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Predicting Yields for Autotrophic and Cometabolic Processes

G. ANDREWS

Center for Industrial Biotechnology and Process Engineering, Idaho National Engineering Laboratory, Idaho Falls, ID 83415

ABSTRACT

The goal of bioprocess engineering is to state how the optimum design and control strategy for a bioprocess follow from the metabolism of the particular microorganism. A necessary step toward this goal is to show how the parameters used in quantitative descriptions of a process (e.g., yield and maintenance coefficients) are related to those describing the metabolism [e.g., Y_{ATP}, (P/O)]. The "yield equation" approach to this problem involves dividing metabolism into the separate pathways for catabolism, anabolism, respiration, and product formation and balancing the production and consumption of reducing equivalents and ATP. The general approach, demonstrated previously for heterotrophic cell growth and products of fermentation, is illustrated by three new examples: the cell yield for chemoautotrophic iron-oxidizing bacteria, the cometabolic degradation of chloroform by methanotrophic bacteria, and the theoretical yield of succinic acid from glucose.

Index Entries: Yield; cometabolism; autotrophic; modeling; succinic acid.

INTRODUCTION

The job of the bioprocess engineer is to show how the optimum design of a bioprocess follows from the characteristics of the metabolism of the particular microorganisms involved. One of the difficulties of this job is that information about metabolism comes from the fields of microbiology and biochemistry, which tend to be descriptive, qualitative

sciences. Bioprocess design, on the other hand, is an intensely quantitative activity involving fixing values for the process variables (bioreactor type and size, flow rates, media composition and concentration, and so forth) that determine process performance. It follows that an essential task of bioprocess engineering is to take the essential features of microbial metabolism and reduce them to a set of rate and yield equations that can then be incorporated into a process model that can provide the required quantitative information.

The author has recently suggested for this purpose the simplified picture of heterotrophic metabolism shown in Fig. 1. It reduces the complexities of metabolism to four essential biochemical pathways: catabolism of the carbon/energy source, anabolism to produce new cell mass, respiration to convert excess electrons into metabolic energy, and the formation of a metabolic product. The respiration pathway disappears for fermentative metabolism, and each extra carbon/energy source or metabolic product means that one more pathway must be considered. Each pathway may contain hydrolysis/dehydrolysis, carboxylation/decarboxylation, amination/deamination, and similar reactions, so each reaction pathway in Fig. 1 contains H_2O, CO_2, NH_3, and so on, as either a reactant or product. The effects of oxidation/reduction reactions are represented by H′, the nomenclature used here for a mole of electrons attached to carriers like NAD. All of the common yield equations for bioprocesses, including the linear growth equation and the Ludeking-Piret equation for single-product fermentations, were derived by simple element and energy (as ATP) balances over the set of reactions shown in Fig. 1 (*1*). Furthermore, the yield and maintenance coefficients, which are normally treated as purely empirical parameters, were related to a number of pseudo-constants describing the metabolism, including the oxidative phosphorylation ratio (P/O), and oxidation/reduction states of the substrates and products. Given a "carbon equivalent" of organic matter written as $CH_hN_nO_oP_pS_s$, the oxidation/reduction state is quantified in terms of the number of available electrons:

$$\gamma = 4 + h - 3n - 2o + 5p + 6s \quad (1)$$

This number is surprisingly constant for the organic fraction of biomass ($\gamma = 4.2 \pm 0.2$) irrespective of the species, growth rate, or the composition of the media on which it is grown.

Much of the previous work represented a generalization of the work of other researchers (*2–4*), but with two additions. First, the nonrespiratory consumption of oxygen by oxygenase enzymes (the ϵ terms in Fig. 1) was included explicitly for the first time. This corrected a discrepancy between the predicted and observed values of the "yield on available electrons" for cell growth on very reduced substrates, like methane. Second, biomass was treated as just another metabolic product and the concept of a pseudo-constant Y^{ATP}, the biomass that can be synthesized using the energy in 1 mol of ATP, was extended to other products. This

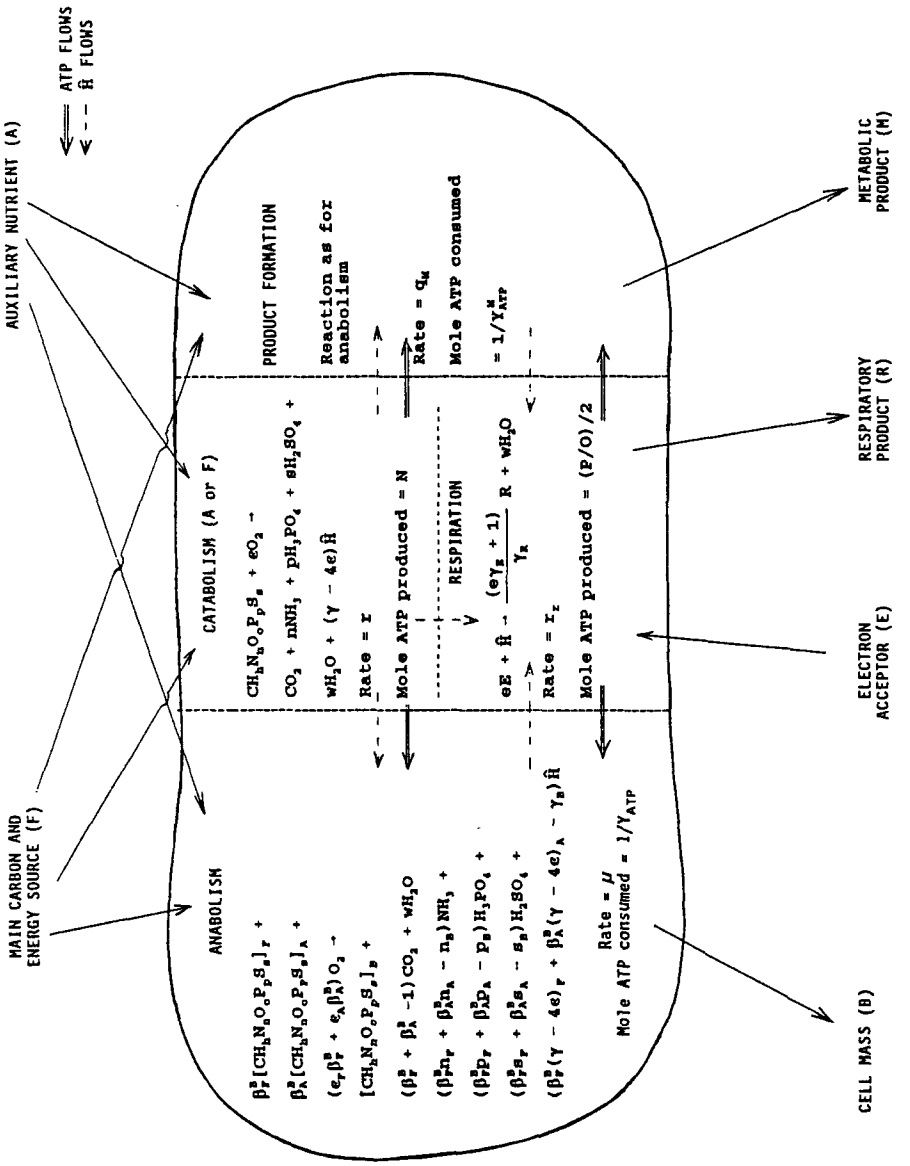

Fig. 1. The "reactions" in one carbon equivalent of biomass.

allowed the yield coefficient to be expressed in a general form valid for biomass or other products:

$$Y = [N_F + (P/O)(\gamma_F - 4\epsilon_F)/2] / [1 / Y^{ATP} + \beta_F N_F + \gamma(P/O)/2] \quad (2)$$

where N_F = mole ATP from substrate-level phosphorylation of a unit of carbon/energy source and β_F = stoichiometric parameter in product formation pathway (*see* Fig. 1). Note that Y is inherently an energy parameter, the numerator being the maximum amount of ATP available from complete catabolism of a unit of the carbon/energy source, and the denominator being the total ATP cost of making a unit of the product (amount actually consumed, $1/Y^{ATP}$, plus the amount forgone by using the carbon/energy source for something other than catabolism).

The objective of this article is to show how the method described previously can be applied to other significant problems. Are the yield predictions for autotrophic microorganisms as good as those for heterotrophs? What information can be obtained about the biodegradation of contaminants by cometabolic pathways? What insights can it provide about the "theoretical yield" of a product? These questions are investigated by studying three examples: the growth yields of iron-oxidizing bacteria, the degradation of chloroform by methanotrophic bacteria, and the manufacture of succinic acid from carbohydrates.

THE GROWTH YIELD OF IRON-OXIDIZING BACTERIA

In chemoautotrophic organisms, the sources of carbon (CO_2) and energy are different, which simplifies the reactions of Fig. 1 and provides an easy illustration of the derivation of Eq. (2). If the excretion of organic matter is insignificant, the reactions for the growth of iron-oxidizing bacteria can be written:

		Rate	ATP produced
Catabolism	$Fe^{2+} + H^+ \rightarrow Fe^{3+} + H'$	q_F	0
Respiration	$4H' + O_2 \rightarrow 2H_2O$	q_o	$2(P/O)$
Anabolism	$CO_2 + nNH_3 + sH_2SO_4 + \gamma H' \rightarrow CH_hN_nO_oS_s + wH_2O$	μ	$-1/Y^{ATP}$

Since the basis of all these calculations is one carbon equivalent of biomass, the rates are given as specific rates (e.g., q_F = mol Fe oxidized/h/C Eq of biomass). Balances are written over this set of reactions for the production and consumption of oxidation/reduction potential (H') and metabolic energy (ATP):

$$q_F = 4q_o + \gamma\mu \quad (3)$$

$$2(P/O)q_o = (\mu / Y^{ATP}) + k \quad (4)$$

where k = maintenance energy requirement in mol ATP/h C Eq of biomass. Eliminating q_o between Eqs. (3) and (4) produces the expected relationship for the observed cell yield on iron:

$$y = (\mu / q_F) = [Y / (1 + Yk_F/\mu)] \quad (5)$$

where Y = yield coefficient = $[(P/O)/2] / [1/Y^{ATP} + \gamma (P/O)/2]$ and k_F = maintenance requirement for iron = $2k/(P/O)$.

This equation for Y is clearly a special case of Eq. (2) when there is no substrate-level phosphorylation ($N_F = 0$) or nonrespiratory consumption of oxygen ($\epsilon_F = 0$) and 1 mol of the energy source generates 1 mol of electrons ($\gamma_F = 1$).

If either (P/O) or Y^{ATP} is known, then the other can be found from the above equation and measured values of the yield coefficient. There is some uncertainty about the exact biochemistry of iron oxidation and ATP generation, so (P/O)/2 is best thought of as total ATP generated/mol of iron oxidized, and is believed to be close to 1. Measured values of Y on the order of 0.05 C Eq/mol therefore, correspond to Y^{ATP} = 0.063 C Eq/mol ATP. Although this is much less than the value of 0.4 C Eq/mol (approx 10 g dry wt/mol ATP) found for heterotrophs, it is in reasonable agreement with the theoretical value for autotrophic bacteria with reversed electron flow, Y^{ATP} = 0.1 C Eq/mol ATP (*5*). It takes far more energy to manufacture biomass from CO_2 than from reduced organic compounds.

THE DEGRADATION OF CHLOROFORM BY METHANOTROPHIC BACTERIA

The reaction of methane with 1 mol of O_2 to form methanol is the first step in the metabolism of methanotrophic bacteria ($\epsilon_F = 1$ in Fig. 1). The methane monooxygenase enzyme that catalyzes this reaction will also gratuitously degrade many of the partially chlorinated aliphatic compounds that are common ground-water contaminants. The degradation pathway for chloroform involves oxidation of the C—H bond followed by spontaneous hydrolysis of the resulting chloro-alcohol via phosgene. Considering the chloroform as an "auxiliary nutrient" identified by subscript A, the reactions of Fig. 1 reduce to:

		Rate	ATP per reaction
Catabolism	$CH_4 + O_2 \rightarrow CO_2 + 4H'$	r	0
Respiration	$4H' + O_2 \rightarrow 2H_2O$	r_r	2(P/O)
Degradation	$CHCl_3 + O_2 + 2H' \rightarrow CO_2 + 3HCl$	q_A	0
Anabolism	$\beta_F(CH_4 + O_2) + nNH_3 + sH_2SO_4 \rightarrow CH_hO_oN_nS_s + wH_2O + (4\beta_F - \gamma)H'$	μ	$-1/Y^{ATP}$

Balances must be written for four species:

Methane	$q_F = r + \beta_F \mu$	(6)
Oxygen	$q_o = r + \beta_F \mu + r_r + q_A$	(7)
H′	$4r + (4\beta_F - \gamma)\mu = 2q_A + 4r_r$	(8)
ATP	$2(P/O)r_r = (\mu / Y^{ATP}) + k$	(9)

Eliminating q_o and the unknown rates r and r_r from these equations gives:

$$q_F = (q_A / 2) + (\mu / Y) + k_F$$

$$\text{with } Y = [2(P/O)] / [\gamma(P/O)/2 + 1/Y^{ATP}] \text{ and } k_F = [k / 2(P/O)] \quad (10)$$

Eliminating q_F, r, r_r gives the oxygen consumption as:

$$q_o = (3q_A / 2) + (\mu / Y^o) + 2k_F$$

$$\text{with } Y^o = (P/O) / [\gamma(P/O)/4 + 1/Y^{ATP}] \quad (11)$$

The form of the cell yield coefficient, Y, is again a special case of Eq. (2), now with no substrate level phosphorylation ($N_F = 0$) and with the values $\gamma_F = 8$ and $\epsilon_F = 1$ appropriate for methane. The presence of chloroform does not alter the value of Y, which is a constant describing the cell's energy metabolism. What it does is add an extra term, q_A, to Eqs. (10) and (11). These are now two equations in four unknowns q_F, q_A, q_o, and μ, so two extra pieces of information (usually two rate equations) are needed to complete a quantitative description of the metabolism. Models for cometabolism by growing cells that contain only one rate equation are doomed to fail.

Equations (10) and (11), or their equivalents for other processes, represent a rational starting point for studying the kinetics of cometabolism, because they are firmly based in what we know about the biochemical pathways. The 2 in the denominator of Eq. (10), for example, reflects the fact that 1/2 mol of methane must be catabolized in order to provide the 2 mol of H′ needed for the action of the methane monooxygenase enzyme on 1 mol of chloroform. To the author's knowledge, however, they have not appeared in the cometabolism literature.

THE PRODUCTION OF SUCCINIC ACID FROM CARBOHYDRATE

Succinic acid is a TCA cycle intermediate whose production pathway from glucose is shown in Fig. 2 (*6*). The key reactions are the so-called anaplerotic reactions by which pyruvate or phosphoenolpyruvate generated by the EMP pathway are carboxylated to oxalacetate. They are catalyzed by single enzymes (pyruvate carboxylase and phosphoenolpyruvate carboxykinase) that exist in most microorganisms to compensate for the removal

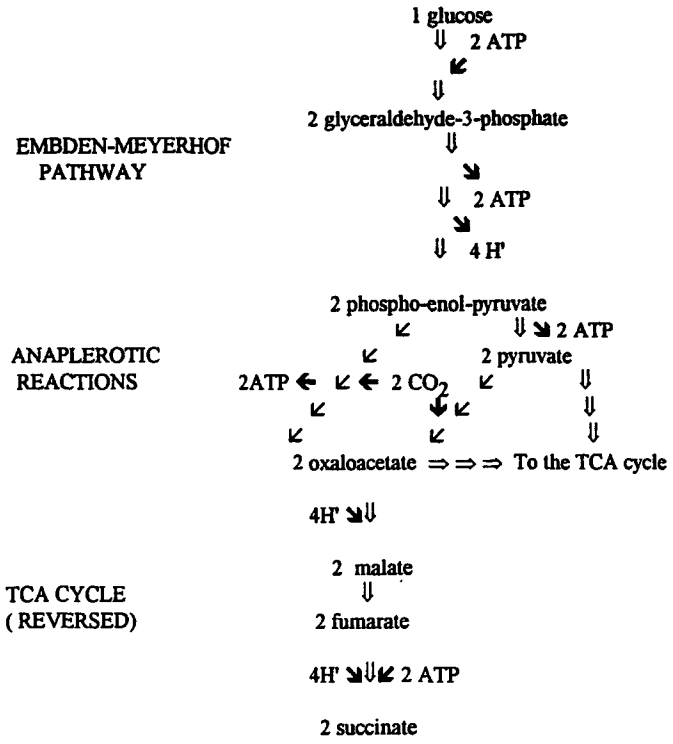

Fig. 2. Pathway for production of succinic acid.

of anabolic precursors, such as glutamic acid, from the TCA cycle. If they did not exist, there would not be enough oxalacetate to condense with acetyl-CoA to form citrate at the start of the TCA cycle. When these enzymes are very active, they produce so much oxalacetate that the last few steps of the TCA cycle can be driven backward, forming 2 mol of maleic, fumaric, or succinic acids from each mole of glucose. This very high yield is one of the attractions of the potential bioprocess. In terms of carbon equivalents, the pathway is:

$$3/4\,CH_2O + 1/2\,H' + 1/4\,CO_2 \rightarrow CH_{3/2}O + 1/4\,H_2O \qquad (12)$$

Thus, the stoichiometric parameter in the product formation pathway (*see* Fig. 1) is $\beta_F^M = 3/4$ (superscript M indicates the product; values for biomass have no superscript). The product formation pathway shown in Fig. 2 has a net ATP consumption of zero so Y_M^{ATP} is infinite. The energetics of cell growth give $Y^{ATP} = 0.4$ C Eq/mol ATP, $N_F = 1/3$ mol ATP/C Eq of glucose via the EMP pathway, and $\epsilon_F = 0$. The β_F for anabolism is another pseudo-constant whose value is not known exactly, but that is close to 1.5 C Eq glucose/C Eq biomass (7). We also know, from the definition in Eq. (1), the oxidation/reduction states of glucose, succinic acid, and (approximately) biomass; $\gamma_F = 4$, $\gamma_M = 3.5$, and $\gamma = 4.2$ if ammonia is the nitrogen source. It follows from Eq. (2) that $Y = 0.60$ and $Y_M = 1.16$ C

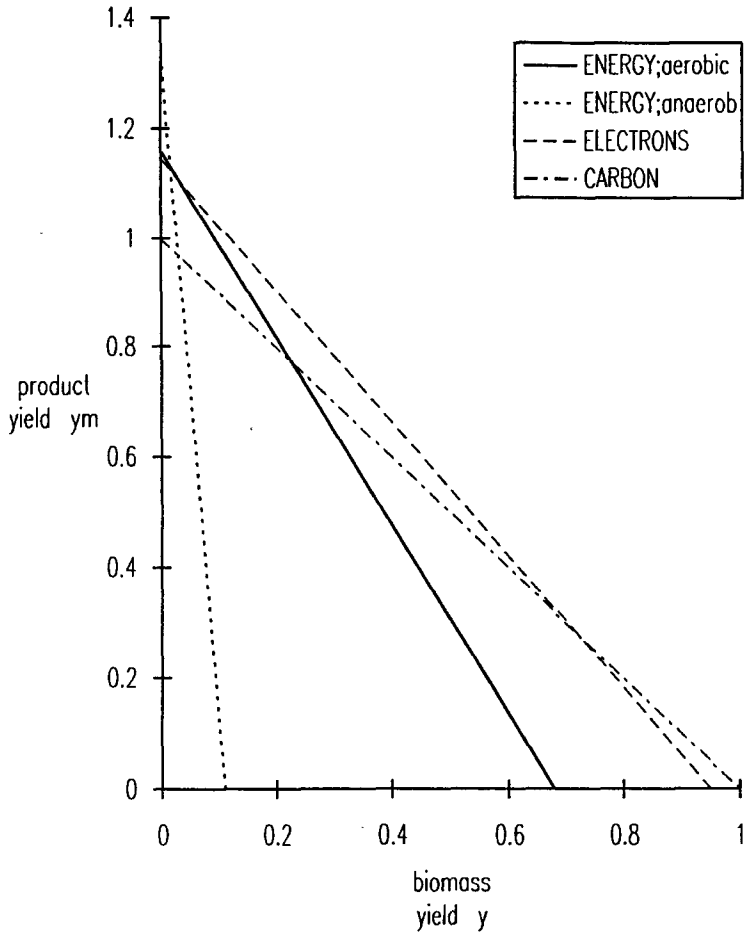

Fig. 3. Yield chart for succinic acid.

Eq/C Eq glucose for aerobic growth with (P/O) = 2, and Y = 0.11, Y_M = 1.33 C Eq/C Eq for anaerobic growth ([P/O] = 0).

Proceeding as before gives a set of equations that can be written as constraints on the observed yields of biomass ($y = \mu/q_F$) and product ($y_M = q_M/q_F$). These upper bounds on the yields arise from the availability of:

Electrons $$(\gamma\, y / \gamma_F) + (\gamma_M\, y_M / \gamma_F) = 1 - (4\, y_o / \gamma_F) \leq 1 \quad (13)$$

Energy (ATP) $$(y / Y) + (y_M / Y_M) = 1 - (k_F / q_F) \leq 1 \quad (14)$$

Carbon $$y + y_M = 1 - y_c \leq 1 \quad (15)$$

Plotting these equations on a graph of y_M vs y, as in Fig. 3, defines a feasible region for process operation. The process must operate inside the "energy" line by an amount equal to the fraction of the carbon/energy source that is used for maintenance (Eq. [14]). Operation outside the "electrons" line is impossible because it implies that oxygen is being pro-

duced rather than consumed (y_o negative in Eq. [13]). The "carbon" line defines conditions where the CO_2 produced by the catabolic and anabolic reactions is all consumed in the product formation reaction (Eq. [12]). Operation outside this line is possible, but only if extra CO_2 is supplied (y_c negative in Eq. [15]). The envelope of feasible yield values is defined for succinic acid by the energy and carbon lines, but other products make other patterns.

Figure 3 illustrates the obvious result that the yields of biomass and product are inversely related. The theoretical yields arise when all of the glucose is devoted to making either cells or product, and are given by the intercepts of the lines with the axes of Fig. 3. There are three possible limits: Y from the availability of energy; γ_F/γ_M from the availability of electrons; and 1 from the availability of carbon (y being defined as a ratio of carbon equivalents). A fourth limit, $1/\beta_F^M$, arises from the pathway. Even if all the glucose were used to make succinic acid, Eq. (12) shows that the yield could not exceed 4/3 (= $1/\beta_F^M$). The theoretical yield is the smallest of these four values. Along the biomass axis, they fall in the order $Y < 1/\beta_F < \gamma_F/\gamma < 1$, which is as expected. Biomass is a very complex "product," and its synthesis consumes a lot of energy, so the yield is energy-limited and the theoretical yield is the same as the yield coefficient. Along the succinic acid axis, they fall in the order $1 < \gamma_F/\gamma_M < Y_M < 1/\beta_F^M$. The theoretical yield is therefore 1 C Eq/C Eq (0.98 g/g), unless CO_2 is added to the process and can be taken up by the cells (evidence indicates that it can be [6]) in which case it rises to 4/3.5 on a carbon equivalent basis or 1.12 g/g. This is typical for the simple, oxidized TCA cycle intermediates. Their synthesis requires little or (in this case) no expenditure of metabolic energy, so yields are high and not affected by the value of Y_M.

The importance of this type of analysis in thinking about potential new bioprocesses can be summarized as follows. It is firmly based in knowledge of the biochemistry, and the more that is known about the pathways and their energetics, the more accurate the analysis can be. It provides a value for the theoretical yield that is essential for preliminary assessments of economic feasibility. More important, by showing what limits the yield, it suggests ways of operating the process. The yield of succinic acid can be improved by adding CO_2, although the benefits must be compared with the cost of the gas and the gas-transfer equipment. If it is not feasible, the best that can be achieved is operation where the "energy" lines in Fig. 3 (corrected for the maintenance requirement) intersect the "carbon" line, and the bioreactor type, media composition, biomass disposal, and so on, can be chosen accordingly. The short distance between this operating point and the "electrons" line means that y_o in Eq. (13) must be small, implying microaerophilic operation.

Anaerobic operation eliminates aeration and reduces the heat generation, so that a simpler, cheaper bioreactor can be used. It also generates less biomass (*see* the "energy anaerobic" line in Fig. 3) leaving more carbon potentially available for succinic acid production. The "electrons" con-

straint remains the same, although the distance from the line to the operating point now represents not the consumption of oxygen, but the formation of a catabolic fermentation product, which consumes some of the available carbon. Two desirable characteristics of this product are obvious: it must be readily separable from succinic acid and it must carry many electrons per unit of carbon (i.e., have large γ). Ethanol is good (the associated CO_2 can, in principle, be incorporated into succinic acid by Eq. [12]), whereas lactic acid is bad. This provides useful guidance for the selection and manipulation of microbial strains for a potential new bioprocess. There is, of course, no guarantee that a facultative, ethanol-fermenting organism that produces high yields of succinic acid can either be found or created by classical mutation or genetic engineering techniques, but the analysis given here shows that a search for this type of organism is dictated by the requirements of process economics.

CONCLUSIONS

The starting point for the design of bioprocess should be knowledge of the biochemical pathways involved. A procedure has been suggested for translating such knowledge into a set of yield equations by means of electron and energy balances over the catabolic, anabolic, respiration, and product formation "reactions." This procedure gives consistent results for the yields of both heterotrophic and chemoautotrophic metabolism, and has suggested new forms of the equations for cometabolic biodegradation of contaminants. It also allows greater insight into the concept of a theoretical yield for biomass and products, and this insight can help in the selection and design of novel bioprocesses.

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