Degradation of Grasses and Straw Cell Walls by Cellulase Preparations as Silage Additives

JianGuo ZHANG*, Sumio KUMAI, Ryohei FUKUMI,
Hiroshi UEDA and Ryohei KOBAYASHI*

*National Grassland Research Institute, Nishinasuno, Tochigi, 329-2793 Japan
College of Agriculture, Ehime University, Matsuyama, Ehime, 790-0905 Japan

Received: September 28, 1998/Accepted February 1, 1999

Synopsis
ZHANG, J. G., S. KUMAI, R. FUKUMI, H. UEDA and R. KOBAYASHI

The effects of three cellulase preparations as silage additives on the degradation of cell walls and sugar production for different materials were investigated. The degradation rates of cell walls were considerably different among materials or cellulase preparations. The degradation rates of cell walls were in the order of bahia grass (Paspalum notatum Flugge), Italian ryegrass (Lolium multiflorum L.) > naked barley straw (Hordeum vulgare L. emand Lam.) > rice straw (Oryza sativa L.) for Acremonium cellulase (AC), Italian ryegrass > bahia grass > naked barley straw > rice straw for Meicelase (MC), and Italian ryegrass > naked barley straw > bahia grass > rice straw for Onozuka cellulase (OC). Most of sugars released by cellulase preparations were glucose and there were also small amounts of xylose, arabinose and galactose, which varied with the sources of the materials or types of cellulase preparations. On the whole, the degrading capacity of cellulase preparations to cell walls was AC > MC > OC, suggesting that AC would be a more efficient additive to silage.

Key words: Cellulase preparation, Cell wall degradation, Grass, Straw, Sugars.

Introduction
Although forages contain almost the same amount of gross energy as do grains, the feeding value of forages is lower and much more variable. This difference is attributed to the high concentrations of cell wall material in forages and the limited digestibility of these cell walls5. To improve the digestibility of forages, various methods have been studied in the world. One of them is cellulase treatment, which can degrade cell walls to produce sugars available. The advantage of this method is especially evident to ensiling6,11,13. During ensilage, added enzymes break down structural polysaccharides, thereby ensuring an adequate supply of substrate (water-soluble carbohydrates) for the rapid establishment and growth of favorable micro-organisms, leading to a good fermentation and preservation. At the same time, it is expected to improve digestibility of silage. On the other hand, majority of cellulase preparations can degrade not only cellulose but also hemicellulose, producing hexoses (mainly glucose) and pentoses (e.g. xylose and arabinose) which would be fermented in the different pathways. Whereas the silage fermentation efficiency greatly depends on the fermentation pathways (homolactic or heterolactic fermentation).

The purpose of the present experiment was to investigate the ability of three cellulase preparations to cell wall degradation, and the types and contents of sugars released from cell walls of different materials, so as to use it effectively for ensiling in various cases.

Materials and Methods
Preparation of materials
Four original materials were bahia grass (Paspalum notatum Flugge), Italian ryegrass (Lolium multiflorum L.), naked barley (Hordeum vulgare L. emand Lam.) straw and rice (Oryza sativa L.) straw. They were obtained from the experimental field of the Agricultural College of Ehime University. Two grasses were harvested at the hay stage of growth, on June 15, 1996. The samples were dried in a forced-air oven at 80°C for 48 h and ground in a mill to pass through a 1.0 mm sieve. After the ground materials were extracted with neutral detergent for 1 h10, they were filtered and the insoluble matter was well washed with hot distilled water, followed by acetone. All insoluble matter was collected and dried for use as cell walls.

Cellulase preparations
Acremonium cellulase-AUS 0301 (AC) and
Meioelase (MC) which commonly used as silage additives were obtained from Melij Seikag Co. AC is a new cellulase preparation derived from a selected strain of Acremonium cellulolyticus, while MC is one with world-wide fame derived from Trichoderma viride. Onozuka cellulase (OC) was from Yakult Chemicals Industry Ltd., which also derived from Trichoderma viride and was extensively used for forage analysis in Japan.

Degradation of cell walls

The effects of sources of cell walls, types of cellulase preparations and incubation time on cell wall degradation were studied. 300 mg of cell wall sample (from bahia grass, Italian ryegrass, naked barley straw or rice straw) and 15.0 ml cellulase solution (200 mg cellulase preparation AC, MC or OC dissolved in acetic acid–sodium acetate buffer solution of 100 ml at pH 4.5) were put in a glass bottle of 20.0 ml. After fixing the cap, they were incubated in two shaking baths with 65 rpm at 40°C, respectively. In order to prevent soluble sugars released from being fermented, 0.5 ml chloroform and 0.5 ml toluene were added each bottle prior to incubation.

Analytical methods

The contents of hemicellulose, cellulose, ADL (acid detergent lignin) and silica of cell walls were determined according to the method described by Abe (1). After incubating for 12, 24 and 36 h respectively, the enzymes were deactivated by heating at 120°C for 20 min. Then, they were filtered with filter papers of known weight, the water-insoluble material was thoroughly washed with distilled water, dried and weighed to determine the degradability of cell walls.

The filtrate was used to determine the components and contents of sugars released by cellulase preparations using high-performance liquid chromatography–HPLC (column: Shodex SUGAR SP 0810; detector: HITACHI L-3300 RI; flowg solvent: distilled water; flowg rate: 1.0 ml/min; temperature: 80°C). Statistical analysis was performed using the analysis of variance and Tukey’s multiple range test was used to test differences between treatment means.

Results

Composition of cell walls

The chemical composition of four materials after extracting with neutral detergent is given in Table 1. Bahia grass contained the highest content of hemicellulose and lower contents of cellulose and ADL. Rice straw had lower content of hemicellulose and higher content of silica than other three materials. In addition, barley straw was of the highest concentrations of cellulose and ADL. While each part of Italian ryegrass was intermediate.

Degradation of cell walls

The types and contents of sugars released from cellulase preparations were apparently different among the sources of cell walls and among cellulase preparations (Tables 2 and 3). AC made all materials release four sugars of arabinose, xylose, glucose and galactose, whereas MC and OC did not release galactose from cell walls of any material. MC did not release arabinose and xylose for rice straw and xylose for bahia grass, and OC did not release arabinose for all materials either.

Apart from rice straw, the degradability of cell walls had only a very small change with the time from 12 to 36 h (Fig. 1) but the total content of sugars evidently increased with the time (Tables 2 and 3). In contrast to two straw materials, the cell walls derived from grasses were more readily degraded and had higher degradabilities. Their degradabilities were bahia grass, Italian ryegrass > barley straw > rice straw for AC and Italian ryegrass > bahia grass > barley straw > rice straw for MC. Whereas OC had a greater impact on barley straw than bahia grass, that is, the degradabilities of cell walls by OC were Italian ryegrass > barley straw > bahia grass > rice straw.

The degradation activity of cellulase preparations to cell walls was AC > MC > OC for any material.

Table 1. Chemical composition of the cell walls derived from various materials.

<table>
<thead>
<tr>
<th></th>
<th>Bahia grass</th>
<th>Italian ryegrass</th>
<th>Barley straw</th>
<th>Rice straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemicellulose (%)</td>
<td>48.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>42.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADL (%)</td>
<td>6.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Silica (%)</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CW : Cell walls, ADL : Acid detergent lignin.
* : Content of each fraction in the original material (% on the basis of material DM).
Rows with different superscripts are significant at P<0.01.
Table 2. The composition of sugars (% CW) released from grass cell walls by cellulase preparations after incubation for 12, 24 and 36 h.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Glucose</th>
<th>Galactose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AC</td>
<td>MC</td>
<td>OC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahia grass</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>1.60 B</td>
<td>1.93 C</td>
<td>25.41 A</td>
<td>0.40 A</td>
<td>29.34 A</td>
</tr>
<tr>
<td></td>
<td>1.92 AB</td>
<td>2.61 A</td>
<td>29.59 A</td>
<td>0.43 A</td>
<td>34.55 A</td>
</tr>
<tr>
<td></td>
<td>2.11 A</td>
<td>3.04 A</td>
<td>32.32 A</td>
<td>0.52 A</td>
<td>37.99 A</td>
</tr>
<tr>
<td>MC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.18 C</td>
<td>0.00 C</td>
<td>14.80 C</td>
<td>0.00 B</td>
<td>14.98 C</td>
</tr>
<tr>
<td></td>
<td>0.20 C</td>
<td>0.00 C</td>
<td>18.91 C</td>
<td>0.00 B</td>
<td>19.11 C</td>
</tr>
<tr>
<td></td>
<td>0.23 C</td>
<td>0.00 C</td>
<td>18.91 C</td>
<td>0.00 B</td>
<td>19.14 C</td>
</tr>
<tr>
<td>OC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.39 C</td>
<td>4.92 D</td>
<td>0.00 B</td>
<td>5.31 D</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.44 C</td>
<td>6.40 D</td>
<td>0.00 B</td>
<td>6.84 D</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.51 C</td>
<td>7.83 D</td>
<td>0.00 B</td>
<td>8.34 D</td>
</tr>
<tr>
<td>Italian ryegrass</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.51 A</td>
<td>4.62 B</td>
<td>26.73 A</td>
<td>0.14 B</td>
<td>33.00 A</td>
</tr>
<tr>
<td></td>
<td>1.63 A</td>
<td>4.89 AB</td>
<td>27.84 A</td>
<td>0.85 A</td>
<td>35.21 A</td>
</tr>
<tr>
<td></td>
<td>1.79 A</td>
<td>6.18 A</td>
<td>28.36 A</td>
<td>0.92 A</td>
<td>37.25 A</td>
</tr>
<tr>
<td>MC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.58 B</td>
<td>1.31 C</td>
<td>19.87 C</td>
<td>0.00 B</td>
<td>21.76 CD</td>
</tr>
<tr>
<td></td>
<td>0.61 B</td>
<td>1.58 D</td>
<td>22.48 BC</td>
<td>0.00 B</td>
<td>24.67 BC</td>
</tr>
<tr>
<td></td>
<td>0.73 B</td>
<td>1.63 D</td>
<td>23.81 AB</td>
<td>0.00 B</td>
<td>26.17 D</td>
</tr>
<tr>
<td>OC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>1.72 D</td>
<td>12.63 D</td>
<td>0.00 B</td>
<td>14.35 EF</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>2.25 D</td>
<td>14.21 D</td>
<td>0.00 B</td>
<td>16.44 EF</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>3.26 CD</td>
<td>15.90 D</td>
<td>0.00 B</td>
<td>18.86 D</td>
</tr>
</tbody>
</table>

CW : Cell walls.  
Columns with different superscripts are significant at P<0.01, within the same material.

Table 3. The composition of sugars (% CW) released from straw cell walls by cellulase preparations after incubation for 12, 24 and 36 h.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Glucose</th>
<th>Galactose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AC</td>
<td>MC</td>
<td>OC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley straw</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.61 B</td>
<td>1.34 BC</td>
<td>16.03 A</td>
<td>0.00 C</td>
<td>17.98 B</td>
</tr>
<tr>
<td></td>
<td>0.82 AB</td>
<td>1.68 B</td>
<td>16.68 A</td>
<td>0.22 B</td>
<td>20.40 AB</td>
</tr>
<tr>
<td></td>
<td>0.91 A</td>
<td>4.29 A</td>
<td>17.70 A</td>
<td>0.60 A</td>
<td>23.50 A</td>
</tr>
<tr>
<td>MC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.08 C</td>
<td>0.82 BC</td>
<td>10.20 BC</td>
<td>0.00 C</td>
<td>11.10 CD</td>
</tr>
<tr>
<td></td>
<td>0.11 C</td>
<td>0.82 BC</td>
<td>10.36 BC</td>
<td>0.00 C</td>
<td>11.29 CD</td>
</tr>
<tr>
<td></td>
<td>0.11 C</td>
<td>0.84 BC</td>
<td>11.95 B</td>
<td>0.00 C</td>
<td>12.90 C</td>
</tr>
<tr>
<td>OC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.51 C</td>
<td>6.99 B</td>
<td>0.00 C</td>
<td>7.50 C</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>1.42 BC</td>
<td>8.18 CD</td>
<td>0.00 C</td>
<td>9.60 CD</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>1.71 A</td>
<td>9.81 BC</td>
<td>0.00 C</td>
<td>11.52 CD</td>
</tr>
<tr>
<td>Rice straw</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.31 B</td>
<td>0.62 C</td>
<td>11.41 B</td>
<td>0.22 B</td>
<td>12.56 B</td>
</tr>
<tr>
<td></td>
<td>0.62 A</td>
<td>1.51 B</td>
<td>14.53 A</td>
<td>0.29 B</td>
<td>16.95 A</td>
</tr>
<tr>
<td></td>
<td>0.74 A</td>
<td>2.09 A</td>
<td>16.34 A</td>
<td>0.51 A</td>
<td>19.84 A</td>
</tr>
<tr>
<td>MC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.00 E</td>
<td>6.79 CD</td>
<td>0.00 C</td>
<td>6.79 CD</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.00 E</td>
<td>8.56 C</td>
<td>0.00 C</td>
<td>8.56 CD</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.00 E</td>
<td>8.62 C</td>
<td>0.00 C</td>
<td>8.62 CD</td>
</tr>
<tr>
<td>OC</td>
<td>12 h</td>
<td>24 h</td>
<td>36 h</td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.18 DE</td>
<td>2.74 E</td>
<td>0.00 C</td>
<td>2.92 E</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.31 DE</td>
<td>3.83 E</td>
<td>0.00 C</td>
<td>4.14 CD</td>
</tr>
<tr>
<td></td>
<td>0.00 C</td>
<td>0.49 CD</td>
<td>4.79 DE</td>
<td>0.00 C</td>
<td>5.28 CD</td>
</tr>
</tbody>
</table>

CW : Cell walls.  
Columns with different superscripts are significant at P<0.01, within the same material.
Fig. 1. The degradation rates of grassess and straw cell walls by cellulase preparations. 
(AC : Acremonium cellulase, MC : Mucilag, OC : Onozuka cellulase)

But xylose released by OC was more than by MC.

Discussion

It is well known that the degrading efficiency of cell walls by cellulases depends on the content and composition of cell wall polysaccharides, and the composition and activity of cellulase preparation itself. Many researchers have already attended to the relationship between the utilization of plant cell walls and the lignin content of cell walls. Majority of them\textsuperscript{2,4,6,10,14} has concluded that lignin content was negatively correlated with digestibility of forages. However, \textit{Fors\textsuperscript{2}} has shown that the rate of cellulose degradation does not change when cell walls are delignified. In current experiment, although Italian ryegrass contained higher concentration of ADL in cell walls than bahia grass, it had obviously higher rates of cell wall degradation by MC and OC compared with bahia grass (Fig. 1). These results indicate that lignin content seems to be not a main limited factor in cell wall degradation, which might be attributed to the structure of cell walls or hemicellulose in cell walls. As presented in Table1, bahia grass contained higher concentration of hemicellulose, while all three preparations used in the present experiment are of low power to degrade hemicellulose, especially with MC and OC. Secondly, the ratio of arabinose to xylose released from bahia grass was lower than that from others (based upon the data in Tables 2 and 3), it appears to hint that there was higher proportion of arabinose in xylan of bahia grass. \textit{Brice} and \textit{Morrison}\textsuperscript{23} have reported that structural features of xylans play a role in the degradation extent of hemicellulose. Xylans with a high degree of arabinose substitution (arabinose to xylose) were degraded by hemicellulases to a lesser extent than less frequently substituted xylans. Besides, it is somewhat surprising that the impact of OC on cell walls of bahia grass was weaker than that of barley straw as well as Italian ryegrass. This possibly shows that the resistance of hemicellulose to cell wall degradation might be more significant on the cellulase preparations with low activity. On the other hand, rice straw with low ADL content had lower degradability than naked barley straw, irrespective of cellulase preparations, which might result from the difference in the content of silica. Rice straw had rather higher content of silica, which is generally considered negative correlation with digestibility. \textit{Van Soest} and \textit{Jones}\textsuperscript{23} have already reported that every unit increase in Si (usually reported as silica) content should be accompanied by a three unit decrease in digestibility.

\textit{AC} released more types and higher amounts of sugars from cell walls than MC and OC, irrespective of cell wall sources, which suggests that each enzyme of AC is of greater activity than that of either MC or OC. As a whole, MC was superior to OC as indicated by the degradability of cell walls. Whereas the amount of xylose liberated by OC was higher than
that by MC from all materials except for that neither of OC and MC released xylose in rice straw, implying that xylanase of OC had a greater activity than that of MC and hemicellulose of rice straw was more difficult to degrade, which probably limited the cell wall degradation of rice straw to some extent. This was also confirmed by the lower content of xylose released by AC from rice straw than that from other materials.

After incubation for 12 h, glucose and xylose continued to increase even in the case that the cell walls were little degraded. It was probably due to the removal of sugars from glucoside with time.

Comparing three preparations, AC could release more glucose from cell walls than MC and OC, which will benefit homolactic fermentation when applied to ensiling. At the same time, AC also liberated relatively higher contents of xylose and arabinose associated with high content of glucose from cell walls, implying that AC, as silage additive, possibly produces more acetic acid than MC and OC as well as untreated silages in difficult ensiling conditions, such as materials with too high moisture and/or too high buffering capacity, and improper temperature, etc.

It was confirmed by the different results in our previous experiments that cellulose additions resulted in several types of silages with a similar, lower or higher content of acetic acid compared with the control.

In conclusion, cellulase preparations could produce fermentable sugars both for the homofermentative and heterofermentative lactic acid bacteria when used as silage addition. Although the applying rate of cellulase preparation to barley straw silage was suggested, in practice it should be decided based upon silage material and cellulase preparation used, because the amounts of sugars released varies with the types of materials and cellulase preparations.

References


* In Japanese only. Translated title by the present authors.

要旨
張 建国*・熊井清雄・福見良平・上田孝史・小林真亮(1999)：セリーレ酵素製剤による牧草および稈類の細胞壁の分解、日農誌 46, 20-25: 小田原水産高等試験場 (329-2793 横枝県西部須賀町)、愛媛大学農学部 (790-0805 松山市樽椛 3-5-7)の3種のセリーレ酵素製剤を供試し、牧草および稈類類の細胞壁の分解率およびに分解に伴う糖の生成に及ぼす影響について検討した。
細胞壁の分解率は供試材料類やセリーレ酵素剤で明らかに差異があり、
められた。各製剤による細胞壁の分解率は、アクレモニウムセルラーゼ（AC）ではバビアグラス、イタリアンライグラス＞ハダカムギ麦穀＞イナワラ、メイセラーゼ（MC）ではイタリアンライグラス＞バビアグラス＞ハダカムギ麦穀＞イナワラ、オノズカセルラーゼ（OC）ではイタリアンライグラス＞ハダカムギ麦穀＞バビアグラス＞イナワラの関係をそれぞれ示した。

各セルラーゼ製剤による生成糖の主体はグルコースであったが、供試材料やセルラーゼ製剤によって微量のキシロース、アラビノースおよびガラクトースが溶出したり、溶出しなかったりした。セルラーゼ製剤の細胞壁に対する分解力はAC＞MC＞OCを示し、ACがサイレージの添加物として最も適しているものと考えられた。

キーワード：藁穂、細胞壁分解、セルラーゼ製剤、糖、牧草。