

A USEFUL EQUATION FOR FERMENTATIONS  
OF BUTYRIC ACID BACTERIA

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Summary The usefulness and uses of an equation which would describe the interrelations among the various products and biomass in fermentations of butyric acid bacteria are discussed. Such a fermentation equation is then derived based on the bacterial biochemistry and general biological regularities. The equation obeys all the constraints of the biochemical topology and thermodynamics. The validity of the equation is demonstrated using experimental data from the literature. The equation is potentially valuable as a predictive and process-control tool in a wide variety of fermentations of butyric-acid bacteria.

INTRODUCTION

There is considerable evidence to postulate that renewable resources (mono- and oligosaccharides from biomass hydrolysis) will provide a significant percentage of the chemical industry's feedstocks in the future (Villet, 1981; Palsson et al., 1981). Butanol, acetone, ethanol, isopropanol, lactic acid and butanediol are some of the main oxychemicals which can be produced by fermentations (Villet, 1981; Palsson et al., 1981; Lipinsky, 1981). Butyric acid bacteria can anaerobically ferment a variety of sugars (hexoses, pentoses and oligosaccharides) (Doelle, 1975; Allcock and Woods, 1982; Mes-Hartree and Saddler, 1982) to produce a variety of organic solvents (butanol, acetone, ethanol, isopropanol, acetoin), carboxylic acids (acetic, butyric and formic acids) and hydrogen (Doelle, 1975; Wood, 1961; Gottschalk, 1979).

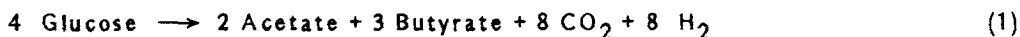
Although most fermentation products of butyric acid bacteria are valuable chemicals, some are more so than others. With current market prices, and on a molar basis, butanol is significantly more valuable than acetone or butyric acid. Also, the high cost of separation and purification would make the production of a single fermentation product highly desirable, if such a capability could be developed. Knowledge of the thermodynamic and biochemical constraints which determine the maximal yield for each fermentation product and calculation of these maximal yields would be of practical and fundamental importance. Rational bounds could be thus established for feasibility studies, and genetic and reactor experimentation. It would be also desirable to know of any possible interrelations

among the amounts of the various fermentation products, biomass and the consumed substrate, in order to check the consistency of experimental data and identify possible sources of experimental error. Perhaps even more importantly, the existence and discovery of precise such interrelations would allow the on-line calculation of the concentrations of some fermentation products which cannot be measured directly, by measuring other fermentation parameters (e.g. biomass, off gases, consumed substrate). These interrelations would play the role of 'gateway sensors' (Humphrey, 1974; Rolf and Lim, 1982), whereby certain significant fermentation parameters can be extracted from a combination of available sensors. Such 'gateway sensors' are very useful in the design and control of fermentation processes (Rolf and Lim, 1982; Hatch, 1982). The interrelations among the various chemical 'species' of a fermentation process can be most conveniently represented by appropriate stoichiometric coefficients of an overall balance equation, which we shall call a fermentation equation.

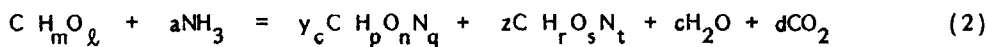
The purpose of this short paper is the fast communication of a fermentation equation, which we believe is valid for fermentations of a wide variety of butyric acid bacteria. We hope that this equation will be of immediate usefulness to the many research groups worldwide which work on the butanol/acetone fermentations. Full details on the derivation, validity and uses of this equation will be given in a forthcoming longer report (Papoutsakis, 1983).

#### A FERMENTATION EQUATION

A number of fermentation equations for butyric acid bacteria have been proposed in the literature, although rarely were they intended to serve the objectives discussed in the preceding section. Wood (1961), for example, proposed, in a very approximate sense, the equation,



for fermentations of *C. butyricum*. This equation does not appear to be valid for other similar carboxylic-acid fermentations. Another, less specific, more recent approach toward the derivation of fermentation equations is to write an equation of the form (Erickson et al., 1978)



where  $CH_mO_\ell$ ,  $CH_pO_nN_q$  and  $CH_rO_sN_t$  represent the elemental compositions of the substrate, biomass and extracellular products, respectively. The coefficients and subscripts of Eq.(2) can then be related by carbon and nitrogen balances, given respectively by,

$$y_c + z + d = 1.0 \quad , \quad y_c q + z t = a \quad (3), (4).$$

Studies of the biomass composition with a variety of microorganisms have shown that the weight fraction of carbon,  $\sigma_b$ , and the reductance degree,  $\gamma_b$ , are relatively constant with coefficients of variation 5% and 4% respectively (Erickson et al., 1979; Erickson, 1980), namely,

$$\sigma_b = 0.462 \pm 0.023 \quad , \quad \gamma_b \equiv 4 + p - 2n - 3q = 4.291 \pm 0.172 \quad (5), (6).$$

Finally, an available electron balance (Erickson et al., 1978) will give,

$$\gamma_s = y_c \gamma_b + z \gamma_p \quad (7)$$

where the reductance degrees of the substrate and product are given respectively by,

$$\gamma_s = 4 + m - 2l \quad , \quad \gamma_p = 4 + r - 2s - 3t \quad (8), (9).$$

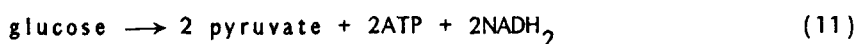
Equations (5) and (6) can be of course replaced by specific equations applying to the biomass of the fermentation of interest. Equation (2), modified according to Eqs. (3)-(9), provides valuable information for many fermentations but perhaps not enough for fermentations with many products, like those of butyric acid bacteria.

Our approach toward the derivation of a more 'advanced' fermentation equation is to use all the available biochemical information on the reaction pathways of the fermentation of interest, in order to assess its needs not only in carbon and nitrogen but also in biosynthetic and reduction energy, and key intermediates. First, we write an equation for the production of cell biomass from the carbon substrate (here, glucose),



The ATP needs of biomass synthesis can be estimated from the applying ATP yield,  $Y_{\text{ATP}}$ . Although the maximal  $Y_{\text{ATP}}$  for growth on glucose has been calculated to be 28.8 (Stouthamer, 1973), the actual  $Y_{\text{ATP}}$  for most bacteria is widely accepted to be 10.5 (Stouthamer, 1969). Equation (5) was also used for the calculation of the 29.7 figure for ATP. The necessary reduction energy ( $\text{NADH}_2$ ) for biomass synthesis is calculated by bringing glucose to the oxidation level of the cell material using Eqs. (5) and (6). The nitrogen source has not been included in Eq. (10) for simplicity and generality. Also, as is customary, no attempt is made at this stage to balance oxygen and hydrogen atoms. Any deviations of the actual  $Y_{\text{ATP}}$  and chemical formula of cell material from those used here, will have only a small effect on the final calculations regarding the production of solvents, carboxylic acids and hydrogen. This is because only 1-10% of the substrate carbon is typically converted into biomass in actual clostridia fermentations.

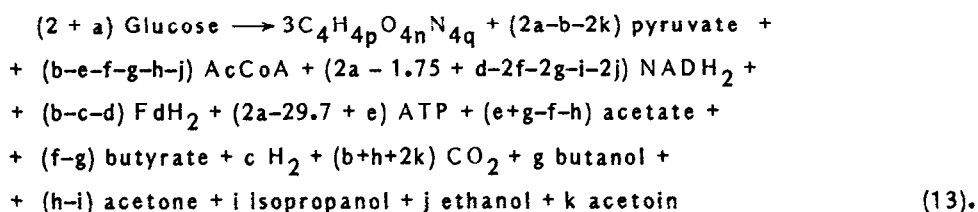
Although there still exist some uncertainties in the bacterial biochemistry of butyric acid fermentations (Papoutsakis, 1983), it is nevertheless possible to assess the needs in biosynthetic and reduction energy of the various fermentation products from key intermediates. Thus, we can write equations which represent single reactions or unique strings of reactions of the bacterial biochemistry toward the production of the final fermentation products. Again, these equations are only balanced for carbon, ATP, and reducing energy,  $\text{NADH}_2$  and ferredoxin,  $\text{FdH}_2$ . For example, for the production of pyruvate from glucose by the phosphoenolpyruvate phosphotransferase system and the Emden-Meyerhof pathway we write,



and for the readily reversible (Gray and Gest, 1965) production of  $\text{H}_2$  through  $\text{FdH}_2$  we write,



The complete set of these equations can be multiplied by different coefficients and added together to produce the following fermentation equation,



For steady-state fermentations or for completed fermentations, since there is no accumulation of pyruvate, acetyl CoA ( $\text{AcCoA}$ ),  $\text{NADH}_2$  and  $\text{FdH}_2$ , the coefficients of these four species can be put equal to zero, thus producing four (4) equations for the eleven (11) unknowns (a-k) of Eq. (13). The coefficient of ATP cannot be equated to zero, because all experimental data show that, in most cases, more ATP is produced through glycolysis than is required for biomass synthesis. It appears then that either ATP is hydrolyzed without performing any significant cellular function or that it is necessary for some other unknown process, whose needs must apparently vary significantly from fermentation to fermentation. The apparent ATP yields shown also in Table 1 has been calculated on the basis of the total produced ATP. The coefficients of the remaining species of Eq. (13) are either positive or non-negative depending on the various products produced in different fermentations. For the 11 unknown variables (a-k) we have thus 4 equations and 13 independent inequality conditions resulting from Eq. (13) and the requirement that b and e must also be positive.

The validity of Eq. (13) with its accompanying equality and inequality conditions has been tested with many complete sets of data from completed batch

fermentations in the literature, provided that these data were adequately satisfying total carbon balances and the CO<sub>2</sub> consistency test. The moles of CO<sub>2</sub> produced per mole of fermentation product have been established rather well for butyric acid fermentations (Doelle, 1975; Gottschalk, 1979). Thus, the total amount of CO<sub>2</sub> which is produced in a fermentation can be calculated from the amounts of the remaining fermentation products. The ratio of the calculated CO<sub>2</sub> amount over the experimental CO<sub>2</sub> amount is a good index of the consistency of the experimental data. According to our calculations, Eq. (13) appears consistent with the experimental data in the literature, within the experimental accuracy of the data at the time that they were obtained, and irrespective of the bacterial strain of the fermentation. Some of the better sample calculations are shown in Table 1. More calculations for a variety of butyric acid clostridia and further discussion on the derivation and uses of Eq. (13) will appear in our extended report (Papoutsakis, 1983).

#### DISCUSSION AND CONCLUSIONS

Clearly, Eq. (13) can be refined further, for improved predictive capabilities, on the basis of more detailed information for specific fermentations. For example, improvements could result from more accurate versions of Eq. (10). In principle, however, fermentation Eq. (13) (and its variants) with the accompanying 4 equality and 13 inequality conditions discussed above, satisfy all the topological and thermodynamic constraints of the bacterial biochemistry, by virtue of its derivation (Papoutsakis, 1983). Moreover, it can be shown that the four equality conditions derived from Eq. (13) contain the carbon and available electron balances of Eqs. (3) and (7). Therefore, Eq. (13) provides more information on the interrelations among the various fermentation products than has been available before. In addition, it can be argued that Eq. (13) is valid under transient fermentation conditions, as well, on the basis of the pseudo-steady-state hypothesis applied to the four intermediate species, AcCoA, pyruvate, NADH<sub>2</sub> and FdH<sub>2</sub>. Thus, it is potentially valuable as a 'gateway' sensor for fermentation process control, and useful for calculating the maximal theoretical yields of the various fermentation products under some specified fermentation conditions (Papoutsakis, 1983). Yet, it is desirable to check its validity on the basis of new experimental data, for potential improvements and extensions.

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TABLE 1: Fermentation of glucose with butyric acid clostridia. Data from: (a) van der Lek (1930); and (b) Kluyver (1931). \*: Values used in calculations

	moles/100 moles glucose fermented					
	(a) <i>C. Acetobutylicum</i>			(b) <i>C. Butyricum</i>		
	Experimental	Calculated		Experimental	Calculated	
Butyrate	36.4	36.4*	35.8	75.4	75.4*	75.6
Acetate	24.1	22.8	24.1*	42.7	43.2	42.7*
H <sub>2</sub>	169	169	170	231	235	234
CO <sub>2</sub>	206	209	209	196	194	194
Butanol	28.9	28.9*	28.9*	0	0*	0*
Acetone	10	10*	10*	0	0*	0*
Ethanol	14.6	14.6*	14.6*	0	0*	0*
Acetoin	5.4	5.4*	5.4*	0	0*	0*
Carbon Recovery (as glucose)	99.4	99.4*	99.4*	97	97*	97*
Apparent Y <sub>ATP</sub>	—	0.35	0.35	—	1.5	1.5
CO <sub>2</sub> Consistency Test	210/206	—	—	193.5/196	—	—
H <sub>2</sub> /CO <sub>2</sub>	0.82	0.81	0.81	1.18	1.21	1.21