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Bioremediation of CCA-C treated wood by *Aspergillus niger* fermentation

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Abstract This study evaluated the potential of the fungus *Aspergillus niger* to remove copper, chromium, and arsenic from waste wood treated with chromated copper arsenate (CCA) wood preservative. The removal of heavy metals by *A. niger* was carried out in two stages. In the first stage, *A. niger* was cultivated in carbohydrates media in order to produce large quantities of oxalic acid. Bioremediation of CCA-treated wood was performed in the second stage through both leaching of heavy metals with oxalic acid occurred during the first stage and possible biosorption of metals onto the binding sites in the cellular structure of *A. niger*. Oxalic acid production by *A. niger* was 13.4 kg/m^3 at pH 6 and in an enriched nitrogen and phosphorus medium. CCA-treated chips exposed to *A. niger* for 10 days showed a decrease in arsenic of 97%. In addition, *A. niger* fermentation removed 49% copper and 55% chromium from CCA-treated chips. This study showed that fungal fermentation and passive metal removal by *A. niger* had a potential in arsenic release from CCA-treated waste wood.

Aufwertung von CCA-behandeltem Holz durch Fermentation mit *Aspergillus niger*

Zusammenfassung Diese Studie untersuchte die Möglichkeit des Pilzes *Aspergillus niger* Kupfer, Chrom und Arsen von Abfallholz, das mit CCA-Holzschutzmittel behandelt worden war, zu entfernen. Die Entfernung von Schwermetallen *durch A. niger* wurde in zwei Stufen durchgeführt. Während der ersten Stufe wurde *A. niger* in Kohlenhydratmedien kultiviert, um große Mengen von Oxalsäure zu produzieren. Die Aufwertung von CCA-behandeltem Holz wurde während der zweiten Stufe durchgeführt. Sowohl das Auslaugen der Schwermetalle mit Oxalsäure als auch die mögliche Biosorption von Metallen an Rezeptoren in der Zellstruktur von *A. niger* fand während der ersten Stufe statt. Die Oxalsäure-Produktion durch *A. niger* betrug 13,4 kg/m^3 bei einem pH-Wert 6 in einem mit Stickstoff und Phosphor angereichertem Medium. Die mit CCA behandelten Späne zeigten nach 10-tägiger Behandlung mit *A.* niger einen Arsenabfall von 97%. Zusätzlich entfernte die *A. niger*-Fermentation 49% Kupfer und 55% Chrom von CCA-behandelten Spänen. Diese Studie zeigte, dass Pilzfermentation und passive Metallentfernung durch *A. niger* eine Möglichkeit bot, Arsen von CCA-behandeltem Holz freizusetzen.

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1 Introduction

Chromated copper arsenate (CCA) is a chemical compound mixture containing inorganic copper, chromium, and arsenic used for wood preservation since the 1940s. CCA-treated wood has been restricted in most European and Asian countries, and the use of CCA-treated wood will be limited to certain industrial and commercial applications in the USA by 2004. An increase in the amount of waste wood treated with CCA wood preservatives is expected within the next few decades due to CCA-treated wood being removed from service after an average service life of 20–40 years. The release of copper, chromium, and arsenic elements into the environment from CCA-treated wood removed and recycled from service, placed in landfills, and incinerated in commercial kilns has become an increasing concern due to their accumulation in forms leading to toxic effects on biotic life. As a result of public concern over CCA-treated waste wood, research on remediation of such waste has

increased during the past decade. Remediation of CCA-treated waste wood prior to recycling, reusing, incineration, and disposal by landfilling will decrease concerns about environmental pollution and the safety of workers involved in such facilities (Kartal and Clausen 2001b, Kartal 2002, 2003, Kartal and Kose 2003).

Previous studies on the chemical and biological remediation of CCA-treated wood showed that certain isolates of fungi and bacteria can readily remediate wood that has been treated with CCA. Clausen and Smith (1998), Clausen (2000), Clausen et al. (2000, 2001), Kartal and Clausen (2001a, b) also showed that the removal of copper, chromium, and arsenic from CCA-treated waste wood increased significantly during oxalic acid extraction. In these studies, oxalic acid has played an important role in partial solubilization of the insoluble metal compounds of CCA wood preservative fixed in the wood. Oxalic acid can be produced in a biotechnological process, because microorganisms such as fungi are capable to secrete oxalic acid at several concentrations into the culture broth. For instance, *Aspergillus niger*, a filamentous fungus, produces not only oxalic acid but also citric acid and gluconic acids (Cameselle et al. 1998). Santoro et al. (1999) showed that *A. niger* produced oxalic acid concentration of 37 kg/m^3 in the optimum substrate concentration. Pazouki et al. (2000) showed that maximum citric acid concentration of 12 kg/m^3 was obtained with *A. niger* using molasses.

The purpose of the present study was to determine the ability of *A. niger* for removing heavy metals from treated waste wood as solid medium instead of aqueous solutions. The bioremediation process consisted of two stages. In the first stage, *A. niger* was cultivated in carbohydrates media in order to produce large quantities of oxalic acid. In the second stage, the fermentation medium and biomass were used as a leaching agent in order to remove heavy metals from CCA-treated wood.

2 Materials and methods

2.1 Wood source

Scots pine (*Pinus sylvestris* L.) poles were obtained from a preservative treatment plant in Adana, Turkey. The poles were previously treated with chromated copper arsenate Type C wood preservative (CCA-C) solution using a full-cell process at a retention of 21 kg/m^3. Sapwood and heartwood portions of the treated poles were separated and the sapwood portions were then chipped with a commercial chipper to approximately 10–17 mm×0.20–0.35 mm×0.15–0.35 mm. The chips were screened and sorted to remove knots and over/under sized chips. The chips were conditioned at 23°C and 65% relative humidity (RH) for two weeks.

2.2 Analysis of preservative retention in the ground wood

The chips were ground to pass a US Standard 40-mesh screen (420 μm). The ground wood samples from the poles were then analyzed for copper, chromium, and arsenic content to determine the initial amounts of the elements in the samples using an ASOMA X-ray fluorescence analyzer (XRF) (ASOMA Instruments, Austin, TX, U.S.A.) according to American Wood Preservers' Association (AWPA) A9-99 test method (AWPA 1999).

2.3 Microorganism and cultivation

The mutant strain *Aspergillus niger* was obtained from The Scientific and Technical Research Council of Turkey (TUBITAK). Spores from an established culture (6–7 days old) incubated on potato/glucose agar (PGA) plates at 28°C were used for the preparation of inocula. The cultures were routinely (every 7–10 days) transferred onto fresh PGA plates by streaking. Before *A. niger* cultures were used for inoculation of liquid growth medium, the fungus was subjected to three transfers on PGA plates.

2.4 Biomass production

Fungal biomass was cultivated in a liquid medium in shaking flasks. The growth medium had the following composition (Camaselle et al. 1998): Sucrose 150 kg/m^3; NH_4NO_3 0.45 kg/m^3; KH_2PO_4 kg/m^3; $MgSO_4$ $7H_2O$ 0.3 kg/m^3; $FeSO_4$ $7H_2O$ 0.1 kg/m^3; $ZnSO_4$ $7H_2O$ 0.25 kg/m^3. The pH of the growth medium was adjusted to 6.0 before autoclaving. The medium was autoclaved for 20 min at 121°C. Spores and mycelium from the PGA spreadplate cultures were transferred to 500 ml Erlenmayer flasks containing 300 ml of growth medium. Cultivation of *A. niger* in the flasks inoculated with spores at a concentration of 10^6 spores/ml was carried out on a rotary shaker at 150 rpm. The culture temperature was maintained at 28°C.

2.5 pH adjustment

During the first two days of the fermentation, pH of the growth medium was maintained at the optimal values of citric acid production (pH: 2.0–3.0). The pH was then shifted after 48 h and adjusted daily to 6 by addition of 5 N NaOH and 2 N HCl. An additional nitrogen and phosphorus source was added into the flasks in order to enhance the oxalic acid production after 48 h fermentation. Nitrogen and phosphorus sources used were NH_4NO_3 (2.5 kg/m^3) and KH_2PO_4 (2.5 kg/m^3), respectively (Cameselle et al. 1998, Santoro et al. 1999).

2.6 Oxalic acid assay

The concentration of oxalic acid produced during the fermentation in the flasks was determined by High Performance Liquid Chromatography (HPLC) (Hewlett Packard 1090).

2.7 Bioremediation of CCA-C treated wood

For the bioremediation process, CCA-C-treated particles were autoclaved for 20 min at 121°C. The particles of 10 g were placed in the flasks containing 300 ml of growth medium incubated with *A. niger* for 15 days as described above. The flasks were agitated for 6 h, 1, 3, 5, 7, and 10 days at 150 rpm on a rotary shaker at 28°C. Uninoculated growth medium (UGM) and deionized (DI) water extraction served as control. Two replicates of 10 g particles were removed at each time interval. The contents in the flasks were filtered through Whatman #4 filter papers using a vacuum pump, and rinsed three times with 300 ml of DI water at 20°C. Bioremediated chips were collected on cheesecloth covered screens, oven-dried at 60°C for 24 h and conditioned at 23°C and 65% RH for two weeks.

Bioremediated chips were ground to pass a US Standard 40-mesh screen (420 μm) and then analyzed for remaining copper, chromium, and arsenic content using an ASOMA X-ray fluorescence analyzer (XRF) (ASOMA Instruments, Austin, TX, U.S.A.) according to the AWPA A9–99 test method (AWPA 1999). The percent reduction of copper, chromium, and arsenic in the sawdust samples was calculated based on the initial amount of elements in the samples.

3 Results and discussion

Oxalic acid production into the medium began quickly after the second day of the fermentation. The oxalic acid secretion was then increased as the fermentation continued with controlled pH in order to maximize oxalic acid production. After 11 days, the production rate was constant and the oxalic acid concentration in the medium reached 13.4 kg/m^3 at the end of the fermentation with a pH adjustment daily. The pH value plays an important role in the oxalic acid production with *Aspergillus niger* fermentation in sucrose medium. In addition to the pH effect, an enriched nitrogen and phosphorus medium is necessary in order to conduct the fermentation towards oxalic acid production (Camaselle et al. 1998). Camaselle et al. (1998) also showed that when the fermentation with *A. niger* was carried out only at a pH higher than 6, the main product was oxalic acid. Under controlled conditions, the oxalic acid production rate and productivity can be greatly increased with better pH control. In our study, the pH shifting technique (fermentation at acid pH in the first two days and then adjusting pH to 6) according to Camaselle et al. 1998 allowed to produce oxalic acid in the shaking flasks.

The results of the X-ray fluorescence spectroscopy analysis for CuO, CrO_3, and As_2O_5 following extraction with deionized (DI) water and uninoculated growth medium (UGM), as well as the fermentation of CCA-treated chips by *A. niger* are given in Table 1. The results are expressed as mg of each component retained per gram of treated wood following exposure condition. The values represent the average of triplicate samples. CCA-treated chips contained 12.1 mg/g CuO, 31.4 mg/g CrO_3, and 20.7 mg/g As_2O_5 before the bioremediation process. In the chips exposed to DI water and UGM extraction for 10 days, the As_2O_5 content remaining in the chips was 17.1 mg/g and 12.1 mg/g, respectively; however, *A. niger* fermentation caused significantly more As_2O_5 removal (0.6 mg/g).

The percentages of copper, chromium, and arsenic elements removed from treated chips following extraction with DI water and UGM, and *A. niger* fermentation are shown in Fig. 1 and Fig. 2, respectively. The total percentage of elements removed from chips with DI water extraction was considerably lower than of those with UGM extraction and *A. niger* fermentation. Extraction with DI water for 10 days removed about 20% copper, 13% chromium, and 18% arsenic from CCA-treated chips. The exposure of treated chips to an extraction with UGM for 10 days enhanced the removal of metals. This remediation process removed about 30%, 32%, and 42% of the initial concentrations of copper, chromium and arsenic, respectively in CCA-treated chips. These results suggested that the UGM containing mainly sucrose had a

Table 1 Amount of CCA components remaining in chips following exposure to deionized water, uninoculated growth medium and *A. niger* fermentation (mg/g)[a]
Tabelle 1 Menge an CCA-Komponenten, die in Spänen verblieben nach Behandlung mit Wachstumsmedium ohne und mit Pilzbeimpfung[a] deionisiertem Wasser ausgesetzt waren

Fermentation time (day)	Exposure conditions								
	Deionized water			Uninoculated growth medium			*A. niger* fermentation		
	CuO	CrO_3	As_2O_5	CuO	CrO_3	As_2O_5	CuO	CrO_3	As_2O_5
6 h	11.19	29.92	19.28	9.41	23.92	15.86	9.53	22.44	14.85
1	11.05	29.08	19.02	9.26	23.04	15.69	7.55	18.54	8.51
3	10.64	28.63	18.73	8.94	22.98	14.56	7.44	16.29	4.29
5	10.64	28.38	18.38	8.71	22.31	13.47	7.31	16.23	2.48
7	10.15	28.23	18.21	8.52	21.40	12.61	6.21	14.46	0.92
10	9.78	27.33	17.06	8.52	21.35	12.10	6.21	14.10	0.63

[a] Values represent the average of triplicate samples.CCA treated wood contained 12.14 mg/g CuO, 31.35 mg/g CrO_3, and 20.71 mg/g As_2O_5 before remediation

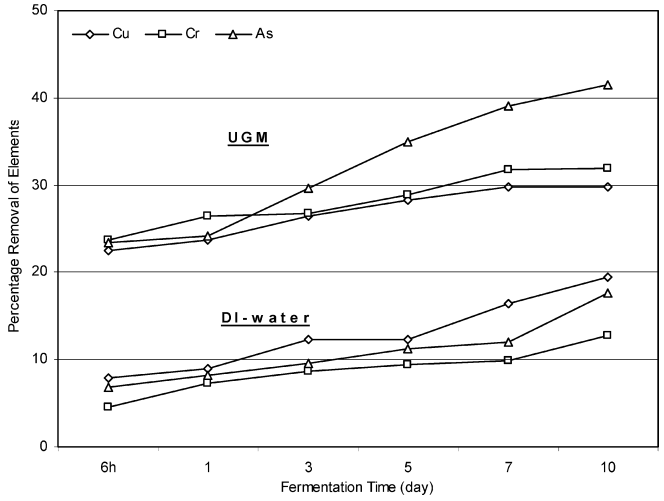

Fig. 1 Percentage removal of elements from CCA-treated wood by DI water extraction and UGM-extraction
Abb. 1 Prozentuale Entfernung von Elementen aus CCA-behandeltem Holz durch DI Wasserextraktion und UGM-Extraktion

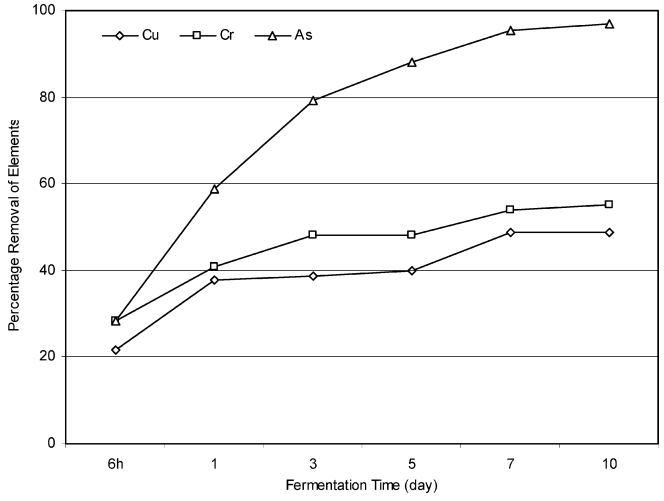

Fig. 2 Percentage removal of elements from CCA-treated wood by *A. niger* fermentation
Abb. 2 Prozentuale Entfernung von Elementen aus CCA-behandeltem Holz durch *A. niger* Fermentation

potential to absorb metals released from CCA-treated wood particles during extraction.

However, the leaching rate and total percentage of arsenic released from treated chips following the *A. niger* fermentation were considerably higher than the extractions with DI water and UGM. The total percentage of arsenic removed from CCA-treated chips by *A. niger* fermentation for 10 days was about 97%, whereas about 18% and 42% of arsenic were released from the chips with DI water and UGM extraction for 10 days, respectively. In other words, the percentage arsenic removed from the chips by *A. niger* fermentation was about 5.4 and 2.3 times greater than that from DI water and UGM-extracted chips, respectively. In addition, the *A. niger*

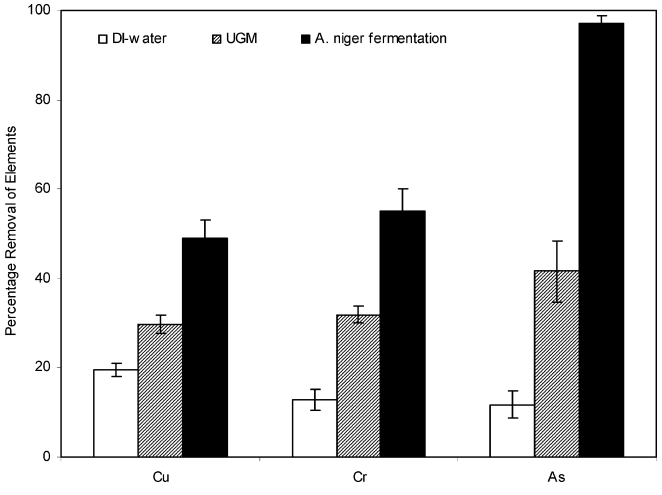

Fig. 3 Percentage of copper, chromium, and arsenic released following DI-water and UGM extraction, and *A. niger* fermentation of CCA-treated chips for 10 days
Abb. 3 Prozentsatz von freigesetztem Kupfer, Chrom und Arsen durch DI-Wasser und UGM-Extraktion sowie *A. niger* 10-tägige Fermentation von CCA-behandelten Spänen

fermentation for 10 days removed about 49% of the copper and 55% of the chromium from CCA-treated chips. Compared to UGM-extracted chips, the copper and chromium removal was about 1.6 and 1.7 times higher in the bioremediated chips by *A. niger*. Figure 3 shows the percentage of copper, chromium, and arsenic following DI-water and UGM extraction, and the *A. niger* fermentation of CCA-treated chips for 10 days.

Kartal and Clausen (2001a, b) showed that 0.8% oxalic acid extraction for 18 h removed 23% copper, 65% chromium, and 74% arsenic from CCA-treated wood particles. In addition, Clausen (2000) found that 1% oxalic acid extraction of CCA-treated wafers for 24 h resulted in about 25% copper, 45% chromium, and 90% arsenic removal. A study by Clausen et al. (2001) showed that 53% copper and 60% arsenic were removed from CCA-treated wood particles under the same extraction conditions. In studies by Kartal (2002) and Kartal and Kose (2003), 1% oxalic acid extraction for 24 h removed about 61% copper, 41% chromium, and 75% arsenic from CCA-treated wood. These results suggested that an oxalic acid extraction was relatively ineffective in the removal of chromium and copper from CCA-treated wood while a considerable amount of arsenic was removed due to the chelating ability of oxalic acid on this element. In our recent study, the first fermentation step was stopped at a final oxalic acid concentration of 13.4 kg/m^3 in the flasks. In the bioremediation process of the study as a second step, an arsenic removal of 97% was achieved due to oxalic acid produced in the flasks. On the other hand, a passive metal removal or biosorption of heavy metals on fungi may have occurred, because of the functional groups present on the fungal cell surface (Kapoor and Viraraghavan 1997). Although oxalic acid is effective in the removal of heavy metals from CCA-treated wood due

to its chelating and reducing capacity (Kartal and Clausen 2001b, Kartal 2002, Kartal and Kose 2003), biosorption of heavy metals from fermentation medium by fungus biomass can play an important role in the bioremediation of treated wood. Kapoor and Viraraghan (1997), Kapoor et al. (1999), and Goyal et al. (2002) also showed that *A. niger* has heavy metal biosorption sites in its structure and is capable of removing heavy metals from aqueous solutions. *A. niger's* ability to produce high amounts of oxalic acid during fermentation under controlled conditions based on pH and effectively removing metals from CCA-treated wood can be used for remediation as an alternative to chemical extraction.

4 Conclusions

Aspergillus niger is able to produce a quite high concentration of oxalic acid using sucrose as a carbon source. Oxalic acid, a chelating and reducing agent, is one of the strongest organic acids. Because it is readily oxidized, it is useful as a reducing agent for bleaching and ink removal. On the other hand, *A. niger* biomass has an ability to absorb heavy metals from aqueous solutions. This study evaluated the removal of copper, chromium, and arsenic from CCA-treated wood using the *A. niger* fermentation. Oxalic acid produced by the fungus during fermentation was used for the removal of metal elements via bioleaching. *A. niger* fermentation removed 97% of arsenic found in the CCA-treated wood. Compared to arsenic, less copper and chromium were removed by an *A. niger* fermentation. We also observed that uninoculated growth medium containing sucrose can be used as a biosorbent for the removal of heavy metals. The ability of *A. niger* to remove heavy metals from CCA-treated wood can be considered as a potential alternative to oxalic acid extraction for the acid extraction of treated wood. However, further research on extraction via dual remediation process using different acids or microorganisms is needed to improve the removal efficiency of other CCA components.

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