Algal Research 33 (2018) 412–418

Contents lists available at ScienceDirect

Algal Research

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

Microwell-assisted filtration with anodic aluminum oxide membrane for Raman analysis of algal cells

Fang-Yu Wen[a], Po-Sheng Chen[a], Ting-Wei Liao[a], Yi-Je Juang[a,b,c,*]

[a] Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
[b] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan, Taiwan
[c] Center for Micro/Nano Science and Technology, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Keywords:
Microalgae
Filtration
Polydimethylsiloxane
Anodic aluminum oxide
Biofuel

ABSTRACT

In this study, rapid and direct Raman analysis of microalgae was demonstrated through measurement of algal cells (or algal paste) prepared by microwell-assisted filtration using an anodic aluminum oxide (AAO) membrane. To obtain the algal paste, an array of polydimethylsiloxane (PDMS) microwells with through holes was placed on top of the AAO membrane, after which a vacuum was initiated and a droplet of algal solution dispensed inside the PDMS microwells for a 5-min filtration process. The results showed that the AAO membrane has the lowest background noise compared to other types of filter papers or membranes. Compared to the droplet evaporation method, the "coffee-ring" effect was effectively suppressed when applying our proposed filtration method. The algal cells became stacked inside the microwells, which led to relatively uniform and strong Raman signals across individual algal paste and consistency among different algal pastes.

1. Introduction

To ensure a successful commercialization of biofuel, it is crucial to increase the lipid content inside the microalgae in short cultivation cycles and efficiently extract the lipids from the algal cells. Various cultivation methods [1–4] and techniques for lipid extraction have been developed, such as coagulating and floating algal cells by electrolysis or chemical agents [5,6] as well as direct lipid extraction via osmotic shock [7]. To quantify the cellular lipids, the conventional approaches of gas or liquid chromatography (GC or LC) coupled with mass spectrometry (MS), lyophilization (or freeze drying), gravimetric separation, agar plates, atomized cell spray, micropipette, and dilution techniques can be applied. However, these approaches are either laborious, time-consuming, complicated in process or costly [8–11]. Near infrared (NIR) spectroscopy, staining methods, and the colorimetric sulfo-phospho-vanillin (SPV) method are good alternatives because of their simpler sample preparation, faster response, lower cost per trial and lower required effort; however, they suffer either limited strain coverage, lower accuracy or inadequate reproducibility [12].

Raman spectroscopy offers several benefits for analyzing the cellular lipids inside algal cells [13,14]. For example, water yields very weak and straightforward Raman signals and, hence, there is no need for sample preparation in general. The samples or living cells can be 3-D spatially characterized and monitored in a label-free and real-time fashion [15]. In recent years, Raman spectroscopy has been used to analyze microalgae at the single-cell level. For example, the iodine value of microalgae cellular lipids [16,17], lipids [18–21], β-carotene [22], nutrient status [23], identification of algae strains [24,25], and so on, can be characterized by Raman spectroscopy. Because of the simplicity and in-vivo analysis of Raman spectroscopy, obtaining real-time information to adjust the cultivation parameters for optimization of the cultivation process and determine the harvest time becomes feasible.

Raman spectroscopy has also been applied to quantify the lipid content inside microalgae at the cell-ensemble level by evaporating a droplet of the algal solution on a gold-coated glass slide [26]. Compared to the single-cell approach, measurement of an ensemble of algal cells is easily performed, the information of which is more straightforward and representative [26]. However, preparation time of the algal paste is relatively long (approximately 1 h) and the evaporation-induced "coffee-ring" effect leads to the deposition of more algal cells near the edge of the paste, which may raise uniformity concerns of the Raman signals.

Filtration by membrane (the so-called membrane process or membrane filtration) is an effective way to separate solids from fluids for a suspension or solution. Among various applications, membrane technology has been integrated into microalgae biorefineries for years [27,28], especially for cell harvesting because it readily scales up and requires less energy; in addition, there are no chemical contaminants,

* Corresponding author at: Department of Chemical Engineering, National Cheng Kung University, No. 1, University Road, Tainan, Taiwan.
E-mail address: yjjuang@mail.ncku.edu.tw (Y.-J. Juang).

https://doi.org/10.1016/j.algal.2018.06.022
Received 31 October 2017; Received in revised form 21 June 2018; Accepted 21 June 2018

which means the recovery of the media can be reused as a water conservation strategy [27,29]. The related issues regarding membrane filtration of microalgae, such as theoretical modeling, critical flux, fouling, and filtration performance, have been discussed [29–34]. In this study, we extended the application of the membrane process to the preparation of the algal paste (*Chlorella vulgaris*) for Raman analysis through microwell-assisted filtration with an anodic aluminum oxide (AAO) membrane. Different membranes and filter papers were used to assess the background signals and the effects of using microwells with through holes on the Raman signals.

2. Material and method

2.1. Sample preparation

Cultivation of *Chlorella vulgaris* is well-documented in the literature [2]. In brief, the microalgae in this study were cultured in a 1-l glass vessel containing Basal medium under 150 rpm agitation with CO_2 aeration (2% and 0.2 vvm). An external light source (14 W fluorescent light, TL5) was mounted on the sides of the vessel with the light intensity adjusted to approximately 9 W/m^2. The cell concentration was determined by optical density measurement at a wavelength of 682 nm (i.e., OD_{682}) using a spectrophotometer (Evolution 60S, Thermo Scientific, USA) after proper dilution with deionized (DI) water. OD_{682} was chosen since a full scan of the microalgal biomass using the spectrophotometer showed that the highest absorption peak was located at the wavelength 682 nm.

To increase lipid production, a two-stage nitrogen starvation strategy was applied [35]. In brief, the algal cells were first grown in the nitrogen-containing basal medium for a certain period of time, and then transferred to another tank containing the same basal medium, but with a reduction in the nitrogen source (KNO_3) concentration to 0.313 g/l (1/4 of its original KNO_3 concentration). The algal cells were then sampled at different time periods for subsequent experiments.

The substrates used for droplet evaporation were single-side polished silicon wafers (Monsanto Electronic Materials Company), glass slides and gold-coated glass slides. Prior to conducting the droplet-evaporation experiments, the substrates were first immersed in acetone and cleaned under sonication for 5 min, followed by immersion in methanol and DI water in sequence with the same procedure, and then blown dry with air. To fabricate the gold-coated glass slides, gold was sputtered (JEOL, 750A) on the cleaned glass slide to form an approximately 100-nm thick gold layer. 5 μl of algal solution was dispensed on the substrates, upon which sessile drops were allowed to evaporate for approximately 1 h under ambient conditions.

2.2. Fabrication of an array of polydimethylsiloxane (PDMS) microwells with through holes

To fabricate an array of PDMS microwells with through holes, an array of polymethylmethacrylate (PMMA) micropillars, 2 mm in diameter and 5 mm in height, was first milled by computer numerical controlled (CNC) machine, followed by pouring a mixture of PDMS base and curing agent (Sylgard 184,Dow Corning) at a ratio of 10:1 onto the substrate. Cleaned glass slides were placed on top of the micropillars and the back-side of the PMMA substrate, after which the whole assembly was clamped tightly. The assembly was then placed in an oven at 65 °C for 4 h. Finally, the array of PDMS microwells with through holes was separated from the PMMA micropillar array.

2.3. Filtration of microalgae and Raman analysis

Fig. 1 shows a schematic of the filtration process with utilization of the microwells. A 5–10 μl droplet of algal solution was dispensed on the filter paper, followed by activation of a vacuum ((ULVAC KIKO, Inc.,G-25SA) for 5 min. The filter papers used in this study were Whatman No. 1 (Whatman, United Kingdom), mixed cellulose ester membrane filters (A020A025A, Advantec) and anodic aluminum oxide (AAO) membranes (Anodisc 25, Whatman, United Kingdom). After filtration, the algal paste on the filter paper underwent Raman analysis (Thermo DXR Raman, Thermo Scientific, USA), using laser light at a wavelength of 532 nm. The exposure time and laser power were 1 s and 1 mW, respectively. The signals were the average of 20 measurements. To assess the uniformity of the Raman signals across the algal paste, spectra were acquired at 10 locations from the edge of the algal paste to the center through line mapping, with 3 measurements taken for each location. To characterize homogeneity among the algal pastes, 3 algal pastes were compared. To verify our method, other common quantification methods such as gas chromatography (GC) and high performance liquid chromatography (HPLC) were compared, for which the average of the Raman signal intensities at the 10 locations across one drop of algal paste was used. In this study, the intensity of the peaks for carotenoids (1524 cm^{-1}) and lipids (1440 cm^{-1}) [24] were analyzed. The PDMS microwell array with through holes was employed by first placing it on top of the filter paper, and then activating the vacuum. Note that the array of PDMS microwells with through holes needs to be in close contact with the filter paper before dispensing the algal solution into the microwells.

2.4. Quantification of cellular content by gas chromatography (GC) and high performance liquid chromatography (HPLC)

To compare the results obtained by our method, the lipid abundance and β-carotene content inside the algal cells were quantified by the standard methods, namely GC and HPLC, respectively. For lipid abundance, the sample was analyzed by GC (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) [35]. Samples were injected into a 30-m long capillary column (Type no. 260M142P, Thermo Fisher Scientific, Waltham, MA) with an internal diameter of 0.32 mm. The flow rate of the carrier gas, helium, was set at 1.3 mL/min. The temperatures of the injector and detector were 250 and 280 °C, respectively. The temperature profile of the oven was programmed as follows: 150 °C for 0.5 min initially, followed by increasing to 180 °C at a rate of 10 °C/min, then to 220 °C at a rate of 1.5 °C/min, and finally to 260 °C at a rate of 30 °C/min and held for 5 min. For the β-carotene content, the dry microalgae was first ruptured, after which the β-carotene was extracted with ether at room temperature for subsequent analysis by HPLC (YMC30 RP-18 column, [4.6 × 250 × 5 μm], Schermbeck, Germany). The detailed composition of the mobile phase and the operational procedure can be found in the literature [36]. The β-carotene was detected by measuring the absorbance at 450 nm, and then quantified with a common standard (Sigma–Aldrich, No. 22040).

3. Results and discussion

3.1. Evaporation of droplet of algal solution on different substrates for Raman analysis

To obtain algal paste for Raman analysis, evaporation of an algal droplet on a substrate is simple and straightforward. However, inhomogeneous deposition of algal cells across the algal paste resulting from the evaporation-induced “coffee-ring” effect [37] leads to variations in the signal intensity from one location to another. Accordingly, searching for “hot spots” on the algal paste, to acquire strong signals, might result in potentially biased data acquisition. To assess the “coffee-ring” effect on the Raman analysis, droplets of algal solution (O.D. = 1) were dispensed on different substrates, as shown in Fig. 2(a). It can be seen that although the algal paste formed a relatively circular ring after evaporation, inhomogeneous deposition of the algal cells was observed under higher magnification, with more algal cells deposited at the edge of the algal paste, as indicated by the darker green color. The Raman analysis result further confirms the inhomogeneity in the distribution of