



## Low molecular-weight silicic acid— inorganic compound complex as wood preservatives

Haruhiko YAMAGUCHI\*

### ABSTRACT

As a purpose to restrain environmental pollution by wood-preservatives, a wood-preservative to use the low-toxicity silicic acid as a main chemical was examined. Silicic acid monomer aqueous solution (SAMS) was compounded with various kinds of metal chloride or boric acid. The decay-resistant ability of agents, which SAMS compounded with metal compound, was large against the decay by *Coriolus versicolor*, but was small against the decay by *Tyromyces palustris*. However, the compounded agent with boric acid showed very large ability against each decays by both rotting fungi. As for the compounded agent with boric acid, even the specimen after the leaching-test maintained large decay-resistant ability. In case of the treated wood with a SAMS—boric acid agent, there was a little leaching of boron in the leaching and decay-test. In other words, by compounding with boric acid in SAMS, the boron could be effectively fixed in wood.

In order to examine the mechanism of the good resistant ability against the decay, the sapwood-powder of *Cryptomeria japonica* D.Don was treated with the same agent. *Tyromyces palustris* was cultured using the liquid medium that the treated wood-powder was mixed. The activity of enzymes in the crude-enzyme liquid, the quantity of the growth of mycelium, and pH of the medium, were assayed. When there was very much proportion of boric acid, the growth of mycelium was completely restrained, and protein was not formed, too. When the proportion of boric acid was small, and the mycelium grew up, the xylanase activity, the mannase activity and the cellulase activity were smaller than them of the control wood-powder. When the powdery boric acid is added to CSAS rather than the boric acid—methanol solution is added to CSAS, it is promising that the treated wood having higher antiweather ability is provided.

### 1. INTRODUCTION

Creosote oil, pentachlorophenol and CCA are widely used as wood-preservatives, and the efficacy of those agents are good. However, these agents have carcinogenicity and/or strong toxic. These are not good in order to keep environment. In order to maintain global environment, wood-preservatives, which have not toxic or have low toxic, are studied lively recently. Various kinds of metal ion inhibit the growth and/or activity of wood-rotting fungi. So, there are many studies that the wood-preservative, which used the metal ion that had toxic to wood-rotting fungi, was examined.

In the previous reports,<sup>1-3)</sup> SAMS, which cation-exchanged sodium-silicate aqueous solution, or the colloidal silicic acid solution (CSAS) was impregnated into the wood, and their solutions changed to the high molecular-weight silicic acid, and then fixed in the wood. In this way, the wood that was compounded with an inorganic compound could be prepared. Because the high molecular-weight silicic acid fixed in wood is superior in water resistance, the dimensional stability and the physical properties of wood improve.

Silicon in the crystal of the high molecular-weight silicic acid can substitute for aluminum, boron and beryllium, which have form the same as silicon.<sup>4)</sup> In this report, this nature of silicic acid was applied, the boric acid or other metallic elements, which showed toxic against wood-rotting fungi, was added to SAMS or CSAS, and the solution was then impregnated into and fixed in wood. The resistant ability for wood-rotting fungi and the inorganic retention capacity of the treated wood was examined. Also, the mechanism of preservation by the low molecular-weight silicic acid—boric acid agents against the decay by the brown-rot fungus, *Tyromyces palustris* was examined.

### 2. EXPERIMENTAL

#### 2.1 Preparation of agents

*Agent to examine the relation between decay-resistant ability and agent:*

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\* Dept. of Forest Product, Kyushu university6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

7 or 10% SAMS was prepared by cation-exchanging 7.0 or 10% (w/v) sodium silicate aqueous solution. 10% SAMS was compounded with an aqueous solution of each compound of Fe, Zn, Co, Cu or B.

*Agents to examine the preservation mechanism:*

Powdery boric acid or 17.9%(w/v) boric acid—methanol solution was added to 10% SAMS or CSAS.

## 2.2 Sample and the cultivation for the examination of preservation mechanism

Wood-powder was prepared from sapwood of *Cryptomeria japonica* D. Don. 4 mL of the agent was added to 1 g of the wood-powder, and then the sample was air-dried. The leaching-test repeated 10 times of next operations. The wood-powder was added distilled-water of 20 times of the weight, were let alone in ambient temperature for 1 h, and then dried at 60°C for 23 h. The treated wood-powder was air-dried.

## 2.3 Assay of quantity of a fixed agent

The weight per gain (WPG1) was calculated from a weight difference of a vacuum-dried specimen before and after the impregnation of agent. WPG2 after the leaching-test was calculated from a difference of weights of the specimen before and after the leaching-test that prescribed in JIS A 9201-1991.

## 2.4 Decay-test

The decay-tests were done according to JIS A 9201-1991 using a brown-rotting fungus, *Tyromyces palustris* and a white-rot fungus, *Coriolus versicolor*. The specimens were incubated at 26 °C for 12 weeks. The extent of the fungal attack was expressed as the weight loss of specimen.

## 2.5 Liquid culture of *T. palustris*

T.L.Highley basal liquid-medium was used as liquid culture medium. When the growth inhibition was measured, 0.5 g of the wood-powder (20-40 mesh) and 50 mL of the liquid medium were put in a 500 mL conical flask. When the enzyme inhibition was measured, 0.8 g of wood powder (passed 200 mesh) and 80 mL of the liquid medium were put in the flask. *Tyromyces palustris*, which was cultured beforehand on a plate medium, was inoculated on the surface of the liquid medium. Standing-calculation for assay of the growth-inhibition was done for 10 d under light exposure, and those for the enzyme-inhibition was done for 14 d.

## 2.6 Assay of each element in which specimen before and after the decay-test includes.

The specimens for the decay-test were ashed at 800 °C in an electric furnace. Ash of each specimen was dissolved in 1N nitric acid, and the quantity of the inorganic elements was measured using ICP-MS (Yokogawa-denki Co. Ltd. PMS2000).

## 2.7 Examination of effect of low molecular weight silicic acid-boric acid agent on the activity of *T. palustris*

### 2.7.1 Assay of growth-inhibition of mycelium

The mycelium and the culture medium were put in a beaker, and a little ion-exchanged water was added to it. The mycelium and the wood-powder are divided in the beaker, and the mycelium was then filtered with the weighted glass-fiber filter paper. The mycelium and the filter paper were dried at 105 °C, and then the weight of mycelium was calculated.

### 2.7.2 Assay of inhibition to enzymatic activity

*Preparation of crude enzyme liquid:* The mycelium and the culture medium were taken out from the cultivation vessel using 0.1 M acetic acid buffer solution (pH 5.5). The mycelium was crushed with an ultrasonic breaker for 30 min (1sec-1sec pulse) while cooling with ice. Solid (mycelium and wood-powder) and liquid (medium including enzymes) were divided with a glass-fiber filter paper. The liquid was added ammonium sulfate to the concentration of 0.9-saturation, and then was done salting-out in a refrigerator. The liquid was centrifuged while cooling (at 0 °C, 10000 rpm, for 30 min), and the precipitate was collected. The precipitate was put in a dialysis membrane, and dialyzed repeatedly against 0.1 M acetic acid buffer solution (pH 5.5) for 3 d in the refrigerator. Contents in the dialysis membrane were centrifuged, and the precipitate was removed. 0.1 M acetic acid buffer solution (pH 5.5) was added to some definite volume to the supernatant. This solution is named the crude-enzyme liquid. The crude enzyme liquid was refrigerated (−30 °C) and stored. The quantity of the protein and the activity of enzyme were assayed.

*Assay of produced protein:*

The quantity of protein in the crude enzyme liquid was assayed by the micro-method of Bio-Rad.

*Assay of enzymatic activity:*

*$\beta$  -1, 4-xylanase activity:*

One mL of 0.1M acetic acid buffer solution (pH 5.5) was added to 1 mL of 1% (w/v)  $\beta$  -1, 4 - xylan-dispersed aqueous solution, and the mixture was kept at 37 °C. 0.5 mL of the crude enzyme liquid was added to this solution, and then the mixture was vigorously agitated. The solution was kept at 37 °C for 15 min. The reaction solution was dipped in boiling water for 5 min, and the enzymes were deactivated. The solution was cooled in an ice bath, and then centrifuged at 5000 rpm for 15 min. One mL of distilled water was added to 1 mL of the supernatant of the solution, and the quantity of reducing sugars, which were produced by the enzyme-reaction, was assayed by Somogyi-nelson method.

Somogyi-nelson method was done as follows; Two mL of alkaline-copper-reagent was added to the above solution, and the mixture was heated at 100 °C for 10 min. The solution was cooled in the ice-bath, 2 mL of arsenic-molybdic-acid reagent is added, and then the mixture was agitated. The solution was centrifuged (at 5000 rpm for 15 min), and the absorbance at 500 nm of the supernatant was assayed. One unit of xylanase activity is the quantity of enzyme to produce 1  $\mu$  mol of xylose for 1 min.

*Mannase activity:*

Glucomannan was used as the substrate. Absorbance in Somogyi-Nelson method was measured at 660 nm. One unit of mannase activity is the quantity of enzyme to produce 1  $\mu$  mol of mannose for 1 min.

*Cx- cellulase (EG) activity:*

Carboxymethyl-cellulose [CMC, degree of substitution (DS) 0.53] was used as substrate. Absorbance in Somogyi-Nelson method was measured at 500 nm. One unit of Cx-cellulase activity is the quantity of enzyme to produce 1  $\mu$  mol of glucose in 1 min.

*C1 -cellulase (CBH) activity:*

It is assumed that the cellulase, which was separated and refined from *Tyromyces palustris*, cannot resolve crystalline-cellulose.<sup>5)</sup> However, in case of this investigation, I thought that C1-cellulase activity can be assayed because of using the partially purified crude-enzyme.

0.5 g of microcrystal-cellulose, 2 mL of the buffer solution and 2 mL of the crude enzyme liquid were mixed, and the mixture was incubated at 40 °C for 24 h. The mixture was boiled for 5 min, and then filtered with a 1G4 glass-filter. The filtrate was centrifuged, and the quantity of reducing-sugar in the supernatant was assayed by Somogyi-nelson method. Absorbance at 500 nm was measured. One unit of C1-cellulase activity is the quantity of enzyme to produce 1  $\mu$  mol of glucose for 1 min.

The cellulose, which was left on the filter paper, was sufficiently washed with distilled water. The left cellulose was dried at 105 °C, the weight loss by the enzyme reaction was calculated afterward.

### 3. RESULTS AND DISCUSSIONS

#### 3.1 Quantity of fixed agent (WPG)

When each metal compound was added to SAMS, the WPG1 were 17-22 %. When boric acid was added to SAMS, the WPG1 was about 15% regardless of quantity of boric acid. WPG2 of the treated wood with an agent of SAMS and metal compound were small. When metal ion of not less than fixed quantity existed, crystal lattice of high molecular-weight silicic acid was moderated, and it was estimated that quantity of the leached metal ion increased. However, when a wood was treated with a compound agent of boric acid and SAMS, the proportion of the leached agent was about 10 %. This proportion is equal to the quantity that leach from the treated wood only with SAMS, and was about 1/4 of the quantity that leach from the treated wood with a compound agent of other metal compound and SAMS. The reason of high anti-leaching ability of the boric acid-SAMS agent was thought that the boron atom was built in the crystal lattice of the high molecular-weight silicic acid by the analogy between the structures of silicon atom and boron atom.

#### 3.2 Decay resistance

In the decay-test using *Tyromyces palustris*, the weight loss of specimen treated with the agent, which SAMS was combined with metal compound such as iron, zinc, cobalt or copper, were not more than 6 % in case of specimen after the leaching-test and were not more than 10 % in case of specimen before the test. On the other hand, in case of wood treated

with the agent, which boric acid compounded with SAMS, the weight loss was 0.8 % regardless of presence of the leaching-test. In case of the specimen that the SAMS—boric acid agent was fixed, the mycelium of *Tyromyces palustris* hardly stuck to the specimen even after the decay-test. The specimen, which was fixed a high molecular-weight silicic acid—boric acid, showed good decay-resistant ability, and even the weight loss of the specimen after the leaching-test was not more than about 0.5 %.

When wood was treated with only CSAS or only SAMS, the weight loss was 4-3 % for the specimen before the leaching-test; these silicic acid solutions had a little decay-resistance. However, both specimens after the leaching-test showed the weight loss of not less than 30%. On the other hand, in case of CSAS and SAMS that boric acid was combined with, the decay-resistant ability improved according to increase of the quantity of added boric acid, too.

### 3.3 Ash in the specimen after the decay-test.

Quantity of ash, which included in the specimen after the leaching-test and after the decay-test using *Tyromyces palustris* or *Coriolus versicolor*, was measured. In case of every specimen treated with SAMS—metal compound agent, the proportion of ash after a decay-test were smaller (2~3 %) than a value of WPG2. Though the fall proportion was smaller than that of a compounded agent with metal, the quantity of ash of a treated specimen with a SAMS—boric acid agent was smaller than the WPG1 and WPG2, too. In other words, chemicals leached in the decay-test from the treated wood with SAMS—boric acid agent.

### 3.4 Constitution ingredient of ash

The quantity of inorganic element in the ash of the specimen, which decayed by *Tyromyces palustris*, was assayed with ICP-MS. The fixed agent in wood-specimen leached during the decay-test, the content of every element in the specimen before the leaching-test decreased by the decay-test.

From the difference of this quantity of decrease between WPG, it was proved that SAMS—boric acid agent was fixed more strongly than SAMS—metal compound agent in wood. In case of SAMS—boric acid agents, both leaching quantity of silicon and boron were small, too. These phenomena were observed in case of both a white-rot fungus, *Coriolus versicolor* and a brown rot fungus, *Tyromyces palustris*.

### 3.5 Mechanism of preservation

#### 3.5.1 Inhibition of mycelium growth

The growth of mycelium of *Tyromyces palustris* was even restrained by only CSAS or SAMS. The ability of growth-repression of SAMS was higher than that of CSAS. Growth of mycelium was inhibited with the augmentation of quantity of the added boric acid, too. In case of the wood-powder after the leaching-test, the agents of series of CSAS inhibited the growth of mycelium than those of SAMS. These reasons estimated that concentration of antimicrobial agent in the liquid medium was low in the agent of series of SAMS, because the agents of SAMS impregnated into the cell wall of wood than the agents of CSAS [diameter of colloid: 4~6 nm, viscosity: 2.5 cP (at 20 °C)] well, and the agents were restrained to leach.

#### 3.2.2 pH of medium

Though the medium was adjusted to pH 5.5 before starting culture, the pH of medium of the agents that added a little quantity of boric acid and of the control wood-powder fell to about pH 3. In case of medium that pH deteriorated, the mycelium bred on the whole medium. However, the drop of pH was small in the medium, which was used the wood-powder treated with the agents that compounding ratio of boric acid was high. This cause estimated that the growth of *Tyromyces palustris* was inhibited by boron.

#### 3.5.3 Quantity of produced protein and enzymatic activity in case of the wood-powder before the leaching-test

##### Quantity of produced protein:

The tendency to produce protein was the same as that of growth inhibition. When the proportion was large, there was a little quantity of the produced protein.

##### Xylanase activity:

The tendency of the xylanase activity was the same as that of growth inhibition. However, the xylanase activities of the control wood-powder and the treated wood-powder with only CSAS, which the growth of mycelium was large, were low. On the other hand, the weight of mycelium could be measured in case of the wood-powder that 25 mL of boric acid—methyl alcohol solution was added to SAMS, the xylanase activity was high. The reason of these phenomena was

estimated that *Tyromyces palustris* created a large quantity of xylanase in the early time in order to resist existence of boron when little boric acid was added.

*Mannase activity:*

The tendency of the mannase activity was the same as that of xylanase activity. The reason was thought that be the same in case of xylanase activity. However, the activity of control wood-powder was high, and the activity of the wood-powder treated with CSAS was low. The reason and whether the activity of the control wood-powder was high or the activity of the wood-powder treated with CSAS was too low were not understood.

*Cx-cellulase (EG) activity:*

The EG activities were high in case of the wood-powder treated with the agent that a few quantities of boric acid was added or in case of the wood powder treated with CSAS or SAMS that boric acid was not added. In other words, in case of the wood treated with the agent of a little addition rate of boric acid, it was assumed that cellulase was formed and the decay of wood moved.

*Action of crude enzyme to microcrystal cellulose:*

*Weight loss of microcrystal cellulose:*

The weight of microcrystal cellulose decreased by the action of the crude enzyme. When the rate of boric acid added to the agent was small, the weight loss of microcrystal cellulose was large.

*C1-cellulase (CBH) activity:*

CBH-activity was assayed using filtrate in the assay of the weight loss of microcrystal cellulose. The tendency of the CBH-activity was the same as those of other enzymes, too. However, the activity was from about 1/5 to 1/50 of those of other enzymes.

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