JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Determination of Alachlor and Its Metabolite 2,6-Diethylaniline in Microbial Culture Medium Using Online Microdialysis Enriched-Sampling Coupled to High-Performance Liquid Chromatography

Chi-Zen Chen,[†] Cheing-Tong Yan,[†,§] Ponnusamy Vinoth Kumar,[†] Jenn-Wen Huang,[‡] and Jen-Fon Jen*[,†]

[†]Department of Chemistry and [‡]Department of Plant Pathology, National Chung Hsing University, Taichung 402, Taiwan

[§]Department of Occupational Safety and Health and Department of Medical Research, Chung-Shan Medical University, Taichung 402, Taiwan

ABSTRACT: In this study, a simple and novel microdialysis sampling technique incorporating hollow fiber liquid phase microextraction (HF-LPME) coupled online to high-performance liquid chromatography (HPLC) for the one-step sample pretreatment and direct determination of alachlor (2-chloro-2′,6′-diethyl-*N* -(methoxymethyl)acetanilide) and its metabolite 2,6-diethylaniline (2,6-DEA) in microbial culture medium has been developed. A reversed-phase C-18 column was utilized to separate alachlor and 2,6-DEA from other species using an acetonitrile/water mixture (1:1) containing 0.1 M phosphate buffer solution at pH 7.0 as the mobile phase. Detection was carried out with a UV detector operated at 210 nm. Parameters that influenced the enrichment efficiency of online HF-LPME sampling, including the length of the hollow fiber, the perfusion solvent and its flow rate, the pH, and the salt added in sample solution, as well as chromatographic conditions were thoroughly optimized. Under optimal conditions, excellent enrichment efficiency was achieved by the microdialysis of a sample solution (pH 7.0) using hexane as perfusate at the flow rate of 4 μL/min. Detection limits were 72 and 14 ng/mL for alachlor and 2,6-DEA, respectively. The enrichment factors were 403 and 386 (RSD < 5%) for alachlor and 2,6-DEA, respectively, when extraction was performed by using a 40 cm regenerated cellulose hollow fiber and hexane as perfusion solvent at the flow rate of 0.1 μL/min. The proposed method provides a sensitive, flexible, fast, and eco-friendly procedure to enrich and determine alachlor and its metabolite (2,6-DEA) in microbial culture medium.

KEYWORDS: alachlor, 2,6-diethylaniline (2,6-DEA), hollow fiber, liquid phase microextraction, HPLC-UV, microbial culture medium

INTRODUCTION

Alachlor (2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide) is an effective pre-emergence and postemergence chloroacetanilide herbicide that has been widely used to control annual grasses and broadleaf weeds in agricultural crops.[1,2] Although it is degraded by environmental soil microorganisms, alachlor and its main metabolite [2,6-diethylaniline (2,6-DEA)], which can diffuse into groundwater and disperse in the environment, cause serious ecological and physiological problems.[3−5] Moreover, alachlor is also known as an extremely toxic endocrine-disrupting chemical and classified as a carcinogen of the B_2 group by the U.S. Environmental Protection Agency (U.S. EPA).[6,7] The potential toxicity of alachlor has been evaluated in a series of rodent chronic bioassays such as in vitro clastogen in Chinese hamster ovary cells[8] and human lymphocytes.[9] However, when toxic pollutants (e.g., alachlor and its main metabolites) exist in the environment, an effect on microbial growth ability is usually observed. Słaba et al.[10] studied the influence of alachlor and zinc on the growth of the filamentous fungus *Paecilomyces marquandii* and its ability to eliminate alachlor and zinc. Since then, cytotoxicity studies of herbicides are often carried out in cell culture. In addition to the DNA fragmentation analysis and immunoblot analysis, a simple, fast, and reliable method to determine alachlor and its metabolite 2,6-DEA in microbial culture medium samples is required in plant pathology studies.

For the analysis of chloroacetanilide herbicide metabolites, high-performance liquid chromatography (HPLC) is preferred because most of the chloroacetanilide metabolites are ionic compounds, which are not sufficiently volatile for analysis by gas chromatography.[11−14] However, an appropriate pretreatment and the enrichment of target species are required prior to HPLC analysis. The conventional pretreatment methods for the analysis of alachlor and its metabolites include liquid−liquid extraction (LLE), solid phase extraction (SPE), pressurized liquid extraction (PLE), and solid phase microextraction (SPME).[15−22] LLE is not efficient for polar species and ionic compounds and is under criticism for using large quantities of organic solvents, thereby causing pollution accompanied by health risks, in addition to the extensive time-consuming cleanup procedures. The use of SPE has eliminated or decreased most of the disadvantages of LLE. However, plugging of the cartridges or disks by high molecular weight species in culture medium limits the application

Received: March 19, 2011
Revised: June 26, 2011
Accepted: June 27, 2011
Published: June 27, 2011

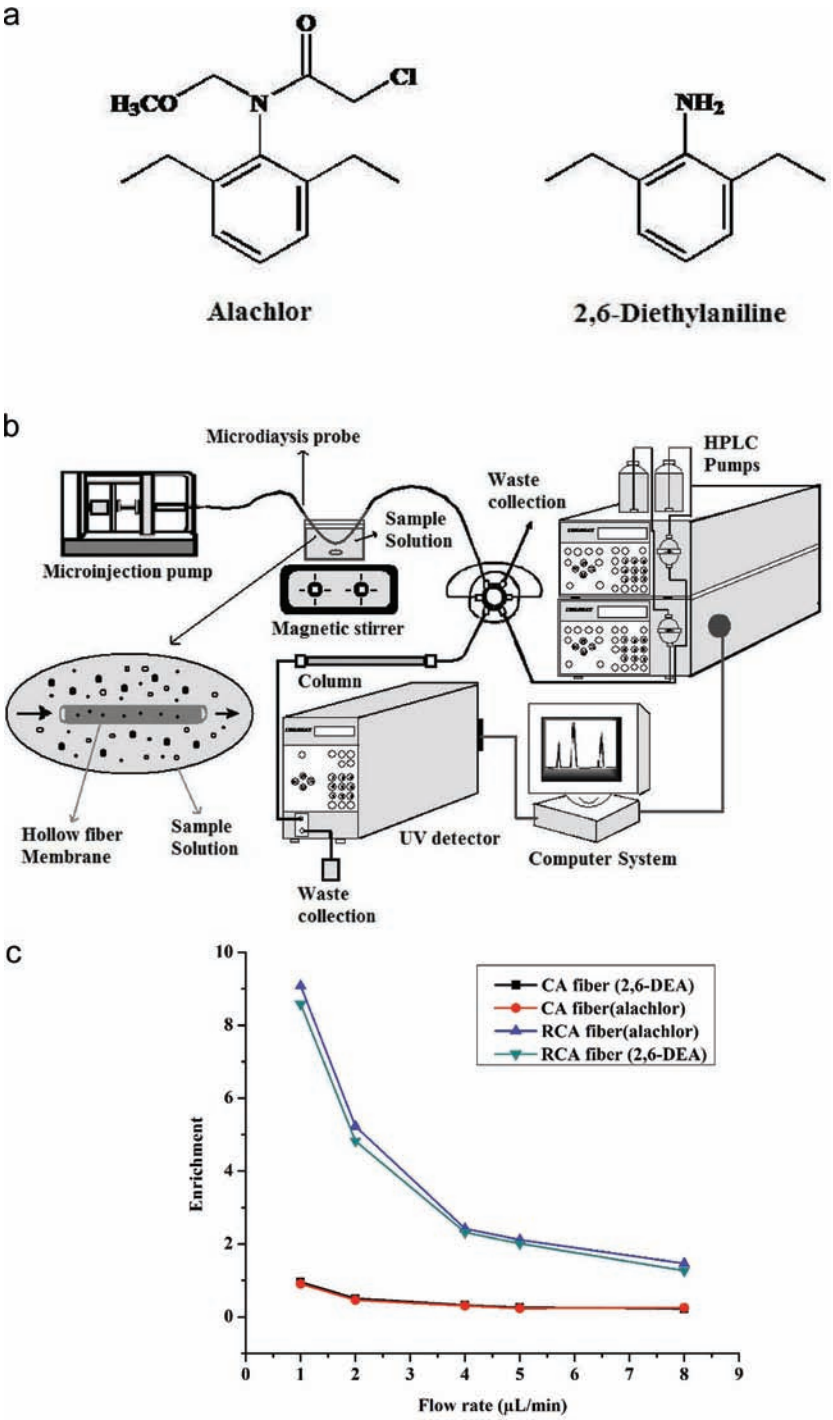

Figure 1. (a) Chemical structures of alachlor and 2,6-diethylaniline. (b) Assembly of the online HF-LPME/HPLC-UV system. (c) Selection of the HF membrane on the online HF-LPME efficiency: sample matrix, 24 g/L PDB cultured medium; length of hollow fiber, 20 cm; sample concentration,10 μg/mL of alachlor and 2,6-DEA.

of SPE in plant pathology and cytotoxicity studies. Although SPME has the advantages of simultaneous solvent-free extraction and preconcentration,[20−22] it is not appropriate for sampling in culture medium because the fiber is easily coated by the high molecular weight species present in samples. Therefore, it is vital to investigate a reliable and eco-friendly method to extract alachlor and its metabolite 2,6-DEA in culture medium.

In the recent decade, an efficient enrichment method was needed for the analysis of complex matrix liquid samples because the amount of analyte is at trace or even ultratrace levels. In recent years, as a novel sample preparation technique, hollow fiber liquid phase microextraction (HF-LPME) has gained considerable attention for biological and environmental sample analysis because it is simple, efficient, and inexpensive, consumes less organic solvent ,and has good sample cleanup ability and high enrichment efficiency.[23] HF-LPME can be carried out either in two- or three-phase mode. Normally, neutral analytes with a high solubility in nonpolar organic solvents can be extracted in a

dx.doi.org/10.1021/jf201129j |J. Agric. Food Chem. 2011, 59, 8078–8085

two-phase system, and acidic and basic analytes can be extracted in a two- or three-phase system.[24]

Microdialysis is a dynamic molecular sampling technique based on analyte diffusion across a semipermeable HF membrane driven by a concentration gradient.[25,26] Microdialysis has been applied to isolate components from sample matrix with the advantages of easy operation, speed, and no or less use of organic solvents. Recently, online microdialysis sampling with HF was established as an LPME technique with high enrichment potential by controlling the status of the sample solution and the conditions of the perfusion stream.[27−29] Thus, interference due to the interaction of analyte with sample matrix species can be decreased through the dilution of the sample solution. Online HPLC with HF microdialysis perfusion sampling provides simplified sample preparation and has been successfully applied to biosamples,[30,31] cosmetic and polymer wastewater samples,[32,33] vegetable seeds,[34] and fermented milk and drinks.[35] However, there is no report related to the application of microdialysis sampling as the cleanup process and enrichment step in the determination of alachlor and its metabolite 2,6-DEA so far. It has the potential to be an alternative to conventional pretreatment processes in the determination of alachlor in culture medium. In this paper, we report for the first time the applicability of the microdialysis sampling technique assembled as a hollow fiber membrane liquid phase microextraction online to HPLC is investigated and examined to develop an eco-friendly process of enrichment for the determination of alachlor and its metabolite 2,6-DEA in microbial culture medium samples. In this study, parameters that influenced the efficiency of enrichment, including the material of hollow fiber and its length, the perfusion solvent and its flow rate, the pH, and the addition of salt in sample solution, as well as the chromatographic behaviors, were studied thoroughly to optimize the online HF-LPME/HPLC-UV technique for the determination of alachlor and 2,6-DEA in culture media.

MATERIALS AND METHODS

Chemicals and Reagents. Ultrapure water was produced using a Barnstead Nanopure water system (New York) for all aqueous solutions. All chemicals and solvents were of ACS reagent grade. Standard stock solutions of 1000 mg/L alachlor (Sinon, Taipei, Taiwan) and 2,6-DEA (Fluka, Buchs, Switzerland) were prepared by dissolving 0.100 g of alachlor and 2,6-DEA individually in 90 mL of HPLC grade methanol (Baker, Phillipburg, NJ) and diluted to 100 mL; the solutions were stored at 4 °C in silanized brown glass bottles with Teflon-lined caps. Fresh working standard solutions were prepared daily by appropriately diluting the stock solution to the studied concentrations with water. The chemical structures of alachlor and its metabolite 2,6-DEA are shown in Figure 1a. Sodium dihydrogen phosphate (NaH_2PO_4) and sodium hydroxide (NaOH) were obtained from Riedel-deHaën (Hanover, Germany) to prepare buffer solution for adjust the pH. The mobile phase was prepared as 50% (v/v) of acetonitrile (ACN) (Merck, Darmstadt, Germany) in water containing 0.01 M phosphate buffer at pH 7.0. All eluents were filtered through a 0.45 μm poly(vinylidene difluoride) (PVDF) membrane filter and degassed ultrasonically.

Apparatus and Instrumentation. HPLC analyses were performed using a Dynamax liquid chromatograph system (Varian, Walnut Creek, CA) equipped with a Dynamax SD-200 solvent delivery system and a Dynamax UV-1 detector with a 20 μL flow cell. Separations were performed on an LC-18 column (25 cm × 4.6 mm inner diameter (i.d.), 5 μm particle size) (Supelco, Bellefonte, PA). The Varian Star chromatography workstation (system control version 5.3) was utilized to perform HPLC operations to obtain the chromatogram and to carry out data calculations. A Rheodyne 7010 injector/switching valve (Rheodyne, Cotati, CA) with a 20 μL sample loop was used as the interface between the HF-LPME system and the HPLC system for sample introduction. The hollow fiber microdialysis system comprised a microinjection syringe pump and its controller and a 1 mL syringe (Bioanalytical System Inc., West Lafayette, IN). A cellulose acetate hollow fiber membrane [Althane AF-220, 5000 Da, i.d. 220 μm, outer diameter (o.d.) 310 μm] was purchased from Baxter/Althin Co. (Ronneby, Sweden). A regenerated cellulose acetate hollow fiber membrane (18000 Da, i.d. 200 μm, o.d. 216 μm, surface area/length 6.3 mm^2/cm, volume/length 0.31 μL/cm) was obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA). A home-assembled hollow fiber membrane probe was prepared and utilized as the HF-LPME enriched-sampling system. By using polyethylene (PE) tubings (i.d. 380 μm, o.d. 1090 μm), the inlet of the hollow fiber for LPME was connected to a syringe pump containing perfusion solvent and the outlet connected to the sample-loop of a switching valve. The online microdialysis sampling coupled HPLC was assembled with minor modifications as in our previous paper.[29,32,34] A schematic diagram of the online HF-LPME/HPLC-UV system used for determination of alachlor and 2,6-DEA in culture medium is presented in Figure 1b.

Preparation of Culture Media and Spiked Samples. Nutrient agar (NA) culture medium was prepared by adding 2.3 g of NA (Difco, Detroit, MI) and 1.5 g of agar (Difco) in a 200 mL flask containing 90 mL of water. After dilution to 100 mL with water, the NA culture medium was sterilized in an autoclave for 1 h. After cooling in a clean bench (HighTen, Taipei, Taiwan), the culture medium was transferred into 125 mL culture medium flasks (about 9 mL for each). Alachlor (10 μg/mL) and 2,6-DEA (5 or 10 μg/mL) were spiked in the culture medium as spiked samples.

Potato dextrose broth (PDB) culture medium was prepared by adding 24 g of PDB (Difco) in a 1000 mL flask containing 900 mL of water. After dilution to 1000 mL with water, the culture medium was transferred into 125 mL flasks (50 mL for each) and sterilized in an autoclave for 1 h. *Rhizopus stolonifer* with a concentration of 5.0×10^5 spores/mL was incubated at 28 °C in PDB culture medium for 96 h to degrade alachlor (100 μg/mL).[36]

Online HF-LPME/HPLC-UV Procedure. After appropriate dilution or adjustment, culture medium containing alachlor and 2,6-DEA was transferred into the dialysis cell (50 mL) for HF-LPME by using hexane as perfusion solvent at 0.1−4 μL/min flow rate. The dialysate was collected online in the sample loop for HPLC analysis. The experiments for each investigation were carried out with five replicates. The enrichment factor (EF) in online HF-LPME is calculated on the basis of the ratio of analyte concentration in the extractant to that in the sample matrix: $EF = C_s/C_i$ (where C_s is the analyte concentration in the extractant and C_i is the analyte concentration in the sample matrix).

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions. Because the online HF-LPME system was a pretreatment step for the HPLC determination, in addition to optimizing the pretreatment system, chromatographic conditions had to be optimized and built up. When referred to the literature,[11,20] a reversed-phase C-18 column had the potential to resolve alachlor and 2,6-DEA from other species and was thus tested herein. From a series of tests, the optimum separation and detection conditions were achieved. Under the optimum conditions (an LC-18 column eluting with 50% (v/v) acetonitrile in 0.01 M phosphate aqueous buffer at pH 7.0 at the flow rate of 1 mL/min), peaks of alachlor and 2,6-DEA in the chromatogram were sharp and symmetric and well separated

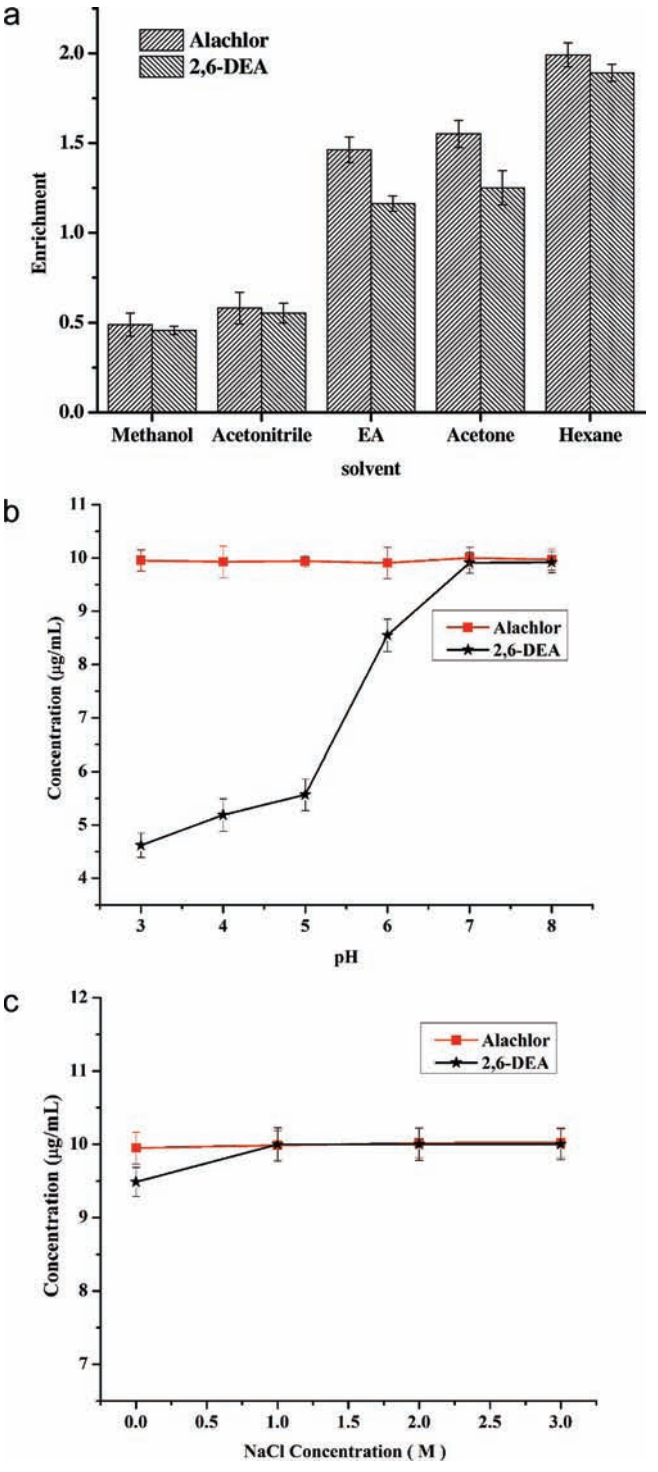

Figure 2. (a) Selection of the perfusion solvent on the online HF-LPME efficiency: sample matrix, 24 g/L PDB cultured medium; length of hollow fiber, 20 cm; flow rate, 4 μL/min; sample concentration, 10 μg/mL of alachlor and 2,6-DEA, N = 5. (b) Effect of sample pH on the online HF-LPME efficiency: sample matrix, 24 g/L PDB cultured medium; length of hollow fiber, 20 cm; flow rate, 4 μL/min; perfusion solvent, hexane; sample concentration, 10 μg/mL of alachlor and 2,6-DEA, N = 5. (c) Effect of salt addition on the efficiency of online HF-LPME. Conditions were the same as in (B) except the sample matrix (NA culture medium at pH 7), N = 5.

within 18 min. On the basis of the UV spectra of alachlor and 2,6-DEA in elution solution, the wavelength for UV detection was set at 210 nm. Under the chromatographic conditions, the retention times of alachlor and 2,6-DEA were 17.18 min (0.30% RSD) and 12.60 min (0.06% RSD), respectively, and the reproducibilities of quantitative detection were 2.78 and 1.23% RSD for three determinations of alachlor (10 μg/mL) and 2,6-DEA (10 μg/mL), respectively.

Selection of Hollow Fiber. Generally, surface characteristics (porous size, wall thickness, and absorption nature) of the hollow fiber influence the extraction of analytes on HF-LPME.[30−34] To choose an appropriate hollow fiber membrane for the online HF-LPME enrichment of alachlor and 2,6-DEA, two different hollow fibers, namely, cellulose acetate (CA) and regenerated cellulose acetate (RCA), were examined under different flow rates (1−8 μL/min) of perfusion in the fortified culture medium sample solutions (10 μL/mL of alachlor and 2,6-DEA). Experimental results, as shown in Figure 1c, revealed that the RCA fiber offers higher extraction efficiency for alachlor and 2,6-DEA than the CA fiber, especially in the low flow rate of perfusion. The RCA hollow fiber was used herein.

Selection of the Perfusion Solvent. In conventional LLE, the polarity of the extraction solvent is one of the main factors affecting the extraction efficiency. The dialysis could be achieved not only by the concentration gradient but also by appropriate selection of perfusion solvent.[30−34] To achieve high extraction efficiency in the online HF-LPME method, perfusion solvent selection is essentially based on polarity, viscosity, and its retention behavior in the chromatographic column. In this study, acetonitrile, acetone, ethyl acetate, methanol, and hexane were selected, and the relative concentration abilities of these solvents were examined in online HF-LPME for alachlor and 2,6-DEA in the fortified sample solution (10 μL/mL of alachlor and 2,6-DEA). Figure 2a indicates that hexane has the highest enrichment potential among the test solvents, followed by acetone and ethyl acetate (EA). In addition, the polarity difference between hexane and culture medium simplified the diffusion of species into perfusate and, thus, gave at better baseline of chromatograms. Hexane was thus used as the perfusion solvent.

Effect of Sample pH. Normally, sample pH is adjusted to improve the extraction efficiency of LLE, LPME, SPE, and SPME, which enables the favorable partition of analytes in their molecular forms into the extraction solvent.[30−34] The enrichment of analytes from a dialysis system depends on the pH of sample solution, thus affecting online HF-LPME efficiency. Figure 2b shows the concentrations of alachlor and 2,6-DEA in perfusate under different pH values of fortified sample solution (10 μL/mL of alachlor and 2,6-DEA). The dialysis efficiency of 2,6-DEA increased with the increase of pH until pH 7.0, and alachlor did not change over the pH range of 3−8. This depicts that only the neutral molecular 2,6-DEA and alachlor were favored to diffuse through the fiber membrane. To obtain good HF-LPME efficiency, the pH of the sample solution was recommended at 7.0. Hence, pH 7 was utilized in the following experiments.

Effect of Salt-Addition in Sample Matrix. A salting-out effect is frequently employed to improve the recovery in extraction processes such as LLE, LPME, and SPME.[30−34] In the present method, various amounts of NaCl were added into the fortified sample solution (10 μL/mL of alachlor and 2,6-DEA) as 0, 1.0, 2.0, and 3.0 M to investigate the effect on diffusion efficiencies of alachlor and 2,6-DEA. A series of tests demonstrated that in NA

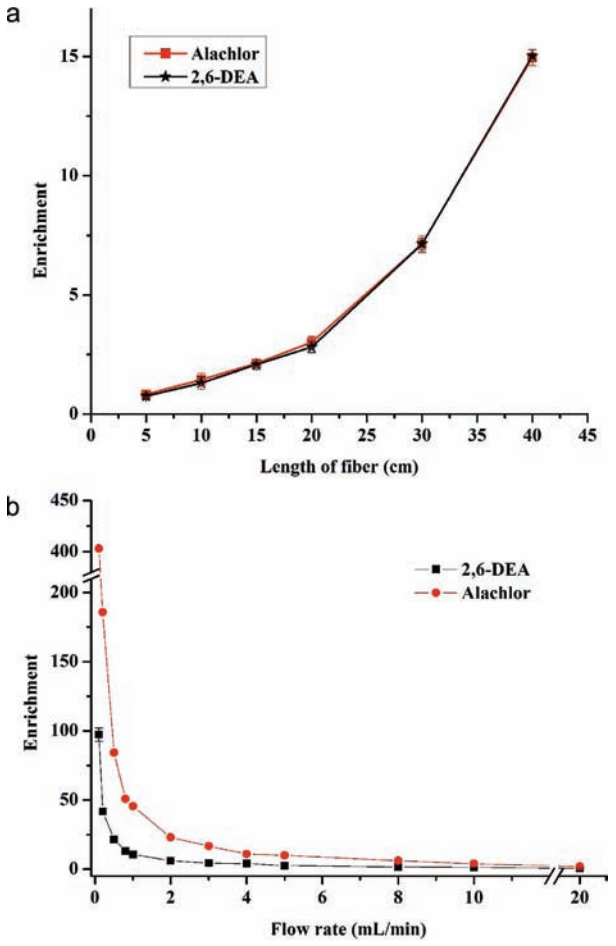

Figure 3. (a) Effect of fiber length on the efficiency of online HF-LPME: sample matrix, 24 g/L PDB cultured medium at pH 7; $N = 5$; flow rate, 4 μL/min; perfusion solvent, hexane; sample concentration, 10 μg/mL of alachlor and 2,6-DEA. (b) Effect of different flow rate at fiber length on the efficiency of online HF-LPME: length of hollow fiber, 20 cm; sample matrix, 24 g/L PDB cultured medium at pH 7; perfusion solvent, hexane; sample concentration, 10 μg/mL of alachlor and 2, 6-DEA.

culture medium, the recovery of 2,6-DEA in the dialysis process increased slightly with the NaCl addition and went to flatness after 1.0 M addition, but it was not significant for alachlor, as shown in Figure 2c. In the PDB culture medium, no significant change of dialysis efficiency for either 2,6-DEA or alachlor occurred due to the PDB culture medium comprising some inorganic salts. Thus, it was not required to add NaCl in the sample solution.

Effect of Fiber Length and Perfusion Flow Rate. As reported in the literature,[29−34] diffusion efficiency and extraction time depend on the length of the hollow fiber and perfusion flow rate. In this study, the extraction efficiency of HF-LPME increased with the length of fiber when 5, 10, 15, 20, 30, and 40 cm were studied. Figure 3a shows that the extraction efficiency of alachlor and 2,6-DEA increased gradually with increase in the length of fiber under the fortified sample solution of 10 μL/mL of alachlor and 2,6-DEA by using hexane as the perfusion solvent at the flow rate of 4 μL/min. A series of tests were carried out under various flow rates from 0.1 to 8 μL/min using hexane as perfusion solvent and 20 cm of hollow fiber in 50 mL of fortified sample solution. Experimental results as shown in Figure 3b revealed that significant enrichment occurred in a low flow rate of perfusion, and enrichment factors could be controlled by the flow rate of perfusion and the length of hollow fiber depending on the requirement of detection sensitivity. The higher the flow rate (8 μL/min) of perfusion, the lower the recovery obtained because of a dilution effect and the increased pressure that reduced the diffusion tendency from the sample solution. Although a low perfusion flow rate (0.1 μL/min) increased the diffusion recovery, it took time to collect enough perfusate to clear the eluent in the sample loop and be injected into the chromatographic system. Therefore, the optimal flow rate of perfusion of 4 μL/min and a hollow fiber length of 20 cm were selected.

Validation of the Method. The applicability of the proposed method (online HF-LPME) was examined for the quantitative determination of alachlor and 2,6-DEA using HPLC-UV by spiking standard solutions of alachlor and 2,6-DEA into the sample matrix under the optimum online HF-LPME conditions (50 mL of sample solution at pH 7, 20 cm of regenerated cellulose acetate hollow fiber using hexane as the perfusion solvent at the perfusion flow rate of 4 μL/min). Calibration plots were built up for alachlor and 2,6-DEA. The plots were specified with equations of $y = 262x + 244$ for alachlor in the range of 1−120 μg/mL and $y = 216x + 62$ for 2,6-DEA in the range of 0.1−80 μg/mL. The linear relationships between the peak area and the spiked quantity were in good agreement with correlation coefficients of 0.9995 and 0.9996 for alachlor and 2,6-DEA, respectively. Detection limits were calculated by dividing 3 times the average background noise by the detection sensitivity (slope of calibration plot), which were 72 and 14 ng/mL for alachlor and 2,6-DEA, respectively. The calibration plots of alachlor and 2,6-DEA established by the direct injection of standard solutions were specified with equations of $y = 92x - 31$ (y is the peak area, and x is the injection concentration) over a concentration range of 1−500 μg/mL for alachlor and $y = 79x + 89$ over a concentration range of 0.1−160 μg/mL for 2,6-DEA. Precision was estimated by performing five enriched samplings of fortified sample solutions with concentrations used for calibrations, and the RSD was <5% ($N = 5$). When samples were fortified with 10 μL/mL alachlor and 2,6-DEA and using a 40 cm hollow fiber under the perfusion flow rate of 0.1 μL/min, the enrichment factors were 403 for alachlor and 386 for 2,6-DEA after the proposed enriched sampling and HPLC-UV analysis.

The proposed method was examined by the analyses of alachlor and 2,6-DEA in the NA culture medium and compared with the chromatograms for those by only filtration with a 0.45 μm PVDF membrane filter. Figure 4 shows the chromatograms of 2,6-DEA and alachlor in NA culture medium via the PVDF filtration and the proposed method by using the fortified sample solution (10 μL/mL of alachlor and 5 μL/mL 2,6-DEA) at pH 7. It is obvious that the baseline of the chromatogram obtained from the proposed online HF-LPME was free from interference of the components in culture media. 2,6-DEA was not identified in the NA culture medium after filtration with the 0.45 μm PVDF membrane filter. The response of the peak area has been enhanced through the online HF-LPME process, and recoveries of 98 and 95% were obtained for alachlor and 2,6-DEA, respectively. This reveals that enrichment occurred in the online HF-LPME process.

The proposed online HF-LPME/HPLC-UV method was also applied to the analysis of degradation of alachlor by *Rhizopus*

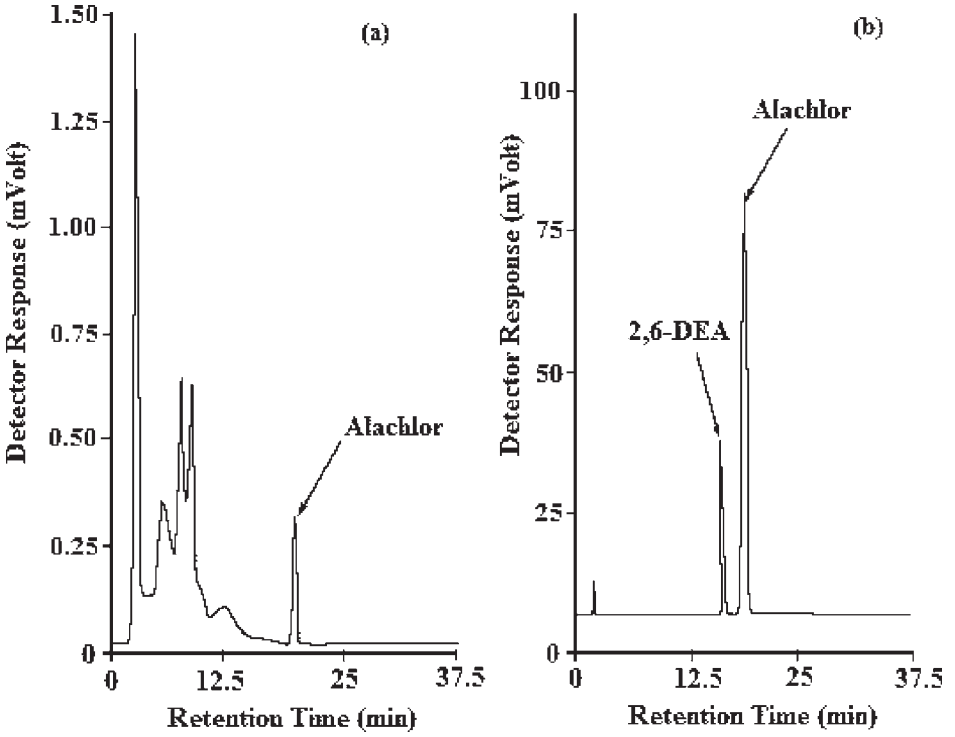

Figure 4. Chromatograms of 2,6-DEA and alachlor in NA culture medium: (a) by PVDF filtration; (b) by the proposed method. Conditions: sample matrix, NA cultured medium at pH 7; flow rate, 0.1 μL/min; perfusion solvent, hexane; sample concentration, 10 μg/mL of alachlor and 5 μg/mL of 2,6-DEA.

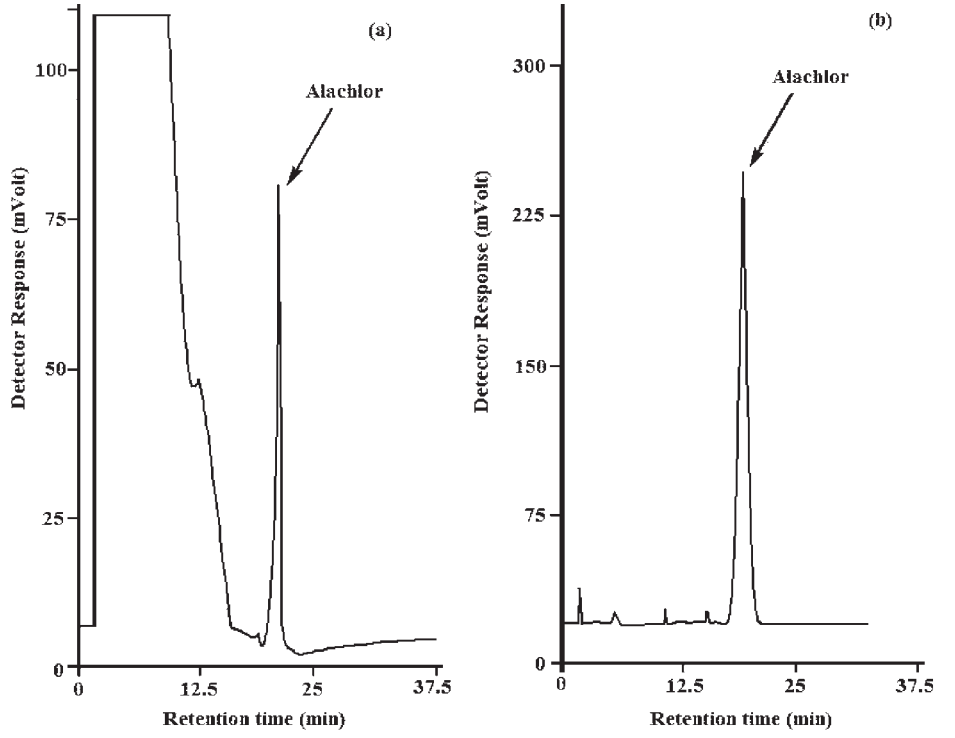

Figure 5. Chromatograms of alachlor in *Rhizopus stolonifer* incubated PBA culture medium: (a) by PVDF filtration; (b) by the proposed method. Conditions: sample, 24 g/L PDB *R. stolonifer* incubated cultured medium at pH 7 and 28 °C to degrade alachlor (100 μg/mL) for 96 h; hexane as the perfusion solvent at a flow rate of 0.1 μL/min.

stolonifer in the PDB culture cell medium as described previously, and the chromatograms were compared with those by only filtration with a 0.45 μm PVDF membrane filter. Figure 5 shows the chromatograms of alachlor in PDB culture medium via PVDF

filtration and the proposed method by using the fortified sample solution (100 μL/mL of alachlor) at pH 7. It is obvious that the baseline of the chromatogram obtained from the proposed online HF-LPME was free from the interference of the components in culture media, and there is no 2,6-DEA peak in the chromatogram. However, 70% of alachlor was degraded in PDB culture medium after 96 h under the incubation conditions. This reveals that the degradation product 2,6-DEA was not in its free form. The response of the alachlor peak area was enhanced, and good enrichment was achieved through the online HF-LPME process.

In summary, this paper has investigated the potential of using online HF-LPME for sample pretreatment and enrichment prior to the determination of alachlor and its metabolite 2,6-DEA in microbial culture media. In the proposed method, a few microliters of organic solvent was utilized to extract alachlor and 2,6-DEA. An excellent enrichment factor (403 for alachlor and 386 for 2,6-DEA) could be achieved by the present method, and the enrichment factors could be adjusted by controlling the length of hollow fiber and the flow rate of perfusion depending on the requirement of detection sensitivity. The results reveal that the present HF-LPME coupled online to HPLC method could be an alternative to determine alachlor and its metabolite 2,6-DEA in microbial culture media with the advantages of easy operation, speed, enrichment potential, flexibility, and less use of organic solvent.

AUTHOR INFORMATION

Corresponding Author

*Phone: +886-4-22853148. Fax: +886-4-22862547. E-mail: jfjen@dragon.nchu.edu.tw.

Funding Sources

We appreciate financial support from the National Science Council of Taiwan under Grants NSC-92-2113-M-005-025 and NSC-98-2113-M-005-016-MY3 and from National Chung Hsing University.

REFERENCES

(1) Sonia, R.; Miranda, L.; Meyer, S. A. Cytotoxicity of chloroacetanilide herbicide alachlor in HepG2 cells independent of CYP3A4 and CYP3A7. *Food Chem. Toxicol.* **2007**, *45*, 871–877.

(2) Ramesh, A.; Maheswari, S. T. Dissipation of alachlor in cotton plant, soil and water and its bioaccumulation in fish. *Chemosphere* **2004**, *54*, 647–652.

(3) Hladik, M. L.; Bouwer, E. J.; Roberts, A. L. Neutral chloroacetamide herbicide degradates and related compounds in Midwestern United States drinking water sources. *Sci. Total Environ.* **2008**, *390*, 155–156.

(4) Ballestros Martin, M. M.; Sánchez Pérez, J. A.; García Sánchez, J. L.; Montes de Oca, L.; Casas López, J. L.; Oller, I. Degradation of alachlor and pyrimethanil by combinet photo-Fenton and biological oxidation. *J. Hazard. Mater.* **2008**, *155*, 342–349.

(5) Qiang, Z.; Liu, C.; Dong, B.; Zhang, Y. Degradation mechanism of alachlor during direct ozonation and O_3/H_2O_2 advanced oxidation process. *Chemosphere* **2010**, *78*, 517–526.

(6) Fava, L.; Bottoni, P.; Crobe, A.; Funari, E. Leaching properties of some degradation products of alachlor and metolachlor. *Chemosphere* **2000**, *41*, 1503–1508.

(7) U.S. EPA. Reregistration Eligibility Decision (RED). Alachlor. Office of Pesticide Programs, EPA-738-R-98-020, 1998.

(8) Lin, M. F.; Wu, C. L.; Wang, T. C. Pesticide clastogenicity in chinese hamster ovary cells. *Mutat. Res.* **1987**, *188*, 241–250.

(9) Ribas, G.; Surralles, J.; Carbonell, E.; Xamena, N.; Creus, A.; Marcos, R. Genotoxicity of the herbicides alachlor and maleic hydrazide in cultured human lymphocytes. *Mutagenesis* **1996**, *11*, 221–227.

(10) Słaba, M.; Szewczyk, R.; Bernat, P.; Długoński, J. Simultaneous toxic action of zinc and alachlor resulted in enhancement of zinc uptake by the filamentous fungus *Paecilomyces marquandii*. *Sci. Total Environ.* **2009**, *407*, 4127–4133.

(11) Kenneth, A.; Hostetler, K. A.; Thurman, E. M. Determination of chloroacetanilide herbicide metabolites in water using high-performance liquid chromatography-diode array detection and high- performance liquid chromatography-mass spectrometry. *Sci. Total Environ.* **2000**, *248*, 147–155.

(12) Logusch, S. J.; Feng, P. C.; Fujiwara, H.; Hutton, W. C.; Wratten, S. J. Enzymatic synthesis of 4-amino-3,5-diethylphenyl sulfate, a rodent metabolite of alachlor. *J. Agric. Food Chem.* **1999**, *47*, 2125–2129.

(13) Rodriaguez-cruz, S.; Lacorte, S. Degradation of alachlor in natural and sludge-amended soils, studied by gas and liquid chromatography coupled to mass spectrometry (GC-MS and HPLC-MS). *J. Agric. Food Chem.* **2005**, *53*, 9571–9577.

(14) Zang, L. Y.; DeHaven, J.; Yocum, A.; Qiao, G. L. Determination of alachlor and its metabolites in rat plasma and urine by liquid chromatography−electrospray ionization mass spectrometry. *J. Chromatogr., B* **2002**, *767*, 93–101.

(15) Johnson, W. E.; Fendinger, N. J.; Plimmer, J. R. Solid-phase extraction of pesticides from water: possible interferences from dissolved organic material. *Anal. Chem.* **1991**, *63*, 1510–1513.

(16) Aga, D. S.; Thurman, E. M.; Pomes, M. L. Determination of alachlor and its sulfonic acid metabolite in water by solid-phase extraction and enzyme-linked immunosorbent assay. *Anal. Chem.* **1994**, *66*, 1495–1499.

(17) Olness, A.; Basta, N. T.; Rinka, J. Redox effects on resin extraction of herbicides from soil. *Talanta* **2002**, *57*, 383–391.

(18) Steen, R. J. C. A.; Freriks, I. L.; Cofino, W. P.; Brinkman, U. A. Th. Large-volume injection in gas chromatography-ion traps tandem mass spectrometry for the determination of pesticides in the marine environment at the low ng/L level. *Anal. Chim. Acta* **1997**, *353*, 153–163.

(19) Azevedo, D. D.; Lacorte, S.; Vinhas, T.; Viana, P.; Barcelo, D. Monitoring of priority pesticides and other organic pollutants in river water from Portugal by gas chromatography −mass spectrometry and liquid chromatography−atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr., A* **2000**, *879*, 13–26.

(20) Gonzalez-Barreiro, C.; Lores, M.; Casaia, M. C.; Cela, R. Optimisation of alachlor solid -phase microextraction from water samples using experimental design. *J. Chromatogr., A* **2000**, *896*, 373–379.

(21) Lambropoulou, D. A.; Sakkas, V. A.; Hela, D. G.; Albanis, T. A. Application of solid-phase microextraction in the monitoring of priority pesticides in the Kalamas River (N.W. Greece). *J. Chromatogr., A* **2002**, *963*, 107–116.

(22) Carabias-Martýnez, R.; Garcýa-Hermida, C.; Rodrýguez-Gonzalo, E.; Soriano-Bravo, F. E.; Hernandez-Mendez, J. Determination of herbicides, including thermally labile phenylureas, by solid-phase microextraction and gas chromatography−mass spectrometry. *J. Chromatogr., A* **2003**, *1002*, 1–12.

(23) Lee, J. Y.; Lee, H. K.; Rasmussen, K. E.; Pedersen-Bjergaard, S. Environmental and bioanalytical applications of hollow fiber membrane liquid-phase microextraction: a review. *Anal. Chim. Acta* **2008**, *624*, 253–268.

(24) Pedersen-Bjergaard, S.; Rasmussen, K. E. Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid−liquid extraction. *J. Chromatogr., A* **2008**, *1184*, 132–140.

(25) Nandi, P.; Lunte, S. M. Recent trends in microdialysis sampling integrated with conventional and microanalytical systems for monitoring biological events: a review. *Anal. Chim. Acta* **2009**, *651*, 1–14.

(26) Kaptein, W. A.; Zwaagstra, J. J.; Venema, K.; Korf, J. Continuous ultraslow microdialysis and ultrafiltration for subcutaneous sampling as demonstrated by glucose and lactate measurements in rats. *Anal. Chem.* **1998**, *70*, 4696–4700.

(27) Kou, D.; Mitra, S. Simultaneous extraction and concentration by on-line hollow fiber membrane extraction. *Anal. Chem.* **2003**, *75*, 6355–6360.

(28) Chiu, H. L.; Lin, H. Y.; Yang, T. C. Determination of ginkgolide A, B, and bilobalide in *biloba* L. extracts by microdialysis-HPLC. *Anal. Bioanal. Chem.* **2004**, *379*, 445–448.

(29) Jen, J.-F.; Chang, C.-T. On-line microdialysis—high-performance liquid chromatographic determination of aniline and 2-chloroaniline in polymer industrial wastewater. *J. Chromatogr., A* **2001**, *930*, 119–127.

(30) Chen, S.-J.; Cheng, F.-C.; Jen, J.-F. On-line microdialysis coupled solid-phase extraction to decrease matrix interference in the HPLC analysis of urinary ketamine and its metabolites. *J. Sep. Sci.* **2010**, *33*, 2010–2016.

(31) Cheng, G.-W.; Wu, H.-L.; Huang, Y.-L. Automated on-line microdialysis sampling coupled with high-performance liquid chromatography for simultaneous determination of malondialdehyde and ofloxacin in whole blood. *Talanta* **2009**, *79*, 1071–1075.

(32) Jen, J.-F.; Liu, T. C. Determination of phthalate esters from food-contacted materials by on-line microdialysis and liquid chromatography. *J. Chromatogr., A* **2006**, *1130*, 28–33.

(33) Lin, C.-H; Wu, H.-L.; Huang, Y.-L. Microdialysis sampling coupled to on-line high-performance liquid chromatography for determination of arbutin in whitening cosmetics. *J. Chromatogr., B* **2005**, *829*, 149–152.

(34) Lin, T.-H.; Huang, J.-W.; Kumar, P. V.; Jen, J.-F. Determination of sinigrin in vegetable seeds by online microdialysis sampling coupled to reverse-phase ion-pair liquid chromatography. *J. Agric. Food Chem.* **2010**, *58*, 4571–4575.

(35) Jen, J.-F.; Wei, M.-C.; Chang, C.-T. Determination of organic acids in fermentation products of milk with high performance liquid chromatography/on-lined micro-dialysis. *Chromatographia* **2001**, *54*, 601–605.

(36) Abdel-Mallek, A. Y.; Hemida, S. K.; Omar, S. A. Fungal succession and decay of herbicide-treated wheat straw. *Folia Microbiol.* **1994**, *39*, 1–566.