Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 32-35

BIOGAS (NATURAL GAS?) PRODUCTION BY ANAEROBIC DIGESTION OF OIL CAKE BY A MIXED CULTURE ISOLATED FROM COW DUNG

K. G. Gollakota* and B. Jayalakshmi

Microbiology and Biochemistry Laboratory
A. P. Agricultural University
Rajendranagar, Hyderabad-500 030, India

Received November 5, 1982

SUMMARY: Starting with cow dung, a mixed culture capable of producing biogas by the anaerobic digestion of castor cake (oil expelled) has been isolated and stabilized. The biogas so produced contains small quantities of ethane, propane and butane in addition to methane and carbon dioxide which are the major constituents. This suggests that the mixed culture contains organisms hitherto unisolated and unidentified which are capable of synthesizing these hydrocarbons through the mediation of the alkyl derivatives of coenzyme M.

The possibility of methane generation from oil cake has long been recognized because any organic matter that can be degraded to organic acids can be a potential source of methane (1). However, there have been very few reports of methane production from oil cakes. Sarson oil cake has been used in a study on biogas production from mixtures of cow dung and agricultural wastes (2). Oil cake (type not specified) is one of the major constituents in the Hungarian process (3). Methane production from mohua cake has also been reported (4).

Because of the urgent need to develop alternate, renewable sources of energy, investigations were undertaken to produce methane from nonedible oil cakes which, in terms of potential availability, are second only to cow dung and are being merely ploughed in as organic manures primarily for their nitrogen content. From cow dung a mixed culture capable of producing biogas (70% methane) from castor cake has been isolated and stabilized. The notable feature of this fermentation is the presence of the lower aliphatic hydrocarbons in addition to methane in the biogas.

MATERIALS AND METHODS

Isolation of mixed culture. Slurry of cow dung (1:1) was allowed to ferment anaerobically at room temperature (20 to 30 $^{\circ}$C) until biogas production started.

*To whom all correspondence should be addressed.

0006-291X/83/010032-04$01.50/0

Starting with such an active culture and following the usual enrichment techniques, we have successfully isolated and stabilized a mixed culture capable of producing biogas from castor cake alone.

Production of biogas. Castor cake is ground and made into a slurry of water (8% solids) and inoculated with the mixed culture and allowed to ferment anaerobically at room temperature. Every 24 hours one fifteenth of the mixture being fermented was replaced with fresh castor cake slurry.

Analysis of biogas. Carbon dioxide content was determined by absorption over 40% potassium hydroxide. For hydrocarbon analysis, the gas was first flushed with 20% potassium hydroxide, 0.5% ammoniacal cuprous chloride and 0.5% potassium periodate to remove carbon dioxide and carbon monoxide. The residual gas was subjected to Janak - carbon dioxide chromatography for detection of the lower aliphatic hydrocarbons (5). This method employs an activated alumina column at room temperature with carbon dioxide as carrier gas at a flow rate of 60 ml/min. The column was previously calibrated with pure gases.

RESULTS

The biogas sample contained 30% carbon dioxide by volume, the rest being a mixture of the lower hydrocarbons. As shown in Figure 1, the hydrocarbon mixture contains 0.46% ethane (retention time 3 min.), 0.19% propane (retention time 6 min.), and 0.23% butane (retention time 10.5 min.) by volume, the rest being methane (retention time 2 min.), not shown in the chromatogram. Occasionally traces of other four-carbon hydrocarbons, probably butylene, could also be detected.

DISCUSSION

Natural gas, the major constituents of which are methane and carbon dioxide, also contains minor amounts of the lower aliphate hydrocarbons. Though biological degradation of organic matter is considered one of the possible mechanisms for the formation of natural gas, it is not considered probable because so far ethane, propane and butane have not been detected among the products of methanogens in nature. We believe we are the first to demonstrate the presence of these hydrocarbons in a biogas sample and this raises some interesting possibilities worth further investigation.

The two well established mechanisms for methanogenesis are 1) reduction of carbon dioxide and 2) transfer of the methyl group of acetate (6). Obviously, hydrocarbons other than methane cannot arise by the reduction of carbon dioxide. This then leaves the possibility of alkyl transfer as the probable mechanism for the formation of ethane, propane and butane by the organism(s) in our mixed culture. This inescapable inference inexorably leads to some musings on the possible role of CoM in the formation of these hydrocarbons. One possibility is the

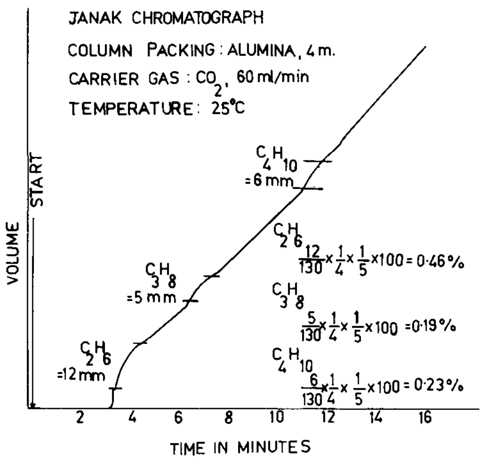

Figure 1: CHROMATOGRAM OF BIO-GAS SAMPLE

reaction between two molecules of methyl CoM mediated by some enzyme system much in the manner of the Wurtz reaction to give ethane. To account for the formation of propane and butane it is necessary to assume the formation of ethyl CoM in addition to methyl CoM and a Wurtz type reaction between them. Another possibility is the formation of a number of alkyl CoM derivatives each giving rise to the corresponding hydrocarbon by transferring the alkyl group to hydrogen in just the same manner as methyl CoM gives rise to methane. No matter what the mechanism involved may be, the mixed culture isolated by us must contain organism(s) hitherto unisolated and unidentified. Vigorous studies are underway to ascertain how generalized this phenomenon is. However, with the limited facilities available, it is hopeless to attempt to isolate the organism(s) or unravel the biochemical mechanism(s). It is hoped that this will interest some of the leading investigators in some of the well equipped laboratories to undertake collaborative studies of this extremely interesting and intriguing phenomenon.

ACKNOWLEDGEMENTS

This work was supported by grants from the Indian Council of Agricultural Research and the Council of Scientific and Industrial Research, New Delhi. The facilities extended by the A. P. Agricultural University and the assistance of the Regional Research Laboratory, Hyderabad in the analysis of the biogas samples are gratefully acknowledged.

REFERENCES

1. Methane generation from human, animal and agricultural wastes (1977), National Academy of Sciences, Washington, D. C., p. 10, 28.

2. Laura, R. D. and Idnani, M. A. (1971) J. Sci. of Food and Ag., India 22, 164-167.
3. Biogas Technology and Utilization (1981) A status report published by the Dept. of Sci. and Tech., Govt. of India, New Dehli, 65-70.
4. Ibid, 64.
5. Jacob, G. and Oberhausen/Rhid (1972) G.I.T., special laboratory (Dowstadt, P. B. 572, Germany) 10, 1139-1145.
6. Wolfe, R. S. (1971) Adv. Microbial Physiol. 6, 107-146.