Impact of carbon and nitrogen feeding strategy on high production of biomass and docosahexaenoic acid (DHA) by Schizochytrium sp. LU310

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HIGHLIGHTS

- Schizochytrium sp. LU310 is an ideal strain for DHA-producing as its impressive productivity.
- Baffled flask enhanced in high cell density cultivation of DHA.
- MSG-feeding combined with glucose feeding strategy was extremely useful for DHA production.
- DHA production was linearly correlated with the concentrations of carbon and nitrogen.

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ABSTRACT

A new isolated Schizochytrium sp. LU310 from the mangrove forest of Wenzhou, China, was found as a high producing microalgae of docosahexaenoic acid (DHA). In this study, the significant improvements for DHA fermentation by the batch mode in the baffled flasks (i.e. higher oxygen supply) were achieved. By applied the nitrogen-feeding strategy in 1000 mL baffled flasks, the biomass, DHA concentration and DHA productivity were increased by 110.4%, 117.9% and 110.4%, respectively. Moreover, DHA concentration of 21.06 g/L was obtained by feeding 15 g/L of glucose intermittently, which was an increase of 41.25% over that of the batch mode. Finally, an innovative strategy was carried out by intermittent feeding carbon and simultaneously feeding nitrogen. The maximum DHA concentration and DHA productivity in the fed-batch cultivation reached to 24.74 g/L and 241.5 mg/L/h, respectively.

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1. Introduction

Docosahexaenoic acid (DHA, 22:6 n-3) has received worldwide attention due to its beneficial physiological functions for humans, for instance, promotion effect for visual acuity and neural development, lowering the incidence of cardiovascular diseases, and reducing risk factors involved in diseases like hypertension, arthritis, arteriosclerosis and thrombosis (Ren et al., 2013; Sijtsma and Swaaf, 2004; Sun et al., 2014). Due to its widespread use, the global DHA market has grown significantly in the recent years and expected to reach $34.7 billion in 2016.

Schizochytrium sp., a thraustochytrid, is an algae-like microorganism that is closely related to heterokont algae (Barclay et al., 1994; Morita et al., 2006; Sijtsma and Swaaf, 2004). It is well-known for producing polyunsaturated fatty acids (PUFAs), among which DHA usually represents 30–40% of the total fatty acids (Yokochi et al., 1998), and thus is considered as a promising and commercially viable alternative DHA feedstock. However, this source of DHA is limited by the high production costs in comparison to the current fish oil prices. In order to reduce the costs of microalgae-based DHA production, higher volumetric productivity must be pursued by culture optimization. A yield of 90–100 g/L DCW with high lipid content (54–63%, w/w) by Aurantiochytrium sp. strain T66 under N starvation and O2 limitation had been reported (Jakobsen et al., 2008). In another case, an intermittent oxygen feeding method to maintain a 50% dissolved oxygen level with a C:N ratio of 1.25 for Schizochytrium limacinum SR21, to achieve DCW at 61.76 g/L and DHA concentration at 20.3 g/L had also been reported (Huang et al., 2012). The literature showed that the cell growth rate, DHA content and DHA productivity of oleaginous microorganisms were mainly influenced by the culture conditions, such as nitrogen concentration, carbon concentration and dissolved oxygen level (de Swaaf et al., 2003; Ganuza and Izquierdo, 2007; Ganuza et al., 2008; Jakobsen et al., 2008; Sun
et al., 2014). It has been suggested that nitrogen limitation could enhance the amount of acetyl-CoA and NADPH, which were the necessary precursors for lipid accumulation (de Swaaf et al., 2003). Recently, Ren et al. (2014) also found that the DHA percentage of TFAs would be decreased after nitrogen exhaustion. However, the phenomenon could be relieved by monosodium glutamate (MSG) addition, which could activate the enzyme of acetyl-CoA carboxylase (Kow lure et al., 2001) and glucose-6-phosphate dehydrogenase (Lan et al., 2002), and thus it ensures the sufficient supply of acetyl-CoA and NADPH for DHA synthesis to enhance the accumulation of lipid and DHA.

The accumulated lipids and fatty acids within oleaginous microorganisms are, therefore, dynamic storage materials and are produced in times of plenty when carbon is sufficient or starvation when carbon is deficient (Ganuza and Izquierdo, 2007; Ratledge, 2002; Wu et al., 2005). Moreover, the high carbon to nitrogen ratio is beneficial to lipid accumulation (Yokochi et al., 1998), while the accumulation of lipid and DHA.

In this study, a new isolated microalga, *Schizochytrium* sp. LU310, used in this work was isolated from the mangrove forest of Wenzhou, Zhejiang, China. It was identified by the morphology and 18S rRNA sequencing using the primers as 18S-F: 5’-TCTCAAGAYTAAAGCCTGCG-3’ and 18S-R: 5’-TTCACCTTCCTTAAACAAATAG-3’. The 18S rRNA sequence was deposited into GenBank under accession number KM245569.

### 2. Methods

#### 2.1. Identification of strain

The microalga, *Schizochytrium* sp. LU310, used in this work was isolated from the mangrove forest of Wenzhou, Zhejiang, China. It was identified by the morphology and 18S rRNA sequencing using the primers as 18S-F: 5’-TCTCAAGAYTAAAGCCTGCG-3’ and 18S-R: 5’-TTCACCTTCCTTAAACAAATAG-3’. The 18S rRNA sequence was deposited into GenBank under accession number KM245569.

#### 2.2. Morphological analysis

A drop of fresh strain LU310 was taken on a slide glass to be observed and recorded by light microscopy (OLYMPUS BX41, Japan) continuously.

#### 2.3. Culture conditions

The seed broth contained (g/L, pH 6.5): glucose, 30; Na₂SO₄, 12; yeast extract, 10; MgSO₄, 2; (NH₄)₂SO₄, 1; KH₂PO₄, 1; K₂SO₄, 0.65; KCl, 0.5; CaCl₂·2H₂O, 0.17 as well as trace element solution (1 ml/L) and vitamin solution (2 ml/L). The trace element solution was composed of (ml/L): FeSO₄·7H₂O, 10; CaCl₂·Pantothenate, 3.2; MnCl₂·4H₂O, 3; ZnSO₄·7H₂O, 3; NiSO₄·6H₂O, 2; CuSO₄·5H₂O, 2; CoCl₂·6H₂O, 0.04; Na₂MoO₄·2H₂O, 0.04. Vitamin solution consisted of (mg/L): thiamine, 9.5; cyanocobalamin, 0.15. Before autoclaving (121 °C, 20 min) the pH of the medium was adjusted to 6.5 with 2 M NaOH. Yeast extract was purchased from OXOID Ltd., UK. If not specifically mentioned, all other chemicals were provided by Sinopharm Chemical Reagent Co., Ltd., China. The trace element solution and the vitamin solution were filter-sterilized (0.2 μm) and added after sterilization. The stored cells were cultured at 28 °C, 170 rpm for 48 h in the seed broth. After three generations of cultivation, the seed culture (4%, v/v) was then transferred to fermentation broth and incubated at 28 °C, 200 rpm for 120 h or more.

#### 2.4. Dry cell weight (DCW), glucose, and nitrogen measurements

3 ml broth was used to determine DCW by gravimetric method. 3 ml broth was transferred to a pre-weighted centrifuge tube and centrifuged at 7000g for 10 min. The cell pellet was washed twice with distilled water and dried at 60 °C until constant (12 h) to analyze the total weight. The supernatant from centrifugation was collected and the vitamin solution was filter-sterilized (0.2 μm) and added after sterilization. The stored cells were cultured at 28 °C, 170 rpm for 48 h in the seed broth. After three generations of cultivation, the seed culture (4%, v/v) was then transferred to fermentation broth and incubated at 28 °C, 200 rpm for 120 h or more.

#### 2.5. Total lipids (TLs)

3 ml fermentation broth was mixed with 4 ml HCl (12 N) and incubated in water bath at 65 °C for 45 min. TLs from the mixture were extracted by three times (15 min of each time) with 5 ml n-hexane, and then the collected extracts were mixed and purified in a rotary vacuum evaporator at 60 °C (Chang et al., 2013).

#### 2.6. Fatty acid composition analysis

Fatty acid methyl esters (FAMEs) were prepared regarding to previous methods (Ren et al., 2009; Yeh and Chang, 2012) with modifications: 5 ml, 0.5 M KOH–methanol was added to a tube containing TLs. The tubes were heated in a water bath at 65 °C for 10 min and added 5 ml 30% BF₃·ether then. The tubes were further heated in a water bath at 65 °C for 30 min and followed of 5 ml hexane was added when the tubes cooled down to room temperature. Arachidic acid methyl (4 g/L, Sigma, USA) as an internal standard was added in the tubes and mixed through a vortex for 1 min, and then settled for separation of two phases after adding 1 ml saturated sodium chloride solution for preventing emulsification. The upper phase containing FAMEs was applied to a gas chromatograph (Agilent GC 7890, USA) equipped with a 100 m × 0.25 mm capillary column (SPäemi-2560, USA). The column was increased from 140 to 240 °C at 3 °C/min and then maintained at 240 °C for further 30 min. The temperature of the injector and detector were both set at 260 °C. Nitrogen was used as the carrier gas at 20 cm/s. Peaks were identified using the authentic standards of fatty acid methyl esters (Sigma, USA). Fatty acids were quantified from the peak areas relative to the peak of the internal standard.
3. Results and discussion

3.1. Identification of new strain

Based on a phylogenetic tree analysis of the 18S rRNA sequence for the most closely related species (Fig. 1), this strain was identified and designated as *Schizochytrium* sp. LU310 (CCDC: KM245569). The new strain was 99.5% similar to the *Schizochytrium* sp. FJU-512 (Genbank Type: Nucleotide AY758384), 89.8% similar to the *Schizochytrium* sp. ATCC20888 (CCDC: DQ367050) with regard to its 18S rRNA sequence.

The morphology of cell was studied by light microscopy (data not shown). The strain LU310 were very similar to *S. limacinum* SR21, especially in the reproduction with continuous binary fission, which made it classified as *Schizochytrium* instead of *Thraustochytrium* (Honda et al., 1998; Morita et al., 2006). Reproduction with continuous binary fission made the strain to be cultivated fast and easily, which can be used as an enormous industrial potential strain.

3.2. The effect of DO on strain LU310

As listed in Table 1, the DHA productivity of *Schizochytrium* sp. LU310 attained in 1000 mL baffled flasks was 194.38 mg/L/h, which was higher when compared with the published value of 117 mg/L/h by *Thraustochytrid* strain 12B (Perveen et al., 2006), 125 mg/L/h by *Schizochytrium* sp. G13/2S (Ganuza et al., 2008), 43.7 mg/L/h by *S. limacinum* SR 21 (Chi et al., 2009) and 119 mg/L/h by *Schizochytrium* sp. CCTCC M209059 (Ren et al., 2010), respectively. The productivity is to our best knowledge to be the highest report for flask-cultured DHA-producing microorganisms.

Baffled flasks and normal flasks were used in fermentation of LU310, because baffled flasks have higher oxygen transfer coefficient ($k_La$) than normal flasks under the identical shaking speed.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Culture modes</th>
<th>250 mL normal flasks</th>
<th>1000 mL normal flasks</th>
<th>250 mL baffled flasks</th>
<th>1000 mL baffled flasks</th>
<th>Increment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCW (g/L)</td>
<td></td>
<td>42.48 ± 1.12</td>
<td>46.80 ± 0.97</td>
<td>52.30 ± 0.13</td>
<td>60.82 ± 0.95</td>
<td>30.0</td>
</tr>
<tr>
<td>TLs concentration (g/L)</td>
<td></td>
<td>21.05 ± 1.38</td>
<td>26.10 ± 1.70</td>
<td>34.47 ± 1.97</td>
<td>39.92 ± 0.85</td>
<td>53.0</td>
</tr>
<tr>
<td>DHA concentration (g/L)</td>
<td></td>
<td>7.27 ± 0.39</td>
<td>8.66 ± 0.43</td>
<td>12.22 ± 0.71</td>
<td>16.33 ± 0.78</td>
<td>88.6</td>
</tr>
<tr>
<td>TLs in DCW (w/w, %)</td>
<td></td>
<td>52.75 ± 5.71</td>
<td>54.64 ± 1.43</td>
<td>67.30 ± 3.58</td>
<td>67.25 ± 1.15</td>
<td>23.1</td>
</tr>
<tr>
<td>DHA in DCW (w/w, %)</td>
<td></td>
<td>17.15 ± 1.37</td>
<td>18.50 ± 0.53</td>
<td>23.87 ± 1.30</td>
<td>27.51 ± 1.21</td>
<td>48.7</td>
</tr>
<tr>
<td>Biomass productivity (g/L/h)</td>
<td></td>
<td>0.44</td>
<td>0.48</td>
<td>1.11</td>
<td>1.21</td>
<td>152.1</td>
</tr>
<tr>
<td>DHA productivity (mg/L/h)</td>
<td></td>
<td>60.58</td>
<td>73.64</td>
<td>162.76</td>
<td>194.38</td>
<td>164.0</td>
</tr>
</tbody>
</table>

* The values were calculated at the cultivation time when DHA was maxima in 1000 mL flasks.
Fig. 2. Time course of fermentation profiles of Schizochytrium sp. LU310 in four independent batch cultures with 1000 mL baffled flasks (square symbols), 1000 mL normal flasks (triangle symbols), 250 mL baffled flasks (circle symbols), and 250 mL normal flasks (diamond symbols): (a) glucose exhaustion (g/L); (b) DCW (g/L); (c) TLs concentration (g/L); (d) DHA concentration (g/L). Experiments were carried out in duplicate trials.
(Gupta and Rao, 2003; Chang et al., 2013). As shown in Fig. 2, cells cultivated in baffled flasks achieved higher biomass, TLs and DHA concentration than that in normal flasks. As indicated in Fig. 2a and e, the glucose and nitrogen consumption rate of cells in baffled flasks were obviously increased compared to that in normal flasks during the fermentation. Fig. 2b showed that cell growth enhanced to a large extent in baffled flasks, especially before 48 h. The highest DCW and biomass productivity up to 60.82 g/L and 1.21 g/L/h were obtained in the 1000 mL baffled flasks. While in the normal flasks (250 or 1000 mL), the final DCW and biomass productivity decreased (Table 1). As baffled flasks supplied more DO than normal flasks, the Schizochytrium sp. processed higher DO supply (baffled flasks) and obtained faster cell growth, which leaded to consume more carbon and nitrogen source. On the contrary, culture in low DO supply (normal flasks), the activity of glucose and nitrogen assimilation was suppressed so that low cell growth was observed. The high cell density was concerned for high production of intracellular products (Ganuza et al., 2008), therefore, baffled flasks culture with high DO was favorable for cell growth and more carbon and nitrogen source used for cell respiration and energy metabolism.

As shown in Fig. 2c and d, lipids began to accumulate accompanied with the cell growth. Previous report showed that lipid accumulation of Schizochytrium sp. was a growth-associated process, but the nitrogen depletion enhanced the accumulation of lipids (Ganuza and Izquierdo, 2007; Ganuza et al., 2008; Jakobsen et al., 2008). But the lipid accumulation was subsequently induced by nitrogen depletion. This particular pattern was also observed in Schizochytrium G13/2S (Ganuza and Izquierdo, 2007). In addition, nitrogen utilization was also considerably affected by different oxygen supply levels. During high DO cultures, nitrogen was consumed much faster which was almost exhausted at 48 h, so that lipids and DHA synthesized much more quickly. However, in low oxygen supply culture, nitrogen was assimilated more slowly which was almost exhausted at 72 h (Fig. 2e), and the DHA production was also showed correspondingly tardy (Fig. 2d).

As summary in Table 1, the highest DCW, lipid and DHA content in DCW were achieved in the high oxygen supply culture (1000 mL baffled flasks), which increased 30.0%, 23.1% and 48.7% higher than the value in the 1000 mL normal flasks, respectively. It indicated that oxygen supply had apparent enhancement on the cell growth and DHA production of Schizochytrium sp. LU310. Previous studies also showed the similar results (Chang et al., 2014; Qu et al., 2011, 2013). For the scale-up of the aerobic fermentation processes by
the organisms that are not sensitive to shear force, the effect of oxygen transfer is normally the most important factor (Qu et al., 2013). The mentionable points were 152.1% and 164.0% improvements in DCW and DHA productivity were observed under 1000 mL baffled flasks than that of the best results controlled by 1000 mL normal flasks. The culture time of this strain also was shortened from 120 h to 84 h, which was quite favorable for the scale-up production of DHA fermentation in the industry. GC analysis showed that the major fatty acids extracted from Schizochytrium sp. LU310 were tetradecanoic acid (C14:0), hexadecanoic acid (C16:0), stearic acid (C18:0), docosapentenonic acid (C22:5), and docosahexaenoic acid (C22:6). And the proportion of other FAs in the normal flasks reached to 23.4% - 28.2% (w/w), which included pentadecylic acid (C15:0), palmitic acid (C16:1), margaric acid (C17:0), oleanic acid (18:1), linolenic acid (C18:3), and ecosapentaenoic acid (C20:5). Fig. 3 illustrated the profiles of fatty acids composition of LU310 grown in the different flasks. The composition of total fatty acids produced by LU310 was quite similar except DHA. The DHA proportion of total fatty acids increased significantly with the increase of DO supply levels. The highest DHA proportion of 40.9% was obtained in 1000 mL baffled flasks. The culture time of this strain also was shortened from 120 h to 84 h, which was quite favorable for the scale-up production of DHA fermentation in the industry.

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3.3. Effects of feeding nitrogen on the fermentation

In order to obtain high cell concentrations with high DHA content, several modes of nitrogen addition were performed during fed batch cultivation. MSG was selected as an effective nitrogen source because MSG addition could accelerate the substrate consumption rate and enhance the accumulation of lipid and DHA, which has a positive influence in the growth of microalgal culture (Ren et al., 2014; Sun et al., 2014). Herein, the feeding concentration of nitrogen (MSG) ranged from 0.4 to 0.8 g/L according to its nitrogen-consuming rate during fermentation. The MSG-feeding time was chosen as the following: (1) 24 h, (before nitrogen depletion), (2) 48 h, (around nitrogen depletion), or (3) 72 h, (after nitrogen depletion for some time). Detailed information and results of MSG-feeding culture were listed in Fig. 4 and Table 2. It demonstrated that MSG feeding could enhance the DCW and DHA production. The 0.4 g/L MSG-feeding on fermentation was better than that of 0.8 g/L MSG-feeding at the same time. When fed with 0.4 g/L MSG at different time, as indicated in Table 2, the DHA concentration and the lipid content in DCW were higher (ranging from 5.5% ~ 8.6% and 2.8% ~ 4.8%, respectively) than that in the culture fed with 0.8 g/L MSG at the same time. According to literature reports, nitrogen limitation may increase the intracellular content of fatty acid acyl-CoA and activate diacylglycerol acyltransferase, which converts fatty acid acyl-CoA to triglyceride (Hsieh and Wu, 2009; Takagi et al., 2000). This is why low nitrogen concentrations increased not only the intracellular lipid content but also the DHA concentration.

When fed with 0.4 g/L MSG at 72 h, as indicated in Table 2, the DHA concentration and the TLs concentration were higher (ranging from 7.5% ~ 18.4% and 11.6% ~ 19.3%, respectively) than that in the culture fed with 0.4 g/L of MSG at 48 and 24 h. The highest DCW of 55.9 g/L was obtained in the culture fed with 0.4 g/L of MSG at 72 h, which was 6.3% and 10.3% more than that fed with 0.4 g/L of MSG at 48 and 24 h, respectively. The possible reason for producing more DHA and biomass with nitrogen feeding during 72 h than the feedings during other phases was that when fed with MSG at 72 h, which not only offered nitrogen source for further cell growth but also yielded more DHA at the end of fermentation, by activating more acetyl CoA carboxylase to accumulate lipids (Hsieh and Wu, 2009).

Sun et al. (2014) reported that the limitation of organic and inorganic nitrogen had different influences on cell growth. Organic nitrogen limitation inhibited the cell growth obviously, but inorganic nitrogen did not. MSG addition could accelerate the substrate consumption rate and enhance the accumulation of lipid and DHA, which has a positive influence in the growth of microalgal culture (Kowluru et al., 2001; Lan et al., 2002; Ren et al., 2014). Thus, MSG-feeding used for culturing Schizochytrium sp. in this study. Although the MSG-feeding strategy was favorable to the DHA production, DCW and DHA productivity were only increased 10.0% and 10.4%. This might be caused by the exhaustion of glucose at 48 h. The deficiency of carbon source made acetyl-CoA not continuously supplied in the cytosol of the cell as a necessary precursor for fatty acid synthetase (FAS), which is the pathway of DHA synthesis relies on (Ren et al., 2009). Therefore, the DHA production was not apparently enhanced. It indicated that MSG addition could relieve the drop tendency of DHA but not deal with the root of the problem. Next, we employed intermittent addition of glucose to improve cultivation of Schizochytrium sp. for higher biomass and DHA content.

3.4. Effects of feeding carbon source on the fermentation

According to the Fig. 2a, glucose was used up at 48 h, so the glucose feeding time was chosen at 48 h. The feeding concentration of carbon (glucose) ranged from 15 to 35 g/L according to the glucose-consuming rate during fermentation was used. The glucose feeding was employed when glucose concentration in the flasks was lower than 1 g/L. The time courses of DCW, DHA concentration, and glucose concentration in the intermittent glucose feeding cultivation were demonstrated in Fig. 5. Compared with the
control group, the experimental groups achieved much higher biomass and DHA. The highest DCW of 80.9 g/L, DHA concentration of 21.06 g/L and DHA productivity of 231.36 g/L/h were obtained by feeding 15 g/L of glucose. These results were 139.7%, 141.3% and 112.2% improvements over the best results controlled by fed

0 g/L glucose (Table 3). The acetyl CoA, a metabolic product of glucose, is the starting material for cell synthesis of FAS, thus to increase the proportion of TLS in cells. The results also showed that the influence of feeding 15 g/L of glucose on the fermentation was better than that of feeding with 35 g/L of glucose. Although the DCW in the intermittent feeding of 35 g/L glucose, also increased obviously (reached to 78.6 g/L), the DHA content in DCW was only 23.47% (w/w), which was much less than that intermittent feeding 15 g/L of glucose. It has reported that too high concentration of carbon source would inhibit cell growth as well as lipid accumulation in DHA cultures (de Swaaf et al., 2003; Ren et al., 2013; Sijtsma and Swaaf, 2004). Wu et al. (2005) also reported when the glucose concentration was more than 40 g/L in the medium, the microorganism synthesized fatty acids and secreted acids into the broth. In the present experiment, glucose was used up at 48 h, which inhibited cell growth. Finally, the best condition was applied of intermittent feeding 15 g/L of glucose at 48 h.

3.5. Effects of couple feeding carbon and nitrogen on DCW and DHA content

Based on preliminary fed-batch results, the highest lipid and DHA content were obtained by cultivation with feeding 0.4 g/L nitrogen during 72 h, while the highest DCW and DHA concentration and DHA productivity were achieved by cultivation with intermittent feeding 15 g/L glucose at 48 h. Thus, an innovative strategy was carried out by intermittent feeding 15 g/L glucose

Table 3

<table>
<thead>
<tr>
<th>Glucose feeding concentration (g/L)</th>
<th>Control</th>
<th>48 h-1</th>
<th>48 h-2</th>
<th>Increment (%)</th>
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<tbody>
<tr>
<td>DCW (g/L)</td>
<td>57.8</td>
<td>80.9</td>
<td>78.6</td>
<td>39.7</td>
</tr>
<tr>
<td>TLS concentration (g/L)</td>
<td>31.73</td>
<td>48.10</td>
<td>43.57</td>
<td>47.1</td>
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<tr>
<td>DHA concentration (g/L)</td>
<td>14.91</td>
<td>21.06</td>
<td>17.84</td>
<td>41.3</td>
</tr>
<tr>
<td>TLS in DCW (w/w, %)</td>
<td>55.78</td>
<td>66.24</td>
<td>50.82</td>
<td>18.8</td>
</tr>
<tr>
<td>DHA in DCW (w/w, %)</td>
<td>25.79</td>
<td>28.77</td>
<td>23.47</td>
<td>11.6</td>
</tr>
<tr>
<td>DHA productivity (mg/L/h)</td>
<td>207.02</td>
<td>232.36</td>
<td>194.30</td>
<td>12.2</td>
</tr>
</tbody>
</table>

* Compared control with the best result in fed-batch culture.

Fig. 5. Time courses of cell growth and carbon consumption for *Schizochytrium* sp. LU310 at different glucose feeding concentration. DCW (square symbols), TLS concentration (unfilled bar charts), DHA concentration (filled bar charts), glucose concentration (triangle symbols), nitrogen concentration (circle symbols): (a) feeding glucose of 0 g/L; (b) feeding glucose of 15 g/L; (c) feeding glucose of 35 g/L.

Fig. 6. Time courses of cell growth, nitrogen and carbon consumption for *Schizochytrium* sp. LU310 in the optimal mode. Open symbols and black bar charts are control, closed symbols and green bar charts are feeding.
when glucose concentration was < 1 g/L and simultaneously feeding 0.4 g/L nitrogen at 72 h. As shown in Fig. 6, the highest DCW of 88.6 g/L, DHA concentration of 24.74 g/L and DHA productivity of 241.1 mg/L/h were achieved in this couple feeding cultivation, which were an increase of 53.3%, 65.9% and 16.5% over the control, respectively. DHA percentage of TLs reached 47.5% when using the novel fermentation strategy, which was an increase of 16.7% over that of the control. Meanwhile, in this process, the DHA concentration and DHA productivity were 51.5% and 24.0% higher than the best results of MSG-feeding, respectively. Similarly, the DHA concentration and DHA productivity were 17.5% and 3.8% higher than the best values obtained in culture with glucose-feeding, respectively. The results clearly indicated that the optimal process with the strategy of nitrogen and carbon addition can promote to produce lipids and DHA effectively. To our best knowledge, it was the highest producing result in the flask-cultured of DHA-producing microalgae.

Regarding to the comparison results in Table 4, although high algal biomass had been achieved in other researches (Huang et al., 2012; Ganuza et al., 2008; Ren et al., 2010), the obtained DHA concentration and DHA productivity were much lower than that of this study (i.e. 24.74 g/L and 241.5 mg/L/h). Obvious differences existed between the reports on the DHA productivity as listed in Table 4, and this probably roots in the diversity of the strains used. The DCW, DHA concentration and DHA productivity in Schizochytrium sp. and the adoption of the culture modes. Our experiment was the highest producing result in the flask-cultured of DHA-producing microalgae.

Table 4

<table>
<thead>
<tr>
<th>Strain in Schizochytrium sp.</th>
<th>Device</th>
<th>DCW (g/L)</th>
<th>DHA (g/L)</th>
<th>DHA productivity (mg/L/h)</th>
<th>References</th>
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<tr>
<td>SR21</td>
<td>Shake-flask</td>
<td>36.0</td>
<td>4.2</td>
<td>35</td>
<td>Yokoi et al. (1998)</td>
</tr>
<tr>
<td>S31</td>
<td>Shake-flask</td>
<td>7.459</td>
<td>0.328</td>
<td>3.42</td>
<td>Wu et al. (2005)</td>
</tr>
<tr>
<td>mangrovei Sk-02</td>
<td>Bioreactor</td>
<td>28.0</td>
<td>6.0</td>
<td>63</td>
<td>Unagui et al. (2007)</td>
</tr>
<tr>
<td>Strain 12B</td>
<td>Shake-flask</td>
<td>30.9</td>
<td>6.8</td>
<td>117</td>
<td>Perven et al. (2006)</td>
</tr>
<tr>
<td>G13/25</td>
<td>Shake-flask</td>
<td>63.3</td>
<td>6.01</td>
<td>125</td>
<td>Ganuza et al. (2008)</td>
</tr>
<tr>
<td>Limacinum SR21</td>
<td>Two-stage in shake-flask</td>
<td>37.9</td>
<td>6.56</td>
<td>43.7</td>
<td>Chi et al. (2009)</td>
</tr>
<tr>
<td>HX-308 (CCTCC M209059)</td>
<td>Stepwise aeration in flask</td>
<td>71.0</td>
<td>17.50</td>
<td>119</td>
<td>Ren et al. (2010)</td>
</tr>
<tr>
<td>A. limacinum SR21 (ATCCMYA-1381)</td>
<td>Bioreactor</td>
<td>61.8</td>
<td>20.3</td>
<td>123</td>
<td>Liu et al. (2010)</td>
</tr>
<tr>
<td>ATCC 20888</td>
<td>AEMR/shake</td>
<td>12.00</td>
<td>2.04</td>
<td>21.26</td>
<td>Huang et al. (2012)</td>
</tr>
<tr>
<td>LU310</td>
<td>Shake-flask</td>
<td>88.6</td>
<td>24.74</td>
<td>241.5</td>
<td>This study</td>
</tr>
</tbody>
</table>

* DHA productivity is not available in the paper and DHA percentage in the lipids used instead.

4. Conclusion

The cell growth rate and DHA accumulation of Schizochytrium sp. LU310 were strongly related to carbon and nitrogen concentration. Based on the experimental observations, the highest DCW and DHA concentration were obtained by cultivation with feeding of 0.4 g/L nitrogen at 72 h and intermittent feeding of 15 g/L glucose when glucose concentration was < 1 g/L, which was the highest production in the flask-culture of DHA-producing microalgae. Consequently, DHA production from microalgae can be successfully accomplished by using an innovative strategy with intermittent feeding moderate glucose combined with limited amounts of MSG during the cultivation.

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References


