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A modified method for calculating practical ethanol yield at high lignocellulosic solids content and high ethanol titer

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ABSTRACT

A modified method for calculating practical ethanol yield in the simultaneous saccharification and fermentation (SSF) at high lignocellulosic solids content and high ethanol titer is proposed considering the liquid volume change caused by high titer ethanol generation and the water consumed during cellulose degradation. This modified method was applied to determine the practical ethanol yields of several practical SSF operations and the results compared to those using the conventional method. The results show that the liquid volume increase with ethanol formation during SSF was approximately five times greater than the volume decrease duo to water consumption during cellulose degradation. Furthermore, the practical ethanol yields calculating using traditional method were underestimated and the underestimated errors increased with the increasing ethanol titer. The present work may provide a convenient and accurate method for calculating practical ethanol yield in a high solids and high ethanol titer SSF systems.

1. Introduction

One of the major technical barriers for commercialization of cellulosic ethanol is the high energy cost of ethanol distillation from the low ethanol titer fermentation broth (Galbe et al., 2007). Therefore, simultaneous saccharification and ethanol fermentation (SSF) at high solids loading of the pretreated lignocellulose feedstock became a common practice to obtain higher ethanol titer (Varga et al., 2004; Jorgensen et al., 2007; Zhang et al., 2010a). At present, most ethanol yield calculation methods for SSF operations at either high or low solids loading were based on Eq. (1) as follows:

$$\text{Ethanol yield } (\%) = \frac{[ETOH]_f - [EtOH]_0}{0.511 \times f \times [Biomass]_0 \times 1.111} \times 100\% \qquad (1)$$

where the term ($[EtOH]_f$ - $[EtOH]_0$) indicates the ethanol produced during the fermentation in grams per liter of the fermentation broth (g/L, w/v) from the beginning of the fermentation ($[EtOH]_0$) to the end ($[EtOH]_f$), $[\text{Biomass}]_0$ is the dry biomass concentration in grams per liter of the fermentation broth (g/L, w/v) at the beginning of the fermentation, f is the cellulose fraction of dry biomass (g/g, w/w), 0.511 is the conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast, 1.111 is the conversion factor for cellulose to equivalent glucose.

In Eq. (1), a hidden assumption is that the liquid volume of the SSF system is constant, thus the term ($[EtOH]_f$–$[EtOH]_0$) used for measuring the produced ethanol is on the same volume basis. However, for a closed SSF system with the recovery of the vaporized water by the condenser of the bioreactor, the total liquid volume does change: it increases with the liquid ethanol generation and decreases with the water consumption in the hydrolysis of cellulose. The volume change caused by glucose released from cellulose could be neglected because almost all the glucose was transformed into ethanol in the final broth (Zhu et al., 2011). The constant volume assumption can only be valid when both the ethanol produced and the water consumed are negligible. When the high ethanol titer from the SSF is required, i.e., 5–7% (w/w) ethanol in the fermentation broth, the high solids loading of lignocellulose feedstock may reach 30–40% by the weight percentage (grams solids in 100 g of the total SSF materials, w/w). The term ($[EtOH]_f$ – $[EtOH]_0$) in Eq. (1) is no longer accurate to apply because the volume unit of $[EtOH]_f$ and $[EtOH]_0$, the liter "L", does not mean the same volume: the "L" in $[EtOH]_f$ indicates the mixture volume of the water (after water loss in enzymatic hydrolysis) and the ethanol produced in the SSF, while the "L" in $[EtOH]_0$ only indicates the initial water volume with minor initial ethanol. In this situation, the ethanol produced and even the water consumed can not be simply ignored and should be taken into account in the ethanol yield calculation.

Most previous ethanol yield calculating methods were based on the constant liquid volume assumption without considering the ethanol produced and the water loss (Suryawati et al., 2008; Faga et al., 2010; Zhang et al., 2010a, 2010b, 2011). As a result, the reported ethanol yields might be lower than they should be, because

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Nomenclature

Parameters	Definitions (Unit)
$[Biomass]$	dry biomass concentration at the beginning of the fermentation (g/g)
$[Biomass]_0$	dry biomass concentration at the beginning of the fermentation (g/L)
$[C_1]$	ethanol concentration in gram ethanol per liter fermentation broth after the SSF ends (g/L)
$[C_1^0]]$	initial ethanol concentration in gram ethanol per liter fermentation broth before the SSF starts (g/L)
$[C_2]$	ethanol concentration in gram ethanol per gram fermentation broth after the SSF ends (g/g)
$[C_2^0]$	initial ethanol concentration in gram ethanol per gram fermentation broth before the SSF starts (g/g)
E	total ethanol produced in the SSF system (g)
E_0	initial ethanol weight before the SSF starts (g)
f	cellulose fraction of dry biomass (g/g)
m	total weight of the SSF system at the beginning of the operation (g)
V_0	liquid volume of the SSF system before the SSF operation (L)
V_f	liquid volume of the SSF system after the SSF operation (L)
W	total water input into the SSF system (g)
W_1	water used in the SSF for cellulose hydrolysis (g)

liquid volume increases with the ethanol produced is always greater than the water consumed in cellulose hydrolysis. What is interesting more, the liquid volume increase with the ethanol generation could not be simply added with water in the slurry because of the shrinkage property of mixing water and ethanol. That means the ethanol–water mixtures have less volume than the sum of their individual components at the given fractions (Kirk and Othmer, 1991). Therefore, the ethanol yield calculation method based on the liquid volume became much more complicated.

Several studies have realized the inaccuracy of the constant liquid volume assumption during glucose yield calculation at high solids loading of pretreated lignocellulosic biomass, and have proposed corresponding solutions in order to improving the traditional calculating methods. Hodge et al. (2009) integrated the variations of liquid density and insoluble solids weight into the glucose yield calculation equation during enzymatic hydrolysis at high solids loading. Roche et al. (2009) converted the sugar concentrations in the liquid (g/L) to mass fractions of the slurry in order to calculate the glucose yield at high solids loading more accurately. Kristensen et al. (2009) calculated the yield of glucose from the cellulose hydrolysis at the high solids loading by taking into account of the mass balance of the whole hydrolysis system. This method corrected the volume inaccuracy and could be practically used for calculation the glucose yield. Zhu et al. (2011) proposed another sugar yield calculating method for high solids enzymatic hydrolysis by taking the liquid volume change caused by the sugar released from cellulose into the consideration. All the above methods made a substantial progress for developing an accurate glucose yield calculation method. However, they were not sufficient and should be modified further to calculate the ethanol yield during SSF at high solids loading because SSF was indeed different from separate enzymatic hydrolysis, which included both the cellulose degradation and the conversion of glucose to ethanol, companied by the CO_2 generation and the liquid volume change.

In this study, a practical method for calculating practical ethanol yield at high ethanol titer and high solids content using easily and accurately measurable parameters as the calculation basis was proposed. This modified method was based on only one easily measurable parameter: the ethanol concentration in the liquid fraction of the fermentation broth in the unit of "g/L" (grams of ethanol in one liter of the mixed ethanol–water solution), which can be accurately measured on HPLC. The other data used in the proposed method include three operational parameters: initial solids content, total water addition, and the initial total weight of the SSF system. All these parameters can be determined accurately for a specific SSF. The proposed method relative accurately calculated the water consumption during cellulose degradation and the liquid volume increase caused by large amount of ethanol generation. The results show that the liquid volume increase due to ethanol formation during SSF was approximately five times greater than the volume decrease duo to water consumption during cellulose degradation, and the practical ethanol yield calculation using the commonly used method was underestimated and the underestimated error increased with the increasing ethanol titer.

2. Methods

2.1. Raw materials

A typical lignocellulose material, corn stover (CS), was selected as the feedstock for SSF operation and the practical ethanol yield calculation. Two CS materials were collected from Jilin, China in 2007, and Shandong, China in 2009, respectively. The Jilin CS was pretreated by uncatalyzed steam explosion and the Shandong CS was pretreated by the dry dilute acid pretreatment. The experimental details and the results of the SSF at the high solids loading of the pretreated CS were described in Zhang et al. (2010a). Briefly, the Jilin CS was pretreated using the steam explosion method at a condition of 210 °C, 2.2 MPa for 4 min using saturated steam. The Shandong CS was pretreated using the dry dilute-acid pretreatment method as described in Zhang et al. (2011) and subsequently detoxified by solid state fermentation with *Amorphotheca resinae* ZN1 for 7 days as described in Zhang et al. (2010b). The dry dilute acid pretreatment for the Shandong CS was conducted at the presoaking ratio of the solid (the dry materials) to the liquid (the sulfuric acid solution) of 2:1 (w/w) at the sulfuric acid usage of 2.5 g per 100 g of the dried CS, then the presoaked CS were pretreated at the conditions of 190 °C, 1.2 MPa, for 3 min. The water content of the resultant pretreated CS was only less than 50% (w/w) and there was no free water in the pretreated materials, thus this pretreatment method was called "dry dilute acid pretreatment".

The cellulase enzyme used was Accellerase 1000 from Genencor International (Rochester, NY, USA), and the activities of cellulase and cellobiase of 65.8 FPU/mL and 152.0 IU/mL respectively were determined using the procedure discussed in details in Zhang et al. (2010a). A thermo- and inhibitor-tolerant baker's yeast mutant strain of *Saccharomyces cerevisiae* DQ1 (CGMCC 2528) was used in all fermentations.

2.2. SSF operation at high solids loading in a helical stirring bioreactor

The SSF of the steam explosion pretreated Jilin CS or the dry dilute sulfuric acid pretreated and biodetoxified CS were both performed in a 5-L bioreactor equipped with a helical impeller (Zhang et al., 2010a), which was designed specially to enable high solids loading operation. In addition, this bioreactor was mounted a condenser for trapping down the vaporized water and ethanol. The SSF was operated at two stages, the prehydrolysis stage started at the beginning and then the real SSF stage was followed until the end of the operation. In the prehydrolysis stage, the CS was fed into the bioreactor within 12 h for prehydrolysis at the enzyme dosage of 7.0–15.0 FPU/g DM, at 50 °C and pH 5.0. The SSF started by adjusting the temperature to 37 °C and inoculating *Saccharomyces cerevisiae* DQ1 seeds (OD_{600} = 11.0) at the dosage of 10% (v/v) into the bioreactor and lasted for another 60–84 h. The pH was maintained during the hydrolysis and SSF stages by addition of 5 M NaOH solution. The samples were withdrawn at regular intervals and centrifuged at 10,000 rpm for 5 min to obtain the supernatant for analysis on HPLC.

2.3. Analysis of the CS composition and liquid samples

The CS composition was determined using Foss 2021 Cellulose Analyzer (Foss A/S, Hillerod, Denmark). Briefly, the composition analysis using Foss 2021 Cellulose Analyzer was the two-step sulfuric acid hydrolysis. First, the CS sample was hydrolyzed using 4.9% (w/w) sulfuric acid solution supplemented with 1.96% hexadecyl trimethyl ammonium bromide (CTAB), in which step the hemicellulose was removed from the sample; then the CS residues were subsequently hydrolyzed by 72% (w/w) sulfuric acid solution, where the cellulose was removed. The original Jilin CS contained 32.1% glucan, 26.4% xylan and 8.1% lignin (w/w, dry weight base). The Shandong CS contained 34.2% glucan, which was slight higher than that of the Jilin CS, largely due to its self-degradation during the longer preservation period, and 31.5% hemicellulose (w/w, dry weight base).

The liquid samples were analyzed to determine the concentrations of sugars, ethanol and inhibitors by HPLC (LC-20AD, RI detector RID-10A, Shimadzu, Japan) using a Bio-Rad Aminex HPX-87H column operating at 65 °C with 0.6 mL/min of 5 mM H_2SO_4 as mobile phase. All samples were filtered through a 0.22 μm filter before injection.

2.4. Analysis on the calculation method of ethanol yield based on Eq. (1)

The basis for most ethanol yield calculation methods was shown in Eq. (1). Practically, for a specific SSF operation, the ethanol yield is calculated on the mass base, rather than the concentration base. Suppose *m* (g) is the total weight of the SSF system at the beginning; [*Biomass*] (g/g) is the dry biomass fraction at the beginning of the fermentation (slightly different from $[Biomass]_0$ in the unit of g/L in Eq. (1), i.e., the solids fraction of the fermentation broth; *E* (g) is the total ethanol produced in the SSF operation. The practical ethanol yield in a specific SSF operation can be calculated by Eq. (2):

$$\text{Practical ethanol yield}(\%) = \frac{E}{0.511 \times f \times [Biomass] \times m \times 1.111} \times 100\% \tag{2}$$

The practical ethanol yield here is defined as the percentage of the theoretical ethanol yield based on the pretreated materials. Assumed that the initial and the final liquid volume of the SSF system are V_0 and V_f (L), then the ethanol produced *E* (g) in the SSF operation can be calculated by Eq. (3):

$$\text{Practical ethanol yield}(\%) = \frac{[EtOH]_f \times V_f - [EtOH]_0 \times V_0}{0.511 \times f \times [Biomass] \times m \times 1.111} \times 100\% \tag{3}$$

Because the direct measurement of the liquid volume of the fermentation broth is difficult, in many cases, generally Eq. (3) is used under two assumptions: (1) the density of the system is assumed to be 1000 g/L, without considering the effects of the solids, ethanol, sugars, and CO_2 release on the density; (2) the fermentation broth volume is assumed to be that of water in the fermentation broth, without considering the effects of the solids, ethanol, and sugars existence on the total volume. When Eq. (3) is used for the practical ethanol yield calculation under the low solids concentration, the two consumptions are approximately valid because the solids content, the ethanol concentration, the sugar residues, and CO_2 release are negligible. However, when high solids loading in SSF is applied to reach high titer ethanol, a larger error may occur using Eq. (3) to calculate the practical ethanol yield.

2.5. Modified calculation for practical ethanol yield

The proposed method includes the following steps: (1) the volume of the ethanol–water mixture was correlated with the ethanol weight per volume concentration "g/L", which was accurately measured on HPLC, and the weight concentration "g/g" using the classic data from the Lange's Handbook of Chemistry (Lange and Dean, 1967); (2) the whole mass balance for ethanol and water were established to give a modified equation for the practical ethanol yield calculation at the high solids and high ethanol titer SSF; (3) after the mass balance was established, finally a modified equation for the practical ethanol yield calculation was developed and further applied to give the corrected yield values using the SSF experimental data.

2.5.1. Correlation of ethanol concentration between the unit "g/L" and "g/g"

The measurable ethanol concentration on HPLC is in grams per litter of the liquid (g/L). Because of the volume shrinking property of the ethanol–water mixing, the liquid volume of the produced ethanol or the consumed water can not be simply added or subtracted to give the final volume. To avoid the difficulties of variable volume, the volume based ethanol titer (g/L) was converted to the mass based concentration (g/g) using the mass balance principle. Lange and Dean (1967) listed the conversion table in "Lange's Handbook of Chemistry" as shown in Table 1, and the correlation between the two units, g/L and g/g, was deduced as shown in Fig. 1 and Eq. (4) (Lange and Dean, 1967).

$$[C_2] = 1.0236 \times 10^{-3} \times [C_1] = \frac{[C_1]}{976.9} \tag{4}$$

where $[C_1]$ is the ethanol concentration in the liquid fraction of the fermentation broth in g/L, and $[C_2]$ is the ethanol concentration in the liquid fraction of the fermentation broth in g/g. The ethanol concentration expressed as g/L and g/g listed in "Lange's Handbook of Chemistry" were measured at 15.6 °C, thus Eq. (4) was only proper for the units conversion of ethanol concentration measured at 15.6 °C. However, the variation of the ethanol solution density was minor with the change of the environmental temperature, which was also listed in "Lange's Handbook of Chemistry" (Lange and Dean, 1967). Taken 4% (w/w) ethanol solution as an example, the ethanol solution density decreased by only 0.28% with the temperature increased from 15 °C to 25 °C. As a result, the deduced Eq. (4) may be appropriately applied at the ambient temperatures with negligible errors.

Table 1
The related data on ethanol concentration in different units, g/L and g/g.

$[C_1]$ (g/L)	$[C_2]$ from literature (g/g) *	$[C_2]$ from correlation (g/g) **	Relative Error (%) ***
39.70	0.0400	0.0406	−1.60
47.60	0.0480	0.0487	−1.51
50.00	0.0505	0.0512	−1.35
98.40	0.1000	0.1007	−0.73
111.10	0.1130	0.1137	−0.64
134.90	0.1378	0.1381	−0.21
146.80	0.1502	0.1503	−0.05
175.40	0.1800	0.1795	0.25
194.40	0.2001	0.1990	0.55

* Data obtained from Lange's Handbook of Chemistry, 10th ed.
** Calculated using Eq. (4).
*** Error $(\%) = \frac{([C_2]_{\text{literature}} - [C_2]_{\text{correlation}})}{[C_2]_{\text{literature}}} \times 100\%$.

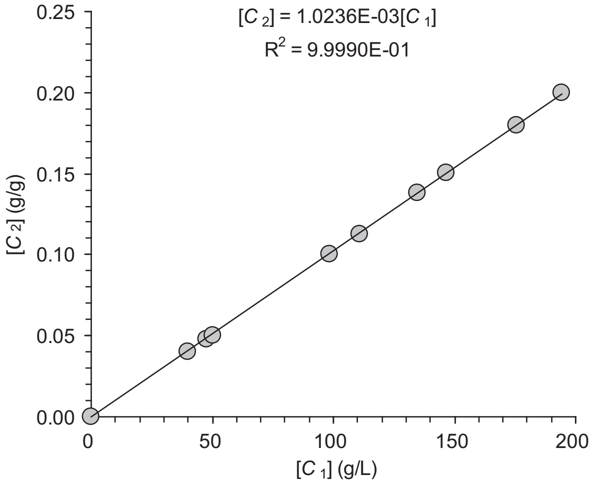

Fig. 1. Correlation between the different ethanol concentration units: $[C_1]$ in g/L and $[C_2]$ in g/g. The original data were shown in Table 1.

2.5.2. Water loss during the SSF operation

The water consumed during cellulose hydrolysis is not negligible at the high solids loading and is calculated based on the stoichiometric reaction equation of Eq. (5)

$$(C_6H_{10}O_5)_n + nH_2O - nC_6H_{12}O_6 - 2nC_2H_5OH - 2nCO_2. \tag{5}$$

$$\begin{array}{cc} n & 2n \\ \frac{W_1}{18} & \frac{E}{46} \end{array}$$

where W_1 (g) is the water consumed in the hydrolysis of cellulose during SSF, E (g) is still the ethanol produced. According to Eq. (5), once n mol water is consumed, there will be 2n mol ethanol produced, and thus:

$$\frac{\frac{W_1}{18}}{\frac{E}{46}} = \frac{n}{2n} \tag{6a}$$

Then:

$$W_1 = \frac{n \times 18 \times E}{2n \times 46} = \frac{E}{5.11} \tag{6b}$$

Suppose W (g) is the total water input into the SSF, then the residual water in the fermentation broth after the SSF is:

$$W - W_1 = W - \frac{E}{5.11} \tag{7}$$

The liquid volume loss in the CO2 tail gas was captured by venting through a bottle containing water. The weight of the bottle was analyzed regularly, and showed almost no increase with time. Therefore, either water or ethanol loss with CO2 vent was minor and could be safely ignored. Furthermore, the following practical ethanol yield calculation equations were only suitable for the closed fermentor or bioreactor systems, not applicable to the open systems such as the SSF in flasks.

2.5.3. Modified practical ethanol yield calculation

Suppose the initial ethanol concentration in the liquid before the SSF start is $[C_1^0]$ in g/L, which equals to the $[EtOH]_0$ defined in Eq. (3), $[C_2^0]$ is the corresponding concentration in g/g, and E_0 (g) is the initial ethanol weight in the SSF system before the SSF start. The following relations can be derived from Eq. (4):

$$[C_2^0] = \frac{[C_1^0]}{976.9} \tag{8}$$

$$E_0 = [C_2^0] \times W = \frac{[C_1^0] \times W}{976.9} \tag{9}$$

The total water and ethanol weight in the fermentation broth after the SSF ends can be expressed as $(E + E_0 + W - \frac{E}{5.11})$, and the ethanol concentration $[C_2]$ in the unit of g/g can be expressed as:

$$[C_2] = \frac{E + E_0}{E + E_0 + W - \frac{E}{5.11}} \tag{10}$$

Thus:

$$E = \frac{(E_0 + W) \times [C_2] - E_0}{1 - 0.804 \times [C_2]} = \frac{([C_2] + [C_2] \times [C_2^0] - [C_2^0]) \times W}{1 - 0.804 \times [C_2]} \tag{11}$$

Substituting Eqs. (4) and (8) into Eq. (11) gives the expression of E as follows:

$$E = \frac{([C_1] + \frac{[C_1] \times [C_1^0]}{976.9} - [C_1^0]) \times W}{976.9 - 0.804 \times [C_1]} \tag{12}$$

Combining Eq. (12) into Eq. (2) gives the modified practical ethanol yield expression as:

$$\begin{aligned} \text{Practical ethanol yield}(\%) &= \frac{E}{0.511 \times f \times [Biomass] \times m \times 1.111} \\ &\quad \times 100\% \\ &= \frac{([C_1] + \frac{[C_1] \times [C_1^0]}{976.9} - [C_1^0]) \times W}{976.9 - 0.804 \times [C_1]} \\ &\quad \cdot \frac{1}{0.511 \times f \times [Biomass] \times m \times 1.111} \\ &\quad \times 100\% \end{aligned} \tag{13}$$

In Eq. (13), the practical ethanol yield can be calculated using the following parameters: (1) the two measurable parameters, the ethanol concentrations at the end and the beginning of the SSF operation, $[C_1]$ and $[C_1^0]$, in the unit of g/L; (2) three operational parameters, the total water added into the SSF operation, W (g); the dry biomass concentration at the beginning of the SSF, [*Biomass*] (g/g); and the total weight of the SSF system at the beginning of the operation (including total solids and liquid), m (g).

The only assumption for using Eq. (13) is that the contribution of the residual sugars, inhibitors and other salts existing in the fermentation broth to the volume shrinkage of mixing water and ethanol was negligible. In the practical SSF operation, the concentrations of these substances in the fermentation broth are much lower, for example, less 10 g/L of sugars, almost no inhibitors, and thus are reasonable to be ignored.

If the initial ethanol concentration before the SSF start, $[C_1^0]$, is negligible, Eq. (13) can be simplified into Eq. (14):

$$\text{Practical ethanol yield }(\%) = \frac{[C_1]\times W}{976.9 - 0.804\times[C_1]} \cdot \frac{1}{0.511\times f\times[Biomass]\times m\times 1.111} \times 100\% \qquad (14)$$

2.5.4. Ethanol mass balance of the modified method

The SSF slurry was vacuum distilled at 50 °C, 0.01 MPa for 90 min to remove the ethanol produced during SSF. The liquid lost during distillation was supplemented with water into the slurry, and this slurry was defined as the original slurry. Then 11.0 ml ethanol solution (the ethanol concentration and the weight of ethanol solution were 0.730 g/mL and 8.7 g, respectively) was added into 146.0 g original slurry. The mixture was mixed rigorously after the ethanol solution addition. The samples before and after ethanol solution addition were centrifuged at 10,000 rpm for 5 min, then the supernatant liquid was analyzed on HPLC.

3. Results and discussion

3.1. Ethanol mass balance calculation of the modified method

The ethanol mass balance was performed according to the following procedure:

The water content of the original slurry was 86.7% (w/w); the ethanol concentration in the liquid supernatant after vacuum distillation was nearly zero; the ethanol concentration in the slurry after ethanol addition was determined to be 58.2 g/L. The ethanol concentration was converted into 0.0596 g/g liquid using Eq. (4). Thus, the overall ethanol mass in the ethanol-slurry system was calculated as:

$0.0695 \times (146.0 \times 86.7\% + 8.7) \approx 8.06$ (g) (the term of $146.0 \times 86.7\%$ was the water mass in the original slurry).

The overall ethanol mass in the original ethanol solution: $11.0 \times 0.730 = 8.03$ (g).

The mass balance of the sample analysis was calculated as: $(8.06-8.03) / 8.03 \times 100\% = +0.37\%$.

The experiment result indicates that the mass balance of the proposed method was valid. The ethanol partition between the liquid phase and the solids residues did exist, but the effect of the ethanol partition on the mass balance was negligible in the practical operation. Therefore, the ethanol concentration in the main body of the liquid phase could be used to approximately represent the total ethanol concentration of the SSF system. Lignin contributed more than 90% of the solid residues in the SSF slurry, while cellulose and hemicellulose contributed the rest 10%. The active groups on the lignin were already deactivated in the intensive pretreatment processing and less possible to selectively adsorb ethanol.

3.2. SSF at different solids loading of the pretreated CS

For the SSF operation at the solids loadings of 15%, 20%, 25%, and 30% (w/w) of the steam explosion pretreated CS, the previous fermentation data were cited (Zhang et al., 2010a). For the SSF operation at the 32.9% and 37.0% (w/w) of the dry dilute acid pretreated CS, the unpublished experimental data were used. The practical ethanol yield results using traditional method Eq. (3) and the modified method Eq. (14) (the initial ethanol concentration $[C_1^0]$ was set to zero because it was not detected on HPLC) are shown in Table 2. The comparisons show that all the practical ethanol yields calculating using traditional method were underestimated, with errors of underestimate of 4.6%, 5.0%, 5.7% and 5.8% observed at increasing solids loading of 15%, 20%, 25% and 30%, respectively. With the further increase of the solids content and the enzyme dosage, the practical ethanol yield calculation differences between these two calculation methods exhibited even larger errors of 5.8%, 8.4%, and 8.5% at solids loading of 30.0%, 32.9% and 37.0%, respectively.

3.3. SSF at different enzyme dosage

Another example was the SSF performed with different enzyme dosages at the 30% solids loading of the steam explosion pretreated CS (Zhang et al., 2010a). The calculation results of practical ethanol yield using Eqs. (3) and (14) are shown in Table 3. Again the initial ethanol concentration $[C_1^0]$ was set to zero because it was not detected on HPLC. It was found that the traditional method was also underestimated and the calculation errors of practical ethanol yield increased with the increasing final ethanol concentration, from 5.6%, 7.8%, to 8.1% with the final ethanol concentration increasing from 40.6, 59.3, to 64.6 g/L, respectively.

The reason for underestimation of the practical ethanol yields using Eq. (3) could be attributed to the assumption of the constant liquid volume during SSF process. In fact, the liquid volume increased with the increasing ethanol formation, and decreased with the water consumption in the cellulose hydrolysis. From Eq. 6b and Tables 2 and 3, we could obviously find out the weight of ethanol formation during SSF was five times greater than that of the water consumed, thus the final liquid volume of the SSF system after fermentation presented an increase trend, and thus the practical ethanol yield calculation taking the volume change into consideration was higher than not.

Considering most microorganisms used for ethanol fermentation could only metabolize the glucose (Olofsson et al., 2008), all equations demonstrated in this study only concerns the conversion of cellulose fraction into ethanol. Thus, a more general equation

Table 2
Comparison of practical ethanol yield between the old and new calculation methods at different solids loading.

[Biomass] (%, w/w)	f (-)	$[C_1]$ (g/L)	m (g)	W (g)	W_1 (g)	E (g)	Practical ethanol yield using Eq. (3) (%)	Practical ethanol yield using Eq. (14) (%)	Relative error (%)
15.0	0.321	24.7	2338.0	1985.5	10.0	51.2	76.7	80.2	−4.6
20.0	0.321	31.0	2340.0	1870.4	11.9	61.0	68.0	71.4	−5.0
25.0	0.321	39.3	2487.0	1864.9	15.2	77.6	64.7	68.4	−5.7
30.0	0.321	40.6	2294.0	1605.4	13.5	69.1	52.0	55.0	−5.8
32.9	0.340	65.3	2225.0	1493.8	20.6	105.5	69.0	74.7	−8.3
37.0	0.340	68.1	2300.0	1450.8	21.0	107.1	60.1	65.2	−8.5

SSF conditions: For [*Biomass*] at 15.0%, 20.0%, 25.0%, and 30.0% of the steam explosion pretreated CS, the enzyme dosage was 7.0 FPU/g DM and the data were cited from Zhang et al. (2010a). For [*Biomass*] at 32.9% and 37.0% of the dry dilute acid pretreated CS, the enzyme dosage was 15.0 FPU/g DM (unpublished data). Each experiment was prehydrolyzed at 50 °C for 12 h at pH 5.0, and then SSF at 37 °C for 60 h or 84 h at pH 5.5.

Table 3
Comparison of practical ethanol yield between the old and new calculation methods at different enzyme loading.

Cellulas dosage (FPU/g DM)	$[C_1]$ (g/L)	m (g)	W (g)	W_1 (g)	E_1 (g)	Practical ethanol yield using Eq. (3) (%)	Practical ethanol yield using Eq. (14) (%)	Relative error (%)
7	40.6	2294.0	1605.4	13.5	69.1	52.0	55.0	−5.8
15	59.3	2395.0	1675.6	20.9	106.9	75.9	81.7	−7.6
30	64.6	2177.0	1523.2	20.8	106.4	82.7	89.4	−8.1

SSF conditions: [*Biomass*] was 30% (w/w) of the steam explosion pretreated CS, *f* was 0.321. The data were cited from Zhang et al. (2010a). Each experiment was prehydrolyzed at 50 °C for 12 h at pH 5.0, and then SSF at 37 °C for 60 h or 84 h at pH 5.5.

should be established when the xylose metabolizing organisms are used for ethanol fermentation.

4. Conclusion

A modified method for calculating practical ethanol yield during SSF at high lignocellulosic solids and high ethanol titer was proposed considering the liquid volume change caused by high titer ethanol generation and the water consumed during cellulose degradation. The modified method was applied to calculate the practical ethanol yields of several practical SSF operations and the results show that the liquid volume increase with ethanol formation was approximately five times greater than the volume decrease duo to water consumption during cellulose degradation, thus the practical ethanol yields using the traditional method were underestimated.

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