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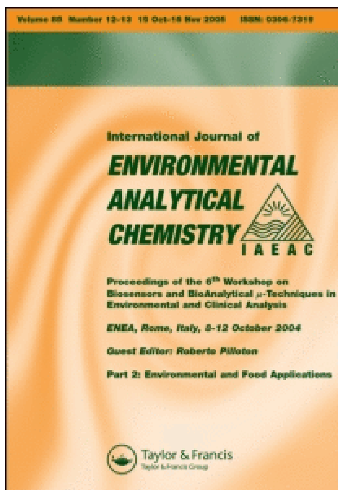

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Volatile fatty acids analysis from pig slurry using high-performance liquid chromatography

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VOLATILE FATTY ACIDS ANALYSIS FROM PIG SLURRY USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A simple and sensitive liquid chromatography method designed to determine the volatile fatty acids (VFA) content in pig slurry sample is proposed. The acids are isolated with a cation exchange column for carbohydrates using an isocratic phosphate eluant coupled with an ultraviolet detector in series. Centrifuged and filtered samples can be injected directly into the liquid chromatographic system. The accuracy of this new liquid-chromatographic chain using spiked solutions ranging between 20 and 5000 mg/L of VFA standard solution varied from 87 to 124%. The precision of the new procedure, expressed as variability, was between 9.2 and 0.6% for a spike solution concentration of 20 and 5000 mg/L, respectively.

Keywords: HPLC; Volatile fatty acid; Pig slurry

INTRODUCTION

Pig production in developed countries is often concentrated in areas with intensive animal production. Animals are housed in confined buildings where slurry is formed by mixing faeces and urine under slatted floors. After a few months, this slurry is generally transferred to a large storage tank outside. Forced by legal constraints for animal waste spreading, storage time has been extended, on a national basis in France, to 4 months, and in Brittany it has been fixed at 6 months [1]. The long storage period favours anaerobic decomposition of animal wastes, where undigested residues like carbohydrates, proteins and lipids contained in the faeces are degraded by microbial activity, and a variety of volatile fatty acids (VFA) are produced, e.g. the acidogenesis phase [2,3]. The concentration ranges of short-chain carboxylic acids (C_2–C_5) are: 2–13 g/L for acetic, 0–3 g/L for propionic, 0–1 g/L for isobutyric, 0–2 g/L

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for butyric, 0–0.5 g/L for isovaleric and 0–0.3 g/L for valeric acid. Usually, total slurry VFA concentrations vary from 5 to 20 g/L [4].

This large quantity of VFAs contained in the slurry may have several environmental and agronomic impacts:

(1) Among the list of roughly 200 compounds which have been identified as odorous compounds in slurry, the VFA group is the largest contributor to odour nuisance [3,5].
(2) Most stored animals wastes are traditionally recycled by spreading onto farmland. This practice may inhibit plant growth [2,6] at levels as low as 0.20 mmol of total VFA per kilogram of soil.
(3) These carboxylic acids are an intermediate in the methanogenic pathway producing carbon dioxide and methane under anaerobic conditions. These gaseous end products are released to the atmosphere, where methane acts as a potent greenhouse gas [7].

Quantification of slurry VFAs is essential for evaluation and reduction of environmental impacts such as odour pollution, plant growth inhibition and methane substrate production. Numerous techniques are available to determine slurry VFAs, the most frequently used being chromatographic techniques followed by colorimetric and titrimetric methods.

Gas chromatography (GC) has long been the preferred method for the separation and quantification of fatty acids. Indeed, this technique, based on the use of a packed or capillary column coupled with a flame ionization detector, allows a high resolution for fatty acid analysis in a complex mixture, including separation of isomers. Limit-analysis determination is possible for sample concentrations lower than 10 mg/L of each VFA [8], and an internal standard is usually added in the sample to correct the reproducibility of the sample volume injection.

Titrimetric [9,10] and colorimetric [11] methods are usually used in the analysis of wastewater. These techniques were developed in parallel with GC methods due to the moderate cost, simple procedure and rapidity of total VFA determination. Several authors have reported that these methods have a detection limit at 40–80 mg/L for the three major fatty acids (acetic, propionic and butyric acids) in wastewater samples [10]. With the titration procedure, a scrupulous attention to pH probe maintenance and calibration was found to be an essential requirement and a high carbonate concentration in the slurry may interfere with VFA determination [10].

With these various analytical methodologies and with a complex physiological fluid like urine–faeces or serum, a sample preparation is required before VFA determination. This preparation involves different steps to purify or concentrate the sample (steam distillation [12], retention by a chromatographic column [13], centrifugation, filtration, ultrafiltration [12], solid-phase microextraction [14] and liquid–liquid extraction). For a single preparation, a combination of several techniques is often used in the analytical procedure.

The aim of this present work is to develop a new high-performance liquid chromatographic (HPLC) method to determine the VFA content in pig slurry. Attention is focused on establishing accuracy, precision and detection limits for six VFAs (acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) added to slurries to assess the effect of the slurry matrix on the outcome of the analysis.

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EXPERIMENTAL

Chemicals and Slurries

High-purity fatty acid standards, orthophosphoric acid and sodium hydroxide were purchased from Sigma Aldrich (USA).

Pig slurry used for all laboratory experiments was collected at a local commercial pig farm. The composition of the slurry (Table I) was determined as follows: for the dry matter the sample was dried at 105°C (Memmert, Germany) until a constant weight was recorded; the total ammonium nitrogen concentration was determined by direct distillation combined with a titration; the total Kjeldhal nitrogen was obtained by acid mineralization followed by a combined distillation and titration (Büchi 324, Switzerland); the chemical oxygen demand was determined by titration after total oxidation; and the total carbon was measured by total oxidation at 1000°C (Skalar, Netherlands). Raw slurry samples were mixed thoroughly before centrifugation at 20 000 *g* and 3°C for 30 min (Avanti J-20, Beckman Coulter, Ireland). The supernatants collected for VFA analysis were diluted five times (1/5 volume per volume) and filtered through a 0.45 μm disk nitro-cellulose filter (Supelco, USA).

In order to obtain a VFA-free slurry, fresh slurry was aerated at a flow rate of 7 L/min of air for 48 h to purge all volatile compounds. Using this short aeration technique, it was possible to produce a raw slurry matrix without volatile compounds [14,15]. The solution of VFAs was neutralized (with sodium hydroxide) and added to the VFA-free slurry to obtain samples with a known amount of VFA. Following this addition of VFA, the slurry sample was centrifuged, diluted and filtered as previously described. Additions were performed to obtain a final concentration of 20, 50, 100, 200, 500, 1000 and 5000 mg of each selected VFA (acetic, propionic, isobutyric, butyric, isovaleric and valeric acid) per litre. Each sample was repeatedly analysed 30 times to determine the accuracy, precision and detection limit of this new HPLC VFA determination method.

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HPLC

VFA was analysed with a Waters (USA) liquid-chromatographic chain (Model 590 pump) equipped with an ultraviolet detector (UV 486) and an automatic autosampler/injector (Wisp 712). A cation-exchange HPLC column for carbohydrates on the H^+ form (Suplecogel 610H, Supelco, USA) was installed in the chromatographic chain [12,16–18]. The column length was 30 cm, and the internal diameter was 7.8 mm. A pre-column with a length of 5 cm and an internal diameter of 4.6 mm composed of the same resin was installed before the analytical column. A six-port,

TABLE I Composition of the pig slurry used in the experiment

Compound	*Pig slurry concentration*
Dry matter (%)	8
Total ammonium nitrogen (mg/g)	3.28
Total Kjeldhal nitrogen (mg/g)	4.92
Chemical oxygen demand (g O_2/L)	25
Total carbon (%)	3.2
Total VFA (g/L)	5.0

two-position, rotary-valve selector (LabPRO, Rheodyne, USA) was positioned between the pre-column and the column, thus allowing partial injection. Organic acids were isolated on an isocratic mobile phase (H_3PO_4 0.1%) at ambient temperature. The total run time was 45 min, and the injection volume was 50 µl. The detector was adjusted at 210 nm, and the signal trace was automatically transferred and integrated with a computer equipped with Millennium software (Waters, USA). Quantification was performed by comparison between the peak area of calibration and peak area of the sample.

Calibration and Quantification

Standard solution mixtures of acetic, propionic, isobutyric, butyric, isovaleric and valeric acids were prepared in pure water at concentrations of 5–10–20–50–100–200–500–1000 mg/L. The linearity of standard curves were determined from an average of 5 injections of each solution mixture. Three calibration curves were used (5–10–20–50; 20–50–100–200; 100–200–500–1000 mg/L) to determine the concentrations of pig slurry VFAs.

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RESULTS AND DISCUSSION

Chromatography Patterns

Typical chromatograms are shown in Fig. 1. Figure 1A represents a standard injection of a mixture of 20 mg/L for each VFAs, while Fig. 1B shows a spiked VFA-free added at a concentration of 20 mg/L of each VFA. Figure 1C shows a routine pig slurry analysis. The Supelcogel 610H satisfactorily separated the VFAs within 34 min. A baseline derivation observed on Fig. 1B during the run did not affect the identification of peaks. Unidentified peaks appeared in the sample chromatogram of spiked VFA-free slurry, probably due to the VFA removal aeration technique, but these peaks did not affect the quantification of the acids added to the slurry. For slurry analysis, the major peak is acetic acid, and the minor peak is valeric acid. Three peaks appeared 37.47, 41.64 and 44.20 min after the VFA peaks with no effect on analysis quality. These compounds were 2-methylvaleric, 4-methylvaleric and caproic, respectively [16]. Without the use of the online pre-column separation, the total analysis time would increase from 34 min to 3 h, with unknown peaks being detected throughout.

Precision and Accuracy

The accuracy and the precision study of the method were determined by spiking a known amount of standard into a VFA-free slurry sample. Thirty replicates were conducted for each sample. The spike recovery rate was calculated to estimate the accuracy of the method, while the precision was estimated using the standard deviation.

To evaluate the VFA removal efficiency of aeration, VFA-free slurry was analysed (data not shown). Two peaks appeared on the chromatogram identified as acetic acid and propionic acid, and the residual concentration of these compounds was 20 and 10 mg/L, respectively. This result shows that although removal of volatile compounds in raw slurry by aeration is an efficient technique, it is difficult to reduce the concentration to almost zero in the aerated slurry because of the large quantities of

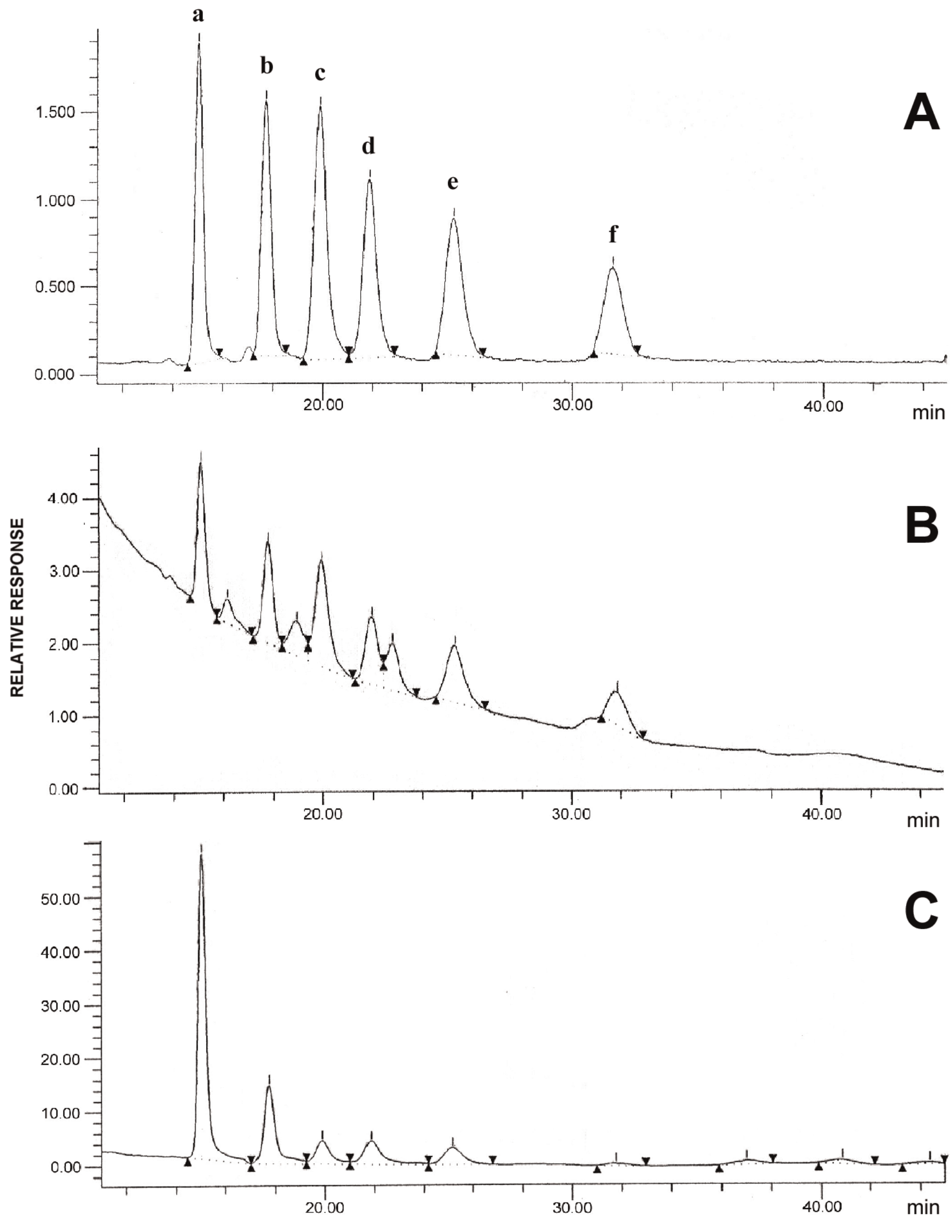

FIGURE 1 Typical chromatograms of VFAs. (A) 20 mg/L VFAs standard solution. (B) Free VFAs slurry samples spiked with 20 mg/L with standards. (C) Routine pig slurry analysis. Peaks: a, acetic acid; b, propionic acid; c, isobutyric acid; d, butyric acid; e, isovaleric acid; f, valeric acid.

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each VFA in the initial raw pig slurry. The residual concentrations of acetic acid and propionic acid remaining in VFA-free slurry were subtracted from the result of spiking solution analysis.

The recovery is expressed as percentage of the VFAs added in the free slurry for the seven levels of concentration (from 20 to 5000 mg/L). The recovery of the spiked slurry sample varied from 87 to 124%. The mean recoveries of total VFAs for each

spike concentration were 104%, 109%, 101%, 100%, 100%, 99% and 97% for 20, 50, 100, 200, 500, 1000 and 5000 mg/L, respectively.

Thirty replicates conducted for the previous accuracy determination study were also used to evaluate the precision of the chromatographic technique. Standard deviations were used as an estimate of the precision of the method. The variability is higher for the lowest concentration, ranging from 5.4 to 9.2% for concentration 20 mg/L, whereas the standard deviation varied between 0.6 and 2.7% for a concentration of 5000 mg/L. The detection limit was determined experimentally for acetic, propionic, isobutyric and butyric acid at 20 mg/L, for isovaleric acid at 50 mg/L and at 100 mg/L for valeric acid in diluted slurries, and the detection limit reached 4, 10 and 20 mg/L, respectively, for a pure acid solution.

CONCLUSION

The proposed analytical procedure for determining VFAs concentrations in pig slurry gives reliable results and works satisfactorily in routine analysis. The main advantage of this method is the simple sample preparation (centrifugation plus filtration) coupled with a direct analysis. Moreover, the detection limits of this new procedure allow further fatty acid quantification in different biological systems.

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