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Efficacy of head space solid-phase microextraction coupled to gas chromatography–mass spectrometry method for determination of the trace extracellular hydrocarbons of cyanobacteria

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ABSTRACT

Hydrocarbons are widespread in cyanobacteria, and the biochemical synthetic pathways were recently identified. Intracellular fatty alka(e)nes of cyanobacteria have been detected by liquid–liquid extraction (LLE) coupled to gas chromatography–mass spectrometry (GC/MS). However, whether fatty alka(e)nes can be released to cyanobacterial culture media remains to be clarified. This work develops a sensitive method for analyzing the trace level of extracellular hydrocarbons in cyanobacterial culture media by head space solid-phase microextraction (HS-SPME) coupled to GC/MS. Headspace (HS) extraction mode using polydimethylsiloxane fiber to extract for 30 min at 50 °C was employed as the optimal extraction conditions. Five cyanobacterial fatty alka(e)nes analogs including pentadecene (C15:1), pentadecane (C15:0), heptadecene (C17:1), heptadecane (C17:0), nonadecane (C19:0) were analyzed, and the data obtained from HS-SPME-GC/MS method were quantified using internal standard peak area comparisons. Limits of detection (LOD), limits of quantitation (LOQ), linear dynamic range, precisions (RSD) and recovery for the analysis of extracellular fatty alka(e)nes of cyanobacteria by HS-SPME–GC/MS were evaluated. The LODs limits of detection (S/N = 3) varied from 10 to 21 ng L−1. The correlation coefficients (r) of the calibration curves ranged from 0.9873 to 0.9977 with a linearity from 0.1 to 50 μg L−1. The RSD values were ranging from 7.8 to 14.0% and from 4.0 to 8.8% at 1.0 μg L−1 and 10.0 μg L−1 standard solutions, respectively. Comparative analysis of extracellular fatty alka(e)nes in the culture media of model cyanobacteria Synechocystis sp. PCC 6803 demonstrated that sensitivity of HS-SPME–GC/MS method was significantly higher than LLE method. Finally, we found that heptadecane can be released into the culture media of Synechocystis sp. PCC 6803 at the later growth period.

1. Introduction

In fact, human society and civilization was completely dependent on fuels, especially petroleum-based fossil fuels. The dependence on petroleum-based fuels can be overcome by using microbial based green conversion technologies, which produce high value biochemicals and high energy biofuels [1]. Long chain alcohols and hydrocarbons are either additives or major components of petroleum. Therefore, hydrocarbons produced from biomass through photosynthetic biological systems have the potential to replace present petroleum-based fuels [2].

Cyanobacteria are a group of prokaryotic microbes which can utilize solar energy and CO_2 to produce a suite of organic compounds [3]. The carbon chain length of hydrocarbons in cyanobacteria was reported to be varying from C15 to C19 with a predominance of C17 [4,5]. Recently, cyanobacteria are becoming more and more attractive to be used as biofuel-producing microbial system due to the identification of the hydrocarbon biosynthetic pathways [6–9]. Unicellular cyanobacteria *Synechocystis* sp. PCC

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6803, genome sequences of which had been published at 1996, had been well developed as a model system for the production of biofuels and high valuable chemicals [10]. Hydrocarbon compositions and contents of *Synechocystis* sp. PCC 6803 have also been reported in several studies. Heptadecane (C17:0) is recognized as one of the major component of fatty alkane in this strain. However, there was still debate on the position of internal carbon–carbon double bond in the other significant hydrocarbon component (heptadecene) [1,6,11,12]. Even so, consensus was reached upon the total hydrocarbon content of wild type of *Synechocystis* sp. PCC 6803. It was about 0.1% of the cell dry weight (300 $\mu g\,L^{-1}$ OD730 absorbance at 730 nm−1, 1500 $\mu g\,L-1$)[13]. It is important to point out here that all the hydrocarbon analysis mentioned above were based on the liquid–liquid extraction (LLE) coupled to GC–MS, and fatty alka(e)nes were all extracted from cyanobacterial cell lysates [1,6,11,14–16]. Thus far, whether hydrocarbons can be released into the cell culture media is still unknown. LLE method, which is largely restricted to the loss of trace level analytes during the purification and concentration, is unsuitable for the extracellular hydrocarbon analysis. Efficient analytical methods are needed to be explored to determine the possible trace amount of hydrocarbon in the culture media.

Solid-phase microextraction (SPME) was developed in the 1990 by Pawliszyn and had been more and more widely used in sample preparation [17–19]. Compared with the traditional sample preparation methods, SPME has significant advantages. Firstly, SPME can integrate sampling, extraction, concentration and sample introduction into one step which makes the sample preparation simple. Secondly, one-step procedure can minimize errors introducing to an analysis and makes the analysis more accurate and reliable. Furthermore, SPME is a solvent-free procedure, and no use of toxic organic solvents is good for pollution and hazards caused by organic solvents. Finally, SPME can be used to detect trace analytes efficiently because of the high sensitivity of SPME.

In this work, the sensitive head space solid-phase microextraction (HS-SPME) was coupled to gas chromatography-mass spectrometry (GC–MS) for the detection of trace level extracellular fatty alka(e)nes in cyanobacterial culture media. The extraction parameters such as extraction mode, the type of fiber, extraction time and extraction temperature were optimized. The optimized HS-SPME-GC/MS method was used to detect the extracellular fatty alka(e)nes in the culture media of *Synechocystis* sp. PCC 6803. The results demonstrated that the extraction efficiency of HS-SPME method was better than traditional LLE method. Due to high sensitivity of HS-SPME-GC/MS method, we found that hydrocarbon can be released into the growth media of *Synechocystis* sp. PCC 6803 at the stationary growth phase.

2. Experimental

2.1. Apparatus

The gas chromatography–mass spectrometry (GC–MS) analysis was performed using a Trace GC Ultra system (Thermo Fisher, USA) equipped with ITQ1100 mass selective detector and Xcalibur software. A split/splitless-type injector was used for sample introduction, with splitless time of 3 min for solid-phase microextraction (SPME) fiber desorption. A DB-5MS fused silica capillary column (30m × 0.25 mm i.d.) with a film thickness of 0.25 μm (Agilent, USA) was used for separation of fatty alka(e)nes. The temperature program used was: 40 °C for 3 min increased at a rate of 15 °C min^{-1}–160 °C and at a rate of 10 °C min^{-1} reached to the final temperature of 250 °C. The injector, MS transfer line and ion source temperatures were 250 °C, 280 °C and 220 °C, respectively. Helium was used as carrier gas at a constant flow of 1 mL min^{-1}. Mass spectra were obtained by electron impact ionization (EI) at 70 eV in the scan range of 50–300 *m/z*.

The SPME holder for manual sampling was bought from Supelco (Dorset, UK). The polydimethylsiloxane (PDMS, 100 μm), polyacrylate (PA, 85 μm) and polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm) were bought from Supelco (Dorset, UK). A magnetic stirrer DF–101 B (Leqing, China) was employed for stirring and temperature control during extraction. Centrifugation was performed using an Allegra X-12 Beckman centrifuge (California, USA).

2.2. Chemicals

The fatty alka(e)nes, including pentadecene (C15:1), pentadecane (C15:0), heptadecene (C17:1), heptadecane (C17:0), nonadecane (C19:1) and eicosane (C20:0, internal standard, IS), used in the experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl acetate and methanol (HPLC grade) was obtained from Burdick & Jackson (Ulsan, Korea). Chloroform (HPLC grade) was from Sinopharm (Beijing, China). The six standard stock solutions including five sample compounds and one internal standard were separately prepared by dissolving 20 mg of each compound in a 10 mL volumetric flask diluted with ethyl acetate at room temperature and stored at 4 °C in the refrigerator.

2.3. SPME procedure

For the SPME method, 10 mL matrix was put into 20 mL headspace sampling vials containing a small magnetic spin bar. Then, the vial was tightly capped with a PTFE septum and a metal cap. A small hot plate-magnetic stirrer with a water bath was employed to adjust the rotation speed and the extraction temperature. Before each extraction, the solutions were stirred under the corresponding temperature condition at the rate of 890–900 rpm for 5 min. Extraction were carried out by exposing all segment of the fiber to the sample solution (DI-SPME) or to the headspace (HS-SPME) for corresponding extraction time. After the extraction, the fiber was immediately pulled back into the needle, removed from the vial and injected into the GC injector kept at 250 °C for desorption. The fiber remained in the injector for 10 min to avoid carryover effects in subsequent analyses. Blanks were run periodically to confirm the absence of contaminants.

2.4. Cyanobacterial culture conditions and sample preparation

The strain of *Synechocystis* sp. PCC 6803 was grown in BG11 culture media, at 30 °C, 30 μmol photons $m^{-2}\,s^{-1}$, with rigorous shaking at 220 rpm. For growth curve and extracellular fatty alka(e)nes analysis, *Synechocystis* sp. PCC 6803 of the exponential phase was diluted to absorbance at 730 nm ~0.05 in three parallel 500 mL flasks containing 300 mL BG11 media, and was bubbled continuously with 5% CO2-air (v/v) for about 12 days.

For extracellular fatty alka(e)nes' analysis, the culture sampled every 3 days were centrifuged (6000*g*, 5 min). Two aliquots of 10 mL supernatant were transferred to 20 mL headspace sampling bottles. Prior to extraction, eicosane was added to the extracellular sample at a concentration of 10 $\mu g\,L^{-1}$ as the internal standard. One of the samples was for HS-SPME extraction, which was extracted at 50 °C for 30 min by SPME fiber. Another sample was for liquid extraction, which was extracted for 2 h at room temperature with 10 mL of chloroform-methanol (v/v, 2:1). The organic phase was separated following centrifugation (3000*g*, 5 min) and evaporated to dryness under nitrogen at 55 °C. The solute was then dissolved in 200 μL of *n*-hexane and a 1 μL aliquot was analyzed by GC–MS.