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Enhanced Extraction of Heavy Metals in the Two-Step Process with the Mixed Culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

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Abstract

For biological extraction of heavy metals from chromated copper arsenate (CCA) treated wood, different bacteria were investigated. The extraction rates of heavy metals using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were highest. The chemical extraction rates were depended on the amounts of pyruvic acid and lactic acid. Especially, the extraction rates using mixed pyruvic acid and lactic acid were increased compared to those of sole one. They were also enhanced in the mixed culture of *L. bulgaricus* and *S. thermophilus*. To improve the extraction of CCA, a two-step processing procedure with the mixed culture of *L. bulgaricus* and *S. thermophilus* was conducted. A maximum of 93% of copper, 86.5% of chromium, and 97.8% of arsenic were extracted after 4 days. These results suggest that a two-step process with the mixed culture of *L. bulgaricus* and *S. thermophilus* is most effective to extract heavy metals from CCA treated wood.

Keywords: copper; chromium; arsenic; lactic acid bacteria, two-step process, mixed culture

1. Introduction

The recycling method for wood waste treated with chromated copper arsenate (CCA) has been a subject of concern of the architectural use of wood. The disposal of CCA-treated wood has becoming a serious issue in many countries, due to increasing levels of contamination from chromium, copper and arsenic. Thus, the need to promote the recycling of the CCA treated wood waste after appropriate pre-treatment has been clearly described in the Construction Materials Recycling Act from the Japanese Ministry of Land, Infrastructure and Transport (JIS K-1570, 1998). Although a sophisticated method using biodegradable chelating agents (s, s-ethylenediaminedisuccinic acid and nitrilotriacetic acid) has recently been reported (Ko et al., 2010), biological extraction is still a useful and cost-effective approach for the removal of heavy metals from CCA-treated wood. In particular, the microbial production of organic acids by fungi is of growing interest in the treatment of pollution and remediation of treated wood (Kartal et al., 2004). However, it can be difficult to maintain appropriate humidity levels for fungal growth and the contamination may cause inhibition of the fungi proliferation. In addition, the fungi may cause transformations of the wood constituents (lignin, cellulose, C, and N). Therefore, this research conducted to evaluate the ability of selected lactic acid bacteria such as *Lactobacillus bulgaricus*, *L. acidophilus*, *L. plantarum*, and *S. thermophilus* to remove the metal components from CCA-treated wood. In addition, a two-step process with the mixed culture of two lactic acid bacteria (*L. bulgaricus* and *S. thermophilus*) was investigated for effective extraction of heavy metals from treated wood.

2. Materials and Methods

2.1. Preparation of CCA-treated wood

The experiment used Southern pine lumber treated with Type III CCA reagent (JIS K-1570, 1998). The outer layer of the CCA-treated wood (30 mm depth) was removed, broken into chips and dried at 60°C for two weeks

to achieve complete fixation of the CCA reagents. The chips were then milled to a powder with a particle size below 40-mesh (420 μm). Finally, a post-heat treatment of drying for one week was also conducted. Southern pine lumber used in this study is composed of 31.2% lignin, 15.8% hemicellulose, 50.4% cellulose, and extractives and ash. The initial metal content in the CCA-treated wood was 6.41 mg/g of Cu, 4.13 mg/g of Cr, and 3.26 mg/g of As.

2.2. Strain and media

The following four lactic acid bacteria (*L. bulgaricus* NBRC13953, *L. acidophilus* NBRC13951, *L. plantarum* NBRC15891 and *S. thermophilus* NBRC13957) were obtained from NITE Biological Resource Center in Japan. To obtain enough cell volume from *L. acidophilus* and *L. bulgaricus*, preculture was performed using media including 5 g polypeptone, 5 g yeast extract, 5 g glucose, 2 g lactose, 0.5 g Tween 80, and 1 g magnesium sulfate heptahydrate in 1 l of deionization water at 37 ˚C. For *S. Thermophilus*, it was performed using media including 100 g skim milk, 100 ml tomato juice, and 5 g yeast extract in 1 l of deionization water at 37 ˚C. In the case of *L. Plantarum,* it was performed using media including 10 g polypeptone, 10 g malt extract, 5 g yeast extract, 20 g glucose, 1 g Tween 80, 2 g dipotassium hydrogenphosphate, 5 g sodium acetate, 2 g ammonium citrate dibasic, 0.2 g magnesium sulfate heptahydrate, and 0.05 g manganese sulfate monohydrate in 1 l of deionization water at 30 ˚C. CCA extraction experiments were established using 300 ml glass stoppered Erlenmeyer flasks containing 100 ml of growth medium and 3 g of milled chips. The growth medium had the following composition in 1 l of deionization water: 3.0 g peptone; 15 g malt extracts; and 40 g glucose. The preculture solution (O.D.600: 0.02–0.03) was inoculated in 100 ml of the growth medium and incubated for 4 days at 120 rpm at 28°C. A two-step processing culture using *L. bulgaricus* and *S. thermophilus* was performed for the extraction of CCA.

Each preculture of the two lactic acid bacteria was inoculated in the reactive liquid as described above and the cultivation was conducted for four days with CCA treated chips for the first processing procedure. The milled chips were then separated by centrifugation and reacted for a further four days in a new liquid medium also containing *L. bulgaricus* and *S. thermophilus*.

2.3 Analyses

The oven-dried milled chips (3 g) were digested using the peroxide-nitric acid method (Illman and Highley, 1996) and analyzed for copper, chromium, and arsenic content using inductively coupled plasma emission spectrometry based on the American Wood Protection Association standard A21-00 (AWPA, 2005). Lactic acid and pyruvic acid were analyzed using an LC-VP HPLC system (Shimadzu, Kyoto, Japan).

3. Results and discussion

3.1. Extraction of CCA from CCA treated wood by lactic acid bacteria

Among various lactic acid bacteria, when *L. bulgaricus* NBRC13953 or *S. thermophilus* NBRC13957 was used, the extraction rates of heavy metals from CCA preservative-treated wood waste were highest (Fig. 1). On the other hand, in the case of other lactic acid bacteria, they were decreased compared to those of *L. bulgaricus* NBRC13953 or *S. thermophilus* NBRC13957. When fungi have been used, relatively long incubation periods (10 days or more) have been reported for CCA extraction (Kartal et al., 2004). However, in this study the lactic acid bacteria were able to extract all three metal ions from the CCA treated wood within four days of culture. These results indicate that *L. bulgaricus* NBRC13953 and *S. thermophilus* NBRC13957 could be useful for eliminating of heavy metals from CCA-treated wood wastes existing in landfill sites. In general, chromium plays a role in the reactions which fix the preservative into the timber, while copper and arsenic are important for the preservative efficiency. The CCA is fixed to the lignin in wood by reducing Cr^{6+} to Cr^{3+} (Lebow and Kartal,

1999). Therefore, the chromium is the strongest binding force to the wood out of the CCA elements. Clausen reported that the strong binding of chromium to the lignin inhibited the extraction of chromium by *B. licheniformis* CC01 (Clausen, 2000). Thus, the poor extraction of chromium may be explained by the different binding forces between the CCA elements and the wood constituents. Lactic acid bacteria produce not only lactic acid but also different concentrations of pyruvic acid from each bacterial strain and different levels of affinity will be formed between these organic acids and the various heavy metals. As shown in Table 1, the concentrations of pyruvic acid and lactic acid produced by *L. bulgaricus* and *S. thermophilus* were higher than those produced by *L. plantarum* or *L. acidophilus*. Thus, the differences in the extraction rate of each heavy metal may be due to the differences in the amounts of organic acid formed.

3.2. Effect of organic acids on the removal rate of CCA

As shown in Table 2. Among those of CCA elements, arsenic was most effectively extracted from the CCA-treated wood. When pyruvic acid or lactic acid concentration was increased, the removal of CCA-elements was increased. Also, when lactic acid was used, the removal of CCA-elements was slightly higher than that of pyruvic acid. Especially, when pyruvic acid concentration was increased from 0.5 to 1.0 g/l, the concentrations of Cu, Cr, and As were increased from 10, 8, and 15% to 35, 18, and 33%. In the case of lactic acid, they were increased from 15, 12, and 25% to 38, 22, and 40%. In addition, the removal rates were increased by 45, 25, and 48% when pyruvic acid (1.0 g/l) and lactic acid (1.0 g/l) are used in combination. These results indicate that the removal rates of CCA-elements from treated wood were strongly affected by pyruvic acid and lactic acid concentration and their combination. However, these extraction rates were lower than those of microbial extraction shown in Fig. 1. The differences in the extraction rate between chemical extraction and microbial extraction may be due to enzymatic reactions. Kartal and Imamura (2003) reported that enzymes such as

hemicellulolytic degrading and ligninolytic enzymes secreted by white-rot fungi play a role in removal or degradation of heavy metals.

3.3. Extraction of CCA in the mixed culture

pH was slowly decreased from 6.4 after culture start to 4.0 after 2 days of culture in the mixed culture (Fig. 2). In the case of extraction rates of CCA, when the mixed culture time was increased from zero to 3 days, the concentrations of Cu, Cr, and As were increased to 65, 55, and 72%, respectively. The CCA extraction rates are most likely improved by the pH decrease as organic acids are produced. When the mixed culture of *L. bulgaricus* and *S. thermophilus* was carried out, the extraction rate of arsenic was improved from 66% to 73%, and the extraction rates of copper and chromium were also improved from 55 to 65% and from 45 to 55%, respectively (data not shown). The results indicate that a mixed culture of *L. bulgaricus* and *S. thermophilus* is the most suitable culture for the effective CCA extraction.

3.4. Extraction of CCA in the two-step process with the mixed culture of L. bulgaricus and S. thermophilus

To improve the extraction of CCA, a two-step processing procedure using *L. bulgaricus* and *S. thermophilus* for 4 days was conducted and results are shown in Fig. 3. In the first step, a maximum of 72% of arsenic, 65% of copper, and 55% of chromium was extracted. In the second step, 92% of arsenic, 80% of copper, and 70% of chromium that remained after the first step was also extracted. Consequentially, the overall extraction rates were 97.8% for arsenic with an initial concentration of 32.3 mg/l, 93% for copper with an initial concentration of 63.5 mg/l, and 86.5% for chromium with an initial concentration of 40.9 mg/l using the two-step processing procedure. Ultimately, the CCA elements were almost completely eliminated from the CCA treated wood chips. After solid/liquid separation, the extracted CCA elements can be easily removed from the liquid phase through precipitation or complexation with sulfide or sulfide-containing materials as well as adsorption to Fe (II)-based

solids (Roberts et al., 2004). The significant improvement in the extraction efficiency using the two-step processing procedure with the mixed culture of *L. bulgaricus and S. thermophilus* seems to be the result of the changing metabolic environment. That is, during the second step, the proliferation and metabolism of the lactic acid bacteria which adhered to the wood during the first step may have been activated. In the control experiment, where the *L. bulgaricus and S. thermophilus* were absent during the second step, only 7% of arsenic, 5% of copper, and 7% of chromium remaining after the first step were extracted (Fig. 2). These results suggested that the growth medium, containing mainly glucose, had the potential to absorb metals released from CCA-treated wood particles during extraction. In the Construction Materials Recycling Act by the Japanese Ministry of Land, Infrastructure and Transport, the maximum values for CCA elements in reusable wood chips have yet to be determined. However, in the German Closed Substance Cycle and Waste Management Act, the maximum values for CCA elements in the manufacture of derived timber are: 0.002 mg/g dry mass for arsenic; 0.02 mg/ g dry mass for copper; and 0.03 mg/g dry mass for chromium (Peek, 1986). Using the two-step process, 97.8% of arsenic, 93% of copper, and 86.5% of chromium were extracted. On the other hand, we examined to eliminate CCA elements from a CCA-treated wood waste from a landfill site in Okinawa, Japan. The CCA-treated wood waste was from more than 30 years old house in Okinawa, Japan. The initial CCA concentrations of the CCA-treated wood waste were as follows: 0.27 mg arsenic ion/g dry CCA-treated wood, 0.48 mg copper ion/g dry CCA-treated wood, and 0.89 mg chromium ion/g dry CCA-treated wood. The experiment was performed by the two-step processing procedure using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. As a result, the concentrations of remaining CCA elements in the wood waste after the two-step processing procedure became below the maximum values indicated in the German Closed Substance Cycle and Waste Management Act (Peek, 1986).

4. Conclusion

The CCA extraction efficiency was improved by mixed culture of two bacteria with the CCA-treated wood being almost completely extracted by a serial proceeding procedure. This result indicates that the two-step process with two bacteria could be useful for eliminating of CCA from CCA-treated wood wastes existing in landfill sites. However, the fundamental information obtained from this study is insufficient for the development of an efficient process for CCA extraction. To meet the requirements of large-scale CCA extraction, therefore, further studies including configuration of a suitable process and optimization of culture conditions for CCA extraction are needed.

Acknowledgements

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References

American Wood-Preservers' Association A21-00. 2005. Standard method for the analysis of treated wood and treating solutions by inductively coupled plasma emission spectrometry, Book of standards. AWPA, Granbury, TX, pp. 210-213.

Clausen, C.A. 2000. CCA removal from treated wood using a dual remediation process. Waste Manage. Res. 18, 485-488.

Illman, B.L., Highley, T.L., 1996. Fungal degradation of wood treated with metal-based preservatives; 27th Annual Meeting of the International Research Group on Wood Preservation, Legosier, Guadeloupe, French West Indies, IRG/WP 96-10163.

Japanese Industrial Standard (JIS K-1570: Wood Preservatives), 1998.

Kartal, S.N., Imamura, Y., 2003. Chemical and biological remediation of CCA-treated waste wood. Wood Research 90, 111-115.

Kartal, S.N., Kakitani, T., Imamura, Y., 2004. Bioremediation of CCA-C treated wood by *Aspergillus niger* fermentation. Holz Roh Werkst. 62, 64-68.

Ko, C.H., Chen, P.J., Chen, S.H., Chang, F.C., Lin, F.C., Chen, K.K., 2010. Extraction of chromium, copper, and arsenic from CCA-treated wood using biodegradable chelating agents. Bioresour. Technol. 101, 1528-1531.

Lebow, S.T., Kartal, S.N., 1999. Fixation effects on the release of copper, chromium, and arsenic from CCA-C treated marine piles. In: Proceedings of 95th Annual Meeting of the American Wood-Preservers' Association, Ft. Lauderdale, Florida, USA, pp. 168-174.

Peek, R.D., 1986. EU directives and national regulations for the recycling and disposal of waste wood. In: Proceedings, Environmental Impacts of Preservative-treated Wood Conference, Orlando, Florida, USA, pp. 210-226.

Roberts, L.C., Hug, S.J., Ruettimann, T., Billah, M.M., Khan, A.W., Rahman, M. T., 2004. Arsenic removal with iron (II) and iron (III) in waters with high silicate and phosphate concentrations. Environ. Sci. Technol. 38, 307-315.

Figure legends

Fig. 1. Results of extraction of CCA from CCA treated wood with lactic acid bacteria. The growth medium comprised: 3.0 g/l peptone; 15 g/l malt extracts; and 40 g/l glucose. The data are mean±S.E. (*n*=3). The initial CCA content in 3 g of CCA-treated wood was 19.23 mg/l for Cu, 12.39 mg/l for Cr, and 9.78 mg/l for As.

Fig. 2. Effect of mixed culture on extraction rate of CCA and pH.

Growth medium is the same as the first experiment. Data shown are mean±S.E. (*n*=3).

Fig. 3. CCA extraction rates from the two-step processing procedure using *L. bulgaricus* and *S. thermophilus*.

The growth medium was the same as the first experiment. The data are mean±S.E. (*n*=3).

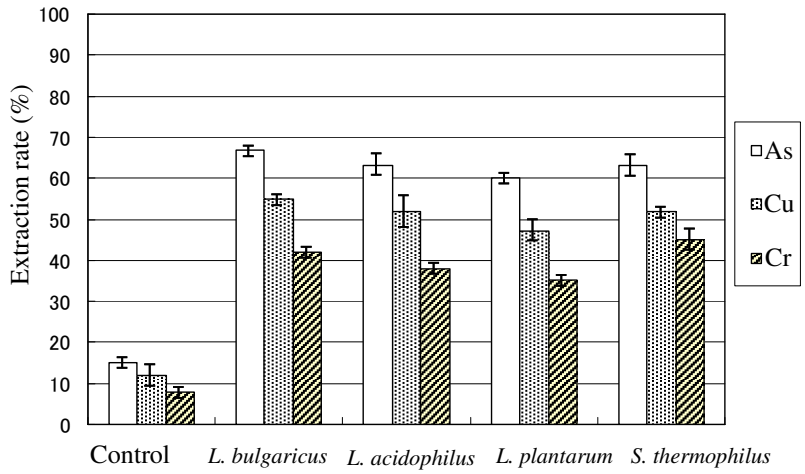

Fig. 1

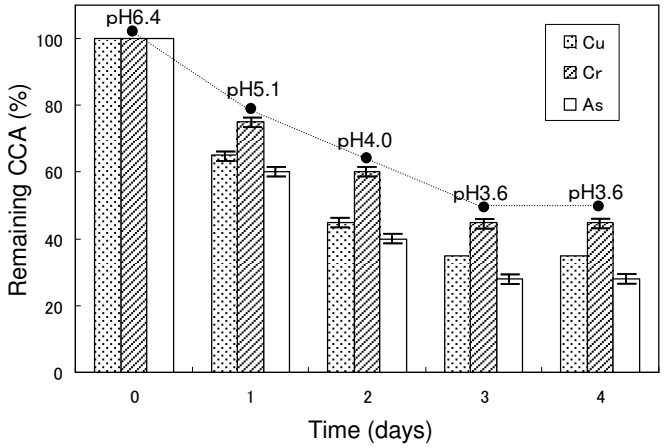

Fig. 2

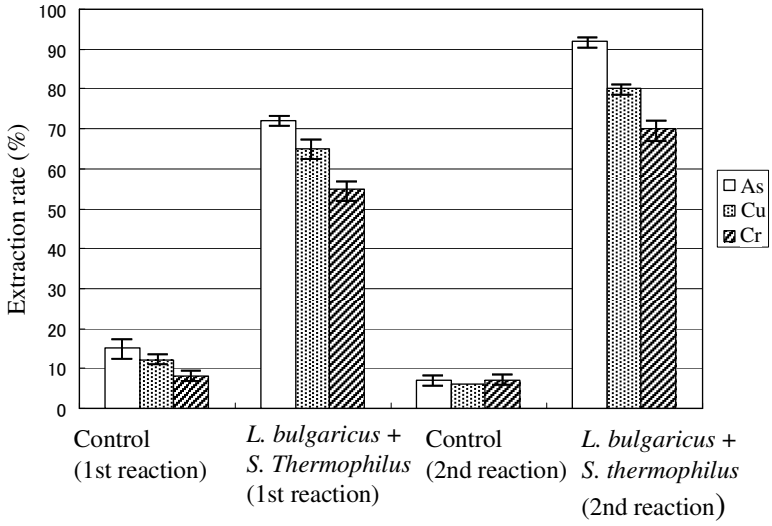

Figure 3

Table legends

Table 1. Comparison of lactic acid bacteria on the concentrations of pyruvic and lactic acid.

Table 2. Effect of organic acids on the removal of CCA-elements from treated wood.

Table 1.

Lactic acid bacteria	Pyruvic acid (g/l)[1)]	Lactic acid (g/l)[1)]
L. acidophilus	1.1±0.03	1.0±0.02
L. bulgaricus	1.3±0.04	1.25±0.01
S. thermophilus	1.2±0.05	1.05±0.02
L. plantarum	0.9±0.02	0.85±0.03

4. Maximum concentration during the culture.
5. Growth medium is the same as the first experiment.
6. Experiments performed in duplicate.

Table 2.

Organic acids	Removal rate (%) of elements		
	Cu	Cr	As
Pyruvic acid (0.5 g/l)	10±0.4	8±0.2	15±0.3
Pyruvic acid (1.0 g/l)	35±0.3	18±0.1	33±0.4
Lactic acid (0.5 g/l)	15±0.5	12±0.2	25±0.5
Lactic acid (1.0 g/l)	38±0.6	22±0.1	40±0.5
Pyruvic acid (1.0 g/l) + Lactic acid (1.0 g/l)	45±0.3	25±0.3	48±0.6

Experiments performed in duplicate.