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## EFFECT OF RICE STRAW ADDITIONS ON PRODUCTION OF ORGANIC ACIDS IN A FLOODED SOIL\*

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### SUMMARY

Disposal of rice straw through soil incorporation may contribute to anaerobic fermentation processes producing concentrations of organic acids which are toxic to rice plants. The present studies were conducted to determine the kind, amount, and time of production of organic acids as a function of rice straw additions (0, 0.25, and 0.5 per cent of soil weight), and temperature (10, 20, and 30°C). Nine samples were taken at 5, and 10 day intervals for 60 days to measure concentrations of organic acids.

Only acetic acid was detected in the incubated soil with rice straw added. The amount and peak production of acetic acid increased with the rate of straw added and temperature. Acetic acid concentrations varied between 10.6 and 22.7  $\mu\text{eq}/20\text{ g soil}$ , and the peak production occurred between 15 and 20 days after incubation. Organic acids were not found in sufficient amounts to affect the growth of rice plants grown in soils that were not previously puddled or in a reduced state.

### INTRODUCTION

Anaerobic intermediary decomposition products of rice straw and crop residues accumulated under flooded soil conditions, often have toxic effects on rice plant growth. Among the toxic products reported are the lower-carbon fatty organic acids. The kind, amount, and rate of organic acid production in flooded soils depend largely on the nature of the organic materials added and the past and present reduction state of the soil.

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Subrahmanyam<sup>11</sup> studied the anaerobic decomposition of several carbohydrates in puddled soils. With sucrose added, lactic acid concentration was increased (679 ppm C) up to the 3rd day and then began to decrease, subsequently soil concentrations of acetic acid (333 ppm C) and butyric acid (513 ppm C) increased. With starch added, the concentrations of all three acids were decreased: lactic (382 ppm C), acetic (128 ppm C), and butyric (62 ppm C). Similarly Yamane and Sato<sup>15 16</sup> found that organic acids were produced rapidly and in large amounts with glucose added, less with starch, and decreased with cellulose and gelatin. The higher the concentration of soluble carbohydrates in the organic residues, the faster the organic acid production and the larger the amounts of organic acids produced.

Green manures are commonly applied in some areas where rice is grown. Green manures decompose faster than the more resistant materials, such as rice straw<sup>10 14</sup>. Motomura *et al.*<sup>9</sup> and Takijima and Sakuma<sup>12</sup> reported the production of acetic acid and butyric acid in puddled soils with milk vetch added. Chandrasekaran and Yoshida<sup>1</sup>, using fresh leaves of *Sesbania*, observed the production of acetic, propionic, butyric, and iso-butyric acids. The peak production, 5 days after green manure addition, was 2.93 meq acetic, 0.41 meq propionic, 0.28 meq butyric, and 0.12 meq isobutyric acid per 100 g soil. Rice straw was more resistant to decomposition than green manures. Gotoh and Onikura<sup>2 3 4</sup> indicate that the production of acetic acid is significantly higher than butyric acid in flooded paddy, and that other acids are produced only in traces. They also showed that the production of acetic acid peaked about 10–14 days after application of rice straw, compared with less than 7 days for easily decomposable organic materials.

The organic acids produced by the decomposition of crop residues serve as energy materials and carbon sources for anaerobic microorganisms. Organic acid formation therefore depends greatly on the activity of the microorganisms responsible for the decomposition. Temperature is also an important factor controlling the activity of microorganisms and hence the occurrence of organic acids.

Mitsui *et al.*<sup>7 8</sup> observed that organic acids were accumulated at low soil temperatures but not at higher temperatures. They explained that the decomposition of organic acids lagged behind production at low temperatures. Yamane and Sato<sup>17</sup> studied the production of organic acids with various carbohydrates added as affected by temperature. With glucose added, the production of acetic and butyric acid, over a 6-week period, proceeded more slowly at 10°C than at 15°C. At 20°C the concentration of acids declined after 4 weeks, while at 25°C, 30°C, and 35°C the time period of peak production was shorter. Results were similar with starch, cellulose, and gelatin as substrate materials.

The present experiment was designed to study the kind, amount, and time of organic acid production with rice straw added to soils which were not puddled or previously kept in a chemically reduced condition. This study was initiated to determine the problems assoc-

iated with the incorporating rice straw into soil and to investigate the occurrence of organic acids which might be harmful to the growth of rice plants. Straw incorporation is becoming an essential practice in areas where rice is direct seeded and where prohibitions prevent burning as a disposal technique.

#### MATERIALS AND METHODS

Twenty grams of air-dried soil were incubated at temperatures of 10, 20 and 30°C in 125-ml Erlenmeyer flasks with rice straw at rates of 0, 0.25, and 0.5 per cent of soil weight. Applied to all flasks prior to straw addition were 50 ppm N as  $(\text{NH}_4)_2\text{SO}_4$ , 25 ppm P, and 31 ppm K as  $\text{KH}_2\text{PO}_4$ . The soil used was a Sacramento clay taken from a depth of 0–30 cm. The soil pH (soil : water = 1 : 5) was 6.7. Mechanical analysis of the soil showed 50.6 per cent clay, 47.6 per cent silt, and 1.8 per cent sand. Total carbon content was 1.64 per cent, and N was 0.098 per cent, resulting in a C : N ratio of 15 : 1. The soil was flooded after treatment with a 4-cm depth of deionized-distilled water. Flasks were incubated in duplicate treatments to provide samples for analysis after 5, 10, 15, 20, 25, 30, 40, 50, and 60-days of incubation.

##### *Measurement of organic acids*

Phosphoric acid was added to the soil suspensions to adjust them to pH 1.0. The suspension was then shaken for 30 minutes and filtered through Buchner filters. The filtrate was analyzed immediately for organic acids with a gas chromatograph, Varian model 1800, equipped with a hydrogen flame detector, by the method of Laskowski and Broadbent <sup>6</sup>.

The organic acids were separated on a 5-foot by 1/8-inch-o.d. glass column packed with 6 per cent FFAP on 80/100 Porapak Q. The carrier gas was  $\text{N}_2$  with a flow rate of 30 ml/min., and air flow 200 ml/min. The column temperature was maintained at 180°C isothermal, while the temperatures of the column inlet and detector were, respectively, 230 and 260°C. The alternator was set at 1, and the range at  $10^{-11}$ . The amount of sample injected was 5  $\mu\text{l}$  and peak areas were measured with an electronic integrator, Varian model 477, with a digital printout. The recorder responses were calibrated by comparing peak areas of the unknowns with those standard quantities of organic acids.

##### *Measurement of redox potential*

The soil, undisturbed and not exposed to the atmosphere, was measured for its redox potential with a platinum and a calomel electrode. Several spots were measured in each flasks for Eh, and the average was recorded. A platinum electrode, properly standardized with quinhydrone standards was used for all treatments.

## RESULTS AND DISCUSSION

Figures 1–3 show the amounts of organic acids produced with time variables up to 60 days as influenced by temperature and the amount of straw added. Only acetic acid was detectable in rice straw treated soil in incubation periods up to 40 days of incubation.

Without straw added, the maximum amount of acetic acid produced was 10.6  $\mu\text{eq}$ , observed at 30°C. The peak acetic acid production at 20 and 10°C respectively was 6.9 and 4.5  $\mu\text{eq}/20\text{ g soil}$ . At all temperatures, acetic acid production peaked on the 15th day. In the 30°C incubation, acetic acid concentration decreased gradually, while at 20 and 10°C there was another (smaller) peak production

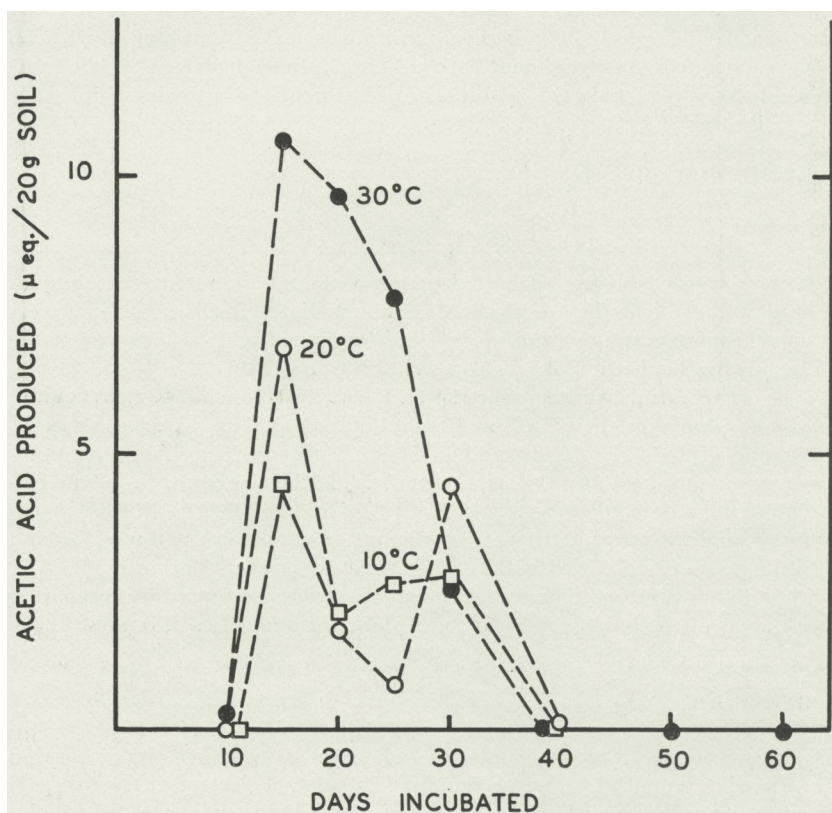


Fig. 1. Effect of temperature on acetic acid production without added rice straw.

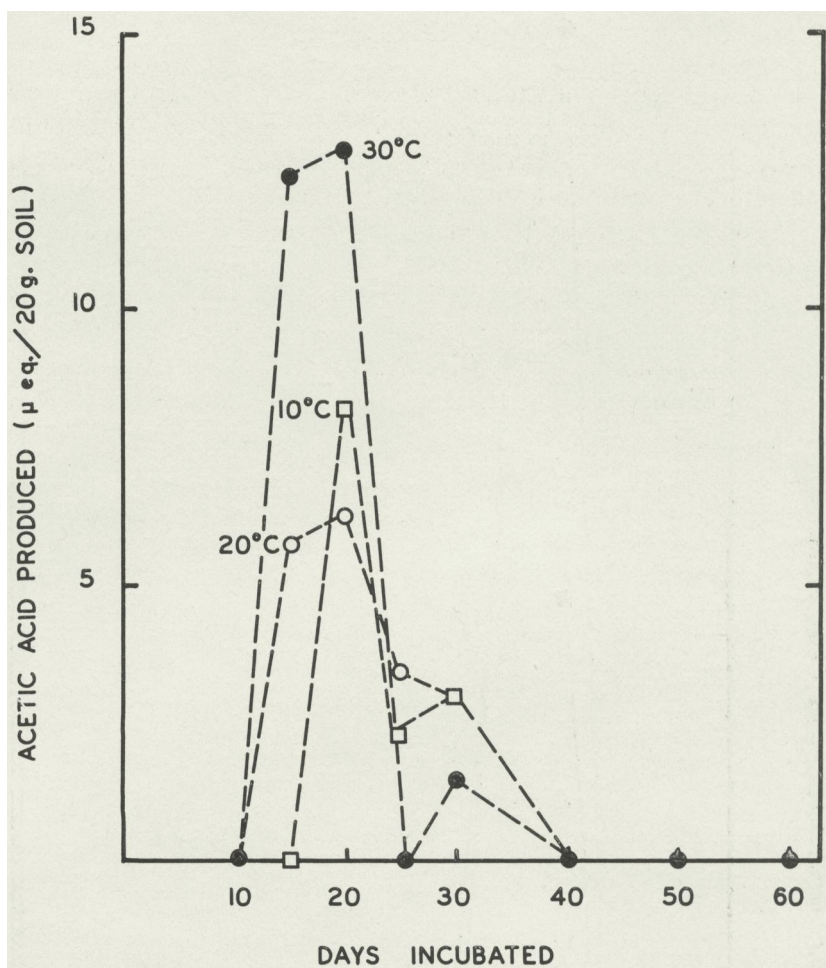


Fig. 2. Effect of temperature on acetic acid production with 0.25 per cent rice straw added.

on the 30th day (Fig. 1). With 0.25 per cent straw addition and 30°C incubation, the peak of acetic acid production was spread between the 15th and 20th days, and the amount was 13  $\mu$ eq. The peak time was similar at 20°C, amounting to 6.5  $\mu$ eq. At 10°C, the peak was on the 20th day (8.2 meq). At all three temperatures a second (small) peak was observed on the 30th day (Fig. 2). Rice straw added at 0.5 per cent and incubated at 30°C produced the largest amount of acetic acid, 22.7  $\mu$ eq on the 15th day. At 20°C,



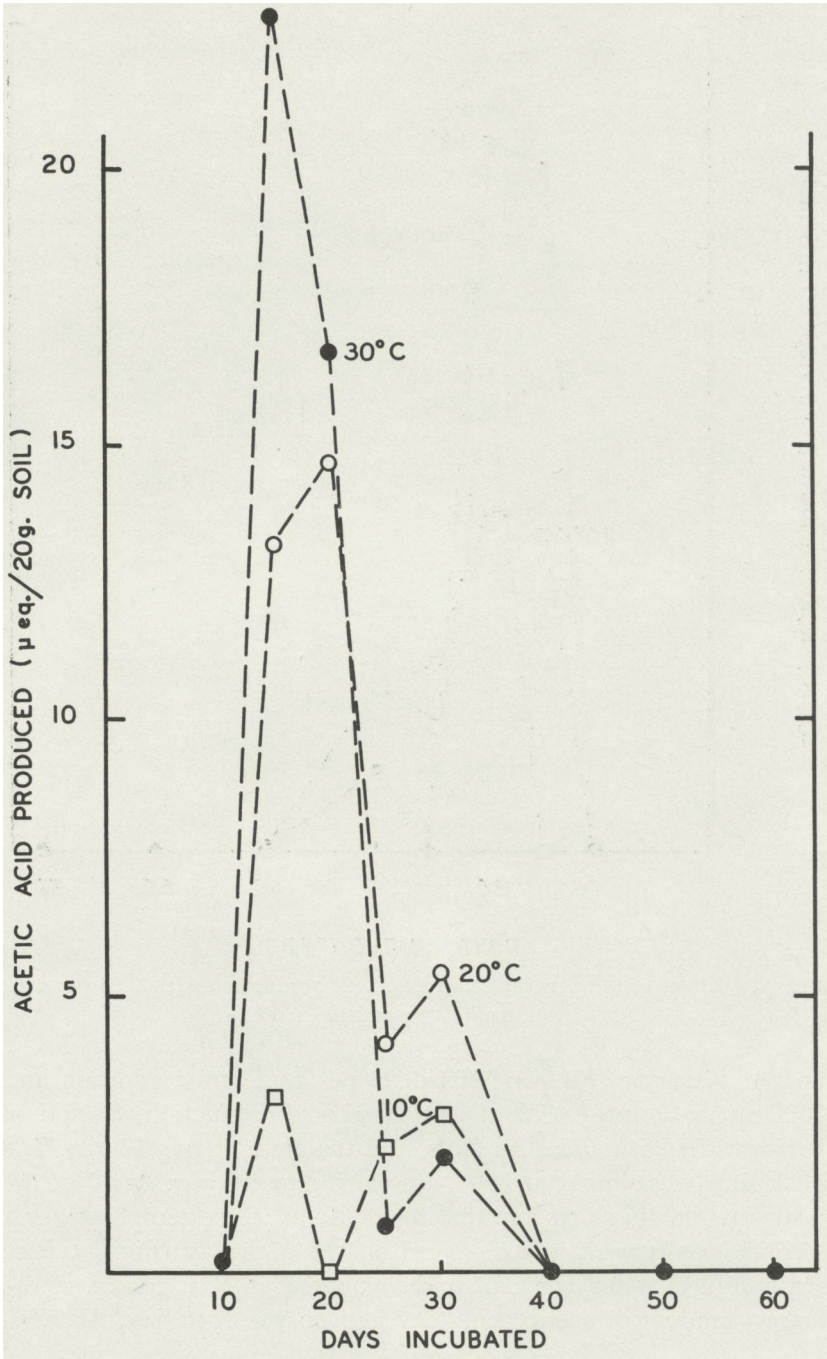


Fig. 3. Effect of temperature on acetic acid production with 0.50 per cent rice straw added.

acetic acid production peaked on the 20th day (14.7  $\mu\text{eq}$ ) as was the case at 10°C also which peaked on the 20th day (3.2  $\mu\text{eq}$ ). As with other rates of rice straw, a second smaller peak was observed on the 30th day at all three temperatures (Fig. 3).

Higher rates of straw addition produced greater amounts of acetic acid, as would be expected since more substrate is provided for the microorganisms. Temperature increases from 10 to 30°C increased the amount of acetic acid produced at a given straw rate. Temperature being a major factor controlling biological activity, increased microorganisms activity and acetic acid production.

At all sampling dates the amounts of acetic acid produced, as influenced by straw additions and incubation temperatures, were found in the range of 0 to 22.7  $\mu\text{eq}/20\text{ g soil}$ . In published literature amounts of organic acids, lower than 20  $\mu\text{eq}$ , are usually reported, as traces<sup>5</sup>. Organic acids in these small amounts were shown to have insignificant effects on plant growth.

There are two main reasons why organic acid production was so low in the present experiment. The first is that the soil used, simulated soil conditions characteristic of California rice soils, which are not flooded prior to rice plantings. These soils generally have a low soil moisture content and are prepared dry for subsequent flooding and direct seeding. Since the experimental soil was air-dried prior to incubation, it took longer on subsequent flooding to attain a low redox potential than would a puddled soil, or one kept under flooded conditions<sup>15 16</sup>.

The redox potential values presented in Table 1 show that without rice straw addition the Eh7 values are all positive, indicating no chemical reduction at any of the three temperatures. When 0.25 per cent straw was added, the Eh7 was still positive at 10°C, whereas at 20°C it was reduced to -95 mv on the 15th day, and at 30°C to -65 mv by the 5th day. Rice straw added at the 0.5 per cent rate reduced the soil to -58 mv at 10°C on the 25th day. At 20°C, 10 days were required to reduce the soil environment to -108 mv, whereas at 30°C the Eh7 was -115 mv by the 5th day.

Even though the redox potential values were still positive without straw added, acetic acid was produced in the incubated soil. The probable reasons for this is that acetic acid is produced through both aerobic and anaerobic microbial processes<sup>11</sup>.

The reason why organic acid production was low is that rice straw



TABLE 1

Eh<sub>7</sub> of Sacramento clay soil amended with rice straw incubated at 10, 20, and 30°C

Treatments		Eh <sub>7</sub> (mv) of soil at various intervals (days)								
Temperature °C	Amount of straw added % of soil weight	5	10	15	20	25	30	40	50	60
10	0	190	185	170	168	170	170	190	180	190
10	0.25	175	180	138	133	138	95	155	65	80
10	0.50	170	165	90	78	-58	-80	-75	-80	-60
20	0	183	160	105	115	80	80	70	50	95
20	0.25	165	75	-95	-60	-80	-68	-20	30	35
20	0.50	140	-108	-153	-138	-155	-140	-115	-65	-65
30	0	138	55	43	50	30	50	45	60	70
30	0.25	-65	-110	-128	-95	-40	-50	-35	-50	10
30	0.50	-110	-170	-185	-170	-138	-115	-145	-145	-165

is resistant to decomposition. Easily decomposable organic materials would likely produce larger amounts of organic acids faster under the same anaerobic conditions <sup>11 12 15 16</sup>. The amounts and rates of organic acids produced were higher for green manures than for rice straw, but lower than for pure carbohydrate materials <sup>1 9 12</sup>. Gotoh and Onikura <sup>2 3 4</sup> also observed a lower level of organic acid production with rice straw additions.

In these experiments, acetic acid was the only organic acid found present in the flooded soil incubated with rice straw. The amount present increased with additions of rice straw and increases in temperature. The peak production occurred on the 15th day with no rice straw, on the 20th day with 0.25 per cent rice straw, and on the 15th–20th day with 0.5 per cent rice straw added. The peak amount of acetic acid produced was 10.6 µeq/20 g soil with no rice straw, 13 µeq with 0.25 per cent rice straw, and 22.7 µeq with 0.05 per cent added rice straw. The amounts produced (0 to 22.7 µeq 20 g soil) were much too low to have significant toxic effects on rice plants. In relation to field conditions, rice straw additions up to 10 tons/ha might be incorporated into non-reduced rice soils without the danger of organic acid toxicity if a period of 15–30 days elapse, between residue incorporation and flooding for the direct seeding of rice.

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