Enhanced lignin biodegradation by consortium of white rot fungi: microbial synergistic effects and product mapping

Tangwu Cui¹#, Bo Yuan¹#, Haiwei Guo², Hua Tian³, Weimin Wang¹, Yingqun Ma¹, Changzhi Li²*, Qiang Fei¹,4

1, School of Chemical Engineering and Technology, Xi’an Jiaotong University, Xi’an 710049, China
2, CAS Key Laboratory of Science and Technology on Applied Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian, 116023, PR China
3, Department of Chemistry, Pennsylvania State University, 215 Chemistry Bldg., University Park, PA 16802, USA
4, Shaanxi Key Laboratory of Energy Chemical Process Intensification, Xi’an Jiaotong University, Xi’an 710049, PR China

# these authors contributed equally to this work.
* corresponding author: Qiang Fei, Changzhi Li

Email address:
Tangwu Cui: tangwu_cui@stu.xjtu.edu.cn
Bo Yuan: boyuan@xjtu.edu.cn
Haiwei Guo: guohaiwei@dicp.ac.cn
Hua Tian: hut3@psu.edu
Weimin Wang: wwm3119116042@stu.xjtu.edu.cn
Yingqun Ma: yingqun_ma@xjtu.edu.cn
Changzhi Li: licz@dicp.ac.cn
Qiang Fei: feiqiang@mail.xjtu.edu.cn

Abstract

Background: As one of the major components in lignocellulosic biomass, lignin has been considered as the most abundant renewable aromatic feedstock in the world. Featuring with mild conditions and diversity, biological degradation of lignin is a promising approach comparing with thermal or catalytic ones.
Results: In this study, a consortium of white rot fungi composed of *Lenzites betulina* and *Trametes versicolor* was employed in order to enhance the ligninolytic enzyme activity of laccase (Lac) and manganese peroxidase (MnP) under microbial synergism. The maximum enzymatic activity of Lac and MnP was individually 18.06 U·mL⁻¹ and 13.58 U·mL⁻¹ along with a lignin degradation rate of 50%, which were achieved from batch cultivation of the consortium. The activity of Lac and MnP obtained from the consortium was all improved more than 40%, compared with monocultures of *L. betulina* or *T. versicolor* under the same culture condition. Our findings of enhanced biodegradation were in accordance with the results observed from scanning electron microscope (SEM) and secondary-ion mass spectrometry (SIMS). Finally, the analysis of heteronuclear single quantum coherence (HSQC) NMR and gas chromatography-mass spectrometry (GC-MS) provided a comprehensive product mapping of the lignin biodegradation, suggesting that the lignin has undergone depolymerization of the macromolecules, side-chain cleavage, and aromatic ring-opening reactions.

Conclusions: Our results revealed a considerable escalation on the enzymatic activities obtained in a short period from the cultivation of the *L. betulina* or *T. versicolor* due to the enhanced microbial synergistic effects, providing a potential bioconversion route for the applications of lignin utilization.

Keywords: White rot fungi; Lignin; Biodegradation; Synergistic effect; Product mapping.

Background

As the major component of lignocellulose, lignin has drawn great attention since its structure of three main phenylpropane units (i.e., sinapyl alcohol, coniferyl alcohol, and *p*-coumaryl alcohol). The degradation of lignin into small molecules is still a challenge due to the complexity of linkages and recalcitrance by various bonds [1, 2]. Therefore, it is critical to explore efficient approaches to degrade lignin polymer via physical, chemical or biological routes [3]. As an example for funneling the mixture of monomers by chemo-catalytic methods to produce value-added products, in 2020, Liao et al. reported a biorefinery process that converts 78 wt% birch into xylochemicals, which is one of the major steps towards full utilization of lignin [4, 5]. In
Recent years, great efforts have been made on lignin biodegradation due to its mild conditions, diversified choices of microorganisms, and high oxidative potentials. Mechanisms of the lignin degradation by major ligninolytic enzymes including laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP) have been extensively studied [6]. The most common bonds in lignin including β-O-4, β-β, 4-O-5, and 5-5’ have all been found to be cleaved by biodegradation methods [7, 8].

White rot fungi (WRF) play an important role in biodegradation of lignin [9]. Catabolism in fungi of Phanerochaete chrysosporium [10], Trametes versicolor [11] and bacteria of Streptomyces viridosporus [12], Sphingomonas paucimobilis [13] have been thoroughly investigated in previous studies, in which the β-ketoadipate (β-KA) pathway has been identified as one of the most common pathways for biodegradation of lignin [14]. The products frequently derived from the process include monophenols, benzenediols based compounds, aromatic hydrocarbons, short-chain acids, etc [15, 16]. Although extensive studies have been carried out for the investigations of enzymes, mechanisms, and pathways of lignin biodegradation, most of them have focused on single fungus/bacterium, which limits the degradation rates of the bioconversion of lignin. On the other hand, lignin degradation by a consortium of fungi or bacteria has shown certain advantages in terms of improved enzymatic activities, novel secondary metabolites, better substrate utilization, higher enzyme diversity, etc [17].

On the purpose of enhancing efficiency of lignin biodegradation, studying the synergistic effects of fungi, and mapping the products from the degradation process, herein a consortium of WRF composed of L. betulina and T. versicolor was investigated in batch cultures with lab-optimized medium. The enzymatic activities of two important ligninolytic enzymes - laccase (Lac) and manganese peroxidase (MnP) [6, 18] secreted by the WRF consortium were examined throughout the cultivation. The performance of lignin biodegradation was determined by calculating the degradation rates and running the scanning electron microscope (SEM) and secondary-ion mass spectrometry (SIMS). Furthermore, detailed product mapping was analyzed by employing heteronuclear single quantum coherence (HSQC) NMR and gas chromatography-mass spectrometry (GC-MS), revealing the catabolic pathways and mechanisms involved in the degradation process.
Results and discussion

Synergistic effects of WRF consortium on ligninolytic enzyme activity and biodegradation

The induction of ligninolytic enzymes was shown to be stimulated when culturing a consortium of WRF due to interspecific interactions [19-22]. Therefore, to investigate the synergistic influence of the WRF consortium on lignin biodegradation, enzymatic activities were analyzed daily during the cultivations with or without the supplement of lignin. As shown in Fig. 1, the maximum enzymatic activity of Lac (18.06 U·mL⁻¹) and MnP (13.58 U·mL⁻¹) was achieved in the cultures with the addition of lignin, which reduced the produce time of ligninolytic enzymes by more than 30%. This finding may be explained that the interspecific interactions between WRFs accelerate a fungal metabolic switch of the formation from primary to secondary metabolites that stimulates the secretion of ligninolytic enzymes [23, 24]. It was believed that ligninolytic enzymes may be regulated differently at the interface of interspecific interactions in response to various stressful conditions. It has been reported that the competition of nutrients or the production of free radicals such as reactive oxidative species (ROS) derived from oxidative stress could cause stimulation to enzymatic inductions [25].

Figure 1 Time courses of Lac and MnP enzymatic activities in the culture of consortium. Dot lines present the cultures without adding lignin; solid lines present the cultures with adding lignin.
To have a comprehensive understanding on the advantages of consortium culture, the activities of Lac and MnP obtained in cocultures were compared with monocultures of *L. betulina* and *T. versicolor*. As shown in Fig. 2, the enzyme activities from cocultures were more than 40% higher than the sum of the values observed in mono-cultivation. This may be possible that cocultivation of interacting fungi elevates expressions of Lac and MnP as well as the induction of novel isoymes. Ligninolytic enzymes such as Lac and LiP could be enhanced to mediate the stress and remove the ROS [22], which were approved by investigating the metabolites at the interaction zones, where novel metabolites were released in response to antagonistic interactions. It was obvious that the enzyme activities for both Lac and MnP obtained in the consortium cultures were higher than most of the previous reports with either monocultures or cocultures (Table 1), indicating an enhanced synergistic effect of the combination of *L. betulina* or *T. versicolor*, which promotes the inductions of ligninolytic enzymes. Nevertheless, the exact synergistic mechanisms of the paired WRFs need to elucidate with more research data.

![Figure 2](image-url)

**Figure 2** Enhanced synergistic effects on enzymatic activities of Lac (a) and MnP (b).
Table 1 Maximum enzymatic activities for Lac and MnP

| Microorganism                                      | Lac, U•mL⁻¹ | MnP, U•mL⁻¹ | Reference |
|----------------------------------------------------|--------------|-------------|-----------|
| L. betulina                                         | 0.026        | 0.03        | [26]      |
| L. betulina                                         | 0.127        | 0.039       | [27]      |
| T. versicolor                                       | 0.525        | 0.107       | [27]      |
| T. versicolor                                       | 0.37         | 0.0039      | [28]      |
| T. versicolor                                       | 0.159        | 0.109       | [29]      |
| Inonotus obliquus                                   | ND           | 159.0       | [30]      |
| Consortium of Trametes sp. AH28-2 and Trichoderma sp. ZH1 | 6.210        | ND          | [22]      |
| Consortium of P. chrysosporium, T. versicolor, Aspergillus niger, Penicillium chrysogenum, Trichoderma harzianum, and P. citrinum | ND           | 66.70       | [31]      |
| Consortium of Rhodotorula mucilaginosa and Pleurotus ferulae JM301 | 10.58        | ND          | [32]      |
| Consortium of Phanerochaete chrysosporium Burdsall and Trichoderma reesei RUT-C30 | <0.6         | 2.39        | [33]      |
| Consortium of L. betulina and T. versicolor         | 18.06        | 12.14       | This study |

ND: not detected

Based on the enzymatic activities data under interspecific interactions, the influence of improved enzymatic activities on degradation products was studied as follows. By analyzing the results of degradation rates, SEM and SIMS, more insights were gained for the quantitative understanding of the biodegradation process. The lignin degradation rate of the consortium was determined to be 50%, which was much higher than that from the cultures of L. betulina (26.6%) and T. versicolor (37.2%), respectively. Moreover, our results was also higher than other reported consortium with 34.1% by Dichomitus squalens [34] or 28.37% by microbial consortia [35]. Subsequently, SEM was performed on the purpose of identifying correlation of surface morphology with biodegradation (Fig. 3). The lignin after treatment by the consortium (Fig. 3c) showed that the particle sizes of the lignin were reduced significantly to below 4 μm, which were smaller than the control sample (Fig. 3a) and the one incubated without microorganisms (Fig. 3b). In addition, more irregular fragments were observed in Fig. 3c, and the particles were more densely packed. This result is consistent with the report by Zhu et al. [36], in which a decrease in particles sizes were also observed with lignin sample treated by Bacillus ligniniphilus.
Figure 3 SEM images for comparisons of lignin samples before and after biodegradation. a: control sample; b: lignin sample (with no microorganisms); c: lignin sample from the consortium.

**Product mapping of the lignin biodegradation by WRF consortium**

The degradation samples were analyzed for revealing the change of molecular weights of the products by SIMS for the first time, which is a state-of-art technique to analyze the topmost surface of the sample, which finds immense potential for applications in various fields. The heterogeneous nature has evidently limited the solubility of lignin without pretreatment. However, pretreatment often leads to a certain degree of bond breakages in lignin sample, rendering inaccuracies in representation of the data to the original sample. SIMS, on the other hand, has resolved this issue since it is performed on solid samples, and GCIB minimizes the damages to the samples. In addition, the high energy GCIB employed offers chemical map of the sample surface at the spatial resolution of 1 micron. Fig. 4 showed the 2D spectra of the lignin sample before (control sample) and after the cultivation of the consortium with adding lignin. The signal intensities of the monomers at m/z 260 were approximately 5 times higher in the control sample than the lignin sample, indicating the diminish of the monomers in the degraded sample. Many signals emerged for the sample after cultivation, ranging from approximately m/z 150 to 450, confirming that lignin macromolecules have been degraded into smaller fragments. These data led to conclusions that the consortium contributed to the improvement of the enzymatic activities and successful biodegradation based upon synergistic effects. Therefore, details of the products and possible pathways and mechanisms were explored by the following analyses.
Figure 4 SIMS analysis of lignin for molecular weights comparisons. a control sample; b lignin sample from the consortium.

Determining the products from the degradation is always one of the most important aspect of the investigations. Detailed product mapping was presented herein with a combination of results from HSQC and GC-MS, which provided a full picture of the products generated from the biodegradation of lignin and showed potential metabolic pathways and mechanisms. The structures of lignin analyzed by HSQC were shown in Fig. 5. The spectra of the control sample (Fig. 5a, δC/δH 50-140/2.0-9.0) could be divided into aromatic rings (δC/δH 90-140/6.0-9.0) and side chain regions (δC/δH 50-90/2.0-6.0). The region for aromatic rings showed multiple signals for Ca-oxidized syringyl (S'), p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) structures, which is the outcome of the natural route for lignin degradation cleaved by Lac and MnP [37].
The region for side chains showed methoxy (OMe) and β-O-4 structures (Aγ). The solvent signal was assigned to Pyridine. Compared with the control sample, the signals for S and S’ structures in the aromatic ring region and Aγ structures in the side chain region diminished (Fig. 5b). In addition, less signals for G and H moieties in the aromatic ring region and OMe moieties in the side chain regions were identified. Our findings are in agreement with previous literatures, in which Lac was found to be responsible for the released aromatic subunits of lignin during biodegradation [37].

![Figure 5 HSQC NMR spectra of lignin. a control sample; b lignin sample treated with the consortium; c corresponding lignin structures.](image)

The signals at ca. (δC/δH 68-70/3.0-3.5) might be attributed to WRF consortium residue structure. The above results indicated that the fungi consortium preferentially removed the S moieties and underwent β-O-4 bonds breakage and cleavage of the aromatic rings, thereby depolymerizing lignin to produce small molecular products. The results for reduction of OMe groups and β-5 bonds breakage were in agreement with the report from Mao et al. [38]. In addition, Zhao et al. also reported an enzymatic and microbial synergistic degradation of lignin, and results also showed that β-O-4 subunits were preferentially removed [39]. These results
suggested several possible mechanisms for lignin degradation, and will be further discussed with GC-MS results.

Products from lignin biodegradation were identified and characterized by GC-MS (Table 2). These products can be categorized into groups including substituted aromatics, small molecule acids and aliphatic acids. Firstly, substituted aromatics are products of side chain cleavage, which was also demonstrated by HSQC results. For example, 4-methylcinnamic acid (Table 2, Entry 21) came from degradation of coumaryl alcohol [40]. Secondly, p-hydroxybenzoic acid (Table 2, Entry 16) was a key product leading to protocatechuic acid, which was the typical intermediate for the protocatechuate branch of the pathway, and may be further metabolized by the β-KA pathway [40]. Similarly, benzoic acid (Table 2, Entry 4) came from degradation of cinnamic acid that leads to catechol, which is a key intermediate for the catechol branch of the pathway that goes into β-KA pathway [41]. Finally, oxalic acid, succinic acid, maleic acid, and other small acids indicated that ring opening occurred via the ortho or meta oxidative ring fission [42, 43], which was in the agreement with the HSQC data where the aromatic regions diminished. A final example is adipic acid (Table 2, Entry 13) [44] as a valuable dicarboxylic acid derived from muconic acid, which comes from the catechol ortho degradation pathway. In conclusion, the GC-MS data showed key intermediates for the side-chain fission and ring-opening, indicating that the β-KA pathway should be the main pathway for the lignin degradation. In addition to β-KA pathway, the upper funneling pathways including protocatechuate pathway and catechol pathway were also found to be present in the biodegradation system by the consortium.

Conclusions

Lignin biodegradation by fungi consortia is a promising strategy, which can overcome many shortcomings from degradation by one single microorganism such as low enzymatic activities, lower enzyme diversity and low substrate utilization. In this study, a considerable escalation on the enzymatic activities were obtained in a short period from the cultivation of the WRF consortium due to the microbial synergistic effects, which was confirmed by analyzing the surface morphology and molecular weights of the products by SEM and SIMS. HSQC spectra exhibited the bond cleavage and diminish of the functional groups, and product mapping was verified by applying GC-MS in order to investigate the possible pathways for the lignin...
The present study provides an advantageous method for the biological treatment of lignin and has great potential in the applications of lignin utilization.

Table 2 Lignin degradation products identified by GC-MS.

| Entry | Retention time (min) | Product Name                             | Product Structure | m/z  |
|-------|----------------------|------------------------------------------|-------------------|------|
| 1     | 12.5                 | Oxalic acid (ethanedioic acid)           | ![Image](image1)  | 90   |
| 2     | 12.7                 | 3-Methyl-1.3-butanediol                   | ![Image](image2)  | 104  |
| 3     | 13.7                 | 2-Methyl-3-hydroxybutyric acid           | ![Image](image3)  | 118  |
| 4     | 14.3                 | Benzoic acid                             | ![Image](image4)  | 122  |
| 5     | 15.1                 | Phenylacetic acid                        | ![Image](image5)  | 136  |
| 6     | 15.2                 | Maleic acid                              | ![Image](image6)  | 116  |
| 7     | 15.3                 | Succinic acid                            | ![Image](image7)  | 118  |
| 8     | 15.8                 | Fumaric acid                             | ![Image](image8)  | 116  |
| 9     | 16.6                 | Glutaric acid                            | ![Image](image9)  | 132  |
| 10    | 17.1                 | α-Hydroxyphenylacetic acid               | ![Image](image10) | 152  |
| 11    | 17.3                 | 4-Hydroxyvaleric acid                    | ![Image](image11) | 118  |
| 12    | 17.8                 | 2-Hydroxybutanedioic acid                | ![Image](image12) | 134  |
| 13    | 17.9                 | Adipic acid                              | ![Image](image13) | 146  |
| 14    | 18.7                 | 2,3-Dihydroxybutanedioic acid            | ![Image](image14) | 150  |
| 15    | 18.9                 | 2-Hydroxyglutaric acid                   | ![Image](image15) | 148  |
| 16    | 19.5                 | p-Hydroxybenzoic acid                    | ![Image](image16) | 138  |
| 17    | 20.9                 | 1,2,3-Propenetricarboxylic acid          | ![Image](image17) | 174  |
| 18    | 21.0                 | 2-Hydroxy-5-methoxybenzoic acid          | ![Image](image18) | 168  |
| 19    | 21.9                 | 2-tert-Butyl-6-methylphenol              | ![Image](image19) | 164  |
| 20    | 23.9                 | Hexadecanoic acid                        | ![Image](image20) | 256  |
| 21    | 24.6                 | 4-Methylcinnamic acid                    | ![Image](image21) | 162  |
| 22    | 25.5                 | 9-Octadecenoic acid                      | ![Image](image22) | 282  |
| 23    | 27.7                 | 2,6-Di-tert-butyl-4-methoxyphenol        | ![Image](image23) | 236  |
Methods

Microorganisms and culture conditions

The *L. betulina* and *T. versicolor* were purchased from China General Microbiological Culture Collection Center (CGMCC). These WRFs were maintained on potato dextrose agar (PDA) plates by a monthly subculture. The lab-modified culture medium (pH 5.4) used for all experiments consists of glucose (20 g·L⁻¹), peptone (5 g·L⁻¹), yeast extract powder (2 g·L⁻¹), KH₂PO₄ (0.5 g·L⁻¹), MnSO₄·7H₂O (0.5 mM), CaCl₂·2H₂O (0.1 g·L⁻¹), succinic acid (1.18 g·L⁻¹), ammonium tartrate (1.84 g·L⁻¹), thiamine (1 mg·L⁻¹), tween-80 (0.5 mL·L⁻¹), trace element (70 mL·L⁻¹). The trace element components consist of MgSO₄·7H₂O (3 g·L⁻¹), MnSO₄·H₂O (0.5 g·L⁻¹), NaCl (1.0 g·L⁻¹), FeSO₄·7H₂O (0.1 g·L⁻¹), CoCl (0.1 g·L⁻¹), ZnSO₄·7H₂O (0.1 g·L⁻¹), CuSO₄·5H₂O (0.1 g·L⁻¹), KAl(SO₄)₂·12H₂O (0.01 g·L⁻¹), H₃BO₃ (0.01 g·L⁻¹), NaMoO₄·2H₂O (0.01 g·L⁻¹). The glass beans (id. 0.5 cm) were applied in all liquid cultures for WRF growth. The other chemicals were used without further purification unless otherwise stated. Lignin was provided by Shandong Longlive Bio-Technology Co., Ltd. It belongs to industry alkaline lignin that was extracted from corn stalk.

A loop of colonies of both strains from PDA plates was transferred in 250mL flasks for seed cultures for 168-192hs. A 10% (v/v) inoculum was used for all experiments using 1000 mL flasks containing 600 mL medium. The seed solutions of *L. betulina* and *T. versicolor* of 6 mL were inoculated into the culture media. The inoculation solution of the consortium was made up of 3 mL of *L. betulina* and 3 mL of *T. versicolor*. Lignin of 1% (g/v) was added into the culture media to study the effect of lignin supplement on WRF growth. All the cultures were performed at 30 °C and 200 rpm in a rotary shaker (ZQZY-70CS, Zhichu, China) with triplicates.

Enzymatic activity assays

Ligninolytic enzymatic activity assays were performed everyday by using a UV spectrophotometer (Persee TU-1800, Beijing, China) in this study. The supernatant was collected from 1 mL culture solution after centrifugation at 8000 rpm for 10 min to remove the cells and residual lignin. Lac activity was determined by monitoring the reaction with ABTS as a substrate at 420 nm with reported procedures [45]. Lac activity was calculated employing Lambert-Beer law, the extinction coefficient (ε) is 36000 M⁻¹cm⁻¹. The manganese
peroxidase (MnP) activity was measured by monitoring the oxidation of 2,6-dimethoxyphenol (2,6-DMP) as the substrate at 470 nm ($\varepsilon_{470} = 49600 \text{ M}^{-1}\text{cm}^{-1}$) [46]. The enzymatic activity was calculated with the UV absorption data according to reported methods [47].

**Determination of degradation rates**

Degradation rates of lignin by employing a single and a mixture of fungi were determined by the Laboratory Analysis Protocol (LAP) from the National Renewable Energy Laboratory (NREL) [48]. In this method, the contents of acid-soluble and acid insoluble lignin need to be determined separately [49]. Before determining the lignin content, the lignin in the biomass needs to be dissolved under alkaline conditions and precipitated under acidic conditions [50]. The lignin degradation rates were determined combing the acid insoluble and soluble portions of the sample according to the NREL protocol.

**Scanning electron microscope (SEM)**

The changes of morphology and structures were observed by SEM. The samples were prepared by centrifuging the culture solution at the end of cultivation at 8000 rpm for 10 min and the precipitate was collected and lyophilized for 48 h to achieve a constant weight. Samples were coated with gold powder by a spray meter beforehand [36]. The samples were subjected to analysis by a TESCAN MALA3 LMH, Czech Republic.

**Secondary-ion mass spectrometry (SIMS)**

Lignin samples after cultivation were centrifuged for 10 min at 8000 rpm. The precipitate was placed at -80°C for 24 h and then freeze dried under reduced pressure. The samples were then grinded into powders. The fungi cells were removed by adjusting the pH to 12 by addition of NaOH (2 mol·L$^{-1}$) and placed at 30°C water bath for 24 h, then centrifuged at 5000 rpm, room temperature for 5 min. The supernatant was transferred and pH was adjusted to 3 by addition of H$_2$SO$_4$ (2 mol·L$^{-1}$), then placed in 30°C water bath for 24 h. The final sample was obtained by centrifuging at 5000 rpm, room temperature and freeze dried again under reduced pressure for 24 h. The lignin sample without fungi was pressurized into a thin layer with a thickness of approximated 5 mm. The top surface ~100 nm of the sample was subjected to SIMS.
measurement with a novel gas cluster ion beam (GCIB) and J105 3D Chemical Imager (Ionoptika, UK) with published procedures [51].

**Heteronuclear single quantum coherence (HSQC) NMR**

Lignin cultivation samples were pretreated by centrifugation, lyophilization, alkaline dissolution, centrifugation, acid precipitation and re-lyophilization. Subsequently 50 mg sample was dissolved in 0.5 mL DMSO-D6 and the solvent peak was used as a reference. 2D HSQC measurements were performed at 25 °C with a Bruker AVANCE III HD 700 MHz spectrometer and the Bruker standard pulse program was used [52]. HSQC cross-signals were analyzed and assigned by comparison with published results.

**Gas chromatography–mass spectrometry (GC-MS)**

Samples for GC-MS analysis were taken at 50 mL aliquots from the cultivation culture and centrifuged at 8,000 rpm for 10 min to remove the cells. The centrifugation condition was and the pH was adjusted to approximately 2 by addition of 6 mol·L⁻¹ HCl to the centrifuged supernatant. Subsequently, the mixture was derivatized according to reported procedures [53]. The derivatized sample (1 μL) was injected into the GC-MS (Thermo Fisher Scientific Trace ISQ, America). The capillary column used was HP-5 with Helium as the carrier gas, and the solvent delay time was 180 s. The injection temperature, ion source temperature, and transfer line temperature were 280, 250, and 200°C, respectively. The column temperature program was set as follows: first heated from room temperature to 50 °C for 5 min, then the temperature was raised at 10 °C·min⁻¹ acceleration rate to 300 °C, then maintained for 5 min. The ionization mass spectrum in the range of 30-550 m/z was recorded in Full Scan mode and 70 eV electron energy, and the results were compared with standard mass spectrometry databases to determine the products after lignin degradation [54].

**Abbreviations**

Lac: laccase; MnP: manganese peroxidase; SEM: scanning electron microscope; SIMS: secondary-ion mass spectrometry; HSQC: heteronuclear single quantum coherence; GC-MS: gas chromatography-mass spectrometry; LiP: lignin peroxidase; WRF: white rot fungi; β-KA: β-ketoadipate; ROS: reactive oxidative
species; \( S' \): Cα-oxidized syringyl; H: \( \rho \)-hydroxyphenyl; G: guaiacyl; S: syringyl; OMe: methoxy; Aγ: \( \beta \)-O-4 structures; PDA: potato dextrose agar; 2,6-DMP: 2,6-dimethoxyphenol; GCIB: gas cluster ion beam.

**Ethical Approval and Consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of supporting data**

Not applicable

**Competing interests**

The authors declare no competing interests.

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**Authors’ contributions**

TWC performed the cultures and drafted this manuscript. BY advised on the experiment design and revised the manuscript. HWG carried out the HSQC and analyzed results. HT carried out the SIMS and analyzed the results. WMW helped the experiment and prepared the manuscript. YQM advised on the design and revised the manuscript. CZL helped the experiment design and revised the manuscript. QF designed the experiments and draft the manuscript.

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Authors’ information

1. School of Chemical Engineering and Technology, Xi’an Jiaotong University, Xi’an 710049, China
2. CAS Key Laboratory of Science and Technology on Applied Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian, 116023, PR China
3. Department of Chemistry, Pennsylvania State University, 215 Chemistry Bldg., University Park, PA 16802, USA
4. Shaanxi Key Laboratory of Energy Chemical Process Intensification, Xi’an Jiaotong University, Xi’an 710049, PR China

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Figure Captions

Figure 1 Time courses of Lac and MnP enzymatic activities in the culture of consortium. Dot lines present the cultures without adding lignin; solid lines present the cultures with adding lignin.

Figure 2 Enhanced synergistic effects on enzymatic activities of Lac (a) and MnP (b)

Figure 3 SEM images for comparisons of lignin samples before and after biodegradation. a control sample; b lignin sample (with no microorganisms); c lignin sample from the consortium.

Figure 4 SIMS analysis of lignin for molecular weights comparisons. a control sample; b lignin sample from the consortium.

Figure 5 HSQC NMR spectra of lignin. a control sample; b lignin sample treated with the consortium; c corresponding lignin structures.