Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga *Chlorella zofingiensis*

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**Highlights**
- The impact of N starvation on algal starch and lipid synthesis was analyzed.
- The initial response to N starvation in cells was massive starch synthesis.
- *C. zofingiensis* tends to produce lipid as a long-term storage upon N starvation.
- The degree of saturation of fatty acids increased in N-deprived *C. zofingiensis*.

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**Abstract**
The aim of this research was to study the metabolic changes of starch and lipid biosynthesis in the microalga *Chlorella zofingiensis* under nitrogen starvation in comparison to nitrogen abundant condition. *C. zofingiensis* showed a rapid growth and kept stable chlorophyll content when grown in nitrogen-replete medium, while a severe inhibition of cell growth and a sharp degradation of chlorophyll occurred under nitrogen depletion. Nitrogen-replete *C. zofingiensis* cells possessed basal levels of starch and lipid. Upon nitrogen starvation, both starch and lipid increased greatly within cells, but starch synthesis preceded lipid accumulation. After 2 days of stress condition, starch was partially degraded, possibly to support lipid synthesis. It was speculated that starch accumulation acted as a quick response to environmental stress, whereas lipid served as long-term energy storage. Additionally, *C. zofingiensis* tends to lower the degree of unsaturation in response to nitrogen starvation which is desirable for biodiesel production.

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1. Introduction

The use of fossil fuels as energy has been widely considered as unsustainable due to depleting resources and the environmental problems associated with fossil fuel consumption. Recently, microalgae have emerged as a promising feedstock for production of biofuels, including bioethanol, biodiesel, green gasoline, methanol, biohydrogen, etc. ([Wijffels and Barbosa, 2010]; [Singh et al., 2011]). However, the development of microalgal biofuels faces great challenges since the production cost of microalgae as a biofuels feedstock is much higher compared to fossil fuels. To enhance the economic feasibility of microalgal biofuels production, many attempts have been made to improve strain performances ([Li et al., 2010a]; [Lv et al., 2010]), culture systems ([Ketheesan and Nirmalakhandan, 2012]; [Zheng et al., 2012]), and harvesting/extraction technologies ([Halim et al., 2012]; [Hanotu et al., 2012]).

Generally, under favorable growth conditions, microalgae mainly synthesize proteins, cell wall carbohydrates and membrane lipids for cell structure, while under certain environmental stress, many microalgae slow down even cease cell division and switch photosynthetic carbon partitioning toward energy-rich storage compounds such as starch and/or lipid, which can be converted into biofuels ([Hu et al., 2008]; [Li et al., 2011]; [Siaut et al., 2011]; [Cakmak et al., 2012]). Therefore, it is important to understand the metabolic pathways and processes that generate these organic macromolecules in order to advance biofuels production.

In comparison with higher plants, starch and lipid biosynthesis have been less studied in microalgae, despite their potential importance in applications such as bioethanol and biodiesel production. Understanding carbon partitioning in an algal cell into lipid and/or starch could be significant for biofuels strain development and designing cultivation strategies. Although the starch and lipid biosynthesis have been respectively investigated in microalgal strain, less attention has been paid to the combination of these two reserves production and accumulation, as well as carbon partitioning in response to environmental stresses.
In recent years, there were increasing reports demonstrating the potential of *Chlorella* for biofuels production (Phukan et al., 2011; Suali and Sarbatly, 2012). Moreover, *Chlorella* has been widely accepted for nutraceutical, feed supplement, bioactive substances like astaxanthin and lutein, and for wastewater treatment. Additionally, *Chlorella* species have high flexibility to adapt to diverse culture conditions and are very promising in commercial applications. *Chlorella* cells can accumulate abundant starch with the content from 21% to 45% of dry weight (DW) (Branyikova et al., 2011; Dragone et al., 2011), as well as lipid with the content from 23% to 60% of DW (Illman et al., 2000; Mujtaba et al., 2012; Pribyl et al., 2012) under various growth conditions. It is thus obvious that *Chlorella* offers a suitable platform to understand the coordination of photosynthetically fixed carbon partitioning between starch and lipid biosynthesis pathways.

To our knowledge, a comprehensive analysis of starch and lipid production and accumulation in *Chlorella* species has not been studied to date. In this scenario, here, we characterized the metabolic changes of starch and lipid biosynthesis in the microalga *Chlorella zofingiensis* under nitrogen starvation condition compared to favorable growth condition. Kinetics of starch and lipid biosynthesis, starch and lipid content, and fatty acid profiles were determined. Starch granules and lipid bodies (LBs) were observed by fluorescent and transmission electron microscopy. Possible correlation between starch and lipid biosynthesis under stress was also discussed. The results contribute to understanding carbon partitioning between starch and lipid as well as choosing cultivation strategies for bioethanol and biodiesel production.

2. Methods

2.1. Strain and culture conditions

*C. zofingiensis* was preserved in our laboratory. Cultures were incubated in a set of air-lift photoreactors (5.0 cm diameter, 60 cm height) under continuous illumination (150 μmol m$^{-2}$ s$^{-1}$) photosynthetically active radiation) at 25 ± 1°C and agitated by aeration enhanced with 1% CO$_2$. Cells were initially grown photoautotrophically to the middle of the logarithmic phase in BG11 medium (Huo et al., 2012). These pre-cultured cells were collected by centrifugation (4000 rpm × 5 min) and resuspended in either regular BG11 or nitrogen-depleted BG11-N medium at an initial OD$_{750}$ = 0.67 ± 0.02 (SD). No nitrogen was detected in BG11-N medium indicating N-starvation condition was realized. Samples for analysis were taken immediately after resuspension (0 day) and at regular intervals. Each experiment was performed in triplicate.

2.2. Growth measurement

For dry weight measurement, an aliquot of culture sample was filtered through a pre-weighed Whatman GF/C filter paper (1.2 μm pore size). Then the filter paper was dried at 80°C in an oven until constant weight and cooled down to room temperature in a desiccator before weighing. The difference between the final weight and weight before filtration was the dry weight of the sample. Cell numbers were counted using a haemocytometer after appropriate dilution. Nitrate analysis was determined by ion chromatography (761 Compact IC, Metrohm), according to the manufacturer's instructions.

2.3. Chlorophyll measurement

Chlorophyll content was determined using DMSO extraction. An aliquot (2 mL) of culture was centrifuged and the pellet resuspended in DMSO and vortexed to extract pigments. Cellular debris was pelleted by centrifugation and chlorophyll a and b levels were determined photometrically, in the supernatant, by measuring optical absorbance at 649 nm and 665 nm. Calculations of total chlorophyll (μg mL$^{-1}$) were performed as described previously (Wellburn, 1994).

2.4. Fluorescent microscopy

For fluorescent microscopy analysis, cells were resuspended in phosphate-buffered saline (PBS). Cells were stained with Nile red (1 μg mL$^{-1}$ final concentration; Sigma) and incubated for 30 min in the dark. The stained cells were visualized by a fluorescent microscope (BX51, Olympus), and representative images were recorded using a digital camera system (DP72, Olympus). The filter set was Semrock Cy3-4040B (excitation 531/40 nm, dichroic mirror 562 nm, emission 593/40 nm; Semrock, NY, USA). Images were processed employing Adobe Photoshop 7.0 (Adobe).

2.5. Transmission electron microscopy

The cells were pelleted by centrifugation (4000 rpm × 5), fixed for 4 h at room temperature with a 4% glutaraldehyde and 3% paraformaldehyde mixture in 0.1 M sodium cacodylate pH 7.4 buffer. After washing three times in 0.1 M sodium cacodylate buffer, the primary fixed cells were then post-fixed with 1% osmium tetroxide in the cacodylate buffer for 2 h at room temperature, and later washed three times with the same buffer. The fixed specimens were dehydrated in a graded ethanol series from 30% to 100% (v/v), and finally washed twice with pure acetone.

The specimens were infiltrated with 25% Spurr resin in acetone for 4 h, 50% resin for 16 h, 75% resin for 8 h and 100% resin for 16 h. Then the 100% resin was changed twice more and the resin polymerized in the oven at 60°C for 48 h. Ultrathin sections were cut with a diamond knife on an Ultracut UCT ultramicrotome (Leica, Vienna, Austria), and then post-stained with 1% uranyl acetate and 1% lead citrate. Grids were examined with JEM-100CXII electron microscope (Japan Electron Optics Laboratories, Tokyo, Japan) at 100 KV.

2.6. Starch analyses

Starch measurement was performed using a modified method used by Branyikova et al. (2011). In brief, 2–4 mg lyophilized biomass was disintegrated by vortexing with 0.5 mL of glass beads (200 μm diameter) for 4 min (2,700 rpm) in 0.25 mL of distilled water. Pigments were extracted three times using 80% ethanol for 15 min at 68°C. For total hydrolysis of starch, 30% perchloric acid was added to the sediment, stirred for 15 min at 25°C and centrifuged. This procedure was repeated three times. The extracts were combined and made up to 10 mL. Thereafter, aliquots of 2 mL of solubilized starch solution were reacted with 5 mL of concentrated sulfuric acid (98 wt%) and 1 mL of phenol (6%, w/v) at room temperature for 10 min. The absorbance was read in a spectrophotometer at 490 nm. Samples were then quantified by comparison to a calibration curve using glucose as the standard.

2.7. Lipid analyses

The lyophilized algal biomass (~20 mg) was transmethylated with 2.5 mL of methanol containing 2% (v/v) H$_2$SO$_4$ at 80°C for 2.5 h (Recht et al., 2012). After the suspension cooled, 1 mL of n-hexane and 1 mL of saturated NaCl solution were added to form separated layers in the tube. The upper n-hexane layer containing fatty acid methyl esters (FAMEs) was removed for analysis by a gas chromatography (GC-2010, Shimadzu, Kyoto, Japan) equipped...
with a flame ionization detector (FID). Samples were injected into a 30 m long RTX-WAX capillary column with 0.25 mm inner diameter and 0.25 μm film thickness. Nitrogen was used as carrier gas. The temperature of the injector and detector were set at 250 °C and 280 °C, respectively. The initial column temperature was maintained at 195 °C for 12 min, then rose to 230 °C at a rate of 10 °C/min, and held at 230 °C for 15 min. The individual fatty acid compositions were identified by chromatographic comparison with authentic standards (Sigma) and were quantified from the peak areas on the chromatogram using heptadecanoic acid (C17:0, Sigma) as the internal standard. The total fatty acid content was calculated by adding all the individual fatty acid contents.

3. Results and discussion

3.1. Cell growth characteristics

To determine the effect of nitrogen on *C. zofingiensis* grown under photoautotrophic conditions, the logarithmic cultures were inoculated into either N-replete or N-depleted medium at an optical density (OD750) of 0.67 and grown under photoautotrophic conditions for 10 days. In N-replete medium, cell density increased approximately 10-fold within 4 days with a maximum of 1.6 × 10⁶ cells mL⁻¹, while N-starved cells increased approximately twofold in number within the first 2 days with a maximum of 5.3 × 10⁵ cells mL⁻¹ (Fig. 1A). The biomass under favorable growth condition increased rapidly from 0.2 to 2.3 g L⁻¹ within 4 days and reached 3.1 g L⁻¹ by day 10, whereas the biomass exposed to stress increased mildly from 0.27 to 0.56 g L⁻¹ within the first 2 days and reached 0.7 g L⁻¹ by day 10 (Fig. 1A). In contrast to cell density, biomass showed a continuous increase throughout the experiment either in N-replete or N-depleted medium, presumably as the cells accumulated storage compounds when growth ceasing (see below). Our study shows that microalgal cell growth is severely inhibited upon nitrogen depletion.

Nitrogen concentration was examined during the experiment (Fig. 2). Under favorable growth condition, nitrate dramatically decreased 38% within the first 2 days, from 1.1 to 0.70 g L⁻¹, and then slowly declined to a final concentration at 0.47 g L⁻¹ on day 10. Nitrogen was always available in N-replete medium during the examined period, which means other factors might limit cell growth (e.g. light intensity). Nitrogen was not detected during N-deficiency although cells could still grow within the first 2 days, probably as algal cells consumed endogenous nitrogen for growth.

Under normal condition, chlorophyll basically maintained slight fluctuation in a certain range from 5.1 to 6.2 μg 10⁻⁷ cells, whereas under N-deficiency, chlorophyll dropped sharply for the first day, from 5.4 to 0.6 μg 10⁻⁷ cells, and then slowed down to finally below the detection limit (Fig. 1B). Chlorophyll breakdown led to the variation in color of culture broths from normally green to yellowish-white by day 10 when cells were moribund. It has been suggested that chlorophyll may be targeted for degradation for nutrient recycling under nutrient depletion (Gaude et al., 2007; Msanne et al., 2012).

3.2. Changes in starch and lipid in *C. zofingiensis* upon N-starvation

Starch and lipid are two major carbon and energy storage compounds appearing in many plants and microalgal cells, particularly in response to stress conditions. In this study, we conducted a time course analysis of metabolic changes under N-starvation compared to those under N-repletion. Under favorable growth condition, starch content was reduced within the first 2 days from 8.2% down to 5.3% of DW, and then increased to 9.7% of DW by day 4. After that, starch content slowly decreased to 4.5% of DW by day 10 (Fig. 3A). While in response to N-starvation, starch content increased sharply on the first day, from 7.6% to a peak 43.4% of DW, which decreased at the following day to 33.1% of DW and then decreased with a slower rate to 18.8% of DW by day 10 (Fig. 3A). When the starch content was calculated on a per cell basis, similar trends were observed with subtle differences. Under normal condition, starch amount was reduced to 6.0 μg 10⁻⁷ cells after 2 days.

![Fig. 1. Changes in algal growth and chlorophyll in *C. zofingiensis* over time.](image1)

![Fig. 2. Nitrate concentration in the broth culture under N-repletion (+N) and under N-depletion (−N).](image2)
from the initial 10 µg L⁻¹ cells. From then on, starch amount moderately increased to approximately 14 µg L⁻¹ cells and remained stable for another two days then slightly reduced to 9.9 µg L⁻¹ cells at the end of the experiment (Fig. 3B). On the contrary, starch accumulated about 7-fold after only 1 day upon N-deprivation, increasing from 8.8 to 62.3 µg L⁻¹ cells, and dropped to 35.1 µg L⁻¹ cells 1 day later, which then slightly fluctuated in a certain extent. By day 10, starch level was 29.4 µg L⁻¹ cells (Fig. 3B).

For the purpose of this work, the lipid content was taken as being equivalent to the fatty acids content. In N-replete medium, total fatty acids (TFA) content was reduced from 6.0% to 2.6% of DW during the first 2 days, and then increased to 8.8% of DW by day 6 and remained constant afterward (Fig. 4A). However, TFA content increased continuously during the progression of N-deficiency, from 6.2% up to 24.5% of DW (Fig. 4A). On the other hand, TFA amount per cell was reduced from 7.4 to 3.0 µg L⁻¹ cells after 2 days, and then gradually increased to 17.1 µg L⁻¹ cells by day 6, which remained stable subsequently when cells were grown in N-replete medium (Fig. 4B). Whereas TFA levels showed a gradual increase as N-deficiency progressed and increased about 7-fold at the end of the experiment. By day 10, TFA level was 52.2 µg L⁻¹ cells (Fig. 4B).

Generally, the variation trend of either starch or lipid showed no significant differences between on a dry weight basis and on a per cell basis in the present study. Nevertheless, we prefer to the per cell basis because it is able to reflect the actual metabolic capacity of starch or lipid in cells, irrespective of other compounds contributing to dry weight. Siaut et al. (2011) found that conclusion drawn from oil content comparisons can be different and even opposite depending on the reference basis and noted that the per cell basis is more appropriate for metabolic engineering comparisons. Hence, the discussion was on a per cell basis in the following context.

Altogether, starch and lipid have little or slight accumulation during the growth except on day 2, when rapid cell division might result in reduced cell inclusion. N-starvation triggered starch and lipid accumulation, but the patterns of these two compounds synthesis were distinct. In order to achieve clearer understanding of starch and lipid production in C. zofingiensis cells, the rates of starch and TFA biosynthesis were calculated upon N-depletion (Table 1). Starch synthesis rate peaked on the first day at 53.27 µg L⁻¹ cells d⁻¹ and decreased to a negative value on day 2, implying sharp starch degradation. From then on, starch synthesis rate was at low negative values, suggesting slight starch degradation (Table 1). Unlike starch synthesis, TFA synthesis rate maintained relatively constant during 6 days, in the range of contributing to dry weight. Siaut et al. (2011) found that conclusion drawn from oil content comparisons can be different and even opposite depending on the reference basis and noted that the per cell basis is more appropriate for metabolic engineering comparisons. Hence, the discussion was on a per cell basis in the following context.

![Graph](image1.png)

**Fig. 3.** Curves of starch in C. zofingiensis cells under N-repletion (+N) and N-depletion (−N). (A) Starch expressed on a dry weight (DW) basis (% of DW); (B) starch expressed on a per cell basis (µg L⁻¹ cells). Each data point represents the average of three independent experiments (±SD).

![Graph](image2.png)

**Fig. 4.** Curves of total fatty acids (TFA) in C. zofingiensis cells under N-repletion (+N) and N-depletion (−N). (A) TFA expressed on a dry weight (DW) basis (% of DW); (B) TFA expressed on a per cell basis (µg L⁻¹ cells). Each data point represents the average of three independent experiments (±SD).

<table>
<thead>
<tr>
<th>Days</th>
<th>Starch synthesis rate (µg L⁻¹ cells d⁻¹)</th>
<th>TFA synthesis rate (µg L⁻¹ cells d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.27 ± 5.58</td>
<td>3.97 ± 0.84</td>
</tr>
<tr>
<td>2</td>
<td>−37.34 ± 7.63</td>
<td>2.78 ± 0.84</td>
</tr>
<tr>
<td>4</td>
<td>−0.08 ± 1.03</td>
<td>4.22 ± 1.07</td>
</tr>
<tr>
<td>6</td>
<td>−4.38 ± 0.26</td>
<td>2.99 ± 0.58</td>
</tr>
<tr>
<td>10</td>
<td>−1.57 ± 0.45</td>
<td>6.20 ± 2.07</td>
</tr>
</tbody>
</table>
et al. (2011) suggested that subcordiformis peaked starch content on a dry weight basis immediately after gaining enough lipid. If biodiesel is desired, it is necessary to remain culture under stress for a long short time to obtain large amounts of starch, while if biodiesel is the pathway. Additionally, the results are of significance for designing beyond a mere competition between lipid and starch synthesis. Therefore, further intensive investigation is undoubtedly needed.

2.2. Pathways of lipid and starch synthesis in algae

Hence, the N-depletion triggered a similar pattern of early synthesis of starch followed by significant lipid accumulation in two fairly different green microalgae C. reinhardtii and Coccocmyxa sp. C-169. Recht et al. (2012) suggested that the major immediate response to stress in Haematococcus pluvialis was intensive production of carbohydrates, which were later converted to fatty acids. It is noted that starch degradation provides metabolites for the production of fatty acids (Li et al., 2011; Rismani-Yazdi et al., 2011). A marine green microalgae Tetraselmis subcordiformis peaked starch content on a dry weight basis immediately following nitrogen exhaustion without lipid analysis (Yao et al., 2012). Two C. reinhardtii wild-type strains first accumulated starch and lipid synthesis was induced together with carbohydrate accumulation under nutrient starvation (Cakmak et al., 2012). Li et al. (2011) suggested that Pseudochlorococcum used starch as a primary carbon and energy storage product and cells shifted the carbon partitioning into neutral lipids as a secondary storage product after N-depletion. Some higher plants also have the similar pattern. For example, in Brassica napus embryos, starch was transiently accumulated and lipid was rising during seed development (Eastmond and Rawsthorne, 2000; Vigeolas et al., 2004).

Starch synthesis from 3-phosphoglycerate requires 6 NADPHs and 9 ATPs for each 18-carbon molecule, which is more energetically and economically compared with lipid synthesis (Li et al., 2011), so it is speculated that starch accumulation acts as a quick response to environmental stress. Nevertheless, as lipid has a much higher (about 2.25 times) energy value compared to starch (Han et al., 1987), it is worthy to believe that algae tend to produce lipid as a long-term storage mechanism in response to prolonged environmental stress.

Given that starch synthesis shares common precursors with lipid synthesis, it is possible that lipid and starch could be interconvertible, which is an important implication for algal strain modification. Then shutting down starch synthesis may be a simple and effective metabolic engineering to enhance TFA production, and vice versa. Several reports on Chlamydomonas reinhardtii wild-type strains and the corresponding starchless mutants have suggested that blocking starch synthesis pathway results in higher oil accumulation capacity (Wang et al., 2009; Li et al., 2010a, 2010b), but recently Work et al. (2010) and Siaut et al. (2011) have the view that complexity of carbon partitioning is beyond a mere competition between lipid and starch synthesis. Therefore, further intensive investigation is undoubtedly needed to elucidate the relationship between starch and lipid biosynthesis pathways. Additionally, the results are of significance for designing cultivation strategies to obtain biofuels efficiently. If bioethanol is desired, the culture should be controlled under stress in a very short time to obtain large amounts of starch, while if biodiesel is desired, it is necessary to remain culture under stress for a long time to gain enough lipid.

3.3. Visualization of lipid bodies and starch granules in C. zofingiensis cells

N-stressed cells often accumulate large amounts of neutral lipids, mainly including triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), sterol ester. After being synthesized, these neutral lipids are deposited in densely packed lipid bodies (LBs) commonly located in the cytoplasm of the algal cell (Hu et al., 2008). Hence LBs can reflect lipid accumulation qualitatively. Nile red, a lipophilic fluorescent dye, emitting bright red fluorescence was used for LB staining. C. zofingiensis cells just possess tiny LBs (Fig. S1, yellow arrow) when grown under N-replete condition, whereas accumulate enormous LBs upon N-deprivation. The number and size of LBs both increased after 6 days of stress. Fluorescent microscopy is a simple tool to monitor lipid storage within cells.

Starch exists in chloroplasts in the form of starch granules. Since starch granules cannot be seen clearly through optical microscopy, transmission electron microscopy (TEM) was adopted subsequently. Several small starch granules and LBs were observed in cells under N-replete condition, implying basal levels of starch and lipid (Fig. S2A). As N-deficiency progressed, both of starch granules and LBs varied. Starch granules drastically increased in size and abundance after 1 day of N-starvation, whereas LBs had no significant change (Fig. S2B). When cells starved for 6 days, the cells were engorged with LBs instead of starch granules. Moreover, the fusion of individual LBs was observed (Fig. S2C). Those changes are in accordance with our quantitative results described above.

3.4. Fatty acid profile in C. zofingiensis cells under N-starvation

Factors such as temperature, irradiance and nutrient availability affect not only lipid content but also fatty acid composition in many algal species (Hu et al., 2008; Khizin-Goldberg and Cohen, 2011; Msanne et al., 2012). Fig. 5 illustrates the changes of fatty acid profile in C. zofingiensis cells during the progression of N-starvation. As N-deficiency progressed, almost all fatty acid species had different increase except C16:2 and C16:4, which had no significant change (Fig. S5A). Among these fatty acid species, C16:0, C18:1 and C18:2 increased most dramatically, from the initial 1.79, 0.14 and 2.06 μg 10^-7 cells up to 12.10, 10.49 and 6.25 μg 10^-7 cells respectively on day 10 under the stress. It should be noted that oleic acid (C18:1) elevated rapidly even within the first day subject to N-deficiency.

It is vital to concern fatty acid composition in percentage of TFA from the perspective of biodiesel production. Fatty acid profile has considerable changes upon N-deficiency (Fig. 5B). C. zofingiensis cells were initially abundant in C16:0 and C18:2, while showed a strongly increased abundance of oleic acid (C18:1) after 1 day of N-starvation. In addition, C16:2 and C18:2 were reduced obviously. As can be seen in Table 2, the cells were rich in polysaturated fatty acid species on day 0 (when cells still grew normally), indicating a predominance of membrane lipids. Subsequently, a decrease in polysaturated species as well as an increase in saturated and monounsaturated species was observed during the progression of N-deficiency (Table 2). In other words, algal cells tend to lower the degree of unsaturation of fatty acids in response to N-starvation. These changes in fatty acid profile were similar in trend to previous reports (Siaut et al., 2011; Ho et al., 2012; Msanne et al., 2012). Polysaturated fatty acids are easily oxidized and thus not desirable for biodiesel production (Siaut et al., 2011). The results show that C. zofingiensis is very suitable for biodiesel production when subject to N-depletion, particularly containing a mass of C18:1 species. Furthermore, there is no significant distinction between day 4 and day 10 of N-starved algal cells in terms of oil quality for biodiesel.

3.5. Biomass, starch and lipid production by C. zofingiensis

To obtain plenty of feedstocks for bioethanol or biodiesel production, it is essential to assess the starch and lipid production po-
potential of C. zofingiensis under different culture conditions. Table 3 summarizes the specific growth rate, biomass productivity, starch/lipid yield and productivity of C. zofingiensis under N-repletion and N-depletion. Though specific growth rate and biomass productivity under N-starvation (0.48 d⁻¹ and 0.20 g L⁻¹ d⁻¹) were much lower than those under N-repletion condition (1.02 d⁻¹ and 0.52 g L⁻¹ d⁻¹), the starch yields under these two conditions were comparable (220.7 mg L⁻¹ for N-repletion vs. 206.3 mg L⁻¹ for N-depletion). Starch productivity under N-starvation (185.1 mg L⁻¹ d⁻¹) was about 3.6 times higher than that under N-repletion (51.3 mg L⁻¹ d⁻¹), indicating that N-stressed condition is a good strategy for rapid and massive production of starch. On the contrary, TFA yield and productivity under N-depletion were half of those under N-repletion respectively, because TFA synthesis rate was much lower than starch synthesis rate during their accumulation phase (Table 1). It is seems that N-stress is not necessarily for lipid production. However, such stress is indispensable in terms of fatty acid profile and oil quality for biodiesel (Fig. 5 and Table 2). Therefore, in order to obtain higher yield and better quality of starch/lipid, two-phase cultivation process might be a good way, which is a nutrient sufficient phase to produce enough biomass followed by a stress phase to induce starch/lipid synthesis (Rodolfi et al., 2009).

4. Conclusions

Nitrogen deprivation led to a severe inhibition of cell growth and a sharp degradation of chlorophyll in C. zofingiensis cells. The initial response of the algal cells to N-starvation was considerable starch synthesis occurred within 1 day. Subsequently, starch was partially degraded, while lipid had a sustained increase trend. It is probably that starch serves as a quick response to stress while lipid serves as long-term energy storage. Hence optimal biofuels production strategies could be acquired by changing cultivation time under stress condition. In addition, increase in saturation of fatty acids renders C. zofingiensis potential for biodiesel production.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.10.092.

References


