Microbial Ecology in Submerged Soils as Revealed by Using TTC (Triphenyl Tetrazolium Chloride) (Part 2)

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ABSTRACT

A few aspects of microbial lives associating with decomposition of rice straw and sesbania leaf in the submerged three Thai paddy soils (one sandy soil and two clayey soils) were examined rather in detail under a microscope using the TTC technique. Water-soluble substances extracted from these plant debris were confirmed to be preferred substrates for soil microorganisms. Removal of these substances somewhat affected the decompostiion process of the plant debris themselves, mode of affection being different between rice straw and sesbania leaf. A succession of microorganisms including portozoa was recognized during decomposition of the plant debris. This succession was considered to proceed from r-strategists to K-strategists. Additional evidence was obtained to support the assumption that sesbania leaf contained some organic compounds toxic to microorganisms and in the initial period of decomposition this compounds were more effective in the sandy soil than in the clayey soils because these compounds were adsorbed by clay particles. Contrary to the widely accepted concept, the decomposition rates of these two plant debris were higher in the clayey soils than in the sandy soil. This may be caused not only by the effect of the toxic substances but also by the difference in microflora. Available information suggested that microbial decomposition of rice straw in the Thai soils were somewhat different from that in Japanese soils.

Key words : TTC technique, Sesbania, organic decomposition

INTRODUCTION

In a previous paper (Patcharapreecha et al.), decomposition processes of rice straw and sesbania leaf in submerged three soils in Northeast Thailand were investigated by examining changes in color and shape at microsites of the soils amended with these plant debris under a microscope using the TTC-technique (Wada 1978). This experiment clarified general features of microbial ecology associating with the decomposition of these plant debris. The same type of experiment was continued to obtain more detailed information on its several aspects: (1) Role of water-soluble substances of the plant debris, (2) succession of protozoa, (3) differences between sandy soil and clayey soils and (4) differences between Thai soils and Japanese soils.

MATERIALS AND METHODS

Soils: Soil samples were the same as those in the previous experiment (Patcharapreecha et al.). They were Roi-Et series (Re soil), Phimai series (Pm soil) and Ratchaburi series (Rb soil).

Plant debris: Air dried chopped rice straw (about 0.5 cm) and air dried sesbania green leaves were collected and hot water-soluble substances (WSS) were extracted from these plant debris by boiling them in water (1 g in 50 ml water) for 1 hr. The residues of the plant debris which were extracted with hot water were used as well as the unextracted plant debris in the experiment. Their pertinent properties are tabulated in Table 1.

Hereafter in this paper, the rice straw and sesbania leaf will be named as "fresh plant debris" to distinguish them from native plant debris originally contained in the soil.

Triphenyl tetrazolium chloride (TTC): The reagent was purchased from Fluka Chemie AG.

Incubation: Nine treatments were set up as shown in Table 2. Seven grams portion of the 3 soil samples were amended with TTC (20 mg for Re soil and 40 mg for Pm and Rb soils) and 500 mg $CaCO_3$ was placed in the lid of a Petri dish and submerged with about 10 ml distilled water (treatments 1-7) or WSS (treatments 8 and 9). A few pieces of the plant debris were immersed into the submerged soil (treatments

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Table 1 Some Properties of Sesbania and Rice Straw.

	Total N %	Water Soluble Organic Substance		
		Organic N %	Organic C %	C/N
Sesbania	3.53	0.778	16.96	21.80
Rice Straw	0.66	0.120	4.52	37.66

2-7). In treatments 4 and 7, a few pieces of rice straw were immersed in one half of the soil and sesbania leaves in another half. The submerged soil of all the treatments were degassed, covered with a bottom of the Petri dish in the similar way as the double layer plate method for cultivating anacrobic microorganisms, wrapped with a piece of plastic film and incubated at room temperature from November 1989 to January 1990.

Observation: Almost every day, all the Petri dishes were observed under a stereoscopic microscope (Nikon, SM2-10) at magnifications x10 - x40. Photographs were taken using an automatic camera system (Nikon, HFX-II).

RESULTS AND DISCUSSION

Roles of water-soluble substances (Effects of removal of hot water-soluble substances)

When the unextracted fresh plant debris were placed in the soil, soil matrix sometimes became redder than the fresh plant debris themselves in the initial period of incubation. This was especially noticeable in the sandy Re soil with unextracted sesbania leaves (Figure 1). This observation suggested (1) water-soluble substances of the fresh plant debris were speaded into the soil matrix by diffusion and preferrentially utilized by microorganisms in the initial period and (2) the spreading of the water-soluble substances was much easier in the sandy soil than in the clayey soils.

Table 2 Detail of the Treatment.

Treatment No.	
1	Control
2	*Sesbania rostrata*
3	Rice straw
4	1/2 Sesbania + 1/2 Rice straw
5	Sesbania (removal of water soluble organic matter)
6	Rice straw (removal of water soluble organic matter)
7	1/2 Sesbania + 1/2 Rice straw (removal of water soluble organic matter)
8	Water soluble organic matter from Sesbania
9	Water soluble organic matter from Rice straw

This consideration was supported by (1) quick reddening of the soil matrix did not occur when the hot water extracted fresh plant debris were placed in the soils (treatments 5 and 6) (Figure 2 and 3), (2) The hot water-extracts contained a rather large amount of organic substances (Table 1) and (3) soil matrix of the treatments 8 and 9, especially 8, became red (Figures 4 and 5).

In the case of rice straw, removal of the hot water-soluble substances made the initial reddening of the main entrances of colonization of the fresh plant debris (Patchar apreecha et al.) became evident. Probably, the hot water-treatment extracted preferable organic substances from inner tissues which were accessible to bacteria: These inner tissues were porous ans may allow bacteria to enter and multiply using the water-soluble substances. Consequently, pioneer nicroorganisms were obliged to grow only at the main entrances of colonization when they tried to attack the hot water-extracted rice straw.

In the case of sesbania leaves, the hot water-extraction not only extracted water-soluble substances but also decreased the amount of the entrapped air. The removal of water-soluble organic substances retarded start of microbial decomposition of the sesbania leaves. This suggested that the pioneer microorganisms in the unextracted sesbania leaves mainly utilized the water-soluble substances at the main entrance of colonization. On the contrary, the absence of entrapped air was helpful to the microbial attack from outside the surface of sesbania leaves.

The hot water-extraction enhanced decomposition of rice straw in the later period of incubation. This was especially so for the sandy soil. On the contrary, the same treatment retarded decomposition of sesbania leaves (Figure 6). That is, in the later period of incubation, the unextracted sesbania leaves were found to become very soft and easily disintegrated by any mechanical disturbance. Almost all the tissues seemed to be already disintegrated except the secondary thickening of the vascular bundles which appeared as if it were springs. In this period, the hot water-treated sesbania leaves did not become soft as yet, probably due to delay of decomposition precesses (Figure 7). In addition, at this stage of decomposition, green lines were recognized inside the main vein (Figure 8). At present,

Figure 1 ***S. rostrata*** **in Roi-Et soil (soil matrix became redder than the fresh plant debris).**

Figure 2 Sesbania removed water soluble substance (Roi-Et soil, 2 days, soil not red).

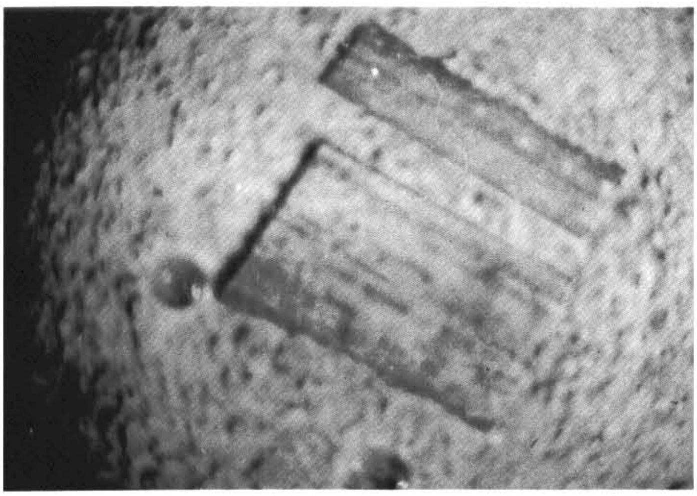


Figure 3 Rice Straw removed water soluble substance (Phimai soil, soil not red).

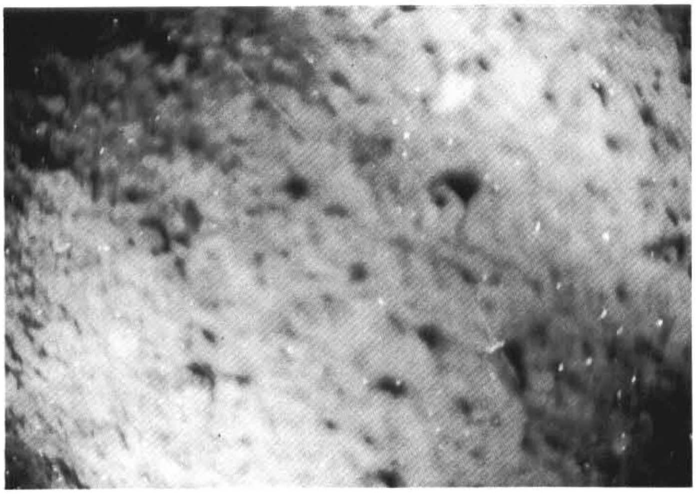


Figure 4 Water soluble substance from sesbania (soil matrix very red).

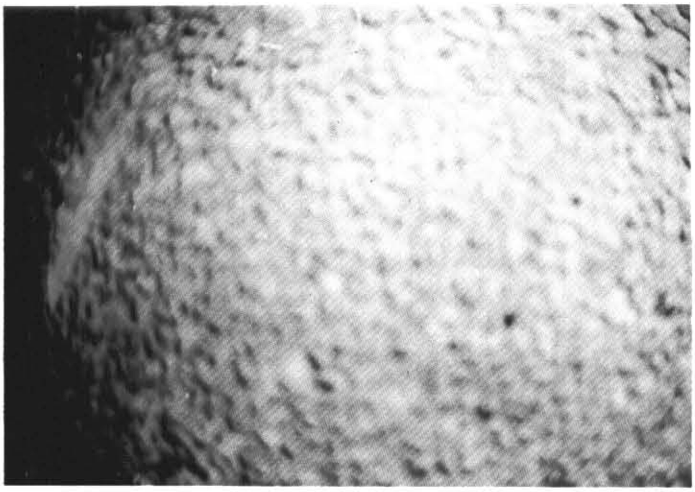
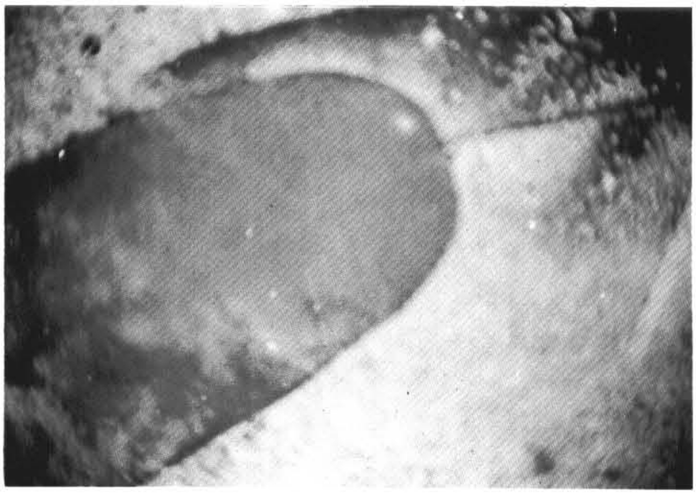


Figure 5 Water soluble substance from rice straw (soil matrix red).



Figrue 6 Sesbania removed water soluble substance in Phimai soil (reterded decomposition).

Figure 7 Sesbania removed water soluble substance in Roi-Et Soil (delay decomposition).

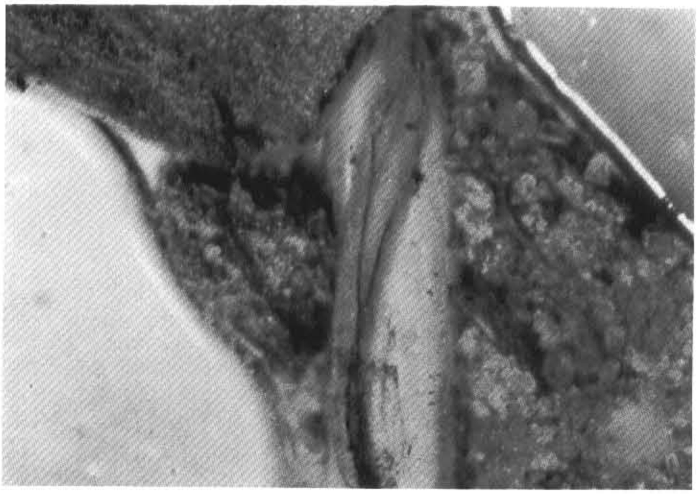


Figure 8 Sesbania in Roi-Et soil (green lines inside the main vein).

Figure 9 Petiole of sesbania became red (Ratchaburi soil, 2 days).

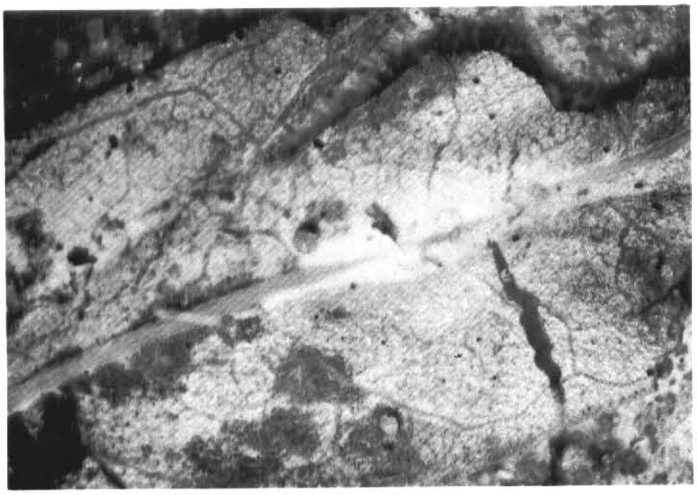


Figure 10 Intacted sesbania (Roi-Et soil, 2 days).

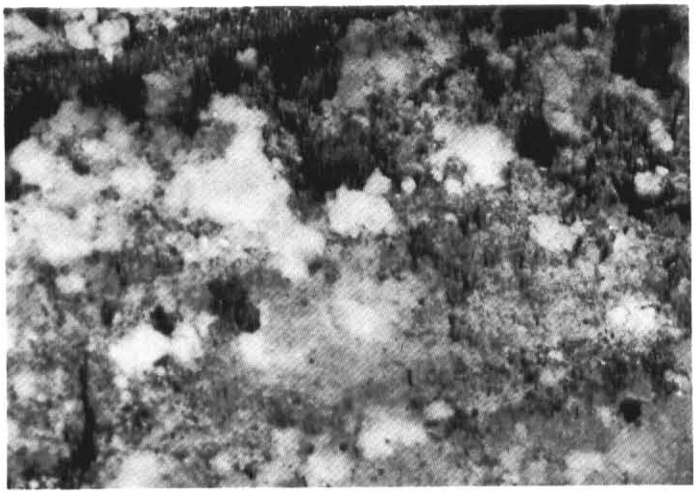


Figure 11 Sulfate reduction became more conspicuous in clayey soil (Phimai soil, 13 days).

identify these lines were not identified.

This apparently contradictory results may be explained in the following way:

1. Rice straw was mainly composed of hemicellulose, cellulose, lignin and silica. The lignin and a part of silica played a role to protect hemicellulose and cellulose against microbial attack. The hot water-treatment removed hemicellulose and silica and partially destructs the protective mechanism of lignin and silica, which make the hot-water extracted rice straw more decomposable than the unextracted rice straw.

2. Sesbania leaves must be mainly composed of protein, hemicellulose and cellulose, lignin content being low. The hot water-treatment may extrect protein and hemicellulose, leaving the cellulose rich residue, which is a poorer substrate than the original sesbania leaves for micro-organisms.

Protozoa and microbial succession

As mentioned above, several kinds of protozoa successively appeared in the submerged soil amended with the fresh plant debris. This is espcecially so for the sandy Re soil amended with rice straw. Probably, the sandy soil provided more sufficient space for ciliata and flagellata to move around.

After a few days of incubation,many tiny protozoa which were completely stained red were quickly moving in the soil solution. They tended to gather near periphery of the Petri dish where O_2-supply must be higher than other places. Probably they were not tolerant to strong reduction.

These tiny protozoa were replaced by either of two other kinds of protozoa. One type of protozoa, probably cilliata, were big in size and were slowly patrolling and picking the fresh plant debris. Only their intestines were stained red. Another type of protozoa were intermediate between the two protozoa mentioned above in terms of size, movement, red-staining and population density.

In the later period, sometines another type protozoa appeared. They were not stained red, their population was very low and their movement was also very slow.

On the basis of the observations mentioned above, microbial succession in the submerged soil amended with the fresh plant debris was supposed to proceed in the following way:

1. In the initial period of incubation, some bacteria, probably typical r-strategists# (De Angelis et al. 1980, Odum 1983), rapidly multiply utilizing water-soluble substances which were abundantly supplied from the fresh plant debris. This bateria were actively grazed by protozoa, also, typical r-strategists, which were small in size and rapidly move around searching for the bacteria. These bacteria and protozoa were well adapted to the enviroments of initial period of incubation when the amount of water-soluble organic matter was abundant and the soil was not strongly reduced.

2. In the middle period of incubation, most of the bacteria were slowly growing on and in the tissues of plant debris because the water-soluble substrates were exhausted in the soil solution. The big protozoa took up the bacteria contained in the decomposed tissues. These bacteria and protozoa had some characteristics of K-strategists (De Angelis et al. 1980, Odum 1983) and were tolerant to strong reduction.

3. If the supply of the water-soluble substances continued in the strongly reduced soil, the middle size anaerobic protozoa, grazers of bacteria in the soil solution, had a chance to work in place of the tiny protozoa.

4. In the later period of incubation when almost all the preferable substrates were depleted, slow-growing bacteria, possibly typical K-strategists, were still working. These bacteria were grazed only by some protozoa which had characteristics of typical K-strategists and tolerant to strongly reduced conditions

In treatments 8 and 9 of the sandy Re soil, the many red stained tiny protozoa were actively swimming only for a few days immediately after incubation. In the case of the clayey soils, treatments 8 and 9 were quickly reduced (sulfate reduction started within a few days) and the red stained tiny protozoa were hardly recognized. These results lend supports to the above mentioned supposition: The red stained tiny protozoa were eating bacteria which were fed on the water-soluble substances and they were not tolerant to strongly reductive state. In addition, this were not tolerant to strongly reductive state. In addition, this results supported the presumption that the toxic organic compounds were not soluble in water.

Differences between the clayey soils and the sandy soil

In the preceding descriptions, we pointed out differences in both decomposition of the fresh plant debris and the microbial lives between the clayey soils and the sandy soil Here, we will sum up and supplement these descriptions.

1. In the initial peirod of incubation, decomposition of sesbania leaves was faster in the clayey soils than in the sandy soil (Figure 9 and 10). This is a violation of the believe that fresh plant debris is decomposed much faster in sandy soils than in clayey soils (Jenkinson 1988). It is assumed that sesbania leaves contained organic compounds which suppressed bacterial growth and that the effect was more pronounced in the sandy soil than in the clayey soils because the organic compounds were adsorbed by clay particles. If this supposition is tenable, It is can be expected that most of the tropical plants, especially leguminous plants, contain some toxic organic compounds to protect themselves against microbial invasion when they are alive and that these toxic substances remain in their debris, resulting in suppression of microbial decomposition.

The decomposition rate of sesbania leaves was higher for the two clayey soils than in the sandy soil even in the later period of incubation. This is a more seriuos violation of the widely accepted belief than that mentioned above. Probably, microorganisms responsible for decomposition are different between the sandy soil and the clayey soils. For instance, ability of cellulose decomposing bacteria was higher in the clayey soil than that of the sandy soil.

2. Even in the later period of incubation, the decomposition rates of the fresh plant debris were higher in the clayey soils than in the sandy soil. This may be caused by defference in microflora between the soils.

3. Sulfate reduction became more conspicuous in the clayey soils (Figure 11) than in the sandy soil. This must be resulted by the fact that content of sulfate ion was less in the sandy soil than in the clayey soils: Probably, sulfate ion was more easily removed from the sandy soil than from clayey soils by leaching.

4. The sandy soil appeared to be less strongly reduced than the clayey soils in the Petri dish. This may be due to difference in the space of macropores between the clayey soils and the sandy soil. The similar situation may be met at the uppermost part of the submerged paddy soil in the field.

Differences in microbial ecology between Thai and Japanese soils

As mentioned above, general features of microbial decomposition of rice straw in the submerged soil are similar between Thai and Japanese soils. However, It is noticed that the following differences in microfauna, microflora and decomposition of rice straw between Thai and Japanese soils.

1. Microfauna

Tested amoeba which were usually present in Japanese paddy soils (Wada 1980) were not recognized in Thai paddy soils as yet. On the contrary, several types of protozoa with different sizes and mobilities, probably ciliata and flagellata, were actively moving in the soil solution of Thai paddy soils but only type of ciliata was predominant in the soil solution of Japanese paddy soils.

2. Microflora

Many sporangia of fungi were present and some of them became red due to formation of red formazan in the presence of TTC after one or two days of incubation. Even under strongly reduced conditions, one species of fungi spread and formed colonies on the surface of rice straw in Japanese soils. Furthermore, sometimes streptomycetes extended hyphae on native plant debris and formed colonies inside bubbles of methane in Japanese soils (Wada 1980). Such fungi and actinomycetes could not be found in Thai soils.

3. The rate and the pattern of decomposition

The decomposition rate of rice straw seemed slower in Thai paddy soils than in Japanese paddy soils.

Further experiments are needed to confirm these differences and to find out their reasons.

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APPENDIX

r-strategist : A member of a young unstable poorly populated ecosystem. It will grow rapidly wasting a lot of resources.

K-strategist: A member of a mature stable densely populated ecosystem. In will grow slowly and keep its life in harmony with other members.