Equations and Calculations of Product Yields and Preferred Pathways for Butanediol and Mixed-Acid Fermentations

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Using the available information of fermentation biochemistry, fermentation (stoichiometric) equations are derived for anaerobic saccharolytic fermentations of butanediol and mixed acids. The equations describe the interrelations among the fermentation products, biomass, and consumed substrate (glucose). The validity of the equations is tested using a variety of batch data from the literature. The validity of the equations is expected to extend to steady-state and transient fermentations, as well. Uses, improvements, and extensions of the equations are also discussed in detail. Among others, it is shown that the equations are useful for checking the consistency of experimental data, for calculating maximal yields and selectivities for the fermentation products, and calculating the extent of utilization of the Embden–Meyerhof–Parnas pathway versus the Hexose Monophosphate pathway of glucose utilization.

INTRODUCTION

In a recent publication,[1] we derived a stoichiometric (fermentation) equation for fermentations of butyric-acid bacteria (butanol/acetone fermentations). The equation was derived on the basis of the existing information on the fermentation biochemistry, an assumed ATP yield and two well-accepted biological regularities.[1] The equation describes the interrelations among the fermentation products, biomass, and consumed substrate (glucose), and was tested using a variety of literature data from completed batch fermentations. That apparently valid fermentation equation was also shown to obey the constraints imposed on growth and product formation by thermodynamics and the biochemical topology. On that basis, it was argued and demonstrated that the equation was useful for checking the consistency of experimental data, for calculating maximal product yields and selectivities, and for predicting experimental data. The latter, coupled with the validity of the equation under both steady-state and transient conditions, as we have argued,[1] renders the equation a useful tool (a "gateway sensor") for on-line monitoring of fermentations. A "gateway sensor" allows the calculation of a number of fermentation parameters using a combination of available sensors.[1,2] It was shown, in fact, that the equation can be used for calculating fermentation parameters which cannot be typically measured directly, such as rates of production of ATP, and reduction energy.[1] The last two physiological parameters were shown to characterize effectively the state of the fermentation,[1,3] and were proven most useful in the fermentor design for solvent production.[3] The equation can be also used to calculate measurable parameters, such as product concentrations, using a combination of available sensors for other fermentation products.[1] Finally, it was shown that the fermentation equation contains all the information that can be derived from carbon, nitrogen and available-electron balances.[1,4-6]

The objective of the present work is to derive, test, and show the practical importance of fermentation equations for fermentations of butanediol and mixed acids, using the methodology we have developed recently.[1] In contrast to the butyric-acid fermentations, the biochemistries of the butanediol and mixed-acid fermentations are known in lesser detail, and thus a number of additional assumptions will become necessary for the derivation of the equations. In a companion article,[7] we derive and test fermentation equations for propionic-acid fermentations and other saccharolytic fermentations where the substrates are pentoses and hexoses (other than glucose) typically obtained from hydrolysis of hemicellulose.[8,9]

The establishment of the thermodynamic and biochemical constraints which determine the theoretically maximal yields of the various fermentation products and the calculation of these maximal yields is of both fundamental and practical importance. Since they determine the rational upper bounds for the productivity and selectivity of a fermentation process, they can be used for feasibility studies and as a guide for genetic and bioreactor-productivity improvements. Production of butanediol and some of the acid products of mixed-acid fermentations are potentially of industrial importance.[10-12] We will argue in

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the discussion section that the fermentation equations may also prove useful for the microbiological characterization and taxonomic classification of some bacteria.

BIOCHEMISTRY OF BUTANEDIOL AND MIXED-ACID FERMENTATIONS

Figure 1 shows the most probable sequences of biochemical reactions and their enzymes which convert glucose to the typical products (acids, alcohols and gases) of the butanediol and mixed-acid fermentations. Typical products are shown in boxes although other intermediates may also accumulate under some fermentation conditions. The reactions leading to the production of glycerol, a typical fermentation product, are not shown in Figure 1, but are discussed in the next section. It should be also understood that not all of the reactions of Figure 1 operate in all microorganisms of the butanediol and mixed-acid fermentations and that, in fact, some products may be formed by variant schemes not shown in Figure 1 as is detailed in the next section. Figure 1 has been drawn on the basis of the most recent available information in the literature.[11,13-18]

Although the physiological significance of the Hexose Monophosphate (HMP) pathway in the conversion of glucose to phosphoenolpyruvate (PEP) or pyruvate is often underplayed[14] or quantitatively poorly understood,[13] it will become clear from the following calculations that it may contribute significantly to the glucose metabolism. Our original calculations utilizing the Embden-Meyerhof-Parnas (EMP) pathway of glucose utilization gave very poor results for many of the butanediol and mixed-acid fermentations. When the HMP pathway (HMPP) was included in the calculations, very good to excellent results were obtained. We then checked the literature for enzymatic studies of the HMPP in bacteria for which our calculations had indicated a significant involvement of the HMPP (the rest of the glucose was presumably metabolized through the EMP pathway). In all cases where any information was available, the enzymatic information confirmed our calculations. Previous quantitative estimations of the extent of involvement of HMPP did not always agree with our calculations. It is also interesting to note that our calculations indicated that the extent of involvement of the HMPP may vary significantly between zero and a large value for a given microorganism, apparently depending upon the fermentation conditions.

Although most enzymatic reactions may operate in either of their forward and reverse directions, most of the reactions of Figure 1 are indicated to operate in their forward direction. Reactions 22 and 23 are a notable exception and they pertain to the interconversion of the various

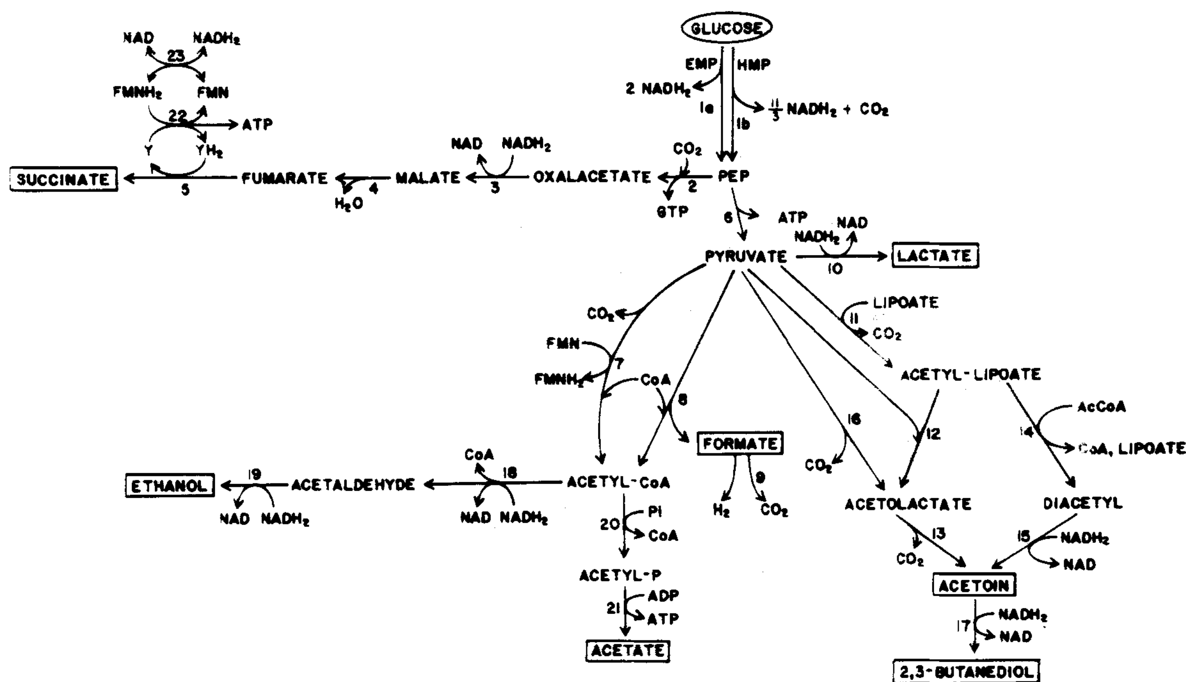


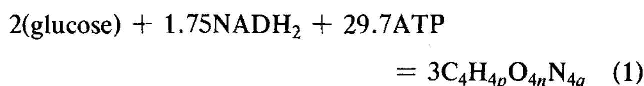
Figure 1. Biochemical pathways of glucose fermentation by butanediol and mixed-acid bacteria. The pathways or enzyme systems for the reactions shown are as follows: (1a) phosphoenolpyruvate phosphotransferase system and the Embden-Meyerhof-Parnas pathway; (1b) phosphoenolpyruvate phosphotransferase system and the Hexose Monophosphate pathway; (2) phosphoenolpyruvate carboxylase (GDP specific); (3) NAD(P)H malate dehydrogenase; (4) fumarate hydratase; (5) succinate dehydrogenase; (6) ATP—pyruvate phosphotransferase; (7) pyruvate-flavin oxidoreductase; (8) pyruvate-formate lyase; (9) formate-hydrogen lyase; (10) lactate dehydrogenase; (11) pyruvate-lipoate oxidoreductase; (12) acetolactate synthase (pyruvate/acetyl lipoate); (13) acetolactate decarboxylase; (14) diacetyl synthase (acetyl lipoate/acetyl-CoA); (15) acetoin dehydrogenase; (16) acetolactate synthase (pyruvate/pyruvate); (17) butanediol dehydrogenase; (18) acetaldehyde dehydrogenase; (19) ethanol dehydrogenase; (20) phosphotransacetylase; (21) acetate kinase; (22) YH_2-FMN oxidoreductase (ATP producing); and (23) NADH-FMN oxidoreductase.

forms of reduction energy, a fundamental assumption of the present calculations. This assumption was crucial in obtaining good agreement between calculated and experimental results, but it is also supported by physiological studies.[11,13-18] Two more reactions were shown in some fermentations to operate in the reverse direction, reaction 7 of Figure 1 and the CO_2 fixation via the HMPP (reaction scheme 1b of Fig. 1). Both will be subsequently discussed in more detail.

DERIVATION OF THE FERMENTATION EQUATIONS

The fermentation equation is derived by summing the various single enzymatic reactions or strings of reactions which lead to the formation of biomass and the various fermentation products from glucose and the other growth-medium nutrients.[1] Relations among the amounts of products and biomass produced and glucose utilized are then obtained by requiring conservation of intermediate species (e.g., pyruvate, acetyl-CoA) and biosynthetic and/or reduction energy. What species may be assumed that are being conserved must be decided and checked on an individual basis for each fermentation. For carbon intermediates, this assumption essentially reduces to our ability to account for all reactions where these intermediates participate. Conservation of reduction energy requires that we can not only account for all enzymatic reactions where it is being produced or required, but also for the reactions which interconvert its various forms ($NADH_2$, $FMNH_2$, FdH_2, etc.) and for possible membrane processes leading to its formation or consumption. Conservation of reduction energy in its various forms was crucial for the validity of the fermentation equation for butyric-acid bacteria.[1] This will prove also the case for the fermentation discussed here and in the companion article.[7] On the other hand, conservation of biosynthetic energy (ATP) has proved[1] and will prove difficult to postulate and implement. This is because ATP can be hydrolyzed (and possibly synthesized) by membrane functions associated with transport of fermentation products or intermediates and other physiological activities (like keeping a necessary ΔpH between the interior of the cells and the growth medium). It is also quite possible that variable amounts of ATP may be hydrolyzed because of purely kinetic reasons (like regeneration of ADP) without performing any physiological function.[1] Such ATP hydrolysis processes deserve a rigorous investigation and may lead to significant fundamental and practical benefits.[1,19]

Production of biomass from glucose can be represented by the following equation[1]:



$$2(\text{glucose}) + 1.75NADH_2 + 29.7ATP = 3C_4H_{4p}O_{4n}N_{4q} \quad (1)$$

with $C_4H_{4p}O_{4n}N_{4q}$ representing the biomass composition. For simplicity we have omitted the nitrogen source in the left-hand side of eq. (1), have omitted ADP and the oxidized form of $NADH_2$, and have made no attempt at this stage to balance the oxygen and hydrogen atoms. We have also assumed that little CO_2 is produced for biomass synthesis from general decarboxylation enzymes not shown in Figure 1.[1] As is detailed in ref. 1, eq. (1) is based on an assumed ATP yield $Y_{ATP} = 10.5$ and the two important biological regularities,[4-6]

$$\sigma_b = 0.462 \pm 0.023 \quad (2)$$

$$\gamma_b = 4 + p - 2n - 3q = 4.291 \pm 0.172 \quad (3)$$

Since complex media were employed for all fermentations whose data are used for the present calculations, the actual Y_{ATP} ought to be higher than 10.5 and closer to the theoretical 28.8 value.[1,20,21] However, the value of 10.5 is used for generality and for making sure that the biosynthetic requirements are amply satisfied. The implications of $Y_{ATP} = 10.5$ will be discussed later.

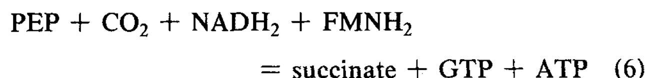
For the production of PEP from glucose through the EMP pathway we write[1,13,14] (reaction string 1a of Fig. 1),

$$\text{glucose} = 2PEP + 2NADH_2 \quad (4)$$

where for simplicity we have omitted the oxidized form of $NADH_2$. The conversion of PEP to pyruvate can be represented by (reaction 6 of Fig. 1),

$$PEP = \text{pyruvate} + ATP \quad (5)$$

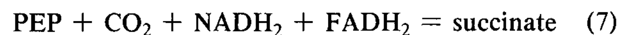
Formation of succinate from PEP through malate and fumarate (reactions 2, 3, 4, 5, and 22 of Fig. 1)[11,13,14,18] can be represented by,



$$PEP + CO_2 + NADH_2 + FMNH_2 = \text{succinate} + GTP + ATP \quad (6)$$

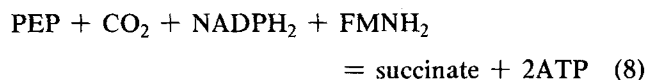
where $FMNH_2$ is the reduced flavin mononucleotide. The carrier YH_2 of reaction 5 (Fig. 1) is not known in all cases but is quite possibly a cytochrome. Again, the oxidized forms of $FMNH_2$ and $NADH_2$, GDP and ADP, have been omitted in eq. (6) and will be omitted in all following equations.

Succinate can be formed from pyruvate (and thus from PEP) via two alternative pathways not shown in Figure 1. The first, which is thought to operate in *Clostridium kluyveri*, *Escherichia coli*, and *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*),[13] leads to the formation of succinate from pyruvate via acetyl-CoA (AcCoA), malonyl-CoA, and succinyl-CoA and can be represented by the equation,



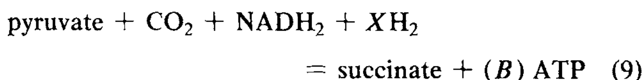
$$PEP + CO_2 + NADH_2 + FADH_2 = \text{succinate} \quad (7)$$

The third possibility for succinate production from pyruvate through malate exists in Enterobacteriaceae, using the enzyme malate dehydrogenase (NADP) (decarboxylating),[13] where the overall reaction can be represented by



$$PEP + CO_2 + NADPH_2 + FMNH_2 = \text{succinate} + 2ATP \quad (8)$$

From a calculation point of view, the three schemes represented by eqs. (6), (7), and (8) differ only in the amount of biosynthetic energy produced. But since typically these fermentations have an ATP surplus and since either of the schemes of eqs. (7) and (8) is expected to operate simultaneously with the scheme of eq. (6), the difference will, in practice, be small. Assuming now that GTP is equivalent to ATP for biosynthetic purposes,[13] the three eqs. (6), (7), and (8) can be represented by the equation,



$$\text{pyruvate} + CO_2 + NADH_2 + XH_2 = \text{succinate} + (B)\,ATP \quad (9)$$

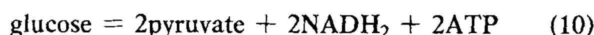
where

$$B = 1 \quad (9a)$$

for succinate production through fumarate (either of the two versions) and,

$$B = -1 \quad (9b)$$

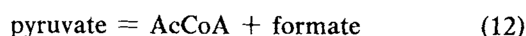
for succinate production through succinyl-CoA. The former we shall call **case 1** and the latter **case 2**. Here, XH_2 represents either or both $FADH_2$ and $FMNH_2$. We may also rewrite eq. (4), in view of eq. (5), as follows,



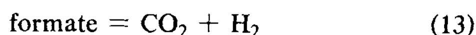
$$\text{glucose} = 2\text{pyruvate} + 2NADH_2 + 2ATP \quad (10)$$

For the reactions leading to the formation of AcCoA, formate, H_2, and lactate (Fig. 1), we write without any further comments,

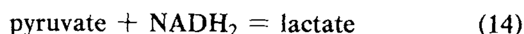
$$\text{pyruvate} = \text{AcCoA} + CO_2 + XH_2 \quad (11)$$



$$\text{pyruvate} = \text{AcCoA} + \text{formate} \quad (12)$$



$$\text{formate} = CO_2 + H_2 \quad (13)$$



$$\text{pyruvate} + NADH_2 = \text{lactate} \quad (14)$$

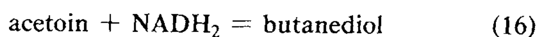
Acetoin (methylacetyl carbinol) may be formed from pyruvate by three reaction schemes, one through diacetyl and two through acetolactate, as shown in Figure 1. Two schemes (reactions 11, 12, and 13 or reactions 16 and 13 of Fig. 1) can be represented by the equation

$$2\text{pyruvate} = \text{acetoin} + 2CO_2 \quad (15)$$

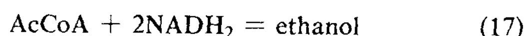
and the third scheme (reactions 11, 14, and 15 of Fig. 1) can be represented by

$$\text{pyruvate} + \text{AcCoA} + NADH_2 = \text{acetoin} + CO_2 \quad (15a)$$

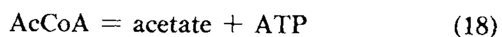
which by virtue of eq. (11) and reaction 23 (Fig. 1) is equivalent to eq. (15), which will be used for further derivations. The production of butanediol (2,3-butanediol), ethanol, and acetate (Fig. 1) will be represented by the following equations:



$$\text{acetoin} + NADH_2 = \text{butanediol} \quad (16)$$

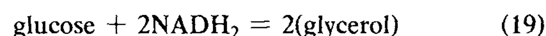


$$\text{AcCoA} + 2NADH_2 = \text{ethanol} \quad (17)$$



$$\text{AcCoA} = \text{acetate} + ATP \quad (18)$$

Glycerol is produced from glucose by reduction of dihydroxyacetone which is formed from dihydroxyacetone phosphate, an intermediate of the EMP pathway.[13] The overall reaction can be represented by the equation



$$\text{glucose} + 2NADH_2 = 2(\text{glycerol}) \quad (19)$$

Assuming that $NADH_2$ can reduce, under proper conditions, FAD and FMN and vice versa (reaction 23 of Fig. 1),[11,16-18] we write,

$$NADH_2 = XH_2 \quad (20)$$

The interconversion of the various forms of reduction energy [eg. (20)] is of crucial importance to the validity of the fermentation equation.

The hexose monophosphate pathway (HMPP) (or pentose phosphate pathway)[13,22] allows the cells to derive more reduction energy from glucose at the expense of producing more carbon dioxide. When not operating exclusively, as in the case of the present fermentations, HMPP is interlinked with the EMP and possibly the Entner-Doudoroff (ED) pathways for economy and versatility.[13] The formation of fructose-6-phosphate (F6P) and glyceraldehyde-3-phosphate (G3P) from glucose-6-phosphate (G6P) can be represented by[13,22]:

$$3(G6P) = 3CO_2 + 6NADPH_2 + 2(F6P) + G3P \quad (21)$$

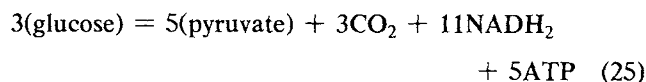
From the EMP pathway, we can write the following equations

$$F6P + ATP = 2(G3P) \quad (22)$$

$$G3P = \text{pyruvate} + 2ATP + NADH_2 \quad (23)$$

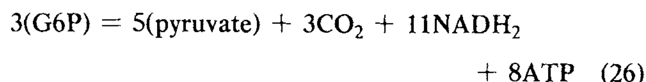
$$\text{glucose} + ATP = G6P \quad (24)$$

Assuming that $NADPH_2$ and $NADH_2$ are equivalent by virtue of the nonenergy requiring transhydrogenases in bacteria,[21] eqs. (21)-(24) can be combined to produce the overall equation



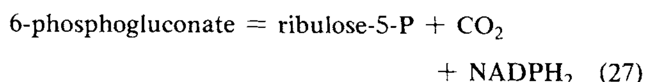
$$3(\text{glucose}) = 5(\text{pyruvate}) + 3CO_2 + 11NADH_2 + 5ATP \quad (25)$$

or its equivalent



$$3(G6P) = 5(\text{pyruvate}) + 3CO_2 + 11NADH_2 + 8ATP \quad (26)$$

We want to argue now briefly that eq. (26) [or eq. (25)] can be used in the reverse direction, in combination with eqs. (24) and (10), to represent CO_2 fixation into biomass and products using some of the enzymes of the HMP or possibly the ED pathways. The reason for this is that some of our calculations indicate that this may indeed be happening. The key reaction of the HMPP,



$$\text{6-phosphogluconate} = \text{ribulose-5-P} + CO_2 + NADPH_2 \quad (27)$$

is known to be a reversible one,[13] although the physiological significance of the reverse reaction remains unknown.

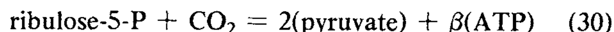
By the reversal of two reactions of the HMPP we can write

$$\text{6-phosphogluconate} + NADPH_2 = \text{G6P} \quad (28)$$

Alternatively, using reactions from the ED pathway, we can have

$$\text{6-phosphogluconate} = \text{G3P} + \text{pyruvate} \quad (29)$$

Combining the reverse of eq. (27) with either eqs. (23) and (29) or eqs. (10), (24), and (28), we obtain



$$\text{ribulose-5-P} + CO_2 = 2(\text{pyruvate}) + \beta(\text{ATP}) \quad (30)$$

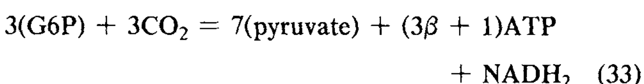
where β is 2 or 3, respectively. A combination of the transketolase, transaldolase, and epimerase reactions of the HMPP can readily lead to the overall reaction

$$2(\text{G6P}) + \text{G3P} = 3(\text{ribulose-5-P}) \quad (31)$$

which when combined with the following reaction from the EMP pathway,

$$\text{G6P} + \text{ATP} = 2(\text{G3P}) \quad (32)$$

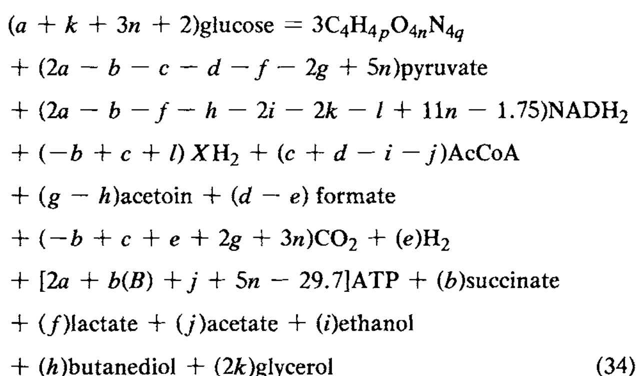
and eqs. (23) and (30) yields



$$3(\text{G6P}) + 3CO_2 = 7(\text{pyruvate}) + (3\beta + 1)\text{ATP} + NADH_2 \quad (33)$$

If $\beta = 3$ [i.e. eq. (28) instead of (29) is employed], eq. (33) can be obtained from combining eqs. (10) and (24) with the reverse of eq. (26). In short, the reverse of eq. (26) [or eq. (25)] in combination with eqs. (10) and (24) can describe CO_2 fixation into biomass or products. If the ED pathway is partially involved [eq. (29)], a somewhat smaller amount of ATP is produced, which is not a critical factor as the calculations will subsequently confirm.

If we now multiply eqs. (9)–(20) and eq. (25) by $b, a, c, d, e, f, g, h, i, j, k, l$, and n, respectively, and add them and eq. (1) together, we obtain,



$$\begin{aligned}(a + k + 3n + 2)\text{glucose} &= 3C_4H_{4p}O_{4n}N_{4q} \\ &+ (2a - b - c - d - f - 2g + 5n)\text{pyruvate} \\ &+ (2a - b - f - h - 2i - 2k - l + 11n - 1.75)NADH_2 \\ &+ (-b + c + l)XH_2 + (c + d - i - j)\text{AcCoA} \\ &+ (g - h)\text{acetoin} + (d - e)\text{ formate} \\ &+ (-b + c + e + 2g + 3n)CO_2 + (e)H_2 \\ &+ [2a + b(B) + j + 5n - 29.7]\text{ATP} + (b)\text{succinate} \\ &+ (f)\text{lactate} + (j)\text{acetate} + (i)\text{ethanol} \\ &+ (h)\text{butanediol} + (2k)\text{glycerol} \quad (34)\end{aligned}$$

For completed or steady-state or transient fermentations (under the pseudo-steady-state hypothesis[1]), since no pyruvate, $NADH_2$, XH_2, or AcCoA accumulate, we set their coefficients equal to zero, thus obtaining the four equations

$$2a - b - c - d - f - 2g + 5n = 0 \quad (35)$$

$$2a - b - f - h - 2i - 2k - l + 11n - 1.75 = 0 \quad (36)$$

$$-b + c + l = 0 \quad (37)$$

$$c + d - i - j = 0 \quad (38)$$

Assuming that acetoin, formate, CO_2, H_2, succinate, acetate, ethanol, and butanediol are final products and are not supplied as substrates to the cells, we must have

$$g - h \geq 0 \quad (39)$$

$$d - e \geq 0 \quad (40)$$

$$-b + c + e + 2g + 3n \geq 0 \quad (41)$$

$$e, b, j, i, h \geq 0 \quad (42)\text{–}(46)$$

If lactate and glycerol are not supplied as substrates, we must also have,

$$f, k \geq 0 \quad (47), (48)$$

Otherwise f and k can be also negative. The coefficient of ATP, assuming that the Y_{ATP} value of 10.5 stands, must also be greater or equal to zero, i.e.,

$$2a + b(B) + j + 5n - 29.7 \geq 0 \quad (49)$$

As will be subsequently seen, most of the calculations satisfy the above inequality with a few exceptions where Y_{ATP} appears to be higher than 10.5 but still considerably lower than 28.8. Since eq. (10) [which was multiplied by "a" before the addition to obtain eq. (34)] is applicable only in the forward direction, we have

$$a > 0 \quad (50)$$

Equations (11) and (20) are applicable in both their forward and reverse directions and therefore c and l can be negative, positive, or zero, their sign indicating the direction of the reactions that eqs. (11) and (20) represent. The reaction of eq. (11) is typically expected to operate in its forward direction, although it is a readily reversible reaction under proper intracellular conditions.[16,18] Similarly, the reaction of eq. (20) can be readily reversed under favorable intracellular conditions as usual thermodynamic calculations can show.[16]

Equation (34), with its accompanying equality and inequality conditions (35)–(50), is what we shall call the fermentation equation for butanediol or mixed-acid fermentations. We have shown in detail[1] that the fermentation equation for butyric-acid bacteria contains both the carbon and available electron balances and that, in fact, provides two more equations than the above two balances. It can be similarly shown that fermentation eq. (34) contains both the carbon and available electron balances and, obviously, provides considerably more information, in the form of additional equations, than the above two balances.

CALCULATION PROCEDURES

According to eq. (34), since from the $(a + k + 2)$ mol glucose 2 mol are converted into biomass, we have

$$a + k + 3n + 2 = \frac{200}{100 - \text{PCRP}} \quad (51)$$

where PCRP is the percentage of glucose recovered into products excluding the biomass. Then, if we define α as,

$$100\alpha \equiv a + k + 3n + 2 \quad (52)$$

eqs. (34)-(38) and eq. (52) can be written in the following equivalent form of algebraic equations:

$$CO_2: -b + c + e + 2g + 3n = Y_1 \equiv \alpha X_1 \quad (53)$$

$$\text{formate: } d - e = Y_2 \equiv \alpha X_2 \quad (54)$$

$$\text{acetoin: } g - h = Y_3 \equiv \alpha X_3 \quad (55)$$

$$\text{ethanol: } i = Y_4 \equiv \alpha X_4 \quad (56)$$

$$\text{lactate: } f = Y_5 \equiv \alpha X_5 \quad (57)$$

$$\text{succinate: } b = Y_6 \equiv \alpha X_6 \quad (58)$$

$$\text{acetate: } j = Y_7 \equiv \alpha X_7 \quad (59)$$

$$\text{butanediol: } h = Y_8 \equiv \alpha X_8 \quad (60)$$

$$\text{hydrogen: } e = Y_9 \equiv \alpha X_9 \quad (61)$$

$$\text{glycerol: } 2k = Y_{10} \equiv \alpha X_{10} \quad (62)$$

$$\text{glucose: } a + k + 3n - 100\alpha = -2 \quad (52a)$$

$$\text{pyruvate: } 2a - b - c - d - f - 2g + 5n = 0 \quad (35a)$$

$$NADH_2: 2a - b - f - h - 2i - 2k - l + 11n = 1.75 \quad (36a)$$

$$XH_2: -b + c + l = 0 \quad (37a)$$

$$\text{AcCoA: } c + d - i - j = 0 \quad (38a)$$

where Y_i is the number of moles of species i and X_i is the number of moles of species i produced per 100 moles glucose fermented. The index $i = 1, 2, \ldots, 10$ has been assigned as shown in eqs. (53)-(62). The above system of eqs. (53)-(62), (52), and (35)-(38) can be rewritten in the following compact matrix form:

$$\mathbf{Lu} = \mathbf{v} \quad (63)$$

where $\mathbf{L}$ can be written in terms of the two submatrices (obtained from the partitioning of $\mathbf{L}$) $\mathbf{L}_A$ and $\mathbf{L}_Y$ as follows,

$$\mathbf{L} = [\mathbf{L}_A \mathbf{L}_Y] \quad (64)$$

where $\mathbf{L}_A$ is the 15×14 matrix of the coefficients of the unknowns, a through l, n, and α, of the left-hand sides of the above set of equations, and $\mathbf{L}_Y$ is a 15×10 matrix whose *only nonzero* elements λ_{ii} are -1 for $i = 1, 2, \ldots, 10$. Parameter $\mathbf{u}$ is the column vector of the 24 unknowns, a through l, n, α, and Y_1 through Y_{10}. Parameter $\mathbf{v}$ is the constant 15-element column vector whose only two nonzero elements, the 11th and the 13th, are equal to -2 and 1.75, respectively.

Equation (63) is a set of 15 algebraic equations with 24 unknowns, a through l, n, α [which is equivalent to the PCRP by virtue of eqs. (51) and (52)] and the product molar amounts Y_1 through Y_{10}. Therefore, given the molar amounts of nine products, the carbon recovery into products (PCRP or α), biomass concentration, the molar amount of the 10th product, glucose consumed, and the extent (or degree of advancement) of the reactions of eqs. (1), (9)-(20), and (25) (as represented by the calculated values of the unknowns a through l and n) can be computed. Alternatively, if the molar amounts per 100 mol glucose fermented of eight products and α or PCRP are given, the amounts of the remaining two products and all the extents of the above reactions can be computed. If the HMPP is not involved in the bacterial biochemistry (i.e. $n = 0$), the amounts of three products and the extents of the various reactions can be computed by measuring the molar amounts, per 100 mol glucose fermented, of seven fermentation products. The solution of the linear set of algebraic equations was obtained using a standard Gauss elimination procedure.

For the calculations reported below, the carbon recovery into products was often recalculated to be consistent with the reported molar-amount data. The rest of the glucose carbon was assumed to form *all* of the biomass produced. Although it is expected that a variable amount of biomass is formed from carbon sources of the complex medium other than glucose (e.g., yeast extract), the reported data do not allow for such possible corrections. We found that this approximation leads to only small errors in most cases.

The amount of excess ATP reported in all the following calculations is the coefficient of ATP in eq. (34) normalized for 100 mol glucose fermented. This amount typically is calculated to be a large positive number, which is only an *indication* of an apparent ATP surplus. In most cases, this is an overestimation of the true excess ATP, because more biomass is typically formed from other carbon sources of the complex media, and also because ATP is most probably used to perform other cellular functions which, as we have discussed in previous sections, cannot be accounted for easily. The apparent Y_{ATP} was calculated on the basis of the total ATP and the biomass calculated from eq. (34) and is *only an indication* of a possible low overall efficiency of substrate utilization.

Finally, a good indication of data consistency is the *gamma ratio*, namely the ratio of the degree of reductance[1,4-6] of the fermentation products to that of the glucose substrate, i.e.,

$$\text{gamma ratio} = \frac{25.746(100 - \text{PCRP}) + \sum_i \gamma_i X_i}{2400} \quad (65)$$

{We would like to bring to the attention of the reader that in the corresponding equation in ref. 1 [eq. (41)] in the term for the degree of reductance of biomass, 6.4365 should be correctly replaced by 25.746.} Again, corrections for the various forms of complex nitrogen and carbon from the growth media utilized, were not possible from the reported data. In the case of low percentages (lower than 85%) of carbon recovered in fermentation products, the effect of incorporation of complex carbon and nitrogen into biomass may lead to computed gamma ratios significantly (i.e. by more than 1%) different from

one, even for otherwise very consistent experimental data, as a careful study of the following calculations can show. The gamma ratio is calculated using all of the experimental data and is shown only in the experimental-data columns in the following tables. For all calculations based on eq. (34) where we use the minimum possible number of experimental data to calculate the remaining data, the gamma ratio is always one as it should ideally be according to eq. (34). This value of the gamma ratio is not shown, for simplicity, in the calculation columns of the following tables.

CALCULATIONS

Validity of Fermentation Equation

As was the case with the butanol/acetone fermentation data,[1] all available *complete* data of butanediol and mixed-acid fermentations date back to 1931–1956. Some of the employed experimental and analytical methods may thus render these data suspicious. To the best of our knowledge, all more recent data are incomplete, with smaller product components and gas data typically missing. We have performed over 450 sets of calculations on 44 complete sets of fermentation data of 16 species and even more strains of butanediol and mixed-acid producing bacteria. The calculations presented in the following tables represent only a small portion of our calculations and are to demonstrate the validity, versatility and usefulness of the fermentation equation. First, we present calculations on butanediol fermentations and then on mixed-acid fermentations. Calculated product components are shown in boldface for easy identification. The rest of the product values were employed in the calculations.

Table I presents calculations of a butanediol fermentation of glucose with *Aerobacter cloaceae*.[23] The first three sets of calculations were performed assuming that the HMPP does not operate and the last three that it does. The predicted product values in both sets of calculations are in excellent agreement with the experimental values. As can be seen from the last three sets of calculations, the moles of CO_2 (per 100 mol glucose fermented) produced through the HMPP [eq. (25)] are very few, indicating that the HMPP was not involved in this fermentation. As a rule, calculations were first performed without the HMPP and then with HMPP. Involvement of the HMPP was accepted if all of the following conditions were met:

(1) the predicted product values were closer to experimental values for calculations with HMPP than without, and

(2) the calculated values of CO_2 moles (per 100 mol glucose fermented) from HMPP [eq. (25)] were *consistent* and *higher in absolute value than 4–5*.

For this set of fermentation data, the amount of excess ATP produced (and therefore the apparent Y_{ATP}) for either of cases 1 or 2 [eqs. (9a) and (9b)] is the same because no succinate was produced [eq. (34)].

Table II presents calculations for a butanediol fermentation of glucose with *Aeromonas hydrophila*.[24] Calculations with the HMPP are as good as without HMPP but

Table I. Fermentation of glucose with *Aerobacter cloaceae*; data are from ref. 23.

	Amount (mol/100 mol glucose fermented)						
		Calculated					
Substance	Experimental	Without HMPP			With HMPP		
Carbon dioxide	173.0	173.0[a]	**172.7**	173.0[a]	**174.2**	**173.3**	173.0[a]
Formate	8.5	8.5[a]	8.5[a]	**8.2**	8.5[a]	8.5[a]	8.5[a]
Acetoin	0.5	0.5[a]	0.5[a]	0.5[a]	0.5[a]	0.5[a]	0.5[a]
Ethanol	65.0	65.0[a]	65.0[a]	65.0[a]	65.0[a]	65.0[a]	65.0[a]
Lactate	3.2	**3.1**	**3.4**	**3.4**	3.2[a]	3.2[a]	3.2[a]
Succinate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Acetate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Butanediol	57.6	**57.8**	57.6[a]	57.6[a]	**57.4**	57.6[a]	**57.7**
Hydrogen	47.0	**43.5**	**43.4**	**43.7**	47.0[a]	**44.6**	**43.7**
Glycerol	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Carbon recovery	92.3	92.3[a]	92.3[a]	92.3[a]	92.3[a]	92.3[a]	92.3[a]
$NADH_2$ from XH_2	—	13.0	13.1	13.1	9.5	11.9	12.8
Excess ATP [case 1, eq. (9a) or case 2, eq. (9b)]	—	70.3	70.3	70.3	69.6	70.1	70.2
CO_2 from HMPP	—	—	—	—	1.9	0.6	0.2
Apparent Y_{ATP} (case 1 or case 2)	—	6.5	6.5	6.5	6.52	6.51	6.50
Gamma ratio	1.002	—	—	—	—	—	—
H_2/CO_2	0.27	0.25	0.25	0.25	0.27	0.26	0.25

[a]Refers to data used in the calculations.

Table II. Fermentation of glucose with *Aeromonas hydrophila*; data are from ref. 24.

	Amount (mol/100 mol glucose fermented)						
		Calculated					
		Without HMPP				with HMPP	
	Experimental						
Substance	1	2	3	4	5	6	7
Carbon dioxide	166.2	**165.9**	**165.9**	**165.8**	**165.9**	166.2[a]	**164.2**
Formate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Acetoin	1.7	1.7[a]	1.7[a]	1.7[a]	1.7[a]	1.7[a]	1.7[a]
Ethanol	52.0	**52.1**	52.0[a]	52.0[a]	**61.3**	**52.0**	52.0[a]
Lactate	23.3	23.3[a]	23.3[a]	**23.4**	23.3[a]	23.3[a]	23.3[a]
Succinate	3.6	3.6[a]	3.6[a]	3.6[a]	3.6[a]	3.6[a]	3.6[a]
Acetate	4.6	4.6[a]	4.6[a]	4.6[a]	4.6[a]	4.6[a]	4.6[a]
Butanediol	54.7	54.7[a]	**54.8**	54.7[a]	**50.1**	54.7[a]	**55.2**
Hydrogen	57.5	**62.1**	**62.2**	**62.1**	57.5[a]	**63.0**	57.5[a]
Glycerol	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Carbon recovery	98.2	98.2[a]	98.2[a]	98.2[a]	98.2[a]	98.2[a]	98.2[a]
$NADH_2$ from XH_2	—	−9.0	−9.2	−9.1	4.9	−10.1	−4.5
Excess ATP [case 1, eq. (9a)]	—	177.9	177.9	177.3	177.9	177.7	178.7
Excess ATP [case 2, eq. (9b)]	—	170.7	170.7	170.7	170.7	—	—
CO_2 from HMPP	—	—	—	—	—	0.5	−2.5
Apparent Y_{ATP} (case 1)	—	1.37	1.37	1.37	1.37	1.37	1.37
Apparent Y_{ATP} (case 2)	—	1.42	1.42	1.42	1.42	—	—
Gamma ratio	0.996	—	—	—	—	—	—
H_2/CO_2	0.35	0.37	0.37	0.37	0.35	0.38	0.35
c, eqs. (11) and (34) (case 1 or case 2)[b]	—	−5.4	−5.6	−5.5	8.5	−6.5	−0.9

[a]Refers to data used in the calculations.
[b]This is normalized for 100 mol glucose.

the values of CO_2 produced from HMPP are neither consistent nor large enough. The HMPP was probably not involved in this fermentation. Since some succinate was produced in this fermentation, the amount of excess ATP is higher for calculations with case 1 than for calculations with case 2, and thus the calculated apparent Y_{ATP} values are higher for case 2 than for case 1. All the other calculated quantities are, as expected, the same with both cases. *All subsequent calculations were performed using case 1 [eq. (9a)].*

The calculations without HMPP (columns 2-5 in Table II) indicate that there was probably an error in the measurement of the H_2 value (gas measurements were typically difficult and often inaccurate). When this somewhat lower experimental value for H_2 was used for calculating other product values (column 5), the calculated values did not agree very well with the experimental ones. This observation can be used to detect systematic errors in one or two experimental values as is detailed in a following section. Finally, note the small but consistent *negative* values predicted for c in this fermentation, indicating that the reaction of eq. (11) was operating in the reverse direction.

Table III presents calculations for a butanediol fermentation of glucose with *Bacillus polymyxa*.[25] The predicted values with or without HMPP agree quite well with the experimental values. For calculations with HMPP the predicted values of CO_2 from HMPP are consistent but very small. Thus, HMPP involvement is not indicated. The same conclusion can be drawn from calculations with a second reported set of fermentation data for *B. polymyxa*.[25]

Tables IV and V present calculations for two butanediol fermentations of glucose with *Bacillus subtilis*.[26] For the fermentation of Table IV, calculated values agree quite well with experimental values for both calculations with or without HMPP. The predicted CO_2 values from HMPP are positive, consistent, but small, and thus HMPP involvement is not indicated. In contrast, for the fermentation of Table V, the predicted values for CO_2 from HMPP are positive, consistent, and reasonably high, indicating involvement of the HMPP. Predicted product values were in good agreement with experimental values for calculations with HMPP and in poor agreement for calculations without HMPP. Calculations with eight more sets of data of *B. subtilis* butanediol fermentations[26] have indicated a variable involvement of HMPP with values of CO_2 produced from HMPP varying between 0 and 18. Previous estimations[13] for *B. subtilis* had HMPP responsible for 26% of the glucose catabolism.

Table VI presents calculations for a butanediol fermen-

Table III. Fermentation of glucose with *Bacillus polymyxa* (formerly *Aerobacillus polymyxa*); data are from ref. 25.

	Amount (mol/100 mol glucose fermented)						
		Calculated					
Substance	Experimental	Without HMPP			With HMPP		
Carbon dioxide	199.6	**199.8**	**199.8**	**199.8**	199.6[a]	199.6[a]	199.6[a]
Formate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Acetoin	2.8	2.8[a]	2.8[a]	2.8[a]	2.8[a]	2.8[a]	2.8[a]
Ethanol	66.2	66.2[a]	66.2[a]	**61.1**	66.2[a]	66.2[a]	**63.7**
Lactate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Succinate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Acetate	2.9	2.9[a]	**0.9**	2.9[a]	2.9[a]	**1.3**	**0.4**
Butanediol	65.1	**62.5**	**63.5**	65.1[a]	**62.6**	**63.4**	65.1[a]
Hydrogen	70.9	**73.9**	70.9[a]	**76.4**	**73.3**	70.9[a]	70.9[a]
Glycerol	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Carbon recovery	101.6	99.9[a]	99.9[a]	99.9[a]	99.9[a]	99.9[a]	99.9[a]
$NADH_2$ from XH_2	—	−4.8	−3.8	−12.4	−4.2	−3.4	−6.8
Excess ATP	—	201.2	199.2	201.2	201.3	199.7	198.9
CO_2 from HMPP	—	—	—	—	−0.3	−0.3	−0.3
Apparent Y_{ATP}	—	0.08	0.08	0.08	0.08	0.08	0.08
Gamma ratio	1.020	—	—	—	—	—	—
H_2/CO_2	0.36	0.37	0.35	0.38	0.37	0.36	0.36

[a]Refers to data used in the calculations.

Table IV. Fermentation of glucose with *Bacillus subtilis*; data are from ref. 26.

	Amount (mol/100 mol glucose fermented)						
		Calculated					
Substance	Experimental	Without HMPP			With HMPP		
Carbon dioxide	117.8	117.8[a]	**117.7**	**120.0**	**119.1**	**119.2**	**119.2**
Formate	1.3	1.3[a]	1.3[a]	1.3[a]	1.3[a]	1.3[a]	1.3[a]
Acetoin	1.6	1.6[a]	1.6[a]	1.6[a]	1.6[a]	1.6[a]	1.6[a]
Ethanol	7.7	7.7[a]	7.7[a]	7.7[a]	**7.5**	7.7[a]	7.7[a]
Lactate	17.6	**21.0**	**21.1**	17.6[a]	17.6[a]	17.6[a]	17.6[a]
Succinate	1.1	1.1[a]	1.1[a]	1.1[a]	1.1[a]	1.1[a]	1.1[a]
Acetate	0.2	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
Butanediol	54.6	**54.6**	54.6[a]	**55.8**	54.6[a]	54.6[a]	**54.5**
Hydrogen	0.2	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
Glycerol	56.8	**53.8**	**53.7**	**54.9**	56.8[a]	**56.7**	56.8[a]
Carbon recovery	98.0	98.0[a]	98.0[a]	98.0[a]	98.0[a]	98.0[a]	98.0[a]
$NADH_2$ from XH_2	—	5.2	5.2	5.2	5.1	5.2	5.2
Excess ATP	—	113.8	113.8	112.7	110.2	110.4	110.2
CO_2 from HMPP	—	—	—	—	1.54	1.48	1.57
Apparent Y_{ATP}	—	2.17	2.17	2.19	2.23	2.23	2.23
Gamma ratio	1.000	—	—	—	—	—	—

[a]Refers to data used in the calculations.

tation of glucose with *Serratia marcescens*.[27] Calculations without HMPP lead to results in poor agreement with experimental values. Calculations with HMPP give values in excellent agreement with experimental ones. The calculated values of CO_2 from HMPP are *negative*, consistent, and sufficiently high. Thus, as it was explained in the preceeding section, CO_2 fixation through HMPP is indicated. The same conclusions could be drawn from calculations with four more sets of butanediol fermentations of *S. marcescens*. Only the fermentation presented in Table XV gave small, positive values for CO_2 from HMPP.

Tables VII and VIII present calculations for two butanediol fermentations of *Klebsiella pneumoniae*[28] to demonstrate the variable involvement of the HMPP for

Table V. Fermentation of glucose with *Bacillus subtilis*; data are from ref. 26.

	Amount (mol/100 mol glucose fermented)							
		Calculated						
Substance	Experimental	Without HMPP		with HMPP				
Carbon dioxide	85.7	85.7[a]	85.7[a]	**85.4**	**85.5**	**85.4**	**85.5**
Formate	15.6	15.6[a]	15.6[a]	15.6[a]	15.6[a]	15.6[a]	15.6[a]
Acetoin	0.6	0.6[a]	0.6[a]	0.6[a]	0.6[a]	0.6[a]	0.6[a]
Ethanol	15.5	15.5[a]	**4.1**	15.5[a]	**15.6**	15.5[a]	15.5[a]
Lactate	54.7	**60.4**	54.7[a]	**54.8**	54.7[a]	54.7[a]	54.7[a]
Succinate	0.3	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
Acetate	1.3	1.3[a]	1.3[a]	1.3[a]	1.3[a]	1.3[a]	1.3[a]
Butanediol	38.0	**41.8**	**47.5**	38.0[a]	38.0[a]	38.0[a]	**38.0**
Hydrogen	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Glycerol	34.4	**23.6**	**29.3**	34.4[a]	34.4[a]	**34.5**	34.4[a]
Carbon recovery	93.0	93.0[a]	93.0[a]	93.0[a]	93.0[a]	93.0[a]	93.0[a]
$NADH_2$ from XH_2	—	0.8	−10.6	0.8	0.9	0.8	0.8
Excess ATP	—	60.1	54.4	46.8	46.8	46.7	46.9
CO_2 from HMPP	—	—	—	7.31	7.31	7.36	7.29
Apparent Y_{ATP}	—	6.65	6.89	7.24	7.24	7.24	7.23
Gamma ratio	0.999	—	—	—	—	—	—

[a]Refers to data used in the calculations.

Table VI. Fermentation of glucose with *Serratia marcescens*; data are from ref. 27.

	Amount (mol/100 mol glucose fermented)							
		Calculated						
Substance	Experimental	Without HMPP		with HMPP				
Carbon dioxide	116.8	**105.4**	**122.5**	**116.8**	**116.8**	116.8[a]	116.8[a]
Formate	48.2	**65.3**	48.2[a]	48.2[a]	48.2[a]	48.2[a]	**48.2**
Acetoin	1.9	1.9[a]	1.9[a]	1.9[a]	1.9[a]	1.9[a]	1.9[a]
Ethanol	46.0	**43.3**	**77.4**	**46.1**	46.0[a]	**46.1**	46.0[a]
Lactate	10.1	10.1[a]	10.1[a]	10.1[a]	10.1[a]	10.1[a]	10.1[a]
Succinate	8.1	8.1[a]	8.1[a]	8.1[a]	8.1[a]	8.1[a]	8.1[a]
Acetate	3.8	3.8[a]	3.8[a]	3.8[a]	3.8[a]	3.8[a]	3.8[a]
Butanediol	64.0	64.0[a]	**46.9**	64.0[a]	**64.1**	**64.0**	**64.1**
Hydrogen	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Glycerol	1.3	1.3[a]	1.3[a]	1.3[a]	1.3[a]	1.3[a]	1.3[a]
Carbon recovery	99.2	99.2[a]	99.2[a]	99.2[a]	99.2[a]	99.2[a]	99.2[a]
$NADH_2$ from XH_2	—	−26.3	24.9	−6.4	−6.5	−6.5	−6.5
Excess ATP	—	197.2	197.2	200.1	200.1	200.1	200.1
CO_2 from HMPP	—	—	—	−8.53	−8.57	−8.55	−8.58
Apparent Y_{ATP}	—	0.60	0.60	0.59	0.59	0.59	0.59
Gamma ratio	0.999	—	—	—	—	—	—

[a]Refers to data used in the calculations.

this potentially industrial microorganism.[12] In Table VII, calculations with or without HMPP are in excellent agreement with experimental values. The value of CO_2 produced from HMPP is calculated to be zero. In Table VIII, calculations without HMPP are in poor agreement with experimental values, while calculations with HMPP give results in agreement with experimental values and *positive*, consistent and high values for CO_2 produced from HMPP. Finally, note that the data of Table VII lead to a calculated apparent Y_{ATP} of 16.24 which is higher than the assumed ATP yield of 10.5 but still lower than the theoretical (and yet practical) maximum value of 28.8.[20,21] Actually, yields closer to 20 should be expected for fermentations on complex media, like *all* the fermentations which are presently discussed. Next we discuss calculations for mixed-acid fermentations.

Table VII. Fermentation of glucose with *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*); data are from ref. 28.

Substance	Amount (mol/100 mol glucose fermented)						
		Calculated					
	Experimental	Without HMPP			With HMPP		
Carbon dioxide	74.9	**74.8**	**74.8**	74.9[a]	**74.8**	74.9[a]	74.9[a]
Formate	60.8	60.8[a]	60.8[a]	**60.7**	60.8[a]	60.8[a]	**60.7**
Acetoin	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Ethanol	48.4	**48.4**	48.4[a]	**48.4**	48.4[a]	**48.3**	48.4[a]
Lactate	6.6	6.6[a]	**6.6**	6.6[a]	6.6[a]	6.6[a]	6.6[a]
Succinate	7.5	7.5[a]	7.5[a]	7.5[a]	7.5[a]	7.5[a]	7.5[a]
Acetate	40.7	40.7[a]	40.7[a]	40.7[a]	40.7[a]	40.7[a]	40.7[a]
Butanediol	27.0	27.0[a]	27.0[a]	27.0[a]	27.0[a]	27.0[a]	27.0[a]
Hydrogen	ND	**21.4**	**21.4**	**21.5**	**21.4**	**21.7**	**21.5**
Glycerol	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Carbon recovery (as glucose)	78.6	78.6[a]	78.6[a]	78.6[a]	78.6[a]	78.6[a]	78.6[a]
$NADH_2$ from XH_2	—	−0.6	−0.6	−0.6	−0.6	−0.9	−0.6
Excess ATP	—	−112.4	−112.4	−112.4	−112.4	−112.4	−112.4
CO_2 from HMPP	—	—	—	—	0.00	0.16	0.00
Apparent Y_{ATP}	—	16.24	16.24	16.24	16.24	16.24	16.24
Gamma ratio	0.980	—	—	—	—	—	—
H_2/CO_2	ND	0.29	0.29	0.29	0.29	0.29	0.29

[a]Refers to data used in the calculations.
ND refers to data not determined.

Table VIII. Fermentation of glucose with *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*); data are from ref. 28.

Substance	Amount (mol/100 mol glucose fermented)					
		Calculated				
	Experimental	Without HMPP		With HMPP		
Carbon dioxide	79.6	**53.9**	**60.0**	**79.5**	79.6[a]	79.6[a]
Formate	68.4	68.4[a]	68.4[a]	68.4[a]	68.4[a]	**68.3**
Acetoin	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Ethanol	51.5	51.5[a]	51.5[a]	51.5[a]	**51.5**	51.5[a]
Lactate	10.1	10.1[a]	**16.5**	10.1[a]	10.1[a]	10.1[a]
Succinate	13.1	**19.5**	13.1[a]	13.1[a]	13.1[a]	13.1[a]
Acetate	51.9	51.9[a]	51.9[a]	51.9[a]	51.9[a]	51.9[a]
Butanediol	19.2	19.2[a]	19.2[a]	19.2[a]	19.2[a]	19.2[a]
Hydrogen	ND	**20.2**	**26.6**	**65.0**	**65.3**	**65.1**
Glycerol	4.2	4.2[a]	4.2[a]	4.2[a]	4.2[a]	4.2[a]
Carbon recovery (as glucose)	87.8	87.8[a]	87.8[a]	87.8[a]	87.8[a]	87.8[a]
$NADH_2$ from XH_2	—	−4.7	−4.7	−43.1	−43.5	−43.1
Excess ATP	—	61.6	55.2	48.8	48.8	48.8
CO_2 from HMPP	—	—	—	19.2	19.3	19.2
Apparent Y_{ATP}	—	7.83	8.04	8.27	8.27	8.27
Gamma ratio	0.946	—	—	—	—	—
H_2/CO_2	ND	0.38	0.44	0.82	0.82	0.82

[a]Refers to data used in the calculations.
ND refers to data not determined.

Tables IX and X present calculations for mixed-acid fermentations of *Escherichia coli*[23,29] to demonstrate the validity of the fermentation equation and the variable involvement of the HMPP. By comparing experimental values with calculated values with or without HMPP, it appears that in the fermentation of Table IX, a significant amount of CO_2 (ca. 12) is produced from HMPP, while in the fermentation of Table X some CO_2 is probably fixed through HMPP. Previous estimations[13] and HMPP responsible for 28% of glucose catabolism in *E. coli*. Such higher percentages of HMPP involvement are predicted for the two mixed-acid fermentations of *Escherichia aurescenes*,[29] as is shown in Tables XI and XII. Calculations without HMPP gave very poor results. Note also that an apparent ATP yield of 12 is calculated for the fermentation of Table XI and that for both fermentations, negative small values are calculated for c, indicating that the reaction of eq. (11) is operating in the reverse direction. Calculations for three more sets of mixed-acid fermentations of *E. aurescenes*[29] indicated a consistently high but variable (7–28%) involvement of HMPP in the catabolism of glucose. Butyrate production in the fermentation of Table XI was assumed to be through AcCoA as in butyric-acid bacteria.[1]

Calculations for butanediol fermentations of *Aerobacter indologenes*[30-32] indicated significant CO_2 fixation through HMPP. Calculations for a butanediol fermentation of *Erwinia carotovora*[33] indicated no involvement of the HMPP. Similarly for a butanediol fermentation of *Serratia kielensis*.[27] Calculations for a mixed-acid fermentation of *Photobacterium fischeri*[34] indicated substantial CO_2 fixation through HMPP. Finally, calculations for a mixed-acid fermentation of *Pseudomonas formicans*[35] indicated that only a small percentage of glucose is catabolized through HMPP producing CO_2.

In conclusion, fermentation eq. (34) appears valid for a wide variety of butanediol and mixed-acid fermentations. Calculations regarding the involvement of the HMPP were in agreement with the existing biochemical information.[13]

Uses of the Fermentation Equation

The validity of fermentation eq. (34) indicates that the assumptions made in the derivation of the equation, namely the assumed reactions of the fermentation biochemistry, the ATP yield and the biological regularities of eqs. (2) and (3) are valid. Given the excess amount of ATP produced in most fermentations, as shown in Tables I–XII, there is very little that can be said about the validity of the ATP yield. However, the other assumptions seem to be valid for many fermentations, with low and high recoveries of carbon in fermentation products, despite difficulties that would be expected from the fact that some nonglucose carbon from the complex media is incorporated into biomass and fermentation products.[1]

Equation (34) can be used to calculate maximal yields for the main products of the butanediol and mixed-acid fermentations.[1] Mathematically, the problem is to solve eq. (63) such that the amount of a given fermentation product [one of the unknowns of eq. (63)] is maximized, subject to the constraints imposed to the unknowns of eq. (63) [namely, eqs. (39)–(50) and any desired additional constraints]. This is the well-known linear programming problem,[36] and can be practically solved using available computer software packages or by intuitive trial and error

Table IX. Fermentation of glucose with *Escherichia coli*; data are from ref. 29.

	Amount (mol/100 mol glucose fermented)						
		Calculated					
Substance	Experimental	Without HMPP		With HMPP			
Carbon dioxide	88.0	88.0[a]	**78.7**	**85.6**	**86.4**	88.0[a]	88.0[a]
Formate	2.4	2.4[a]	2.4[a]	2.4[a]	2.4[a]	2.4[a]	2.4[a]
Acetoin	0.1	0.1[a]	0.1[a]	0.1[a]	0.1[a]	0.1[a]	0.1[a]
Ethanol	49.8	49.8[a]	**42.9**	49.8[a]	**50.7**	**49.9**	**51.7**
Lactate	79.5	**79.8**	79.5[a]	**80.4**	79.5[a]	79.5[a]	79.5[a]
Succinate	10.7	**5.9**	10.7[a]	10.7[a]	10.7[a]	10.7[a]	**9.8**
Acetate	36.5	**45.8**	**48.2**	36.5[a]	36.5[a]	36.5[a]	36.5[a]
Butanediol	0.3	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
Hydrogen	75.0	75.0[a]	75.0[a]	75.0[a]	75.0[a]	**79.7**	75.0[a]
Glycerol	1.4	1.4[a]	1.4[a]	1.4[a]	1.4[a]	1.4[a]	1.4[a]
Carbon recovery	91.7	91.7[a]	91.7[a]	91.7[a]	91.7[a]	91.7[a]	91.7[a]
$NADH_2$ from XH_2	—	12.3	2.9	−1.8	−1.0	−6.4	1.0
Excess ATP	—	110.4	117.6	102.0	102.0	101.3	101.2
CO_2 from HMPP	—	—	—	11.7	11.7	14.0	11.2
Apparent Y_{ATP}	—	5.54	5.37	5.74	5.74	5.76	5.76
Gamma ratio	0.996	—	—	—	—	—	—
H_2/CO_2	0.85	0.85	0.95	0.88	0.87	0.91	0.85

[a]Refers to data used in the calculations.

Table X. Fermentation of glucose with *Escherichia coli*; data are from ref. 23.

Substance	Experimental	Amount (mol/100 mol glucose fermented) Calculated Without HMPP			With HMPP		
Carbon dioxide	44.0	**46.0**	**46.9**	44.0[a]	**46.9**	**46.9**	44.0[a]
Formate	2.3	2.3[a]	2.3[a]	2.3[a]	2.3[a]	2.3[a]	2.3[a]
Acetoin	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Ethanol	43.0	43.0[a]	43.0[a]	43.0[a]	43.0[a]	43.0[a]	43.0[a]
Lactate	86.0	86.0[a]	**88.6**	86.0[a]	**84.7**	86.0[a]	86.0[a]
Succinate	31.0	**32.7**	31.0[a]	**33.8**	31.0[a]	31.0[a]	**30.8**
Acetate	43.0	**38.1**	**37.2**	**37.0**	43.0[a]	**41.1**	43.0[a]
Butanediol	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Hydrogen	41.0	41.0[a]	41.0[a]	**38.0**	41.0[a]	41.0[a]	**35.0**
Glycerol	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Carbon recovery	100.0	99.9[a]	99.9[a]	99.9[a]	99.9[a]	99.9[a]	99.9[a]
$NADH_2$ from XH_2	—	5.0	5.9	6.0	11.7	9.8	17.9
Excess ATP	—	269.1	266.5	269.1	274.3	271.7	275.1
CO_2 from HMPP	—	—	—	—	−5.81	−3.91	−8.93
Apparent Y_{ATP}	—	0.06	0.06	0.06	0.06	0.06	0.06
Gamma ratio	1.010	—	—	—	—	—	—
H_2/CO_2	0.93	0.89	0.87	0.86	0.87	0.87	0.80

[a]Refers to data used in the calculations.

Table XI. Fermentation of glucose with *Escherichia aurescens*; data are from ref. 29.

Substance	Experimental	Amount (mol/100 mol glucose fermented) Calculated (with HMPP)				
Carbon dioxide	106.2	**103.7**	**103.1**	**103.1**	**104.9**	106.2[a]
Formate	5.2	5.2[a]	5.2[a]	5.2[a]	5.2[a]	5.2[a]
Acetoin	0.2	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
Ethanol	56.9	**57.5**	56.9[a]	56.9[a]	56.9[a]	**60.0**
Lactate	52.7	52.7[a]	**53.3**	52.7[a]	52.7[a]	**50.2**
Succinate	10.6	10.6[a]	10.6[a]	10.6[a]	10.6[a]	10.6[a]
Acetate	37.3	37.3[a]	37.3[a]	**38.2**	37.3[a]	37.3[a]
Butanediol	0.7	0.7[a]	0.7[a]	0.7[a]	0.7[a]	0.7[a]
Hydrogen	90.9	90.9[a]	90.9[a]	90.9[a]	**94.6**	90.9[a]
Glycerol	0.3	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
Butyrate	0.2	0.2[a]	0.2[a]	0.2[a]	0.2[a]	0.2[a]
Carbon recovery	84.0	84.0[a]	84.0[a]	84.0[a]	84.0[a]	84.0[a]
$NADH_2$ from XH_2	—	−11.6	−12.2	−11.3	−15.9	−9.1
Excess ATP	—	−29.5	−29.5	−28.2	−30.0	−23.5
CO_2 from HMPP	—	22.73	22.73	21.82	24.58	22.73
Apparent Y_{ATP}	—	11.98	11.98	11.91	12.01	11.98
Gamma ratio	0.997	—	—	—	—	—
c, eqs. (11) and (34)[b]	—	−1.0	−1.6	−0.7	−5.3	1.5
H_2/CO_2	0.86	0.88	0.88	0.88	0.90	0.86

[a]Refers to data used in the calculations.
[b]This is normalized for 100 mol glucose.

for the present simple case. Some of the results are shown in Table XIII, for an assumed 95% carbon recovery in products.

According to our calculations, acetoin and butanediol production is not limited by the availability of reduction energy, and thus the employment of HMPP would not increase their maximal yields. If the reaction of eq. (11) can be assumed to operate in the reverse direction ($c < 0$), then 95 mol of either acetoin or butanediol can be produced from 100 mol glucose fermented (Table XIII).

Table XII. Fermentation of glucose with *Escherichia aurescens*; data are from ref. 29.

Substance	Amount (mol/100 mol glucose fermented)				
	Experimental	Calculated (with HMPP)			
Carbon dioxide	4.6	4.6[a]	**5.8**	4.6[a]	4.6[a]
Formate	85.0	**86.8**	85.0[a]	85.0[a]	**89.8**
Acetoin	0.6	0.6[a]	0.6[a]	0.6[a]	0.6[a]
Ethanol	42.7	**41.2**	**41.5**	**40.3**	42.7[a]
Lactate	64.2	64.2[a]	64.2[a]	**65.4**	**62.2**
Succinate	8.5	8.5[a]	8.5[a]	8.5[a]	8.5[a]
Acetate	36.5	36.5[a]	36.5[a]	36.5[a]	36.5[a]
Butanediol	0.7	0.7[a]	0.7[a]	0.7[a]	0.7[a]
Hydrogen	0.6	0.6[a]	0.6[a]	0.6[a]	0.6[a]
Glycerol	0.8	0.8[a]	0.8[a]	0.8[a]	0.8[a]
Carbon recovery	80.1	80.1[a]	80.1[a]	80.1[a]	80.1[a]
$NADH_2$ from XH_2	—	−18.2	−16.0	−17.3	−19.6
Excess ATP	—	−97.7	−97.4	−97.4	−98.2
CO_2 from HMPP	—	19.64	18.72	18.72	21.10
Apparent Y_{ATP}	—	15.68	15.65	15.65	15.72
Gamma ratio	1.010	—	—	—	—
c, eqs. (11) and (34)[b]	—	−9.7	−7.6	−8.8	−11.2
H_2/CO_2	0.13	0.13	0.11	0.13	0.13

[a]Refers to data used in the calculations.
[b]This is normalized for 100 mol glucose.

Table XIII. Theoretically maximal yields for primary products of glucose fermentations with bacteria of butanediol and mixed-acid fermentations. A 95% carbon recovery in products was assumed.

Substance	Amount (mol/100 mol glucose fermented) — Product of Maximum Yield										
	Without HMPP		Without HMPP		Without HMPP	With HMPP		Without HMPP	With HMPP	Without HMPP	
	Butanediol	Butanediol[b]	Acetoin	Acetoin[b]	Succinate	Succinate[b]	Succinate	Glycerol	Glycerol	Lactate	Formate
Carbon dioxide	190.0	190.0	190.0	190.0	0.0	0.0	0.0	64.8	72.7	2.2	0.0
Formate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**190.0**
Acetoin	0.0	0.0	**95.0**	**48.6**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethanol	0.0	60.4	0.0	92.8	45.3	39.5	0.0	0.0	0.0	0.0	92.8
Lactate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**187.8**	0.0
Succinate	0.0	0.0	0.0	0.0	**95.0**	**122.8**	**142.5**	0.0	0.0	0.0	0.0
Acetate	0.0	0.0	0.0	0.0	49.7	0.0	37.1	64.8	37.1	2.2	97.2
Butanediol	**95.0**	**64.8**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydrogen	90.6	60.4	185.6	92.8	0.0	39.4	138.1	0.0	0.0	0.0	0.0
Glycerol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**125.2**	**141.0**	0.0	0.0
Carbon recovery	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0
$NADH_2$ from XH_2	−90.6	0.0	−185.6	0.0	0.0	−122.6	−280.6	64.8	37.1	2.2	0.0
Excess ATP	115.8	115.8	115.8	115.8	260.4	210.8	210.8	55.3	0.0	117.9	212.9
Apparent Y_{ATP}	4.10	4.10	4.10	4.10	2.33	2.73	2.73	6.01	10.50	4.05	2.71
c, eqs. (11) and (34)[a]	−90.6	0.0	−185.6	0.0	95	0.0	−27.3	64.8	44.1	2.2	0.0
CO_2 from HMPP	—	—	—	—	—	83.3	142.5	—	35.6	—	—

[a]This is normalized for 100 mol glucose.
[b]This carries the additional requirement that c [eqs. (11) and (34)] is non-negative.

Otherwise, smaller maximal yields are calculated. Succinate production appears to be limited by the availability of CO_2 and thus employment of the HMPP would be beneficial. If a negative c value is permitted, up to 142 mol succinate can be produced from 100 mol glucose (Table XIII). Glycerol production appears to be limited by the availability of reduction energy and thus a higher yield is obtained with the HMPP. Similarly, lactate production could be slightly higher than the value shown in Table XIII, if the HMPP were employed in the calculations. If CO_2 fixation

through the HMPP is assumed, higher yields could be obtained for some of the products of Table XIII.

Equation (34) can be also used to check the consistency of experimental data, or detect a systematic error in a product measurement.[1] Assuming that a significant measurement error exists in the value of a product, if this value is used to calculate other product values, the agreement with the experimental values would be poor. If, however, correct experimental values are used for calculations, good agreement with experimental values would be obtained except, of course, for the product whose experimental value contains the measurement error. That was the case with the H_2 values in Table II and also Table XIV which presents calculations for a butanediol fermentation of *Serratia plymuthicum*.[27] Calculations with HMPP indicate also a possible error in the H_2 value, and since essentially no CO_2 is calculated to be produced from HMPP, implying no HMPP involvement, these calculations are not shown in Table XIV. Calculations for three more sets of butanediol fermentation data with *S. plymuthicum*[27] have indicated a small and variable amount of CO_2 (0–7.5) to be produced through HMPP. All calculations have indicated an error in the experimental values of hydrogen. In the butanediol fermentation of Table XV, the measurement error appears to be in the CO_2 value, which when used in the calculations of the last three columns of Table XV produce values in poor agreement with experimental ones.

The use of fermentation equations as "gateway sensors" for on-line monitoring of fermentation processes has been discussed in detail earlier.[1] The fermentation equation is most useful for such applications in the form of eq. (63). We should point out here that it is possible to use eq. (63) in order to calculate the "best" values of certain desired fermentation parameters (according to the least-squares method, for example) by employing more experimental data values than the minimum number required for the solution of eq. (63). For example, let's assume that we want to calculate the "best" values of N (where $N < 15$) of the 24 unknowns of eq. (63), using the experimental values of the remaining (24-N) unknowns. Let $\mathbf{x}$ be the column vector of these N unknowns. Then we can rewrite eq. (63) as follows:

$$\mathbf{Ax} = \mathbf{y} \tag{66}$$

where $\mathbf{A}$ is a $15 \times N$ known matrix and $\mathbf{y}$ a known vector with 15 components containing experimental data. Equation (66) is a set of 15 equations with N unknowns. In general, because of experimental errors, eq. (66) is a set of inconsistent equations. That is, it is not generally possible to find $\mathbf{x}$ such that eq. (66) is satisfied. If the vector $\mathbf{r}$ of the residuals resulting from the experimental errors is then added to eq. (66), we obtain the more precise relationship,

$$\mathbf{Ax} = \mathbf{y} + \mathbf{r} \tag{67}$$

The least-squares method[36] computes the best value of $\mathbf{x}$ by minimizing the sum of squares of the residuals, $\mathbf{r}^T\mathbf{r} = (\mathbf{y} - \mathbf{Ax})^T(\mathbf{y} - \mathbf{Ax})$. Thus, the best value of $\mathbf{x}$ is the solution of the problem[36],

$$\mathbf{A}^T\mathbf{Ax} = \mathbf{A}^T\mathbf{y} \tag{68}$$

Finally, fermentation equations may be useful in the classification of microorganisms by checking, on the basis of reliable experimental data, if the microorganism satis-

Table XIV. Fermentation of glucose with *Serratia plymuthicum*; data are from ref. 27.

	Amount (mol/100 mol glucose fermented)						
		Calculated (without HMPP)					
Substance	Experimental 1	2	3	4	5	6	7
Carbon dioxide	145.2	**144.9**	145.2[a]	145.2[a]	**162.8**	145.2[a]	145.2[a]
Formate	3.3	3.3[a]	3.3[a]	3.3[a]	3.3[a]	3.3[a]	3.3[a]
Acetoin	3.6	3.6[a]	3.6[a]	3.6[a]	3.6[a]	3.6[a]	3.6[a]
Ethanol	50.5	50.5[a]	**51.0**	**50.8**	50.5[a]	**32.9**	50.5[a]
Lactate	33.9	33.9[a]	**33.6**	33.9[a]	**16.0**	**33.6**	**68.8**
Succinate	7.1	7.1[a]	7.1[a]	**6.9**	7.1[a]	7.1[a]	**−10.5**
Acetate	4.5	4.5[a]	4.5[a]	4.5[a]	4.5[a]	4.5[a]	4.5[a]
Butanediol	46.5	**46.6**	46.5[a]	46.5[a]	**55.5**	**55.5**	**37.9**
Hydrogen	58.7	**49.8**	**49.7**	**49.8**	58.7[a]	58.7[a]	58.7[a]
Glycerol	1.7	1.7[a]	1.7[a]	1.7[a]	1.7[a]	1.7[a]	1.7[a]
Carbon recovery	99.0	99.0[a]	99.0[a]	99.0[a]	99.0[a]	99.0[a]	99.0[a]
$NADH_2$ from XH_2	—	−5.2	−4.6	−4.7	−14.1	−31.6	3.5
Excess ATP	—	193.0	193.0	192.9	193.0	193.0	175.5
Apparent Y_{ATP}	—	0.75	0.75	0.75	0.75	0.75	0.82
Gamma ratio	1.010	—	—	—	—	—	—
H_2/CO_2	0.40	0.34	0.34	0.34	0.36	0.40	0.40

[a]Refers to data used in the calculations.

Table XV. Fermentation of glucose with *Serratia marcescens*; data are from ref. 27.

	Amount (mol/100 mol glucose fermented)					
Substance	Experimental	Calculated (with HMPP)				
Carbon dioxide	103.8	**107.5**	**107.8**	103.8[a]	103.8[a]	103.8[a]
Formate	48.5	48.5[a]	48.5[a]	48.5[a]	**54.1**	**59.6**
Acetoin	0.3	0.3[a]	0.3[a]	0.3[a]	0.3[a]	0.3[a]
Ethanol	40.8	**39.0**	40.8[a]	**18.6**	**38.1**	40.8[a]
Lactate	15.7	15.7[a]	15.7[a]	15.7[a]	15.7[a]	**12.0**
Succinate	3.0	3.0[a]	3.0[a]	3.0[a]	3.0[a]	3.0[a]
Acetate	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Butanediol	57.9	57.9[a]	**56.9**	**69.0**	57.9[a]	57.9[a]
Hydrogen	0.0	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Glycerol	6.1	6.1[a]	6.1[a]	6.1[a]	6.1[a]	6.1[a]
Carbon recovery	90.7	90.7[a]	90.7[a]	90.7[a]	90.7[a]	90.7[a]
$NADH_2$ from XH_2	—	−12.5	−10.6	−32.8	−19.0	−21.8
Excess ATP	—	38.4	38.2	40.2	37.5	36.5
CO_2 from HMPP	—	3.69	4.20	−1.86	6.47	9.25
Apparent Y_{ATP}	—	8.22	8.23	8.13	8.26	8.30
Gamma ratio	1.010	—	—	—	—	—
c, eqs. (11) and (34)[b]	—	−9.5	−7.7	−29.9	−16.0	−18.8

[a]Refers to data used in the calculations.
[b]This is normalized for 100 mol glucose.

fies the equation of a given class of microorganisms, and calculating the extent to which it uses a given pathway or reaction.

DISCUSSION

Fermentation eq. (34) and its variants are expected to be valid for completed batch, steady-state and transient fermentations.[1] Improvements could result from employing more accurate values of ATP yields, by accounting for the complex carbon incorporated into biomass and products, by further elucidation of the fermentation biochemistry, and by incorporating maintenance requirements for uses in continuous fermentations. Although some of these improvements may result in loss of generality, this loss can be compensated for by the improved predictive capabilities of the equation, for on-line monitoring and control of fermentations.

The derivation procedure of the fermentation eq. (34) may give the false impression that the equation satisfies only the stoichiometric or topological requirements of the biochemistry of growth and product formation. This is not true, however. The derivation procedure also takes into account the energetic requirements of growth and product formation by considering the ATP and reduction energy requirements of individual reactions or assemblies of biochemical reactions. In fact, the ATP and reduction-energy yields or requirements of such single or assemblies of reactions is a much more accurate index of the amount of biologically useful energy that may be produced or required than the net free-energy change.[1,16,20,21] A comparison of eqs. (7) and (8), for example, shows the large differences in the efficiency of production of biosynthetic energy by the two routes of the same overall reaction. Since it is also indeed quite probable that anaerobic (but also aerobic[37]) fermentations may hydrolyze a significant amount of ATP without performing any significant physiological function, conservation of biosynthetic or free energy may not be necessarily the goal or basis of operation for biological growth. As such, ATP conservation, ATP yields, and maintenance requirements may only poorly characterize such fermentations.

As a final note, we want to emphasize that eq. (34) is independent of and says nothing about the rates of production of biomass and fermentation products, or the interaction between biomass growth and product formation according to some particular kinetics. There is, in fact, plenty of experimental evidence to indicate that such an interaction does not necessarily follow any particular kinetics in such multiproduct fermentations.

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NOMENCLATURE

a,b,c,d,e,f g,h,i,j,k,l,n	stoichiometric coefficients or unknowns, in eqs. (34) and (63)
A	the 15 × N matrix of eq. (66)
AcCoA	acetyl-CoA
B	constant in eq. (9)
ED	Entner-Doudoroff (pathway)

EMP	Embden-Meyerhof-Parnas (pathway)
$FADH_2$	reduced form of flavin adenine dinucleotide
FdH_2	reduced form of ferredoxin
$FMNH_2$	reduced form of flavin mononucleotide
F6P	fructose-6-phosphate
G3P	glyceraldehyde-3-phosphate
G6P	glucose-6-phosphate
HMP, HMPP	hexose monophosphate (pathway)
$\mathbf{L}$	the matrix of eq. (63)
$\mathbf{L}_A$	matrix in eq. (64)
$\mathbf{L}_Y$	matrix in eq. (64)
PEP	phosphoenolpyruvate
PRCP	percentage of substrate carbon recovered in products; in eq. (51)
$\mathbf{r}$	the 15-component vector of residuals in eq. (67)
$\mathbf{u}$	the unknown vector in eq. (63)
$\mathbf{v}$	the known vector in eq. (63)
$\mathbf{x}$	the N-component column vector of eq. (66)
X_i	mol species i produced/100 mol glucose fermented
XH_2	$FADH_2$ or $FMNH_2$ or both, in eq. (9)
$\mathbf{y}$	the 15-component column vector of eq. (66)
Y_{ATP}	ATP yield, g dry biomass/mol ATP utilized in biomass synthesis
Y_i	mol species i
YH_2	electron carrier of reaction (5) in Figure 1
α	the variable defined in eq. (52)
β	constant in eq. (30)
γ_b	reductance degree of biomass; eq. (3)
γ_i	reductance degree of species i; eq. (65)
λ_{ij}	the elements of matrix $\mathbf{L}_Y$
σ_b	weight fraction of carbon in biomass; eq. (2)

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