Fibrous Material in Feedlot Waste Fermented by *Trichoderma viride*¹

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*Trichoderma viride* QM9123 fermented fiber isolated from feedlot waste at concentrations up to 16.7% solids. The fermented fiber solids decreased by 32%, and carbohydrate decreased by 60%. Cellulolytic enzyme production was better with fiber substrates that had been alkali pretreated and had a lower hemicellulose-to-cellulose ratio.

Griffin et al. (2) demonstrated that whole feedlot waste at 2.5% concentration is a complete and convenient medium for the production of cellulolytic enzymes by *Trichoderma viride* (5). However, higher concentrations were inhibitory to the fungus. We have attempted to eliminate this inhibition and improve the overall amount of feedlot waste digestion through fermentation of fibers isolated from the whole waste. Three samples of fiber (Table 1) were isolated and compared with whole waste as a fermentation substrate.

Isolated fiber differed from the whole waste in that no inhibition was detectable at substrate concentrations up to 16.7%, but nutrient deficiencies appeared. With the alkali-washed Ariz:OH fiber, all of the nutrient supplements described by Mandels and Weber (4) were necessary for good enzyme production. Enzyme production was also influenced by the amount and kind of nitrogen nutrients added to the fermentation flasks (Fig. 1). At a semisolid substrate level of 16.7%, enzyme production reached a maximum at ammonium sulfate-urea and peptide concentrations of 200 and 15 mg per flask, respectively.

In all three samples, approximately 60% of the carbohydrate disappeared (Table 2) and only 68% of the solid was recoverable by centrifugation. Lignin content, which is generally 13 to 20%, increased to 20 to 28% in the fermented fibers, and there was a 0.5 to 1.8% net increase of insoluble nitrogen. Significant difference in the fermentation of cellulose appeared among the samples, however, which correlated with the hemicellulose content of fibers. Hemicellulose is defined here as those sugars not found in the cellulose fraction (Tables 1 and 2). When the ratio of hemicellulose to cellulose was relatively large (e.g., 1.33 for the Illinois fiber), the hemicellulose fraction appeared to be degraded preferentially. For example, the ratios of hemicellulose to cellulose were 1.33, 0.82, and 0.36 for the Illinois, Arizona, and alkali-treated Arizona fibers, respectively. These ratios became 0.87, 0.84, and 0.53 upon fermentation of the fibers.

Also, cellulolytic enzyme production was inverse to the hemicellulose content of fibers. Cellulolytic activity described as milligrams of glucose released per flask per hour (2) was 120, 160, and 250 for the Illinois, Arizona, and alkali-treated fiber substrates, respectively. Presumably, a smaller quantity of cellulolytic enzyme is needed with hemicellulose-enriched substrates to give growth.

The sequence of fermentation events starting from a spore inoculum is shown in Fig. 2. Between days 3 and 7, 60 to 70% of the digestible nutrients was consumed, whereas cellulolytic

| Sample           | Total Carbohydrate | Cellulose | Lignin | Nitrogen |
|------------------|--------------------|-----------|--------|----------|
| Illinois fiber   | 63                 | 27        | 13     | 1.5      |
| Arizona fiber    | 36                 | 22        | 13     | 3.4      |
| Ariz:OH fiber    | 42                 | 31        | 20     | 2.3      |
| Whole FLW        | 25                 | 10        | 7      | 3.6      |

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FIG. 1. Amounts of ammonium sulfate-urea nitrogen supplement (A) and peptone (B) required by Trichoderma viride cultures grown on 16.7% feedlot waste fiber to produce (O) cellulolytic enzyme and (×) total protein in the supernatant after centrifugation. Supernatant protein was calculated by a colorimetric method described by Layne (3), and cellulolytic enzyme was assayed by the procedure of Griffin et al. (2). Except for either the nitrogen supplement or peptone, each 500-ml cultural flask contained 10 g of fiber in 50 ml of nutrients (163 mg of ammonium sulfate, 35 mg of urea, 15 mg of peptone, and 25 mg of Tween 80 adjusted to a final pH 4.5 with H₃PO₄), and the spore-inoculated culture was incubated aerobically at 28 C for 10 days.

TABLE 2. Change in fiber composition caused by T. viride fermentation

| FLW sample | Loss of insoluble components (%) | Total carbohydrate | Cellulose |
|------------|----------------------------------|-------------------|-----------|
| Illinois fiber | 56 | 46  |           |
| Arizona fiber | 56 | 61  |           |
| Ariz:OH fiber | 60 | 65  |           |

* Each flask with 5.00 g of feedlot waste (FLW) fiber contained 50 ml of nutrient supplements (4) in excess. All cultures were incubated aerobically at 28 C for 13 days with periodic adjustment to pH 5.0 with H₃PO₄.

* Recovery of insoluble components by centrifugation.

enzyme was detectable only during the later days. Other soluble proteins appeared after the release of cellulolytic enzyme and may have been released upon cellular death and lysis.

T. viride seemed to be as effective as Thermoaclimomyces (1) in reducing the volume of waste solids. Furthermore, the fiber substrates could be fermented at high concentrations, and cellulolytic enzyme was produced conveniently in the fermentation broth.

FIG. 2. Time course of Arizona feedlot waste fiber (16.7%, manure sifted and washed) fermentation by T. viride when measured by supernatant protein (×), cellulolytic enzyme (○), and total solids (△) recovered per flask. Total solids include insoluble residue recovered by centrifugation and supernatant solids after lyophilization.
LITERATURE CITED

1. Bellamy, W. D. 1974. Single cell proteins from cellulosic wastes. Biotechnol. Bioeng. 16:869–880.
2. Griffin, H. L., J. H. Sloneker, and G. E. Inglett. 1974. Cellulase production by *Trichoderma viride* on feedlot waste. Appl. Microbiol. 27:1061–1066.
3. Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. Methods Enzymol.
4. Mandels, M., and J. Weber. 1969. The production of cellulases. Adv. Chem. Ser. 95:391–414.
5. Sloneker, J. H., R. W. Jones, H. L. Griffin, K. Eskins, B. L. Bucher, and G. E. Inglett. 1973. Processing animal wastes for feed and industrial products, p. 13–38. In G. E. Inglett (ed.), Processing agricultural and municipal wastes. Avi Publishing Co., Westport, Conn.