Biochimica et Biophysica Acta, 936 (1988) 406–412
Elsevier

BBA 42902

Effect of macromolecular composition of microorganisms on the thermodynamic description of their growth

Marcel M. Mulder*, Hanneke M.L. van der Gulden, Pieter W. Postma and Karel van Dam

Laboratory of Biochemistry, University of Amsterdam, Amsterdam (The Netherlands)

(Received 7 June 1988)
(Revised manuscript received 13 September 1988)

Key words: Thermodynamics; Macromolecular composition; Chemostat; Growth model; (*K. aerogenes*)

Most models describing bacterial growth, including the original mosaic non-equilibrium thermodynamic (MNET) description, do not take into account that the macromolecular composition of the cells varies with growth rate. The MNET description of bacterial growth is extended to account for such a variation in macromolecular composition of the cells in order to make the MNET description more generally applicable. *Klebsiella aerogenes* NCTC 418 was cultured in a chemostat under glucose- or ammonia-limited conditions to determine the macromolecular composition at varying growth rate. The dilution rate has a strong influence on the macromolecular composition of the cells. Under glucose-limited conditions an increase of the RNA content of the cells was observed with increasing growth rate. The RNA content of the cells was much lower under ammonia-limited conditions of the cells than under glucose-limited conditions but also showed an increase with increasing growth rates. Under ammonia-limited conditions, the polysaccharide content strongly decreased with increasing growth rate. The other cellular components changed relatively less with changing growth rate. It is shown that the slope of the line relating catabolism to anabolism varies very little due to variation of the macromolecular cell composition with growth rate, at least under the tested conditions.

Introduction

Most growth models proposed sofar, consider a bacterium as a more or less constant unit and do not account for variation in cell composition with growth rate. However, the cell composition as a function of growth rate was studied already by Herbert [1,2] and he reported that the ratio of

* Present address: Institute for Livestock Feeding and Nutrition Research (IVVO), P.O. Box 160, 8200 AD Lelystad, The Netherlands.

Abbreviation: MNET, mosaic non-equilibrium thermodynamic.

Glossary: J_p^c, (minus the) rate of ATP synthesis coupled to anabolism;
J_p^a, rate of ATP synthesis coupled to anabolism;
n_p^c, (minus the) number of moles ATP produced per C-mol catabolic substrate consumed;
n_p^a, (minus the) theoretical number of moles ATP hydrolyzed per C-mole substrate consumed;
J_c, the rate of catabolism;
J_a, (minus the) rate of anabolism;
J_p^l, the rate of uncoupled ATP hydrolysis;
L_p^l, specific activity of uncoupled ATPase;
L_c, specific activity of catabolic enzyme system;
L_a, specific activity of anabolic enzyme system.

Correspondence: K. van Dam, Laboratory of Biochemistry, University of Amsterdam, P.O. Box 20151, 1000 HD Amsterdam, The Netherlands.

different cell components may change significantly. For instance, a continuous culture of *Klebsiella aerogenes* under glycerol-limited conditions showed an increase in the amount of RNA with increasing growth rate from 7% of the bacterial dry weight at a growth rate of 0.1 h^{-1} to 17.5% of the dry weight at a growth rate of 0.8 h^{-1} [1]. Other examples of varying cell composition as a function of growth rate are given by Russell [3] and Bremer and Dennis [4]. Herbert and coworkers [5] formulated a general empirical formula for biomass: $CH_{1.95}O_{0.45}N_{0.25}P_{0.03}$. Similar average formulae can be given for protein ($CH_2O_{0.4}N_{0.2}$), nucleotides (RNA/DNA) ($CH_{1.4}O_{0.7}N_{0.5}P_{0.1}$), lipids ($CH_2O_{0.1}$) and polysaccharide ($CH_2O$).

We wanted to investigate what consequences variation in cell composition with growth rate would have on the measured and calculated relationship between catabolism and anabolism. Therefore, we studied the dependence of the cell composition on the dilution rate for *K. aerogenes* under glucose- and ammonia-limited conditions in the chemostat.

In the original MNET description of bacterial growth [6–9] the bacterial cell was also considered as a unit of constant composition and the model did not account for specific variations in composition. It is the purpose of this paper to integrate the present knowledge of cell composition as a function of growth rate in a modified MNET model.

Theory

In the model, as it was presented by Westerhoff and coworkers [6] the metabolism of a bacterial cell consists of three separate fluxes: (i) a catabolic flux in which catabolic substrates are converted into endproducts, coupled to production of energy-rich molecules; (ii) an anabolic flux, in which the energy-rich compounds are used together with anabolic substrates for the formation of new cells; and (iii) a flux that consumes energy-rich compounds not coupled to the formation of new biomass.

In order to account for the variations in the cell composition with growth rate, the anabolic flux can be divided into a number of different fluxes, each with its own theoretical number of moles ATP hydrolysed per C-mol anabolic product (n_p^a). In this section, we will divide the anabolic flux of the original model into i different fluxes to obtain a more general model. In the results section we show results of this extended description for ($i = $) 5 components: protein, DNA, RNA, lipid and polysaccharide.

Analogous to the model of Westerhoff et al. [6] we write for the rate of ATP formation:

$$J_p^c = n_p^c J_c \tag{1}$$

for the rate of ATP dissipation:

$$J_p^{ai} = n_p^{ai} J_{ai} \tag{2}$$

The $(i + 2)$nd equation is the leak flow:

$$J_p^1 = L_p^1 (\Delta G_p - \Delta G_p^{\#}) \tag{3}$$

$\Delta G^{\#}$ is a factor introduced to account for the region of the flux–force relation in which the measurement is made; in the linear part $\Delta G^{\#}$ is a constant, in the saturating regions ($\Delta G - \Delta G^{\#}$) is a constant. The flux–force relations (we simplify by assuming the γ-factors of the original description to be 1) are given by:

$$J_c = L_c[(\Delta G_c - \Delta G_c^{\#}) + n_p^c(\Delta G_p - \Delta G_p^{\#})] \tag{4}$$

and

$$J_{ai} = L_{ai}[(\Delta G_{ai} - \Delta G_{ai}^{\#}) + n_p^{ai}(\Delta G_p - \Delta G_p^{\#})] \tag{5}$$

All elemental flows (Eqns. 1–3) are summed. The total ATP (phosphate) flux is now given by:

$$J_p = J_p^c + \sum_{i=1}^{i=i} J_p^{ai} + J_p^1 = 0 \tag{6}$$

Substituting Eqns. 1–3 in Eqn. 6 yields:

$$0 = n_p^c J_c + \sum_{i=1}^{i=i} n_p^{ai} J_{ai} + L_p^1(\Delta G_p - \Delta_p^{\#}) \tag{7}$$

$$J_c = \sum_{i=1}^{i=i} \frac{n_p^{ai}}{n_p^c} \times - J_{ai} - \frac{L_p^1(\Delta G_p - \Delta G_p^{\#})}{n_p^c} \tag{8}$$

For any of the anabolic flows (for instance $i = 1$) Eqn. 5 can be rewritten as:

$$(\Delta G_p - \Delta G_p^{\#}) = \frac{J_{a1}/L_{a1} - (\Delta G_{a1} - \Delta G_{a1}^{\#})}{n_p^{a1}} \tag{9}$$

Eliminating $\Delta G_p - \Delta G_p^{\#}$ from Eqns. 8 and 9 yields:

$$J_c = \sum_{i=2}^{i=i} \frac{n_p^{ai}}{n_p^c} \times -J_{ai} - L_p^1 \frac{J_{a1}/L_{a1} - (\Delta G_{a1} - \Delta G_{a1}^{\#})}{n_p^{a1} n_p^c} \quad (10)$$

$$J_c = \frac{n_p^{a1}}{n_p^c}\left(1 + \frac{L_p^1}{L_{a1}(n^{a1})^2}\right) \times -J_{a1} + \sum_{i=2}^{i=i} \frac{n_p^{ai}}{n_p^c} \times -J_{ai} + \frac{L_p^1}{n_p^{a1} n_p^c}(\Delta G_{a1} - \Delta G_{a1}^{\#}) \quad (11)$$

We introduce the following: $J_{ai} = \alpha_i J_a$, i.e., flux J_{ai} makes up a fraction α_i of the total flux J_a. Substituting this in Eqn. 11 gives:

$$J_c = \left(\frac{n_p^{a1}}{n_p^c}\alpha_1\left(1 + \frac{L_p^1}{L_{a1}(n_p^{a1})^2}\right) + \sum_{i=2}^{i=i} \frac{n_p^{ai}}{n_p^c}\alpha_i\right) \times -J_a + \frac{L_p^1}{n_p^{a1} n_p^c}(\Delta G_{a1} - \Delta G_{a1}^{\#}) \quad (12)$$

An analogous formula can be obtained by eliminating ΔG_p after rewriting Eqn. 4 and substituting in Eqn. 8:

$$J_c\left(1 + \frac{L_p^1}{L_c(n_p^c)^2}\right) = \sum_{i=1}^{i=i} \frac{n_p^{ai}}{n_p^c} \times -J_{ai} + \frac{L_p^1}{(n_p^c)^2}(\Delta G_c - \Delta G_c^{\#}) \quad (13)$$

Substituting the parameters for the different cell components gives:

$$J_c\left(1 + \frac{L_p^1}{L_c(n_p^c)^2}\right) = \sum_{i=1}^{i=i} \frac{n_p^{ai}}{n_p^c}\alpha_i \times -J_a + \frac{L_p^1}{(n_p^c)^2}(\Delta G_c - \Delta G_c^{\#}) \quad (14)$$

Eqn. 12 will be used under catabolite-limited conditions because the anabolic substrates will be present in excess and the last term may be considered as a constant. Under anabolite-limited conditions, Eqn. 14 will be more appropriate because, under such conditions, the catabolic substrates will be present in excess and the last term may again be considered to be constant. The parameters α_i are experimentally determined.

Materials and Methods

Organism. In all experiments *Klebsiella aerogenes* NCTC 418 was used.

Growth conditions. All experiments were carried out in continuous culture as described before [10]. For the glucose-limited culture a simple salts medium was used as specified by Evans et al. [11] with 25 mM glucose as sole energy and carbon source. For the ammonia-limited culture the amount of glucose was the same but the concentration of ammonium chloride was decreased from 100 mM under glucose-limited conditions to 7.5 mM in an ammonia-limited culture. Bacterial dry weight was measured by the procedure of Herbert et al. [12].

Determination of cell components. Cells were divided into five different components: protein, DNA, RNA, lipid and polysaccharide. Protein was determined with the method of Lowry, modified according to Peterson [13]. Bovine serum albumin (Sigma) was used as a standard.

DNA was determined according to the method of Fiszer-Szafarz et al. [14]. As a standard purified DNA of *Escherichia coli* (Sigma) was used.

RNA was determined by the orcinol method as described by Herbert et al. [5].

Lipids were determined according to the procedure described by Herbert et al. [5], slightly modified as follows: cells were centrifuged and the pellets were resuspended in 5 ml 6 M HCl. This suspension was heated in boiling water for 1 h. After the samples had been cooled, 15 ml ethyl ether was added. The tubes were well shaken. The ethyl ether fraction was put in a weighed bottle. Extraction with ethyl ether was performed three times. After evaporation of the ethyl ether to the air, the bottles were dried over P_2O_5 and NaOH and then weighed again. The amount of lipids is given as the difference in weight of the bottles before and after the extraction.

Polysaccharides were determined by the anthrone colour reaction as described by Herbert et al. [5]. All cell components should sum up to a total of 100% of the dry weight; a margin of 10% in this value was considered acceptable. The amount of the various cell components was experimentally determined at different growth rates and the values incorporated in the refined model.

Results

In Fig. 1 the refined MNET model is shown for the case of $i = 5$ anabolic fluxes (see Theory section). The variation of the relative amount of cell components under glucose- and ammonia-limited conditions was investigated as a function of the growth rate of *K. aerogenes* NCTC 418 in the chemostat.

Samples were taken to determine the cell composition at various growth rates. Under glucose-limited conditions, the bacterial dry weight was 2 $g \cdot l^{-1}$ at all growth rates. Under ammonia-limited conditions, the bacterial dry weight was about 1 $g \cdot l^{-1}$ at all growth rates.

In Fig. 2 the results are shown of the determination of the various cell components in percentages of the bacterial dry weight as a function of the growth rate under glucose-limited conditions. Except for the RNA content, all other cell components show a decrease with increasing growth rate. The lines relating the protein, RNA, DNA, lipid and polysaccharide contents with the dilution rate (J_a) are (J values are given in percent; best fit by linear regression):

$$J_c = -16.6(\pm 3.5) \cdot J_a + 70.6(\pm 1.6) \text{ (protein)}$$

$$J_c = 19.9(\pm 2.3) \cdot J_a + 9.6(\pm 1.1) \text{ (RNA)}$$

$$J_c = -3.4(\pm 0.2) \cdot J_a + 5.3(\pm 0.1) \text{ (DNA)}$$

$$J_c = -3.9(\pm 0.8) \cdot J_a + 12.6(\pm 0.3) \text{ (lipid)}$$

$$J_c = -0.4(\pm 0.4) \cdot J_a + 2.7(\pm 0.2) \text{ (polysaccharides)}$$

Ammonia is used as a substrate for DNA, RNA and protein synthesis. Therefore, one might expect to find differences in cell composition under ammonia-limited conditions, as compared to glucose-limited conditions. To investigate whether the macromolecular cell composition of the cells was influenced by the type of limitation, *K. aerogenes* was also grown under ammonia-limited conditions. The results are shown in Fig. 3. The lines relating the protein, RNA, DNA, lipid and polysaccharide contents with the dilution rate (J_a) are, in this case (J values are given in percent):

$$J_c = -8.2(\pm 2.5) J_a + 69.9(\pm 1.1) \text{ (protein)}$$

$$J_c = 12.0(\pm 1.2) J_a + 4.5(\pm 0.6) \text{ (RNA)}$$

$$J_c = -2.1(\pm 0.2) J_a + 4.4(\pm 0.1) \text{ (DNA)}$$

$$J_c = 4.1(\pm 1.9) J_a + 7.5(\pm 0.9) \text{ (lipid)}$$

$$J_c = -11.2(\pm 1.1) J_a + 12.5(\pm 0.5) \text{ (polysaccharides)}$$

From these lines the fraction of each cell component at each growth rate can be calculated.

Stouthamer [15] calculated an ATP requirement for the synthesis of the various cell components. In Table I these data are given as a multiple of the ATP requirement for protein synthesis, and can be incorporated together with the data calculated from the experimentally determined cell components under glucose-limited conditions in Eqn. 12. We take protein synthesis as flux 1, because the relative proportion of protein is the most constant. Eqn. 14 is more useful under ammonia-limited

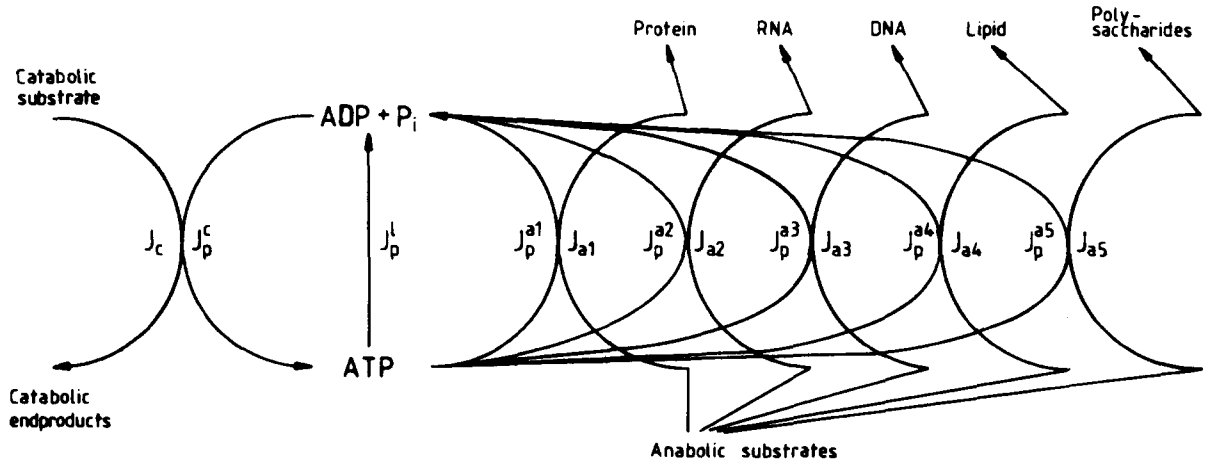

Fig. 1. Scheme of microbial growth (see text).

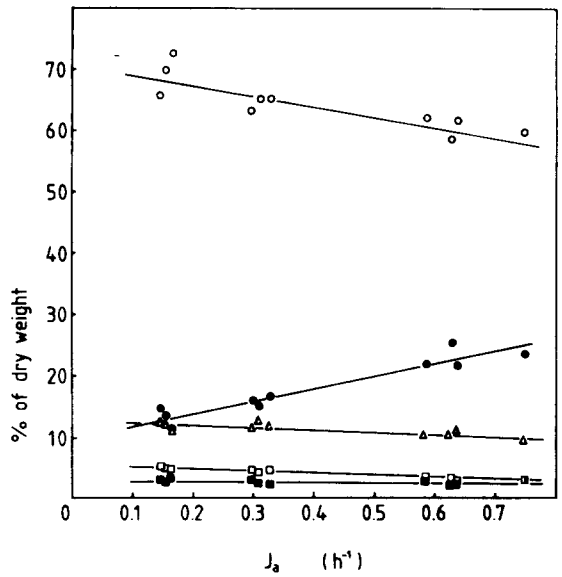

Fig. 2. Relationship of the various macromolecular cell components as a function of the dilution rate under glucose-limited conditions. ○, % protein; ●, % RNA; □, % DNA; △, % lipid; ■, % polysaccharides.

conditions for reasons mentioned in the Theory section and in this equation the data calculated from the experimentally determined cell compo-

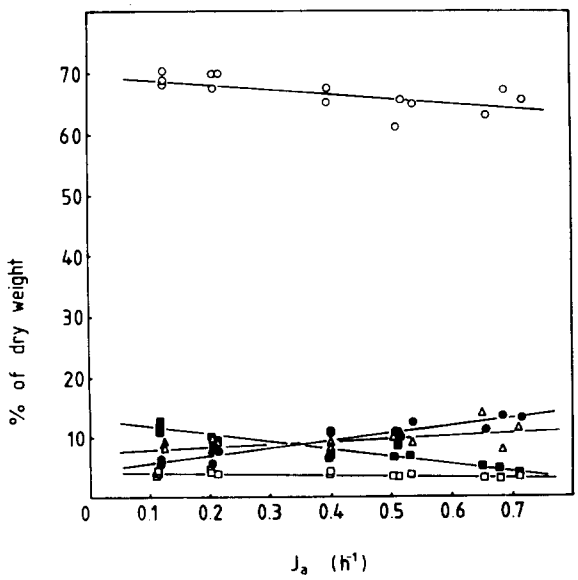

Fig. 3. Relationship of the various macromolecular cell components as a function of the dilution rate under ammonia-limited conditions. ○, % protein; ●, % RNA; □, % DNA; △, % lipid; ■, % polysaccharides.

TABLE I

ATP REQUIREMENT FOR THE FORMATION OF MACROMOLECULAR CELL COMPONENTS AS A MULTIPLE OF THE ATP REQUIREMENT FOR PROTEIN SYNTHESIS

Relative ATP requirements are calculated from Stouthamer [15], Table IV. 391.1 mol ($\times 10^{-4}$) ATP are required for the synthesis of 1 g of protein.

$n_p^{a1} = 1.0 \cdot n_p^{a1}$	protein
$n_p^{a2} = 0.95 \cdot n_p^{a1}$	RNA
$n_p^{a3} = 0.84 \cdot n_p^{a1}$	DNA
$n_p^{a4} = 0.04 \cdot n_p^{a1}$	lipid
$n_p^{a5} = 0.32 \cdot n_p^{a1}$	polysaccharides

nents under ammonia-limited conditions together with the data of Table I are incorporated.

Under glucose-limited conditions it could now be calculated that the slope of the line relating catabolism to anabolism according to the refined MNET model changed only slightly with increasing growth rate:
for $J_a = 0.1\ h^{-1}$

$$J_c = \frac{n_p^{a1}}{n_p^c}\left(0.85 + 0.67\frac{L_p^1}{L_{a1}(n_p^{a1})^2}\right) \times - J_a + \frac{L_p^1}{n_p^{a1} n_p^c}(\Delta G_{a1} - \Delta G_{a1}^{\#})$$

and for $J_a = 0.7\ h^{-1}$

$$J_c = \frac{n_p^{a1}}{n_p^c}\left(0.88 + 0.59\frac{L_p^1}{L_{a1}(n_p^{a1})^2}\right) \times - J_a + \frac{L_p^1}{n_p^{a1} n_p^c}(\Delta G_{a1} - \Delta G_{a1}^{\#})$$

Under ammonia-limited conditions, the change in the slope of the line relating catabolism to anabolism was also small:
for $J_a = 0.1\ h^{-1}$

$$J_c\left(1 + \frac{L_p^1}{L_c(n_p^c)^2}\right) = 0.82\frac{n_p^{a1}}{n_p^c} \times - J_a + \frac{L_p^1}{(n_p^c)^2}(\Delta G_c - \Delta G_c^{\#})$$

and for $J_a = 0.7\ h^{-1}$

$$J_c\left(1 + \frac{L_p^1}{L_c(n_p^c)^2}\right) = 0.81\frac{n_p^{a1}}{n_p^c} \times - J_a + \frac{L_p^1}{(n_p^c)^2}(\Delta G_c - \Delta G_c^{\#})$$

Discussion

The results presented in this paper show that the macromolecular cell composition is dependent on the limiting substrate of the culture, extending previous findings [1,5]. Under glucose-limited conditions, the cell does not have enough carbon substrate for the formation of storage materials. Therefore, the polysaccharide content of the cells was, as expected, maintained at a low level of 2–3% of the dry weight. Under ammonia-limited conditions, where there is enough carbon substrate present, the cells take up glucose and partly store it in the form of glycogen. At increasing growth rates more glucose is needed to maintain a sufficient energy level for growth and thus relatively less glucose is available for the formation of storage materials. Therefore, the amount of polysaccharides decreases with increasing growth rate down to a level found under glucose-limited conditions.

The DNA content of the cells (in mg per g dry weight) hardly changed with growth rate. The slight change that was observed may be due to varying cell size. The lipid content of the cells did not change much either and was about 10% of the dry weight. These results are in good agreement with previous findings of Herbert [1,2], Russell [3] and Bremer and Dennis [4].

From the linear regression equations one can calculate the percentage of the five different macromolecules at each growth rate. Combining these data with the formulae for the various macromolecules (see Introduction), we can calculate an empirical formula for the biomass. For the glucose-limited culture one obtains $C_4H_{7.3}O_{1.7}N_{1.05}P_{0.1}$ at growth rate 0.1 h^{-1} and $C_4H_{7.0}O_{1.9}N_{1.16}P_{0.16}$ at growth rate 0.7 h^{-1}. For the ammonia-limited culture one obtains $C_4H_{7.55}O_{1.7}N_{0.89}P_{0.07}$ and $C_4H_{7.36}O_{1.74}N_{0.98}P_{0.11}$ at a growth rates 0.1 h^{-1} and 0.7 h^{-1}, respectively. Evidently, the gross chemical composition of biomass differs not too strongly from that determined by Herbert et al. [5] ($C_4H_{7.8}O_{1.8}N_{1.0}P_{0.1}$), even though the relative contribution of different components changes significantly.

From Figs. 2 and 3 it can be concluded that the protein content showed relatively the smallest change with increasing growth rate. Therefore, we chose to express the ATP requirement of the other cell components relative to that for protein, in Eqn. 12. In this equation it is shown that the 'maintenance' term, i.e., substrate utilization at zero growth rate, is only dependent on the Gibbs' free energy difference of protein synthesis. The other parameters (L_p^1, n_p^{a1} and n_p^c) are considered to be constants.

Most classical growth models assume a constant cell composition with increasing growth rate. In a first approach, the MNET description for bacterial growth also assumed a constant cell composition. In this paper we have shown that the MNET description can be refined to incorporate the variations in cell composition with growth rate. From the more detailed equations, it must be concluded that no longer a linear relation exists between J_a and J_c if the cell composition varies.

In this paper we measured the cell composition at various growth rates and incorporated the values in the refined MNET model. From this, it is concluded that the change in cell composition fortunately only has a very small effect (in the order of a few percent) on the slope of the line describing the relation between catabolism and anabolism under our conditions. Only under very unusual circumstances, for instance under conditions were there is an excessive lipid production (see Table I), this change might be larger. It should be emphasized that the MNET model provides, to our knowledge, the only available method to draw this important quantitative conclusion.

Acknowledgements

The authors are indebted to Mr. A.F. Hartog for valuable suggestions for measuring lipid and DNA contents. This work was in part supported by the Netherlands Organisation for the Advancement of Pure Research (N.W.O.) under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).

References

1 Herbert, D. (1961) Symp. Soc. Gen. Microbiol. 11, 391–416.
2 Herbert, D. (1976) A publication for the society of Chemical Industry London (Dean, A.C.R., Ellwood, D.C., Evans, C.G.T. and Melling, J, eds.), pp. 1–30, Ellis Horwood Publisher, Chichester.

3 Russell, J.B. (1986) J. Bacteriol. 168, 694–701.
4 Bremer, H. and Dennis, P.P. (1987) in *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology. (Neidhardt, F.C., Ingraham, J.L., Brookslow, K., Magasanik, B., Schaechter, M. and Umbarger, H.E. eds.), American Society for Microbiology, Vol. 2, pp. 1527–1524, Washington, DC.
5 Herbert, D., Phipps, P.J. and Strange, R.E. (1971) in Methods in Microbiology (Norris, J.R. and Ribbons, D.W., eds.), Vol. 5b, 209–344, Academic Press, London.
6 Westerhoff, H.V., Lolkema, J.S., Otto, R. and Hellingwerf, K. (1982) Biochim. Biophys. Acta 683, 181–220.
7 Westerhoff, H.V. and Van Dam, K. (1987) Thermodynamics and Control of biological Free-energy Transduction, Elsevier, Amsterdam.
8 Van Dam, K., Mulder, M.M., Teixeira de Mattos, M.J. and Westerhoff, H.V. (1988) in Physiological models in Microbiology, CRC Series Mathematical Models in microbiology (Prosser, J. and Bazin, M.J., eds.), Vol. 1, pp. 25–48.
9 Mulder, M.M. (1988) Ph. D. Thesis, University of Amsterdam, Elinkwijk, Utrecht.
10 Mulder, M.M., Teixeira de Mattos, M.J., Postma, P.W. and Van Dam, K. (1986) Biochim. Biophys. Acta 851, 223–228.
11 Evans, C.G.T., Herbert, D. and Tempest, D.W. (1970) in Methods in Microbiology (Norris, J.R. and Ribbons, D.W., eds.), Vol. 2, 277–327, Academic Press, London.
12 Herbert, D., Phipps, P.J. and Tempest, D.W. (1965) Laboratory Practice 14, 1150–1161.
13 Peterson, G.L. (1977) Anal. Biochem. 83, 346–356.
14 Fiszer-Szafarz, B., Szafarz, D. and Guevara de Murillo, A. (1981) Anal. Biochem. 110, 165–170.
15 Stouthamer, A.H. (1977) Symposium of the Society for Gen. Microbiol. Microbial energetics (Haddock, B.A. and Hamilton, W.A., eds.), Vol. 27, pp. 285–315, Cambridge University Press, Cambridge.