SUPPLEMENTARY PDF FILE FOR

Ammonia Fiber Expansion (AFEX) Pretreatment of Lignocellulosic Biomass

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SUMMARY OF SUPPLEMENTAL DOCUMENT CONTENTS:

Here, we present here additional supplementary protocols for conducting AFEX pretreatment of corn stover at the lab-scale using a 0.6-liter or 5-gallon Parr reactors by either delivering the anhydrous liquid ammonia to the reactor gravimetrically or volumetrically using a pump, respectively. In addition, we have provided a rough outline of the protocol used to conduct packed bed AFEX using MBI's custom-built, commercial scale, 1-ton AFEX reactor system with integrated ammonia recovery. Bench-scale or lab-scale AFEX (using either the 5 gallon or 0.6 liter Parr reactors) was carried out at 1.0 g ammonia per g dry biomass, 0.6 g water per g dry biomass, for 30 min at 100 ± 5 °C. Pilot-scale packed bed AFEX was carried out at 0.6:1 ammonia-to-biomass loading, 60% moisture loading, for 30 min at 100 ± 5 °C based on packed bed AFEX method outlined in detail elsewhere.[1]

Our goal is to provide supplemental operating procedures for the safe and consistent operation of pressurized reactors for performing AFEX pretreatment on cellulosic biomass like corn stover. These supplemental protocols can be used to scale-up the AFEX process using larger Parr-type stirred stainless-steel reactors based on user requirements.

SUPPLEMENTAL PROTOCOL 1 TO PERFORM AFEX USING 5-GALLON PARR REACTOR WITH AMMONIA DELIVERY USING PUMP:

PROTOCOL STEPS:

1. Adjusting Biomass Moisture Content

1.1 See supplemental **Table S1** below outlining all major equipment and materials necessary to perform bench or lab scale AFEX pretreatment.

1.2 Estimate total moisture content of biomass using a moisture analyzer, or an oven set at 103 °C. The moisture content of the biomass used here was estimated to be 10% (on total weight basis).

1.3 Combine 750 g of total weight biomass (or 675 g of dry weight of biomass) and water in the amounts according to the desired AFEX pretreatment conditions in a plastic container, mix well using spatula or by hand wearing nitrile gloves. Here, to achieve final desired moisture content of 40% on total weight basis, add 195 g of water to the original biomass.

2. Pretreatment Reactor and Auxiliary Equipment Setup

2.1 Make sure that the following equipment is securely plugged in by careful visible inspection: Lewa pump skid, Parr controller, and Julabo water bath (Set temperature to 4 °C). Check that the fume hood ventilation system is turned on. Directly underneath the pump head, the inlet side of the pump, fill the plastic pail reservoir with crushed ice. Do not run pump until the temperature gauge reads 40 °C or below.

Table S1 Major equipment and materials necessary to perform AFEX benchscale pretreatment using 5 gallon Parr reactor with volumetric ammonia delivery capabilities

Name of Materials/Equipment	Company	Catalog Number/s	Comments/Description
5 Gallon Pressure Reactor	Parr Instrument Company (Moline, IL)	NS4557	5 Gallon high pressure mixing reactor made with 316 Stainless Steel, equipped with an ammonia inlet valve and an exhaust valve. Maximum pressure of the vessel is 1900 psig at 225 °C.
Reactor PID Controller	Parr Instrument Company (Moline, IL)	4843	Temperature and mixing speed controller.
Respirator with filters	Northern Safety Co., Inc. (Utica, NY)	161706 and 3943	Full-face respirator fitted with unexpired methylamine filter to prevent exposure to anhydrous ammonia
Diaphragm Pump	American Lewa, Inc. (Rochester, NY)	EKM5-1-20MM	Diaphragm pump for ammonia delivery. Power: 0.5 HP; RPM: 1730 min-1; Diaphragm material: PTFE.
Julabo recirculating chiller/water bath	Julabo GmbH (Allentown, PA)	CD-200F	We need a recirculating chiller water bath at 4 C to keep ammonia pump skids cooled down.
Portable single gas direct readout ammonia monitor	Honeywell Analytics Ltd. (Lincolnshire, IL)	ToxiPro 544521VD Single Gas Polycarbonate Ammonia (NH3) Detector monitors	For detection of ammonia concentration in lab to prevent accidental exposure
Coriolis Mass Flow Measuring System	Endress + Hauser (Chalfont, PA)	Model Proline Promass 80	Mass flow meter for liquid ammonia.
Microprocessor Control	Kessler-Ellis Products Co. (Eatontown, NJ)	Batch Control II - BT 28A3AZE Ver. 8.8	Liquid ammonia mass flow controller.
Back Pressure Regulator	Tescom Corporation (RJM Sales, Scotch Plains, NJ)	26-2300 Series - 26-2363-24	Back pressure regulator installed downstream of the mass flow meter. Operation range: 10-250 psig. Body: Stainless Steel. Seals: Teflon.
Corn Stover	National Institute of Standards and Technology	Standard Reference Material RM 8412	Cellulosic biomass feedstock that needs to be pretreated. Corn stover can be procured from other sources if not available at NIST.
Nalgene Plastic Utility Boxes	VWR	36212-361	To be used for mixing biomass with water
Heat protective gloves	VWR	75836-506	Kevlar® Nomex® heat resistant gloves protect up to 260°C during handling of hot reactor vessel
Cold protective gloves	VWR	89217-722	Keep hands warm and dry in temperatures as low as –31°C when handling liquid ammonia
Crushed ice	-	-	Need crushed ice to keep Lewa pump skids cool
Earplugs	VWR	55533-030	Ear plugs to protect hearing when releasing ammonia at end of pretreatment
Spatulas with PVC Handles	VWR	82027-516	Large spatula to mix and handle biomass
Crescent and open wrench	VWR	62510-100	Suitable tool kit to open/close Parr reactor etc
Distilled water	-	-	To mix into biomass to adjust moisture content

2.2 Open the utility cold water valve to the reactor internal heat transfer coils and verify that water is flowing through the reactor and out to the drain. Put the reactor in the carrier. Align the reactor base with an agitator and raise the reactor base by pressing up on the control stick until there is a four-inch gap between the upper and lower part of the reactor bomb.

2.3 Check that the O-ring located in the top part of the reactor is free of debris and thinly coated with vacuum grease. Reapply grease, if needed, to ensure reactor lid seals properly.

3. Loading Biomass into Pretreatment Reactor

3.1 Add the moist and well-mixed biomass into the reactor. Once the biomass has been added, raise the reactor base until the bottom of the reactor is sealed with the top of the reactor.

3.2 When the reactor lid is seated against the O-ring, place the collar around the Parr reactor, and seal the reactor using a wrench. Use the rotational control stick to rotate the vessel from the vertical position to the horizontal position.

3.3 Ensure that all of the following manual valves on the reactor head or vessel are closed to begin with (based on reactor head design): (a) for ammonia release after run is completed, (b) for loading ammonia into the reactor, (c) for loading nitrogen into the reactor, and (d) for removing contents from the underside of the reaction vessel.

3.4 Connect the nitrogen tank line into the nitrogen inlet. Open the nitrogen tank valve. Charge the vessel with high pressure regulated nitrogen to 65 psi or 4.4×10^5 Pa to prevent the lines from freezing during addition of ammonia. Open the manual valve connecting the tank to reactor. Adjust regulator until the desired pressure has been reached; then close the valve and disconnect. Close the nitrogen tank valve.

3.5 Open the valve located directly on top of the ammonia tank and the ammonia outlet/inlet valves to the reactor. Check all fittings for tightness by visual inspection.

3.6 Record the temperature and pressure readings prior to heating. Preheat the mantle with the set point at 105 °C, on full power for 30 min prior to addition of ammonia to reactor. If step is skipped, the targeted temperature during pretreatment after ammonia addition will likely not be achieved.

3.7 Turn on and off the mantle power (manually or using temperature controller if available) depending on the temperature during the course of the process.

3.8 Turn on the Parr controller display and heater. Press button I and press the Reset button on the back of the controller. Set heater to desired mantle temperature, raise mantle, and begin preheating.

Table S2 Lewa pump settings to be used to transfer desired ammonia to AFEX reactor

Desired Ammonia Loading (g)	Pump Setting
100	0.22
150	0.33
200	0.44
250	0.55
300	0.66
350	0.77
400	0.88
450	0.99
500	1.1
600	1.32
700	1.54
800	1.76

4. Loading Ammonia into Pretreatment Reactor

4.1 For addition of liquid ammonia into the reactor, use a pre-calibrated Lewa pump skid. See supplementary **Table S2** outlining details on pump settings to be used to transfer desired ammonia. The amount of ammonia to be added will depend on the AFEX pretreatment process conditions to be tested.

4.2 To program the pump skid equipment; first press the menu button on the pump display to PRESET option and then press enter. Next, enter the desired amount of ammonia to be batched (in grams) and press enter again.

4.3 If the PREWARN setting is equal to or greater than the PRESET, observe a display of PREWRONG indicating that the user must increase the PREWARN setting. To change this, press the menu button until it reads PREWARN, then press enter and adjust the PREWARN value so it is less than the preset value. Press enter.

4.4 To load ammonia into the reactor, turn the three-way valve towards the 5-gallon reactor (Valve midway down line from pump skid). To start the Run Mode, press “A” and then press the START button.

Note: When started, the pump turns on and the counter begins to calculate the amount of ammonia being administered in grams per min. When complete, the pump will automatically turn off and display the amount that was dispensed for that batch run.

4.5 To stop a batch, press “B”, to temporarily suspend the current batch process by de-energizing the PRESET and the PREWARN switches. Press start, “A”, to continue process.

4.5.1 To repeat a batch, press the CLR button then repeat step.

4.5.2 To change the batch size – with the current PRESET flashing on the display, type a new set point using the keypad. This number becomes the PRESET.

4.5.3 To display the batch total or rate – with the current PRESET flashing, press “ENT” to place the PRESET value in memory and use the “C” button to toggle between the batch total and the rate.

5. AFEX Pretreatment Operation & Biomass Recovery

5.1 After the desired amount of ammonia has been delivered, close the bonnet and needle valves on the ammonia line. Turn the agitator on by rocking the switch to the on position. Slowly ramp speed up to the second hash mark on the speed knob to set the desired agitation speed of 100 rpm (rotations per minute).

5.2 When the reactor is within 5 °C of the set point, start the reaction time. If unable to reach the target temperature within 5 min of the ammonia pump stopping, abort the run.

5.3 If the target temperature is obtained within the time criteria, record the temperature and pressure of the system. This is the initial temperature reading. Record the temperature and the pressure of the reactor every three min.

5.4 To regulate the temperature of the reactor, turn the mantle off and on, and raise and lower the mantle as needed during the run (if automatic temperature controller is not available). The following 'Quality Control Criteria' have been established based on target temperature for AFEX pretreatment. If after reaching the set point, the reactor temperature goes outside ± 10 °C from the set point, the experiment must be aborted. If the target temperature (within 5 °C) is not reached within 5 min after ammonia pumping, abort the experiment.

5.5 When the desired pretreatment reaction time is complete, set the agitation to zero and turn off the heater jacket.

5.6 To arrest the reaction, slowly open the ball valve to release the ammonia directly into the fume hood. Wear earplugs to prevent damage to hearing. To access the reactor inside a walk-in fume hood, wear a respirator.

5.7 Flush the reactor vessel with nitrogen to remove bound ammonia from the vessel before opening the reactor to recover the pretreated samples. Wait for sufficient period of time. Note that this time depends on reactor size. For example, a 5-gallon reactor will likely require waiting for 15-30 min to purge most of the ammonia from the vessel.

5.8 Before opening reactor, verify that the reactor is fully depressurized. Using thermo-protective gloves, as needed, loosen and remove the collar. Lower the reactor cylinder using the pneumatic control stick. While wearing a respirator, remove the biomass from the reactor cylinder inside the fume hood.

6. Reactor Shutdown and Cleanup

6.1 Clean out the reactor and lid.

6.2 Close all the ammonia valves. Turn off the Parr reactor controller. Turn off the pump skid controller. Turn off the cooler for the pump skid. Close the utility cold water valves.

REPRESENTATIVE RESULTS & DISCUSSION FOR SUPPLEMENTARY PROTOCOL 1:

Figure S1. Nine different steps involved in producing AFEX (or Ammonia Fiber Expansion) treated biomass in a lab-scale 5-gallon pressure reactor. 1) Weighing biomass; 2) Adding water; 3) Mixing water with biomass; 4) Loading biomass in the reactor; 5) Applying vacuum and loading ammonia; 6) Running the reaction; 7) Removing the reactor head; 8) Unloading the AFEX-treated biomass; 9) Bagging biomass once dry.

In **Figure S1**, the following nine steps are summarized. (1) Add the desired amount of biomass into a pre-weighed plastic tub. Details regarding moisture analysis of biomass are available online on the National Renewable Energy Laboratory (NREL, Golden, Colorado, USA) website highlighting commonly used laboratory analytical procedures for standard biomass analysis (https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html). The moisture content of the biomass used here was estimated to be 10% (on total weight basis). Corn stover stalks were finely milled using standard hammer/knife mill to pass through a 5 mm sized screen, as outlined elsewhere[2], prior to performing bench-scale AFEX. (2) The required amount of water has to be mixed with biomass to raise the moisture content. Based on the moisture loading needed, the required amount of water is mixed with biomass. Here, to achieve final desired moisture content of 40% on total weight basis, we add 195 g of water to 750 g of total weight biomass (or 675 g of dry weight of biomass). (3) The biomass is mixed well to evenly distribute the moisture. (4) Moist biomass is loaded immediately into the 5-gallon pressure reactor. (5) The reactor head is tightened to seal the reactor and vacuum is applied for few min to remove air from inside the reactor. Next, an appropriate amount of ammonia is loaded into the reactor using the pump skid. Here, we are looking to add 1 kg of ammonia per kg of dry weight biomass. Therefore, the pump setting was set to deliver 675 g of ammonia to the reactor. (6) The reactor is then tilted from vertical position to horizontal position and mixed using a motorized auger for even heating of biomass using heating jacket. The operator user can use vertical mixing if access is limited to upright positioned reactor. At the end of the reaction time, the pressure is rapidly released, and ammonia is vented inside a fume hood. (7) The reactor bolts are loosened, and the head is removed from the reactor base. (8) AFEX treated biomass is then transferred into a plastic tub and left inside the fume hood to remove residual traces of ammonia overnight. (9) Lastly, AFEX treated biomass dry (<10% moisture) is then packaged the next day into zip-lock bags for storage at room temperature (or deli fridge for long-term storage) until further use. Representative enzymatic hydrolysis results for the 5-gallon AFEX reactor treated corn stover are highlighted in Figure 4.

In order to operate this larger bench scale AFEX reactor equipment, there should always be two operators present at all times for sake of safety. Operators must wear safety glasses at all times when operating the reactor and ear protection (ear plugs or protective ear muffs) during venting. Operators must wear protective heat resistant gloves when opening the reactor following the reaction. Cold-protective gloves must be available for use in the event of liquid ammonia leaks. Operators must be certified for full-face portable respirators. These should be equipped with unexpired methylamine cartridges and worn when inside the walk-in hood and opening the reactor following a run or unloading biomass from the reactor. Any residual ammonia in the reactor should be allowed to evaporate inside a fume hood. Prior to entering the fume hood, the operator should insert the portable ammonia monitor behind the plastic screen to determine the atmospheric concentration of ammonia. At concentrations above 35 ppm (STEL or Short Term Exposure Limit which is the employee's 15 minute time-weighted average exposure which shall not be exceeded at any time during a work day), operators must wear a respirator when entering the hood. At concentrations above 50 ppm, operators should not enter the hood. In the event of exposure to anhydrous ammonia, the operator first evacuates the premises for access to fresh air and immediately flush the affected area with water for at least 15 min. In the event of a catastrophic failure in which ammonia levels exceed the Immediately Dangerous to Life/Health or IDHL (IDHL is maximum concentration of a substance from which one could escape within 30 min without escape-impairing symptoms or irreversible effects) of 300 ppm (or the upper limit of sensor detection) inside the main work area, operators must evacuate the room and not attempt to contain the leak.

Having positive pressure in the reactor after loading the biomass and sealing the reactor will hamper the ammonia loading. De-pressurizing the reactor by applying vacuum (or adding steam to wet the biomass to targeted moisture content and purge air) prior to loading ammonia will help remove air from the reactor and subsequently make it easier to load liquid or gaseous ammonia. Temperature and pressure sensors are very important for monitoring the system. If any of those sensors fail, they must be replaced prior to beginning experiments. An operator should be present close to the reactor and ammonia delivery system during the course of pretreatment process to oversee the operation. Stainless steel vessels (conventional alloys) are highly recommended. Mild steel or aluminum reactors will corrode over time when exposed to ammonia. If the operator realizes during the course of the pretreatment that too much or too little ammonia was added, that pretreatment run should be aborted. It may be dangerous to add or try to remove ammonia during the course of the reaction.

SUPPLEMENTAL PROTOCOL 2 TO PERFORM AFEX USING MBI's PACKED BED 1-TON PILOT SCALE REACTOR SYSTEM WITH INTEGRATED AMMONIA RECYCLE:

Summary of critical equipment specifics for performing packed bed AFEX process on a pilot scale is provided in **Table S3** below. Additional details can be made available by MBI/MSU[1] and a representative image of the MBI pilot AFEX equipment is shown in **Figure S2A** below.

PROTOCOL STEPS:

1. Biomass preparation

Ground corn stover with particle size around 1-2 inches and the required amount of water for adjusting the moisture to 20 ±2% (based on total weight) are added to a ribbon mixer. The contents of the mixer are then dispensed into baskets (**Fig. S2B**) and packed to the target density (80-100 kg/m^3) using the in-house fabricated basket packer device (**Fig. S2C**). Baskets were fabricated by MBI team using stainless steel perforated sheet, with 16.5"diameter and 14-3/4 inch height. Seven baskets are needed for each reactor.

2. Load biomass

The tops of MBI's pilot-scale reactor vessels are equipped with hinged T-Bolt closures and the packed baskets are lowered in from the top. Baskets' outer diameters are ¼" to 1/8" smaller than the reactors' inner diameters, so that the baskets fit snugly within the reactors. The baskets sit on top of each other. Once all seven baskets are loaded into the reactor, the T-Bolt closure is sealed.

3. Pre-steaming

Once the reactor is sealed, the pre-steaming step starts by opening the bottom valve on the reactor and introducing a low pressure (15 psig) steam to the reactor from the top. During the pre-steaming process, air is forced out of the reactor through the bottom port and displaced by saturated water vapor. At the same time, condensation of steam heats and moistens the biomass. The process continues until a temperature sensor at the bottom of the reactor reaches 80°C, at which point the steam is turned off and the valves at both the top and bottom of the reactor are closed. The average temperature in the reactor is approximately 95°C at this point, and the pressure remains at or near atmospheric.

4. Ammonia addition

After pre-steaming, the reactor bottom valve is closed and ammonia vapor is added to the reactor through a port located above the top of top basket. Ammonia added to the reactor may be compressed vapor recovered from depressurization and steam stripping of the opposite reactor, or may be fresh vapor generated by pumping anhydrous liquid ammonia from a storage tank into a steam-jacketed vaporizer. As fresh ammonia vapor is added, the pressure in the reactor vessel rises. When the pressure reaches 300 psig, the ammonia pump is turned off and the pressure in the reactor is allowed to decrease until the pressure drops to 250 psig. At that point, the pump is turned back on and more ammonia is added. This process continues until the desired amount of ammonia is added. When recovered ammonia vapor obtained from the opposite reactor is added, the pressure typically rises to between 200 and 250 psig, then fresh ammonia vapor is added to reach 300 psig as described above.

Table S3 Major equipment and materials necessary to perform AFEX pilotscale pretreatment using packed bed AFEX reactor with volumetric ammonia delivery capabilities

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Major equipment of AFEX pilot plant			
Pilot plant Packed bed AFEX reactor	Kennedy Tank and manufacturing Co, Indianapolis, IN	Custom made	Diameter: 18", Height: 144", Material: 304 stainless steel, Maximum pressure: 490psig@400˚F, Top closure type: Sypris tube turns T-bolt, Bottom head: Standard weight pipe cap, Tank shell: 1/2" thick rolled and welded stainless steel plate
Compressor	Fisher Refrigeration , INC	Frick model RXF 15H	Type: Screw compressor, Suction pressure: 0 psig, Discharge pressure: 300psig, flow 3lb/min
Vaporizer	Enerquip, LLC	Custom made	Inlet: Liquid anhydrous ammonia, 300 psig , Ambient temperature of TK-1A1, lowest temperature of -18˚ C; Outlet:Gasseous anhydrous ammonia, 300 psig, Minimum 55 °C; Flow Rate: 2.35 kg/min; Utility: Saturated 75 psig steam
Ribbon mixer	Colorado Mill Equipment	RB-2000	Mixing capacity: $60ft^3$, Motor: 7.5 HP, Agitator RPM: 18
Baskets	Designed and fabricated by MBI	Custom made	304 stainless steel perforated sheet, with 16.5"diameter and 14-3/4 inch height
Basket packing device	Designed and fabricated by MBI	Custom made	Capable of compressing the biomass to density abount 100 kg/m3
Ammonia pump	Hydra-Cell	M-03	with metalic head, Model#M03EASJHFEHA

Figure S2. (A) Picture showing original packed bed AFEX reactor constructed at MBI-MSU. Next, picture shows the type of wire mesh basket used to load untreated biomass, before loading in to the pilot plant tubular reactor. Here, (B) depicts baskets fabricated by MBI, and (C) depicts manual basket packing device designed and fabricated by MBI.

5. Soak time

Once all ammonia is added to the reactor, it is allowed to soak. Valves at the top and bottom of the reactor are closed, and the biomass becomes pretreated. During this time, the pressure within the reactor gradually decreases, as ammonia vapor enters the liquid phase. Likewise, the temperature gradually decreases as heat is lost to the ambient air. The residence time of this soaking period can be between 30 min and several hours. In pilot scale operations, the residence time is approximately one hour, which is the amount of time needed to allow the second reactor to cool sufficiently to open and remove the treated biomass baskets and add new untreated baskets.

6. Depressurization

After soaking, the reactor is depressurized. Typically, depressurization of a reactor takes place just as the opposite reactor has finished pre-steaming. A flow control valve at the bottom of the reactor vessel is opened slowly and the pressure is released by allowing the ammonia vapor to flow into the top of the opposite reactor, until the two reactor vessels are at equal pressure. At this stage, a compressor is engaged, drawing the pressure in the reactor to below 20 psig. A condenser and liquid trap located on the suction side of the compressor minimizes intake of water vapor into the compressor. Ammonia vapor removed from the reactor during depressurization is re-pressurized by the compressor and is added to the opposite reactor.

7. Steam stripping

Ammonia remaining in the reactor vessel after depressurization is removed via steam stripping. During steam stripping, steam is admitted to the top of the reactor vessel. Condensation of steam on the biomass releases heat which drives evaporation of ammonia vapor. The stripped ammonia vapor has lower temperature and higher density than the incoming steam, and is thus pushed out the bottom of the reactor as steam penetrates downward through the biomass. Ammonia vapor is drawn from the bottom of the reactor by the compressor, and the compressed ammonia vapor is added to the opposite reactor. Once the temperature at the bottom of the reactor reaches 100°C, it is assumed that all ammonia has been stripped. At this point, the steam flow is stopped and the bottom valve is shut off. The pressure in the reactor remains below 20 psig during steam stripping.

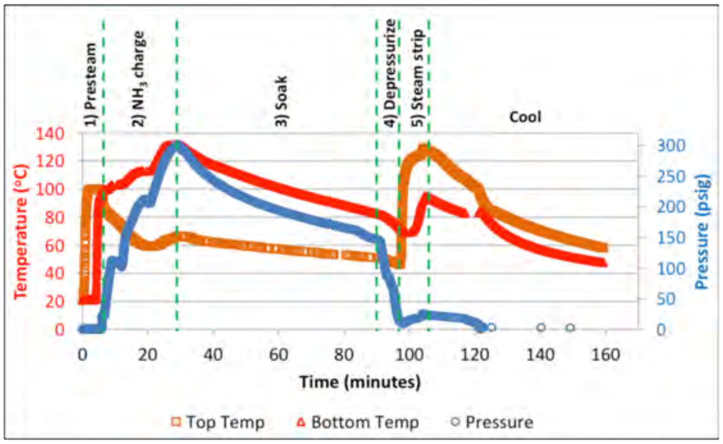
8. Unloading the reactor

At the end of steam stripping the baskets are too hot (about 100°C) for manual handling and are allowed to cool with air sweeping through the reactor for approximately 1-1.5 hours. The reactor is allowed to cool to 60°C before opening the T-Bolt closure, at which point the baskets can be removed using a hoist. The AFEX treated biomass is approximately 40 to 45 percent moisture at this stage and must be dried prior to storage.

REPRESENTATIVE RESULTS & DISCUSSION FOR SUPPLEMENTARY PROTOCOL 2:

Pretreatment efficacy for the pilot-scale AFEX reactor has been demonstrated to match the 5-gallon and 200 mL lab-scale batch reactions, achieving comparable yields of hydrolyzed sugars using commercial cellulase cocktails (Figure 4). **Figure S3A** shows the temperature and pressure profile within the packed bed pilot-scale reactor during a typical AFEX run. All steps except for loading the reactor and unloading the reactor are shown. This custom designed system enables control for two reactors, a condenser, compressor, and ammonia vaporizer, as well as inlet ports for steam, ammonia, and air and an outlet port to a scrubber connected to a waste vessel. This control panel is used to open or close each of the valves that are involved in the processing steps for both reactors. Overall bed cycle time at pilot scale is approximately 110 minutes to perform a single cycle of packed bed AFEX pretreatment. Duration of each step is presented in **Figure S3B**. Lastly, **Figure S3C** shows representative glucose and xylose hydrolysis yields from more than 500 pilot scale AFEX treatment runs of corn stover.

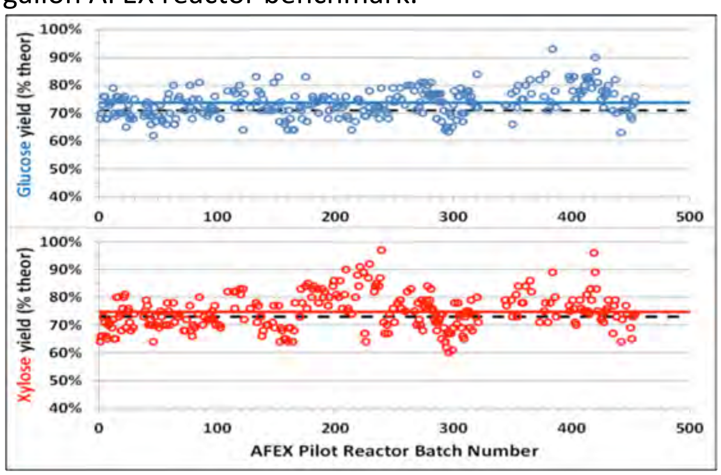
Figure S3. (A) Temperature and pressure profiles within a pilot-scale reactor during a typical run.



(B) Cycle time based on packed bed AFEX pilot plant runs.

Steps	NH_3 Charge	Makeup NH_3 Add → Soak	DePressurize → Steam Strip	Unload → Reload → PreSteam
Time (minutes)	19	36	19	36
	← Reactor Cycle Time = 110 minutes →			

(C) Trend showing the glucose and xylose yield from more than 500 pilot scale runs of corn stover. The solid line represents the average while the dashed line represents the laboratory scale 5-gallon AFEX reactor benchmark.



SUPPLEMENTAL PROTOCOL 3 TO PERFORM AFEX USING 0.6-LITER PARR REACTOR:

In the absence of a suitable pump to transfer of ammonia from the bulk ammonia cylinder to the reactor, anhydrous ammonia can be transferred gravimetrically. The following protocol summarizes this approach using standard Parr reactors (ranging in volume from 0.1-0.6 L), and details are similar to what has been published previously[3,4]. Here a 0.6 L reactor was operated at Rutgers University to demonstrate how to perform AFEX pretreatment. Equipment details are summarized in **Table S4** and representative pictures of the reactor presented in **Figure S4**.

1. **Adjusting Biomass Moisture Content**

1.1 Estimate the total moisture content of biomass, as illustrated in the 5-gallon reactor protocol.
1.2 Add 28.3 g of water to the original biomass (e.g., 60 g of total weight biomass or 55.2 g of the dry weight of biomass with 8% initial moisture) to achieve a final desired moisture content of 60% on a total weight basis. Mix well by hand wearing nitrile gloves.

2. **Pretreatment Reactor and Auxiliary Equipment Setup**

2.1 Ensure that the Parr controller is securely plugged in, and heating coils are functional. Check that the fume hood ventilation system is operating correctly.
2.2 Check that the captured O-ring located in the top part of the reactor is free of debris and thinly coated with vacuum grease. Reapply grease, if needed, to ensure reactor lid seals properly.

3. **Loading Biomass into Pretreatment Reactor**

3.1 Add the moist and well-mixed biomass into the reactor. Once the biomass has been added, put the reactor lid on the reactor vessel.
3.2 When the reactor lid is seated against the O-ring, place the collar around the Parr reactor, and seal the reactor using a wrench.
3.3 Ensure that all of the following manual valves on the reactor head or vessel are closed, to begin with (based on reactor head design): (a) for ammonia release after run is completed, (b) for loading ammonia into the reactor, (c) for loading nitrogen into the reactor, and (d) for removing contents from the underside of the reaction vessel.
3.4 Leak test of the reactor. Connect the nitrogen tank line into the inlet of the reactor. Open the nitrogen tank valve. Charge the vessel with high pressure regulated nitrogen to 200 psi. Isolate the reactor by closing the valve and disconnect. Close the nitrogen tank valve. Wait for 15 minutes to see the pressure drop. If the pressure drops less than 5 psi, continue the move to the next step. Otherwise, depressurize the reactor, dismantle the reactor, clean the seals and check the valves. Repeat steps from 3.2 to 3.4 again.
3.5 Depressurize the reactor opening the vent valve slowly.
3.6 Connect the reactor inlet to the ammonia tank. Open the valve located directly on top of the ammonia tank and the ammonia outlet/inlet valves to the reactor. Check all fittings for tightness by visual inspection.
3.6 Record the temperature and pressure readings before heating. Preheat the mantle with the setpoint at 105 °C, on full power for 30 min before addition of ammonia to the reactor. If the step is skipped, the targeted temperature during pretreatment after ammonia addition will likely not be achieved.
3.7 Turn on and off the mantle power (manually or using a temperature controller if available)

depending on the temperature during the process.
3.8 Turn on the Parr controller display and heater. Press button I and press the Reset button on the back of the controller. Set heater to desired mantle temperature, raise mantle and begin preheating.

4. **Loading Ammonia into Pretreatment Reactor**
4.1 Before ammonia addition, weigh the reactor filled with biomass to find the initial weight.
4.2 For the addition of liquid ammonia into the reactor, use a time-based withdrawal. The amount of ammonia to be added will depend on the AFEX pretreatment process conditions to be tested. This approach is based on a trial-and-error method and the user will need to run some preliminary experiments to determine the optimal time for obtaining the desired ammonia loading in their respective pretreatment system setup.
4.3 Stop the loading of ammonia after a specified time. Weigh the reactor. Subtract the initial weight to find out ammonia addition into the reactor. If the charged amount of ammonia is less than the required amount. Calculate the ammonia loaded per minute to approximate the time required for further addition. Add ammonia to the reactor for up until the calculated time.
4.4 If the ammonia is loaded more than the required amount. Adjust the ammonia loading by venting the excess ammonia through the vent valve. Here, for a 1:1 ammonia to dry weight biomass loading a total of 55.2 g of ammonia needs to be added to the reactor.

5. **AFEX Pretreatment Operation & Biomass Recovery**
5.1 After the desired amount of ammonia has been delivered, close the needle valves on the ammonia line.
5.2 When the reactor is within 5 °C of the setpoint, start the reaction time. If unable to reach the target temperature within 5 min of the ammonia addition, abort the run.
5.3 If the target temperature is obtained within the time criteria, record the temperature and pressure of the system. This is the initial temperature reading. Record the temperature and the pressure of the reactor every three min.
5.4 To regulate the temperature of the reactor, turn the mantle off and on, and raise and lower the mantle as needed during the run (if automatic temperature controller is not available). The following 'Quality Control Criteria' have been established based on target temperature for AFEX pretreatment. If after reaching the set point, the reactor temperature goes outside ± 10 °C from the setpoint, the experiment must be aborted. If the target temperature (within 5 °C) is not reached within 5 min after ammonia loading, abort the experiment.
5.5 When the desired pretreatment reaction time is complete, turn off the heating mantle.
5.6 To arrest the reaction, slowly open the ball valve to release the ammonia directly into the fume hood.
5.7 Before opening the reactor, verify that the reactor is fully depressurized. Using thermo-protective gloves, as needed, loosen the collar and remove the collar. Remove the biomass from the reactor cylinder inside the fume hood.

6. **Reactor Shutdown and Cleanup**
6.1 Clean out the reactor and lid.
6.2 Close all the ammonia valves. Turn off the Parr reactor controller.

Table S4 Major equipment and materials necessary to perform AFEX benchscale pretreatment using 0.1-0.6 L Parr reactor with gravimetric ammonia delivery option

Name of Materials/Equipment	Company	Catalog Number/s	Comments/Description
0.1-0.6 L Pressure Reactor System	Parr Instrument Company (Moline, IL)	Series 4560 Mini Reactor system with integrated heating mantle and controller	100-600 mL range high pressure mixing reactor made with 316 Stainless Steel, equipped with an ammonia inlet valve and an exhaust valve. Maximum pressure of the vessel is 2000 psig at 225 °C. Temperature and mixing speed controller integrated with reactor system.
Weighing Balance	OHAUS	e.g., OHAUS Model D31P60BR5	Weighing balance to weigh reactor before and after ammonia addition
Portable single gas direct readout ammonia monitor	Honeywell Analytics Ltd. (Lincolnshire, IL)	ToxiPro 544521VD Single Gas Polycarbonate Ammonia (NH3) Detector monitors	For detection of ammonia concentration in lab to prevent accidental exposure
Corn Stover	National Institute of Standards and Technology	Standard Reference Material RM 8412	Cellulosic biomass feedstock that needs to be pretreated. Corn stover can be procured from other sources if not available at NIST.
Nalgene Plastic Utility Boxes	VWR	36212-361	To be used for mixing biomass with water
Heat protective gloves	VWR	75836-506	Kevlar® Nomex® heat resistant gloves protect up to 260°C during handling of hot reactor vessel
Cold protective gloves	VWR	89217-722	Keep hands warm and dry in temperatures as low as –31°C when handling liquid ammonia
Earplugs	VWR	55533-030	Ear plugs to protect hearing when releasing ammonia at end of pretreatment
Distilled water	-	-	To mix into biomass to adjust moisture content

Figure S4. Different steps involved in producing AFEX (or Ammonia Fiber Expansion) treated biomass using a lab-scale 0.6 L Parr reactor using gravimetric method for ammonia delivery.

1. Adjust corn stover biomass moisture and mix contents well prior to addition into standard 0.6L Parr reactor
2. Add wet biomass to Parr reactor and seal reactor lid shut along with all ammonia entry/exit valve ports
3. Weigh the sealed reactor with loaded biomass prior to charging ammonia using a weighing balance
4. Pre-heat biomass in reactor to ~60 ^{0}C prior to charging in ammonia for target temperatures > 100 ^{0}C
5. Charge the liquid ammonia directly from ammonia cylinder (with dip-tube) into 0.6L-Parr reactor

6. Weigh reactor after charging ammonia and adjust by venting or adding ammonia according to desired loading
7. Pretreatment begins at desired temperature and ammonia loading for fixed total residence time
8. Monitor reactor pressure to check for any leaks during the entire process prior to ending the AFEX pretreatment run
9. Discharge ammonia carefully inside fume hood from reactor using vent valve to end AFEX pretreatment run
10. AFEX pretreated biomass is next removed from reactor for drying in fume hood overnight to ensure ammonia removal

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