

**Evaluation of white rot fungi pretreatment of mushroom residues for volatile fatty acid production by anaerobic fermentation**

**Feedstock applicability and fungal function**

Fang, Wei; Zhang, Xuedong; Zhang, Panyue; Carol Morera, Xavier; van Lier, Jules B.; Spanjers, Henri

**DOI**

[10.1016/j.biortech.2019.122447](https://doi.org/10.1016/j.biortech.2019.122447)

**Publication date**

2020

**Document Version**

Final published version

**Published in**

Bioresource Technology

**Citation (APA)**

Fang, W., Zhang, X., Zhang, P., Carol Morera, X., van Lier, J. B., & Spanjers, H. (2020). Evaluation of white rot fungi pretreatment of mushroom residues for volatile fatty acid production by anaerobic fermentation: Feedstock applicability and fungal function. *Bioresource Technology*, 297, Article 122447. <https://doi.org/10.1016/j.biortech.2019.122447>

**Important note**

To cite this publication, please use the final published version (if applicable). Please check the document version above.

**Copyright**

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

**Takedown policy**

Please contact us and provide details if you believe this document breaches copyrights. We will remove access to the work immediately and investigate your claim.

***Green Open Access added to TU Delft Institutional Repository***

***'You share, we take care!' – Taverne project***

**<https://www.openaccess.nl/en/you-share-we-take-care>**

Otherwise as indicated in the copyright section: the publisher is the copyright holder of this work and the author uses the Dutch legislation to make this work public.



# Evaluation of white rot fungi pretreatment of mushroom residues for volatile fatty acid production by anaerobic fermentation: Feedstock applicability and fungal function

Wei Fang<sup>a,b,c</sup>, Xuedong Zhang<sup>b,d</sup>, Panyue Zhang<sup>c,\*</sup>, Xavier Carol Morera<sup>e</sup>, Jules B. van Lier<sup>b</sup>, Henri Spanjers<sup>b</sup>

<sup>a</sup> State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, PR China

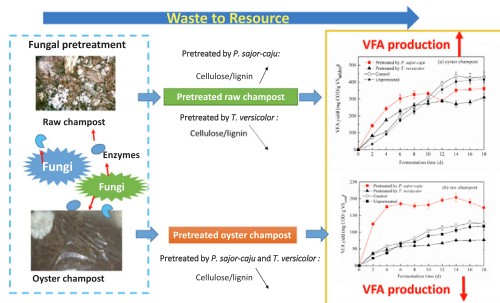
<sup>b</sup> Department of Water Management, Section Sanitary Engineering, Delft University of Technology, PO Box 5048, 2600 GA Delft, the Netherlands

<sup>c</sup> Beijing Key Lab for Source Control Technology of Water Pollution, Beijing Forestry University, Beijing 100083, China

<sup>d</sup> Veolia Water Technologies Techno Center Netherlands B.V., Tanthofdreef 21, 2623 EW Delft, the Netherlands

<sup>e</sup> Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, E-08017 Barcelona, Spain

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Keywords:

Fungal pretreatment  
White rot fungi  
VFA production  
Mushroom residues  
Anaerobic fermentation

## ABSTRACT

White rot fungi using *P. sajor-caju* and *T. versicolor* was examined to pretreat raw champost (lignin-rich) and oyster champost (cellulose-rich) for enhancement of fermentative volatile fatty acid (VFA) production. Results showed that the efficiency of pretreatment and VFA production was influenced by the fungal strains and substrates. *P. sajor-caju* pretreatment showed preferential lignin degradation on raw champost and obtained the maximum VFA yield ( $203 \pm 9$  mg COD/g  $VS_{added}$ ), which increased by 60% and 74% compared to that of control and unpretreated champost, respectively. For cellulose-rich oyster champost, however, fungal pretreatment decreased VFA yield compared to unpretreated champost. Further mechanisms analysis demonstrated the two strains grow and secreted ligninolytic enzymes, which substantially influenced the characteristics of two champosts such as cellulose/lignin ratio and morphology in different extents. *P. sajor-caju* was highly efficient to lignin-rich champost on selectively degrading lignin and further enhancing digestibility such as VFA production.

\* Corresponding author.

E-mail address: [panyue\\_zhang@bjfu.edu.cn](mailto:panyue_zhang@bjfu.edu.cn) (P. Zhang).

<https://doi.org/10.1016/j.biortech.2019.122447>

Received 23 October 2019; Received in revised form 15 November 2019; Accepted 16 November 2019

Available online 18 November 2019

0960-8524/ © 2019 Elsevier Ltd. All rights reserved.

## 1. Introduction

Spent mushroom residues, also known as champost, is a lignocellulosic byproduct of commercial mushroom industry (Pelkmans et al., 2016). Due to limited sustainable disposal strategies, the production of champost goes far beyond the current treatment capacity, leading to substantial champost being discarded as wastes (Kapu et al., 2012). The conversion of lignocellulosic biomass into biofuel by anaerobic fermentation or digestion has been widely considered as a suitable and promising treatment method for waste treatment and bioenergy recovery (Wan and Li, 2012; Yang et al. (2015a,b)). However, lignocellulosic biomass is not fully biodegradable during the anaerobic digestion processes because of its complex structure (Mustafa et al., 2016). Cellulose and hemicellulose are easily fermented, whereas lignin is relatively resistant to microbiological degradation under anaerobic conditions (Hamelinck et al., 2005; Wang et al., 2017). When the linkages between lignin and carbohydrates are broken or removed, highly lignified carbohydrates become more accessible and readily degradable by bacteria during anaerobic digestion (Grabber, 2005; Rouches et al., 2016). Recently, the carboxylate platform concept based on volatile fatty acids (VFAs) has been proposed and attracted more and more attention, because VFAs may serve as organic resources to produce higher value-added products, rather than methane (Agler et al., 2011). Hence it is attractive to enhance the conversion of lignocellulosic biomass to VFAs via pretreatment (Wang et al., 2019).

White rot fungi as a biological pretreatment method has attracted more and more attention due to a low consumption of chemicals and energy, compared with conventional methods (Harms et al., 2011). As lignocellulolytic microorganisms, white rot fungi are able to decompose lignin by their enzymes. Many species of white rot fungi, which are highly-selective lignin degraders, have been employed and examined in their ability to improve the biodegradability of lignocellulosic residues for methane or ethanol production (Singh et al., 2014; Wan & Li, 2010; Zhao et al., 2014). Nevertheless, to date, there is no report on whether fungal pretreatment could enhance VFA production from anaerobic fermentation of champost. Furthermore, improvement in delignification and digestibility of biomass by fungi also largely depends on the characteristics of fungal strains and substrates. For example, Tuyen et al. (2012) reported that fungal strains of white rot fungi were highly selective for lignin degradation and improved biogas production from wheat straw. Hence, it is necessary to identify specific combinations of fungal strains and champost to substantially improve the biodegradability of champost and further to elucidate the mechanisms involved in the fungal pretreatment of champost.

Above all, the objectives of this study are to evaluate feedstock applicability and fungal function during fungal pretreatment of champost (oyster and raw champost) for improving volatile fatty acid production by anaerobic fermentation. The VFA yield and composition were monitored to assess the performance of fungal pretreatment on lignocellulosic champost. Then the applicability of champost based on their basic characteristics and selective function of fungi were discussed. Finally, the detailed mechanisms involved in the effect of fungal pretreatment on fermentative VFA production were deciphered through understanding fungal growth, characteristics of champost structural properties (chemical composition and microstructure) and ligninolytic enzyme activities.

## 2. Materials and methods

### 2.1. Fungal strains and substrate preparation

The fungal strains *Trametes Versicolor* (*T. versicolor*; strain MES 11914) and *Pleurotus Sajor Caju* (*P. sajor-caju*; strain MES 03464) employed in this study were selected due to their known specialty in degradation of lignin, and obtained from the fungal stock collection of Wageningen University and Research (Wageningen, the Netherlands).

The fungi were pre-cultured on 3% malt extract agar medium at 25 °C for 10 d. After adding five pieces of colonized agar (1.5 × 2.0 cm) into sterilized sorghum grains, the inoculated sorghum was incubated at 25 °C until full colonization, and subsequently stored in a fridge at 4 °C until further use.

Oyster champost and raw champost were harvested from a mushroom research plant at Wageningen University, and used as substrate to be pretreated by the fungi. The oyster champost was collected from cultivation of oyster mushroom (*Pleurotus ostreatus*). The substrate for oyster mushroom growth was wheat straw mixed with calcium carbonate and other supplements. After one or two flushes of oyster mushroom harvest, the substrate was discarded as oyster champost. The raw champost was from cultivation of *Agaricus bisporus*. The substrate was a mixture of wheat straw with horse manure, chicken manure, gypsum and water. After two flushes of mushroom production, the substrate was discarded as raw champost. These substrates were chopped into pieces with a size of between 2 and 3 cm before pretreatment.

### 2.2. Fungal pretreatment

The fungal pretreatment of champost was carried out in 1.2 L autoclavable polypropylene containers (Combiness, Nazareth, Belgium). 100 g (based on dry matter) champost was weighed into containers with filter cover and autoclaved for 1 h at 121 °C. When the temperature of autoclaved containers decreased to ambient temperature, prepared spawn was inoculated to champosts at a weight ratio of spawn to substrate of 0.1, and mixed in a laminar flow hood (EF/B2, Telstar, Spain) under sterile conditions. Afterwards, the champosts were incubated at 25 °C with relative humidity of 70% in a climate controlled chamber (HPP110, Memmer Company, Germany) for 6 weeks. Samples for analysis were collected every week under sterile conditions in a laminar hood. All batch tests of fungal pretreatment were carried out in triplicate.

### 2.3. Anaerobic fermentation of pretreated mushroom champosts

The batch test for VFA production was carried out using anaerobic bottles with a working volume of 400 ml. Anaerobic fermentation was carried out with a total solid (TS) content of 15% and an inoculum-to-substrate ratio of 2 (TS basis) at 30 °C without pH control. The inoculum used in this study was taken from an anaerobic digester of a municipal sewage treatment plant (Harnaschpolder, Den Hoorn, the Netherlands). The TS and volatile solid (VS) of inoculum were 2.73% and 1.92%, respectively. Before anaerobic fermentation 19 mmol/L 2-bromoethanosulfophate (2-BES) was added to the bottles with substrates and inoculum together to inhibit methanogens, and the bottles were flushed with nitrogen gas for 2 min to obtain anaerobic conditions. Samples were extracted from the bottles every two days for analyses. After sampling, each bottle was flushed with nitrogen gas for 2 min to guarantee the anaerobic conditions. The champost without autoclaving and fungal pretreatment was named as unpretreated group, and the champost with autoclaving and without fungal pretreatment was named as control group. All the tests were operated in triplicate. The autoclaved champost was used as a control group.

### 2.4. Analytical methods

The determination of TS and VS was carried out following the standard methods (APHA, 2005). The content of cellulose, hemicellulose and lignin was determined using a fibretherm fibre analyzer (Gerhardt, Bonn, Germany) (Vansoest et al., 1991). The ratio of carbon to nitrogen was calculated based on the content of total carbon and total nitrogen, which was analyzed with an elemental analyzer (vario MACRO cube, Elementar, German). To determine SCOD and VFA, the samples collected during anaerobic fermentation were first centrifuged at 12,000 × g for 15 min using a centrifuge (Thermal Scientific, USA),

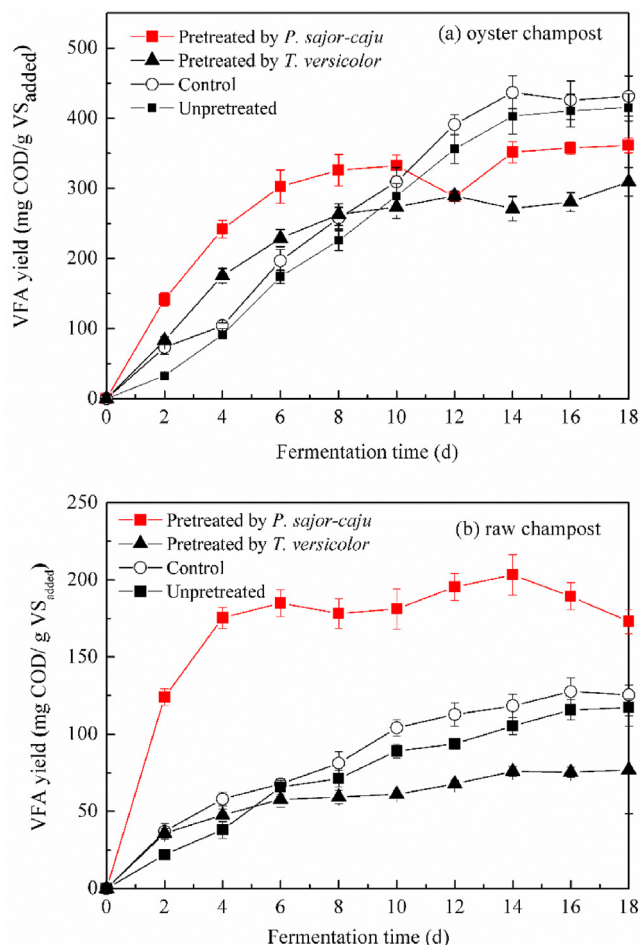


Fig. 1. Effect of fungal pretreatment on VFA yield over time during anaerobic fermentation (a) oyster champost, (b) raw champost.

and then filtered by syringe membrane filters (0.45  $\mu\text{m}$ , Whatman, Germany). The VFA was measured with a gas chromatograph (GC, Agilent Technology 7890A, USA) with a flame ionization detector and a capillary column (25 m  $\times$  320  $\mu\text{m}$   $\times$  0.5  $\mu\text{m}$ , Agilent 19091F-112). The ergosterol content was determined according to Fang et al. (2018).

The microstructure of champosts before and after pretreatment was characterized with a scanning electron microscope (SEM), (Quanta 200, America). Ligninolytic enzymatic activity and ergosterol content were measured according to Fang et al. (2018). The functional groups in champosts were analyzed using a Nicolet Impact 400 fourier transform infrared spectroscopy (FTIR) spectrometer equipped with a DTGS detector (Perkin-Elmer, the Netherlands).

### 3. Results and discussion

#### 3.1. Performance of anaerobic fermentation of raw and oyster champost with fungal pretreatment

##### 3.1.1. VFA production

Fig. 1 shows the VFA yields during anaerobic fermentation of two champosts. Although the pretreatment of cellulose-rich oyster champost by *P. sajor-caju* and *T. versicolor* increased VFA production within the first 8–10 days of fermentation, they resulted in a decrease of VFA yield throughout the anaerobic fermentation period (Fig. 1a). Maximum VFA yield of  $437 \pm 21$  mg COD/g VS<sub>added</sub> was achieved in the control, which was 17% and 29% higher than that of pretreated oyster champost by *P. sajor-caju* and *T. versicolor*, respectively. However, the VFA yield of  $415 \pm 13$  mg COD/g VS<sub>added</sub> from the unpretreated

oyster champost was even higher than that from other lignocellulosic biomass with physical and chemical pretreatment (Rughoonundun et al., 2012; Zhou et al., 2013). Fang et al. (2016) reported that the VFA yield from a spent mushroom compost was higher than that of other lignocellulosic biomass, which was probably due to substrate decomposition by bacteria and lignocellulolytic enzymes released by mycelia during mushroom cultivation. Hence, oyster champost is a potential feedstock for bioenergy or biochemical production such as VFA.

In contrast to the impact on pretreatment of cellulose-rich oyster champost, *P. sajor-caju* showed obviously positive effect on the pretreatment of lignin-rich raw champost for VFA production (Fig. 1b). The fungal pretreatment using *P. sajor-caju* significantly enhanced the VFA yield from raw champost, and the maximum VFA yield reached to  $203 \pm 9$  mg COD/g VS<sub>added</sub>, which was 60% and 74% higher than that of the control and unpretreated raw champost, respectively. On the other hand, fungal pretreatment by *T. versicolor* decreased the VFA yield by 39% and 35%, compared with that of the control and unpretreated, respectively. The detailed mechanisms of difference in VFA production between different substrates and different fungi were discussed in the Section 3.2 and Section 3.3.

##### 3.1.2. VFA composition

It is of great interest and importance to analyze the produced VFA compositions, because the VFA composition in fermentation products may influence their further applications (Chen et al., 2004). Fig. 2a shows the percentage of individual acetate, propionate, butyrate, valerate and hexanoate in all tests at their highest concentrations. The fungal pretreatment insignificantly changed VFA composition in

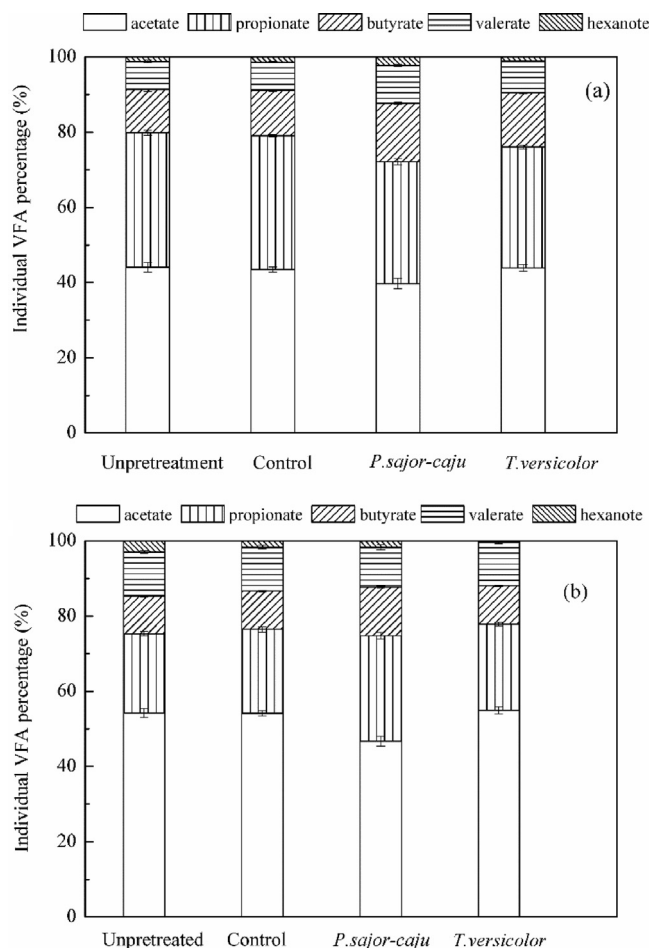


Fig. 2. Effect of fungal pretreatment on individual VFA composition of (a) oyster champost, (b) raw champost.

fermentation broth from oyster champost. Acetate was the predominant species in the VFAs fermented from oyster champost, ranging from 40% to 48%. The percentage of propionate ranged between 32% and 36% regardless of the fungal pretreatment, the control or untreated groups. Both of butyrate and valerate accounted for 10%–15% in all the tests. The fraction of hexanoate was less than 3.0%, which was the lowest VFA species from the oyster champost.

Fig. 2b shows that acetate and propionate were also dominant products, accounting for above 70% in all VFAs produced from raw champost. The results were consistent with a previous study that reported acetate and propionate are dominant in produced VFA from anaerobic fermentation without pH control (Fang et al., 2017). However, in this study a higher fraction of acetate (46%–54%) and a lower fraction of propionate (22%–28%) were achieved from raw champost, compared to that from oyster champost. The difference probably resulted from different compositions of these two champosts (Morgan-Sagastume et al., 2011). However, the fungal pretreatment did not substantially change the VFA composition. Similarly, Yu et al. (2014) found that the most abundant VFA species were acetate and propionate, no matter whether the lawn grass was pretreated by different pretreatment methods or not. It is worth noting that recovery of the produced VFA from fermentation broth is a major challenge due to the complex components and the low concentration of VFA in fermentation broth. Hence, it is necessary to investigate efficient recovery methods to enable bio-based high-value products in the future.

### 3.2. Applicability of mushroom champost

Table 1 shows the characteristics of two types of mushroom champost. Both oyster and raw champost presented high VS, viz. 86.8% and 84.0% of TS, respectively, indicating a high content of organic matters in the mushroom residues. Compared with raw champost, oyster champost has a higher cellulose and hemicellulose content of 48.3% and a lower lignin content of only 9.4% (based on dry matter). Besides, the carbon/nitrogen ratio (C/N) of oyster champost was 32.6, which is in the suitable range for anaerobic digestion (Fang et al., 2020). Abundant cellulose and hemicellulose in the oyster champost were the important carbon and energy sources for growth and metabolism of anaerobic microorganisms, which lead to the high biodegradability and VFA production from oyster champost in control and untreated group (Fig. 1a). Meanwhile, the lower lignin content and higher cellulose and hemicellulose content of oyster champost could provide more contact area and opportunity for anaerobic microorganisms and related enzymes. In contrast, raw champost presented a higher lignin content of 24.6% and a lower cellulose and hemicellulose content of only 11.6% (Table 1). Therefore, it is not unexpected that a lower VFA yield was achieved from raw champost than oyster champost due to their different compositions.

Most importantly, the basic characteristics of champost will influence the applicability of fungal pretreatment on raw and oyster

**Table 1**  
Characteristics of oyster champost and raw champost.

Parameters	Oyster champost	Raw champost
TS <sup>a</sup> (g/kg)	44.92 ± 0.82	22.13 ± 0.97
VS <sup>a</sup> (g/kg)	25.71 ± 0.42	12.54 ± 0.64
VS/TS (%)	86.8 ± 0.0	84.0 ± 0.0
Total carbon <sup>(b)</sup> (%)	43.7 ± 0.5	36.3 ± 0.3
Total nitrogen <sup>(b)</sup> (%)	1.3 ± 0.3	2.9 ± 0.2
C/N	32.6 ± 0.3	12.4 ± 0.2
Total phosphorus <sup>(b)</sup> (%)	0.16 ± 0.02	0.62 ± 0.03
Cellulose <sup>(b)</sup> (%)	30.5 ± 0.3	7.1 ± 0.2
Hemicellulose <sup>(b)</sup> (%)	17.8 ± 0.5	4.5 ± 0.2
Lignin <sup>(b)</sup> (%)	9.4 ± 0.4	24.6 ± 0.4

Note: <sup>a</sup> represents wet weight; <sup>b</sup> represents dry weight. Data are expressed as averaged value ± standard deviations of triplicates.

champost. Generally, the ligninolytic fungi mainly rely on digestible polysaccharides in lignocellulosic biomass as their nutrient and energy sources through exposure of fungal cellulases and hemicellulases. And then lignin is degraded during secondary metabolism under limited nutrients (Ramamamy et al., 1985). Meanwhile, it has been documented that fungal pretreatment seems to be particularly efficient on the degradation of lignin in lignin-rich biomass (Rouches et al., 2016). As reported by Van Kuijk et al. (2015), during the early colonization phase of white rot fungi, lignin was selectively degraded, followed by a fruiting stage in which polysaccharides were degraded. Hence, it is reasonable to infer that the cellulose and hemicellulose in oyster champost could be firstly and easily utilized by fungi during pretreatment, which might reduce the amount of cellulose and hemicellulose for VFA production by anaerobic bacteria. While the *P. sajor-caju* might mainly degrade the lignin in lignin-rich raw champost during pretreatment, which reduced the lignin and change the lignocellulosic structure to improve the VFA production from raw champost. Additionally, the different C/N ratios of both champosts could also affect fungal growth (Zhang et al., 2002) and production of lignocellulosic enzymes (Curvetto et al., 2002; Zhang et al., 2002).

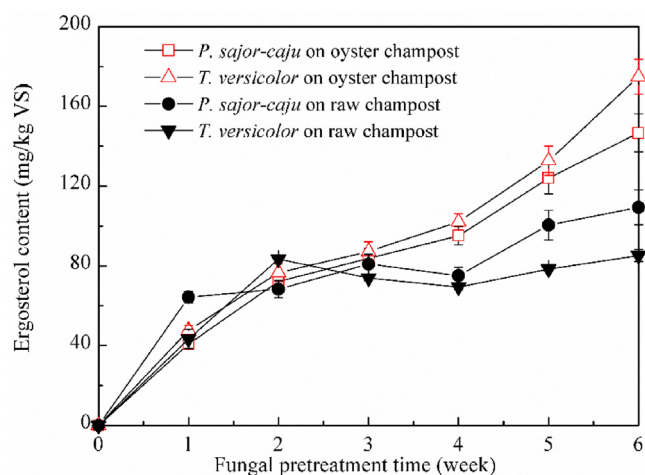
### 3.3. Fungal function for pretreatment of mushroom residues

#### 3.3.1. Fungal growth

An effective fungal pretreatment requires a good colonization by mycelium. And content of ergosterol has been widely used to quantify fungal cells in soil or organic residues (Bååth, 2001). As shown in Fig. 3, the *P. sajor-caju* and *T. versicolor* rapidly and constantly grew on oyster and raw champost, but the ergosterol content on oyster champost was higher than that on raw champost after 3 weeks of inoculation. The highest ergosterol content of 175 mg/kg TS was observed from *T. versicolor* on oyster champost by the end of the pretreatment. The visual observation also confirmed the growth of *P. sajor-caju* and *T. versicolor* on oyster champost and raw champost. The differences in ergosterol content between raw and oyster champost could be attributed to the different composition. Especially abundant cellulose and hemicellulose from oyster champost as carbon and energy source more obviously enhanced the growth of fungi, which might also cause the excess consumption of cellulose and hemicelluloses and further decrease of VFA production (Fig. 1a).

#### 3.3.2. Influence of fungal species on change of chemical composition of champost

Degradation of organic matters was inevitably as a result of fungal growth. As shown in Table 2, the autoclaving process resulted in higher cellulose, hemicellulose and lignin content in champosts than those in



**Fig. 3.** Ergosterol content of fungal strains on oyster and raw champosts.

**Table 2**  
Compositions of untreated and pretreated oyster and raw champost.

Substrate	Conditions	Cellulose (% dry weight)	Hemicellulose (% dry weight)	Lignin (% dry weight)	Cellulose/lignin ratio
Oyster champost	Untreated	30.5	17.8	9.4	3.24
	Autoclaved	52	19.6	16.8	3.09
	Pretreated by <i>P. sajor-caju</i>	40.9	14.6	13.3	3.07
	Pretreated by <i>T. versicolor</i>	39.1	16.7	15.0	2.61
Raw champost	Untreated	7.1	4.5	24.6	0.29
	Autoclaved	16.6	7.9	36.8	0.45
	Pretreated by <i>P. sajor-caju</i>	22.4	7.6	24.3	0.92
	Pretreated by <i>T. versicolor</i>	9.1	5.2	38.7	0.24

the untreated champosts. The results could be explained by the removal or dissolution of ash and extractives in function of autoclaving (Fang et al., 2018).

The strain of *P. sajor-caju* showed different impacts on oyster champost and raw champost. For instance, the highest ratio of cellulose to lignin for raw champost of 0.92 was observed in the sample pretreated by *P. sajor-caju*, which was 3.2 times of that from the untreated raw champost. It indicated that fungal pretreatment of raw champost by *P. sajor-caju* presented a significant delignification, but a weak degradation of cellulose. FT-IR analysis indicated that the peak around at 2900 cm<sup>-1</sup> representing C-H stretching in methylene and methyl groups in lignin decreased after 6 weeks of inoculation of *P. sajor-caju* (Gao et al., 2016). The preference of *P. sajor-caju* to lignin degradation on lignocellulosic biomass has been also observed by other studies (Bisaria et al., 1997; Fang et al., 2018). When the bonds between lignin and the carbohydrates were removed, highly lignified raw champost could be broken down anaerobically (Grabber, 2005). The ability of selective degradation of lignin by *P. sajor-caju* could improve the accessibility of microbes and enzymes to the cellulose. Several previous studies also confirmed that the lower lignin content was beneficial to the higher bioenergy production in the form of methane and/or ethanol (Rouches et al., 2016; Wan & Li, 2012; Zhao et al., 2014). However, the *T. versicolor* consumed a considerable amount of cellulose from raw champost, resulting in a lower cellulose-to-lignin ratio and poorer biodegradability of raw champost as aforementioned.

In contrast, the content of cellulose and hemicellulose in oyster champost decreased after pretreatments by *P. sajor-caju* and *T. versicolor*, while the lignin content increased. And the ratio of cellulose to lignin decreased in 6 weeks from 3.24 in the untreated champost to 3.07 and 2.61, respectively. The results were different from that for the pretreatment of raw champost, which could be attributed to the effect of different components of both champosts on fungal growth and production of lignocellulolytic enzymes. Owaied et al. (2015) observed that the productivity and biological efficiency of *Pleurotus ostreatus* is higher with a mixture of 50% wheat straw and 50% cardboard than wheat straw alone due to the differences in content of cellulose, hemicellulose and lignin. The decrease of VFA yield from pretreated oyster champost might be attributed to the considerable consumption of easy accessible cellulose and hemicellulose during fungal pretreatment (Tuyen et al., 2012; Van kuijk et al., 2015), even though the increase of surface area and porosity of oyster champost would improve the initial VFA production rate. Tuyen et al. (2012) also demonstrated that wheat straw lost 44.8% and 45.0% cellulose and hemicellulose with pretreatment by *T. versicolor* for 49 d.

On the other hand, the pretreatment by *T. versicolor* resulted in an obvious increase of lignin content in oyster and raw champost, reaching the lowest cellulose/lignin ratio compared to the control, autoclaved and *P. sajor-caju*-pretreated group. The *T. versicolor* has a good capacity of lignin degradation according to other studies (Van kuijk et al., 2015; Tuyen et al., 2012), but presented only limit effectiveness on lignin degradation in this study. This differences may be because of the different culture conditions, substrates and inoculums/substrate ratios.

### 3.3.3. Morphological observation

SEM can be used to observe morphological changes in oyster and raw champost before and after fungal pretreatments. The untreated oyster and raw champost presented a compact but slightly rigid structure, probably because of the decomposition and permeation by mycelia during mushroom cultivation. Substantial fungal mycelia were visible in both champosts, which was consistent with the results in fungal growth analysis (Fig. 3). Moreover, the structure of lignocellulosic fibers was broken down and exposed because of hyphal penetration at different extents, which also could change the champost compositions (Table. 2). In addition, the fungal pretreatment using *P. sajor-caju* and *T. versicolor* improved the structure disruption in oyster and raw champost, leading to loosening the champost structure with a simultaneous increase in surface area and porosity. Thus, fungal pretreatment could be conducive to the enzymatic hydrolysis and acidification of raw and oyster champosts during anaerobic fermentation process. Mustafa et al. (2016) reported that the specific surface area of rice straw increased by 42% after fungal pretreatment by *Pleurotus ostreatus* for 30 d, indicating that more biomass fragments became accessible to enzyme active sites, compared with the untreated rice straw.

### 3.3.4. Ligninolytic enzyme activities in champosts during fungal pretreatment

White rot fungi degrade lignin with the help of various secreted ligninolytic enzymes by different mechanisms. As shown in Fig. 4, the fungi species presented different ligninolytic enzyme activities in oyster and raw champost based on laccase and manganese peroxidase (MnP) during fungal pretreatment. For example, the laccase activity in oyster champost was higher than that in raw champost, no matter which fungus was employed. After one week pretreatment, the laccase activity of *T. versicolor* in oyster champost reached the maximum about 312 U/gTS, which was higher than that produced by *T. versicolor* in raw champost. This result indicated that the oyster champost might be beneficial for more laccase release by selected *T. versicolor* during the pretreatment process.

The dominant function enzyme for raw champost delignification by *P. sajor-caju* was MnP, while laccase was still the dominant ligninolytic enzyme for *T. versicolor*. The maximal MnP activity of 110 U/g TS was achieved at the 5th week of pretreatment, while the *T. versicolor* rapidly produced laccase and the maximal laccase activity of 312 U/g TS was achieved in the first week. In fact, the laccase presented lower redox potential compared to the other ligninolytic enzymes, therefore degraded only compounds with low redox potential, resulting in the oxidation of less recalcitrant aromatic compounds, compared to MnP (Fang et al., 2018). This is a possible reason why the *P. sajor-caju* showed a stronger ability of delignification than the *T. versicolor*. Besides, some chelator associated with MnP such as alkylitaconic acids could inhibit hydroxyl radicals produced with lignin degradation to attack cellulose (Fang et al., 2018; Rahmawati et al., 2005). The results of higher cellulose and lower lignin content of oyster and raw champost after fungal pretreatment by *P. sajor-caju* were shown in Table 2.

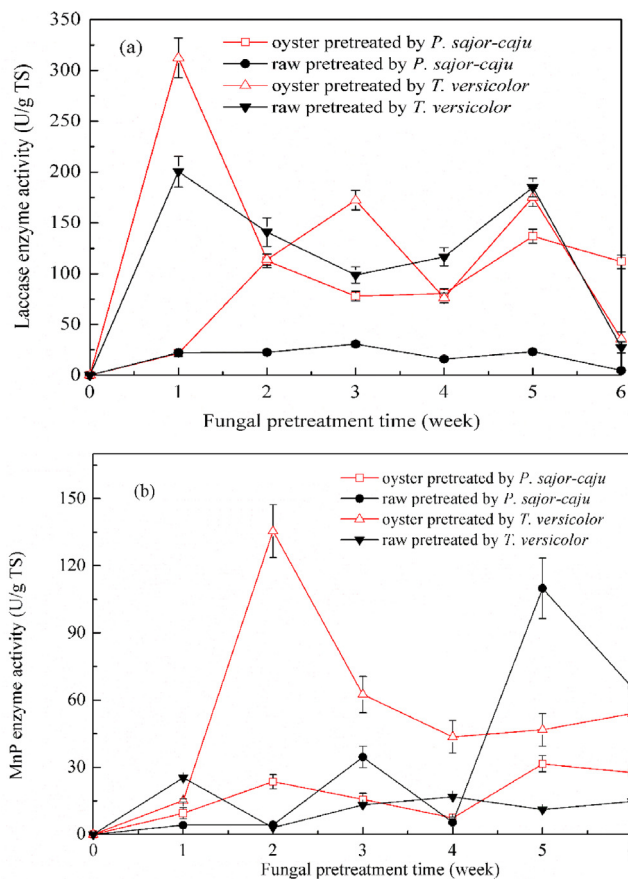


Fig. 4. Enzyme activities in champost during fungal pretreatment (a) Laccase, (b) MnP.

#### 4. Conclusions

The type of fungal strain and characteristics of substrates substantially affected the performance on physical and chemical characteristics of champost and VFA production in solid-state anaerobic fermentation. Fungal pretreatment using *P. sajor-caju* caused significant degradation of lignin but had limited effect on cellulose in lignin-rich champost, consequently improving VFA production. However, *P. sajor-caju* and *T. versicolor* resulted in decrease of VFA yield from cellulose-rich champost, due to higher cellulose degradation during pretreatment. Hence, although fungal pretreatment such as *P. sajor-caju* can pretreat lignin-rich lignocellulosic biomass for improving VFA production, it is important to ensure preferential lignin degradation during pretreatment.

#### CRediT authorship contribution statement

**Wei Fang:** Conceptualization, Writing - original draft, Writing - review & editing, Visualization. **Xuedong Zhang:** Conceptualization, Writing - review & editing. **Panyue Zhang:** Supervision, Writing - review & editing. **Xavier Carol Morera:** Visualization. **Jules B. Lier:** Writing - review & editing. **Henri Spanjers:** Supervision, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

The authors acknowledge the program “Increasing the utilization of organic waste and low value feeds with help of lignin degrading fungi” supported by the Dutch Technology Foundation, and express their gratitude to China Scholarship Council for providing the PhD Fellowship Awards (No. 201506130051) to Wei Fang.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2019.122447>.

#### References

- Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* 29 (2), 70–78.
- Bååth, E., 2001. Estimation of fungal growth rates in soil using <sup>14</sup>C-acetate incorporation into ergosterol. *Soil. Biol. Biochem.* 33 (14), 2011–2018.
- Bisaria, R., Madan, M., Vasudevan, P., 1997. Utilisation of agro-residues as animal feed through bioconversion. *Bioresour. Technol.* 59 (1), 5–8.
- Chen, Y.G., Randall, A.A., McCue, T., 2004. The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid. *Water Res.* 38 (1), 27–36.
- Curvetto, N.R., Figlas, D., Devalis, R., Delmastro, S., 2002. Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N-NH<sub>4</sub><sup>+</sup> and/or Mn(II). *Bioresour. Technol.* 84 (2), 171–176.
- Fang, W., Ye, J., Zhang, P., Zhu, X., Zhou, S., 2017. Solid-state anaerobic fermentation of spent mushroom compost for volatile fatty acids production by pH regulation. *Int. J. Hydrogen Energ.* 42 (29), 18295–18300.
- Fang, W., Zhang, X.D., Zhang, P.Y., Wan, J.J., Guo, H.X., Dara, S.M., Xavier, C.M., Zhang, T., 2020. Overview of key operation factors and strategies for improving fermentative volatile fatty acid production and product regulation from sewage sludge. *J. Environ. Sci.* 87, 93–111. <https://doi.org/10.1016/j.jes.2019.05.027>.
- Fang, W., Zhang, P., Zhang, X., Zhu, X., van Lier, J.B., Spanjers, H., 2018. White rot fungi pretreatment to advance volatile fatty acid production from solid-state fermentation of solid digestate: Efficiency and mechanisms. *Energy* 162, 534–541.
- Fang, W., Zhang, P.Y., Gou, X.Y., Zhang, H.B., Wu, Y., Ye, J., Zeng, G.M., 2016. Volatile fatty acid production from spent mushroom compost: Effect of total solid content. *Int. Biodeter. Biodegr.* 113, 217–221.
- Gao, Z., Fan, Q., He, Z., Wang, Z., Wang, X., Sun, J., 2016. Effect of biodegradation on thermogravimetric and chemical characteristics of hardwood and softwood by brown-rot fungus. *Bioresour. Technol.* 211, 443–450.
- Grabber, J.H., 2005. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. *Crop Sci.* 45 (3), 820–831.
- Hamelink, C.N., Van Hooijdonk, G., Faaij, A.P., 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle-and long-term. *Biomass Bioenerg.* 28 (4), 384–410.
- Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat. Rev. Microbiol.* 9 (3), 177–192.
- Kapu, N.U.S., Manning, M., Hurley, T.B., Voigt, J., Cosgrove, D.J., Romaine, C.P., 2012. Surfactant-assisted pretreatment and enzymatic hydrolysis of spent mushroom compost for the production of sugars. *Bioresour. Technol.* 114, 399–405.
- Morgan-Sagastume, F., Pratt, S., Karlsson, A., Cirne, D., Lant, P., Werker, A., 2011. Production of volatile fatty acids by fermentation of waste activated sludge pretreated in full-scale thermal hydrolysis plants. *Bioresour. Technol.* 102 (3), 3089–3097.
- Mustafa, A.M., Poulsen, T.G., Sheng, K.C., 2016. Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid-state anaerobic digestion. *Appl. Energy* 180, 661–671.
- Owaid, M.N., Abed, A.M., Nassar, B.M., 2015. Recycling cardboard wastes to produce blue oyster mushroom *Pleurotus ostreatus* in Iraq. *Emir. J. Food. Agr.* 27 (7), 537.
- Pelkmans, J.F., Vos, A.M., Scholtmeijer, K., Hendrix, E., Baars, J.J., Gehrmann, T., Reinders, M.J., Lugones, L.G., Wosten, H.A., 2016. The transcriptional regulator c2h2 accelerates mushroom formation in *Agaricus bisporus*. *Appl. Microbiol. Biotechnol.* 100 (16), 7151–7159.
- Rahmawati, N., Ohashi, Y., Watanabe, T., Honda, Y., Watanabe, T., 2005. Ceriporiol A, an extracellular metabolite of *Ceriporiopsis subvermiformis*, suppresses the depolymerization of cellulose by the fenton reaction. *Biomacromolecules* 6 (5), 2851–2856.
- Ramasamy, K., Kelley, R.L., Reddy, C.A., 1985. Lack of lignin degradation by glucose oxidase-negative mutants of *Phanerochaete-chrysosporium*. *Biochem. Biophys. Res. Co.* 131 (1), 436–441.
- Rouches, E., Herpoël-Gimbert, I., Steyer, J.P., Carrere, H., 2016. Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: A review. *Renew. Sust. Energ. Rev.* 59, 179–198.
- Rughonundun, H., Mohee, R., Holtzapfel, M.T., 2012. Influence of carbon-to-nitrogen ratio on the mixed-acid fermentation of wastewater sludge and pretreated bagasse. *Bioresour. Technol.* 112, 91–97.
- Singh, R., Shukla, A., Tiwari, S., Srivastava, M., 2014. A review on delignification of



- lignocellulosic biomass for enhancement of ethanol production potential. *Renew. Sust. Energ. Rev.* 32, 713–728.
- Tuyen, V.D., Cone, J.W., Baars, J.J.P., Sonnenberg, A.S.M., Hendriks, W.H., 2012. Fungal strain and incubation period affect chemical composition and nutrient availability of wheat straw for rumen fermentation. *Bioresour. Technol.* 111, 336–342.
- Van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H., Cone, J.W., 2015. Fungal treated lignocellulosic biomass as ruminant feed ingredient: A review. *Biotechnol. Adv.* 33 (1), 191–202.
- Vansoest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy. Sci.* 74 (10), 3583–3597.
- Wan, C.X., Li, Y.B., 2012. Fungal pretreatment of lignocellulosic biomass. *Biotechnol. Adv.* 30 (6), 1447–1457.
- Wan, C.X., Li, Y.L., 2010. Microbial pretreatment of corn stover with *Ceriporiopsis subvermispora* for enzymatic hydrolysis and ethanol production. *Bioresour. Technol.* 101 (16), 6398–6403.
- Wang, S.Q., Tao, X., Zhang, G.M., Zhang, P.Y., Wang, H.J., Ye, J.P., Li, F., Zhang, F., Mohammad, N., 2019. Benefit of solid-liquid separation on volatile fatty acid production from grass clipping with ultrasound-calcium hydroxide pretreatment. *Bioresour. Technol.* 274, 97–104.
- Wang, S.Q., Li, F., Zhang, P.Y., Jin, S.G., Tao, X., Tang, X., Ye, J.P., Mohammad, N., Wang, H.J., 2017. Ultrasound assisted alkaline pretreatment to enhance enzymatic saccharification of grass clipping. *Energ. Convers. Manage.* 149, 409–415.
- Yang, L., Xu, F., Ge, X., Li, Y., 2015a. Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renew. Sust. Energ. Rev.* 44, 824–834.
- Yu, L., Bule, M., Ma, J.W., Zhao, Q.B., Frear, C., Chen, S.L., 2014. Enhancing volatile fatty acid (VFA) and bio-methane production from lawn grass with pretreatment. *Bioresour. Technol.* 162, 243–249.
- Zhang, R.H., Li, X.J., Fadel, J.G., 2002. Oyster mushroom cultivation with rice and wheat straw. *Bioresour. Technol.* 82 (3), 277–284.
- Zhao, J., Zheng, Y., Li, Y., 2014. Fungal pretreatment of yard trimmings for enhancement of methane yield from solid-state anaerobic digestion. *Bioresour. Technol.* 156, 176–181.
- Zhou, A., Guo, Z., Yang, C., Kong, F., Liu, W., Wang, A., 2013. Volatile fatty acids productivity by anaerobic co-digesting waste activated sludge and corn straw: Effect of feedstock proportion. *J. Biotechnol.* 168 (2), 234–239.
- Yang, G., Zhang, G., Wang, H., 2015b. Current state of sludge production, management, treatment and disposal in China. *Water Res.* 78, 60–73.