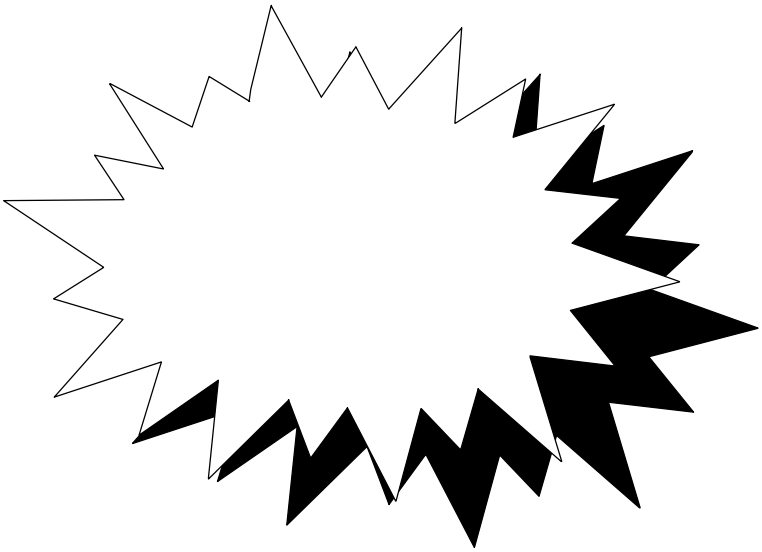

Chemical Analysis and Testing Task

Laboratory Analytical Procedure

LAP-018

Procedure Title: **Determination of Insoluble Solids of Pretreated Biomass Material**

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Determination of Insoluble Solids in Pretreated Biomass

Laboratory Analytical Procedure #018

1. Introduction

1.1 Pretreated biomass samples are composed of water-soluble and water insoluble components. These two fractions are analyzed separately. Analytical results are then mathematically recombined in the computation of the mass balance on the pretreatment process. To separate the water soluble portion from the sample a thorough water extraction is performed leaving a portion of solid material called the insoluble solids fraction of the sample. This method describes two reliable procedures for determining both the percent insoluble solids and percent fraction insoluble solids in a sample of hydrolyzate slurry from pretreated biomass.

2. Scope

2.1 This procedure is intended to determine the percentage of water insoluble solids in a pretreated biomass sample after all soluble components have been extracted with aggressive water washing.

2.2 All analyses shall be performed according to guidelines established by the Ethanol Quality Insurance Plan.

3. References

3.1 NREL Ethanol Project Laboratory Analytical Procedure #012, "Standard Test Method for Moisture, Total Solids, and Total Dissolved Solids in Biomass Slurry and Liquid Process Samples.

4. Terminology

4.1 **Pretreated Biomass**-Biomass which has been chemically and/or thermally altered to change the structural composition.

4.2 **Hydrolyzate slurry**-The liquid and solid material in a sample resulting from biomass pretreatment.

4.3 **Hydrolyzate Liquor**- Liquid portion of hydrolyzate slurry.

4.4 **Washed Solids-**Water insoluble portion of hydrolyzate slurry.

4.5 **Pressate**-Liquid product from pretreated biomass pressed via centripetal force, manual or hydraulic pressure.

4.6 **Filtrate**-Hydrolyzate liquid product from hydrolyzate slurry which has been placed in a Buchner funnel and vacuum filtered.

4.7 **Oven Dried Weight**-The moisture-free weight of a biomass sample as determined by Lap-001, Standard Method for Determination of Total Solids in Biomass.

4.8 **Insoluble Solids (IS)-**The oven dried weight of water insoluble solids divided by the weight of whole hydrolyzate slurry sample (as received).

4.9 **Fraction Insoluble Solids (FIS)-**The oven dried weight of water insoluble solids divided by the *oven dried weight* of the whole hydrolyzate slurry.

5. Significance and Use

5.1 The percent insoluble solids and the percent fraction insoluble solids are used to combine the liquid and solid compositions of the pretreated biomass in the mass balance determination.

6. Interference

6.1 Technique is critical to minimizing the amount of material lost during the wash steps. Care should be taken when separating the liquid from the solids or results will not meet standard quality assurance requirements.

6.2 Hydrolyzate slurries separate quickly. Special attention is recommended when taking samples .To obtain a representative sample thoroughly mix prior to taking a sample.

7. Apparatus *Procedure A (Centrifugation)*

7.1 Analytical balance readable to 0.1 mg.

7.2 Convection ovens with temperature control to 45 °C ± 3 and 105 °C ± 3.

7.3 Analytical balance readable to 0.01g.

7.4 Desiccator.

7.5 Centrifuge refrigerated to 4°C and rotor specified to hold 300 ml. capacity bottles and rated at least 9,000 rpm.

7.6 Aluminum foil weighing dish.

7.7 Centrifugation bottles with wide opening, caps with seals and 300 ml capacity/reservoir.

7.8 Biochemical analyzer (YSI) for measurement of glucose (optional).

7.9 Eppendorf microcentrifuge tubes and transfer pipettes (optional).

8. Apparatus *Procedure B (Filtration)*

8.1 Whatman GF/D 2.7um glass microfiber FilterCup (1600R823) with FilterCup stem (1600R900). An alternate is Buchner funnels (two-part, polypropylene) with GF/D glass microfiber filter paper.

8.2 Glass microfiber filter paper sized for the Buchner funnel chosen.

8.3 1000 ml vacuum flask.

8.4 Filtration set-up including vacuum source and vacuum adapters for Buchner funnels.

8.5 Items 1-4 as described in section 7.

8.6 Teflon coated spatulas.

9. Reagents and Materials

9.1 pH paper (range 2-9).

9.2 Electronic pipette and disposable pipettes in the range of 25 ml.

9.3 2,000 ml flask or beaker.

9.4 YSI dextrose standards 2.0 g/L, 9.0 g/L (optional).

9.5 Water 18 megaohm deionized.

10. ES&H Considerations and Hazards

10.1 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

11. Procedure A *(Centrifugation)*

11.1 Record weight of *centrifuge bottle and cap* to nearest the 0.01g on an analytical balance and tare.

11.2 Add 25-50 grams of sample. Record *weight of sample as received* to the nearest 0.01g.

11.3 Add 175-200 grams of deionized water. Shake vigorously for 60 seconds. (Note: If collecting liquor for further analysis, do so prior to this step).

11.4 Cap and spin in centrifuge at 4°C for 20 minutes at 9,000 rpm.

11.5 Carefully remove bottles from centrifuge to minimize disturbance of sample pellet.

11.6 Remove liquid portion by decanting without disturbing sample pellet. Use electronic or manual pipetter to remove small amounts of liquid until small traces are left.

11.7 Repeat steps 10.3-10.6 three more times or until sample pH is at least 4.5-7.0. (For some applications it may be necessary to test the amount of glucose present by biochemistry analyser in determining if another wash is necessary. Glucose remaining in the last wash should not exceed more that 0.5 g/L.)

11.8 Record weight of *bottle, cap and sample* to the nearest 0.01g. Then subtract weight of bottle and cap from this recorded weight to caluculate and record the *weight of washed*

sample.

11.9 Perform total solids on the as received and washed material as described in Lap-001.

11.10 Store remaining sample in refrigerator or freezer as appropriate to maintain sample integrity.

12. Procedure B *(Filtration)*

12.1 Record weight of buchner funnel filter cup with glass filter paper and record to the nearest 0.01g on analytical balance and tare.

12.2 Add 25-50 grams of sample. (Record *weight of sample as received*).

12.3 Gradually pour 200-250 ml of deionized water. Stir gently to mix sample. Allow vacuum filtration to proceed slowly.

12.4 Use pH sticks to test the pH of the filtrate and sample.Repeat steps 12.2-12.3 three more times or until sample pH is at least 4.5-7.0. (For some applications it may be necessary to test the amount of glucose present by biochemistry analyser in determining if another wash is necessary. Glucose remaining in the last wash should not exceed more than 0.5 g/L.)

12.5 Let process sit under vacuum for 5 minutes to remove excess water. Remove filter cup and place on paper towel to allow water accumulation on filter cup bottom to escape.

12.6 Weigh filter cup with glass fiber filter paper and sample. Record weight. Subtract the weight of the filter cup and filter to get the *wet weight of the washed sample*. Then place in 105°C oven for overnight.. *(If alternate Buchner funnel is used check with manufactuer for temperature limitations. Use 45°C as oven temperature if necessary and dry to a constant weight.)*

12.7 Remove filter cup with sample from oven and place in desiccator for five minutes. Weigh filter cup + filter + sample and record. Subtract weight of filter cup and filter paper to get dry sample weight. Record *dry weight of washed sample* to the nearest 0.01 grams.

12.8 Perform total solids on the as received material as described in Lap-001. The % total

solids on the washed sample is calculated from *dry weight of washed sample* in step 12.7.

12.9 Discard or store sample as necessary.

13. Calculations

13.1 Calculate the percent insoluble solids and fraction insoluble solids for each sample on a percent dry weight basis. Total solids by LAP-001, on the as received and the washed sample will be necessary for each procedure. However, in *Procedure B (Filtration)* the percent total solids of the washed material is calculated based on the whole sample weight.

Procedure A (Centrifugation)

$$\textit{Dry Weight of Washed sample} = \frac{\textit{(Weight of washed sample) (\% Total solids of washed sample)}}{100}$$

$$\textbf{\textit{\% Insoluble Solids}} = \frac{\textit{Dry weight of washed sample}}{\textit{Weight of sample as received}} \; X\ 100$$

$$\textit{Dry Weight of Sample As Received} = \frac{\textit{(Weight of Sample As Received) X (\% Total Solids As Received)}}{100}$$

$$\textbf{\textit{\% Fraction Insoluble Solids}} = \frac{\textit{Dry Weight of Washed sample}}{\textit{Dry weight of sample as received}} \; X\ 100$$

13.2 Percent insoluble solids and percent fraction insoluble solids can be calculated as described in Procedure A provided the percent total solids are calculated as demonstrated below. The percent total solids of the washed sample are based on the entire sample that is washed and dried in the filter cup.

Procedure B (Filtration)

Weight of washed sample = (Weight of filter cup, filter, & washed sample) – (Weight of filter cup & filter)

***% Total Solids on As Received Sample** = As described in LAP 001*

$$\textbf{\% Total Solids on Washed Sample} = \frac{\text{Dry Weight of the Washed Sample}}{\text{Wet Weight of Washed Sample}} \times 100$$

14. Report

14.1 Report the result as a percentage with two decimal places.

14.2 For replicate analyses of the same sample, report the average, standard deviation, and relative percentage difference.

15. Precision and Bias

15.1 Analysis in one laboratory of a method verification standard showed a sample recovery of 95.6% (IS) and 100.48 (FIS) with a coefficient of variation of 1.28% (IS) and 1.52% (FIS) for Procedure B (Filtration). In Procedure A (Centrifugation) the recovery values were 96.49% (IS) and 100.09 (FIS) with a coefficient of variation of 1.56% (IS) and 1.78% (FIS). Estimation of standard deviation from duplicate numbers was 2.46 (IS) and .03(FIS) for Procedure A(centrifugation) and 0.09(IS) and 0.006)(FIS) for Procedure B(filtration).

15.2 Statistical analysis for several different types of biomass material gave an estimation of standard deviation of 0.59(IS) for Procedure A and 0.10(IS) Procedure B. Statistical analysis comparing both procedures indicated no significant difference between the two methods, assuming a 95% confidence level.

16. Quality Control

16.1 *Reported significant figures:* Report all data to two significant figures.

16.2 *Replicates:* Run all samples in duplicate or triplicate if time permits.

16.3 *Blank:* Not applicable.

16.4 *Relative percent difference criteria:* % RPD should not exceed 6%.

16.5 *Method verification standard:* A method verification standard should be run with each sample set. Solka Floc can be processed as the MVS by both procedures. Use 10-15g sample size when selecting Solka Floc as the MVS.

16.6 *Calibration verification standard:* Not Applicable.

16.7 *Sample size*: A minimum of 25-50 grams should be processed in these procedures. It is possible scale up the process depending on equipment and time constraints.

16.8 *Sample storage* Samples shall be stored in the refrigerator.

16.9 *Standard storage: Not Applicable.*

16.10 *Standard preparation:* Not Applicable.

16.11 *Definition of a sample set*: Any number of samples analyzed together and recorded together within the limitation of instrumentation and time requirements.

16.12 *Control charts:* A control chart should be kept for the method verification standard.