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Extrusion Processing for Ammonia

Fiber Explosion (AFEX)

presented by

Justin Weaver

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of the requirements for

MS degree in ChE

Major professor

Date May 15, 1998



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**EXTRUSION PROCESSING FOR AMMONIA
FIBER EXPLOSION (*AFEX*)**

**By
Justin Weaver**

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

MASTER OF SCIENCE

Department of Chemical Engineering

1998

ABSTRACT

EXTRUSION PROCESSING FOR AMMONIA FIBER EXPLOSION (AFEX)

By

Justin Kaine Weaver

The Ammonia Fiber Explosion (AFEX) process, previously run only in a batch reactor, has been adapted to run in a twin screw extruder. The extruder has been modified to accommodate the process. A port has been machined for the injection of ammonia, the mixing zone of the extruder has been adjusted to help trap the ammonia inside the extruder barrel, the discharge screws of the extruder have been altered to promote biomass transport, and the extruder outlet has been constructed to promote the AFEX process. The extrusion laboratory has also been adapted to work safely with ammonia.

An effective treatment has been indicated by physical evidence, an increase in sugar concentration after enzymatic hydrolysis, and an increase in ruminant digestibility. The sugar yield of extrusion ammonia treated corn fodder has increased up to 250% over completely untreated material. The ruminant digestibility has increased up to 32% over completely untreated material.

DEDICATION AND ACKNOWLEDGEMENTS

This thesis is dedicated to the many family members, friends, and professional advisors that have encouraged and guided me through the last 24 years. Without them, I would truly not be where I am today.

Sincere thanks go to Dr. Bruce Dale for taking me on, showing tremendous patience, and giving excellent guidance when necessary. He let me find the answers on my own and I appreciate that.

I would also like to acknowledge the following people for their help on this project, specifically: Dr. Ramani Narayan for use of his laboratory and equipment, Ken Seybold for his guidance and direction in the initial stages of this project, John Glassbrook for his expertise in all things mechanical, Guy Stone, Jr. and Clay Blacksher who, among others, made my time in Texas both educational and enjoyable, Chris Saffron for his friendship and many hours of assistance, Weston Twigg for his friendship and help with equipment photos, and the generous support of Michigan State University.

Furthermore, I must thank the Chemical Engineering office staff for all of the excellent work they do and the tremendous amount of help they supply. Hats off to Faith, Lindsay, Julie, and Candy.

A special thanks goes to my wife, Susan, my mother, Phyllis, and my grandmother, Mildred. The combined effect of these women on my life is immeasurable.

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CHAPTER 1

Introduction

The “most abundant organic material on earth” is lignocellulosic biomass (21). This term includes, but is not limited to, plant material such as agricultural and forestry wastes (21). The world produces approximately 1.5×10^{11} dry tons of this material every year (21). While some developing countries derive significant energy reserves (20) from this material, it is largely underutilized.

Most plants are consist of approximately 35-50% cellulose and 20-35% hemicellulose (21). Cellulose is composed completely of polymerized glucose, while hemicellulose is a complex linkage of a mixture of sugars containing primarily xylose (21). If these sugars could be effectively released, they could be used as starting material for fermentation to ethanol and many other important industrial chemicals. Furthermore, these sugars could be used as potential feed supplements or alternative food sources for animals.

Unfortunately, hemicellulose and cellulose are fairly resistant to enzymatic hydrolysis. They are also only moderately digestible¹ by ruminant² animals, such as cattle, sheep, and goats, and are virtually indigestible to humans. Significant amounts of research are being done to develop an effective pretreatment that will increase the sugar yield resulting from the enzymatic hydrolysis or ruminant digestion of lignocellulosics.

Previous work has been done using a batch reactor and liquid anhydrous ammonia to increase both the enzymatic and ruminant digestibility of this material by explosive depressurization. This increase results from an increase of surface area of the cellulose, which makes the material more susceptible to microbial breakdown, a reduction in the apparent lignin³ content of the material, the decrystallization of cellulose, and the partial hydrolysis of hemicelluloses.

The primary goal of this research was to determine if an extruder could be used to facilitate the Ammonia Fiber Explosion (AFEX) Process, previously performed only in a batch reactor. If the AFEX process could be performed in an extruder, a preliminary study of the most effective conditions would be done. Effectiveness of the treatment was defined as an increase in enzymatic or ruminant *in situ* digestibility.

¹ Microorganisms present in the rumen produce cellulase that breaks down the cellulose.

² Ruminant animals are even-toed, hoofed animals that chew a cud.

³ Lignin is a virtually indigestible portion of plant material which gives mechanical strength and is known to inhibit utilization of cellulose and hemicellulose.

CHAPTER 2

Literature Search - Plant Components and Utilization

Given the high reliance of the United States on Middle East petroleum reserves, and the volatile nature of this portion of the world, not to mention the environmental problems associated with burning fossil fuels, an alternative fuel and source for it must be discovered (17). One natural alternative is ethanol. This is a very clean burning fuel that is easily produced from sugar fermentation. The primary feedstock for this conversion in the United States is corn starch (21). For the full potential of this fuel to be realized, other sources of fermentable sugars must be obtained. One obvious source of this sugar is from lignocellulosic biomass reserves. These are relatively underutilized, especially in the United States, and offer an incredible supply of renewable lignocellulosic sugars.

Unfortunately, the total reserve of biomass cannot be utilized. Consideration must be given to soil loss due to erosion (both wind and water induced) and nutrient loss. Larson states the amount of biomass¹ available for removal was as high as 60%, but as low as 36% (12) in his study. Obviously, the amount of material available for removal will depend on geographic location, soil type, crop type, extent of supplemental mineral fertilization, and a host of other factors.

The term biomass refers to a large class of organic materials, consisting mostly of plant material (20). These materials consist primarily of 3 constituents: cellulose,

hemicelluloses, and lignin (21). Cellulose typically constitutes 35-50% of biomass, hemicelluloses account for 20-35%, and a significant portion of the remaining material is lignin (20).

Cellulose is a linear polymer of glucose with up to 10,000 repeating units (21). Hemicelluloses refer to a large class of compounds, consisting primarily of xylose (21). Lignin, which gives plants mechanical strength, is a poorly defined substance whose composition and structure may vary between plants (7).

In a plant, cellulose chains are surrounded by hemicelluloses and lignin. These compounds shield the cellulose from cellulase enzymes and may even absorb some of these enzymes (21). If the lignin and hemicellulose sheath can be removed or reduced, effective microbial degradation can occur to obtain glucose, short chain glucose linkages, xylose, and other simple sugars. Microorganisms are then available to convert these sugars to ethanol.

Literature Search - Extrusion

The primary focus of extrusion research is polymer and food processing. Since lignocellulosic biomass has no determinable viscosity and an exceedingly variable density (depending on moisture, settling, particle size distribution, etc.), much of this work was not helpful. The most helpful information was of a general sort, and much of this information could not be implemented because of physical limitations of the process.

While this work represents the first research in continuous AFEX processing, experimentation with similar goals has been performed with an extruder. The research

¹ In this study, corn was the major product under consideration.

was conducted at the USDA's North Regional Research Center in Peoria, Illinois by two separate groups. Both investigators looked at the effect that alkaline extrusion conditions can have on the digestibility of wheat straw. Gould (9) treated wheat straw in a single screw extruder using alkaline hydrogen peroxide. Effectiveness of the treatment was demonstrated as increased ruminant *in situ* digestibility as used in this work². Gould reports results that show digestibility was "increased...to a level comparable to that observed for cereal grains (9)". Carr performed similar trials on wheat straw using a variety of alkaline chemical mixtures in both a twin (4) and single (3) screw extruder. He reports that a sodium hydroxide treatment in the twin screw extruder provides a much more effective and efficient means of removing pentosans, solubilizing lignins, and increasing cellulose accessibility (4) than either the batch reactor or single screw extruder.

Several references were also found which related to extrusion cooking in a twin screw extruder. The two most applicable investigated the effect that the screw configuration can have on cooking either rice or wheat flour. Results showed primarily that residence time of the material as well as product quality (22) can vary significantly with changes in the screw profile. Additionally, small changes in the screw profile can affect the way the energy put into the system is distributed (8).

Unfortunately, none of this information proved overly valuable. Again, the concepts were important, but those ideas that were applicable to this work could not necessarily be put into practice on the equipment used. For example, the information on energy input as related to screw profile could prove valuable to future work. However,

² The *in situ* method used in this research will be described in the "Results" section.

the machine used was severely limited in many aspects, one of which was the allowable screw configuration³.

Batch AFEX

The most significant source of information for this work was that published on the batch AFEX process. This material gave guidance with regard to the desired effects of the treatment and treatment conditions. Again, however, guidance was of a general sort.

The batch AFEX process consists of a pressure vessel fitted with heating and cooling capabilities. Biomass samples are placed in the container which is then sealed and purged. Liquid ammonia is added and the entire system is heated to the reaction temperature and allowed to equilibrate for a predetermined length of time. Once the treatment time has elapsed, the pressure is rapidly released through a ball valve to a blowdown tank which absorbs the ammonia vapors with water. This rapid pressure release is referred to as the “explosion” throughout this work⁴. The treated material is then allowed to air dry.

The batch AFEX process increases enzymatic hydrolysis and *in situ* ruminant digestibility in three principal ways. First, the ammonia plasticizes the cellulose, and upon explosion, blows the fibers apart increasing the surface area available for microbial attack. This effect also helps decrystallize the cellulose. Crystalline cellulose is much more resistant to microbial degradation than amorphous cellulose (21). Second, the AFEX treatment demonstrates a reduction in apparent lignin content. Lignin is a completely indigestible material that adds mechanical strength to plant fibers, and can

³ This is discussed further in Chapter 3.

inhibit digestion of cellulose and hemicelluloses (7). Finally, partial hydrolysis of the hemicelluloses occurs, thereby exposing more cellulose to hydrolysis.

⁴ Please note that there is no explosion in the classical sense. The term is used to describe the powerful and rapid depressurization that is observed.

CHAPTER 3

Ammonia Safety

Ammonia¹ is a common industrial and agricultural chemical. As an anhydrous liquid, it is injected directly into the ground as a fertilizer and is a well known refrigerant. It is also used to manufacture nitric acid, explosives, and solid fertilizers.

Ammonia is a colorless gas with a pungent odor which is readily recognizable. Davis lists (6) 5 ppm as the least perceptible odor, 20 to 50 ppm as a readily detectable odor, 100 ppm as noticeable irritation of the eyes and nasal passages, 400 ppm as severe irritation, 1700 ppm as lethal at 1/2 hour exposure, and 10,000 ppm as immediately fatal. Due to a moderately high IDLH (Immediately Dangerous to Life and Health) level of 500 ppm, it is classified as an “Acute Toxic Hazard (6),” and must be handled with extreme care.

The compound boils at -33°C (-28°F) under atmospheric pressure and has a room temperature (21°C, 70°F) vapor pressure of 8.8 atm (129 psia) (6). Pure ammonia vapor is lighter than air, but depending on atmospheric conditions, a mixture of ammonia and air may be heavier than air (6). Because of the high heat of vaporization of ammonia, a concentrated vapor cloud may form a fog due to water condensation in the air (6).

¹ Much of the information on safety was taken from Davis (6). It is a very encompassing manual on ammonia.

The flammability limits of the gas in air are from 16% to 25% by volume and 15% to 79% in pure oxygen (6) at atmospheric pressure. While some sources label ammonia in these conditions as flammable, the National Safety Council (15) has deemed the mixture “difficult to ignite”. Furthermore, combustion requires an intense heat source with a temperature above 650°C when the mixture is in the presence of iron for combustion, and above 850°C when iron is not present. The presence of oil can lower the ignition temperature somewhat, but not significantly (15).

Ammonia as a gas or liquid is highly soluble in water. At 0°C, the compound is nearly 43% soluble (by weight) in water, and is 14% soluble at 60°C (10). The reaction between ammonia and water generates ammonium hydroxide, a caustic compound. This makes ammonia a contact hazard, as well as an inhalation hazard. The gas or liquid can be absorbed by moisture on the skin and lead to caustic burns. Anhydrous liquid ammonia is also dangerous because the high heat of vaporization of the compound may lead to the skin freezing.

Ammonia is a stable compound, but is very reactive. It will react with organic and inorganic acids to give salts, many of which are used as fertilizers. It is known to react with all of the halogens and some of the interhalogens violently, sometimes producing explosive products (1). Also, gold, silver, and mercury and their salts can react with ammonia to generate explosive, shock sensitive compounds (6).

Most common metals (stainless steels, aluminum, brass, copper, bronze and others) demonstrate excellent corrosion resistance to anhydrous liquid ammonia, especially at room temperature and below (16). However, when a small amount of water

is added, the resistance of many metals becomes unsatisfactory. Stainless steels, Hastelloy, carbon steel and aluminum demonstrate excellent corrosion resistance to ammonia under normal temperatures and all concentrations (16).

To develop a safe environment in which to work with ammonia, the Office of Radiation, Chemical, and Biological Safety (ORCBS) at Michigan State University (MSU) was contacted. After their inspection of the site, several recommendations were made, and followed.

First, the laboratory in which the extruder was placed was modified. The room already contained a large drop down hood of sorts. This “hood” was a sheet metal enclosure, dropping approximately 1.5 ft from the ceiling. Inside this enclosure were two large ventilation ducts with an estimated flow of 1500 ft³/min apiece. The modification was made by further enclosing the area by installing vinyl curtain around the entire perimeter of the hood. The curtain was hung from the existing hood and reached nearly to the ground², virtually enclosing the entire ammonia operation. This modification was made to contain any ammonia leaks. Testing of this enclosure was done with a smoke bomb that emitted 10,000 ft³ of smoke in 1 minute, none of which was observed to escape.

Secondly, a horizontal fume hood was constructed by a local contractor and attached to one of the ventilation ducts to concentrate ventilation immediately over the extruder. The hood had face dimensions of 3’x 4” which was sufficient to effectively ventilate the biomass feed port, the extruder outlet, and the ammonia injection point.

² The manufacturer recommends a slight gap at the floor level to allow for air flow.

Finally, an eye wash station was installed in the room, outside the safety curtain, and two full-face respirators with removable ammonia cartridges were purchased for use during all experimentation. A safety program was developed and reviewed with each person assisting in any experimentation outlining emergency procedures and safety protocols. The safety document stipulated, among other things, that 1) no work was to be done alone with the ammonia cylinder open including leak testing and 2) respirators were to be worn (at least with neck strap) at all times when inside the enclosure when ammonia cylinder was open. The safety document also included emergency procedures for inhalation, skin or eye contact, and ingestion of ammonia.

To minimize the potential for ammonia exposure due to catastrophic failure of the ammonia cylinder, only 50 pound ammonia cylinders were used. By calculating the volume of the room as 2324 ft³, a total failure of a full ammonia cylinder would result in an immediate ammonia concentration of 345 ppm in the enclosure. While this concentration would be irritating, it is well below the IDLH of 500 ppm.

The ammonia cylinder was secured to a rack that was constructed underneath the open room vent. The piping system featured a safety shutoff valve immediately after the cylinder and a purge line to vent the ammonia pressure when experimentation was complete and to blow any residual ammonia vapors out of the line. Lines were initially leak tested with a 35% (wt) hydrochloric acid solution. Ammonia leaks were evidenced by formation of ammonium chloride, a white fog-like precipitate, when the acid vapor was sprayed on connections. Further confirmation of sealed connections was obtained by carefully smelling for ammonia in the room.

Throughout experimentation, no problems were encountered with ammonia. By continually wearing the respirator when inside the curtain, all problems were easily avoided. Ammonia cartridges were changed periodically, or whenever the odor was detected while wearing the respirator. While the odor was certainly present at times outside the safety curtain (often being entrained in the fibers of clothes or dissolved in the wet biomass attached to equipment that needed rinsing), no significantly hazardous occurrences were observed.

Probably the most prevalent hazard observed was dealing with residual ammonia. The amount of ammonia dissolved in a small amount of water that can produce a significant airborne concentration is unexpectedly small. Well after the ammonia cylinder had been closed off and all of the transport lines purged, ammonia dissolved in the wet biomass had the potential to create moderately high concentrations of ammonia in the surrounding air.

Equipment Used

The main piece of equipment used was a Baker-Perkins 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. Heating was supplied by electric cartridge heaters in three zones along the barrel and the die. Cooling was supplied in the corresponding

zones by chilled water fed through cored barrel sections³. The die was air cooled. All interior surfaces of the instrument were nitrided with a 63 μ -inch finish which provided excellent corrosion resistance. All agitator parts were made of heat treated alloy steel. The corrosion resistance of a single piece of the agitator was determined prior to any experimentation and no corrosion was observed through the experimentation⁴.

As mentioned previously, stainless steels show excellent corrosion resistance to ammonia and ammonia solutions. For this reason, 316 SS was used as a material of construction whenever possible. The pump used to deliver ammonia was an American Lewa reciprocating diaphragm metering pump. The pump head was made of 316 SS with Teflon seals. The pump was capable of metering 0.23 to 23.0 GPH. The drive supplied 3 HP. The pump was calibrated with water and the necessary conversions were done to determine the amount of liquid ammonia fed using a specified stroke length and speed setting. Volumetric output of the pump was determined to be linearly related to the product of stroke length and motor speed.

All tubing, fittings, and valves used were constructed with 316 SS. The tubing used was 3/8" OD with 0.065" walls, rated to a working pressure of 6500 psig. All fittings were rated to the burst pressure of the tubing⁵. All of the tube-to-tube connections were Swagelok compression fittings, but many of the fittings between tubing and

³ The final cooling zone had minimal effect if any. Untreated water had been used for cooling previously, and the barrel core was almost completely plugged with mineral deposits. Several unsuccessful attempts were made to remove the blockage.

⁴ Many of the surfaces in contact with ammonia actually appeared to have been cleaned.

⁵ The pressure ratings of the materials used are well in the safe range. This was done to insure safety while working with ammonia.

equipment (pump head, pressure regulator, ammonia injection port, etc.) were NPT. All NPT fittings were sealed with Teflon Tape.

Biomass was fed into the extruder using a twin screw feeder produced by the K-Tron Institute. The feeder featured a mixing arm in the material hopper to continually provide biomass to the feed screws by eliminating “dead” zones. Material was continually turned over and replaced at the bottom of the hopper. This was very important when feeding the biomass mixture, which tended to fluff. The feeder was calibrated by feeding a material with a known moisture content for a known period of time and determining the mass fed for different screw speeds. Samples that contained different amounts of moisture were calibrated individually.

A schematic process flow diagram is given in the appendix, as well as several equipment photos, that illustrates the final state of all process equipment and peripherals.

Samples Used

The primary material investigated in this project was corn fodder or stover⁶. This includes all above ground portions of a corn plant except the grain and cob. Due to the time of year in which the research was started, very little corn fodder was available so wheat straw was substituted. However, only preliminary trials were done with the wheat straw such as determining the ability to flow with and without ammonia, etc.

Corn fodder was eventually obtained in a large square bale. This material was coarsely chopped using a tractor mounted grinder. The material was then dried to less

⁶ The sample material is also referred to as biomass.

than 5% moisture in an oven and further milled to pass a 2 mm screen in a rotary knife mill. The material was stored in plastic bags inside cardboard drums.

Adaptation of Equipment

This particular extruder was primarily used for processing of polymers. Thus, it was necessary to make several modifications to the existing equipment. First, a port needed to be machined to allow the injection of ammonia. There were two positions on the barrel, typically used as secondary feed ports for polymer blending, that allowed this adaptation to be easily implemented. The first was approximately half way down the length of the extruder barrel, and the second was located more than three-quarters down the length of the barrel. The first point was chosen to maximize the equilibration time of the biomass and ammonia. The equilibration time was approximately 1 minute with the die block and a die in use, but depended on feed rate, ammonia load, die temperature, etc. Another reason for choosing the first point was that the final position was very near the die block, where the biomass compacted. Injecting ammonia at this point would cause problems with pumping⁷ due to the biomass plugging the injection hole.

Second, the screw configuration was modified significantly⁸ in an effort to minimize ammonia loss through the feed port. Additionally, the discharge screws at the end of screw shaft were modified. Originally, these screws were flat on the end as illustrated in the lower screw in Figure 3-1. However, this design would force the biomass into the die block, where it would subsequently plug due to its resistance to flow.

⁷ Even the earlier position had some problems with biomass being forced into the injection port and causing problems with pumping. Several instances of ammonia building in the tubing between the check valve and the extruder and then rapidly blowing into the extruder barrel were observed.

⁸ This is discussed in more depth in the "Parametric Variation" section.

By building the end of the screw into a more conical shape, illustrated by the upper screw in Figure 3-1, the biomass was directed into the die block and eventually out through the block, rather than plugging.

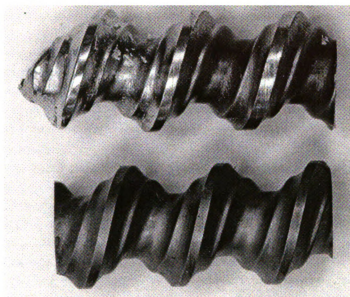


Figure 3-1 - Camelback Discharge Screw

The ammonia used was supplied in 50 lb. cylinders equipped with a dip tube to ensure liquid delivery. Initially, ammonia was introduced through a piping system that was controlled by a needle valve. The pressure in the cylinder was used to force the ammonia into the extruder. As the project progressed, the need for a pump was realized and the system was modified to accommodate it. The pump allowed for more control and accuracy in the delivery of ammonia. A check valve was installed just prior to the injection port to maintain the pressure on the ammonia in the tubing after the pump to help insure liquid delivery. Once the pressure generated by the pump reached the cracking pressure of the check valve, the ammonia flowed directly into the extruder. The reseal pressure was set above the vapor pressure of ammonia to prevent any leakage

through the check valve. Due to the corrosive nature of ammonia, especially with water present, several bypass valves were implemented to allow the tubing to be purged after use to prevent internal corrosion.

CHAPTER 4

Research Progression

The first step in this research was to determine whether biomass would flow through the extruder or not. Initial trials with a die were less than encouraging, and so the entire die assembly, including the die block, was removed. This allowed the biomass to flow relatively easily through the instrument. The flow rate was limited somewhat due to the screw configuration of the machine. It was originally set up with a polymer processing configuration that featured a very restrictive mixing zone. This placed limits on the amount of biomass that could be fed through the machine. To promote more effective biomass transport in the extruder, the mixing zone configuration was changed to a 45° feed forward (FF) design¹.

The next step was to add ammonia. The flow of ammonia was initially controlled in two ways. First, a regulator was implemented to reduce the outlet pressure of the liquid ammonia. Then, the actual flow of ammonia was controlled with a needle valve. Flow rates were determined by weight difference of the ammonia cylinder. The ammonia cylinder was attached to a rack which was constructed under the open ventilation duct inside the enclosure.

Ammonia was injected through a small hole typically used for polymer blending. This hole was fit with an insert to maintain the inner barrel profile when only one feed

material was used. A replica insert was machined, cored, and tapped with NPT threads to allow ammonia injection. Initial trials with this injection port showed some ammonia leakage as evidenced by foaming around the port. This was eliminated by inserting a Teflon sheet around the port.

Once it was verified that ammonia and biomass would flow together through the extruder, increasingly restrictive dies were used. First, not even the die block was used. However, as more confidence was gained, the block was added, as well as a die soon afterwards. The flow area with the die block was reduced nearly 80%. In other words, the outlet of the die block was 20% of the area of the inlet. Several explosions were obtained with only the block in use which gave very good results. However, a die was eventually implemented under the presumption that the increased pressure generated from a smaller orifice would provide a more effective treatment.

Before any work was done with a die, a pump was purchased to deliver ammonia. The pump offered greater control over the flow of ammonia and increased precision with regards to delivery amount. The pump and control module were mounted on a cart to allow easy transport. The pump was calibrated using water, and the necessary conversions were made to determine the amount of ammonia delivered. The delivery system was also modified. Flexible, braided SS, Teflon-lined tubing, rated to 1800 psig working pressure and 7200 psig burst pressure, was implemented to make connections simpler. Since the pump was a diaphragm pump delivering liquid to an essentially atmospheric pressure chamber, the only restriction to flow of the pressurized liquid was a

¹ Discussion of screw configuration is considered in greater depth in the *Parametric Variation* section.

steel sealing ball. The pressure of ammonia was more than adequate to lift this ball off the seat, so a check valve was installed between the pump and the extruder. The check valve had an adjustable cracking pressure up to 150 psig. This is well above the vapor pressure of ammonia at room temperature, but not so high as to provide a significant restriction. A slight pressure generated by the pump (over the vapor pressure of ammonia) would be enough to open the seal, but as soon as the pressure was released (end of stroke), the valve would reseal and stop the ammonia flow. A bypass valve was also implemented for purging the ammonia out with air and vice versa. This feature also helped in priming the pump with liquid ammonia. All cylinders purchased featured a dip tube which should ensure liquid delivery, but some problems were noticed. By opening the bypass valve, the flow of liquid ammonia was started and many pumping problems were alleviated.

Much work has been done on the design of extrusion dies. However, the biomass mixture is unlike any polymer or food which can be extruded through a standard die because of its non-fluid behavior. Polymer and food extrusion dies are designed using physical properties such as density and viscosity (13), neither of which apply to ground corn fodder effectively.

Thus, dies were designed intuitively. Several preliminary dies were tried with minimal success. The first dies attempted were machined using a ball end mill to give a circular compression with a slit outlet. These dies² were minimally effective and led to biomass compaction and no flow. The other major type of die investigated implemented

² One die was milled and the slit width was gradually increased.

a more gradual contraction. These were initially machined by hand and the rough edges and interior surfaces also created problems with biomass flow through the restriction. The die that proved most useful was machined with a 5° end mill to give a smooth, gradual contraction of 40% of the inlet area (i.e. outlet was 60% of the area of the inlet). With this die, explosions could be consistently obtained.

The explosions typically came at regular intervals³. Depending on the ammonia load and temperature, the steady state operation was either continuous or periodic. Higher levels of ammonia (>1.5 mass ammonia/mass biomass⁴) prompted the material to slowly discharge for 2-3 minutes and then violently discharge from the die. Pressure was observed to build to ~300 psig, and torque approached 50%, until the explosion where pressure dropped to 0 psig and ~11% torque (typical no load value). Alternatively, lower amounts of ammonia (<1.5 M/M) demonstrated continuous minor explosions where small explosions were constantly observed. These smaller explosions were characterized by small popping noises accompanied by small pieces of biomass shooting off of the main plug or fracturing and expansion of the main plug coming through the die.

To improve the process performance, several modifications were made to aspects of the operation at various times through the experimentation. For example, Teflon sheeting was used to minimize extruder pressure loss through the die. It was observed several times that two critical metal-metal interfaces (the interface of the extruder and die block, as well as between the die block and the die itself) were allowing a portion of the

³ Some trials showed reproducibility of ±15 seconds.

⁴ Ammonia loads are given in a mass ratio. For example, an ammonia load of 3 signifies 3 pounds (or kilograms, or grams, etc.) of ammonia to 1 pound (or kilograms, or grams, etc.) of dry biomass. Hereafter, ammonia loads are given M/M units, connoting mass to mass ratio.

high pressure ammonia to escape as evidenced by slight foaming at the interface. The Teflon sheeting was cut to fit around the orifice that it sealed and was then installed between the two surfaces. After the installation of these seals, no further foaming was observed.

Parametric Variation

The main parameters varied were temperature, feed rate, and ammonia loading. Water content of the biomass was also varied, but with little success. Moisture content higher than 60%⁵ led to the water being squeezed out of the biomass and flowing back to the biomass feed port. This created problems with feeding the biomass into the extruder due to foaming in the feed port caused by the ammonia vaporizing. The foaming wetted the inner surfaces of the feed funnel and the biomass stuck to the sides of the funnel resulting in eventual plugging. Alternatively, moisture levels lower than 60% did not allow for effective equilibration with the ammonia, and hence, no explosions were obtained. Thus, all trials referred to in the *Results* section were obtained at 60% moisture.

The effect of temperature was tested by changing the treatment temperature. Typically, the first zone, centered on the biomass feed port, was unheated⁶ and cooling was shut off⁷. The second zone, at the ammonia injection point was heated slightly, depending on the die set point. Too much heating will vaporize the ammonia and result in a less effective treatment. The third zone was usually heated to a temperature near the average of the setpoints of the second zone and the die. The die temperature was

⁵ Biomass moisture levels are given as the percent of total mass. So, 60% moisture would be 60 grams of water in 100 grams of a biomass and water mixture.

⁶ In this case, heating refers to heating above room temperature.

considered the reaction temperature. It appeared that there was an upper bound to the die temperature as no explosions were obtained above a set point of 70° C. This is probably due in part to partial vaporization of the ammonia in the third heating zone. The die temperature necessarily caused a temperature rise in the third zone by conductive heat transfer. This would lead to an increased amount of ammonia vaporization and a less effective treatment due to the ammonia being forced out through the biomass port. Because the ammonia was not trapped as effectively as desired, the vapor was essentially free to leave the chamber. Lower temperature treatments would not vaporize as much ammonia and are probably not subject to this effect.

Feed rate of the biomass was tested. This parameter varied somewhat and appeared to be dependent on atmospheric conditions with some trials allowing a very high feed rate and others a very low feed rate for the same moisture level material. Allowable feed rate varied up to 10 units⁸ for 60% moisture biomass. One observation about this problem focused on mixing the sample. Sample material was prepared by weighing out the biomass and water separately, and then adding the water to the biomass and mixing. The biomass was completely wetted upon thorough mixing, but invariably formed clumps of material. These were broken up as much as possible, but would often reform in the mixer and persist through the feeder screws, as evidenced by large plugs of material being fed followed by little or no flow. Once these plugs reached the extruder screws, they were too large to fit into the screw flights and would simply roll on the top

⁷ The cooling ability of the first zone was removed because it created condensation around the feed port that led to clogging of the feed.

⁸ The feeder speed was set as high or low gear, and then a percentage. High gear was always used at about 20%.

of them. Material added tended to build in the feed funnel and plug. This problem was alleviated to a certain extent by forcing the material into the screws with a short length of stiff tubing. However, it was impossible to tend to the pump, ammonia cylinder, extruder control console, process outlet, lab notebook, and process feed continually, and plugs were common.

The final parameter tested was the ammonia load. The ammonia load was varied from 0.5 to 2.0 M/M. The calculated ammonia load is only an estimation of the actual treatment amount. The extruder used had a relatively small L/D (10:1, D=30 mm), with a measured barrel length of about 15.25" and the only practically allowable point for ammonia injection was approximately half way down the barrel. This created some problems with ammonia flowing back through the biomass feed port.

Several efforts were made, by altering the screw configuration, to create a zone of high mixing prior to the ammonia injection point that would effectively provide a plug that would restrict ammonia flow back to the feed port. Unfortunately, the first three pieces of screw flighting were permanently attached to the screw shaft due to the stresses of heating and cooling. These first sections were all forward transport screws, illustrated in Figure 4-1, which typically ran at 50% capacity and did not provide an effective restriction. Thus, with a limited amount of the screw shaft available to implement the mixing zone, only 5 mixing paddles could be used.

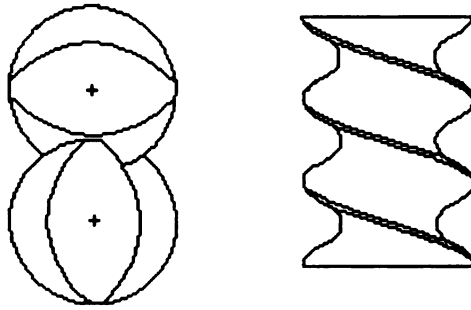


Figure 4-1 - Forward Transport Screw

There were other limitations with regard to the mixing zone. Paddles, illustrated in Figure 4-2, are aligned on an individual screw shaft at various angles. When paddles are aligned at angles from 0 - 90°⁹, the forward transport of the mixing zone increases as the angle approaches 45° from either direction, and decreases as the angle diverges away from 45°. At 0° there is no mixing, and at 90° there is maximum mixing. Paddles

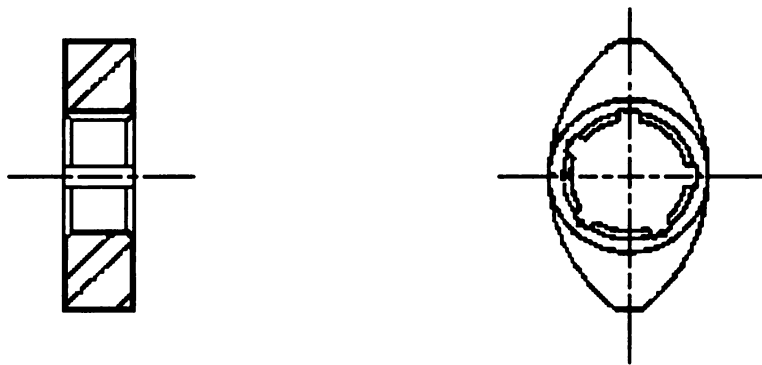


Figure 4-2 - Mixing Zone Paddle

⁹ Angles are determined by looking down the shaft from the discharge end, i.e. towards the feed port. The angle given is the angle of the outermost paddle to the next one down the shaft. For example, paddles aligned at 30° have one paddle on the shaft, and the next one closer to the outlet rotated 30° clockwise with respect to the previous.

aligned at negative angles are reversing and force the flow in the opposite direction.

Thus, an alignment of 90° or a negative angle would provide the most effective restriction.

Several mixing zone alignments were tried, but most of them were torque limited. In other words, only a small amount of biomass could be fed through the machine before the torque would approach its maximum value¹⁰. Thus, a 45° mixing zone was implemented. This allowed for a good plug to be formed¹¹ and also permitted a large flow of biomass. Unfortunately, the length of the mixing zone was not long enough to provide a significant restriction for the ammonia vapors. So, some of the injected ammonia was lost through the feed port due to vaporization. The actual amount of ammonia effectively contacted with the biomass was probably less than the amount injected, but all runs were categorized by the amount of ammonia injected.

¹⁰ Torque limits are dependent on rotation speed of the shafts.

¹¹ Inspection of the screws after filling showed that the mixing zone was ~90% full, while other sections were ~50% filled.

CHAPTER 5

Physical Results

In the batch AFEX process, a significant amount of fiber splitting was observed (6). This has been observed in the extrusion process as well. Completely Untreated (CU) biomass is shown in Figure 5-1 and Figure 5-2 at 125x magnification¹. The material has little, if any splitting of the fibers. Compare these photos with those photos of Extrusion Ammonia Treated (EAT) biomass in Figure 5-3 and Figure 5-4 at the same magnification. These photos illustrate the desired fiber splitting obtained in the batch AFEX treatment. The photos of the EAT material represent the photos that best exemplify the desired treatment. The entire sample processed did not experience this splitting.

Other results are observed in the batch process as well. The batch treated material² exhibits a greater “water-holding capacity (6)” than CU material. This seems to be replicated in the extrusion process in that the EAT material would readily sink in the buffer solution used in the enzymatic hydrolysis procedure. The CU material would float on the surface of the solution, even after being left in the liquid for 8 or more hours. The only way to effectively wet the CU material was by shaking the mixture. Another

¹ Maximum possible magnification available.

² The material studied to obtain this data was alfalfa.

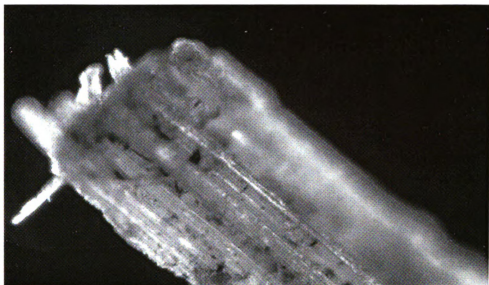


Figure 5-1 - Untreated Biomass

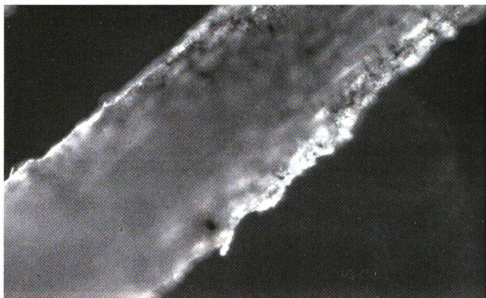


Figure 5-2 - Untreated Biomass

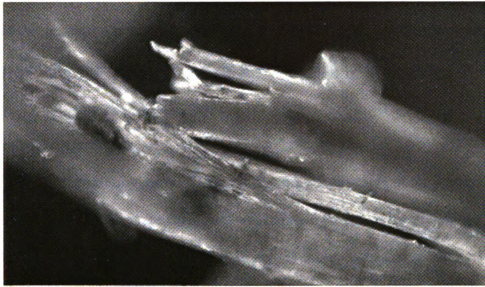


Figure 5-3 - Treated Biomass

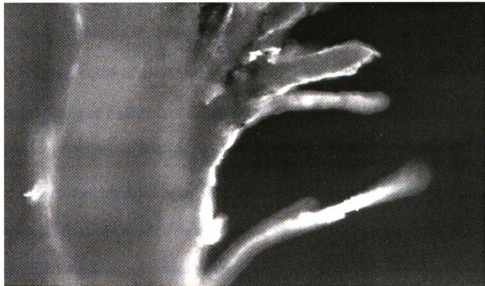


Figure 5-4 - Treated Biomass

result observed in the batch treatment was the decrease of bulk density (5). Due to the nature of this process, physical compaction of the biomass, this variable was not investigated. Any attempts to “unpack” the material would compromise the sample integrity.

Enzymatic Hydrolysis

Initially, the primary means of quantifying the effectiveness of the process was enzymatic hydrolysis. Since the main goal was to prove the extrusion technology worked, maximum sugar concentrations were not the focus, but rather relative digestibility.

All samples were hydrolyzed under the same conditions. The conditions chosen were based on enzyme manufacturer recommendations. The enzyme loading was selected based on standard hydrolysis techniques. All samples were hydrolyzed at 50° C in a 0.05 M citrate buffer of pH 4.8 with a cellulase loading of 15 IU/g biomass and a β -glucosidase loading of 1 mL/mL of cellulase at 5% wt. solids. The cellulase used was Celluclast and the β -glucosidase used was Novo 188, both by Novo Nordisk.

Samples were thoroughly dried in an oven, and 5 g of each sample was placed in a 250 mL Erlenmeyer flask of known weight. Approximately 70 g of citrate buffer was added and the material was allowed to soak overnight. The following day, each flask was adjusted to pH 4.8 using either the 0.05 M sodium citrate or 0.05 M citric acid solution used to prepare the buffer³. Once the sample pH was properly adjusted, buffer was added to make the total solution mass 90 g. A 10 mL volume of the enzyme mixture was then

³ The sample were invariably above the desired pH, so only the acid solution was added.

added to each flask. Samples were covered with Parafilm and aluminum foil and placed in a water bath preheated to 50° C. Samples were lightly agitated between sample points, which were taken up to 48 hours after starting the hydrolysis. Typically, samples were taken at 1, 3, 8, and 24 hours.

The primary analysis took place on a lead HPLC column (Interaction Chromatography, San Jose, CA) which analyzed for glucose, xylose, galactose, arabinose, and mannose. All samples run through the lead column had to be neutralized with lead carbonate. This was the most informative data gained as individual peaks were identified, but this analysis could not be used throughout the research. Late in the summer of 1997, the column crashed several times when analyzing the EAT samples. Initially, this was believed to be a column problem as no interferences had been observed before. Two new columns crashed consecutively while running different batches of EAT material⁴. Due to the repeated column problems and the high cost associated with these columns, an alternative means of analysis was used. They were done on an acid column which analyzed for glucose and a composite sugar peak which represented the sum of xylose, mannose, and galactose concentrations. The only preparation required for these samples was a micro-syringe filtration. Finally, a YSI (Yellow Springs Instrument Co., Yellow Springs, OH) instrument was also used to give a quick estimate of glucose concentrations.

⁴ It was originally believed that the problems were caused because of an extended shelf life which may have allowed for bacteria growth inside the column. However, the problems persisted with newer columns as well.

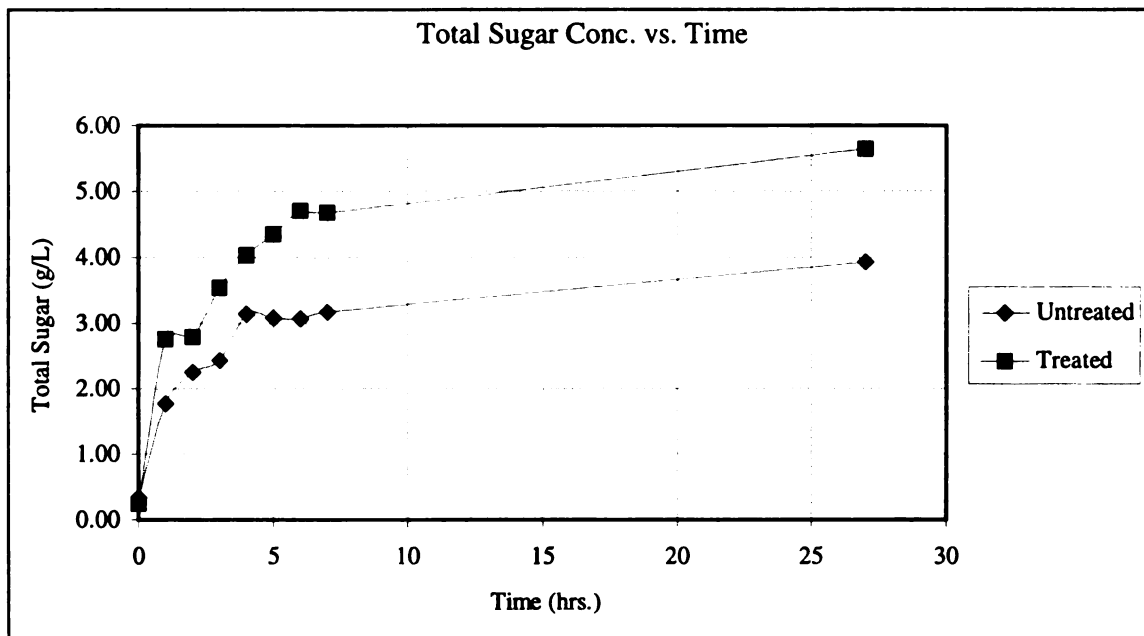


Figure 5-5 - Total Sugar Concentration vs. Time for CU material and EAT material with no die or die block.

Preliminary samples were generated without the use of the die block or die. This material gave a total sugar⁵ concentration 1.44 times greater than the CU material after 27 hours of enzymatic hydrolysis. The EAT material showed a consistently higher degree of digestibility than the CU material, as illustrated in Figure 5-5. Figure 5-5 also shows that the initial rate of digestion of the EAT material was higher than CU material as well.

Use of the die block alone gave an explosion that resulted in a total sugar concentration after enzymatic hydrolysis for 24 hours of 2.4 times that of the CU sample. The temperatures and ammonia loads are given in Table 5-1. The glucose concentration after enzymatic hydrolysis of this material was 2.1 times that of the CU material after the same amount of hydrolysis time. The total sugar concentration after enzymatic hydrolysis

⁵ The sum of the concentrations of glucose, xylose, galactose, arabinose, and mannose.

of this material was also 2.0 times greater than the Extrusion Treated (ET - no ammonia used in treatment) biomass and the corresponding glucose concentration was 2.5 times that of the ET biomass. Three time points were taken for this hydrolysis. When analyzed on the lead column, the second timepoint data was under range and is thus not useable. No chart is presented for this reason.

Table 5-1 - Treatment matrix for samples generated using a die block.

Sample	Temperature	Ammonia Load	Moisture
A - Unprocessed	N/A	N/A	N/A
B - Untreated	No Heating	N/A	60%
C - Explosion	No Heating	1.1	66%
D - Non Explosion	40	1.2	60%
E - Explosion	40	1.2	60%

Further trials with the die block alone gave more encouraging results. The treatment conditions are summarized in Table 5-2. Total sugar concentrations were

Table 5-2 - Treatment matrix for samples generated using a die block

Sample	Temperature	Ammonia Load	Moisture
A- Blank	N/A	N/A	N/A
B - Untreated	No Heating	N/A	60%
C - Unprocessed	N/A	N/A	N/A
D - Explosion	50	~2:1	60%
E - Explosion	50	~2:1	60%

as much as 3.5 times greater than the CU material and 3.4 times greater than ET material after 24 hours of hydrolysis. The concentration versus time data is summarized in Figure 5-6.

Once the die was manufactured, the best results obtained showed a total sugar concentration 2.4 times greater than the CU material after 6 hours of enzymatic hydrolysis⁶. The glucose concentration at this point was 2.3 times greater than that obtained from CU material.

In general, the most effective treatments for enzymatic hydrolysis seemed to be runs with a mild die temperature. Some heating is required to promote an effective explosion, but too much heat promotes ammonia vaporization and loss in this system. With a lower temperature, the ammonia will remain in contact with the biomass longer and a more effective treatment is the result.

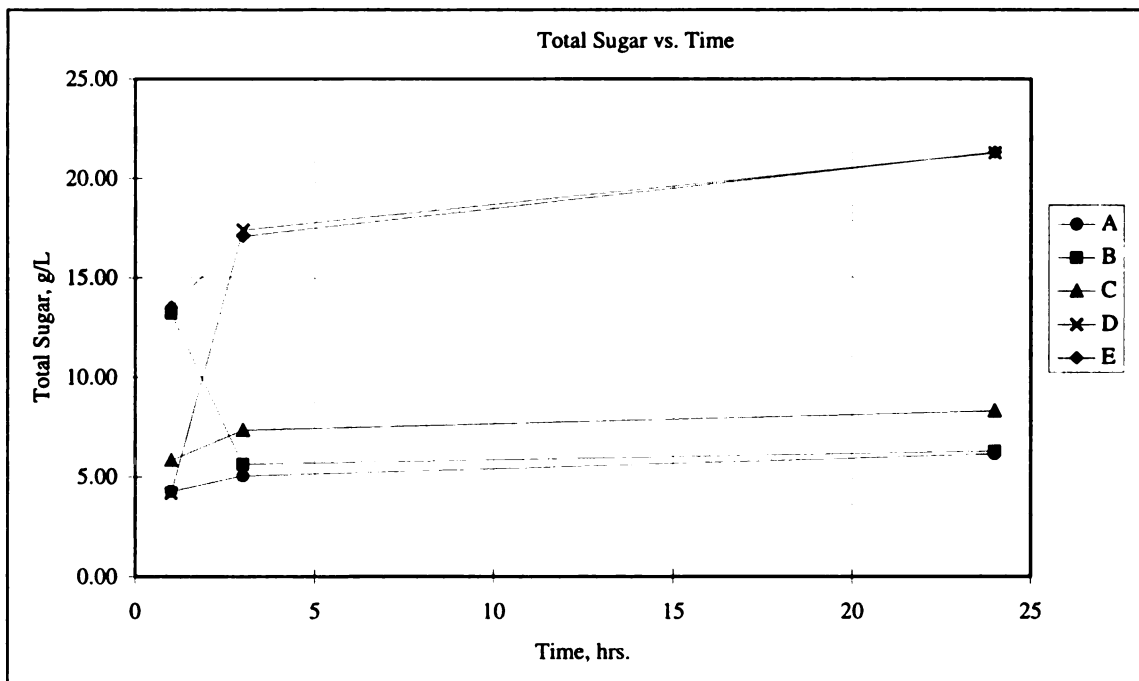


Figure 5-6 - Total Sugar Concentration vs. Time

⁶ This particular hydrolysis was only ran 6 hours total. Other trials were run with a die, but this was the data lost when the lead column crashed.

CHAPTER 6

In Situ Ruminant Digestibility - Background Information

After enzymatic digestibility results like those cited in Chapter 5, the ruminant digestibility of the material in an *in situ* trial was desired. To accomplish this, several samples were generated using the die with different temperature treatments and ammonia loads. The samples were taken to the Texas A & M University Animal Science Department and analyzed by *in situ* digestibility methods.

In situ digestibility is done using a fistulated steer. This is an animal which allows direct access to the rumen by means of a surgically inserted tube, called a cannula. To add the cannula, a patch of skin is removed which is slightly smaller in diameter than the cannula. A similarly sized hole is created in the rumen wall, and the two surfaces are surgically attached. The cannula is inserted, and capped to minimize exposure to oxygen which would kill many of the rumen bacteria.

The animal used in this trial was named "Red¹." Red, the 15 year old, 2000 lb. mobile fermentor is a Santa Gertrudis. This breed originated on the King ranch in Texas by crossbreeding Brahman and Shorthorn cattle. Due to the composition of the original stock, and subsequent breeding practices, the breed is estimated to be approximately five-eighths Shorthorn and three-eighths Brahman (2). A deep, cherry-red color is

¹ Photos of Red and more information about the *in situ* digestion process can be seen at <http://asnet.tamu.edu/cine>

characteristic of the breed. Red is shown in Figure 6-1. The cannula which allows access to the rumen is clearly visible.

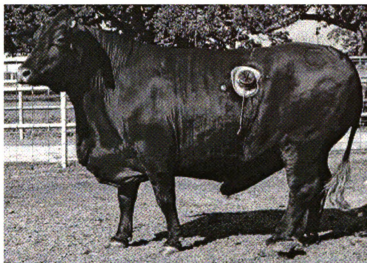


Figure 6-1 - Red

The idea behind the *in situ* trial is to place a known amount of material into the animal's rumen and determine the amount remaining after a specified time interval. To eliminate potentially confusing conversions, all determinations were made on a dry matter basis². Thus, the material was placed in small, non-digestible, permeable bags of known weight and dried 24 hours to determine the mass of dry matter. These small bags were grouped by removal time and were placed in larger bags. Each of the larger bags was secured to a rope that was weighted on the end and then the entire assembly was placed in the rumen. Typically, before the samples can be placed in the rumen, some of the material already in the rumen must be removed to create space for the samples. A portion of the rope was left outside the animal to ease sample collection and prevent rumen

² This eliminates conversions associated with accounting for moisture in wet material.

passage. In the first trial, a total of 171 sample bags enclosing 533 grams (1.2 lb.) of sample and 667 grams (1.5 lb.) of total mass, placed in 9 larger bags, were used. The second trial used 120 sample bags enclosing 398 grams (0.9 lb.) of sample and 482 grams (1.1 lb.) of total mass, placed in 9 larger bags.

The bags were then removed at specified time points³ (0, 3, 6, 12, 24, 48, and 96 hours), thoroughly rinsed, and dried. The digestibility of the materials was then determined as percent of the sample weight lost on a dry matter basis. The weight loss of the material was called the dry matter digestibility, or DMD.

$$DMD = \frac{\text{Original Sample Weight} - \text{Digested Sample Weight}}{\text{Original Sample Weight}} * 100$$

Equation 6-1 - Dry Matter Digestibility

Results

Two *in situ* trials were run. The first focused on extrusion treated materials, with the control being a sample extruded at 50°C with no ammonia. The treatment conditions are summarized in Table 6-1. The second trial attempted to compare the extrusion process to the batch AFEX process, as well as the original unprocessed material. These parameters are summarized in Table 6-2, along with whether the treatment was by the extruder or the batch reactor.

³ The 0 hr time point is obtained by holding the samples in the rumen fluid for 1 minute.

Table 6-1 - Treatment matrix for *in situ* trial 1.

Sample	Temperature (°C)	Ammonia	Moisture
A - ET	50	N/A	60%
B	No Heat	1.0	60%
C	55	1.5	60%
D	65	0.8	60%
E	65	1.0	60%
F	65	1.5	60%
G	65	1.6	60%
H	65	2.0	60%
I	55	1.5	60%

When generating samples for the second trial in the batch reactor, all three samples were heat damaged so the results may not be indicative of the actual performance of the batch process. They can, however, provide a rudimentary comparison.

Table 6-2 - Treatment matrix for *in situ* trial 2.

Sample	Temperature (°C)	Ammonia	Moisture	Batch or Extrusion
C	55	1.5	60%	Extruded
H	65	2.0	60%	Extruded
J - CU	N/A	N/A	N/A	N/A
N	80	1.0	60%	Batch
X	80	1.5	60%	Batch
Y	90	1.0	60%	Batch

Results of the first and second *in situ* trials are shown in Figure 6-2 and Figure 6-3 respectively. The 48-hour digestibility of the trials is tabulated in Table 6-3 and Table 6-4. In both tables, the results are ordered with respect to decreasing digestibility. The first trial showed that the EAT material was up to 77.4% digestible as compared to the

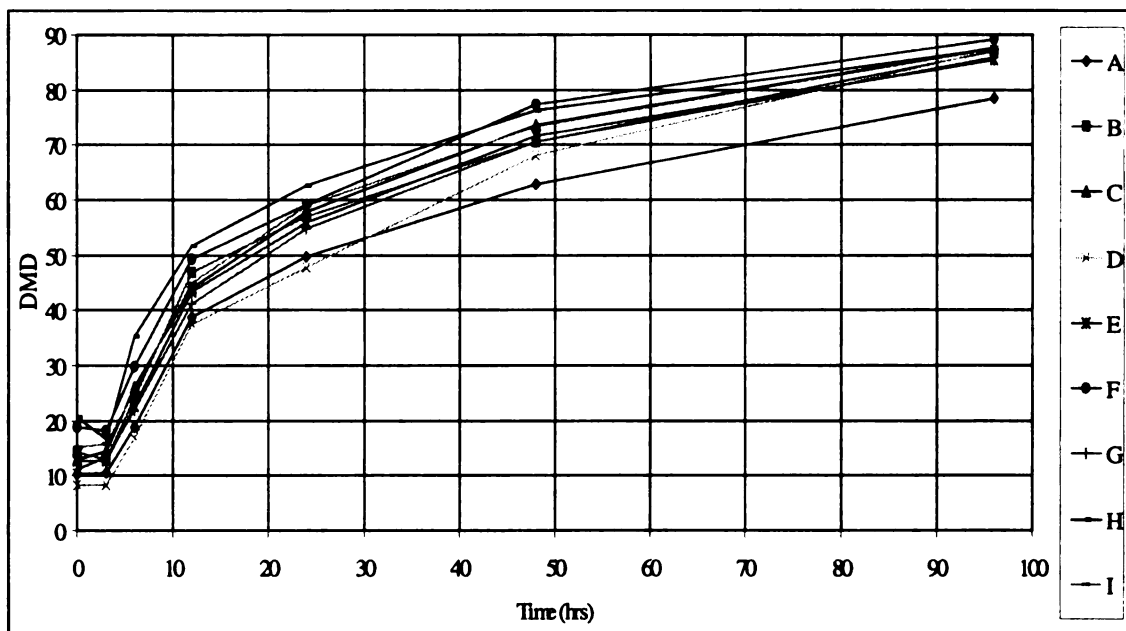


Figure 6-2- Dry Matter Digestibility, Trial 1

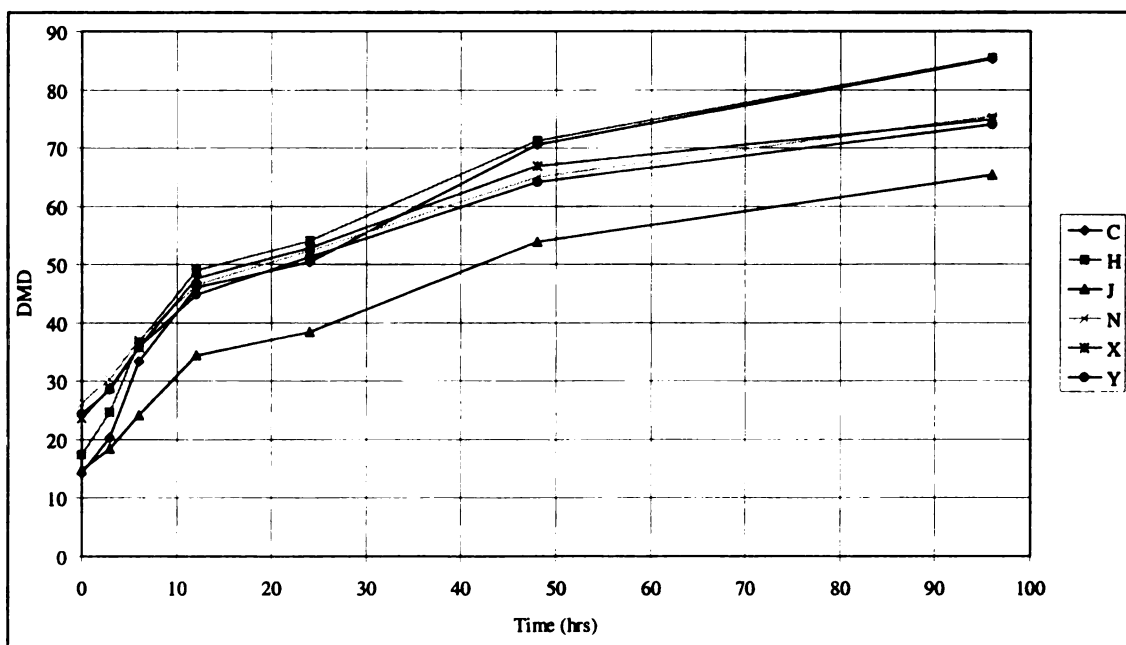


Figure 6-3 - Dry Matter Digestibility, Trial 2

digestibility of 63.0% of the ET control at 48 hours⁴. The second trial gave a maximum digestibility of 71.2% for the EAT material, compared to 53.8% digestible CU material⁵.

Table 6-3 - 48 Hour Dry Matter Digestibility for Trial 1

ID	DMD	NH ₃ Load	T (°C)
F	77.4	1.5	65
H	76.1	2.0	65
C	73.5	1.5	55
I	73.4	1.5	55
E	71.6	1.0	65
G	70.5	1.6	65
B	70.4	1.0	0
D	68.0	0.8	65
A	63.0	N/A	50

Table 6-4 - 48 Hour Dry Matter Digestibility for Trial 2

ID	DMD	NH ₃ Load	T (°C)	B or E
H	71.2	2.0	65	E
C	70.5	1.5	55	E
X	67.0	1.5	80	B
N	65.0	1.0	80	B
Y	64.1	1.0	90	B
J	53.8	0.0	N/A	N/A

Notes: DMD is the dry matter digestibility, reported as a percent.

NH₃ Load is the mass of ammonia injected per mass of biomass treated.

T (°C) is the Die Setpoint.

At 48 hours, the effects of temperature and ammonia loading are not extremely clear 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

⁴ Typical rumen passage is 48 hours or less.

M/M and temperature 65° C, was only the 6th 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 0.05% points and have an average digestibility of 73.5%. Samples *C* and *I* were generated at an ammonia load of 1.5 M/M with a temperature of 55° C. Throughout the first trial, samples *F* and *H* were consistently the most digestible, with sample *H* being the most digestible at all time points except 48 and 96 hours.

The composition of each material at each sample point was determined as well using the Van Soest procedure (19) modified by the Texas A & M Animal Nutrition department. Assays were used to determine the amount of Neutral Detergent Fibers (NDF), Acid Detergent Fibers (ADF), Lignin, and Insoluble Ash. The NDF assay gave the amount of cellulose, hemicelluloses, lignin, and ash in a sample. The ADF procedure removed the hemicelluloses, the lignin procedure removed the cellulose, and the ash procedure removed the lignin.

Cellulose and hemicelluloses are both considered somewhat digestible while lignin is considered indigestible (7). Additionally, lignin reduces the digestibility of the other components (7), so a reduction in apparent lignin content is desired. This reduction has been achieved in the batch AFEX procedure and is realized in the extrusion treatment as well. Results show that the apparent lignin content of the samples treated in the extruder were reduced up to 27.7% as compared to the extruded control, with an average

⁵ The two runs did not occur simultaneously in the steer, and thus are not directly compared.

of 12.5% reduction. Samples *C* and *H* also showed an average reduction in lignin content of 15.6% when compared to the completely untreated material.

The rate at which digestion occurs is important as well. In all cases, the initial rate of digestion⁶ for all treated samples was higher than either the completely unprocessed material, or the material extruded with no ammonia. In each *in situ* trial, the maximum rate of digestion was 2.2 times that of the control in the trial (untreated control in trial 1, unprocessed control in trial 2). The maximum observed rate was shown by sample *H* in trial 1 at 6.16 %/hr.

The data also show that both hemicellulose and cellulose fractions decrease over time, while lignin content increases, illustrating that digestion of cellulose and hemicelluloses was occurring. In trial 1 (see Figure 6-4 and Figure 6-5), the untreated control consistently showed the highest content of hemicelluloses. The cellulose content of the untreated control material was the lowest observed through 24 hours and was near the median at 48 hours. As the figures show, the point at which the concentration of hemicellulose reaches its minimum, the cellulose concentration is maximized. The next time point shows that the fraction of hemicelluloses has increased and the cellulose fraction has decreased. This seems to imply that the hemicelluloses are digested until the

⁶ In this case, the initial rate of digestion was determined as the rate of digestion between hours 3 and 6. In many cases, the samples gained weight from the 0 to 3 hour samples which does not provide acceptable data. This may be explained by insufficient washing of the 3 hour samples which would leave dust, soluble fractions, and microbes trapped in the bag. Another explanation is that the material used to fill the 0 hour bags contained more dust than the 3 hour sample, and would thus allow for more material to be rinsed out.

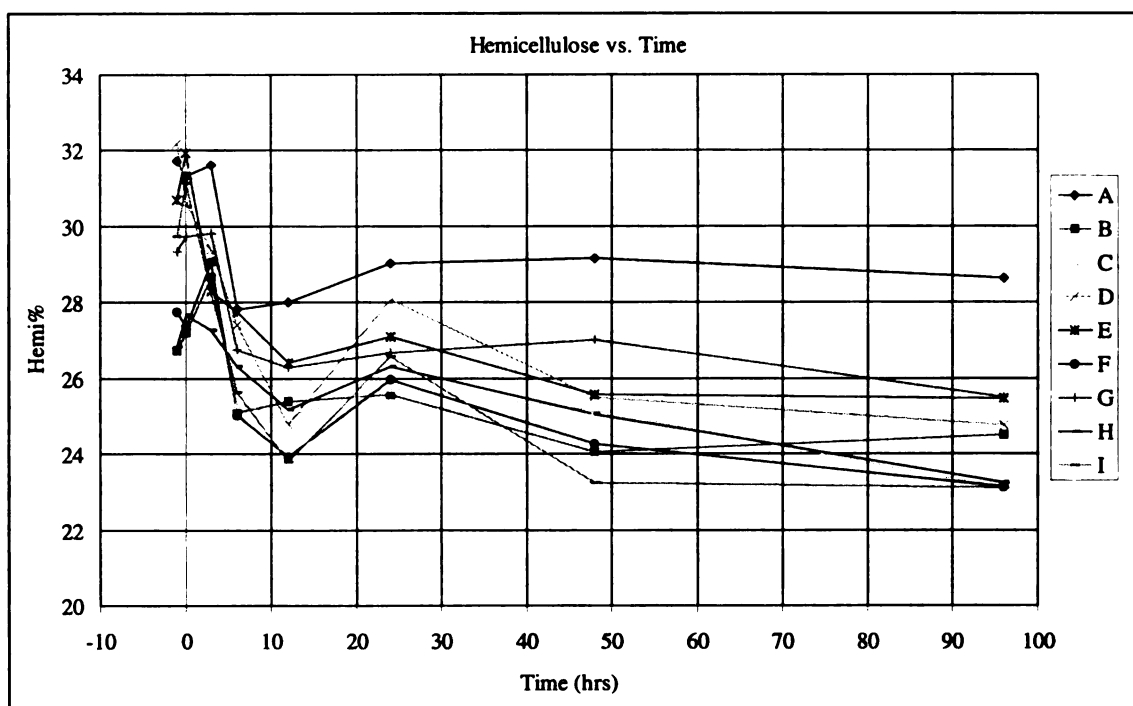


Figure 6-4 - Hemicelluloses vs. Time, Trial 1

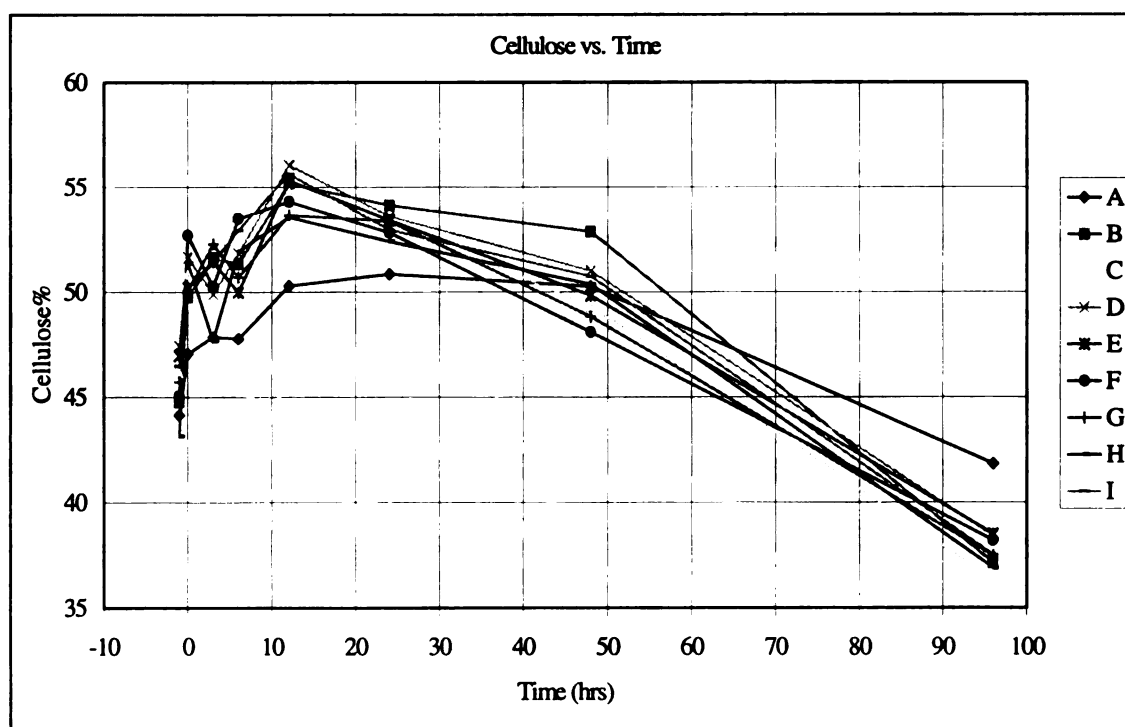


Figure 6-5 - Cellulose Concentration vs. Time, Trial 1

cellulose has been sufficiently reduced to allow for effective digestion. Digestion of hemicelluloses has not necessarily stopped, but now the cellulose is competing for available enzymes and both materials are being digested.

The second trial results are summarized in Figure 6-6 and Figure 6-7. This data shows similar trends to those observed in the first trial. The CU material had the highest hemicellulose content after 48 hours of digestion, and the lowest cellulose concentration. The batch samples had an average fraction of hemicelluloses of 20.3% compared to 25.9% for the EAT samples and 30.0% for the CU material. The cellulose fractions of the EAT material showed an average of 51.2% compared with 48.2% for the batch process and 46.6% for the CU material.

Trends similar to those noticed in trial 1 are apparent for the extrusion treated material in trial 2. The increase in cellulose and decrease in hemicelluloses concentrations observed follow similar trends. One noticeable difference between the batch and extrusion samples is that the concentration of hemicelluloses of the batch samples continually decreases and does not demonstrate the concentration increase seen in the extrusion material. Due to the heat damage suffered by each of the batch samples, it is difficult to say whether this observation is a result of the treatment or the damage the samples received. Considering that the damage was charring, this may have led to a partial degradation of the fibers which could moderately enhance the digestibility.

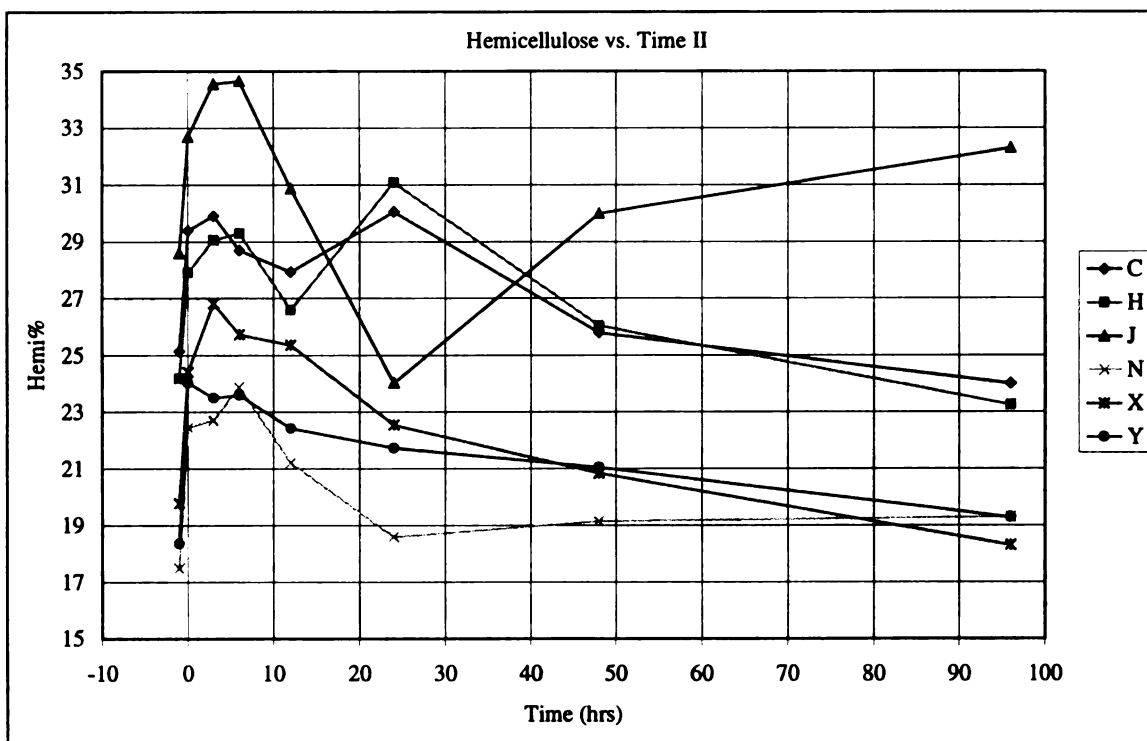


Figure 6-6 - Hemicelluloses vs. Time, Trial 2

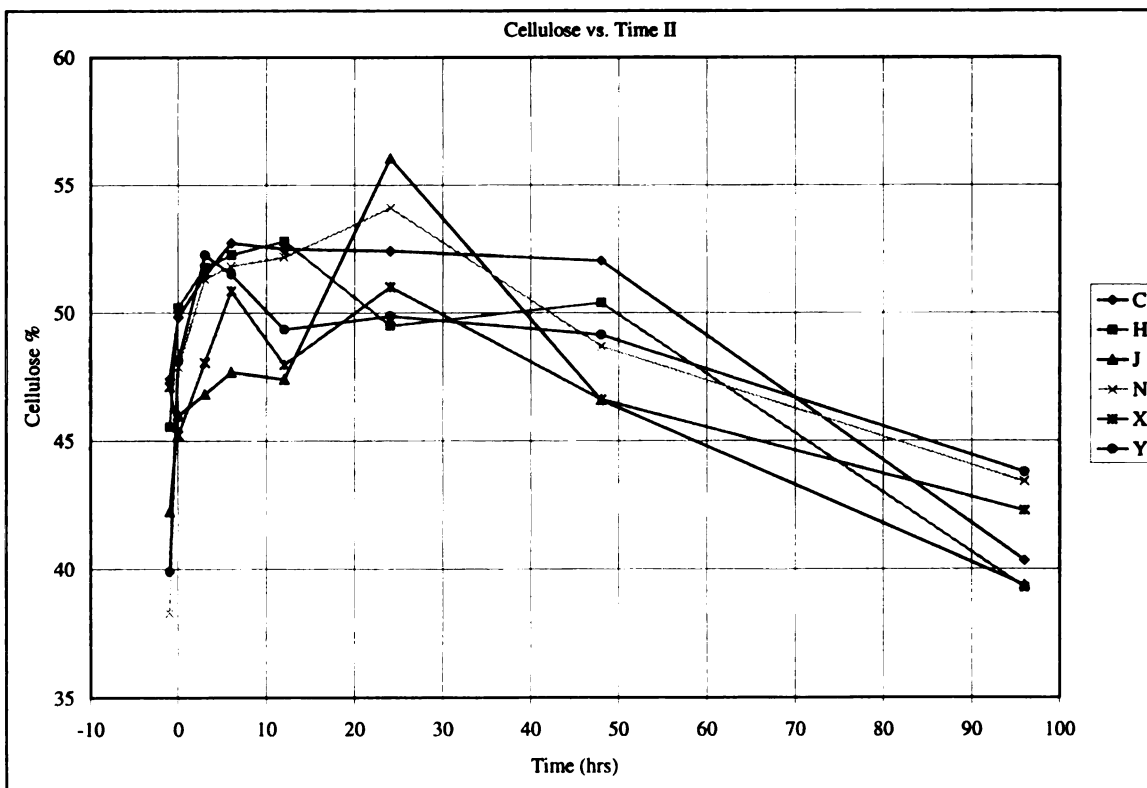


Figure 6-7 - Cellulose vs. Time, Trial 2

CHAPTER 7

Process Improvements

While the process under study did produce promising results, there were several aspects of the process that need improvement in future work. Many of the improvements need to be made before the operation could be performed on a production scale.

Perhaps the main problem that must be addressed is how to contain the ammonia. The only way this will be practically possible is by forming a biomass plug that minimizes the amount of ammonia backflow. This is important for two primary reasons. First is operator safety. The less ammonia released, the safer the operation will be. Second is process economics. If ammonia is being released into the atmosphere, it is unavailable for recycling and recompression. This means that the ammonia will have to be replaced more often which will drive up the operating cost. Environmental regulations will not allow for this discharge either.

There are two obvious solutions to this problem. First, the place in which the operation occurs can be ventilated to a separation system. Given that ammonia will already be separated from the product and reused, it may be possible to combine the two ammonia streams. However, due to the large amount of air required to properly ventilate a large facility, this option has a drawback in that the cost of the separation system will likely increase. The increased flow of gas through the system will necessarily increase the capital and operating costs of the system. The separation system will be required to

process a larger volume of gas, and all the associated conduits that will need to be constructed of appropriate materials which may cost more. It is also likely that the separation efficiency will decrease with the increased flow. Obviously, a ventilation system will have to be implemented, but if the ammonia can be contained at the source, the cost of the ventilation system can be kept to a minimum.

The second option to decrease the ammonia release is to prevent loss from the extruder. This can be facilitated by altering the screw configuration and the injection point of ammonia. An extruder with a larger L/D will allow for a large mixing zone after the biomass feed, and given the large number of agitator components available, numerous combinations can be attempted to achieve the desired result. Furthermore, most newer extruders come in modules. Thus, they have several interchangeable barrel sections that will allow for the ammonia injection point to be moved to many positions along the length of the barrel. By experimenting with the screw configuration and injection point, ammonia loss can be minimized while achieving a high degree of mixing and a large residence time.

Another important modification that must be made is to the biomass feed mechanism. This work was done using a twin screw feeder that dropped the biomass into the feed port by gravity. While this worked, it was far from optimum. If the feed funnel became moist for any reason (ammonia backflow, biomass falling on the funnel, cooling water flow through the barrel, etc.), the biomass would tend to stick to the moist spots until the funnel was completely plugged. This was usually watched and avoided by constantly forcing the biomass into the screw flights manually, but this is not a practical solution for a pilot or production scale process. Another consequence of this style of

feeding was that the feed screw flights did not run at capacity. When the biomass was mixed with water, it resulted in a moist puffy mixture. This material would not pack effectively into the screw flight and thus the process operated at less than optimum capacity.

The best solution to this problem would be to implement a constant pressure solids feeder. This would force the biomass into the extruder and help pack the screw flights full. This would probably help minimize ammonia losses as well. By filling the screws, a more effective plug would be created. Also, if the biomass feed were “piped” directly into the extruder with no opening at the feed port, this would provide a further restriction to ammonia loss, not to mention the conduit full of biomass carrying the material from the feeder to the extruder. Feeders of this type are available commercially. Some are equipped with flexible augers that would allow the entire system configuration to be modified if necessary.

A collection/separation drum will also be necessary. A preliminary design was created, but never implemented due to the small scale. The drum constructed consisted of a large barrel, which could be connected to the extruder outlet with a flexible ammonia resistant tube. Another tube was led out of the drum to the ventilation system. While the drum was never used in processing, it was used several times to contain ammonia vapors being released while drying samples. The connection intended for the extruder was simply left open to the atmosphere. A similar drum will be necessary to contain the explosions obtained in a pilot scale operation. The main consideration is how to direct the biomass mixture into the drum without creating a clog. This will depend on several factors, most notably the angle at which the material leaves the extruder, but also product

temperature, moisture and ammonia content, pressure generated, etc. If the material comes out at a downward angle, it can be channeled the same direction with a deflector plate. If the material is directed out of the extruder horizontally, it may be advantageous to implement a blower and a hydrocyclone immediately afterwards to begin the separation process. Once an actual process is begun, the best design will need to be evaluated.

Other process modifications that should be considered are means of breaking up the sample after discharge from the machine. Because of the compaction in the die, the material tends to form large pellets. When the material is discharged from the extruder, the pellets are still wet and soft and are easily broken up into a material similar to the feed biomass. However, if left as pellets, the material becomes very hard upon drying. They can be softened again when immersed in water, but the material tends to remain clumped together. Depending on the ultimate fate of the material, this may or may not be a problem.

Further Research

While the results of this investigation provided very encouraging results, they were far from optimized. Several of the factors involved need to be further investigated on a newer, more powerful machine.

First, different ammonia treatments need to be more fully investigated. As mentioned previously, much of the ammonia injected into the system was lost through the biomass feed port. Newer extruders come with a much larger variety of adjustable screw profile units. These could be implemented to create a much more effective restriction to hold ammonia inside the extruder, resulting in a much more effective treatment.

Additionally, a machine with a larger L/D would have more barrel length available to create a biomass plug and more barrel length for equilibration and reaction.

Investigating the effect of moisture level is necessary as well. This parameter was investigated somewhat but with few clear results due to system constraints. Too much water in the biomass caused the feed port to plug and too little did not supply any explosions. A larger machine, with a more effective mixing zone, would contain the liquid ammonia much better. This would lead to increased equilibration, which might generate explosions at lower moisture levels. Due to the high solubility of ammonia in water, a higher moisture level may lead to more rapid and effective equilibration. On the other hand, the problems observed with higher moisture levels may be difficult to alleviate under the current configuration. Biomass will always be compacted in the die block and water will be squeezed out at that point as well. However, increased optimization of the operating parameters may minimize this effect.

Another parameter that needs to be investigated more fully is the treatment temperature. Again, the small size of the extruder used prevented a full study on this parameter. The maximum temperature which gave explosions was 65°C , while the most effective treatments seemed to occur at lower temperatures. This does not correspond with the data produced in the batch reactor and may depend on the amount of ammonia escaping or mechanical energy put into the system. Different temperature profiles could be explored as well.

A fourth parameter to study would be the effect of varying die sizes and designs. Only one die was used effectively in this research and it provided a contraction of 40% of the flow area. The explosions obtained were certainly violent, with observed pressures as

high as 300 psig. However, a larger contraction of area would provide a further restriction to flow and may generate a more powerful and effective explosion. Additionally, less ammonia may be required for an effective treatment if a significant amount of “mechanical” pressure can be generated. The outlet restriction may be balanced with the ammonia loss as well. If the die provides too effective a restriction, vaporizing ammonia will take the path of least resistance which would probably be back to the feed port. Another design alternative would be to provide the contraction followed by a length of non-constrictive flow.

There are still other parameters that can be considered. The screw speed of an extruder can be varied over a wide range. The biomass itself could be milled to several different particle sizes. Obviously, different plant materials could also be investigated.

Scale-Up

To scale this process up, several considerations besides those already given must be examined. First is the type of extruder. Second, a separation and ammonia recompression system must be implemented. How will this operate? What type of pump should be used? What other process variables must be considered?

First, a twin screw extruder is the most logical choice of equipment. They provide superior mixing, using less power to provide a higher throughput, and are suitable for a wide variety of tasks. A single screw extruder¹ could be used with a lower capital cost and some of the same features, but the twin screw machine provides better overall processing capabilities. Some extruders (single and twin screw) are equipped with

¹ It was attempted to run the biomass through a single screw extruder, but this test failed.

degassing capabilities. These may warrant investigation, but due to the explosive nature of the process, it would seem that these units would plug easily. However, if the process were optimized such that the explosions were more continuous and controlled, this may prove an effective accessory.

Second, the best type of pump is probably a diaphragm metering pump. This is the type of pump used in this research. These pumps provide excellent control over flow and have a wide range of flowrates that can be accurately metered. They tend to have a higher capital cost than other types of pumps, but provide exacting accuracy in delivery volume, continuously linear variability in stroke length, and are virtually leak free. Another nice feature of these pumps is that they are able to handle gases. They are not intended to pump gases, but they will not burn up if cavitation occurs.

Another consideration for pilot or process scale operation is an ammonia detection system. These are commercially available and can detect very low concentrations of the gas. Several detector types are in common use², the most practical being an electrochemical (diffusion type) detector (18). While these are not completely selective for ammonia³, they offer rapid response, a higher selectivity than solid state detectors, and a lower price than more advanced types of detectors (18).

Several materials of construction are available for process piping and other pieces of equipment. Aluminum, carbon steel, and 316 SS offer corrosion resistance to anhydrous ammonia of less than 0.002" per year (16) at temperatures below 200°C. Hastelloy and 304 or 307 SS all show corrosion of less than 0.02" per year for the same

temperatures. If ammonium hydroxide or ammonia gas is used, Hastelloy or 316 SS seem to offer the best corrosion resistance to all conditions.

Another major factor that must be considered is a separation system. Because this may be a relatively open system, the separation will involve ammonia, water, and air. The ammonia can be absorbed into water and then the solution can be distilled to remove the ammonia. Several of these systems are available commercially.

Scale-Up Calculations

To have an economically viable process, a large throughput must be available. Using the equations given in the extruder manual, plus some general considerations in other texts, scale-up factors have been determined. Since all of the work done in extruder scale-up pertains to food or polymer processing, these factors can only be considered approximate⁴. The appropriate laboratory trials should be done to supplement and verify the calculations. All calculations are based on a desired throughput of 300 lb/hr.

Typically, the scale-up of a twin screw extruder is done on geometric terms (11). If two extruders are the same in all respects except the screw diameter⁵, a factor equal to the cube of the ratio of screw diameters estimates the throughput of the larger diameter machine (14). To estimate scale changes with different screw speeds or motor sizes, another important factor is required, the specific mechanical energy of the material. This is calculated as

² Thompson (18) discusses installation of an ammonia detection system and gives a good overview of the variables involved, maintenance and calibration suggestions, etc.

³ According to Thompson, organic compounds such as amines can give false alarms.

⁴ Interestingly, very little work has been done in scale-up considerations for twin screw extruders, most of the information available is for single screw machines.

⁵ This includes screw profile, screw speed, agitator clearance, etc.

$$SME = \frac{Gross\ HP - No\ Load\ HP}{Throughput}$$

Equation 7-1 - Specific Mechanical Energy

where Gross HP is calculated as

$$Gross\ HP = Torque\ Fraction * RPM * \frac{Motor\ Rating}{Rating\ RPM}$$

Equation 7-2 - Gross Horsepower

and No Load HP is calculated the same way using the Torque Fraction observed with no material in the instrument. The extruder used had a No Load Torque Fraction of 0.11. The motor was rated at 3 HP at 500 RPM, and the screw speed used was 100 RPM. This gave a No Load HP of 0.066 HP. Similarly, since the consistently maximum observed Torque Fraction was 0.50, the Gross HP was 0.3 HP. Based on the maximum practical throughput of 14 dry g/min material (35 wet g/min) which corresponds to 1.85 dry lb/hr (4.63 wet lb/hr), the SME was 0.13 HP•hr/lb for dry biomass. Theoretically, the maximum output of this extruder at 100 RPM would be

$$\dot{m}_{max} = \frac{1.0 * 100\ RPM * \frac{3\ HP}{500\ RPM} - 0.066\ HP}{0.13\ HP \cdot hr / lb} = 4.1\ lb / hr$$

Note that a higher throughput is possible on the machine by increasing the screw speed, but the residence time, and hence the treatment time, will decrease. Furthermore, changing the screw speed will change the SME.

If the SME is assumed relatively constant⁶ at higher screw speeds, a new throughput can be estimated. Since extruder motors are typically constant torque, the screw speed is directly related to torque in a linear fashion. For example, on the extruder used, 100% torque is observed at 3 HP at 500 RPM. Similarly, 100% torque is observed at 1.5 HP and 250 RPM. In other words, the ratio of HP to Screw Speed at a Torque Fraction of 1.0 is constant for all operational screw speeds. That said, the new throughput can easily be estimated by adjusting the operating screw speed in both the Gross and No Load Torque calculation. For example, at a Torque Fraction of 1.0 at 250 RPM, the throughput would be

$$\dot{m} = \frac{1.0 * 250 \text{ RPM} * \frac{3 \text{ HP}}{500 \text{ RPM}} - 0.1 * 250 \text{ RPM} * \frac{3 \text{ HP}}{500 \text{ RPM}}}{0.13 \text{ HP} \cdot \text{hr} / \text{lb}} = 10.4 \text{ lb} / \text{hr}$$

on the same extruder assuming a No Load Torque Fraction of 0.1.

To go to a larger machine, the desired throughput is ratioed with the maximum obtainable throughput⁷ at a screw speed⁸ of 100, calculated as 4.11 lb/hr, to obtain a scale up factor of 73. The cube root is taken to give the ratio of screw diameters necessary for this scale-up, and then multiplied by the current screw diameter to give the estimated screw diameter of the new machine. These values are 4.2 and 125 mm respectively. Thus, an extruder with a screw diameter of 125 mm would be necessary to achieve 300 lb/hr of throughput for the same size motor at 100 RPM.

⁶ The SME will be assumed constant for this material for all further calculations.

⁷ Maximum attainable throughput is used in anticipation of the improved performance of the new extruder and treatment system.

⁸ This screw speed is selected as it was used throughout all of the trials. As illustrated earlier, the throughput produced by any screw speed can be estimated and scaled as well.

Many extruders manufactured today use much higher powered motors than 3 HP⁹.

Power ratings of 50 HP are not uncommon on machines with 30 mm screws, and much larger motors are available for larger diameter machines. For example, assume a motor rated at 50 HP at 500 rpm was used on the current extruder. If the screw speed (100 RPM) and Fractional Torque (1.0) remain the same, the machine would be expected to have a maximum throughput of 69 lb/hr if the No Load Torque is assumed to be 0.1.

$$\dot{m} = \frac{(1.0 - 0.1) * 100 \text{ RPM} * \frac{50 \text{ HP}}{500 \text{ RPM}}}{0.13 \text{ HP} \cdot \text{hr} / \text{lb}} = 69 \text{ lb} / \text{hr}$$

Thus, an extruder with the same size motor would only need a screw diameter of 49 mm to achieve the desired output.

$$\left(\frac{300 \text{ lb} / \text{hr}}{69 \text{ lb} / \text{hr}} \right)^{1/3} * 30 \text{ mm} = 49 \text{ mm}$$

In general, the estimated screw diameter required to process 300 lb/hr of biomass in an extruder operating at S RPM and 1.0 Torque Fraction with motor size P at N RPM and No Load Torque Fraction of 0.1 is given by

$$D = \left(\frac{300 \text{ lb} / \text{hr} * 0.13 \text{ HP} \cdot \text{hr} / \text{lb}}{P \text{ HP}} * \frac{N}{S} * 0.9 \right)^{1/3} * 30 \text{ mm}$$

Equation 7-3 - Estimated Screw Size

Again, these numbers must be examined with actual laboratory experimentation.

They are meant more as a general guide rather than an absolute reference. The SME of

⁹ This fact causes some concern for accurate scaling. All of the vendors contacted expressed some concern about the age of the machine and the applicability of the data obtained to scale-up.

the material is dependent on screw speed, agitator configuration, temperature profiles, etc. and must be verified with actual laboratory data. In gathering information for scaling up, it was suggested that the material be run in a newer machine to obtain more accurate scaling information. This was attempted, but failed. Due to safety, ammonia could not have been used in the trial, and any data obtained would only be acceptable for scaling up the extrusion of the biomass alone. Furthermore, the attempt itself failed due to a persistently clogged feed port.

While these numbers are not exact, they do provide a basis for scaling up and a typical extruder can be priced out. B & P Process Equipment quoted a 50 mm extruder with a 50 HP motor as \$278,000. This machine has an L/D of 25 and can be extended up to 40 L/D for \$24,000 per 5 L/D. A comparable machine from the Berstorff Corporation is quoted at \$220,000. It has an L/D of 48, 44 mm screws, and a 73 HP motor.

Larger extruders are available as well. Since the larger machines typically come with larger motors, using the calculations method above, many of these machines have extremely high throughputs available. This illustrates why a pilot scale trial with one of the companies is so important. The age of the machine and the nature of the biomass mixture prevent the scale-up from being typical. A full scale pilot trial would provide a wealth of information that would help determine the proper size extruder and motor. The only foreseeable problem with a pilot plant trial would be preparing the site for use with ammonia. Significant modifications were made to the laboratory used in this research to ensure safety. Similar modifications may have to be made if ammonia is to be used in the pilot testing.

In addition to purchasing the extruder, a site check must be done to ensure that the site can accommodate the weight of the instrument. Proper utilities for all process equipment will need to be installed as well. Training on the instrument may be required. Spare agitator shafts and parts will be necessary as well. A biomass feed system, an ammonia pump, at least one separation system will be required for solid vapor separation, and at least one separation system for ammonia recovery will be necessary.

CHAPTER 8

Conclusions

As the cited results indicate, an extruder can be used to facilitate the AFEX process. The total sugar yield from enzymatic digestibility of the corn fodder has been increased up to 250%, and the *in situ* ruminant digestibility has increased 32% (from 53.8% digestible to 71.2%) over the completely untreated 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% digestible to 77.4%) over the material that was extruded with no ammonia. In the trial that provided a direct comparison between the batch and extrusion processes, the extrusion process gave results that compare well with the batch process (recall the batch samples were slightly damaged). This leads to the conclusion that the extrusion process can be made as effective as the batch process has proven to be.

Other results of the trials are encouraging as well. Fiber splitting has clearly been accomplished. A reduction in apparent lignin is also desired and has been achieved with an average decrease of 11.9% (maximum reduction of 20.9% from 8.42% to 6.66%) from the material extruded with no ammonia and the completely untreated material. A high rate of *in situ* digestion has been observed as well. The highest rates of digestion between the 3 and 6 hour points were 2.2 times the rate of digestion experienced by the control used in each trial. Finally, the reduction of cellulose and hemicellulose in the extrusion

treated material observed during the trials, coupled with the increase in lignin and ash content, implies utilization of these constituents.

These facts imply that the extrusion AFEX process can be used to effectively pretreat biomass samples to increase enzymatic and *in situ* ruminant digestibility. While more research is necessary to optimize the process, it is readily apparent that this method can be used as an effective pretreatment technique for biomass hydrolysis.

APPENDICES

APPENDIX A

EQUIPMENT PHOTOGRAPHS

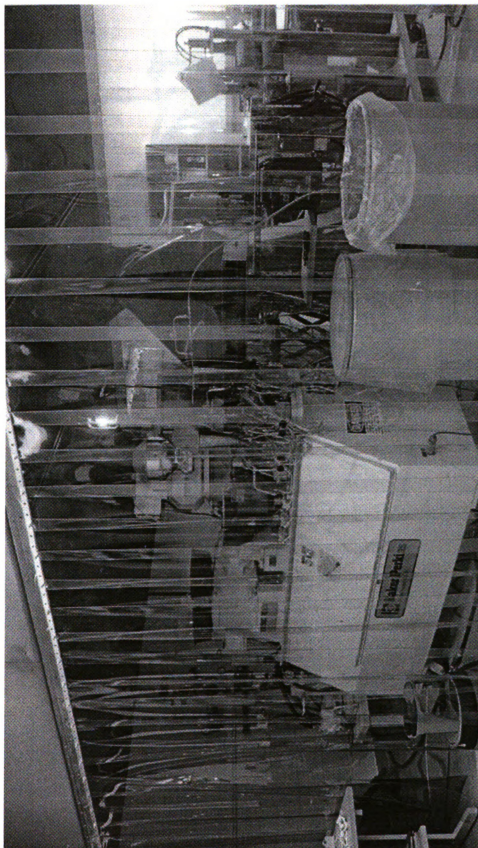


Figure A-1 - Extrusion Laboratory Photograph

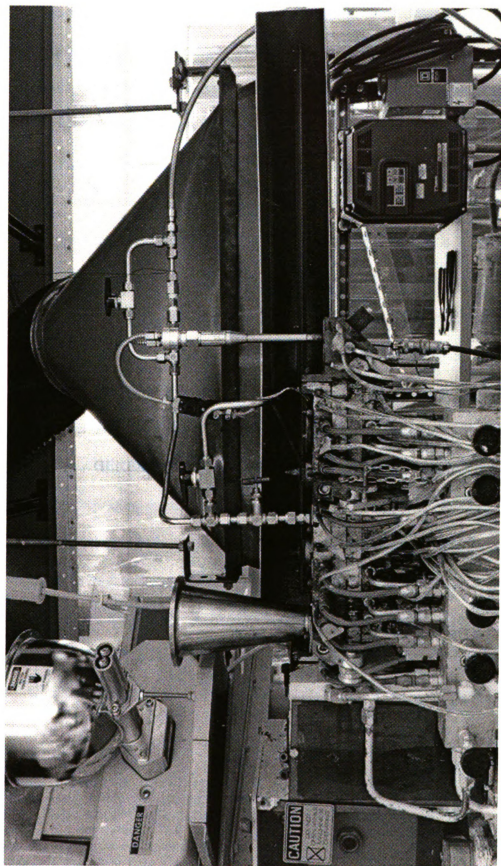


Figure A-2 - Extruder Setup Photograph

APPENDIX B

GENERAL PROCEDURES FOR EXTRUSION AFEX

Once all of the piping is connected, all valves are closed. While wearing the full face respirator, the ammonia cylinder is cracked open to fill the first section of tubing with ammonia. This section is then checked for leaks by puffing the vapor over a 38% (wt) solution of hydrochloric acid onto tubing connections. If no white fog¹ is seen, the connection can be tested by carefully smelling for ammonia. This is best done by standing at arms length from the connection and “waving” the vapor in the area towards oneself. If no ammonia is detected, one can move slightly closer until it is obvious no ammonia is escaping. If no ammonia is detected, the valve is opened and the next section of tubing is checked as illustrated until the entire tubing system is deemed safe.

Once the tubing is leak free, the outlet valves are shut and the cylinder can be opened fully. A slow flow of ammonia can be run through the vent line² to prime the ammonia pump. Calculations will have been done previously to determine the flow rate of ammonia and biomass necessary to achieve the desired treatment. The pump is set to operate at the desired stroke length and speed, and the biomass feed is set similarly.

Once the extruder has reached the desired operating temperature, the ammonia and biomass flows are started. As the process equilibrates, it may sometimes be necessary to adjust the flow of ammonia or biomass to meet system constraints³.

When processing, the feed port must be continually watched so that it does not plug. Ammonia flow into the machine is evidenced by fogging at the biomass feed port and is audible.

¹ A white precipitate, ammonium chloride, forms if ammonia is present.

² A vent/purge line into the hood is extremely important when working with ammonia so that the excess material can be safely removed. Also, if a check valve is implemented, a bypass line around the check valve is important for the same reason.

³ For example, biomass feed rate may need to be reduced if the feed port continually plugs.

When experimentation is complete, the ammonia cylinder is shut off and the injection port valve is closed. Ammonia in the lines is bled out through the bypass valve and the purge line. Once all of the liquid ammonia has left the tubing, the tubing is disconnected from the cylinder and blown out with air, making sure that all lines are thoroughly vented.

Treated material is allowed to air dry several days under the hood, and is then stored in plastic bags or bottles.

APPENDIX C
PROCESS FLOW DIAGRAM

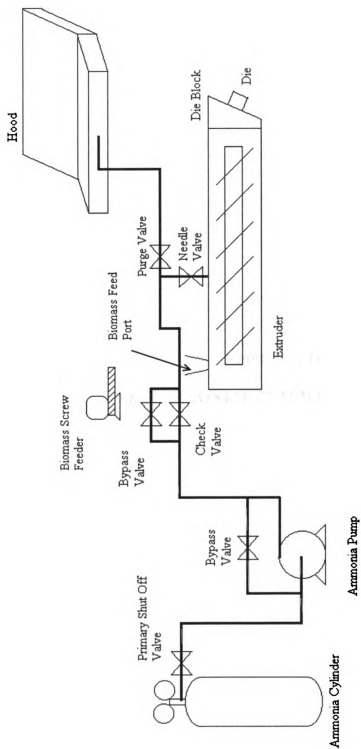


Figure C-1 - Process Flow Diagram

APPENDIX D
VENDOR CONTACTS AND INFORMATION

Extrusion Equipment

American Leistritz Extruder Corporation
169 Meister Avenue
Somerville, NJ 08876
(908) 685-2333
Charlie Martin - National Sales Manager

B & P Process Equipment and Systems
1000 Hess Street
Saginaw, MI 48601
(517) 757-1300
Peter M. Giles - Sales Manager

Berstorff Corporation
8200 Arrowridge Boulevard
P.O. Box 240357
Charlotte, NC 28224
(704) 523-4353
Jeff Karigan - Field Sales Manager

Werner and Pfleiderer Corporation
663 East Crescent Avenue
Ramsey, NJ 07446
(201) 327-6300
William L. Novak - Sales Engineer

Solids Feeding Equipment

Flexicon Corporation
1375 Stryker's Road
P.O. Box 5269
Phillipsburg, NJ 08865
(908) 859-4700

K-Tron America
Routes 55 & 553
P.O. Box 888
Pitman, NJ 08071-0888
(609) 589-0500

Acrison, Inc.
20 Empire Blvd.
Moonachie, NJ, 07074
(201) 440-8300

Ammonia Distillation

**Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201
(614) 424-6424**

**Organics Limited
The Barclay Centre
University of Warwick
Science Park, Coventry, CV4 7EZ**

APPENDIX E
HPLC METHOD

The carbohydrate system consisted of an Interaction Chromatography Inc. (San Jose, CA) lead-form ion-evaluation column (CHO-682, 300 x 7.8 mm), with a guard column and operated at 90°C. The eluent was degassed Milli-Q water with a helium sparge. The flow rate was 0.4 mL/min. Analysis time was 75 minutes per sample. The detector was a Waters (Waters Chromotography Division, Milford, MA) refractive index detector with an internal temperature of 35°C. All data was collected and processed with Turbochrom (PE Nelson, Cupertino, CA) analytical software.

REFERENCES

REFERENCES

1. Aldrich Chemical Company, Material Safety Data Sheet for Anhydrous Ammonia, Milwaukee, 1996.
2. Burditt, L., Breeds of Livestock - Santa Gertrudis Cattle, [Online] Available <http://www.ansi.okstate.edu/breeds/cattle/santgert/>, April 28, 1998.
3. Carr, M. and Doane, W., *Biotechnology and Bioengineering*, 26, pgs. 1252-1257, 1984.
4. Carr, M. and Doane, W., *6th Symposium on Biotechnology for Fuels and Chemicals*, "Pretreatment of Wheat Straw in a Twin-Screw Compounder", John Wiley and Sons, Chichester, 1984, pgs. 187-195.
5. Dale, B. and Moreira, M., *4th Symposium on Biotechnology for Fuels and Chemicals*, "A Freeze-Explosion Technique for Increasing Cellulose Hydrolysis", No. 12, John Wiley and Sons, 1982, pgs. 31-43.
6. Davis, D., *Prevention Reference Manual: Chemical Specific, Volume 4: Control of Accidental Releases of Ammonia (SCAQMD)*, U.S. Environmental Protection Agency, Research Triangle Park, 1987.
7. Ensminger, M., *Feeds and Nutrition*, Ensminger Publishing Company, Clovis, 1990.
8. Erdemir, M., Edwards, R., and McCarthy, K., *Food Science and Technology*, 25, pgs. 502-508, 1992.
9. Gould, J. and Jasberg, B., *Biotechnology and Bioengineering*, 33, John Wiley and Sons, Chichester, pgs. 233-236, 1987.
10. Kirk-Othmer, *Encyclopedia of Chemical Technology*, 4th Edition, John Wiley and Sons, New York, 1992.
11. Kokini, J., Ho, C., and Karwe, M., *Food Extrusion Science and Technology*, pg. 465-472, Marcel Dekker, New York, 1992.
12. Larson, W.E., *Effects of Tillage and Crop Residue Removal on Erosion, Runoff, and Plant Nutrients*, Soil Conservation Society of America, Special Publication No. 25, "Crop Residues: Energy Production or Erosion Control?", 1979, pgs. 4-6.
13. Michaeli, W., *Extrusion Dies : Design and Engineering Computations*, MacMillan, New York, 1984.
14. MPC/V-30 Manual, Baker Perkins Inc., Saginaw.
15. National Safety Council Data Sheet on Anhydrous Ammonia, National Safety Council, Chicago, 1979.
16. Schweitzer, P.A., *Corrosion Resistance Tables: 2nd Edition*, Marcel Dekker Inc., New York, 1986.

17. Sperling, D., *Alternative Transportation Fuels*, Quorum Books, New York, 1989.
18. Thompson, J. and Sekula, L., *Ammonia Plant and Related Facilities Safety: Volume 32*, "Successful Application of Ammonia Detectors", pgs. 77-85, Technical Manual Published by The American Institute of Chemical Engineers, New York, 1992.
19. Van Soest, P., Robertson, J., and Lewis, B., *Journal of Dairy Science*, 74, pg. 3583-3597, 1991.
20. Wereko-Brobby, Charles Y. and Hagen, Essel B., *Biomass Conversion and Technology*, John Wiley and Sons, Chichester, 1996
21. Wyman, Charles E., *Handbook on Bioethanol: Production and Utilization*, Taylor and Francis, Washington DC, 1996.
22. Yeh, A. and Hwang, S., *International Journal of Food Science and Technology*, 27, pgs. 557-563, 1992.

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