Fungal biodegradation of CCA-treated wood and removal of its metal components

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A R T I C L E   I N F O

Article history:
Received 6 August 2011
Received in revised form 20 March 2012
Accepted 24 March 2012
Available online 8 May 2012

Keywords:
Fungal bioprocessing
Biodegradation
Metal removal
Brown-rot fungi
CCA-treated wood wastes

A B S T R A C T

In the present study, 5 isolates of brown-rot fungi were used for fungal bioprocessing (FB) of chromated copper arsenate (CCA)-treated wood wastes: Antrodia vaillantii SEL8501, Fomitopsis palustris TYP0507 and TYP6137, and Crustoderma sp. KUC8065 and KUC8611. The isolates showed notable capacity for the degradation of treated wood and removal of CCA components via the American Wood Protection Association soil block test. Among them, Crustoderma sp. KUC8611 effectively decayed the treated wood, causing a mass loss of up to 60%. F. palustris caused extensive leaching of CrO3 of up to 79% and As2O3 of up to 87%, but only moderate leaching of CuO of up to 50%. This high capacity for removal of CrO3 and As2O3 showed a strong logarithmic relationship with the amount of oxalic acid produced in the decayed wood. The majority of metals removed from treated wood during the decay process were deposited in the soil and feeder strip. Further investigation will be required to establish the capability of selected fungi for FB of full-sized lumber treated with CCA.

1. Introduction

Over the last 60 yr, chromated copper arsenate (CCA) has been one of the most effective waterborne preservatives. CCA-treated wood has been widely used in many structures, including residential decks, public playgrounds, and building materials (Barray et al., 2009). However, numerous studies have reported the adverse effects of chromium and arsenic on human health (Kwon et al., 2009). Similarly, many studies have reported that harmful heavy metals, such as copper, chromium, and arsenic, can be leached from construction and demolition debris landfills (Townsend et al., 2004; Saxe et al., 2007; Jambeck et al., 2008; Dubey et al., 2009). Although landfill sites equipped with a liner can prevent the metal leaching into soil, there is a shortage of landfill space. Accordingly, alternative disposal methods should be developed. Incineration has also been used for the disposal of CCA-treated wood waste in many countries, including Korea, but this process produces fly ash contaminated with metals.

To alleviate the problems caused by landfill disposal and incineration of CCA-treated wood wastes, fungal bioprocessing (FB) using brown-rot fungi may be an effective method for pre-treatment. FB can be used to simultaneously decrease the volume of waste and remove metals from waste. A number of studies have investigated FB for the remediation of CCA-treated wood waste (Illman et al., 2000; Clausen and Green III, 2003; Choi et al., 2009). FB is considered a cost effective and easy method for reducing the volume of waste and removing metal components from the waste (Illman et al., 2000). To improve the efficiency of FB, it is important to identify useful fungi, as these could play a key role in the FB of CCA-treated wood wastes. Studies on FB have screened for useful fungi for biodegradation. Several brown-rot isolates, including Antrodia vaillantii, Fomitopsis palustris, Meruliporia incrassata, and Wolfiporia cocos, have been proposed as candidates for FB of CCA-treated wood waste (De Groot and Woodward, 1999;
Illman et al., 2000; Clausen and Green III, 2003). Among them, 2 isolates of *M. incrassata* TFFH-294 and Mad-563 showing superior biodegradation of treated wood wastes are able to reduce the mass of treated wood blocks by 37% and 24%, respectively (Illman et al., 2000). Recently, Choi et al. (2009) proposed that *Crustoderma* sp., which was isolated from CCA-treated wood waste, is more effective for FB than previously reported isolates.

To date, the fate of metals during FB has not been thoroughly investigated. It has been reported that some copper-tolerant fungi overproduce oxalic acid, which can affect metal movement during FB of CCA-treated wood (Clausen and Green III, 2003). The objectives of this study were to investigate the ability of several CCA-tolerant fungi to degrade CCA-treated wood and to confirm the removal of metal components from CCA-treated wood during the FB process. This study attempted to elucidate the basis for the removal of metal components by measuring the amount of oxalic acid produced during the decay of CCA-treated wood. Finally, the migration of metals removed from the treated wood during the decay process was also investigated.

### 2. Materials and methods

#### 2.1. Fungal isolates

A total of 35 brown-rot fungi were obtained from the Research Institute for Sustainable Humanosphere, Kyoto University, Japan. Fungi were transferred from stock cultures to 2% malt extract agar (MEA) plates and allowed to grow in the dark for 7–14 d at 27 °C prior to the bioassay and wood decay test for assessing CCA-tolerance and degradation efficiency. The fungi’s CCA tolerance was determined using an in vitro bioassay, described as a “choice test” by Leithoff et al. (1995).

Five brown-rot isolates, *Gyromitron sacchari* GYV9595 (previously named *Hyphrophoropsis aurantia*), an unknown Polyporales sp. LAS6497, *A. vaillantii* SEL8501, and 2 isolates of *P. palustris* TYP0507 and TYP6137, were identified as highly tolerant to CCA. The efficiencies of these isolates in degrading CCA-treated wood were measured. Two brown-rot fungi, *Crustoderma* sp. KUC8065 and KUC8611, which were reported to have a strong ability to degrade CCA-treated wood (Choi et al., 2009), were also included in this study. The optimum growth temperatures of each fungal isolate were also examined, and these values were used for testing CCA-treated wood decay.

#### 2.2. Preparation of CCA-treated wood samples

Sapwood blocks (19 x 19 x 19 mm) of *Pinus radiata* (Radiata pine, RP), *Tsuga heterophylla* (Western hemlock, WH), and *Cryptomeria japonica* (Japanese cedar, JC) were prepared from air-dried boards and were vacuum impregnated with CCA-C solution containing 18.5% CuO, 47.5% CrO₃, and 34.0% As₂O₅ to a target retention of 4.0 kg m⁻³. After treatment, the samples were wrapped in plastic bags and conditioned for 2 d at 60 °C to allow complete fixation of the CCA components. The untreated controls were also prepared from the air-dried boards. The wood blocks were then air-dried at room temperature for more than 7 d.

To determine the CCA retention of treated wood, fine sawdust samples were obtained from CCA-treated blocks and digested using the peroxide-nitric acid method to dissolve the CCA elements according to American Wood Protection Association (AWPA) A07-04 (AWPA, 2005a). The CCA elements in solution were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) according to AWPA A21-00 (AWPA, 2005b). The initial retention of CCA and the elemental contents are shown in Table 1.

<table>
<thead>
<tr>
<th>Wood species</th>
<th>Retention* (kg m⁻³)</th>
<th>CuO</th>
<th>CrO₃</th>
<th>As₂O₅</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiata pine</td>
<td>0.75 (0.02)</td>
<td>2.03</td>
<td>0.07</td>
<td>1.34</td>
<td>4.12</td>
</tr>
<tr>
<td>Western hemlock</td>
<td>0.73 (0.03)</td>
<td>2.00</td>
<td>0.01</td>
<td>1.28</td>
<td>4.01</td>
</tr>
<tr>
<td>Japanese cedar</td>
<td>0.76 (0.06)</td>
<td>2.01</td>
<td>0.11</td>
<td>1.33</td>
<td>4.03</td>
</tr>
</tbody>
</table>

*Values represent the average of 3 replicates; values in parentheses are the standard deviations.

#### 2.3. Decay test

The capacity of the selected fungal isolates to degrade CCA-treated wood was evaluated as the percentage of mass loss according to AWPA E10-01 (AWPA, 2005c). All treated and non-treated blocks were steam-sterilized for 30 min by autoclaving at 121 °C. A feeder strip (RP sapwood) was placed on the culture soil (Seung-jin Fertilizer, Korea) in culture bottle and was also steam-sterilized. As a reference, 68 mL of distilled water was added to the culture bottle containing 150 g of the culture soil prior to sterilization. Two fungal plugs, which were removed from the pre-culture 2% MEA media, were inoculated on each edge of the feeder strip and incubated for a minimum of 3 wk until the feeder strip was completely covered by mycelium. Two wood blocks were then placed on the feeder strip. All bottles were incubated for 12 wk at the optimum growth temperature determined for each fungal isolate. Following incubation, the test blocks were removed from the soil bottles, brushed free of mycelium, dried at 60 °C for at least 48 h, and re-weighed to determine the mass loss. The mean percentage of mass loss was calculated for each of the 5 groups of treated and non-treated blocks for each of the test fungi.

#### 2.4. Analysis of CCA elements in decayed treated blocks

After the 12-wk fungal exposure, the CCA elements retained in the decayed treated blocks were measured to evaluate the amount of the CCA elements removed during the decay process using the same procedures as for determining the initial CCA retention of the CCA-treated wood.

#### 2.5. Oxalic acid assay

Each decayed block was extracted according to the method proposed by Hunt et al. (2004). Five milliliters of 0.1 N NaOH was added to a 15-mL centrifuge tube containing 0.1 g of milled decayed wood sample, maintained at 65 °C for 1 h, and centrifuged at 21,000 g for 10 min. The supernatant was acidified with 0.25% H₂SO₄ and filtered through a 0.45-μm filter. The oxalic acid in the filtrate was determined using high performance liquid chromatography according to the method proposed by Kim et al. (2009).

#### 2.6. Migration of CCA elements

The CCA elements in the soil, feeder strip, and fungal hyphae covered with decayed treated wood were analyzed after the decay test to confirm the transport of the CCA elements from the decayed treated wood during the decay process. The metal contents of the soil after the 12-wk fungal exposure were determined using ICP-OES after digestion using the USEPA Method 3050B (USEPA, 1996). Fungal hypha and feeder strips were digested and were then analyzed using the same procedures as for determining the initial retention of CCA in the CCA-treated wood. This was performed for 2 target brown-rot isolates, *F. palustris* TYP0507 and *Crustoderma* sp. KUC8611, and one wood species, RP, treated with CCA.
3. Results and discussion

3.1. Fungal biodegradation of CCA-treated wood

Five isolates of brown-rot fungi, A. vaillantii SEL8501, F. palustris TYP0507, and TYP6137, and Crustoderma sp. KUC8065 and KUC8611, degraded at least 10% of the original dry weight of the 3 wood species (Table 2). Of the 3 wood species, RP had the greatest mass losses in both treated and untreated blocks. Thus, the RP used in this study was considered to have the lowest natural durability against decay.

Crustoderma sp. KUC8611 and KUC8065, which are strong degraders of CCA-treated wood (Choi et al. 2009), caused the highest percentages of degradation of treated RP; they degraded CCA-treated RP sapwood by 60% and 54%, respectively (Table 2) and CCA-treated WH and JC by up to 23% and 16%, respectively. Choi et al. (2009) reported that Crustoderma sp. KUC8611 and KUC8065 markedly degraded CCA-treated RP and could degrade Douglas fir and WH treated with CCA by more than 34%. Therefore, Crustoderma sp. KUC8611 and KUC8065 may be valuable for the FB of CCA-treated wood waste, especially those comprised of RP. F. palustris TYP0507 and TYP6137 also demonstrated significantly great capacities for decaying CCA-treated wood. F. palustris TYP0507 degraded CCA-treated RP, WH, and JC by 53%, 28%, and 30%, respectively. The difference in the degradability of CCA-treated wood between the fungal strains observed in this study, using Crustoderma sp. and F. palustris, can be explained by the intra-species degradability variations, as reported by Arango et al. (2009). A. vaillantii SEL8501 also caused more than 10% mass losses of all CCA-treated wood species. For all experiments using RP and some using WH, the CCA-treated wood was decayed to a higher degree than the untreated controls by the above 5 isolates (i.e., two isolates of Crustoderma sp., two isolates of F. palustris, and A. vaillantii).

Further studies on extracellular enzymes are underway in order to investigate the possible causes for this result.

G. sacchari GYV9595 and an unknown Polyporales sp. LAS6497 grew on CCA-treated wood but caused only slight mass losses (8% or less) (Table 2). Based on this observation, it can be concluded that not all CCA-tolerant fungi can decay CCA-treated wood. Thus, in order to select fungal isolates for FB of CCA-treated wood, it is important to measure their actual capacities for decaying CCA-treated wood.

3.2. CCA metal removal during the decay process

The percentages of metals removed from the CCA-treated radiate pine (a), Western hemlock (b), and Japanese cedar (c) sapwood blocks during the decay process.


![Fig. 1. Percentages of metals removed from the CCA-treated radiate pine (a), Western hemlock (b), and Japanese cedar (c) sapwood blocks during the decay process.](image)

F. palustris TYP0507 and TYP6137 removed significant amounts of CrO3 (up to 79%) and As2O5 (up to 87%) from the 3 treated wood species but only moderate amounts of CuO (up to 50%). A. vaillantii SEL8501 also removed up to 70% of the CrO3 and 72% of the As2O5 but less than 24% of the CuO. Although 2 strains of Crustoderma sp. leached reasonable amounts of CrO3 and As2O5 from the CCA-treated RP, these amounts were relatively low compared to their great ability to degrade CCA-treated wood. The differences in the ability of the fungal isolates to extract CCA might be due to differences in the amount of oxalic acid produced during the decay process. Therefore, the amount of oxalic acid produced in the decayed blocks was determined. Further, the relationship between the

**Table 2**

Average percentage of mass loss in CCA-treated and untreated wood samples.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Isolate no.</th>
<th>GenBank acc. no.</th>
<th>Radiata pine Treated (%)</th>
<th>Western hemlock Treated (%)</th>
<th>Japanese cedar Treated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gyrodontium sacchari</td>
<td>GYV9595</td>
<td>JQ410895</td>
<td>2 (1) D</td>
<td>3 (3) D</td>
<td>3 (2) E</td>
</tr>
<tr>
<td>Unknown Polyporales</td>
<td>LAS6497</td>
<td>EU024961</td>
<td>8 (4) D</td>
<td>3 (1) D</td>
<td>3 (2) E</td>
</tr>
<tr>
<td>A. vaillantii</td>
<td>SEL8501</td>
<td>EU024959</td>
<td>46 (11) C</td>
<td>18 (1) C</td>
<td>10 (4) D</td>
</tr>
<tr>
<td>Fomitopsis palustris</td>
<td>TYP0507</td>
<td>EU024964</td>
<td>53 (7) ABC</td>
<td>28 (4) A</td>
<td>30 (7) A</td>
</tr>
<tr>
<td>Fomitopsis palustris</td>
<td>TYP6137</td>
<td>JQ410896</td>
<td>51 (7) BC</td>
<td>27 (3) A</td>
<td>30 (7) A</td>
</tr>
<tr>
<td>Crustoderma sp.</td>
<td>KUC8065</td>
<td>AY858355</td>
<td>54 (15) AB</td>
<td>22 (2) B</td>
<td>16 (5) C</td>
</tr>
<tr>
<td>Crustoderma sp.</td>
<td>KUC8611</td>
<td>EU024960</td>
<td>60 (6) A</td>
<td>23 (3) B</td>
<td>11 (4) D</td>
</tr>
</tbody>
</table>

Values represent the average of 10 replicates; values in parentheses are the standard deviations.

Numbers followed by the same letter in each column are not significantly different (a = 0.05) according to the Duncan's method.
percentages of CCA metals removed and the amount of oxalic acid produced was investigated.

3.3. Oxalic acid production and its relationship with metal removal

Table 3 shows the amounts of oxalic acid produced in the decayed wood samples during the decay process. As expected, the fungal isolates that removed the highest amounts of CrO$_3$ and As$_2$O$_5$ from CCA-treated wood produced relatively large amounts of oxalic acid during the degradation process. F. palustris produced the largest amount of oxalic acid (up to 35.5 mg g$^{-1}$), followed by A. vaillantii SEL8501 (up to 17.8 mg g$^{-1}$), and then the 2 strains of Crustoderma sp. (up to 11.9 mg g$^{-1}$).

A correlation analysis was performed using SAS version 9.1.3 (SAS Institute, USA), which demonstrated that the production of oxalic acid and the rate of CuO removal. A regression analysis demonstrated high logarithmic relationships between the production of oxalic acid and CrO$_3$ ($R^2 = 0.898$–0.945) and As$_2$O$_5$ ($R^2 = 0.712$–0.923) removal. This indicates that the removal of CrO$_3$ and As$_2$O$_5$ from CCA-treated wood during the decay process was considerably affected by fungal production of oxalic acid. However, the production of oxalic acid does not appear to affect the leaching of CuO from the decaying treated woods. Clausen (2000) found that increasing the concentration of oxalic acid from 0% to 1% reduced the amount of arsenic (1.50–0.53 mg g$^{-1}$) and chromium (3.58–1.61 mg g$^{-1}$) in a CWA wafer during a dual remediation process using Bacillus licheniformis CC01 and oxalic acid. However, the amount of copper remaining in the CWA wafer was unchanged by increasing the concentration of oxalic acid. Choi et al. (2009) also reported that more than 90% of arsenic and chromium were extracted from CCA-treated sawdust in liquid media containing a high amount of oxalic acid (4.0 g L$^{-1}$) produced by an unknown Polyporales sp. LAS6497, but there was only moderate extraction of copper (51%). In a study by Kartal et al. (2004), Coniophora puteana removed more copper than did Laetiporus sulphureus from CCA-treated sawdust by liquid fermentation, although L. sulphureus produced more oxalic acid than C. puteana. These results suggest that the removal of CrO$_3$ and As$_2$O$_5$ from CCA-treated wood is significantly affected by fungal production of oxalic acid. There may not be a correlation between oxalic acid production and CuO removal, because the water-insoluble compound, copper-oxalate, forms when copper reacts with high concentrations of oxalic acid (Kazi and Copper, 1998; Clausen, 2000; Katikian et al., 2007, 2009).

3.4. Migration of CCA elements

Metals removed from the treated wood samples during the decay process mainly migrated into the soil; the remaining metals were mainly deposited in the feeder strip (Table 4). In the fungal hypha of F. palustris TYP0507, small quantities of metal (less than 0.21 mg) were adsorbed, while 2.0 mg of the CuO was adsorbed onto the fungal hypha of Crustoderma sp. KUC8611. The metal adsorption capacity by mycelium varies by the kinds of metal and fungal isolate. In the present study, metal adsorption by fungal hypha participated in the removal of active CCA ingredients from treated wood, as previously reported (Chou et al., 1973; Pohleven et al., 1999; Trivedi and Patel, 2007). Because fungal isolates that inhabited in feeder strip might continuously produce oxalic acid, it is possible that the metals in the feeder strip could also be extracted and accumulated in the soil over time. Consequently, a technique for recovering metals from the soil after FB should be developed to prevent secondary environmental pollution with heavy metal and for the reuse of the metals.

4. Conclusions

Herein, 5 isolates of brown-rot fungi, A. vaillantii SEL8501, F. palustris TYP0507 and TYP6137, and Crustoderma sp. KUC8605 and KUC8611, were evaluated as candidates for the FB of CCA-treated wood...
wood waste. Certain fungal species, such as Crustoderma sp. and F. palustris, effectively decayed treated wood waste, causing mass losses of up to 60 and 53%, respectively. During the decay of treated wood, fungal species removed large amounts of CrO₃ and As₂O₅. Furthermore, the amounts of CrO₃ and As₂O₅ removed were significantly correlated with the amount of oxalic acid produced by the fungi during the decay of CCA-treated wood.

In order to use CCA-treated wood waste as the feedstock for thermochemical conversion processes, reducing waste volume by FB could be considered disadvantageous. However, the metals that remain in fly ash after incineration can also cause environmental contamination if not removed prior to incineration. Landfills are not an environmentally sound disposal method, since the capacities of landfills are limited, and it is difficult to obtain public approval for new landfill facilities. Therefore, FB, which simultaneously removes metals from treated wood and reduces the volume of treated wood waste, could be a valuable pre-treatment method for solving the problems associated with current disposal methods. However, it is important that an easy method for recovering and to reuse of metals collected in the soil during FB should be developed. Finally, for the practical application of FB, further studies must elucidate the decay capabilities of selected fungi on full-sized lumber treated with CCA.

Acknowledgments
This work was supported by a Korea Research Foundation Grant (KRF-2008-313-F00056), funded by the Korean Government, and by a Forest Science & Technology Project (Project No. S1210101110100) provided by the Korea Forest Service of the Korean Government.

References
AWPA, 2005c. Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures. AWPA E10-01, American Wood Protection Association, Grandbury, TX.