

ORIGINAL ARTICLE

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## Development of acid soil conditioning agent from lignin by ozone treatment I

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**Abstract** A purified softwood kraft lignin was modified by ozone treatment and its activity as an acid soil conditioning agent, mainly focusing on elimination of aluminum toxicity, was assayed by planting experiments. The growth of radish root was examined in nutrient solution containing  $\text{CaCl}_2$  and  $\text{AlCl}_3$  at pH 4.8 with and without modified kraft lignins. The modified kraft lignins that absorbed 1.8 and 3.9 moles of ozone per C6-C3 unit ( $M_w$  180) showed two effects: the elimination of aluminum toxicity and the acceleration of root growth. The effect on the elimination of aluminum toxicity was observed even with modified kraft lignin that absorbed 1.0 mole of ozone per C6-C3 unit. The high molecular weight part of the modified kraft lignin that absorbed 3.9 moles of ozone per C6-C3 unit also proved to be effective not only in elimination of aluminum toxicity but also in acceleration of root growth. The acceleration effect of ozone-treated lignins on root growth was also observed under the absence of aluminum in planting experiments.

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

### Introduction

Acidification of soil is a serious problem, because poor plant growth occurs in acid soil. There are several reasons for poor plant growth in acid soil, but aluminum toxicity is believed to be one of the main reasons for it.<sup>1,2</sup> Aluminum ion eluted from acid soil matrix causes serious damage to

plant roots and causes inhibition of plant growth. The authors believe that lignin has advantages as an agent for remediation of extensive tracts of acid soil, because lignin is the second most abundant biomass component after cellulose, is reproducible, and forms part of the soil matrix.

The use of lignin derivatives as an acid soil conditioning agent, especially focused on elimination of aluminum toxicity, has been studied in detail.<sup>3–8</sup> These studies used lignins modified by alkaline oxygen and radical sulfonation treatments as an acid soil conditioning agent. The lignins were effective not only in elimination of aluminum toxicity but also in acceleration of root growth.<sup>3–5,7</sup> In particular, the lignin modified with alkaline oxygen was more efficient than the other lignin, for both favorable effects.<sup>3,5</sup> They suggested that muconic acid-type, catechol-type, and phenolic-type structures in such modified lignins should be important for the elimination of aluminum toxicity.<sup>5</sup>

In the present study, the authors focused on ozone treatment for the preparation of modified lignin as acid soil conditioning agent. If it is assumed that oxidation is necessary for the preparation of acid soil conditioning agent from lignin, ozone treatment is advantageous for its rapid and highly efficient oxidation of lignin. Ozone can oxidize both phenolic- and non-phenolic-type structures in lignin,<sup>9,10</sup> while alkaline oxygen treatment cannot oxidize non-phenolic-type structures in lignin.<sup>11</sup> It has been reported that ozone treatment can efficiently convert the aromatic ring of lignin model compounds to a muconic acid-type structure,<sup>10,12–15</sup> which was suggested to be an important structure for the elimination of aluminum toxicity.<sup>5</sup> Hatakeyama et al.<sup>12</sup> subjected vanillyl alcohol and veratryl alcohol to ozone treatment and examined their degradation products. They reported that muconic acid-type structures constituted 36% and 67% of the ether extracts of degradation products from vanillyl alcohol and veratryl alcohol, respectively.

Aromatic rings are degraded with ozone to low molecular weight products including oxalic acid.<sup>16,17</sup> Kratzl et al.<sup>15</sup> reported the formation of oxalic acid by ozone treatment of lignin model compounds in  $\text{CHCl}_3$ . Tsutsumi et al.<sup>18</sup> reported that 20% (mol/mol) of oxalic acid was formed

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from *threo*-veratrylglycerol by oxidation with ozone followed by alkaline treatment. Gas chromatography (GC) analysis of degradation products of  $\beta$ -1 dilignol model compound with ozone followed by alkaline treatments revealed that oxalic acid was the principal product (37% peak area).<sup>18</sup> It is reported that oxalate is effective in eliminating aluminum toxicity by complex formation with Al.<sup>8,19–26</sup> It is known that oxalate is exuded from the roots of some aluminum-tolerant plants in response to Al in the soil.<sup>19–23</sup> The activity of ozone-treated lignins for elimination of aluminum toxicity is discussed in this report.

## Experimental

### Modification of kraft lignin by ozone treatment

Crude softwood kraft lignin from Tokai Pulp and Paper was purified by aqueous dioxane/diethyl ether method:<sup>27</sup> the crude softwood kraft lignin (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. Precipitated lignin was collected by filtration and washed with diethyl ether. Collected lignin was dried successively in air overnight and then in vacuo at room temperature overnight. The yield of purified kraft lignin (KL) was 72%.

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 oxidized by bubbling with oxygen containing ozone (ca. 3.7%) at a rate of 0.5 l/min at 0°C with stirring for a set time (0.5, 1, 2, or 4 h). Residual ozone after the ozone treatment was removed by continued oxygen bubbling for a further 10 min. Ozone consumption was measured by iodometric titration. The reaction mixture was evaporated to dryness. The residual amount of acetic acid was removed by repeated evaporation with ethanol. The KL modified with ozone for 4 h was suspended in water and freeze-dried. The residue was dried under reduced pressure at 45°C overnight. The yields of all ozone-treated KLs were 97%–111% on KL. The lignins modified with ozone for 0.5, 1.0, 2.0 and 4.0 h were labeled as 0.5O<sub>3</sub>, 1.0O<sub>3</sub>, 2.0O<sub>3</sub>, and 4.0O<sub>3</sub>, respectively.

### Measurement of ozone consumption with KL

A solution of 10% potassium iodide was placed downstream of the reactor used for ozone treatment. The ozone consumption with KL (1 g) was determined by iodometric titration of the trapped ozone in the 10% potassium iodide solution, which passed through the reactor without reacting with KL during ozone treatment. The potassium iodide solution (10%, 250 ml) was renewed every 15 min and was used for iodometric titration. Blank test was run without KL.

### Dialysis of fraction 4.0O<sub>3</sub>

The modified KL 4.0O<sub>3</sub> was subjected to dialysis against distilled water for 3 days. The membrane used was Size 27

dialysis membrane [Wako, molecular weight cutoff (MWCO) 12 000–14 000]. The dialysate obtained was evaporated and freeze-dried. The yield of residue was 27% and the residue was labeled as 4.0O<sub>3</sub>D.

### Modification of kraft lignin by alkaline oxygen treatment

KL was modified with alkaline oxygen according to Katsumata et al.<sup>5</sup> with some modifications. KL (6 g) was dissolved in 1 M NaOH solution (300 ml) and subjected to alkaline oxygen treatment under 0.3 MPa at 70°C for 4 h. The reaction mixture was applied to a cation-exchange column (Dowex 50w x 4, H<sup>+</sup> form). The pH of the eluate was adjusted to neutral and evaporated and finally freeze-dried. The yield of the residue was 90.8% on KL. This residue was labeled as O<sub>2</sub>C.

### Determination of oxalic acid in lignin samples

KL and O<sub>2</sub>C were dissolved in 10 ml of 0.1 M NaOH solution or distilled water and applied to the cation-exchange resin column (H<sup>+</sup> form, C-112, Kurita Water Industry). The eluate was evaporated and freeze-dried. The residues were used for oxalic acid determination.

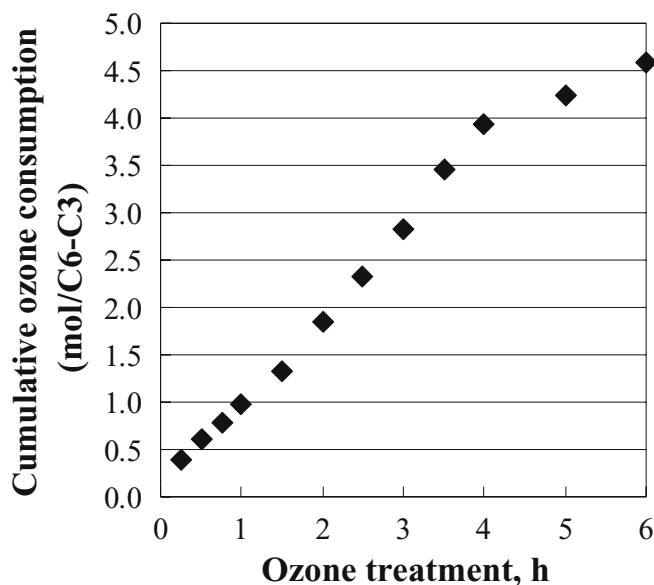
The content of oxalic acid in lignin samples was determined by the following method. The sample (10 mg) was subjected to trimethylsilylation with 300  $\mu$ l of dimethyl sulfoxide (DMSO) containing a calculated amount of erythritol as an internal standard, 200  $\mu$ l of hexamethyldisilazane (HMDS), and 200  $\mu$ l of trimethylchlorosilane (TMCS) at 60°C for 30 min. The upper layer of the reaction mixture, which separated into two phases after the reaction, was subjected to GC analysis using an Inert Cap 1 column (GL Sciences, 30 m  $\times$  0.25 mm i.d.). The temperatures for the injection and the detector were 260°C and 280°C, respectively. The column temperature was programmed as follows: holding for 4 min at 100°C, rising by 4°C/min to 230°C. The calibration curve of oxalic acid was prepared using erythritol as an internal standard. Peak identification was done by comparison of retention times with those of authentic compounds on GC and by GC-mass spectrometry.

### Infrared spectroscopy analysis

Fourier transform infrared (FT-IR) spectra of KL and ozone-treated KLs were recorded on a Jasco spectrometer (FT/IR-410) on KBr tablets.

### Planting experiment

Our planting experiment was conducted according to Wang et al.<sup>7</sup> with some modifications. 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. The seeds were rinsed with distilled water and then kept in distilled water overnight in a refrig-



**Fig. 1.** Relationship between cumulative ozone consumption by purified kraft lignin (KL) and time of ozone treatment. A molecular weight of 180 was used for a C6-C3 unit

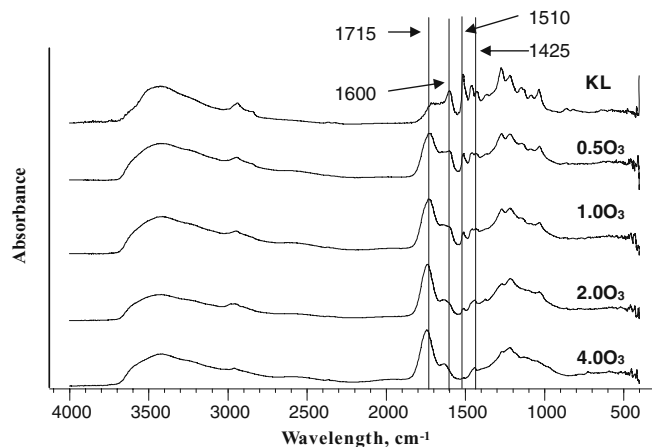
erator. The seeds were germinated on moist filter papers at 23.5°C for 1 day in darkness and for 3 days under a regimen of 16 h of light and 8 h of darkness.

Ten young seedlings were transferred to a nylon mesh holder that was floating on 700 ml of nutrient solution. A solution with 1.2 mM  $\text{CaCl}_2$  at pH 4.8 was used as nutrient solution. The planting experiment was conducted with and without  $\text{AlCl}_3$  (37.5  $\mu\text{mol/l}$ ) and lignins (10–250 ppm) at 23.5°C with continuous air bubbling. The pH of nutrient solution was maintained at pH 4.8 by adding dilute aqueous hydrochloric acid and/or sodium hydroxide two times a day during the planting experiment. The primary root lengths were measured after 3 days of growth. The results were evaluated statistically after removing extreme values using Dixon's Q method.<sup>28</sup>

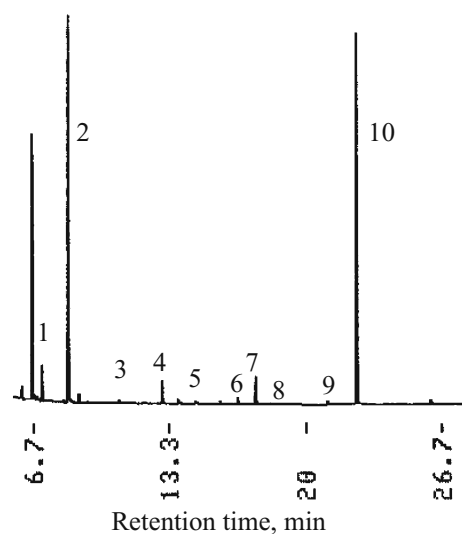
## Results and discussion

Ozone consumption with KL during ozone treatment was measured by iodometric titration. The result is shown in Fig. 1. Ozone consumption by KL increased almost linearly for up to 4 h of ozone treatment, and then the rate of ozone consumption decreased thereafter. Using a molecular weight of 180 for a C6-C3 unit of lignin for convenience, about 1 mole of ozone was consumed every hour for a C6-C3 unit of KL for up to 4 h of ozone treatment. The amount of ozone consumed per C6-C3 unit of KL was 0.6, 1.0, 1.8, and 3.9 moles for 0.5, 1.0, 2.0, and 4.0 h of ozone treatment, respectively.

Figure 2 shows the IR spectra of ozone-treated KLs. The aromatic ring was rapidly degraded with ozone, as shown by the decreasing intensity of the bands at 1600, 1510, and 1425  $\text{cm}^{-1}$  and by the increasing intensity of the bands at



**Fig. 2.** Infrared (IR) spectra of KL and ozone-treated KLs.  $0.5\text{O}_3$ , KL modified with ozone for 0.5 h;  $1.0\text{O}_3$ , KL modified with ozone for 1 h;  $2.0\text{O}_3$ , KL modified with ozone for 2 h;  $4.0\text{O}_3$ , KL modified with ozone for 4 h

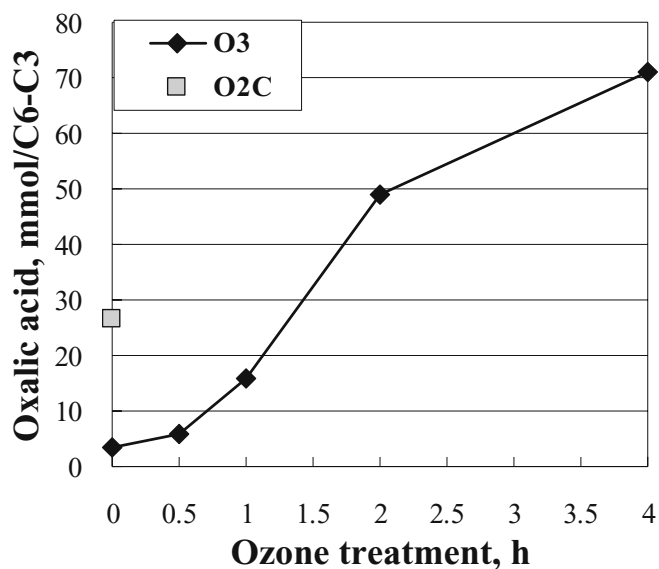


**Fig. 3.** Gas chromatogram of trimethylsilylated products obtained from  $4.0\text{O}_3$ . The numbered peaks correspond to the following identified compounds: 1, glycolic acid; 2, oxalic acid; 3, malonic acid; 4, glyoxylic acid; 5, succinic acid; 6, threonolactone; 7, tartronic acid; 8, erythronolactone; 9, malic acid; 10, erythritol (internal standard)

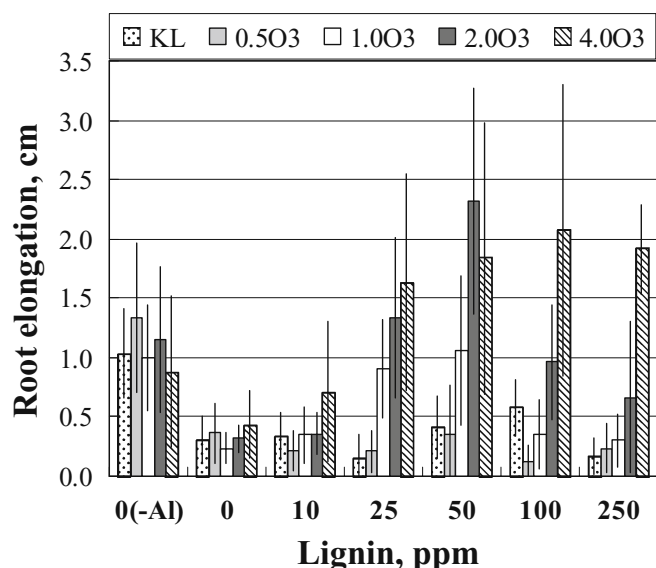
1710–1730  $\text{cm}^{-1}$  as the treatment progressed. This spectroscopic evidence indicated the formation of ester and carboxylic acid groups.

The content of oxalic acid was determined for the KLs modified with ozone and with alkaline oxygen ( $\text{O}_2\text{C}$ ). Figure 3 shows the gas chromatogram of trimethylsilylated derivatives from  $4.0\text{O}_3$ . Oxalic acid was the main peak on gas chromatograms of all ozone-treated KLs. The minor products found on the gas chromatograms were similar to those reported by Tsutsumi et al.<sup>18</sup>

The oxalic acid contents of ozone-treated KLs and  $\text{O}_2\text{C}$  are shown in Fig. 4. It is clear that the content of oxalic acid increased as the ozone treatment progressed. The KLs modified with ozone for more than 2 h contained more oxalic acid than  $\text{O}_2\text{C}$ .



**Fig. 4.** Oxalic acid contents of ozone-treated KLs ( $O_3$ ) and KL modified by alkaline oxygen treatment ( $O_2C$ ). A molecular weight of 180 was used for a C6-C3 unit



**Fig. 5.** Effect of ozone-treated KLs on root elongation in planting experiment. Culture solution contained 1.2 mmol/l  $CaCl_2$  and 37.5  $\mu\text{mol/l}$   $AlCl_3$  at pH 4.8. 0(-Al) indicates culture solution without aluminum. Growth period was 3 days ( $n = 10$ ). Error bars represent  $\pm$  SD

Figure 5 shows the effect of ozone-treated KLs on root elongation in the planting experiment. Aluminum toxicity was evaluated by inhibition of root growth, because the effects of aluminum toxicity first appear on growth of the plant root.<sup>1,22,23</sup> KL and the KLs modified with ozone for 2 h or less were almost insoluble in the nutrient solution, but KL treated for 4 h was almost soluble.

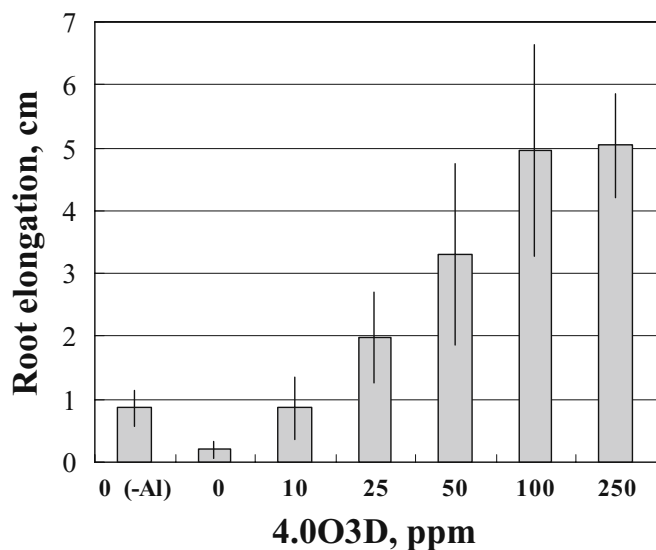
As shown in Fig. 5, KL itself did not have any positive effect on removal of aluminum toxicity. It was, however, observed that the root growth was recovered when 1.0O<sub>3</sub>, 2.0O<sub>3</sub>, and 4.0O<sub>3</sub> were used in a certain dosage range.

Assessment of significance by the *t*-test method<sup>28</sup> showed that there was no significant difference between root elongation under the condition without aluminum [0(-Al)] and those of 1.0O<sub>3</sub>, 2.0O<sub>3</sub>, and 4.0O<sub>3</sub> at a dosage of 25 ppm ( $P = 0.05$ ). If the elimination of aluminum toxicity is defined as the recovery of root growth up to the level of that under the condition without aluminum [0(-Al)], this was attained with 1.0O<sub>3</sub> and 2.0O<sub>3</sub> at the dosage of 25–50 ppm and 4.0O<sub>3</sub> at the dosage of 25–250 ppm.

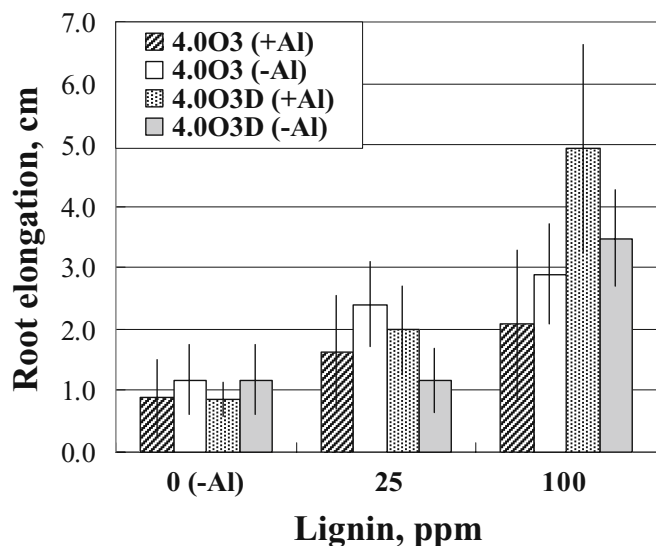
It is interesting to note that root elongation was accelerated with 2.0O<sub>3</sub> at a dosage of 50 ppm and with 4.0O<sub>3</sub> at a dosage of 50–250 ppm more than that under the condition without aluminum [0(-Al)]. Significance test by the *t*-test method<sup>28</sup> showed that there was significant difference between root elongation under the condition without aluminum [0(-Al)] and those of 2.0O<sub>3</sub> at a dosage of 50 ppm and 4.0O<sub>3</sub> at a dosage of 50–250 ppm ( $P = 0.05$ ). This means that 2.0O<sub>3</sub> and 4.0O<sub>3</sub> have two effects: elimination of aluminum toxicity and acceleration of the root growth. These two effects were also observed for the lignin modified with alkaline oxygen.<sup>3,5,7</sup>

It has been reported that aluminum toxicity was eliminated when an amount of oxalic acid twice that of  $AlCl_3$  was added to the culture solution in planting experiments.<sup>8,20,26</sup> The dosages of ozone-treated KLs needed to secure an amount of oxalic acid twice that of  $AlCl_3$  under the planting experiment in Fig. 5 (37.5  $\mu\text{mol/l}$   $AlCl_3$ ) were calculated based on the content of oxalic acid in each sample as shown in Fig. 4. They were 2248, 847, 276, and 190 ppm for 0.5O<sub>3</sub>, 1.0O<sub>3</sub>, 2.0O<sub>3</sub>, and 4.0O<sub>3</sub>, respectively. These figures show that an amount of oxalic acid twice that of  $AlCl_3$  was only attained at dosages of 250 ppm of 4.0O<sub>3</sub> and 2.0O<sub>3</sub> in this experiment. The oxalic acid contents were considerably low in the other cases. This fact shows that the elimination of aluminum toxicity with ozone-treated KLs cannot be explained only by the action of oxalic acid.

Hue et al.<sup>24</sup> reported that some low molecular weight organic compounds could be excellent Al detoxifiers if the positions of hydroxyl and carboxyl groups in the molecule were favorable for the formation of stable 5- or 6-membered chelate ring structures with Al. This finding may imply that the low molecular weight part of ozone-treated KL has the ability to eliminate aluminum toxicity, because low molecular weight organic compounds, such as oxalic acid, malonic acid, and malic acid, were found to be present in the ozonized samples, as shown in Fig. 3. It is, however, not known if the high molecular weight part of ozone-treated KL is also effective. The high molecular weight part of 4.0O<sub>3</sub> was prepared by dialysis (4.0O<sub>3</sub>D) to address this question. The effect of 4.0O<sub>3</sub>D on root elongation in planting experiment is shown in Fig. 6. Significance test by the *t*-test method<sup>28</sup> showed that there was no significant difference between the root elongation under the condition without aluminum [0(-Al)] and that of 4.0O<sub>3</sub>D at a dosage of 10 ppm. However, at dosages in the range of 25–250 ppm, there was significant difference between the result for no aluminum [0(-Al)] and that for 4.0O<sub>3</sub>D ( $P = 0.05$ ). These results indicate that the high molecular weight part of 4.0O<sub>3</sub> was more effective not only in the elimination of aluminum



**Fig. 6.** Effect of high molecular weight dialysate of  $4.0O_3$  ( $4.0O_3D$ ) on root elongation in planting experiment. Culture solution contained  $1.2\text{ mmol/l CaCl}_2$  and  $37.5\text{ }\mu\text{mol/l AlCl}_3$  at pH 4.8. Growth period was 3 days ( $n = 10$ ). Error bars represent  $\pm$  SD



**Fig. 7.** Effect of  $4.0O_3$  and  $4.0O_3D$  on root elongation in planting experiment with and without aluminum. Culture solution contained  $1.2\text{ mmol/l CaCl}_2$  at pH 4.8 (-Al) or  $1.2\text{ mmol/l CaCl}_2$  and  $37.5\text{ }\mu\text{mol/l AlCl}_3$  at pH 4.8 (+Al). Growth period was 3 days ( $n = 10$ ). Error bars represent  $\pm$  SD

toxicity, but also in the acceleration of root growth than that under the condition without aluminum [0(-Al)].

Acceleration of root growth was observed for  $2.0O_3$ ,  $4.0O_3$ , and  $4.0O_3D$  for certain dosage ranges (Figs. 5 and 6). It is, however, not clear if root growth was accelerated by interaction between aluminum and lignin. Katsumata et al.<sup>5</sup> reported that an acceleration effect was observed for lignin modified with alkaline oxygen on root growth in the presence and the absence of aluminum. A planting experiment was carried out with use of  $4.0O_3$  and  $4.0O_3D$  with and without aluminum. As shown in Fig. 7,  $4.0O_3$  and  $4.0O_3D$  at dosages of 100 ppm also accelerated the root growth in spite

of the presence or absence of aluminum. Significance test by the  $t$ -test method<sup>28</sup> showed that there was significant difference between the root elongation under the condition without aluminum [0(-Al)] and those of  $4.0O_3(+Al)$ ,  $4.0O_3(-Al)$ ,  $4.0O_3D(+Al)$ , and  $4.0O_3D(-Al)$  at dosages of 100 ppm ( $P = 0.05$ ).

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