

Fermentation Equations for Propionic-Acid Bacteria and Production of Assorted Oxychemicals from Various Sugars

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Fermentation (stoichiometric) equations are derived for anaerobic fermentations of propionic-acid bacteria (of both the *Propionibacterium* and acrylate pathways) and for production of various oxychemicals (butanol, acetone, isopropanol, butanediol, butyrate, acetate, propionate, succinate, lactate, and acrylate) from pentoses, hexoses, and cellobiose. The derivations of the equations are based on the fermentation biochemistries of the various bacterial classes. The validity of the equations is tested using fermentation data from the literature. The equations are shown to be valuable, among other uses, for calculating maximal yields and selectivities of the various fermentation products, as "gateway sensors" for monitoring of the fermentations, and for calculating the extents of the various intracellular reactions of the fermentation biochemistry.

INTRODUCTION

Fermentations of propionic-acid bacteria are of potential industrial importance for production of a number of carboxylic acids (malic, fumaric, succinic, propionic, acrylic) which are typical end products or key intermediates of these fermentations.¹⁻³ Since few quantitative studies have been published on these fermentations, little is known about expected optimal yields and selectivities of the various products. The objective of this work is to derive fermentation equations^{4,5} for propionic-acid bacteria on the basis of existing information on their biochemistry and some necessary additional assumptions, and to test these equations using literature data. Since the validity of these equations essentially confirms the assumptions made for the biochemistry and the bioenergetics of the fermentations,^{4,5} the equations can be then used for calculating maximal product yields and selectivities for the various products.

Many bacteria of the butyric-acid, butanediol, mixed-acid, and propionic-acid fermentations can utilize various pentoses, hexoses, and cellobiose for production of assorted oxychemicals. In view of the existing interest to fully utilize all the sugars produced from biomass hydroly-

sis,^{6,7} we shall derive, test, and use fermentation equations for sugars other than glucose to calculate maximal yields and selectivities for the products of the aforementioned fermentations. Other uses of the fermentation equations have been discussed in detail earlier.^{4,5}

A fermentation equation is a stoichiometric equation which is derived on the basis of, and is thus satisfying, the biochemistry and bioenergetics of the fermentation, and which, by definition, provides the maximum information on the interrelations among the amounts of products and biomass produced and substrates utilized.^{4,5} The procedures for the derivation and testing of fermentation equations have been detailed previously.^{4,5}

This article has been subdivided in sections as follows: "Biochemistry of Propionic-Acid Fermentations," "Equations for Propionic-Acid Fermentations," "Calculations for Propionic-Acid Fermentations," "Biochemistry and Equations for Fermentations of Sugars Other than Glucose," "Calculations for Fermentations of Sugars Other than Glucose," and "Discussion and Conclusions."

BIOCHEMISTRY OF PROPIONIC-ACID FERMENTATIONS

Drawn on the basis of the most recent available information,^{2,8-13} Figure 1 presents the most probable sequence of enzymatic reactions leading to the formation of propionate, succinate, acetate, and lactate as primary products of glucose fermentation by propionibacteria. Lactate and pyruvate may also be fermented as substrates, while malate, fumarate and pyruvate may accumulate as products under proper fermentation conditions. Propionibacteria possess most or some of the enzymes of the Hexose Monophosphate pathway (HMPP),¹⁰ and are thus expected to catabolize glucose by a combination of the Embden-Meyerhof-Parnas (EMP) pathway and HMPP. The degree of involvement of each pathway is not known. Reactions 15 and 16 (Fig. 1), which interconvert the various forms of reduction energy, are crucial to the deriva-

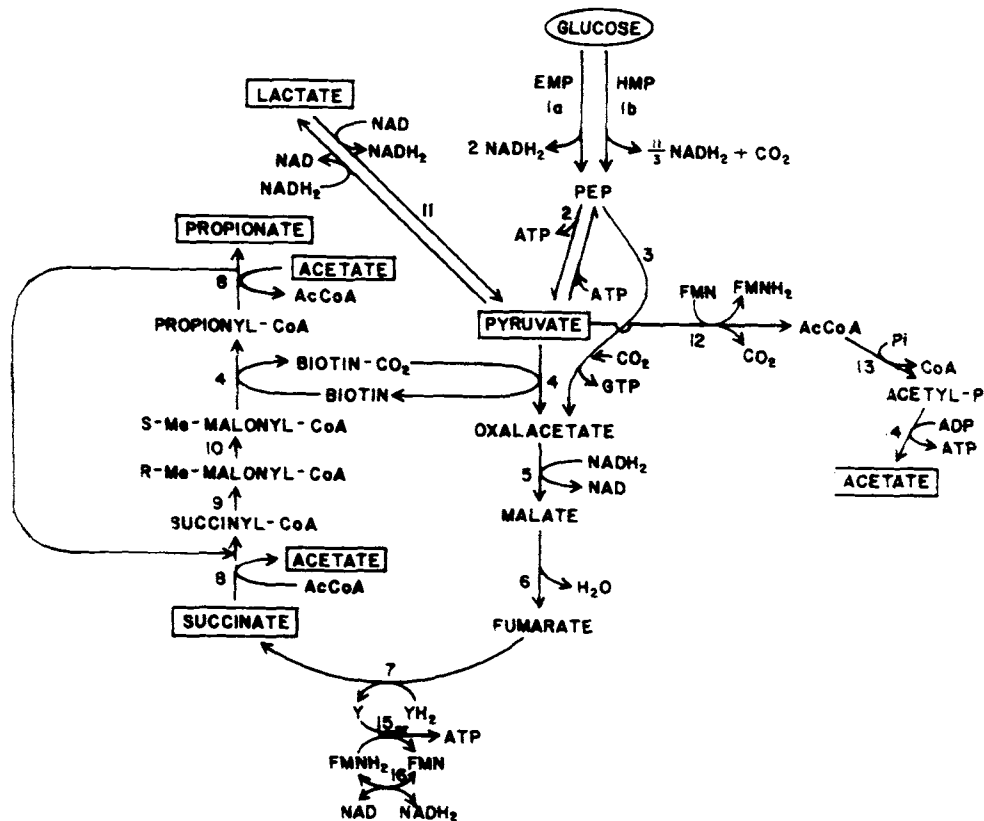


Figure 1. Biochemical pathways of glucose fermentation by *Propionibacterium* and related species. The pathways or enzyme systems for the reactions shown are as follows: (1a) phosphoenolpyruvate phosphotransferase system and the Embden-Meyerhof-Parnas pathway, (1b) phosphoenolpyruvate phosphotransferase system and the Hexose Monophosphate pathway, (2) ATP: pyruvate phosphotransferase, (3) PEP carboxylase (GDP specific), (4) (S)-methylmalonyl-CoA-pyruvate transcarboxylase, (5) malate dehydrogenase, (6) fumarate hydratase, (7) succinate dehydrogenase, (8) CoA transferase, (9) (R)-methylmalonyl-CoA mutase, (10) methylmalonyl-CoA racemase, (11) lactate dehydrogenase, (12) pyruvate-flavin oxidoreductase, (13) phosphotransacetylase, (14) acetate kinase, (15) YH_2 -FMN oxidoreductase (ATP producing), and (16) NADH-FMN oxidoreductase.

tion of the fermentation equation and have been discussed in detail previously.⁵ Since the conversion of pyruvate to oxalacetate is coupled to the formation of propionate (reaction 4 of Fig. 1), when succinate accumulates, its formation must necessarily proceed directly through phosphoenolpyruvate (PEP) and oxalacetate (reactions 3, 5, 6, and 7 of Fig. 1).

Figure 2 presents, according to the most recent available information,^{3,8-10} the acrylate pathway of propionate and acetate formation from pyruvate, lactate and acrylate. Lactate and pyruvate may also accumulate as products. The acrylate pathway is known to occur only in *Clostridium propionicum* and *Megasphaera (Peptostreptococcus) elsdenii*.^{9,10} The electron carrier (Y/YH_2) of reaction 7 (Fig. 2) is probably a flavoprotein which can be reduced in principle by either $NADH_2$ or FdH_2 . The reduction of ferredoxin by $NADH_2$ and vice versa has been discussed previously.⁴ Finally, only indirect evidence exists^{3,8-10} for reaction 6 of Figure 2.

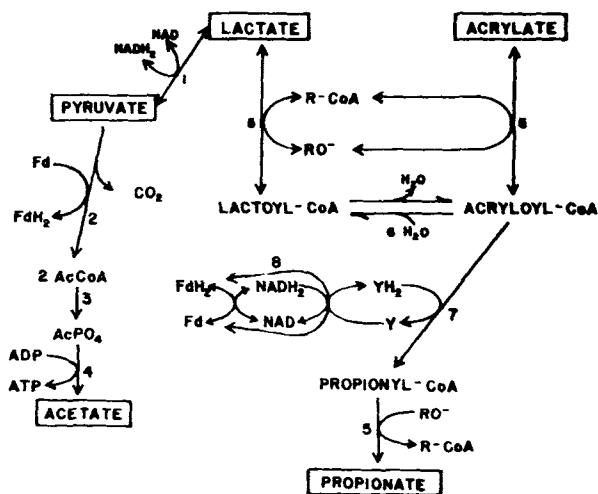


Figure 2. The acrylate pathway of propionic-acid bacteria. The enzymes for the reactions shown are as follows: (1) lactate dehydrogenase, (2) pyruvate-ferredoxin (or flavodoxin) oxidoreductase, (3) phosphotransacetylase, (4) acetate kinase, (5) CoA transferase, (6) lactoyl-CoA dehydratase, (7) acyl-CoA dehydrogenase, and (8) YH_2 -ferredoxin-NAD oxidoreductases.

EQUATIONS FOR PROPIONIC-ACID FERMENTATIONS

The fermentation equation is derived by summing all the enzymatic reactions which lead to the formation of biomass and the fermentation products from the carbon substrate and the other growth nutrients.^{4,5} Then, by requiring conservation of the intermediate species (e.g., pyruvate, acetyl-CoA) and the reduction energy, we can obtain algebraic relations for the amounts of biomass and products formed and substrate(s) utilized.^{4,5} The biosynthetic requirements of biological growth must, of course, be satisfied. Conservation of biosynthetic energy (ATP), however, cannot be easily implemented because (1) there are a number of transport or membrane processes requiring or synthesizing ATP, and (2) ATP may be hydrolyzed without performing any significant physiological function.^{4,5}

Since propionibacteria can grow on a number of sugars, pyruvate, and glycerol, the equation for biomass production in this case will be written on pyruvate as follows,^{4,5}

$$4(\text{pyruvate}) + 5.75\text{NADH}_2 + 33.7\text{ATP} = 3\text{C}_4\text{H}_{4p}\text{O}_{4n}\text{N}_{4q} \quad (1)$$

where $\text{C}_4\text{H}_{4p}\text{O}_{4n}\text{N}_{4q}$ represents the elemental composition of biomass. Equation (1) was derived assuming, (1) an ATP yield $Y_{\text{ATP}}^G = 10.5$ for growth on glucose, (2) two important biological regularities pertaining to the degree of reductance and carbon content of biomass, and (3) that pyruvate is formed from glucose through the EMP pathway.^{4,5} The last assumption combined with the assumed $Y_{\text{ATP}}^G = 10.5$ is then equivalent to assuming that the ATP yield for growth on pyruvate is $Y_{\text{ATP}}^P = 9.25$. Either of the two ATP-yield values are very conservative and in fact represent average values expected for growth on totally synthetic media. All the fermentation data that will be used subsequently have been obtained for fermentations on complex media where Y_{ATP}^G values closer to the theoretical 28.8 value can be expected.^{14,15} For simplicity, we have omitted the nitrogen source in the left-hand side of eq. (1), and also ADP and NAD in the right-hand side, and have made no attempt at this stage to balance the oxygen and hydrogen atoms.^{4,5} These simplifying rules will be followed in all the following equations. Finally, in writing eq. (1) we have assumed that little CO_2 is produced for biomass synthesis from general decarboxylation enzymes not shown in Figure 1.

Glycerol metabolism apparently proceeds via dihydroxyacetone and dihydroxyacetone phosphate,⁸ and thus the overall reaction for formation of pyruvate from glycerol, using some portion of the EMP pathway, can be represented by,

$$\text{glycerol} = \text{pyruvate} + \text{ATP} + 2\text{NADH}_2 \quad (2)$$

Pyruvate formation from glucose through the EMP pathway (reaction string 1a of Fig. 1) can be represented by^{4,5}

$$\text{glucose} = 2(\text{pyruvate}) + 2\text{NADH}_2 + 2\text{ATP} \quad (3a)$$

When the HMPP (reaction string 1b of Fig. 1) is employed, the overall reaction can be represented by⁵

$$3(\text{glucose}) = 5(\text{pyruvate}) + 3\text{CO}_2 + 11\text{NADH}_2 + 5\text{ATP} \quad (3b)$$

We have shown previously⁵ that eq. (3b) can also be used in the reverse direction, combined with eq. (3a), to represent CO_2 fixation through the HMPP. Our calculations, which are presented below, have indicated that this may indeed be happening in some *Propionibacterium* fermentations.

Production of propionate from pyruvate (reactions 4-10 and 15 of Fig. 1) can be represented by,

$$\text{pyruvate} + \text{NADH}_2 + \text{FMNH}_2 = \text{propionate} + \text{ATP} \quad (4)$$

while production of succinate from PEP (reactions 3, 5-7, and 15 of Fig. 1), in view of reaction 2 of Figure 1, can be represented by

$$\text{pyruvate} + \text{CO}_2 + \text{NADH}_2 + \text{FMNH}_2 = \text{succinate} + \text{ATP} \quad (5)$$

where we have taken GTP to be equivalent to ATP for biosynthetic purposes.⁸ The formation of lactate from pyruvate and vice versa can be represented by

$$\text{lactate} = \text{pyruvate} + \text{NADH}_2 \quad (6)$$

and for acetate formation from pyruvate (reactions 12, 13, and 14 of Fig. 1), we write

$$\text{pyruvate} = \text{acetate} + \text{ATP} + \text{FMNH}_2 + \text{CO}_2 \quad (7)$$

Finally, reaction 16 of Figure 1 can be represented by

$$\text{NADH}_2 = \text{FMNH}_2 \quad (8)$$

Next, we multiply eqs. (2)-(8) by $a, b_1, b_2, c, d, e, f,$ and $g,$ respectively, and add them together along with eq. (1) to obtain

$$\begin{aligned} & 3\text{C}_4\text{H}_{4p}\text{O}_{4n}\text{N}_{4q} - (b_1 + 3b_2)\text{glucose} \\ & - a(\text{glycerol}) - e(\text{lactate}) \\ & + (2b_1 + 5b_2 - c - d + e - f + a - 4)\text{pyruvate} \\ & + (2b_1 + 11b_2 - c - d + e - g + 2a - 5.75)\text{NADH}_2 \\ & + (f + g - c - d)\text{FMNH}_2 \\ & + (2b_1 + 5b_2 + c + d + f + a - 33.7)\text{ATP} \\ & + (3b_2 + f - d)\text{CO}_2 + c(\text{propionate}) \\ & + d(\text{succinate}) + f(\text{acetate}) = 0 \end{aligned} \quad (9)$$

Requiring conservation of NADH_2 and FMNH_2 , we obtain, respectively,

$$2b_1 + 11b_2 - c - d + e - g + 2a - 5.75 = 0 \quad (10a)$$

$$f + g - c - d = 0 \quad (10b)$$

The coefficient of pyruvate is negative if pyruvate is a substrate, or non-negative if it is a product, namely,

$$2b_1 + 5b_2 - c - d + e - f + a - 4 < 0$$

(if pyruvate is a substrate) (11a)

$$2b_1 + 5b_2 - c - d + e - f + a - 4 \geq 0$$

(if pyruvate is a product) (11b)

The coefficient of glucose is negative if glucose is a substrate, or zero if it is not,

$$-(b_1 + 3b_2) \leq 0 \quad (12)$$

The coefficients of glycerol, lactate, and CO₂ are negative if they are used as substrates,

$$-a < 0 \quad (\text{if glycerol is a substrate}) \quad (13a)$$

$$-e < 0 \quad (\text{if lactate is a substrate}) \quad (14a)$$

$$3b_2 + f - d < 0 \quad (\text{if CO}_2 \text{ is fixed}) \quad (15a)$$

or non-negative if they are products,

$$-a \geq 0 \quad (\text{if glycerol is a product}) \quad (13b)$$

$$-e \geq 0 \quad (\text{if lactate is a product}) \quad (14b)$$

$$3b_2 + f - d \geq 0 \quad (\text{if CO}_2 \text{ is a product}) \quad (15b)$$

The coefficients of ATP, propionate, succinate, and acetate must be non-negative,

$$2b_1 + 5b_2 + c + d + f + a - 33.7 > 0 \quad (16)$$

$$c, d, f \geq 0 \quad (17)-(19)$$

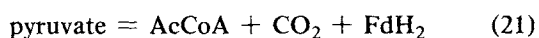
Since the overall reaction represented by eq. (3a) can take place only in the forward direction, we must also have,

$$b_1 \geq 0 \quad (20)$$

Finally, b_2 and g can be positive, negative, or zero. Equation (9) with its accompanying equality and inequality conditions (10)-(20) is what we shall call the fermentation equation for fermentations of the *Propionibacterium* pathway.

For the derivation of the fermentation equation for the acrylate pathway, we shall assume, without loss of generality, that pyruvate can be formed from glucose through the EMP pathway, and thus use eq. (3a). Although no known microorganism which employs the acrylate pathway can catabolize glucose or other sugars, it is conceivable that such microorganisms can be either isolated or produced by some type of gene transfer. We shall use the fermentation equation to examine such possibilities at the theoretical level. Equation (3a) was chosen instead of eq. (3b) because *Clostridia* typically utilize the EMP pathway exclusively.

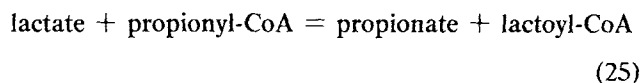
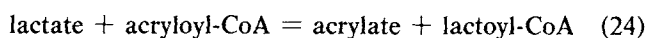
Production of lactate from pyruvate and vice versa can be represented by eq. (6). For the formation of acetyl-CoA (AcCoA) from pyruvate (reaction 2 of Fig. 2), we write



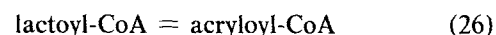
Acetate formation for AcCoA (reactions 3 and 4 of Fig. 2) can be represented by



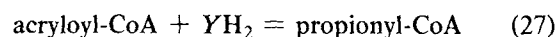
The CoA transferase reactions (reactions 5 of Fig. 2) for the formation of lactoyl-CoA can be represented by,



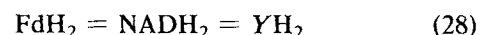
All the rest of the CoA transferase reactions can be obtained by combining pairs of eqs. (23), (24), and (25). For reaction 6 of Figure 2, we write



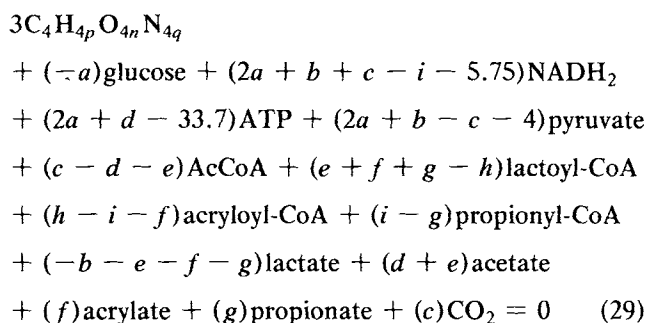
and reaction 7 of Figure 2 can be represented by



Finally for the electron-transfer reactions (step 8 of Fig. 2), we write,



For simplicity, in view of eq. (28), we shall use NADH₂ as the only carrier of reduction energy in all reactions above. If we now multiply reactions (3a), (6), and (21)-(27) by a , b , c , d , e , f , g , h , and i , respectively, and add them together along with eq. (1), we obtain



From the conservation of NADH₂, AcCoA, lactoyl-CoA, acryloyl-CoA, and propionyl-CoA, we obtain, respectively

$$2a + b + c - i - 5.75 = 0 \quad (30)$$

$$c - d - e = 0 \quad (31)$$

$$e + f + g - h = 0 \quad (32)$$

$$h - i - f = 0 \quad (33)$$

$$i - g = 0 \quad (34)$$

The rest of the species of eq. (29) must have negative coefficients if they are substrates, positive coefficients if products, or zero coefficients if they are neither substrates nor accumulating products. Thus, we have

$$-a \leq 0 \quad (35)$$

$$2a + b - c - 4 \leq 0 \quad (36)$$

$$-b - e - f - g \leq 0 \quad (37a)$$

(if lactate is a substrate)

$$-b - e - f - g \geq 0 \quad (37b)$$

(if lactate is a product)

$$d + e \geq 0 \quad (38)$$

$$f \leq 0 \quad (\text{if acrylate is a substrate}) \quad (39a)$$

$$f \geq 0 \quad (\text{if acrylate is a product}) \quad (39b)$$

$$g \geq 0 \quad (40)$$

$$c \geq 0 \quad (41)$$

and, as we have discussed above, for the coefficient of ATP we must have

$$2a + d - 33.7 \geq 0 \quad (42)$$

If, however, the ATP yield is higher than the assumed 10.5 value, but less than the maximal value of 28.8, the coefficient of ATP may have an apparently negative value, which will be understood to imply a higher value for the actual ATP yield. Equation (29), accompanied by the equality and inequality conditions eqs. (30)–(42), is the fermentation equation for the acrylate pathway.

All the fermentation equations that have been derived in this section and that will be derived subsequently, can be shown⁴ to contain both the carbon and available electron balances. Obviously, they also provide considerably more information, in the form of additional equations,⁵ than the above two balances.

CALCULATIONS FOR PROPIONIC-ACID FERMENTATIONS

The calculation procedure involves the reformulation of each fermentation equation into a system of linear algebraic equations and the solution of that system using the least possible information from experimental data and a

Gauss elimination numerical scheme.⁵ A number of implicit assumptions and clarifications that are useful in the interpretation of the calculated figures have been discussed in detail earlier.⁵ The gamma ratio, the ratio of degree of reductance of the fermentation products and biomass to that of the substrates is an indication of the data consistency when carefully interpreted,⁵ and is shown in the experimental data columns of the following tables. Note that in all calculations according to the fermentation equations, the gamma ratio is one (1) and is thus not shown in the calculation columns of the following tables.

Complete experimental data on propionic-acid fermentations are considerably more scarce than butanol, butanediol, and mixed-acid fermentation data,^{4,5} and they date back from 1928 to 1961. Since good accuracy was not always the objective of or necessary for the reported fermentation data, and in view of the complex analytical procedures that were employed, some of them may contain large experimental errors and should therefore be used with care. Nevertheless, we found that most of the available data obeyed the fermentation equations, as is demonstrated by the following sample of calculations.

Table I presents data and calculations for a glucose fermentation by *Propionibacterium arabinosum*.¹⁶ The first two sets of calculations shown were performed assuming that the HMPP does not operate, and gave values in poor agreement with the experimental data. The last three sets of calculations were performed with the HMPP operating in conjunction with the EMP pathway and gave values in good to excellent agreement with experimental ones. The calculations indicate that 60% of the glucose was catabolized through the HMPP.

Table II shows data and calculations for a glucose fermentation by *Propionibacterium pentosaceum*.¹⁶ Calculations with and without the HMPP are presented which indicate that the HMPP is responsible again for 60% of the glucose catabolism. All of our calculations for glucose

Table I. Fermentation of glucose with *Propionibacterium arabinosum*; data are from ref. 16.

Substance	Amount (mol/100 mol C ₃ fermented)						
	Experimental	Calculated					
		Without HMPP		With HMPP			
Propionate	69.6	55.9	61.9	69.6 ^a	69.6 ^a	69.3	68.7
Acetate	8.9	32.4	32.4	8.4	8.9 ^a	8.9 ^a	8.9 ^a
Carbon dioxide	31.8	25.8	31.8 ^a	32.6	32.6	32.5	31.8 ^a
Succinate	6.6	6.6 ^a	0.6	6.6 ^a	6.3	6.6 ^a	7.3
Carbon recovery	94.9	94.9 ^a	94.9 ^a	94.9 ^a	94.9 ^a	94.9 ^a	94.9 ^a
NADH ₂ from FMNH ₂	—	−30.1	−30.1	−67.8	−67.0	−67.0	−67.0
Excess ATP	—	151.9	151.9	131.4	131.8	131.8	131.8
CO ₂ from HMPP	—	—	—	30.8	30.2	30.2	30.2
Apparent Y _{ATP}	—	2.04	2.04	3.23	3.19	3.19	3.19
Gamma ratio	1.000	—	—	—	—	—	—

^a Refers to data used in the calculations.

fermentations by *P. arabinosum*, *P. pentosaceum*, and *P. shermanii*¹⁶ have indicated that the HMPP is responsible for 24–60% of the glucose catabolism. The percent of the HMPP involvement in the glucose catabolism varied also among different fermentations of the same strain. *Propionibacterium petersonii*¹⁶ was, however, an exception, as the calculations of Table III indicate, in that it appeared to employ the HMPP in a very small extent (12%). Accordingly, calculations without the HMPP gave results also in good agreement with experimental values. Calculations for another glucose fermentation by *P. petersonii* indicated an even smaller (10%) involvement of the HMPP.

Calculations for a glycerol fermentation by *P. pentosaceum*¹⁰ are shown in Table IV. The calculations may indicate a small CO₂ fixation through the HMPP, however that small an amount (4 mol CO₂/100 mol glycerol fermented) could be entirely due to small errors in the data used in the calculations. Note that in this fermentation

CO₂ is utilized as a cosubstrate of glycerol. Employment of the HMPP for fermentations with substrates other than glucose is indeed possible by the reactions of gluconeogenesis as is discussed in detail below.

Cytophaga succinicans and *Micrococcus lactilyticus* are believed to employ the *Propionibacterium* pathway, as well.¹⁰ Formate and/or hydrogen are also formed. For our calculations, we have assumed that formate and hydrogen can be formed from pyruvate by lyases as in the butanediol and mixed-acid fermentations.⁵ Hydrogen could be also formed by a hydrogenase.⁴ Both mechanisms, however, give the same results. Table V presents data and calculations for a glucose fermentation by *C. succinicans*.¹⁷ CO₂ is also used as a cosubstrate. No HMPP involvement is indicated, since essentially no CO₂ is calculated to be produced through the HMPP. Similarly, calculations for a fermentation of lactate by *M. lactilyticus*¹⁸ indicated no HMPP involvement.

In conclusion, the validity of the fermentation equation

Table II. Fermentation of glucose with *Propionibacterium pentosaceum*; data are from ref. 16.

Substance	Amount (mol/100 mol C ₃ fermented)						
	Experimental	Calculated					
		Without HMPP			With HMPP		
Propionate	63.9	49.8	63.9 ^a	63.3	61.4	63.9 ^a	63.9 ^a
Acetate	8.3	31.9	31.9	8.3 ^a	8.3 ^a	8.3 ^a	7.2
Carbon dioxide	26.2	21.4	35.4	28.1	26.2^a	28.7	28.4
Succinate	10.5	10.5 ^a	-3.6	10.5 ^a	12.4	9.9	10.5 ^a
Carbon recovery	92.2	92.2 ^a	92.2 ^a	92.2 ^a	92.2 ^a	92.2 ^a	92.2 ^a
NADH ₂ from FMNH ₂	—	-28.5	-28.5	-65.5	-65.5	-65.5	-67.2
Excess ATP	—	126.5	126.5	106.3	106.3	106.3	105.4
CO ₂ from HMPP	—	—	—	30.3	30.3	30.3	31.7
Apparent Y _{ATP}	—	3.16	3.16	5.00	5.00	5.00	5.14
Gamma ratio	1.010	—	—	—	—	—	—

^aRefers to data used in the calculations.

Table III. Fermentation of glucose with *Propionibacterium petersonii*; data are from ref. 16.

Substance	Amount (mol/100 mol C ₃ fermented)						
	Experimental	Calculated					
		Without HMPP			With HMPP		
Propionate	57.4	54.3	54.1	57.0	57.4 ^a	57.4 ^a	55.5
Acetate	27.0	31.8	31.8	27.0 ^a	26.4	27.0 ^a	27.0 ^a
Carbon dioxide	25.8	25.9	25.8 ^a	27.3	27.5	27.7	25.8 ^a
Succinate	5.9	5.9 ^a	6.1	5.9 ^a	5.9 ^a	5.5	7.5
Carbon recovery	92.0	92.0 ^a	92.0 ^a	92.0 ^a	92.0 ^a	92.0 ^a	92.0 ^a
NADH ₂ from FMNH ₂	—	-28.3	-28.3	-35.9	-36.9	-35.9	-35.9
Excess ATP	—	124.6	124.6	120.5	119.9	120.5	120.5
CO ₂ from HMPP	—	—	—	6.2	7.0	6.2	6.2
Apparent Y _{ATP}	—	3.25	3.25	3.51	3.55	3.51	3.51
Gamma ratio	1.000	—	—	—	—	—	—

^aRefers to data used in the calculations.

Table IV. Fermentation of glycerol with *Propionibacterium pentosaceum*; data are from ref. 10.

Substance	Amount (mol/100 mol C ₃ fermented)						
	Experimental	Calculated					
		Without HMPP			With HMPP		
Propionate	55.8	57.8	55.8 ^a	62.2	56.2	61.4	55.8 ^a
Acetate	2.9	0.0	0.0	0.0	2.9 ^a	2.9 ^a	3.5
Carbon dioxide	-37.7	-42.1	-44.1	-37.7 ^a	-43.0	-37.7 ^a	-43.1
Succinate	42.1	42.1 ^a	44.1	37.7	42.1 ^a	36.8	42.1 ^a
Carbon recovery	101	99.9 ^a	99.9 ^a	99.9 ^a	99.9 ^a	99.9 ^a	99.9 ^a
NADH ₂ from FMNH ₂	—	-99.9	-99.9	-99.9	-95.4	-95.4	-94.4
Excess ATP	—	199.1	199.1	199.1	201.6	201.6	202.1
CO ₂ from HMPP	—	—	—	—	-3.8	-3.8	-4.5
Apparent Y _{ATP}	—	0.04	0.04	0.04	0.04	0.04	0.04
Gamma ratio	0.997	—	—	—	—	—	—

^aRefers to data used in the calculations.**Table V.** Fermentation of glucose with *Cytophaga succinicans*; data are from ref. 17.

Substance	Amount (mol/100 mol C ₃ fermented)						
	Experimental	Calculated					
		Without HMPP			With HMPP		
Propionate	0.0	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
Acetate	39.0	40.1	39.7	39.6	39.7	39.8	39.0 ^a
Carbon dioxide	-39.0	-39.5	-39.1	-39.0^a	-39.0^a	-39.0^a	-39.8
Succinate	59.0	58.6	59.0 ^a	59.1	59.0 ^a	59.0 ^a	59.2
Formate	21.0	21.0 ^a	19.8	19.4	19.7	19.6	21.0 ^a
Hydrogen	0.0	0.0 ^a	0.0 ^a	0.0 ^a	0.1	0.0 ^a	0.0 ^a
Carbon recovery	98.7	98.7 ^a	98.7 ^a	98.7 ^a	98.7 ^a	98.7 ^a	98.7 ^a
NADH ₂ from FMNH ₂	—	-39.5	-39.1	-39.0	-39.1	-38.8	-41.2
Excess ATP	—	187.7	187.7	187.7	187.7	187.9	186.8
CO ₂ from HMPP	—	—	—	—	—	-0.1	0.5
Apparent Y _{ATP}	—	0.57	0.57	0.57	0.57	0.56	0.58
Gamma ratio	0.997	—	—	—	—	—	—

^aRefers to data used in the calculations.

for the *Propionibacterium* pathway seems to be confirmed by the data of a variety of fermentations and microorganisms. The extent of involvement of the HMPP seems to be quite variable even among different fermentations of the same strain.

Complete data for fermentations of the acrylate pathway are very limited. Tables VI and VII present calculations for fermentations of pyruvate, lactate, and acrylate by *Clostridium propionicum*.¹⁹ Note that the fermentations were performed with washed cells grown on alanine, and that, therefore, the excessively high ATP yield of 34 calculated in Table VII should be interpreted to imply accumulation, very probably intracellularly, of large amounts of precursors and intermediates of biosynthesis, other than the excreted primary metabolites shown in Table VII. The agreement between calculated and experimental values is indeed very good.

Assuming a 95% carbon recovery in products and that the fermentation equations are valid, theoretically maximal yields for the main products of propionic-acid fermentations can be computed as has been discussed earlier.⁵ Table VIII shows calculated maximal yields for propionate and succinate from glucose fermentations through the *Propionibacterium* pathway, with and without the HMPP. Note that the calculations, for comparison purposes, are based on 100 mol of three-carbon skeletons (C₃) fermented. Maximal yields of succinate by *Propionibacterium* pathway fermentations are considerably lower than the yields from mixed-acid fermentations.⁵

Theoretically maximal yields for propionate and acrylate production from pyruvate, lactate, and glucose via the acrylate pathway are presented in Table IX. Note that when lactate is the substrate, no acrylate can be produced because of the biosynthetic requirements and the required

Table VI. Fermentation of pyruvate and lactate by washed cell suspensions of *Clostridium propionicum* grown on alanine; data are from ref. 19.

Substance	Amount (mol/100 mol C ₃ fermented)						
	Pyruvate			Lactate			
	Experimental	Calculated		Experimental	Calculated		
Propionate	33.3	32.0	32.7	66.0	66.6	66.0 ^a	66.0
Acetate	66.7	66.3	66.7 ^a	33.0	33.3	33.0	33.0 ^a
Carbon dioxide	61.6	66.3	66.7	36.5	33.3	33.0	33.0
Lactate	0.0	0.0 ^a	0.0 ^a	-100.0	-100.0 ^a	-100.0 ^a	-100.0 ^a
Acrylate	0.0	0.0 ^a	-1.1	0.0	0.0 ^a	0.9	0.9
Carbon recovery	98.3	98.3 ^a	98.3 ^a	100.2	99.9 ^a	99.9 ^a	99.9 ^a
Excess ATP	—	52.0	52.4	—	32.5	32.2	32.2
Apparent Y _{ATP}	—	2.00	1.99	—	0.23	0.24	0.24
Gamma ratio	1.020	—	—	1.05	—	—	—

^aRefers to data used in the calculations.

Table VII. Fermentation of acrylate by washed cell suspensions of *Clostridium propionicum* grown on alanine; data are from ref. 19.

Substance	Amount (mol/100 mol C ₃ fermented)				
	Experimental		Calculated		
Propionate	58.2	56.8	55.9	58.2 ^a	52.9
Acetate	29.1	31.0	30.6	31.8	29.1 ^a
Carbon dioxide	30.6	31.0	30.6 ^a	31.8	29.1
Lactate	0.0	0.0 ^a	1.3	0.0 ^a	0.0 ^a
Acrylate	-100.0	-100.0 ^a	-100.0 ^a	-102.2	-94.2
Carbon recovery	87.8	87.8 ^a	87.8 ^a	87.8 ^a	87.8 ^a
Excess ATP	—	-71.7	-72.2	-71.0	-73.7
Apparent Y _{ATP}	—	30.62	31.07	29.92	32.67
Gamma ratio	1.000	—	—	—	—

^aRefers to data used in the calculations.

Table VIII. Theoretically maximal yields of primary products of glucose fermentations with *Propionibacteria* and related species. A 95% carbon recovery in products was assumed.

Substance	Amount (mol/100 mol C ₃ fermented)			
	Product of maximum yield			
	With HMPP		Without HMPP	
	Propionate	Succinate	Propionate	Succinate
Propionate	81.1	39.5	62.6	30.2
Acetate	0.0	0.0	32.4	32.4
Carbon dioxide	41.7	0.0	32.4	0.0
Succinate	0.0	41.7	0.0	32.4
Carbon recovery	95.0	95.0	95.0	95.0
NADH ₂ from FMNH ₂	-81.1	-81.1	-30.2	-30.2
Excess ATP	125.1	125.1	152.9	152.9
Apparent Y _{ATP}	3.98	3.98	2.00	2.00
CO ₂ from HMPP	41.7	41.7	—	—

Table IX. Theoretically maximal yields of primary products of lactate, pyruvate, and glucose fermentation with bacteria of the acrylate pathway.

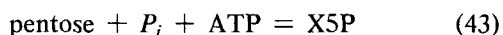
Substance	Amount (mol/100 mol C ₃ fermented)				
	Product of maximum yield				
	Pyruvate		Lactate	Glucose	
	Propionate	Acrylate	Propionate	Acrylate	Propionate
Propionate	29.3	0.0	62.6	0.0	62.6
Acetate	65.7	51.1	32.4	1.1	32.4
Carbon dioxide	65.7	51.1	32.4	1.1	32.4
Lactate	0.0	0.0	-100.0	0.0	0.0
Acrylate	0.0	43.9	0.0	93.9	0.0
Carbon recovery	95.0	95.0	95.0	95.0	95.0
Excess ATP	23.6	9.0	-9.7	59.0	90.3
Apparent Y_{ATP}	5.93	7.63	12.03	3.85	2.94

conservation of reduction energy. When pyruvate is the substrate, acrylate can be produced, but its yield is still relatively low because of insufficient reduction energy. For the theoretical possibility that a microorganism can utilize glucose and employ the acrylate pathway, excellent yields for acrylate and propionate can be obtained. Still, however, better yields for propionate can be obtained from the *Propionibacterium* fermentations, as a comparison of yields in Tables VIII and IX would show.

BIOCHEMISTRY AND EQUATIONS FOR FERMENTATIONS OF SUGARS OTHER THAN GLUCOSE

Hydrolysis of hemicellulose typically yields the pentoses xylose and arabinose, the hexoses mannose, galactose and glucose and the disaccharide cellobiose.^{6,7}

The utilization of pentoses (xylose, arabinose, ribose) proceeds through the following four steps^{2,6,8-10}: (1) isomerization and/or phosphorylation in position 5, (2) epimerization to D-xylulose-5-P (X5P), (3) conversion of D-xylulose-5-P to glyceraldehyde-3-P (G3P) and fructose-6-P (F6P) via the HMPP, and (4) degradation by the EMP pathway to PEP or pyruvate. If we let "pentose" represent xylose, arabinose or ribose, the first two steps can be represented by the overall reaction



Step 3, which requires the action of an epimerase, an isomerase, a transaldolase, and transketolases, can be represented by



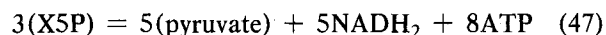
Fructose-6-P can be converted to pyruvate via the EMP pathway, resulting in the overall reaction



Glyceraldehyde-3-P can also be converted to pyruvate via the EMP pathway by the overall reaction



Combination of eqs. (44)-(46) results in the following equation:



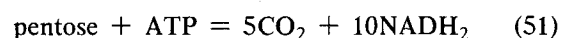
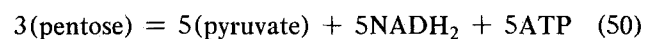
Xylulose-5-P can also be completely degraded to CO₂ to produce reduction energy (NADH₂) if necessary. This degradation scheme requires gluconeogenesis whereby essentially two moles of glyceraldehyde-3-P combine to produce one mole of glucose-6-P. Glucose-6-P is then converted to CO₂ and ribulose-5-P. All of these reactions are part of the HMPP,^{8,20} and the overall degradation reaction can be represented by



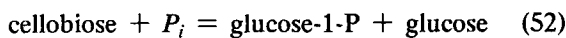
Finally, ribulose-5-P can be combined with CO₂ via the reverse action of some of the HMPP enzymes to eventually form glucose-6-P, which then is converted to pyruvate via the EMP pathway. The overall reaction can be represented by,⁵



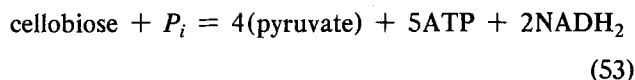
Note that if eq. (47) is combined with the reverse of eq. (48), we can obtain eq. (49) except that for every 5 mol CO₂ fixed, an additional 1 mol ATP would be produced than the correct amount of ATP predicted by eq. (49). If we allow for this correction at the end of the calculations, the combination of eq. (47) with the forward or reverse reaction represented by eq. (48) can be used to represent the conversion of xylose-5-P to pyruvate, with or without CO₂ release or with CO₂ fixation. Finally, we can rewrite eqs. (47) and (48), in view of eq. (43), as follows:



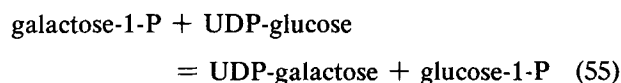
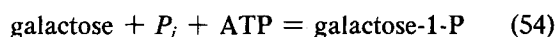
Cellobiose uptake is initiated by a phosphorylytic cleavage catalyzed by a phosphorylase,⁹



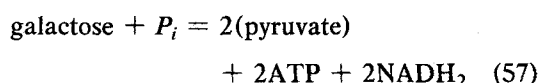
Glucose-1-P is converted to glucose-6-P by a phosphoglucosyltransferase and thus enters the EMP pathway. Glucose is phosphorylated to glucose-6-P either by a hexokinase or by a phosphotransferase using acetyl-P and producing acetate. Acetate is then phosphorylated with ATP to regenerate acetyl-P.^{8,9} Thus, the overall reaction can be represented by



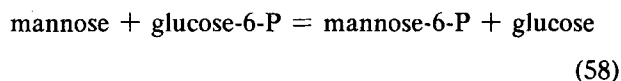
Galactose is converted to glucose-1-P by the consecutive action of three inducible enzymes, galactokinase, uridylyltransferase, and an epimerase, catalyzing, respectively, the three reactions⁹



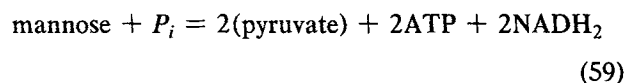
Glucose-1-P is converted to glucose-6-P as discussed above, and thus enters the EMP pathway. The overall reaction can be represented then by,



Mannose is phosphorylated to mannose-6-P with glucose-6-P by a phosphotransferase yielding glucose,



Mannose-6-P is converted to fructose-6-P by an isomerase and thus enters the EMP pathway. Glucose is phosphorylated as discussed above. The overall reaction therefore is



It can be thus seen that all three hexoses, glucose, galactose, and mannose, can be converted via the EMP pathway to 2 mol pyruvate yielding also 2 mol each of NADH₂ and ATP. Therefore, all the fermentation equations that have been derived earlier^{4,5} and in this work for glucose will also be valid for galactose and mannose, as will be all of the maximal yields that have been calculated for the various fermentation products.

From eq. (53), it can be seen that, *per six-carbon skeleton*, cellobiose also produces 2 mol pyruvate and 2 mol NADH₂ but 2.5 mol ATP, i.e. 0.5 mol ATP more than the hexoses. However, we have seen that all the saccharolytic fermentations we have discussed here and earlier^{4,5} are not limited by the ATP supply. Therefore, all the theoretical product yields that were calculated earlier^{4,5} and here (Tables VIII and IX) on glucose can be used to calculate yields on cellobiose by simply multiplying them by two.

The fermentation equations for cellobiose can be easily derived as has been discussed earlier^{4,5} by employing eq. (53). Since cellobiose degradation proceeds via glucose-6-P, it is obvious that cellobiose can also be fully or partially degraded to CO₂ via the HMPP as has already been discussed above and earlier.^{4,5}

Remaining to be discussed, as an example, are the derivation of a fermentation equation for pentoses, verification of the pentose equations from experimental data through calculations, and calculations of maximal product yields for fermentations on pentoses.

We shall derive a fermentation equation for pentose degradation and utilization via the *Propionibacterium* pathway discussed above. The procedure is the same as the one employed to derive the equation with glucose as a substrate. Equations (3a) and (3b) are now replaced by eqs. (50) and (51), respectively. The resulting equation, which corresponds to eq. (9) for glucose utilization is

$$\begin{aligned} 3C_4H_4O_4N_4 - (3b_1 + b_2)\text{pentose} - a(\text{glycerol}) \\ - e(\text{lactate}) + (5b_1 - c - d + e - f + a - 4)\text{pyruvate} \\ + (5b_1 + 10b_2 - c - d + e - g + 2a - 5.75)\text{NADH}_2 \\ + (f + g - c - d)\text{FMNH}_2 \\ + (5b_1 - b_2 + c + d + f + a - 33.7)\text{ATP} \\ + (5b_2 + f - d)\text{CO}_2 + c(\text{propionate}) + d(\text{succinate}) \\ + f(\text{acetate}) = 0 \end{aligned} \quad (60)$$

The accompanying equality and inequality relations for eq. (60) are the same as for eq. (9), except as follows. Equation (10a) is replaced by

$$5b_1 + 10b_2 - c - d + e - g + 2a - 5.75 = 0 \quad (61)$$

Relations (11a) and (11b) are replaced by

$$\begin{aligned} 5b_1 - c - d + e - f + a - 4 < 0 \\ (\text{if pyruvate is a substrate}) \end{aligned} \quad (62a)$$

$$\begin{aligned} 5b_1 - c - d + e - f + a - 4 \geq 0 \\ (\text{if pyruvate is a product}) \end{aligned} \quad (62b)$$

Relations (10b), (13), (14), and (17)–(20) remain unchanged. Relations (12), (15a), and (15b) are replaced, respectively, by,

$$-(3b_1 + b_2) \leq 0 \quad (63)$$

$$5b_2 + f - d < 0 \quad (\text{if CO}_2 \text{ is fixed}) \quad (64a)$$

$$5b_2 + f - d \geq 0 \quad (\text{if CO}_2 \text{ is a product}) \quad (64b)$$

Finally, eq. (16) is replaced by

$$5b_1 - b_2 + c + d + f + a - 33.7 \geq 0 \quad (65)$$

Similarly, fermentation equations for butyric-acid, butanediol, mixed-acid, and acrylate-pathway fermentations with pentose substrates can be derived, utilizing the information that was presented above and earlier.^{4,5} The details will be left to the reader.

CALCULATIONS FOR FERMENTATIONS OF SUGARS OTHER THAN GLUCOSE

Complete data for fermentations of sugars other than glucose are significantly more limited than the fermentations we have discussed above or earlier.^{4,5}

Table X presents data and calculations for a butanediol fermentation of D-xylose by *Aeromonas hydrophila*.²¹ Calculations assuming that no CO₂ is produced through the HMPP give results in good agreement with experimen-

tal values. Accordingly, calculations assuming that CO₂ can be produced from HMPP did not result in any better agreement with the experimental values, and the calculated amount of CO₂ produced from HMPP was too small to indicate any significant decarboxylation through the HMPP.⁵ Calculations for a butanediol fermentation of D-xylose by *Bacillus polymyxa*²² indicated that a significant amount of CO₂ (15 mol/100 mol pentose fermented) is produced through the HMPP.

Table XI presents data and calculations for two butane-

Table X. Fermentation of D-xylose with *Aeromonas hydrophila*; data are from ref. 21.

Substance	Amount (mol/100 mol pentose fermented)					
	Experimental	Calculated				
		With no CO ₂ from HMPP	With CO ₂ from HMPP	With no CO ₂ from HMPP	With CO ₂ from HMPP	With CO ₂ from HMPP
Carbon dioxide	134.7	138.4	137.6	134.7 ^a	134.7 ^a	134.7 ^a
Formate	0.0	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
Acetoin	2.6	2.6 ^a	2.6 ^a	2.6 ^a	2.6 ^a	2.6 ^a
Ethanol	48.9	48.9 ^a	48.9 ^a	45.2	46.0	46.5
Lactate	20.4	20.4 ^a	21.2	20.4 ^a	22.3	20.4 ^a
Succinate	1.1	1.1 ^a	1.1 ^a	3.0	1.1 ^a	2.3
Acetate	9.3	9.3 ^a	6.6	9.3 ^a	9.3 ^a	9.3 ^a
Butanediol	39.0	38.0	39.0 ^a	39.0 ^a	39.0 ^a	39.0 ^a
Hydrogen	53.9	58.3	53.9 ^a	57.4	53.9 ^a	53.9 ^a
Carbon recovery	96.6	96.6 ^a	96.6 ^a	96.6 ^a	96.6 ^a	96.6 ^a
NADH ₂ from XH ₂	—	-1.2	0.5	-5.9	0.3	-0.3
Excess ATP	—	129.3	126.7	131.2	130.2	131.2
CO ₂ from HMPP	—	—	—	—	-2.7	-2.1
Apparent Y _{ATP}	—	2.49	2.53	2.47	2.48	2.47
Gamma ratio	1.010	—	—	—	—	—

^aRefers to data used in the calculations.

Table XI. Fermentation of D-xylose and D-ribose with *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*); data are from ref. 23.

Substance	Amount (mol/100 mol pentose fermented)						
	D-xylose		D-ribose				
	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated	
Carbon dioxide	46.0	45.7	45.7	60.0	62.9	62.9	64.4
Formate	72.3	72.3 ^a	72.3 ^a	59.4	59.4 ^a	59.4 ^a	59.4 ^a
Acetoin	0.0	0.0 ^a	0.0 ^a	0.0	0.0 ^a	0.0 ^a	0.0 ^a
Ethanol	55.6	55.6 ^a	55.6	59.6	59.6 ^a	58.1	59.6 ^a
Lactate	3.3	3.3 ^a	3.3 ^a	3.5	3.5 ^a	3.5 ^a	2.0
Succinate	6.0	6.0 ^a	6.0 ^a	11.0	11.0 ^a	11.0 ^a	11.0 ^a
Acetate	61.0	61.0 ^a	61.0 ^a	54.6	54.6 ^a	54.6 ^a	54.6 ^a
Butanediol	3.7	3.7	3.7 ^a	10.3	9.6	10.3 ^a	10.3 ^a
Hydrogen	N.D.	32.8	32.8	N.D.	40.1	40.9	40.9
Carbon recovery	80.0	80.0 ^a	80.0 ^a	88.7	88.7 ^a	88.7 ^a	88.7 ^a
NADH ₂ from XH ₂	—	5.5	5.5	—	3.7	1.5	2.9
Excess ATP	—	-47.2	-47.2	—	73.6	73.6	73.6
Apparent Y _{ATP}	—	11.12	11.12	—	6.32	6.32	6.32

^aRefers to data used in the calculations.

Note: ND refers to data not determined.

diol fermentations of D-xylose and D-ribose by *Klebsiella pneumoniae*.²³ The calculations were performed assuming that no CO₂ is produced through the HMPP, and the calculated values are in excellent agreement with the experimental ones. However, calculations for two butanediol fermentations of D- and L-arabinose by *K. pneumoniae*²⁴ have indicated that a significant amount of CO₂ (10–17 mol/100 mol pentose fermented) were produced through the HMPP.

Table XII presents data and calculations for a fermentation of L-arabinose by a *Propionibacterium* species.¹⁰ Despite the excessive amount of carbon recovered in products and the poor gamma ratio, the calculated values, assuming that CO₂ is produced through the HMPP, are in

reasonable agreement with experimental ones. Approximately 20 mol CO₂ (per 100 mol pentose fermented) were calculated to be produced through the HMPP. As expected then, calculations (column 2 of Table XII) assuming that no CO₂ is produced from the HMPP gave values in poor agreement with the experimental data.

In conclusion, from calculations based on the limited available data on pentose fermentations, the fermentation equations appear to be generally valid. Therefore, they can be at least used for calculating theoretical maximal yields for the various products of pentose fermentations. Such calculations for butyric-acid, butanediol (or mixed-acid), and propionic-acid fermentations are presented, respectively, in Tables XIII, XIV, and XV. Qualitatively,

Table XII. Fermentation of L-arabinose with a *Propionibacterium* species; data are from ref. 10.

Substance	Amount (mol/100 mol arabinose fermented)				
	Experimental	Calculated			
	1	2 ^b	3 ^c	4 ^c	5 ^c
Propionate	98.3	81.8	91.1	90.6	91.1
Acetate	33.8	48.3	32.0	33.8 ^a	33.8 ^a
Carbon dioxide	38.2	33.5	38.2 ^a	38.2 ^a	38.2 ^a
Succinate	14.8	14.8 ^a	14.8 ^a	14.3	14.8 ^a
Lactate	21.6	21.6 ^a	21.6 ^a	21.6 ^a	20.4
Glycerol	0.0	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
Carbon recovery	105	99.9 ^a	99.9 ^a	99.9 ^a	99.9 ^a
NADH ₂ from FMNH ₂	—	−29.0	−74.0	−71.1	−72.1
Excess ATP	—	186.1	291.9	294.0	294.7
Apparent Y _{ATP}	—	0.04	0.04	0.04	0.04
CO ₂ from HMPP	—	—	21.0	18.7	19.2
Gamma ratio	1.060	—	—	—	—

^aRefers to data used in the calculations.

^bThis is with no CO₂ produced from HMPP.

^cThis is with CO₂ produced from HMPP.

Table XIII. Theoretically maximal yields for primary products of pentose fermentations with butyric-acid bacteria. A 95% carbon recovery in products was assumed.

	Amount (mol/100 mol pentose fermented)				
	Product of maximum yield				
	Butanol	Acetone	Butyrate	Acetate	Isopropanol
Butyrate	0.0	0.0	79.2	0.0	0.0
Acetate	1.8	0.0	0.0	158.3	0.0
Hydrogen	0.0	313.0	154.7	313.0	233.9
Carbon dioxide	158.3	237.5	158.3	158.3	237.5
Butanol	78.3	0.0	0.0	0.0	0.0
Acetone	0.0	79.2	0.0	0.0	0.0
Ethanol	0.0	0.0	0.0	0.0	0.0
Acetoin	0.0	0.0	0.0	0.0	0.0
Isopropanol	0.0	0.0	0.0	0.0	79.2
Carbon recovery	95.0	95.0	95.0	95.0	95.0
NADH ₂ from FdH ₂	158.3	−154.7	3.6	−154.7	−75.5
Excess ATP	106.6	184.0	184.0	263.1	184.0
Apparent Y _{ATP}	3.85	2.64	2.64	2.00	2.64
H ₂ /CO ₂	0.0	1.82	0.98	1.98	0.98

Table XIV. Theoretically maximal yields for primary products of pentose fermentations with propionic-acid bacteria. A 95% carbon recovery in products was assumed.

Pathway	Amount (mol/100 mol pentose fermented)					
	<i>Propionibacterium</i> pathway				Acrylate pathway	
	Propionate ^a	Succinate ^a	Propionate ^b	Succinate ^b	Acrylate ^a	Propionate ^a
Product of maximum yield						
Propionate	135.2	65.8	104.3	50.3	0.0	104.3
Acetate	0.0	0.0	54.0	54.0	1.8	54.0
Carbon dioxide	69.4	0.0	54.0	0.0	1.8	54.0
Succinate	0.0	69.4	0.0	54.0	—	—
Lactate	0.0	0.0	0.0	0.0	0.0	0.0
Glycerol	0.0	0.0	0.0	0.0	—	—
Acrylate	—	—	—	—	156.5	0.0
Carbon recovery	95.0	95.0	95.0	95.0	95.0	95.0
NADH ₂ from FMNH ₂	-135.2	-135.2	-30.2	-30.2	—	—
Excess ATP	194.6	194.6	152.9	152.9	59.0	90.3
Apparent Y _{ATP}	2.45	2.45	2.00	2.00	3.85	2.94
CO ₂ from HMPP	69.4	69.4	—	—	—	—

^aThis is with no CO₂ produced through the HMPP.

^bThis is with CO₂ produced through the HMPP.

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

Substance	Amount (mol/100 mol pentose fermented)							
	Product of maximum yield							
	Butanediol ^c	Butanediol ^{c,d}	Acetoin ^c	Acetoin ^{c,d}	Succinate ^e	Succinate ^{d,e}	Succinate ^c	Lactate ^c
Carbon dioxide	158.3	158.3	158.3	158.3	0.0	0.0	0.0	1.8
Formate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetoin	0.0	0.0	79.2	40.5	0.0	0.0	0.0	0.0
Ethanol	0.0	50.3	0.0	77.3	0.0	32.9	37.8	0.0
Lactate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	156.5
Succinate	0.0	0.0	0.0	0.0	118.8	102.3	79.2	0.0
Acetate	0.0	0.0	0.0	0.0	0.0	0.0	41.4	1.8
Butanediol	79.2	54.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydrogen	75.5	50.3	154.7	77.3	115.1	32.9	0.0	0.0
Carbon recovery	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0
NADH ₂ from XH ₂ ^a	-75.5	0.0	-154.7	0.0	-233.9	-102.3	0.0	1.8
Excess ATP	96.5	96.5	96.5	96.5	151.9	161.7	217.0	98.3
Apparent Y _{ATP}	3.90	3.90	3.90	3.90	2.92	2.80	2.26	3.85
c ^{a,b}	-75.5	0.0	-154.7	0.0	-115.1	0.0	79.2	1.8
CO ₂ from HMPP	—	—	—	—	118.8	69.4	—	—

^aSee ref. 5.

^bNormalized for 100 mol pentose.

^cThis is with no CO₂ produced from the HMPP.

^dThis is with the additional restriction that *c* is non-negative^a.

^eThis is with CO₂ produced from the HMPP.

the calculated values behave similarly to the values calculated for glucose fermentations, as has been discussed in detail above (Tables VIII and IX) and earlier.^{4,5}

DISCUSSION AND CONCLUSIONS

A variety of complete fermentation data, although still somewhat limited, allowed a fairly thorough testing of the

fermentation equation for *Propionibacterium* fermentations. However, more complete data will be necessary for testing the equations for the acrylate pathway and the utilization of the various pentoses, cellobiose, and other hexoses. Such data would be particularly desirable for pentose fermentations by butyric-acid bacteria in view of the renewed interest in the butanol/acetone fermentation.

Some very recent data on butanol/acetone fermentations of pentoses are unfortunately incomplete.^{7,25}

Once confidence in single-substrate fermentation equations is attained, equations for multi-substrate fermentations can be derived in a similar fashion. After they have been thoroughly tested, such equations may become a useful tool in process monitoring and control as "gateway sensors."⁴ As we have argued earlier,^{4,5} fermentation equations are expected to be valid for steady-state and completed as well as transient fermentations by virtue of the pseudo-steady-state hypothesis. In our laboratory we have found that the equation for butanol/acetone fermentations⁴ is indeed valid for both completed and transient fermentations and is thus a useful tool for process monitoring and control. Perhaps of equal significance to us was the contribution of the equation in understanding the biochemical control mechanisms of the butanol/acetone fermentation.²⁶ By analyzing an extensive amount of experimental data, and correlating the computed extents of some key intracellular reactions with the experimentally observed extracellular events, we were able to construct a plausible scenario for the biochemical-control mechanisms of the fermentation. We were subsequently able to test such a scenario and we currently use it for reactor and process optimization. Extensions and improvements of the fermentation equations have been discussed earlier in detail.^{4,5}

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NOMENCLATURE

$a, b_1, b_2, c, d, e, f, g, h, i$	stoichiometric coefficients or unknowns, in eqs. (9), (29), and (60)
AcCoA	acetyl-CoA
AcPO ₄	acetyl phosphate in Figure 2
EMP	Embden-Meyerhof-Parnas pathway
Fd/FdH ₂	ferredoxin
FMN/FMNH ₂	flavin mononucleotide
F6P	fructose-6-P
G3P	glyceraldehyde-3-P
HMPP	hexose monophosphate pathway
PEP	phosphoenolpyruvate
R(RO ⁻ , R-CoA)	acetyl, acrylyl, or propionyl in Figure 2
R-Me-Malonyl-CoA	(R)-methylmalonyl-CoA in Figure 1

S-Me-Malonyl-CoA	(S)-methylmalonyl-CoA in Figure 1
UDP	uridine diphosphate
X5P	D-xylulose-5-P
Y/YH ₂	electron carrier system in Figures 1 and 2
Y _{ATP} ^G	ATP yield for growth on glucose, g dry biomass/mol ATP utilized for biomass synthesis
Y _{ATP} ^P	ATP yield for growth on pyruvate, g dry biomass/mol ATP utilized for biomass synthesis.

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