Full Length Research Paper

Enhancement of biomass and hydrocarbon productivities of *Botryococcus braunii* by mixotrophic cultivation and its application in brewery wastewater treatment

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Accepted 9 December, 2011

Successful production of mixotrophic algae allows the integration of both photosynthetic and heterotrophic components during the diurnal cycle. This reduces the impact of biomass loss during dark respiration and decreases the amount of organic substances utilization during growth; these features infer that mixotrophic production can be an important part of the microalgae to biofuels process. Mixotrophic mode provides an effective way to cultivate *Botryococcus braunii* with high cell density and short cultivation cycle because exogenous carbon sources, in especial, organic carbon sources, such as sodium acetate, glucose and sucrose can stimulate the biomass production of the alga remarkably. The biomass and hydrocarbon volumetric productivities were promoted significantly by the mixotrophic cultivation of *B. braunii* compared with the photoautotrophic group, even though the hydrocarbon contents under mixotrophic conditions were not increased in pace with the biomass contents. The present study suggested that coupling the cultivation of mixotrophic microalgae and organic wastewater treatment is a potential way to produce microalgae biomass, accumulate hydrocarbon and remove organic and inorganic salts.

Key words: *Botryococcus braunii*, mixotrophic cultivation, growth characteristics, biomass production, hydrocarbon accumulation, brewery wastewater treatment.

INTRODUCTION

Microalgal cells can trap light energy as the energy source and assimilate CO₂ as the carbon source; moreover, organic substrates can also be utilized as the carbon and energy sources by many microalgae (Yang et al., 2000). Many algal organisms are capable of using either metabolism process (autotrophic or heterotrophic) for growth, meaning that they are able to photosynthesis as well as ingest prey or organic materials (Zhang et al., 1999). Growth is influenced by the media supplement with glucose during the light and dark phases; hence, there is less biomass loss during the dark phase (Andrade and Costa, 2007). Chojnacka and Noworyta (2004) compared *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. They found that mixotrophic cultures reduced photosynthesis and improved growth rates over both autotrophic and heterotrophic cultures. Successful production of mixotrophic algae allows the integration of both photosynthetic and heterotrophic components during the diurnal cycle. This reduces the impact of biomass loss during dark respiration and decreases the amount of organic substances utilization during growth. These features infer that mixotrophic production can be an important part of the microalga-to-biofuels process (Brennan and Owende, 2010).

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The green alga *Botryococcus braunii* has high hydrocarbon content, ranging from 15 to 75% of dry weight, as long chain unsaturated hydrocarbons (Sawayama et al., 1994). This characteristic has attracted increasingly more attention in the last two decades in attempts to exploit *B. braunii* for renewable biofuel (Kojima and Zhang, 1999). Many studies have been conducted to determine the culture conditions for *B. braunii* biomass production and hydrocarbon accumulation (Metzger and Largeau, 2005). Recently, the impact of carbon, nitrogen, phosphorus and other nutrients on the growth of *B. braunii* CHN 357 have been studied (Wang et al., 2003; Yang et al., 2004a) and the growth and lipid content of three *B. braunii* strains from China, United Kingdom and Japan were also compared at three temperatures (20, 25 and 30°C), three irradiances (60, 100 and 300 Wm⁻²) and four salinities (0, 0.15, 0.25, and 0.5 M NaCl), the study showed that the environmental requirement varied among the three *B. braunii* strains (Li and Qin, 2005). The effect of nitrogen limitation in a medium on the composition of intracellular lipids in the alga *B. braunii* Kütz IPPAS H-252 indicated that under nitrogen limitation, the alga cells accumulated lipids in the form of oleic acid-rich triacylglycerols (Zhila et al., 2005); however, its industrial cultivation has not been realized due to the economical and technical barriers, especially its slow growth rate and low biomass concentration (Yang et al., 2004b; Banerjee et al., 2002).

In this study, the effects of carbon sources, sodium acetate and glucose content on the biomass production and hydrocarbon accumulation of *B. braunii* under mixotrophic cultivation were investigated and particularly, the growth characteristics and hydrocarbon production of these green algae in brewery wastewater medium were analyzed.

**MATERIALS AND METHODS**

**Strain and cultivation**

*B. braunii*, obtained from the Culture Collection of Algae from the Institute of Hydrobiology (Chinese Academy of Sciences), was grown on Chu 13 × 2 medium (Yamaguchi et al., 1987). Cultivation of *B braunii* was carried out in 500 ml Erlenmeyer flasks containing 150 ml Chu 13 × 2 medium (pH 7.2) at 25 ± 2°C with light illumination (2500 lux; 12:12 h light:dark cycle) and shaking under 100 rpm in an illumination incubator for 15 days. For mixotrophic culture, the medium was supplemented with different inorganic and organic carbon sources (Table 1).

*B. braunii* was also cultured in untreated BWW (brewery wastewater) medium (obtained from Gansu Resources Breweries Co., Ltd., pH=7.6), or BWW supplied with 0.50 g/L potassium nitrate as the nitrogen source (triplicate were performed for both cultures), respectively. Algal stock culture was inoculated to the medium to give a 10% (v/v) concentration. All solutions were autoclaved at 121°C for 20 min prior to use.

**Table 1.** Carbon sources employed for the autotrophic and mixotrophic cultivation of *B. braunii.*

<table>
<thead>
<tr>
<th>Code</th>
<th>Carbon sources</th>
<th>Content (g/L)</th>
<th>Code</th>
<th>Carbon sources</th>
<th>Content (mM)</th>
<th>Code</th>
<th>Carbon sources</th>
<th>Content (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>2</td>
<td>NaHCO₃</td>
<td>1 SA₁₀</td>
<td>3</td>
<td>CH₃COONa</td>
<td>1 SA₂₀</td>
</tr>
<tr>
<td>4</td>
<td>Na₃-Citric acid</td>
<td>1 SA₄₀</td>
<td>5</td>
<td>Glucose</td>
<td>1 SA₆₀</td>
<td>6</td>
<td>Sucrose</td>
<td>1 SA₁₀₀</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>-</td>
<td>2</td>
<td>G₁</td>
<td>10</td>
<td>3</td>
<td>G₃</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>Sodium acetate</td>
<td>1 SA₄₀</td>
<td>5</td>
<td>Glucose</td>
<td>1 SA₆₀</td>
<td>6</td>
<td>Sucrose</td>
<td>1 SA₁₀₀</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>-</td>
<td>2</td>
<td>G₁</td>
<td>10</td>
<td>3</td>
<td>G₃</td>
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<td>1 SA₆₀</td>
<td>6</td>
<td>Sucrose</td>
<td>1 SA₁₀₀</td>
</tr>
</tbody>
</table>

**Determination of algal growth and biomass concentrations**

The cultures were harvested and the cells were washed with distilled water after centrifugation at 5000 rpm for 10 min; then the pellet was dried at 60°C for 48 h to give the dry cell weight. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight.

At the end of each run, specific growth rate (µ, day⁻¹) of *B. braunii* at the exponential phase was calculated according to the equation:

\[ \mu = \frac{(\ln X_t - \ln X_0)}{(t_t - t_0)} \]

The biomass doubling time (G, day) calculated using:

\[ G = (\ln 2) / \mu \]

The maximum biomass concentration (X_max, mg/l) was recorded and the productivity (P, mg/L/day) calculated from the equation:

\[ P = (X_t - X_0) / (t_t - t_0) \]

Where X_t and X_0 are the dry cell weight concentration (mg/L) at time t, and 0, respectively (Andrade and Costa, 2007).

**Hydrocarbon extraction and determination**

Dried algal biomass was homogenized and pestle with n-hexane for 30 min and centrifuged. The supernatants were taken into pre-weighted glass vial. The extraction process was repeated three times and the solvents were pooled and evaporated under 40°C, then the glass vial was dried completely at 70°C. The quantity of residue was measured gravimetrically (Dayananda et al., 2005).

**Carbohydrate estimation**

The occurrence of dissolved polysaccharides in BWW and the spent medium was checked by phenol-sulphuric acid method (Rao and Pattabiraman, 1989).
Effect of carbon sources on the growth and the biomass content of *B. braunii*.

**Figure 1.**

**Table 2.** Effect of carbon sources on the growth parameters ($\mu, G, X_{\text{max}}, P$) of *B. braunii*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>NaHCO$_3$</th>
<th>CH$_3$COONa</th>
<th>Na$_3$-Citric acid</th>
<th>Glucose</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$, day$^{-1}$</td>
<td>0.222±0.008$^a$</td>
<td>0.250±0.004$^b$</td>
<td>0.340±0.001$^a$</td>
<td>0.312±0.002$^c$</td>
<td>0.613±0.004$^d$</td>
<td>0.595±0.004$^d$</td>
</tr>
<tr>
<td>G, day</td>
<td>3.13±0.11$^e$</td>
<td>2.78±0.05$^d$</td>
<td>2.04±0.01$^b$</td>
<td>2.22±0.02$^c$</td>
<td>1.13±0.01$^a$</td>
<td>1.17±0.01$^a$</td>
</tr>
<tr>
<td>$X_{\text{max}}$, mg/L</td>
<td>22.33±2.52$^a$</td>
<td>33.00±2.00$^a$</td>
<td>117.33±1.53$^c$</td>
<td>79.33±2.52$^b$</td>
<td>250.00±10.00$^a$</td>
<td>211.00±7.00$^a$</td>
</tr>
<tr>
<td>$P$, mg/L/day</td>
<td>1.52±0.18$^a$</td>
<td>2.29±0.14$^a$</td>
<td>8.31±0.11$^c$</td>
<td>5.60±0.18$^b$</td>
<td>17.79±0.71$^a$</td>
<td>15.00±0.50$^c$</td>
</tr>
</tbody>
</table>

Values are mean ± SD, *n*=3; mean values in the same line with different letters in the superscript are significantly different (*p*<0.05).

### Nitrite analysis

Nitrite was determined according to the standard methods for the examination of water and wastewater (Rand et al., 1975).

### Statistical analysis

Data were expressed as mean ± standard deviation (SD) from three independent parallel experiments. The analysis of variance was performed by ANOVA and significant differences among the means of samples were analyzed by Turkey’s test with a 95% confidence level.

### RESULTS AND DISCUSSION

**Effect of carbon sources and content on the biomass and hydrocarbon production of *B. braunii***

To determine the utilization of carbon source types, the effect of different inorganic and organic carbon sources on the growth of *B. braunii* were investigated. *B. braunii* was grown in Chu 13 × 2 medium containing the different carbon sources at 1.0 g/L concentration, under an irradiance of 2500 Lux. Over the course of each culture, the biomass concentrations were determined.

The experimental results are summarized in Figure 1 and Table 2; the algal cells specific growth rates and biomass concentrations were significantly enhanced by the addition of glucose, sucrose, sodium acetate, and sodium citrate at 1.0 g/L concentration, respectively, but except to sodium bicarbonate. Furthermore, the cultures added with glucose and sucrose reached exponential and stationary phases early. The specific growth rates, maximum biomass concentrations and productivities of *B. braunii* cultured under supplemented with glucose, sucrose, sodium acetate, sodium citrate and sodium bicarbonate were much higher than that of the control, meanwhile, the doubling times were shortened by the addition of carbon sources. The facilitation effects caused by organic carbon sources were higher than inorganic carbon sources. The maximum biomass concentration and productivity of *B. braunii* were 250 mg/L and 17.79 mg/L/day for cells cultured under glucose concentration of 1.0 g/L, while 22.33 mg/L and 1.52 mg/L/day for control
cells culture. These results indicated that the *B. braunii* can utilize variety of carbon sources, especially, the organic carbon sources such as saccharide and acetate better than the inorganic carbon sources.

To investigate the effect of the organic carbon source content on the growth behaviour of *B. braunii*, sodium acetate was added in Chu 13 × 2 medium containing different concentration (from 10 to 100 mM). The laboratory findings are showed in Figure 2 and Table 3; in all cultures, the algal cells specific growth rates and biomass concentrations were significantly promoted by the addition of sodium acetate from 10 to 100 mM compared with the control group. The maximum specific growth rate, biomass concentration and productivity of *B. braunii* under mixotrophic cultivation (supplement with glucose and illumination) were higher than the values of control (0.201 day⁻¹, 25 mg/L, 1.23 mg/L/day, respectively), while, the doubling times of the algal cells were shortened by the addition of glucose.

However, higher glucose content (20 g/L) resulted in cell growth inhibition of *B. braunii*, with specific growth rate of 0.345 day⁻¹ and maximum biomass concentration

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**Table 3.** Effect of sodium acetate content on the growth parameters (μ, G, X_max, P) of *B. braunii*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>SA10</th>
<th>SA20</th>
<th>SA40</th>
<th>SA80</th>
<th>SA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ, day⁻¹</td>
<td>0.191±0.004ᵃ</td>
<td>0.281±0.001ᵇ</td>
<td>0.269±0.002ᵈ</td>
<td>0.255±0.001ᵇ</td>
<td>0.255±0.002ᵇ</td>
<td></td>
</tr>
<tr>
<td>G, day</td>
<td>3.63±0.07ᵈ</td>
<td>2.47±0.01ᵃ</td>
<td>2.58±0.02ᵇ</td>
<td>2.62±0.02ᵇ</td>
<td>2.68±0.01ᵇ</td>
<td>2.72±0.02ᶜ</td>
</tr>
<tr>
<td>X_max, mg/L</td>
<td>42.33±2.52ᵃ</td>
<td>178.33±2.52ᵇ</td>
<td>147.67±4.51ᵈ</td>
<td>138.00±3.61ᶜ</td>
<td>125.67±2.08ᵇ</td>
<td>118.67±3.06ᵇ</td>
</tr>
<tr>
<td>P, mg/L/day</td>
<td>2.52±0.16ᵃ</td>
<td>11.02±0.16ᵇ</td>
<td>9.10±0.28ᵈ</td>
<td>8.50±0.23ᶜ</td>
<td>7.73±0.13ᵇ</td>
<td>7.29±0.19ᵇ</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n=3; mean values in the same line with different letters in the superscript are significantly different (p<0.05).
of 248 mg/L. Above results suggested that glucose is more suitable for *B. braunii* culture with the purpose of high density. The results also indicated that stepwise addition of glucose can avoid the inhibitory action of high substrate content at the initial culture period.

The influence of cultivation modes (photoautotrophic and mixotrophic) and glucose content on the biomass production and hydrocarbon content and productivity of *B. braunii* were investigated. The cultures were carried out in 500-ml Erlenmeyer flask with shaking at 100 rpm, 25°C, light flux density 2500 Lux, light-dark cycle 12 h/12 h, inoculation at 10% (v/v), and cultured 15 days. As shown in Table 5, the biomass concentrations were significantly enhanced by the addition of glucose, sodium acetate and sucrose compared with control (0.12 g/L), and the maximum values of 0.28, 0.58 and 0.51 g/L were obtained under the carbon sources contents at 5 g/L, respectively; however, the hydrocarbon contents for *B. braunii* under mixotrophic cultivation were not grew in pace with the biomass enhancement. The hydrocarbon

Table 4. Effect of glucose content on the growth parameters ($\mu$, G, $X_{\text{max}}$, P) of *B. braunii*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>G1</th>
<th>G5</th>
<th>G10</th>
<th>G15</th>
<th>G20</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$, day$^{-1}$</td>
<td>0.201±0.005$^a$</td>
<td>0.336±0.001$^a$</td>
<td>0.351±0.001$^c$</td>
<td>0.351±0.001$^c$</td>
<td>0.350±0.002$^c$</td>
<td>0.345±0.001$^b$</td>
</tr>
<tr>
<td>G, day</td>
<td>3.45±0.09$^b$</td>
<td>2.06±0.01$^a$</td>
<td>1.97±0.01$^a$</td>
<td>1.97±0.01$^a$</td>
<td>1.98±0.01$^a$</td>
<td>2.01±0.01$^a$</td>
</tr>
<tr>
<td>$X_{\text{max}}$, mg/L</td>
<td>25.00±2.00$^a$</td>
<td>215.33±4.51$^b$</td>
<td>275.33±4.51$^d$</td>
<td>276.33±5.13$^d$</td>
<td>272.00±6.56$^d$</td>
<td>248.00±4.00$^d$</td>
</tr>
<tr>
<td>P, mg/L/day</td>
<td>1.23±0.16$^c$</td>
<td>9.69±0.83$^c$</td>
<td>14.81±2.52$^b$</td>
<td>15.38±2.09$^b$</td>
<td>15.29±2.06$^b$</td>
<td>13.56±1.69$^b$</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n=3; mean values in the same line with different letters in the superscript are significantly different (p<0.05).

Table 5. Biomass and hydrocarbon content and productivity of *B. braunii* under photoautotrophic and mixotrophic cultivation with different carbon sources.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Chu 13 × 2)</th>
<th>Sodium acetate (5 g/L)</th>
<th>Glucose (5 g/L)</th>
<th>Sucrose (5 g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass, g/L</td>
<td>0.12±0.02$^a$</td>
<td>0.28±0.03$^b$</td>
<td>0.58±0.02$^c$</td>
<td>0.51±0.03$^c$</td>
</tr>
<tr>
<td>Biomass productivity, mg/L/day</td>
<td>7.78±1.02$^a$</td>
<td>18.89±1.68$^b$</td>
<td>38.67±1.33$^d$</td>
<td>33.78±1.68$^d$</td>
</tr>
<tr>
<td>Hydrocarbon, %</td>
<td>19.85±0.58$^c$</td>
<td>14.58±1.00$^b$</td>
<td>11.03±0.80$^a$</td>
<td>9.14±1.00$^a$</td>
</tr>
<tr>
<td>Hydrocarbon productivity, mg/L/day</td>
<td>1.55±0.22$^a$</td>
<td>2.77±0.43$^b$</td>
<td>4.26±0.16$^c$</td>
<td>3.08±0.19$^b$</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n=3; mean values in the same line with different letters in the superscript are significantly different (p<0.05).
content of control (19.85%) was higher than the mixotrophic groups, and the culture added with sucrose content at 5 g/L obtained the lowest hydrocarbon content of 9.14%. However, taking into account the productivity of biomass and hydrocarbon, the mixotrophic cultivation displayed its superiority. The biomass and hydrocarbon productivities were highly enhanced by the mixotrophic culture compared with the control of 7.78 and 1.55 mg/L/day, respectively. The maximum productivity of biomass (38.67 mg/L/day) and hydrocarbon (4.26 mg/L/day) were obtained at 5 g/L glucose content. As shown in Table 6, the addition of glucose in Chen et al. (2006) reported that supplementation of photosynthetic metabolisms at high production rates. Mixotrophy and photoheterotrophy allow mixotrophic, photoheterotrophic or heterotrophic growth of algae. Mixotrophy and photoheterotrophy allow microalgal cells to synthesize simultaneously characteristic compounds of both heterotrophic and photosynthetic metabolisms at high production rates. Chen et al. (2006) reported that supplementation of acetate to the mixotrophic culture of Spirulina platensis led to a significant enhancement in biomass concentration, chlorophyll a, lutein, β-carotene, phycocyanin and allophycocyanin production when compared to the photoautotrophic culture, and the optimal acetate concentration of 4.0 g/L was tested. Although heterotrophic growth and photosynthesis has been reported to occur simultaneously and independently in mixotrophic Spirulina cultures (Marquez et al., 1993), the presence of organic carbon can alter both the photosynthetic and heterotrophic metabolism of Chlorella (Villarejo et al., 1995) and decreases production of photosynthetic pigments as compared with the amounts present in the absence of organic carbon source (Ogbonna and Tanaka, 1998). In the present study, the experiments indicated that the utilization variety of carbon sources for B. braunii and organic carbon sources such as saccharide and acetate were better than inorganic carbon sources for biomass production. The specific growth rates, maximum biomass concentrations and productivities of B. braunii cultured under mixotrophic conditions were much higher than that of autotrophic groups. The optimal sodium acetate concentration of 10 mM (0.82 g/L) for mixotrophic cultivation of B. braunii was obtained. The growth characteristics and the hydrocarbon content of B. braunii were very well under mixotrophic cultivation. The biomass and hydrocarbon productivities of B. braunii in BWW during batch culture for 20 days. As shown in Figure 4A, with an initial potassium nitrate concentration of 0.50 g/L, the algal growth was enhanced due to the appropriate concentration of nitrate. After 20 days of cultivation, a biomass of 0.109 g/L and hydrocarbon content of 9.36 mg/L obtained in crude BWW (Figure 4B). Dissolved polysaccharides concentration of 1,250 mg/L in crude BWW was removed by the algae and the final polysaccharides concentration was 135 mg/L.
after 20 days, showing that 89.20% of the initial carbohydrate was utilized by microalgal cultivation.

In BWW supplemented with nitrate, the final biomass of 0.205 g/L and hydrocarbon content of 24.44 mg/L were obtained, respectively (Figure 4A). Nitrate of 512.2 mg/L was utilized by the algae. The final nitrate concentration was 2.8 mg/L after 20 days, indicating that 99.46% of the initial nitrate was utilized by the microorganism; meanwhile, the results from the Figure 4B indicated that 97.76% of the carbohydrate in BWW supplemented with potassium nitrate was utilized, which higher than the remove rate in crude BWW group (p<0.05). The productivity of biomass and hydrocarbon for B. braunii in the presence of nitrate (0.50 g/L) showed a 1.88-fold and 2.61-fold increase over that in BWW without any modification at the end of cultivation (20 days), respectively. The above results indicated that the untreated nitrogen-free BWW was not suitable for B. braunii culture directly.

Wastewater treatment by microalgal culture has several major advantages: it rests on the principles of natural ecosystems and is therefore not environmentally hazardous; it causes no secondary pollution, as long as the biomass produced is reused; and it allows efficient recycling of nutrients (Martínez et al., 2000). B. braunii was able to grow well in secondarily treated sewage (STS) in a batch system. The growth in STS was as good as in the common artificial medium of modified Chu 13 and its hydrocarbon contents were high enough at 53 and 40% compared with 58% in the case of Chu 13 medium (Sawayama et al., 1992).

Their results showed the possibility of using STS as a medium to grow B. braunii and for removal of nitrogen and phosphorus by algal consumption in STS. B. braunii also grew well in piggery wastewater pretreated by a membrane bioreactor with acidogenic fermentation (An et al., 2003). A dry cell weight of 8.5 mg/L and hydrocarbon level of 0.95 g/L were obtained, and nitrate was removed at a rate of 620 mg/L.

These results indicated that pretreated piggery wastewater provides a good culture medium for the growth and hydrocarbon production by B. braunii. The present case shown the algal cells grew better, produced hydrocarbons, and removed carbohydrates with the addition of 0.50 g/L potassium nitrate in crude BWW, which suggested the growth of B. braunii in BWW was limited to the availability of nitrogen source. These results also indicated good prospects for being able to cultivate B. braunii to reduce the amount of organic nutrients in BWW.

Moreover, to improve hydrocarbons productivity, microalgae can be harvested and inoculated in nitrogen-free media, especially in the later phase of growth, resulting in the accumulation of reserve hydrocarbons in the bioreactor (Su et al., 2011). Although nitrogen-free conditions can reduce the algal biomass production. Thus, stepwise addition of nitrogen in fed batch cultivation can avoid the problem of growth restriction and hydrocarbons accumulation of algae. For inorganic and organic nutrients consumption, B. braunii can utilize soluble carbohydrate and nitrate effectively as long as it was cultured in BWW, which demonstrated that the productivity of biomass and hydrocarbons can be improved by cultivating the green algae in BWW supplied with appropriate level of nitrogen. The present work indicated that it is a potential way to cultivate B. braunii under mixotrophic conditions with the purposes of biomass production, hydrocarbons accumulation and
Conclusion

Mixotrophic mode provides an effective way to cultivate *B. braunii* with high cell density and short cultivation cycle because exogenous carbon sources, in especial, organic carbon sources, such as sodium acetate, glucose and sucrose can stimulate the biomass production of the alga remarkably. The biomass and hydrocarbon volumetric productivities were promoted significantly by the mixotrophic cultivation of *B. braunii* compared with the photoautotrophic group, even though the hydrocarbon contents under mixotrophic conditions were not increased in pace with the biomass contents.

The present study suggested that coupling the cultivation of mixotrophic microalgae and organic wastewater treatment is a potential way to produce microalgae biomass, accumulate hydrocarbon and remove organics and inorganic salts from BWW.

ACKNOWLEDGEMENTS

Financial support was provided by National Science Found for Distinguished Young Scholars of China (Grant No. 20625308) and Research Fund for Young Teachers of Northwest Normal University (Grant No. NWNU-LKQN-10-30).

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