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Individual volatile fatty acids determination by chromogenic derivatization coupled to multi-syringe chromatography

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ABSTRACT

In this paper, a new multisyringe chromatography (MSC) system is proposed for a simple and accurate measurement of individual volatile fatty acids (VFA) in anaerobic treatment processes. The determination method is based on the derivatization of VFA with *N*-(1-naphthyl) ethylenediamine (EDAN) followed by the separation of VFA derivatives on an Onyx C18 monolithic column (25 mm × 4.6 mm i.d.). Chromatographic separation conditions have been investigated and were found to be optimal with a mixture of acetonitrile and formic acid 0.1% (ratio 35/65), providing good separation of C_2–C_5 VFA in 8 min. Optimization of the derivatization reaction was also carried out with special attention paid to the buffering capacity of the reaction medium, so as to be able to deal with samples of various characteristics in terms of alkalinity or of VFA concentration range. Individual VFA could be quantified between 0.05–2.5 g L^{-1} with LOD of 0.01–0.02 g L^{-1}. Overall procedure time was about 18 min for one analytical cycle, which fulfils the requirement of real-time monitoring of an anaerobic digester. Validation of the system developed has been assessed by application of the procedure to sludge samples from various origins, and comparative results with gas chromatography analyses showed satisfactory correlation ($R^2 > 0.98$).

1. Introduction

Anaerobic digestion of organic wastes, wastewaters or sewage sludges has gained considerable importance over the past decades, due mainly to its high organic removal efficiency and biogas production potential [1,2]. However, process failure due to the sensitivity of the bacterial community involved in the process towards operating conditions has been the main limiting criteria to its widespread application for a long time.

Several control parameters for achievement of process stability and optimal biogas production have been proposed in the literature related to anaerobic digestion efficiency [3,4]. The most common indicators for monitoring of this process are biogas production and composition [5], pH [6], alkalinity and volatile fatty acids (VFA) [7,8]. The main feature of a suitable control parameter should be its ability to prevent digester failure by indicating as soon as possible an imbalanced state in the digester, regardless of the nature of the digester input (organic load, buffering capacity and others). To this end, it has been widely acknowledged that VFA monitoring is one the key parameter for optimal process control [9]. VFA concentration range depends on process configuration, microorganisms and digester input. Typical anaerobic digestion of organic sludge from wastewater treatment processes requires detection of VFA concentrations in the range 1–5000 mg L^{-1} [10], acetic and propionic acids accounting for over 90% of all VFA [11,12].

VFA monitoring can rely either on total VFA or on individual VFA measurements. Total VFA concentration has been so far the most commonly used VFA parameter due to the availability of relatively simple on-line or on-site analytical devices [13–15], despite possible inaccuracies of analytical methods like titration [16] or near-infrared spectroscopy [17]. Nevertheless, several recent studies have underlined that measurements of individual VFA concentrations can be more relevant for actual process state knowledge [9], particularly propionate concentration which has been cited as the most effective indicator of stress status of the process [4,18–20]. Additionally, other research topics such as bioplastics production from anaerobically fermented effluent have a strong interest in the knowledge of individual VFA composition [21].

Individual VFA concentrations can be determined by gas chromatography, liquid chromatography or capillary electrophoresis [22–24]. Although laboratory measurements are now routine tasks, on-line implementation of these analytical techniques can be much more troublesome, even if on-line gas chromatography coupled to headspace chromatography [25] or sample filtration [26] has been proposed. The complexity and high cost of the analytical equipment required strongly hinders the development

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of such on-line devices and there is a need for a relatively simple and low-cost alternative for measurement of individual VFA.

In previous studies, our research team has developed an alternative procedure for the determination of total VFA by use of batch procedures or by microplate assays [27,28]. The analytical methodology is based on a specific derivatization of VFA via a two-step mechanism [28]: (i) activation step with two activating agents (*N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide and 7-Aza-1-Hydroxybenzotriazole) under acidic conditions (pH 3.5–4); (ii) amidation step with *N*-(1-naphthyl) ethylenediamine (primary amine with UV-absorbing or fluorescent properties) and pH > 7 (see Supplementary materials: Fig. S1). VFA derivatives are then selectively detected by spectrophotometric or fluorimetric measurement. This method allowed thus only total VFA determination.

In the present paper, an innovative flow-based analytical procedure for the determination of individual VFA is presented. This procedure is based on previously-mentioned chromogenic derivatization of VFA combined with the use of a C_{18} monolithic column for separation of VFA derivatives and sequential determination of individual VFA. The unique properties of monolithic columns, namely fast mass transfer and low column back pressure, have enabled the recent development of multisyringe chromatography (MSC) or sequential injection chromatography (SIC) which have found some applications in pharmaceutical and environmental analysis [29–34]. Development and application of our MSC system for individual VFA analysis are presented herein.

2. Experimental

2.1. Reagents

All chemicals and solvents were of analytical or HPLC reagent grade and used without further purification. 15 g L^{-1} stock solution of the activating reagent *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC) (Sigma-Aldrich, Saint-Quentin Fallavier, France) was prepared by dissolving appropriate amount in absolute ethanol (Sigma-Aldrich). 6 g L^{-1} of a secondary activating reagent (7-Aza-1-Hydroxybenzotriazole, HOAT) (Genscript Corporation, Piscataway, USA) and 0.5 g L^{-1} of the derivatizing reagent (*N*-(1-naphthyl) ethylenediamine, EDAN) (Sigma-Aldrich) were prepared as a mixed solution in ultrapure water (Millipore, resistivity > 18 MΩ cm) at pH 3.6. EDC and (HOAT+EDAN) solutions were both prepared in amber glass bottles. The basic phosphate solution was made of a 500 mM solution of potassium dihydrogen phosphate (KH_2PO_4) prepared with a 0.7 M NaOH solution. Stock solutions of carboxylic acid standards (10–50 g L^{-1}, depending on respective solubility) (Acros Organics, Geel, Belgium) were prepared in ultrapure water. A standard mixed solution with C_2-C_5 VFA at 5 g L^{-1} and pH set at 6 was used for further dilutions as required.

Acetonitrile (MeCN) (Sigma-Aldrich) was used as the mobile phase organic modifier and buffered by 0.1% formic acid (HCOOH) (Acros Organics), pH 3. Two mobile phases were used during experiments. The mobile phase 1 was MeCN/[HCOOH 0.1% pH 3]: 35/65 and was used for VFA separation. A second mobile phase (mobile phase 2) composed of MeCN 100% was used for the cleanup of the monolithic column.

Suprapure hydrochloric acid (HCl) (Sigma-Aldrich) was used in the initial experiments and for all pH adjustments. Each mobile phase was filtered through a 0.45 μm PES membrane.

2.2. Sample preparation

The whole real samples were stored at −18 °C before analysis. Sludge samples preparation included centrifugation at 12,000 rpm for 10 min and subsequent filtration through 0.45 μm PES filters. Samples were analyzed on the same day they were prepared.

2.3. Gas chromatography analyses

VFA were determined under classical gas chromatography conditions using a Varian CP-3800 gas chromatograph (GC) with a free fatty acid phase (FFAP) 25 m fused silica capillary column of 0.1 μm film thickness and an i.d. of 0.25 mm (Varian CP-WAX 58-CB). The GC oven temperature was programmed to be ramped from 115 to 140 °C within 5 min, and a final temperature of 140 °C was then held for 1.25 min. Ghost peaks was prevented by a double injection of 1 μL of 1% formic acid blank solution between two samples, with oven temperature held at 160 °C for 3 min at every formic acid injection [35]. The injector and detector temperature were set at 290 °C and 300 °C, respectively. Helium was used as carrier gas at a pressure of 1.4 bar. A flame ionization detector (FID) was used and quantification was performed using the internal standard method. Limits of detection between 5 mg L^{-1} (valeric acid) and 20 mg L^{-1} (acetic acid) were obtained. All sample analyses were performed in duplicate.

2.4. Instrumentation and software

The set-up of the MSC system is outlined in Fig. 1. It comprised a multisyringe burette (BU-4-S, Crison Instruments, Alella, Barcelona, Spain) used as a propulsion unit equipped with four syringes (Hamilton, Bonaduz, Switzerland), two 5 mL (mobile phase 1 and 2), one 2.5 mL (HOAT+EDAN reagent) and one 1 mL (EDC reagent). Standard solenoid valves on the head of syringes 1 and 2 (containing mobiles phases) were replaced by two-way connectors made of Delrin® (Sciware, Palma de Mallorca, Spain) in order to resist to organic solvents. The latter connected respective syringes with new solenoid valves (V1 and V2, Takasago MTV-3-1/4UKGH, Takasago, Japan) which are situated outside the syringe module and allow a maximum operating pressure of 6 bars. V3 and V4 were standard solenoid valves. The manifold was constructed with 0.8 mm internal diameter poly(tetrafluoroethylene) (PTFE) tubing.

Syringes 1 and 2 were connected to one port of a 6-port injection valve module (Crison Instruments) equipped with a 125 μL loop. Some experiments were carried out with a 50 μL injection loop but results were better with a larger sample volume. We found that a sample volume of 125 μL was a good compromise between broadness of the peaks, peaks separation and analytical performance of the system. The three other ports of the injection valve were connected to the mixing tank, the monolithic column and waste.

The set-up was also constituted by three solenoid micro-pumps (MP1-MP3) (Bio-Chem Valve Inc., Boonton, NJ, USA). The indicated stroke volume for all micro-pumps is 20 μL. Micro-pumps were computer-controlled by means of an MCFIA/MPFS system (SCI-WARE, Palma de Mallorca, Spain) which has eight digital 12 V output channels.

Derivatization procedure was performed in a 2.5 mL lab-made polymethyl methacrylate (PMMA) mixing tank (1.4 cm i. d. × 2.2 cm height), equipped with a small magnetic stirrer (0.9 cm) for homogenization of the reaction mixture (see Supplementary materials: Fig. S2).

The separation of VFA derivatives was performed on an Onyx™ monolithic C18 column (25 mm × 4.6 mm, Phenomenex, USA) with a 10 mm × 4.6 mm guard column of the same material and from the same supplier. The detection system was composed of an ultra violet-visible light source (UV) DH-2000 Deuterium Tungsten Halogen Light Sources (wavelength range 215–2000 nm, Ocean Optics, Dunedin, FL, USA) with only deuterium light source used, a 30 μL inner volume flow-cell with 10 mm light path (Hellma) and

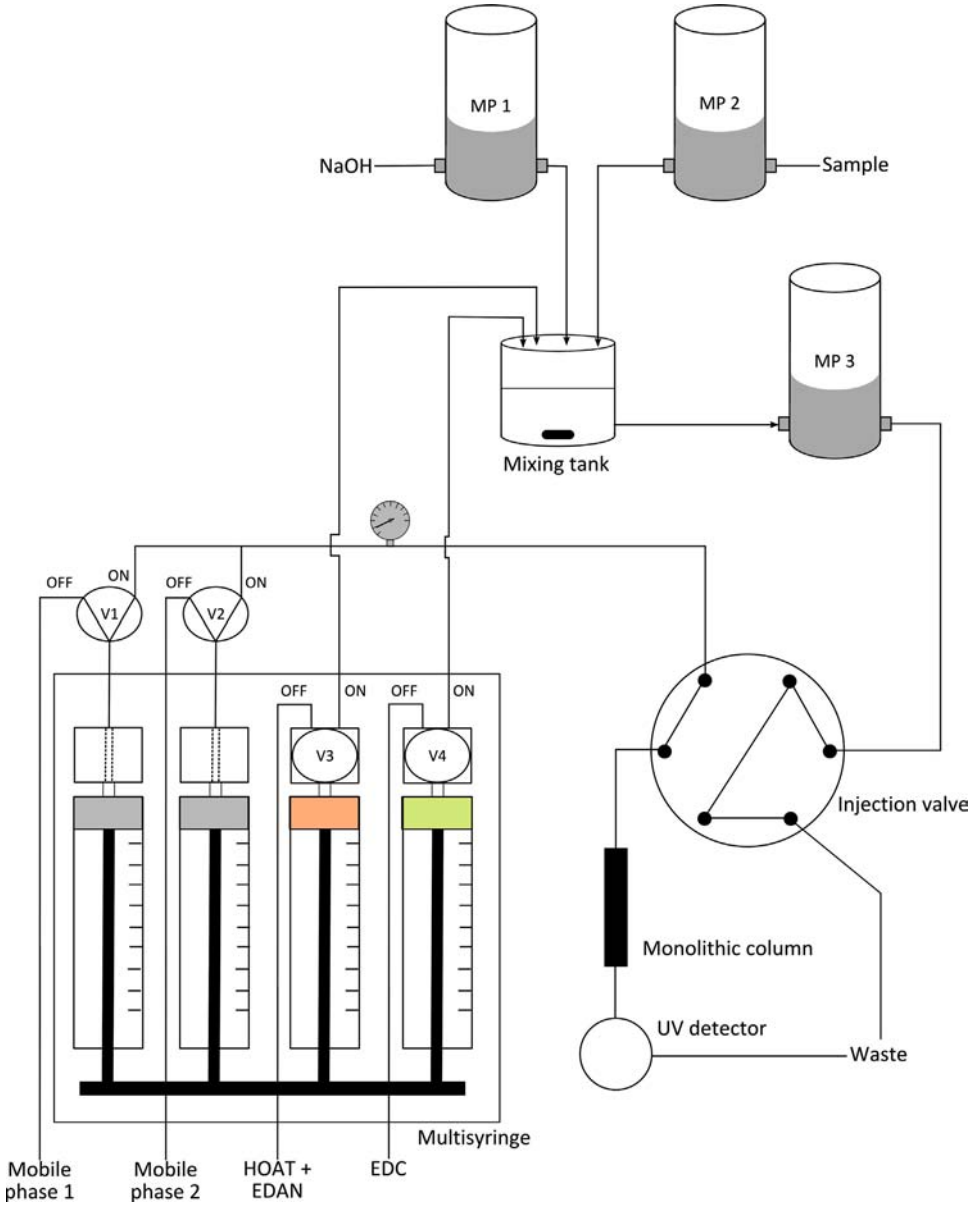

Fig. 1. Schematic depiction of the MSC manifold used for individual VFA determination. *V*=solenoid valve; MP1, MP2 and MP3=Solenoid micro-pump; "NaOH"=solution of KH_2PO_4 500 mM in NaOH 0.7 M.

a USB 2000 miniature fiber-optic spectrometer detector (detector range: 200–1100 nm, Ocean Optics) connected to a computer via a USB interface. The analytical wavelength was set at 327 nm. Two FC-UV600-2 optical fibers (Ocean Optics) were used to guide the light from the source through the flow cell and further to the spectrophotometric detector. System control, data acquisition and processing were performed using the software package Autoanalysis 5.0 (Sciware, Palma de Mallorca, Spain).

2.5. MSC procedure

The operational details of the MSC system are listed in Table 1 and summarized as follows:

1. [HOAT+EDAN] solution (step 2), pre-treated sample (step 3) and EDC solution (step 4) are successively dispensed in the mixing tank for the activation step of the derivatization reaction. After 300 s of mixing, a basic phosphate solution (KH_2PO_4 500 mM in NaOH 0.7 M="NaOH" on Fig. 2) is added to the mixing tank (step 7) to promote the amidation step of the reaction.
2. After 60 s, injection valve is switched to "Load" position to fill the sample loop with the reaction mixture in the mixing tank (step 9). Mobile phase 1 (MeCN/[HCOOH 0.1% pH 3]: 35/65) is then dispensed at 0.75 mL min^{-1} through injection valve in "Inject" position to perform the chromatographic separation of VFA derivatives (step 11–13, with step 12 for mobile phase reloading in syringe 1) while absorbance measurement is carried out at 327 nm.
3. All syringes are reloaded to their initial position (step 15) and the mixing tank is washed with the next sample to be analyzed (step 16 and 17), so as to be ready for the next analytical cycle.

Syringe containing mobile phase 2 (MeCN) is used only when real samples are injected, with 3 mL of acetonitrile injected in the flow system (flow-rate: 1 mL min^{-1}) to wash the monolithic column from hydrophobic compounds potentially contained in real samples, followed by 5 mL of mobile phase 1 for reequilibration of

Table 1
Detailed multi-syringe chromatography protocol for the sequential determination of volatile fatty acids in environmental samples.

Step	V1	V2	V3	V4	InjectionValve	Mode	Volume (mL)	Flow-rate (mL min^{-1})	MP1 (μL)	MP2 (μL)	MP3 (μL)
1	START LOOP (n=2)										
2	OFF	OFF	ON	OFF	INJECT	DISPENSE	1	15	–	–	–
3	OFF	OFF	OFF	OFF	INJECT	–	–	–	–	100	–
4	OFF	OFF	OFF	ON	INJECT	DISPENSE	0.1	15	–	–	–
5	OFF	OFF	OFF	OFF	INJECT	PICK UP	3	15	–	–	–
6	WAIT 300 s										
7	OFF	OFF	OFF	OFF	INJECT	–	–	–	100	–	–
8	WAIT 60 s										
9	OFF	OFF	OFF	OFF	LOAD	–	–	–	–	–	1300
10	START MEASUREMENT										
11	ON	OFF	OFF	OFF	INJECT	DISPENSE	5	0.75	–	–	–
12	OFF	OFF	OFF	OFF	INJECT	PICK UP	1.8	15	–	–	–
13	ON	OFF	OFF	OFF	INJECT	DISPENSE	1.8	0.75	–	–	–
14	STOP MEASUREMENT										
15	OFF	OFF	OFF	OFF	INJECT	PICK UP	5	15	–	–	–
16	OFF	OFF	OFF	OFF	INJECT	–	–	–	–	500	–
17	OFF	OFF	OFF	OFF	INJECT	–	–	–	–	–	500
18	STOP LOOP										

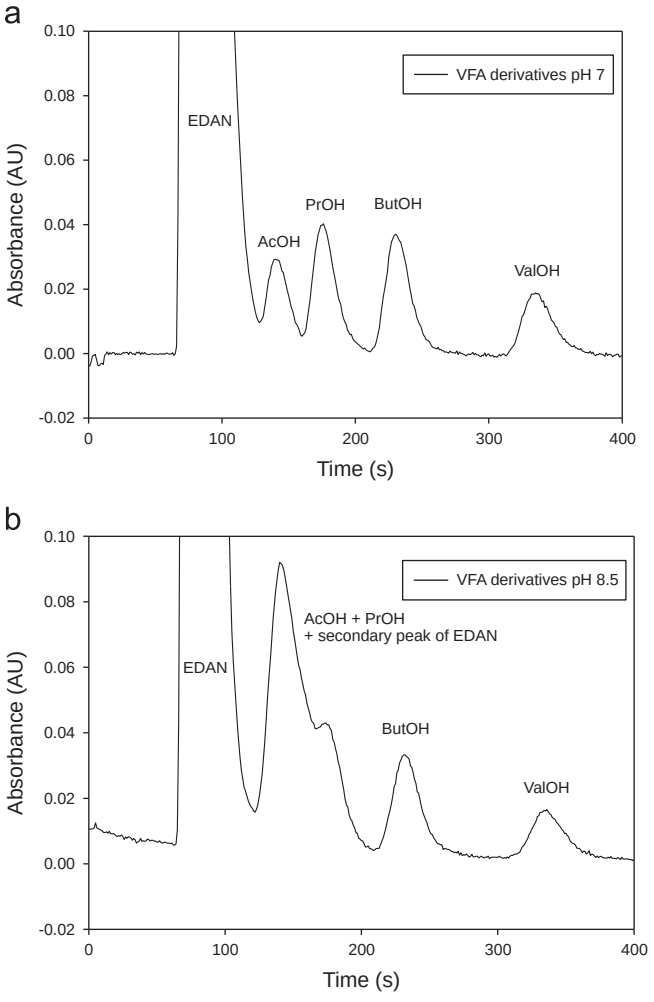

Fig. 2. MSC chromatogram obtained from a standard mixture of VFA at 1 g L^{-1} and derivatized "off-line". Final pH of VFA derivatives solution: (a) pH 7; (b) pH 8.5. Mobile phase: MeCN/HCl 1 mM : 45/55, flow-rate 0.75 mL min^{-1}, UV detection at 327 nm.

the column. This procedure should be implemented every five real samples to avoid clogging of the monolithic column.

The derivatization procedure could be performed on a second sample while the first sample being separated but this solution has not been used for now in order to simplify the description of MSC procedure.

3. Results and discussion

3.1. Study of chromatographic conditions

For the optimization experiments, VFA derivatives were formed "off-line" via application of the derivatization conditions and corresponding reaction performed in a test tube. Derivatives were then directly injected in the chromatographic system.

3.1.1. Optimization of the MSC mobile phase

MSC system was first used with pre-formed VFA derivatives of acetic acid (AcOH), propionic acid (PrOH), n-butyric acid (n-BuOH) and n-valeric acid (n-ValOH) directly injected in the sample loop. The eluent composition selected for these experiments was a classical MeCN/HCl 1 mM mixture [36], and provided the chromatograms shown on Fig. 2a. EDAN peak was isolated from VFA derivatives, which were well separated from each other, depending on the length of the VFA carbon chain (C_2–C_5).

As can be seen in Fig. 2a and b, the final derivative pH has an important influence on the first two peaks (AcOH and PrOH). When the derivative pH is slightly too basic, a large secondary peak of EDAN appears in the area of the AcOH and PrOH peaks.

In the previous study [27], we showed that the EDAN amidation step should be carried out at a pH greater than 8. In this study with the MSC system, the final pH of VFA derivatives should not exceed 7.5 because of this secondary peak, implying use of a stronger acidic eluent.

Considering this pH issue and that the normal operating pH for the monolithic column should be in the range 2–7.5, we decided to use a stronger acidic buffer for the aqueous phase and we turned our attention to formic acid (0.1%, pH 3). We first checked that the presence of formic acid (carboxylic acid with only one carbon) in the mobile phase did not lead to the appearance of a non-desired peak in the chromatogram, which was not the case. Indeed, our previous study [28] demonstrated that formic acid response to the derivatization protocol was very low, and formic acid is only used here in the eluent after the derivatization reaction is complete.

The combination of formic acid with acetonitrile enabled us to avoid the presence of the secondary peak even with reaction mixture pH slightly too basic (chromatograms similar to Fig. 2a

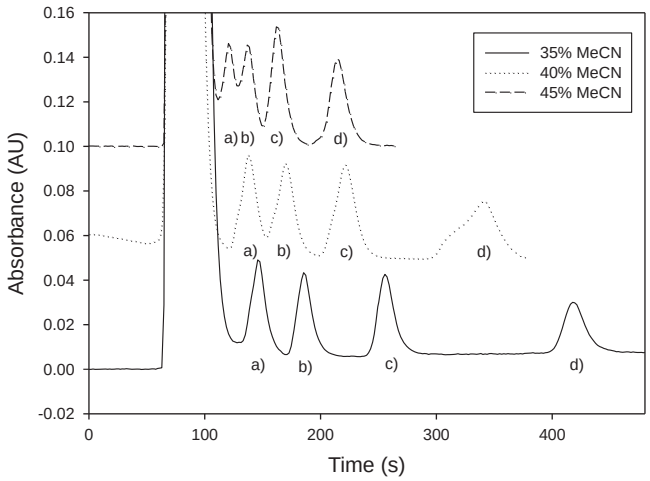

Fig. 3. Influence of the eluent acetonitrile content on the chromatographic separation of a standard mixture of VFA at 1 g L^{-1} and derivatized "off-line". Mobile phase: acetonitrile/[formic acid 0.1% pH 3], flow-rate 0.75 mL min^{-1}, UV detection at 327 nm. Peaks (a) AcOH; (b) PrOH; (c) ButOH; (d) ValOH.

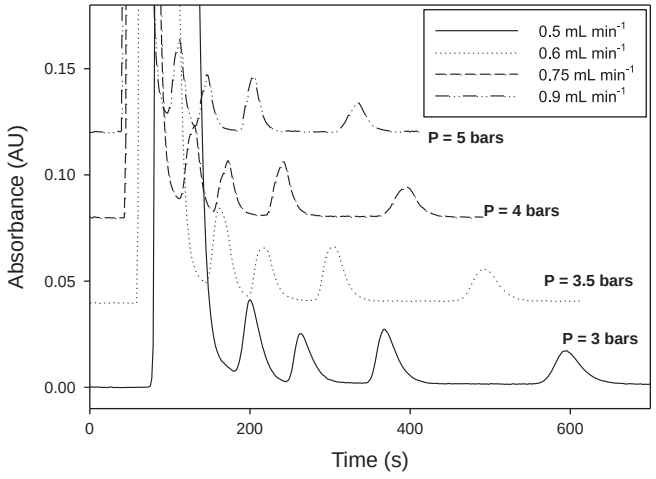

Fig. 4. Influence of the eluent flow-rate on the chromatographic separation of a standard mixture of VFA at 1 g L^{-1} and derivatized "off-line". Mobile phase: acetonitrile/[formic acid 0.1% pH 3]: 35/65, UV detection at 327 nm. P represents the back pressure in the MSC system.

even with a final pH of 8.5). An investigation on the optimal composition of this acetonitrile/formic acid eluent was thus conducted to reach an effective separation of VFA derivatives (Fig. 3). Our experiments demonstrated that a ratio acetonitrile/[formic acid 0.1% pH 3]: 35/65 provided the best separation for the four VFA derivatives and the first EDAN peak. A lower acetonitrile content could probably improve this separation, but at the expense of a longer retention time for the last peak (ValOH), and we thus decided to keep this ratio for further experiments.

Other buffers for pH between 4 and 7 were also tested for the aqueous phase of the eluent (phosphate, 2-(N-morpholino)ethanesulfonic acid (MES)), but had a little influence on the chromatogram obtained and the best results remained those obtained with formic acid.

Noteworthy, n-ButOH and i-ButOH derivatives were eluted at the same retention time and were therefore integrated together as "C_4-VFA" (four carbons in the side chain). i-ButOH yields a response 11% superior to that of n-ButOH (see Supplementary materials: Table S1). i-ValOH and n-ValOH also provided two overlapping peaks with a 9% response factor between the two ValOH isomers (see Supplementary materials: Table S1). i-ValOH and n-ValOH were also considered together as "C_5-VFA".

3.1.2. Eluent flow-rate

Influence of eluent flow-rate with previously-optimized eluent composition was investigated in the range 0.5–0.9 mL min^{-1} (Fig. 4). System back pressure was measured for each flow rate using acetonitrile-formic acid mobile phase. It was observed that increasing flow-rate led to increasing back pressure. The results obtained for the chromatographic separation of VFA derivatives using MSC were compared to those obtained using HPLC. The height equivalent to a theoretical plate (HETP) for AcOH peak were 42 μm with classic HPLC system (analysis performed in lab with a VWR Hitachi ELITE LaChrom, equipped with an L-2130 pump, an L-2200 autosampler and an L-2400 DAD; the column used was a Supelco Lichrospher RP18 250 mm × 4.6 mm i.d.) and 55 μm with MSC. The HETP could not be improved by increasing flow rate; 0.75 mL min^{-1} was thus selected as the optimal flow-rate regarding peak separation, retention times and system back pressure (P=3.5 bars with this flow-rate). We preferred to avoid back-pressures over 5 bars (for 0.9 mL min^{-1} for instance) to ensure potentially better long-term operation of our MSC system.

3.2. Optimization of the derivatization reaction

The derivatization protocol had already been optimized "off-line" in previous studies when using microplates [28]. The first "activation step" is best performed under acidic conditions with two activating agents (HOAT and EDC), and the second "amidation step" under slightly basic conditions (pH > 7) with EDAN as the chromogenic or fluorescent reagent. Nevertheless, the preferential and simpler use of absorbance detection instead of fluorescence and a wider analytical range required for this specific analytical flow system by wastewater treatment plants managers (0.1–10 g L^{-1} total VFA) prompted us to re-evaluate some of the reaction conditions.

3.2.1. Phosphate buffer

In the microplate procedure, phosphate ions were introduced with HOAT (dissolution of HOAT powder in a phosphate buffer) in the first step of the protocol with the purpose to contribute to the buffering of the reaction mixture. New experiments aimed at testing the buffering capacity with increasing phosphate concentrations in the HOAT solution (with pH of reaction mixture carefully controlled and properly fixed) resulted in lower peak areas, which means that phosphate ions have a detrimental effect on the reaction yield when present in large amounts in the first step as is the case with buffer solutions. The peak area loss was about 10% with 10 mM phosphate buffer and more than 40% with 50 mM.

Moreover, phosphate has a very limited buffering capacity at pH 3.6–4 (activation step) since its pKa is around 2.1. We thus decided to prepare the [HOAT+ EDAN] solution in pure water, HOAT being the buffering agent for the activation step (pKa=3.3).

Nevertheless, phosphate buffering capacity is important for the amidation step (optimal pH between 7 and 8) and phosphate ions were therefore introduced with the basic solution aimed at increasing the pH for the amidation step. This basic solution was optimized to reach the suitable pH range and pH buffering capacity, and was finally made with potassium dihydrogen phosphate (KH_2PO_4) prepared at 500 mM in NaOH 0.7 M. Phosphate ions added in the second step of the reaction had no detrimental effect on the resulting peak areas.

3.2.2. HOAT concentration

As stated above, HOAT is responsible for the buffering capacity on the activation step. An accurate pH study had already been

conducted in a previous work [28] and demonstrated that the optimum pH range for this activation step was between 3.6 and 4.1 (after addition of sample which might result in a pH increase, depending on sample pH and alkalinity). Various HOAT solutions were thus prepared with concentrations ranging from 2 to 6 g L^{-1} and initial pH was set at 3.6. Subsequent addition of 100 μL of a 10 g L^{-1} standard solution of AcOH at pH 7 was made in 1 mL of these HOAT solutions. pH values measured in these solutions were 5.2, 4.4 and 4.1 for HOAT concentration of 2, 4 and 6 g L^{-1} respectively. HOAT concentration is a key factor for reaching suitable buffering capacity and an HOAT concentration of 6 g L^{-1} is sufficient to buffer extreme VFA concentrations in the analytical sample. Solutions with concentration above 6 g L^{-1} are moreover difficult to prepare due to the limited solubility of HOAT.

3.2.3. Reaction kinetics

Time-dependence of both activation and amidation steps has been re-assessed with the global analytical system and previously optimized conditions (Fig. 5). Experiments were carried out with a 0.5 g L^{-1} AcOH standard solution.

Optimal reaction times are thus 5 min for activation step (Fig. 5a) and 1 min for amidation step (Fig. 5b). Reaction with other VFA provided similar results.

3.3. Analytical features

The analytical features of the protocol were evaluated under the optimal experimental conditions. Regression equations, calibration ranges, LODs, LOQs and retention times of the analytes are listed in Table 2. The linearity was validated in the range 0–1 g L^{-1} for each VFA, using 6 calibration points in standard solutions. Linear regression coefficients ranged from 0.9988 to 0.9997. A second-order polynomial regression was also validated for the range 0–2.5 g L^{-1} for each analyte (see Supplementary materials: Fig. S3).

The detection limit (L_D) and quantification limit (L_Q) were estimated using the classical 3σ and 10σ approaches respectively, i.e. calculation of L_D and L_Q through analysis of the standard deviation of blank measurements ($n=10$). Limits of detection obtained with MSC system were in the range 0.01–0.02 g L^{-1}. These limits of detection were comparable with those obtained by HPLC-UV [37] or GC-FID [35] but were obviously higher than those obtained using more complex methods such as HS-SPME-GC-MS [22] or LLE-RP-HPLC-UV [24]. However limits of detection and quantification were low enough for our analytical purposes and specific demands from industrials working in the methanation and biogas production field. Moreover, sensitivity of the proposed method is comparable with that obtained with the reference method by GC-FID in our laboratory. Satisfying relative standard deviations (RSD) between 4 and 5% were obtained.

Regarding sample throughput, the overall procedure took about 18 min, including derivatization reaction, chromatographic separation and preparation for a new analytical cycle, allowing the analysis of 3 samples/h. The analysis time per sample in gas chromatography was shorter (approximately 12 min; 5 samples/h) but the present method provided additional merits of low instrumental and operational costs. Moreover, this new method can be implemented directly on site for control of biogas process.

3.4. Validation on real samples

A comparative study between our MSC system and GC-FID analysis (reference method for VFA) has been conducted on real sludge samples ($n=6$). These samples originated from various industrial facilities: sewage sludge samples collected in Marseille's sewage sludge treatment plant before and after fermentation tank, co-digestion sludge samples from a wastewater treatment plant

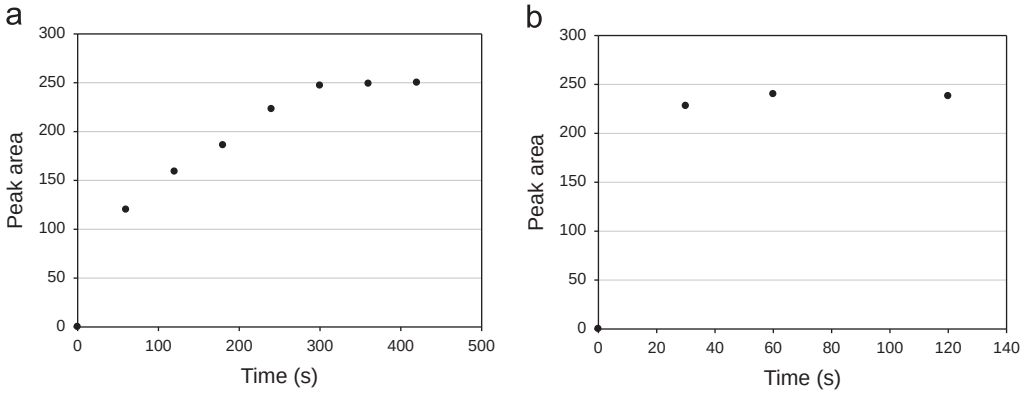

Fig. 5. Time-dependence of the on-line derivatization reaction for a 0.5 g L^{-1} AcOH standard solution. (a) activation step; (b) amidation step.

Table 2
Analytical features of the developed MSC system.

Analytical parameter	AcOH	PrOH	ButOH	ValOH
Limit of detection (gL^{-1})	0.019	0.011	0.010	0.015
Limit of quantification (g L^{-1})	0.061	0.035	0.032	0.048
Linear calibration range (g L^{-1})	0.06–1	0.03–1	0.03–1	0.05–1
Linear regression equation[a]	$A=(466.4\pm4.5)[C]+(2.1\pm2.3)$	$A=(467.2\pm3.4)[C]+(2.3\pm1.7)$	$A=(537.7\pm4.3)[C]+(3.1\pm2.1)$	$A=(485.8\pm2.3)[C]+(0.3\pm1.1)$
R^2	0.9988	0.9993	0.9991	0.9997
RSD (%)[b]	4.9	4.5	3.9	5.1
Retention time (s)	146	186	256	420
RSD of retention times (%)	1.22	1.08	0.92	0.67

[a] A was area of the peak; [C] was the analyte concentration (g L^{-1}).
[b] RSD was calculated on a 0.5 g L^{-1} standard; $n=6$ replicates.

Table 3
Comparative results between MSC system and gas chromatography (GC) analysis on various sludge samples (g L^{-1}, mean ± standard deviation, $n=2$ replicates).

	C2		C3		C4		C5	
	GC	MSC	GC	MSC	GC	MSC	GC	MSC
Sample 1	1.89 ± 0.06	1.68 ± 0.10	0.49 ± 0.01	0.45 ± 0.01	0.99 ± 0.03	0.95 ± 0.02	0.25 ± 0.01	0.29 ± 0.01
Sample 2	0.95 ± 0.04	0.82 ± 0.02	0.69 ± 0.02	0.62 ± 0.02	0.29 ± 0.01	0.24 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
Sample 3	0.08 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	N.D.	N.D.	N.D.	N.D.
Sample 4	0.85 ± 0.10	0.78 ± 0.05	0.24 ± 0.01	0.21 ± 0.01	0.51 ± 0.03	0.48 ± 0.02	0.13 ± 0.01	0.14 ± 0.01
Sample 5	0.11 ± 0.01	0.18 ± 0.01	2.00 ± 0.08	1.98 ± 0.10	0.06 ± 0.01	0.04 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Sample 6	0.43 ± 0.20	0.43 ± 0.03	1.73 ± 0.10	1.62 ± 0.04	0.14 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01

N.D.: Not Detected.

(Saint-Thibault des Vignes, France) and sludge samples from methanation facilities treating household wastes (Montpellier, France). Pre-treated samples were directly injected in the MSC system and in the GC-FID in duplicate. Results for individual C_2–C_5 VFA concentrations are detailed in Table 3 (see chromatograms of two real samples as examples in Supplementary materials: Fig. S4).

Individual VFA concentrations ranged from 30 to 2000 mg L^{-1}. The results exhibited a slight underestimation of our MSC procedure compared to GC-FID, for all VFA except C_5 (lowest concentrations). Results obtained with MSC system are nevertheless well-correlated with the reference method (Wilcoxon p value < 0.05). As expected, AcOH and PrOH (C_2 and C_3 VFA) exhibited the highest concentrations in the whole sludge samples, AcOH being the major VFA in Marseille's and Saint-Thibault's samples and PrOH in Montpellier's sample (from an anaerobic reactor that apparently led to digester failure). Regarding C_4 and C_5 VFA, n-ButOH and i-ValOH were the major compounds detected by the GC system, respectively.

4. Conclusions

We have demonstrated that individual VFA could be determined by a MSC procedure, thanks to the combination of a derivatization strategy coupled to the selectivity offered by the use of a monolithic column. This low-pressure system enables quantitative and selective detection of individual VFA from C_2–C_5 in the range 0.05–2.5 g L^{-1} with a sample throughput of about 3 samples h^{-1}. Results on filtered real sludge samples with our MSC procedure and GC reference method were well-correlated and demonstrated the applicability of our analytical technique for on-site or on-line analyses. To meet this specific demand, the on-line filtration issue of sludge samples on industrial sites is under study at the present time. The first results obtained with a filtration at 0.8 μm indicate that this type of flow-based analytical device represents a promising alternative for relatively simple and low-cost on-line analyzer that could help for real-time monitoring and process control.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.06.019.

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