Research paper

Improved methods for the determination of drying conditions and fraction insoluble solids (FIS) in biomass pretreatment slurry

Amie Sluiter[*], Justin Sluiter, Ed Wolfrum, Michelle Reed, Ryan Ness, Christopher Scarlata, Jeanette Henry

National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO, 80401, USA

ABSTRACT

Accurate and precise chemical characterization of biomass feedstocks and process intermediates is a requirement for successful technical and economic evaluation of biofuel conversion technologies. The uncertainty in primary measurements of the fraction insoluble solid (FIS) content of dilute acid pretreated corn stover slurry is the major contributor to uncertainty in yield calculations for enzymatic hydrolysis of cellulose to glucose. This uncertainty is propagated through process models and impacts modeled fuel costs. The challenge in measuring FIS is obtaining an accurate measurement of insoluble matter in the pretreated materials, while appropriately accounting for all biomass derived components. Three methods were tested to improve this measurement. One used physical separation of liquid and solid phases, and two utilized direct determination of dry matter content in two fractions. We offer a comparison of drying methods. Our results show utilizing a microwave dryer to directly determine dry matter content is the optimal method for determining FIS, based on the low time requirements and the method optimization done using model slurries.

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1. Introduction

The potential positive impacts of alternative fuels, in this case biofuels, has been long established [1] [2] [3]. Good analytical chemistry is fundamental to research, development, and production process control. Most biofuel feedstocks require preprocessing to begin the deconstruction of lignocellulosic biomass before upgrading the carbohydrate and lignin intermediates to fuels or products [4]. One common approach is pretreatment, where the feedstock is subjected to chemical, aqueous, or mechanical degradation at a specified temperature and residence time. These processes typically produce solid-liquid slurries. The consistency of the slurry is dependent on the type of pretreatment used, and can range from a thick paste to damp clumps and particulates.

Slurries contain two phases and have historically been separated prior to characterization with wet chemical analytical methods. The amount of insoluble solids in the slurry is termed the fraction insoluble solids (FIS). The solid phase contains much of the lignin, cellulose, and insoluble ash. The solubilized material, or soluble solids, is another class of material in the slurry that typically is comprised of sugars, low molecular weight lignin, products of carbohydrate depolymerization, inorganic compounds, acetic acid and extractive materials [5]. The soluble and insoluble fractions are characterized separately, and the overall composition of the slurry is determined from these individual characterizations [6].

Previous work has examined the effect of primary measurement uncertainties on calculated yield values of the unit operations comprising biomass conversion to ethanol, and the resulting uncertainty in process economics [7]. This work showed that the FIS value is the single largest contributor to enzymatic hydrolysis and fermentation yield uncertainty, which influences cost uncertainty in the overall process. Because of this influence, we determined that improvements in FIS measurements would be very valuable.

Two FIS determination methods have been published by NREL. The first is the "wash" method, a NREL Laboratory Analytical Procedure that involves centrifugation to separate the liquor and solid, then exhaustive washing of the solids by sequential washing/centrifugation/decanting steps to remove liquor entrained in the solid phase [8]. This procedure results in a solid phase that is free of

any soluble components, which is referred to as washed solids [9] [10]. The dry matter (DM) content of both the washed solids and the original whole slurry are then determined, typically by oven drying. FIS is then calculated as:

$$FIS_{wash} = \frac{Weight_{dry\ washed\ solids}}{Weight_{dry\ slurry}} \qquad (1)$$

Note that in Eq. (1), the denominator can also be the weight of the wet slurry, generally used when determining values for which the slurry remains intact, such as enzymatic hydrolysis or fermentation. However, here we are considering the dry weights of both fractions, typically used for mass balance around pretreatment processes.

This method typically requires 6-10 separate washing/centrifugation steps to produce a decant glucose concentration below 0.05 g/L. An efficient analyst can perform the wash FIS procedure on 6 to 12 samples in a single day (not including drying time), depending on slurry volume, liquor carbohydrate concentration, and sample consistency. This method has several drawbacks. Results from this method are highly technique dependent, since it requires the analyst to decant wash water while retaining any fine material present. It is also time intensive, and throughput is limited to the available centrifuge space and run time.

The second method, referred to as the "no-wash" method, calculates FIS from measurements of the dry matter content of the whole slurry and of the separated liquor phase:

$$FIS_{no-wash} = \left(\frac{DM_{whole\ slurry} - DM_{liquor}}{1 - DM_{liquor}} \right) \div DM_{whole\ slurry} \qquad (2)$$

The development of this equation, and data comparing the wash and no-wash FIS methods, have been published [11]. The derivation of this equation is somewhat lengthy, and the theory and derivations can be found in the referenced publication. This method offers the advantage of eliminating the laborious washing steps to produce the washed solids, although a liquor separation step is required. An efficient analyst can process up to 20 samples over the course of two nonconsecutive days, again not including time required for drying. The drying techniques used in this method were not previously optimized.

In this work we compare the traditional wash method to two no-wash methods: the previously described published method and one no-wash method utilizing optimized drying methods. We recognize that all FIS calculation methods are subject to difficulties in homogenizing the slurry in order to take a representative sample. This difficulty is outside the scope of this work; here we focus on the effect of different drying procedures on FIS calculations using either Eq. (1) or Eq. (2).

Measuring the dry matter (DM) content of a given samples is conceptually trivial: the DM is simply the difference in weight before and after drying divided by the original ("wet") weight. When using a pan balance, the appropriate equation is:

$$
\begin{aligned}
DM &= \frac{Weight_{dry\ sample}}{Weight_{sample\ as\ received}} \\
&= \frac{\left(Weight_{weigh\ pan\ plus\ dry\ sample} - Weight_{weigh\ pan} \right)}{\left(Weight_{weigh\ pan\ plus\ sample\ as\ received} - Weight_{weigh\ pan} \right)}
\end{aligned}
$$
$$(3)$$

The numerator of Eq. (3) is typically called the "oven dry weight" (ODW) of a sample. The DM value can be multiplied by 100 and expressed as a percentage, and is sometimes referred to as the "%

solids" value. The moisture or water content of a sample is often calculated as 1-DM.

However, measuring the water content of a sample is surprisingly difficult, particularly when dealing with samples that contain soluble acids or bases (e.g., H_2SO_4, NaOH), or volatile materials. Acids and bases will increase in concentration during drying and potentially catalyze further chemical reactions of other soluble compounds, and volatile compounds such as acetic acid or furans can evaporate along with water [12]. The standard methods for the determination of DM (or alternatively, moisture content) use either a conventional or vacuum oven, and were developed for feedstocks, not the pretreated biomass slurries of interest in this work [13–15,9]. A number of papers have been published on the subject demonstrating the difficulty of drying complex sample matrices, including biases between methods, sample degradation, and side chemical reactions [16–19].

Oven methods are not the only drying methods commonly applied. Techniques like Karl Fischer coulometry titration, toluene distillation, lyophilization, infrared driers, and microwave driers are also used [17]. Some of these methods were ruled out for this study. Previous work in our laboratory showed that coulometry determinations were unsuitable for solid and slurry analysis, as the solids interfere with the titrations. During previous toluene distillations, the liquors charred, so that method was ruled out as well. Previous attempts to dry liquor via lyophilization resulted in incomplete drying even after five days [12]. Infrared drying resulted in incomplete liquor drying and charred slurry solids.

In this work we examined oven and microwave drying options. We first consider the effects of drying method on the determination of DM in liquor and whole slurry samples. We then use these results to compare wash and no-wash FIS calculations based on these drying methods.

2. Materials and methods

2.1. Oven drying

Both conventional and vacuum oven systems were used. We investigated oven temperatures of 105 °C, 80 °C, 60 °C, and 40 °C in a variety of combinations with oven type. Vacuum oven pressures were 7−10 psi, varying only with house vacuum pressures that are not controllable. Disposable aluminum weighing pans were used for all oven drying experiments. Weigh pans that were used as received are referred to as "air dry" pans, while weigh pans that were dried at 105 °C for a minimum of 24 h and stored in a desiccator until use are referred to as "oven dry pans".

2.2. Microwave drying

We developed two drying methods for the microwave dryer, one for liquors and one for whole slurries. The liquor method used less power to dry samples, while the slurry method required more power to dry slurries quickly. We used two microwave moisture analyzers (LMA200PM, Sartorius, Bohemia, NY).

Liquors were analyzed by putting two glass fiber sample pads (69MA0325, Sartorius) on the weighing module, taring the unit, and then using a plastic transfer pipet (282, Samco Scientific) to add 2 g ± 0.25 g of liquor directly on the pads and starting the unit. The liquor moisture determination method was run in low range standard analysis mode, with a 3-stage drying profile (30% power for 9 min, 100% power for 1 min, 100% power until weight did not change more than 2 mg in 30 s), 0.1 mg weight resolution, and 0.01% percent resolution.

Slurries were analyzed by putting two sample pads on the weighing module, taring the unit, removing the pads and

separating them, adding 2 g ± 0.25 g of slurry directly on one pad, covering the sample with the other pad, placing in the unit, and starting. Care was taken to place the slurry in a thin even layer to prevent hot spots. The slurry moisture determination method was run in low range, standard analysis mode, 3-stage drying (50% power for 6 min, 75% power for 1 min, 80% power until weight did not change more than 2 mg in 30 s), 0.1 mg weight resolution, and 0.01% percent resolution. A maximum weight of 2.25 g of slurry was used to prevent potential combustion of the sample.

2.3. Materials

We used a set of 55 pretreated corn stover slurries in this study. All three FIS methods were performed on a subset of these 55 samples. Corn stover slurries were obtained from 2012 to 2014 NREL pretreatments, and included dilute-acid or steam pretreatments. Liquor samples were obtained from the whole slurry by either centrifugation or manual bench top pressing (3851, Carver, Inc., Wabash, IN), and then filtering to remove any residual solids.

We utilized synthetic liquor solutions to perform optimization experiments, since the matrix could be simplified or made more complex as needed. The base solution was 1, 5, or 10% w/v glucose and/or NaCl. Neutral solutions were made up with water, while acidic solutions were made up with a 1%w/w H_2SO_4 solution.

3. Results and discussion

3.1. Comparison of drying methods - synthetic liquors

Synthetic liquor samples containing 1, 5, or 10% w/v glucose, NaCl, or glucose/NaCl mixtures were dried for three days with various combinations of temperature and oven type, using "air dry" and "oven dry" aluminum weigh pans. Each liquor sample was analyzed in triplicate. Figs. 1–3 show the results, with a recovery calculated as measured weight/expected weight. A recovery of 1.0 indicates all of the water was removed and all dry matter accounted for. A recovery of less than 1.0 indicates that less dry material remained than expected, and suggests sample volatilization or degradation, while a recovery greater than 1.0 indicates that more dry matter remained than expected and suggests incomplete
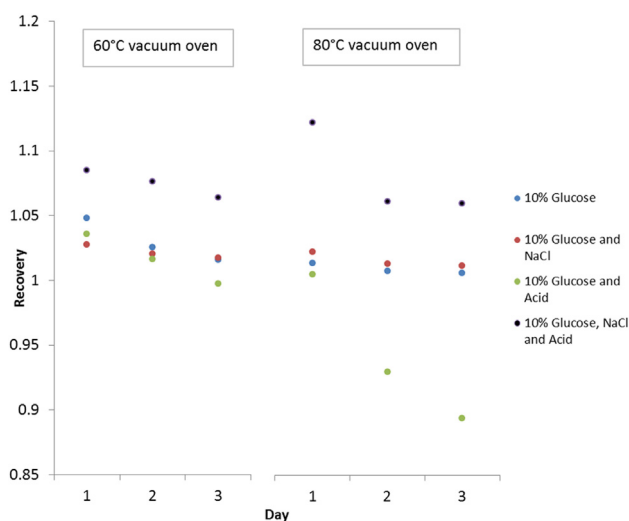
Fig. 2. Results of drying synthetic liquor samples of varying glucose, NaCl, and H_2SO_4 concentrations in the 60 °C vacuum oven and 80 °C vacuum oven for three days. Recovery was calculated as measured weight/expected weight. All points are averages of triplicate measurements. Blue line is perfect recovery. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
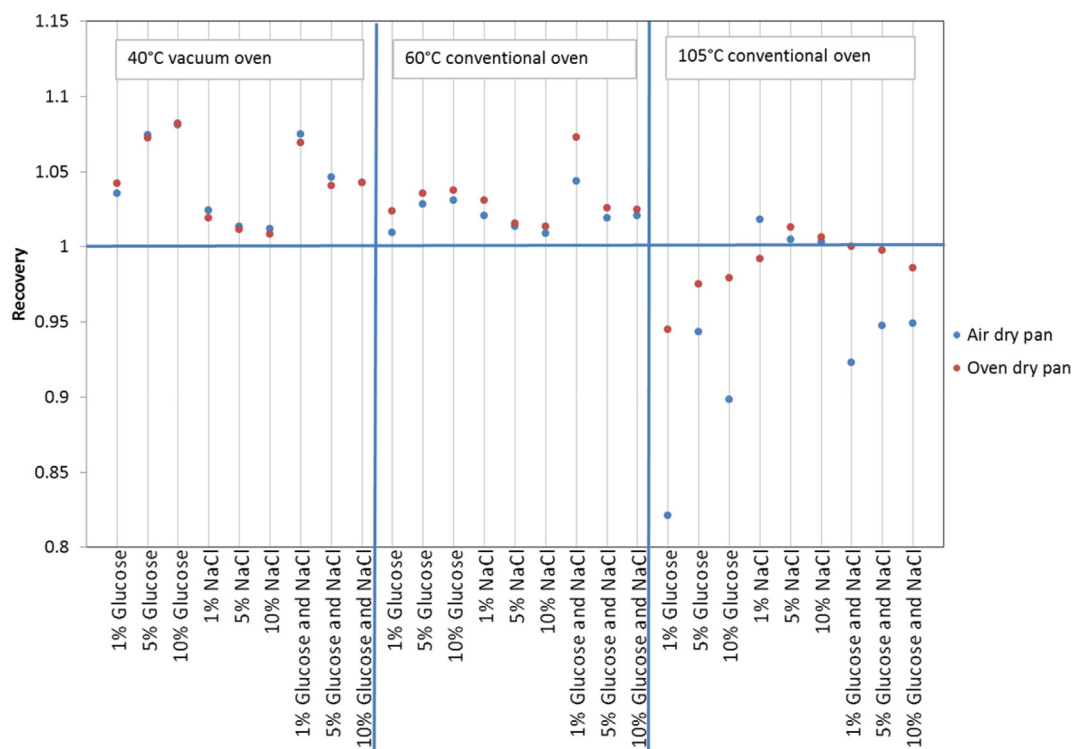
Fig. 1. Results of drying synthetic liquor samples with varying glucose and NaCl concentrations for three days. Oven conditions include 105 °C conventional oven, 40 °C vacuum oven, and 60 °C conventional oven. Results using air dried pans and 105 °C dried pans are referred to as wet and dry, respectively. Recovery was calculated as measured weight/ expected weight. All points are averages of triplicate measurements.
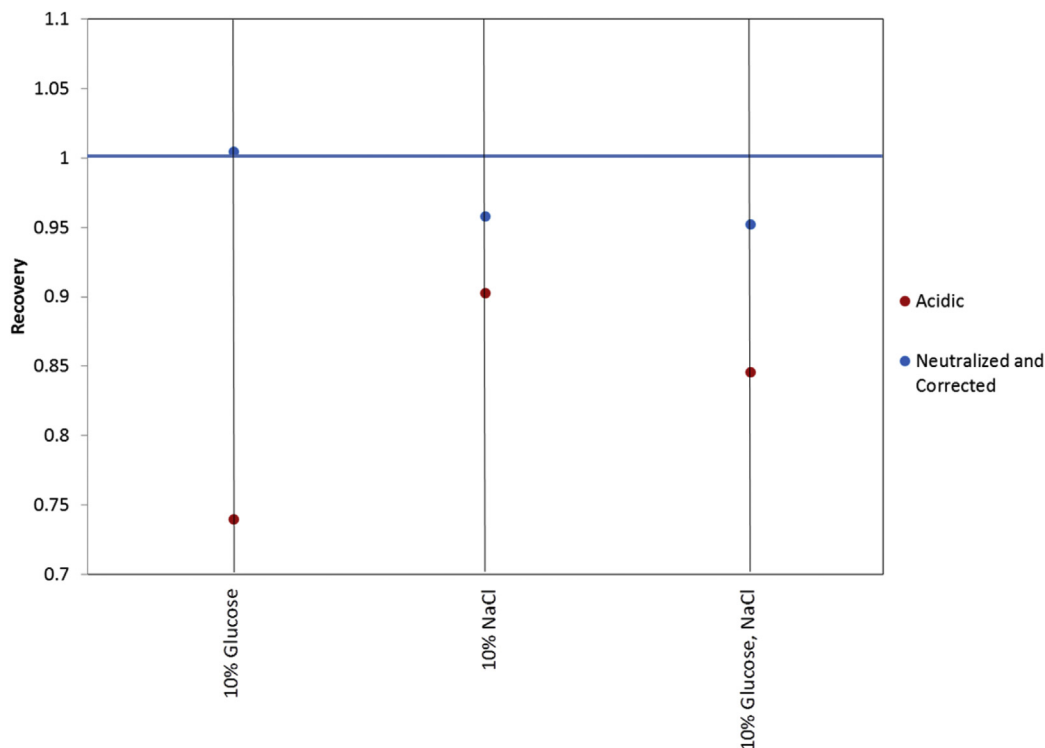
Fig. 3. Results of drying a variety of synthetic liquor samples with acidic and neutral pH in an 80 °C vacuum oven for three days. Acidic samples were originally 1% H2SO4, and were neutralized with NaOH. Recovery was calculated as measured weight/expected weight. All points are averages of triplicate measurements. Blue line is perfect recovery. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

drying.

The data in Fig. 1 show the three standard oven methods used for drying: 105 °C conventional oven, 40 °C vacuum, and 60 °C conventional oven. The results indicate that oven drying at 105 °C results in recoveries lower than the other two oven methods, and less than 1.0 for the majority of the glucose-containing samples. These results are not surprising, since carbohydrates, including glucose, begin to degrade at 100 °C [17]. Drying in the 60 °C oven or the 40 °C vacuum resulted in recoveries greater than 1.0 for all samples tested. Samples dried at 60 °C were more accurate (recoveries closer to 1) and precise than the other two methods. All three methods were statistically significantly different (p = 0.05). We also found using ANOVA (data not shown) that using "oven dry" aluminum weigh pans, pans dried at 105 °C in an oven for a minimum of 24 h and stored in a desiccator, significantly improved accuracy as compared with air dry pans, pans used as received, and all subsequent data are shown with oven dried pans.

Since the results shown in Fig. 1 suggest that neither the 105 °C nor the 40 °C vacuum oven were appropriate for drying liquors, we investigated conditions between these two extremes: 60 °C and 80 °C vacuum ovens. We also added acid as a variable to mimic the conditions of real liquor, which are often acidic. We chose 1% H_2SO_4 as a representative acid concentration that is generally used during pretreatment. Recovery was measured every 24 h for four days, with samples being returned to the oven between measurements, and all samples analyzed in triplicate.

Fig. 2 shows the results of these experiments. The 60 °C vacuum oven demonstrates insufficient drying, with the exception of the acidic glucose solution. As the neutral glucose solution did not show this behavior, we attribute the decrease in recovery below 1.0 in the acidic glucose solution to the acid concentrating during drying, causing reactions that degrade glucose. This trend is more apparent at 80 °C, where the glucose and acid samples quickly drop

below a recovery of 1.0. For samples without acid present, the recovery was improved at higher drying severity, with the best recovery at 80 °C. Samples of NaCl with and without acid showed a recovery slightly above 1.0 in both the 60 °C and 80 °C vacuum oven. However, the salt and acid together showed a recovery of 1.15–1.2. We believe this to be a result of reaction chemistry between the salt, acid, and aluminum pan. This conclusion is further backed up by the blackening observed on the aluminum pans where the solution dried.

From these results we determined that high temperature drying of acidic solutions with carbohydrates present leads to biased results. To mitigate the degradation effects of acid, we neutralized the acidic solutions with a dilute sodium hydroxide solution (4.16% w/w) prior to drying in a vacuum oven at 80 °C. Fig. 3 shows the effect of neutralizing acidic solutions that contain glucose and/or salt. As before, we see that glucose dried in an acidic solution is degraded, but neutralization of the samples improves recovery substantially. The addition of the neutralization step complicates the calculation of DM since we need to account for the addition of sodium hydroxide solution to the liquor and the production of water from the neutralization reaction between NaOH and H_2SO_4. We can modify Equation (3) to account for this. This correction is shown in Eqs (4)–(7). Upon correction, the neutralized samples, particularly those containing glucose, come much closer to a recovery of 1.0.

$$DM = \frac{\left(Weight_{dry\ pan plus dry\ sample} - Weight_{dry\ pan}\right)}{Weight_{sample\ as\ prepared}}$$
$$= \frac{Dry\ solids(g)_{corrected}}{Wet\ solids(g)_{corrected}} \qquad (4)$$

$$Dry\ solids(g)_{corrected} = Measured\ dry\ solids\ - Na^+(g)\ +\ H^+(g) \tag{5}$$

$$H^+(g) = \frac{1}{23}\ x\ Na^+(g) \tag{6}$$

$$Wet\ solids\ (g)_{corrected} = Measured\ wet\ solids(g)$$
$$- NaOH\ solution(g) \tag{7}$$

If an oven drying technique is required, we recommend drying neutralized liquors in an 80 °C vacuum oven, and accounting for the base and loss of water upon neutralization, which provides the most accurate data for liquors containing both acid and carbohydrates.

3.2. Comparison of drying methods — whole slurries and isolated liquors

Having tested methods on synthetic samples, we analyzed a set of whole slurries, dilute-acid or steam pretreated corn stover, to examine the applicability of these methods to process relevant samples. In whole slurry samples most of the concern in drying comes from the reactions in entrained liquor, as much higher temperatures are required to affect structural polymeric carbohydrates. Fig. 4 shows the comparison of drying slurries and isolated pretreatment liquors at 40 °C under vacuum and using the microwave developed slurry method.

The first plot in Fig. 4 describes drying whole slurry. The R^2 value is 0.99, and an f-test shows the variances to be equal. Paired t-tests (p = 0.05) demonstrate that both methods have equivalent population means. These results indicate that drying whole slurry is not as condition-sensitive as drying liquors. Therefore, oven drying acidic slurries at ambient pressure or under vacuum at a temperature of 60 °C or below is recommended to minimize liquor reactions, with 60 °C maximum chosen based on results from synthetic sample degradation.

The second plot in Fig. 4 illustrates the difference in drying isolated pretreatment liquors at 40 °C under vacuum and using the microwave developed liquor method. The correlation between the results from the vacuum oven and the microwave oven is very high ($R^2 = 0.99$). A small but statistically significant difference in the slope can be measured, but the intercept is not statistically different from zero (p = 0.05). The microwave generally provides higher total solids values, likely because the oven method is not completely drying the liquor samples. This is consistent with the results we saw when drying synthetic liquors.

3.3. Microwave drying

The oven-drying data suggest that carbohydrate degradation in the presence of acid is greater at longer times, such as three days. We concluded that a faster option might be helpful, so we investigated a microwave drying system with drying times less than ten minutes. We also added volatiles to some of the synthetic solutions, to more closely mimic true liquors. We included furfural and hydroxymethylfurfural (HMF), carbohydrate degradation products common in acid pretreated liquors and slurries [20]. We added 0.25 g/L and 1.27 g/L, respectively.

Fig. 5 shows the recovery of synthetic liquors after drying in the microwave oven. A comparison of samples identical in composition except for the absence or presence of volatile components shows that the samples with volatile components recover less dry matter than expected (red data). When the volatiles are removed from the calculation of the *expected* dry matter (blue data or DM* in Fig. 5), the DM recoveries are much closer to 1.0. From this we conclude that volatiles are completely or almost completely removed by the microwave. These assumptions were validated by measuring the dry matter content of solutions of volatiles alone; the apparent DM values were zero (data not shown). The DM* measurement still shows less than perfect recovery. Decreasing this uncertainty is an area for future improvement.

Once the expected dry matter weight is accounted for in the calculations, it is apparent that the microwave drier provides equivalently accurate data compared to oven methods (Figs. 3 and 5), with no need to neutralize the samples prior to drying.

Fig. 6 shows a comparison of the microwave method to the optimized oven methods (80 °C vacuum oven with neutralization for liquors and ambient pressure 60 °C oven for slurries) for the determination of dry matter content in three pretreated whole slurry associated liquor samples. The replication between methods validates the microwave as a viable option for measuring dry matter. The slight variance in slurry A is due to the heterogeneity of the sample, as it contained large chunks of biomass that were difficult to representatively subsample at a small scale. The microwave methods developed for slurries and liquors work for both acidic and neutral samples, and are the recommended drying methods.

3.4. Fraction insoluble solids (FIS) calculations

Since the FIS calculations in equations (1) and (2) use the dry solids content of either slurry or the slurry and liquor fractions, it is reasonable to discuss how these drying methods affect the calculated values of FIS.

To determine the FIS standard deviation for all three methods, seven random samples were analyzed in triplicate. Whole slurry samples, washed solids, and liquors were all dried in a vacuum oven at 40 °C for three days, as per the standard procedures [21], for the wash and no-wash oven methods. Equations (1) and (2) were used to calculate the wash and no wash FIS values, respectively. Slurry and liquor samples were analyzed for DM using the microwave methods described above. Equation (2) was used to calculate no wash FIS.

The mean, pooled standard deviation, and minimum and maximum standard deviations are shown in Table 1. The microwave method is close to the wash method, while the no wash oven method has a smaller pooled standard deviation. Although the precision of the microwave method is not comparatively the best value, we believe the accuracy of the method to be greater, as described below.

As we cannot determine the true FIS value in an unknown, we must rely on best practice methods developed for accuracy in the previous experiments. The microwave method demonstrates a range of standard deviations similar to the wash method, but it has been optimized using known samples, and we can assume it provides the most accurate values. The three FIS techniques are compared in Fig. 7: wash using a conventional 40 °C vacuum oven, no-wash using a 40 °C vacuum oven, and microwave. The wash method did not correlate well with the no-wash vacuum oven method or the microwave method, with R^2 values of 0.39 and 0.48, respectively. The two no-wash methods (vacuum oven and microwave) were more highly correlated ($R^2 = 0.71$). As the wash method and no-wash using a 40 °C vacuum oven do not correlate well with the microwave method, we determined that neither of these FIS methods is ideal, as compared to the optimized
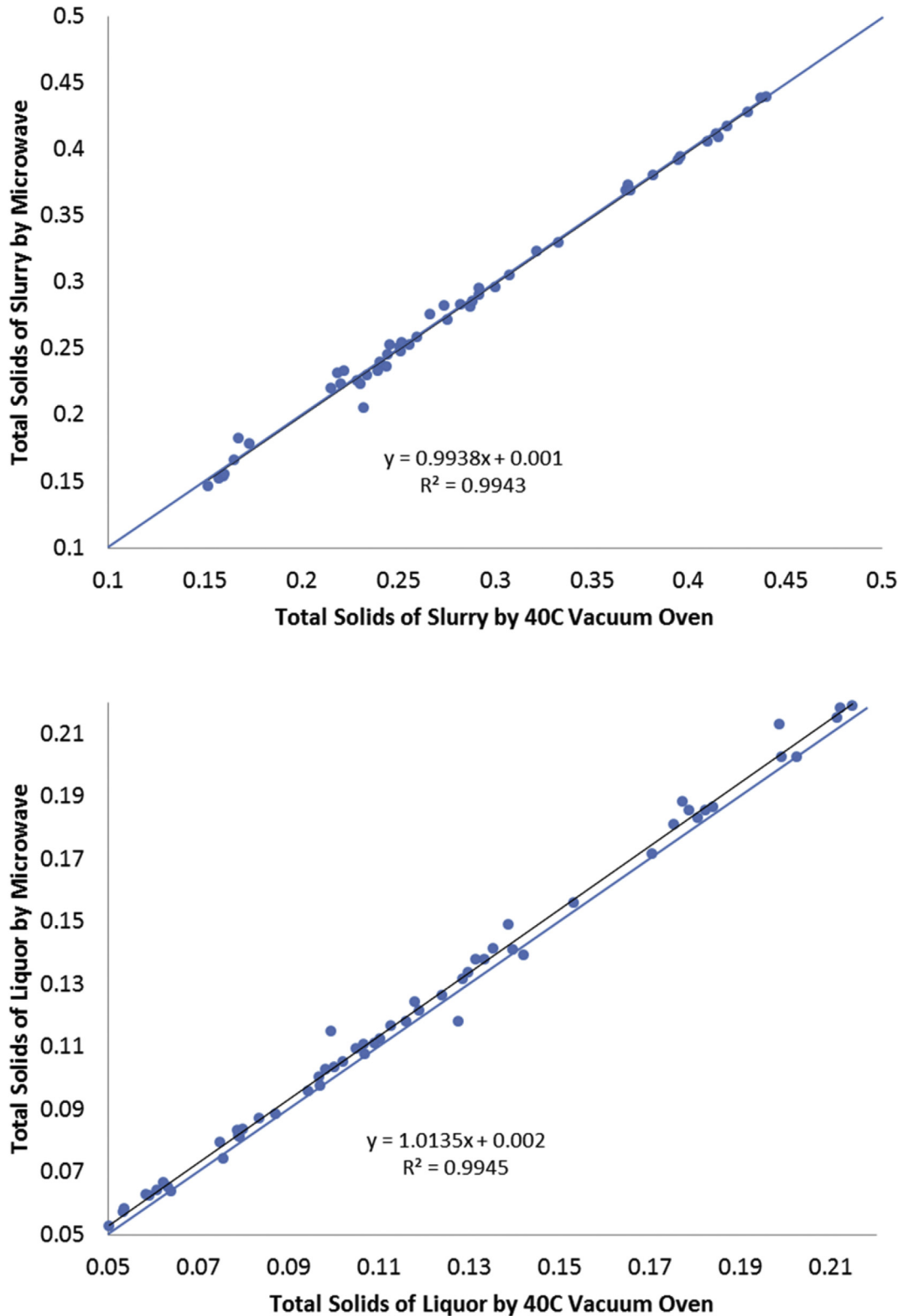
$y = 0.9938x + 0.001$
$R^2 = 0.9943$

$y = 1.0135x + 0.002$
$R^2 = 0.9945$

Fig. 4. Comparison of total solids measurement (wt %) for slurries (top plot) and liquors (bottom plot) dried in a 40 °C vacuum oven and microwave. Blue line is line of perfect fit and black line is regression line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microwave method. The microwave method has been optimized using synthetic liquors, where the other methods have not, and the microwave method is a fast process (8–10 min per dry matter analysis), which eliminates or minimizes sample degradation.

4. Conclusion

In conclusion, calculation of the FIS value using the "no-wash" equation and using dry matter measurements from microwave
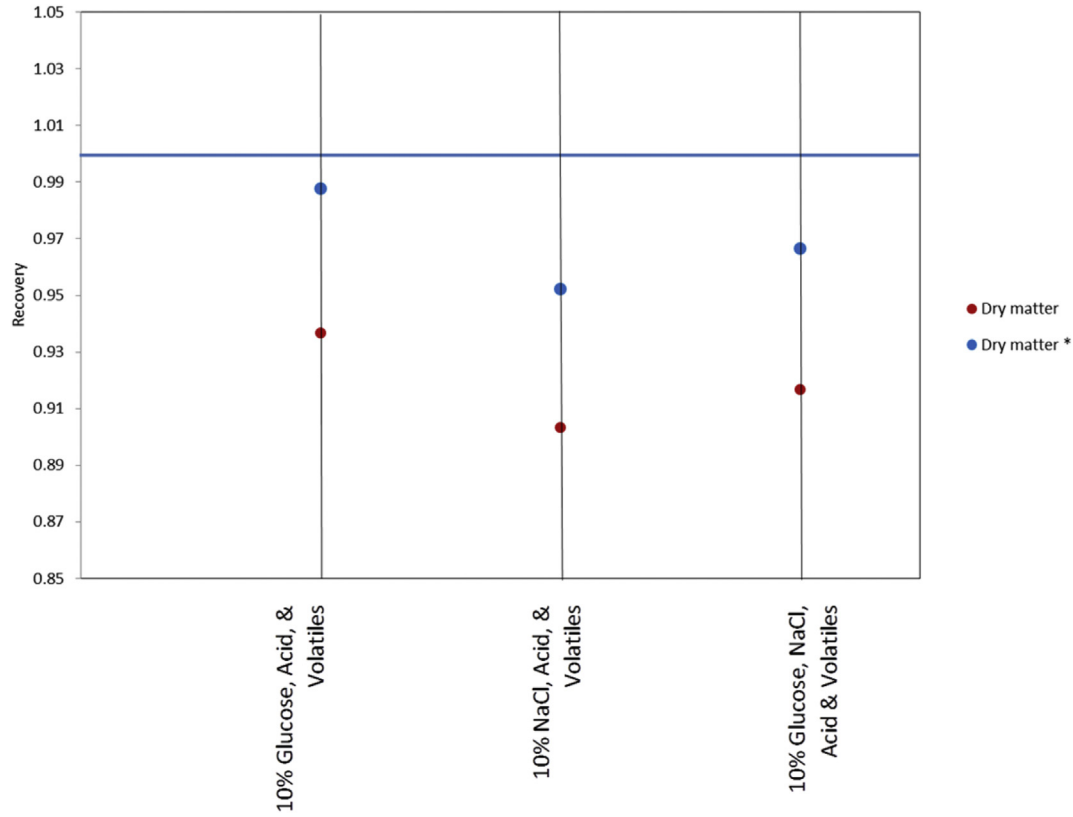
Fig. 5. Results of drying a variety of synthetic liquor samples using the microwave method. Recovery was calculated as measured weight/expected weight. DM is dry matter. DM* is dry matter calculated with the volatile weights removed from the equation. All points are averages of triplicate measurements.
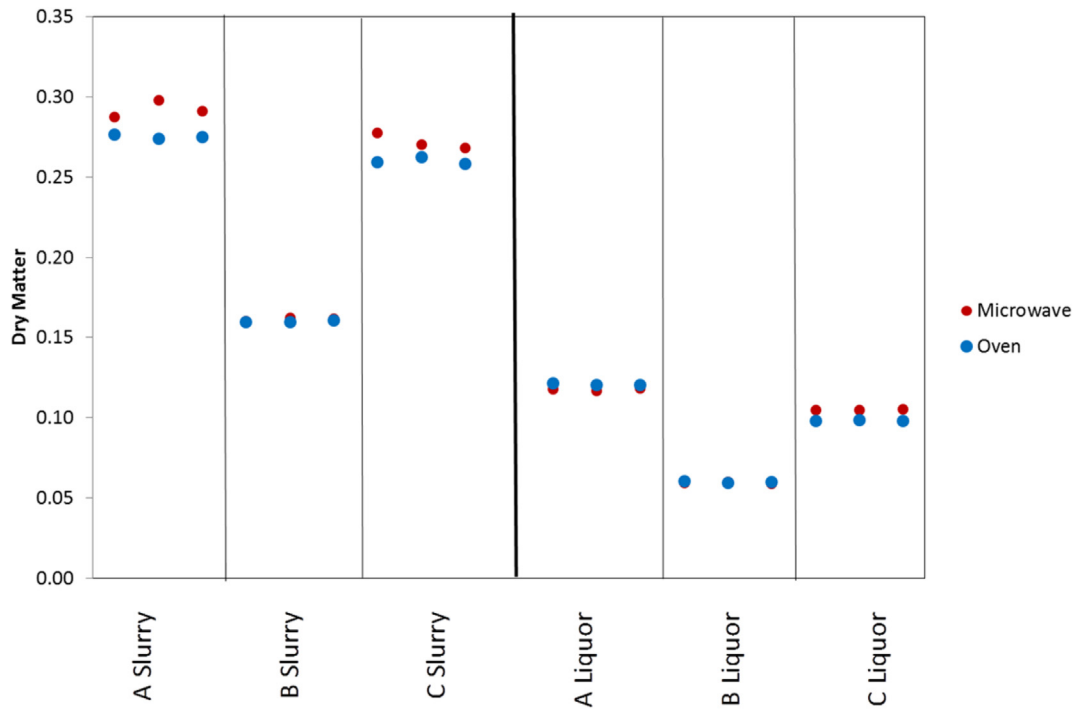

Fig. 6. Results of drying three slurry samples, both whole slurry and isolated liquor. Microwave methods as described in the text used for liquor and slurry, as appropriate. Liquors were neutralized and dried in a vacuum oven at 80 °C and corrected for neutralization, and solid samples were dried in an oven at 60 °C. Triplicate measurements are shown to illustrate reproducibility.

Table 1
FIS results for seven randomly chosen samples, calculated using wash, no wash oven, and microwave methods. The mean, pooled standard deviation, and minimum and maximum standard deviations are shown.

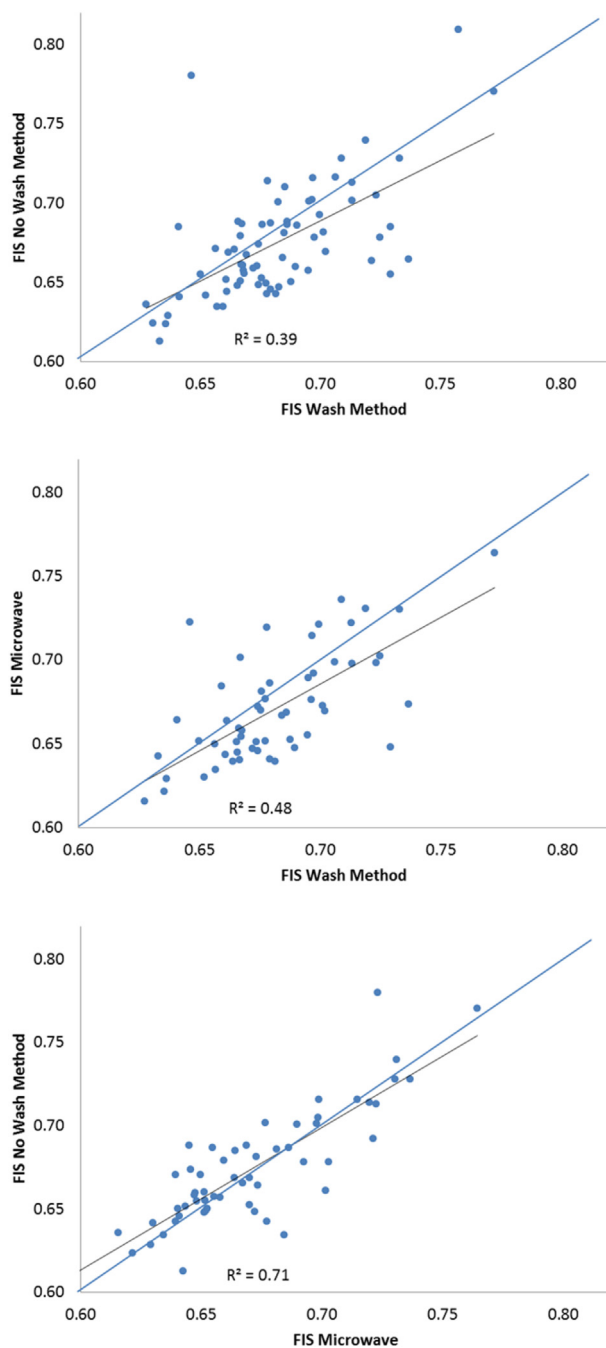|	Mean	Pooled SD	Min SD	Max SD
Wash method	0.669	0.014	0.002	0.025
No wash- oven	0.673	0.005	0.002	0.009
Microwave	0.673	0.013	0.004	0.026

Fig. 7. Comparisons of wash, no wash, and microwave FIS methods. Blue line is target fit, black line is regression line. All values are % fraction insoluble solids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

drying was the fastest and, because it was optimized using known samples, the most accurate, method for determining FIS values. The microwave also has the benefit of eliminated or minimized sample degradation during drying. Sample drying is most accurate when the samples can be neutralized or exposed to heat for a minimal amount of time, as with the microwave. If a microwave drier is not available, acidic liquors can be neutralized with NaOH, dried in a vacuum oven at 80 °C, and corrected for neutralization. Solid samples can be run in an oven at 60 °C. None of these methods take into account the loss of volatiles from true slurries and liquors; this is an area for improvement in future methods. Slurries and liquors are complex matrices with many constituents and may behave in slightly different ways depending on pretreatment severity and type.

Acknowledgements

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