Supporting Text

Carbon recovery calculation

n. Carbon recovery was calculated based on measured concentrations of cellulose, fermentation products, cellulase (determined by Elisa as described above), and protein, assuming that one more of CO_2 is produced for each mol acetic acid and ethanol produced. The carbon recovery for microbial conversion, CR_M, was found by using the following formula:

$$CR_M = \frac{3*(\Delta E/46 + \Delta A/60)}{6*(C_o - C)/180} + f_{C/X}\frac{\Delta P_P - \Delta E_P}{12 * f_{P/X}} + f_{C/P}\frac{\Delta E_P + (\Delta P_t - \Delta P_p)}{12} \quad [1]$$

The carbon recovery for SSF, CR_{SSF}, was calculated by using Eq. **2**:

$$CR_{SSF} = \frac{3*(\Delta E/46 + \Delta A/60)}{6*(C_o - C)/180} + f_{C/X}\frac{\Delta P_P - \Delta E_P}{12 * f_{P/X}} + f_{C/P}\frac{(\Delta P_T - \Delta P_P) - (\Delta E_T - \Delta E_p)}{12} \quad [2]$$

Variables in Eqs. **1** and **2** are defined as follows:

E = ethanol (g per liter)

A = acetic acid (g per liter)

C_o = initial (batch) or feed (continuous) cellulose, g of glucose equivalent per liter

C = cellulose, g per liter

P_p = pellet protein, g per liter

P_T = total protein, g per liter

E_P = pellet cellulosome, g per liter

$f_{C/X}$ = mass fraction of carbon in cells (unitless)

$f_{P/X}$ = mass fraction of protein in cells (unitless)

$f_{C/P}$ = mass fraction of carbon in protein (unitless)

In these equations, Δ denotes final concentration minus initial concentration for batch culture and output concentration minus input concentration (equal to zero) for continuous culture. The first term represents carbon in fermentation products, the second term represents carbon in cells, and the third term represents noncellular protein produced by the organism in the fermentor. A value of $f_{C/X}$ equal to 0.46 was used based on typical values for microorganisms compiled by Papoutsakis (1). An $f_{P/X}$ value of 0.52 was measured for *C. thermocellum* (2) and is also representative of bacteria generally (3). A value of $f_{C/P}$ equal to 0.5 is used based on the typical elemental protein composition reported by Lehninger (4). It may be noted that carbon recoveries are quite insensitive to the values used for $f_{C/X}$, $f_{P/X}$, and $f_{C/P}$ since most substrate carbon is converted to fermentation products rather than cells or noncellular protein.

Factors suggesting that any differences between cellulases present under the conditions examined are not large. Cellulase was recovered from cell-free broths present at the end batch cultures (for batch SSF experiments) and in effluents from steady-state continuous fermentors (for continuous SSF experiments). Cellulase present in batch and continuous SSF experiments had very similar specific activity in the absence of metabolically active cells (Table 2), and our estimates for the degree of enzyme-microbe synergism (Table 1) give no indication that evaluation of this parameter is sensitive to whether data are taken from batch or continuous cultures. The high activity recovery obtained with the affinity digestion procedure used for cellulase purification from broth supernatants (≥80%) (2, 5) is consistent with the purified cellulase obtained

being representative of crude unfractionated cellulase and is not consistent with preferential enrichment of a minor (nonrepresentative) cellulase component. Affinity-digestion purified cellulases from Avicel-grown cultures appear very similar with respect to SDS/PAGE banding patterns and the absorbance response in the ELISA assay used for cellulase quantification regardless of whether the cellulase was prepared from the supernatant or pellet or from batch cultures with incomplete or complete cellulose utilization; these cellulase preparations also exhibited very similar cellulase specific activity (2). Two detailed studies have concluded that the major characteristics of the *C. thermocellum* cellulase system coincide with those of cellulase purified from extracellular broths (2, 6).

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