Chapter 5

EFFECTS OF SOIL PROPERTIES, ORGANIC MATERIALS AND TEMPERATURE ON THE KINETICS OF ALCOHOLS IN SUBMERGED SOILS

SUMMARY

Alcohols (methanol and ethanol) were formed in significant amounts in submerged soils only when 1% green manure (Gliricidia sepium) was added, and the addition of rice straw (0.25%) did not favor the formation of alcohols. In soils amended with 1% green manure, the order in which peak concentrations of alcohols were formed seems to reflect how easily each soil is reduced. Alcohol formation attained peaks earliest in a calcareous soil with high amount of easily decomposable organic matter and low amount of active iron, and last in an acidic ferruginous soil. Metabolism of alcohols in submerged soils was very fast. Even in an acidic ferruginous soil, methanol concentration attained a peak after 3 days at 35°C and after 6 days at 20°C. Ethanol disappeared faster, and attained a peak after 1 day at 35°C and after 4 days at 20°C in the acidic ferruginous soil. Formed amounts of methanol and ethanol at 35°C were at most 8.4 ppm and 5.3 ppm, respectively, and much smaller than acetic acid (75 ppm) which was formed during the same period.

INTRODUCTION

Methanol, ethanol, n-propanol, and n-butanol were detected and quantified in soils treated with leaves of sugarcane and Crotalaria jungcia by Wang et al. (1967). Isopropanol, isobutanol, sec-butanol (Adamson et al, 1975) and 2, 3-butane-diol (Kubota and Furusaka, 1981) were detected in soils treated with glucose and incubated anaerobically.

Alcohols were found in both submerged and aerobic soils (Wang et al., 1967), but formation and decomposition of alcohols proceeded more slowly in aerobic soils than in submerged soils. Alcohols were not detected in soils which did not receive glucose or other carbonaceous amendments (Adamson et al., 1975; Kubota and Furusaka, 1981). In spite of above informations, kinetics of alcohols have not been studied so intensively as volatile fatty acids, probably because they are less toxic and formed amounts are low. Effects of temperature and soil properties also have not been clarified well. In this study, kinetics of alcohols were investigated in 3 soils amended with green manure and incubated at 20°C and 35°C. Volatile fatty acids were also determined in the same sample.

MATERIALS AND METHODS

Ten gram of soil sample (Pila clay loam, Maahas clay, and Luisiana clay) which had been air dried and passed through a 2 mm sieve was weighed into a 50 ml conical flask. Properties of soil samples are shown in Table 1 of Chapter 2. One hundred milligram of green manure (Glyricidic sepium), 25 mg of green manure or 25 mg of rice straw was added to the soil and mixed well. The soil was then submerged by 20 ml of water. The air in the head space was purged by argon and the flask was sealed by parafilm. It was incubated at 20°C and 35°C in an incubator. After designed period of incubation for 1 - 9 days, the content of the flask was transferred to a centrifuge tube and centrifuged at 7000 rpm for 15 min. The supernatant was filtered through Whatman No. 42 filter paper, and 2 µl of the filtrate was injected to a gas-chromatograph directly.

Gas-chromatography

A Varian aerograph Model 1868 equipped with FID was used. This instrument features on-column injection and direct connection of the column to the detector. A glass column (6 ft x 6 mm O. D. x 2 mm I. D.) packed with Porepak QS (80/100 mesh) was used. Nitrogen was used as carrier gas at a flow rate of 50 ml/min. Flow rates of hydrogen and air were 30 ml and 300 ml, respectively. The temperature of injector, column oven, and detector was maintained at 200°C, 100°C, and 260°C, respectively.

Volatile fatty acids formed together with alcohols were also determined for samples incubated at 35°C. The Porapak QS column for alcohol analysis was not good for volatile fatty acid analysis because their peaks were very broad. A stainless steel column (6 ft x 1/8 inch O. D.) packed with Chromosorb 101 (80/100 mesh) was connected with a stainless steel trap (13 cm x 1/8 inch) packed with Porapak QS (80/100 mesh). The trap was inserted into injection port of the gas chromatograph, and 5 µl of the aqueous sample was directly injected into this trap. This column gave satisfactory peaks of volatile fatty acids (acetic, propionic, isobutyric, butyric and isovaleric acids). Other conditions for gaschromatography for volatile fatty acid analysis were same as those for alcohol analysis.

RESULTS AND DISCUSSION

When rice straw was added to soils at 0.25% level, no alcohol formation was observed, and 0.25% of green manure also resulted in too low alcohol concentrations in soils to be determined correctly. Therefore, kinetics of alcohols were studied only in soils added with 1% of green

manure. Kinetics of alcohols in 3 soils are shown in Figure 17 (at 35°C) and Figure 18 (at 20°C). Methanol and ethanol were the only alcohols detected in the water extract. Formation of methanol in Pila clay loam, Maahas clay, and Luisiana clay at 35°C attained peaks 1, 2, and 3 days after submergence, and peak concentrations were 1.30, 1.29 and 1.10 mmol/kg, respectively. When soils were incubated at 20°C, methanol was formed more slowly, but accumulated in slightly larger amounts than at 35°C. Methanol concentration at 20°C attained a peak 2, 4, and 6 days after submergence in Pila clay loam, Maahas clay, and Luisiana clay, respectively. The peak concentrations were 1.40, 1.45 and 1.72 mM for Pila, Maahas and Luisiana, respectively. The order in which peak concentrations were formed seems to reflect how easily each soil is reduced. In Pila clay loam, a reduced state developed rapidly due to the low content of active Fe^{3+}, while it developed most slowly in Luisiana clay due to its high content of active Fe^{3+}. It was obviously shown in the kinetics of Eh in these 3 soils in Chapter 2.

Formation of ethanol at 35°C was observed only within 2 days of incubation in all those soils. The amount was largest in Luisiana clay (0.576 mmol/kg), followed by Maahas clay (0.087 mmol/kg) and Pila clay loam (0.007 mmol/kg). Ethanol formation in Pila clay loam was insignificant at 35°C. Probably the peak of ethanol formation in Pila clay loam passed off within 24 hours at 35°C. Ethanol concentration at 20°C attained a peak after 1 day in Pila clay loam and Maahas clay, and after 4 days in Luisiana clay. Peak concentrations were 1.10, 1.11, and 0.79 mmol/kg for Pila, Maahas, and Luisiana soils, respectively. Ethanol concentration in Pila clay loam decreased remarkably after 2 days, but it showed a

still high value after 2 days in Maahas clay. Ethanol concentration was also slightly higher at 20°C than at 35°C.

Volatile fatty acids in submerging water were also determined for the samples incubated at 35°C. Besides alcohols, acetic acid, propionic acid, isobutyric acid, butyric acid, and isovaleric acid were detected in soil solution (Table 9). Large amount of acetic acid (0.5 - 0.9 mmol/kg soil) was detected already after 1 day of incubation. Acetic acid was the dominant fatty acid which accounted for more than 70% of total volatile fatty acid. The concentration of acetic acid fluctuated daily. Concentrations of volatile fatty acids showed maximum values after 3 days of incubation in all these soils. Isovaleric acid was not detected on the first day of incubation, but it became a second abundant fatty acid after 3 days of incubation. Isovaleric acid is a specific product of proteolytic clostridia which utilize amino acids as electron donors and acceptors (Takeda and Furusaka, 1975). Its formation is favored in soils amended with large amount of organic nitrogen source such as green manure. Concentrations of volatile fatty acids in submerging water decreased in the following order in most cases; acetic acid >> isovaleric acid > propionic acid > butyric acid > isobutyric acid.

Compared with volatile fatty acids, turn over of alcohols was very fast and accumulated amounts of alcohols were much less than that of volatile fatty acids. Practically, alcohols may not cause any physiological effect to rice plant grown on submerged soils.

Though butanol was not detected in this study, it may be a specific product when glucose and special plant material were added to soils (Wang et al., 1967). Predominance of methanol over ethanol, and their short period of prevailence conformed to the former results (Wang et al., 1967, Kubota and Furusaka, 1981).

LITERATURE CITED

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Table 9. Kinetics of alcohols and volatile fatty acids in submerged soils applied with green manure (10 g/kg soil) at 35°C.

	Days after submergence			
	1	2	3	4
	mmol/kg soil			
Pila clay loam				
Methanol	1.31	0.00	0.01	0.00
Ethanol	0.11	0.00	0.00	0.00
Acetic acid	4.63	0.63	6.28	2.12
Propionic acid	0.37	0.19	0.03	0.89
Isobutyric acid	0.02	0.15	0.02	0.04
Butyric acid	0.01	0.08	0.70	0.65
Isovaleric acid	0.00	0.11	0.98	0.71
Maahas clay				
Methanol	1.15	1.29	0.02	0.00
Ethanol	0.09	0.00	0.00	0.00
Acetic acid	3.38	1.51	3.49	0.82
Propionic acid	0.54	0.14	0.32	0.33
Isobutyric acid	0.04	0.07	0.00	0.01
Butyric acid	0.04	0.07	0.10	0.26
Isovaleric acid	0.00	0.18	0.37	0.27
Luisiana clay				
Methanol	0.17	0.96	1.10	0.55
Ethanol	0.58	0.03	0.00	0.00
Acetic acid	2.74	2.25	4.12	0.90
Propionic acid	0.19	0.09	0.30	0.33
Isobutyric acid	0.02	0.07	0.02	0.02
Butyric acid	0.02	0.08	0.11	0.19
Isovaleric acid	0.00	0.14	0.23	0.29

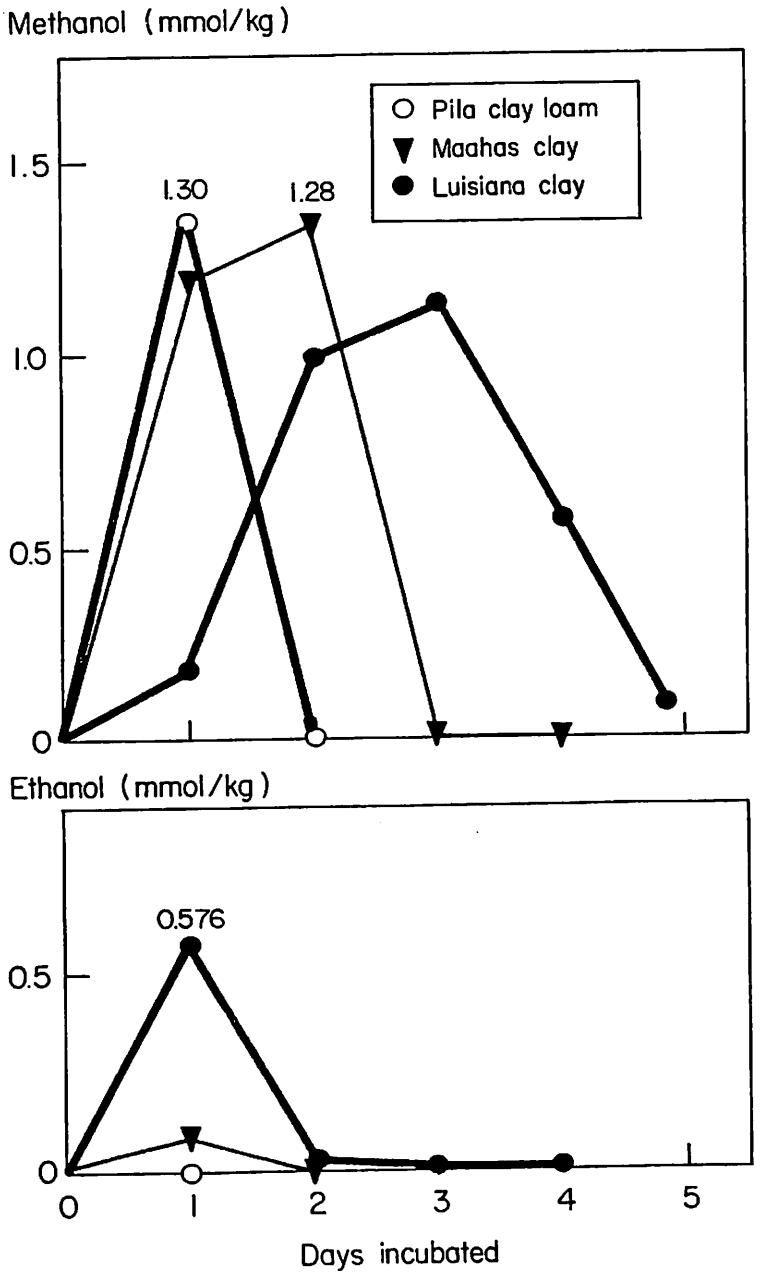

Fig. 17 Kinetics of alcohols in 3 submerged soils treated with green manure (1%) at 35°C.

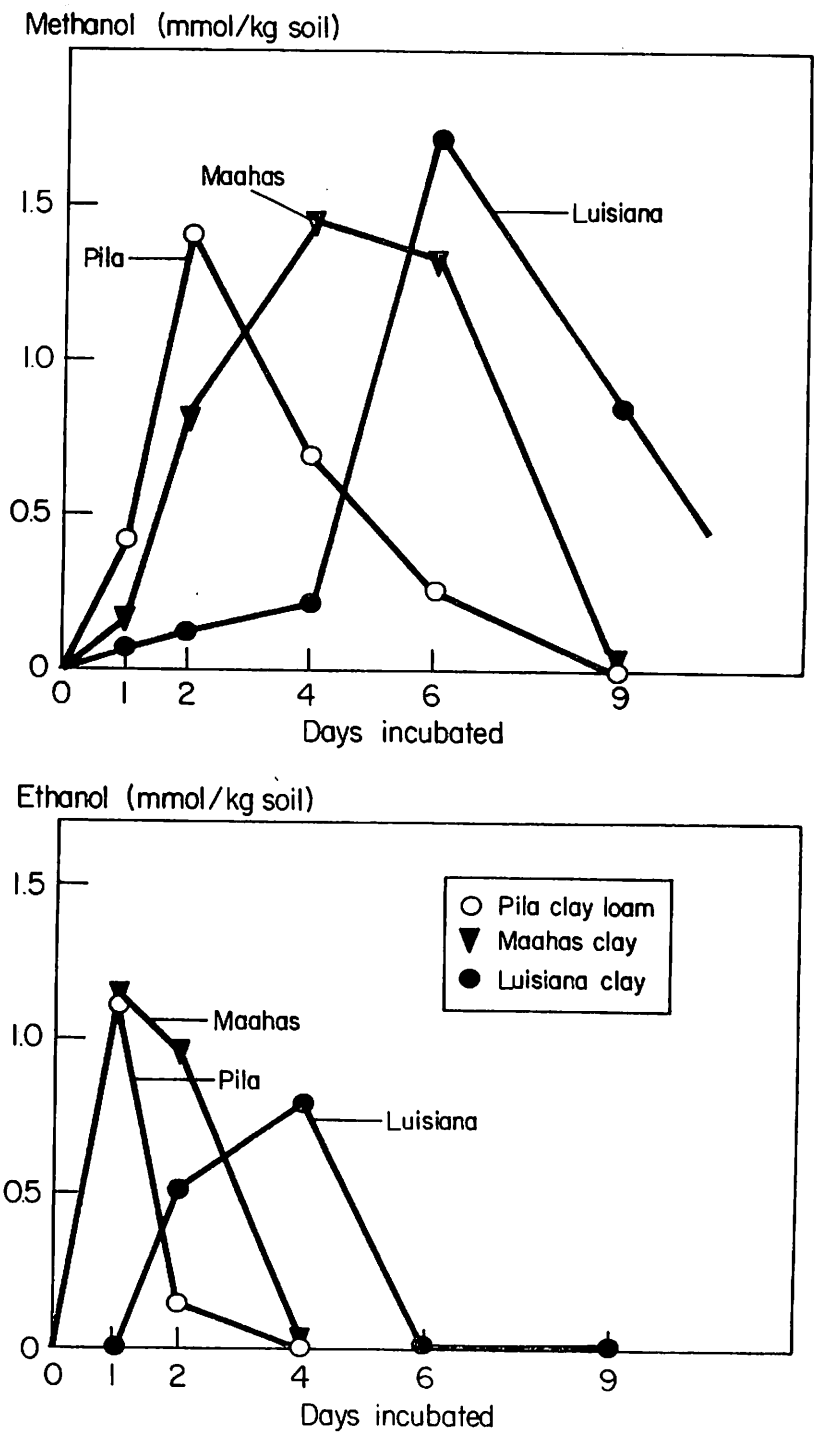

Fig. 18. Kinetics of alcohols in 3 submerged soils added with green manure (1%) at 20°C.

Chapter 6

FORMATION OF ALDEHYDES IN SUBMERGED SOILS APPLIED WITH GREEN MANURE

SUMMARY

Kinetics of aldehydes were investigated in 3 soils applied with green manure (Gliricidia sepium) and incubated anaerobically at 20°C and 35°C. Rapid analysis of trace amounts of aldehydes were accomplished by the extraction and gas liquid chromatography techniques by Selim (1977). The major aldehyde was acetaldehyde, but the amount formed was very low -- at most 0.1 mmol at 35°C or 0.05 mmol/kg soil at 20°C. Two peaks were observed in the formation of acetaldehyde. The first was after 1 day and the second was after 4-6 days of incubation at 35°C. After the second peak was attained, acetaldehyde did not disappear rapidly, but decreased gradually over 4 weeks.

INTRODUCTION

Aldehydes and ketones have been detected in soils under anaerobiosis of glucose (Adamson et al 1975). Acetaldehyde and butyraldehyde were formed in very small amounts representing about 0.01% of the added glucose, while acetone and methylethylketone were formed in larger amounts representing at most 0.4% of the added glucose. In an effort to identify reducing organic substances in the leachate from submerged rice soil, Okazaki et al (1981) detected formaldehyde, acetaldehyde, propionaldehyde, n-butyraldehyde, and n-valeraldehyde at concentrations of 0.03, 0.05, 0.04, 0.01, and

0.01 mmol/kg soil, respectively, from a excessively eluviated wetland rice soil. They consider that these aldehyde play important roles in the dissolution and removal of Fe and Mn from subsurface horizons of excessively eluviated wetland rice soils.

Except for the above mentioned reports, there have been no information on the behavior of aldehydes in anaerobic soils. Neither an accurate and rapid method for the routine analysis of aldehydes in submerged soil has been elaborated yet

Method by Adamson et al (1975) which is devised for the volatile compounds in gaseous phase may not be applied to submerged soil which contains too much water, and the method of Okazaki et al (1980) which is composed of steam volatilization, 2, 4-dinitrophenylhydrazone formation, and collection of precipitate seems to be semi micro procedure and require large amount of sample. By the method we adopted in this study, very low concentration of aldehydes (less than 0.1 mmole/kg) in the water extract of submerged soils could be determined taking only 10 ml of sample solution. We applied this method for studying the kinetics of aldehydes in 3 paddy soils added with green manure (1%) and incubated under submerged condition at 20°C and 35°C.

MATERIALS AND METHODS

Incubation

Three paddy soils (Pila, Maahas, Luisiana) differing in pH, organic matter content, active iron content and some other properties (Table 1) were used. Dried leaves of Gliricidia sepium (C:45.8%,

N : 3.21%, C/N 14.3) was used as green manure. To 10g of soils, 100 mg of green manure was mixed in a 50 ml conical flask. The soil was submerged by 20 ml of distilled water, and the flask was capped by a silicon rubber septum. The headspace gas in the flask was replaced to argon through a needle injected through the septum. This sample was incubated at 20°C or 35°C for required period from 1 to 28 days.

Method of analysis

Derivatization of aldehydes by 2,4-dinitrophenylhydrazine and the two step solvent extraction of dinitrophenylhydrazones were carried out following the method of Selim (1977). After incubation, soil and water in the flask was transferred into a centrifuge tube and centrifuged at 7000 rpm for 15 minutes. The obtained supernatant was then filtered through Whatman No. 42 filter paper. Ten ml portion of the filtrate was taken into a 50 ml conical flask by a volumetric pipet. One ml of 0.25% 2, 4-dinitrophenyl-hydrazine in 6 N HCl and 10 ml of isooctane were added to this. This was stirred for 30 minutes by a magnetic stirrer and then transferred to a separatory funnel. The lower aqueous layer was taken into a conical flask, and extracted by another 10 ml of isooctane by stirring for 10 minutes by a magnetic stirrer. The isooctane layer was separated by a separatory funnel. The two isooctane extracts were combined together and transferred into a separatory funnel and extracted with 10 ml of acetonitrile 2 times. Lower acetonitrile layers were combined together, to which

50 µl of 0.12% butyraldehyde- 2, 4-dinitrophenylhydrazone in acetonitrile solution was added as an internal standard, and then evaporated to dryness at 50°C by a rotary evaporator. The dried residue was transferred to a 3 ml Reacti Vial with 2-3 ml of CH_2Cl_2, which was evaporated off under a stream of nitrogen after transfer. Finally the volume of the sample was made up to 1 ml by adding 1 ml of CH_2Cl_2 to the vial. Five microliter of this solution was injected to gas liquid chromatography.

Gas liquid chromatography

A varian aerograph model 1868 equipped with a FID detector was used. This instrument features on-column injection and direct connection of the column to the detector. A glass column (6 ft x 6 mm O.D. x 2 mm I.D.) packed with 2% Silicon OV-17 on Uniport HP (60/80 mesh) was used. Nitrogen was used as carrier gas at the flow rate of 50 ml/min. Flow rates of hydrogen and air for FID detector were 30 ml and 300 ml/min, respectively. The temperature of injector and detector was maintained at 300°C. The temperature of column oven was programmed to maintain 230°C for 5 min after injection, then increase at the rate of 8°C/min for the following 5 min and then maintain 270°C constantly for the rest of time.

RESULTS AND DISCUSSION

Gaschromatogram of 2, 4-dinitrophenylhydrazones of carbonyl compounds extracted from the water extract of submerged paddy soils is shown in Fig. 1. Peaks of formaldehyde and acetaldehyde were identified. The peak of propionaldehyde or acetone was very small. Butyraldehyde was not detected in the samples, therefore it was used as an interanl standard. One peak which appeared before formaldehyde and three peaks between propionaldehyde and butyraldehyde have not been identified yet.

The major aldehyde in submerged paddy soils was acetaldehyde, but the amount formed was very low - - at most 0.1 mmol at 35°C or 0.05 mmol at 20°C per kg of soil. The greater amount of acetaldehyde at 35°C than at 20°C may be explained by higher microbial activity at 35°C. Because it is an unstable intermediate, the acetaldehyde detected in soil should be regarded to be formed within a few days before analysis.

The kinetics of acetaldehyde in 3 submerged soils at 20°C and 35°C are compared in Figure 2. In Maahas clay and Luisiana clay, the formation of acetaldehyde showed two peaks: the first was after 1 day and the second was after 4-6 days of incubation. After the second peak was attained, acetaldehyde did not disappear rapidly as alcohols did in these soils. Acetaldehyde decreased gradually over 4 weeks. Several precursors are known for acetaldehyde in bacterial metabolism (Doelle 1975). In fermentation, pyruvate, acetyl-CoA, ethanol, and acetate are assumed to be important. Acetaldehyde

formed in the first peak 1 day after incubation might have come from pyruvate, acetyl-CoA, or ethanol which were produced through the glycolysis of carbohydrates in green manure. The acetaldehyde formed in the second peak might have come from acetic acid accumulated in the soil.

The amounts of formaldehyde and propionaldehyde formed were much lower than that of acetaldehyde and insignificant. Therefore, their kinetics were not followed in this study.

From their very low concentration in submerged soils, aldehydes may not participate in any injury of wetland rice.

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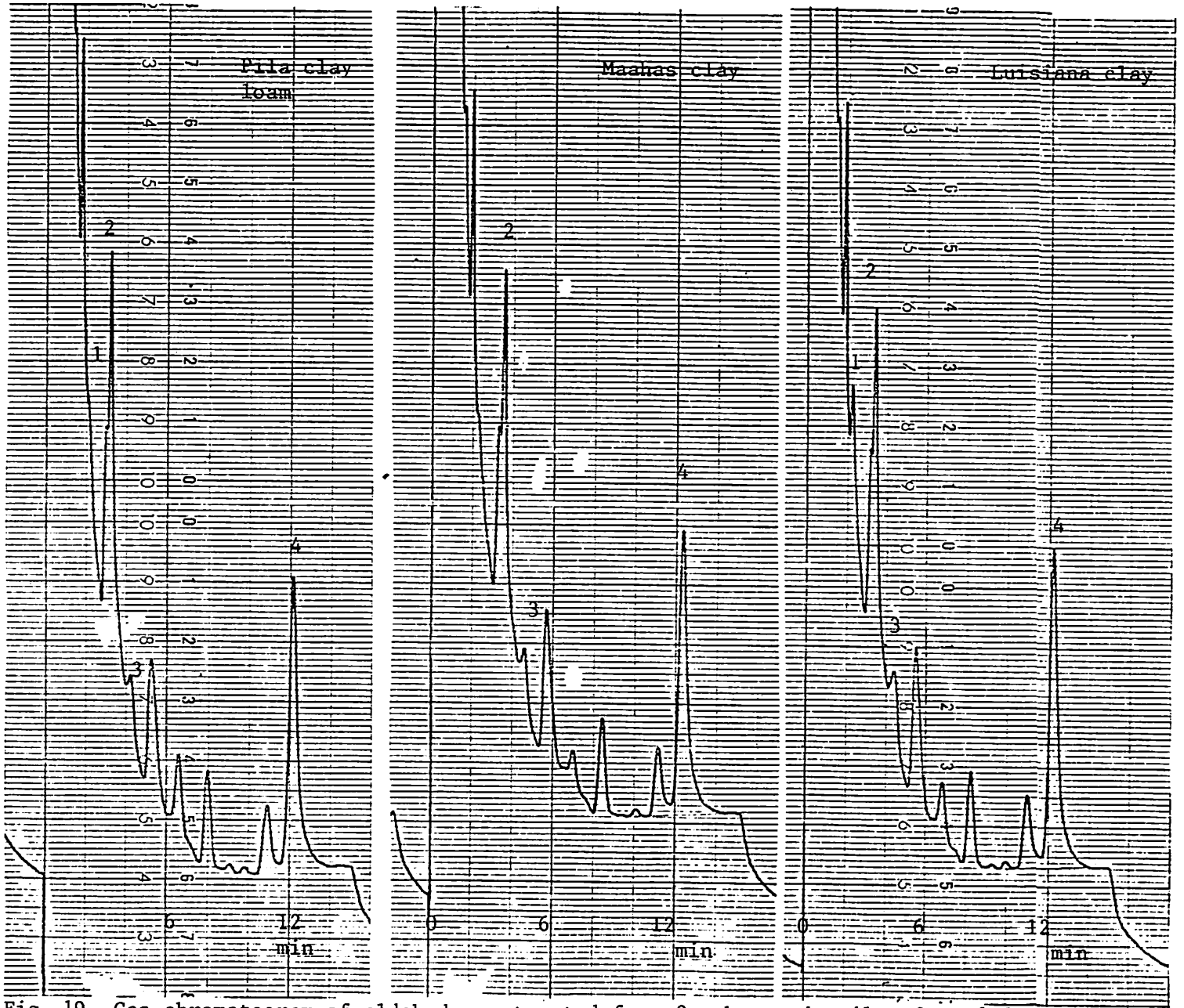

Fig. 19. Gas chromatogram of aldehydes extracted from 3 submerged soils after 6 days of incubation at 35°C. 1:formaldehyde, 2:acetaldehyde, 3:propionaldehyde, 4:butyraldehyde (internal standard).

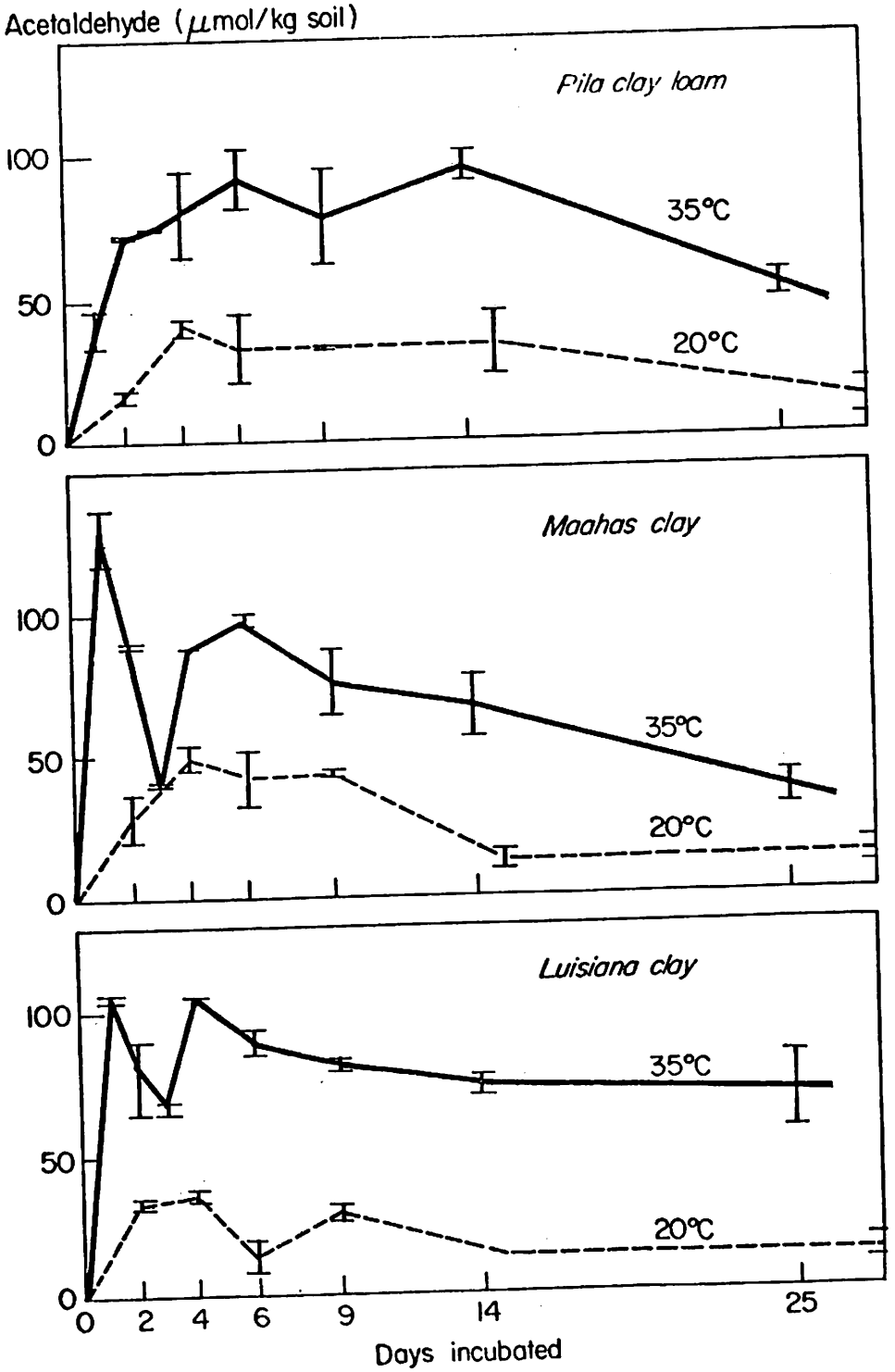

Fig. 20. Kinetics of acetaldehyde in submerged soils applied with green manure (1%) at 20 and 35°C