Hydrothermal processing and enzymatic hydrolysis of sorghum bagasse for fermentable carbohydrates production

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Abstract

Untreated and hydrothermally treated sorghum bagasse (SB) was hydrolyzed to simple sugars by the synergistic action of cellulases and hemicellulases produced by the fungi *Fusarium oxysporum* and *Neurospora crassa*. Synergism between the two lignocellulolytic systems was maximized with the application of higher fraction of *N. crassa* enzymes.

Hydrothermolysis of SB was studied at a wide range of treatment times and temperatures. At intense pretreatment conditions (210ºC for 20 min; $logR_0$=4.54), the residual hemicellulose percentage was 17.45%, while formation of inhibitory products, 5-hydromethyl-furfural (HMF), furfural, acetic and formic acid, (0.21, 0.51, 3.36 and 1.80 g/l, respectively) remained in acceptable levels.

Maximum conversion of cellulose and total polysaccharides of the untreated SB were 23.18% and 18.79% respectively. Combining hydrothermal treatment and enzymatic hydrolysis of released oligosaccharides and insoluble solids resulted in improvement of cellulose (approximately 15% increase) and total polysaccharides (2-fold) hydrolysis compared to that of untreated SB.

Keywords: Sorghum bagasse; cellulases; hemicellulases; hydrothermal pretreatment; severity factor; enzymatic hydrolysis; cross-synergism

1. Introduction

Lignocellulosic biomass such as agricultural and forestry residues and dedicated crops provides a low-cost and uniquely sustainable resource for production of many organic fuels and chemicals that can reduce greenhouse gas emissions, enhance energy security, improve the economy, dispose of problematic solid wastes, and improve air quality (Wyman, 2003).

Processing of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/purification (Taherzadeh and Karimi, 2008). The inherent properties of native lignocellulosic materials make them resistant to enzymatic attack. In general, two approaches aimed at enhancing lignocelluloses degradation can be considered: (a) pretreatment of the substrate (Taherzadeh and Karimi, 2008; Jørgensen et al., 2007) and (b) the use of "advanced" enzyme formulations that act synergistically on lignocellulosic biomass (Selig et al., 2008; Kumar and Wyman, 2009).

Physical, physico-chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials (Taherzadeh and Karimi, 2008; Jørgensen et al., 2007). Among the different physico-chemical pretreatments liquid hot water (LHW) is one of the hydrothermal pretreatment methods applied for pretreatment of lignocellulosic materials since several decades ago in e.g. pulp industries. The major advantages are no addition of chemicals different from water thus making the whole process environment-friendly. Furthermore, hemicelluloses can be converted into hemicellulosic sugars at good yields with low by-product generation, leading to solutions of sugar oligomers and/or sugars that can be utilized for a variety of practical purposes (Taherzadeh and Karimi, 2008).

Several studies have been carried out on hydrothermal treatment as a method of pretreating lignocellulosic materials like wheat straw (Kabel et al., 2007; Kaparaju et al., 2009; Østergaard-Petersen et al., 2009), corn stover (Öhgren et al., 2006, 2007), sugar cane bagasse (Laser et al., 2002), switchgrass (Hu and Wen 2008; Suryawati et al., 2008), barley husk (Palmarola-Adrados et al., 2005) and wood (Söderström et al., 2004).

Enzymatic hydrolysis of pretreated lignocellulosic materials is carried out using mainly commercial cellulases. The use of enzyme formulations containing ''accessory'' enzymes to degrade any remaining hemicelluloses and synergize with cellulases which are typically responsible for a significant portion of sugar production during the enzymatic conversion of biomass is another approach aiming at enhancing degradation of biomass polysaccharides. Synergism between cellulase with non-cellulase enzymes, including primarily hemicellulases has be reported in the hydrolysis of lignocellulosic materials (Selig et al., 2008; Kumar and Wyman, 2009).

Utilization of sorghum bagasse (SB), the solid residue obtained after extraction of sugars from sweet sorghum stalks, is important for the economy of the global use of the crop. Scenarios for SB exploitation are based on enzymatic hydrolysis of polysaccharides and co-fermentation of glucose and xylose to ethanol (Gnansounou et al., 2005; Dogaris et al., 2009). However, previous attempts to take advantage of this valuable bioethanol industry derivative were associated with relatively low ethanol yields (Dogaris et al., 2009; Ballesteros et al., 2004).

The main objective of the present work was to improve depolymerization of lignocellulosic components from sorghum bagasse in order to create a suitable source of sugars for subsequent conversion to valuable bio-based products. Maximization of biopolymers disintegration was attempted by applying the enzymatic systems derived from ethanol producing fungi (*N. crassa* and *F. oxysporum*), the chemical-free hydrothermal pretreatment method, as well as their combination.

2. Materials and Methods

2.1. Microorganisms

The wild-type strain F3 of *Fusarium oxysporum* (Christakopoulos et al., 1989) and *Neurospora crassa* DSM 1129 from Deutsche Sammlung von Microorganismen und Zellkulturen GmbH (Germany) were the microorganisms used in the present study.

2.2. Reagents and solid substrates

Carboxymethyl cellulose, birchwood xylan, Avicel and *p*-nitrophenyl glycosides were obtained from Sigma Chemical (St Louis, MO, USA). All other chemicals were analytical grade. Wheat straw (WS), wheat bran (WB), corn cobs (CC) and sorghum bagasse (SB) were prepared as described (Dogaris et al., 2009).

2.3. Preparation of enzymes

Submerged culture of alkali-treated CC and WB (3% and 0.3% w/v, respectively) was used for enzyme production by *F. oxysporum* (Mamma et al., 1996), while the enzymatic system of *N. crassa* was produced under solid state cultivation of WS and WB (5:1 w/w ratio) (Dogaris et al., 2009). Following removal of suspended materials and fungal biomass, enzymes were concentrated by ultrafiltration (Amicon chamber 8400) using a Diaflo PM10 membrane with 10 kDa exclusion size (Millipore, Billerica, USA) and used in the subsequent experiments. Main activities of the cellulolytic and hemicellylolytic systems obtained from *N. crassa* DSM 1129 and *F. oxysporum* F3 are presented on Table 1.

2.4. Enzyme assays

Endoglucanase (1,4-*β*-D-glucan 4-glucanohydrolase EC 3.2.1.4), cellobiohydrolase (1,4-*β*-D-glucan cellobiohydrolase EC 3.2.1.91) and β-1,4-endoxylanase (1,4-*β*-D-xylan xylanohydrolase EC 3.2.1.8) activities were assayed on carboxymethyl cellulose, Avicel and birchwood xylan, respectively (Dogaris et al., 2009). Total cellulase activity was measured against filter paper by the standard IUPAC method (Ghose, 1987). The activities of *β*-glucosidase (*β*-D-glucoside glucohydrolase, EC 3.2.1.21) and *β*-xylosidase (1,4-*β*-D-xylan xylohydrolase, EC 3.2.1.37) were determined spectrophotometrically using the related *p*-nitrophenyl glycosides (Dogaris et al., 2009). Enzyme activities from *F. oxysporum* and *N. crassa* were assayed at 50ºC and pH 6.0 and 5.0, respectively. Inactivated enzyme samples (after boiling at 100 °C for 15 min) were used as reference.

2.5. Enzymatic hydrolysis of SB

The enzymatic systems produced by *N. crassa* [E1] and *F. oxysporum* [E5], as well as mixtures of these systems where the *N. crassa* to *F. oxysporum* ratio varied (75:25 [E2]; 50:50 [E3]; 25:75 [E4]), were applied for hydrolysis of raw and pretreated SB. Reaction conditions were as described elsewhere (Dogaris et al., 2009). Briefly, the enzyme preparations (60 U of endoglucanase activity) supplemented a suspension of SB (0.8 g) in 50 mM citrate-phosphate buffer, pH 5.5 (10 ml final reaction volume). Hydrolysis was carried out at 30 ± 1°C and 250 rpm. Samples were withdrawn periodically, centrifuged (10000x g for 10 min), and analysed for sugars. Experiments were carried out in duplicate.

The experimental data obtained from SB hydrolysis were fitted to the model proposed by Holtzapple et al. (1984):

$$x = x_{max} \cdot \left(\frac{t}{t_{1/2} + t} \right) \tag{1}$$

where x is the percentage degree of conversion of polysaccharides (cellulose or total polysaccharides) to simple sugars (saccharification), x_{max} is the maximum degree of saccharification, t is the time of enzymatic saccharification, and $t_{1/2}$ is the time required to achieve 50% of x_{max}.

The following equations were used for cross-synergism calculations:

$$(synergism)_j = \frac{m_{sj}}{m_{CLj}} \tag{2}$$

Where m_{sj} is the degree of cellulose or total polysaccharides hydrolysis of cellulase mixture j, while m_{CLj} is given by:

$$m_{CLj} = \sum_{i=1,2} r_{ij} m_i \tag{3}$$

Where r_{ij} is the volume ratio of enzymes i in mixture j and m_i is the degree of cellulose or total polysaccharides hydrolysis of cellulase i.

2.6. Hydrothermal pretreatment of SB

Microwave digestion equipment (speed wave™ MWS-2, Berghof Instruments GmBH, Germany) was employed for substrate pretreatment. The system was equipped with an IR sensor, which enabled remote, contact-free and thus absolutely contamination-free temperature determination. The microwave power was set at 700 W. The charge of solids, measured by the liquor to solid ratio (LSR), was kept at 6.7 g g^{-1}. The experimental settings of hydrothermal pretreatment, temperature and pretreatment time, were: 160°C (10, 20, 30 min), 180°C (10, 20, 30 min), 200°C (3, 5, 8, 10, 12, 15, 20, 25, 30 min) and 210°C (10, 20, 30 min). The combined effect of both variables, that is a measure of the intensity in a given treatment, was estimated by the severity factor R$_0$ (Overend and Chornet, 1989), which is defined by the following equation:

$$\log(R_0) = \log\left(t \cdot \exp\left(\frac{T - 100}{14.75} \right) \right) \tag{4}$$

where t: reaction time (min) and T: temperature (°C).

2.7. Crystallinity index (CrI)

Modification of the crystallinity index (CrI) of cellulose after hydrothermal treatment of sorghum bagasse was determined by an X-ray Diffractometer (Siemens D5000), as described elsewhere (Kalantzi et al, 2008).

2.8. Carbohydrates analysis

Total reducing sugars were estimated by the 3,5-dinitrosalicylic acid method (Miller, 1959). High performance anion-exchange chromatography (HPAEC) was applied for carbohydrates analysis. The equipment consisted of a quaternary gradient pump (Jasco PU-1580I, Jasco Ltd, UK) and a Rheodyne injector controlled by a Borwin software. Separation of sugars was accomplished with a CarboPack PA1, (4x250 mm) column equipped with a guard column (Dionex Corporation, USA). For mono- and di-saccharides analysis (glucose, galactose, xylose, arabinose, cellobiose and xylobiose) the column was eluted isocratically with 17.5 mM NaOH at a flow rate of 1 ml/min. For xylo-oligosaccharides analysis the column was eluted at 1 ml·min^{-1} with Solvent A: 60 mM NaOH and solvent B: 60 mM NaOH containing 300 mM sodium acetate, using a linear gradient. In both cases eluted sugars were monitored by a pulse amperometric detector (HPAEC-PAD) (Dionex Corporation, USA). Fucose (50 μM) was added as internal standard. All analyses were performed in duplicate.

2.9. Degradation products

5-Hydroxymethyl-2-furfural (5-HMF) and furfural, as well as formic and acetic acid, which have been observed among the degradation products of lignocellulose, were quantified by an HPLC system (Jasco PU987, USA). Furfurals were determined according to a modified method of Ameur et al. (2006) using a C_{18} reversed phase column (Nucleosil 100-5, 250x4.6 mm, Macherey-Nagel, Germany) and a UV detector that was set at 280 nm (Waters 486). Mobile phase consisted of methanol/water (20/80) and the flow rate was adjusted to 1 ml·min^{-1}.

Organic acids were analyzed using an Aminex HPX-87H (Bio-Rad, USA) ion moderated partition chromatography column, in combination with a refractive index

detector (Waters 410). The flow rate of the mobile phase (5 mM H_2SO_4) was adjusted at 0.6 $ml \cdot min^{-1}$ and the temperature at 50 °C (Dogaris et al., 2009).

2.10. Cellulose and hemicellulose determination of pretreated SB

Carbohydrate content (cellulose and hemicellulose) of pretreated materials was determined by the NREL protocol (Sluiter, 2006).

3. Results and Discussion

3.1. Enzymatic hydrolysis of untreated SB

Sorghum baggase (SB), the solid residue obtained after extraction of simple sugars from sweet sorghum stalks is rich in cellulose (40.4%, w/w) and hemicellulose (35.5%, w/w) (Dogaris et al., 2009). The concentrations of the main carbohydrates released from SB by *N. crassa* and *F. oxysporum* multi-enzyme lignocellulose degrading systems and their combinations, are cited in Table 2. Regardless of the composition of the enzymatic mixture, glucose was the major monosaccharide determined during SB hydrolysis, which in conjunction with the low level of final cellobiose concentration, indicate inclusive cellulolytic systems for both fungi. Cellulases from *F. oxysporum* (E5) compose a more efficient system for cellulose depolymerization than the corresponding system produced by *N. crassa* (E1). The concentration of glucose that was liberated with the application of E5 was approximately 60% higher than the corresponding value obtained with E1, which could be associated with the relatively higher cellobiohydrolase activity in *F. oxysporum* blend of cellulases. The importance of cellobiohydrolase activity during hydrolysis of defined or complex cellulosic materials is well documented (Lynd et al, 2002).

The group of non-cellulosic carbohydrates detected in enzymatic hydrolysates of SB included xylose, arabinose and galactose, since arabinoxylan comprises the major hemicellulosic component of this agricultural by-product, and is accompanied by various types of galactan (Goto et al., 1991). The amount of simple sugars released from SB by *F. oxysporum* hemicellulolytic system was 36% higher than the corresponding value achieved with the application of *N. crassa* hemicellulases. Low molecular weight oligosaccharides with more than two xylose units could not be detected in hydrolysates. However, substantial accumulation of xylobiose was recorded during treatment of SB by both fungal systems and their combinations, ranging between 3.5 and 6.3 g/l, after 51 h of hydrolysis. Increased amounts of xylobiose during hydrolysis of SB by *N. crassa* enzymatic system has been related to inefficiency of *β*-xylosidase activity (Dogaris et al., 2009).

Synergism between the two lignocellulolytic systems examined in the present study was recorded during enzymatic depolymerization of SB (Table 3). Hydrolysis of cellulose was carried out much more effectively (up to 38%) when both fungal systems were present. This is in accordance with previous results where synergy between *F. oxysporum* and *N. crassa* cellulases was reported during hydrolysis of cellulosic substrates, such as ball-milled Avicell, cotton and filter paper (Tarantili et al., 1996). Similar values were estimated when the degree of synergy was quantified with respect to the amount of total polysaccharides released. The level of synergism was maximized with the application of higher fraction of *N. crassa* enzymes (system E2) (Table 3).

Fitting the hyperbolic model of cellulose hydrolysis (Eq. 1) to the experimental data obtained during treatment of SB by *F. oxysporum* and *N. crassa* systems was also attempted. The values of x_{max} and $t_{1/2}$ that were estimated for the enzymatic

combinations examined in the present study are presented in Table 4. Based on the correlation coefficient values ($R^2 > 0.96$), the proposed model can be used efficiently to predict the maximum degree of cellulose and total polysaccharides conversion. In accordance with previous observations, the synergistic effects between the two systems were reflected in the x_{max} values obtained for their combinations. As it can be concluded from $t_{1/2}$ values, in addition to increased conversion of SB, the application of *F. oxysporum* and *N. crassa* mixtures led to faster hydrolysis of substrate, in comparison with single-enzymatic system treatments. Combining equivalent amounts of the two systems (E3) could substantially reduce (more than 50%) the time required for SB cellulose hydrolysis by separate components.

3.2. Hydrothermal pretreatment of SB

A wide range of treatment times and temperatures were applied for hydrothermal processing of SB. The effect of the intensity of pretreatment, which can be measured by the severity factor ($logR_0$), on the outcome of the process, is presented in Figure 1. The fraction of solubilized substrate was insignificant at $logR_0$ values lower than 3.5, but amplified radically when higher intensity treatments were implemented. The same pattern was observed for liberation of total reducing sugars. Sigmoidal equations (Eqs 5-6) could describe efficiently the dependence of SB solubilization and concentration of released reducing sugars on the severity factor of the hydrothermal process:

$$WL = \frac{42.0919}{\left(1 + e^{-\left(\frac{SF - 3.8452}{0.3104}\right)}\right)} \qquad \text{Eq. 5}$$

($R^2 = 0.90$, standard error of estimates=4.7, P<0.0001)

$$TRS = \frac{13.8982}{\left(1 + e^{-\left(\frac{SF - 3.9022}{0.1409}\right)}\right)}$$ Eq. 6

(R²=0.90, standard error of estimates=2.2, P<0.0001)

where *WL* is the solubilization of initial SB (%, w/w), *TRS* is total reducing sugars yield (g per 100 g of SB) and *SF* is the severity factor (logR_0).

Similar findings have been reported for the effect of the severity factor on solubilization of xylan after hydrothermal treatment of raw materials such as agricultural by-products (corn stalks, bagasse) and poplar wood (*Populus tremoloides* and *Populus deltoides*) (Garrote et al., 1999).

Compositional analysis of the fiber fraction resulted after hydrothermal treatment showed that the level of cellulose depolymerization was relatively low, while extensive hydrolysis of hemicellulose was recorded at the harsher pretreatment (210°C for 20 min; logR_0=4.54) (Table 5). Hemicellulose hydrolysis resulted in a large number of xylo-oligosaccharides at the liquid fraction, after hydrothermal treatment. The pattern of released xylooligosaccharides shifted towards lower molecular weight products with the application of more intense pretreatment conditions (210°C for 20 min; logR_0=4.54), although complete depolymerization to xylose was not possible (Figure 2). Xylobiose was the major product of SB hydrolysis with a maximal yield (approximately 5.20 g/l) observed when most severe conditions were implemented (210°C for 20 min; logR_0=4.54). Carasco et al., (1994) reported 5% degradation of cellulose after pretreatment of SB at 230°C for 0.5 min (logR_0=3.53), while steam exploded SB at 210°C for 2 min (logR_0=3.54) resulted in 20% cellulose solubilization (Ballesteros et al., 2004). Furthermore, Sipos et al.,

(2009) reported 6.3% and 71.7% cellulose and hemicellulose degradation respectively after steam explosion of SB at 200°C for 5 min ($logR_0$=3.64).

The concentrations of the major inhibitors formed during hydrothermal pretreatment of SB, in relation to the severity of the process, are depicted in Figure 3. The amounts of inhibitory products increased drastically when harsher pretreatments were applied ($logR_0$>4.25). Maximal production of inhibitors was observed at the most intense conditions examined (210°C for 20 min; $logR_0$=4.54). The highest concentrations of HMF (0.21 g/l), furfural (0.51 g/l), formic acid (1.80 g/l) and acetic acid (3.36 g/l) correspond to 0.14 g, 0.34 g, 1.20 g and 2.24 g per 100 g of SB, respectively. In accordance with the relatively lower degree of cellulose hydrolysis, the concentrations of HMF were lower than the corresponding values observed for furfural. The values of inhibitors concentrations achieved in the present study were lower than those reported for steam-pretreated wheat straw (Kabel et al., 2005), corn stover (Öhgren et al., 2006, 2007), softwood (Söderström et al., 2004), switchgrass (Suryawati et al., 2008) and barley husk (Palmarola-Adrados et al., 2005).

The crystallinity of raw SB was found 48.16% and increased to 58.38% at the harsher pretreatment conditions (210°C, 20min, $logR_0$=4.54) indicating significant structural variations in response to compositional changes. Removal of amorphous materials (lignin, hemicelluloses) from lignocellulosics and rearrangement of remaining components have been associated with improved crystallinity (Öztürk et al., 2009). In accordance with previous findings, higher CrI values were observed following steam explosion of sweet sorghum bagasse (Carasco et al., 1994). Furthermore, Inoue et al., (2008) reported increased crystallinity for eucalyptus chips treated with hot-compressed water, while similar results were obtained for delignified corn stover (Kim and Holtzapple, 2006).

3.3. Combined hydrothermal pretreatment and enzymatic hydrolysis of SB

The proposed method included an initial step of SB hydrothermal treatment followed by simultaneous hydrolysis of released oligosaccharides and insoluble solids with the application of *N. crassa* and *F. oxysporum* enzymatic systems. Pretreatment conditions (210°C for 20 min, $logR_0$=4.54) were selected so as to attain the highest degree of substrate solubilization and production of sugars, while maintaining acceptable levels of degradation products.

Implementation of the combined scheme was associated with improvement of substrate hydrolysis (Table 3). Regardless of the enzymatic mixture examined, the level of liberated sugars from pretreated SB was considerably higher (up to 100% increase) than the corresponding values observed for untreated substrate. In accordance with previous findings presented for raw SB hydrolysis, the enzymatic system produced by *F. oxysporum* exhibited superior performance. The concentration of reducing sugars released by system E5 (*F. oxysporum* system) was 20.5 g/l, while the corresponding value obtained with the application of *N. crassa* system (E1) was 15.8 g/l. Cross-synergism between the two fungal systems could be also detected for hydrolysis of hydrothermally treated SB. The highest level of synergy (1.41), which was observed for liberation of sugars by system E3, was higher than the corresponding value achieved with untreated substrate (1.30 for system E2) (Table 3). Hydrothermal pretreatments of raw materials improve their behavior as substrates for enzymatic hydrolysis due to favorable structural modifications including increased available surface area and higher pore volume (Garrote et al., 1999).

Maximal values of cellulose and total polysaccharides conversion after enzymatic hydrolysis of pretreated SB were 26.7 and 36.8% respectively. This is in contrast to the findings regarding hydrolysis of untreated SB, where higher conversion of

cellulose, as compared to total polysaccharides, was recorded (Table 3). Despite the higher CrI values determined for hydrothermally treated samples, hydrolysis of cellulose was only marginally affected. The results agree with those reported by Kim and Holtzapple (2006) where the increased crystallinity of pretreated corn stover had only minor effects on subsequent enzymatic hydrolysis.

Carbohydrates analysis at the end of the combined process showed that the majority of products derived from hemicellulose depolymerization (Table 6). The major sugars detected in hydrolysates of pretreated SB were xylose and xylobiose, at concentrations as high as 2.78 g/l and 9.85 g/l, respectively. The results demonstrated the suitability of both systems to convert the xylooligosaccharides released from hydrothermal pretreatment of SB into simpler assimilable sugars.

The combined process involving hydrothermal treatment of SB followed by simultaneous hydrolysis of released oligosaccharides and insoluble solids resulted in significant improvement of cellulose (approximately 15% increase) and total polysaccharides (2-fold) hydrolysis compared to that of untreated SB.

The results obtained in the present study compare favorably to those described for various sorghum bagasse pretreatments. Carrasco et al. (1994) reported much lower yields (approximately 26% solubilization of solids based on initial cellulose and xylan), following acid pretreatment of SB for 120 min. Similar levels of solids solubilization (37.5%) has been reported after steam explosion of the same substrate at 210 ºC for 2 min (Ballesteros et al. 2004). Furthermore, the results of the present study are comparable to those reported in literature for other pretreated substrates since the higher saccharification yields are obtained using uneconomically high cellulase loadings (10-30 FPU/g of substrate) supplemented in several cases with *β*-glucosidase (20-60 Units/g of substrate) and at lower solid content (Kabel et al., 2007;

Østergaard Petersen et al., 2009; Öhgren et al., 2006; Hu and Wen, 2008; Söderström et al., 2004).

4. Conclusions

The lignocellulolytic systems produced by *F. oxysporum* and *N. crassa* could act synergistically for effective disintegration of sorghum bagasse carbohydrates. Hydrothermal treatment of raw sorghum bagasse resulted in extended hemicellulose solubilization. Despite significant compositional changes and structural variations, the concentration of inhibitors formed during the process was low.

The combined process involving hydrothermolysis of SB followed by enzymatic hydrolysis of released oligosaccharides and insoluble solids resulted in improvement of cellulose (approximately 15% increase) and total polysaccharides (2-fold) hydrolysis compared to untreated SB. Both enzymatic systems could be used at economically low titers for conversion of agroindustrial by-products into simple sugars.

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Table 1. Enzymatic activities of *F. oxysporum* F3 and *N. crassa* DSM 1129.

Enzyme	Activity (Units/ml)	
	N. crassa DSM 1129	*F. oxysporum* F3
Endoglucanase	69.41	26.45
Exoglucanase	0.15	0.61
β-Glucosidase	6.76	3.70
Filter Paper Activity	0.56	0.79
Endoxylanase	54.05	73.60
β-Xylosidase	0.04	0.07

Table 2. Mono- and di-saccharides release after 51 hours hydrolysis of untreated SB by the synergistic action of *N. crassa* DSM 1129 and *F. oxysporum* F3 enzymatic systems.

Coded	Mono- and di-saccharides concentration (g/l)					
name	Arabinose	Xylose	Galactose	Glucose	Xylobiose	Cellobiose
E1	0.47 ± 0.01	0.32 ± 0.01	0.14 ± 0.02	5.35 ± 0.01	3.53 ± 0.32	n.d.*
E2	0.61 ± 0.01	0.82 ± 0.04	0.18 ± 0.01	8.29 ± 0.12	5.15 ± 0.15	0.09 ± 0.00
E3	0.64 ± 0.03	0.89 ± 0.03	0.21 ± 0.00	8.22 ± 0.24	6.34 ± 1.57	n.d.
E4	0.75 ± 0.09	1.18 ± 0.13	0.29 ± 0.02	8.32 ± 0.31	5.52 ± 0.69	0.12 ± 0.05
E5	0.73 ± 0.05	1.11 ± 0.32	0.30 ± 0.06	7.91 ± 0.05	3.92 ± 1.49	0.14 ± 0.04

Conditions: SB concentration 8% (w/v), hydrolysis temperature 30ºC, hydrolysis time 51 h.

*n.d.: not detected

Data are means ± SD of three independent experiments

Coded names: *N. crassa* to *F. oxysporum* ratio 100:0 [E1]; 75:25 [E2]; 50:50 [E3]; 25:75 [E4]; 0:100 [E5]

Table 3. Cross-synergism in cellulose and total polysaccharides hydrolysis for the untreated and hydrothermally treated SB.

Coded name	Enzymatic hydrolysis of untreated SB				Hydrothermal treatment of SB+enzymatic hydrolysis			
	Hydrolysis of:		Synergism in the hydrolysis of:		Hydrolysis of:		Synergism in the hydrolysis of:	
	Cellulose	Total polysaccharides	Cellulose	Total polysaccharides	Cellulose	Total polysaccharides	Cellulose	Total polysaccharides
E1	14.69 ± 0.01	11.35 ± 0.01	-	-	19.54 ± 0.68	22.69 ± 0.74	-	-
E2	22.88 ± 0.30	17.00 ± 0.15	1.38 ± 0.02	1.30 ± 0.01	25.14 ± 0.86	33.46 ± 0.00	1.18 ± 0.16	1.37 ± 0.02
E3	22.57 ± 0.76	18.31 ± 0.30	1.23 ± 0.05	1.24 ± 0.03	26.52 ± 0.00	36.77 ± 0.64	1.15 ± 0.02	1.41 ± 0.18
E4	23.18 ± 0.99	18.79 ± 0.17	1.14 ± 0.04	1.14 ± 0.02	26.69 ± 0.92	31.14 ± 0.44	1.08 ± 0.11	1.12 ± 0.07
E5	22.12 ± 0.24	18.12 ± 0.21	-	-	26.52 ± 0.02	29.50 ± 0.79	-	-

Data are means $\pm$ SD of three independent experiments

Coded names: *N. crassa* to *F. oxysporum* ratio 100:0 [E1]; 75:25 [E2]; 50:50 [E3]; 25:75 [E4]; 0:100 [E5]

Table 4. Fitted X_{max} and $t_{1/2}$ values for untreated SB hydrolysis.

Coded name	Cellulose		Total polysaccharides	
	x_{max} *(%)*	$t_{1/2}$ *(h)*	x_{max} *(%)*	$t_{1/2}$ *(h)*
E1	18.87 ± 0.02	16.06 ± 0.02	12.93 ± 0.02	9.61 ± 0.04
E2	24.21 ± 0.81	5.19 ± 0.67	18.06 ± 0.49	3.17 ± 0.06
E3	24.13 ± 0.86	5.13 ± 0.42	19.43 ± 0.39	3.20 ± 0.08
E4	25.41 ± 0.81	6.18 ± 0.29	20.00 ± 0.71	3.60 ± 0.61
E5	29.66 ± 0.27	18.37 ± 0.35	18.82 ± 0.25	2.92 ± 0.05

Coded names: *N. crassa* to *F. oxysporum* ratio 100:0 [E1]; 75:25 [E2]; 50:50 [E3]; 25:75 [E4]; 0:100 [E5]

Table 5. Composition of fiber fraction of the pretreated sorghum bagasse.

Pretreatment		$logR_0$	Carbohydrate content (%, w/w)	
Temperature (ºC)	Time (min)		Cellulose[a]	Hemicellulose[b]
160	20	3.07	40.52 (96.14 %)	28.42 (76.72 %)
180	20	3.66	40.81 (94.04 %)	29.20 (76.59 %)
200	20	4.25	53.91 (90.12 %)	20.84 (39.59 %)
210	20	4.54	58.36 (88.66 %)	10.05 (17.45%)

[a] estimated from the amount of glucose, [b] estimated from the amount of xylose, arabinose, galactose and acetate content

Data are means of three independent experiments and the standard deviation was below 5% in all cases.

Data in parenthesis show the residual percentages based on their mass in raw bagasse

Table 6. Mono- and di-saccarides release after 96 hours hydrolysis of hydrothermally treated SB by the synergistic action of *N. crassa* DSM 1129 and *F. oxysporum* F3 enzymatic systems.

Coded	Mono- and di-saccharides concentration (g/l)					
name	Arabinose	Xylose	Galactose	Glucose	Xylobiose	Cellobiose
E1	0.96 ± 0.15	1.28 ± 0.20	0.49 ± 0.09	7.12 ± 0.25	5.33 ± 0.82	0.32 ± 0.05
E2	0.92 ± 0.10	2.40 ± 0.25	0.53 ± 0.06	9.16 ± 0.45	9.85 ± 0.55	0.49 ± 0.05
E3	1.20 ± 0.11	2.47 ± 0.22	0.58 ± 0.05	9.34 ± 0.06	6.84 ± 0.61	0.44 ± 0.04
E4	1.40 ± 0.36	2.43 ± 0.63	0.60 ± 0.09	9.39 ± 0.16	4.80 ± 0.74	0.30 ± 0.08
E5	1.46 ± 0.26	2.78 ± 0.49	0.75 ± 0.07	9.01 ± 0.40	5.14 ± 0.35	0.33 ± 0.06

Conditions: Hydrothermal treatment: 210ºC for 20 min (logR$_0$=4.54), enzymatic hydrolysis: 30ºC, for 96 h, initial SB concentration 8% (w/v)

Data are means ± SD of three independent experiments

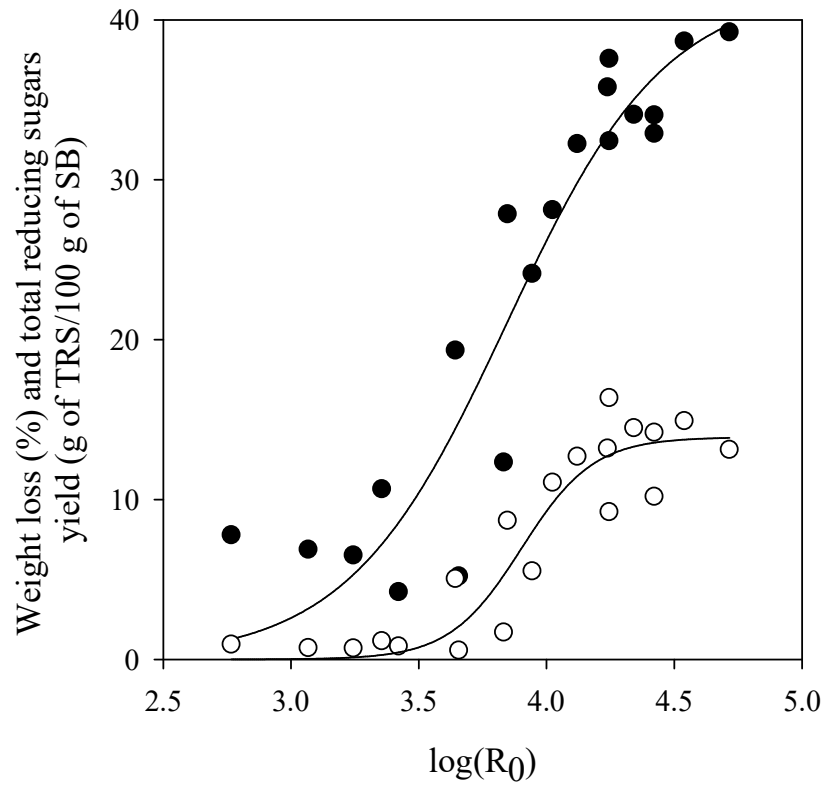Coded names: *N. crassa* to *F. oxysporum* ratio 100:0 [E1]; 75:25 [E2]; 50:50 [E3]; 25:75 [E4]; 0:100 [E5]

Figure 1. Effect of severity factor on (●) solid removal and (○) total reducing sugar

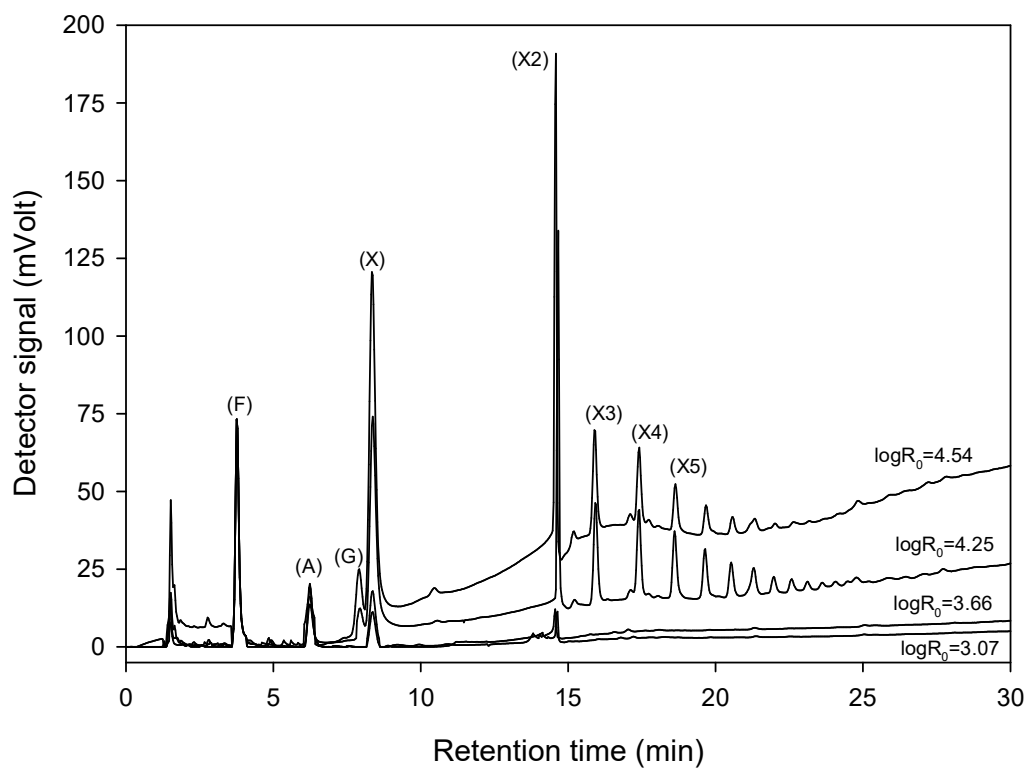release during hydrothermal treatment of sweet sorghum bagasse.

Figure 2. HPAEC-PAD analysis of sugars in hydrolysates derived from sweet sorghum bagasse following hydrothermal treatment at different $logR_0$ (3.07, 3.66, 4.25 and 4.54). F: fucose, A: arabinose, G: glucose, X: xylose, X2: xylobiose, X3:xylotriose, X4: xylotetraose, X5: xylopentaose.
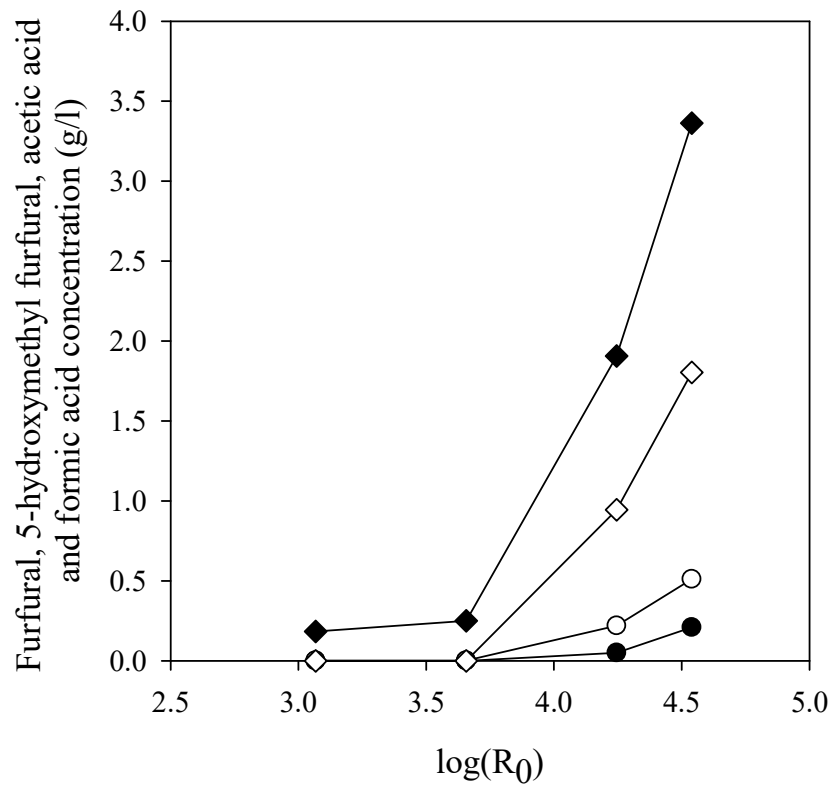
Figure 3. Effect of severity factor on (●) 5-HMF, (○) furfural, (◆) acetic acid and (◇) formic acid formation during hydrothermal treatment of sweet sorghum bagasse.