Supporting Text

Justification and Explanation for Eq. 1. Eq. **1** is as presented (1), except that: (*i*) ATP synthesis resulting from the Embden-Meyerhoff pathway and the action of acetate kinase are represented in terms of fermentation product yields, and (*ii*) ATP consumption for synthesis of cells and noncellular protein (including cellulase) is represented in terms of flows leaving the fermentor rather than rates of generation within the fermentor. The latter change is made to avoid implying that cells attached to cellulose have the same rates of growth and cellulase synthesis as nonattached cells in a cellulose-grown continuous culture, which may not be the case. It may be noted that the rate at which cells leave the fermentor per unit volume (g cells/liter·hr) is equal to DX, which at steady state is also equal to the volumetric rate of cell production within the fermentor. Thus, the rate that ATP is consumed for cell synthesis per unit volume (mol ATP/liter·hr) is equal to $DX/Y_{X/ATP}^{True}$, and the cell-specific rate of ATP consumption for growth (mol ATP/g cell·hr) is $D/Y_{X/ATP}^{True}$. By a similar line of reasoning, the cell-specific rate of ATP consumption for synthesis of cellulase and noncellulase extracellular protein (mol ATP/g cells·hr) is $DY_{E/X}/Y_{E/ATP}^{True}$, which can also be expressed as $DY_{E/X}/(RY_{X/ATP}^{True})$, where $R = Y_{E/ATP}^{True}/Y_{X/ATP}^{True}$. Coupled ATP-limited metabolism is expected for anaerobic fermentation of carbohydrates in the presence of nonlimiting quantities of required nutrients other than the carbon and energy source (2).

Evidence That $\alpha = 1$. Values of $Y_{X/ATP}^{True}$ are expected to be very similar if not the same for both cellobiose and cellulose, because all evident differences between metabolism of these substrates, cellodextrin transport, cellulase synthesis, and phosphorolytic cleavage, are accounted for in the bioenergetic model (Eq. **1**). We also expect, based on mechanistic considerations, that the actual value of α is an integer. Hypotheses were tested using a paired *t* test, as described (3). Based on the data in Table 2, the hypothesis that Y_{ATP} values are equal for cellobiose and cellulose can be rejected at $P = 0.01$ for the following cases: $\alpha = 2$ and $n = 2$ for both substrates, $\alpha = 1$ and $n = 2$ for both substrates, $\alpha = 2$, $n = 2$ for cellobiose, and $n = 4.2$ for cellulose.

The hypothesis that $Y_{X/ATP}^{true}$ for cellobiose is $\leq Y_{X/ATP}^{true}$ for cellulose is also rejected at P = 0.05 for the case where $\alpha = 1$, $n = 2$ for cellobiose and $n = 4.2$ for cellulose. Thus, we conclude that $\alpha = 1$ is consistent with the data, whereas $\alpha = 2$ is not, regardless of the value of n. We also conclude that $n = 2$ for cellobiose and 4.2 for cellulose is consistent with the data, whereas $n = 2$ for both substrates is not, regardless of the value of α.

Quantification of Intracellular Cellodextrins. ^{14}C-labeled cellodextrins in pellets obtained after microbial hydrolysis of ^{14}C-cellulose may exist in several forms in the experiments reported: (a) Cellodextrins not associated with cells that are adsorbed to the surface of cellulose and (b) cell-associated cellodextrins that are adsorbed to the cell surface; and (c) cell-associated cellodextrins taken up from the growth medium. The quantity of cell of cell-associated cellodextrins (b + c) is calculated by measuring the total quantity of each ^{14}C-cellodextrin quantified in the pellet and subtracting from this total ^{14}C-cellodextrins a control carried out under identical conditions, except that purified affinity digestion-purified cellulase with activity equal to that used in microbial experiments is added in lieu of cells. This control provides an estimation of the amounts of ^{14}C-labeled cellodextrins adsorbed to cellulose (form a, above) that are not cell-associated. Significant accumulation of cellodextrins on the cell surface (form b, above) is considered unlikely in light of the high affinity of the *Clostridium thermocellum* cellodextrin transport system (4).

Primary Data and Calculations for Figs. 1, 2, and 4. Fig. 1. Quantities presented in Fig. 1 in the text are calculated from data in Table SOM1. Abscissa values are calculated based on Eq. 4.

$$Y_{E/X} = \frac{NCP}{X} \qquad [\mathbf{4}]$$

where

NCP = noncellular protein (supernatant protein + pellet cellulase measured by ELISA), and *X* = cell concentration (exclusive of cellulase, g dry weight/liter).

Values for q_{ATP}, plotted on the ordinate in Fig. 1, are calculated based on Eq. **1** with yields for product P_i from Table 3 calculated using Eq. **5**, and r_S^X (mol glucose equivalent/g cell/hr) calculated by using Eq. **6.**

$$Y_{i/Glu} = \frac{P_i}{S_{in} - S_{out}} \qquad [\mathbf{5}]$$

P_i = concentration of ith product (mol/liter)

S_{in}, S_{out} = feed substrate concentration (*m*, in or out as indicated)

$$r_S^X = \frac{(S_{in} - S_{out})D}{X} \qquad [\mathbf{6}]$$

Carbon recovery (CR) was calculated for this and following Tables 3-5 using Eq. **7**.

$$CR = \frac{3*(EtOH + HAc)}{(S_{in} - S_{out})} + 0.485*X/12 + 0.457*NCP/12 \quad [\mathbf{7}]$$

Fig. 2. Quantities presented in Fig. 2 are calculated from data in Table 4. Abscissa values are assumed values of *n.* Ordinate values are calculated based on Eq. **2** with $\alpha = 1$, $Y_{X/ATP}^{True}$ = 16.44 g cells/mol ATP, and *m* = 3.27 mmol ATP/g cell/hr, as determined from Fig. 1; r_S^X calculated using Eq. **6**. G_{ATP}^{P-T} for cellulose is calculated from data in Table 7 (below), consistent with Eq. **3** from the text.

Fig. 4. Quantities presented in Fig. 4 are calculated from data in Table 5, consistent with Eq. **1** with $Y_{X/ATP}^{True}$ = 16.44 g cells/mol ATP, *m* = 3.27 mmol ATP/g cell · hr, *n* = 4.2, and

$\alpha = 1$. Rates of ATP formation (Fig. 4*A*) and consumption (Fig. 4*B*) for various metabolic processes are calculated as follows, with product yields calculated using Eq. **5** and r_S^X calculated using Eq. **6**.

Metabolic process Rate (mol ATP/hr per g cell)

ATP supply

Glycolysis: $\frac{(Y_{E/Glu} + Y_{Ac/Glu})D}{X}$

Acetate kinase: $\frac{Y_{Ac/Glu}D}{X}$

Phosphorolytic cleavage: $r_S^X f\left(\frac{n-1}{n}\right)$

ATP demand

Sugar transport: $r_S^X \frac{\alpha}{n}$

Growth: $\frac{D}{Y_{X/ATP}^{True}}$

Noncellulase extracellular protein synthesis: $\frac{D(NCP - E)/X}{0.82 * Y_{X/ATP}^{True}}$

Cellulase synthesis: $\frac{DE/X}{0.82 * Y_{X/ATP}^{True}}$

Maintenance: m

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