

Determination of Insoluble Solids in Pretreated Biomass Material

Laboratory Analytical Procedure (LAP)

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Technical Report
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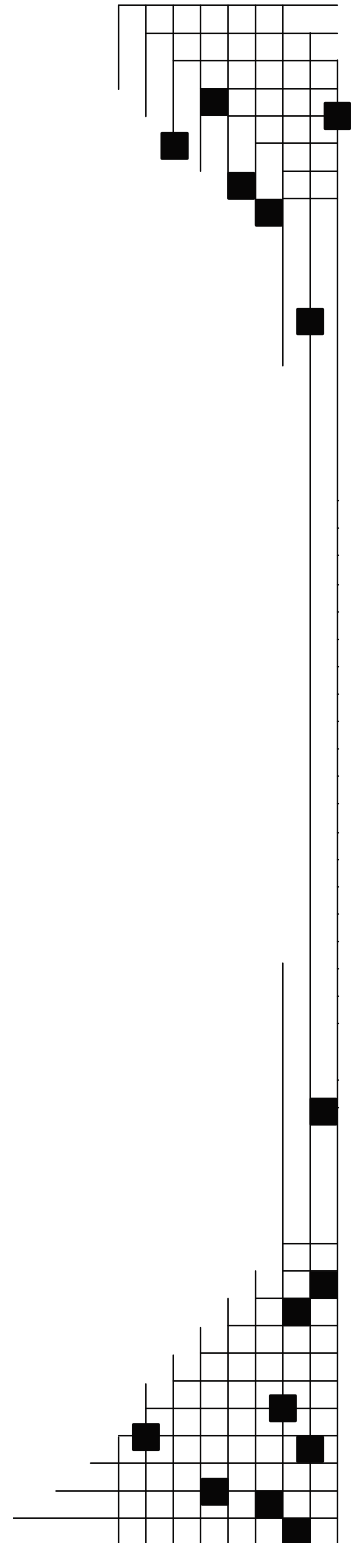
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Procedure Title: Determination of Insoluble Solids in Pretreated Biomass

Laboratory Analytical Procedure

1. Introduction

- 1.1 Pretreating biomass samples results in slurry, a mixture of liquid and solid material. For analytical purposes the liquid and solid material must be separated. The analytical results on each fraction are mathematically combined at the conclusion of the analyses to obtain mass balance. The following procedure covers two methods of separating these fractions. The centrifugation method is useful for severely pretreated samples, while the filtration method can be used for mildly pretreated “chunky” material.
- 1.2 The solid fraction must also be water washed to remove any residual liquor prior to analysis, so as to not confound the solids analysis.
- 1.3 This method may also be applied to enzymatically digested pretreated solids to close mass balance around enzymatic digestion.
- 1.4 This procedure may also be referred to as Fraction Insoluble Solids (FIS).

2. Scope

- 2.1 This procedure is used to separate the liquid and solid fractions of a pretreated biomass slurry. It is used to calculate the weight percent of insoluble material in biomass slurry. It is also used to remove any residual liquor from the solid fraction.
- 2.2 All analyses should be performed in accordance with an appropriate laboratory specific Quality Assurance Plan (QAP).

3. Terminology

- 3.1 *Oven dry weight (ODW)*- the weight of biomass mathematically corrected for the amount of moisture present in the sample at the time of weighing
- 3.2 *Pretreated biomass*- biomass that has been chemically or thermally altered, possibly changing the structural composition
- 3.3 *Slurry*- the combined liquid and solid material resulting from biomass pretreatment
- 3.4 *Liquor*- the liquid fraction of a biomass slurry
- 3.5 *Washed solids*- the water insoluble portion of a biomass slurry, water rinsed to remove any residual liquor to less than 0.05 g/L glucose.
- 3.6 *Fraction insoluble solids (FIS)*- the oven dry weight of the washed solids, referred to as a weight percentage of the slurry
 - 3.6.1 *Fraction insoluble solids dry (FISD)*- the oven dry weight of the washed solids, referred to as a weight percentage of the dry slurry weight
 - 3.6.2 *Fraction insoluble solids slurry(FISS)*- the oven dry weight of the washed solids, referred to as a weight percentage of the wet slurry weight

4. Significance and Use

- 4.1 This procedure is used, in conjunction with other procedures, to determine the chemical composition of biomass materials.

5. Interferences

- 5.1 Slurry can separate quickly. Thoroughly mix slurry prior to sampling to ensure a representative sample.
- 5.2 Centrifugation method is recommended unless slurry sample is composed of large chunky material, as the filter can quickly clog with fine material when using the filtration method. Extensive filtering time can concentrate the liquor and should be avoided. If filtration is difficult, the centrifugation technique described in 10.1 should be used.
- 5.3 Technique is critical to minimizing the amount of material lost during solids washing. When decanting liquid for the centrifugation method, minimize the loss of fine solid material.

6. Apparatus and Materials

- 6.1 Analytical balance, accurate to 0.1mg
- 6.2 Convection drying oven, with temperature control of $45 \pm 3^{\circ}\text{C}$
- 6.3 Convection drying oven, with temperature control of $105 \pm 3^{\circ}\text{C}$
- 6.4 Desiccator containing desiccant
- 6.5 YSI analyzer with appropriate membranes or equivalent glucose quantification method
- 6.6 Additional equipment required for centrifugation method
 - 6.6.1 Centrifuge refrigerated to 4°C with rotor specified to hold 500 ml bottles and rated at least 9,000 rpm
 - 6.6.2 Centrifuge bottles, 500 ml capacity, with wide mouth, and caps with seals.
 - 6.6.3 2L flask or beaker for wash water collection
 - 6.6.4 Aluminum foil weigh pans
 - 6.6.5 Glass pipettes with bulbs
- 6.7 Additional equipment required for filtration method
 - 6.7.1 Buchner funnel filters (extra large size) with glass microfiber filter paper, 30 μm or less pore size
 - 6.7.2 1 L vacuum flasks
 - 6.7.3 Vacuum source and funnel adaptors
 - 6.7.4 Spatulas

7. Reagents

- 7.1 Reagents
 - 7.1.1 Water, 18 megaohm deionized

8. ES&H Considerations and Hazards

- 8.1 Slurry may contain hazardous material. Handle accordingly.
- 8.2 Ensure that centrifuge bottles are well balanced to prevent centrifuge malfunction. Inspect each bottle and rubber o-ring for cracks prior to use to prevent leaking.
- 8.3 Follow all applicable NREL chemical handling procedures

9. Sampling, Test Specimens and Test Units

- 9.1 Vigorously shake or vortex the sample to suspend any solids. Transfer the whole slurry to a larger volume container if necessary to ensure proper mixing to achieve sample homogeneity.

9.2 Care must be taken to ensure a representative sample is taken for analysis at each step.

When measuring volumes for analysis, the sample should be at room temperature.

9.3 Store samples in sealed containers so the volatile component concentration remains consistent. Samples should be stored in a refrigerator until ready to use.

10. Procedure

Separate the slurry into liquid and solid components. Perform either 10.1 or 10.2.

Note: Centrifugation method is recommended unless slurry sample is composed of large chunky material, as the filter can quickly clog with fine material when using the filtration method. Extensive filtering time can concentrate the liquor and should be avoided. If filtration is difficult, the centrifugation technique described in 10.1 should be used.

If LAP “Determination of Total Solids in Biomass” is to be performed on whole slurry, aliquot a portion of whole slurry and set aside for this analysis.

10.1 Centrifugation method.

10.1.1 Record the weight of a labeled centrifuge bottle with labeled cap to the nearest 0.01 g on an analytical balance. Tare the balance. Add 100-150 g of homogenized slurry sample. Record the weight of the sample to the nearest 0.01 g. Tightly cap bottle.

10.1.2 Repeat step 10.1.1 for the remaining centrifuge bottles, ensuring that the total weight of each bottle with sample is equal to all other centrifuge bottles. Check centrifuge specifications for allowable weight differences between bottles. Analyze samples in duplicate.

10.1.3 Spin bottles in centrifuge at 4°C for 20 minutes at 9,000 rpm. Bottles can be spun for 10 minutes at 10,000 rpm if centrifuge is rated at least 10,000 rpm. Gently remove the bottles, taking care to not disturb the solid pellet.

10.1.4 Decant the liquor without disturbing the pellet. Residual liquid may be removed with a pipette. Save and refrigerate the liquor for later analysis. If little or no liquor is obtained from centrifugation, a pneumatic or hydraulic hand press may be used to press liquor out of the whole slurry.

10.1.5 To wash the solids, rinse the solids from the side of the bottle into the bottom of the bottle. Break up the pellet with a spatula; rinse the solids off of the spatula into the bottle. Add 100-400 ml deionized water to each bottle, ensuring that the total weight of each bottle and cap with sample is equal to all other centrifuge bottles. Each bottle should be no more than $\frac{3}{4}$ full. Cap tightly and shake each bottle vigorously for at least 30 seconds.

10.1.6 Repeat step 10.1.3. Decant the liquor without disturbing the pellet. Residual liquid may be removed with a pipette. Dispose of the wash water appropriately.

10.1.7 Repeat steps 10.1.5 and 10.1.6 until glucose remaining in wash is less than 0.05 g/L, as determined by YSI or equivalent method.

10.1.8 Record the weight of the bottle, cap, and washed sample. Perform LAP “Determination of Total Solids in Biomass”, convection oven method, on a portion of the homogenized solids. Store remaining solids in refrigerator or freezer, or dry, as appropriate.

10.2 Filtration method.

- 10.2.1 Record the weight of a Buchner funnel with filter paper to the nearest 0.01 g on an analytical balance. Tare the balance. Add 100-150 g of homogenized slurry sample. Record the weight of the sample to the nearest 0.01 g. Analyze samples in duplicate.
 - 10.2.2 Filter the sample using vacuum filtration. Filtration should last no longer than five minutes. Extensive filtering time can concentrate the liquor and should be avoided. If filtration is difficult, the centrifugation technique described in 10.1 should be used instead. Slurries with very little liquor may require a pneumatic or hydraulic hand press to press liquor out of the whole slurry.
 - 10.2.3 Save and refrigerate the liquor for later analysis.
 - 10.2.4 While sample is still under vacuum, add approximately 200 ml deionized water. Stir the sample, taking care to not disturb the filter paper. Filter the sample.
 - 10.2.5 Repeat step 10.2.3 until glucose remaining in wash is less than 0.05 g/L, as determined by YSI or equivalent method.
 - 10.2.6 Record the weight of the funnel, filter paper, and washed sample. Perform LAP “Determination of Total Solids in Biomass” on a portion of the homogenized solids. Store remaining solids in refrigerator or freezer, or dry, as appropriate.
- 10.3 If FISS is required, perform LAP “Determination of Total Solids in Biomass”, convection oven method, on a portion of the whole slurry.

11. Calculations

- 11.1 Calculate the amount of fraction insoluble solids in the slurry, utilizing LAP “Determination of Total Solids in Biomass”, convection oven method, to obtain the oven dry weight (ODW) of the washed solids and the ODW of the slurry, if necessary.

$$\%FISS(\text{Fraction Insoluble Solids Slurry}) = \frac{ODW_{\text{washed-solids}}}{\text{Weight}_{\text{bottle, cap, \& slurry}} - \text{Weight}_{\text{bottle \& cap}}} \times 100$$

$$\%FISD(\text{Fraction Insoluble Solids Dry}) = \frac{ODW_{\text{washed-solids}}}{ODW_{\text{slurry}}} \times 100$$

- 11.2 To report or calculate the relative percent difference (RPD) between two samples, use the following calculation

$$RPD = \left(\frac{X_1 - X_2}{X_{\text{mean}}} \right) \times 100$$

Where:

X_1 and X_2 = measured values
 X_{mean} = the mean of X_1 and X_2

- 11.3 To report or calculate the root mean square deviation (RMS deviation) or the standard deviation (st dev) of the samples, use the following calculations.
First find the root mean square (RMS), of the sample using

$$RMS = x_m = mean = \sqrt{\left(\frac{\sum_{i=1}^n x_i}{n}\right)^2}$$

Then find the root mean square deviation, or standard deviation, using

$$RMS\ deviation = \sigma = stdev = \sqrt{\frac{\sum_{i=1}^n (x_i - x_m)^2}{n}}$$

Where:

x_m =the root mean square of all x values in the set

n=number of samples in set

x_i =a measured value from the set

12. Report Format

12.1 Report percent insoluble solids to two decimal places. RPD and RMS deviation may also be reported.

13. Precision and Bias

13.1 Determined by data quality objectives and laboratory specific Quality Assurance Plan.

14. Quality Control

14.1 Reported Significant Figures or Decimal Places: Determined by data quality objectives and laboratory specific Quality Assurance Plan.

14.2 Replicates: Duplicate analyses, at minimum.

14.3 Blank: None.

14.4 Relative percent difference criteria: Determined by data quality objectives and laboratory specific Quality Assurance Plan.

14.5 Calibration verification standard: None.

14.6 Sample size: 50-200 g

14.7 Sample storage: Store slurry and liquor samples in the refrigerator in sealed containers so the volatile component concentration remains consistent. Wet solids should be stored in sealed containers in the refrigerator or freezer. Solid samples with less than 10% moisture by weight may be stored in sealed containers at room temperature.

14.8 Standard storage: None.

14.9 Standard preparation: None.

14.10 Definition of a batch: Any number of samples which are analyzed and recorded together. The maximum size of a batch will be limited by equipment constraints.

14.11 Control charts: None.

15. Appendices

15.1 None.

16. References

16.1 NREL LAP “Determination of Total Solids in Biomass”.

16.2 NREL Ethanol Project CAT Task Laboratory Analytical Procedure #018, “Determination of Insoluble Solids of Pretreated Biomass Material”.