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# Potential use of soybean hulls and waste paper as supports in SSF for cellulase production by *Aspergillus niger*

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ABSTRACT

Cellulase has by vast applications in the biofuel, pulp and paper, detergent and textile industries. The three components of the enzyme complex (endoglucanase, exoglucanase and β-glucosidase) can effectively depolymerize the cellulose chains in lignocellulosic substrate. Solid-state fermentation (SSF) by fungi is a preferable production route for cellulase because of its low cost, among other advantages. This work describes the cellulase production by *Aspergillus niger* NRRL3 grown on SSF. SSF was carried out on soybean hulls and waste paper as supports. The effect of the support on cellulase production was assessed under a completely randomized factorial design. The support-time interaction was significant for all the variables studied. Both materials were characterized in terms of water absorption index and critical humidity point. Samples of culture were analyzed with scanning electron microscopy (SEM) to study spores and fungal growth.

Maximum endoglucanase activity was found at 96 h using soybean hulls as support (5914.29 U $L^{-1}$), being four times higher than that obtained using waste paper at the same fermentation time. The exoglucanase activity in soybean hulls was maximal at 96 h (4551.19 U $L^{-1}$), being 9.6 times higher than that obtained in waste paper at the same time. The maximum β-glucosidase activity in soybean hulls (984.01 U $L^{-1}$) was reached at 96 h, being 1.7 times greater than that obtained in waste paper. Besides, the use of soybean hulls provided high volumetric productivities at shorter times, which may decrease production costs considering a scaled process.



## 1. Introduction

Cellulase refers to an enzyme complex which hydrolyzes glycosidic linkages and which includes: endocellulase (EC 3.2.1.4.), exocellulase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) (Jecu, 2000).

Cellulase participates in a large proportion in the world market for enzymes. Indeed, cellulase represents approximately 20% of the enzyme global market and it is the world's third largest industrial enzyme by dollar volume. The increasing demand of cellulase is related to its relevant use in the textile, food and drink, pulp and paper, biofuel, detergent and animal feed industries (Dave et al., 2013; Silva et al., 2013).

Commercially, conversion of cellulosic biomass requires the utilization of cellulases, making the process expensive. Therefore, cellulase production from a broad range of microorganisms has been studied.

Cellulases are produced by several microorganisms as they are required for their growth and development under certain conditions. *Trichoderma reesei* cellulases are characterized by a high proportion of endoglucanase and exoglucanase but a low proportion of β-glucosidase. For this reason, other microorganisms such as the genus *Aspergillus* were analyzed. *Aspergillus niger* has been widely used because it produces the three fundamental enzymes required for cellulolysis. Fermentation studies are needed to evaluate the productivity of cellulases (Sohail et al., 2009).

In previous works the use of the proven technology of submerged fermentation has been widely reported (Meinicke et al., 2012; Prajapati et al., 2014; Rustiguel et al., 2015; Vendruscolo et al., 2013) due to better monitoring and ease of handling. However, SSF (Solid-state fermentation) offers an alternative for low-cost enzyme production and it has different advantages such as greater yields, lower investment costs and lower energy demand (Gupta et al., 2015).

In order for the substrate to enable microbial growth and metabolism, it is necessary to maintain adequate moisture conditions, which imitate those of natural growth (Orzua et al., 2009). Water availability in SSF affects microbial growth and metabolism and determines water activity, a physicochemical parameter referred

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to as the relationship between the water vapor pressure of the substrate and the water vapor pressure of pure water. The chemical constitution and structure of the supports employed in SSF influence the values of water activity, which can fluctuate from 0.80 to 0.99 to achieve an effective fungal metabolism (Martins et al., 2011). Different materials have been used as solid supports for SSF, such as rice and wheat bran (Khandeparkar and Bhosle, 2006), coffee by-products (Machado et al., 2012), mango peels (Buenrostro-Figueroa et al., 2010), coconut husks (Orzua et al., 2009), corn cobs (Mussatto et al., 2009b) and grape skins (Botella et al., 2007) among others. Some of these derivatives have been used as substrates and supports to obtain products of industrial interest, including antibiotics, flavor and aroma compounds, organic acids, pigments, bioactive molecules and a wide variety of enzymes (Martins et al., 2011).

In previous works, cellulase production under SSF has been evaluated by determining total cellulase activity (Latifian et al., 2007; Silva et al., 2013). However, this method does not determine the cellulase activity of the three components of the cellulase complex individually and it does not allow elucidation on whether the three components have been adequately produced.

The aim of this study was to produce cellulase enzyme by growing *A. niger* NRRL3 on SSF using different materials as carbon sources and as growth supports. An individual monitoring of each of the three components of the cellulase complex was carried out. In addition, materials were characterized in terms of water absorption index and critical humidity point. Samples of culture were analyzed with scanning electron microscopy (SEM) to study fungal growth.

## 2. Materials and methods

### 2.1. Physico-chemical characterization of the supports

The supports used in this work included: soybean hulls (acquired from agricultural regions of Argentina) and waste paper (acquired from the printing industry). The physico-chemical parameters studied were: critical humidity point (CHP) (Mussatto et al., 2009b), packing density (PD) (Santomaso et al., 2003) and water absorption index (WAI) (Orzua et al., 2009).

The CHP was assayed by adding 1 g of sample in a thermo-balance at 130 °C until a constant weight was obtained. The PD was determined by adding 10 g of sample in a graduated tube, and the sample was stirred until no volume changes were observed for 5 min.

For the analysis of WAI, 1.5 g of a sample of support and 15 mL of distilled water were placed in a centrifuge tube. The mix was homogenized at 25 °C for 1 min and centrifuged at 3000 g for 10 min. The supernatant was removed, and the WAI was determined from the weight of the remaining gel and calculated as g gel/g dry weight.

### 2.2. Microorganism and culture

Strains of *A. niger* NRRL3 were provided by the culture collection of Agricultural Research Service, USDA (USA for donating the strain of *A. niger*). The strain was maintained at −20 °C in 10% w/v glycerol. Spores from *A. niger* NRRL3 were activated in potato glucose agar medium at 30 °C for five days. The culture spores were collected with sterile solution of 0.1% Tween-80 and quantified through a calibration curve from *A. niger* NRRL3.

### 2.3. Solid-state fermentation

Cellulase production experiments were performed in 250 mL flasks considered as bioreactors, which were sterilized with an homogeneous mixture containing the following fermentable mass: 15 g of each support (soybean hulls and waste paper) was mixed with 45 mL of Mandels culture medium with the following composition (g $L^{-1}$): $(NH_4)_2SO_4$ (1.4), $KH_2PO_4$ (2.03), $CaCl_2$ (0.30), $MgSO_4 \cdot 7H_2O$ (0.30), peptone (1.00), $FeSO_4$ (0.005), $MnSO_4 \cdot 4H_2O$ (0.0016), $ZnSO_4 \cdot 7H_2O$ (0.0014), $CoCl_2 \cdot 6H_2O$ (0.02), urea (0.30), yeast extract (0.25), $CuSO_4 \cdot 5H_2O$ (0.001). The medium pH was adjusted to pH 4.6 and then autoclaved 121 °C for 15 min. The fermentable mass was aseptically inoculated with $1 \times 10^6$ spores/mL of Mandels culture medium. The SSF was carried out at 30 °C for 120 h. Enzymatic extract (EE) was obtained by adding 20 mL of 0.1 M acetate buffer (pH 4.8) to each reactor. Fermented material was compressed and filtered. Cellulase activity and soluble protein were determined on the EE. Biomass was determined from fermentable mass. Cellulase volumetric productivity (U $L^{-1}$ $h^{-1}$) was calculated as the ratio among cellulase activity value and fermentation time.

### 2.4. Analytical methods

Protein content was estimated by the Warburg and Christian method. Fungal biomass was determined according to the glucosamine method described by Blix (1948).

The samples were subjected to acid hydrolysis in order to remove glucosamine from fungal cell wall. Glucosamine mixed with acetylacetone forms a pyrrole compound which reacts with p-dimethylaminobenzaldehyde, obtaining a product that can be measured at 530 nm. A calibration curve was performed under the same experimental conditions of the samples. The fungal biomass was expressed as mg glucosamine per gram of sample.

Assays for the activity of individual enzyme components, i.e., endoglucanase, exoglucanase and β-glucosidase are briefly described as follows:

Endoglucanase. Carboxymethylcellulose (CMC, 1%) solution was prepared in 50 mM sodium citrate buffer (pH 5.3). CMC solution was incubated with enzymatic extract at 50 °C for 10 min. A milliliter of 3,5-Dinitrosalicylic acid (DNS) reagent was added, it was incubated for 10 min at 100 °C and the absorbance was read at 560 nm. The reducing sugar concentration produced from the enzymatic reaction was then measured and used to calculate the endoglucanase activity.

Exoglucanase. Enzymatic extract was added to Whatman filter paper # 1 in 0.1 M sodium acetate buffer (pH 4.8). After incubation at 50 °C for 60 min, 1 mL of 1% DNS reagent was added, it was incubated at 100 °C for 10 min and the absorbance was read at 560 nm. The reducing sugar concentration produced from the enzymatic reaction was then measured and used to calculate the exoglucanase activity.

β-Glucosidase activity was estimated using 4-Nitrophenyl β-D-glucopyranoside (Sigma-Aldrich, Co., St. Louis, USA) as a substrate. The reaction mixture consisted of 4-Nitrophenyl β-D-glucopyranoside 9 mM and enzymatic extract in 0.2 M acetate buffer (pH 4.6). After incubation at 50 °C for 10 min, the enzyme reaction was stopped by adding 0.1 M $Na_2CO_3$. The p-nitrophenol liberated was measured at 400 nm. A calibration curve of p-nitrophenol was carried out at the same experimental conditions as the samples.

### 2.5. Scanning electron microscopy

Visualization of spore and fungal growth was done using SEM (Environmental Scanning Electron Micro-scope) FEI QUANTA 200 F Feg. Samples were dehydrated and analyzed at 5 keV under low vacuum at 0.10 mbar with a Lector LFD (large field detector). Then the samples dehydrated were coated with electrolytic gold and analyzed under high vacuum at a pressure of 6.2 $10^{-4}$ mbar with an ETD (Everhardt Thornley Detector) and BSED (back-scattered detector).

### 2.6. Experimental design and data analysis

The effect of the support on cellulase production was evaluated under a completely randomized factorial design. The treatments were the combination of two factors levels: Support (soybean hulls and waste paper) and Time (determined at 0, 24, 48, 72, 96 and 120 h). Biomass production, total proteins, cellulase activity, cellulase specific activity, volumetric and specific productivities were determined. Data were analyzed by Two-Way Parametric or Non-Parametric ANOVAs as appropriate. A transformation of data was used when the assumptions of normality and homoscedasticity, requirements for a classical analysis, were not met: Aligned-Rank Transformation (Wobbrock et al., 2011). Data were analyzed using the ARTool packages and lsmeans of R 3.2 software (Kay and Wobbrock, 2014; Lenth and Hervé, 2015; Team, 2015). Means treatments were compared using Tukey's multiple range procedure. A p-value less than 0.05 was regarded as significantly different.

## 3. Results and discussion

### 3.1. Physico-chemical characterization of the supports

The SSF process demands low water content materials to make fungal growth and development easier. The support properties play an important role in the growth rates of microorganisms (Manpreet et al., 2005). WAI and CHP are highly important parameters when selecting the type of material used as support in SSF (Mussatto et al., 2009b; Orzua et al., 2009). WAI specifies the sample capacity to absorb water, subjected to the availability of hydrophilic groups which bind water molecules (Mussatto et al., 2009a). The highest WAI value was observed by use of soybean hulls, which was 37% higher than those obtained with waste paper. According to Robledo et al. (2008), materials with high WAI are more suitable since they cooperate with microbial growth and development. CHP is defined as the percentage of water bound to the support and cannot be utilized by microorganisms for their metabolic functions. The supports must have low CHP to promote microorganism cultivation. CHP high values are not favorable due to the large percentage of water attached to the support (Martins et al., 2011). Table 1 shows the CHP values obtained in the present work for each support assayed. Both supports tested have CHP values under 40%, a limit recognized for the growth of *A. niger* in SSF (Moo-Young et al., 1983). The high WAI and low CHP values found in soybean hulls and waste paper make them excellent materials to be used as supports on SSF. For the SSF processes, PD is another important parameter to consider since it indicates the material compaction degree. Low PD values are required since an increase in packing density can reduce the void space between particles and, thus, a concomitant reduction in the area of exchange with the surrounding atmosphere (Barrios-González et al., 1993). The lowest PD value (Table 1) was obtained with soybean hulls (0.1786 g/cm$^3$).

**Table 1**
Water absorption index (WAI), critical humidity point (CHP) and packing density (PD) of different materials.

| Water absorption index (WAI), critical humidity point (CHP) and packing density (PD) of different materials | | | |
|---|---|---|---|
| **Support** | **WAI (g/g dw)** | **CHP (%)** | **PD (g/cm$^3$)** |
| Soybean hulls | 4.69 ± 0.06 | 11.00 ± 0.68 | 17.86 $10^{-2}$ ± 4.52.$10^{-3}$ |
| Waste paper | 3.41 ± 0.16 | 9.17 ± 0.71 | 20.50 $10^{-2}$ ± 7.07 $10^{-3}$ |

Data are shown as the mean ± standard deviation (SD).

### 3.2. Cellulase production in SSF

During the fermentation process, various materials can supply carbon and nutrients or can be used as growth support. Fungi adapt to SSF since their hyphae can grow over the surface and penetrate into the interstitial spaces and colonize the solid support (Graminha et al., 2008).

Glucosamine ($C_6H_{13}NO_5$) is an amino sugar that is found primarily in the exoskeleton of crustaceans and arthropods, and it is part of the cell wall of fungi and many other organisms, representing the most abundant monosaccharide, and thus, its quantification allows estimation of the biomass content in the culture (Blix, 1948).

In this work, *A. niger* NRRL3 presents a biomass production reaching the maximum value at 120 h in soybean hulls (Fig. 1). This value is eight times higher than that obtained at 120 h in waste paper. This difference could be due to the presence of certain components in the soybean hulls, which are suitable for the growth of fungus.

Endoglucanase activity was assayed for both supports. In Fig. 2 (A), it can be seen that the endoglucanase activity is higher in soybean hulls at all times studied. The same situation was found for exoglucanase and β-glucosidase activities (Fig. 2B, C).

Maximum endoglucanase activity was found at 96 h (5914.29 U L$^{-1}$), being four times higher than that obtained using waste paper at the same fermentation time. Endoglucanase activity values obtained in this study are 25–50-fold higher than those reported by Acharya et al. (2008), who used SSF of pretreated sawdust at 9.6% concentration and an *A. niger* natural isolate wild-type strain.

The exoglucanase activity in soybean hulls was maximal at 96 h (4551.19 U L$^{-1}$), being 9.6 times higher than that obtained in waste paper at the same time. Fig. 2(C) shows that the maximum β-glucosidase activity in soybean hulls (984.01 U L$^{-1}$) was reached at 96 h, being 1.7 times greater than that obtained in waste paper.

Therefore, cellulase activities markedly increased up to 96 h. There was no difference between 96 and 120 h. A similar pattern was obtained by Brijwani et al. (2010) when they performed SSF of soybean hulls supplemented with wheat bran using a co-culture of *T. reesei* and *Aspergillus oryzae*.

Cellulase activities obtained in this work using soybean hulls as support were two to three times higher than those reported by Shin et al. (2000). They employed SSF using different types of materials as a carbon source and *T. reesei* Rut C-30 strain.

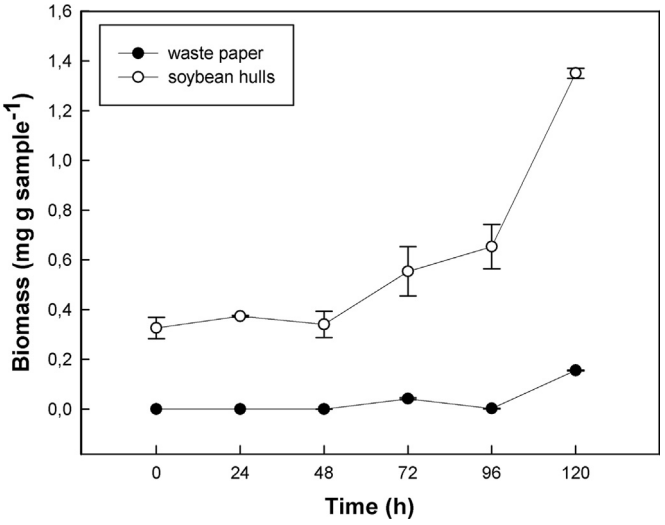


**Fig. 1.** Time course of biomass production.

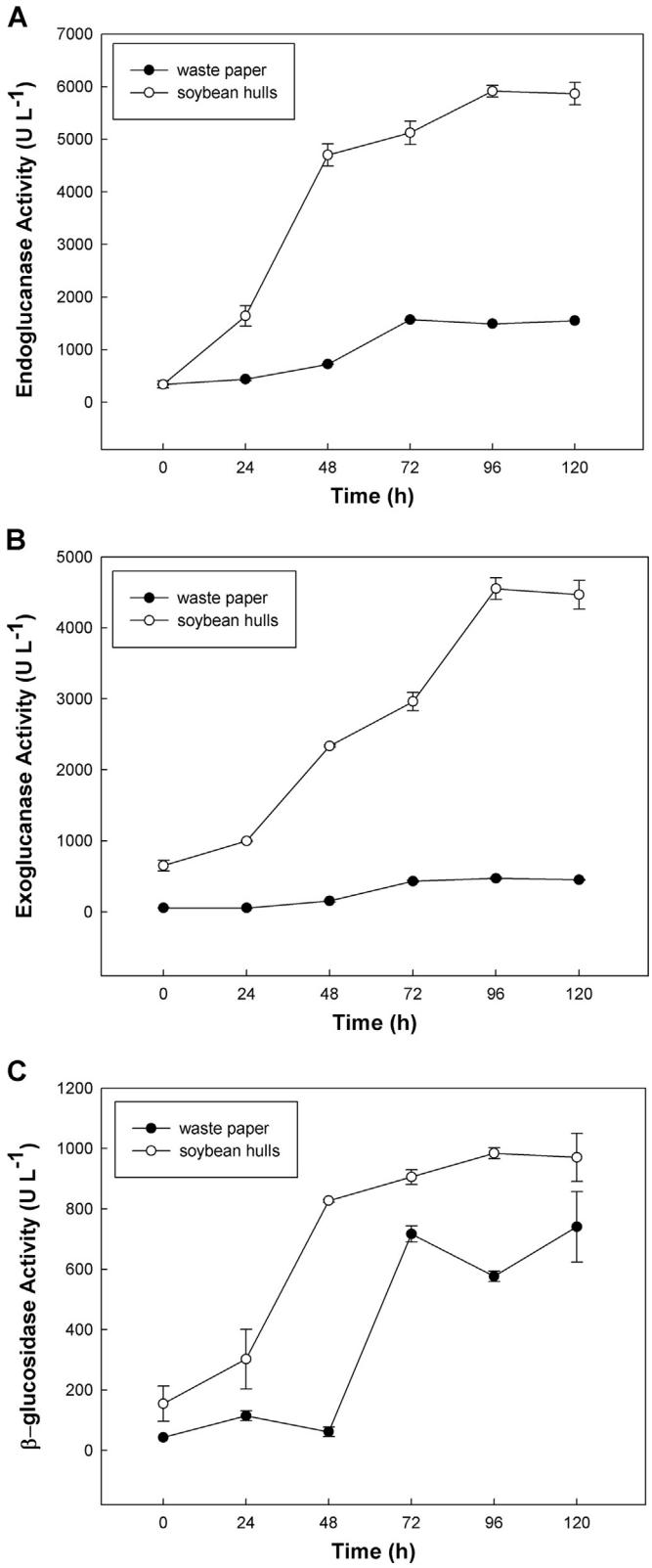


**Fig. 2.** Time course of endoglucanase activity (A). Time course of exoglucanase activity (B). Time course of β-glucosidase activity (C).

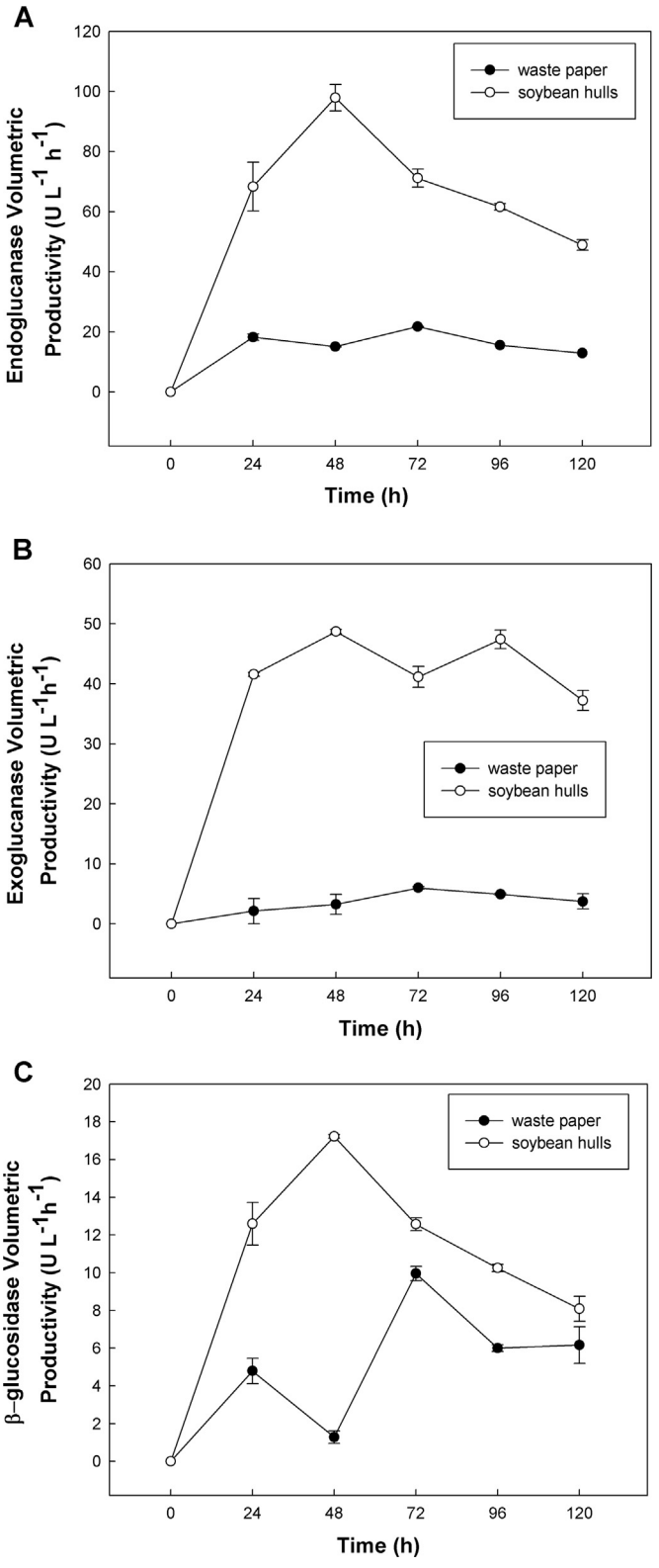


**Fig. 3.** Time course of endoglucanase volumetric productivity (A). Time course of exoglucanase volumetric productivity (B). Time course of β-glucosidase volumetric productivity (C).

Moreover, they have reported a maximum cellulase activity after seven days of fermentation. This fermentation time is greater than that obtained in this work, which would increase costs considering a scaled process. On the other hand, when Shin et al. (2000) carried out SSF using steam-exploded wood as support, they reported 140 U $L^{-1}$ of β-glucosidase activity at six days of fermentation. These values are five to seven times lower than those reached in this work using waste paper and soybean hulls respectively. These results evidence a lower production of β-glucosidase by *T. reesei* compared to that of *A. niger*.

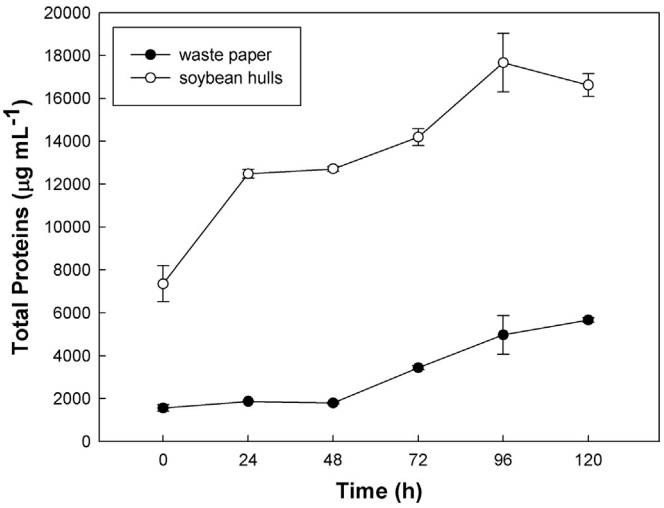


**Fig. 4.** Time course of total proteins.

These results suggest that the soybean hulls support contains the proper nutrients for optimal production of cellulolytic complex. The highest WAI value and the lowest PD obtained in soybean hulls can favor cellulase production and fungal growth and development. The use of soybean hulls provides the highest volumetric productivity for the three enzymes that make the cellulolytic complex for short production times (Fig. 3A–C). The maximum volumetric productivity was found for the three enzymes at 48 h of production in SSF using soybean hulls as support. Besides, the use of agro-industrial residues as support contributes to decreasing environmental problems (Adeoye et al., 2015).

According to Fig. 4, total proteins in soybean hulls were higher than those obtained in waste paper at all fermentation times studied. The maximum was reached at 96 h of fermentation (17,667.41 μg $mL^{-1}$).

Other authors (Chen and Wayman, 1991) have used waste newspaper as substrate for cellulase production by *T. reesei* under submerged fermentation. They estimated protein content as an indicator of cellulase production. At six days of fermentation they reported a protein concentration 18 times lower than that obtained in the present work at five days of fermentation using waste paper as support. Besides, they carried out pretreatments on the waste newspaper, which increases the costs of the overall process. Furthermore, previous works (Wood et al., 1997) have made SSF on waste office paper but they used ultrasound on SSF process and this fact does not contribute to the energy saving process.

Also, Shin et al. (2000) carried out SSF for cellulase production using waste newspaper and office paper as supports. These substrates had been subjected to various pretreatments, which represent a disadvantage as mentioned above. Besides, they reported a maximum of cellulase activity at seven days of fermentation. In our work, endoglucanase, exoglucanase and β-glucosidase activities peaked in just 72 h when waste paper was employed as support.

Similarly, previous research has studied different pretreatments on soybean hulls (Corredor et al., 2008; Yoo et al., 2011). Brijwani and Vadlani (2011) investigated the effect of pretreatment on the physicochemical characteristics of soybean hulls and production of cellulolytic enzymes in SSF by *T. reesei* and *A. oryzae* cultures. They observed that enzyme production in both mono and mixed cultures of *T. reesei* and *A. oryzae* was significantly reduced in alkali-pretreated soybean hulls and in acid-pretreated soybean hulls compared to the native substrate. Besides, the elimination of pretreatment technology prevents the production of fermentation inhibitors (Klinke et al., 2004), simplifies the process and reduces costs. In addition, most pretreatment processes degrade proteins chemically, eliminating its nutrient and commercial value for animal feed. In this work, SSF was carried out on soybean hulls in the absence of any thermochemical pretreatment, thus having the advantages mentioned above. These benefits may be more significant in the potential application of this method in scaling up.

### 3.3. Scanning electron microscopy

Environmental scanning electron microscopy showed that *A. niger* NRRL3 grew by invading and penetrating the soybean hulls support (Fig. 5). Soybean hulls have a rough surface with some micropores (Fig. 5 A). In Fig. 5(A), the micropore is 3.63 μm and 1.14 μm long wide. In Fig. 5(D), a 12.18 μm-wide conidiophore with a 66.60 μm-wide and 45.73 μm-high conidial head is observed. Spores with 2.57 μm in diameter can be seen in Fig. 5(E).

### 3.4. Statistical analysis

The support-time interaction was significant for all the variables studied (Table 2).

According to the results of statistical analysis, even though biomass production was higher in soybean hulls at all times in the descriptive study, there were no significant differences at times 24, 72, 96 h between both supports.

No significant differences were found for endoglucanase and exoglucanase activities at 48 and 72 h. Moreover, significant differences were detected between both supports for β-glucosidase activity at times 48, 72, 96, 120 h. In each case, the activity values were higher in soybean hulls at all times studied.

Because of volumetric productivity values obtained, the support of soybean hulls is very interesting for cellulase production. Although, at certain times, there were no significant differences, volumetric productivity of three enzymes was higher with soybean hulls than with waste paper at all times studied. No significant differences were found for endoglucanase volumetric productivity at times 24, 72 and 96 h. Significant differences were detected for exoglucanase volumetric productivity at all times studied except at 0 h of SSF. No significant differences were found for β-glucosidase volumetric productivity at times 24, 72, 96 and 120 h.

For endoglucanase specific productivity, a significant difference between both supports was detected only at 24 h. While exoglucanase specific productivity was higher in soybean hulls at all times studied, no significant differences were detected between both supports for the desired significance level. In addition, significant differences between both supports were detected at 24 and 72 h for β-glucosidase specific productivity.

Although total proteins were higher in soybean hulls at all times studied, significant differences were detected at 0 and 96 h.

There were significant differences between both supports for endoglucanase specific activity at 0, 24 and 72 h. While exoglucanase specific activity was higher in soybean hulls, statistical significant differences were detected at 48, 96 and 120 h. Furthermore, significant differences between both supports were detected for β-glucosidase specific activity at 24, 72, 96 and 120 h.

The results of this study suggest that soybean hulls are a potential material for cellulase production under SSF. According to the results obtained from the physico-chemical analysis, soybean hulls showed a high WAI value, being four times higher than that obtained by Robledo et al. (2008), when they evaluated the use of pomegranate residues as supports in SSF for ellagic acid production. Moreover, when Orzua et al. (2009) tested ten agro-industrial wastes, they obtained CHP values above 20%. In this work, soybean hulls showed a CHP value of only 11%. This low value of CHP is

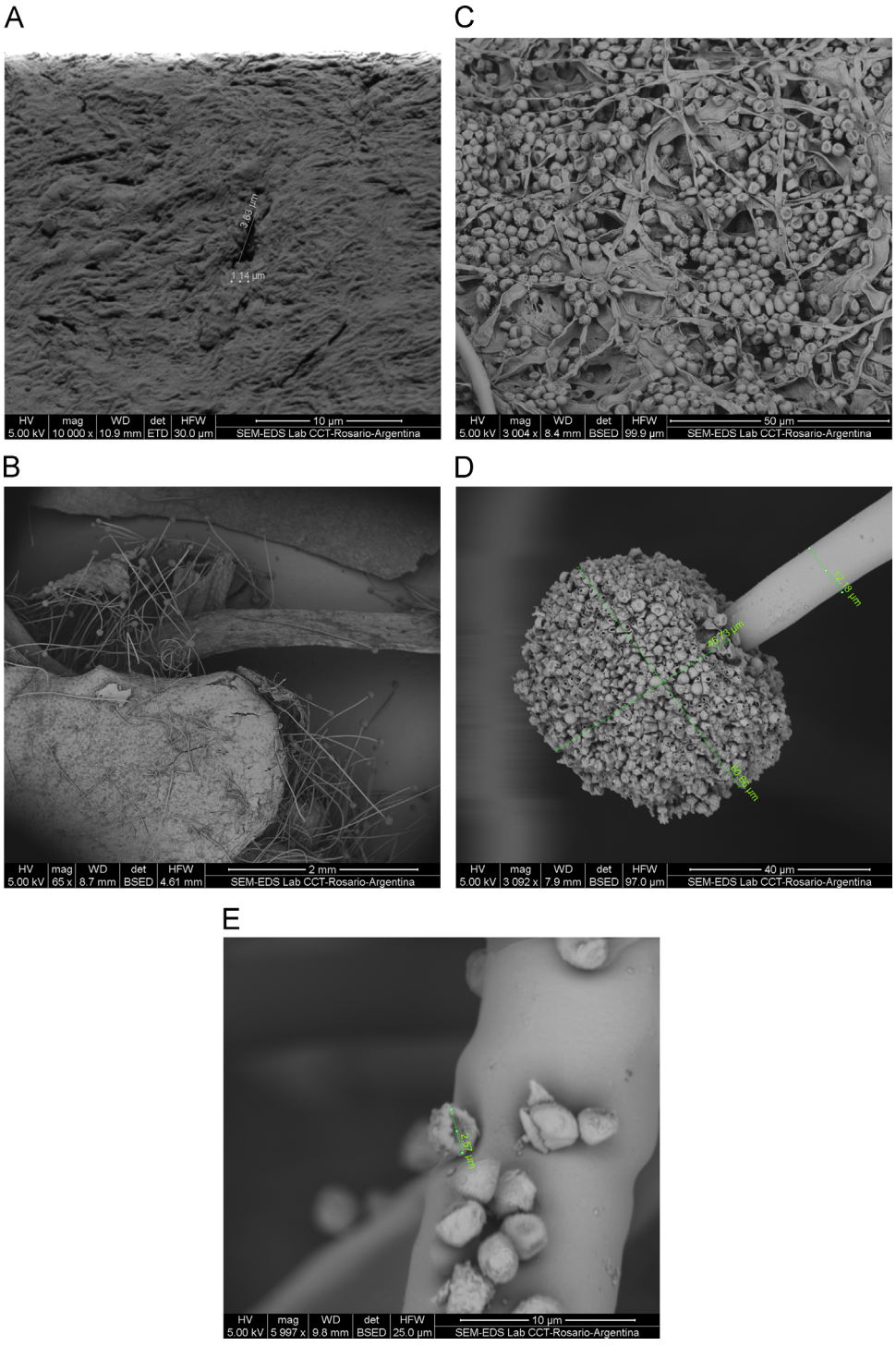


**Fig. 5.** SEM of soybean hulls (A). SEM of *A. niger* NRRL3 · growth on soybean hulls (B and C). SEM of conidiophore and conidial head (D). SEM of spores on soy hulls (E).

favorable for fungal growth and represents an additional advantage for selecting soybean hulls as support.

Furthermore, SEM evidenced that *A. niger* NRRL3 grew by invading the soybean hulls support.

Besides, the volumetric productivity of the three components of the cellulase complex reached its maximum in just 48 h, which is a promising result for future research.

The selection of an appropriate substrate is a key aspect in SSF (Pandey, 2003). Since Argentina is a major soybean producer and given that soybean hulls are an agro-industrial waste widely available, soybean hulls would represent an attractive substrate to be used in SSF.

## 4. Conclusions

In SSF processes, it is important to take into account aspects such as cost and availability of the support, environmental impact, as wells as process cost. According to the results obtained from the physico-chemical analysis (WAI, CHP and PD), soybean hulls and waste paper have great potential to be used as supports in SSF process. However, the use of soybean hulls provided higher volumetric productivities at shorter times.

The development of a bioprocess for cellulase production using soybean hulls as support would offer economic and environmental

**Table 2**
ANOVA results for the effect of support and time on cellulase production.

| $F_{values}$ | Responses | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Biomass** | **En.A.** | **Ex.A.** | **β.A.** | **En.V.P.** | **Ex.V.P.** | **β.V.P.** | **En.S.P.** | **Ex.S.P.** | **β.S.P.** | **Proteins** | **En.S.A.** | **Ex.S.A.** | **β.S.A.** |
| **Support** | 36.9*** | 37.0*** | 36.3*** | 200.3*** | 38.7*** | 4370.0*** | 36.4*** | 38.4*** | 36.4*** | 99.9*** | 36.3*** | 30.7*** | 113.2*** | 172.0*** |
| **Time** | 16.2*** | 45.9*** | 46*** | 146.4*** | 31.9*** | 267.0*** | 10.3*** | 320.1*** | 11.5*** | 67.6*** | 30.0*** | 106.0*** | 21.2*** | 82.3*** |
| **Support*time** | 27.1*** | 80.3*** | 64.5*** | 19.6*** | 27.1*** | 188.0*** | 14.5*** | 26.8*** | 3.2* | 32.7*** | 22.0*** | 18.6*** | 5.3** | 44.0*** |

En.A.: *Endoglucanase Activity.* Ex.A.: *Exoglucanase Activity.* β.A.: β-*Glucosidase Activity.* En.V.P.:*Endoglucanase Volumetric Productivity.* Ex.V.P.: *Exoglucanase Volumetric Productivity.* β.V.P.: β-*Glucosidase Volumetric Productivity.* En.S.P.: *Endoglucanase Specific Productivity.* Ex.S.P.: *Exoglucanase Specific Productivity.* β.S.P.: β-*Glucosidase Specific Productivity.* En.S.P.: *Endoglucanase Specific Activity.* Ex.S.P.: *Exoglucanase Specific Activity.* β.S.P.: β-*Glucosidase Specific Activity.*

* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

benefits compared with chemical methods.

This work has highlighted that solid-state fermentation is a valuable method in order to obtain the three components of the cellulase complex that can effectively saccharify lignocellulosic wastes like soybean hulls. It is not necessary to produce the enzymes separately since *A. niger* NRRL3 produces the three components of the cellulase complex in sufficient quantities. This would have a positive impact on the economy of the industries that require the use of the cellulase complex.

Future studies should aim at developing a scale-up operation of cellulase production from soybean hulls under SSF, which would be helpful to local industries in order to achieve high productivity at low costs.

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