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Development of a fluorescence-based microplate method for the determination of volatile fatty acids in anaerobically digested and sewage sludges

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ABSTRACT

This paper presents a simple, accurate and multi-sample method for the determination of volatile fatty acids (VFAs) thanks to a 96-well microplate technique. A procedure using an activating reagent of the carboxylic function (water-soluble carbodiimide EDC) and a fluorescent amino labeling reagent (*N*-(1-naphthyl)ethylenediamine, EDAN) allows the formation of an isoindole derivative that needs to be separated from initial fluorescent amine for efficient VFAs determination. Isolation of these fluorescent VFA-derivatives was carried out by use of the fluorescent quenching of EDAN with *o*-phthaldialdehyde (OPA). Quenching was most efficient at pH around 7 and by heating at 40 °C within the microplate reader. This optimized procedure has been applied to various carboxylic acids and other organic compounds, demonstrating that VFA exhibit the highest fluorescence responses with homogeneous results for the main ones (acetic, propionic and butyric acid, all mass concentration expressed as acetic acid equivalents). This protocol was calibrated against acetic acid and determination of VFA was thus possible in the range 3.9–2000 mg L^{-1} (acetic acid equivalents). Subsequent application to real samples (sewage sludges or anaerobically digested samples) and comparison to gas chromatography analyses gave accurate results, proving the great potential of our high-throughput microplate-based technique for the analysis of VFA.

1. Introduction

Monitoring of volatile fatty acids (VFAs) has gained considerable importance in the past years, both for environmental or industrial purposes. VFAs are key intermediates and metabolites in many biological processes and the presence of VFA in a sample matrix is often indicative of a bacterial activity. Consequently, VFA monitoring and study can be of great interest in various fields such as rumen fermentation study [1], biohydrogen production [2], polyhydroxyalkanoate production [3], landfill operation [4], biological wastewater treatment plant carrying out anaerobic digestion [5], phosphorus removal [6] or denitrification [7], or for sewage sludge treatment plants [8] and anaerobic treatment facilities [9,10].

Anaerobic digestion is a widespread process routinely used for sewage sludge treatment or organic waste stabilization for instance. It enables both high organic removal efficiency and a net energy benefit through the production of biogas (methane). However, it is frequently acknowledged that successful control of anaerobic treatment facilities is rather delicate; indeed, efficient transformation of organic matter to biogas depends on several parameters potentially affecting the proper development and activity of the bacterial community involved in the process [11,12]. Among these parameters, VFA concentration (C_2–C_5 carboxylic acids) is of particular importance regarding bacterial development and pH stability inside the digesters, sudden increase in their concentration causing possible pH drop and inhibition of acetogenic or methanogenic bacteria, leading to digester failure.

Therefore, there is a need for the development of simple, fast and accurate methods for measuring VFA in anaerobic systems. Currently, the most common techniques used for VFA determination include ionic or gas chromatography for individual VFA [13,14] and titration methods [15,16] for total VFA concentration. Chromatographic techniques, using derivatization steps with labeling reagents [17–19], require expensive equipment, maintenance, and the need for skilled technicians, and therefore they would not be generally available on-site for routine measurements. Titration methods have proven to be accurate in several studies of anaerobic digesters, although they are prone in some cases to systematic errors, are limited to particular conditions regarding VFA concentration/total alkalinity ratios or require the parallel knowledge of other digester's parameters [16]. Moreover, another limitation common to all traditional techniques is their ability to perform

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only single-sample analyses that generally require duplicates or triplicates to provide accurate results, resulting in time-consuming procedures.

Recently, our laboratory has devised a new method for VFA analysis based on a spectrofluorimetric measurement of derivatized VFA with a fluorescent amine reagent, namely *N*-(1-naphthyl)ethylenediamine (EDAN) [20]. The developed protocol included an extraction step with an organic solvent, step allowing us to overcome the interferences between EDAN and the resulting amide.

This extraction step is not a problem when on-line analysis systems are used [21] but it is rather difficult to implement in a microplate format (with simultaneous fluorescence lecture of organic and aqueous phases when using a microplate reader), therefore we have developed an original quenching step of the fluorescent amino reagent in the aqueous phase using *ortho*-phthaldialdehyde (OPA) to replace this step of organic extraction.

The second innovation of the present paper is the implementation of the whole analytical procedure into a microtiter plate, to take thus advantage of great possibilities offered by microtiter plate assays, namely low sample and reagent consumptions, automation possibilities, fast preparation of microplates and multi-sample analyses. Despite these recognized advantages, microtiter plate assays have been seldom applied to environmental analysis [22–24] and we can reasonably assume it will be an expanding field of study in the coming years.

Development, design and preparation of these ready-to-use microplates for VFA analysis are presented herein.

2. Experimental

2.1. Materials and reagents

N′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC), formic, succinic and lactic acid (solution in water, 85%) were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). *N*-(1-naphthyl)ethylenediamine (EDAN), acetic, propionic, butyric, valeric, *i*-butyric and *i*-valeric acid were obtained from Fisher Scientific (Illkirch, France). 7-Aza-1-hydroxybenzotriazole (HOAT) was from Genscript Corporation (Piscataway, USA). *o*-Phthaldialdehyde (OPA) and denaturated ethanol were received from Acros (Illkirch, France).

2.2. Real sample origins and preparation

Sewage sludge samples were collected in Sormiou's sewage sludge treatment plant (Marseille, France) before and after fermentation tanks, centrifuged at 10.000 rpm min^{-1} for 20 min and filtered through 0.8 μm PES filters. "Vinci sludge" samples were received from Vinci Environnement methanisation facilities treating household wastes (Montpellier, France) and were prepared by the same procedure.

2.3. Microplate instrumentation

Microplate fluorescence measurements were carried out on a microplate reader (Infinite M200 model, Tecan Austria GmbH, Salzburg, Austria) equipped with an excitation and emission double monochromator (bandwidth 9 nm and 20 nm for excitation and emission monochromator, respectively). Fluorescence detection was performed from above the microplate wells (top configuration), at λ_{ex} = 335 nm and λ_{em} = 442 nm. Operating temperature was set to 40 °C. The shaking mode was orbital with shaking amplitude of 3 mm (shaking operated in the microplate reader). Other parameters were as follows: gain: 100; number of flashes: 5; integration time: 20 μs. Fluorescence intensities were expressed in arbitrary units (a.u.).

Black 96 conical-bottom well microplates made with polypropylene were used (Nunc, Illkirch, France), with 450 μL as the maximal well volume.

2.4. Gas chromatography analysis

VFA were determined under classical GC 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 incremented from 115 to 140 °C within 5 min, and a final temperature of 140 °C was then held for 1.25 min. 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 ionisation detector (FID) was used and quantification was performed using internal standard method. All sample analyses were performed in triplicate. VFA concentrations were transformed into acetic acid equivalents for comparison purpose.

2.5. Fluorescence quenching with OPA

EDAN (1.5 mg/mL) and HOAT (2 mg/mL; secondary activating reagent), were dissolved in a 10 mmol L^{-1} phosphate buffer. This solution was then partitioned and pH set between 5 and 9 with 0.5 M NaOH. 17.5 μL of an OPA solution (17.1 mg/mL EtOH) was added to 200 μL of these solutions in the wells of a microplate, and the microplate was shaken for 13 min at 40 °C. Fluorescence intensities were recorded every minute. After having selected optimal quenching pH thanks to this experiment, solutions containing various quantities of OPA (8.6–34.3 mg/mL EtOH) were prepared and tested similarly.

Finally, temperature influence was studied with temperature ranging from 25 to 40 °C and optimal quenching parameters.

2.6. Optimization of the derivatization protocol

HOAT (2 mg/mL) was dissolved in a 10 mmol L^{-1} phosphate buffer and pH was subsequently set to the desired pH values (between 3.4 and 4.4, depending on the experiment) with a pH meter properly calibrated. 150 μL of HOAT was injected in a well of a microplate, followed by 8 μL of acetic acid standard solutions (500 mg L^{-1}) or ultrapure water (for blank measurements) and 17.5 μL of EDC (28.6 mg/mL EtOH). After variable-length shaking (temperature: 40 °C for the whole procedure), 50 μL of EDAN (6 mg/mL of a 50 mmol L^{-1} borate buffer, pH 8.6) were added, then submitted to another shaking period. Finally, 17.5 μL of OPA (22.9 mg/mL EtOH) was pipetted in the wells and the mixture was shaken for 13 min and then scanned for fluorescence.

2.7. Ammonium interference study

A procedure similar to that described above was used, with a HOAT solution whose pH value was fixed at 3.7, and activation (after EDC addition) and amidation (after EDAN addition) steps of 4 and 2 min, respectively. Ammonium chloride was added to acetic acid standard solution (500 mg L^{-1}) so as to obtain final ammonium concentration of 1.5 g L^{-1} (expressed as NH_4^+). Variable volumes (8–20 μL) of this modified standard solution were then transferred into the wells to assess the influence of ammonium ions on the fluorescence measurements. Quenching effects were

calculated against ammonium-free acetic acid standard solution (500 mg L^{-1}).

2.8. Optimized microplate protocol applied to standards and real samples

Sludge samples were diluted in a 40 mmol L^{-1} phosphate buffer (pH 2.1), by a diluting factor of two or four depending on the sample (two-fold dilution for sewage sludge samples, four-fold for other samples). 8 μL of these diluted solutions were then used according to the procedure described above in the optimization study (HOAT pH, 3.7; activation step, 4 min; amidation step, 2 min). Blank and standard solutions were prepared in a 30 mmol L^{-1} phosphate buffer solution containing 0.25 g L^{-1} NH_4^+ (pH 6.3). Average blank measurement was subtracted from average fluorescence intensity measured for standard solutions or samples. Blank and sample analyses were performed in triplicates, standards in duplicates.

2.9. Reagent stability tests

Reagent solutions were prepared as described before (HOAT, EDC, EDAN and OPA) and stored at +4 °C in a refrigerator. The optimized protocol was applied every 8 days on an acetic acid solution (2000 mg L^{-1}) and on a blank solution (duplicate analyses, both solutions prepared in a 30 mmol L^{-1} phosphate buffer solution containing 0.4 g L^{-1} of NH_4^+ at pH 6.3). Fluorescent measurements were compared over a period of 40 days.

3. Results and discussion

3.1. EDAN fluorescence quenching

Several potential chemical quenchers of amines were tested, but most of them proved either poorly efficient (phthalic anhydride, benzoquinone, naphthoquinone sulfonic acid) or too efficient due to complete quenching of both fluorescent amine and derivatized compound (sodium hypochlorite, sodium nitrite + HCl, toluene sulfonyl chloride). Only OPA gave satisfactory results, although it seemed to require longer reaction time (up to 1 h) and gave seemingly non-quantitative results under the conditions used in our first experiments. We thus decided to investigate further this quenching method.

OPA is a known reagent for quenching of fluorescent amines. It was used by Kobayashi in 1994 [19] at 30 °C for 30 min in a borate buffer (pH 9.5). Re-examining the reaction mechanism between OPA and amines, we thought that reaction pH could be critical to the quenching efficiency, reaction between aldehydes and amines being usually favoured by slightly acidic conditions (acid catalysis).

Accordingly, we first studied the influence of pH values on the fluorescence quenching (so on the resulting fluorescence intensity) (Fig. 1). Our initial experiments showed that previously described conditions (pH 9.5) led to a very long reaction time, while lowering pH gave much better results, and we thus focused on a pH range varying from 5 to 8.5 (final pH after OPA-quenching). Fig. 1A displays a very rapid decrease of fluorescence intensity for pH values between 6 and 7, at the opposite of more acidic or more basic conditions. Likewise, residual fluorescence intensity after 13 min (Fig. 1B) was at its lowest value for the same range of pH (6 < pH < 7.5, best choice: 6.5–7). Residual fluorescence could be explained by the presence of fluorescent impurities in EDAN commercial reagent (98% purity). Although 13 min could seem to be a relatively long reaction time, it was selected to reach the maximum fluorescence stability, bearing in mind that 96 samples can be analyzed on a single plate and therefore ensuring high sample throughput.

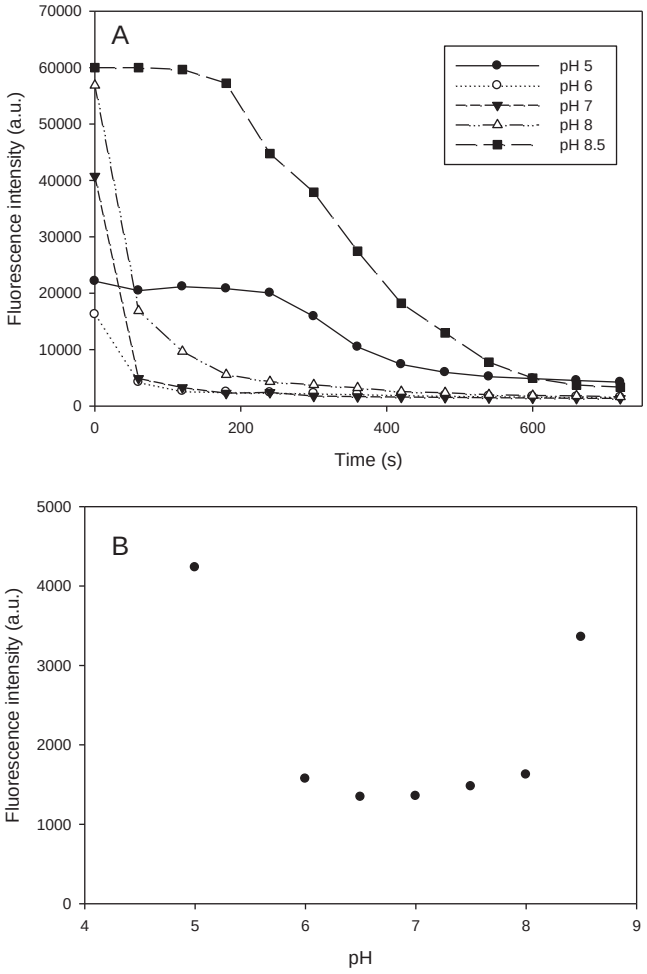


Fig. 1. Influence of pH values on the fluorescence quenching of EDAN by OPA (T = 40 °C). (A) Fluorescence intensity evolution over quenching period (13 min). (B) Residual fluorescence intensity after 13 min (end of quenching).

Two other parameters had to be assessed in terms of fluorescence quenching efficiency: OPA/EDAN molar ratio and reaction temperature (Fig. 2). OPA was most efficient at ratios above 2/1, that is to say for at least two molar equivalents compared to EDAN. Although 4/1 provided the best results, 3/1 (4 mg OPA for 3 mg EDAN) was selected for reagent consumption and stability reasons. The highest temperature able to be reached by the microplate reader (40 °C) gave the best quenching results. This is in agreement with the fact that aldehyde/amine reactions are traditionally favoured by heating of the reaction mixture.

3.2. Optimization of the derivatization protocol

Time-dependence of both activation and amidation steps has then been assessed. Reaction times were first investigated with the following pH values: 3.7 for the carbodiimide-activation and 6.5–7 for the amidation and OPA-quenching steps (measured after final quenching). A suitable borate buffer was used to both dissolve EDAN and induce pH increase so as to avoid an additional basification step with sodium hydroxide. EDC was dissolved in ethanol owing to its instability in water solution and high solubility and stability in alcohols.

As can be seen on Fig. 3, reaction durations are low (4 min for activation with EDC and HOAT and 1 or 2 min for amidation with EDAN); fluorescence intensities being stable afterwards. This fact is very important for a 96-well microplate application, as the

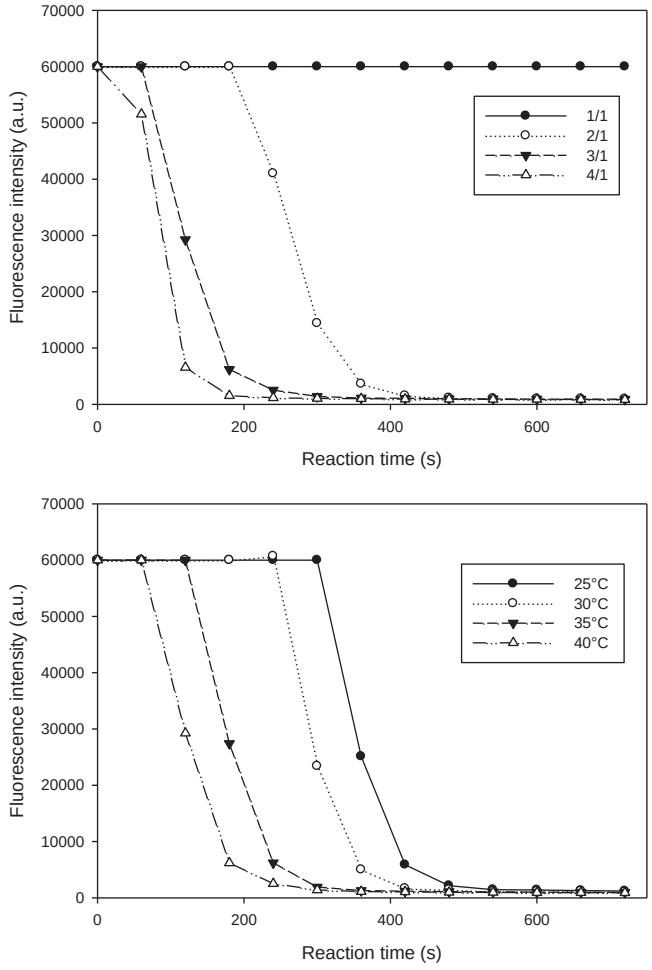


Fig. 2. (A) Influence of OPA/EDAN ratio on fluorescence quenching of EDAN by OPA (T = 40 °C, pH = 6.5). (B) Influence of the temperature on fluorescence quenching of EDAN by OPA (OPA/EDAN = 4/3; pH = 6.5).

numerous pipetting required for a complete microplate can cause reaction delay among the microplate wells.

We have also decided to focus more accurately on the pH activation step (between 3.4 and 4.4), in order to know the right pH range where homogeneous results could be obtained (sample addition might result in a slight and variable pH modification of the reaction medium, depending on sample pH and buffering capacity). Results have shown that optimum reaction pH was between 3.6 and 4.1. Special care is thus necessary to ensure that pH remains between these values after sludge sample addition, regardless of sample type or origin.

Table 1
Ammonium interferences on VFA measurements. Sample added was a standard solution containing acetic acid and ammonium at a concentration of 500 mg L^{-1} and 1.5 g L^{-1}, respectively. The quenching effect is linked to the decrease of fluorescence intensity, expressed in %, in presence of corresponding amount of ammonium.

Sample volume added (μL)	Quenching effect (%)
20	11.9
17	9.8
14	8.3
11	6.0
8	5.5

3.3. Ammonium interference

Ammonium ions can interfere either by competitive reaction during the amidation reaction or during the OPA-quenching. Initial experiments had demonstrated a fluorescence quenching effect (fluorescence decrease in the presence of ammonium) that has prompted us to test influence of sample volume added on quenching effect. Indeed, lower volumes entails lower quantities of ammonium ions present in the reaction medium. Sample volumes injected were thus varied in the range 8–20 μL (Table 1), with sample corresponding to a standard solution containing acetic acid and ammonium at concentration of 500 mg L^{-1} and 1.5 g L^{-1}, respectively. 1.5 g L^{-1} was selected as a relatively high concentration of NH_4^+ for common sewage sludge samples [25]. Quenching effect was clearly non-negligible (11.9%) when 20 μL of sample was injected prior to the reaction. This effect could be reduced up to 5.5% through sample volume reduction, with 8 μL as the minimum volume limit for convenient pipetting.

A possible explanation lies in the simultaneous formation of an OPA-NH_4^+ adduct during EDAN quenching which can quench some part of the fluorescence measured. Reaction wells containing NH_4^+ were characterized by a slightly red coloration, this gave evidence of the formation of this OPA-NH_4^+ adduct. Another experimental observation that supported this hypothesis was the result obtained for blank measurements with ammonium solutions containing no VFA: the same type of quenching effect (data not shown) was observed although no competitive reaction with VFA and EDAN could occur. OPA-NH_4^+ adduct is therefore the most likely explanation for this quenching phenomenon.

3.4. Influence of real samples on pH variations

Sewage sludge samples had pH values between 6.5 and 8.5; therefore, injection of a defined volume of these sludge samples in the initial HOAT solution was likely to be responsible in an increase of pH value before EDC injection, potentially leading to a detrimental effect on the activation step. Indeed, it was observed that injection of only 8 μL of sample in a 150 μL HOAT solution (pH

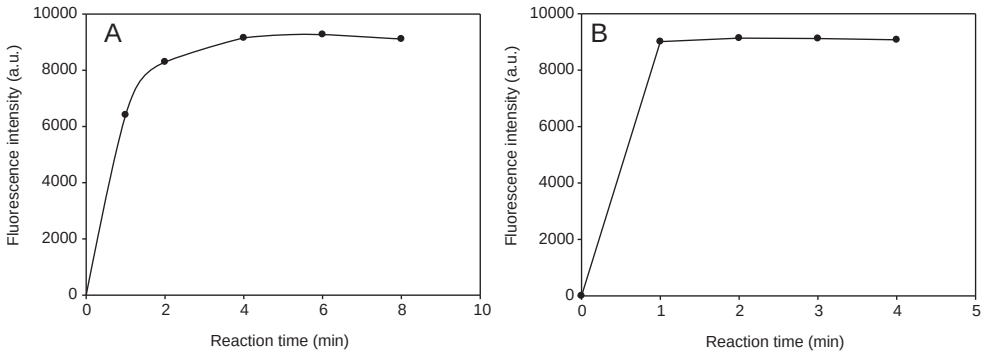


Fig. 3. Time-dependence of the microplate derivatization procedure (experiment carried out with a 500 mg L^{-1} acetic acid). (A) Activation step. (B) Amidation step.

3.7) could lead to a pH variation from 3.7 up to 5.3. As stated in the above-mentioned optimization experiments, it is necessary to remain in a strict pH range (3.6–4.1) to get optimum fluorescence results and a prior acidification was thus required. This "acidification step" was devised to be of general application for all sludge samples, so as to avoid individual acidification through pH measurements. An acidic 40 mmol L^{-1} phosphate buffer with pH set at 2.1 was used, with a dilution factor of 2 or 4 depending on sludge type: 2 for samples with rather low dry matter content (<10%) and 4 for other samples with higher dry matter content. pH measurements after addition of these diluted samples in the reagent solution were thereafter in the optimum range for activation step. This dilution factor was then taken into account for the calculation of VFA content of sludge samples.

3.5. Analytical features

Standard solutions were prepared in order to match as closely as possible composition of sludge samples (following dilution with acidic phosphate buffer, and trying to take into account possible interferences by ammonium ions). A 30 mmol L^{-1} phosphate buffer (pH 6.3) containing 0.25 g L^{-1} ammonium (as NH_4^+) was thus selected. Blank measurements were performed using this solution, with ten measurements for LOD calculation and three replicates for standard protocol. Blank average was then subtracted from measured fluorescence intensity to obtain calibration curves. As it is well-known that acetic acid is the most frequently encountered VFA in anaerobic processes, it was selected as the reference compound for our study and its calibration curve has thus been used to transcript fluorescence emission intensities measured with real samples into VFA concentrations (expressed as acetic acid equivalents).

The calibration graph under the previously conditions quoted is linear and given as $FI = 1673 \times C$ in the range 0–2000 mg L^{-1} (FI is the fluorescence intensity of the resulting amide and C is acetic acid concentration, expressed in mg L^{-1}), with a correlation coefficient (r^2) of 0.996. Relative standard deviation (RSD) was assessed on the 1000 mg L^{-1} standard solution with six replicates, giving a satisfying result of 4.6%. A detection limit (LOD) of 3.9 mg L^{-1} was calculated from the calibration curve ($\mu_B + 3\sigma_B$; μ_B, average of the blank; σ_B, standard deviation of the blank; $n = 10$). One should keep in mind that a dilution factor of two or four was applied, giving actual LOD of 7.8 mg L^{-1} or 15.6 mg L^{-1}, depending on the sample.

3.6. Application of the protocol to various organic compounds

The optimized protocol on acetic acid (summarized in Table 2) was applied to a wide range of organic compounds likely to have a fluorescent response under the reaction conditions (Fig. 4). Among them are VFA (propionic, butyric, . . .), other carboxylic acids (formic, lactic, tartric, . . .), carbohydrate (glucose) and amino acids (glycine, alanine, cysteine). VFA were clearly the most reactive compounds towards the derivatization protocol, with the exception of succinic acid which led to a high fluorescence response. Nevertheless, succinic acid is seldom encountered in sewage sludges or as degradation intermediate in anaerobic processes and should not have a significant contribution to the fluorescence measurements. Other non-VFA carboxylic acids, such as formic or lactic acids, gave very low fluorescence intensities, as well as carbohydrate or amino acids.

Total VFA concentrations are frequently expressed as acetic acid equivalents when measurement result is a mass concentration (mg L^{-1} in most cases), so as to compare it with results expressed as molar concentration. The formula used to convert VFA mass concentration from normal mass concentration (mg L^{-1}) to acetic acid equivalents (mg L^{-1} acetic acid equivalent) is the following:

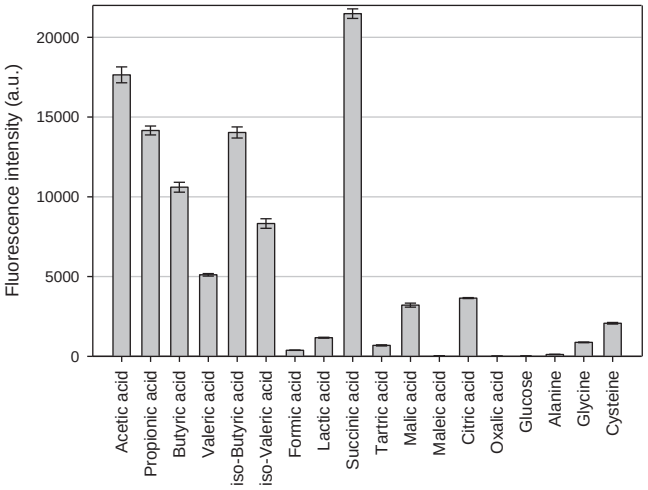


Fig. 4. Fluorescence measurements after application of the optimized protocol on various organic compounds (each organic compound was present at a concentration of 1000 mg L^{-1}).

[VFA] (mg L^{-1} acetic acid eq.) = [VFA] (mg L^{-1}) $\times$ ($M_{Acetic\ acid}/M_{VFA}$). Experiments were thus performed using VFA solutions with a 1000 mg L^{-1} acetic acid equivalents concentration. As can be seen on Fig. 5, acetic, propionic and butyric acid gave a similar fluorescent response, whereas valeric, iso-butyric and iso-valeric acids exhibited either lower or higher values. Considering the large prevalence of acetic, propionic and butyric acid in anaerobically digested samples, it is reasonable to conclude that VFA measurements with our fluorescent method can be correctly expressed as acetic acid equivalents.

3.7. Reagent stability tests

Regular experiments carried out for several days with the same reagents have demonstrated that rather stable results could be obtained after application of the optimized procedure. The less stable reagent seemed to be the fluorescent amine (EDAN), with the reagent solution turning from transparent to slightly reddish after a few days at room temperature, probably due to air sensitivity of the amine in aqueous solution. This phenomenon seemed to be less pronounced when the solution was stored in

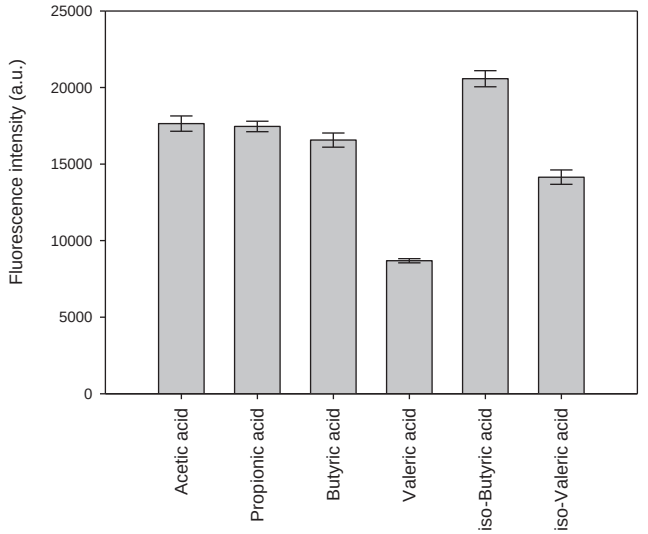
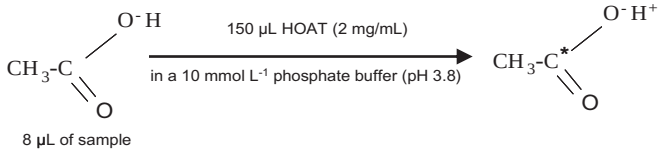
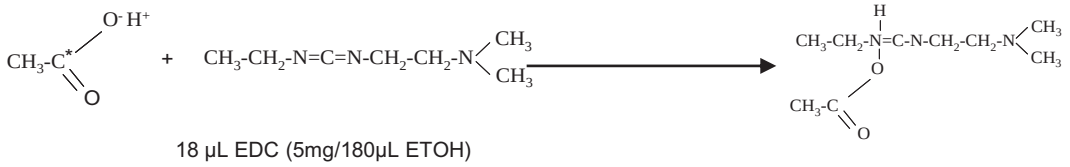
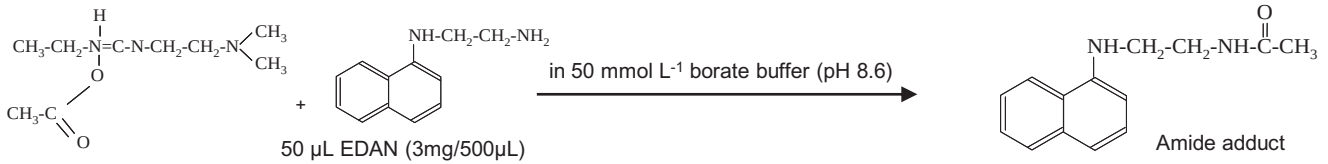


Fig. 5. Fluorescence intensities of various VFA. The whole carboxylic acids are at a concentration of 1000 mg L^{-1}, expressed as acetic acid equivalent.

Table 2
Schematic of the whole optimized analytical determination of VFA at the microplate scale.

Step	Reaction	Duration
Pre-activation of carbon bearing –OH group		Instan-taneous
Activation of pre-activated carbon bearing –OH group		4 min
Fluorescent labeling =amide formation		2 min
Fluorescence quenching of initial EDAN		12 min
Fluorescence reading	λ_{ex} = 335 nm; λ_{em} = 442 nm	

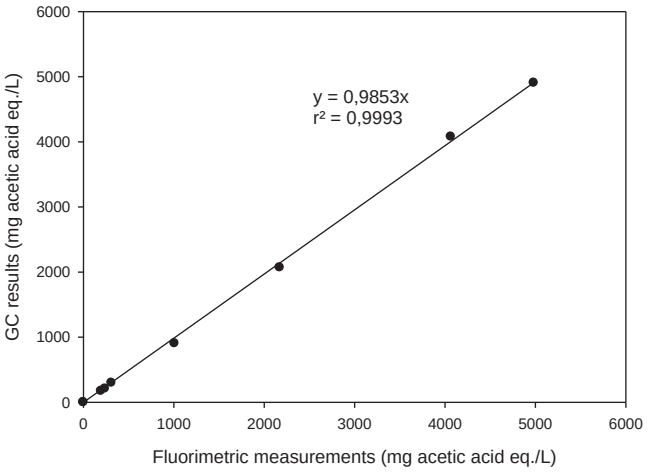


Fig. 6. Comparison between GC and fluorimetric measurements for several sludge samples.

the refrigerator, and it was thus decided to perform stability tests by keeping the reagents at +4 °C. The experiments were conducted every 8 days both on blank solution and acetic acid (2000 mg L^{-1}). Results obtained exhibited stable fluorescence measurements over a period of 40 days, although blank intensity increased by around 15%. From a practical point of view, we recommend to change EDAN solution at least every month, as it turns slowly reddish even at +4 °C and is responsible for the blank fluorescence increase. Other reagents seem perfectly stable for at least 2 months when stored in the refrigerator.

3.8. Application to real sample analyses

Validation of our methodology has been assessed by application to real sludge samples and comparison with GC measurements. Sludge samples were analyzed on the same day both by microplate measurement and GC analysis (Fig. 6). All results were expressed as acetic acid equivalent so as to compare results obtained with the two different analytical techniques.

As can be seen, VFA concentrations ranged from 0 (for some old samples kept in the fridge for some months) to 5000 mg L^{-1} (acetic acid equivalent). It was interesting to analyze older samples that had no more VFA to check whether other remaining compounds could result in some fluorescent intensity, which was not the case. The more concentrated samples were that from Vinci methanisation facilities which had a high siccity value (around 25%). Regarding individual VFA concentrations (GC data not shown), acetic and propionic acid were in large majority for samples after fermentation tanks (>90%), while other VFA such as butyric and iso-valeric acid also had a significant contribution to total VFA concentration before fermentation (after thickener). RSD for our fluorimetric measurements ranged from 3.9 to 9.0% (3 replicates).

The linear regression line on Fig. 6 shows a very good regression coefficient and a slope very close to unity, proving accuracy of our alternative methodology. The slope value under unity demonstrates a very slight overestimation of VFA concentrations by our fluorimetric procedure, probably due to the presence of non-VFA compounds that can result in minor fluorescence (lactic, formic acid...).

4. Conclusions

The present paper reports the development and the application of a microplate assay for the determination of VFA in various fermentation processes, and especially for application in biogas production where monitoring of VFA is necessary to prevent digester failure. Overall results are nevertheless very promising for a broad range of VFA concentrations, demonstrating that our microplate strategy can be applied successfully to the fast determination of VFA for a large number of samples (up to 24 per microplate with blank, calibration and triplicate analyses of samples on the same microplate within 15 min). This kind of analytical device can provide very interesting alternatives to expensive and time-consuming chromatographic methods, and therefore lead to new prospects in the field of laboratory or on-site analyses.

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