J Wood Sci (2009) 55:121–125
DOI 10.1007/s10086-008-1010-4

ORIGINAL ARTICLE

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Development of acid soil conditioning agent from lignin by ozone treatment II

Received: April 21, 2008 / Accepted: December 3, 2008 / Published online: January 10, 2009

Abstract Kraft lignins (KLs) modified with ozone were saponified with 0.1 M NaOH at room temperature overnight and the abilities of the modified KLs in reducing aluminum toxicity in acidic soil were assayed by planting experiments with radish (*Raphanus sativa* var. *radicula* Pers.). The abilities of ozone-treated KLs in reducing aluminum toxicity and accelerating root elongation increased markedly by saponification. Furthermore, KL treated with 0.1 M NaOH at room temperature overnight also effectively reduced aluminum toxicity and accelerated root elongation. These results suggest that KL contains chemical structures that contribute toward these favorable effects. Saponified ozone-treated KLs reduced aluminum toxicity at lower dosages than the alkaline-treated KL.

Key words Aluminum toxicity · Ozone · Lignin · Soil-conditioning agent · Acid soil

Introduction

Aluminum is one of the most abundant elements in soil and it comprises about 7% of the earth's crust. Aluminum usually exists in insoluble form at neutral pH, but phytotoxic aluminum ion is eluted by acidification of the soil.[1] Plant roots suffer serious damage from eluted aluminum ion.[1–3] It is known that elongation of plant roots is inhibited with Al^{3+} at concentrations as low as the micromoles-per-liter level within 1 h.[1]

It has been reported that lignins modified with alkaline oxygen or radical sulfonation reduced aluminum toxicity.[4–9] We reported in our previous article[10] that kraft lignin (KL) modified with ozone reduced aluminum toxicity. Oxalic acid, which is known to be an excellent aluminum detoxifier,[9,11–19] is formed by ozone treatment of KL and lignin model compounds.[10,20–22]

Katsumata et al.[4,6,7] suggested that the reduction of aluminum toxicity with lignin that had been modified by alkaline oxygen treatment was due to formation of a complex with aluminum. They confirmed the formation of complexes between aluminum and KL modified with alkaline oxygen by potentiometric titration and ^{27}Al nuclear magnetic resonance (NMR) spectroscopy.[7] It was reported that carboxyl groups play an important role for formation of complexes with aluminum[23–25] and reducing aluminum toxicity.[16,23] Tam and McColl[24] examined the aluminum binding affinity of organic ligands at pH 4.5 by an ion-pairing chromatography technique. They reported that carboxyl groups are one of the important functional groups for binding with aluminum.[24] Schnitzer and Skinner[25] reported that selective blocking of carboxyl groups significantly reduced metal retention capacity of soil organic matter. Hue et al.[16] reported that some low molecular weight organic compounds could be excellent Al detoxifiers if they have carboxyl and hydroxyl groups that are favorably positioned for the formation of stable five-membered or six-membered chelate ring structures with Al.

It was reported that methyl oxalate[20] and muconic acid derivatives including methyl ester and lactones[26–29] were formed by ozone treatment of lignin model compounds. Kaneko et al.[30] suggested the presence of ester bonds in ozone-treated thiolignin based on the fact that its molecular weight was decreased with saponification because of transformation of ether linkages to ester linkages during ozone treatment.[20,26,27,29,31] Kratzl et al.[20] indicated the presence of ester bonds in ozone-treated lignins by comparison of methoxyl and carboxyl contents before and after saponification.

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This report was presented in part at the 57th Annual Meeting of the Japan Wood Research Society, Hiroshima, Japan, August 2007, and at the 52nd Lignin Symposium, Utsunomiya, Japan, November 2007

We also confirmed increases of ester or carboxyl groups in KL by infrared (IR) spectroscopy with progress of the ozone treatment.[10] It was suggested that the ability of ozone-treated KL to reduce aluminum toxicity is improved due to cleavage of ester bonds by saponification. The water solubility of ozone-treated KLs is also expected to be improved by saponification of ester groups. The activity of saponified ozone-treated KLs on reduction of aluminum toxicity is discussed in this report.

Experimental

Modification of KL with ozone

Crude softwood KL from Shimada Mill of Tokai Pulp and Paper (Shizuoka, Japan) was purified in a method using aqueous dioxane (95%, v/v)/diethyl ether.[10,32] The crude softwood KL (20 g) was dissolved in 200 ml of dioxane–water (95:5, v/v) and insoluble material was removed by filtration. The filtrate was added dropwise to 1800 ml of diethyl ether with stirring. The precipitate was collected by filtration and washed with diethyl ether. The collected precipitate was dried successively in air overnight and then in vacuo at room temperature overnight. The yield of purified KL was 72%.

Modification of KL with ozone was reported in detail in the previous article.[10] The purified softwood KL (1 g) was suspended in 60 ml of a solvent mixture consisting of acetic acid/water/methanol (16:3:1, v/v/v) and was oxidized by bubbling with oxygen containing ozone (ca. 3.7%, w/w) at a rate of 0.5 l min^{-1} using an ED-OG-R3Lt ozone generator (Ecodesign, Saitama, Japan) for 0.5, 1.0, 2.0, and 4.0 h at 0°C with stirring. The reaction mixture was bubbled with oxygen for a further 10 min and then evaporated to dryness. The lignins modified with ozone for 0.5, 1.0, 2.0, and 4.0 h were labeled as 0.5-O_3, 1.0-O_3, 2.0-O_3, and 4.0-O_3, respectively. The ozone consumptions of KL measured by iodometric titration were 0.6, 1.0, 1.8, and 3.9 moles per monomeric (C6-C3) unit of KL for 0.5, 1.0, 2.0, and 4.0 h of ozone treatment, respectively.[10] A molecular weight of 180 was assumed for the C6-C3 unit.

Alkaline treatment of KL and ozone-treated KLs

KL or ozone-treated KL (400 mg each) was dissolved in 0.1 M NaOH solution (100 ml) and held at room temperature overnight. The solution was applied to a cation-exchange column (H^+ form, 1.0 eq l^{-1}, C-112, Kurita Water Industry, Tokyo Japan). After neutralization of the eluates with diluted NaOH, they were concentrated in vacuo and freeze-dried. The yield of the product lay within the range 95%–118% based on the starting materials. The products from KL, 0.5-O_3, 1.0-O_3, 2.0-O_3, and 4.0-O_3 were labeled as KLA, 0.5-O_3A, 1.0-O_3A, 2.0-O_3A, and 4.0-O_3A, respectively. After the alkaline and ion exchange treatments of 0.5-O_3, part of the eluate was concentrated and freeze-dried without neutralization. The yield of the product was 90% on 0.5-O_3 and the product was labeled as 0.5-O_3A-I. The products were dissolved in the nutrient solution prepared for the planting experiment described later, except for 0.5-O_3A-I.

Determination of oxalic acid in ozone-treated KLs

The ozone-treated KL (40 mg) was saponified in 0.1 M NaOH solution (10 ml) and sodium ion was removed as described above. The eluates after passing an ion exchange column were evaporated without neutralization, and then freeze-dried. The products were used for determination of oxalic acid content.

Oxalic acid content of KL and ozone-treated KLs before and after saponification were determined by gas chromatography (GC) after trimethylsilyl derivatization as described in our previous article.[10] Oxalic acid was a major peak on gas chromatograms of all modified KLs. Glycolic acid, malonic acid, glyoxylic acid, succinic acid, threonolactone, tartronic acid, erythronolactone, and malic acid were detected as minor peaks on the gas chromatograms.

Determination of methoxyl content in KL and ozone-treated KLs

Ozone-treated KL (40 mg) was saponified in 0.1 M NaOH solution (10 ml) as described above. The solution was neutralized with diluted HCl and evaporated to dryness. The products were used for methoxyl content determination.

Methoxyl contents of KL and ozone-treated KLs before and after saponification were determined according to the method of Goto et al.,[32] except for the use of *n*-propyl iodide as internal standard instead of ethyl iodide. The presence of sodium chloride did not disturb the methoxyl content determination in this method.

Infrared spectroscopy analysis

Fourier transform-infrared (FT-IR) spectra of KL and modified KLs were recorded on an FT/IR-410 spectrometer (Jasco, Tokyo, Japan) using a KBr disk containing 1% finely ground sample.

Planting experiment

The method for the planting experiment was reported in detail in our previous article.[10] Seeds of radish (*Raphanus sativa* var. *radicula* Pers.) were sterilized in 70% ethanol aqueous solution for 30 s and then in 1% sodium hypochlorite solution for 10 min. Ten young seedlings were transplanted on a nylon mesh holder that was floating on 700 ml of the nutrient solution. An aqueous solution of 1.2 mM $CaCl_2$ was used as nutrient solution. The pH of the nutrient solution was adjusted to pH 4.8 with diluted HCl and/or NaOH before the planting experiment. The planting experiment was carried out under the absence and presence of

$AlCl_3$ (37.5 μM) and lignin samples (5–250 mg l^{-1}) at 23.5°C with continuous air bubbling. The pH of the nutrient solution was adjusted to 4.8 with diluted HCl and/or NaOH twice a day during each planting experiment. The primary root lengths were measured with a ruler after a 3-day growth period.[12] Abnormal values were removed using Dixon's Q method (n = 10).[33] The results were evaluated statistically by t-test for assessment of significance.[33]

Results and discussion

Oxalic acid and methoxyl group content

It has been reported that ester bonds are formed by ozone treatment of lignin[30,31] and lignin model compounds.[20,26–29] We confirmed the increase in the number of ester or carboxyl groups in KL by IR spectroscopy with the progress of ozone treatment.[10] It was expected that the ability of ozone-treated KLs to reduce aluminum toxicity would be improved due to cleavage of ester bonds by saponification, because carboxyl groups are one of the important functional groups for reduction of aluminum toxicity.[16,23] The content of oxalic acid and methoxyl group in ozone-treated KLs were determined before and after saponification.

It was reported in our previous article[10] that the oxalic acid content increased in KL with the progress of ozone treatment. It can be expected that more oxalic acid will be released by saponification of ozone-treated KL if oxalic acid is attached through an ester group (Fig. 1). In order to confirm this, the oxalic acid content of ozone-treated KLs before and after saponification was examined (Fig. 2). The oxalic acid content of ozone-treated KLs increased by saponification, indicating that part of the oxalic acid in ozone-treated KLs was attached to the lignin macromolecule by ester bonds.

Some of the methoxyl groups are expected to be attached to the ozone-treated KLs through ester bonds (Fig. 1), because formation of ester bonds has been reported by ozone treatment of lignin[30,31] and lignin model compounds.[20,26–29] Therefore, the content of methoxyl groups in ozone-treated KLs was determined by hydriodic acid treatment before and after saponification.[32,34] The results are shown in Fig. 3. The methoxyl content per monomeric (C6-C3) unit of KL, for which the unit molecular weight was assumed as 180, was calculated based on the amount of methyl iodide formed from each sample. As expected, the content of methoxyl group in each sample decreased after saponification, showing that the methyl ester in ozone-treated KLs was cleaved by saponification and an appreciable number of carboxyl groups were formed.

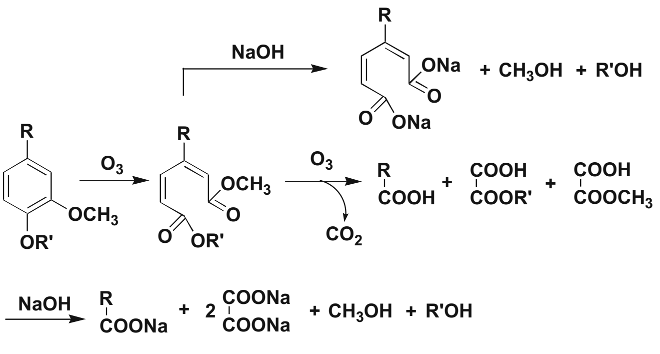

Fig. 1. Oxidative degradation of aromatic ring with ozone followed by saponification

Effect on root elongation

Original KL and ozone-treated KLs were subjected to alkaline treatment. The alkaline solutions were applied to a

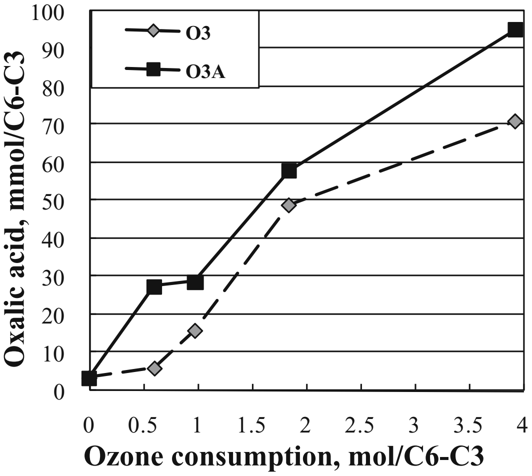

Fig. 2. Oxalic acid content of ozone-treated kraft lignins (KLs) before and after saponification. O_3, Ozone-treated KLs before saponification.[10] O_3A, Ozone-treated KLs after saponification. A molecular weight of 180 was used for a C6-C3 unit

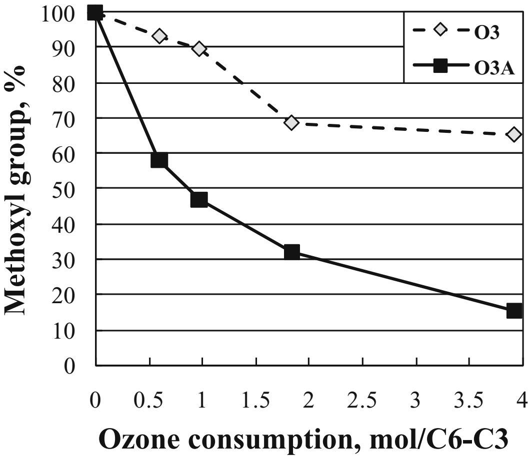

Fig. 3. Changes in methoxyl content of ozone-treated KLs before and after saponification. Changes in methoxyl group content were based on the methoxyl content of KL (0.63/C6-C3). Data were calculated based on a molecular weight of 180 for the C6-C3 unit

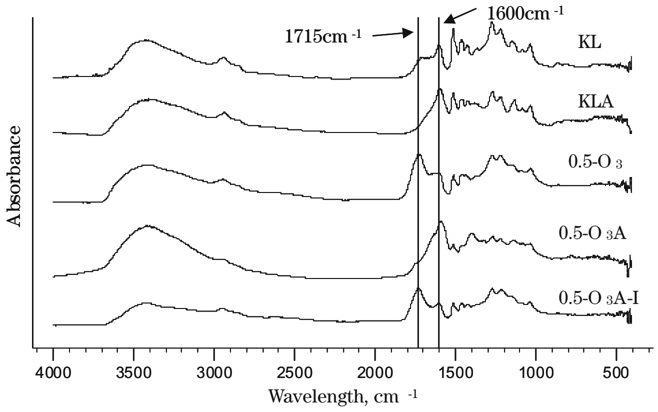

Fig. 4. Infrared (IR) spectra of KL, alkaline-treated KL (*KLA*), lignin treated with ozone for 30 min (*0.5-O_3*), alkaline-treated 0.5-O_3 (*0.5-O_3A*), and alkaline-treated 0.5-O_3 without neutralization after ion-exchange (*0.5-O_3A-I*)

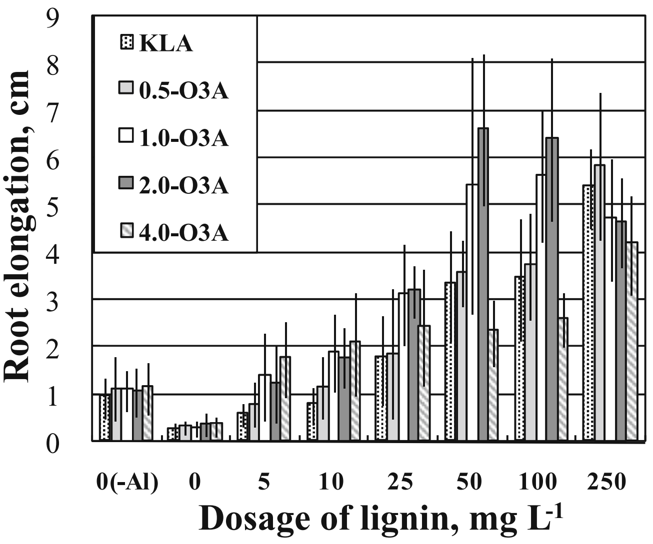

Fig. 5. Effect of saponified ozone-treated KLs on root elongation of radish (*Raphanus sativa* var. *radicula* Pers.) in hydroponic solution containing 1.2 mM $CaCl_2$ and 37.5 μM $AlCl_3$ at pH 4.8. *0(-Al)*, Hydroponic solution without aluminum. Growth period was 3 days ($n = 10$); *error bars* represent ± standard deviation

cation-exchange column. The eluates, for which the pH values were within the range 2.1–3.2, were neutralized with diluted NaOH and evaporated to dryness. The yields of the products were 95%–118% based on the starting materials. The products obtained after alkaline treatment become completely soluble in the nutrient solution used for the planting experiment. IR spectra of 0.5-O_3 and 0.5-O_3A are shown in Fig. 4. The band around 1710–1730 cm^{-1} for 0.5-O_3 was shifted to the region at 1600 cm^{-1} in 0.5-O_3A, indicating the conversion of ester or carboxyl groups to its sodium salt. The same behavior was also observed in the IR spectra of 1.0-O_3A, 2.0-O_3A, and 4.0-O_3A. These spectra showed that ester bonds hardly remained in saponified ozone-treated KLs.

We reported in our previous article[10] that the KLs that consumed 1.0, 1.8, and 3.9 moles of ozone per C6-C3 unit reduced aluminum toxicity, and those that consumed 1.8 and 3.9 moles of ozone per C6-C3 unit accelerated root elongation. The recovery of root elongation back to the level of the condition without aluminum was defined as reduction of aluminum toxicity, and promotion above the level of the condition without aluminum was defined as acceleration of root elongation.

Figure 5 shows the effect of saponified ozone-treated KLs on root elongation in the planting experiment. Saponification improved the ability of ozone-treated KLs (0.5-O_3, 1.0-O_3, and 2.0-O_3) to reduce aluminum toxicity and to accelerate root elongation (Fig. 5). These abilities of saponified ozone-treated KLs appeared at lower dosages than for the originals (see Fig. 5 in previous article[10]). Saponified 0.5-O_3 also reduced aluminum toxicity and accelerated root elongation, which did not show any effects before saponification.[10] However, the ability of 4.0-O_3 to accelerate root elongation was nearly the same before and after saponification. These results could be explained by the fact that the ozone-treated KLs became water soluble on saponification, whereas 4.0-O_3 was water soluble even before saponification. It can be considered that water solubility is an important factor for acceleration of root elongation.

Alkaline-treated KL (KLA) also reduced aluminum toxicity and accelerated root elongation effectively, although original KL did not show any effects.[10] This fact suggests that KL itself potentially has some chemical structures that are effective in reducing aluminum toxicity and accelerating root elongation, although the abilities were lower than those of saponified ozone-treated KLs as shown in Fig. 5.

KLA was soluble in water, but the original KL was not water soluble. A preliminary experiment showed that KLA was soluble in water even at the concentration of 1 g l^{-1}. The IR spectra of KL and KLA were very similar, but the band around 1710–1730 cm^{-1} in the KL spectrum shifted to 1600 cm^{-1} in the KLA spectrum (Fig. 4) indicating that the conversion of free carboxyl group to its sodium salt in KLA. These facts suggest that water solubility and/or the state of the carboxyl group are important for reducing aluminum toxicity in the case of KL. It was reported that carboxyl content of softwood kraft lignin was 0.8 and 0.89 meq g^{-1}.[35,36] It is, however, not clear if these factors are also important for saponified ozone-treated KL to reduce aluminum toxicity. To confirm this fact, insoluble saponified ozone-treated KL (0.5-O_3A-I) was prepared from 0.5-O_3 according to the method described in Experimental part and used in the planting experiment. The IR spectrum of 0.5-O_3A-I in Fig. 4 clearly shows the band of the free carboxyl group (1710–1730 cm^{-1}). The effects of 0.5-O_3, 0.5-O_3A-I, and 0.5-O_3A on root elongation in the planting experiment are shown in Fig. 6. It is observed that 0.5-O_3A-I effectively reduced aluminum toxicity and accelerated root elongation, although it was almost insoluble in the nutrient solution. It indicates that the different behavior between 0.5-O_3A-I and KLA was attributable to the introduction of carboxyl groups by ozone treatment.

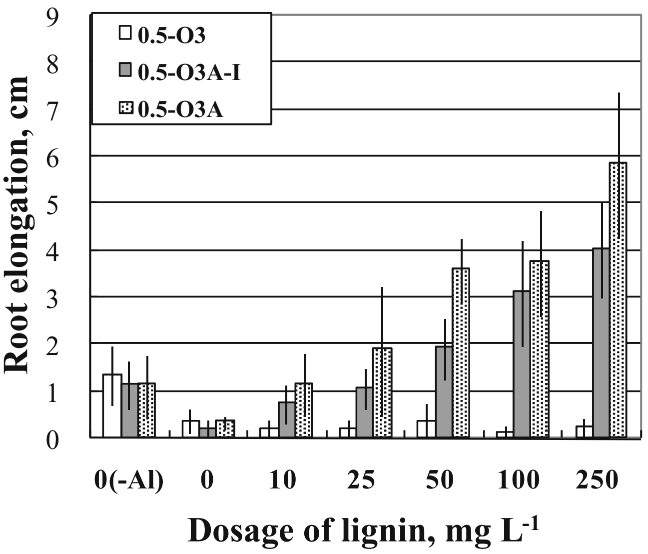

Fig. 6. Effect of 0.5-O$_3$, 0.5-O$_3$A-I, and 0.5-O$_3$A on root elongation of radish (*Raphanus sativa* var. *radicula* Pers.) in hydroponic solution containing 1.2 mM CaCl$_2$ and 37.5 µM AlCl$_3$ at pH 4.8. *0(-Al)*, Hydroponic solution without aluminum. Growth period was 3 days (n = 10)

Acknowledgments This study was financially supported by the 21st Century COE (Centers of Excellence) Program for the research project "Development of New Bioremediation Systems" from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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