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Extrusion Processing for Ammonia Fiber Explosion (AFEX)

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Abstract

The ammonia fiber explosion (AFEX) process, previously run only in a batch reactor, has been adapted to run on a twin-screw extruder. The sugar yield of AFEX material after enzymatic hydrolysis has been increased up to 3.5 times over that of completely untreated material. The ruminant digestibility of corn fodder has been increased up to 32% (from 54–71%) over completely untreated material, and 23% (from 63–77%) over material extruded with no ammonia. Extrusion-treated material proved more digestible by the ruminant at 48 h than material treated in the batch reactor.

Index Entries: AFEX; extrusion; ammonia; hydrolysis; ruminant digestibility.

Introduction

The success and application of the batch AFEX process is well-documented *(1)*. This biomass-pretreatment technique takes advantage of the explosive depressurization of anhydrous liquid ammonia effectively to blow the plant fibers apart. This treatment enhances enzymatic and ruminant digestibility in at least two ways. First, the surface area available to microbial attack is increased and second, the reduction in apparent lignin allows for more effective utilization of the cellulose and hemicelluloses contained in the plant fibers. This process also increases digestibility by decrystallizing cellulose and hydrolyzing hemicelluloses.

As this process continues to grow into a full-scale production process, large-scale production equipment is desired. One means of facilitating this goal is to use an extruder. This type of machine is attractive for the AFEX process because extruders offer excellent temperature control, provide thorough mixing, are capable of high throughputs, and are adaptable to many different process modifications.

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The goal of this research was to adapt the AFEX process to run in an extruder and to perform a preliminary extruder optimization study. Included in this goal are maintaining a high degree of enzymatic and ruminant digestibility and demonstrating the fiber-splitting effect observed in the batch process.

Methods

Material

The main material studied was corn fodder (or stover). This includes all portions of the corn plant except the grain and cob. The material was obtained in a large square bale. The fodder was coarsely ground, dried to <5% moisture in a large oven, and milled to pass a 2-mm screen.

Equipment

The extruder used was a Baker-Perkins (Saginaw, MI) MPC/V-30 twin-screw compounder (or extruder). This instrument supplied 3 HP at 500 rpm. The barrel diameter was 30 mm with a length to diameter ratio (L/D) of 10:1. The screws were co-rotating and self-wiping with a variable profile. The instrument was supplied with controls for both heating and cooling. Electric cartridge heaters in three zones along the barrel and in the die block supplied heating. Cooling was supplied along the barrel length by chilled water fed through cored barrel sections. The die and die block were air-cooled. All interior surfaces of the instrument were nitrided with a 63 μ-inch (1.6 μm) finish that provided excellent corrosion resistance. All agitator parts were made of heat-treated alloy steel. The corrosion resistance of a single agitator piece was confirmed prior to any experimentation and no corrosion was observed throughout the experimentation.

The pump used to deliver ammonia was an American Lewa (Holliston, MA) reciprocating-diaphragm metering pump. The pump head was made of 316 Stainless Steel with Teflon seals. The pump was capable of metering 0.23–23.0 GPH (0.24–24.2 cm^3/s). The drive supplied 3 HP (2.24 kW). The pump was calibrated by recording the mass of water pumped at a specific stroke length and motor rpm. The mass of water was converted to a volume, and it was found that the volumetric output of the pump was directly related to the product of stroke length and rpm. Knowing this, a simple conversion was done to determine the mass of ammonia delivered at a specified stroke length and motor rpm.

All tubing, fittings, and valves used were of 316 SS. The tubing used was 3/8" (9.52 mm) optical density (OD) with 0.065" (1.65 mm) walls, rated to 6500 psig (44.9 MPa). All fittings were rated to the burst pressure of the tubing. Most of the connections were Swagelok compression fittings, but a few were NPT. Those connections that were NPT were sealed with Teflon tape.

Biomass was fed into the extruder using a twin-screw feeder produced by the K-Tron Institute (Pitman, NJ). The feeder was calibrated by feeding material for a known period of time and determining the mass. Samples that contained different amounts of moisture were calibrated individually. The material hopper of the feeder featured a mixing arm that continually supplied material to the feeding screws and eliminated any dead zones.

Sample Analysis

Initially, the primary means of quantifying the effectiveness of the process was enzymatic hydrolysis. Because the main goal was to prove the extrusion technology worked, maximizing sugar concentrations was not the focus, but rather, demonstrating increases in digestibility relative to controls. Thus, all samples were hydrolyzed in a pH 4.8 citrate buffer with a cellulase loading of 15 IU/g and a β-glucosidase loading of 1 mL/mL of cellulase. The cellulase used was Celluclast and the β-glucosidase used was Novo 188, both supplied by Novo Nordisk (Franklinton, NC). All samples were hydrolyzed at 50°C in an agitated water bath in a 5% (dry weight/volume) solution for up to 48 h. The primary analysis took place on a lead-based high-performance liquid chromatography (HPLC) column for glucose, xylose, galactose, arabinose, and mannose. Other analyses were done on an acid-based column that gave a glucose and composite sugar peak that consisted of xylose, mannose, and galactose concentrations. Finally, a Yellow Springs Instruments (YSI) instrument was used for glucose concentrations.

Ruminant digestibility was done according to Texas A & M Animal Nutrition Department guidelines. Several samples were generated with different temperature treatments and ammonia loads. The treated material was placed in a small permeable bag of known weight and dried to determine the mass of dry matter. The bags were then placed in a large nylon bag, secured to a rope, and placed in the rumen of a fistulated steer (an animal that has had a tube, or cannula, surgically inserted that allows access to the animal's rumen). By removing the bags at specific intervals (0, 3, 6, 12, 24, 48, and 96 h), thoroughly rinsing, and drying, the digestibility of the materials was determined as percent of material weight lost. The weight loss of the material in a specific bag is called the dry-matter digestibility, or DMD.

Fiber analysis was also done for each time in the digestibility trial. The procedure used was based on the Van Soest procedure *(2)* and was modified by the Texas A & M Animal Nutrition Department. Assays were used to determine the amount of neutral detergent fibers, acid-detergent fibers, lignin, and ash in each sample. Each fiber sample consists of neutral detergent solubles, hemicelluloses, cellulose, lignin, and ash. The neutral-detergent fiber analysis removes the neutral-detergent solubles, the acid detergent fiber assay removes the hemicelluloses, the lignin procedure removes the cellulose, and the ash procedure removes the lignin. Individual fiber components are then determined by differences in weight following each assay.

Modification of Equipment

Before research could begin, several modifications to the extruder and the laboratory were necessary to handle safely anhydrous ammonia. Based on suggestions from the Office of Radiation, Chemical, and Biological Safety at Michigan State University, the area surrounding the extruder was enclosed with vinyl strip curtains to contain any ammonia releases. This area enclosed supplemental room-ventilation units that provided continual air circulation and replacement. Additionally, a fume hood was installed directly over the extruder to remove any vapors generated in that area. Finally, full-face respirators with removable ammonia cartridges were purchased and worn during all experimentation.

To inject liquid ammonia, a small port was machined to fit into a secondary feed port on the extruder. There were two possible locations for this injection point, located at approx one-half and three-fourths of the length down the barrel. The first was chosen to maximize biomass ammonia equilibration time and to avoid problems with pumping caused by biomass compaction near the extruder outlet.

Much work was done modifying the screw profile of the extruder as well. A zone of high mixing was created just prior to the ammonia-injection point to minimize the amount of ammonia released from the biomass feed port. Several designs were tried, but owing to torque limitations and the relatively small amount of barrel length available for modification, a zone of 45°, feed-forward mixing paddles was used. These provided an acceptable restriction to ammonia backflow, but allowed for a high throughput of biomass.

The discharge screws on the extruder were modified as well. Originally, these screws were flat on the end nearest the discharge. When the die block was in use, the biomass would plug inside of it. The discharge screws were modified to be more conical at the discharge end. This modification effectively directed the biomass into the restriction and promoted flow.

As experimentation progressed, increasingly restrictive orifices were used. Initially, no restriction was used. Then the die block was added. This addition allowed for outlet temperature control as well as providing a restriction of nearly 80% of the inlet area. In other words, the die block outlet area was ~20% of the inlet area. The final restriction added was the die. Several preliminary dies were attempted without success. The die that proved most successful was machined with a 5° end mill to provide a smooth gradual contraction of 40% of the inlet area (the outlet of the die was 60% of the area of the inlet).

Results

Physical Results

In the batch AFEX process, a significant amount of fiber splitting was observed *(3)*. This has been observed in the extrusion process as well (figures not shown). Completely untreated (CU; material that has neither been extruded nor ammonia-treated) biomass has little, if any splitting of the fibers. Material treated by extrusion and ammonia (EAT) clearly shows the desired fiber splitting as obtained in the batch AFEX treatment.

Other significant effects are observed in both the batch and extrusion ammonia processes as well. The batch-treated (BT) material (alfalfa in the cited research) exhibits a greater "water-holding capacity" than CU material *(3)*. This effect is reproduced in the extrusion process in that the EAT material readily sinks and absorbs the buffer solution used in the enzymatic hydrolysis procedure. In contrast, the CU material floats on the surface of the solution, even after being left in the liquid for 8 h or more. The only way effectively to wet the CU material was by shaking the mixture.

Enzymatic Hydrolysis

Material was initially processed with no outlet restriction on the extruder. Enzymatic hydrolysis of this material for 27 h gave a total sugar concentration (the sum of the concentrations of glucose, xylose, galactose, arabinose, and mannose) 1.4 times greater than the sugar concentration obtained from CU material. The die block was subsequently added to the extruder outlet to provide a restriction to flow and thereby presumably create a higher pressure and pressure drop, and hence a more effective treatment.

Addition of the die block gave an explosion that resulted in a total sugar concentration after enzymatic hydrolysis for 24 h of 2.4 times that of the CU sample. The glucose concentration of this material was 2.1 times that of the CU material after the same length of time. The same material gave a total sugar concentration 2.0 times greater than the biomass extruded without ammonia (extrusion treated; ET) and the corresponding glucose concentration was 2.5 times that of the ET material. Further trials with the die block gave total sugar concentrations as much as 3.5 times greater than the CU material and 3.4 times greater than ET material after 24 h of hydrolysis. Enzymatic hydrolysis results are summarized in Table 1.

Apparently, extrusion under these conditions has little effect on sugar production in the absence of ammonia. Also, increases in glucose concentration as measured by the YSI analyzer track quite closely with increases in total sugars as measured by HPLC. If we focus on shorter reaction times, we see that total sugar concentration for EAT material was 2.4 times greater than for the CU material after 6 h of enzymatic hydrolysis, whereas the glucose concentration at this time point was 2.3 times greater than that obtained from CU material.

In general, the most effective treatments for enzymatic hydrolysis were obtained for lower temperature runs. The higher the die temperature used, the less effective the treatment. This may be owing in part to more of the ammonia escaping the extruder due to increased vaporization at a higher temperature. At lower temperatures, the ammonia will remain in contact

Table 1
Comparison of Extrusion Ammonia Treatments with Two Controls by Enzymatic Hydrolysis

Temp (°C)	Ammonia load[a]	Time (h)[b]	Die block?[c]	Die used	Total sugar Conc vs CU[d]	Glucose Conc vs CU[d]	Total sugar Conc vs ET[d]	Glucose Conc vs ET[d]
9	~2	27	No	No	1.44	1.30	—	—
50	~2	24	Yes	No	2.40	2.13	1.99	2.46
40	0.86	24	Yes	No	3.48	2.78	3.39	2.43
45	2.8	6	Yes	Yes	2.42	2.00	—	—

[a] Ammonia load is given as units of mass of ammonia/mass of biomass.
[b] Time is the hydrolysis time.
[c] The Die block and Die columns indicate whether or not they were used.
[d] Concentrations are given as the ratio of the specified sugar(s) to the control (CU or ET) given at the top of the column. For example, the last sample had a total sugar concentration after 6 h of hydrolysis 2.42 times greater than the sugar concentration obtained by hydrolyzing completely untreated material for 6 h.

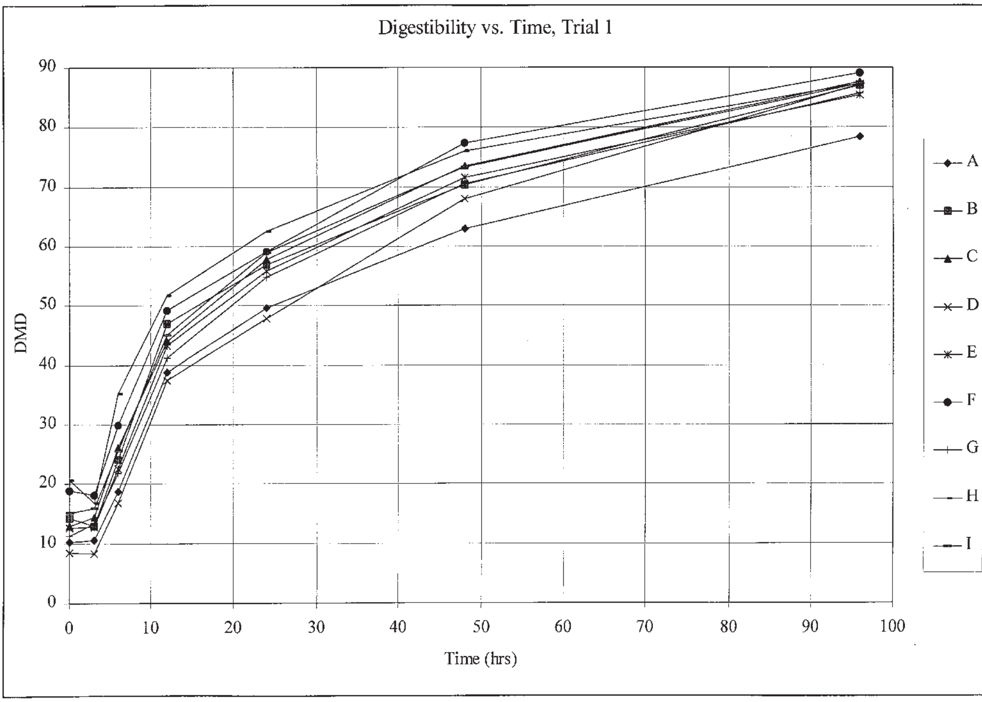

Fig. 1. Dry matter digestibility, trial 1.

with the biomass longer and a more effective treatment results. If this hypothesis is correct, it will be essential to minimize ammonia losses from the extruder in a commercial-scale process. This hypothesis of ammonia losses at higher extruder temperatures is supported by the observation that higher temperatures (up to about 90°C) increase the effectiveness of batch ammonia treatments *(5)*, but not in these extrusion runs. However, it is also possible that the higher ammonia treatment levels (2 kg ammonia/kg corn stover and greater) caused excessive plasticization of the biomass and thereby diminished the fiber disruption effect of the explosion and decreased yields, as we have observed in batch trials *(5)*.

In Situ *Ruminant Digestibility*

Two *in situ* trials were run. The first focused on EAT material, with the control being ET material extruded with a die temperature of 50°C. The second trial compared the EAT samples to the BT materials, as well as the original CU material. All three BT samples were overheated, so the results with these samples may not indicate the potential performance of the batch process, but should still provide some basis for comparison with the extruded materials.

Results of the first and second *in situ* trials are shown in Figs. 1 and 2. The 48-h digestibility of the samples, as well as the treatment conditions,

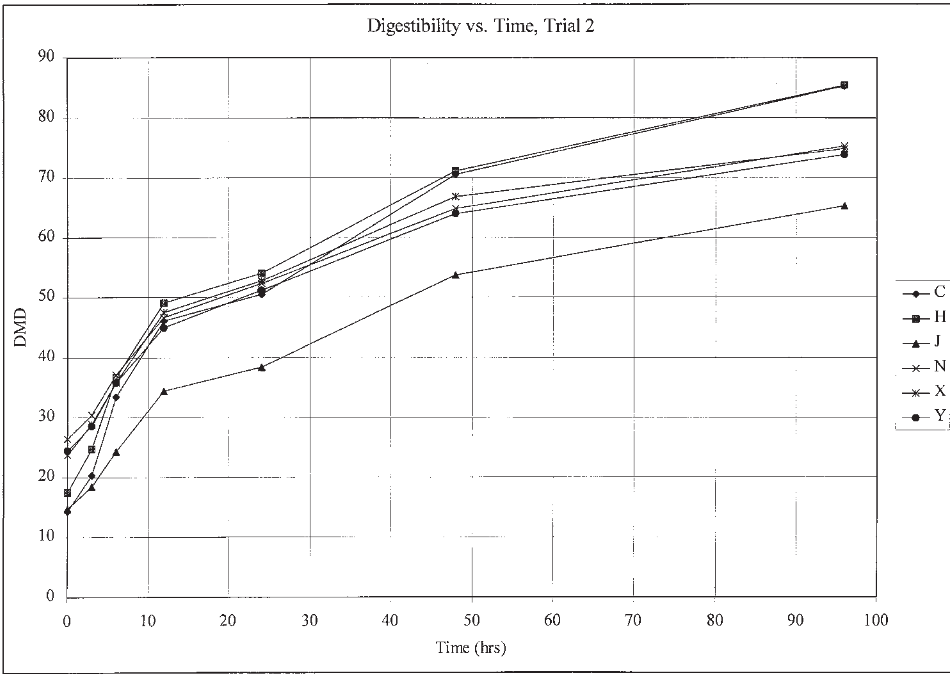

Fig. 2. Dry matter digestibility, trial 2.

are tabulated in Tables 2 and 3. The first trial showed that the digestibility of the EAT material at 48 h was up to 77.4% digestible as compared to the digestibility of 63.0% of the ET control (Fig. 1). The second trial gave a maximum digestibility of 71.2% for EAT material, compared to 53.8% digestible for CU material (Fig. 2). The first and second trials were taken on different days and hence are not expected to show the same digestibility, given probable differences in the metabolic state of the animal. Comparisons within a trial are therefore the most significant.

At 48 h, the relative effects of temperature and ammonia loading on digestibility are not easily distinguished in trial 1. The two most effective treatments obtained are at 65°C with an ammonia load of 1.5 and 2.0 (Samples F and H, respectively). The digestibility of F (77.4%) was only slightly greater than H (76.1%). However, sample G, with an ammonia load of 1.6 mass ammonia/mass biomass and a temperature of 65°C, was only the sixth most digestible material, at 70.5%. The third and fourth most effective treatments (Samples C and I, respectively) are duplicate samples and show excellent agreement in that they are separated by only 0.1 percentage points of digestibility after 48 h. Samples C and I were generated at an ammonia load of 1.5 mass ammonia/mass biomass with a temperature of 55°C. Throughout the first trial, samples H and F were consistently the most digestible, with sample H being the most digestible at all time points except 48 and 96 h.

Table 2
48-H Dry Matter Digestibility for Trial 1

ID	DMD[a]	NH_3Load[b]	T (°C)[c]	Treatment
F	77.4	1.5	65	EAT
H	76.1	2.0	65	EAT
C	73.5	1.5	55	EAT
I	73.4	1.5	55	EAT
E	71.6	1.0	65	EAT
G	70.5	1.6	65	EAT
B	70.4	1.0	0	EAT
D	68.0	0.8	65	EAT
A	63.0	0.0	50	ET

[a]DMD, dry-matter digestibility, reported as a percent.
[b]NH_3Load, mass of ammonia injected/mass of biomass treated.
[c]T (°C), die setpoint.

Table 3
48-H Dry Matter Digestibility for Trial 2

ID	DMD[a]	NH_3Load[b]	T (°C)[c]	Treatment
H	71.2	2.0	65	EAT
C	70.5	1.5	55	EAT
X	67.0	1.5	80	BT
N	65.0	1.0	80	BT
Y	64.1	1.0	90	BT
J	53.8	—	—	CU

[a]DMD, dry-matter digestibility, reported as a percent.
[b]NH_3Load, mass of ammonia injected/mass of biomass treated.
[c]T (°C), die setpoint.

The rate at which digestion occurs is important as well. In this case, the initial rate of digestion was determined as the rate of digestion between h 3 and 6. This was done because many of the samples gained weight between 0 and 3 h. This is probably owing to insufficient washing of the bags that could leave dust and microbes trapped inside. Insufficient washing probably also explains the negligible digestion occurring between times 0 and 3 h in trial 1.

As previously mentioned, the material was also analyzed for fiber composition at each time point as well. By determining the amount of each component in the fiber, insight can be gained as to the relative rates of digestion of each and how these are affected by treatment conditions. Cellulose and hemicelluloses are both considered somewhat digestible, whereas lignin is considered indigestible and is known to hinder the digestion of other components *(4)*.

Thus, a reduction in apparent lignin content is desired, and this has been achieved in batch AFEX reactor studies *(6)* and in this study. The lignin content of the EAT samples in trial 1 was reduced by as much as 28% as compared to the ET material, with an average of 12.5% reduction. Samples C and H in trial 2 also showed an average reduction in lignin content of 15.6% when compared to the CU material. Owing to the thermal damage to the BT samples, an effective comparison is not possible with EAT material.

The data also show that both the amount of hemicelluloses and cellulose decrease over time for these solid samples, illustrating that digestion of these biomass components is occurring. The hemicelluloses are digested most rapidly, especially over the first 12 h. The cellulose fibers are digested more slowly until approximately 12 h of digestion, at which point the rate of cellulose digestion increases (data not shown).

In all cases, the initial rate of digestion for all EAT-treated samples was higher than either the CU material or the ET material. In each *in situ* trial, the maximum rate of digestion was 2.25 times that of the control in the trial (ET in trial 1, CU material in trial 2). The maximum observed initial rate was demonstrated by sample H in trial 1 at 6.16%/h.

Discussion

As the aforementioned results indicate, an extruder can be used to carry out the AFEX process. The total sugar yield as measured by enzymatic digestibility of the corn fodder has been increased up to 250%, and the *in situ* ruminant digestibility has increased by as much as 32% (from 53.8–71.2%) over the CU sample. Additionally, the total sugar yield from enzymatic digestibility of the corn fodder has been increased up to 240%, and the *in situ* ruminant digestibility has increased 23% (from 63.0 to 77.4% digestible) over the ET material. The extrusion process (with ammonia) generally compares well with the batch ammonia process, leading to the conclusion that the extrusion process can probably be made as effective as the batch AFEX process has proven to be.

Other results of the trials are encouraging as well. A reduction in apparent lignin is desired and has been achieved with an average decrease of 12.5% (maximum reduction of 27.7% from 5.88 to 4.25%) from the ET material, and an average reduction of 15.6% (maximum reduction of 23.2% from 8.98 to 6.90%) from CU material. A high initial rate of digestion has also been observed. The highest initial rates of digestion were approx 2.3 times the initial rate of digestion experienced by the controls used in the respective trials. Finally, the fraction of cellulose and hemicelluloses in the remaining solid material is reduced during the digestion process as compared with the CUT and ET materials, thereby further demonstrating the effectiveness of ammonia combined with extrusion treatment for increasing the rate and extent of sugar production from lignocellulosic materials.

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References

1. Holtzapple, M. T., Jun, J., Ashok, G., Patibandala, S. L., and Dale, B. E. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 59–74.
2. Van Soest, P. J. (1991), *J. Dairy Sci.* **22,** 3583–3597.
3. Dale, B. E. and Moreira, M. J. (1982), *Biotechnol. Bioeng. Symp.* **12,** 31–43.
4. Ensminger, M. E. (1990), *Feeds and Nutrition*, 2nd ed., Ensminger, Clovis, CA.
5. Moniruzzaman, M., Dale, B. E., Hespell, R. B., and Bothast, R. J. (1997), *Appl. Biochem. Biotechnol.* **67,** 113–126.
6. Ferrer, A., Byers, F. M., Sulbaran de Ferrer, B., and Dale, B. E. (1998), *J. Animal Feed Sci. Tech.* (Submitted).