

# Induction of cellulases and hemicellulases from *Neurospora crassa* under solid-state cultivation for bioconversion of sorghum bagasse into ethanol

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## Abstract

The cellulolytic and hemicellulolytic system from the mesophilic fungus *Neurospora crassa* was produced under solid-state cultivation (SSC) of wheat straw and wheat bran mixtures. Following optimization of nitrogen source, pH and initial moisture of the growth medium, yields as high as 492.8, 1.08, 26.7, 297.8 and 0.132 (in U g<sup>-1</sup> of carbon source) were obtained for endoglucanase, exoglucanase,  $\beta$ -glucosidase, xylanase and  $\beta$ -xylosidase, respectively. The potential of the multienzyme system was demonstrated for hydrolysis of sorghum bagasse (SB) into fermentable carbohydrates. *N. crassa* cells were found able to assimilate the majority of the released sugars and generated limited levels of other metabolic products during simultaneous saccharification and fermentation of this valuable substrate into ethanol.

## Keywords

*Neurospora crassa*; Solid-state cultivation; Cellulase; Hemicellulase; Wheat straw; Sorghum bagasse; Bioethanol

## 1. Introduction

In view of recent developments in crude oil market prices, the use of alternative, non-petroleum based sources of energy is expected to rise sharply in the coming years (Kumar et al., 2008). Lignocellulosic biomass (energy crops) and wastes (forest, agricultural, and municipal) could offer a huge renewable resource for second generation biofuels production (Tengerdy and Szakacs, 2003). Efficient and inexpensive production of cellulolytic and hemicellulolytic systems for enzymatic breakdown of such materials could accelerate the forthcoming changes and provide a solution that is consistent with the increasing public environmental concern (Zhang et al., 2006). Meanwhile, commercial cellulases and hemicellulases are used in a different, though wide, range of applications, including detergents and textile industry, pulp and paper industry, animal feeding, extraction of fruit and vegetable juices, and starch processing (Bhat, 2000; Beg et al., 2001; Polizeli et al., 2005).

Numerous fungi have been identified to degrade cellulose and hemicellulose, but only a few exhibit both depolymerase and fermentative capacity (Lezinou et al., 1995). *Neurospora crassa* is able to synthesise and secrete high levels of all three enzyme types involved in cellulose degradation (Yazdi et al., 1990), as well as endoxylanase and  $\beta$ -xylosidase activities (Mishra et al., 1984; Deshpande et al., 1985). In addition, it is a well-known ethanol producing microorganism that has been used for fermentation of agricultural residues (Rao et al., 1985). The application of biological systems for direct microbial conversion of lignocellulosic materials into ethanol is highly advantageous due to significant reductions in both capital and operation cost (Cardona and Sánchez, 2007).

Production of enzymes by solid-state cultivation (SSC) represents an attractive alternative over conventional submerged cultivation (Viniegra-González et al., 2003). The advantages of SSC, which have been claimed by many workers, include higher productivity per reactor volume, lower cost and space requirements, simpler equipment and easier downstream processing (Pérez-Guerra et al., 2003). The use of SSC for induction of cellulolytic and hemicellulolytic enzymes provides a means to exploit various agro-industrial by-products, such as wheat bran, wheat straw, corncobs, sugar beet pulp, apple pomace, and cassava waste (Pandey et al., 1999).

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 (Negro et al., 1999). Scenarios for SB exploitation are based on enzymatic hydrolysis of polysaccharides and co-fermentation of glucose and xylose to ethanol (Gnansounou et al., 2005). However, previous attempts to take advantage of this valuable bioethanol industry derivative were associated with relatively low ethanol yields (Ballesteros et al., 2004).

The aim of the present study was to enhance cellulases and hemicellulases production by *N. crassa* under SSF of various agricultural by-products. Following medium optimization, the potential of the enzymic system was investigated for hydrolysis of sorghum bagasse to monomeric sugars and further conversion to bioethanol.

## 2. Materials and methods

### 2.1. Microorganism

The microorganism used in the present study was *N. crassa* DSM 1129 and supplied by DSMZ (Deutsche Sammlung von Microorganismen und Zellkulture, Germany). Stock cultures were maintained on potato dextrose agar slants at 4°C.

### 2.2. Reagents

Carboxymethyl cellulose, birchwood xylan and *p*-nitrophenyl glycosides were obtained from Sigma Chemical (St Louis, MO, USA). All other chemicals were analytical grade.

### 2.3. Solid substrates

Wheat straw (WS), wheat bran (WB), corn cobs (CC) and fresh sweet sorghum stalks were chopped to less than 3 mm diameter particles. SB was prepared following double extraction of sugars from sweet sorghum in 15% (w/v) aqueous suspension at 50°C for 45 min, filtration, extensive washing and drying at 70°C overnight.

### 2.4. Media and growth conditions

Solid state cultivation (SSC) was carried out in 100-ml Erlenmeyer flasks containing 2.5 g of dry carbon source moistened with Toyama's mineral medium (in g l<sup>-1</sup>: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10; KH<sub>2</sub>PO<sub>4</sub>, 3; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5; CaCl<sub>2</sub>, 0.5) (Toyama and Ogawa, 1978). Following heat sterilization (121°C) for 20 min, each flask was inoculated with 1 ml spore suspension (approximately 5.8x10<sup>7</sup> conidia) and incubated at 30°C under static conditions. Experiments were carried out in duplicate.

A step by step optimization procedure regarding the effect of important parameters (carbon and nitrogen source, initial growth pH and moisture) on enzymes production was employed. Ammonium sulphate, ammonium phosphate, potassium nitrate, urea, yeast extract, peptone and corn steep liquor were the nitrogen sources examined. Their elemental concentration was 0.04 g nitrogen per g of carbon source. The initial culture pH ranged between 4.0 and 7.0. The moisture level (60.0, 70.0, 80.0 and 87.5% w/w) was adjusted with the addition of the appropriate amount of Toyama's mineral medium.

### 2.5. Enzyme extraction

After suitable periods of time, enzymes were extracted from the fermented growth medium with 10-fold (v/w) citrate-phosphate buffer pH 5.0 (50 mM) by shaking (250 rpm) at 28°C for 60 min. The suspended materials and fungal biomass were separated by centrifugation (12,000 x g at 4°C for 15 min) and the clarified supernatant was used for enzyme activity measurements.

### 2.6. Enzyme assays

Endoglucanase (EG), exoglucanase (EXG) and xylanase (XYL) activities were assayed on carboxymethyl cellulose, Avicel and birchwood xylan, respectively, as described (Kalogeris et al., 1999). Measurement of released reducing sugars was accomplished by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The activities of  $\beta$ -glucosidase ( $\beta$ -GLU) and  $\beta$ -xylosidase ( $\beta$ -XYL) were determined spectrophotometrically using the respective *p*-nitrophenyl glycosides as substrates (Kalogeris et al., 1999). All assays were carried out at 50°C and pH 5.0. Blanks with inactivated

enzyme (after boiling for 15 min) were used as a reference. One unit (U) of enzyme activity was defined as the amount of enzyme liberating 1  $\mu$ mole of product per min.

### 2.7. Chemical analysis

The moisture, ash and crude fat content of sorghum bagasse were determined according to standard methods (AOAC, 1990). Pectic polysaccharides, cellulose, hemicellulose and acid insoluble lignin content were determined as described previously (Mamma et al., 2008). Analysis was carried out in duplicate.

### 2.8. Enzymatic hydrolysis of SB

The cellulolytic and hemicellulolytic system of *N. crassa* DSM 1129 was produced under the optimal conditions determined for SSC. Crude enzyme was extracted from fermented WS/WB mixtures after 7 days of growth and concentrated using an Amicon PM-10 membrane. The retentate supplemented a suspension of substrate in 50 mM citrate-phosphate buffer, pH 5.5 (8% w/v final SB concentration), so as to create a cellulase concentration corresponding to 6 U/ml of endoglucanase activity. Microbial contamination was prevented by the addition of sodium azide (0.02% w/v). Hydrolysis was performed at  $30 \pm 1^\circ\text{C}$  in a rotary shaker (250 rpm). Samples were withdrawn periodically, centrifuged (10,000 x g for 10 min), and analysed for sugars as described in the following section. Total reducing sugars were estimated by the 3,5-dinitrosalicylic acid method (Miller, 1959). Experiments were carried out in duplicate.

### 2.9. Conversion of SB into ethanol

Sorghum bagasse was converted into ethanol with the application of a two-phases process where enzymes production (first phase) was combined with simultaneous saccharification and fermentation of the substrate (second phase). Production of the cellulolytic and hemicellulolytic system from *N. crassa* was accomplished by fungal cells grown aerobically under the optimal conditions found for SSC. During the second (ethanol production) phase, fungal mycelia and the in situ produced cellulolytic and hemicellulolytic enzymic systems were supplemented with sterilized sorghum bagasse to a final concentration of 8%. The initial activity of cellulase was adjusted at 6 U/ml (based on endoglucanase) with the addition of citrate-phosphate buffer (50 mM) pH 5.5. Fermentation was carried out in a rotary shaker operating at  $30^\circ\text{C}$  and 80 rpm using 100 ml Erlenmeyer flasks provided with special rubber stoppers, which ensured anaerobic conditions and allowed release of produced carbon dioxide.

### 2.10. Analysis of sugars

The carbohydrates generated by the action of *N. crassa* enzymic system on SB were determined by HPAEC. The analysis system consisted of a quaternary gradient pump (Jasco PU-1580I, Jasco Ltd., UK), and a Rheodyne injector controlled by a Borwin software. Separation of sugars (20  $\mu$ l injection volume) was accomplished with a CarboPack PA1, (4x250 mm) column equipped with a guard column (Dionex Corporation, USA). The column was eluted isocratically with 17.5 mM NaOH at a flow rate of 1 ml/min. Eluted sugars were monitored by a pulse amperometric detector (HPAEC-PAD) (Dionex Corporation, USA). Fucose (50 $\mu$ M) was added as internal standard. All analyses were performed in duplicate.

### 2.11. Metabolite profiling

The extracellular metabolites pattern of *N. crassa* cells grown on SB was analyzed by column liquid chromatography (HPLC). A Jasco (PU-987) HPLC system equipped with an ion moderated partition chromatography column, Aminex HPX-87H (Bio-Rad), was used in combination with a Waters (410) refractive index detector. The flow rate of the mobile phase (5 mM H<sub>2</sub>SO<sub>4</sub>) was adjusted at 0.6 ml/min and the temperature at 50 °C (Panagiotou et al., 2005).

## 3. Results and discussion

### 3.1. Effect of carbon source

Among the lignocellulosic materials examined in the present work, wheat straw (WS) was the most effective carbon source for production of both cellulolytic and hemicellulolytic activities by *N. crassa* (Fig 1). It is well known that the type and composition of the carbohydrates present in WS are suitable for induction of cellulases and hemicellulases from filamentous fungi under SSC [Kalogeris et al., 2003]. The use of wheat bran (WB), a nutrient-richer intermediate of the wheat processing industry, resulted in satisfactorily high enzyme levels but was less effective than WS. Implementation of corn cobs (CC) and sorghum straw (SS) particles as substrates was associated with significantly lower enzyme titres. Unsuitability of solid materials to support enzyme production under SSC could be attributed to inappropriate physical properties hindering microorganism growth, such as particles size, geometry and compactness (Krishna, 2005).

WS and WB combinations exhibited superior performance than those obtained when each one of these carbon sources was examined individually (Fig. 1). The addition of WB substantially improved endoglucanase and endoxylanase activities; 58% and 71% higher values than those achieved with WS, respectively. Maximum enzyme activities were observed for the highest WB/WS ratio tested (1 to 5 w/w), and this condition was adopted in subsequent experiments. Induction of cellulolytic and hemicellulolytic activities as a result of incorporation of WB into growth media is well documented. Both mesophilic and thermophilic fungi have been reported to produce enhanced levels of lignocellulose degrading enzymes under SSC of WB and straw mixtures (Jecu, 2000; Jatinder et al., 2007).

### 3.2. Effect of nitrogen source

The results obtained when a number of nitrogen sources supplemented the growth medium are presented in Fig. 2. The levels of enzyme activities were generally lower when complex organic sources, such as peptone, yeast extract and corn steep liquor were employed. Comparable high endoxylanase activities could be obtained with both organic (urea) and inorganic (potassium nitrate and ammonium sulphate) nitrogen. Ammonium sulphate was by far the most effective nitrogen source for combined cellulases and hemicellulases production by *N. crassa* under SSC, and it was selected for further studies.

These findings are in contrast to previous works where the same fungus was grown under submerged culture in the presence of complex and more expensive nitrogen sources. A combination of peptone, yeast extract and malt extract was implemented so as to induce the cellulolytic and xylanolytic systems of *N. crassa* (Mishra et al., 1984; Rao et al., 1985). Furthermore, replacement of inorganic nitrogen by yeast extract improved substantially cellulases production; approximately

two-fold enhancement for endoglucanase and exoglucanase activities and more than three-fold for  $\beta$ -glucosidase activity (Yazdi et al., 1990).

### 3.3. Effect of initial culture pH

Since controlling the pH of a multi-phase growth medium is not an easy task, the majority of small scale SSC studies deal with the effect of the initial pH of the moist solid substrate on process outcome (Rajoka et al., 2005). The effect of initial pH value on enzyme production by *N. crassa* cells grown under SSC is depicted in Figure 3. Production of all three components of *N. crassa* cellulose-degrading system was maximized at acidic pH values. The highest endoglucanase and exoglucanase activities were observed at pH 5.0, while similarly high  $\beta$ -glucosidase activities were obtained at pH 4.0 and 5.0. The optimum endoxylanase activity was observed at the lowest initial pH value examined (pH 4.0), whereas production of  $\beta$ -xylosidase was practically unaffected by the pH value of the growth medium.

The best pH values found in the present work differ significantly from those reported for enzymes production by *N. crassa* cells grown in submerged cultures. When the latter technique was employed, optimal exoglucanase and total cellulase activities were recorded at neutral conditions (pH 6.5-7.0), while a more alkaline environment (pH 8.0) was favourable for endoglucanase and  $\beta$ -glucosidase production (Yazdi et al., 1990; Romero et al., 1999). In addition, a higher pH value (5.0) than the optimum value determined in this work, was applied for maximal endoxylanase production under submerged cultivation (Mishra et al., 1984). The results indicate that the effect of essential growth variables is dependent on the type of cultivation technique which is employed for enzymes production.

### 3.4. Effect of initial moisture content

The moisture content of the growth medium is one of the most important variables affecting the outcome of an SSC process (Gervais & Molin, 2003). Cultivation of microbial cells in excess of water can lead to particles sticking, limited gas exchange and higher vulnerability to bacterial contamination, while low moisture levels are connected with reduced microbial growth, enzyme stability, substrate swelling and diffusion of nutrients (Prior et al., 1992).

The cellulolytic and hemicellulolytic activities secreted by *N. crassa* DSM 1129, when the moisture level of the growth medium varied, are shown in Table 1. The highest enzyme activities were obtained when the initial moisture content of the growth medium was 70.5%. Similar moisture levels have been reported to optimize the production of cellulolytic activities when WS-WB mixtures were used as carbon source [Jecu, 2000]. Higher moisture levels (80%) were necessary for maximal cellulases and hemicellulases production when the same strain was grown on industrial by-products of the citrus-processing industry under SSC (Mamma et al., 2008). The optimum moisture content for enzyme production under SSC is dependent upon the water binding properties of the substrate as well as the microorganism used (Mamma et al., 2008).

### 3.5. Application of optimal conditions

The time course of cellulolytic and hemicellulolytic activities obtained under optimal SSC conditions (type of carbon and nitrogen source, initial moisture and pH of the growth medium) for *N. crassa* DSM 1129, is depicted in Fig. 4. The highest endoglucanase and  $\beta$ -glucosidase activities (492.8 and 26.7 U per g of substrate, respectively) were observed at the eighth day of fermentation, whereas maximal exoglucanase activity (1.1 U per g of substrate) was recorded quite early (second

day of fermentation). Optimum endoxylanase (297.8 U per g of substrate) and  $\beta$ -xylosidase (0.13 U per g of substrate) activities were achieved at the sixth and eighth day of fermentation, respectively. After maximal values were reached, most of the enzyme activities displayed fast reduction rates, which is not uncommon during production of enzymes under SSC (Techapun et al., 2003).

Quantitative comparison between lignocellulose-degrading enzyme activities reported in the literature is not always possible since no standard assaying methods have been adopted yet. Cellulolytic and hemicellulolytic activities produced under SSC by fungal strains capable for one-step conversion of lignocellulose to ethanol, is presented in Table 2. High cellulase activities were achieved in the present study; related endoglucanase and  $\beta$ -glucosidase activities were much higher than those reported in previous works (more than 60% and 300%, respectively). The highest endoxylanase activity was associated with *F. oxysporum* cells grown in the presence of corn stover. Improved  $\beta$ -xylosidase production was achieved under SSC of *N. crassa* on WS-WB mixtures, since the corresponding activity was more than 40% higher when compared to those cited in the literature for ethanol producing fungi.

### 3.6. Hydrolysis of sorghum bagasse

The potential of the multi-enzyme lignocellulose degrading system produced by *N. crassa* under SSC was examined during hydrolysis of SB. Compositional analysis of this important agro-industrial by-product is presented in Table 3. The low lignin content that was estimated for SB (approximately 3.9 %), is advantageous, since inhibition in both enzyme production and activity has been reported for lignin-rich materials (Jørgensen & Olsson, 2005). The cellulosic and hemicellulosic fragments of SB represent 40.4% and 35.5% (w/w) of the material, respectively. Despite its remarkable prospective as a source of fermentable sugars, the use of SB is hindered by its resistance to hydrolysis. Even when employing steam explosion pre-treatment, solubilization of substrate was 35% (w/w), which is equivalent to the yields observed for recalcitrant woody materials (Ballesteros et al., 2004).

The proportions of SB cellulose and hemicellulose that were converted by *N. crassa* enzymic system to their corresponding constituent carbohydrates were 14.7% and 20.3%, respectively, which is noteworthy considering that no pre-treatment method was employed. The time course of the main carbohydrates derived from SB hydrolysis by *N. crassa* cellulases and hemicellulases is depicted in Figure 5. Glucose comprised the major product of SB hydrolysis, verifying the ability of *N. crassa* cellulolytic system to depolymerize cellulose into its monomeric building blocks. Formation of glucose was considerably faster than the production of hemicellulosic constituents, and its final concentration ( $5.4 \text{ g l}^{-1}$ ) was reached after 51 hours of hydrolysis. On the contrary, the rate of xylose release was much lower and it had not been completed even after 75 hours of hydrolysis. Substantial accumulation of xylobiose leading to concentrations as high as  $5.4 \text{ g l}^{-1}$ , could be recorded early in hydrolysis, followed by its gradual disruption into two xylose units. The pattern of xylan degradation products could be explained by *N. crassa*  $\beta$ -xylosidase inefficiency. Other simple sugars that were detected during SB hydrolysis included galactose and arabinose. As it has been reported, bifunctional endoxylanases secreted by *N. crassa* are capable of hydrolyzing 1,3- $\alpha$ -L-arabinofuranosyl branch points and release arabinose as an end product of xylan hydrolysis (Mishra et al., 1984).

### 3.7. Conversion of sorghum bagasse into ethanol

Microbial cells grown under SSC of WS-WB mixtures as well as the in situ generated hydrolases were used for simultaneous saccharification and fermentation of SB. The concentrations of carbohydrates observed during anaerobic growth of *N. crassa* on SB were significantly lower than those recorded when the substrate was treated with the cell-free multi-enzyme system at the same conditions. Therefore, the fungus was able to metabolize the majority of the fermentable sugars which were liberated by its cellulolytic and hemicellulolytic components. Negligible glucose, xylose and galactose levels during fermentation demonstrate that the microorganism consumed these sugars as soon as they were produced. On the contrary, the amount of xylobiose which could be detected in the course of fermentation, (7.0% of the amount produced by SB hydrolysis) which once more stresses the importance of  $\beta$ -xylosidase incompetence. A considerable level of arabinose (17.4% of the amount produced by SB hydrolysis) was present until the late hours of the process, indicating that assimilation of the specific pentose by the fungus was not preferable. Analysis of remaining solids revealed that the ratio of cellulose to hemicellulose increased in the course of fermentation from 1.1 (initial value) to 3.7 (end of anaerobic process), which is indicative of an extended assimilation of the hemicellulosic fragment of SB

The time course of the metabolites formed by *N. crassa* during SB fermentation is presented in Figure 6. Ethanol was the major end-product (81.5%), followed by acetic acid (8.1%), xylitol (5.7%) and glycerol (4.7%). The proportion of SB that was converted to non-ethanol metabolic products, was much lower than the corresponding value observed for cellulose fermentation by *F. oxysporum*, where acetic acid generation comprised more than 35% of the overall metabolites production (Panagiotou et al., 2005). Accumulation of xylitol has been associated with imbalanced redox between nicotinamide cofactors, which is developed in the early steps of xylose degradation pathway for both *N. crassa* and *F. oxysporum* strains (Panagiotou et al., 2002, Woodyer et al., 2005). Despite extensive assimilation of released sugars and low levels of side-products, the yield of ethanol production was not satisfactory (approximately 4.7 g of ethanol per 100 g of SB). Further investigation concerning the application of various pretreatments on SB could enhance its enzymic digestibility and improve its direct conversion to ethanol.

## 4. Conclusions

*N. crassa* DSM 1129 was able to secrete high levels of cellulolytic and hemicellulolytic activities under SSC. Optimization of important cultivation variables using a combination of WS-WB particles as carbon source, resulted in the highest endoglucanase,  $\beta$ -glucosidase and  $\beta$ -xylosidase activities ever reported for ethanol producing fungi. Hydrolysis of untreated sorghum bagasse by the fungal enzymic system was associated with the formation of its monomeric constituent carbohydrates. The microorganism was able to assimilate the majority of the released sugars during direct conversion of sorghum bagasse into ethanol with limited levels of other metabolic products.

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**Table 1.** Effect of initial moisture content on cellulases and hemicellulases production by *N. crassa* DSM 1129.

Moisture content (%, w/w)	Enzyme activity (U/g <sub>substrate</sub> )				
	Endoglucanase	Exoglucanase	$\beta$ -Glucosidase	Xylanase	$\beta$ -Xylosidase
60.0	106.0 $\pm$ 2.7	0.30 $\pm$ 0.01	16.8 $\pm$ 0.3	142.9 $\pm$ 2.9	0.093 $\pm$ 0.002
70.5	492.8 $\pm$ 10.3	1.08 $\pm$ 0.03	26.7 $\pm$ 0.4	297.8 $\pm$ 7.4	0.132 $\pm$ 0.003
80.0	387.4 $\pm$ 9.1	1.18 $\pm$ 0.03	17.3 $\pm$ 0.3	230.9 $\pm$ 5.0	0.106 $\pm$ 0.003
87.5	268.4 $\pm$ 5.9	0.67 $\pm$ 0.02	7.4 $\pm$ 0.2	68.6 $\pm$ 1.3	0.050 $\pm$ 0.001

**Table 2.** Cellulolytic and hemicellulolytic activities produced by ethanol-producing fungal strains under SSC of lignocellulosic biomass

Organism	Substrate	Enzyme activities (Units g <sup>-1</sup> substrate)					Reference
		EG	EXG	$\beta$ -GLU	XYL	$\beta$ -XYL	
<i>N. crassa</i> DSM 1129	Brewing spent grain	56.0	4.2	1.6	1073	0	Xiros et al. (2008)
<i>N. crassa</i> DSM 1129	Orange peels	138.5	n.d.	7.9	56.8	n.d.	Mamma et al. (2008)
<i>F. oxysporum</i> F3	Orange peels	69.5	n.d.	0.9	28.9	0.09	Mamma et al. (2008)
<i>F. oxysporum</i> BAFS 768	Olive-mill residue	1.0	1.2	0.1	1.5	n.d.	Sampedro et al. (2007)
<i>F. oxysporum</i> F3*	Corn stover	304.0	4.1	0.14	1840.0	0.04	Panagiotou et al. (2003)
<i>N. crassa</i> DSM 1129	Wheat straw/wheat bran	492.8	1.1	26.7	297.8	0.13	Present work

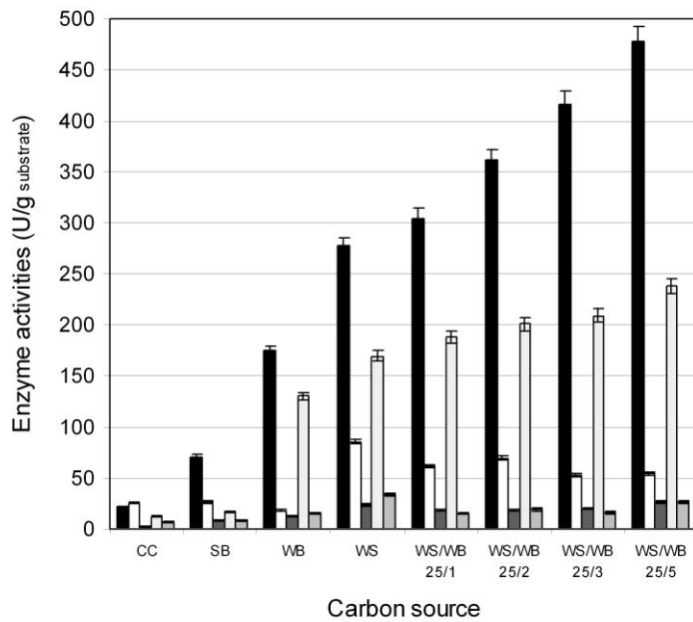
\* grown on SSF bioreactor, n.d. : not determined

**Table 3.** Composition of sorghum bagasse\*.

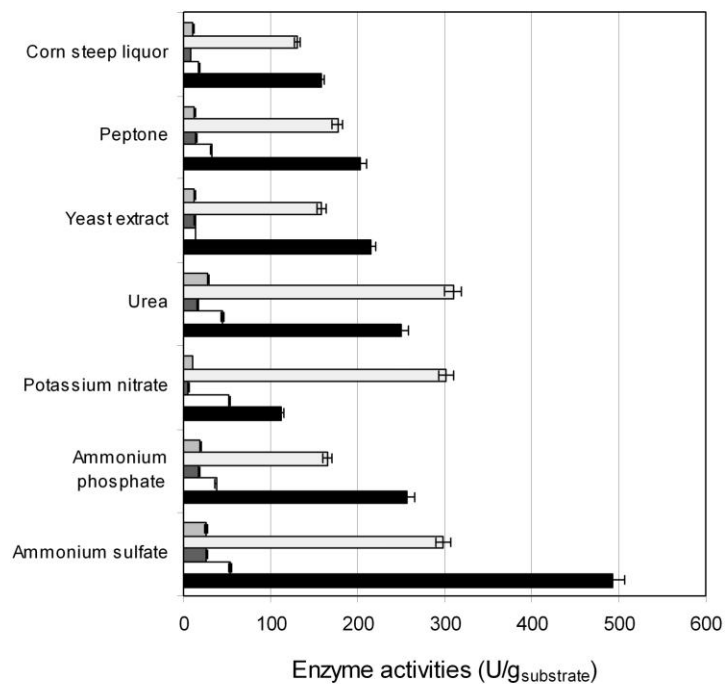
<b>Component</b>	<b>% (w/w dry basis)</b>
Water soluble materials	3.6 ± 0.1
Crude fat	10.4 ± 0.3
Pectin	4.3 ± 0.1
Cellulose	40.4 ± 1.0
Hemicellulose	35.5 ± 0.9
Acid insoluble lignin	3.9 ± 0.3
Ash	0.2 ± 0.01

\* Moisture content 3.8% (w/w)

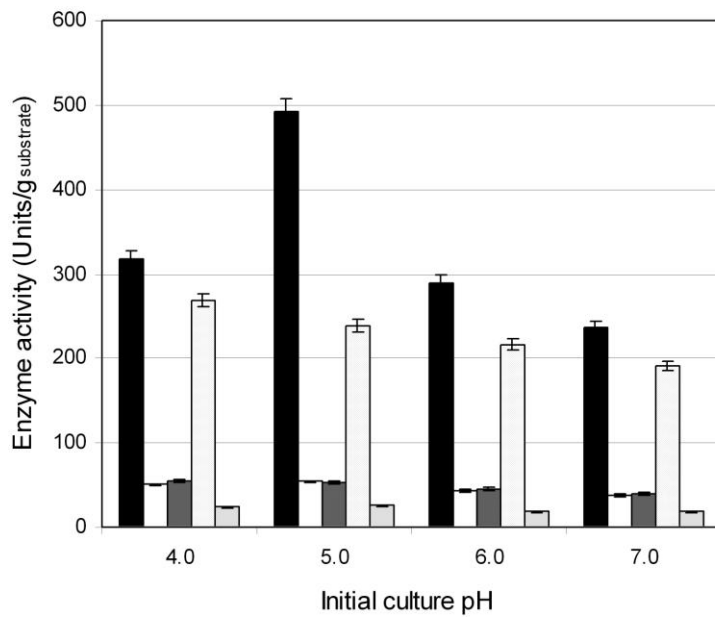
## Figures



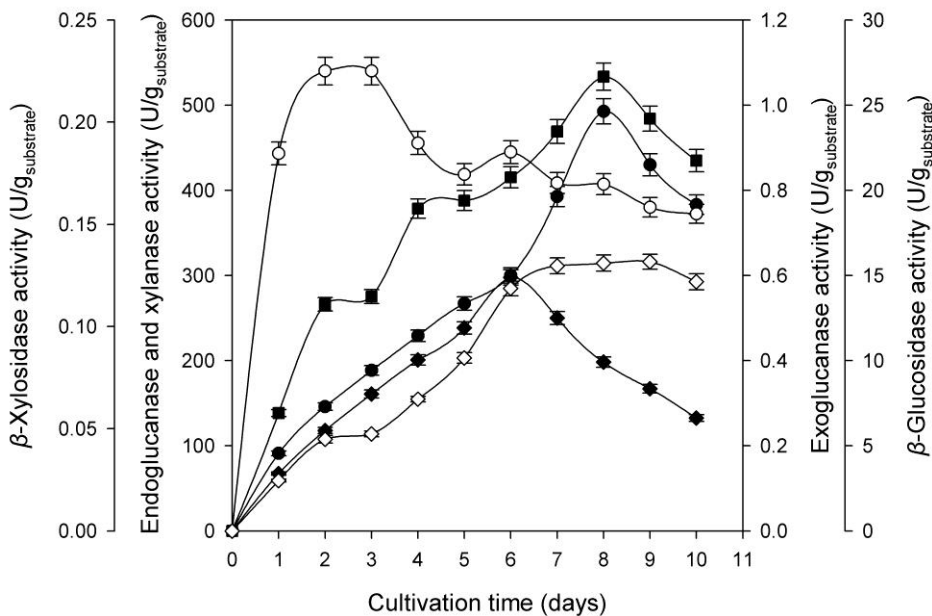
**Figure 1.** Effect of carbon source on cellulases and hemicellulases production by *N. crassa* DSM 1129. CC: corn cobs, SB: sorghum baggasse, WB: wheat bran and WS: wheat straw. Symbols: (■) endoglucanase, (□) exoglucanase (x50), (⊙) xylanase, (▨) β-glucosidase and (▩) β-xylosidase (x200).



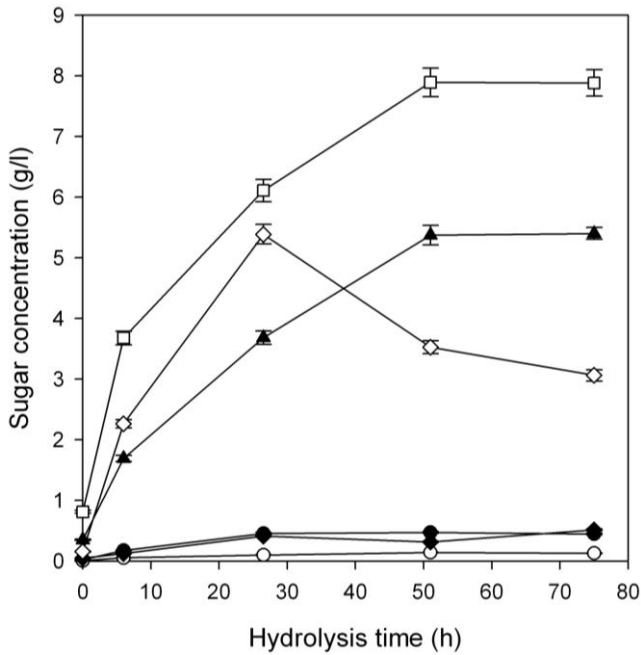
**Figure 2.** Effect of inorganic and organic nitrogen sources on cellulases and hemicellulases production by *N. crassa* DSM 1129. Symbols: (■) endoglucanase, (□) exoglucanase (x50), (⊙) xylanase, (▨) β-glucosidase and (▩) β-xylosidase (x200).



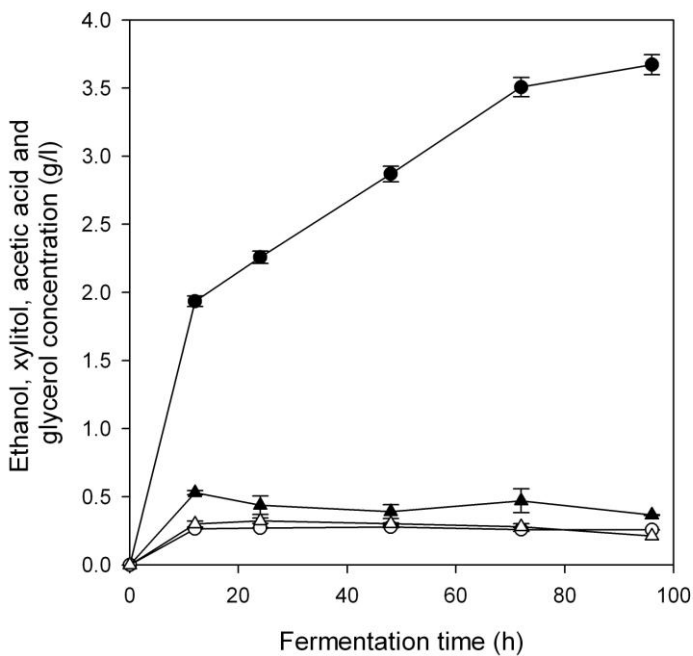
**Figure 3.** Effect of initial culture pH on cellulases and hemicellulases production by *N. crassa* DSM 1129. Symbols: (■) endoglucanase, (□) exoglucanase (x50), (⊙) xylanase, (▨)  $\beta$ -glucosidase and (◊)  $\beta$ -xylosidase (x200).



**Figure 4.** Endoglucanase (●), exoglucanase (○),  $\beta$ -glucosidase (■), xylanase (◆), and  $\beta$ -xylosidase (◇) activities produced by *N. crassa* DSM 1129 grown on WS/WB mixture (5/1, w/w) under SSF [nitrogen source: ammonium sulphate, initial culture pH 5.0 and initial moisture content: 70.5% (w/w)].



**Figure 5.** Time course of total reducing sugars ( $\square$ ), arabinose ( $\bullet$ ), galactose ( $\circ$ ), glucose ( $\blacktriangle$ ), xylose ( $\blacklozenge$ ) and xylobiose ( $\diamond$ ) released during hydrolysis of SB by cellulases and hemicellulases from *N. crassa* DSM 1129.



**Figure 6.** Metabolic profile of *N. crassa* DSM 1129 during simultaneous saccharification and fermentation of sorghum bagasse. Symbols: ethanol ( $\bullet$ ), xylitol ( $\circ$ ), acetic acid ( $\blacktriangle$ ), glycerol ( $\triangle$ ).