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SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF PRETREATED BIOMASS: IMPROVING MASS BALANCE CLOSURE

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SUMMARY

The successful implementation of simultaneous saccharification and fermentation (SSF) technology for biomass conversion to ethanol requires competent analysis of complex biomass process streams, often obtained at extremes of pH. In this study, optimal conditions for handling biomass samples recovered from acid and alkaline pretreatments prior to traditional compositional analysis were developed. Methods for processing slurries from SSF were also determined. In both cases, a mixed wastepaper feedstock was used to test improved handling procedures and to document recommended performance.

INTRODUCTION

Simultaneous saccharification and fermentation (SSF) is recognized as an efficient approach to the cost-effective conversion of biomass to fuel ethanol. This methodology takes advantage of the relief in end-product inhibition realized by conducting cellulose hydrolysis and glucose fermentation in the same well-stirred vessel (Wilke et al. 1976; Wright et al. 1988). In SSF, ethanol is produced by concurrent saccharification and yeast fermentation, utilizing the glucose produced from the hydrolysis of the cellulosic content in biomass.

Experimental variance encountered in the determination of the total mass composition of native woody biomass found in paper ranges from a standard deviation of (s) = 4.2 for pine (a good softwood model) to s = 6.7 for poplar (a good hardwood

model) based on a recent nine-laboratory, International Energy Agency (IEA)-sponsored round-robin study (Agblevor et al. 1992). The same study showed an ability to close mass balance no better than 91.4% for poplar and 93.6% for pine. Although some specialty laboratories are able to achieve lower values of variance and mass balance closure for native (untreated) woody tissue approaching 98% (Vinzant et al. 1993), compositional analysis of processed biomass is usually more difficult. In the present study, we have targeted three important biomass process intermediates that present chemical analysis challenges: compositional analysis of acid pretreatment solids, compositional analysis of alkaline pretreatment solids, and analysis of fermenter broth (solids). Recommendations are given for analytical protocols based on performance with wastepaper feedstock.

MATERIALS AND METHODS

Pretreatment. Dilute acid pretreatment was conducted on 500-g milled paper samples in a PARR 2-g high-pressure stirred reactor. The reactor vessel used was constructed of Carpenter Cb20-3 stainless steel alloy to ensure minimal contamination of the pretreated product. The condition for pretreatment of all paper blend samples followed the method applied by Grohmann et al. (1985) to aspen wood and wheat straw. In all cases, the sulfuric acid was added at the attainment of final reaction temperature (20 min from initiation of heating) using a Beckman model 110A high performance liquid chromatography (HPLC) pump. Alkaline pretreatment was accomplished with similar addition of 0.1 M NaOH to the PARR reactor charged with mixed paper following the method used by Tatsumoto et al. (1988) to pretreat aspen wood. When pretreatment was complete, the reactor was removed from the heating mantle with an electric hoist and transported to a sink containing an ice water bath. Cool-down usually required only 4-5 min. The treated solids were separated from the liquor with a muslin screen and washed preliminarily with approximately 5 L of distilled water or left unwashed. Samples to be analyzed as washed samples were washed exhaustively with distilled water (i.e., against flowing water for 2 d).

Mixed Wastepaper Feedstocks. The feedstock used for this study was a proprietary blend of waste paper. The paper blend was subjected to knife milling using an Allsteel 30-hp mill equipped with a 1/4-in. rejection screen and gravity collection.

Pretreated Mixed Wastepaper and Fermenter Broth Analysis. The procedure used to analyze the native and pretreated paper for carbohydrates is based on the sequential hydrolysis of the samples with 72% and 4% sulfuric acid to ensure conversion of all polymeric sugars to monomers (Moore and Johnson 1967). After neutralization of the acidic digestion mixture with calcium carbonate and filtration, the samples were analyzed for neutral sugars by ion-moderated partition (IMP) chromatography using Aminex HPX-87P and HPX-87C columns with distilled water as the eluant and refractive index detection. Acid-insoluble lignin was determined by the Klason method (Moore and Johnson 1967; TAPPI Standard Method T13). The filtrate generated from this method was measured spectrophotometrically at A_{205} for acid-soluble lignin (TAPPI

Standard Method T222). Total solids were determined by oven drying at 105°C and ash contents were determined after ignition at 575°C (TAPPI Standard Method Tos-63).

Where necessary, dried samples were knife milled to a 40-mesh consistency using a Wiley/Thomas Micromill.

Procedure A. A portion of the unwashed pretreated solids (i.e., in acid liquor) was washed repeatedly with Nanopure deionized (DI) water. The remaining unwashed solids, the washed solids, and the hydrolysis liquor were all analyzed using the methods described above. Where appropriate, samples were milled to reduce the particle size of the material. In addition to the complete compositional analysis of the unwashed solids and the washed solids samples, a lyophilized unwashed solids sample was analyzed in parallel for total carbohydrates. Composition of the calculated (combined) slurry sample was estimated from summation of the contents of the liquid (inferred from dry wt) and solids components.

Procedure B. Each slurry sample from alkaline pretreatment was extensively washed with DI water prior to analysis. After the initial drying of the sample, at either 45°C or at 105°C, it was necessary to grind the sample to reduce the particle size of chunks found in these pretreated samples. The samples were then redried prior to proceeding with the compositional analyses.

Procedure C. Fermentation samples were divided into two portions, with one portion taken through the additional process step of first centrifuging and then washing the pelleted material. The whole slurry, the washed solids, the liquor (supernatant), and the first wash were all analyzed using the methods given above. In addition to the complete compositional analysis of the whole slurry and the washed solids samples, corresponding lyophilized samples were also analyzed in parallel for total carbohydrates.

RESULTS AND DISCUSSION

Procedure A. Compositional Analysis of Acidified Pretreatment Solids. Typically, and prior to fermentation (SSF), pretreated solids are analyzed after being washed free of hydrolysis liquor. The resulting slurries are essentially neutral in pH. However, because fermentations will also be performed on unwashed pretreated solids, it was necessary to carefully determine the composition of these unwashed slurries. Drying such an acidified sample (i.e., pH 1.8) was expected to cause degradation of the sugars of interest, even if the drying was conducted at 45°C. It was therefore necessary to evaluate the extent of this degradation, and if deemed significant, develop analysis methods to avoid this degradation.

The following table compares the results of representative total carbohydrate determinations for the "as received" unwashed sample, washed solids, and lyophilized unwashed solids, as well as calculated whole sample composition. To obtain the calculated values, the contribution of the liquor (i.e., in terms of total sugars) was added to the carbohydrate composition of the washed solids, as determined by the apportionment of solids and liquid in the "as received" unwashed solids sample itself. It is clear from Table I that the analysis of the unwashed solids sample, regardless of whether the sample was oven dried or lyophilized, underestimates the amount of glucose in the sample. The value for xylose for the washed solids sample appears low, because the contribution of xylose in the liquor was not taken into account. The minor sugars results are all comparable.

Table I

Sample, dried weight basis	Glucose	Xylose	Arabinose	Galactose	Mannose
Projected whole sample composition (calculated from washed solids plus liquor)	80.03%	6.80%	0.11%	0.43%	4.37%
Composition of unwashed solids	78.99%	7.13%	trace	0.49%	4.88%
Composition of washed solids	79.74%	6.09%	trace	0.43%	4.37%
Composition of lyophilized unwashed solids	76.09%	6.39%	trace	0.42%	4.42%

Recommendation: It was concluded from this study that unwashed, pretreated solids from mixed wastepaper cannot be adequately analyzed without first being divided into liquor and washed solids components. It is believed that hydrolysis liquor entrained in the unwashed solids is acidic enough to catalyze the destruction (reversion) of carbohydrates, particularly glucose, leading to depressed carbohydrate values. The effect of acid on the minor sugars was more difficult to quantitate, because these sugars are present as soluble monomers in the liquor, as well as in the pretreated solids fraction, whereas the majority of the glucose is present as insoluble fiber. It was clear that extensive water washing of the unwashed pretreatment solids removed a large portion of the acid, decreasing the possibility for sugar degradation.

Part B. Compositional Analysis of Alkaline Pretreatment Solids. Sodium hydroxide pretreatment of the wastepaper feedstocks is also a focus of many research efforts. At the outset, it was felt that unless the solids were washed free of alkali, problems would be encountered in compositional analysis, particularly in the acid hydrolysis steps (i.e., excess salt loadings resulting from neutralization with acid and the HPLC analysis problems this would induce). Therefore, a DI water wash step was incorporated as part of the basic procedure.

Recommendation: The alkaline pretreated solids must be adequately washed free of alkali prior to analysis. After drying, the samples tend to aggregate and should be milled to approximately 60 mesh for consistency. Mass closures were very good, ranging from 97% to 101% for the samples used in this study (data not shown).

Part C. Compositional Analysis of Fermentation Solids and Slurries. Analysis of fermentation samples represents a particularly difficult analytical challenge because of the complexity of the sample, as well as the presence of significant levels of proteins (Chum and Gellerstedt 1991). Proteins are known to interfere with the analysis of carbohydrates, particularly if acidic conditions are encountered at elevated temperatures. Several approaches were investigated in order to develop a method that could be used to handle samples generated from fermentation experiments. The following table compares the results of the total carbohydrate determinations for the "as received" whole slurry (fermenter broth) samples, as well as calculated whole slurry compositions. To obtain these calculated values, the contribution of the liquor (i.e., in terms of total sugars) was added to the carbohydrate composition of the washed solids as determined by the apportionment of solids and liquid in the "as received" whole slurry sample itself. It is clear from Table II that the analysis of the whole slurry sample underestimates the amount of glucose in these samples.

Table II

Composition of whole sample, dried weight basis	Glucose	Xylose	Arabinose	Galactose	Mannose
Sample #1 - calculated composition (washed solids plus liquor)	54.36%	12.44%	1.92%	1.24%	6.94%
Sample #1 - "as received" composition	43.97%	10.60%	1.00%	1.37%	5.84%
Sample #2 - calculated composition (washed solids plus liquor)	56.77%	12.68%	2.18%	1.23%	6.83%
Sample #2 - "as received" composition	40.38%	9.56%	1.52%	1.19%	6.51%

In Table III, an estimate of mass balance has been calculated for each of the solid samples analyzed, including the calculated whole slurry composition. Again it is clear from the lower-than-expected mass balances for the "as received" whole slurry samples (e.g., totals of around 90% instead of closer to the ideal total of 100%) that the analyses of these samples underestimates the amount of glucan present, whether the sample was oven dried or lyophilized.

Table III

Sample	GN	XN	AN	GAN	MN	LKL	LAS	AT	Total
Whole slurry, calculated	48.57	9.11	1.41	1.12	6.25	19.68	0.95	12.86	99.95
Whole slurry, oven dried	39.57	9.33	0.88	1.23	5.26	18.87	2.00	14.71	91.85
Whole slurry, lyophilized	39.13	9.92	1.72	1.06	5.69	*	*	*	93.10
Washed solids, oven dried	48.43	7.96	1.41	1.12	6.25	19.68	0.95	12.86	98.66
Washed solids, lyophilized	48.87	8.53	1.69	1.17	5.99	*	*	*	99.75
Whole slurry, calculated	51.09	11.16	1.92	1.11	6.15	19.78	0.95	13.05	105.21
Whole slurry, oven dried	36.34	8.41	1.34	1.07	5.86	17.53	1.99	13.34	85.88
Whole slurry, lyophilized	36.25	9.89	1.56	1.16	5.47	*	*	*	87.19
Washed solids, oven dried	50.34	8.08	1.37	1.11	6.15	19.78	0.95	13.05	100.83
Washed solids, lyophilized	47.33	8.18	1.71	1.13	5.89	*	*	*	98.02

* Because these specific analyses were not requested for the lyophilized samples, the results from the corresponding oven dried sample have been used to calculate a mass balance.

All results are reported on % dry weight basis.

AN=araban; AT=total ash; GN=glucan; GAN=galactan; LAS=acid soluble lignin; LKL=Klason lignin; MN=mannan; XN=xylan

Recommendation: It can be concluded from this study that the whole slurry sample of fermenter broth cannot be adequately analyzed without first being processed into liquor and washed solids components. It is believed that protein may be binding specifically to the cellulose fibers, protecting it from complete hydrolysis and leading to depressed glucose values. The effect of protein on the minor sugars is more difficult to determine because these sugars are present in the liquor, as well as in the insoluble hemicellulosic fraction, whereas the majority of the glucose is present as insoluble fiber. It is clear that extensive water washing of the fermentation solids removes a large portion of the protein, decreasing the opportunity for protein-sugar interactions.

In conclusion, optimal assessment of the efficiency of SSF technology requires that the precise quantity of fermentable sugar be known in the experiment. The ability to close total mass balance provides assurance that this condition is met. Because of the intrinsically high errors expected from the analysis of untreated woody biomass alone (Agblevor et al. 1992), minimization of similar or larger variances in the analysis of pretreated feedstock and the components in the final SSF broth have the potential to severely confound competent analysis of fermentability. We have provided insight into the improved handling of selected process stream samples notorious for posing analysis problems in the hope that overall experimental error can be reduced.

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