

APPENDIX

VOLATILE PRODUCTS AND LOW-MOLECULAR WEIGHT
PHENOLIC PRODUCTS OF THE ANAEROBIC DECOMPOSITION
OF ORGANIC MATTER

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SUMMARY

The kinetics of volatile products including volatile fatty acids, alcohols, aldehydes, ketones, volatile S compounds, and low molecular phenolic acids in submerged soils are reviewed. Incubation of soils at high temperature reduces the accumulation of volatile fatty acids. The formation of alcohols, aldehydes, ketones, and volatile S compounds was minimal. The kinetics of volatile products in submerged soils were greatly affected by the amounts of easily decomposable organic matter and oxidizing substances such as ferric compounds in soils, and by temperature.

Concentrations of phenolic acids in paddy soils are considered to be low enough to cause no injury even when organic materials are incorporated. However, a locally developed adverse environment around the fragment of decomposing plant residue should be taken into consideration in both the cases of volatile fatty acids and phenolic acids formation.

Although organic manures are assuming increasing importance in rice production, there is little information on the decomposition of organic matter in tropical rice soils. In wetland rice soils, organic matter usually undergoes anaerobic decomposition. Besides their beneficial effects, organic manures may cause adverse effects to crops due to their decomposition products, especially during anaerobic decomposition. Anaerobic decomposition includes anaerobic respiration and fermentation. Through fermentation, pyruvic acid, the key metabolite of carbohydrate metabolism, is transformed into an array of substances that include alcohols, diols, glycerol, aldehydes, ketones, volatile and nonvolatile organic acids, CO_2 , H_2 , CH_4 , and C_2H_2 (Ponnamperuma 1972). Most of these products have been identified collectively in anaerobic soils. Some of them are toxic to plants and soil biota depending on their concentrations. Most of these products are volatile or gaseous.

Besides fermentation products, phenolic substances are also considered to be potentially harmful substances in soils. Phenolic substances are very abundant in soils. Humic acid and fulvic acid can be regarded as high molecular weight phenolic acids due to their high contents of carboxylic and phenolic hydroxyl groups (Tsutsuké and Kuwatsuka 1978). Low molecular weight phenolic compounds are

present in plant residues, cleaved or microbially formed from humic substances or lignin

VOLATILE FATTY ACIDS

Kinetics

The formation of volatile fatty acids resulting from the decomposition of organic matter in soil has drawn the attention of researchers for a long time. Takaishi et al (1909) studied the formation of organic acids during the decomposition of organic manure in soils. Their work elucidated the formation of formic acid, acetic acid, butyric acid, and lactic acid during the decomposition processes of oil cake, rice bran, and compost. Acharya (1935a) incubated rice straw with soil and followed the decomposition under anaerobic conditions. He observed that the first phase of the decomposition was the rapid formation of organic acids, followed by the formation of CH_4 . The decomposition products detected were acetic acid, butyric acid, CO_2 , CH_4 , and negligibly small amounts of H_2 . In his further experiments, Acharya (1935b,c) studied the effects of temperature, pH, and aeration on the course of decomposition. Maximum decomposition of rice straw was observed at 30-35°C at pH 8. Outside this temperature range and above and below pH 8, accumulation of organic acids and retardation of gas formation were observed.

Takijima (1960a) detected organic acids in paddy soils in the following order: acetic > butyric > formic > fumaric > propionic > valeric > succinic and lactic. Formation of branched fatty acids such as iso-butyric and iso-valeric acids in paddy soils was detected by Takeda and Furusaka (1975b). Formation of volatile and gaseous products in submerged soils is almost entirely due to facultative and obligate anaerobic bacteria. Pyruvic acid, the final product of the EMP, HMP, ED, and phosphoketolase pathways, is converted to many kinds of organic compounds depending on the kinds of bacteria and on the environmental conditions during fermentation (Ponnamperuma 1972). Takeda and Furusaka (1970) used anaerobic incubation processes to conduct extensive surveys of bacteria isolated from paddy soil. The number of strict anaerobes was always about 10% of the facultative anaerobes, and the strict anaerobes were mostly clostridia. Among the facultative anaerobes, Enterobacteriaceae predominated during nonsubmerged periods, and Aeromonas and coccoid bacteria predominated during flooding (Watanabe and Furusaka 1980).

Acetic acid, which is accumulated in the greatest amount among the organic acids in submerged soil, can be produced by both facultative and obligate anaerobes through many classes of carbohydrate fermentation. It is also a product of proteolytic clostridia (Doelle 1975). Pyruvate is

usually transformed via acetyl-CoA and acetylphosphate to acetate. A pathway by which CO_2 is converted to acetic acid is also known in some species of clostridia. In the former pathway, one ATP is produced, but no NADH is consumed; in other words, no intermediate is working as an electron donor. Therefore, acetic acid formation is usually accompanied by the formation of other products by which NADH is consumed. Such examples are propionic acid fermentation by *Propionibacterium*; butyric acid fermentation by clostridia; and ethanol, lactate, and succinate formation by mixed acid producers among the Enterobacteriaceae (Doelle 1975). However, acetic acid surpasses other fermentation products in amount in submerged soils. As noted by Neue and Scharpenseel (this conference), this may be attributed to the interspecies transfer of H_2 liberated from ADH using ferredoxin as an intermediate carrier.

Formic acid is produced concomitantly when acetyl-CoA is produced from pyruvic acid and Coenzyme A by facultative anaerobes belonging to the Enterobacteriaceae, but it is not produced by saccharolytic clostridia (Doelle 1975). Its formation keeps step with acetic acid, but the amount formed is very low (Gotoh and Onikura 1971, Yamane and Sato 1970). This may be because formic acid is further decomposed to H_2 and CO_2 , and the CO_2 is then used in the synthesis of other products like succinate and CH_4 (Doelle 1975).

Butyric acid and propionic acid are usually produced concomitantly with acetic acid by saccharolytic clostridia and propionibacteria. Strains of Propionibacterium increased in a submerged soil after prolonged submergence (Watanabe and Furusaka 1980).

The very small amounts of branched fatty acids such as isobutyric and isovaleric acids in paddy soils are considered to be products of proteolytic clostridia (Takeda and Furusaka 1975 a,b).

Volatile fatty acids are intermediate products which are transformed to CO_2 by oxidation while the reduction of Fe^{+3} is going on in submerged soils (Kamura et al 1963). After the reduction of Fe^{+3} ceases the volatile fatty acids are used by methanogenic bacteria as substrates to produce CH_4 . In methanogenesis in submerged soils, CH_4 is formed preferentially from the methyl group of acetic acid, and the formation of CH_4 from CO_2 is of less importance (Takai 1970).

In soils with a high content of active Fe, only small amounts of volatile fatty acids accumulate, while the contrary is true in soils with a low content of active Fe (Takai et al 1957). Asami and Takai (1970) also found that the formation of volatile fatty acids and CH_4 is repressed remarkably when amorphous $\text{Fe}(\text{OH})_3$ is added to the soil and then submerged. There was a highly significant positive

correlation between the amounts of Fe^{+2} and CO_2 formed within the first 2 weeks of submergence. Moreover, when acetic acid was added to a well reduced soil, a large amount of CH_4 was formed, but when acetic acid and amorphous $\text{Fe}(\text{OH})_3$ were added together to the reduced soil, a large amount of CO_2 was formed instead of CH_4 . From these results, Asami and Takai assumed the coupling of the oxidation of acetic acid and the reduction of Fe in submerged soils.

An increase in volatile fatty acid formation owing to the addition of fresh organic matter has been reported in many papers, including those cited above (Chandrasekaran and Yoshida 1973, Gotoh and Onikura 1971, Lynch 1978, Yamane and Sato 1970, Rao and Mikkelsen 1977).

Temperature greatly affects the kinetics of volatile fatty acids (Yamane and Sato 1967, Cho and Ponnampereuma 1971). Higher temperature accelerates both production and destruction of volatile fatty acids. However, methanogenesis, which accompanies the destruction of acids, is much more accelerated than the production of acids at a temperature higher than 25°C (Yamane and Sato 1967). As a result, the accumulation of organic acids is diminished at higher temperatures.

The kinetics of volatile fatty acids were also studied using three soils (Table 1) treated with three kinds of

organic materials (Table 2) and incubated anaerobically at 20°C and 35°C (Tsutsuki and Ponnampereuma, unpublished). The kinetics of acetic acid in three submerged soils treated with rice straw (0.25%) at 20°C are shown in Figure 1. In Pila clay loam, a large amount of acetic acid accumulated within 2 weeks of submergence. A low active Fe content and large amounts of easily decomposable organic matter in the soil were assumed to be the cause of the large accumulation within such a short incubation period. In Maahas clay, organic acid accumulation was low at both temperatures, even when organic matter was added to the soil. A slight accumulation was seen after 2 weeks of submergence. In Louisiana clay, organic acid accumulation lasted longer than in the other soils. When rice straw or green manure was added to this soil at 20°C, acetic acid accumulation showed two peaks -- at the first and the sixth weeks of incubation. Louisiana clay is a heavy clay and contains a large amount of active Fe. The second peak implies that acetic acid could accumulate after most of the active Fe^{+3} in the soil had been reduced. The acetic acid formed in the first week might have come from strictly anaerobic sites which developed locally in the soil.

In Figure 2, the accumulations of isovaleric acid in Pila soil treated with rice straw and green manure (both 0.25%) are compared with controls at 20°C and 35°C.

Isovaleric acid accumulation was prominent when green manure was applied to the soil. Addition of rice straw slightly enhanced the accumulation of isovaleric acid. Pila clay loam produced a significantly larger amount of isovaleric acid than the other soils. At 35°C, the accumulation of isovaleric acid was earlier and lower than at 20°C.

Isovaleric acid is produced by clostridia utilizing amino acids as electron donors and acceptors (Takeda and Furusaka 1975a). Such a metabolism would become active where the content of other electron acceptors like Fe^{+3} compounds is low and proteinous material is abundant. This is the reason why isovaleric acid was produced in large amounts in Pila clay loam (low active Fe content) treated with green manure, which contains a substantial amount of N. Rice straw did not enhance the formation due to its low N content.

Volatile fatty acid accumulation in fields

Studies on volatile fatty acid accumulation in arable lands have been closely connected with the evaluation of volatile fatty acids as factors in phytotoxicity. As the phytotoxicity of organic acids is discussed in another session, some field data which might be suggestive in solving this problem are cited below.

Yamane and Sato (1970) found that large amounts of acetic acid (13.5 mmol/kg soil) and butyric acid (5.5 mmol/kg soil) were produced when Italian ryegrass (10.6 t/ha

of shoot and 19.3 t/ha of root) was incorporated into rice fields. However, within 2-3 weeks after incorporation, volatile fatty acids almost disappeared.

Gotoh and Onikura (1971) conducted a field experiment to examine whether organic acids accumulate to a toxic level in a rice field applied with rice straw. They detected a maximum of 0.31 me/kg acetic acid (0.91 mM in soil solution) 4 weeks after the incorporation of rice straw (15 t/ha). From the low concentration of volatile fatty acids detected they concluded that the main factor governing plant growth in the early stages was probably N deficiency, with volatile fatty acids being a minor factor. In warm areas such as Kyushu, where the experiment was conducted, they assumed that volatile fatty acids derived from the decomposing rice straw would probably not cause injury to plants. Though toxicity is considered to be further alleviated in submerged rice soils due to the neutrality of soil pH, Chandrasekaran and Yoshida (1973) suggested that volatile fatty acids can inhibit the growth of rice plants even in a neutral soil because the pH around the rice root is much lower than the pH outside the rhizosphere.

An adverse environment developing in the vicinity of decomposing plant residue was found responsible for the poor establishment of direct drilled winter oats associated with the presence of unburnt straw in the drill slits

(Agricultural Research Council Letcombe Laboratory 1976). The same type of inhibition may occur if rice is direct seeded where applied rice straw or other organic matter has not decomposed well. The distribution of added organic matter in soil is heterogenous, and the concentrations of volatile fatty acids are highest and the pH lowest at the site of decomposing organic debris itself. It was found that the acetic acid concentration declined exponentially with distance from the straw surface (Agricultural Research Council Letcombe Laboratory 1977).

ALCOHOL FORMATION

Methanol, ethanol, n-propanol, and n-butanol were detected and quantified in soils treated with leaves of sugarcane and Crotalaria juncia by Wang et al (1967a). Isopropanol, iso-butanol, 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 1967a), 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), and they were formed in significant amounts in submerged soils only

when 1% green manure (*Glycidia sepium*) was added; addition of rice straw did not favor the formation of alcohols in submerged soils (Tsutsuki and Ponnampereuma, unpublished).

The kinetics of alcohols in three soils at 35°C are shown in Figure 3. The formation of methanol in Pila clay loam, Maahas clay, and Louisiana clay attained a peak 1, 2, and 3 days after submergence, 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 high content of easily decomposable organic matter and the low content of active Fe^{+3} , while it developed more slowly in Louisiana clay due to its high content of active Fe. Formation of ethanol was observed only within 2 days of incubation in all those soils. The amount was largest in Louisiana clay, followed by Maahas clay and Pila clay loam in that order. Ethanol formation in Pila clay loam was insignificant at 35°C.

When the soils were incubated at 20°C, methanol and ethanol were formed more slowly, but accumulated in slightly larger amounts than at 35°C. At 20°C, methanol content attained a peak 2, 4, and 6 days after submergence in Pila clay loam, Maahas clay, and Louisiana clay, respectively. The accumulated amounts of methanol at the peaks were between 1.4 and 1.7 mmol/kg soil. Ethanol content attained a peak

after 1 day in Pila clay loam and Maahas clay, and after 4 days in Luisiana clay. The accumulated amounts were 0.8 1.1 mM, which were also slightly larger than those at 35°C.

Kinds and amounts of alcohols formed in soils vary with type of soil and with the kind of amended organic matter. Ethanol and butanol were formed in large amounts when glucose was added to soils (Adamson et al 1975, Kubota and Furusaka 1981). When leaves of sugarcane, Crotalaria, and Glydicia were added to soils, methanol was formed in the greatest amount, followed by ethanol. Butanol formation was favored in soils with higher organic C content or amended with Crotalaria (Wang et al 1967a).

According to Kubota and Furusaka (1981) methanol, ethanol, and butanol were detected only within the first few days of incubation. The same result was obtained by Tsutsuki and Ponnampereuma (unpublished). However, those alcohols were detected even after 4 or 8 weeks of incubation by Wang et al (1967a).

Kubota and Furusaka (1981) detected 2,3-butane-diol and its precursor acetoin in submerged soils even without glucose, but addition of glucose enhanced and increased the production. They concluded that carbohydrates are metabolized through the 2,3-butane-diol fermentation pathway as well as through volatile fatty acids under anaerobic soil conditions.

ALDEHYDES AND KETONES

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 an excessively eluviated wetland rice soil. They consider that these aldehydes play important roles in the dissolution and removal of Fe and Mn from subsurface horizons of excessively eluviated wetland rice soils.

The kinetics of aldehydes were also studied by Tsutsuki and Ponnampereuma (unpublished) in submerged soils amended with green manure (1%) and incubated at 20°C and 35°C. Formaldehyde, acetaldehyde, and propionaldehyde were detected. The major aldehyde was acetaldehyde, but the amount formed was very low -- at most 0.1 mmol at 35°C or 0.05 mmol at 20°C. Because it is an unstable intermediate, the amount of acetaldehyde detected in soil should be regarded as the amount formed within a few days before

analysis. The greater amount of acetaldehyde at 35°C than at 20°C may be explained by higher microbial activity at 35°C.

In Maahas clay and Luisiana clay, the formation of acetaldehyde showed two peaks; the first was after 1 days and the second was after 4-6 days of incubation. After the second peak was attained acetaldehyde did not disappear rapidly like alcohols, but 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 might have come from pyruvate, acetyl-CoA, or ethanol derived from the carbohydrates in green manure, and 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.

VOLATILE SULFUR COMPOUNDS

In anaerobic media, the main changes involving S are the reduction of SO_4^{2-} to sulfide and the dissimilation of the amino acids cystine and methionine to H_2S , thiols, ammonia, and fatty acids (Ponnamperuma 1972).

Methylmercaptan has been found in submerged soils (Takai and Asami 1962, 1963; IRRI 1965). Mercaptans showed

concentration peaks within 2 weeks of flooding and then declined (IRRI 1965). Higher temperature enhanced the disappearance of methylmercaptan (Asami and Takai 1963). The addition of green manure and stable manure enhanced the formation of methylmercaptan, while the addition of Fe_2O_3 interfered with the accumulation of methylmercaptan (Asami and Takai 1963).

Sensitive gas chromatographic techniques using a flame photometric detector enabled the analysis of trace amounts of volatile S gases (Banwart and Bremner 1974). Though the main change of S in anaerobic soils is the reduction of SO_4^{2-} to sulfide, no trace of H_2S was detected over submerged soils treated with SO_4^{2-} or S-containing organic materials (Banwart and Bremner 1976a, 1976b). However, a considerable amount of H_2S was detected from animal manures decomposed without soil (Banwart and Bremner 1975). Not only gaseous H_2S but also water soluble H_2S are almost chemically undetectable in submerged soils (IRRI 1965). This is due to their removal as insoluble sulfides, chiefly FeS , and to their instability in the air phase (Ponnampruma 1972).

According to Banwart and Bremner (1976a), S volatilized from unamended and SO_4^{2-} -treated soils, chiefly in the form of CH_3SCH_3 (55-100%), associated with small amounts of COS , CS_2 , CH_3SH , and CHSSCH_3 . The amended

SO_4^{2-} was not transformed to volatile S compounds. More S volatilized under submerged soil conditions than under aerobic conditions. However, the amount of S volatilized from unamended and SO_4^{2-} -amended soils was very small (0-84 ug S/kg soil) and did not account for more than 0.05% of the total S in unamended soils.

The release of volatile S compounds from soils treated with S-containing organic materials was also studied by Banwart and Bremner (1976b). Most of the S volatilized from soils treated with sewage sludges was in the form of CH_3SCH_3 and CH_3SSCH_3 , whereas most of the S volatilized from soils treated with animal manures and plant materials was in the form of CH_3SH and CH_3SCH_3 . Small amounts of COS and CS_2 were associated with these compounds. Submerged soil conditions and the addition of S-containing organic materials favored the formation of volatile S compounds. When the application rate of organic matter to the soils was 1%, amounts of S volatilized were 0.27 mg/kg for animal manure treated soils, 0.38 mg/kg for sewage sludge treated soils, and 4.36 mg/kg for plant material treated soils, on the average.

Minami et al (1981) also analyzed volatile S compounds evolved from rice soils treated with rice straw, compost, and cystine (Table 3). In the soil treated with rice straw, H_2S was detected in the greatest amount among the

S-containing products by shaking the flask immediately before analysis. Dimethyl sulfide (CH_3SCH_3) was the second most abundant S-containing product; CH_3SH , CH_3SSCH_3 , COS , and CS_2 were formed in smaller amounts. The addition of compost did not increase the formation of volatile S compounds from soil as compared with the control.

PHENOLIC ACIDS

As Takijima (1964) suggested, injury of the rice plant in peat soils or in soils amended with fresh organic matter cannot be explained only by volatile fatty acids; some other toxic substance may participate in the injury.

Aromatic organic acids have much higher toxicity than aliphatic acids. Phenolcarboxylic acids exhibited 50% inhibition of the growth of the roots of rice seedlings at concentrations of 0.6-3 mM (Takijima 1960b). The lowest concentrations of phenolic acids causing injury to various plants have been found to range between 0.1 and 0.01 mM by many researchers (Takijima 1963).

Walters (1917) may have been the first researcher to recognized phenolic acids as the cause of a soil-borne plant disease. He found 22 ppm of p-hydroxybenzoic acid and 1.7 ppm of benzoic acid in the soil from an orange orchard which was badly affected with the citrus disease commonly known as "die back." Later, Winter (1955) studied the occurrence of