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Ammonia Fiber Expansion (AFEX) as spin off technology from 2nd generation biofuel for upgrading cereal straws and stovers for livestock feed

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ABSTRACT

Ten cereal straws and stovers from India were treated using the ammonia fiber expansion (AFEX) technique to explore the effectiveness of the AFEX technique for releasing sugars from structural carbohydrates and for the upgrading of cereal crop residues as livestock feed. Recovery of glucose and xylose in AFEX treated material was about three times the recovery in untreated material. AFEX treatment increased recovery of glucose between 60 and 85% and of xylose between 50 and 85% of their theoretical yields. AFEX treatment increased average crude protein (CP) by 260% (CP content: 62 vs 161 g/kg). Cell wall content as estimated by NDF decreased on average by 47 g/kg (NDF: 656 vs 609 g/kg) while cellulose contents estimated as ADF apparently increased by 23 g/kg (ADF: 443 vs 466 g/kg). Lignin contents estimated as ADL did not significantly differ between untreated and treated material. Measured after 24 h of incubation, AFEX treatment consistently and significantly increased *in vitro* gas production (42.9 vs 33.3 ml/200 mg), *in vitro* apparent digestibility (493 vs 630 g/kg) and true digestibility (624 vs 755 g/kg) and *in vitro* metabolizable energy content (6.9 vs 8.6 MJ/kg). Treatment changes in digestibility estimated based on *in vitro* gas production generally agreed with gravimetric estimates based on undigested residues, making it unlikely that the effect of AFEX treatment on digestibility was overestimated by unrecovered soluble but un-fermentable substrate. Increases in CP content and *in vitro* digestibilities upon AFEX treatment were unrelated to CP content and *in vitro* organic matter digestibilities (IVOMD) of untreated base material, though increases in IVOMD upon treatment tended ($P = 0.07$) to be lower in material with high (> 530 g/kg) baseline IVOMD.

1. Introduction

Lignocellulosic biomass from forest, agricultural wastes and crop residues is the most abundant renewable biomass on earth with a total annual production of about 10–50 billion metric tons (Sanchez and Cardena, 2008). About 3.8 billion metric tons are contributed by crop residues with cereals contributing 74%, sugar crops 10%, legumes 8%, tubers 5% and oil crops 3% (Lal, 2005).

Abbreviations: AFEX, ammonia fiber expansion; MP, amegapascal; LMC, low and middle income countries; MBI, Michigan Biotechnology Institute

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Cellulose is the major constituent in lignocellulosic biomass ranging from about 300–550 g/kg followed by hemicelluloses which constitutes about 150–350 g/kg and lignin which constitutes about 60–300 g/kg (Ivetic and Antov, 2013). Cellulose is a linear polymer of cellobiose which itself is made up of a glucose to glucose dimer in the β 1–4 glucan configuration. This β 1–4 glucan configuration conveys molecular stability to cellulose when compared to starch, a glucose to glucose dimer in the α 1–4 glucan configuration (Van Soest, 1994). Thus lignocellulosic biomass is, in its essence, not that different from the primary products of cereals, the starch in grains, even though their respective accessibility to mammalian digestive enzymes is very different (Van Soest, 1994).

The work on 2nd generation bio-fuels (bio-fuels based on lignocellulosic biomass rather than on grains as in 1st generation biofuel) has attracted US multi-billion dollars of investment during the last two decades (Blümmel et al., 2014a). It may be feasible to utilize spin-offs from 2nd generation bio-fuel technologies to upgrade lignocellulosic biomass for animal feeding by increasing the accessibility of sugars in plant cell walls. This includes pretreatment approaches that render the lignin-hemicellulose-cellulose matrix more accessible to hydrolysis by rumen microbial and/or external enzymes and advances in enzyme technologies which made enzymes more stable, more affordable and targeted and substrate specific. Of the numerous mechanical, chemical, and biological approaches applied in 2nd generation bio-fuel technologies fiber expansion approaches using moderate temperature and pressure in an alkaline environment (ammonia) generating only solid substrate seem to be the most promising (Dale et al., 2010; Blümmel et al., 2014a). One such pretreatment, the Ammonia Fiber Expansion (AFEX) pretreatment was tested in the present work for its upgrading ability using ten cereal straws and stovers from wheat, rice, sorghum, pearl millet and maize. During AFEX treatment, ammonia vapor is added to the biomass under moderate pressure (100–400 psi) and temperature (70–200 °C) before rapidly releasing the pressure and recovering more than 95% of the ammonia used in the process. About 2% of the ammonia that is not recovered in the process is chemically bound to the biomass, contributing to the crude protein content of the treated samples (Campbell et al., 2013).

2. Material and methods

2.1. Straws and stovers investigated

Ten cereal straws and stovers from India were investigated consisting of two rice straws (cultivar names: Aditya and Vardhan), three sorghum stovers (cultivar names: CSV-22, ICGV 93046 and Zaheerabad), one wheat straw (nondescript purchased from a fodder market), two pearl millet stovers (cultivar names 86M88 and HHB-67) and two maize stovers (cultivar names: NK 6240 and 9125). Except for wheat, each crop straw and stover were chosen to include cultivars with higher and lower *in vitro* digestibility (IVOMD) and metabolizable energy (ME) content.

2.2. Analysis of cellulose, xylan, galactan, arabinan and insoluble lignin at MBI

Representative sub-samples of all the 10 straws and stovers were collected from an amount of about 20 kg each and were processed for cellulose, xylan, galactan, arabinan, and insoluble lignin analysis in triplicates following the laboratory analytical procedures developed by the National Renewable Energy Laboratory (Sluiter et al., 2012).

2.3. AFEX treatment

The 10 straws and stovers in particle sizes of 2–5 cm were AFEX treated using Michigan Biotechnology Institute (MBI) lab scale (Fig. 1) packed bed AFEX system. All samples were subjected to the same AFEX conditions: 20% initial moisture, 1 kg of ammonia per kg of dry biomass and 30 min soak time. Four runs (beds) of AFEX were conducted for each crop residue. The samples were moistened to about 20% by weight by spraying with deionized water, and then packed into stainless steel baskets at bed density of about 80–100 kg (dry) per m³. A photo of a basket packed with biomass is shown in Fig. 1 inset; 6 baskets were inserted into each reactor tube. Baskets containing biomass were assembled into the reactor tubes and AFEX treated using a cycle of five steps: 1) Pre-steam, 2) Ammonia charge, 3) Soak, 4) Depressurize, and 5) Steam strip (Campbell et al., 2013).

Process conditions during the packed bed AFEX cycle varied only slightly from bed to bed. Starting with biomass at 20–25% moisture (based on the total weight) and 22–25 °C, the pre-steaming step increased bed moisture to 35–40% and temperature to 90–100 °C. After addition of all the ammonia (1 kg ammonia per kg of dry biomass), the pressure was 1.4 MPa, temperature at the top of the reactor was 40–45 °C, and temperature at the bottom was 100–110 °C. During the soak period, significant heat was lost from the beds to the surrounding air. By the end of the soak period, top and bottom bed temperatures typically had dropped to 35–45 °C. Depressurization dropped the bed pressure to atmospheric, top temperature to 30–35 °C, and bottom temperature to 10–20 °C. By the end of steam stripping, top and bottom temperatures were 90–100 °C, and moisture was 50–55% (Campbell et al., 2013). During the depressurization and steam stripping steps more than 95% of the charged ammonia was recovered and transferred to the next bed for treating the next batch of biomass. Following bed-to-bed ammonia transfer, makeup ammonia was added to the next bed as part of its ammonia charging step, to compensate for residual ammonia not removed from the previous bed. Using a convection oven, the treated samples were dried to less than 10% moisture. The dried samples then were stored in dry condition to prevent spoilage for future analysis.

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