Journal of Chromatography, 236 (1982) 503–507
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,417

Note

Determination of formic acid in aqueous fermentation broth by headspace gas chromatography

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(Received September 28th, 1981)

During the anaerobic digestion of complex macro-molecules free fatty acids are formed initially, with subsequent conversion to methane. The types and levels of fatty acid present give an indication of the state of the fermentation. Hence it is valuable to be able to quantify these acids in samples which are usually highly coloured or cloudy.

The C_2–C_6 fatty acids can conveniently be determined by gas chromatography (GC)[1] using a flame ionisation detector but the determination of formic acid is more difficult.

The detection and determination of formic acid have been achieved by using thin-layer chromatography[2,3], column partition chromatography[4], spectrophotometry[5], ion chromatography[6] and GC. Column and thin-layer chromatography, whilst often giving good results are laborious, demanding assiduous sample preparation, condition control and long development time for optimum separation.

The spectrophotometric method put forward by Lang and Lang[5] has been used in a modified form in this laboratory for 3 years but whilst it is quick and sensitive (lower limit of detection 15 ppm), it is not feasible when dealing with highly coloured or cloudy solutions.

Ion exclusion chromatography avoids these particular disadvantages, but the equipment is currently very expensive and it may be difficult to obtain sufficient resolution from other organic acids.

GC may be used but a major drawback exists in that formic acid gives almost no response when using a flame ionisation detector. Free formic acid has been determined gas chromatographically using an automatic titrator[7], a katharometer[8] and an argon detector[9] but these methods are not applicable to the determination of low levels of formic acid in the presence of substantial quantities of other fatty acids.

Formic acid has been derivatised and thus determined in a FID detector but most of the derivative procedures are long and complicated[10–12]. However, Sizova *et al.*[13] put forward a method for the determination of formic and acetic acids and formaldehyde in aqueous media. This has been modified, such that formic acid may be determined in the presence of other fatty acids, down to a lower detection limit of 50 ppm.

0021-9673/82/0000–0000/$02.75

EXPERIMENTAL

Chemicals

Ethanol, Synthetic grade was obtained from BP Chemicals, methanol, Ultra grade, toluenesulphonic acid, formic acid and acetic acid, all Analar reagents, from Hopkins & Williams. A 5% (w/v) Toluenesulphonic acid solution was made up in methanol.

Instrumentation

A Pye 104 gas chromatograph, fitted with a heated FID and a wide range amplifier was used. Initially stainless steel columns (1.8 m × 4 mm I.D.) packed with 20% Carbowax 400 on Chromosorb W AW DMCS (80–100 mesh) were utilised, the oven temperature being 80°C. Latterly glass columns (1.5 m × 4 mm I.D.) packed with acetone extracted Porapak Q or QS were utilised. The oven temperature was 100°C and the injection port and detector 200°C and 260°C respectively. The carrier gas was helium, flowing at 30 ml/min.

Derivatisation

The broth samples were spun at a relative centrifugal force of 12,000 × *g* for 10 min, to provide a supernatant solution for analysis. The reaction was carried out in 1-oz McCartney bottles fitted with neoprene septa inside the metal caps.

A 1.4-ml volume of Ultra grade methanol was pipetted into the bottle; 0.5 ml of 5% toluenesulphonic acid in "Ultra" methanol were added followed by 0.5 ml of the sample supernatant. The bottles were capped, shaken momentarily by hand and heated at 80°C for 30 min in a DRI-BLOCK heater. A beaker, inverted over the bottles produced a draught-free enclosure which was easily removed for sampling. A 0.5-ml volume of the headspace vapour from each bottle was injected into the gas chromatograph using a nylon syringe. The syringe was washed with methanol and dried between injections to minimise carry-over and cross-contamination. The use of Ultra-grade methanol for the analysis is preferred as it gives a lower blank.

Quantitation

Standard solutions of formic acid were prepared over the range of concentrations 0.2–1.0 g/l. These were derivatised and the headspace samples injected as described above. A calibration graph was constructed by plotting the peak height obtained against the formic acid concentration. A typical result is shown in Fig. 3.

Thereafter a standard was included with each batch of samples to check that the calibration was still valid.

RESULTS AND DISCUSSION

The initial paper by Sizova *et al.*[13] described a method whereby formic and acetic acids and formaldehyde were reacted with ethanol in the presence of *p*-toluenesulphonic acid as a catalyst, forming ethyl formate, ethyl acetate and diethoxymethane respectively. When the solution from a mixture of formic acid and formaldehyde treated this way was chromatographed on a Carbowax 400 column it produced the chromatogram shown in Fig. 1. It was found that frequent injections of the acidic solution rapidly destroyed the column.

In order to overcome this problem and noting that the reaction products are volatile, it was decided to try a headspace method of analysis. Use of ethanol as the esterifying alcohol necessitated the use of heated sampling syringes to overcome the problems of condensation. However, the use of methanol as the esterification alcohol minimises this problem since the boiling point of methyl formate is 31.5°C.

The chromatogram given by the use of the headspace method on a standard solution of formic acid at a concentration of 1 g/l is shown in Fig. 2.

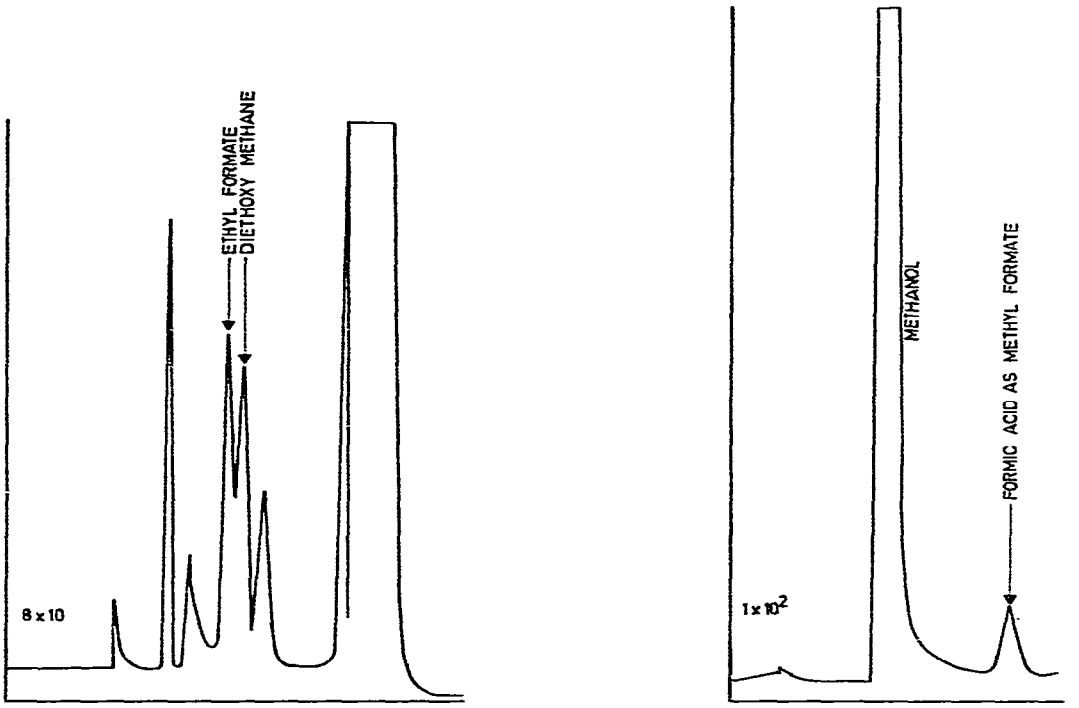

Fig. 1. Chromatogram of formic acid and formaldehyde reacted according to Sizova's method. Column: 1.8 m × 1/4 in., stainless steel. 20% Carbowax 400 on Chromosorb W AW DMCS (80–100 mesh). Temperatures: oven, 80; injector, 200; detector, 260°C. Chart speed: 0.25 in./min. Carrier gas (helium) flow-rate: 30 ml/min.

Fig. 2. Headspace analysis of formic acid at the level of 1 g/l. Column: 6 ft. × 2 mm I.D., Porapak QS (80–100 mesh). Oven temperature: 100°C other details as in Fig. 1.

Control of the temperature during equilibriation of the gas phase, and the salts concentration in the liquid phase are the two primary requirements for consistent headspace analysis. The simple draught shield described, minimises the effect of temperature variation. The high level of *p*-toluenesulphonic acid fixes the salts level of the final solution such that variations in the salts level of individual broth samples have a negligible effect on the partition of the volatile components.

The calibration graph (Fig. 3) demonstrates good linearity for the method up to 1 g/l indicating a consistent partition of methyl formate into the vapour phase, and good sampling of that phase by the syringe technique described.

The minimum detection level of formate is 0.02 g/l with this sytem. If greater

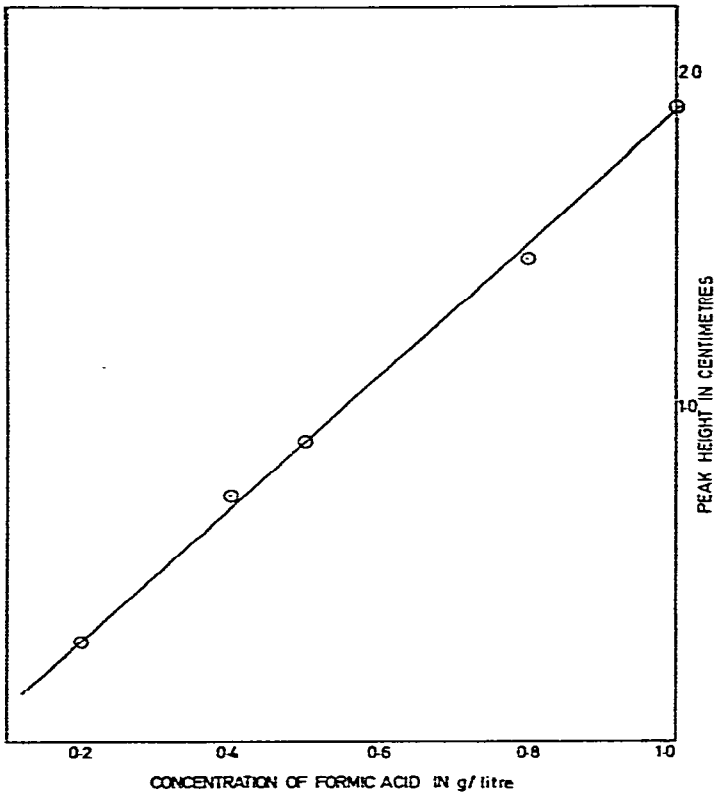

Fig. 3. Calibration graph for the determination of formic acid in aqueous solution.

sensitivity is required a column offering better resolution of the methyl formate would be required.

The standard addition technique should be used if samples are encountered with very variable salt concentrations.

Sizova's original work covered samples within the concentration range 3–15 g/l. Using this headspace method over the range 0.1–1.0 g/l and with standards run with each batch of samples, it has been found that the repeatability between triplicate analysis is of the order of 5%.

It should be noted that both nylon and glass syringes are compatible with this method, but the ester has a pronounced solvent effect with many other plastics.

This headspace modification of Sizova's method has provided a particularly advantageous technique for the measurement of formic acid in fermenter broths. The sample manipulations are simple, and directly applicable to coloured, heterogeneous broths, total analysis time is 45 min and standard GC apparatus can be used with no ghosting problems as often experienced with the free acid.

The method has been used routinely in this laboratory for 2 years in the analysis of anaerobic effluent digester systems.

ACKNOWLEDGEMENT

Permission to publish this paper has been given by The British Petroleum Company Limited.

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