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Sequential accumulation of starch and lipid induced by sulfur deficiency in *Chlorella* and *Parachlorella* species

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HIGHLIGHTS

- Starch and lipid accumulation occurred in 4 Chlorellaceae spp. under sulfur deficient conditions.
- Lipids accumulated subsequent to a reduction in stored starch.
- A change in illumination cycle is sufficient for *Chlorella lobophora* to induce lipid accumulation.
- Fatty acid compositions were altered in *Chlorella lobophora* and *Parachlorella kessleri* by sulfur depletion.

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ABSTRACT

The influence of sulfur deficiency on biomass production was analyzed in the four Chlorellaceae species, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorella lobophora*, and *Parachlorella kessleri*. Culturing under sulfur-deficient conditions promoted transient accumulation of starch followed by a steady increase in lipid storage. Transmission electron microscopy indicated an increase and decrease in starch granules and subsequent enlargement of lipid droplets under sulfur-deficient conditions. Chlorellaceae spp. accumulated 1.5–2.7-fold higher amounts of starch and 1.5–2.4-fold higher amounts of lipid under sulfur-deficient conditions than under sulfur-sufficient conditions. More than 75% of the fatty acids that accumulated in Chlorellaceae spp. under the sulfur-sufficient condition were unsaturated and culturing under sulfur-deficient conditions increased the saturated fatty acid content from 24.3% to 59.7% only in *P. kessleri*. These results indicate that the sequential accumulation of starch and lipid is a response to the sulfur depletion that commonly occurs in Chlorellaceae spp.

1. Introduction

Due to high growth rates and photosynthetic efficiency, microalgae have recently received much attention as a potential renewable energy resource. Lipid production can be increased under heterotrophic and mixotrophic culture conditions (Miao and Wu, 2004; Heredia-Arroyo et al., 2010; Shen et al., 2010), nitrogen depletion (Liang et al., 2009; Hsieh and Wu, 2009), phosphorus depletion (Rodolfi et al., 2009), high salt concentrations (Takagi et al., 2006), and high iron concentrations (Liu et al., 2008) in some microalgae. Similarly, starch accumulation can be induced by treatments such as nitrogen depletion (Dragone et al., 2011), sulfur depletion (Brányiková et al., 2011), high light intensity (Brányiková et al., 2011), or high CO_2 concentrations (Izumo et al., 2007). However, the mechanisms regulating starch and lipid accumulation in response to altered growth environments and the interrelationship between stored starch and lipid remain unclear.

Supplementation of a culture with additional carbon or nitrogen increases total biomass, starch and lipid, but the increase in cost is unacceptable to maintain competition between biofuels and fossil fuels. However, depletion of macroelements is a simple and an effective way to increase the relative starch and lipid contents per unit cell dry weight. However, this approach reduces biomass

Abbreviations: TAP, tris-acetate phosphate; DMSO, dimethyl sulfoxide; FAMEs, fatty acid methyl esters; FA, fatty acid; TEM, transmission electron microscopy.

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