SPIN-OFF TECHNOLOGIES FROM 2ND GENERATION BIOFUEL: POTENTIAL GAME CHANGERS FOR UPGRADING CEREAL STRAWS AND STOVERS FOR LIVESTOCK FEED IN INDIA

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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). Cellulose is the major constituent in lignocellulosic biomass ranging from about 300 to 550 g/kg followed by hemicelluloses which constitutes about 150 to 350 g/kg and lignin which constitutes about 60 to 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). Considering the huge quantities of lignocellulosic biomass available and the high nutritive quality of their hexose and pentose sugars, it comes as no surprise that attempts to upgrade lignocellulosic biomass for livestock fodder reach back to the beginning of the 20th century (Fingerling and Schmidt, 1919; Beckmann, 1921).

The work on second generation bio-fuels (bio-fuels derived from lignocellulosic biomass) was motivated by reasons very similar to those of the early animal nutritionists: the abundance of lignocellulosic biomass and its content of basic sugars. 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. Key processes (see also Fig. 1) in 2nd generation biofuel that matter for livestock feed resources are: 1) post harvest collection and mechanical pre-treatment of lignocellulosic biomass; 2) physical-chemical-biological pre-treatment to disrupt lignin-hemicelluloses-cellulose matrices, partially hydrolyze weaker linkages of pentoses in hemicelluloses structure and make hexoses in cellulose more susceptible to enzymatic hydrolysis; and 3) design and application of targeted and tailored enzyme cocktails. For animal nutritionist pre-treatment technologies up to the generation of glucose (or equivalents) are interesting, from here on rumen microbes and mammalian enzymes can take over (see also dotted line in Fig.1). The current paper explores the impact of three 2nd generation biofuel technologies on the fodder quality of a wide range of Indian cereal straws and stovers: 1) Steam treatment, 2) Ammonia Fiber Expansion (AFEX); and 3) NaOH– H_2O_2 treatment.

- Efficient harvest and collection of high volume-low density
- biomass
- Balance central versus decentralized approach
- Optimize physical form-transport-susceptibility to pre- treatment-voluntary feed intake
- Swell and disrupt hemicellulose-cellulose-lignin matrix
- Partially hydrolyze xylan structure
- Increase surface and porocity of fiber structure
- Unclear benefit for ruminant nutrition, more research with new
- enzymes/enzyme cocktails needed
- Demand/potential for monogastric nutrition
- “One pot” complete enzymatic conversions

Spin-off technologies investigated

(a) Steam explosion developed by Nargajuna Fertilizer and Chemicals Research and Development Center (R&D)

Steam explosion is a promising pre-treatment potentially effective without pH interventions if partially hydrolyzed hemicelluloses are recovered. In the case of steam treatments application to upgrading lignocellulosic biomass for feeding preceded use in 2nd generation bio-fuel technologies for example treating wood to feed to ruminants (Bender *et al*., 1970). However, opportunities from steam explosion appear to increase with the exploration of its use as pre-treatments in 2nd generation bio-fuel technologies,

particularly when partially hydrolyzed hemicelluloses is not separated from the solid phase and no chemicals are used. In a collaboration between the International Livestock Research Institute (ILRI) and Nagarjuna R&D maize stover from a superior dual purpose hybrid, a superior dual purpose sorghum variety andtwo sorghum stovers purchased from fodder market were steam-treated using intermittent live steam injection to heat stovers to 160°C for 10 minutes. After 10 min the stovers were exploded into a receiver tank. After drying steam treated samples and untreated control samples were ground to pass a 1 mm mesh. Two hundred mg of substrate was weighted into glass syringesof the Hohenheim gas production test and gas volume and *in vitro* true degradability were determined after 48 h of incubation.After 48 h of incubation, steam treatment on average increased *in vitro* gas production significantly by 10% and *in vitro* trueorganic matter degradability by 14% or 8.9 percentage units (71.8 vs. 62.9%), respectively (see also Table 1).Details of this work have been reported by Dhanalakshmi *et al.* (2015).

(b) Ammonia Fiber Expansion (AFEX) technology developed by the Michigan Biotechnology Institute (MBI)

This technique was developed by Dale and Weaver (2000). During AFEX treatment, ammonia vapor is added to the biomass under moderate pressure (100 to 400 psi) and temperature (70 to 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). In a collaboration between ILRI and MBI 10 cereal straws and stovers from India 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. 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: 443vs 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 (39.8 vs 29.0 mL/200 mg), *in vitro* apparent digestibility (63.0 vs 49.3%) and true digestibility (77.0 vs. 60.2%) and *in vitro*metabolizable energy content (8.6 vs 6.9 MJ/kg). Measured after 48 h of incubation, AFEX treatment increased *in vitro* gas production from 42.9 to 51.5 mL and *in vitro*true digestibility from 65.1 to 84.4%). 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. Details of this work have been reported by Blümmel *et al.* (2018).

(c) Indian Institute for Chemical Technology (IICT) Alkaline (NaOH) – Oxidative (H_2O_2) treatment

In a collaboration between ILRI, IICT and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) 4 pearl millets stovers (Hybrid KH 16, ICMV 05555, ICMV 05777, ICMV 15111), three sorghum stover (ICSV 25333, ICSV 25335, IS 27246), two maize stover (9125, NK 6240) and two non-descript wheat straws (New Delhi fodder market, Uttrakhand farmer field) were treated. The treated stovers and straws had high water content ranging approximately fom 40 to 80%. Washing further increased the water content to consistently above 80%. Organic matter content was consistently lower in treated than in untreated material suggesting residual NaOH from treatment. Organic matter content was higher in treated and washed than in treated material suggesting that washing removed residual NaOH. Nitrogen content significantly decreased with treatment and washing becoming almost complexly removed in treated and washed material. The cell wall components NDF, ADF and ADL were differently affected by the treatments. Neutral detergent fiber recovery significantly decreased in treated material by 5.8 percent units but was increased by washing relative to untreated material by 11.5 percent units. Acid detergent fibre recovery was significantly increased by 6.2% units by washing and by 18.3% units by the washing of treated material. Acid detergent lignin recovery was approximately halved by the treatments. Treatment increased all *in vitro* fermentation, digestibility and ME measurements. *In vitro* gas volumes measured after 24 h and 48 h of incubations were more than 100 and 68%, respectively, higher in treated than in untreated material. *In vitro* apparent digestibility after 24 h and *in vitro* true digestibility after 48 h were 58 and 68%, respectively, higher in treated compared to untreated material. Metablizable energy was improved by 64% by the treatment. Washing of treated material had only a significant effect on gas volumes measured after 48 h of incubation otherwise there was no significant difference between treated and treated and washed material in *invitro* variables.

(d) Comparison of effectiveness of steam, AFEX and NaOH-H_2O_2 treatment on in vitro measurements and influence of baseline straw and stover quality

Increases in *in vitro* GP and true IVOMD measured after 48 h of incubation were greatest upon NaOH-H_2O_2 treatment followed by AFEX and finally steam treatment (Table 1).

Table 1. Summary of effects of steam, ammonia fiber expansion and NaOH-H_2O_2 treatment on *in vitro* gas production (GP) and true *in vitro* digestibility[-1] after 48 h of incubation. U = untreated; T = Treated

Spin-off technology	n	*In vitro* GP after 48 h (mL/200 mg)		True IVOMD after 48 h (%)	
		U	T	U	U
Steam Treatment	4	48.6	53.6	62.9	71.8
AFEX Treatment	10	42.9	51.5	65.1	84.4
NaOH-H_2O_2 Treatment	11	39.7	66.7	55.9	94.1

[-1]The average difference between true and apparent IVOMD is about 12.9 percentage units (van Soest, 1994). Increments in digestibility are similar independent of expression as apparent or true digestibility.

NaOH-H_2O_2 treatment on average increased true IVOMD by 38.2 percentage units from 55.9 in untreated straws and stovers to 94.1% after treatment. Such an increase is amazing and converts the straws and stovers in essence into concentrate feeds. The true IVOMD measurement is gravimetric in nature and calculated from the truly undegraded residue; all substrate not recovered is supposed to have been digested. This might not always be the case particularly in treated feed stuffs where some undigestible substrate might have been solubilized and so not recovered in the incubation residue (Blümmel *et al*., 2005). However, the increase in true IVOMD of 68% agrees with the average increase *in vitro* GP of 66% (66.7 vs 39.7 mL) and GP reflects generation of fermentation products and is so not gravimetric in nature. Put differently, the increases in straws and stover quality seem to be real. In AFEX and NaOH-H_2O_2 treatments increments in true IVOMD were significantly inversely correlated to the true IVOMD of un-treated straws and stovers (Figure 2). In other words, higher quality straws and stover benefit less from treatment than lower quality ones. It is important to realize that upon AFEX and NaOH-H_2O_2 treatments true IVOMD's approached 100%, an absolute ceiling that constraints increments in true IVOMD. A practical conclusion appears to be that baseline IVOMD does not really matter when very effective treatments are applied.

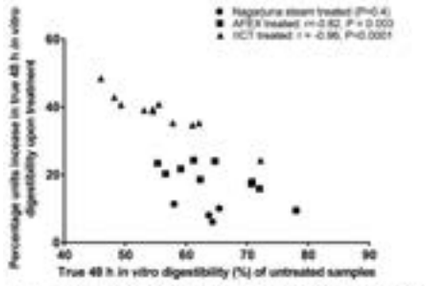

Figure 2: Relationship between true *in vitro* digestibility (IVOMD) of untreated straws and stovers and percentage units increase in true IVOMD upon treatment

Cost-benefit estimates of 2nd generation biofuel technologies for upgrading ligno cellulosic biomass as feed

The kind of straws and stovers investigated for this work are widely traded for urban and peri-urban dairy production in India. An interesting feature of this fodder markets is that price premiums exist for quality differences between straws and stovers from the same crop traded at the same time and place for example because of perceived cultivar or management differences (Blümmel and Rao, 2006; Teufel *et al*., 2010). Fig. 3 and 4 summarize data from ILRI sorghum fodder market surveys over the past 5 years in Hyderabad in India focusing on the two sorghum stovers most widely traded: Andhra (a higher quality stover) and Telangana (a lower quality stover).The average cost of the higher and lower quality stover per kg (dry matter) were 9.8 (16 cents US) and 7.5 (12 cents US) Rs, respectively (Fig. 3).The average (apparent) IVOMD was 44.7 and 40.3% in the higher and lower quality stover, respectively, see Figure 4. In other words and average difference in (apparent) IVOMD of 4.4 percentage units was associated with a price difference of Rs.2.3/kg (4 cents US).

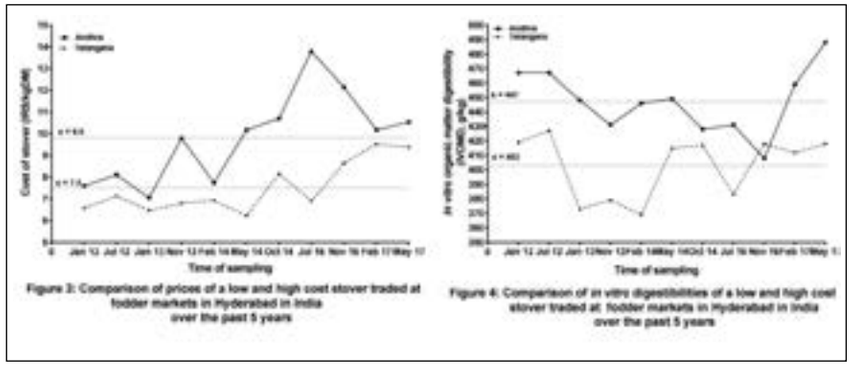

Figure 3: Comparison of prices of a low and high cost stover traded at fodder markets in Hyderabad in India over the past 5 years

Figure 4: Comparison of in vitro digestibilities of a low and high cost stover traded at fodder markets in Hyderabad in India over the past 5 years

The costs of AFEX treatment in India are estimated at 3 to 5 Rs./ kg of stover (Bringi, personal communication) which is currently equivalent to about 5 to 8 cents US. AFEX treatment increased the (apparent) IVOMD of sorghum stover on average by 14.5 percentage units (calculated from Blümmel *et al*., 2018) By extrapolation this would be equivalent to a value increase of 7.6 Rs/kg (14.5/4.4 times 2.3). Assuming the average cost of AFEX treatment to be 4 Rs/kg, the cross cost: benefit ratio from AFEX treatment of sorghum stover would be about 1: 2. Similar calculation are now under way to calculate cost- benefits from steam and NaOH - H_2O_2 treatments.

Considering the huge quantities of lignocellulosic biomass available and the high nutritive quality of their hexose and pentose sugars, it comes as no surprise that attempts to upgrade lignocellulosic biomass for livestock fodder reach back to the beginning of the 20th century (Fingerling and Schmidt, 1919; Beckmann, 1921). These and later

attempts included chemical, physical and biological treatments, but chemical treatments received maximum attention of researchers, particularly the use of hydrolytic agents such as NaOH and $NH_4$for ensilaging (Jackson, 1977; Sundstol and Coxworth, 1984; Owen and Jayasuriya, 1989). However, comparative little uptake of these technologies was observed, even though considerable efforts were expanded by the international research community and development practitioners. For example, Owen and Jayasuriya (1989) listed and reviewed 12 major international conferences addressing the improved use of lignocellulosic biomass for livestock feed from 1981 to 1988 and concluded that large scale adoption of treatment interventions was very rare and did not continue once project activities ceased, despite the many efforts expended on simplifying treatment technologies and use of locally available materials and inputs. This is unfortunate since crop residues are the major feed resources for livestock in low and middle income countries (LMCs) and for example in India comprise about 70% of the feed resources (NIANP, 2012; Blümmel *et al*., 2014b). Even in specialized and commercialized urban and peri-urban dairy systems crop residues usually contribute more than 50% of the feed resources explaining their intensive trading. The work presented in this paper suggests that some spin-off technologies from 2nd generation biofuel offer real possibilities for significantly upgrading ligno-cellulose biomass for livestock feed.

As shown by Vogel and Sleper (1994), differences of 3 to 5 percentage units (30 to 50 g/kg) in forage digestibility were associated with 17 to 24% differences in livestock productivity. Surveying commercial sorghum stover fodder traders in India, Blümmel and Rao (2006) observed that a mean difference of 5% units (47 to 52% or 50 g/kg) in *in vitro* stover digestibility was associated with price premiums of 25% and higher. Anandan et al. (2010) collaborated with Miracle Feed and Fodder Pvt Ltd (Shah, 2007) that commercially produced total mixed rations (TMR) consisting of about 50% of sorghum stover to assess differences 5 percentage units in digestibility in the basal sorghum stover. Milk potential was more than 5.5 and 7 kg/d higher in dairy buffalo and dairy cattle, respectively when offered TMR with higher quality sorghum compared to TMR with lower quality sorghum stover, this as a result of higher quality TMR and higher voluntary feed intake (Anandan *et al*., 2010). Thus intuitively small differences (i.e. 50 g/kg or 5 percentage units in digestibility) of basal diets can have significant effects on livestock productivity and also explain the price premiums paid at crop residue fodder markets (Fig. 3 and 4).The findings presented in Table 1 need to be seen in this light. Increments in true IVOMD upon steam, AFEX and NaOH-H_2O_2 were 8.9, 19.3 and 38.2 percentage units, respectively (Table 1). Particularly AFEX and NaOH-H_2O_2treatment will have potentially very significant impact on fodder quality of cereal straws and stovers, in effect turning cereal crop residues into concentrates and feed resource boundaries between ruminants and monogastric animals could largely dissolve. These improvements are far superior to the ensilage methods based on pH change by NaOH and NH_4reviewed by Jackson (1977);

Sundstol and Coxworth (1984) and Owen and Jayasuriya (1989). It is important to point out that these findings, as promising as they are, need to be corroborated and supported by animal experimentation to confirm that 1) voluntary feed intake is not negatively affected but corresponds to improved *in vitro* digestibility and 2) treatmentsare safe for the animal and the produced milk and meat are safe for human consumption. We also want to stress that the three pre-treatments explored are not exhaustive and other potentially useful spin-off technology from 2nd generation biofuel are out there and need to be investigated. Animal nutritionist can and should leverage from these multi-billion investments into 2nd generation biofuel in collaboration with the private sector. Small and medium enterprises using applying these spin-off technologies would: 1) very significantly increase fodder quality of crop residues thereby increasing livestock productivity at decrease feed costs; and 2) generate employment and income opportunities for rural population; and 3) decreasing environmental hazards from regional burning of crop residues (mainly rice straw) in parts of South and South East Asia.

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(Please contact the corresponding author for details of references)