COMMUNICATION TO THE EDITOR

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Application of a Reaction Model to Improve Calculation of the Sugar Recovery Standard for Sugar Analysis

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ABSTRACT: A kinetic model was applied to improve determination of the sugar recovery standard (SRS) for biomass analysis. Three sets of xylose (0.10–1.00 g/L and 0.999–19.995 g/L) and glucose (0.206–1.602 g/L) concentrations were measured by HPLC following reaction of each for 1 h. Then, parameters in a kinetic model were fit to the resulting sugar concentration data, and the model was applied to predict the initial sugar concentrations and the best SRS value (SRS_p). The initial sugar concentrations predicted by the model agreed with the actual initial sugar concentrations. Although the SRS_e calculated directly from experimental data oscillated considerably with sugar concentration, the SRS_p trend was smooth. Statistical analysis of errors and application of the *F*-test confirmed that application of the model reduced experimental errors in SRS_e. Reference SRS_e values are reported for the three series of concentrations.

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Accurate measurement of carbohydrates, such as glucan and xylan in cellulosic materials, is critical for mass balance of these components for process development (Sluiter and Sluiter, 2010). The primary standard method for sugar determination is reported in technical report NREL/TP—510-42623 authored by Sluiter et al. (2006), and has been widely applied for structural carbohydrate analysis of biomass (Laser et al., 2002; Shen and Wyman, 2011; Shi et al., 2011; Xu and Cheng, 2011). The method is based on acid catalyzed hydrolysis of structural carbohydrates to monomeric sugars at 121°C. However, because sulfuric acid degrades sugars during the procedure, the sugar concentration measured by HPLC after the reaction is complete is divided by a sugar recovery standard (SRS) to better estimate the initial amounts of structural carbohydrates present in the samples of interest. SRS is determined by measuring the sugar concentration left after 1 h of reaction with dilute sulfuric acid and dividing that value by the concentration present initially. The standard approach recommends establishing SRS values for low, medium, and high sugar concentrations, for example, 10, 50, and 100 g/L of xylose, and applying the SRS value corresponding to that at a sugar concentration closest to that measured for the biomass being analyzed (Sluiter et al., 2006). However, it is often difficult to choose the proper standard sugar concentrations for SRS determination prior to performing the actual analysis because the actual sugar content of biomass is not known. It is also unclear what difference between the SRS concentration and the measured sugar concentration is acceptable. In addition, all sugars are exposed to acid at the start for the SRS samples, while those from the biomass being analyzed are released over time, reducing their relative exposure to acid and temperature. On top of all these concerns, SRS values determined for individual sugar concentrations vary significantly from one experiment to the next and among investigators due to experimental errors. These factors cause inaccuracies in determination of the composition of structural carbohydrates in biomass and associated mass balances and in comparisons of biomass compositions for different

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investigations. Thus, a key issue is how to obtain a series of continuous initial sugar concentrations within any concentration interval from sugar concentrations released in a fixed time, for example, 1 h, and calculate a series of SRS values. A kinetic model is a powerful tool to meet this objective. On the other hand, an SRS value determined by a single experimental concentration is often unreliable due to experimental errors. An improved approach to eliminate experimental errors of each point systematically can be achieved by modeling a series of experimental data points obtained from application of the standard SRS procedure.

However, kinetic models are typically based on fitting rate constants so that predicted and actual concentrations of a reactant such as sugar are as close as possible at various times starting from a known initial reactant concentration. In the present SRS approach, the determination of SRS values is based on following sugar reactions after just one time (e.g., 1 h for the autoclave procedure) starting from a range of known sugar concentrations initially. With appropriate modifications, basic chemical kinetic modeling principles can be applied to calculate SRS values.

Against this background, the objectives of this study were to (1) develop a chemical kinetic model to improve SRS calculations, (2) calculate SRS values for three series of glucose and xylose reference concentrations, and (3) validate the fitted model constants by statistical methods.

When exposed to sulfuric acid at high temperatures, sugar (x) will degrade as follows:

$$\text{Sugar}(x) \rightarrow \text{Degradation products}(y) \tag{1}$$

Assuming this to be a homogenous reaction according to the law of mass action, the sugar reaction rate can be expressed in terms of the following empirical relationship:

$$\frac{dx}{dt} = -kx^n \tag{2}$$

where x is the sugar concentration (mmol/L) at any time, k is a rate constant ($(\text{mmol/L})^{1-n}\,\text{s}^{-1}$), and n is the reaction order. Although HPLC analysis measures mass concentrations, molar units were employed in Equation (2) because chemical kinetics are based on molecular collisions. Integrating Equation (2) with the initial concentration $x = x_0$ at $t = 0$ results in:

$$x_0 = [x^{1-n} + kt(1-n)]^{1/1-n} = (x^b + a)^{1/b} \tag{3}$$

where a and b are constants calculated as $a = kt(1-n)$, and $b = 1-n$. Applying a nonlinear regression method to the series of reactant concentrations measured over time t starting from a series of initial reactant concentrations x_0, the constants a and b in Equation (3) can be determined. Thus, the predicted sugar recovery standard, SRS_p, can be calculated for any initial sugar concentration by the following equation:

$$\text{SRS}_\text{p} = \frac{x}{x_{0\text{p}}} = \left(1 - \frac{a}{x_{0\text{p}}^b}\right)^{1/b} \tag{4}$$

where $x_{0\text{p}}$ is the initial sugar concentration predicted from the measured concentrations over time by Equation (3). If we express $x'_{0\text{p}}$ in mass concentration units of g/L, we obtain:

$$\text{SRS}_\text{p} = \left[1 - \frac{a\text{MW}^b}{(1,000x'_{0\text{p}})^b}\right]^{1/b} \tag{5}$$

where MW is the sugar molecular weight. The predicted SRS_p value can then be compared to SRS_e values calculated by the conventional method of comparing sugar concentrations after 1 h to those initially:

$$\text{SRS}_\text{e} = \frac{x}{x_0} \tag{6}$$

A nonlinear regression method was applied to determine the constants a and b in Equation (3) from sugar concentrations measured after reacting the series of initial sugar concentrations in Table I for 1 h; these values are shown in Table II. The initial low (Fig. 1A) and high (Fig. 1B) measured sugar concentrations (points in the figures) and the initial sugar concentrations (lines in the figures) predicted by the model (Eq. 3) are plotted against the measured sugar concentrations after 1 h reaction time in Figure 1. Thus, the predicted initial sugar concentrations are in very good agreement with the initial sugar concentrations. However, there are differences between the experimental SRS_e (Eq. 6) and predicted SRS_p (Eq. 4) values (Fig. 2). The greatest deviations between experimentally determined and predicted SRS values were particularly apparent at very low sugar concentrations, which may be because the HPLC analysis had the greatest errors at such low concentrations. The irregular oscillations in experimental SRS_e values in Figure 2 suggest that individual SRS_e values are unreliable for correcting sugar concentrations, while the SRS_p values from the model vary smoothly. The latter is consistent with the expectation that SRS values should be continuously smooth versus initial concentration, and the former behavior can be attributed to experimental errors in the SRS_e values. Thus, the model obviously reduced the impact of such experimental errors. Table III gives predicted SRS_p

Table I. The initial low and high sugar concentrations used.

Low xylose (g/L)	0.100	0.204	0.402	0.598	0.800	1.000
Low xylose (mmol/L)	0.667	1.36	2.68	3.99	5.33	6.67
Low glucose (g/L)	0.206	0.608	1.001	1.201	1.410	1.602
Low glucose (mmol/L)	1.14	3.38	5.56	6.67	7.83	8.90
High xylose (g/L)	0.999	2.004	4.004	7.995	15.992	19.995
High xylose (mmol/L)	6.66	13.36	26.69	53.3	106.6	113.5

Table II. The constants in Equation (3) and statistical values in Equations (7) and (8).

	k ((mmol/L)$^{1-n}$ min^{-1})	n	a	b	R^2	F
Low xylose	0.001154	1.477	−0.0330	−0.4768	1.00	58,871
Low glucose	0.001271	0.866	0.01022	0.1340	1.00	21,205
High xylose	0.001786	1.084	−0.00904	−0.08431	1.00	52,045

values for the three series of xylose and glucose concentration as references.

The tedium of sugar analysis would be reduced if it were not necessary to determine SRS values each time carbohydrate analyses are conducted. One would expect that the degradation kinetics of pure chemicals can be reproducibility predicted if internal and external heat and mass transfer are unimportant. Because SRS reactions are in small vessels

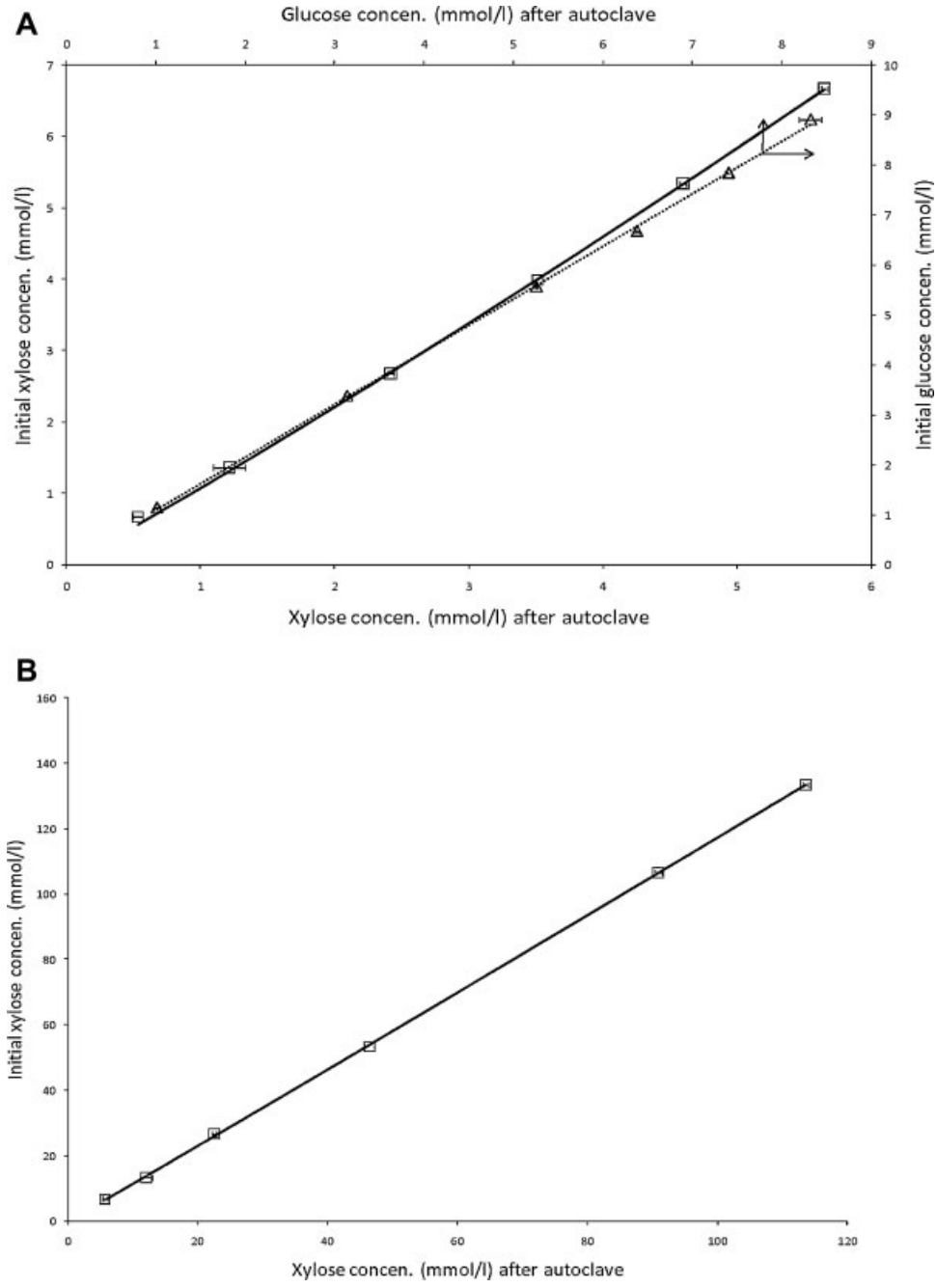

Figure 1. Initial low (**A**) and high (**B**) sugar concentrations (points) and predicted initial xylose (square) and glucose (triangle) concentrations (lines) (Eq. 3) versus measured sugar concentrations after reaction for 1 h at 121°C.

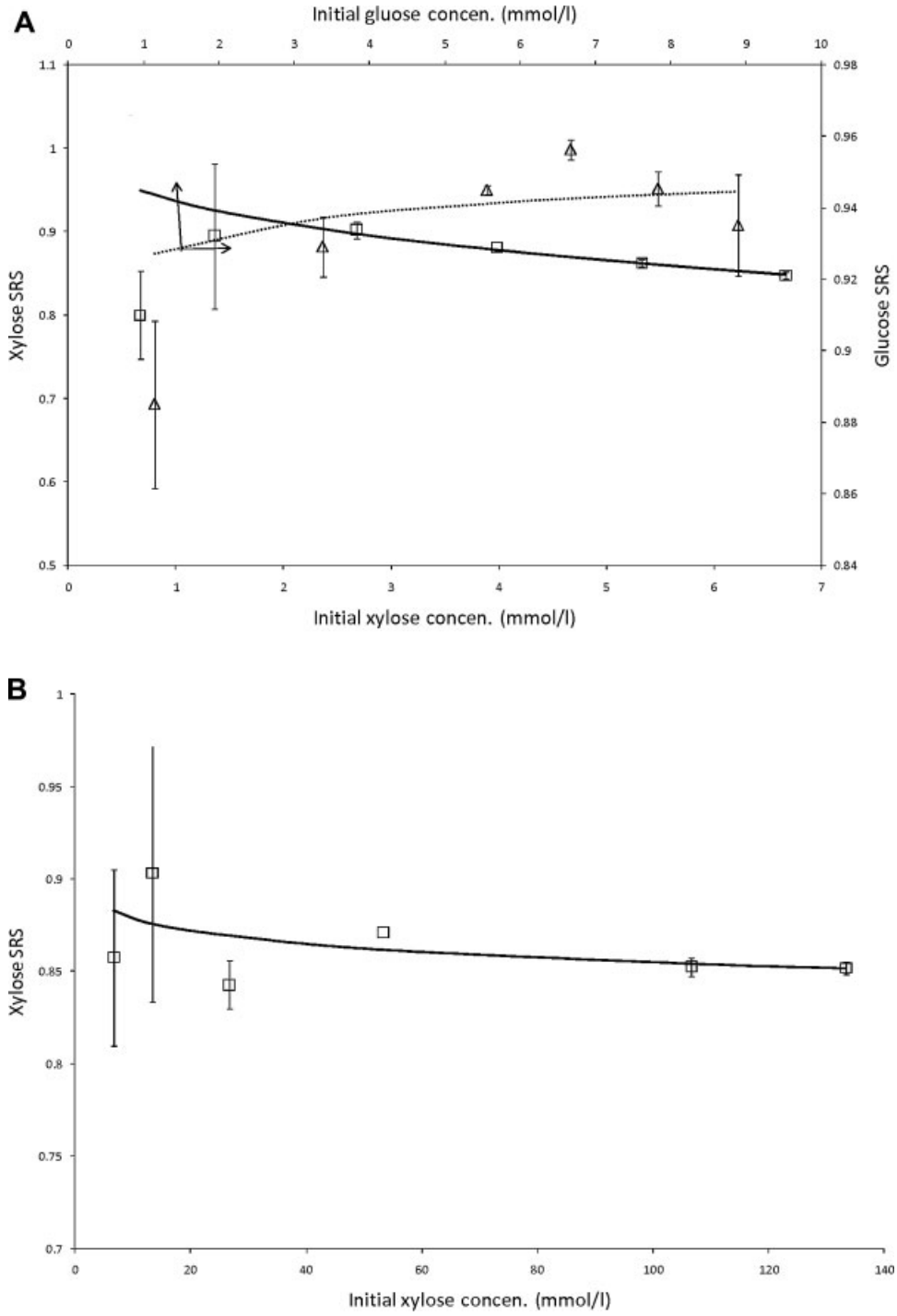

Figure 2. Experimental SRS_e (points) for low (**A**) and high (**B**) xylose (square) and glucose (triangle) concentrations by Equation (6) and the predicted SRS_p (lines) (Eq. 5) versus the initial sugar concentrations.

containing homogenous dilute solutions in water, internal heat and mass transfer should be minimal. Because the SRS reactors are heated in boiling water, external heat and mass transfer resistances should also be small. Thus, we expect that SAS_p values can be applied to all biomass analyses that employ the same HPLC column. However, heat up and cool down time during SRS runs must also be consistent for this approach to apply, with steam heating by an autoclave likely to provide better control of these times than electrical heating.

Two criteria were applied to assess the validity of the model constants regressed from experimental points: (1) R^2

Table III. SRS_p of three series of sugar concentrations calculated by model (Eq. 5).

		Low glucose		High xylose	
	SRS_p	(g/L)	SRS_p	(g/L)	SRS_p
0.1	0.9453	0.2	0.9272	1.0	0.8824
0.2	0.9250	0.3	0.9309	2.0	0.8758
0.3	0.9101	0.4	0.9335	3.0	0.8718
0.4	0.8979	0.5	0.9354	4.0	0.8689
0.5	0.8874	0.6	0.9369	5.0	0.8665
0.6	0.8782	0.7	0.9381	6.0	0.8646
0.7	0.8698	0.8	0.9392	7.0	0.8630
0.8	0.8621	0.9	0.9401	8.0	0.8616
0.9	0.8550	1	0.9410	9.0	0.8603
1.0	0.8484	1.1	0.9417	10	0.8592
		1.2	0.9423	11	0.8581
		1.3	0.9429	12	0.8572
		1.4	0.9435	13	0.8563
		1.5	0.9440	14	0.8554
		1.6	0.9445	15	0.8547
				16	0.8539
				17	0.8533
				18	0.8526
				19	0.8520
				20	0.8514

$(1 - [\text{sum of square between experimental values and predicted values}]/[\text{sum of squares of experimental values}])$ and (2) the *F*-test. The former was determined by Equation (7):

$$R^2 = 1 - \frac{\sum_{i=1}^{m} (y_{ei} - y_{pi})^2}{\sum_{i=1}^{m} y_{ei}^2} \tag{7}$$

where y_{ei} and y_{pi} are the experimental and predicted values, respectively, and m is the number of experimental points. Values for F were calculated by the following:

$$F = \frac{\left[\sum_{i=1}^{m} y_{ei}^2 - \sum_{i=1}^{m} (y_{ei} - y_{pi})^2\right]/M}{\left[\sum_{i=1}^{m} (y_{ei} - y_{pi})^2\right]/(m-M)} \tag{8}$$

where M is the number of model constants. The R^2 and F values calculated for the experiments reported above are listed in Table II. Because the R^2 values are all equal to 1 and the F values are greater than $4 \times F_{(M,\ m-M,\ 1-\alpha)} = 4 \times F_{(2,\ 4,\ 0.95)}$ (4×6.94) $(1 - \alpha$ is the confidence, usually taking 0.95), the regressed constants can be considered to be reliable (Draper and Smith, 1981).

The kinetic model developed here smoothly predicted SRS_p values for three series of xylose (low and high) and glucose (low) concentrations after reaction for 1 h. This method can predict SRS values for any sugar concentrations, reduce errors for SRS values calculated from experimental data, and improve compositional analysis of structural sugars in biomass. The model constants fit to the degradation data were shown to be reliable based on statistical analysis of errors and the *F*-test.

Materials and Methods

Seventy-two percent sulfuric acid was purchased from Sigma-Aldrich (St. Louis, MO). D-(+)-glucose and xylose (both Sigma-Aldrich, 99.5%) were used as standards for HPLC analysis. Calcium carbonate (Fisher Scientific, Pittsburgh, PA 99.0%) was used to neutralize acid in samples before HPLC analysis.

SRS values are employed for two sugar analysis situations: (1) composition analysis of raw biomass, involving determination of SRS at low sugar concentrations, and (2) sugar, and particularly xylose, analysis after post hydrolysis involving determination of SRS for high sugar concentrations. Therefore, a series of low concentrations of xylose and glucose and another series of high concentrations of xylose were prepared for SRS measurements. These initial concentrations are shown in Table I.

Consistent with the NREL standard procedure (Sluiter et al., 2006), 3 mL of 72 wt% sulfuric acid was added to 84 mL of sugar solutions at the concentrations in Table I to form 4 wt% acid solutions. These solutions were then autoclaved (HA-300 MII, Hirayama, Saitama, Japan) at 121°C for 1 h and further prepared for HPLC analysis of the sugars. Duplicates of each sample were autoclaved.

The lower glucose and xylose concentrations were measured using a Waters HPLC model 2695 system equipped with a 2414 refractive detector, a Waters 2695 autosampler, a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA), and Millenium32 chromatography manager 3.2 software (Waters Co., Milford, MA). The column temperature was 85°C, and the mobile phase was deionized water at a flow rate of 0.6 mL/min. An Agilent HPLC equipped with a RI detector and a Bio-Rad Aminex HPX-87H column (Bio-Rad Laboratories) was employed at 65°C for analysis of the high xylose concentrations. The mobile phase was 0.005 M sulfuric acid at a flow rate of 0.6 mL/min. Identities of the compounds were authenticated by comparing their retention times with those of pure compounds (Sigma-Aldrich). Before injecting into the HPLC, acid in the filtrates was neutralized with calcium carbonate to pH 5–6, and then the suspensions were centrifuged at 15,000 rpm for 5 min through a 0.2 μm member filter. The supernatants were used for HPLC analysis.

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References

Draper NR, Smith H. 1981. Applied regression analysis, 2nd edn. New York, USA: John Wiley & Sons. Inc. p 129–133.

Laser M, Schulman D, Allen SG, Lichw J, Antal MJ, Jr., Lynd LR. 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresour Technol 81(1):33–44.

Shen JC, Wyman CE. 2011. A novel mechanism and kinetic model to explain enhanced experimental xylose yields for dilute sulfuric acid compared to hydrothermal pretreatment of corn stover. Bioresour Technol (in press; available on line DOI: 10.1016/j.biortech.2011.04.001).

Shi J, Pu Y, Yang B, Ragauskas A, Wyman CE. 2011. Comparison of microwaves to fluidized sand baths for heating tubular reactors for hydrothermal and dilute acid batch pretreatment of corn stover. Bioresour Technol 102(10):5952–5961.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. 2006. In Laboratory Analytical Procedures No. TP-510-42623, National Renewable Energy Laboratory, Golden, CO.

Sluiter J, Sluiter A. 2010. In Laboratory Analytical Procedures No. TP-510-48087, National Renewable Energy Laboratory, Golden, CO.

Xu JL, Cheng JJ. 2011. Pretreatment of switchgrass for sugar production with the combination of sodium hydroxide and lime. Bioresour Technol 102(4):3861–3868.