Bioresource Technology 116 (2012) 74–79

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

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

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ARTICLE INFO

Article history:
Received 28 December 2011
Received in revised form 27 March 2012
Accepted 29 March 2012
Available online 13 April 2012

Keywords:
Modified practical ethanol yield calculation
Simultaneous saccharification and fermentation (SSF)
Lignocellulose
High solids loading
High ethanol titer

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 ($[EtOH]_f$), $[\mathrm{Biomass}]_0$ 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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http://dx.doi.org/10.1016/j.biortech.2012.03.100

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