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Detailed Material Balance and Ethanol Yield Calculations for the Biomass-to-Ethanol Conversion Process

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ABSTRACT

Applying material balance calculations to the evaluation and optimization of lignocellulosic biomass conversion processes is fundamentally important. The lack of a general framework for material balance calculations and inconsistent compositional analysis data have made it difficult to compare results from different research groups. Material balance templates have been developed to follow accurately the distribution of carbon in lignocellulosic substrates through the pretreatment and simultaneous saccharification and fermentation (SSF) processes, and provide information on overall carbon recovery, recovery of individual sugars, and solubilization of biomass components. Based on material balance considerations, we developed equations that allow us to compute overall ethanol yields for biochemical conversion of biomass correctly.

Index Entries: Carbon balance; dilute-acid pretreatment; simultaneous saccharification and fermentation; enzymatic conversion; overall yield.

Nomenclature: C-mol, amount (mass) of a substance containing 1 mol of the element carbon (g); $[C_5]$, concentration of pentose sugars in hydrolysate (g/L); $[C_6]$, concentration of hexose sugars in hydrolysate (g/L); d_L, density of liquor (SSF or hydrolysate) (g/L); $[E]$, ethanol concentration (g/L); f_s, fraction of insoluble solids (g insoluble solids/g slurry); L_f, mass-loss factor of insoluble solids from pretreatment (g pretreated solids/g raw solids); m, mass of whole slurry (g); m_L, mass of liquid phase (g); m_s, mass of insoluble solids (g); Q_{C5}, mass fraction of pentose sugars in raw substrate (g/g dry mass); Q_{C6}, mass fraction of hexose sugars in raw substrate (g/g dry mass); Y_E, ethanol yield (g/100 g hexose sugar). Subscripts: L, liquid-phase composition or mass; *max*, maximal potential yield; 0, raw substrate or beginning of SSF (t = 0); p, substrate or solids compositions after pretreatment; s, solids composition or mass.

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THE BIOMASS-TO-ETHANOL TECHNOLOGY

Cellulosic biomass, the most abundant natural polymer on earth, is a renewable resource that can be transformed efficiently into commodity and specialty chemicals using biotechnology *(1)*. In the United States, biomass feedstocks include energy crops, agricultural and forestry residues, pulp and paper industry waste streams, and municipal solid waste. Ethanol-from-biomass production, in particular, is seen as a high-potential technology that offers several advantages to the country and the environment. By replacing gasoline with ethanol as transportation fuel, urban air pollution and global warming may be ameliorated, the US economy will become less dependent on foreign oil, and jobs will be created in the agricultural sector.

Cellulosic biomass typically consists of cellulose (40–45% [w/w]), hemicellulose (15–20% [w/w]), and lignin (25–30% [w/w]), but its composition varies considerably among different feedstocks. Although rich in carbohydrates (cellulose and hemicellulose), biomass is an insoluble substrate with a complex structure: cellulose fibers are embedded in a sheath of hemicellulose and lignin, and held together by hydrogen and van der Waals bonds. Nevertheless, despite its compact structure, cellulose can be converted first to fermentable sugars (primarily glucose) and eventually to ethanol or other chemicals using cellulose-hydrolyzing cellulase enzymes and ethanol-producing microorganisms.

There are several ways to increase the digestibility of cellulose before it is exposed to enzymes: mechanical, physical, or chemical pretreatment. The use of dilute sulfuric acid (0.5–1% [w/w]) and high temperatures (160–200°C) has significantly improved the accessibility of cellulose to cellulase *(2)*. During the process, a significant fraction of hemicellulose is hydrolyzed to its components: the pentoses xylose and arabinose and the hexoses mannose, galactose, and some glucose. At the same time, part of the lignin is also solubilized to phenolic compounds, along with small amounts of cellulose, which yields glucose. Clearly, the selected pretreatment conditions will determine the efficiency of the pretreatment process. The release of readily fermentable pentose monomers (primarily xylose) is highly desirable, as opposed to the unfermentable xylose oligomers. Measuring the concentration of each component is essential to selecting the optimal conditions. Similarly, the pretreatment conditions will determine the degree of lignin solubilization and recondensation, which directly affects the digestibility of the remaining solids that are enriched in cellulose.

Although sugar release is desirable, free sugars are vulnerable to degradation under the high-temperature acidic conditions of pretreatment; xylose degrades to furfural and glucose to hydroxymethyl furfural (HMF). Those compounds, along with lignin-derived phenolics and hemicellulose- derived acetic acid, are known inhibitors of cell metabolism and may have a detrimental effect on the fermentability (simultaneous saccharification and fermentation [SSF] performance) of the pretreated biomass. In addition, their formation represents a waste of biomass carbon that needs to be accounted for when ethanol yields are calculated.

Following the pretreatment, the cellulosic content of biomass is converted to ethanol using the SSF process. The SSF combines the enzymatic hydrolysis of polymeric cellulose to monomeric glucose with the fermentative conversion of glucose to ethanol in the same environment. The hydrolytic step is catalyzed by the synergistic action of endoglucanases, exoglucanases, and β-glucosidases *(3)*. Endos and

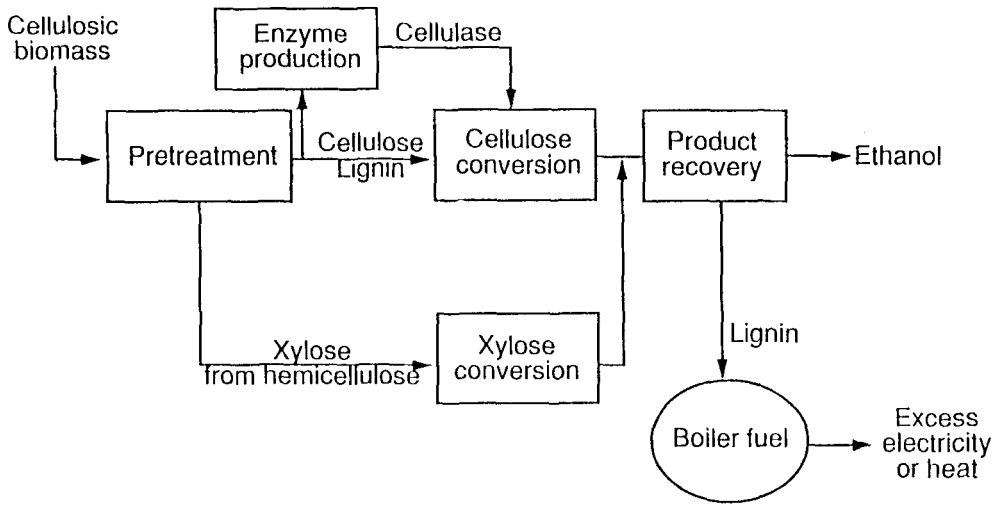

Fig. 1. Simplified schematic of the NREL biomass-to-ethanol conversion process.

exos (collectively referred to as cellulase) randomly attack cellulose chains to produce (primarily) cellobiose, as well as oligosaccharides and glucose. The dimeric and oligomeric sugars are then hydrolyzed to glucose by β-glucosidases. Finally, fermentative microorganisms convert glucose to ethanol, which is recovered through downstream processing. Depending on the SSF conditions and the characteristics of the microorganism, other metabolic byproducts, such as glycerol and organic acids, may also be synthesized. To the extent that they cannot be recovered as high-value products to generate revenue, those byproducts constitute a diversion of carbon to low-value biogass from anaerobic digestion, and their formation should be minimized.

Pretreatment and SSF are at the heart of the bioconversion process (Fig. 1): collectively, they account for the largest portion of the ethanol production cost, almost 45% (*4*). The efficiency of the pretreatment determines the extent of xylose recovery and the quality of the resulting xylose-rich liquor (hydrolysate) with regard to sugar and inhibitor concentration. On the other hand, the efficiency of the SSF process is equally important, since it determines the yield and productivity of ethanol from solubilized sugars and insoluble cellulose. Because ethanol is the major value-added product of the process, measuring the loss of biomass carbon to other products and calculating ethanol yield based on the actually utilized raw material are imperative for a technically sound process design. Only by calculating the combined efficiency of these two sequential operations (pretreatment and SSF), can we determine the overall feasibility and economic viability of the biomass-to-ethanol conversion technology with high accuracy and reliability.

To date, this reality has been largely ignored because of the complexity of the biomass conversion system. As a result, high SSF yields are erroneously considered equivalent to high overall biomass yields and analytical errors remain unchecked because of the complete lack of carbon balance calculations. This article presents, for the first time, a concise analysis of the accounting aspects and engineering calculations of biomass conversion by (1) formulating a comprehensive carbon balance spreadsheet for the pretreatment and SSF operations to determine carbon distribu-

tion, and (2) developing mathematical formulas to calculate product yields for the pretreatment, SSF, and overall process. Such calculations are a necessary precondition for any credible scale-up, optimization, and commercialization study.

EXPERIMENTAL PROCEDURES

Substrate

Yellow poplar wood *(Liriodendron tulipifera)* sawdust, milled to pass through a 4-mm screen, was pretreated with dilute sulfuric acid (0.40% [w/w]) at 150–190°C for 15 min/stage according to a two-stage, reverse-flow percolation process *(5)*. The pretreated biomass was employed in the SSF process at a concentration of 12% solids (8% cellulose) on a dry-wt basis. The composition of raw and pretreated biomass is given in Table 1.

Enzyme

Cellulolytic enzyme, synthesized by a *Trichoderma reesei* strain (CPN International, Jupiter, FL), was employed at a concentration of 25 IFPU/g cellulose. The cellulase and β-glucosidase volumetric activities of the enzyme preparation were 88 and 230 IFPU/mL, respectively, when measured according to the IUPAC methods *(6)*.

SSF

The SSF runs were performed with 12% of pretreated sawdust in yeast-extract, peptone (YP) medium (10 g/L yeast extract, 20 g/L peptone) of initial pH 5.0 at 38°C in 250-mL flasks equipped with stoppers and carbon dioxide traps to simulate anaerobic conditions. In all cases, agitation was maintained at 150 rpm. The pretreatment liquor was partially detoxified by an overliming treatment *(7)* before use. Inocula were prepared in the same media supplemented with 50 g/L glucose under aerobic conditions. A preadaptation step of the inoculum was carried out in 20% (v/v) of the hydrolysate before the SSF run. The fermentative microorganism was *Saccharomyces cerevisiae* D_5A, a strain developed at NREL from baker's yeast *(8)*. A 5% (v/w) inoculum was used to initiate each SSF run.

Chemical Analysis

The biomass was analyzed to determine its glucan, mannan, galactan, xylan, arabinan, lignin, and ash content, according to National Renewable Energy Laboratory (NREL) standard procedures. Fermentation samples were filtered through 0.45-μm nylon filters, and analyzed by high-performance liquid chromatography (HPLC) for the presence of cellobiose, monomeric, and total sugars, and metabolites (ethanol, organic acids, and glycerol). Total sugars were determined after a secondary hydrolysis of the pretreatment or fermentation liquors with 4% (w/w) sulfuric acid for 1 h at 121°C. The HPLC unit 1090LC (Hewlett-Packard, Avondale, PA) was equipped with a refractive index detector. All compounds except for sugars were analyzed using a Bio-Rad Aminex HPX-87H column (Bio-Rad, Hercules, CA), operated at 65°C using 0.2-μm filtered 0.01*N* sulfuric acid as eluent at a flow rate of 0.6 mL/min. Sugars were analyzed using a Bio-Rad Aminex HPX-87C column for glucose, xylose, and arabinose, or a Bio-Rad

Table 1
Pretreatment Carbon Balance

TWO-STAGE SAWDUST PERCOLATION (2x12")

Process: Percolation
Catalyst: Sulfuric Acid
Feedstock: Yellow Poplar
Run: INHPOP16

CONDITIONS

Temperature (°C) :	150-190
Time (min) :	15
Concentration (wt%) :	0.4015

TOTAL MASS BALANCE

	Raw Feed	*Unwashed Pretreated*
Wet Solids Mass (g):	383.8	580.7
% Dry Solids (%):	59.8	22.9
Dry Solids Mass (g):	229.4	133.0
Liquor (mL):	3370.0	3370.0
Solids Solubilized (%):		42.0

Researcher : R. Torget
Run Date : 20-Mar-95
CAT Reports : 95-004
95-005

Carbon Balance: Pretreatment

Component	Unpretreated		Pretreated							
	Dry Biomass		*In Solids*			*In Liquor*			*Total*	***Recovery***
	(% dry weight)	(C-mole)	(% dry weight)	(C-mole)	(% C in Feed)	(g/L)	(C-mole)	(% C in Feed)	(C-mole)	(% C in Feed)
Glucose	46.55	3.556	65.61	2.906	*81.7*	4.43	0.497	*14.0*	3.416	96.0
Mannose	3.66	0.280	0.09	0.004	*1.4*	2.11	0.236	*84.5*	0.240	85.9
Galactose	0.49	0.037	0.00	0.000	*0.0*	0.35	0.039	*104.9*	0.039	104.9
Xylose	17.59	1.344	0.74	0.033	*2.4*	11.30	1.269	*94.4*	1.353	100.7
Arabinose	0.89	0.068	0.00	0.000	*0.0*	0.56	0.062	*91.6*	0.062	91.6
Acetic Acid	4.70	0.359	0.00	0.000	*0.0*	2.76	0.309	*86.1*	0.309	86.1
Formic Acid										
Levulinic Acid										
Lignin	26.65	2.709	31.33	1.846	*68.1*	6.04	0.901	*33.3*	2.748	101.4
Furfural						0.30	0.052	*3.7*		
HMF						0.08	0.013	*0.3*		
Total	*91.83*	***8.354***	*91.11*	*4.789*	*57.3*		*3.379*	*40.5*	***8.168***	97.8

C - RECOVERY: **97.8%**

Aminex HPX-87P column if cellobiose, mannose, and galactose were also to be determined. Both columns were operated at 85°C using 0.2-μm filtered and degassed HPLC-grade water at a flow rate of 0.6 mL/min. The HPLC-based measurements of glucose and ethanol concentration were confirmed using a YSI glucose analyzer (YSI, Yellow Springs, OH) and a gas chromatograph (Model 5890, Hewlett-Packard), respectively.

MATERIAL BALANCE CALCULATIONS

A necessary first step in assessing the feasibility and potential of new technologies is a techno-economic evaluation of various process scenarios. Such evaluations usually identify the critical parameters of the process and dictate minimal performance criteria for its feasibility. The objective of laboratory bench-scale or demonstration-scale work is then to provide experimental evidence for whether or not the assumed process performance is attainable. Therefore, the reliability of the experimental process data is critical for the unbiased assessment of the technology.

In the biomass-to-ethanol process, the pretreatment of the lignocellulosic feedstock before enzymatic hydrolysis, and the subsequent enzymatic hydrolysis and fermentation of the resulting sugars to ethanol are the essential parts of the process. Technoeconomic evaluation of the NREL biomass- to-ethanol process has identified the SSF step as being the most critical for overall process performance *(4)*. Effective pretreatment, however, can have a large impact on the economics by improving the effectiveness of the enzymatic hydrolysis. Therefore, laboratory evaluations of the biomass- to-ethanol process need to consider pretreatment and SSF steps concurrently.

The efficacy of the various pretreatment methods is typically evaluated based on the following criteria:

1. The yield or recovery of the main sugars in soluble form;
2. The rate and extent of the pretreated substrate's enzymatic digestibility; and
3. The rate and degree of the solubilized and insoluble sugars fermentability through SSF for ethanol production.

The last criterion is the most direct measure of the conversion process performance, and is designed to assess both the digestibility of the solids and the fermentability of the prehydrolysate (pretreatment liquor). This is typically limited by the presence of inhibitors, such as phenolic fragments from solubilized lignin, acetic acid from hemicellulose, and the sugar degradation products furfural and hydroxymethylfurfural (HMF). The concentration of inhibitors increases with the pretreatment severity and the degree of solubilization of the cellulosic, hemicellulosic, and lignaceous components of the feedstock.

The need for a general framework under which pretreatment and SSF experimental data for different feedstocks can be consistently evaluated is therefore of primary importance, as stated in the pretreatment literature *(10,11)*. A comprehensive framework should include:

1. Material balance evaluation;
2. Sugar recovery information; and
3. SSF and overall process ethanol yields.

Material balance calculations are essential for verifying the integrity and consistency of the compositional analysis data both after pretreatment and after SSF, especially in light of reported difficulties associated with the accurate determination of the composition of pretreated lignocellulosic feedstocks and SSF residues *(9,12,13)*. The information on sugar yields and recoveries can then be readily obtained from the material balance calculations around the pretreatment process. Similarly, the SSF and overall process ethanol yields can be calculated from SSF and pretreatment mass balance information, and the produced ethanol.

In an effort to adopt a consistent evaluation procedure to facilitate comparison of results between different groups throughout NREL's biomass-to-ethanol project, a set of EXCEL templates was developed to perform material balance calculations around the pretreatment, SSF, and overall conversion processes. These templates are described next. In addition, a set of formulas developed to calculate ethanol yields correctly for the overall conversion process involving pretreatment and SSF is described in the following section.

Pretreatment Carbon Balance

The material balance for the pretreatment process was followed both in terms of total mass and for the individual components, in terms of carbon. This choice for the individual component balances was made (1) to avoid complications with the water balance resulting from the various hydration and dehydration reactions, and (2) to illustrate clearly changes in the distribution of carbon in the liquid and solid phases that occur between the various carbon-containing substances during the course of the pretreatment process. All major components of the biomass hemicellulosic, cellulosic, and lignaceous factions were considered for individual component balances. These include:

1. The hexose sugars, glucose, mannose, and galactose;
2. The pentose sugars, xylose and arabinose;
3. Lignin;
4. Acetic acid, which results from hydrolysis of the acetyl groups of hemicellulose *(14)*;
5. The sugar degradation products furfural and HMF *(15,16)*; and
6. 4-oxopentanoic or levulinic acid and formic acid, the former resulting from degradation of HMF *(17)*, and the latter mainly from HMF *(17)* and to a lesser degree from furfural *(18)*.

The template for the pretreatment carbon balance is shown in Table 1. The type of pretreatment, pretreatment equipment, catalyst, feedstock, and pretreatment conditions are listed on the top of the template for easy reference. The first part of the spreadsheet consists of the total mass balance calculations. The information needed here is the dry solids loading and liquor volume (catalyst solution) used and the corresponding amounts recovered at the end of the pretreatment. From this data, the hydrolyzed biomass fraction (an important design parameter) is calculated.* In addition to providing this information, the accuracy of the total mass balance is critical, since it affects the balance calculations on the individual

*The percent solubilized solids is calculated as $(1\text{-}m_{s/p}/m_{s,0}) \times 100$, where $m_{s/p}$ and $m_{s,0}$ denote the dry solids mass after and before pretreatment, respectively.

Table 2
Molecular and C-Mol Weights

Component	C	H	O	N	OMe	Formula weight, g	C-mol weight, g
Glucose	6	12	6			180.16	30.03
Mannose	6	12	6			180.16	30.03
Galactose	6	12	6			180.16	30.03
Xylose	5	10	5			150.13	30.03
Arabinose	5	10	5			150.13	30.03
Glycerol	3	8	3			92.10	30.70
Acetic acid	2	4	2			60.05	30.03
Lactic acid	3	6	3			90.08	30.03
Succinic acid	4	6	4			118.09	29.52
Lignin	9	8.05	2.70		1.41	203.09	22.57
Furfural	5	4	2			96.09	19.22
HMF	6	6	3			126.11	21.02
Formic acid	1	2	2			46.03	46.03
Levulinic acid	5	8	3			116.12	23.22
Cellulose	6	10	5			162.14	27.02
Cellobiose	12	22	11			342.30	28.53
Cell mass (D5A)	3.874	6.657	2.081	0.752		97.07	25.06
Ethanol	2	6	1			46.07	23.03
Carbon dioxide	1		2			44.01	44.01

components. Therefore, extra attention needs to be given to obtaining the needed mass and moisture content of the untreated and pretreated solids.

The second and main part of the spreadsheet consists of the balance calculations on individual components. Compositional analysis data for the raw solids, pretreated solids, and pretreatment liquor, together with the total mass balance information, are used in these calculations. The solids composition is typically reported on a dry-wt basis, with the sugar concentrations reported as monomeric equivalents. For the particular sample shown in Table 1, the analyzed components make up 91.8% of the raw dry biomass, after applying weight adjustments for anhydro-sugars and acetyl groups,** ash is 0.6%, and the remaining 7.6% is most likely uronic acids and extractives. Total soluble sugar concentrations, including both monomeric and oligomeric forms, are measured in the liquid streams in grams per liter. To use a common basis for the calculations, the compositions of the solids and liquid streams were converted to carbon moles (C-mol), using information from the total mass balance and the C-mol weights of the components,† which are summarized in Table 2. A C-mol is the amount of a substance containing

**These adjustments are needed to account for gain in mass owing to water addition during hydrolysis and are 0.9, 0.88, and 0.7 g polymer/g monomer for hexoses, pentoses, and acetic acid, respectively.

†The number of C-mol of a component x in the solids and liquor is calculated as $Q_s\, m_x/(CMW)_x$ and $[C_x]\, V_L\, /\, (CMW)_x$, respectively, where *(CMW)* denotes the C-mol weight of component *x* (*see* Abbreviations for definition of the remaining symbols).

1 mol of the element carbon *(27)*, i.e., it equals the molecular mass divided by the number of carbon atoms in the molecule. A compositional formula for hardwood (beech) lignin *(19)* was used to calculate the molecular weight of yellow poplar lignin. A slightly different formula has been reported for softwood lignin *(19)*, resulting in a 7% lower molecular weight.

An advantage of using C-mol as the basis for the calculations is that the C-mol ratio of the various components within a stream reflects the distribution of carbon within that stream. In the example of Table 1, more than 60% of the carbon in the pretreated solids is in the form of glucan, and about 38% of the carbon comes from lignin. Furthermore, the fraction of each component that becomes solubilized as a result of the pretreatment is calculated as the fraction of the total C-mol of that component appearing in the liquid stream. For example, the data of Table 1 show that 14–18% of the glucan, 94% of the xylan, and 33% of the initial lignin are solubilized. Furthermore, based on the summary carbon balance information,[††] 57% of the initial carbon remains in the solids after pretreatment, which agrees well with the 42% solubilization that is calculated from the total mass balance data (*see above*, discussion on total mass balance calculations).

For the nonreacting components, the total recovery is calculated as the fraction of the initial carbon recovered in the solids and liquid streams after pretreatment. An exception is glucose and xylose recovery, for which the carbon associated with HMF and furfural, respectively, is added to the amounts of glucose and xylose recovered in the pretreatment solids and liquor to calculate overall recoveries. We therefore assume that all HMF comes from glucose dehydration and similarly that all furfural comes from xylose. This assumption is not entirely correct, however, since all hexoses have been reported to produce HMF when treated in dilute sulfuric acid and all pentoses to produce furfural, among other products, in acidic media *(20)*. However, the reactivity of individual sugars in dilute acid varies according to the approximate order xylose > arabinose > mannose > glucose ≈ galactose *(21)*, and different sugars give different yields of HMF and furfural, with arabinose giving much lower furfural yield than xylose and mannose and galactose significantly lower yields of HMF than either glucose or fructose *(20)*. Therefore, the above assumption is rather justifiable. Similarly, since levulinic acid and formic acid are degradation products of HMF, the carbon associated with these components is being added to the recovery of glucose. Finally, the overall carbon recovery for the pretreatment process is calculated as the percentage of the total initial carbon recovered in all components in both the liquid and solids streams.

The individual component and overall carbon recoveries shown in Table 1 are typical of the percolation process *(5)*. Larger losses on a percentage basis are observed with the minor sugars and are mainly attributed to analytical error. The recoveries from batch dilute sulfuric acid pretreatment are comparable to those reported here, except for xylose, for which losses of 10–25% are usual. Such losses are mainly caused by the formation of polymeric humic compounds that result from furfural degradation *(22)*, which can be analyzed as lignin and therefore upset the lignin balance as well.

[††]Calculation of the "Total" in Table 1 involves different calculations depending on the column. For the "% dry weight" columns, it is calculated as explained in the above footnote.[‡] For the "C-mol" columns, it is a straight sum, and for the "% C in feed" columns, it is the ratio of the total C-mol in the solids or liquid streams over the total C-mol in the raw substrate.

SSF Carbon Balance

As with the pretreatment balance, the SSF material balance was followed in terms of carbon. During SSF, the polymeric sugars are hydrolyzed by cellulolytic enzymes and released in the liquor as soluble sugars, which are then fermented to ethanol by the ethanologenic organism. The conversion of the major sugars, both soluble and polymeric, and the production of ethanol and major byproducts are followed through the SSF process. Lignin is also included in the balance for consistency verification purposes, since it is expected to be neither produced nor consumed during SSF. The SSF balance spreadsheet is shown in Table 3. Various concentrations are converted to the common basis of C-mol/kg slurry using the mass fraction of insoluble solids before and after SSF.[§] Because this information is critical to the accuracy of the overall balance, it needs to be determined as accurately as possible.

In addition to the pretreated solids and liquor, the compositions of which are known (*see* Table 1), other streams, including the yeast inoculum, growth media, and enzyme solution, contribute to the overall carbon entering the SSF. The SSF solids residue composition and the concentrations of residual sugars, ethanol, glycerol, and the main organic acid byproducts in the SSF liquor also need be measured at the end of the SSF. Carbon dioxide evolution is typically measured using head-space CO_2-analyzer measurements or mass spectrometry *(23)*. At a smaller scale, where it is often impractical to perform such measurements accurately, CO_2 production can be estimated stoichiometrically based on the amounts of ethanol and byproducts formed during fermentation. For yeast, the anaerobic fermentation pathway produces equimolar amounts of ethanol and CO_2, and 2 mol of CO_2 are produced/mol succinate. Carbon dioxide does not accompany acetate or glycerol formation *(24)*. Measuring cell mass concentration in the presence of solids exhibits well-known challenges; several techniques have been developed and applied with varying degrees of success *(25)*. One promising technique currently under development uses two-color fluorescence staining to detect differentially yeast cells in the presence of solids *(26)*. Although colony forming unit (CFU) measurements is a very time- intensive and often inaccurate technique for solids suspensions, this technique is currently used to determine cell mass in SSF. A calibration curve of CFU vs dry cell mass is used to convert to the appropriate concentration units.

The balance on the individual components consists of three major parts:

1. Carbon in solids and liquid streams entering SSF;
2. Carbon in solids, liquid, and gaseous streams leaving SSF; and
3. Sugar conversions and product formation balance.

Unlike the pretreatment balance, mass closure on individual components cannot be determined for the SSF because of the biotransformations that occur during this process. However, the yields of the fermentation products based on the consumed sugars can be determined, providing useful process information and, at the same time, a material balance for product formation. Because the distribution of product yields is characteristic of a given organism for fixed growth conditions, they can be used to detect contamination in SSF and quantify its impact on ethanol

[§]The compositions of a component x in the solids and liquid streams are converted to C-mol/g slurry as $Q f_s / (CMW)_x$ and $[C_x] (1 - f_s) / d_L (CMW)_x$, respectively (*see* † and Abbreviations for definition of symbols).

Table 3
SSF Carbon Balance

SSF CARBON BALANCE

Enzyme: 25 FPU/g cellu (CPN)
Organism: Yeast D5A
Feedstock: Yellow Poplar
Pretreatment: Percolation
Run: INHPOP16
Sample: 48 hrs

Researcher : T.K. Hayward
Run Date : 5-Apr-95
CAT Reports : 95-0010
95-0011

SOLIDS BALANCE

	In	Out
Lignin (%):	32.28	51.97
Insoluble Solids (%):	12.00	7.10

PERFORMANCE PARAMETERS

Cellulose Conversion:	63.6%
Overall C6-Sugar Conversion:	60.8%
Overall C5-Sugar Conversion:	21.2%
Ethanol Process Yield (% theor):	57.4%
Ethanol Metabolic Yield (% theor):	94.4%

Carbon Balance: SSF

Component	Carbon In							Carbon Out							Conversion	Yield
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	100 (In-Out)/In	100 x g / g C6 cons
	(% dry wt)	(C-mole/Kg Sln)	(% Total In)	(g/L)	(C-mole/Kg Sln)	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln)	(% Total Out)	(g/L)	(C-mole/Kg Sln)	(% Total Out)	(C-mole/Kg Sln)	(%)	
Cellobiose				1.00	0.031		0.031				0.43	0.014		0.014		
Glucose	75.04	2.999	*94.3*	6.20	0.182	*5.7*	3.181	46.17	1.092	*91.5*	3.28	0.101	*8.5*	1.194	62.5	
Galactose	0.00	0.000	*0.0*	0.39	0.011	*100.0*	0.011	0.00	0.000	*0.0*	0.36	0.011	*100.0*	0.011	1.3	
Mannose	0.65	0.026	*30.8*	1.99	0.058	*69.2*	0.084	1.08	0.026	*32.8*	1.69	0.052	*67.2*	0.078	7.6	
Xylose	0.86	0.034	*38.8*	1.85	0.054	*61.2*	0.089	0.62	0.015	*19.2*	1.99	0.062	*80.8*	0.076	13.8	
Arabinose	0.04	0.002	*8.1*	0.62	0.018	*91.9*	0.020	0.05	0.001	*13.2*	0.25	0.008	*86.8*	0.009	54.3	
Lignin	32.28	1.717	*82.7*	9.22	0.359	*17.3*	2.076	51.97	1.636	*79.0*	10.57	0.435	*21.0*	2.071	0.2	
Ethanol				1.10	0.042		0.042				32.40	1.307		1.307		48.3
Cell Mass				0.31	0.011		0.011				1.94	0.072		0.072		2.5
Carbon Dioxide												0.638		0.638		46.5
Glycerol				0.32	0.009		0.009				1.37	0.041		0.041		1.6
Acetic Acid				2.72	0.080		0.080				2.70	0.084		0.084		0.2
Lactic Acid				1.18	0.034		0.034				1.36	0.042		0.042		0.4
Succinic Acid				0.44	0.013		0.013				0.76	0.024		0.024		0.5
Total	*101.19*	*4.778*	*84.1*		*0.904*	*15.9*	***5.681***	*95.08*	*2.770*	*48.9*		*2.891*	*51.1*	***5.661***		*100.0*

C - RECOVERY: **99.6%**

process yields. These calculations are shown in the last column of Table 3 The important SSF process parameters, including conversions of glucan, total hexose and pentose sugars, and ethanol yields based on total (process yield) and consumed (metabolic yield) hexose sugars, are summarized on the top section of the spreadsheet. Finally, overall carbon closure is determined as the percentage of total carbon in the SSF recovered in the solids residue and the fermentation products.

ETHANOL YIELD CALCULATIONS

The ethanol yields of the biomass-to-ethanol technology should be based on the raw biomass fed to the pretreatment reactor because the cost of feedstock is a significant component of the production cost. Hence, to evaluate the overall performance of the biomass conversion process, ethanol yield calculations need to consider the efficiency of the pretreatment and SSF operations, not only the fermentation step. Any losses of fermentable carbon either in the form of sugar degradation products or as part of unused hydrolysate should be accounted for, because in reality they decrease the ethanol yield from the purchased biomass. Based on this rationale, we first present yield calculations for the SSF process, and then formulate overall yield expressions.

SSF Ethanol Yields

In high solids fermentation processes, such as SSF, failing to account for the amount of suspended insoluble solids can bias mass balance closure and the determined yields. In an effort to address these issues comprehensively expressions for the ethanol yield from raw and pretreated substrates were developed based on the following assumptions:

1. All hexoses (glucose, mannose, galactose), which are the products of hydrolysis of the cellulosic and hemicellulosic components of lignocellulosic substrates, can be fully utilized by the organism;
2. The yields for the overall process are based on the total hexose content of the raw substrate, as fed to the pretreatment process; and
3. A significant amount of insoluble solids is present in the SSF media, and therefore, yield calculations need to correct for the reduction in the liquid volume.

The ethanol yield based on the total hexose content is therefore defined as:

$$Y_E = \text{g EtOH produced / g C6 sugars in raw substrate} \quad (1)$$

The maximal theoretical (stoichiometric) yield of ethanol from hexoses is 0.511 g ethanol / g C6 sugar.[§§] The ethanol yield can be expressed as a percent of the stoichiometric yield as $(Y_E / 0.511) \times 100$. This yield is always <100% as part of the sugars is converted to cell mass and other byproducts by the organism.

The mass fraction of the insoluble solids in the SSF medium is denoted by f_s, where $f_s = m_s/m$ in g insoluble solids/g slurry. As slurry, we define the liquid–solid mixture of pretreated biomass exiting the pretreatment process and entering the

[§§]It is worth pointing out that, because the Gibbs free energy for the conversion of glucose to ethanol is negative, the stoichiometric yield is also thermodynamically achievable.

SSF process. The mass fraction of the liquid phase, which includes any dissolved solids, is therefore $1 - f_s$. The total solids fraction consists of insoluble solids, dissolved solids, and cell mass. A typical dry-wt determination of an SSF sample provides a measure of the total solids fraction. The fraction of insoluble solids f_s can be determined with a standard dry-wt determination procedure, if the SSF residue is thoroughly washed before drying; washing is essential to removing any dissolved solids from the sample. The contribution from the cell mass, expressed as dry cell weight, to the total insoluble solids in an SSF system needs to be quantified with alternative methods such as CFUs.

Some cellulosic materials, such as office paper, can be converted to ethanol efficiently without pretreatment. The vast majority of biomass species, however, require pretreatment to varying degrees of severity. Based on Eq. (1), the following calculations provide the appropriate SSF ethanol yield formulas for both feedstock categories.

SSF of Raw Substrate

The amount of ethanol present in the SSF flask or reactor at any time is equal to the residual ethanol concentration in the liquid phase of the SSF suspension multiplied by the volume of the liquid phase, i.e., $[E]\ m_L/d_L$ or $[E]\ m\ (1 - f_s)/d_L$. The total amount of hexoses available for ethanol production at the beginning (t = 0) of the SSF is $f_{s,0}\ m_0\ Q_{C6,0}$. Therefore, the ethanol yield from raw (unpretreated) substrate is:

$$Y_E^{SSF} = [E]m(1 - f_s)\ /\ f_{s,0}\ m_0\ d_L\ Q_{C6,0} \tag{2}$$

in (g EtOH) / (g hexoses in raw substrate). Typically, the density of the liquid phase is taken to be equal to that of water.

If all solids are assumed to have been completely solubilized at the end of the SSF (i.e., $f_s = 0$) and there is no mass loss (e.g., via evaporation) during the course of the fermentation (i.e., $m = m_0$), Eq. (2) simplifies to the following form:

$$Y_E^{SSF} = [E]\ /(f_{s,0}\ Q_{C6,0} \times 1000) \tag{3}$$

Any mass loss during SSF will have a significant effect on yield calculations. For example, a 10% mass loss from an SSF residue with 6% (w/w) insoluble solids, which is a typical case, will result in a 20% overestimation of the yield, if Eq. (3) is used instead of Eq. (2).

SSF of Pretreated Substrate

Equation (2) can also be used to calculate the SSF ethanol yield from pretreated substrate. If only washed solids are used in the fermentation, Eq. (2) applies unmodified, but the term $Q_{C6,0}$ now refers to the washed pretreated biomass composition. Although not realistic from a process design standpoint, washed solids are often used at bench scale to eliminate the inhibitory effects of acetic acid, HMF, furfural, and phenolics on cell metabolism and SSF performance.

If the pretreatment hydrolysate is also used in the SSF, the yield calculation will have to account for the hexose sugars present in the hydrolysate. In this case, the ethanol yield for the SSF conversion of unwashed pretreated solids is:

$$Y_E^{SSF,Pr} = [E]\ m\ (1 - f_s)\ /[f_{s,0}\ m_0\ d_L\ Q_{C6,0} + [C_6]_0\ m_0\ (1 - f_{s,0})] \tag{4}$$

in (g EtOH produced)/(g hexoses in pretreated slurry), where the slurry is the sum of solid residue and hydrolysate.

Overall Ethanol Yields from Pretreated Substrate

The ethanol yield in the SSF process defines the efficiency of that operation. To determine the carbon utilization efficiency of the overall process (pretreatment and SSF), we need to base all calculations on the raw biomass that enters the pretreatment reactor and thus take into account any carbon losses that occur during pretreatment.

SSF of Washed Pretreated Solids

Pretreatment of the substrate effectively solubilizes part of the insoluble solids (*see* Table 1). Therefore, we need to account for the carbon lost in those sugars in the ethanol yield calculation from washed solids. The amount of ethanol produced is found in the same way as in the previous case, but the total amount of hexoses available for ethanol production at the beginning of the SSF is $(f_s / L_f)\, m_0\, Q_{C6}$, where L_f is a mass-loss factor expressing the insoluble solids fraction recovered after the pretreatment process (i.e., $1 - L_f$ is the insoluble solids fraction that becomes solubilized by pretreatment). Thus, the ethanol yield from the pretreated solids, after accounting for solubilization, is:

$$Y_E^{Pr,w} = [E]\, m\, (1 - f_s) \,/\, [\,(f_{s,0}/L_f) m_0\, d_L\, Q_{C6,0}] \qquad (5)$$

in (g EtOH produced)/(g hexoses in raw biomass).

SSF of Unwashed Pretreated Solids and Hydrolysate

Except for sugar degradation products, the pentosans and hexosans that become solubilized by the pretreatment process appear as soluble sugars in the pretreatment liquor (hydrolysate). To minimize the cost of ethanol production, all available sugars (just the hexose sugars for the present analysis), including those in the hydrolysate, should be converted to ethanol. To evaluate the fermentability of those sugars, SSF experiments are routinely set up using the unwashed pretreated solids and the corresponding hydrolysate to make up the SSF suspensions. Because all yields are based on the hexose content of the raw substrate according to Eq. (1), the ethanol yield for unwashed pretreated substrate is again given by Eq. (5). If the hydrolysate does not contain significant amounts of sugar degradation products, the ethanol yield will be higher, because more sugars are made available for fermentation.

When the SSFs are run with unwashed substrate and pretreatment hydrolysate, only a portion of the generated hydrolysate can be utilized. Figure 2 illustrates a typical case. Assuming that 20% of the insoluble solids are solubilized during pretreatment (i.e., $L_f = 0.8$), 125 g of raw substrate need to be pretreated to generate 100 g of pretreated solids in the SSF. If the pretreatment is carried out at a 5% (w/w) solids loading, 2375 g of prehydrolysate will also be generated. If the SSF is run at a 10% (w/w) solids level, only 900 g of the hydrolysate are needed/100 g of pretreated solids. Therefore, only 38% of the generated hydrolysate is actually used in the fermentation test. This is because dilute-acid batch pretreatment experiments are usually carried out at lower solids levels (5% [w/w]) than SSF (10% [w/w]) to achieve good mixing with small-scale pretreatment equipment. Consequently, even when pretreatment hydrolysate is added to the unwashed pretreated solids for SSF evaluation, the yields calculated according to Eq. (5) underestimate the potential ethanol yield for the overall pretreatment-SSF process, because part of

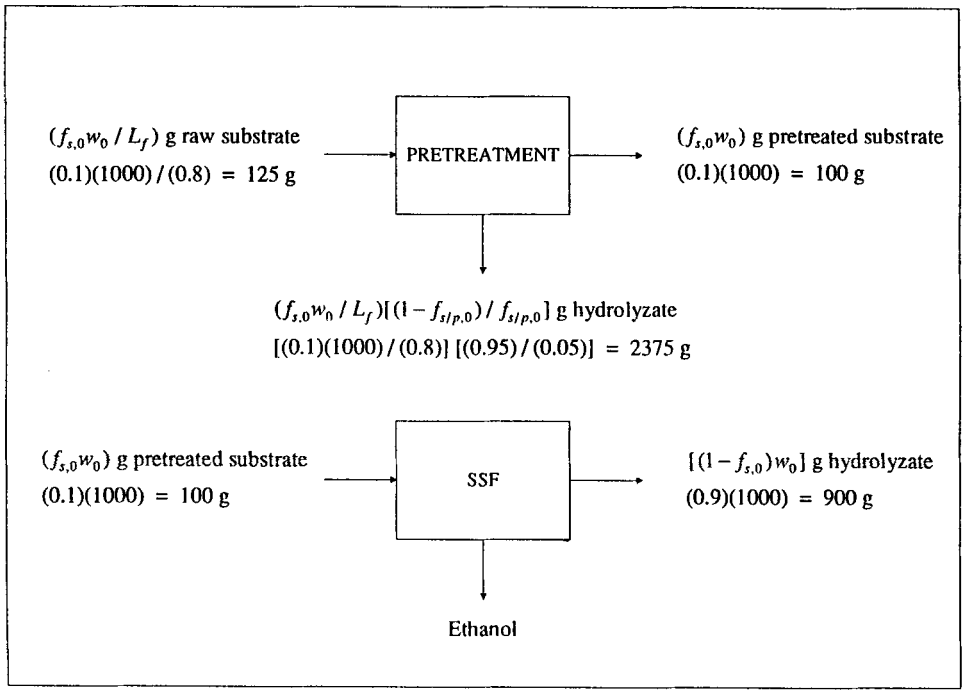

Fig. 2. Mass balance around a typical laboratory-scale pretreatment and SSF processes. Only part of the pretreatment liquor is used in the SSF.

the sugar content of the raw biomass is not made available for fermentation. Ways to account for these effects are presented in the following sections.

Maximal Potential SSF Yield for Pretreated Biomass

If the hydrolysate from a particular pretreatment does not inhibit the organism, the SSF ethanol yield determined from the conversion of the unwashed solids and hydrolysate will approximate the maximal overall process yield (i.e., the yield from converting raw substrate to ethanol). However, if the hydrolysate is inhibitory, the above SSF yield will considerably underestimate the maximal yield that can be potentially attained by an appropriately adapted organism. Therefore, a maximal potential yield can be defined based on the observed yield from the washed residue and the assumption that the organism can utilize all the free hexoses in the hydrolysate, which amount to $[C_6]_0\, m_{L,0}/d_L$ at the maximal theoretical yield of 0.511 g/g. Depending on the assumed degree of utilization of the generated hydrolysate, several expressions can then be formulated to calculate the maximal potential yield.

Use enough hydrolysate to make up SSF suspension: As mentioned in the previous section, if the pretreatment process is run at a lower solids level than the SSF process, only part of the hydrolysate can be used to set up the SSF. In this case, the maximal potential yield is defined as:

$$Y_{E,max}^{Pr} = [\,[E]m(1-f_s) + 0.511[C_6]_0 m_0\,(1-f_{s,0})\,]/[(f_{s,0}/L_f)m_0 d_L Q_{C6,0}] \qquad (6a)$$

because only $m_0\,(1-f_{s,0})$ g liquor can be utilized in this case (assuming that no moisture is associated with the remaining $m_0 f_{s,0}$ g of insoluble solids). Based on the

assumptions that led from Eq. (2) to Eq. (3), the following approximate expression c an be obtained:

$$Y_{E,max}^{Pr} = [\,[E] + 0.511\,[C_6]_0\,(1 - f_{s,0})\,]/[(f_{s,0}/L_f)Q_{C6,0} \times 1000] \quad (6b)$$

This case is representative of the typical bench-scale evaluation of pretreated substrates.

Use all hydrolysates generated per given amount of pretreated solids: The maximal yields calculated by the above formulas are not appropriate for processes in which all the sugars in the pretreatment hydrolysate are expected to be utilized, as for example in the NREL base-case design where the whole pretreated slurry is fed to the fermentation tanks without an intervening separation step *(4)*. If all the hydrolysate generated by pretreating a given amount of solids is used in the SSF, the maximal potential overall yield is given by an equation similar to Eq. (6a). More specifically, if $m_{s/p,0}$ g of dry solids are pretreated generating $m_{L/p}$ g of hydrolysate with $[C_6]_0\ m_{L/p}\ /\ d_L^{'}$ g of free hexose sugars, the maximal amount of ethanol that can be produced is $0.511\ [C_6]_0\ m_{L/p}\ /\ d_L$ g and the yield is now given by:

$$Y_{E,max}^{Pr} = [\,[E]m(1 - f_s) + 0.511\,[C_6]_0\,m_{L/p}]/[(f_{s,0}\ /\ L_f)\,m_0\,d_L\,Q_{C6,0}] \quad (7)$$

If, however, only a portion of the pretreated solids equal to $f_{s,0}\,m_0$ g is used in the SSF, the hydrolysate that corresponds to the amount of the pretreated solids used is $f_{s,0}\ m_0\ /\ m_{s/p,0})\ m_{L/p}$, and in this case, the maximum potential yield is given by:

$$Y_{E,max}^{Pr} = [\,[E]m(1 - f_s) + 0.511\,[C_6]_0\,(f_{s,0}\,m_0/m_{s/p})m_{L/p}]/[(f_{s,0}/L_f)m_0 d_L Q_{C6,0}] \quad (8a)$$

An approximate equation similar to Eq. (6b) can also be derived for this case:

$$Y_{E,max}^{Pr} = [\,[E](1 - f_s) + 0.511\,[C_6]_0\,(f_{s,0}/f_{sp})]/[(f_{s,0}/L_f)Q_{C6,0} \times 1000] \quad (8b)$$

where $f_{s/p}$ is the fraction of insoluble solids after pretreatment.

Simultaneous Saccharification and Cofermentation Ethanol Yields

All the above formulas for SSF yield calculations can be generalized to apply to a cofermentation process in which hexose and pentose sugars are converted to ethanol. In this case, the ethanol yield is still given by Eq. (1), but is now based on total available sugars. As the stoichiometric yield for ethanol production from pentose sugars is again 0.511 g/g, the remaining formulas are still valid, if the correct compositions are used. More specifically, Q_{C6} needs to be replaced by (Q_{C5} + Q_{C6}) and $[C_6]$ by ($[C_5]$ + $[C_6]$) in all the above formulas.

CONCLUSIONS

A comprehensive framework for evaluating process data and performing the engineering calculations for the biomass-to-ethanol conversion process has been developed. The need for such an analysis scheme has long been recognized *(10,11)*, and it is becoming more urgent as the technology nears commercialization.

The developed framework emphasizes the need to consider the overall process performance when evaluating laboratory data, which are typically obtained from studies on individual unit operations instead of on the integrated process. In addition to providing a consistent platform for conversion and yield calculations, the presented framework is also valuable in directing experimental efforts more effectively by pointing out critical measurements and potential inconsistencies in

the analytical data. Although a base-case process, which involves a pretreatment and an SSF step for conversion of hexose sugars, was considered in the present analysis, the results can be readily extended to other process configurations, e.g., an SSF process with recycle or cofermentation processes with simultaneous pentose and hexose sugar conversion.

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