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Short Communication

Direct aqueous injection gas chromatography on a potassium fluoride crystal hydrate-containing sorbent

Determination of volatile organic solvents in the fermentation broth of *Clostridium* strains

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ABSTRACT

For the direct aqueous injection gas chromatographic determination of volatile organic solvents (acetone, ethanol, butanol) in culture spent media of *Clostridium acetobutilicum*, a sorbent which contains potassium fluoride crystal hydrate and a conventional stationary phase, Triton X-305, is used. Analysis is carried out using a stainless-steel column packed with the developed sorbent. The total time of analysis is less than 5 minutes at a column temperature of 65°. The column is stable for at least 1 year.

INTRODUCTION

The biotechnological production of volatile organic solvents, such as acetone, ethanol, isopropanol and butanol, is based on anaerobic fermentation of different strains of *Clostridium* on carbohydrate-rich media, such as molasses, starch, hydrolysed wood, straw and corn. Solvents such as acetone and butanol can be obtained at concentrations up to 20 g/l [1–4].

For the selection of suitable strains of microorganisms and optimization of the fermentation conditions, fast and convenient analytical methods are necessary. Usually gas chromatography (GC) is used for this purpose. The samples are aqueous systems which consist of the different components, including microbial cells, high-molecular-mass compounds such as proteins and nucleic acids, low-molecular-mass, non-volatile compounds including amino acids, sugars and salts and volatile organic compounds such as aldehydes, alcohols and lower carboxylic acids. Quantitative isolation of the volatile fraction from such matrix is complex and time consuming [5–7]. The preferred method of analysis is based on the direct injection of aqueous samples into the chromatographic column. When the direct injection of aqueous solutions is necessary, columns packed with porous polymeric sorbents (*e.g.*, Porapak, Chromosorb "Century" series, Tenax GC) are mostly used [8,9].

The utilization of such sorbents leads to specific problems. Owing to the very high tendency for dispersive interactions, the determination of organic solvents in *Clostridium* spent media on porous poly-

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mers is usually carried out at relatively high temperatures (120–200°C) [1–4]. At these temperatures thermolabile compounds in samples of natural origin may degrade, with the formation of volatile products.

Recently, we demonstrated the possibility of using sorbents that contain potassium fluoride crystal hydrate (m.p. 42°C) and conventional stationary phases for the direct aqueous injection GC of polar compounds such as alcohols and amines [10]. With such columns it was possible to determine polar compounds in aqueous solutions with high sensitivity and under mild analytical conditions.

The aim of this work was to use these sorbents for the rapid analysis of the production of the volatile organic solvents acetone, ethanol and butanol by *Clostridium* strains.

EXPERIMENTAL

A stainless-steel column (200 cm × 3 mm I.D.) with a sorbent which contains 5% of Triton X-305 and 5% of KF · $2H_2O$ (per unit weight of solid support) on Chromosorb W AW (60–80 mesh) was packed and conditioned as described previously [10].

A Varian Model 3600 gas chromatograph with a flame ionization detector was used for the measurements. The incorporated data handling system with a thermal printer–plotter served as a recorder–integrator. The temperatures of the column, injector and detector were 65, 120 and 250°C, respectively, the flow-rate of helium carrier gas was 30 ml/min and the flow-rates of hydrogen and air were 30 and 300 ml/min, respectively.

C_2–C_4 alcohols and acetone were obtained from Merck or Aldrich and used as received. The test compounds were injected as aqueous solutions using 10- and 50-μl microsyringes (Hamilton).

Different strains of *Clostridium* were cultivated as described previously [1]. Samples of fermentation media were centrifuged using a Model 5414S microfuge (Eppendorf). The volume of sample injected was usually 1 μl. Quantification of samples was effected using the external standard method. A standard mixture was injected at the start and end of the working day.

RESULTS AND DISCUSSION

Preliminary experience with model mixtures demonstrated that the developed sorbent is suitable for the separation of volatile compounds produced by *Clostridium* strains. On a 2-m column packed with Triton X-305–KF · $2H_2O$, up to a baseline separation of solutes of interest (acetone, ethanol and butanol) is obtained within 5 min at a column temperature of 65°C, which is about 100°C lower than when polymeric sorbents are used (Fig. 1). A linear dependence between concentration of volatile compounds and peak areas was achieved in the range 0.1–20 g/l. Therefore, it is possible to exclude the stage of sample dilution and inject the samples directly. With this direct injection of supernatants of centrifuged samples, the total time of analysis is decreased and the precision increases.

Fermentation media of different strains of *Clostridium acetobutilicum* were used to test the ability of the studied columns for direct aqueous injection GC of biotechnological samples (Fig. 2). Usually 1-μl samples were injected but it was possible to inject up to 50 μl of fermentation broth without any visible change in the properties of the column.

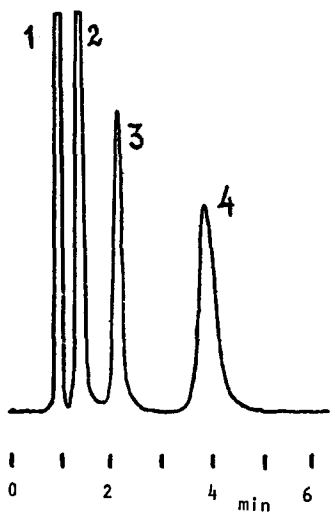

Fig. 1. Separation of a model mixture. Column: 200 cm × 3 mm I.D. stainless steel. Sorbent: 5% Triton X-305 and 5% KF · $2H_2O$ on Chromosorb W AW (60–80 mesh). Column temperature: 65°C. Sample: 1 ml of aqueous solution containing *ca.* 1 g/l of each of the test compounds. Total analysis time: 5 min. Peaks: 1 = acetone; 2 = ethanol; 3 = propanol; 4 = butanol.

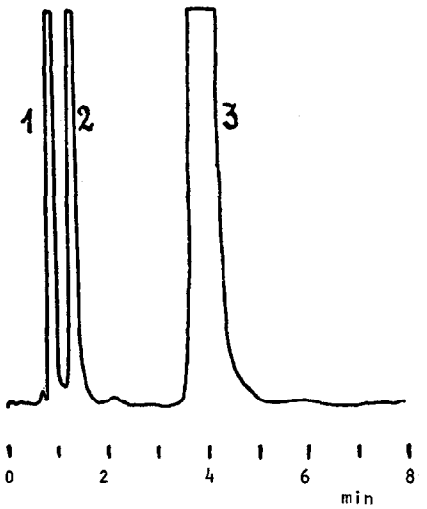

Fig. 2. Analysis of samples of fermentation broth of an industrially used *Clostridium* strain. Column and temperature as in Fig. 1. Sample: 1 ml of fermentation broth. Peaks: 1 = acetone; 2 = ethanol; 3 = butanol.

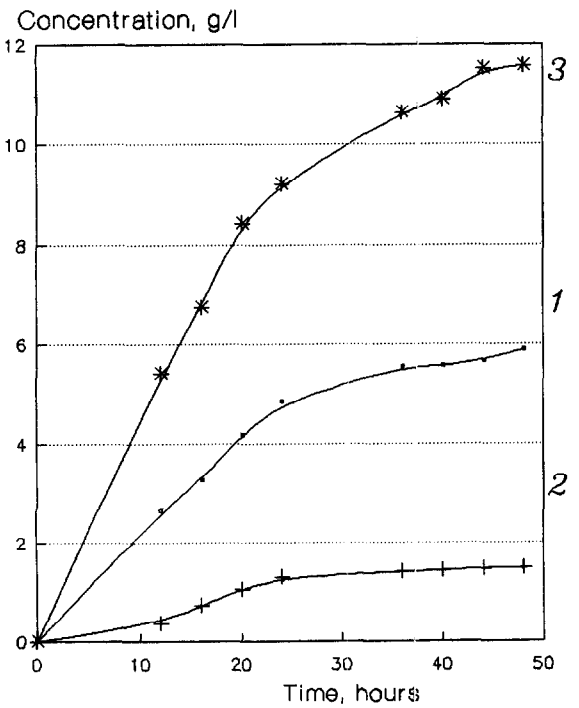

Fig. 3. Kinetics of the accumulation of organic solvents by *Clostridium acetobutilicum*. 1, Acetone; 2, ethanol; 3, butanol.

With this column about 50 strains of *Clostridium* obtained from soil samples were tested. Among these, strains producing up to 10 g/l of butanol were found. This column also was used to test the influence of fermentation conditions on the production of volatile solvents by industrially used strains of *Clostridium acetobutilicum*. The kinetics of the production of organic solvents with these strains were studied (Fig. 3). It was found that tested strains produce most of the organic solvents during first 36 h of fermentation.

During a 1-year period about 500 samples of fermentation media were analysed on a column packed with Triton X-305–KF · $2H_2O$ without visible deterioration of the peak shape or symmetry.

CONCLUSIONS

A direct aqueous injection GC method for polar volatile solvents in fermentation media of *Clostridium* strains using a sorbent which contains potassium fluoride crystal hydrate and Triton X-305 has been developed. A rigid stainless-steel column was used instead a fragile glass column. Considering the possibility of injecting samples of fermentation broth directly and carrying out GC under relatively gentle conditions, this sorbent may be recommended for the determination of volatile polar compounds in different biotechnological samples. Such columns may also be useful in environmental pollution control, forensic studies and other tasks connected with the GC analysis of aqueous samples.

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