

## Holocellulose and Lignin Content Measurement

### Holocellulose

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The major components of lignocellulosic biomass are cellulose, hemicellulose, and lignin. Holocellulose is composed of cellulose and hemicellulose. Cellulose and hemicellulose are polysaccharides, which could be hydrolyzed to a low degree of polymerization for further biological and chemical utilization. There are a variety of methods for quantifying holocellulose, such as sodium chlorite treatment, peracetic acid treatment, or other treatments. These treatments are commonly used for removal of wood lignin. Sodium chlorite treatment induces acidolysis of lignin, and the lignin is separated from biomass. This delignification method allows you to isolate and purify holocellulose.

#### 1. Materials

- ① Biomass: **Mongolian Oak** (1 group), **Larch** (2 group), **Rapeseed stalk** (3 group) → (40 mesh)
- ② Equipment: Water bath, Oven(105°C), Desiccator, Aspirator

#### 2. Methods

- ① After drying a glass filter (1G3) in oven (105°C, 24 h), cool it down in desiccator and weigh a ODW (oven dry weight) the glass filter.
- ② Insert a fat-removed sample (1.25 g) in Erlenmeyer flask (volum: 250 ml).
- ③ Add H<sub>2</sub>O (75 mL), NaClO<sub>2</sub> (0.5 g), CH<sub>3</sub>COOH (0.1 mL), and react in water bath (80°C) up to 1 hour.
- ④ Repeat the process ③ (softwood: four times, hardwood: three times, and grass: three times).
- ⑤ Filter the sample with glass filter (1G3) in aspirator (wash with H<sub>2</sub>O (250mL) + Acetone (25mL) in sequence).
- ⑥ After drying the glass filter in oven (105°C, 24 h), cool it down in desiccator and weigh a ODW (oven dry weight) the glass filter.
- ⑦ Calculate the content of Holocellulose in the sample (based on dry weight of raw material).

$$\text{Holocellulose content (\%)} = \frac{(\text{ODW glass filter} + \text{Holocellulose (g)}) - \text{ODW Glass filter (g)}}{\text{ODW sample(g)}} \times 100$$

- ※ In this calculation, ODW sample weight is needed for measuring the lignin content. But actually you use fat-removed sample for weighing. Therefore you have to modify weight of fat-removed sample to weight of ODW sample. In order to calculate weight of ODW sample, you need to consider
- ① **moisture content** and
  - ② **extractives content**.

$$\text{Moisture content (\%)} = \frac{\text{ADW sample (g)} - \text{ODW sample (g)}}{\text{ADW (air dried weight) sample(g)}} \times 100$$

## Lignin

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A various methods have been proposed for determination of lignin content. One of the most common methods to measure the lignin content is Klason lignin method. Klason lignin method is widely applied to promote carbohydrate hydrolysis using sulfuric acid. The lignin isolated by this treatment is converted to acid-insoluble lignin, or more commonly called Klason lignin. This method is basically composed of two steps: ① treatment of the lignified material with 72% (w/w) sulfuric acid up to 1 hour at 30°C followed by ② dilution of the suspension to 4% (w/w) sulfuric acid and boiling to complete the hydrolysis. During these steps, sulfuric acid attributes to chemical swelling of cellulose fibril, solubilization, and hydrolysis of hemicelluloses and cellulose.

### 1. Materials

- ① Biomass: **Mongolian Oak** (1 group), **Larch** (2 group), **Rapeseed stalk** (3 group) → (0.5mm)
- ② Equipment: Water bath, Oven (105°C), Desiccator, Auto-clave, Aspirator

### 2. Methods

- ① After drying a glass filter (1G4) in oven (105°C, 24 h), cool it down in desiccator and weigh a ODW (oven dry weight) the glass filter.
- ② Insert a fat-removed sample (0.3 g) in Erlenmeyer flask (volum: 50 ml).
- ③ Stir the sample with 3 ml of 72% (w/w) sulfuric acid solution using a glass rod.
- ④ React in water bath (30°C) up to 1 hour.
- ⑤ Dilute with distilled water (84 mL) carefully, and transfer the sample (4% (w/w) sulfuric acid solution) to Erlenmeyer flask (volum: 250 ml).
- ⑥ React the sample in auto-clave (121°C) up to 1 hour.
- ⑦ After cooling it down, filter the sample with glass filter (1G4) in aspirator (wash with distilled water (250 ml)).
- ⑧ After drying the glass filter in oven (105°C, 24 h), cool it down in desiccator and weigh a ODW (oven dry weight) the glass filter.
- ⑨ Calculate the content of Lignin in the sample (based on dry weight of raw material).

$$\text{Lignin content (\%)} = \frac{(\text{ODW glass filter} + \text{Lignin (g)}) - \text{ODW Glass filter (g)}}{\text{ODW sample(g)}} \times 100$$

- ※ In this calculation, ODW sample weight is needed for measuring the lignin content. But actually you use fat-removed sample for weighing. Therefore you have to modify weight of fat-removed sample to weight of ODW sample. In order to calculate weight of ODW sample, you need to consider ① **moisture content** and ② **extractives content**.
- ※ **Report should be written by MS words (10 points, line spacing 1) or Hancom office (10 points, line spacing 120).**
- ※ **Writing procedure of report (in Korean): 1. Introduction, 2. Materials and methods, 3. Results and discussion, 4. Conclusions, 5. References**
- ※ **You should report 1<sup>st</sup> week data and 2<sup>nd</sup> week data together, share data with other groups and compare components of samples respectively.**
- ※ **Assignment should be appended to report. (If you copy and paste, you can not get a grade)**
- ※ **If you take the average of data, you have to calculation the standard deviation.**
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