

Methane Potential and Enzymatic Saccharification of Steam-exploded Bagasse

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To evaluate the biofuel potential of bagasse, an abundant co-product in sugarcane-based industries, the effect of steam explosion on the efficiency of enzymatic saccharification and anaerobic digestion was studied. Bagasse was steam exploded at four different severity levels, and the impact of pretreatment was evaluated by analyzing the release of glucose after enzymatic saccharification with Cellic CTec2 and by analyzing methane production during anaerobic batch digestions. Increasing the severity of pretreatment led to degradation of xylan and the formation of pseudo-lignin. The severity of pretreatment was correlated with the enzymatic release of glucose; at optimal conditions, > 90% of the glucan was released. The highest methane yield (216 mL/gVS) was 1.3 times higher than the yield from untreated bagasse. More importantly, the pretreatment dramatically increased the rate of methane production; after 10 days, methane production from pretreated material was approximately twice that of the untreated material. To assess the possibility of developing combined processes, steam-exploded bagasse was enzymatically pre-hydrolyzed and, after the removal of released sugars, the remaining solid was subjected to anaerobic digestion. The results indicated that, in terms of total heating value, combined ethanol and biogas production is as beneficial as producing only biogas.

Keywords: Methane; Steam explosion; Anaerobic digestion; Enzymatic saccharification; Cellulase; Bioethanol

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INTRODUCTION

Because of the accumulation of greenhouse gases in the atmosphere and limited fossil fuel reserves, a transition away from fossil fuels is necessary (Horn 2013). The production of conventional oil has already peaked (Alekklett *et al.* 2010), and it is thus of particular importance to develop liquid fuel substitutes. In this regard, residual lignocellulosic materials from agriculture, forestry, and industry can play major roles as biomass sources for biofuel production. Sugarcane bagasse is the solid residue remaining after sugarcane has been processed for extracting the sugar juice. It is an abundant waste resource that can be used for the production of biomethane (Badshah *et al.* 2012) and bioethanol (Rabelo *et al.* 2011b). The annual global production of dry cut sugarcane is about 328 teragram (Tg) (328 million tonnes). After the sugars have been extracted, about 180 Tg of dry sugarcane bagasse remains, which could be used to produce about 51 gigalitres (GL) of bioethanol (Kim and Dale 2004). However, bagasse is mostly burned for process heat in sugar mills and distilleries (Alonso Pippo *et al.* 2011). Recently, the concept of the biorefinery has received considerable attention as an alternative route for

biomass exploitation, based on integrated combined processes for the simultaneous generation of energy, fuels, and value-added products (Ragauskas *et al.* 2006). As an abundant agricultural waste product, bagasse could be used for production of significant amounts of fuels. While both bioethanol and biomethane production have been demonstrated from bagasse, studies on integrated production of both fuels are lacking.

Bagasse is a lignocellulosic biomass primarily composed of cellulose (38 to 45%), hemicelluloses (23 to 27%), and lignin (19 to 32%) (Canilha *et al.* 2012). Today new enzyme cocktails developed for saccharification of lignocellulose contain high β -glucosidase activity and lytic polysaccharide monooxygenases, LPMOs (Vaaje-Kolstad *et al.* 2010; Quinlan *et al.* 2011; Cannella *et al.* 2012; Horn *et al.* 2012), and may also include better and more stable cellulases. Overall these improvements have led to considerably more efficient enzyme cocktails (Cannella and Jørgensen 2014). Despite this, the enzymatic processing of lignocellulosic biomass usually requires some physical-chemical treatment (Ramos 2003; Galbe and Zacchi 2012) for the process to be sufficiently efficient. Steam explosion (SE) is one of the most widely used pretreatment methods for lignocellulosic biomass (Alvira *et al.* 2010) and has been applied to a range of raw materials (Sassner *et al.* 2005; Mabee *et al.* 2006; Horn and Eijsink 2010; Vivekanand *et al.* 2012; Bondesson *et al.* 2013). This process involves high-temperature steam treatment followed by mechanical disruption of the biomass fibers by a rapid pressure drop (explosion). Recent studies on hardwoods have shown that steam explosion pretreatment improves not only enzymatic saccharification, but also biogas production through anaerobic digestion (Horn *et al.* 2011a; Bondesson *et al.* 2013; Menardo *et al.* 2013; Vivekanand *et al.* 2013). Thus, for the co-production of bioethanol, via enzymatic saccharification, and biogas from bagasse steam explosion may be a suitable pretreatment step for both fuels.

In the present study, steam explosion of bagasse was investigated as a pretreatment method for both enzymatic saccharification and biogas production. A combined process in which sugars were removed enzymatically before biogas production was also investigated. Concomitantly, the effects of steam explosion on the chemical composition and morphology of the biomass were analyzed. Possible correlations between the results of these analyses and the outcome of the biochemical conversion processes are discussed in this work.

EXPERIMENTAL

Raw Material

Bagasse from sugar cane (*Saccharum officinarum*) was supplied by Borregaard (Sarpsborg, Norway). The material was dried at room temperature, then milled to pass a 10-mm sieve (SM2000, Retsch, Haan, Germany) and stored at room temperature in plastic bags. The dry matter content (DM) of the milled material was 93.8%.

Steam Explosion Pretreatment

Pretreatment was performed as previously described (Horn *et al.* 2011b) using a steam explosion facility at the Norwegian University of Life Sciences designed by Cambi AS (Asker, Norway). The steam was generated by a 25 kW electric boiler, and it was mixed with the biomass in a 20 L pressure vessel. The pressure (and temperature) in the vessel were kept constant at the chosen set point by adding steam to the vessel through an

automatic air-actuated valve. Bagasse samples weighing 300 g were pretreated at 195, 205, 215, and 225 °C with a 10-min residence time. Each pretreatment was ended by activating an air-actuated ball valve at the bottom of the vessel which caused a rapid pressure drop (explosion) and release of the pretreated biomass to the flash tank. The pretreated biomass was collected in a removable bucket at the bottom of the flash tank. Prior to each pretreatment, the pressure vessel was preheated for 10 min at the temperature to be used for pretreatment. The pretreated fractions were stored at 4 °C prior to the saccharification and digestion experiments. Water contents of the material before and after pretreatment (20 to 30 %) are reported in Table 1. All added water came from steam injected into the pressure tank during the steam explosion procedure.

Unwashed samples were used in all experiments and analyses. The DM content of the steam-exploded material was in the range of 20 to 30% (Table 1).

Severity Factor

The temperature and residence time variables in steam explosion can be combined in a single reaction ordinate (Ro), typically reported as the “severity factor” $\log Ro$ (Overend and Chornet 1987). This severity factor is defined by the following equation:

$$\log(Ro) = \log\{t \cdot \exp[(T-100)/14.75]\} \quad (1)$$

where t is residence time in minutes and T is the temperature in °C.

Enzymatic Saccharification

Saccharification of bagasse samples was carried out in triplicate using 30-mL reaction volumes in 50-mL screw-capped centrifuge tubes with shaking at 130 rpm at 50 °C. Fifty millimolar citrate-phosphate buffer was used to adjust the pH to 4.5. The reaction tubes were preheated at 50 °C before the enzymes were added. The substrate concentration in the tubes was 50 g DM/L. The cellulase cocktail used in this study was Cellic CTec2 (Novozymes, Bagsvaerd, Denmark) containing 120 FPU/mL (70.5 mg protein/mL). The standard enzyme dose was 9.4 mg protein per g of dry matter substrate (16 FPU per g substrate). The protein concentration in Cellic CTec2 was measured with the Bradford assay (Bio-Rad, Hercules, CA, USA). For analysis of glucose release, samples from the reaction tubes were collected and immediately centrifuged at 4 °C, after which the supernatants were frozen at -20 °C until analysis.

To evaluate the biomethane potential of the solid residue remaining after enzymatically removing sugars, a larger saccharification experiment was designed. In this experiment, the reactions took place in 500-mL bottles with a liquid working volume of 200 mL, using the conditions described above. Samples from the reaction tubes were collected and immediately centrifuged at 10,000 g (at 4 °C), after which the supernatants were frozen at -20 °C until analysis. The solid residue contained 26.2% DM and was stored at 4 °C until the biogas experiments were initiated. The volatile solid (VS) content was 87.1% of the DM.

Biogas Inoculum

The microbial inoculum for the biogas trials was obtained from a local biogas plant (Biowaz, Tomb, Norway) that runs large-scale, continuous anaerobic digestion of cow manure and food waste at mesophilic temperature. Prior to the experiments, the inoculum was incubated anaerobically (37 °C, 10 days) to reduce endogenous biogas

production. The inoculum was then diluted with water and divided into 400-mL aliquots, which were added to 555-mL batch bottles. Diluted inoculum had a DM concentration of 1.2%, a pH of 7.6, and an ammonium concentration of 932 ppm.

Biogas Production

The bagasse samples were digested anaerobically in sealed batch flasks. The batch fermentation for biogas production was performed in triplicate, which included untreated bagasse, four different steam-exploded samples, an enzymatically-treated sample, and inoculum alone as a control. For this, 0.60 g of VS substrate was added to and mixed with the inocula in the batch bottles. Subsequently, the bottles were flushed with nitrogen and closed with rubber stoppers and aluminum screw caps before being transferred to a shaker (Multitron Standard, Infors HT, Switzerland) for incubation (37 °C, 90 rpm, 41 days).

Analysis

Compositional analysis

Analyses of the Klason lignin and carbohydrate contents (Table 1) in unwashed bagasse samples before and after steam explosion were carried out according to NREL/TP-510-42618. After acid hydrolysis, monosaccharides were detected and quantified using high-performance anion exchange chromatography (HPAEC) with a Carbowac PA20 column. The elemental composition of carbon, hydrogen, and nitrogen was determined by combustion using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA). Dry matter and ash contents were determined by drying or burning the samples at 105 °C and 550 °C overnight. The VS content was calculated by subtracting the ash from the DM content.

Determination of soluble sugars

HPLC analyses of glucose and xylose concentrations were completed using a Dionex Ultimate 3000 system (Dionex, Sunnyvale, CA, USA) with a refractive index detector. Sugar concentrations were quantified by running standards. The HPLC samples were prepared by diluting samples from the reactions five-fold with the mobile phase (5 mM H₂SO₄), followed by centrifugation and filtration (0.2 µm Sarstedt Filtropur S). Samples (10 µL) were applied to a Rezex RFQ-Fast Fruit H+ column (7.8 × 100 mm analytical column; Phenomenex, Torrance, CA, USA) conditioned at 82 °C; the flow rate was 1.0 mL min⁻¹.

Gas composition and calculation

Gas composition analysis and calculations were performed as described in a report by Vivekanand *et al.* (2013). In short, biogas production was monitored twice a week by measuring the pressure generated in the flask digesters using a digital pressure transducer (GMH 3161, Greisinger Electronic, Germany) fitted with a needle that can be used to directly penetrate the rubber stoppers of flask digesters. The biogas composition was analyzed using a gas chromatograph (3000 Micro GC, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD).

FTIR spectroscopy

Fourier transform infrared spectroscopy (FTIR) of pretreated and untreated bagasse samples was performed to evaluate chemical changes during pretreatment.

Samples were dried at 40 °C overnight and ball-milled to a fine powder. The spectra (40 scans) of the samples were recorded in the 4000- to 400-cm⁻¹ range using a Spectrum BX FTIR spectrophotometer (Perkin Elmer, England). The spectra were recorded in transmittance mode, automatically baseline corrected, and normalized using the associated software. The spectra were truncated to a fingerprint region of 800 to 1800 cm⁻¹.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to observe the morphological differences between untreated and pretreated samples using a JSM-840 scanning electron microscope (JEOL USA Inc., Peabody, MA). Dried samples were mounted on aluminum stubs (d = 10 mm) using double-coated tape. They were then coated with gold/palladium, and images were recorded.

Statistical analysis

Data sets for cellulose conversion yield and biogas yield were analyzed by one-way analysis of variance (ANOVA) ($\alpha = 0.05$) using Microsoft Excel. ANOVA was also used when comparing biogas production data for selected samples.

RESULTS AND DISCUSSION

Chemical and Physical Effects of Steam Explosion

Bagasse was pretreated at four different steam explosion conditions. Temperatures ranged from 195 to 225 °C with a fixed residence time of 10 min. Temperature and residence time variables can be combined into a single “reaction ordinate” (Ro) (Overend and Chornet 1987), often reported as the “severity factor” $\log Ro$. The severity factor in this pretreatment series varied from 3.8 (195 °C, 10 min) to 4.6 (225 °C, 10 min; Table 1). As commonly observed in steam explosion pretreatment of lignocellulosic materials (Ballesteros *et al.* 2004; Horn and Eijssink 2010), higher severity factors led to the production of darker and stickier biomass with less visible residual fiber structure.

Table 1. Compositional Analysis of Bagasse Samples

Pretreatment	DM (%)	Severity ^a	C (%)	N (%)	H (%)	O ^b (%)
Untreated	93.8	-	46.5	0.25	4.8	48.4
195 °C (10 min)	20.6	3.8	47.6	0.32	4.4	47.7
205 °C (10 min)	29.8	4.1	49.5	0.29	4.7	45.5
215 °C (10 min)	25.9	4.4	50.6	0.35	4.6	44.4
225 °C (10 min)	19.7	4.6	50.3	0.33	4.3	45.1

Compositional data are expressed as percentage of dry matter.

^a Severity was calculated according to equation: $\log(Ro) = \log(t \cdot \exp((T-100)/14.75))$, where t is residence time (minutes) in pretreatment and T is the temperature in °C.

^b Oxygen was calculated by subtracting C, N, and H values from 100%.

Table 2 shows that increasing the severity led to an accumulation of lignin in the pretreated samples, which is primarily caused by production of pseudo-lignin from xylan degradation products (Sannigrahi *et al.* 2011; Kumar *et al.* 2013; Vivekanand *et al.* 2013). During steam explosion, the xylan is depolymerized to xylose, which subsequently is dehydrated to furfural. Furfural may then become repolymerized to an acid-insoluble pseudo-lignin, which increases the Klason lignin value (Kumar *et al.* 2013; Vivekanand *et al.* 2013). There is a clear correlation between increased severity, increased lignin content, and decreased xylan content (see Table 2). Although C6 sugars also may lead to the formation of pseudo-lignin (*via* HMF), C5 sugars (primarily xylose) are the main source of pseudo-lignin formation from bagasse because they are more labile under the pretreatment conditions (Ramos 2003), as demonstrated by the carbohydrate contents depicted in Table 2.

Lignin accumulation (Table 2) is accompanied by accumulation of carbon and loss of oxygen (Table 1). This may be attributed to the transformation of xylan (45.4% C) to more carbon-rich lignin (about 60% C; (Das 2011)) *via* furfural (62.5% C). Thus, steam explosion not only makes cellulose more accessible for subsequent biological processing; it also leads to substantial changes in the chemical composition of the raw material (Hu *et al.* 2012; Kumar *et al.* 2013; Sannigrahi *et al.* 2011; Vivekanand *et al.* 2012; Vivekanand *et al.* 2013).

Table 2. Carbohydrate Composition of Bagasse Samples

Pretreatment	Arabinan	Galactan	Glucan	Xylan	Lignin
Untreated	1.3 ± 0.2	0.3 ± 0.1	40.1 ± 5.2	17.1 ± 3.5	26.3 ± 1.2
195 °C (10 min)	0.7 ± 0.1	0.1 ± 0.0	39.7 ± 5.8	12.7 ± 1.5	26.5 ± 0.5
205 °C (10 min)	0.2 ± 0.1	0.1 ± 0.0	41.2 ± 8.2	7.2 ± 1.4	30.7 ± 0.3
215 °C (10 min)	0.1 ± 0.0	ND ± 0.0	38.0 ± 5.4	2.3 ± 0.3	36.0 ± 1.0
225 °C (10 min)	0.2 ± 0.0	ND ± 0.0	39.8* ± 0.7	1.8 ± 0.0	46.0 ± 0.3

Carbohydrate contents are expressed as percentage of dry matter. The amount of polysaccharides was calculated using the masses of anhydrous sugars. Enzymatic hydrolysis of this material in a reaction run at 50 g/L DM could theoretically yield $50 \times 0.398 \times 180 / 162 = 22.1$ g/L soluble glucose. Lignin was quantified gravimetrically as Klason lignin and includes pseudo-lignin.

Comparison of the FTIR spectra of untreated bagasse and the sample from the highest severity treatment (Fig. 1) shows that the spectra are similar for wave numbers higher than 1200 cm^{-1} . Absorbance in the $1000\text{-}1200\text{-cm}^{-1}$ region is characteristic of cellulose (Cao and Tan 2004). The spectra of the samples show clear differences in this region. Both have an absorbance maximum around 1032 cm^{-1} , but the absorbance is much stronger for the pretreated bagasse sample. This shows that the C-O and C-C bonds of cellulose were much more exposed after pretreatment, indicating more accessible cellulose (Zhang *et al.* 2011).

SEM images of untreated and steam-exploded (severity 4.6) samples revealed notable structural modifications following pretreatment (Fig. 2). While the untreated material had a compact and smooth morphology, the pretreated sample showed ruptured fibers and gave the impression of being more porous. The increased roughness of the surface was probably also due to the formation of pseudo-lignin, which is known to appear as spherical droplets on the surface of pretreated lignocelluloses (Sannigrahi *et al.*

2011; Kumar *et al.* 2013). The SEM pictures clearly show that pretreatment led to an increased surface area, which typically leads to higher enzyme efficiency due to the better access of enzymes to the substrate (Selig *et al.* 2007; Donohoe *et al.* 2008). However, pretreatment is a balance between more accessible fibers and pseudo-lignin formation, which has been reported to retard enzymatic cellulose degradation (Kumar *et al.* 2013).

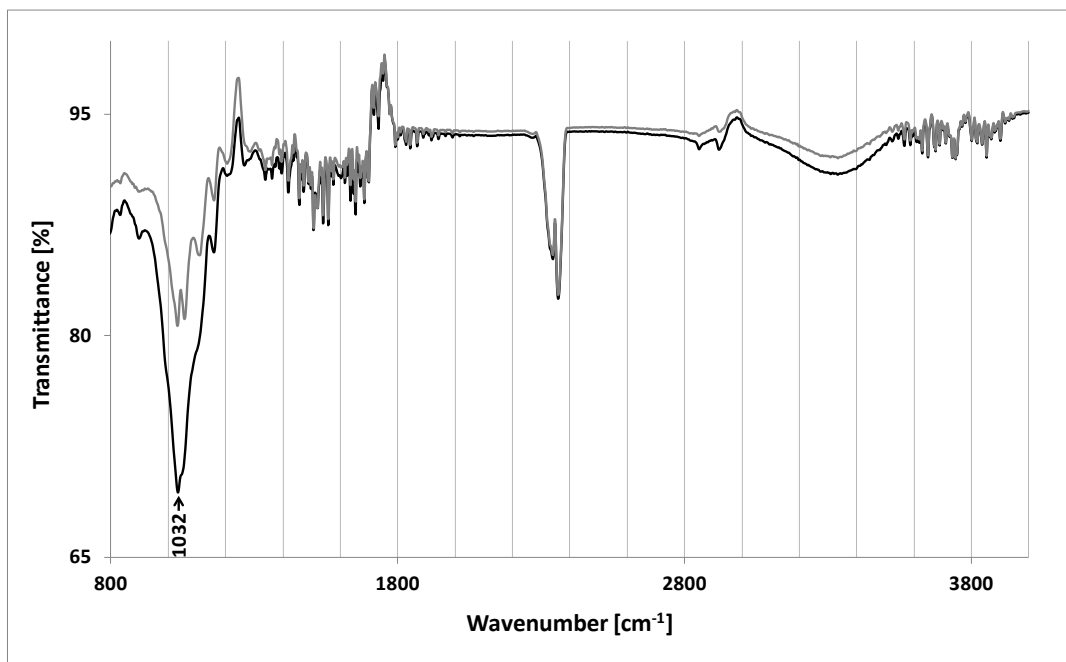


Fig. 1. FTIR analysis of bagasse. The figure shows the spectra recorded for untreated bagasse (gray line) and bagasse pretreated at severity 4.6 (black line).

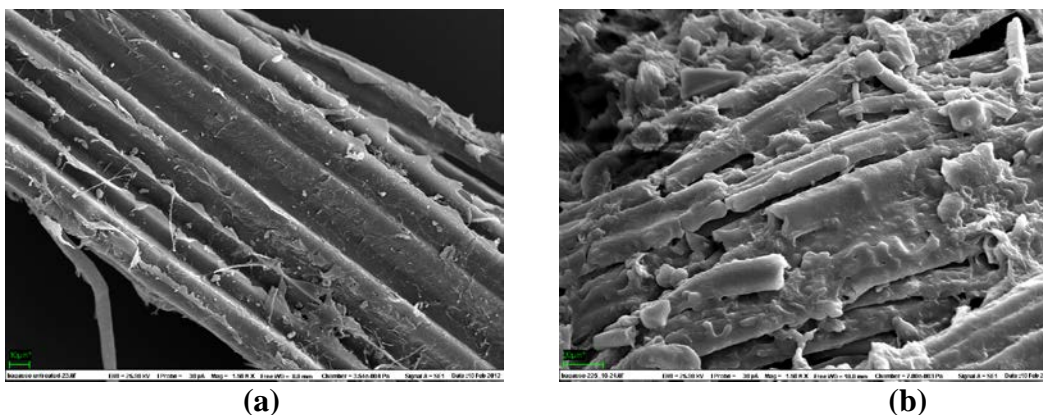


Fig. 2. Scanning electron microscopy images of (a) untreated bagasse and (b) steam-exploded bagasse (severity 4.6)

Enzymatic Saccharification

The pretreated bagasse samples were hydrolyzed using a commercial cellulase preparation (Cellic CTec2). Figure 3 shows the effect of pretreatment severity on enzymatic conversion of cellulose to glucose. In this set of experiments, the final glucose yield after hydrolysis increased with increasing severity. The two highest severities yielded solubilization of 93% of the cellulose. However, increasing the severity had little effect on initial glucose release, as monitored after 4 h of hydrolysis.

Using the same conditions, the material pretreated at the highest severity was saccharified on a larger scale (200 mL compared to 30 mL) to provide prehydrolyzed biomass for biogas tests (see below). In this case, the glucose yield after 24 h was lower (66 %) than it was with saccharification on a smaller scale (93 %). The reason for this might be less optimal mixing in the larger tubes, mass transfer limitations and/or less efficient oxygen transfer due to a smaller surface area/volume ratio. Current commercial cellulase preparations contain lytic polysaccharide monoxygenases, which require oxygen for their activity (Cannella *et al.* 2012; Horn *et al.* 2012).

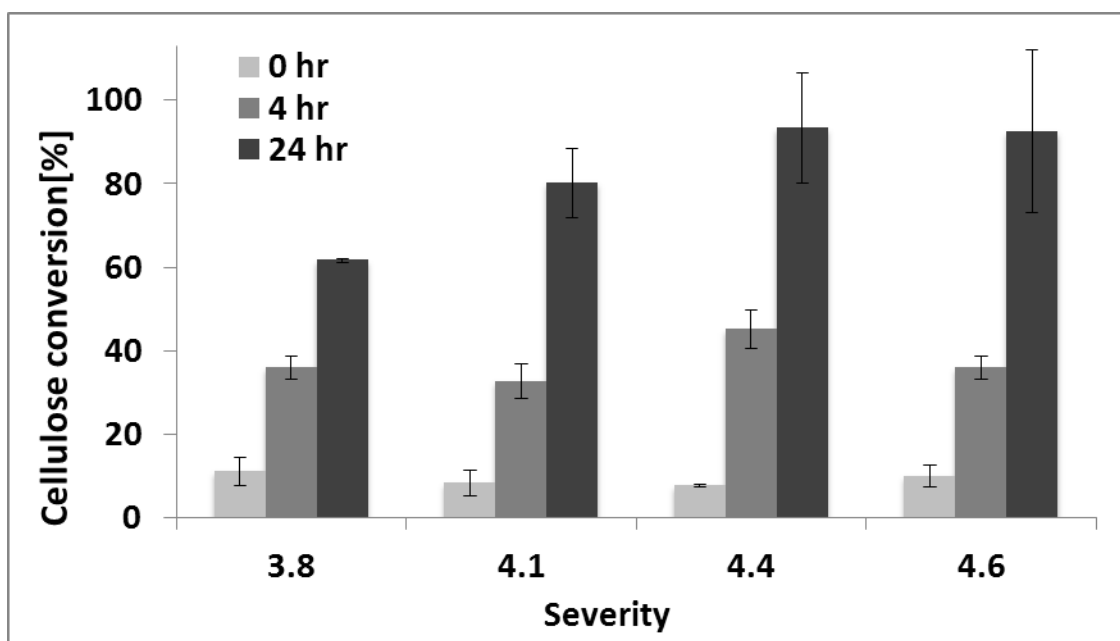


Fig. 3. Enzymatic conversion of cellulose to glucose from differently pretreated bagasse samples upon incubation with Cellic CTec2. The bars show % of maximum theoretical yield if all cellulose is converted to glucose. The figure shows average values derived from three independent experiments. Single-factor ANOVA analysis of the 24 h data set showed that $P=0.14$.

Biogas Production

Untreated and pretreated bagasse samples were anaerobically digested in batch flasks for 41 days. The final methane content in the produced biogas was in the range of 54 to 57% in all experiments, and the final pH and ammonium concentration were 7.2 to 7.4 and 1050 to 1150 ppm, respectively. Figure 4 shows the specific accumulated methane yields (endogenous methane production from the inoculum has been subtracted) from different samples during incubation (90 rpm, 37 °C). Flasks containing pretreated bagasse generally showed much quicker methane production than flasks containing untreated material. For example, the accumulated methane yield from the most severely pretreated material after 10 days (190 mL *per g* VS) was higher than the yield obtained from untreated material after 41 days (162 mL *per g* VS). In fact, in the former case, 88% of the total yield after 41 days was released after 10 days, as compared to 60% for untreated material. Compared to the untreated sample all pretreatments yielded significantly ($P<0.05$) more methane after 10 days of digestion. The highest final methane yield was 216 mL *per g* VS for the sample pretreated at severity 4.6, which is 1.3 times higher than the yield obtained for untreated bagasse. Regarding final methane yield this pretreatment was the only one significantly different from the untreated sample

($P < 0.05$). Methane yields of up to 260 mL *per g* VS of steam exploded bagasse have been reported, but these results were obtained with bagasse that had a lower lignin content than the biomass utilized here (De Paoli *et al.* 2011).

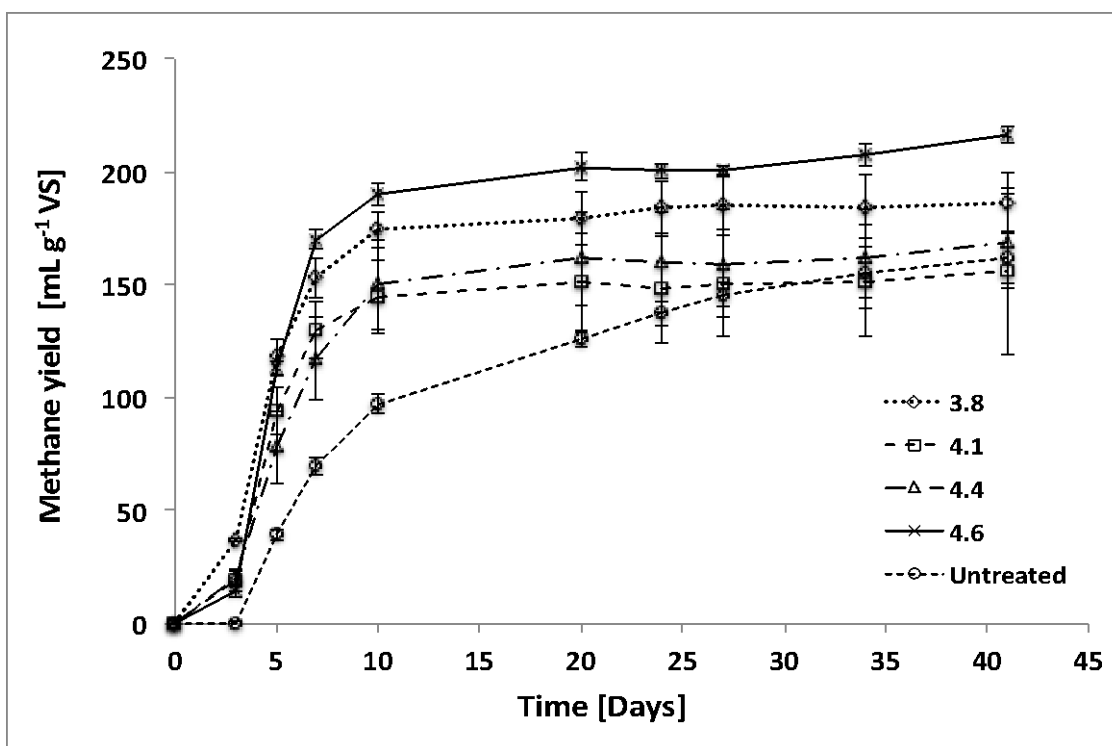


Fig. 4. Methane production from untreated and pretreated sugarcane bagasse. The figure shows cumulative methane yields for up to 41 days. The values represent the average from 3 independent experiments. Endogenous methane production from inoculum has been subtracted. Single-factor ANOVA analysis of the data set for final methane yield showed that $P=0.03$.

Notably, while the material pretreated at the highest severity of 4.6 showed the highest methane production rate and yield, the data did not show a straightforward correlation between these parameters. For example, the material pretreated at the lowest severity showed the second highest methane production rate and yield. This probably reflects the fact that both cellulose and xylan (Barakat *et al.* 2012) are substrates for biogas production, and the pretreatment affects these polysaccharides differently. Cellulose accessibility generally increased in the severity range used in this study, as demonstrated for saccharification efficiency (Fig. 3). Xylan, on the other hand, tended to be degraded to furfural and polymerized to pseudo-lignin, seen as xylan loss (Table 2) and lignin accumulation (Table 1). Thus, optimal pretreatment for biogas production is a balance between substrate accessibility and substrate degradation.

To obtain a glimpse of the potential of combined ethanol and biogas production, pretreated bagasse (severity 4.6) was enzymatically hydrolyzed and soluble sugars (glucose and xylose) were removed prior to biogas production. Figure 5 shows the methane yields obtained from steam-treated bagasse with and without enzymatic removal of sugars. As expected, the enzymatically-pretreated bagasse showed lower biogas production (124 mL *per g* VS) than the non-treated bagasse (216 mL *per g* VS). Analysis of the sugar-containing liquid removed from the enzymatically treated sample showed that 69% of the cellulose was removed as glucose, while 81% of the xylan was found as

soluble xylose. It should be noted that no attempts were made to inactivate residual enzymes in the enzymatically-pretreated bagasse. Thus enzymes might still have been active after thawing the frozen samples and may thus have contributed to extra sugar release during biogas production. However, this contribution is probably low due to non-optimal conditions for the enzymes in the biogas digester.

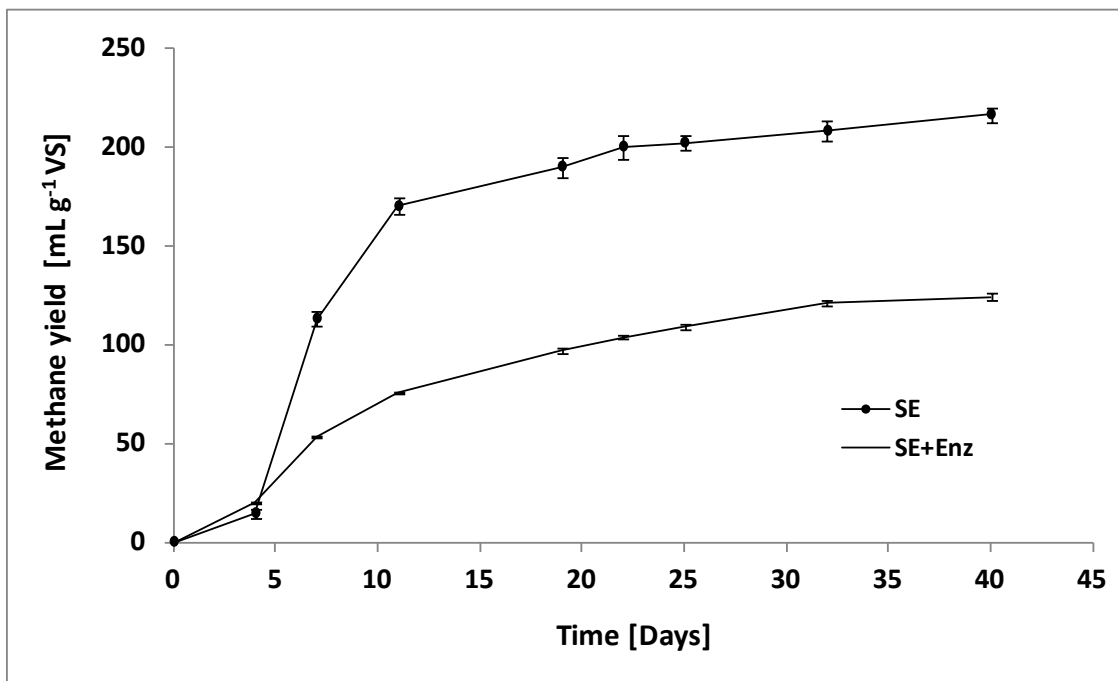


Fig. 5. Effect of sugar removal on methane production. The figure shows methane production from pretreated bagasse (severity 4.6) with and without prior enzymatic removal of sugars. The values represent the average from three independent experiments. Endogenous methane production from inoculum has been subtracted.

*SE: Steam Explosion, Enz: Enzyme

Using the figures from the above analyses, it can be calculated that anaerobic digestion of 1000 kg of pretreated bagasse would yield 7688 MJ of methane (Fig. 6). If sugars are removed enzymatically prior to anaerobic digestion, only 3030 MJ of methane will be produced. However, in this case, the extracted sugars may be fermented to 4083 MJ of ethanol. This implies that the total amount of heating power obtained in gas and liquid form is slightly (8%) higher in a methane-only process compared to a combined process. However, several other additional factors need to be taken into account. A combined biogas-ethanol process is a more complex and more expensive process, since *e.g.* cellulases and yeast are needed for ethanol production. Furthermore, while steam explosion is needed for efficient enzymatic saccharification and ethanol production, methane production may take place without such a pretreatment. However, while a process without pretreatment reduces the final methane yield by a modest 21 %, there is a large negative effect on the speed of the digestion process (Fig. 4). A faster digestion process will make it possible to design smaller digestion plants, which reduces costs, while the pretreatment step will add extra costs. All of these factors have to be considered when choosing the best process option.

In a similar experiment for steam-exploded oat straw, Dererie *et al.* (2011) estimated production of 9.5 MJ/kg for the combined process and 7.4 MJ/kg for methane alone. However, fermentation by yeast was carried out on the total saccharified fraction, and ethanol was removed by distillation (*i.e.*, no extraction of sugars). Thus, yeast cells, pentose sugars, and buffer components followed as additional substrates in the subsequent anaerobic digestion, which likely contributed to higher methane yields (Dererie *et al.* 2011). In another study, the energy from bagasse combustion (15.1 MJ/kg) was compared to the production of different energy carriers. In the best scenario, a combination of bioethanol production, biogas production, and residue combustion yielded 10.7 MJ/kg, where ethanol contributed 5.4 MJ/kg (Rabelo *et al.* 2011a).

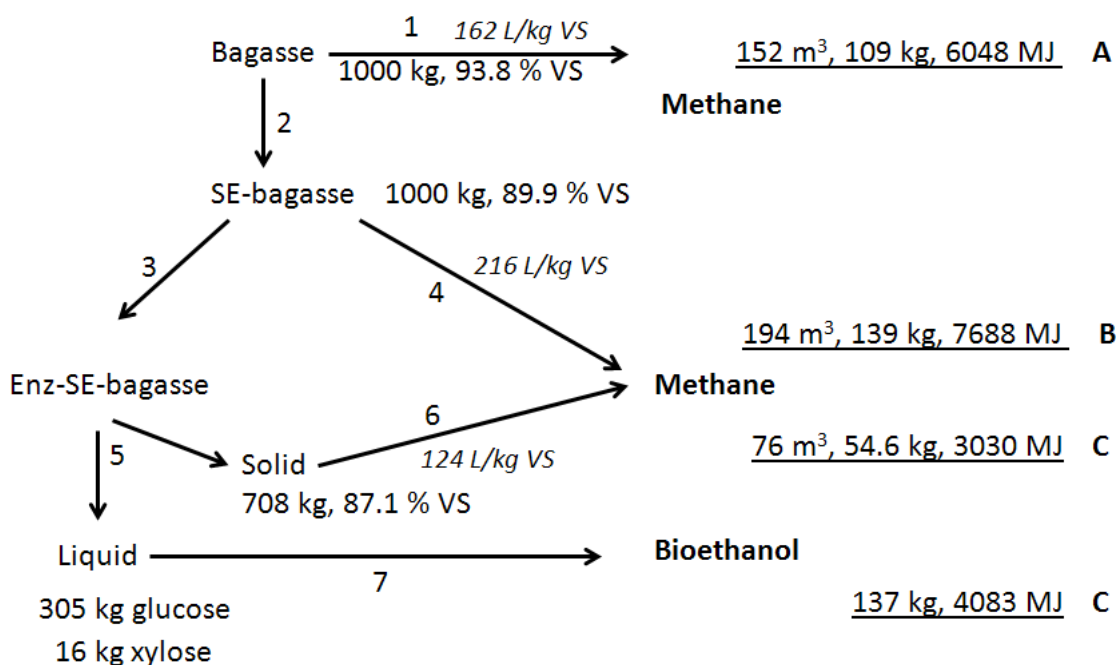


Fig. 6. Process scheme for methane production and combined methane and ethanol production. Three potential processes are depicted: A) methane production from untreated bagasse, B) methane production from steam-exploded bagasse, C) methane and ethanol production from steam exploded bagasse. The numbers indicate the following processes: 1) anaerobic digestion, 2) steam explosion; severity 4.6, 3) enzymatic saccharification, 4) anaerobic digestion, 5) solid/liquid separation, 6) anaerobic digestion, 7) fermentation (ethanol yield from glucose estimated at 0.45 kg/kg). Higher heating values for ethanol and methane are 29.8 and 55.5 MJ/kg, respectively.

CONCLUSIONS

1. This work shows that steam explosion is an efficient pretreatment of bagasse and that similar conditions may be used for optimizing both enzymatic saccharification and methane production.
2. All the pretreatments increased the speed of the anaerobic digestion process, but only the most severe pretreatment yielded significantly higher final methane yield than the untreated biomass.

- Finding the optimal energy mix to be derived from bagasse (heat, methane, ethanol) requires further studies and will depend on factors such as pretreatment costs, plant size, and the selling price of methane and ethanol.

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