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Detection and quantitation of lipid in the microalga *Tetraselmis subcordiformis* (Wille) Butcher with BODIPY 505/515 staining

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HIGHLIGHTS

- BODIPY 505/515 staining is a promising method for neutral lipid determination in *T. subcordiformis*.
- A concentration of 0.276 μg ml^{-1} and staining for 6 min was optimal.
- High concentrations of nitrogen improved cell growth but decreased lipid volume.
- The optimum $NaNO_3$ concentration for lipid production was 120 mg L^{-1}.
- A correlation coefficient of $R^2 = 0.934$ for BODIPY 505/515 staining and gravimetric analysis of lipids was obtained.

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ABSTRACT

BODIPY 505/515, a lipophilic bright green fluorescent dye was tested for lipid detection in the microalga *Tetraselmis subcordiformis*. A concentration of 0.28 μg ml^{-1} and staining for 6 min was optimal. Lipid bodies stained with BODIPY505/515 had a characteristic green fluorescence. Their volumes were determined using the sphere volume formula. Lipid accumulation under different nitrogen concentrations was analyzed. With an increase in $NaNO_3$ concentration from 0 to 240 mg L^{-1}, the maximum algal concentration increased from 8.23 ± 0.62 ($\times 10^5$ cells ml^{-1}) to 1.61 ± 0.13 ($\times 10^6$ cells ml^{-1}), while the maximum volume of intracellular neutral lipid decreased from 9.78 ± 1.77 μm^3 cell^{-1} to 6.00 ± 0.59 μm^3 cell^{-1}. A comparison of the lipid contents measured by BODIPY 505/515 staining and the gravimetric method showed a positive correlation coefficient of $R^2 = 0.93$. BODIPY 505/515 staining is a promising method in lipid quantitation in *T. subcordiformis*.

1. Introduction

Lipid droplets, recognized as dynamic organelles in most eukaryotic cells, contain oils that are of great importance for the food and energy industries and play crucial roles in cellular energy homeostasis and lipid metabolism (Guo et al., 2008). Lipid droplets have a unique physical structure as they vary greatly in size with diameters ranging from 1 μm to 100 μm. The droplets are surrounded by a protein-decorated phospholipid monolayer that envelops a neutral lipid core of triacylglycerols (TAGs) and sterol esters. TAG is the major form of energy storage. Both TAG and sterol esters serve as reservoirs of membrane lipid components (Walther and Farese, 2009; Farese and Walther, 2009). Under nitrogen starvation or high irradiance, the accumulation of oleic acid in lipids, mainly in triacylglycerols (TAGs) by *Haematococcus pluvialis*, was linearly correlated with the accumulation of astaxanthin monoesters (Zhekisheva et al., 2002).

Recently much research has been conducted to produce microalgal lipids as a source of biofuel (Hsieh and Wu, 2009). For mass production of biofuel, proper species selection and culture conditions optimization are important. The lipid content in some microalgae can be increased under stress conditions such as nitrogen deprivation (Li et al., 2008), high light intensity (Khotimchenko and Yakovleva, 2005), and low temperature (Renaud et al., 2002). Among these factors, nitrogen concentration has the strongest influence on lipid accumulation in various microalgae (Li et al., 2010).

The gravimetric method has been traditionally adopted for quantification of lipid content in organisms, although this method

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