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Factors Derived from Studies of Aerobic Growth in Minimal Media

W. R. MAYBERRY, G. J. PROCHAZKA, AND W. J. PAYNE
Department of Microbiology, University of Georgia, Athens, Georgia 30601

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There has been considerable interest in the proportionality of yields of cells to energy generated by bacteria in both aerobic and anaerobic cultures. For example, M. J. Johnson (4) recently reviewed many of the factors involved in the conversion, by aerobic microorganisms, of noncrop chemicals to cellular protein as a potential source of food and fodder. Our own recent studies of yields from aerobic cultures (7) have provided data to indicate a new factor to consider with regard to yields. We observed that bacteria growing on single organic compounds as sole sources of carbon and energy in minimal, mineral media produce 3.14 ($\pm$0.11) g (dry weight) of cells per "available electron" (av e^-) in the substrate. That this value is not inconsistent with the findings of other investigators was made obvious by recalculating many data from the literature on the basis of yield per av e^-; our calculations provided a value of 3.0 ($\pm$0.28). This factor was derived from considerations of experiments done with various pseudomonads, *Escherichia coli*, *Aerobacter*, and *Arthrobacter* with batch as well as continuous cultures (3, 4, 8, 10, 11).

The concept of "degree of reduction of carbon," defining the particular influence on yields of cells, was suggested by I. C. Gunsalus and C. W. Shuster (2). We have refined this concept for computational purposes in the following way: those electrons in a compound not involved in orbitals with oxygen are considered "available" for (i) transfer to oxygen or (ii) synthetic reductive reactions. We can now report additional observations of several relationships between (i) our factor, (ii) the heat of combustion (or formation) of dried cells, (iii) the entropy term for this type of aerobic growth, and (iv) approximation of *Y*(ATP).

Elemental analyses of cells harvested from culture on the substrates listed in Table 1 were obtained with an F & M model 185 C-H-N Analyzer. Mean values were: carbon, 45.6%; hydrogen, 7.6%; nitrogen, 12.1%; ash, 3.0%; and oxygen (by difference), 31.7%. These values indicated a simple molecular formula (neglecting ash) of $C_4H_8O_2N$, with a unit weight of 102. Analysis with a Parr oxygen bomb calorimeter revealed a mean heat of combustion of 5.27 kcal per g of cells.

If the values for heats of combustion reported by M. S. Karasch (5) for the compounds used here, as well as for representatives of several other classes of organic compounds, are divided by the number of available electrons in each, we obtain the heat of combustion per equivalent of available electron. These values range from 25.5 kcal per equivalent for some dicarboxylic acids to 28.2 for ether glycols. The mean for 18 representative compounds is 26.53 $\pm$ 0.28, which is in good agreement with the value of 26.05 kcal per equivalent for the transfer of electrons to oxygen from a "methane-type bond" (6).

If we burn, in theory, one formula weight of cells to CO_2, N_2, and water, we find that 20 equivalents of electron are transferred, or 0.196 per g. Multiplying this by the mean heat of combustion that we derived provides a calculated heat of combustion for dried cells of 5.20 kcal per g, as compared with a value of 5.27 kcal per g as determined by calorimetry.

The factor 0.196 equivalents of electron per g of cells (or, preferably, 0.199, if the experimental value 5.27 kcal per g is divided by 26.53 kcal per equivalent of electron) should approximate, if multiplied by the *Y*(sub) values in Table 1, the equivalents of electron incorporated into cells per mole of substrate utilized. Similarly, multiplication of the values representing moles of O_2 consumed per mole of substrate utilized (O_2/sub) by 4 (number of electrons required to reduce 1 mole of O_2) should approximate the equivalents of electron transferred to O_2 per mole of substrate utilized. Assuming that all of the organic substrate disappearing from the media in this type of growth is used either for synthesis of cells or for generation of energy, the sum of these two values should approach the number of av e^- per mole of substrate (av e^-/mole) supplied at the onset. Calculations of these approximations are seen in Table 1. We conjecture that the slightly high values are due to, in addition to experimental error, variable contributions of electrons occur-

TABLE 1. *Yields and distribution of substrate electrons which are incorporated into cells or transferred to oxygen*[a]

Substrate	Y(sub)[b]	O_2/sub[b]	Y(av e^-)[b,c]	Equiv e^-/mole sub (cells)	Equiv e^-/mole sub (to O_2)	Total av e^- (calculated)	Av e^-/mole sub (actual)
Benzoate[d]	86.8	3.46	2.89	17.3	13.8	31.1	30
Phenylglyoxylate[d]	102.2	4.06	3.20	20.4	16.2	36.6	32
Phenylacetate[d]	111.0	3.73	3.08	22.1	14.9	37.0	36
Succinate plus acetate[d]	72.2	2.54	3.28	14.4	10.2	24.6	22
Acetate[d]	23.5	1.11	2.94	4.7	4.4	9.1	8
Succinate[d]	42.3	1.40	3.02	8.4	5.6	14.0	14
1-Dodecanol[d]	217.0	7.41	3.01	43.2	29.6	72.8	72
Diethylene glycol[e]	58.0	3.10	2.90	11.6	12.4	24.0	20
Triethylene glycol[e]	103.1	4.40	3.44	20.6	17.6	38.2	30
Tetraethylene glycol[e]	129.9	6.01	3.25	25.8	24.0	49.8	40

[a] Abbreviations: Y(sub) = yield in g (dry cells)/mole of substrate utilized; O_2/sub = moles of oxygen consumed/mole of substrate utilized; Y(av e^-) = yield in g (dry cells)/equivalent of available electrons utilized; equiv e^-/mole sub (cells) = equivalents of electrons retained in cells/mole of substrate utilized; equiv e^-/mole sub (to O_2) = equivalents of electrons transferred to oxygen/mole of substrate utilized; av e^-/mole sub = equivalents of available electrons/mole of substrate.

[b] These values are the midpoints of the respective 95% confidence intervals.

[c] For 216 individual determinations, Y(av e^-) = 3.03 = m (3.14) = 3.25.

[d] Test organism was *Pseudomonas* $C_{12}B$.

[e] Test organism was bacterium TEG-5.

ring as a result of incorporation of ammonium ions into synthesis of amino acids; a portion of these ions may then be oxidized and a portion assimilated.

Since synthesis of cellular materials is a process entailing a diminution in entropy, it seems logical that, in this type of growth, energy expended in the transfer of electrons from substrate to O_2 contains all of the net entropy term plus sufficient excess to drive the endergonic reactions of synthesis, and the entropy term can be calculated. This can be accomplished by multiplying our yield factor of 3.14 g per av e^- by 5.27 kcal per g, which indicates that 16.55 kcal per av e^- are entrapped in the synthesis of cells [this may be considered free energy or ΔF by the concept of E. P. Odum (9)]. Assuming that 26.53 kcal per av e^- is a good approximation of enthalpy of combustion (ΔH) for a large variety of organic compounds and applying the second law of thermodynamics ($\Delta H - \Delta F = T\Delta S$), we thus obtain a difference of very nearly 10 kcal per av e^- as the entropy term ($T\Delta S$).

An additional set of interesting observations relates our yields to the well-known Y(ATP) value of 10.5 derived from several anaerobic systems (1). If the $Y(O_2)$ values for each substrate are rank-ordered and made relative to an arbitrary value of 1.00 for acetate and a P:O ratio of 1 is assumed for acetate, a list of values for assumed ATP produced per mole of O_2 consumed may be formulated. When this is done, the Y(ATP) for each substrate can be calculated to approximate 10.7 very closely. For example, the $Y(O_2)$ for succinate is 31.4; relative to acetate it is 1.47, and the assumed ATP/O_2 is 2.94. The calculated Y(ATP) is then 10.68. Values for the other substrates can also be calculated to approximate 10.7.

It is thus apparent that study of the "budgeting" of electrons in the substrate for transfer to oxygen for energy or incorporation into biosynthesis can provide useful new data for evaluating aerobic growth in minimal media.

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