

ORIGINAL ARTICLE

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Characterization of lignin fragments in alkaline oxygen-stage waste liquor as soil-conditioning agent

Received: February 9, 2004 / Accepted: June 15, 2004

Abstract We characterized the lignin fragments in the alkaline oxygen delignification-stage waste liquor from a pulp and paper mill as a soil-conditioning agent. Chemical reactions of this lignin fragment should be very similar to those of alkaline-oxygen treatment of kraft lignin. Alkaline oxygen delignification-stage waste liquor was subjected to gel filtration chromatography and four different molecular weight fractions (F1, F2, F3, and F4) were obtained. These four fractions were used in plant growth experiments. A very clear positive effect in removing the aluminum (Al) toxicity to root growth of radish was found for all four fractions. When aluminum concentration in the nutrient solution was as low as 0.5 ppm, root elongation was practically inhibited, but if 12.5 ppm of lignin fragment was added, the toxic effect was practically removed and root elongation better than that in the control was observed. However, each fraction showed different performance in removing aluminum toxicity, and the highest molecular weight fraction, which contained some aromatic structures, showed better performance in removing Al toxicity. The reason for their different behaviors on Al toxicity was also examined.

Key words Alkaline oxygen delignification-stage waste liquor · Al toxicity · Soil-conditioning agent · Al detoxification

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Parts of this report were presented at the 12th International Symposium on Wood and Pulp Chemistry, Madison, USA, June 2003 and the 48th Lignin Symposium, Fukui, Japan, November 2003

Introduction

Soil acidification resulting from either continuous use of ammonia- and amide-containing fertilizer¹ or nitrogen fixation by legumes² aggravates Al toxicity and contributes to an increase in the damaged soil area.³ Furthermore, acid precipitation is increasing soil acidity⁴ and enhancing Al solubility in many locations worldwide,⁵ thus aggravating Al toxicity in acid-sensitive soil.

Aluminum is a major constituent of most of soil and under an acidic environment becomes mobilized into toxic forms, and thereby limits crop productivity in many arable soils.⁶ In 1918 aluminum was suggested as a factor that may cause yield decline of *Hordeum vulgare* and *Secale cereale* grown on acid soils.⁷ Over the years following the initial report, aluminum has become recognized as the most growth-inhibiting factor in many acid soils throughout the world.⁸

The initial and most dramatic symptom of Al toxicity is inhibition of root growth, because Al accumulates preferentially in the root tips at the site of cell division and cell elongation.⁹ The injured roots usually become susceptible to drought. The problem of Al toxicity may be affecting about 40% and perhaps up to 70% of the world arable land that is used for food and biomass production.¹⁰

Aluminum toxicity studies for plants have been carried out for many years, and many techniques to remediate Al-contaminated soil have been developed. However, these techniques are reported to be inefficient as well as expensive and labor intensive. Therefore, the problem is not only economic in nature, but also requires technical feasibility and compatibility with conventional soil management practices.¹¹ Hence, an alternative or a supplemental approach to the problem is needed. Because of the structural similarity of degraded lignin and humic substance,¹² partially oxidized lignin was expected to decrease metal toxicity and maintain good soil conditions. In our previous studies,^{13–16} a high molecular weight fraction of alkaline oxygen-treated kraft lignin was proved to have positive effect for removing aluminum toxicity.

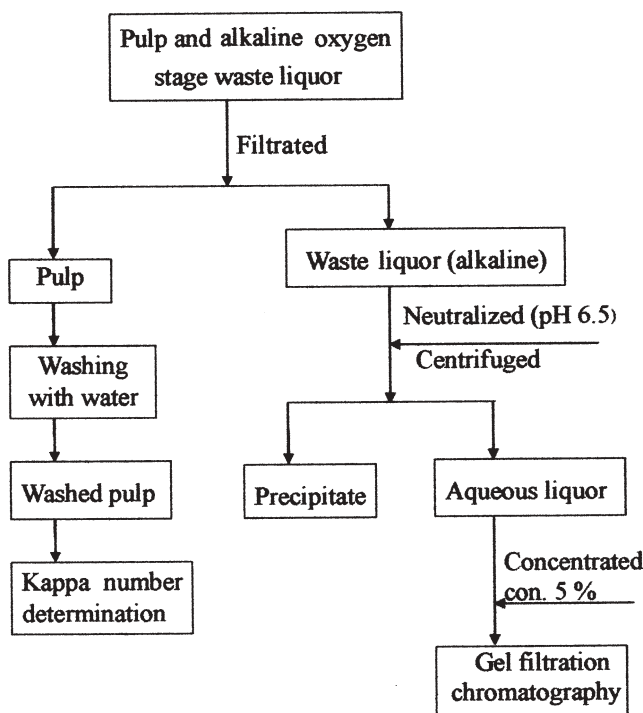


Fig. 1. Scheme for sample preparation

The objectives of the present study are to evaluate the possibility of utilizing lignin fragments obtained from alkaline oxygen delignification-stage waste liquor as a soil-conditioning agent and to examine the behaviors of different molecular weight fractions of those lignin fragments.

Materials and methods

Materials

Waste liquor from the alkaline oxygen delignification stage of the hardwood bleached kraft pulp (LBKP) production process was kindly provided by Ishinomaki Mill, Nippon Paper Industries.

Sample preparation

Figure 1 shows the procedure for sample preparation. After the separation of some precipitates, the waste liquor of the alkaline oxygen delignification stage was mildly acidified to pH 6.5 with dilute HCl, and then centrifuged to remove freshly formed precipitates. The supernatant was concentrated and subjected to gel filtration chromatography (GFC) on Sephadex G-25 (M), using water as eluent. The column was 6.5cm wide, 114cm long, with a volume of 3781 cm³. Distribution of lignin fragments was measured by UV absorbance at 280nm. As shown in Fig. 2, the waste liquor sample was separated into four fractions (F1, F2, F3, and F4). Fraction F1 was a relatively high molecular weight fraction, while the others were of relatively low molecular

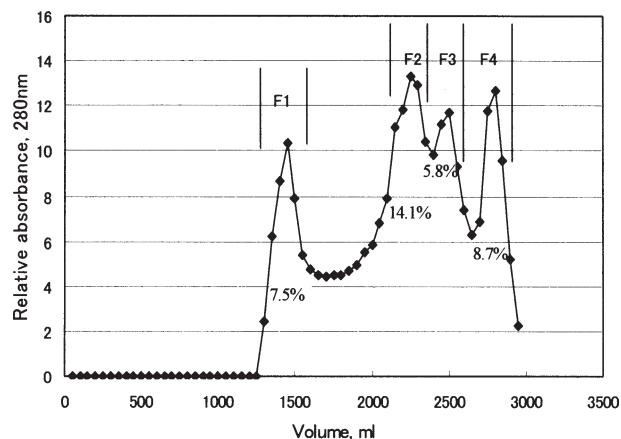


Fig. 2. Gel filtration chromatography curve of waste liquor sample. Gel: Sephadex G25 (M); column: 6.5cm (d) × 114cm (h); eluent: water. Percentage of each fraction was calculated based on absorbance at 280nm

weight. The highest molecular weight fraction (F1) made up 7.5% of the sample, whereas F2, F3, and F4 accounted for 14.1%, 5.8%, and 8.7%, respectively. These four fractions were used separately for the plant experiments.

Potentiometric and conductometric titrations

Titration experiments were conducted at room temperature using a computer-assisted titration system (Comitite-550, Hiranuma) consisting of two burettes, a pH electrode, conductometric titration electrode, and temperature compensating electrode. The two burettes were filled with standard 0.1M HCl and 0.1M KOH. Experiments were performed by titration with base (forward titration) and with acid (backward titration) under a nitrogen atmosphere. The titration curves showing pH and conductivity versus volume were recorded simultaneously.

Culture solution

In order to avoid the precipitation of Al by other nutrients, calcium solution was used as the basal growth medium. Stock solutions of aluminum and calcium were prepared by dissolving AlCl₃·6H₂O and CaCl₂·2H₂O separately in deionized water. Different amounts of stock solutions were added to deionized water, to which a solution of a lignin fragment fraction was added to prepare a culture solution. Aluminum concentration in the culture solution was adjusted from 0ppm to 10ppm. The growth solution was initially adjusted to pH 4.8.

Growth conditions

Phytotoxicity of Al is characterized by rapid inhibition of root elongation and subsequent decrease in the uptake of nutrients and water.¹⁷ The abilities of lignin fragments to stop Al toxicity to plants were examined by measuring the

root elongation, for which radish was used as the test plant. Radish seeds were germinated on a moist filter paper for 4 days. Young seedlings were transferred to the nylon mesh holders which were floating on 700 ml of the culture solution and then placed in a growth chamber (LH-100-RDS, NKS). During the growth experiment, pH of the growth solution was readjusted every 12h with HCl or NaOH to keep the pH constant. After the growth period of 3 days with or without Al, the primary root length was measured.¹⁵

Three conditions for plant experiment (condition I–III)

Condition I was a control experiment and a fraction obtained by GFC was directly applied to the plant experiment. A fractionated sample was charged to an ion-exchange column to protonate its acidic groups, and then neutralized with $\text{Ca}(\text{OH})_2$ and NaOH, to prepare lignin samples for conditions II and III, respectively.

Analysis of chemical characteristics

A Shimadzu UV-240 spectrometer was used for the ultraviolet (UV) spectroscopy of lignin fragments. Fourier transform-infrared (FT-IR) spectra were recorded on a Jasco FT-IR 615 spectrometer on KBr tablets. Proton nuclear magnetic resonance (NMR) spectra of lignin fragments were recorded on a Bruker AC 300 NMR spectrometer in D_2O with dioxane as internal standard. Sodium content in lignin fragments was determined by inductively coupled plasma (ICP) emission (argon) spectroscopy on an Ultima T (Jobin Yvon).

Results and discussion

Chemical characteristics of each fraction

Figure 2 shows that GFC was successful in separating the waste liquor sample into four fractions of different molecular weight. Points worthy of investigation are how the chemical composition of these four fractions vary with apparent molecular weight, and the structural differences between the fractions. To answer these questions, the UV spectrum of each fraction was examined. As shown in Fig. 3, F1 has a clear shoulder at 280nm, but F2 and F3 have a maximum at around 260nm. On the other hand, F4 has two shoulders at around 280nm and 360nm. Because a typical UV spectrum of natural lignin is known to have an absorption maximum at 280nm, F1 seems to still retain, at least partly, the aromatic structures in the original lignin. This is also confirmed by a broad signal at around 7ppm in the ^1H NMR spectrum in Fig. 4.

When the FT-IR spectra of F3 and sodium oxalate (shown in Fig. 5) were compared, every peak in the spectrum of oxalate was confirmed in that of F3. A peak at 1640cm^{-1} was assigned to asymmetric stretching of COO^- and two peaks at 1341cm^{-1} and 1326cm^{-1} were assigned to

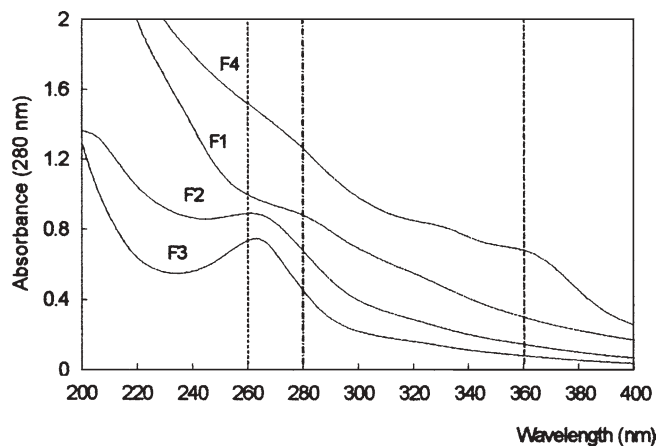


Fig. 3. Ultraviolet spectra of fractionated samples

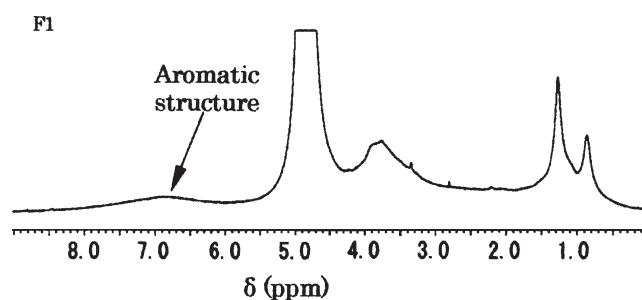


Fig. 4. Proton nuclear magnetic resonance spectrum of F1

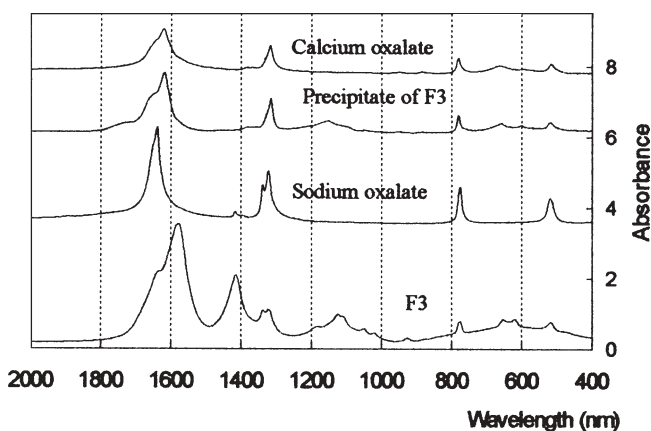


Fig. 5. Fourier transform-infrared spectra of F3 and model compounds

COO^- symmetric stretching. This evidence indicates the existence of sodium oxalate in F3.

During the alkaline oxygen treatment, ring-opening reactions to introduce carboxylic acid groups and side-chain elimination reactions are known to occur in lignin macromolecules.¹⁴ Carboxylic acid and phenolic hydroxyl groups are believed to be important ionizable sites present in humic substance and are supposed to play important roles in forming complexes with metals.¹² Therefore, the content and characteristics of carboxylic acid and phenolic hydroxyl

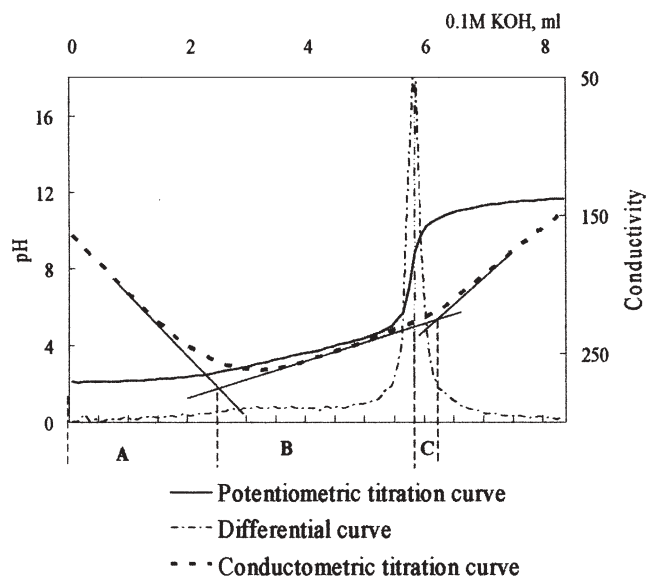


Fig. 6. Forward potentiometric and conductometric titration curves of F3. A, Strongly acidic group; B, weakly acidic group I; C, weakly acidic group II

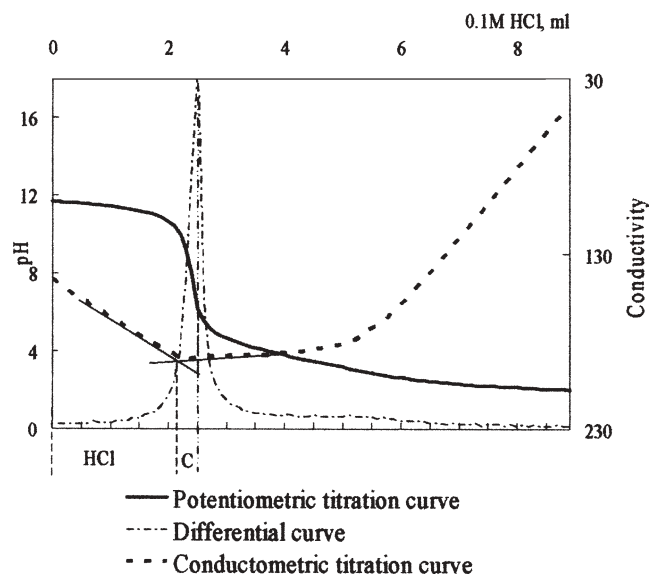


Fig. 7. Backward potentiometric and conductometric titration curves of F3

Table 1. pKa values of acidic groups in separated fractions

Sample	Strongly acidic group	Weakly acidic group I	Weakly acidic group II
F1		3.5, 5.02	9.12
F2	2.36	4.60	9.49
F3	2.36	4.20	9.61
F4	2.21		6.18

Table 2. Acidic groups in separated fractions (mol/200g)

Sample	Strongly acidic group	Weakly acidic group I	Weakly acidic group II	Total
F1		0.39	0.29	0.68
F2	0.58	0.96	0.27	1.81
F3	0.76	1.42	0.20	2.38
F4	2.01	1.11		3.12

groups are keys for better understanding of the function of each lignin fraction as a soil-conditioning agent.

Potentiometric and conductometric titrations, which can be used to measure both the type and the quantity of acidity, were conducted for each fraction. Figures 6 and 7 are the forward and backward titration curves of F3. The forward conductometric titration curve with dilute KOH solution was divided into three parts: strongly acidic group, pKa around 2.21 to 2.36; weakly acidic group I attributed to carboxylic group (—COOH), pKa around 4.2 to 5.02; and weakly acidic group II probably because of phenolic hydroxyl (—OH) group, pKa around 6.18 to 9.61 (see Table 1). A pronounced change in conductance around the first inflection point was observed. The stoichiometric point can be determined by a peak in the differential curve. However, in the pH range of 10–11, no clear change in the conducto-

metric and potentiometric titration curves was observed. It was not possible to differentiate between the weakly acidic group II and excess alkali by the forward titration. Therefore, the backward titration was performed with dilute HCl solution (Fig. 7).

The acidic group content of each fraction is shown in Table 2, indicating that with the decrease of molecular weight the content of acidic groups increased. F3 contains more acidic groups than the higher molecular weight fractions of F1 and F2. This phenomenon can be explained by the fact that at least one carboxylic acid group was introduced by the ring-opening reaction during alkaline oxygen treatment which leads to the depolymerization of lignin to some extent.¹⁴ The different structural characteristics of separated fractions may affect their functions as soil-conditioning agents.

Function of removing Al toxicity

Toxicity of Al on the growth of plants must depend on many factors, such as the species of plant, forms of Al, concentration of dissolved Al, and concentration of soil organic matter.¹⁸ In our experiment, when Al concentration in the growth solution was changed from 0 to 10 ppm, root growth was heavily inhibited in the presence of 0.5 ppm Al (data not shown here). The color of the root tip darkened slightly and almost no root elongation was observed at that Al level. Therefore, the following plant experiments mainly focused on the 0.5-ppm level.

The root elongation of radish in the presence of different fractions can be seen in Fig. 8. Figure 8A (condition I) shows the positive effect of F1 in the presence of 0.5 ppm Al. When 12.5 ppm of F1 was added to the Al-containing solution, Al toxicity was practically removed. F2 also shows efficient amelioration of the Al toxicity (see Fig. 8B, condition I). It is interesting to note that the root growth was much better than that of the control (without Al), and that the density of the lateral root (secondary root) also increased. As shown in Fig. 8D (condition I), 100 ppm of F4 was needed to alleviate the Al toxicity at 0.5 ppm Al, but, in the cases of F2 and F3, only one eighth of the dosage was enough to attain a similar effect. The reason why these four fractions show such different Al detoxifying abilities should be attributed to their different chemical characteristics.

Special behaviors of fraction 3 (F3)

As discussed above, the four fractions (F1–F4) showed different performance in removing Al toxicity. In the case of F3, the efficiency in removing Al toxicity was observed to decrease with the increase of its dosage. In order to understand this behavior of F3, further plant experiments were conducted under two different conditions (Fig. 8) and the results were compared with those of the original condition (condition I).

The decreased efficiency was not alleviated under condition II, in which Na^+ in the original sample (condition I) was replaced by Ca^{2+} . The limited root growth with F3 under condition II was unexpected, because decreased growth with increased F3 was thought to be due to the negative effect of the high Na^+ concentration. This might be due to the precipitation of some lignin fragments in the culture solution under condition II. The decreased efficiency with increased F3 under condition III was tentatively assumed to be due to sodium ion content or some other components in F3.

Concerning the precipitation mentioned above, it is important to note that the presence of a remarkable amount of oxalic acid in F3 was confirmed by FT-IR spectra. The similarity between the FT-IR spectra of calcium oxalate and the precipitate of F3, as shown in Fig. 5, strongly suggests that the precipitate is mainly calcium oxalate, although a broad extra band around 1150cm^{-1} may indicate some contamination. We, therefore, assumed that the decreased root

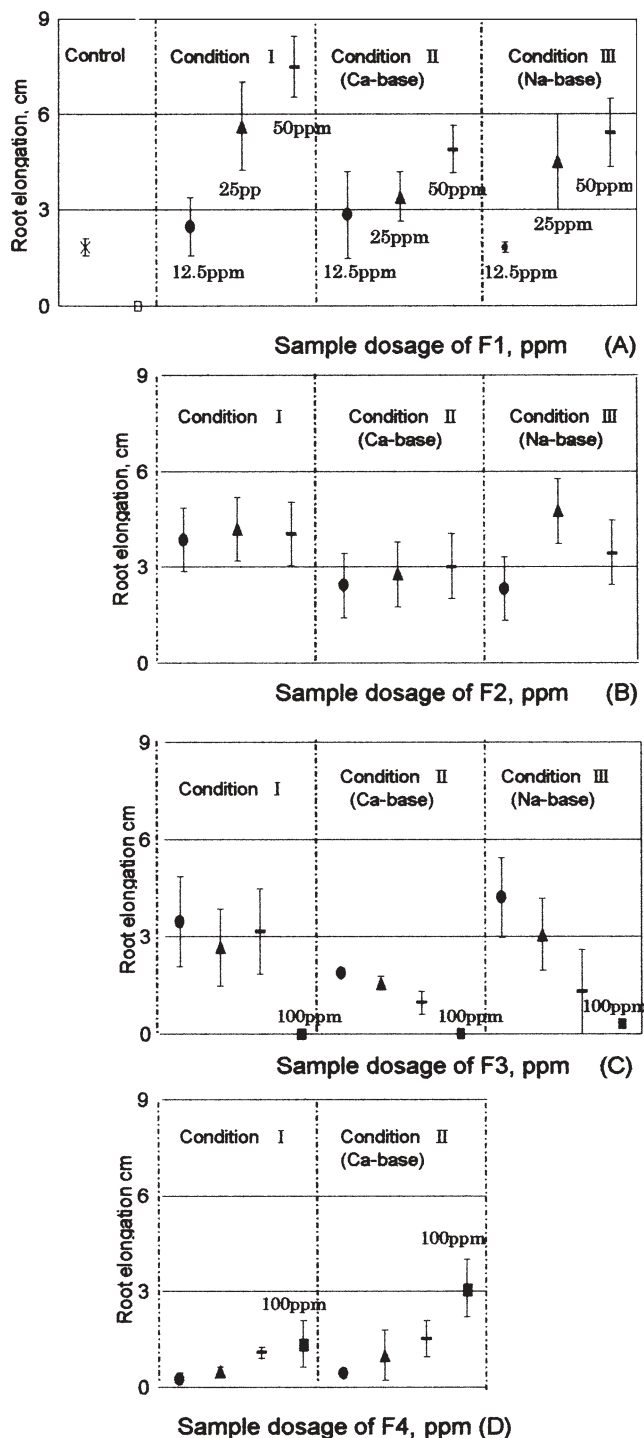


Fig. 8A–D. Root elongation of radish under different conditions for **A** fraction F1, **B** fraction F2, **C** fraction F3, and **D** fraction F4. Crosses, Control (no Al, no lignin); open squares, Al; circles, Al (0.5 ppm) + sample (12.5 ppm); triangles, Al (0.5 ppm) + sample (25 ppm); bars, Al (0.5 ppm) + sample (50 ppm); filled squares, Al (0.5 ppm) + sample (100 ppm).

elongation may be explained by the precipitation of oxalic acid which otherwise has a positive effect in removing Al toxicity.¹⁹ This is probably the reason why root growth under condition II was generally much lower than that observed under conditions I and III.

Table 3. Sodium content in separated fractions (%)

Sample	F1	F2	F3	F4
Na ⁺ content	4.49	16.89	24.15	28.88

Effect of the concentration of sodium ion on root growth

The effect of the concentration of sodium ion on root growth was examined by the use of aqueous NaCl solution. When the concentration of sodium ion in culture solution was 45 ppm, root elongation of radish was obviously suppressed, and it was fatally inhibited at 160 ppm. The sodium content in each fraction determined by ICP is listed in Table 3, indicating that sodium concentration in the culture solution with 100 ppm of F3 should be only 24 ppm. Sodium content in F3 could not be the reason for the decreasing efficiency with the dosage of F3. In other words, there might be some components in F3 which have negative effect on root growth. In this context, effects of low molecular weight compounds including oxalic acid in F3 are now under investigation.

Conclusions

Lignin fragments in the alkaline oxygen delignification-stage waste liquor showed clear ability in removing Al toxicity and to improve the growth of plants.

Different molecular weight fractions obtained by GFC have different overall chemical compositions and different efficiencies in removing Al toxicity. The highest molecular weight fraction, which still has some aromatic structures, shows better performance in removing Al toxicity. Lower molecular weight fractions containing higher amounts of acidic groups also showed positive effects, but the effect was not improved remarkably with increased dosage. In particular, fraction F3, rich in oxalic acid, showed decreased performance with increased dosage.

Acknowledgment We thank Ishinomaki Mill, Nippon Paper Industries for offering us the sample of waste liquor from the alkaline oxygen delignification stage.

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