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White rot fungi pretreatment to advance volatile fatty acid production from solid-state fermentation of solid digestate: Efficiency and mechanisms

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ABSTRACT
Anaerobic digestion has been widely applied throughout the world for lignocellulosic biomass treatment and energy recovery. However, the solid digestate from anaerobic digestion still contains a rather large fraction of poorly anaerobic degradable lignocellulosic fibers due to inhibition of lignin, which deeply limits the bioenergy production from lignocellulosic biomass. Therefore, a novel fungal pretreatment method using P. sajor-caju and T. versicolor was investigated to advance the solid-state fermentation of solid digestate and improve the production of fermentative volatile fatty acids (VFAs). The results showed that a maximum VFA yield of 240 mg COD/g VS was obtained from solid digestate pretreated by P. sajor-caju in 6 weeks, which was 1.17-fold and 1.24-fold higher than that of the autoclaved group and raw substrate, respectively. The mechanisms indicated that these fungal strains could grow on the solid digestate and secrete ligninolytic enzymes such as laccase and manganese peroxidase to degrade lignin in different extents. Besides, fungal pretreatment substantially changed the solid digestate characteristics such as cellulose/lignin ratio and the presence of specific functional groups. Moreover, fungal pretreatment using P. sajor-caju effectively damaged the structure and increased surface area and pore size of the solid digestate, which is beneficial to further VFA production.

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1. Introduction

As an efficient and relatively simple biotechnology, anaerobic digestion has been widely applied throughout the world for the stabilization of, and energy recovery from lignocellulosic wastes [1,2]. However, the conversion efficiency of anaerobic digestion process largely depends on the presence and intertwining of recalcitrant fibrous matters in the substrate biomass [3]. After digestion, the solid digestate that is separated from the liquid fraction by dewatering contains a rather large fraction of the poorly anaerobic degradable lignocellulosic fibers. The observed recalcitrance of these polymers is due to their complex, polymorphic structure and high degree of aromaticity of the lignin part. Therefore, solid digestate is usually considered to be unsuited for further conversion into more value-added products and generally finds its way to application in the agricultural sector for soil conditioning or animal bedding, or the solid digestate is incinerated [4].

However, with suitable pretreatment the residue still could be further utilized and converted into higher value-added products. Various pretreatment methods such as physical, chemical and biological ones have been widely studied [5–7]. One biological method uses white rot fungi, which is considered a potentially economical and environment friendly approach compared with traditional physical and chemical methods, due to its low consumption of energy and chemicals. Owing to their complex non-specific extracellular enzymes, white rot fungi have the ability to change their chemical composition and degrade lignocellulosic biomass [8].

Recently, production of volatile fatty acids (VFAs) instead of
2. Materials and methods

2.1. Fungal strains and spawn

Two white rot fungi, *Trametes Versicolor* (T. versicolor, strain MES 11914) and *Pleurotus Sajor Caju* (P. sajor-caju, strain MES 03464), were employed in this study. The fungi were initially cultured and incubated on 3% malt extract agar medium (pH ≈ 5.0) and at 25 °C until mycelia covered most of surface of plates. Then spawn was prepared by adding five pieces of colonized agar (1.5 × 2.0 cm) into sterilized sorghum grains and incubating at 25 °C until grains were colonized by the mycelia. Afterwards, the spawn was stored in a fridge at 4 °C for further use.

2.2. Solid digestate and inoculum

Solid digestate used in the present study as substrate was collected from a co-digestion biogas plant located in Waalwijk, the Netherlands, which mainly treats agricultural residues such as wasted fruits and vegetables residues. The substrate was initially dried at 60 °C for 24 h, and subsequently chopped into small pieces with a size between 2 and 3 cm. Inoculum used in this study was collected from an anaerobic digester of a local WWTP (Harnaschpolder, Den Hoorn, the Netherlands) that treats primary and secondary sludge. Table 1 summarizes the basic characteristics of the solid digestate and the inoculum.

2.3. Fungal pretreatment

Around 100 g (dry matter) of substrate was weighed into autoclavable polypropylene containers (1.2 L) with a filter cover (Combines, Nazareth, Belgium). The substrate and containers were sterilized by autoclaving for 30 min at 121 °C. After the autoclaved containers were cooled to room temperature, the spawn was added to the sterilized substrate at a weight ratio of 0.1 (0.1 g wet weight of spawn per g dry matter of substrate) and mixed under sterile conditions. Then, the mixed samples were incubated at 25 °C and at a relative humidity of 70% in a climate-controlled chamber (HPP110, Memmer Company, Germany). Samples for analysis were taken under sterile condition every week during pretreatment.

### Table 1

<table>
<thead>
<tr>
<th>Components</th>
<th>Solid digestate</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solid (TS) (g/kg)</td>
<td>296.3 ± 0.2</td>
<td>27.3 ± 0.1</td>
</tr>
<tr>
<td>Volatile solid (VS) (g/kg)</td>
<td>257.1 ± 0.2</td>
<td>19.2 ± 0.1</td>
</tr>
<tr>
<td>VS/TS (%)</td>
<td>86.80 ± 0.01</td>
<td>70.01 ± 0.10</td>
</tr>
<tr>
<td>pH</td>
<td>ND</td>
<td>7.85 ± 0.03</td>
</tr>
<tr>
<td>Total carbon (%)</td>
<td>44.1 ± 0.5</td>
<td>ND</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>18.1 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>Hemicellulose (%)</td>
<td>14.6 ± 0.5</td>
<td>ND</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>31.1 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>2.4 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>C/N</td>
<td>18.2 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>Total phosphorus (%)</td>
<td>0.95 ± 0.02</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND represents not determined. The data are shown as average ± standard deviations of three replicates.

* Represents the measurements based on dry weight.

b Represents the measurements based on wet weight.

2.4. Batch assays of anaerobic fermentation

Anaerobic fermentation was operated with a TS content of 15% (solid-state), and a ratio of inoculum to substrate of 2:1 (based on TS). To inhibit the activity of methanogens, we added 19 mmol/L of 2-Bromoethanosulfophate (2-BES) in all the test bottles before fermentation. Batch tests for VFA production were carried out using a Batch Test System (Bioprocess Control, Lund, Sweden) with a working volume of 400 ml at 30 °C. The experiment was divided into four groups, named raw substrate, control group, pretreated by *P. sajor-caju* and pretreated by *T. versicolor*. Raw substrate was original solid digestate without autoclave and fungal pretreatment. The control group was autoclaved solid digestate without fungal pretreatment. The group of pretreated by *P. sajor-caju* and *T. versicolor* was solid digestate autoclaved and pretreated by *P. sajor-caju* and *T. versicolor*, respectively.

Samples for the determination of the VFA were extracted from the bottles every two days and analyzed. The mixture samples were poured into centrifuge tubes of 15 ml from each fermentative bottle. After the sample collection, each bottle was sealed with a rubber stopper and flushed nitrogen gas into bottom of bottles for 5 min to replace the oxygen in the headspace, and then connected with Batch Test System again. The collected samples in centrifuge tubes were used for the chemical analysis. All the tests were carried out in triplicate.

2.5. Analytical methods

2.5.1. Determination of ergosterol concentration in fungal biomass

Ergosterol was used as a fungal biomarker. Ergosterol was extracted using an alkaline extraction protocol and determined according to procedure with minor modifications [13]. The modified procedure was as follows: 1.0 g moist sample was put into 15 ml of test tubes with 4 ml 10% KOH in methanol. After 15 min sonication, the test tubes went through a heat treatment (i.e. keeping the tubes in a water bath at 70 °C for 90 min) to release the esterified ergosterol. Subsequently, ergosterol was extracted by adding 1 ml Milli-Q water and 2 ml hexane. The tubes were stirred vigorously for 30 s and centrifuged for 10 min at 4000 r/min, and 1 ml liquid of top phase was transferred into small glass tubes. This step was repeated once more by adding 1 ml hexane for completely removing KOH. After evaporation of the hexane in a water bath at 50 °C in the fume hood for at least a whole night, 1 ml methanol was added into the samples in glass tubes. Afterwards, the samples were sonicated for 4 min and then the liquid was filtered into a vial by using 0.2 µm syringe filter. Then the ergosterol extracted from samples was analyzed using a high performance liquid
chromatography (HPLC) with C18 column (250 mm × 4.6 mm, Alltech Allsphere ODS-25 μ).

2.5.2. Extracellular enzymatic activity

Extracellular enzymatic activity was analyzed by UV spectrometry (Genesys 105 UV–vis). 1.0–1.5 g sample was suspended at a sample-to-distilled water ratio of 1:20 (w/v) on a shaker at 160 r/min for 30 min. Afterwards, the mixed sample was centrifuged at 12000 × g for 15 min and then the supernatant was filtered using a syringe membrane with 0.45 μm pore size. Laccase activity was measured by the oxidation of 2,6-dimethoxynaphthalen as described in the study of (Cruz-Morato et al., 2013) [14]. Manganese peroxidase (MnP) activity was measured by monitoring the enzyme’s oxidation of Mn (II) to Mn (III) [15].

2.5.3. Fourier transform infrared spectroscopy (FTIR) analysis

Analysis by fourier transform infrared spectroscopy (FTIR) was carried out to determine the functional groups of substrate before and after fungal pretreatment. Prior to the FTIR analyses, the samples were dried in an oven at 105 °C for 8 h and then ground by a cutting mill (Retsch SM 2000, Germany) with a mesh size of 0.2 mm. The prepared samples were analyzed using a Nicolet Impact 400 FTIR spectrometer equipped with a DTGS detector (Perkin–Elmer, the Netherlands). Spectral range was from 4000 to 600 cm\(^{-1}\) with a spectral resolution of 4 cm\(^{-1}\).

2.5.4. Other analysis

TS and VS were determined on a weight basis according to the standard methods (APHA, 2005) [16]. Samples for analysis of soluble chemical oxygen demand (SCOD), ammonium (NH\(_4\)-N), phosphate (PO\(_4\)-P) and VFA were first centrifuged at 12000 × g for 15 min with a centrifuge (Thermal Scientific, USA), and then the centrifuges were filtered by syringe membrane with 0.45 μm pore size. Their SCOD, NH\(_4\)-N and PO\(_4\)-P were measured using kits (Hach–Lange, Germany) according to Zhang et al. (2016) [17]. The contents of cellulose, hemicellulose and lignin were determined according to the method described by van Soest et al. (1991) using a Fibretherm Fibre Analyzer (Gerhardt, Bonn, Germany) [18]. pH was measured by an Orion 370 PerpHecT Meter (Thermo Fisher Scientific, USA).

The VFA concentration in the filtrate was measured by Gas Chromatography equipped with a 25 m × 320 μm × 0.5 μm Agilent 19091F-112 column and flame ionization detector. Helium was used as carrier gas with a flow rate of 1.8 ml/min. The 0.3 ml filtrate samples were collected in 1.5 ml vial with 1.2 ml internal standard pentanol solutions (320 mg/L), and 10.5 μl 98% H\(_3\)PO\(_4\) was added to adjust the pH to 3.5 ± 0.5. The temperature for the injection port, column and detector was set at 240, 240 and 250 °C, respectively.

VFA yield was calculated by Equation 1:

\[
\text{VFA yield (mg COD/g VS\text{added})} = \left(\frac{\text{VFA}_x - \text{VFA}_i}{\text{VS\text{added}}} \times \frac{V}{\text{VFA}_i}\right) \times V\text{added}
\]

where VFA\(_x\) represents the VFA concentration in the anaerobic fermentation test (mg COD/L); V represents the volume of fermentation system and VFA\(_i\) represents the VFA concentration in inoculum test (mg COD/L); VS\text{added} represents the amount of VS of substrate (g) in the test. The conversion factors between individual VFA and COD used in this study were 1.07 g COD/g for acetate, 1.51 g COD/g for propionate, 1.82 g COD/g for butyrate, 2.04 g COD/g for valerate, and 2.20 g COD/g for hexanoate.

3. Results and discussion

3.1. Fermentative VFA production from solid digestate

3.1.1. Effect of fungal pretreatment on VFA yield during anaerobic fermentation

VFA yields in anaerobic fermentation under different conditions are shown in Fig. 1. VFA production in the group with the raw substrate slowly increased during fermentation and the maximum VFA yield obtained was only 107 mg COD/g VS\text{added}, because most easily biodegradable components had already been converted to biogas in the methanogenic process in the original digestion plant. The VFA yield of autoclaved sample (110 mg COD/g VS\text{added}) was slightly lower than the control group, but the difference was not statistically significant at 95% level. However, the VFA yield from the solid digestate pretreated by P. sajor-caju was substantially enhanced and a maximum yield of 240 mg COD/g VS\text{added} was obtained at the 14th day of anaerobic fermentation. Evidently, the pretreatment by P. sajor-caju significantly improved the anaerobic biodegradability of solid digestate and further increased the VFA yield by approximately 1.17-fold and 1.24-fold compared with that of the control and raw solid digestate, respectively. Over time during fermentation, the VFA yield slightly decreased to 217 mg COD/g VS\text{added} from 14th days onwards to the end of fermentation, because the 2-Bromoethanosulfophate used for inhibition of methanogens might be consumed or its concentration substantially might reduce, which could lead to VFA consumption and methane production of VFA consumption and conversion into methane.

In contrast, the maximum VFA yield from solid digestate pretreated by T. versicolor was only 101 mg COD/g VS after 18 days of anaerobic fermentation, which was 9% and 6% lower compared to the control group and the raw solid digestate, respectively. A comparable result was observed in a previous study [19], which reported that using T. versicolor to treat wheat straw resulted in a reduction in biogas production by 20%, compared to the control group (autoclaved but un-inoculated straw). An explanation for the reduction could be that in the presence of T. versicolor a higher degradation of cellulose simultaneously occurred than the degradation of lignin during pretreatment [19].

A summarized comparison of VFA yields from different substrates with or without fungal pretreatment is given in Table 2. As shown in Table 2, although the solid digestate used in the present study had been anaerobically digested, the VFA yield from solid digestate test significantly increased by approximately 1.17-fold and 1.24-fold compared with that of the control and raw solid digestate, respectively.
digestate after pretreatment using *P. sajor-caju* was still comparable to that obtained from previous studies, in which the VFA was produced from anaerobic fermentation of different types of raw and pretreated lignocellulosic biomass and even activated sludge. Therefore, fungal pretreatment offers a novel and promising approach to improve the biodegradability of refractory organic materials under anaerobic conditions and further VFA production.

An efficient VFA product chain requires a minimal input of chemicals for pH control. In this study, the initial pH in all tests was in a range of 7.8–8.1. Without pH control, the final pH of mixed broth decreased to 7.1, 6.8, 6.4 and 6.6 after 18-day fermentation with raw digestate, autoclaved digestate, *P. sajor-caju* pretreated digestate and *T. versicolor* pretreated digestate as substrate, respectively. Apparently, the lowest pH was obtained from fermented sample pretreated by *P. sajor-caju* mainly because of higher VFA production. Besides, the pH values were always above the limited value of 6.0 for anaerobic fermentation of lignocellulosic biomass during the whole fermentation process [20]. Thus, it is not necessary to dose chemicals to control the pH for comparable VFA yield from solid digestate with fungal pretreatment.

### 3.1.2. Effect of fungal pretreatment on VFA profile

The profiles of individual VFAs from raw, autoclaved (control group) and pretreated substrates were analyzed and are shown in Fig. 2. Acetate was the most prevalent VFA products at all tests, accounting for more than 47% by the end of fermentation, followed by propionate of up to 23%. Butyrate and valerate was respectively below 15% and 14% of the total VFA products. Whereas, hexanoate took a fraction less than 2% and was the lowest VFA fraction in all tests. After the substrate was pretreated by *P. sajor-caju* and *T. versicolor*, the propionate content increased to 30% and 27%, respectively, and valerate content decreased, compared to the control group. The results can be probably explained by reduction in chain elongation during fermentation process [24]. Compared to raw substrate, acetate content in mixed VFA products with fungal pretreatment by *P. sajor-caju* and *T. versicolor* only decreased 0.2% and increased 3.0%, respectively. Hence, the total acetate and propionate were dominant VFA species in any investigated tests, with a total range of more than 70% by the end of fermentation. Specifically, the total contents of acetate and propionate increased to 78%, higher about 7% than that of raw solid digestate. The high content of micromolecular organic acids in the produced VFA mixtures indicates that the proposed pretreatment method might be suitable and potentially beneficial for possible subsequent bioprocesses, such as downstream bio-refinery and/or biological nutrient removal processes in WWTPs [25].

### 3.1.3. Ammonium and phosphate release after anaerobic fermentation

Pretreatment with *P. sajor-caju* significantly enhanced the VFA production from solid digestate, however, a number of ammonium and phosphate were released from organic materials during anaerobic fermentation of solid digestate (as shown in Table 3). The NH$_4^+$-N concentration at all tests was in a range of 1067–1121 mg/L after 18-day anaerobic fermentation, corresponding to a NH$_4^+$-N release of 9.34–13.88 mg/g VS$_{added}$. The PO$_4^{3-}$-P concentration at all tests was in a range of 314–336 mg/L, corresponding to a PO$_4^{3-}$-P release of 0.92–1.59 mg/g VS$_{added}$. For the purpose of maximizing the efficient application of VFAs and resource recovery, the NH$_4^+$-N and PO$_4^{3-}$-P are suggested to be separated from the fermentation broth. Various technologies may be employed to recover the resources from the fermentation broth, such as ammonium stripping or the formation of struvite as a fertilizer [26], but the influence of fermentation broth characteristics should be further detailedly

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investigated.

3.2. Why did fungal pretreatment influence VFA production of solid digestate?

3.2.1. Fungi growth during fungal pretreatment

A good colonization is a prerequisite for an effective fungal pretreatment of lignocellulosic materials. Ergosterol has been widely used to quantify fungal biomass in soil or organic matters because it is an important component in the cellular membrane of almost all fungi, which was not commonly produced by other organisms [27]. Thus, in this study, ergosterol content was employed to quantify the fungal biomass growth of each species. As shown in Fig. 3, both fungi used in the present study were able to grow on the substrate. As shown in Fig. S1, visual observation also confirmed the growth of P. sajor-caju and T. versicolor in solid digestate. P. sajor-caju grew well on substrate in the first 2 weeks of inoculation and a substantial increase of P. sajor-caju mycelia was observed. However, a slight decrease in the fungal biomass was observed from week 2 to week 4 of the inoculation. In the present study, ergosterol content of P. sajor-caju after 6 weeks was 225 mg/kg VS, which was obviously higher than the ergosterol content (120 mg/kg DM) for the same fungus specie (P. ostreatus) with wheat straw as substrate [13]. The higher ergosterol content might result from the difference in culture substrates. The ergosterol content of T. versicolor in treated substrate had a different pattern. It is only first week that a substantial increase in ergosterol content of T. versicolor was observed. Afterwards, the ergosterol content of T. versicolor decreased from 111 to 26 mg/kg VS during the second week, and further slowly increased to 54 mg/kg VS by the end of 6 weeks of inoculation. Different growth stages could influence ergosterol content in fungi, but the content is not always positively related with degradation of components in substrate [13,28].

3.2.2. Effect of fungal pretreatment on chemical composition of solid digestate

Fungal growth on substrate is accompanied by degradation of organic matter. Therefore, the effects of fungal pretreatment on the chemical composition of solid digestate was studied and presented in Table 4. The autoclave process had a significant impact on the composition of solid digestate. The sum of cellulose, hemicellulose and lignin content in solid digestate (dry substrate) increased from 63.8% to 83.6% after autoclave process, which indicated that some ash fractions in solid digestate were removed or dissolved.

The lowest lignin content of 22.4% was observed in P. sajor-caju after 6 weeks of inoculation, which was about 38% and 28% lower than the autoclaved and raw substrate, respectively. The lignin content in solid digestate after the pretreatment by T. versicolor declined only to 27.5%. It means that compared to T. versicolor, P. sajor-caju had a stronger efficiency in degradation of lignin in solid digestate. The fungal pretreatment especially by P. sajor-caju could break lignin-carbohydrate linkage and release more cellulose and hemicellulose from the complex structure of solid digestate, which will be beneficial to anaerobic fermentation because cellulose is an easy degradable substrate for anaerobic microbes [29]. Comparing compositions of solid digestate after the pretreatment by P. sajor-caju with those of the raw substrate, the cellulose content increased from 18.1% to 34.1%. The higher cellulose content could be explained by the smaller loss of cellulose relative to the loss of other polymers in substrate. However, T. versicolor presented a lower ability of lignin degradation and a higher ability of cellulose degradation than P. sajor-caju, resulting in no improvement in the anaerobic fermentation of solid digestate [19]. As shown in Fig. 4 and Table S1, the peaks around 2922 cm$^{-1}$ corresponding to C–H stretching in methyl and methylene groups in lignin decreased with P. sajor-caju [30], while the intensity of the peaks around 1300 cm$^{-1}$ that are assigned to the C–O–C groups in cellulose.

Table 4

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cellulose (%) dry weight</th>
<th>Hemicellulose (%) dry weight</th>
<th>Lignin (%) dry weight</th>
<th>Cellulose/lignin ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw substrate</td>
<td>18.1 ± 0.3</td>
<td>14.6 ± 0.5</td>
<td>31.1 ± 0.4</td>
<td>0.58</td>
</tr>
<tr>
<td>Control (autoclaved)</td>
<td>31.1 ± 0.8</td>
<td>17.6 ± 0.3</td>
<td>34.9 ± 0.7</td>
<td>0.89</td>
</tr>
<tr>
<td>Pretreated by P. sajor-caju</td>
<td>34.1 ± 0.9</td>
<td>13.6 ± 0.2</td>
<td>22.4 ± 0.2</td>
<td>1.52</td>
</tr>
<tr>
<td>Pretreated by T. versicolor</td>
<td>26.9 ± 0.5</td>
<td>14.5 ± 0.5</td>
<td>27.5 ± 0.3</td>
<td>0.98</td>
</tr>
</tbody>
</table>
cellulose and hemicellulose did not substantially change after 6 weeks inoculation [31]. On the other hand, the fungal pretreatment did not noticeably change the hemicellulose content in the substrate, compared with cellulose and lignin content. The cellulose/lignin ratio is a useful parameter to evaluate the biodegradability of substrate [32,33]. In the present study, a maximum cellulose/lignin ratio of 1.52 was observed after pretreatment by P. sajor-caju. The results indicated that the lignin was more degraded than the cellulose during P. sajor-caju pretreatment, presumably implying the P. sajor-caju preferred the lignin degradation rather than cellulose utilization. Furthermore, the fungal pretreatment using P. sajor-caju selectively degraded lignin, thus de-encrusting the cellulosic fibers and enhancing the availability of binding sites of feedstock for hydrolytic enzymes during anaerobic fermentation [34,35]. These results were the generally positive reasons for the significant improvement of VFA production from the solid digestate pretreated by P. sajor-caju (Fig. 1). The similar preference of P. sajor-caju to lignin when grown on lignocellulosic biomass has been previously confirmed by other studies, who concluded that the higher lignin removal was positively related with better anaerobic digestion in terms of biogas production [36]. The low cellulose/lignin ratio of solid digestate pretreated by T. versicolor demonstrated that T. versicolor degraded more cellulose simultaneously with the lignin degradation, which resulted in a low cellulose recovery [19].

3.2.3. Ligninolytic enzymatic activities in solid digestate during fungal pretreatment

Fig. 5 depicts activities of two dominant ligninolytic enzymes in the substrate during the fungal pretreatment. However, lignin peroxidase was not detected in the fungal pretreatment system, which might be attributed to that Pleurotus species (such as, P. sajor-caju) are not able to secret lignin peroxidase. Different levels of laccase and manganese peroxidase (MnP) activity were detected after one week of inoculation, which has not been reported previously in the literature on degradation of solid digestate. (Fig. 1). The similar preference of P. sajor-caju to lignin when grown on lignocellulosic biomass has been previously confirmed by other studies, who concluded that the higher lignin removal was positively related with better anaerobic digestion in terms of biogas production [36]. The low cellulose/lignin ratio of solid digestate pretreated by T. versicolor demonstrated that T. versicolor degraded more cellulose simultaneously with the lignin degradation, which resulted in a low cellulose recovery [19].

![Fig. 5. Enzymatic activities during the pretreatment by two fungi: (a) laccase activity; (b) MnP activity.](image-url)
was weaker than that by *P. sajor-caju* as shown in Fig. 6(d) and there was no enough surface area for enzyme reaction, which might be the reason that the VFA production from the solid digestate by *T. versicolor* was not enhanced.

### 3.2.5. pH variation during fungal pretreatment of solid digestate

pH is an important parameter that can significantly impact fungal growth and production of non-specific extracellular enzymes [37]. The pH variations in substrate during fungal pretreatment are shown in Fig. 7. In this study, solid digestate presented an initial pH ranging from 6.6 to 6.9, but the pH dramatically decreased by about one unit during the growth of *Pleurotus ostreatus* and *T. versicolor* as shown in Fig. 6. In this study, solid digestate presented an initial pH ranging from 6.6 to 6.9, but the pH dramatically decreased by about one unit during the growth of *Pleurotus ostreatus* and *T. versicolor* as shown in Fig. 6(d) and there was no enough surface area for enzyme reaction, which might be the reason that the VFA production from the solid digestate by *T. versicolor* was not enhanced.

Fig. 6. SEM images of solid digestate (a) raw, (b) after autoclaving, (c) pretreated by *P. sajor-caju*, and (d) pretreated by *T. versicolor*.

The pH variations in substrate during fungal pretreatment of solid digestate are shown in Fig. 7. In this study, solid digestate presented an initial pH ranging from 6.6 to 6.9, but the pH dramatically decreased by about one unit during the first week. Afterwards, the pH in *P. sajor-caju* and *T. versicolor* fluctuated slightly, and then slowly decreased and stabilized at 4.9 and 4.7, respectively, by the end of the pretreatment trial. The general pH profile was similar to a previous study of Dong (et al., 2013), who observed a pH decreased from 5.5 to 3.2 during the growth of *Pleurotus ostreatus* on sugarcane bagasse for three weeks [44]. The decrease in pH might be attributed to the production of organic acids, such as carboxylic and alkylitaconic acids, from the degradation of lignin and hemicellulose by white rot fungi [45]. Chen (et al., 1983) investigated the breakdown of spruce wood lignin by typical white rot fungi (*P. chrysosporium*) and stated that more than 35% of the products with low molecular weight were aromatic carboxylic acids. Therefore, the pH variation may be used as an indicator of the degradation of solid digestate [46].

### 4. Conclusions

A novel pretreatment method using white rot fungi to improve solid-state fermentative VFA production from solid digestate was examined and successfully developed. The present study demonstrated the fungal pretreatment using *P. sajor-caju* during 6 weeks of inoculation substantially enhanced the VFA yield by 1.17-fold and 1.24-fold, compared with the control group with autoclaved substrate and the group with raw substrate, respectively. The VFA yield of 240 mg COD/g VS from solid digestate pretreated by *P. sajor-caju* was comparable to the reported data from different biomass without anaerobic digestion before. In contrast, the pretreatment using *T. versicolor* slightly decreased the VFA yield. The mechanisms analysis indicated that fungal pretreatment using *P. sajor-caju* effectively damaged the structure and changed the characteristics of solid digestate owing to the secretion of ligninolytic enzymes for lignin degradation. Therefore, the fungal pretreatment using *T. versicolor* is a potential and feasible approach to enhance the degradability of solid digestate and conversion of solid digestate into high value added products, such as VFAs. However, the fungal pretreatment should be further optimized when applied to the utilization of solid digestate because of the long fungal pretreatment. Additionallly, it is interesting to further investigate efficient fungal strains which are preferential to delignification during pretreatment of different solid digestate. Suitable and efficient fungal strains should be selected according to the characteristics of substrate and required degradation degree of different components of biomass.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.energy.2018.08.082.

### References


[5] Behera S, Arora R, Sandhapogal N, Kumar S. Importance of chemical pre-
treatment for bioconversion of lignocellulosic biomass. Renew Sustain Energy
fermentation and mechanical pretreatment for lignocellulosic deconstruction: an
innovative strategy for biofuels and volatile fatty acids recovery. Appl Energy
2015;147:67–73.
production by a modified biological pretreatment in anaerobic fermentation
[8] Shirikavand E, Baroutian S, Gapes DJ, Young BR. Pretreatment of lignocellulosic
[9] Marshall CW, LaBelle EV, May HD. Production of fuels and chemicals from
lignocellulosic biomass degradation using Phanerochaete chrysosporium. Bio-
treatment of lignocellulosic biomass: importance of fungal species, colonization and
[14] Kramer-Morató C, Fernández-Clement L, Rodríguez-Mozaz S, Barceló D, Marco-
[15] Pasczyński A, Crawford RL, Huyh V-B. Manganese peroxidase of phaner-
[16] APHA. Standard methods for the examination of water and wastewater. DC
microbial community changes in a digester treating sludge from a brackish
[18] van Soest PJ. Robertson J, Lewis B. Methods for dietary fiber, neutral deter-
[20] Asanuma N, Hino T. Tolerance to low pH and lactate production in rumen
[22] Bäth K. Estimation of fungal growth rates in soil using 14-C-acetate incor-
gravimetric and chemical characteristics of corn stover by different white-
[26] Lalak J, Kasperszycza A, Martyniak D, Tys J. Effect of biological pretreatment
[27] Zhong W, Zhang Z, Luo Y, Sun S, Qiao S, Xiao M. Effect of biological pre-
[29] Zhao J, Zheng Y, Li Y. Fungal pretreatment of yard trimmings for enhancement of
pretreatment of lignocellulosic biomass as ruminant feed ingredient: a review. Bio-
[31] Tavares A, Coelho M, Agapito M, Coutinho J, Xavier A. Optimization and
[34] Ruggoniundun H, Mohr B, Holtzapple MT. Influence of carbon-to-nitrogen
ratio on the mixed-acid fermentation of wastewater sludge and pretreated
[35] Liu XL, Liu H, Chen JH, Du GC, Chen J. Enhancement of solubilization and
[36] Agler MT, Wrenn BA, Zinder SH, Angenent LT. Waste to bioproduction with
[37] Tong J, Chen Y. Recovery of nitrogen and phosphorus from alkaline
fermentation liquid of waste activated sludge and application of the
[38] Huang WW, Huang WL, Yuan T, Zhao ZW, Cai W, Zhang ZY, et al. Volatile fatty
acids (VFAs) production from swine manure through short-term dry anaer-
obic digestion and its separation from nitrogen and phosphorus resources in the