Bioresource Technology 128 (2013) 107–112

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluation of FT-IR and Nile Red methods for microalgal lipid characterization and biomass composition determination

Guo-Dong Feng [a], Fang Zhang [a], Li-Hua Cheng [b,*], Xin-Hua Xu [b], Lin Zhang [a], Huan-Lin Chen [a]

[a] Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China
[b] Department of Environmental Engineering, Zhejiang University, Hangzhou 310058, China

HIGHLIGHTS

- FT-IR and Nile Red were compared with gravimetry for microalgal lipid determination.
- Carbohydrate and protein measured by FT-IR were compared with DNS and Bradford.
- Nile Red staining could locate intracellular and extracellular lipid droplets.
- Guideline of methods selection for microalgal lipid characterization is proposed.

ARTICLE INFO

Article history:
Received 3 April 2012
Received in revised form 20 August 2012
Accepted 28 September 2012
Available online 12 October 2012

Keywords:
Microalgal lipid
FT-IR
Nile Red (NR)
Nannochloropsis sp., *Botryococcus braunii*
FACHB-357

ABSTRACT

To characterize lipid content of microalgal cells rapidly and accurately, the gravimetric determination, FT-IR and Nile Red (NR) staining were investigated on six typical eukaryotic and prokaryotic algae species. FT-IR and Nile Red were relative quantification methods and a standard curve was required in contrast to the gravimetric method. The FT-IR method determined the lipid, carbohydrate and protein contents simultaneously assuming that the algal cells only consisted of those three components. The Nile Red method was a relatively rapid method for neutral lipid content characterization by spectrofluorometry and could locate lipid body of the algal cell by fluorescence microscopy. According to sample sources and processing purposes, the gravimetric determination was preferable for large-scale cultivation with low-frequency monitoring, while FT-IR and Nile Red were suitable for general laboratory cultivation with medium-frequency monitoring, in particularly Nile Red was appropriate for small samples when high-frequency screening was required.

1. Introduction

Lipid content is one of the key indicators to evaluate microalgae for biodiesel production. As the intracellular lipid content depends on algal species, growth phase and culture conditions, its rapid and simple characterization is of great significance for high-throughput screening, culture condition optimization and harvesting decisions.

Several methods have been developed for algal lipid content analysis such as gravimetric determination, FT-IR and Nile Red staining. The traditional gravimetric method is relatively time-consuming and uses organic solvents such as chloroform/methanol or ether for extraction followed by the evaporation of the solvent (Bligh and Dyer, 1959; Feng et al., 2005; Widjaja et al., 2009; Lee et al., 2010).

FT-IR (Fourier Transform Infrared Spectroscopy) can also be used for the lipid content determinations in alga as lipid, carbohydrate and protein have their own absorbance at specific frequency regions in the mid-infrared zone (Lv et al., 2010).

Alternatively, the lipid-selective fluorescent dye Nile Red (NR) has been used for *in vivo* lipid staining and quantitative analysis of microalgae through spectrofluorometry (Eltgroth et al., 2005; Elsey et al., 2007; Chen et al., 2009, 2011; Huang et al., 2009; Doan and Obbard, 2011). Since the fluorescence intensity is affected by factors, such as emission wavelength, dye concentration, cell density and staining time, those conditions usually need to be optimized for *in vivo* lipid staining. Penetration of the dye into the cell is often facilitated with dimethyl sulfoxide (DMSO) or microwaves (Chen et al., 2009, 2011). Eltgroth et al. (2005) observed the stained lipid body in two algal cells of *Isochrysis galbana* and *Emiliania huxleyi* using a fluorescence microscope.

Usually, only one of the methods for lipid content analysis is carried out, without the comparisons of their accuracy and the experimental requirements such as biomass composition monitoring or lipid localization to our best knowledge.

In the present study, gravimetric determination, FT-IR analysis and Nile Red staining were utilized for lipid content screening of

* Corresponding author. Tel./fax: +86 571 88982025.
E-mail address: chenglihua@zju.edu.cn (L.-H. Cheng).

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http://dx.doi.org/10.1016/j.biortech.2012.09.123