Journal of Chromatography, 259 (1983) 508–510
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,619

Note

Rapid gas chromatographic analysis of residual hydrocarbons in fodder yeast

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(Received December 14th, 1982)

About 2,000,000 tons of protein for animal feeding are produced microbiologically, *i.e.*, by culturing microorganisms on various substrates, including oil hydrocarbon fractions. The residual hydrocarbon content is an essential characteristic of the commercial product.

To analyze the content of residual hydrocarbons in the final product, the method recommended by IUPAC is usually employed[1]. It is based on alkaline hydrolysis of microbial biomass, paraffin extraction with hexane and gas chromatographic (GC) analysis of the extract. Biomass hydrolysis is necessary because the bulk of hydrocarbons immobilized in cell biopolymer structures is not extracted unless cell material is destroyed. Quantitative analysis by the IUPAC method takes about 5–6 h and, hence, it is not suitable for immediate control of the product quality.

We propose a method of residual hydrocarbon analysis which is simpler and may be automated[2]. It is based on hydrocarbon release due to thermal destruction of the microbial biomass without pyrolysis of hydrocarbons, and sorption of the products of thermal destruction on aluminium oxide. The hydrocarbons are selectively desorbed with a suitable solvent, *e.g.*, normal paraffins C_6–C_8.

EXPERIMENTAL

A known amount of the internal standard, *e.g.*, *n*-docosane dissolved in *n*-hexane, was placed in a glass reaction vial (4 × 50 mm), followed by a sample of biomass (usually *ca.* 10–30 mg). The vial was then gently tapped to compact the sample had become dark brown. The vial was then cooled to room temperature and 0.15–0.2 ml *n*-hexane or other volatile hydrocarbon were added. The contents of the vial were stirred with a rigid steel wire for 0.5 min; after precipitation of alumina, an aliquot of the sample (usually 0.5–2 μl depending on the hydrocarbon concentration) was removed and analyzed by GC. The analysis was performed using a Hewlett-Packard Model 5830 instrument equipped with a flame ionization detector and a glass column coated with OV-101 (10 m × 0.2 mm). Other conditions: carrier gas, helium; injection with splitting ratio 1:50; temperature program, from 120 to 240°C at 8°/min.

0021-9673/83/$03.00

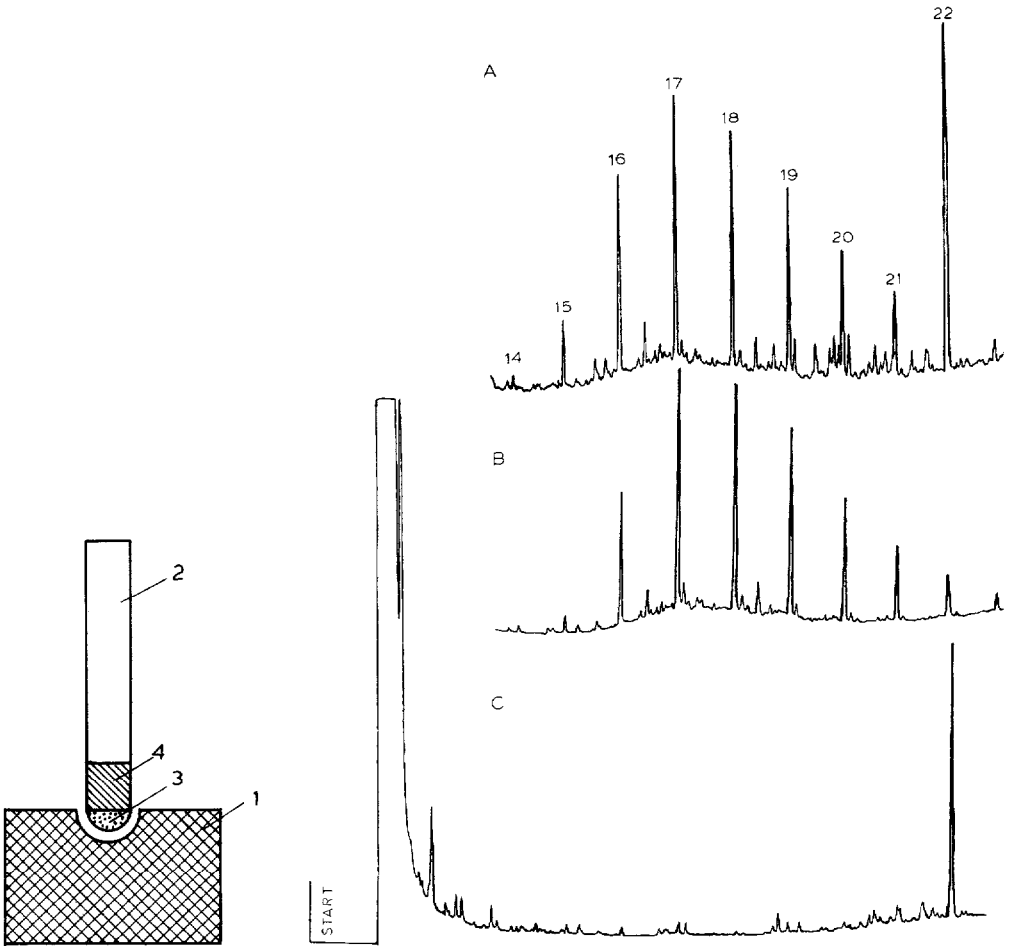

Fig. 1. Apparatus for hydrocarbon isolation by thermal desorption method. 1 = Heater block; 2 = reaction vial; 3 = biomass sample; 4 = alumina.

Fig. 2. Chromatograms of residual hydrocarbons isolated from fodder yeast by thermal desorption method (A) and the IUPAC method (B), and of a sample isolated from yeast *Hansenula polymorpha* DL-1 obtained by thermal desorption (C). Amounts: lyophilized biomass of *H. polymorpha* DL-1, 26 mg; *n*-docosane (C_{22}) internal standard, 18.2 μg; alumina, 70 mg. Peak numbers correspond to the number of C atoms in paraffin molecules.

RESULTS AND DISCUSSION

Gas chromatograms of residual hydrocarbons isolated from the same sample of yeast biomass (Kstov Plant, the Estonian SSR) analyzed by our method and by the IUPAC method[1] are shown in Fig. 2A and B respectively. The distribution of *n*-hydrocarbons in both chromatograms is similar, except for the lower contents of *n*-pentadecane and *n*-hexadecane in Fig. 2B and some additional minor components, which do not influence the quantitative analysis of *n*-hydrocarbons, Fig. 2A. The

total amount of *n*-paraffins determined by the thermal desorption method and the IUPAC method was 0.138% and 0.127% (w/w) respectively.

The analysis reproducibility is not more than ± 5% (relative). As was demonstrated in blank experiments, hydrocarbons were not destroyed during the analysis. The chromatogram shown in Fig. 2C also indicates that no *de novo* production of hydrocarbons occurs during thermal desorption of yeast biomass. This chromatogram is for a sample of hydrocarbons isolated from yeast *Hansenula polymorpha* DL-1 cultures in a mineral medium supplemented with methanol as substrate. The preparation of the medium and growth conditions were as previously described[3].

CONCLUSIONS

Values of the residual hydrocarbon content determined by our method are greater than those by the IUPAC method (see Table I).

TABLE I

PERCENTAGES OF RESIDUAL *n*-PARAFFINS IN FODDER YEAST DETERMINED BY THERMAL DESORPTION AND IUPAC METHODS

Method	*Sample No.*				
	1	*2*	*3*	*4*	*5*
Thermal desorption	0.49	0.28	0.44	0.12	0.19
IUPAC	0.48	0.19	0.56	0.10	0.14

REFERENCES

1 *Total Residual Hydrocarbons and Residual Aromatic Hydriocarbons; IUPAC Inform. Bulletin Technol., Report N 12*, 1974, p. 20.
2 V. K. Eroshin, E. B. Danilova and G. K. Skryabin, *Mikrobiologiya*, 36 (1965) 77.