the cultivation of microalgae; only when cell density is in the optimal range can the highest biomass productivity be achieved. In our study, *Chlorella* sp. XQ-200419 was cultured in a simulated open circular pond instead of the

### Table 1. The relationship between culture depth, initial cell density, and maximum biomass productivity of *Chlorella* sp. XQ-200419.

<table>
<thead>
<tr>
<th>Culture depth (cm)</th>
<th>Culture medium</th>
<th>Initial cell density (OD₅₄₀)</th>
<th>Initial biomass density (g/m²)</th>
<th>Achieved biomass productivity (g/m²/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd day</td>
</tr>
<tr>
<td>4</td>
<td>2× BG-11</td>
<td>0.50</td>
<td>7.0</td>
<td>9.67</td>
</tr>
<tr>
<td>4</td>
<td>5× BG-11</td>
<td>0.95</td>
<td>17.6</td>
<td>12.21</td>
</tr>
<tr>
<td>10</td>
<td>2× BG-11</td>
<td>0.50</td>
<td>17.6</td>
<td>11.23</td>
</tr>
</tbody>
</table>

Initial cell density: the cell density of the algal suspension at the beginning of culture, expressed as OD₅₄₀.

Initial biomass density: the areal biomass density of the algal suspension at the beginning of culture, expressed in g/m².
flat plate photobioreactor used for *Spirulina platensis*. Although the changes in biomass productivity followed a similar pattern in both studies, there was also a significant effect noted at different culture depths in our study. Whereas a sharp decrease in areal productivity was observed right after reaching the maximum for the 2 and 4 cm cultures, the maximal productivity of the 6, 8, and 10 cm cultures was retained for 2–4 days, and then started to decrease. Therefore, instead of having an optimal biomass density like shallower cultures, there was actually a range of optimal biomass densities for deeper cultures. The highest biomass productivity could be achieved when cell densities fall into this range (Fig. 2).

It is generally accepted that the light path of a flat plate or tubular photobioreactor affects the growth and biomass productivity of *Chlorella* through light distribution in the photobioreactor and the movement frequency of the algal cells between the surface and the bottom zones [6, 17]. Additionally, in our opinion, biomass density is also a critical factor affecting growth and biomass productivity. Under suitable conditions of temperature, light intensity and liquid circulation, there is a range of optimal biomass densities, and areal biomass productivity is able to reach its maximum when the biomass density is in the optimal range; otherwise, productivity will decrease. The hypothesized influence of culture depth or light path and cell density on areal biomass productivity is actually the influence of areal biomass density on biomass productivity (Table 1). Therefore, the key to achieving the highest areal productivity is the maintenance of optimal biomass density. We have termed this conclusion “the range of optimal biomass density theory.”

Based on this theory, in continuous or semi-continuous cultures, areal biomass productivity obtained in shallow culture systems with high cell densities, established by increasing areal biomass densities, can be achieved in deep pond cultures with low cell densities. Compared with deep pond cultures, the shallow culture system is a water- and energy-saving mode of microalga cultivation. Besides the advantage of low cost, this system also improves the energy input–output ratio, which plays an important role in achieving net energy output and promoting the commercialization of production of microalgal biodiesel.

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