Full Length Article

Mechanisms of Three Fungal Types on Humic-Like Substances Formation during Solid-State Fermentation of Corn Straw

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Abstract

In order to explore the dynamics of humus-like substances (HLS) formation, corn straw was inoculated with three fungi in a laboratory solid-state fermentation experiment, carried out for 25 days at 25°C. Corn straw was inoculated with: (i) *Phanerochaete chrysosporium*, (ii) *Trichoderma harzianum* and (iii) *T. reesei*, and (iv) un-inoculated corn straw (CK). Humification degree (PQ) was computed. The results demonstrated that fungi play an important role in the process of transforming corn straw residues to HLS. The highest decomposition rate of corn straw and the highest humic acid-like (HAL), and water-soluble substance appeared in the last day of fermentation. While, fluvic acid-like (FAL) and humin-like contents decreased with the duration of the experiment. The total organic carbon content of the corn straw decreased by 9.31, 7.71 and 15.92% under *P. chrysosporium, T. harzianum* and *T. reesei* treatments, respectively. The PQ values of *T. reesei, T. harzianum* and *P. chrysosporium* treatments increased by 33.43, 31.59 and 7.78%, respectively, compared with CK. Changes in HLS composition is accordance with the polyphenol theory, in which the FAL is formed first, followed by HAL in later stages of straw decomposition. Compared with all treatments, the *T. harzianum* and *T. reesei* treatments were more conducive to the improvement of HAL contents, whereas the *P. chrysosporium* appeared to favour accumulation of FAL content. © 2020 Friends Science Publishers

Introduction

About 350 million tons of corn straw is produced annually by China's agricultural activity, accounting for almost 50% of straws' production. Turning these agricultural waste products into a valuable resource and source of fertilizer has become a critical focus of research in the field of biomass resource utilization. Solid-state fermentation, as green technology, remains one of the best methods for agricultural waste treatment, and can achieve efficient biotransformation (Zhang *et al.* 2016). Tang *et al.* (2010) observed that its humification process is similar to that in soil. It primary begins with mineralization of organic matter which is gradually transformed into humus (Wang *et al.* 2015), a final product in decomposition of organic materials. This process is mainly mediated by the action of microorganisms.

Corn straw is composed of lignin, cellulose and hemicellulose. In the solid-state fermentation, the fungi have a strong ability to decompose and degrade these compounds due to two mechanisms: (i) production of extracellular enzymes by the fungi and (ii) and mechanical perforation of the straw by the fungal hyphae which facilitate the decomposition. Under these conditions, the hydrolysis rate

of organic matter is accelerated, thusly, increasing the decomposition of refractory organic matter (Johansson *et al.* 1989; Fu 2012). Therefore, fungi hold a primordial role in the mineralization and decomposition of corn straw in solid-state fermentation.

Vijai *et al.* (2014) reported that *Trichoderma* specie is a beneficial fungus, because of their strong ability to break down cellulose, promotes the fermentation and utilization of organic waste (Cruz-Quiroz *et al.* 2017), and degrade complex molecular compounds (Sánchez–Monedero *et al.* 1999). The *Trichoderma reesei* in particular has been shown to hold an excellent lignocellulase production capacity (Xin and Geng 2009; Ortiz *et al.* 2015) and can enhance formation of humic acid-like (HAL) content in a relatively short period (Yang *et al.* 2019). Moreover, Yang *et al.* (2019) demonstrated that *T. reesei* during the fermentation process enhanced corn straw humification degree and fermented corn straw product could be a good source of fertilizer. Latifian *et al.* (2007) used rice bran as a substrate and showed that the optimal conditions for cellulase production occurred at temperatures of 25–30°C and a moisture content of 55–70%. Castro *et al.* (2010) demonstrated that cellulose degradation within 72 h, was

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rich in β-glucosidase activity, when bagasse was treated with *T. harzianum* in a solid-state fermentation. Apart from corn straw, Zeng *et al.* (2014) showed that peanut shell powder and corn cob can also be used by *T. harzianum* under solid-state fermentation. In another study Zhang *et al.* (2017) reported that *Phanerochaete chrysosporium* is capable of degrading lignin by excreting extracellular oxidases such as lignin peroxidase, manganese peroxidas, and lactase. Huang *et al.* (2006) concluded that corn straw treated with *P. chrysosporium* decreased significantly the lignin content within 15 days, while Chen *et al.* (2019) inoculated a spore solution of *P. chrysosporium* to maize straw and canola residue, and observed a lignin degradation rate up to 64.3%. The lignin and cellulose have a profound contribution to the formation of humic substances (HS). Therefore, dynamic changes in these compounds will affect HS turn-over rate.

Polyphenol theory is prevalent in the interpretation of corn straw degradation and humus formation process. Organic matter is first decomposed into simple organic compounds under microbial action. Under the action of enzymes, polyphenolsare are oxidized to quinones (Yu *et al.* 2003) and the latter are condensed with the nitrogen-containing compound to form HS. On the other, the lignin theory suggests that lignin is the basic structural unit of humic formation, by first forming humic-acid (HA), then through further oxidation fuvlic-acid (FA) is formed (Thevenot *et al.* 2010). Many researchers have investigated the subject and found that fungi can degrade and transform both lignin and cellulose, and form quinone compounds (Bahri *et al.* 2008). In the early studies, Tuomela *et al.* (2002), used ^{14}C labeled lignin to track its decomposition by *P. chrysosporium*. They observed that *P. chrysosporium* was able to degrade lignin and the ^{14}C was used to synthesize newly formed HS.

The corn straw is our key raw material for simulating humification in which HSs are our final product. In this study, three types of fungi (*P. chrysosporium*, *T. harzianum* and *T. reesei*) were chosen based on their ability to decompose and mineralize corn straw. A simulation of the short-term humification process was performed in a solid-state fermentation and we monitored dynamic changes of HS contents in the residue for 25 days. The aim of this study was; (i) to provide a new reference to clarify the fungal mechanisms involved in the formation and transformation of humus, and (ii) determine fungi with the best efficient utilization effect on corn straw transformation.

Materials and Methods

Site description and corn straw sampling

The sampling site of corn straw is presented by a corn cropland located in Jilin Agricultural University, Nanguan District, Changchun City, Jilin Province, China (N43° 48′ 43.5′, E125° 23′ 38.50′). The region is

characterized by four distinct seasons: a cold wet spring; short warm summer; sunny, dry and windy fall; and a long cold winter. The mean annual temperature is 4.8°C, and the mean annual rainfall is 618 mm. The highest rainfall occurs between July and August. Black soils (Chinese Soil taxonomy) are the main soil of the region and are classified as Argiudolls according to the USDA Soil Taxonomy (United States Department of Agriculture 2014).

The corn cultivar was Zhongjin 368 type (Beijing Golden Grain Seed Co., Ltd.), which was planted at the end of April 2017 and harvested in early October. After harvest, the whole corn straw was naturally air-dried and cut into 0.5 cm segments. The basic properties of corn straw were: 376.4 g kg^{-1} organic C, 7.22 g kg^{-1} total nitrogen, 7.7 g kg^{-1} total phosphorus, 4.5 g kg^{-1} total potassium, and a C/N ratio of 61.8.

Preparation of fungi and fungal liquid

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

Each of the three strains of fungi was inoculated to a solid bevel tube containing 30 mL of potato dextrose agar (PDA). The solid bevel tubes were placed in an incubator at 28°C for 72 h to obtain mature microbial spores (mycelium). Spore solution preparation process was as follows: 2 mL of sterile distilled water was added to the inoculated solid bevel tube and vortexed for 2 min. The solution was transferred to a sterile tube, and the concentration was calculated through a haemocytometer and diluted to a final concentration of 1×10^7 spores/mL. Finally, the spore suspension was transferred to liquid medium at a ratio of microbial liquid to liquid medium of 1:10. The culture was incubated on a reciprocating incubator at 100 rpm and 30°C for 6 days. The mycelium-containing broth was used as a backup.

The PDA medium was prepared from the 200 g peeled potato, which was boiled for 30 min in 1 L of distilled water, and filtered. Thereafter, 20 g L^{-1} of glucose and 20 g L^{-1} of agar were added. Meanwhile, the liquid medium was also prepared from peeled potato (200 g L^{-1}) boiled for 30 min and filtered; then 20 g L^{-1} of glucose was added.

Solid-state fermentation and collection

The fermentation was conducted in a BIOTECH-30SS solid fermentation tank (Baoxing Biological Engineering Equipment Co., Ltd., Shanghai, China). The tank had a volume of 30 L with a sterilizing function, automatic stirring, controlled humidity and temperature, and air intake

feature. 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 (particle size = 0.5 cm) was sterilized in the solid fermenter. The sterilization was conditioned at 121°C for 25 min. After the sterilization, corn straw was thoroughly mixed with each microbial liquid containing the spore mycelia (0.6 L) and the mineral salt solution (3.75 L). Each treatment was replicated three times and randomized. The fermentation was set at 30°C, 60% humidity and 6.0 rpm.

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

Sampling and analysis

Small portions of straw samples were collected from each bevel tubes on the first day and every 5 days. Before each sampling, corn straw and the liquid were uniformly mixed. The monitoring lasted for 25 days and the samples were analyzed separately for each day of collection. Collected corn straw samples were oven-dried at 55°C for 6 h, then fine grounded to pass through a 0.25 mm sieve.

Analytical methods

Extraction and determination protocol of humus in corn straw was based on the modified method of soil humus composition (Kumada *et al.* 1967). Water-soluble substances (WSS) were extracted from corn straw with distilled water under permanent shaking for 1 h. The Humic-like substances (HLS) were extracted with a mixture of 0.1 *M* sodium hydroxide and 0.1 *M* sodium pyrophosphate. The humic acid-like (HAL) and fulvic acid-like (FAL) were separated with 0.5 M sulfuric acid. The residues left were considered as humin-like (HML).

The carbon content of the total organic carbon (TOC), WSS, humus-like extracted (HLE), HAL and HML components of corn straw was determined by potassium dichromate oxidation method (Lu 2000), and the carbon content of FAL was determined by difference in HLE and HAL. The PQ (%) value was computed as:

$$PQ(\%) = \frac{HAL}{HAL + FAL} \times 100$$

and relative content of WSS was calculated as:

$$WSS(\%) = \frac{WSS}{TOC} \times 100$$

Data processing and statistical analysis

Microsoft Office Excel 2017 was used for data processing

and statistical analysis was performed by SPSS Statistics 22.0. Significance differences among treatment means were evaluated using the least significant difference test with DUCAN adjustment at *P* < 0.05.

Results

Change in TOC in corn straw during fermentation

The TOC content decreased with fermentation time across all treatments (Fig. 1). Corn straw treated with fungi showed an exponential decrease in TOC content, while non-treated corn showed gradual decrease. After 5 days, *T. reesei* showed a significant decrease in TOC content compared with other treatments. Between the 5th, 10th, and 15th days, no significant difference was observed in TOC content between *P. chrysosporium* and *T. harzianum.* At the end of the fermentation period, the TOC of *T. reesei*, *P. chrysosporium* and *T. harzianum,* respectively decreased by 15.92, 9.31 and 7.71% compared with that of CK.

Effects of fungi on humic-like substances in corn straw composition

The carbon content at the first day after inoculating corn with *P. chrysosporium*, *T. harzianum* and *T. reesei* increased in the following order: HML>WSS>FAL>HAL (Table 1). The C content in FAL of the three fungal inoculations were higher than that of CK throughout the fermentation time, except that of *T. harzianum* and *T. reesei* which was lower than CK in the 20th and 25th days. The C content of HAL showed an inverse trend, that is, HAL was markedly higher in corn straw treated with fungi than CK during the fermentation period but not always (Table 1). The C content of WSS did not exhibit distinct distribution pattern, some treatments increased WSS to a particular day and started to decrease again. However, the C content in HML appeared to decrease throughout fermentation period in all treatments. The C content in HAL of *T. harzianum* and *T. reesei* treatments exceeded that of FAL on the 15th and 20th days. Meanwhile, CK and *P. chrysosporium* showed greater FAL carbon contents than HAL C content throughout the fermentation period (Table 1)

During the fermentation, the relative contents of WSS under the three fungi inoculations showed different degrees of increasing trend meanwhile that of CK decreased slowly (Fig. 2a). The organic C in corn straw inoculated with fungi was transformed into water-soluble organic C at varying degrees and was stabilized after 20 days. At the end of fermentation, the relative content of WSS in *T. reesei*, *T. harzianum* and *P. chrysosporium* increased by 6.07, 3.84 and 2.38%, respectively, compared with CK.

The relative contents of HAL under the four treatments increased with time, and the magnitude of change followed the order of: *T. reesei*>*T. harzianum*>*P. chrysosporium*> CK (Fig. 2b). At day 25, the HAL of *T.*

Table 1: Effect of different fungi on humic-like substance in corn straw

Treatments	Time	WSS	HAL	FAL	HML
	(d)	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})	(g kg^{-1})
CK	0	68.28 ± 1.28a	23.86 ± 0.98c	41.44 ± 0.37d	224.44 ± 1.79a
	5	67.73 ± 1.20a	25.89 ± 0.67b	44.13 ± 0.66d	221.25 ± 1.52ab
	10	67.11 ± 0.92a	26.98 ± 1.43b	45.83 ± 0.82c	219.92 ± 0.99b
	15	66.42 ± 0.94ab	29.17 ± 0.16ab	46.64 ± 0.99c	218.87 ± 0.95c
	20	65.98 ± 1.33b	30.26 ± 0.94a	47.91 ± 0.26b	216.87 ± 1.22d
	25	65.01 ± 0.69b	31.49 ± 0.67a	48.81 ± 0.26a	215.37 ± 1.35e
P. chrysosporium	0	68.28 ± 1.76a	22.84 ± 1.40c	46.33 ± 0.77c	222.60 ± 0.35e
	5	69.40 ± 1.10a	23.45 ± 1.01c	47.09 ± 0.72c	217.70 ± 1.84e
	10	69.57 ± 0.97a	25.40 ± 1.22c	50.33 ± 0.74b	212.08 ± 1.51d
	15	69.19 ± 1.02a	29.13 ± 1.83b	50.71 ± 0.59b	197.99 ± 4.33c
	20	69.67 ± 1.46a	36.22 ± 1.74a	53.89 ± 1.33a	185.97 ± 0.44ab
	25	70.05 ± 2.27a	37.71 ± 1.85a	54.73 ± 0.99a	176.24 ± 1.33a
T. harzianum	0	68.28 ± 0.91c	23.00 ± 2.88f	46.08 ± 0.26b	224.57 ± 0.67a
	5	69.50 ± 2.80c	25.19 ± 2.59e	47.11 ± 0.59a	217.81 ± 0.71b
	10	70.83 ± 0.99bc	31.69 ± 1.46d	48.08 ± 0.97a	207.63 ± 2.43c
	15	72.62 ± 2.79abc	38.85 ± 0.88c	45.50 ± 1.01bc	191.10 ± 4.00d
	20	75.91 ± 3.15ab	51.06 ± 2.39b	37.89 ± 0.91d	183.81 ± 5.78d
	25	76.42 ± 3.97a	59.06 ± 2.90a	31.99 ± 0.66e	178.08 ± 6.05d
T. reesei	0	68.28 ± 1.26c	23.54 ± 0.79f	46.25 ± 0.11b	223.61 ± 0.28a
	5	69.90 ± 2.29c	34.04 ± 1.51e	47.66 ± 1.49a	196.29 ± 3.71b
	10	71.89 ± 2.54b	44.33 ± 1.31d	45.94 ± 1.13c	170.27 ± 3.11c
	15	74.68 ± 3.35b	52.55 ± 1.60c	41.29 ± 0.88d	158.48 ± 3.34d
	20	76.16 ± 3.02ab	59.20 ± 1.58b	38.75 ± 0.54e	141.98 ± 0.72e
	25	76.62 ± 2.81a	68.03 ± 2.54a	33.27 ± 1.98f	133.66 ± 3.62f

Mean±standard deviation. Values sharing same letter(s) within each treatment are not significantly different at *P*>0.05. CK represent a corn straw not inoculated with fungi; The *T. reesei, T. harzianum*, and *P. chrysosporium* denotes corn straw treated with these fungi and incubated for 25 days. WSS is water soluble substance, HAL denotes humic acid like, FAL represent fluvic acid like, and HML is humin like
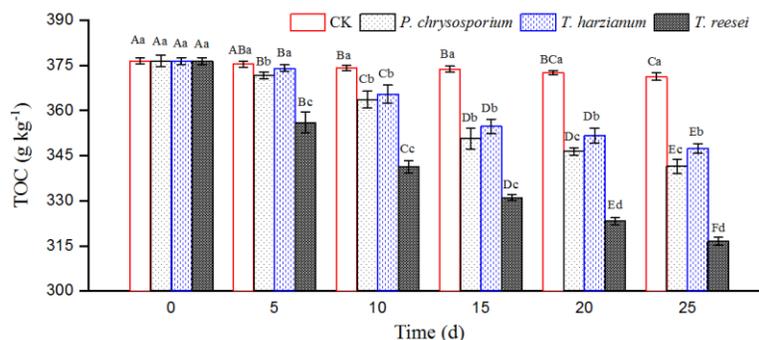
Fig. 1: The TOC content of corn straw over the course of the fermentation

The mean total organic carbon (TOC) content of corn straw over the course of the fermentation plus standard deviation. Bar means followed by different capital letter-cases are significantly different during the fermentation period for the same treatment at *P* < 0.05. Means followed by different small letters are significantly different among each day of fermentation per treatment

reesei, T.harzianum and *P. chrysosporium* respectively increased by 15.24, 10.89 and 4.98% compared with CK treatment.

The relative contents of FAL under CK and *P. chrysosporium* increased with time (Fig. 2c), while that of *T. harzianum* and *T. reesei* treatments decreased after day 10. At the end of fermentation period, *T. reesei* and *T. harzian* treatments decreased FAL by 1.78 and 2.70%, respectively. While *P. chrysosporium* increased FAL by 3.72% compared with CK.

The carbon content of HML decreased along with the fermentation process (Fig. 2d). The magnitude change followed the order of: *T. reesei>T.harzianum>P. chrysosporium>* CK. The three fungi treatments

respectively, decreased HML by 17.18, 8.41 and 7.50% compared with CK treatment in day 0.

Effects of different fungi inoculant on the PQ of humic-like substances

The PQ value represents the percentage of HAL in HLE which can reflect the degree of humification of straw residues (Fig. 3). The PQ values of corn straw inoculated with fungi showed different degrees of increasing trend, implying that the formation rate of HAL is eventually higher than FAL. The humification degree was highest in *T. reesei* treatment followed by *T. harzianum*, *P. chrysosporium* and CK. At the end of fermentation, the PQ values of *T. reesei*,
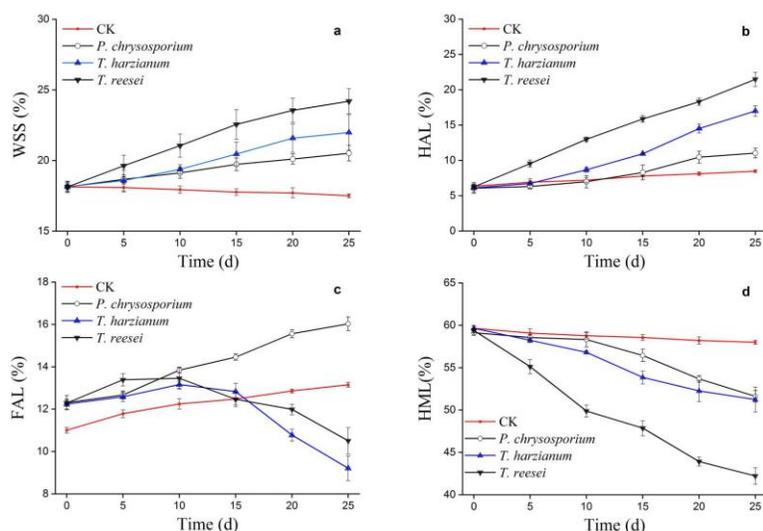
Fig. 2: Changes of relative content of each HLS component in different treatments: WSS (**a**); HAL (**b**); FAL (**c**); HML (**d**)
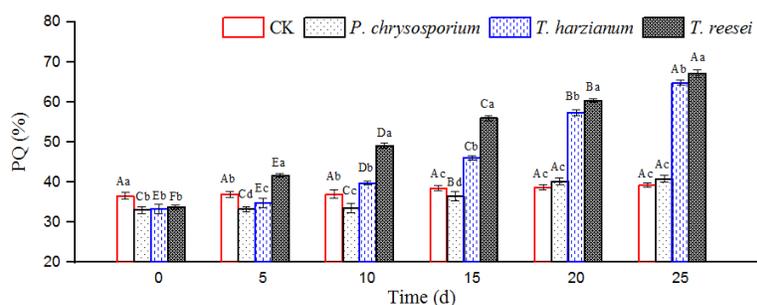

Fig. 3: Changes in PQ value under different treatments
The mean PQ value of corn straw over the course of the fermentation plus standard deviation. Bar means followed by different capital letter-cases are significantly different during the fermentation period for the same treatment at $P < 0.05$. Means followed by different small letters are significantly different among each day of fermentation per treatment

T. harzianum and *P. chrysosporium* respectively increased from 33.73, 33.27 and 33.01 in 0 day to 67.16, 64.86 and 40.78% in the 25th dat. The CK treatment showed a slight change in PQ (%), increasing from 36.52 to 39.21%.

Discussion

The TOC content of corn straw decreased during the 25 days of fermentation, which could be related to dynamic changes in C content of humus components. Hu *et al.* (2017) suggested that the C content of HAL in organic matter play an important role in SOC storage. Similar to our study Li *et al.* (2015), found that microbial inoculant significantly reduced TOC and HML but increased the content of HA in fermented organic materials. In the present study, the highest C content of HAL in corn straw across all treatments was measured in the 25th day and *T. reesei* showed greatest HAL than other treatments. Yang *et al.* (2019) also observed greater HAL content in straw inoculated with *T. reesei*. This could be ascribed to greater polymerization of FAL into a more complex and relatively stable HAL fraction and is consistent with the Polyphenolic theory of humus formation.

It could mean the *T. reesei* and *T. harzianum* in our study preferentially utilized HAL as a source of C and energy, thusly enabling synthesis of HAL fractions. These suggestions are supported by a rapid decline in FAL content in corn straw treated with *T. reesei* and *T. harzianum*.

At the early stages of fermentation (0–10 days), the C content in FAL across all treatments was nearly 2 times higher than that in HAL, and humification degree (PQ) was less than 50%. Tuomela *et al.* (2002) showed that the FA content in immature compost is high than HA content and Hu *et al.* (2019), found that corn straw application resulted to effective accumulation of FA in the soil. However, as straw decomposition progressed, a gradual decrease in FAL content in corn straw inoculated with *T. reesei* and *T. harzianum* and increase in HAL and PQ was observed, which coincides with previous studies (Yang *et al.* 2019). Zhang and Dou (2005) investigated the fermentation of corn straw for 15 days and also found that, in the initial stage of straw decomposition HA was produced at a lower rate than FA. However, over time FA was slowly transformed into HA, and Wei *et al.* (2018) showed that formed HA could be in a loosely bound from or stable from. In the present study

the results suggest that *T. reesei* and *T. harzianum* were more effective in the transformation of FA into stable HA fraction. While, *P. chrysosporium* favoured accumulation of both HAL and FAL contents with the duration of fermentation. Huang (2006) discussed that the degradation of lignin by *P.chrysosporium* occurs in the secondary metabolic stage, and the degradation is dominated by oxidation. Therefore, it could mean corn straw was firstly decomposed into FA, or directly decomposed into CO_2, and then FA was converted to HA fraction.

The utilization of HML C is the leading cause to the decrease in TOC content. Before the 10th day, the three treatments except *T. reesei* has used less than 3% of HML as source of C and energy, which confirms that HML is a stable fraction. Another alternative would suggest that microorganisms proliferate at an extremely rapid rate in the early stages of fermentation and a part of microbial biomass carbon might have replenished C losses in HML which has been utilized by the microorganisms. Thusly, changes in HML were slightly insignificant in the first 10 days of fermentation. According to Paul (2002) part of dead microorganisms are not recycled through the microbial pool and hold the possibility of entering humus C. Our results further showed that in the later stages of straw fermentation, the relative content of HML decreased rapidly, because the readily available sources of C were nearly depleted. Therefore, microorganisms could have started to utilize the HML as a source of C and energy. Among the three fungal strains, the *T. reesei* demonstrated the strongest ability to utilize and transform HML, while *P. chrysosporium* and *T. harzianum* showed seldom difference.

In the present study, we found that the composition of corn straw HLS in the solid fermentation process possessed high C content in WSS. The *T. reesei*, *T. harzianum* and *P. chrysosporium* inoculants can effectively degrade the C found primarily in HLS, which leads to the conversion of the small molecular substrate detected in a "free state" in WSS. Because *T. reesei* has the strongest ability to reduce HLS contents, its corresponding WSS increment was also the largest compared with treatments. It is well established that WSS is an important C substrate for microbes (Dou *et al.* 2007). Gregorich *et al.* (2003) pointed out that WSS is easily utilized by microbes shortly after inoculation. However, in this study, the increase in WSS content after fungi inoculation could be attributed to the degradation preference by fungi. The fungi inoculants might have preferred to degrade complex molecules, resulting in the continuous accumulation of new WSS during corn straw decomposition. The increase in WSS in fungi treatments tended to be steady on the days 20 and 25, which confirmed the idea of easy consumption of WSS proposed by Gregorich *et al.* (2003).

Conclusion

In the 25 day solid-state fermentation, the three fungi inoculants significantly increased the relative content of WSS, FAL in corn straw and reduce the relative content of HML. The *T. reesei* have the most significant effect on humus formation. During decomposition and transformation of corn straw into humus, FAL is formed first, then later transformed into HAL. *P.chrysosporium* preferentially promote formation of FAL, while the two species of *Trichoderma* preferentially promote formation of HAL. In the last day of fermentation, *T.* reesei improved corn straw humification, HAL, and WSS carbon contents by 40.62, 53.71 and 15.15%, respectively, compared with CK. Fungi can effectively promote the transformation of corn straw into humus, and their transformation dynamics is consistent with polyphenol theory. The *T. reesei* has the best ability to synthesize HAL and WSS fractions in HLS.

Acknowledgement

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