

MORPHOLOGICAL STUDY ON THE DIGESTION OF RICE STRAW BY TREATMENT WITH AMMONIA AND SULPHUR DIOXIDE

Y. H. Song¹, M. Shimojo² and I. Goto²

Dept. of Animal Science, College of Animal Agriculture
Kangweon National University, Chuncheon 200-701, Korea

Summary

Rice straw treated with anhydrous ammonia and/or sulphur dioxide was incubated with rumen liquor for 24 hours and 48 hours to investigate the changes in cell wall structure caused by the treatments and bacterial degradation using scanning electron microscopy (SEM). A less significant tissue loss of untreated rice straw was inspected after incubated for 24 hours and 48 hours. Sulphuration decreased the thickness of sclerenchyma and apparently removed parenchyma tissues. Ammoniation degraded the phloem, and the lignified inner portion of the cell wall was completely, however, little collapsed epidermis and vascular bundles. Ammonia and Sulphur dioxide combined treatment removed the inner layer from outer layer. The extent of apparent degradability following combination treatment was the largest due to the enhanced microbial degradation of sclerenchyma and parenchyma cells.

(Key Words: Morphological, Microscopy, Rice Straw, Ammonia, Sulphur Dioxide)

Introduction

Increase in digestibility *in vitro* and *in sacco* of cereal straw by treatment with ammonia and/or sulphur dioxide is caused by enhanced degradation of fibrous materials by rumen microbes (Song et al., 1991a, 1991b). Studies on the ammoniation and sulphuration of rice straw have revealed the improvement of nutritive value, however, little information is available for ultrastructural changes in the treated straw during incubation with the rumen microbes. Scanning electron microscopy (SEM) is useful for surface analysis of specific tissue loss over time from the exposed surface of forage section and thereby provides relative data on the rate and extent of tissue digestion among various forages (Akin, 1979).

The objective of this study was to investigate by SEM the morphological changes during incubation with goat rumen fluid in rice straw treated with ammonia and sulphur dioxide.

Materials and Methods

Rice straw (japonica type, var. Nihonbare) collected at the Kyushu University Farm in 1989 was dried for 48 hr at 70°C. The midportion of the straw cut into about 2-3 cm length. The straw was moistened with water (450 g/kg DM) and incubated at 20°C for 4 days in a polyvinyl jar.

Sulphur dioxide (40 g/kg DM) was injected into the jar for storing at 70°C for 3 days or anhydrous ammonia (30 g/kg DM) at 20°C for 28 days, and a combination treatment of ammoniation followed by sulphuration also was performed. The treated straw were aired 3 days to exclude excess chemicals and cut into cross sections of 2 to 5 mm in length.

The straw digestibility was determined with rumen liquor (Minson and McLeod, 1972) from three Tokara goats fitted with rumen cannulae and fed on alfalfa hay.

Digested samples were retrieved at specified intervals and prepared for scanning electron microscopy (WET-SEM) by a modified method of Akin and Amos (1975). The intact and incubated materials were washed with distilled water and then kept in a refrigerator to restore for a few days. Thereafter fractions were placed in 4% glutaraldehyde in 0.1 M cacodylate buffer

¹Address reprint requests to Dr. Y. H. Song, Dept. of Animal Science, College of Animal Agriculture, Kangweon National University, Chuncheon 200-701, Korea.

² Department of Animal Science, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

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and then postfixed in 1.5% buffered osmium tetroxide at 4°C for 3 days. Specimens were washed in buffer and dehydrated, for 5 minutes in 65%, 10 minutes in 75%, 15 minutes each in 85, 95, 99% and four times in 100% ethanol (V/V). Specimens were dried in a desiccator and then observed in a Akishi Beam Tech. (ABT-3 2) at 15kV.

Results and Discussion

1. Micrographs of cross section of untreated and treated rice straw stems before incubation

Figure 1 shows untreated rice straw stems before incubation, and this micrograph showed

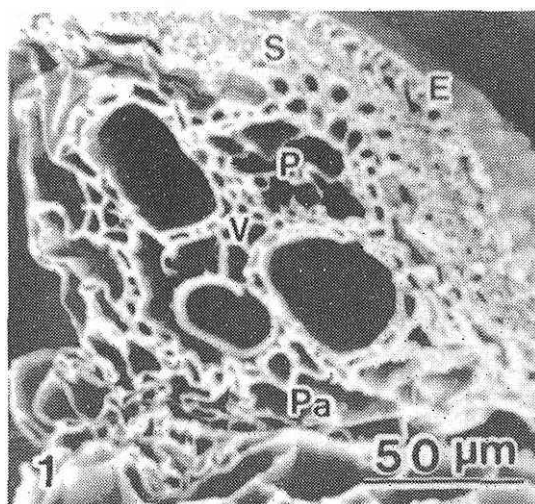


Figure 1. Cross section of untreated rice straw. The epidermis tissue (E), parenchyma (Pa), phloem (P), sclerenchyma (S) and vascular tissue (V) maintained structural integrity.

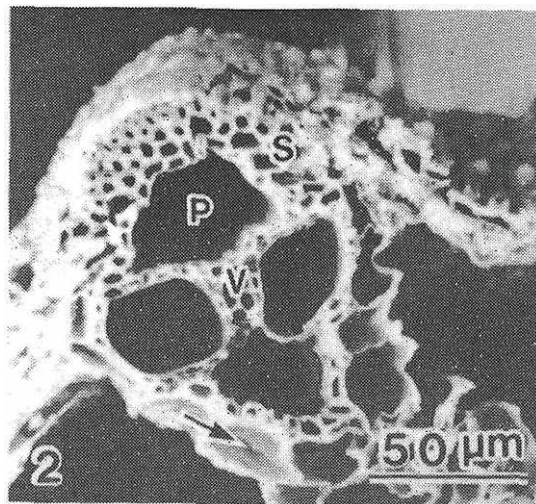


Figure 2. Untreated rice straw incubated with rumen liquor for 24 hr. The parenchyma cell (arrow), vascular tissue and sclerenchyma were partially collapsed.

2. Micrographs of cross sections of untreated and treated straw stems incubated for 24 hr by rumen liquor

Incubation for 24 hr of untreated straw (figure 2) showed digestion of starch granules, partially ruptured parenchyma (Harbers et al., 1982), vascular bundle sheath and phloem. Dekker et al. (1972) reported that starch in cell wall was completely degraded by rumen bacteria and protozoa. The thickness of sclerenchyma was decreased and distortion of the phloem and vascular bundle occurred after 24 hr incubation

that all tissue cell wall were intact and maintained structural integrity. Treated straws were not incubated showed slightly thinned parenchyma by SO_2 (figure 4), ruptured vascular bundle sheath and phloem by NH_3 (figure 7) and small bundle sheath and sclerenchyma distortion giving them a fragile appearance after treatment by NH_3 and SO_2 (figure 10). Starch granules are intact in parenchyma (Itoh et al., 1981) and were not affected in treated straws (figure 4, figure 7, figure 10, arrow). Ammoniation and/or sulphuration had no effect on the localization of silica in the epidermis (Itoh et al., 1981) thus rumen microorganisms would not be expected to penetrate these walls (Harbers et al., 1982).

of SO_2 treated straw (figure 5).

The parenchymal tissues of sulphurated stems appeared to be more extensively degraded than those of untreated stems. The parenchyma, phloem, vascular bundle sheath, sclerenchyma and epidermis cells in stems were more distorted by ammoniation (figure 8). The inner layer, including the vascular bundle, was completely removed from the outer layer by ammoniation and sulphuration (figure 11). Lignified vascular tissue was not completely degraded, only separated from the epidermis. Cutinized epidermis and lignified tissue

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usually resist microbial degradation (Akin et al., 1973). The small vascular bundle sheath was easily collapsed by 24 hr incubation. Cells in combi-

nation treatment of straw stems were more damaged than by ammoniation alone (Itoh et al., 1981; Harbers et al., 1982).

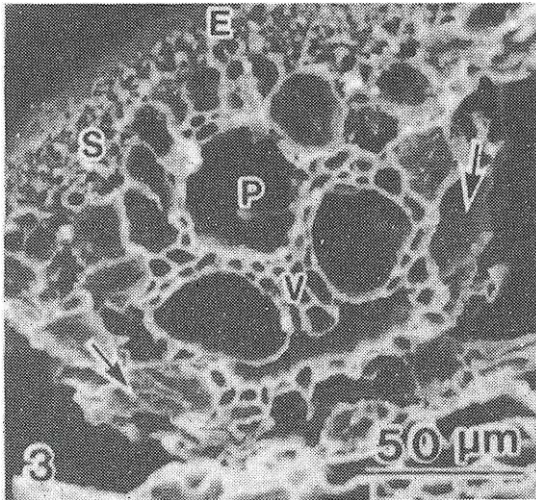


Figure 3. Untreated rice straw incubated with rumen liquor for 48 hr. The parenchyma was greatly removed (arrow).

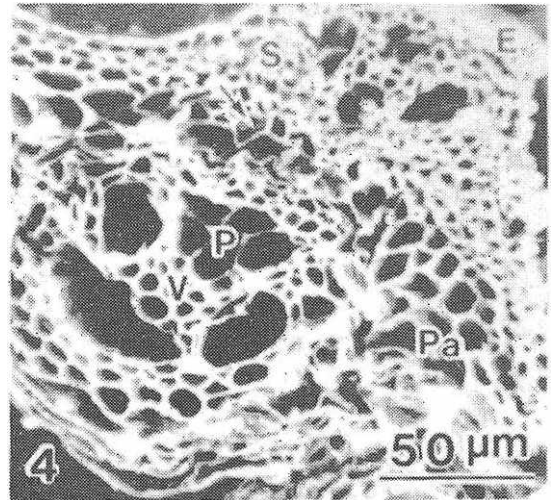


Figure 4. The 4% sulphurated rice straw. It shows a slight shrinkage of parenchyma however, starch granules were not affected in contrast to control.

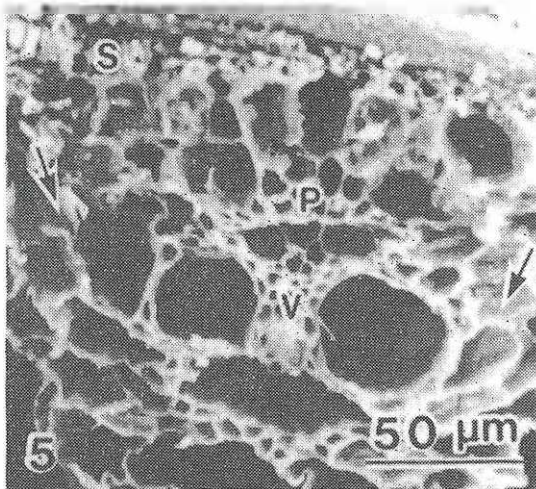


Figure 5. The 4% sulphurated rice straw incubated with rumen liquor for 24 hr. The parenchyma was more collapsed than untreated rice straw for 24 hr (arrow). The thickness of sclerenchyma was decreased.

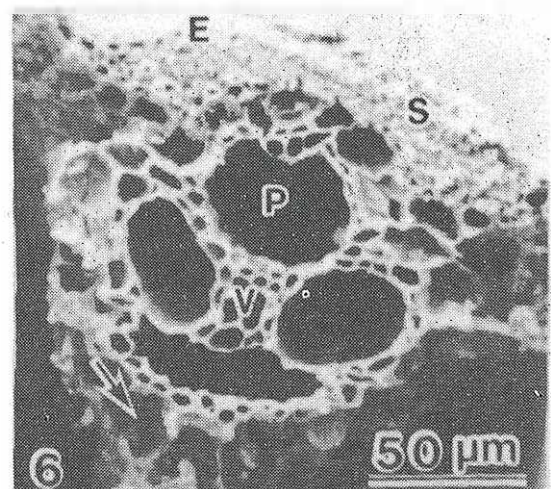


Figure 6. The 4% sulphurated rice straw incubated with rumen liquor for 48 hr. The parenchyma was completely removed (arrow).

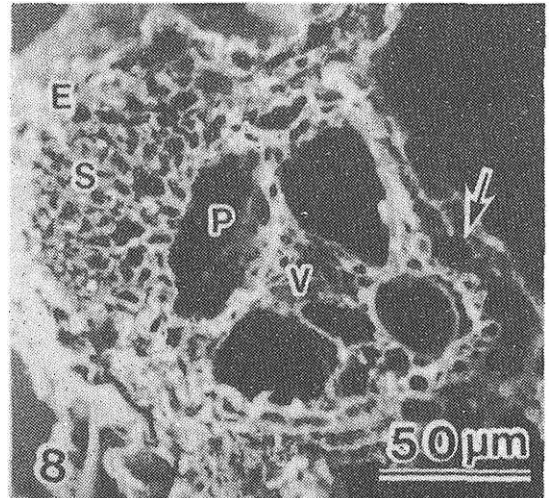
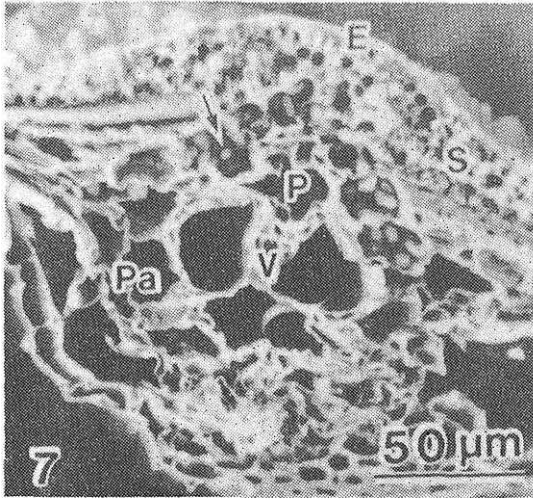


Figure 7. The 3% ammoniated rice straw, The cells of parenchyma and vascular bundle sheath were collapsed. Starch granules were found in thin-walled parenchyma (arrow).

Figure 8. The 3% ammoniated rice straw incubated with rumen liquor for 24 hr. It shows a severe destruction and most of the parenchyma completely removed (arrow). Vascular bundle sheath and sclerenchyma were remarkably decreased.

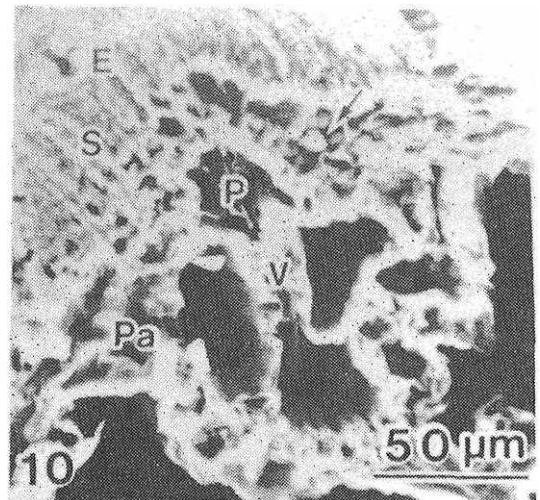
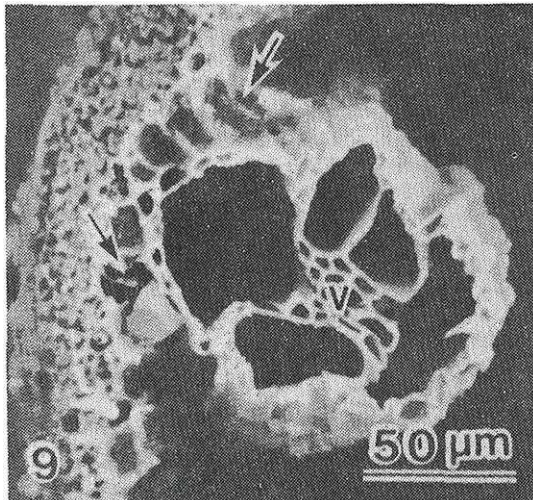


Figure 9. The 3% ammoniated rice straw incubated with rumen liquor for 48 hr. The sclerenchyma, parenchyma (arrow) and vascular bundle were degraded and cell wall constituents were digested.

Figure 10. The 3% ammoniated and 4% sulphurated rice straw. It shows severe destruction and most parenchyma collapsed, however, starch granules were found (arrow).

3. Micrographs of cross section of untreated and treated rice straw stems incubated for 48 hr incubation in rumen liquor

After incubation for 48 hr in rumen liquor, untreated straw showed no collapsed sclerenchyma

of outer epidermis (figure 3). However, the parenchyma was extensively distorted, and the phloem and vascular bundle sheath were more ruptured than by 24 hr incubation. In sulphuration, the parenchyma was completely removed

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from the section, and only large vascular bundle sheaths left attached to the outer mechanical layer (figure 6). Additional structural changes were observed in ammoniation straw (figure 9), which showed little collapsed epidermis and a large remainder of the rigid portions of the vascular bundle. The inner bundle sheath was attacked and degraded extensively in the phloem region, and the lignified inner portion of the cell wall

was completely removed (figure 9, arrow).

However, Harbers et al. (1982) reported ammoniation had no effect on the highly lignified sclerenchyma, vascular tissues and epidermis of wheat straw. In the combined treatment with ammoniation and sulphuration, 48 hr incubation in rumen fluid brought about more tissue degradation than the other treatment and little epidermis seemed to be left (figure 12).

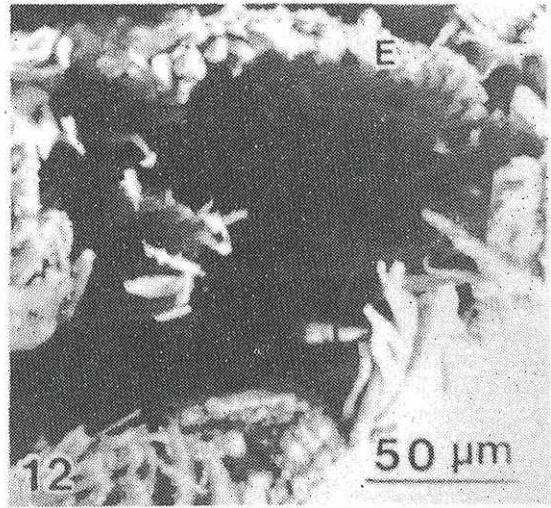
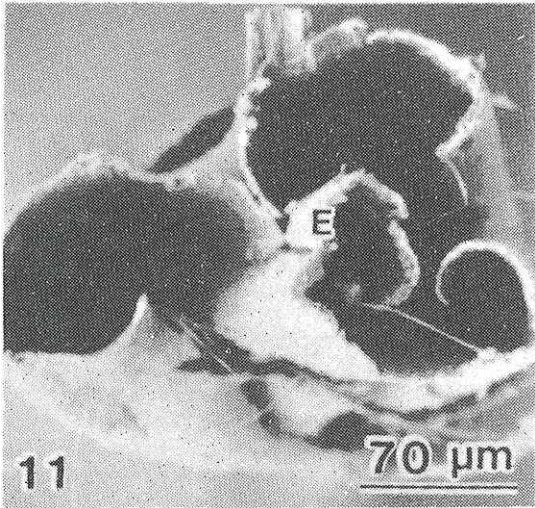


Figure 11. Combined treatment of rice straw incubated with rumen liquor for 24 hours. The inner layer was removed from the outer layer.

Figure 12. Combined treatment of rice straw incubated with rumen liquor for 48 hours. There seems to be nothing other than the cutinized epidermis.

Generally, the unligified tissues of a forage grass are easily broken down by rumen bacteria, which was also shown in a SEM study according to the comparison of incubated preparations (Cheng et al., 1977). The moderately resistant outer bundle sheath and epidermal tissues are broken down more slowly by the activity of adherent bacteria, and the highly lignified vascular and sclerenchyma tissue are left intact and have very few adherent bacteria (Cheng et al., 1977). It was shown that the microbial attack on cell wall constituents was affected by the deposition of lignin and silica in the tissues and by the steric disposition of various tissues. Phloem was disintegrated less quickly than parenchyma, although neither tissues were lignified. The explanation for this difference may be that the lignified tissues enclosing phloem hinder the microbial invasion

(Kawamura, 1973).

The bacterial digestion of parenchymal potential nutrients, including starch in parenchyma cells, are finally performed by rumen microbes (Itoh et al., 1981). Microbial degradation of the parenchyma cell wall of rice straw was apparently highly increased by the combination of ammoniation and sulphuration. The cell wall of parenchyma, which was not lignified, was readily digested compared with the epidermis and mechanical tissues such as vascular bundle and sclerenchyma containing silica and lignin (Kawamura, 1973).

In the rice plant, silica is localized in the epidermis, vascular bundle and bundle sheath and in the sclerenchyma linking them (Yoshida et al., 1962a, 1962b). It is considered that the quantities of lignin and silica in these materials affect the rate of microbial degradation. The rate and extent

of microbial digestion of rice straw is promoted by sulphuration or ammoniation and further increased by their combination. Another treatment may be needed for degradation of the structure of epidermis and bundle sheath, including lignin and silica.

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