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Enhancing starch production of a marine green microalga *Tetraselmis subcordiformis* through nutrient limitation

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HIGHLIGHTS

- Limited NO_3^- or SO_4^{2-} induced starch accumulation in *Tetraselmis subcordiformis*.
- Maximum starch productivity and content were 0.62 g L^{-1} d^{-1} and 62.1% respectively.
- Photosynthetic activity was a prerequisite to sustain starch accumulation.
- Balanced nutrition was necessary to achieve high starch concentration and content.

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ABSTRACT

Microalgal starch is a potential feedstock for biofuel production. The effects of KNO_3 and $MgSO_4$ concentrations and light intensity on biomass and starch production by the marine microalga, *Tetraselmis subcordiformis*, were investigated. Under 200 µmol m^{-2} s^{-1} irradiance and sulfur-deprived conditions, a starch productivity of 0.62 g L^{-1} d^{-1} and a starch content of 62.1% based on dry weight (DW) was achieved. A starch content of 54.3% was achieved under low irradiance and nitrogen starvation, which was 6.5% higher than that under nutrient- and light-sufficient conditions. Photosynthetic activity was indispensable for starch accumulation. It is difficult to reach high starch productivity and starch concentration simultaneously. Proper nutrient concentrations are necessary to achieve high starch productivity or starch concentration based on the target. The high starch productivity and starch content suggest that *T. subcordiformis* is a promising microalgal starch producer.

1. Introduction

Starch constitutes the primary photosynthetic carbon sink in many species of microalgae (Li et al., 2011, 2010). Bioethanol has been produced from microalgal starch (John et al., 2011), but an increase in microalgal starch production is necessary to make the process more economic. Starch accumulation in microalgae can be induced via macroelement (nitrogen, sulfur, or phosphorus) limitation (Ball et al., 1990). Starved for nitrogen, microalgae cease cell division and switch photosynthetic carbon partitioning toward starch synthesis (Klein, 1987). When subjected to nitrogen-limited Tris–acetate-phosphorus (TAP) medium, *Chlamydomonas reinhardtii* can mixotrophically accumulate starch from 35% to 58% of dry weight (DW) (Thu et al., 2009; Eriksen et al., 2007; Li et al., 2010; Choi et al., 2010). *Chlorella vulgaris* also showed starch accumulation under nitrogen-depletion conditions, with starch contents from 38% to 48% of DW (Brányiková et al., 2011; Behrens et al., 1989; Dragone et al., 2011). Deprivation of sulfur in *C. reinhardtii* caused cell expansion, regulated protein degradation, and a nearly ten-fold increase in cellular starch content (Zhang et al., 2002). *Dunaliella salina* altered the partition of photosynthate between starch and protein, and induced a ten-fold increase in starch/protein ratios under sulfur-deprived conditions (Cao et al., 2001). A sulfur-limited culture of *C. vulgaris* maintained a high starch content (60%) in laboratory culture and a starch content of 50% was obtained in an outdoor pilot-scale experiment (Brányiková et al., 2011). Strikingly, when cytoplasmic protein synthesis was inhibited, starch productivity of this microalga was 2.5 g L^{-1} d^{-1}. Under sulfur deprivation, the starch productivity of

Abbreviations: DW, dry weight; ASW, artificial seawater; ASW-N-S, nitrogen- and sulfur-deprived artificial seawater; +N+S, nitrogen- and sulfur-sufficient conditions; −N, nitrogen-deprived conditions; −S, sulfur-deprived conditions; HI, high irradiance; LI, low irradiance.

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