



# Temperature modulation of fatty acid profiles for biofuel production in nitrogen deprived *Chlamydomonas reinhardtii*

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## HIGHLIGHTS

- ▶ Algae often produce higher levels of polyunsaturated fatty acids than is desirable for biodiesel.
- ▶ Temperature shifts can alter the fatty acid profile of storage lipids under nitrogen starvation.
- ▶ 32 °C was the optimal temperature for fatty acid content and composition for biodiesel production.
- ▶ Carbohydrate and lipid storage were both affected by shifting temperature.
- ▶ Environmental factors can manipulate algal feedstocks potentially reducing the refining cost.

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## ABSTRACT

This study investigated the changes in the fatty acid content and composition in the nitrogen-starved *Chlamydomonas reinhardtii* starchless mutant, BAF-J5, grown at different temperatures.

The optimal temperature for vegetative growth under nitrogen sufficient conditions was found to be 32 °C. Shifting temperature from 25 to 32 °C, in conjunction with nitrogen starvation, resulted in BAF-J5 storing the maximum quantity of fatty acid (76% of dry cell weight). Shifting to temperatures lower than 25 °C, reduced the total amount of stored fatty acid content and increased the level of desaturation in the fatty acids. The optimal fatty acid composition for biodiesel was at 32 °C. This study demonstrates how a critical environmental factor, such as temperature, can modulate the amount and composition of fatty acids under nitrogen deprivation and reduce the requirement for costly refining of biofuels.

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## 1. Introduction

The mass cultivation of microalgae as a biomass feedstock for liquid biofuels is being assessed worldwide. Microalgae have high photosynthetic productivity and growth rates compared to terrestrial plants and can accumulate energy rich compounds such as polysaccharides and lipids. Microalgal lipids are of particular interest for nutrition (Mansour et al., 2005) and as a replacement for petroleum based transportation fuels such as diesel and jet fuel (Lee Chang et al., 2012). Therefore, a greater understanding of the biological processes and environmental factors that influence the storage of these metabolites is required. For mass cultivation in the open, seasonal changes in light and temperature have the potential to significantly affect algal growth and modulate productivity. These environmental factors could directly impact the economic viability for industrial-scale biofuel production.

The eukaryotic green alga *Chlamydomonas reinhardtii* is an important model for the study of photosynthesis (Grossman, 2000) and lipid metabolism in microalgae (Lohr et al., 2005). The availability of a sequenced genome with mature annotation (Merchant et al., 2007) and metabolic databases (Lopez et al., 2011) enables high-throughput techniques like transcriptional, proteomic and metabolomic profiling to be applied to understanding lipid metabolism. The availability of strain collections and mutant libraries also facilitates the elucidation of lipid metabolism in microalgae (Boyle et al., 2012; Miller et al., 2010; Nguyen et al., 2011; Wang et al., 2011). The metabolic consequences of changes to environmental conditions or induced by functionally removing a gene or disrupting its regulation may have unanticipated consequences. Therefore it is important to directly identify and quantify the metabolites to fully understand the biology.

*Chlamydomonas reinhardtii* normally stores carbon in starch granules and lipid droplets when nitrogen deprived (Ball et al., 1990; Miller et al., 2010; Moellering and Benning, 2010; Msanne et al., 2012; Nguyen et al., 2011). However, in the low-starch

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