Solid-Phase Microextraction in Combination with GC–FID for Quantification of the Volatile Free Fatty Acids in Wastewater from Constructed Wetlands

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Abstract

A headspace solid-phase microextraction (HS-SPME) followed by gas chromatography coupled to flame ionization detector (GC–FID) is developed for the direct determination of volatile fatty acids (VFAs) in wastewater from constructed wetlands. Through the investigation on extraction behavior of different coatings for VFAs and the chromatographic behavior of VFAs in a Stabilwax-DA capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) using nitrogen as carrier gas, the polyacrylate fiber is selected as the satisfactory coating for VFAs analysis, and optimum chromatographic conditions are obtained. The oven temperature is programmed at 70°C for 2 min, then a 4°C/min rate until 180°C, holding 2 min, and then a 20°C/min rate until 200°C, holding the final temperature for 3 min. The various parameters including extraction time, extraction temperature, pH value, ion strength, sample volume, and desorption conditions are investigated for the optimization of HS-SPME performance for VFAs in the wastewater. The experimental results show that the linear dynamic ranges are 10–45000 µg/L, and over two to four orders of magnitude depending on different acids. The detection limit (3σ) for VFAs are low to µg/L levels and the relative standard deviations are less than 10%, and the recoveries are between 85% and 117%. By using 2-ethylbutyric acid as internal standard, the proposed method is successfully applied to the determination of VFAs in wastewater from the constructed wetlands.

Introduction

Constructed wetlands are among the recently proven efficient technologies for wastewater treatment. Recently, there has been growing interest in using constructed wetlands for removing pollutants from agricultural and highway runoff (1,2), river water, or industrial and domestic wastewaters (3–5). The wastewater treatment relies on biological, chemical, and physical processes in a natural environment. However, the available information on the performance of these systems is limited to common contaminants, such as suspended solids, COD, BOD_5, and nutrients (6). At present, very little information is available on specific degradation intermediates of organic contaminants, such as volatile fatty acids (VFAs), and it mainly deals with specific contaminants such as pesticides (7), pharmaceuticals (8), and surfactants (9). As the degradation intermediates of carbohydrates, proteins and fats, the short chain VFAs in wastewater have been proved to strongly affect the efficiency of biological nutrient (phosphorus and nitrogen) removal processes. VFAs, such as acetic and propionic acids, have been demonstrated to be the most suitable substrates to support enhanced biological phosphorus removal (10). Studies also showed that VFAs (acetic, butyric, and propionic acids) can reduce nitrate formation with no effect on ammonia oxidation during activated sludge treatment (11,12). A recent study demonstrated that VFAs could stimulate aerobic denitrification during biotransformation of nitrogen in activated sludge (13). Unfortunately, knowledge regarding the fate and dynamics of VFAs and their interactions in constructed wetland systems is highly limited.

Due to the importance of VFAs in water treatment systems, the development of a fast and reliable analytical method for VFA analysis is especially important and necessary for better understanding of the carbon–cycle processes related to VFAs and their role in the complex network of transformation processes, better understanding of the "black box" mechanism, and for optimum design and operation of wetland systems. For fatty acid measurement, several methods have been reported, such as Fourier transform Raman spectrometry (14), gas chromatography (GC), high-performance liquid chromatography (HPLC) with fluorescence (15) or electrochemical detection (16), among which the GC with flame ionization detection (FID) was the major analytical method for fatty acid determination using liquid–liquid extraction (17,18), distillation (19), or purge-and-trap (20) as sample pretreatment methods. Due to the high polarity, volatility, and solubility in water of VFAs, direct analysis of these compounds is problematic. Generally, a derivatization step is selected to obtain non-polar VFAs derivatives followed by GC or GC–mass spectrometry (21–23), at the cost of analyte loss, contamination potential, and poor reproducibility. Although the direct aqueous injection (24) has been suggested as a non-deriva-

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tization method for VFA analysis by GC–FID, the contamination of the GC injection port and column with sample matrix components that interfere with analysis and degrade chromatographic performance increases the incidence of maintenance.

Solid-phase microextraction (SPME) is a modern extraction technique which is cost-effective, time efficient, and solvent-free. It makes the use of a fused fiber coated with a thin layer of a polymer to extract analytes from aqueous, the headspace in equilibrium with the aqueous or solid, or a gaseous phase. The analytes are partitioned between a sample matrix and the fiber coating until equilibrium is reached. This technique is well suited to combine with GC for analysis because the analytes sorbed onto fiber coating can be desorbed directly in the hot GC injector, which eliminates the use of toxic organic solvents in sample preparation. In recent years, a lot of research has been done involving the use and application of SPME–GC in environmental, food, and pharmaceutical analysis (25). The application of SPME to the analysis of VFAs has also been previously reported (26–32). Regarding the direct determination of VFAs by non derivatization-based SPME, however, there is limited literature dealing with the issue to date. Pan et al. (26) used direct SPME for VFA analysis but no real water sample applications. In 1999, Yo (27) reported a carbowax-divinylbenzene (CW-DVB) fiber-based SPME sample enrichment method for VFAs in a pig farm by GC–MS. More recently, Ábalos et al. reported a polydimethylsiloxane-carboxen (PDMS-CAR) fiber-based SPME method for the analysis of VFAs by GC–FID using hydrogen as carrier gas (28) and GC coupled to chemical ionization mass spectrometry (29). Unfortunately, due to the interfering components from the matrix co-extracted with the PDMS-CAR fiber, accurate determination of acetic acid in real samples is problematic when using the non-selective GC–FID. In order to realize the analysis of faecal short-chain fatty acids, Mills et al. (30) proposed an indirect method using PA-based in-fiber SPME derivatization with 1-pyrenyldiazomethane. The derivatization, with benzyl bromide in water phase followed by SPME, was also developed for acetic acid analysis using GC–FID (31). More recently, Pinho et al. (32) reported a polyacrylate (PA)-based SPME-GC–MS method for analyzing C_3–C_{13} free fatty acids in ewe cheese. However, only four acids; namely, butyric, hexanoic, octanoic, and decanoic, were identified and quantified; acetic, propanoic, and pentanoic acids were identified but not quantified (32).

The previously mentioned literature survey demonstrates that there is only one paper using an SPME-based method for direct analysis of VFAs in real wastewater samples by GC–FID (28); however, accurate determination of acetic acid in real samples is problematic due to the interfering components from the matrix co-extracted with the PDMS-CAR fiber (28). According to the discussion mentioned previously, the major objectives of this study were to (i) develop a selective headspace SPME-GC–FID method for direct determination of VFAs without derivatization, (ii) investigate the extraction behavior of different coatings for VFAs and the chromatographic behavior of VFAs, to improve the resolution for VFA analysis, (iii) optimize the SPME performance by studying the effect of extraction time, extraction temperature, pH value, ion strength, sample volume, and desorption conditions, and (iv) use the proposed analytical method for VFA determination in real wastewater from constructed wetlands.

Experimental

Chemicals and materials

All reagents used were of analytical-reagent grade unless specified otherwise. Doubly deionized water was used throughout the study. All reagents were obtained from Chongqing Chemical Reagents Company (Chongqing, China), except for VFAs, which were from Merck (Schuchardt OHG, Germany). The VFA standards (99.99%) used in this study are acetic acid, propionic acid, butyric acid, pentanoic acid, isopentanoic acid, hexanoic acid, and 2-ethylbutyric acid (internal standard). All standards were used as received. The SPME holder and fibers were purchased from Supelco Ltd. (Bellefonte, PA). The SPME fibers used in this study are PA, 85 µm; PDMS-DVB, 65 µm; CW-DVB, 65 µm; and PDMS-CAR, 75 µm. All headspace SPME extraction was performed in 40-mL glass vials (Supelco).

The standards used for calibration were prepared from a stock solution containing acetic, propionic, butyric, pentanoic, isopentanoic, and hexanoic acids. Stock standard solutions of each analyte (1250–2500 µg/mL) were prepared in doubly deionized water. The working standard solutions were obtained by proper diluting of the individual stock solutions with doubly deionized water. All the standard solutions were stored at 4°C in the darkness.

Sample collection

The water samples were from the 120 m^2 pilot-scale subsurface constructed wetland system which is located in Southwest University campus (Beibei, Chongqing, Southwest China). Predominantly domestic sewage from the students' dormitory and restaurant undergoes solids removal and primary clarification prior to discharge to one facultative lagoon (22 m^3); effluent from the lagoon passes through 8 parallel vegetated gravel wetland beds (each with 15 m^2) which were planted with *Cyperus alternifolius* at 6 plants/m^2. Effluent from the lagoon was equally distributed to the 8 research wetland beds by means of a weir with eight holes flowing to each bed. Between the lagoon effluent and the weir, there is a valve and a flow meter. The design parameters for the wetland beds, such as four length to width ratios (1:1, 1.5:1, 2:1, and 2.5:1), two water levels (70 and 50 cm), and two granular medium sizes (20 mm and 12 mm) were evaluated for their effects on the performance of the wetland beds. Water levels were approximately 70 and 50 cm for deep and shallower beds, respectively. Length to width ratios of 1:1, 1.5:1, and 2:1 correspond to a water level of 70 cm. A length to width ratio of 2.5:1 corresponds to a water level of 50 cm. The wetland effluent is discharged into the Longfeng River, a branch of the Jialing River.

Samples were collected from influent and effluent of treatment wetland beds. The water was normally sampled once a week between 9 a.m. and 11 a.m. in the sampling day. A total of 30 sampling campaigns were carried out, of which 3 corresponded to a hydraulic loading rate (HLR) of 12 cm/d, 4 to 24 cm/d, 10 to 38 cm/d, 7 to 51 cm/d, 3 to 65 cm/d, and 3 to 86 cm/d. Grab samples of the influent and the effluents of the beds were taken using plastic containers. Samples were kept refrigerated at 4°C until analysis.

General procedure for headspace SPME and GC–FID analysis

Twenty milliliters of doubly deionized water or sample were placed into a 40-mL vial together with a proper amount of NaCl (baked at 400ºC for 6 h before use), and then the vial was sealed with a PTFE lined septum and spiked with standard mixture of VFAs (under conditions of method development) and the internal standard (i.e., 2-ethylbutyric acid) by injection through the septum. Next, the pH was adjusted to 1.5 by injecting HCl. Extraction was performed in the headspace along proper time under magnetic stirring using SPME fibers. Extraction temperature was controlled by a Type 85-2 thermostated water bath. Fiber desorption was performed in the injector of a Trace GC (ThermoElectron, Waltham, MA). For the analysis of VFAs with GC–FID, the GC conditions were as follows: analytical column Stabilwax-DA (30 m × 0.32 mm i.d., 0.25 µm film thickness, Restek, Bellefonte, PA); the oven temperature was programmed at 70ºC for 2 min, then a 4ºC/min rate until 180ºC, holding 2 min, and then a 20ºC/min rate until 200ºC, holding the final temperature for 3 min; carrier gas was the high purity nitrogen (99.999%) at 1.2 mL/min, and the FID was held at 280ºC. All headspace SPME extractions were performed in triplicate.

Results and Discussion

Considerations on the coating selection for VFAs analysis

The polarity of SPME fiber coating is a key factor controlling the extraction efficiency: by choosing the correct type of coating, extraction of specific compounds can be enhanced (26). Generally, PA coating is polar absorbent and is applicable to polar organic compounds such as VFAs (26, 30–32) and organophosphorous pesticides (33). CW-DVB is polar absorbent and is applicable for polar organic compounds such as alcohols, ketones, nitroaromatics (33). CAR-PDMS coating is bipolar absorbent and can be applied to volatile organic compounds (VOCs) and hydrocarbons (28, 33). PDMS-DVB coating is bipolar absorbent and can be applied to polar and semi-volatile organic compounds and amine compounds (33). In this study, we evaluated the extraction efficiency and selectivity of four fiber coatings (i.e., PA, CW-DVB, PDMS-DVB, and PDMS-CAR) for the studied fatty acids using VFAs standards and wastewater samples. The experimental results show that the CW-DVB coating can extract much more of analytes than other coatings, and PDMS-DVB, PDMS-CAR, and PA can give a satisfactory peak area (Figure 1). This result was in agreement with that of Yo (27) who used a CW-DVB fiber-based SPME sample enrichment method for volatile fatty acids in pig farm wastewater samples. Pan et al. (26) found PA coating showed the best performance as compared to other two tested fibers (PDMS, CW-DVB) for the extraction of free C_2–C_{10} acids in aqueous solution. According to Ábalos et al. (28), PDMS-CAR fiber was selected for VFA preconcentration among the evaluated five coatings (PA, CW-DVB, PDMS-DVB, PDMS-CAR-DVB, and PDMS-CAR). Our experimental results showed that when performing the constructed wetland water sample analysis, PA gave the best selectivity for the studied VFAs. In contrast, CW-DVB and other coatings co-extracted interfering components from the matrix. Also, considering that the mechanical stability of the PA fiber was better than that of the CW-DVB fiber (31), finally, the PA fiber was chosen for further optimization of the analytical procedure.

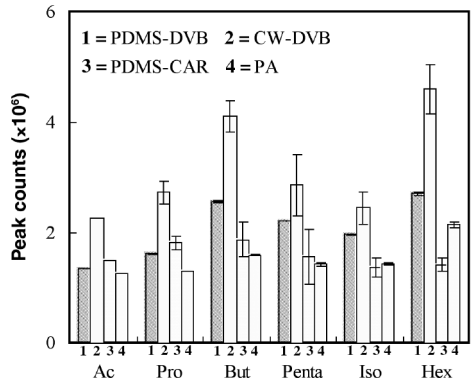

Figure 1. Effect of different SPME fiber coatings on VFA extraction performance using 20 mL of VFA mixed standards: Ac, Pro, But, Penta, Iso, and Hex represented acetic, propionic, butyric, pentanoic, isopentanoic, and hexanoic acid, respectively. Concentration of the VFA mixed standard solutions: Ac, 2500 µg/L; Pro, 1250 µg/L; But, 1250 µg/L; Penta, 300 µg/L; Iso, 300 µg/L; Hex, 300 µg/L; 2-ethylbutyric acid (I.S.), 500 µg/L. Extraction temperature, 25°C; extraction time, 20 min; pH value, 1.5; sodium chloride, 3.5 g. Fiber desorption conditions: 250°C for 3 min. Error bars represent one standard deviation for three measurements.

Effect of extraction temperature

Headspace SPME is an equilibrium process of adsorption and desorption. Temperature is an important factor for analyte extraction. The increase of temperature may accelerate analytes to escape from the aqueous phase, and speed up the analytes' move to fiber; it is in favor of adsorption. Higher temperatures will lead the analytes' desorption from fiber; and lower temperatures prevent volatilization of the target analytes. The effect of extraction temperature on headspace SPME performance using VFA mixed standards was examined at 20, 25, 35, and 45°C. Results are shown in Figure 2. As can be seen for C_2–C_4 fatty acids and pentanoic acid, the peak area increases with the temperature up to 25°C, above which the peak area decreases. However, for isopentanoic acid and hexanoic acid, the response increases with the temperature. When the real sample analysis was performed, all the peak areas increased with temperature in the span of 20°C to 45°C, as shown in the inset of Figure 2. This result is somewhat consistent with that of Mills et al. (30), who observed that the peak areas of C_1–C_3 fatty acids plateaued at 40°C, but those of C_4 and C_5 fatty acids continued to increase at 60°C when performing analysis of faecal short-chain fatty acids by PA-based SPME. Finally, 50°C was selected as an optimal temperature. Ábalos et al. (28) investigated the effect of two temperatures, 25°C and 35°C, on extraction efficiencies of the PDMS-CAR fiber for VFAs; they found that good extraction performance could be obtained at a relatively low temperature (25ºC), although the peak areas were higher at 35°C than at 25°C. Considering that C_2–C_4 fatty acids were major fatty acids in wastewater from the constructed wetland in our case (see latter) and that PA fiber showed a poor performance at higher tempera-

tures due to potential damage of the PA coating in the headspace of a higher concentration of HCl at the higher extraction temperature; finally, 25ºC was chosen as the extraction temperature for VFA analysis in the present study.

Effect of extraction time

The extraction time is decided mainly by the equilibrium rate of the solid-liquid-gas phase of analytes. The effect of extraction time was tested at 10, 20, 30, 40, and 50 min for the mixed standards. As can be seen from Figure 3, the adsorption sensitivity generally increased with increase of extraction time, although there is no obvious improvement for C_2–C_4. This may be due to the fact that the mass transfer of the analytes should be very fast and the equilibrium should be more rapidly established when the sample is stirred vigorously with a magnetic stir bar. Pan et al. (26) observed a similar trend for propionic and butyric acids. Ábalos et al. (28) reported an optimal extraction time of 20 min with the PDMS-CAR fiber for a minimum analysis time without affecting the reproducibility of the whole procedure. A similar trend was also observed when performing the real water sample analysis (Figure 3 inset). Considering the sensitivity, reproducibility, and major fatty acid components in this study, the extraction time was set at 20 min.

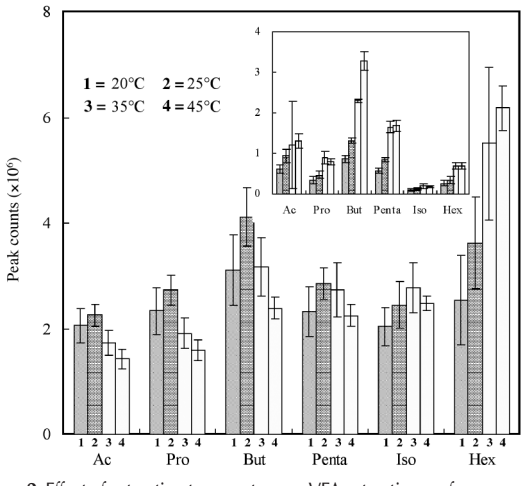

Figure 2. Effect of extraction temperature on VFA extraction performance by a PA fiber using 20 mL of VFA mixed standards. Other conditions were the same as in Figure 1. Inset: Effect of extraction temperature on VFA extraction performance by a PA fiber using 20 mL of real water sample from constructed wetland.

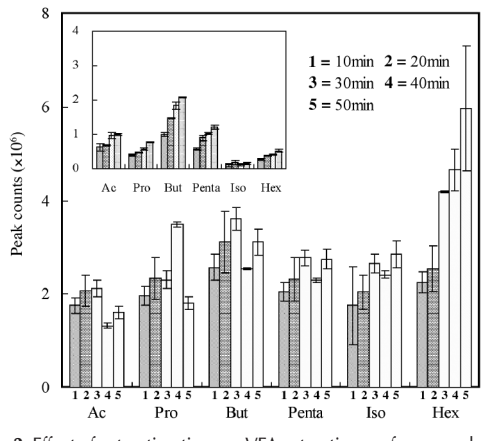

Figure 3. Effect of extraction time on VFA extraction performance by a PA fiber using VFA mixed standards. Other conditions were same as in Figure 1. Inset: Effect of extraction time on VFA extraction performance by a PA fiber using 20 mL of real water sample from constructed wetland.

Effect of acid and salt effect

The pH of the matrix is an important factor for VFA extraction by headspace SPME because VFA extraction efficiency depends on it. In most cases, the VFA extraction efficiency by headspace SPME increases with a decrease in matrix pH value. A similar tendency was observed in this study. The examined pH ranges were from 1.5 to 7.0. Results shown in Figure 4 indicate that the amount of VFAs extracted from the mixed standards aqueous solution by the PA-coated fiber decreased with an increase in pH values. The pH effect enhancement of the extraction of VFAs was previously evaluated by Pan et al. (26). They found that by lowering the pH of the sample matrix, acids were mainly present in their neutral forms, which have a greater affinity for the fiber. They reported the optimal pH was 1.5. Results shown in the inset of Figure 4 demonstrate that the effect of acid on extraction efficiencies of the studied VFAs was much more significant when performing in the wetland wastewater, showing that the fatty acids in wastewater are mainly present in an ionic state, especially for C_2–C_5 fatty acids. Considering the potential loss of fiber coating at pH 1.0 (26), the effect of pH below 1.5 was not studied. Finally, a pH of 1.5 was selected in the present work for further study.

The salt-out effect by the addition of salt into the sample matrix increases the amount of most of the acids extracted with fibers because the water molecules prefer to solvate the salt ions; the addition of saturated salt into the sample matrix will decrease the solubility of the acids of the neutral form. The magnitude of the increase depends on the solubility of the acids. It is reported that the addition of lithium sulphate increased recoveries of faecal short-chain fatty acids using headspace GC (34). However, when performing analysis of faecal short-chain fatty acids by in fiber SPME derivatisation with 1-pyrenyldiazomethane, sodium chloride was suitable because lithium sulphate caused rapid disintegration of the fiber coating (30). Pinho et al. reported that

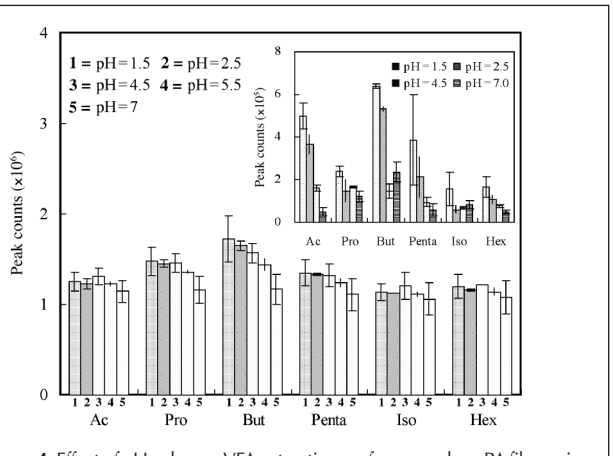

Figure 4. Effect of pH value on VFA extraction performance by a PA fiber using VFA mixed standards. Other conditions were same as in Figure 1. Inset: Effect of pH value on VFAs extraction performance by a PA fiber using 20 mL of real water sample from constructed wetland.

sodium sulfate had a better extraction efficiency for short chain free fatty acids in cheese with PA coated fiber (32). Pan et al. reported the use of sodium chloride as the salt-out reagent for the determination of fatty acids using SPME (26). In this regard, sodium chloride was selected to investigate the salt-out effect in the present study. The examined NaCl amount ranges were from 0 to 5.5 g. As shown in Figure 5, the peak area increases with an increasing addition of salt. However, there are no significant changes in the peak areas for C_2–C_4 acids by using 3.5 to 5.5 g NaCl when performing in the mixed VFA standards aqueous solution. When the real sample analysis was performed, as shown in the inset of Figure 5, for C_4–C_5 VFAs, the peak areas increased significantly when sodium chloride exceeded 3.5 g. For acetic acid, the peak of extraction was observed at NaCl 3.5g. Considering that acetic acid and propionic acid were the two major VFA components in our case, the amount of salt was set at 3.5 g.

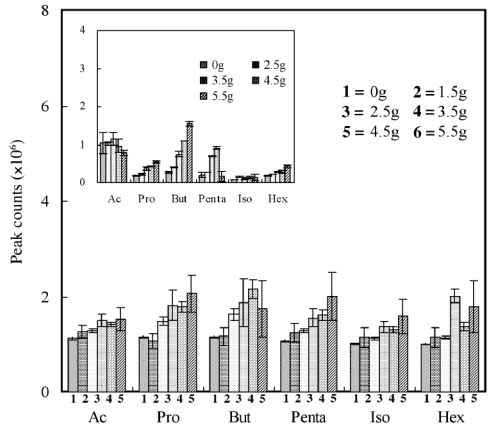

Figure 5. Effect of sodium chloride on VFA extraction performance by a PA fiber using VFA mixed standards. Other conditions were same as in Figure 1. Inset: Effect of sodium chloride on VFA extraction performance by a PA fiber using 20 mL of real water sample from constructed wetland.

Effect of desorption conditions

The analytes adsorbed onto the fiber coating can be desorbed directly in the hot GC injector. Therefore, injector temperature and desorption time have to be optimized in SPME analysis in order to avoid the carryover effect. Four different desorption conditions were considered (i.e., 200°C for 3 min, 200°C for 5 min, 250°C for 3 min, and 250°C for 5 min). The experimental results indicate that under the desorption condition of 250°C for 3 min, the carryover of C_2–C_6 acids is lower than 5%.

Performance of the proposed method for VFAs measurements

Under the optimum conditions given previously, a series of standard solutions with 2-ethylbutyric acid as internal standard were prepared and extracted by headspace SPME. The reproducibility of the method was determined by performing the extraction of nine standard solutions with the same concentration (1500 µg/L C_2–C_4 acids, 300 µg/L C_5–C_6 acids). The linear dynamic ranges together with correlation coefficients for each of analytes are given in Table I. As can be seen from Table I, there was a linear correlation between the peak area and concentration of six VFAs from 10–45000 µg/L; the detection limits of the target compounds for an extraction of 20 mL pure water samples were in the range of 3–467 µg/L. Detection (LOD) and quantity (LOQ) limits listed in Table I were calculated from the calibration

Table I. Comparison of the Performance of the Developed Method for VFA Analysis with that of Other SPME–GC Methods

Compounds (acid)	Acetic	Propionic	Butyric	Pentanoic	Isopentanoic	Hexanoic	References
Linear range (µg/L)	1500–40000	70–45000	10–35000	55–6000	15–6000	15–5500	This work
	1095–45350	55–46450	5–36240	70–10660	–	10–9720	28
Correlation coefficient (r^2)	0.9826	0.9908	0.9937	0.9973	0.9974	0.9997	This work
	0.9867	0.9876	0.9894	0.9920	0.9896	0.9910	30
	0.996	–	–	–	–	–	31
LOD (µg/L)	467	62	19	12	3	3	This work
	760	280	122	3.1	–	0.5	26
	675	54	6	46	–	19	28
LOQ (µg/L)	1245	165	51	32	8	8	This work
	6340	224	55	153	–	64	28
RSD%	9.8	4.9	6.2	6.6	6.5	8.9	This work
	5	4	3.1	2.9	–	3	26
	11.2	5.6	11.5	8.2	–	10.4	28
	3.3	2.4	5.0	12.7	9.8	–	30
	15.6	–	–	–	–	–	31
Recoveries (%)	99.9–115.1	96.1–112.4	104.2–117.2	97.3–107.5	93.7–101.7	84.6–89.1	This work
	78.5–113.9	77.3–111.3	86.1–122.3	85.6–120.0	85.7–120.2	–	27
	82.3–97.8	93.3–102.4	82.2–107.9	95.5–107.2	77.1–92.9	85.6–100.3	30

curves by considering the peak area corresponding to three and ten times the signal-to-noise ratio, respectively, of a procedural blank. As can be seen, acetic acid has a higher LOD and LOQ, which is probably attributed to its strong polarity and volatility (28). The relative standard deviations were less than 10% (in the range of 4.9–9.8%). Also, the figures of merit for extraction and analysis of the VFA compounds by other researchers using SPME–GC are listed in Table I; as can be seen, the linear dynamic ranges were almost same as those reported by Ábalos et al. (28). However, the LOD of 3–467 µg/L for the studied VFAs obtained by the proposed method was lower than 19–675 µg/L using PDMS-CAR coating fiber, except for butyric acid (28). For C_2–C_4 acids, the LOD of 19–467 µg/L for the studied VFAs obtained by the proposed method was lower than 122–760 µg/L (26) with PA fiber. For C_5–C_6 acids, the LOD by the proposed method is higher than that by Pan et al. (26).

Analysis of real water samples from the constructed wetland

For real sample analysis, Table I shows the results of VFA recovery tests made on the spiked real water samples. The recovery for spiked real water samples ranged from approximately 85 to 117%. Compared with the results reported by Yo (27) and Mills et al. (30), the recovery obtained by the proposed method was acceptable for real sample determination. Thus, the proposed method can be used for the routine estimation of VFAs in real water samples. As stated previously, through careful considerations on the selection of fiber coating and chromatographic conditions, resolution for VFA analysis could be improved, especially for acetic acid determination. Results shown in Figure 6 indicate that the present method using PA coating fiber for headspace SPME and using nitrogen as carrier gas for VFA determination has good selectivity for acetic acid analysis, which excludes interference from real water samples. As an illustration of analytical application, the proposed method was applied to free VFA determination in real water samples. The water samples were from the pilot scale subsurface constructed wetlands treating wastewater from the students' dormitory and Restaurant of Southwest University. The treatment system planted with Cyperus alternifolius has eight outlets which were designed for gaining insight into the mechanism of organic pollutant removal, through investigation of the degradation intermediates of organic contaminants (such as VFAs, alkylsulfides, and alkylamines) in relationship with the electronic acceptors (dissolved oxygen, sulfate, nitrite, and nitrate). Also, the effect of design parameters such as length to width ratio, medium size, etc. and operational parameters such as water level, HLR, etc. on

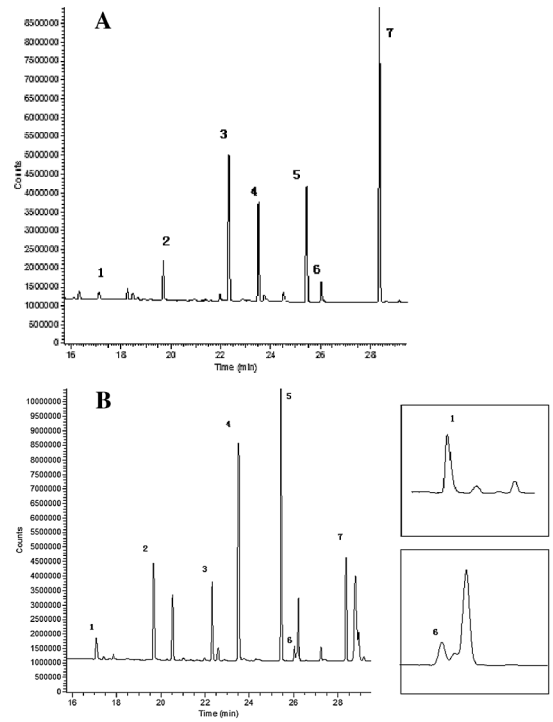

Figure 6. The chromatogram of standard solution containing C_2–C_6 acids (A) and real wastewater sample from the constructed wetland (B). Inset: acetic acid peak and internal standard peak. Peak identification: acetic acid (1); propionic acid (2); butyric acid (3); pentanoic acid (4); isopentanoic acid (5); 2-ethylbutyric acid (I.S.) (6); hexanoic acid (7).

Table II. Averages and Standard Deviations of the VFAs in the Influent and Effluents of Wetland Systems (Average Percentage Removal in Parentheses)

Compound (acid)	Concentration (µg/mL)								
	Influent	Effluent 1	Effluent 2	Effluent 3	Effluent 4	Effluent 5	Effluent 6	Effluent 7	Effluent 8
Acetic	134 ± 151	56 ± 61 (58)	45 ± 52 (66)	45 ± 61 (66)	46 ± 57 (66)	48 ± 70 (65)	69 ± 107 (48)	54 ± 81 (60)	77 ± 127 (42)
Propionic	14 ± 12	2.7 ± 3.0 (81)	3.3 ± 4.2 (77)	2.4 ± 3.3 (83)	3 ± 4 (79)	2.4 ± 3.2 (83)	5.6 ± 8.7 (61)	3.3 ± 5.2 (76)	5.1 ± 8.5 (64)
Butyric	4.8 ± 4.5	2.8 ± 0.8 (42)	1.9 ± 1.8 (61)	1.6 ± 3.2 (67)	1.7 ± 2.3 (65)	2.0 ± 3.8 (58)	1.6 ± 2.5 (67)	1.1 ± 1.7 (78)	1.3 ± 1.8 (74)
Pentanoic	2.7 ± 1.8	0.7 ± 1.4 (73)	0.8 ± 1.3 (71)	0.7 ± 1.4 (73)	0.8 ± 1.4 (69)	0.8 ± 1.5 (72)	1.2 ± 1.5 (56)	0.8 ± 1.1 (70)	1.2 ± 1.3 (57)
Isopentanoic	0.8 ± 0.6	0.1 ± 0.3 (84)	0.14 ± 0.29 (81)	0.1 ± 0.3 (86)	0.1 ± 0.3 (85)	0.1 ± 0.3 (84)	0.15 ± 0.23 (80)	0.1 ± 0.2 (86)	0.11 ± 0.11 (85)
Hexanoic	0.3 ± 0.2	0.07 ± 0.07 (74)	0.09 ± 0.10 (67)	0.09 ± 0.09 (69)	0.08 ± 0.08 (70)	0.13 ± 0.38 (53)	0.14 ± 0.16 (51)	0.09 ± 0.06 (69)	0.12 ± 0.12 (58)

its performance is considered. The results for 30 sampling campaigns with an approximately weekly pattern are shown in Table II. Results show that acetic acid was the most important acid in the target analytes, followed by propionic acid and butyric acid. The wetland system can remove VFAs efficiently. The highest average percentage removal is obtained for isopentanoic acid; the lowest is for acetic acid. This may be due to the fact that VFAs with more than three carbonic chains can biodegrade and transform to form acetic acid in the anaerobic condition of the subsurface constructed wetland.

In order to know more about the effects of design parameters and operational parameters on the performance of the subsurface constructed wetland, statistical analysis was carried out with a SYSTAT10 soft package. The pairwise comparison of the averages of the effluent acetic acid concentrations gives no significant statistical differences ($p > 0.05$) between beds. The analysis of variance (ANOVA) statistical analysis performed on 30 campaign data indicated that the length to width ratios (1:1, 1.5:1, 2:1, and 2.5:1) and water level produced no significant differences ($p > 0.05$) on the average effluent VFA concentrations. According to the ANOVA test, medium size produced significant differences ($p < 0.05$) in terms of effluent pentanoic acid and hexanoic acid. However, for other studied VFAs, medium size caused no significant differences ($p > 0.05$). According to the ANOVA test, the HLR caused significant statistical differences ($p < 0.05$) on the average effluent VFA concentrations, except for butyric and isopentanoic acids.

The preliminary results shown previously demonstrate that the major factor controlling the performance of the subsurface constructed wetland system for the studied VFAs is the HLR. Relatively, the medium size, length to width ratio, and water level show minor effects on the performance of the subsurface constructed wetland system for the studied VFAs. Our results concerning the relationship between concentrations of the target analytes and parameters investigated, such as design parameters and operational parameters, are consistent with the results indicated by the EPA's report (35), in which the effect of design parameters and operational parameters on removal of common contaminants such as suspended solids, COD, BOD_5, and nutrients were discussed. Further study is now in progress to investigate the fate and dynamics of VFAs and their interactions in constructed wetland systems.

Conclusion

Through the investigation on extraction behavior of different coatings for VFAs and the chromatographic behavior of VFAs in a Stabilwax-DA capillary column using nitrogen as carrier gas, an improved headspace SPME-GC–FID method was developed for direct determination of VFAs without derivatization. After optimizing all the experiment parameters, the proposed method was applied to the determination of short chain VFAs in wastewater from constructed wetlands, which can promote the study on the fate and dynamics of VFAs and their interactions in constructed wetland systems.

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