STUDY ON DEGRADATION MECHANISM OF CORN STRAW BY DIFFERENT FUNGI IN NON-SOIL ENVIRONMENT

ZHANG, Y. F. – DOU, S.* – ZHANG, D. D. – NDZELU, B. S. – MA, R. – YE, S. F.

College of Resource and Environmental Science, Jilin Agricultural University
Changchun 130118, Jilin Province, China

**Corresponding author*
e-mail: dousen1959@126.com

(Received 18th Feb 2022; accepted 20th May 2022)

Abstract. Studies on optimizing the best composting conditions using microorganisms producing enzymes have a long history, yet few studies have emphasized the similarities and differences in the decomposition mechanisms of different fungi during the same conditions in non-soil environments. The degradation and transformation of corn straw treated with three different fungi (*Trichoderma reesei*, *Phanerochaete chrysosporium* and *Trichoderma harzianum*) under 25-day solid-state aerobic composting was investigated, including total organic carbon (TOC) and C/N ratios, lignocellulose, enzyme activities and scanning electron microscope (SEM) observation. The present study illustrated that under the same non-soil environment, *T. reesei* had the best general degradation and utilization ability among the three fungi, with a cumulative TOC consumption of 58.5 $g \cdot kg^{-1}$ in 25 days and a cumulative decrease of 5.8 in C/N. *T. reesei* and *P. chrysosporium* were more suitable for further exploration of the mechanism of corn straw degradation and transformation in soil environment than *T. harzianum*.
Keywords: *total organic carbon, Trichoderma reesei, Phanerochaete chrysosporium, Trichoderma harzianum, C/N ratio*

Introduction

Corn straw is composed of 25-35% cellulose, 20-40% hemicellulose, and 10-25% lignin (Martinez et al., 2004), which are chemically bonded and entangled to form a plant cell wall harsh to degrade (Heck et al., 2002; Sánchez, 2009). With vigorous agricultural activity, a large amount of corn straws is produced annually as relatively redundant agricultural waste. The Food and Agriculture Organization of the United Nations (FAO) estimates that one-third of all food produced is lost or wasted (Hebrok and Boks, 2017; Salihoglu et al., 2017). Hence reasonable organic waste management has become a global environmental challenge, especially in developing countries (Guo et al., 2016; Onwosi et al., 2017). Methods for better use of corn straw have become a research hotspot. Straw returning has been shown to be an effective means to improve soil fertility and manage crop residues. However, the decomposition process of straw residues when directly returned to the field is slow and is affected by soil microorganisms, enzymes, soil texture, and pH. As a result, several physicals, chemical, and biological technologies to treat corn straw have been developed to achieve a beneficial use of this ubiquitous waste product (Guo et al., 2013; Wang et al., 2015; Zou et al., 2016). Solid-state aerobic composting is regarded as an efficient utilization method under non-soil environment, which involves the process of degrading and transforming the straw organic matter through microbial intervention under optimized physical and chemical conditions. The process consists of both organic matter mineralization and humification (formation of Humic substances). The final product of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

composting can be used as an organic fertilizer and soil improver (Maeda et al., 2010; Jurado et al., 2015).

Fungi are the predominant micro-organisms that degrade lignocellulose, and the efficiency and rate of fungal degradation of corn straw is generally much higher than that of bacteria. In the common ecological concept, the selection of organisms that increases the intrinsic growth rate is called r-selection; the selection of organisms that is conducive to the increase of competitiveness is called K-selection (Pianka, 1970; Blagodatskaya et al., 2007). K-strategists can degrade complex insoluble organic substances due to the diversity of metabolic pathways, providing the minimal substrate flux necessary for slow growth. In the soil environment, fungi that are K-strategic microorganisms mainly decompose refractory organic matter, while bacteria that are r-strategic microorganisms prefer to use active organic matter (Dorodnikov et al., 2009; Grover et al., 2015). Fungi are also more susceptible to decomposition and therefore contribute more to soil organic matter. The mechanism of fungal degradation of lignocellulose is mainly due to two mechanisms: (i) production of extracellular enzymes by the fungi, and (ii) mechanical perforation of the straw by the fungal hyphae which facilitate the decomposition. Studying the use of fungi in corn straw can achieve maximum utilization benefits and value. *Trichoderma* is a genus that secretes and forms cellulase systems that researchers know best so far (Vijai et al., 2014). *Trichoderma reesei* (*T. reesei*) has been investigated in the degradation of cellulose from renewable substrates such as spruce, bagasse, waste paper, cow dung, and willow (Shin et al., 2000; Sørensen et al., 2014; Sukumarana et al., 2009; Wen et al., 2005). Tangnu et al. (1981) did not find changes of cellulase yield and final yield of *T. reesei* in the pH range of 4-6. Xia and Cen (1999), Latifian et al. (2007) also reported similar phenomena about *T. reesei* MCG77. But for filamentous fungi, the optimal pH of exoglucanase and β - glucosidase is 5-6 (Prasetyo et al., 2009). Li et al. (2013) found that 5.0 was the best pH of *T. reesei* for hyphal branching and cellulase production. Nazanin et al. (2018) found that the enzyme activity of *T. reesei* was the highest at 30 °C. *Trichoderma harzianum* (*T. harzianum*) is also the most widely used fungus of the genus *Trichoderma*. The optimal growth temperature of *T. harzianum* proposed by Zhang and Yang (2015) is 30 °C. Compared with *T. reesei*, some *T. harzianum* strains produced a cellulolytic complex with higher β-glucosidase and endoglucanases activities, and the xylanase activity of some *T. harzianum* strains was higher than *T. reesei*. However, the efficiency of simultaneous cellulose and hemicellulose hydrolysis by the secretome of *T. harzianum* was still low (Hu et al., 2014; Zhao et al., 2016). Castro et al. (2010) reported endoglucanase and cellulase activities and demonstrated that cellulose degradation occurred within 72 h under 30 °C when bagasse was pre-treated with *T. harzianum* IOC-3844. Paz et al. (2018) observed that *T. harzianum* EM0925 growing on corn stalk had high content of lignocellulosic enzymes and high capacity of producing enzymes without any additives. Zhang et al. (2020b) found that the cellulase activity and xylanase activity of *T. harzianum* had the highest relative activities at pH = 5. *Phanerochaete chrysosporium* (*P. chrysosporium*) is a fungal species capable of degrading lignin by excreting extracellular oxidases such as lignin peroxidase (LiP), manganese peroxidase (MnP), and lactase (Lac) (Law et al., 2003; Tamagawa et al., 2005; Bak et al., 2009; Lin et al., 2015). Rodríguez et al. (2012) used outside and inside corn cob to study ligninolytic enzymes produced by *P. chrysosporium* ATCC 24725 during solid state fermentation conditions (pH 4.5-5) and achieved a maximum MnP activity of 96

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

$U \cdot L^{-1}$. It was the optimum value for the growth of oxidin peroxidase by *P. chrysosporium* when the pH was 4.5-5 (Aksu and Donmez, 2003; Cetin and Donmez, 2006; Liu et al., 2015). Zhang et al. (2019a) showed that the metabolism of *P. chrysosporium* was more effectively promoted at 30 °C.

Studies on optimizing the best composting conditions using pure microbial producing enzymes have a long history, but few studies have emphasized the similarities and differences in the decomposition mechanisms of different fungi during the same condition in non-soil environments. This work reports choosing *T. reesei*, *P. chrysosporium*, and *T. harzianum* to determine the similarities and differences in the decomposition mechanism of corn straw in non-soil environments. Through the background investigation of three kinds of fungi, we found and chose the common best condition (pH = 5, temp = 30 °C) to compare the degradation differences of three kinds of fungi on corn straw. It promotes the further screening of strains that can effectively transform corn straw and provides a theoretical basis for future applications in the soil environment.

Materials and methods

Site description and corn straw sampling

The sampling site of corn straw (N43°48′43.5″, E125°23′38.50″) presented by maize cropland located in Jilin Agricultural University, Nanguan District, Changchun City, Jilin Province, China. The area is in a temperate continental semi-humid region and receives a mean annual rainfall of 618 mm, a mean annual relative air humidity of 68%, and an average annual temperature of 5.1 °C. The highest average temperature of 23.1 °C occurs in July and August, while the lowest average temperature of -10.6 °C occurs in November and December. Black soils (Chinese Soil taxonomy) is the main soil in the region and is classified as Argiudolls by the United States Department of Agricultural Soil Taxonomy (Soil Survey Staff, 2014).

The corn cultivar Zhongjin 368 type (Beijing Golden Grain Seed Co., Ltd.), was planted at the end of April 2016 and harvested in early October. The basic properties of the corn straw are shown in *Table 1*. After the harvest, the whole corn straw was naturally dried and cut into 0.5 cm segments.

Table 1. *Basic properties of corn straw*

Organic material	Total organic carbon ($g.kg^{-1}$)	Total nitrogen ($g.kg^{-1}$)	Total phosphorus ($g.kg^{-1}$)	Total potassium ($g.kg^{-1}$)	C/N ratio (mol)
Corn straw	376.4	7.22	7.7	4.5	60.8

Preparation of microorganisms and microbiological liquid

Three fungal strains used in this study: *T. reesei* MCG77, *P. chrysosporium* ATCC 24725 were purchased from the American Type Culture Collection (ATCC). The *T. harzianum* was isolated and purified from fresh soil at the Jilin Agricultural University experimental field after one year of straw returning (Provided by The Microbial laboratory of Environment and Resource Department, Jilin Agricultural University).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

The three strains of fungi were placed in a solid bevel tube containing 30 ml of potato dextrose agar (PDA). The solid bevel tube was incubated at 28 °C for 72 h to obtain mature microbial spores (mycelium). The method for preparing a spore solution was given the same as Zhang et al. (2020a). The concentration was calculated through a haemocytometer and diluted to a final concentration of 1×10^7 CFU mL^{-1}. The spore suspension was transferred to a liquid medium considering a ratio of 1:10. The culture was incubated at 30 °C and 100 rpm for six days. PDA medium: potato (peeled, 200 $g\cdot L^{-1}$) boiled for 30 min and filtered; glucose: 20 $g\cdot L^{-1}$; agar: 20 $g\cdot L^{-1}$. Liquid medium: potato (peeled, 200 $g\cdot L^{-1}$) boiled for 30 min and filtered; glucose: 20 $g\cdot L^{-1}$.

Solid-state fermentation

This fermentation was conducted in a BIOTECH-30SS solid fermentation tank (Shanghai Baoxing Biological Engineering Equipment Co., Ltd). This tank has a volume of 30 L, a sterilizing function, automatic stirring, controlled humidity and temperature, and air intake. A KQ-C type automatic steam generator (Shanghai Fengxian Xiexinji Power Plant) was used to generate steam for sterilization.

Prior to the fermentation, 1.5 kg of corn straw powder (segments size = 0.5 cm) was sterilized in the solid fermenter. The sterilization was conditioned at 121 °C for 25 min. After the sterilization, the microbial liquid containing the spore mycelia (0.6 L) and the mineral salt solution (3.75 L) were mixed. The fermentation was set at 30 °C, 60% humidity and 6.0 rpm.

Corn straw was inoculated with: (i) *Phanerochaete chrysosporium* (*P. chrysosporium*), (ii) *Trichoderma harzianum* (*T. harzianum*), (iii) *Trichoderma reesei* (*T. reesei*), and (iv) un-inoculated corn straw (CK). The CK treatment was prepared without microbial inoculation on corn straw and designated as a control.

The mineral salt solution contained at a final pH of 5 was prepared by adding: urea 4.2 $g\cdot L^{-1}$, ammonium sulfate 19.6 $g\cdot L^{-1}$, calcium chloride 0.028 $g\cdot L^{-1}$, potassium dihydrogen phosphate 28 $g\cdot L^{-1}$, magnesium sulfate 4.2 $g\cdot L^{-1}$, ferrous sulfate 0.07 $g\cdot L^{-1}$, manganese sulfate 0.021 $g\cdot L^{-1}$, zinc sulfate 0.019 $g\cdot L^{-1}$, cobalt chloride 4.2 $g\cdot L^{-1}$, and yeast extract 7 $g\cdot L^{-1}$.

Sample collection

The samples were homogenously mixed before the fermentation process. The experiment was performed in triplicates: 3 samples per treatment were collected from the fermenter from three random positions every day. At each sampling, the straw and the liquid were uniformly mixed. The monitoring lasted for 25 days with the samples analysed separately. The fermentation process was performed under the same experimental condition for each treatment.

Analytical methods

Scanning electron microscope observation

A small amount of straw residues sample was taken and fixed on the sample stage with conductive double-sided tape, and gold plating was performed under vacuumed conditions. After forming a conductive film on the surface of the material, the samples of corn straw residues were observed by scanning electron microscope (SEM, SHIMADZU S-550, operating voltage: 15 kV).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

Measuring residual rates of corn straw

To determine the water content of straw fermentation products, an empty aluminium box was placed in Oven at 105 °C for 30 min. After cooling, the mass of the empty box was recorded. Three parallel samples were then weighed from the mixture up to 5 g each (~0.01 g). The sample was added to the aluminium box and the combined weight was recorded. The aluminium box with the sample was then placed in an oven at 85 °C. The mixture was placed in a desiccator, cooled for 20 min, and weighed again. The straw residual rate (n%) was calculated as follows:

$m_{n(\text{dry})}$: mass of the sample and the aluminium box after drying (g) during the nth day.

$m_{n(\text{box})}$: mass of aluminium box (g) during the nth day.

The dry weight of the nth day was measured and recorded as R_n:

$$R_n = m_{n(dry)} - m_{n\,(box)} \quad \text{(Eq.1)}$$

The straw residual rate of the nth day was measured and recorded as n%:

$$n\% = R_n / R_0 \times 100\% \quad \text{(Eq.2)}$$

TOC (total organic carbon) content and C/N ratios of corn straw residues

Weighed 0.25 g of dried (55 °C for 6 h) and fine ground corn straw sieved at 0.25 mm. The TOC content was determined using the potassium dichromate volumetric method (Lu, 2000).

The elemental composition of the residue of the straw after solid-state fermentation was analysed using an Elemental Vario EL III elemental analyser from Germany in the C/H/N mode. The contents of C, and N elements were measured. The C/N ratio was calculated based on the result of element composition as follows:

$$C / N = C(g \cdot kg^{-1}) / N(g \cdot kg^{-1}) \quad \text{(Eq.3)}$$

Determination of lignin and cellulose in corn straw

The determination of straw cellulose and lignin content was based on Sluiter's work (Sluiter et al., 2008). Exactly, 0.3 g sample was digested with 3 mL of 72% H_2SO_4 and heated at 30 °C for 1 h. The mixture was then transferred to a 500 mL reagent bottle, and 84 mL of deionized water was added. The mixture was then sterilized in an autoclave at 121 °C for 1 h. The solution was filtered using vacuum filtration and the residue weight and glucose content were measured. The cellulose and lignin contents were calculated using the formula described by Anuradha and Valli (2011).

Determination of enzyme activity

Determination of cellulase activity: a Whatman filter paper (6 × 1 cm) was folded and put into the bottom of a sterile test tube, added into which 1 mL of citrate buffer (pH = 4.8). 0.5 mL diluted spore suspension was then added following five different concentrations: 0.04, 0.05, 0.057, 0.1, 0.2 g·mL^{-1}. After 60 min at 50 °C, added 3 mL of 3,5-dinitrosalicylic acid (DNS), added, thereafter, the mixture was heated in a boiling

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

water bath for 5 min, then cooled immediately. 200 μL of the above solutions were diluted afterwards with 2.5 mL deionized (DI) water. Absorbance was measured on a visible spectrophotometer (SP-722E) at OD540, and the glucose content was calculated according to the NREL method (Sluiter et al., 2008). The ratio of 0.37 corresponding to the enzyme concentration, releasing 2 mg glucose, is defined as the cellulase activity (Ghose et al., 1987).

Determination of xylanase activity: 1.0 g of xylan (Production company is Sigma, level is AR) was dissolved into 80 mL of 50 mM citrate buffer (pH = 4.8), balanced at 60 °C water bath for 1 h, then transferred to another water bath at 100 °C until boiling. The mixture was homogenized using magnetic stirrer during the cooling process. The solution was left overnight and then refilled with citrate buffer (pH = 4.8) to 100 mL to prepare xylan stock with concentration 10 $mg \cdot mL^{-1}$. Stored it at 4 °C up to one week. After a week, 1.8 mL of the stocked xylan and 200 μL of fermented sample solution was diluted to 1:10 with deionized water, where pipettes into 15 mL test tube, and then placed in a water bath at 50 °C for 5 min. Exactly, 3.0 mL of DNS reagent was added into the test tube, boiled for another 5 min, and then cooled immediately. Absorbance was measured at OD540. The amount of enzyme to produce 1.0 μmol xylose per minute was defined as a unit of xylanase activity (Bailey et al., 1992).

The β-glucosidase activity was measured by cellobiose method. 200 μL fermented sample solution was diluted to 1:200 with citrate buffer (pH = 4.8). 1.0 mL of the above solution and 1.0 mL of 15 $mmol \cdot L^{-1}$ cellobiose were pipette into a sterile centrifuge tube, then placed in a water bath at 50 °C for 30 min, and then transferred to 100 °C water bath for 5 min. The tube was then removed and cooled. The mixed solution was used to measure glucose content with a glucose kit (Huili Biotech, China). Another 1.0 mL of the diluted sample solution and added 1.0 mL of citrate buffer into a sterile centrifuge tube as an enzyme blank. The ratio of the corresponding enzyme concentration to 0.0926 when the glucose content in the sample is 1 $mg \cdot ml^{-1}$ was defined as the glucosidase activity. For details, see the previous report (Anuradha and Valli, 2011).

Determination of the total fungal content

The fungal quantity was measured on the samples at 0 d, 5 d, 10 d, 15 d, 20 d, and 25 d after the start of the culture in each group. Weigh 10 g of each sample to be tested and put it in 90 mL of sterile water. After shaking and culturing for 20 min, shake thoroughly for 15-20 min, leave it for 30 min, and separate the suspension into layers. The dilution coating plate counting method was based on Pitt and Hocking (2009). Total fungal counts were expressed as colony forming units per gram (CFU g^{-1}).

Data processing

Microsoft Office Excel 2017 was used for data analysis and processing. Mean values and standard deviations of triplicate measurements were reported in this study. Significant differences among treatment means were evaluated using the least significant difference test with TUKEYs adjustment at $P < 0.05$. The SPSS 19 for Windows® software (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. The figures were drawn using the Origin 2019b software.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

Results

Scanning electron microscope (SEM) observation of the corn straw after different treatments

Figure 1 shows the Scanning Electron Microscopy results of corn straw residues of different treatments in solid-state aerobic composting for 25 days (O: Original corn straw; A: CK; B: *T. reesei*; C: *T. harzianum*; D: *P. chrysosporium*). The surface of untreated corn straw was smooth and free of fungi (*Fig. 1O*). In SEM observation of the treated corn straw residue, due to the invasion of fungi, the original structure on the surface of the corn straw was destroyed in a large area, and many cracks appeared. These filamentous fungi mechanically attacked and swelled corn straw, invaded the interior through narrow cracks, and eventually decomposed the straw. Fungi can be distributed in communities to participate in decomposition. CK (*Fig. 1A*) treatment still maintained the original shape of the straw, and no obvious mechanical damage was observed. The corn straw treated by *T. reesei* (*Fig. 1B*) was severely damaged both in internal and external; *T. harzianum* (*Fig. 1C*) damaged the surface of the corn straw to a certain extent, but the erosion of the internal structure is not apparent; *P. chrysosporium* (*Fig. 1D*) penetrated the external pores of corn straw and destroyed its internal structure.

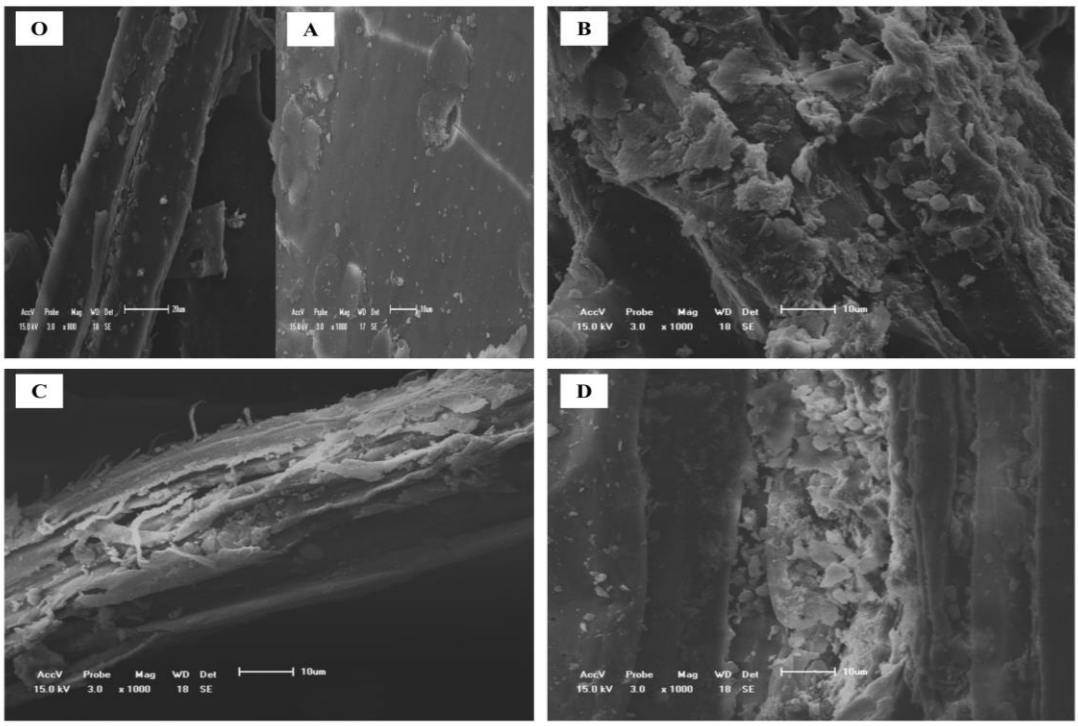

Figure 1. *Electron microscopy results of corn straw residues under different treatments in a solid-state aerobic composting for 25 days. The treatments represented by the capital letters in the figure are as follows: O: Original corn straw; A: CK, B: T. reesei; C: T. harzianum; D: P. chrysosporium*

Dynamic changes of residual rates

The residual rate of the straw residues gradually decreased with fermentation time regardless of treatments (*Fig. 2*). The most significant reduction in water was noted for

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

the *T. reesei* treatment on 16-20 d, with an average reduction of 1.66% per day and residual rate of 78.6 ± 0.77% at 25 d (*Fig. 2*). The *P. chrysosporium* and *T. harzianum* were completely fermented by 13-16 d in which a significant decrease was observed by respectively 1.31% and 1.16% per day. CK treatment decreased slowly throughout the entire culture process, with an average reduction of 0.31% per day. At the end of the fermentation, the total weight loss of straw under the four treatments can be ordered as the following: *T. reesei* > *P. chrysosporium* > *T. harzianum* > CK (*Fig. 2*). The straw residue rates were respectively 78.6 ± 0.8%, 84.6 ± 0.8%, 86.1 ± 1.7%, and 92.2 ± 1.2%. (Notes: Number of technical replications is three.)

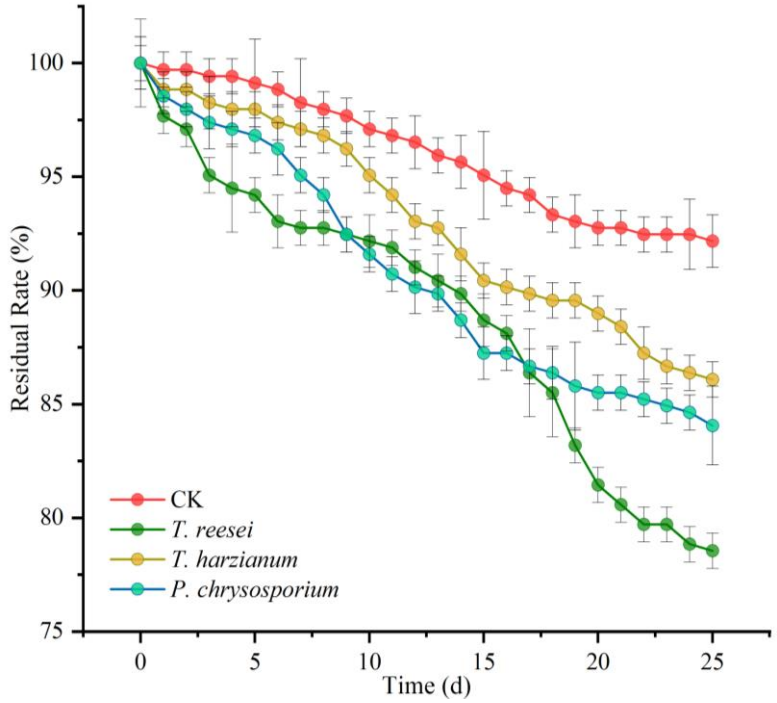

Figure 2. *The corn straw residual rates of different treatments over time under solid-state aerobic composting. Results are means ± SD (n = 3) on every single time-point*

Changes of total organic carbon and C/N ratios in corn straw residues

TOC and C/N ratio are essential indicators to characterize the degradation and transformation of corn straw (Bernal et al., 1998; Bertoldi et al., 1983). Through *Figure 3* we can observe the degradation and transformation of corn straw by different treatments during the 0-25 days. According to the statistics of the TOC distribution of different treatments, it showed that TOC of CK treatment was almost unchanged, always concentrated at 370 $g \cdot kg^{-1}$; TOC of *T. reesei* treatment in 5-25 days were all lower than 350 $g \cdot kg^{-1}$; *T. harzianum* and *P. chrysosporium* treatments had similar distributions, with TOC concentrated between 340 $g \cdot kg^{-1}$ and 370 $g \cdot kg^{-1}$, respectively. According to the statistics of the distribution of C/N ratios of different treatments, it showed that the distribution of CK treatment was concentrated at 24-26, with a little change. The C/N ratios of *T. reesei* after 10 d were all lower than 22; and the C/N ratio of *P. chrysosporium* treatment had a more significant decline than *T. harzianum*. The TOC and C/N ratios of the corn straw under the three fungal treatments gradually

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

decreased with the duration in fermentation time. The *T. reesei* had the best ability to reduce TOC and C/N in the four treatments. While, *P. chrysosporium* showed a stronger ability to degrade corn straw TOC than *T. harzianum*.

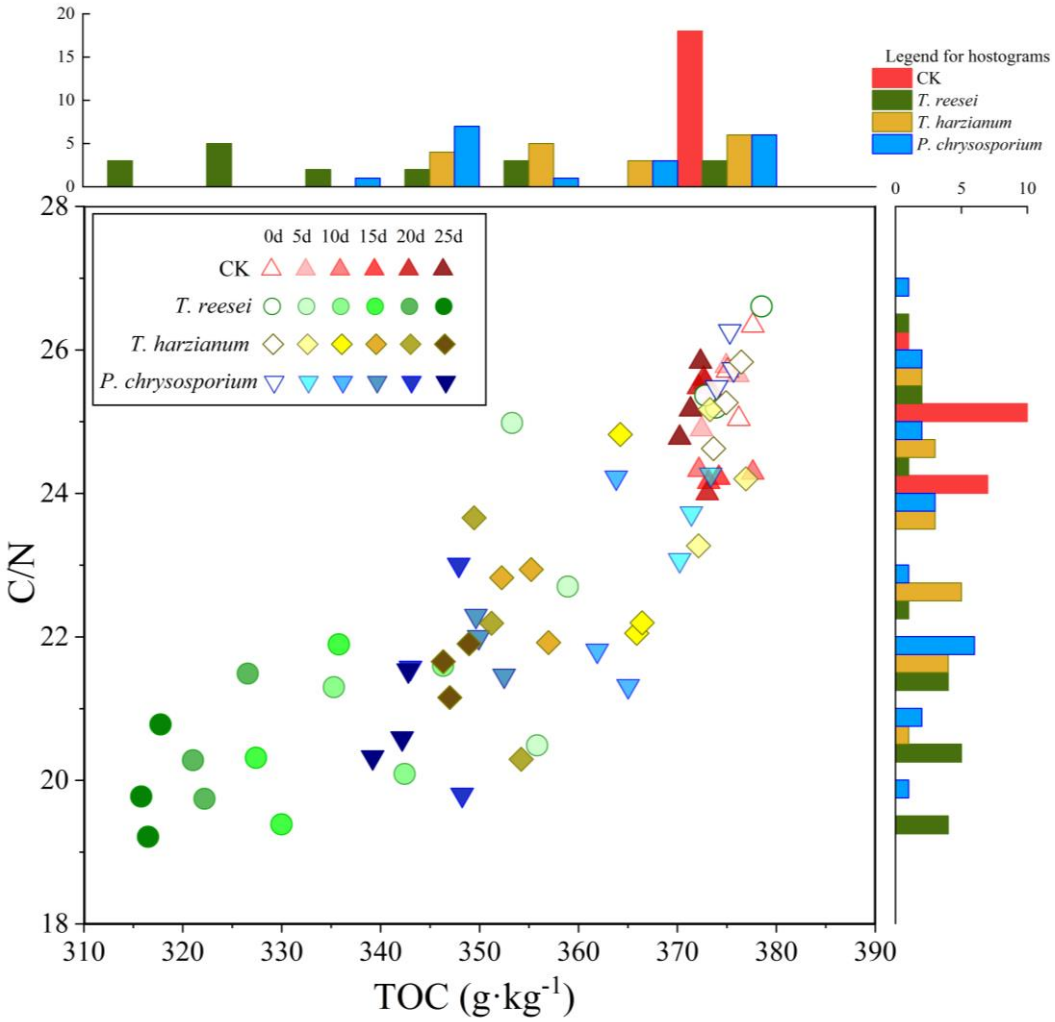

Figure 3. *The TOC contents and C/N ratios of corn straw treated by different treatments over the course of the solid-state aerobic composting. The upper histogram shows the data amounts of different treatments in different TOC content ranges. The histogram on the right shows the data amounts of different treatments in different C/N ratios ranges*

Cumulative degradation rates of cellulose and lignin

The cumulative degradation rates of cellulose and lignin of corn straw treated by different fungi are shown in *Figure 4*. The degradation of CK treatment was far poorer than that of the other three fungal treatments in 25 days, and its cellulose degradation was less than 5% with nearly no lignin degradation. In the treatments inoculated with fungi: The *T. reesei*'s cellulose showed the best degradation ability, and the cumulative degradation reached 70.28%; *T. harzianum*'s ability to degrade cellulose and lignin was at a medium level among the three fungi treatments; Lastly, the *P. chrysosporium* had the best lignin degradation ability, which can total 66.75%.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

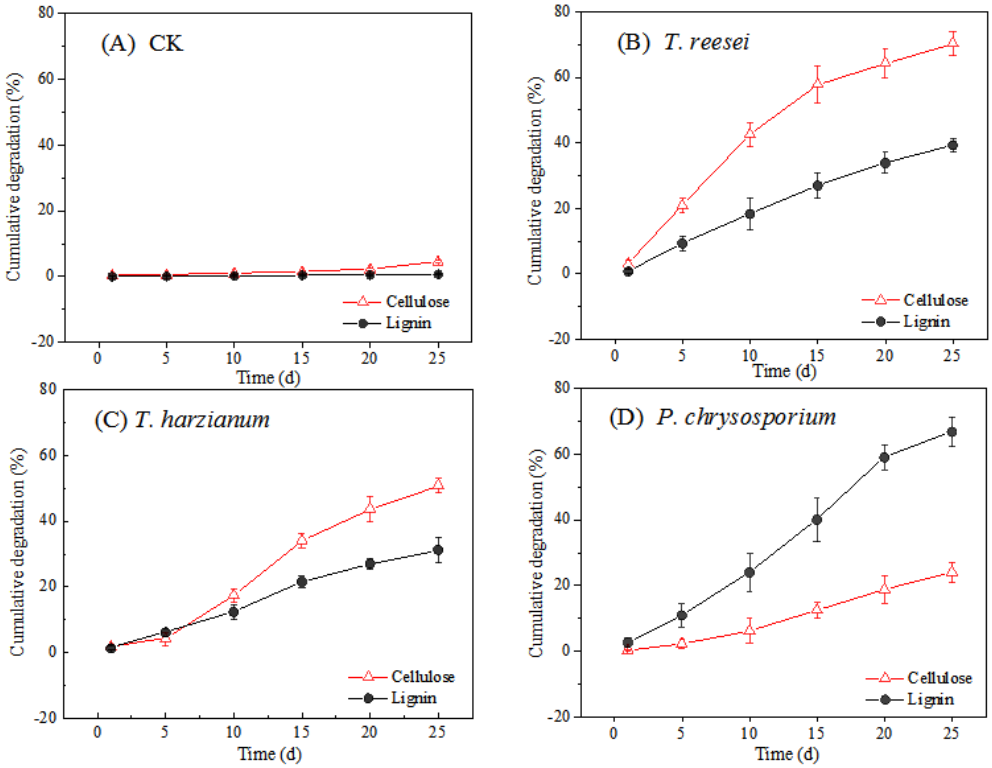

Figure 4. *The changes in the cumulative degradation rates of cellulose and lignin under different treatments. Results are means ± SD (n = 3) on every single time-point of each treatment. A: CK; B: T. reesei; C: T. harzianum; D: P. chrysosporium*

Enzyme activity

Cellulase, xylanase, and β-glucosidase, which were produced by *T. reesei*, *P. chrysosporium* and *T. harzianum* during fermentation of corn straw are shown in *Figure 5*. Throughout the composting process the cellulase and β-glucosidase activities of *T. reesei* were much higher than those of *P. chrysosporium* and *T. harzianum*. However, the xylanase activity of *T. reesei* was just slightly higher than *P. chrysosporium* and *T. harzianum*.

The peak values under *T. reesei* of cellulase, xylanase, and β-glucosidase activities were respectively, 8.45 FPU·mL^{-1}, 0.61 IU·mL^{-1}, and 80.3 IU·mL^{-1}. The peak values under of cellulase, xylanase, and β-glucosidase activities were 6.12 FPU·mL^{-1}, 0.68 IU·mL^{-1}, and 71.3 IU·mL^{-1}, respectively. The peak values under *T. harzianum* of cellulase, xylanase, and β-glucosidase activities were 5.15 FPU·mL^{-1}, 0.54 IU·mL^{-1}, and 68.5 IU·mL^{-1}, respectively.

Total fungal content

Figure 6 records the changes in the total fungal contents under different treatments during the 25-day solid-state aerobic composting. The fungal contents of *T. reesei* and *T. harzianum* before the 15th day was higher than that of *P. chrysosporium*. Among the treatments, *T. reesei* treatment peaked at 32.4 ± 2.1 CFU·g^{-1} on the 5th day, and *T. harzianum* treatment peaked on the 10th day 27.2 ± 2.1 CFU·g^{-1}. At the 15th day, the *P. chrysosporium* reached a peak of 24.3 ± 1.3 CFU·g^{-1}, and exceeded the other two

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

treatments at the same time. In the whole process, the total fungal contents of T. *harzianum* were always lower than that of *T. reesei* (*Fig. 6*).

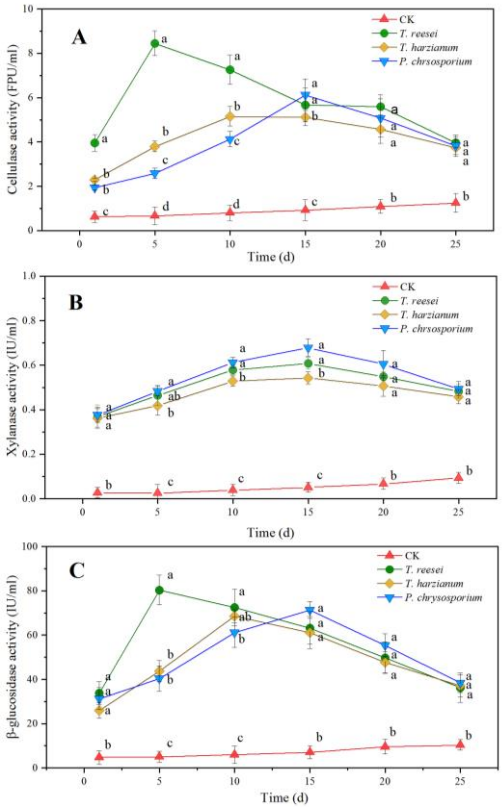

Figure 5. *The activity changes of enzymes at different fermentation time. (a: cellulase; b: xylanase; c: β-glucosidase). Error bars represent the standard deviations of the mean (n = 3). Different lowercase letters mean significant differences (P < 0.05) among different treatments at the same time*

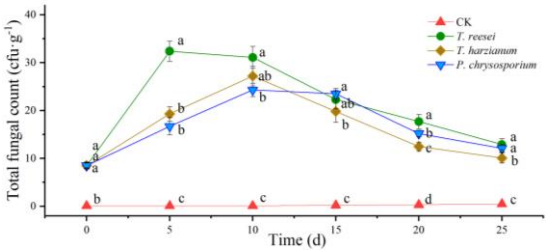

Figure 6. *Total fungal counts of different time during 25-day solid-state aerobic composting. Error bars represent the standard deviations of the mean (n = 3). Different lowercase letters mean significant differences (P < 0.05) among different treatments at the same time*

Discussion

In the early stages of cultivation, corn straw degradation rate was fast which is due to the expansion and growth of fungi's population (*Fig. 6*), and the subsequent increase of enzyme-producing content (*Fig. 5*), and the area of the matrix. As the substrates accumulate, the gap between the straws becomes smaller, the carbon source and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

ventilation gradually decrease, which inhibits the growth (*Fig. 6*) of the strain to some extent. The decomposition rate of *T. reesei* increased quickly in the first 16 days, which may be due to the ability of *T. reesei* to destroy the substrate and multiply more effectively than the other two fungi, and the total fungal contents at 20-25 d were also the highest among the three treatments (*Fig. 6*). The results of the damage degree of corn straw by the four treatments observed by SEM (*Fig. 1*) were consistent with the results of residual rate (*Fig. 2*).

Generally, an initial carbon-to-nitrogen ratio of 25 to 30 is considered to be the best ratio for aerobic composting (Kumar et al., 2010; Silva et al., 2014). In this study, we adjusted the initial matrix C/N ratio to 25.8 by adding an exogenous N-containing mineral culture solution. Wu et al. (2017b) reported that an initial C/N ratio of 25 is favorable for the formation of high-quality and low-toxic compost. The C/N ratio of the three fungal treatments decreased significantly compared to the CK treatment (*Fig. 3*). This indicates that fungi utilized carbon as a source of energy, and it further proved that adding NH_4 + - N and NO_3 - N to the compost can supplement the depleted N source, stimulate the activity of microorganisms in time, and promote decomposition and transformation to form HS (Humic substances) precursor process (Wu et al., 2017a). The *T. reesei* treatment had the most obvious significant decrease in the C/N ratio during the whole composting process (*Fig. 3*), which is due to its nitrogen mineralization rate being lower than the carbon mineralization rate. The decomposition and utilization of TOC by *T. reesei* were much higher than the other two fungi. The degradation rate of organic carbon in corn straw during the fermentation process showed a tendency to first increase and then decrease. This may be related to the growth status of fungi (*Fig. 6*). Fungal microorganisms can to grow rapidly with straw as a carbon source in the early stages. During the late stages of fermentation, with the carbon source availability decreasing, the microbial enzyme activities gradually decrease, and the decomposition is slowed (Jiang et al., 2015). Notably, carbon sources play a crucial role in the production of enzymes since carbohydrates and their derivatives generate most cellulolytic enzymes (Adav et al., 2012). Among all treatments, the *T. reesei* was more effective in utilizing the carbon source in the straw, indicating that *T. reesei* can produce a large amount of degradable corn straw enzyme in the aerobic composting process (*Fig. 5*).

Lignin is the most challenging component to degrade and transform in corn straw. Under the same culture conditions in the present study, the degree of utilization of lignin components in corn straw by *P. chrysosporium* was much higher than that of the other two fungi (*Fig. 4*). This indicated that *P. chrysosporium* was more conducive in degrading lignin in corn straw residues. Several other studies have shown that inoculation of P. chrysosporium in mixed compost of sludge, straw and bran effectively promoted biodegradation of lignocellulose and encouraged the formation of HA (humic acid) (Zhang et al., 2018). Even so, the TOC content of *P. chrysosporium* treatment remained higher than that of *T. reesei* treatment at the end of the culture process. This indicated that during mineralization and decomposition of lignin components by *P. chrysosporium* corn straw derived-C was not entirely converted into CO_2 and moisture. Rather, other C sources (non-lignin) were synthesized or left undecomposed. These C sources may be lignin monomers, or other pure compounds (HS precursors) may have been synthesized by *P. chrysosporium*. Relevant studies have shown that *P. chrysosporium* can first crack the lignin and the C─O bond connected to the aromatic ring, split it into lignin monomer or small molecules of lignin (Wang et al., 2015). In the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

theory of lignin (Stevenson, 1994), it has been described that HS is synthesized from compounds derived from lignin, which is the raw material and skeleton of HS precursors (Campitelli and Ceppi, 2008; Kulikowska, 2016). This may be the reason why TOC content of *P. chrysosporium* was still high at the end of composting, even though xylanase activity was high and the lignin content was low.

Among the three enzyme activities measured in this study (*Fig. 5*), the cellulase activity and β-glucosidase enzyme activity of *T. reesei* were the highest among all the treatments. When Lee et al. (2011) fermented 1:1 mixed bagasse and palm mash with *T. reesei*, they observed that the cellulase activity reached its highest level in 5 days. Similarly, Mekala et al. (2008) used bagasse as a substrate for *T. reesei* in the solid-state fermentation, and found that the cellulase activity peaked at 72 h. Furthermore, Zhao et al. (2011) used water hyacinth as a substrate for fermentation, and reported that the cellulase activity increased four-fold after 7 days of fermentation by *T. reesei*. From previous published research, it appears that cellulase activity reached the highest between 3 to 5 days. Our work also supports these results, and implies that *T. reesei* may adapt quickly into a new environment at the early stages. Although the cumulative degradation rates of lignin by *T. harzianum* was lower than the other two fungi in the early stages of fermentation (*Fig. 4C*), *T. harzianum* still had strong degradation ability of cellulose (*Fig. 4C*). In other studies, it was found that *T. harzianum* can use domestic sewage as a substrate to produce cellulase (Libardi et al., 2017). Similarly, Rocha et al. (2013) found that under optimal conditions, the cellulase and β-glucosidase activities of *T. harzianum* IOC-3844 reached the highest after 42 h. While both *Trichoderma* were more inclined to degrade cellulose, the xylanase activity of *T. reesei* was higher than that of *T. harzianum* (*Fig. 5*). Hu et al. (2014) and Zhao et al. (2016) had the similar results.

Even though the results of our study showed that the general degradation and utilization of *T. reesei* was the best among the three fungi. But, did *T. reesei* mineralize corn straw into simple compounds during the composting process? Does it synthesize substances such as HS? Although not tested in the present, previous related studies have shown that *T. reesei* can produce more HA in 8 days (Yang et al., 2019) and increase the relative HAL (humic acid -like) content in solid-state fermentation (Yang et al., 2019; Zhang et al., 2019b). It can be found from the results of this study that the decrease in TOC content under *T. reesei* at the first 0-10 d was much higher than that in the 10-25 d, and the C/N in 10-25 d also had a slower decline (*Fig. 3*). The reasons for this phenomenon are: on one hand, due to the slower growth and degradation of the C source itself, and the other important reason is that during the cultivation process, some fungal metabolites and fungal residues remain as N sources. In the culture substrate, the circulating input of the N source was maintained for the living fungi to use (Bernal et al., 2009; Jiang et al., 2015), thereby decreasing C/N ratio. Therefore, the residues produced by the fungi using corn straw have a potential for improving soil fertility and could be used as a soil conditioner.

Conclusion

Under the same non-soil environment and culture conditions, *T. reesei* and *T. harzianum* still maintained their preference for cellulose degradation and utilization, and the ability of *P. chrysosporium* to degrade lignin was more substantial. *T. reesei* had the best general degradation and utilization ability among the three fungi, with a cumulative

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

TOC consumption of 58.5 $g \cdot kg^{-1}$ in 25 days and a cumulative decrease of 5.8 in C/N. In summary, we can infer that the comprehensive treatment capacity of fungi in corn straw is: *T. reesei* > *P. chrysosporium* > *T. harzianum* > CK. Indeed, *T. reesei* and *P. chrysosporium* were more conducive to the decomposition of corn straw than *T. harzianum*. The *T. reesei* and *P. chrysosporium* are more suitable for further exploration of the mechanism of corn straw degradation and transformation in soil environment.

Acknowledgements. The study was supported by the National Natural Science Foundation of China (42077022) and the Key Research and Development Program of Jilin Province (20200402098NC). We are very grateful to Jianying Zhou for her contribution to Data curation and Validation.

REFERENCES

[1] Adav, S. S., Chao, L. T., Sze, S. K. (2012): Quantitative secretomic analysis of *Trichoderma reesei* strains reveals enzymatic composition for lignocellulosic biomass degradation. – Molecular & Cellular Proteomics 11: M111.012419.
[2] Aksu, Z. and Donmez, G. (2003): A comparative study on the biosorption characteristics of some yeasts for Remazol blue reactive dye. – Chemosphere 50: 1070-1075.
[3] Anuradha, J. S., Valli, N. C. (2011): Utilization of pretreated bagasse for the sustainable bioproduction of cellulase by Aspergillus nidulans MTCC344 using response surface methodology. – Industrial Crops and Products 34: 1564-1571.
[4] Bailey, M. J., Biely, P., Poutanen, K. (1992): Interlaboratory testing of methods for assay of xylanase activity. – Journal of Biotechnology 23: 257-270.
[5] Bak, J. S., Ko, J. K., Choi, I. G., Park, Y. C., Seo, J. H., Kim. K. H. (2009): Fungal pretreatment of lignocellulose by Phanerochaete chrysosporium to produce ethanol from rice straw. – Biotechnology Bioengineering 104(3): 471-482.
[6] Bernal, M. P., Paredes, C., Sánchez-Monedero, M. A., Cegarra, J. (1998): Maturity and stability parameters of composts prepared with a wide range of organic wastes. – Bioresource Technology 63: 91-99.
[7] Bernal, M. P., Alburquerque, J. A., Moral. R. (2009): Composting of animal manures and chemical criteria for compost maturity assessment. A review. – Bioresource Technology 100: 5444-5453.
[8] Bertoldi, M. D., Vallini, G., Pera., A. (1983): The biology of composting: a review. – Waste Management and Research 1: 157-176.
[9] Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T. H., Kuzyakov, Y. (2007): Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. – Applied Soil Ecology 37: 95-105.
[10] Campitelli, P., Ceppi, S. (2008): Effects of composting technologies on the chemical and physicochemical properties of humic acids. – Geoderma 144: 325-333.
[11] Cetin, D., Donmez, G. (2006): Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions. – Enzyme and Microbial Technology 38: 926-930.
[12] De Castro, A. M., Ferreira, M. C., Da Cruz, J. C., Pedro, K. C. N. R., Carvalho, D. F., Leite, S. G. F., Pereira., N. (2010): High-yield endoglucanase production by *Trichoderma harzianum* IOC-3844 cultivated in pretreated sugarcane mill by product. – Enzyme Research 2010(854526): 1-8.
[13] Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Fangmeier, A., Kuzyakov, Y. (2009): Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO_2 depends on soil aggregate size. – FEMS Microbiology Ecology 69: 43-52.
[14] Ghose, T. K. (1987): Measurement of cellulase activities. – Pure Applied Chemistry 59: 257-268.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

[15] Grover, M., Maheswari, M., Desai, S., Gopinath, K. A., Venkateswarlu, B. (2015): Elevated CO_2: Plant associated microorganisms and carbon sequestration. – Applied Soil Ecology 95: 73-85.
[16] Guo, H., Chang, J., Yin, Q., Wang, P., Lu, M., Wang, X., Dang, X. (2013): Effect of the combined physical and chemical treatments with microbial fermentation on corn straw degradation. – Bioresource Technology 48: 361-365.
[17] Guo, X., Huang, J., Lu, Y., Shan, G., Li, Q. (2016): The influence of flue gas desulphurization gypsum additive on characteristics and evolution of humic substance during co-composting of dairy manure and sugarcane pressmud. – Bioresource Technology 219: 169-174.
[18] Hebrok, M., Boks., C. (2017): Household food waste: drivers and potential intervention points for design - an extensive review. – Journal of Cleaner Production 151: 380-392.
[19] Heck, J. X., Hertz, P. F., Ayub., M. A. Z. (2002): Cellulase and xylanase productions by isolated Amazon Bacillus strains using soybean industrial residue based solid-state cultivation. – Brazilian Journal of Microbiology 33: 213-218.
[20] Hu, J., Arantes, V., Pribowo, A., Gourlay, K., Saddler., J. N. (2014): Substrate factors that influence the synergistic interaction of AA9 and cellulases during the enzymatic hydrolysis of biomass. – Energy Environment Science 7: 2308-2315.
[21] Jiang, J., Liu, X., Huang, Y., Huang., H. (2015): Inoculation with nitrogen turnover bacterial agent appropriately increasing nitrogen and promoting maturity in pig manure composting. – Waste Management 39: 78-85.
[22] Jurado, M. M., Suárez-Estrella, F., López, M. J., Vargas-García, M. C., López-González, J. A., Moreno, J. (2015): Enhanced turnover of organic matter fractions by microbial stimulation during lignocellulosic waste composting – Bioresource Technology 186: 15-24.
[23] Kulikowska, D. (2016): Kinetics of organic matter removal and humification progress during sewage sludge composting. – Waste Management 49: 196-203.
[24] Kumar, M., Ou, Y. L., Lin, J. G. (2010): Co-composting of green waste and food waste at low C/N ratio. – Waste Management 30: 602-609.
[25] Latifian, M., Hamidiesfahani, Z., Barzegar, M. (2007): Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. – Bioresource Technology 98: 3634-3637.
[26] Law, W. M., Lau, W. N., Lo, K. L., Wai, L. M., Chiu., S. W. (2003): Removal of biocide pentachlorophenol in water system by the spent mushroom compost of Pleurotus pulmonarius. – Chemosphere 52: 1531-1537.
[27] Lee, C. K., Darah, I., Ibrahim, C. O. (2011): Production and optimization of cellulase enzyme using aspergillus niger USM AI 1 and comparison with *Trichoderma reesei* via solid state fermentation system. – Biotechnology Research International. https://doi.org/10.4061/2011/658493.
[28] Li, C., Yang, Z., He, R., Zhang, C., Zhang, D., Chen, S., Ma., L. (2013): Effect of pH on cellulase production and morphology of *Trichoderma reesei* and the application in cellulosic material hydrolysis. – Journal of Biotechnology 168: 470-477.
[29] Libardi, N., Soccol, C. R., Góes-Neto, A., De Oliveira, J., Vandenberghe, L. P. de S. (2017): Domestic wastewater as substrate for cellulase production by *Trichoderma harzianum*. – Process Biochemistry 57: 190-199.
[30] Lin, B., Lyu, J., Lyu, X., Yu, H., Hu, Z., Lam, J. C. W., Lam, P. K. S. Characterization of cefalexin degradation capabilities of two Pseudomonas strains isolated from activated sludge. – Journal of Hazardous Materials 282: 158-164.
[31] Liu, J., Song, Y., Liu, Y., Ruan, R. (2015): Fungal Pretreatment of Effluent from Piggery Anaerobic Digestion by *Phanerochaete chrysosporium*. – CLEAN-Soil Air Water 43: 1190-1196.
[32] Lu, R. K. (2020): Analytical Methods for Soil and Agro-chemistry. – China Agricultural Science and Technology Press, Beijing (in Chinese).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

[33] Maeda, K., Hanajima, D., Morioka, R., Osada., T. (2010): Characterization and spatial distribution of bacterial communities within passively aerated cattle manure composting piles. – Bioresource Technology 101: 9631-9637.
[34] Martinez, D., Larrondo, L. F., Putnam, N., Gelpke, M. D. S., Huang, K., Chapman, J., Helfenbein, K. G., Ramaiya, P., Detter, J. C., Larimer, F., Coutinho, P. M., Henrissat, B., Berka, R., Cullen, D., Rokhsar, D. (2004): Genome sequence of the lignocellulose degrading fungus Phanerochaete chrysosporium strain RP78. – Nature Biotechnology 22: 695-700.
[35] Mekala, N. K., Singhania, R. R., Sukumaran, R. K., Pandey, A. (2008): Cellulase production under solid-state fermentation by *Trichoderma reesei* RUT C30: statistical optimization of process parameters. – Applied Biochemistry and Biotechnology 151: 122-131.
[36] Nazanin, D., Zohreh, H. E., Parisa, H. (2018): Optimization of cellulase production under solid-state fermentation by a new mutant strain of *Trichoderma reesei*. – Food Science and Nutrition 7: 572-578.
[37] Onwosi, C. O., Igbokwe, V. C., Odimba, J. N., Eke, I. E., Nwankwoala, M. O., Iroh, I. N., Ezeogu, L. I. (2017): Composting technology in waste stabilization: on the methods, challenges and future prospects. – Journal of Environmental Management 190: 140-157.
[38] Paz, A., Outeirino, D., Perez, G. N., Domínguez, J. M. (2018): Enzymatic hydrolysis of brewer's spent grain to obtain fermentable sugars. – Bioresource Technology 275: 402-409.
[39] Pianka, E. R. (1970): On r-and K-selection. – American Naturalist 104: 592-597.
[40] Pitt, J. I., Hocking, A. D. (2009): Fungi and Food Spoilage. 3rd Ed. – Blackie Academic and Professional, London.
[41] Prasetyo, J., Sumita, S., Okuda, N., Park, E. Y. (2009): Response of Cellulase Activity in pH-Controlled Cultures of the Filamentous Fungus Acremonium cellulolyticus. – Applied Biochemistry Biotechnology 16: 52-61.
[42] Rocha, V. A. L., Maeda, R. N., Santa Anna, L. M. N., Pereira Jr, N. (2013): Sugarcane bagasse as feedstock for cellulase production by *Trichoderma harzianum* in optimized culture medium. – Electronic Journal of Biotechnology 16(5).
[43] Rodríguez-Rodríguez, C. E., Jesús García-Galán, M., Blánquez, P., Díaz-Cruz, M. S., Barceló, D., Caminal, G., Vicent, T. (2012): Continuous degradation of a mixture of sulfonamides by Trametes versicolor and identification of metabolites from sulfapyridine and sulfathiazole. – Journal of Hazardous Materials 213-214: 347-354.
[44] Salihoglu, G., Salihoglu, N. K., Ucaroglu, S., Banar., M. (2017): Food loss and waste management in Turkey. – Bioresource Technology 248: 88-99.
[45] Sánchez, C. (2009): Lignocellulosic residues: biodegradation and bioconversion by fungi. – Biotechnology Advances 27: 185-194.
[46] Shin, C. S., Lee, J. P., Lee, J. S., Park, S. C. (2000): Enzyme production of *Trichoderma reesei* rut C-30 on various lignocellulosic substrates. – Appled Biochemistry Biotechnology 84-86: 237-245.
[47] Silva, M. E. F., de Lemos, L. T., Nunes, O. C., Cunha-Queda, A. C. (2014): Influence of the composition of the initial mixtures on the chemical composition, physicochemical properties and humic-like substances content of composts. – Waste Management 34: 21-27.
[48] Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. (2008): Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure (LAP). – National Renewable Energy Laboratory (NREL), Denver.
[49] Soil Survey Staff.: Keys to Soil Taxonomy. 12th Ed. – Natural Resources Conservation Service, USDA-Natural Resources Conservation Service, Washington, DC.
[50] Sørensen, A., Andersen, J. J., Ahring, B. K., Teller, P. J., Lübeck, M. (2014): Screening of carbon sources for beta-glucosidase production by Aspergillus saccharolyticus. – International Biodeterioration & Biodegradation 93: 78-83.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

[51] Stevenson, F. J. (1994): Humus Chemistry: Genesis, Composition, Reactions. Second Ed. – John Wiley and Sons, New York.
[52] Sukumarana, R. K., Singhaniaa, R. R., Mathewa, G. M., Pandey, A. (2009): Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. – Renewable Energy 34: 421-424.
[53] Tamagawa, Y., Hirai, H., Kawai, S., Nishida, T. (2005): Removal of estrogenic activity of endocrine-disrupting genistein by ligninolytic enzymes from white rot fungi. – FEMS Microbiology Letters 244: 93-98.
[54] Tangnu, S. K., Blanch, H. W., Wilke, C. R. (1981): Enhanced production of cellulase, hemicellulase, and β-glucosidase by *Trichoderma reesei* (Rut C-30). – Biotechnology and Bioengineering 23: 1837-1849.
[55] Vijai, K. G., Monika, S., Alfredo, H. E., Upadhyay, R. S., Irina, D., Maria, G. T. (2014): Biotechnology and Biology of *Trichoderma*. – Elsevier's Science & Technology Rights Department, Oxford, UK.
[56] Wang, W., Ling, H., Zhao, H. (2015): Steam explosion pretreatment of corn straw on xylose recovery and xylitol production using hydrolysate without detoxification. – Process Biochemistry 50: 1623-1628.
[57] Wen, Z., Liao, W., Chen, S. (2005): Production of cellulase by *Trichoderma reesei* from dairy manure. – Bioresource Technology 96: 491-499.
[58] Wu, J., Zhao, Y., Qi, H., Zhao, X., Yang, T., Du, Y., Zhang, H., Wei, Z. (2017a): Identifying the key factors that affect the formation of humic substance during different materials composting. – Bioresource Technology 244: 1193-1196.
[59] Wu, S., Shen, Z., Yang, C., Zhou, Y., Li, X., Zeng, G., Ai, S., He, H. (2017b): Effects of C/N ratio and bulking agent on speciation of Zn and Cu and enzymatic activity during pig manure composting. – International Biodeterioration and Biodegradation 119: 429-436.
[60] Xia, L., Cen, P. (1999): Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. – Process Biochemistry. 34: 909-912.
[61] Yang, Y., Wang, L., Zhang, Y., Li, L., Shi, X., Liu, X., Ren. X., Dou, S. (2019): Transformation of corn stalk residue to humus-like substances during solid-state fermentation. – Sustainability 11: 6771.
[62] Zhang, J. D., Yang, Q. (2015): Optimization of solid-state fermentation conditions for Trichoderma harzianum using an orthogonal test. – Genetics and Molecular Research 14: 1771-1781.
[63] Zhang, C., Xu, Y., Zhao, M., Rong, H., Zhang, K. (2018): Influence of inoculating white-rot fungi on organic matter transformations and mobility of heavy metals in sewage sludge based composting. – Journal of Hazardous Materials 344: 163-168.
[64] Zhang, T., Cai, L., Xu, B., Li, X., Qiu, W., Fu, C., Zheng, C. (2019a): Sulfadiazine biodegradation by Phanerochaete chrysosporium: mechanism and degradation product identification. – Chemosphere 124418.
[65] Zhang, Y., Dou, S., Ye, S., Zhang, D. (2019b): Specificity of humic acid-like (HAL) substance from Trichoderma reesei inoculated corn straw. – Journal of Agro-Environment Science 38: 2184-2192.
[66] Zhang, Y., Dou, S., Hamza, B., Ye, S., Zhang, D. (2020a): Mechanisms of three fungal types on humic-like substances formation during solid-state fermentation of corn straw. – International Journal of Agriculture and Biology 24: 970-976.
[67] Zhang, Y., Yang, J., Luo, L., Wang, E., Wang, R., Liu, L., Liu, J., Yuan, H. (2020b): Low-Cost Cellulase-Hemicellulase Mixture Secreted by *Trichoderma harzianum* EM0925 with Complete Saccharification Efficacy of Lignocellulose. – International Journal of Molecular Sciences 2020(21): 371.
[68] Zhao, S. H., Liang, X. H., Hua, D. L., Ma, T. S., Zhang, H. B. (2011): High-yield cellulase production in solid-state fermentation by *Trichoderma reese*i SEMCC-3.217 using water hyacinth (Eichhornia crassipes). – African Journal of Biotechnology 10: 10178-10187.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568

[69] Zhao, X., Xiong, L., Zhang, M., Bai, F. (2016): Towards efficient bioethanol production from agricultural and forestry residues: exploration of unique natural microorganisms in combination with advanced strain engineering. – Bioresource Technology 215: 84-91.
[70] Zou, H., Ye, X., Li, J., Lu, J., Fan, Q., Yu, N., Zhang, Y., Dang, X., Zhang, Y. (2016): Effects of straw return in deep soils with urea addition on the soil organic carbon fractions in a semi-arid temperate cornfield – PLoS One 11: e0153214.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2004_35513568