SHORT COMMUNICATION

J. Gen. Appl. Microbiol., **14**, 447–450 (1968)

THE UTILIZATION OF METHANE, ETHANE AND PROPANE BY SOIL MICRO-ORGANISMS

P.G. BRISBANE AND J.N. LADD

C.S.I.R.O. Division of Soils, Adelaide, South Australia

(Received June 11, 1968)

The bacteria obtained from soils enriched with gaseous alkanes have been studied by various workers (1–4). The substrate specificity of these bacteria is of considerable interest as LEADBETTER and FOSTER (5) have shown that *Pseudomonas methanica* will not grow on ethane, propane and butane but will co-oxidise these alkanes whilst growing on methane. This paper describes the utilization of mixtures of gaseous alkanes by the micro-flora of a wide range of soils.

Duplicate top-soil samples were collected from seventeen sites at eleven locations near Adelaide. At three sites sub-soils were also sampled. Within ten days of collection, the soils (Table 1) were assayed for their ability to oxidise gaseous alkanes using the following method.

Each sample of soil was sieved and mixed. A sub-sample (20 g) was placed in a bottle (250 ml capacity), covered with 30 ml of mineral salt solution and sealed with a rubber bung fitted with a glass tube stoppered by a SUBASEAL* (6, 7). The bottles were evacuated and filled with a gas mixture containing 0.6% methane, 0.3% ethane and 0.2% propane in air and incubated as sets of shaken and still cultures at 25° for 69 days. After 21 and 45 days' incubation, all bottles were re-evacuated and refilled with the mixture of gaseous substrates. In addition, five soils were incubated as shaken cultures with individual alkanes at concentrations of 0.6% methane, or 0.3% ethane or 0.2% propane in air. Controls contained alkane(s) and mineral salt solution but no soil.

At least once each week a 250 μ l sample of the atmosphere within each bottle was removed with a hypodermic syringe, via the Subaseal stopper, and injected into a F & M model 609 flame ionization gas chromatograph. The alkanes were separated at room temperature on a column containing 30% (w/w) silicone oil DC 200 supported on Chromasorb P. Alkane concentrations were estimated by measuring peak heights (6, 7).

* Subaseal stoppers obtained from W. Freeman & Co., Ltd., Barnsley, Yorks, U.K.

Table 1. Properties of soils.

No.	Location	Soil Group	Sampling depth (cm)	pH	Clay ^a (%)	Organic carbon ^a (%)	Field moisture ^a (%)
1	Litchfield	Lateritic podzolic	0-5	5.9	14	2.3	17
2	"	"	0-5	5.8	13	6.2	35
3	Urrbrae	Pond mud	0-5	7.9	49	2.5	126
4	"	Red brown earth	0-5	7.7	16	2.4	8
5	"	"	60-65	6.7	59	0.6	18
6	Murray Bridge	Neutral peat	0-5	7.2	43	6.1	38
7	O'Halloran Hill	Grey clay	0-5	8.0	36	3.1	41
8	"	"	60-65	8.7	35	1.6	30
9	Mt. Compass	Siliceous sands	0-5	5.1	5	2.4	19
10	"	"	0-5	5.6	2	0.8	5
11	"	"	0-5	5.7	6	4.6	14
12	"	Acid peat	0-5	4.5	26	40.0	196
13	Mt. Magnificent	Yellow podzolic	0-5	5.2	10	5.1	32
14	Claremont	Wiesenboden	0-5	6.9	31	0.4	26
15	"	"	60-65	7.8	62	1.1	35
16	Bull's Creek	Terra Rossa	0-5	6.5	37	5.4	33
17	"	Black earth	0-5	5.4	27	11.0	53
18	Kyeema	Alluvium	0-5	6.3	31	2.7	28
19	Page's Flat	Humic grey	0-5	6.8	11	4.9	89
20	Monarto	Solonized brown soil	0-5	7.7	1	0.1	1

^a Percentages are expressed on the basis of weight of dry soil.

As there were three separate gassings during the experimental period, graphs were constructed showing the alkane concentration at various times during the incubation. The utilization of an alkane by a particular soil microflora was estimated by measuring the area under the curve and subtracting this from the area under the control curve, this difference being expressed as a percentage of the control curve. Duplicates were averaged. The results for shaken suspensions are set out in Tables 2 and 3.

The unshaken soil suspensions were less active than shaken suspensions, but the relative activities of the various soils were similar. There was no close relationship between any of the measured soil properties and alkane utilization. Top-soils were more active than sub-soils, and soils that were wet or moist when sampled were more active than dry soils.

There was some variation in the alkane utilization by duplicate samples from the same soil, but in any particular bottle which contained a mixture of alkanes, methane and ethane were utilized simultaneously, whereas propane was utilized at a later stage. A co-variance analysis of the data summarised

Table 2. Utilization* of mixtures of gaseous alkanes.

Soil No.	Methane utilization	Ethane utilization	Propane utilization
1	22	25	10
2	36	33	13
3	84	79	53
4	21	28	18
5	4	7	7
6	83	79	68
7	56	55	42
8	32	32	12
9	71	70	36
10	52	49	15
11	49	48	20
12	86	84	50
13	54	54	31
14	52	42	5
15	6	8	6
16	59	58	37
17	45	47	28
18	59	60	34
19	68	68	37
20	3	9	9
Standard error of mean	10	8.4	5.9

* Area under a time $\times$ concentration curve, expressed as % of control.
Average of two replicates.

Table 3. Utilization* of single gaseous alkanes.

Soil No.	Methane Utilization	Ethane Utilization	Propane Utilization
1	10	18	0
3	73	28	53
4	55	17	15
7	61	13	43
9	75	20	4
Standard error of mean	8.6	9.3	7.0

* Area under a time $\times$ concentration curve, expressed as % of control.
Average of two replicates.

in Table 2 showed significant between-soil correlations for the utilization of methane and ethane ($r=0.99$), methane and propane ($r=0.88$), and ethane and propane ($r=0.84$). When the differences due to soils were removed, the

relationship between methane and ethane remained high ($r=0.96$), but was considerably lowered between methane and propane ($r=0.27$), and ethane and propane ($r=0.33$).

The average utilization of alkanes by the five soils tested both with mixed and single gases is shown in Table 4. When methane was present as the sole alkane substrate, or as part of a mixture of alkanes, methane oxidation was similar. Likewise, propane utilization was similar whether propane was present as the sole substrate or as part of a mixture of alkanes. By contrast, ethane was utilized relatively poorly when supplied as the sole substrate. It is concluded that when low concentrations of mixed gaseous alkanes are added to soil, methane-utilizing microorganisms will co-oxidise ethane, whereas propane appears to be utilized by different microorganisms. When ethane is the sole substrate it is utilized by those bacteria that grow on ethane, and they are less abundant than methane-utilizing bacteria.

Table 4. Average utilization* of alkanes by five soils.

Substrate(s)	Methane Utilization	Ethane Utilization	Propane Utilization
Mixed alkanes	51	51	32
Single alkane	55	19	23

* Area under time $\times$ concentration curves, expressed as percentages of controls.

Ethane-utilizing bacteria are found in large numbers over certain oil fields (1, 2, 8), and it is generally assumed that this is due to their growth on ethane. If ethane is co-oxidised by *Pseudomonas methanica* in these soils (methane-utilizing bacteria are plentiful in these areas (8)), then it is likely that the actual substrates for ethane-utilizing bacteria are acetic acid and acetaldehyde and ethanol (5).

We wish to acknowledge the assistance received from Mr. A.R.P. Clarke and Mr. S. McLeod who performed the soil analyses. Mr. K. Cellier analysed the data, and Mr. R. Maude and Mr. G. Blackburn selected and described the soils. Mr. M. Amato provided skilled technical assistance.

REFERENCES

- 1) P.G. BRISBANE and J.N. LADD, *Ann. Rev. Microbiol.*, **19**, 351 (1965).
- 2) A.A. KARTSEV, Z.A. TABASARANSKII, M.I. SUBBOTA and G.A. MOGILEVSKII, *Geochemical Methods of Prospecting and Exploration for Petroleum and Natural Gas*, Univ. California Press, Berkeley (1959).
- 3) J.W. FOSTER, *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **28**, 241 (1962).
- 4) H. IIZUKA and K. KOMAGATA, *J. Gen. Appl. Microbiol.*, **2**, 103 (1965).
- 5) E.R. LEADBETTER and J.W. FOSTER, *Arch. Mikrobiol.*, **35**, 92 (1960).
- 6) P.G. BRISBANE and M. AMATO, *Soil Biology*, **6**, 39 (1966).
- 7) P.G. BRISBANE, M. Sc. (Agric.) *Thesis, University of Western Australia*, (1965).
- 8) J.B. DAVIS, R.L. RAYMOND and J.P. STANLEY, *Appl. Microbiol.*, **7**, 156 (1959).