A theoretical study on the amount of ATP required for synthesis of microbial cell material

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The amount of ATP required for the formation of microbial cells growing under various conditions was calculated. It was assumed that the chemical composition of the cell was the same under all these conditions. The analysis of the chemical composition of microbial cells of Morowitz (1968) was taken as a base. It was assumed that 4 moles of ATP are required for the incorporation of one mole of amino acid into protein. The amount of ATP required on account of the instability and frequent regeneration of messenger RNA was calculated from data in the literature pertaining to the relative rates of synthesis of the various classes of RNA molecules in the cell. An estimate is given of the amount of ATP required for transport processes. For this purpose it was assumed that 0.5 mole of ATP is necessary for the uptake of 1 g-ion of potassium or ammonium, and 1 mole of ATP for the uptake of 1 mole of phosphate, amino acid, acetate, malate etc. The results of the calculations show that from preformed monomers (glucose, amino acids and nucleic acid bases) 31.9 g cells can be formed per g-mole of ATP when acetyl-CoA is formed from glucose. When acetyl-CoA cannot be formed from glucose and must be formed from acetate, Y_{ATP}^{MAX} is only 26.4. For growth with glucose and inorganic salts a Y_{ATP}^{MAX} value of 28.8 was found. Addition of amino acids was without effect on Y_{ATP}^{MAX} but addition of nucleic acid bases increased the Y_{ATP}^{MAX} value to that for cells growing with preformed monomers. Under these conditions 15–20% of the total ATP required for cell formation is used for transport processes. Much lower Y_{ATP}^{MAX} values are found for growth with malate, lactate or acetate and inorganic salts. During growth on these substrates a greater part of the ATP required for cell formation is used for transport processes. The calculated figures are very close to the experimental values found.

The interrelations between Y_{ATP}^{MAX} and Y_{ATP}, the specific growth rate (μ), the maintenance coefficient (m_e) and the P/O rate are given. From a review of the literature evidence is presented that these parameters may vary under different growth conditions. It is concluded that in previous studies on the relation between ATP production and formation of cell material these effects have unjustly been neglected.

INTRODUCTION

The relationship between ATP formation and formation of microbial cell material can be studied in two ways. In the experimental approach the amount of cell material formed during the conversion of a certain amount of substrate is measured. If the ATP yield during the conversion of this amount of substrate is known, Y_{ATP} can be calculated. This term was introduced by Bauchop and Elsden (1960) and was defined as the amount of dry weight of organisms produced per g-mole of ATP. At first it was thought that Y_{ATP} was a biological constant and that no more than about 10 g of cells could be obtained per g-mole of ATP (for reviews see Stouthamer, 1969; Payne, 1970; Forrest and Walker, 1971). However in a number of recent publications other Y_{ATP} values have been reported, e.g. for *Lactobacillus casei* (de Vries et al., 1970) and for *Zymomonas anaerobia* (McGill and Dawes, 1971). Therefore it cannot be maintained that Y_{ATP} is a general biological constant (Stouthamer and Bettenhaussen, 1973).

In the second approach the macromolecular composition of the cells is taken as a base and subsequently the amount of ATP required to form cell material from this composition is calculated. In the past, several authors have tried to perform such calculations. Gunsalus and Shuster (1961) calculated a value of 0.03 g-moles ATP per gram of cells formed from preformed monomers. This value was corrected by Forrest and Walker (1971) to 0.036 moles of ATP per gram of cells. The lower value found by Gunsalus and Shuster (1961) was mainly due to their assumption that three ATP were required per mole of amino acid incorporated into protein, whereas Forrest and Walker (1971) assumed an ATP requirement of 5 moles per amino acid. Furthermore Forrest and Walker (1971) concluded that provision of preformed monomers confers no energetic advantage to an organism, if it is capable of synthesizing monomers from hexose and simple inorganic salts.

Originally a wide gap existed between the Y_{ATP} values calculated on account of the amount of ATP required for the formation of cell material (Gunsalus and Shuster, 1961; Forrest and Walker, 1971) and the Y_{ATP} values determined experimentally. However microorganisms also need a certain amount of energy for maintenance (Pirt, 1965; van Uden, 1969). A new term has been defined, Y_{ATP}^{MAX}, which is the dry weight of organisms produced per g-mole of ATP after correction for the ATP required for maintenance (Stouthamer and Bettenhaussen, 1973). Only very few Y_{ATP}^{MAX} values have been determined till now (de Vries et al., 1970; Stouthamer and Bettenhaussen, 1973). The Y_{ATP}^{MAX} values are very close to those calculated theoretically (Gunsalus and Shuster, 1961; Forrest and Walker, 1971). This suggested that Y_{ATP}^{MAX} might be a biological constant and that differences in Y_{ATP} for various microorganisms were due to differences in main-

tenance coefficient and specific growth rate (Stouthamer and Bettenhaussen, 1973).

Unfortunately there are several errors in the previous theoretical calculations:

a. No account was made for the ATP required for transport processes. Nearly all nutrients are taken up in the cell from the extracellular medium by an active process, which requires a net input of energy.

b. New data have accumulated on the ATP required for polymerization reactions in the formation of cellular macromolecules.

c. In the calculation of the amount of ATP needed for the synthesis of monomers from glucose by known biosynthetic pathways the ATP produced by substrate phosphorylation during the conversion has not, or insufficiently been accounted for.

d. No account was made for the ATP required for NADPH formation from NADH by transhydrogenation.

Therefore new calculations are given in this paper. For the cell composition the very detailed analysis of Morowitz (1968) was used. For the biosynthetic pathways the books of Mandelstamm and McQuillen (1968) and of Mahler and Cordes (1966) were consulted together with a number of new review articles. Furthermore the influence of the composition of the medium on the amount of ATP required for cell synthesis was calculated. The results show that in previous studies this influence has strongly been neglected.

CALCULATIONS

Composition of microbial cells. The analysis of the composition of microbial cells by Morowitz (1968) is given in Table 1. The content of the various classes of macromolecules calculated from these data is given in Table 2.

The polysaccharide content was calculated as polyglucose, although it is known that the cells contain more complex polysaccharides, e.g., those present in the mucopeptide and in the lipopolysaccharide layers of the cell wall. This simplification does not much affect the figure calculated for the ATP required for the formation of polysaccharide.

In their calculation Forrest and Walker (1971) assumed that the lipid in the bacterial cell was a triglyceride with C20 fatty acids. However it seems more reasonable to assume that the lipid is phosphatidylethanolamine with 2C16 fatty acids, since it has been reported that this is the main phospholipid in gram-negative bacteria (Reaveley and Burge, 1972; Goldfine, 1972). The molecular weight of this compound is 670.

Table 1. Monomer composition of microbial cells (From data of Morowitz, 1968)

Compound	Amount (moles $\times 10^{-4}$/g)	Compound	Amount (moles $\times 10^{-4}$/g)
Alanine	4.54	Serine	3.02
Arginine	2.52	Threonine	2.52
Aspartate	2.01	Tryptophan	0.50
Asparagine	1.01	Tyrosine	1.01
Cysteine	1.01	Valine	3.02
Glutamate	3.53	Hexose	10.26
Glutamine	2.01	Ribose	4.47
Glycine	4.03	Deoxyribose	0.96
Histidine	0.50	Thymine	0.24
Isoleucine	2.52	AMP	1.40
Leucine	4.03	GMP	1.40
Lysine	4.03	CMP	1.40
Methionine	2.01	UMP	1.15
Phenylalanine	1.51	Glycerol	1.40
Proline	2.52	Fatty acid	2.80

Table 2. Content of various macromolecules calculated from the data of Table 1 in microbial cells.

Macromolecule	Amount (g/100 g cells)
Polysaccharide	16.6
Protein	52.4
Lipid	9.4
RNA	15.7
DNA	3.2
Total	97.3

The sum of the various macromolecules yields 97.3% (Table 2). It is known that the cells contain in addition about 1.5% of potassium and 0.2% of magnesium (Tempest, Dicks and Hunter, 1966). The phosphorus content of the cells (about 2.4%) exceeds that in phospholipid, RNA and DNA. Consequently we may conclude that the result of Table 2 which indicates that various macromolecules make up 97.3% of the total dry weight, is very satisfactory.

In the following sections an analysis will be given of the amount of ATP required to form cells of this fixed composition under various growth conditions.

The amount of ATP required for the formation of microbial cells from preformed monomers. The amount of ATP required for the formation of cell material from preformed monomers is given in Table 3. The ATP required for the formation of

the various macromolecules will be treated in the following sections. The results indicate that 26.4 g cells per g-mole of ATP can be formed from glucose, amino acids, nucleic acid bases and acetate and 31.9 g cells per g-mole of ATP from glucose, amino acids and nucleic acid bases. In the latter case acetyl-CoA necessary for lipid synthesis is formed from glucose. Although the calculations differ in many aspects from those of Forrest and Walker (1971) the final figures are similar.

The amount of ATP required for polysaccharide biosynthesis. The synthesis of polysaccharide from glucose has an ATP requirement of 2 moles/mole of glucose. Glucose transport in most microorganisms is accomplished by the phosphoenolpyruvate phosphotransferase system by which glucose is accumulated from the extracellular medium as glucose-6-phosphate (Kaback, 1970, 1972; Harold, 1972). However in some organisms in which the glycolytic system does not operate in the breakdown of glucose the phosphoenolpyruvate phosphotransferase system does not function. This has been shown for *Azotobacter vinelandii*, in which glucose uptake is dependent upon respiration (Barnes, 1972).

Table 3. ATP requirement for the formation of microbial cells from preformed monomers (glucose, amino acids and nucleic acid bases). Formation of lipids from acetate or glucose are separately considered.

Macromolecule	Amount of monomer moles × 10^{-4}/g cells	ATP required per monomer moles/mole		ATP required moles × 10^{-4}/g cells	
		Lipids formed from			
		Acetate	Glucose	Acetate	Glucose
Polysaccharide	10.26	2	2	20.52	20.52
Protein	47.85	4	4	191.40	191.40
Lipid	1.40	33	1	46.20	1.40
RNA[1]	4.60	5	5	23.00	23.00
DNA	0.96	6	6	5.76	5.76
Turnover RNA				13.90	13.90
Total				300.78	255.98
ATP required for transport of					
Amino acids				47.85	47.85
Acetate				22.40	–
Potassium ions				1.92	1.92
Phosphate				7.74	7.74
Total ATP requirement				380.69	313.49
$Y_{ATP}^{MAX} = \frac{10\,000}{\text{Total ATP requirement}}$				26.4	31,9

[1] For the calculation of the RNA content the UMP content (Table 1) has been taken as basis.

In this case more than 2 moles of ATP are required for the formation of polysaccharide from glucose. This latter possibility will not be treated in this paper. The amount of ATP required for polysaccharide formation is therefore polysaccharide content $\times$ 2. According to the data in Table 1 this amounts to 2 $\times$ 10.26 = 20.52 moles $\times$ 10^{-4}/g (Table 3).

The amount of ATP required for protein synthesis. For the calculation of the energy expenditure for protein biosynthesis Forrest and Walker (1971) assumed that 5 moles of ATP were required for the incorporation of 1 mole of amino acid into protein. This figure was based on utilization of three high-energy bonds in direct peptide-bond formation and utilization of two high-energy bonds for the regeneration of messenger-RNA, which has a very limited lifetime. In this paper it is assumed that 4 moles of ATP are needed for the incorporation of one mole of amino acid into protein: 1 ATP is involved in the activation of the amino acid and is converted into AMP, and 2 GTP are involved in peptide-bond formation on the ribosome (Lengyel and Söll, 1969; Lucas-Lenard and Lipmann, 1971). The ATP required for the rapid breakdown and regeneration of messenger RNA will be calculated separately when the amount of ATP needed for RNA synthesis is estimated. The requirement for ATP for the uptake of amino acids from the extracellular medium will also be treated separately. Consequently the ATP required for protein biosynthesis from preformed amino acids is 4 $\times$ total amino acid content. According to the data in Table 1, the protein in one g of cells contains 47.85 moles $\times$ 10^{-4} amino acids. The ATP required for protein biosynthesis is therefore 4 $\times$ 47.85 = 191.4 moles $\times$ 10^{-4} per g cells.

The amount of ATP required for lipid biosynthesis.

a. *Lipids synthesized from acetate.* The main phospholipid in the bacterial cell was assumed to be phosphatidylethanolamine with two C16 fatty acids. For the formation of this compound 33 moles of ATP are required. The formation of glycerolphosphate from glucose requires 1 mole of ATP, 2 are required for reconversion of CMP into CTP which is needed for the attachment of ethanolamine to the glycerolphosphate. The synthesis of a C16 fatty acid requires 8 moles of ATP for the conversion of acetate to acetyl-CoA and 7 moles of ATP for the elongation of acetyl-CoA to palmityl-CoA. Synthesis of two C16 fatty acids thus requires 30 moles of ATP. The amount of ATP required for the formation of ethanolamine is unknown, but may be neglected since this simple compound is present in small amounts. Furthermore 28 moles of NADPH are required per mole of lipid. Consequently the total amount of NADPH required for lipid biosynthesis is 39.2 g-moles $\times$ 10^{-4}/g cells. The calculations for the amounts of ATP required for NADPH formation and acetate transport will be dealt with separately.

b. *Lipids synthesized from glucose.* Most microorganisms can convert glucose into acetyl-CoA. Only among the homofermentative lactic acid bacteria some are known to require acetate as a growth factor. During the conversion of glucose into acetyl-CoA by the glycolytic system there is a net gain of 1 mole ATP per mole of acetyl-CoA formed. The formation of two C16 fatty acids is thus accompanied by a net gain of 2 moles of ATP. Consequently, the formation of 1 mole of phosphatidylethanolamine with two C16 fatty acids requires only 1 mole of ATP per mole.

In Table 3 therefore separate calculations have been given for the ATP required for lipid biosynthesis starting with either acetate or glucose. It is evident that these values differ greatly. Forrest and Walker (1971) calculated only the amount of ATP required for lipid synthesis from acetate. Since most microorganisms can synthesize acetyl-CoA from glucose, the lesser amount of ATP required for lipid biosynthesis seems to be more valid.

The amount of ATP required for RNA synthesis from nucleic acid bases. The present calculation differs considerably from that of Forrest and Walker (1971). They assumed three moles of ATP to be required for the conversion of a nucleic acid base to the corresponding trinucleotide with an average molecular weight of 300. The reaction sequence in the formation of a nucleoside triphosphate is however:

Ribose-5-phosphate + ATP → phosphoribosyl-pyrophosphate + AMP.

Nucleic acid base + phosphoribosyl-pyrophosphate → nucleosidemonophosphate + pyrophosphate.

Purine transport has been shown to occur by a group translocation mechanism (Kaback, 1972; Harold, 1972). Thus nucleic acid bases are converted to the nucleoside monophosphate by adenine phosphoribosyltransferase concurrently with the transport inside the cell. Therefore we assume that 5 moles of ATP are required for the incorporation of 1 mole of nucleic acid base into RNA: 3 moles are needed for the formation of phosphoribosyl-pyrophosphate and 2 for the conversion of the nucleoside monophosphate into the corresponding nucleoside triphosphate. The data in Table 1 indicate that 4.60×10^{-4} moles of nucleic acid base are incorporated into RNA per g of bacterial cells. Consequently, for the synthesis of RNA 23 moles $\times 10^{-4}$ of ATP/g cells are required (Table 3).

For estimating the influence of the decay of messenger RNA on the amount of ATP required for RNA synthesis the results of Norris and Koch (1972) were used. These authors concluded from their results that in batch cultures of *E. coli* 60% of the RNA formed was messenger RNA, which amounted to only 3–4.5% of the total amount of RNA in the cell. This small amount of messenger RNA relative to the total amount of RNA is neglected. The decaying messenger RNA yields nucleoside monophosphate. Consequently 2 moles of ATP are

consumed for the regeneration of nucleoside triphosphate needed for the synthesis of new messenger RNA. The amount of ATP needed for the decay of messenger RNA is therefore $^6/_4 \times 4.6 \times 2 = 13.90$ moles $\times 10^{-4}$ of ATP/g cells. Our estimate is much lower than the previous one of Forrest and Walker (1971), who calculated 109 moles $\times 10^{-4}$ of ATP per g cells. The latter value seems excessively high when it is taken into account that messenger RNA must be instable in order to enable the bacterial cell to rapidly regulate protein synthesis, thereby avoiding the biosynthesis of unnecessary proteins. The amount of ATP needed for messenger RNA synthesis as estimated by Forrest and Walker (1971) would be a high price for the regulation of protein synthesis. Its unlikeliness can also be deduced in another way. In messenger RNA three bases code for one amino acid. As indicated above the decay of messenger RNA yields nucleoside monophosphates, which need 2 moles of ATP for the conversion to nucleoside triphosphates. If 2 moles of ATP were needed to account for the instability of messenger RNA (Forrest and Walker, 1971), each molecule of messenger RNA would function only three times in protein biosynthesis. It is known that the actual figure is much higher.

The amount of ATP required for DNA synthesis from nucleic acid bases. Forrest and Walker (1971) assumed that four ATP are required to convert a nucleic acid base into a deoxytrinucleotide with an average molecular weight of 280. Since the conversion of a nucleic acid base into a deoxyribonucleoside triphosphate requires one more mole of ATP than the conversion of a nucleic acid base into a ribonucleosidetriphosphate, a total ATP requirement of 6 moles of ATP per mole of nucleic acid base incorporated into DNA was used in this paper. The data of Table 1 indicate that 0.96 $\times 10^{-4}$ g-moles of nucleic acid base are incorporated into DNA per gram of bacterial cells for which 5.76 g-moles $\times 10^{-4}$ of ATP are required.

The amounts of ATP required for transport processes. It is very difficult to give exact data for the amounts of ATP required for transport processes. According to recent views the uptake of amino acids, ammonium- and potassium ions and of inorganic phosphate is energy-dependent (Kaback, 1972; Harold, 1972). However there is no agreement on the mechanism. In membrane vesicles uptake of amino acids is linked to oxidation of D(–) lactate by the respiratory chain (Kaback, 1970, 1972). ATP derived from oxidative phosphorylation is *not* an intermediate in the coupling of respiration and transport in these membrane vesicles. According to Harold (1972) energy-rich intermediates or an energized state of the membrane are involved. This view is strongly supported by the failure to detect respiration-linked transport in mutants defective in oxidative phosphorylation and deficient in the Mg^{2+}–Ca^{2+}-activated ATPase. (Schairer and Haddock, 1972; Simoni and Shallenberger, 1972). This supports the orig-

inal conception of transport by chemiosmotic processes (Mitchell, 1970). According to the chemiosmosis hypothesis the hydrolysis of one mole of intracellular ATP is associated with the extrusion of two protons, by which a membrane potential is generated. Accumulation of K^+ and NH_4^+ is attributed to electrogenic porters. Consequently the uptake of two K^+ or NH_4^+ ions is associated with the hydrolysis of one mole of ATP. The uptake of phosphate is more complicated and requires 1 mole of ATP per mole of phosphate (Mitchell, 1970; Harold, 1972). The uptake of malate similarly requires 1 mole of ATP. However the amount of ATP associated with the uptake of one mole of amino acid is not yet known. Therefore we assume that 1 mole of ATP is required for the uptake of one mole of amino acid, acetate etc. Since the amounts of ATP required for transport processes are only estimates, they are given separately in the Tables 3, 5 and 6. This will make correction easier, when later more definite data for the amounts of ATP required for transport processes are available.

The amount of ATP required for NADPH synthesis. NADPH is needed for the formation of lipids from acetate and furthermore in the formation of amino acids from glucose and inorganic salts (see later). In higher organisms, the formation of NADPH from NADH is carried out by an energy-dependent transhydrogenase. In microorganisms NADPH is formed by the first two reactions of the hexose monophosphate pathway and by the isocitrate dehydrogenase. The study of mutants of *E. coli* blocked in the hexose monophosphate pathway has shown that the principal role of this pathway is the provision with NADPH (Fraenkel, 1968). In most organisms it can easily be calculated that the production of NADPH during glucose breakdown will be sufficient to meet the demands of biosynthesis. In *E. coli* an energy-dependent transhydrogenase is formed only during growth with glucose in minimal medium (Bragg, Davies and Hou, 1972) and is repressed by the presence of amino acids. A mixture of 2 amino acids gives already partial, and a mixture of 4 amino acids complete repression. From these data it can be calculated that only small amounts of ATP are required for NADPH formation. During growth on other substrates (lactate, acetate and malate) sufficient NADPH may be produced by the isocitrate dehydrogenase.

Under some conditions, however, much more NADPH may be required. In *A. vinelandii* NADPH is the electron donor in nitrogen fixation (Benemann et al., 1971), however the transhydrogenase is not energy-dependent (Chung, 1970). The same properties have been reported for the enzymes of *Pseudomonas fluorescens* (Colowick et al., 1952) and *Chromatium* (Keister and Hemmes, 1966). These observations are the basis for our assumption that, in microorganisms, formation of NADPH to be used for biosynthetic processes does not, as a rule, require any ATP.

The amount of ATP required for the formation of microbial cells from glucose and inorganic salts. The amounts of ATP required for the formation of polysaccharide and lipid (when acetyl-CoA is formed from glucose) and for the turnover of messenger-RNA are equal to those required in the presence of preformed monomers. The amounts of ATP required for the formation of the monomers listed in Table 1 were calculated as the net change in the amount of ATP in the formation of these compounds (Table 4). For this purpose it was assumed that glucose is broken down along the glycolytic pathway. Only the change in ATP occurring under anaerobic conditions was calculated. Consequently only ATP formation from substrate phosphorylation has been considered. During some of the conversions NAD(P)H is produced, which may be oxidized under aerobic conditions. Oxidative phosphorylation may occur during this oxidation. However under aerobic conditions oxygen uptake during growth is mostly measured, and growth yields are expressed as Y_0 values (g dry weight of organisms/g-atom oxygen taken up) as described by Hadjipetrou et al. (1964) and Stouthamer (1969). Consequently ATP production during oxygen uptake in the formation of monomers is accounted for in this way.

The ATP requirement for the formation of amino acids and nucleoside monophosphates is given in Table 4. Unfortunately the results differ in many aspects from those of Forrest and Walker (1971). The complete calculation of the ATP required for synthesis of cell material from glucose and inorganic salts is given in Table 5.

The amount of ATP required for amino acid biosynthesis from glucose and inorganic salts. The calculation given in this paper differs from that of Forrest and Walker (1971) for the following amino acids: the aspartate family of amino acids (aspartate, asparagine, isoleucine, methionine, threonine), the glutamate family of amino acids (glutamate, glutamin, proline, arginine) and glycine, serine, cysteine, histidine. As examples the synthesis of serine and glycine which starts with 3-phosphoglycerate and the synthesis of asparate which starts with phosphoenol-pyruvate are mentioned. No net change in ATP occurs during the conversion of glucose into 3-phosphoglycerate (precursor of serine, glycine and cysteine) and phosphoenolpyruvate (precursor of aspartate) by the glycolytic pathway. Glutamate is formed from phosphoenolpyruvate, acetyl-CoA and carbon dioxide. During the formation of acetyl-CoA from glucose 1 mole of ATP is generated per mole of acetyl-CoA. Consequently the conversion of 1 mole of glucose into 1 mole of α-ketoglutarate is accompanied by an ATP gain of 1 mole of ATP. In the synthesis of arginine and uracil carbamylphosphate is involved; its synthesis in microorganisms needs 1 ATP in contrast to that in higher organisms, where 2 ATP are required (Mahler and Cordes, 1966). In the synthesis of arginine acetyl-CoA is converted into acetate. The formation of

Table 4. ATP formation or consumption during the formation of precursors for the synthesis of macromolecules from glucose and inorganic salts by known biochemical pathways.

Precursor	ATP formed (+) or consumed (−) moles/mole precursor	Amount (g-moles × 10^{-4} /g cells)	Total ATP (g-moles × 10^{-4} /g cells)
Alanine	+1	4.54	+4.54
Arginine	−3	2.52	−7.56
Aspartate	0	2.01	0
Asparagine	−2	1.01	−2.02
Cysteine	−3	1.01	−3.03
Glutamate	+1	3.53	+3.53
Glutamine	0	2.01	0
Glycine	0	4.03	0
Histidine	−7	0.50	−3.50
Isoleucine	−1	2.52	−2.52
Leucine	+3	4.03	+12.09
Lysine	0	4.03	0
Methionine	−4	2.01	−8.04
Phenylalanine	−2	1.51	−3.02
Proline	0	2.52	0
Serine	0	3.02	0
Threonine	−2	2.52	−5.04
Tryptophan	−5	0.50	−2.50
Tyrosine	−2	1.01	−2.02
Valine	+2	3.02	+6.04
Total amino acids			−13.55
AMP	−10	1.15	−11.50
GMP	−11	1.15	−12.65
CMP[1]	−5	1.15	−5.75
UMP	−4	1.15	−4.60
Total RNA precursors			−34.50
dAMP[1]	−11	0.24	−2.64
dGMP[1]	−12	0.24	−2.88
dCMP[1]	−6	0.24	−1.44
dTMP	−7	0.24	−1.68
Total DNA precursors			−8.64

[1] Calculated as the nucleoside monophosphates, although some are directly formed as the corresponding di- or triphosphates inside the cell.

1 mole of acetyl-CoA from glucose is associated with the net production of 1 mole of ATP. During the conversion of acetyl-CoA into acetate the energy-rich bond of acetyl-CoA is lost during arginine synthesis. Therefore no net change in ATP is assumed to occur during the conversion of acetyl-CoA into acetate. In

Table 5. ATP requirement for the formation of microbial cells from glucose and inorganic salts and the influence of various additions (amino acids and nucleic acid bases).

Macromolecule	ATP required (moles $\times 10^{-4}$/g cells)			
	No addition	Amino acids	Nucleic acid bases	Amino acids plus bases
Polysaccharide	20.52	20.52	20.52	20.52
Protein				
amino acid formation	13.55	0	13.55	
polymerisation	191.40	191.40	191.40	191.40
Lipid	1.40	1.40	1.40	1.40
RNA				
nucleoside monophosphate formation	34.50	34.50	13.80	13.80
polymerisation	9.20	9.20	9.20	9.20
DNA				
deoxynucleoside monophosphate formation	8.64	8.64	3.84	3.84
polymerisation	1.92	1.92	1.92	1.92
Turnover mRNA	13.90	13.90	13.90	13.90
Total	295.03	281.48	269.53	255.98
ATP required for transport of				
Ammonium ions[1]	42.42	10.41	32.0	0
Amino acids	0	47.85	0	47.85
Potassium ions	1.92	1.92	1.92	1.92
Phosphate	7.74	7.74	7.74	7.74
Total ATP requirement	347.1	349.4	311.19	313.49
Y_{ATP}^{MAX}	28,8	28,6	32,1	31,9

[1] On basis of the data in Table 1 the N content of the cells is 11.8%. Per g cells there are 64.0 g-atom N $\times 10^{-4}$ in amino acids and 20.84 g-atom N $\times 10^{-4}$ in nucleic acids.

the synthesis of histidine one mole of 5-amino-1-(5′-phosphoribosyl)- imidazole-4-carboxamide is formed from ATP. The amount of ATP needed to convert this compound again into ATP is added to the requirement calculated for histidine biosynthesis. For the formation of the amino acid mixture from glucose and inorganic salts a requirement of 110.3 moles $\times 10^{-4}$ NADPH per g cells can be calculated. As stated earlier no ATP requirement is assumed for the formation of NADPH in cells growing with glucose and inorganic salts. The uptake of ammonium ions, needed for the formation of amino acids and nucleic acid bases, is assumed to require, as mentioned earlier, 0.5 mole of ATP per mole of ammonia.

The amount of ATP required for nucleic acid synthesis from glucose and inorganic salts. Forrest and Walker (1971) calculated that 12 moles of ATP are required for the formation of 1 mole of AMP from glucose and inorganic salts. In the present paper only a requirement of 10 moles is calculated, since no allowance has been made for the ATP needed for the generation of formyl- and

methenyl-tetrahydrofolates. These are formed in sufficient amounts during the conversion of serine into glycine to make possible the formation of methionine, purines and thymine. The amount of ATP needed for the formation of the nucleoside monophosphates from glucose is included in Table 4. For the formation of RNA and DNA these nucleoside monophosphates must be converted into the corresponding triphosphates, for which 2 moles of ATP are required.

Influence of additions to the growth medium on the amount of ATP required for the formation of microbial cells. The amount of ATP required for the formation of microbial cells from glucose and inorganic salts is given in Table 5 (first column). It is evident that nearly the same result is obtained as when all monomers are provided (Table 3; last column, acetyl-CoA formed from glucose). However, the amount of ATP required for the formation of the various macromolecules under the latter conditions differs a great deal from that required under the former. Most markedly it differs for the formation of nucleic acids: in the presence of monomers (Table 3) RNA and DNA synthesis requires 28.76 moles $\times 10^{-4}$, and in the presence of glucose and inorganic salts (Table 5) 54.26 moles $\times 10^{-4}$ of ATP per g cells. The amounts of ATP required for transport processes are about the same under both conditions, though different nutrients are transported.

When various nutrients are added to the minimal medium two effects may be expected:

a. A larger part of the glucose may be broken down and less will be used for the formation of cell material. Consequently the total ATP production from the amount of glucose provided will increase.

b. The amount of ATP required for the formation of the cellular macromolecules stands midway between that in the second column of Table 3 and that in the first column of Table 5. The results are shown in Table 5. It is clear that the addition of nucleic acid bases considerably decreases the amount of ATP needed for cell synthesis. The effect of the addition of amino acids however is negligible. These results thus disagree with the conclusion of Forrest and Walker (1971) that provision of preformed monomers confers no energetic advantage to an organism if it is capable of synthesizing monomers from hexose and simple inorganic salts.

Formation of cell material from other substances. For this purpose it is necessary to calculate the amount of ATP needed for the synthesis of all the listed cell components from the substance involved. Therefore it is necessary to know the pathway by which the relevant substance is assimilated. However there is one difference between growth with glucose and growth with other substances as carbon source. Glucose is converted into glucose-6-phosphate during uptake;

whereas the uptake of most other substances is energy-dependent. As examples, the formation of cell material from lactate, malate and acetate will be treated in this paper. Lactate is converted into pyruvate and the formation of many components from pyruvate involves reversal of the glycolytic system. The first reaction in this process is the pyruvate kinase reaction: pyruvate + ATP → phosphoenolpyruvate + AMP + inorganic phosphate. Acetate is assimilated by the glyoxylate cycle. The formation of one mole of C_4 dicarboxylic acid requires two moles of acetyl CoA for which 4 ATP are required, two for the transport of acetate and two for its conversion into acetyl-CoA. The results of these calculations are given in Table 6. The amount of ATP required for the formation of cell material from lactate is much higher than for the formation from glucose. Similarly more ATP is needed for the formation of cell material from acetate than from lactate. The amount of these substrates needed for the formation of all of the compounds listed in Table 1 was calculated. This is the amount of substrate which must be transported into the cell. Therefore it is

Table 6. ATP requirement for the formation of microbial cells from lactate, malate or acetate and inorganic salts.

Macromolecule	ATP required (moles × 10^{-4}/g cells)		
	Lactate	Malate	Acetate
Polysaccharide			
G6P formation	61	41	82
polymerisation	10	10	10
Protein			
amino acid formation	148	94	236
polymerisation	191	191	191
Lipid	27	25	50
RNA			
nucleoside monophosphate formation	62	47	78
polymerisation	9	9	9
DNA			
deoxynucleoside monophosphate formation	14	11	17
polymerisation	2	2	2
Turnover mRNA	14	14	14
Total	538	444	689
ATP required for transport of			
Carbon source	148	148	254
Ammonium ions	42	42	42
Potassium ions	2	2	2
Phosphate	8	8	8
Total ATP requirement	738	644	995
Y_{ATP}^{MAX}	13.4	15.4	10.0

evident that with these substrates more ATP is required for transport processes than during growth with glucose.

DISCUSSION

In this paper the results of a theoretical study on the amount of ATP required for cell synthesis are given. A considerable improvement over previous calculations (Gunsalus and Shuster, 1961; Forrest and Walker, 1971) was obtained. One should realize that there are a number of uncertainties in the calculations. Especially as regards the amount of ATP required for transport processes we need more data; when these have become available the calculations may need revision. However, from these calculations a number of figures emerge which can be verified experimentally and may then contribute to our knowledge of the relation between ATP production and formation of cell material.

For all these calculations it was assumed that the chemical composition of the cells is not changed by changes in the growth conditions, though it is known that it may vary. As long as no large amounts of storage materials are formed, such changes will only slightly influence Y_{ATP}^{MAX}. It is evident however that differences in cell composition of different species may be reflected by differences in Y_{ATP}^{MAX} values.

Here it seems appropriate to compare some values calculated in this paper with the experimental Y_{ATP}^{MAX} values. For *L. casei* a value of 24.3 was found (de Vries et al., 1970), which is very close to the figure (26.4) in Table 3 (first column) for an organism growing on a medium with preformed monomers. For *A. aerogenes*, growing in minimal medium with glucose, values of 25.4 and 27.8 were found, which are close to the figure in Table 5.

We shall now discuss the various factors on which the growth yield depends.

In a previous communication (Stouthamer and Bettenhaussen, 1973) the following equation was derived:

$$q_{ATP} = \frac{\mu}{Y_{ATP}^{MAX}} + m_e = \frac{\mu}{Y_{ATP}}$$

in which μ = specific growth rate (hr^{-1}),
q_{ATP} = the specific rate of ATP production (g-moles ATP/g dry weight of organisms/hr),
Y_{ATP}^{MAX} and Y_{ATP} as defined previously,
m_e = maintenance coefficient (g-moles ATP/g dry weight of organisms/hr).

For an aerobic organism, converting glucose by the glycolytic system and

excreting some acetate under aerobic conditions the equation

$$q_{ATP} = 2q_{glu} + q_{ac} + q_{o_2} \cdot 2P/O$$

can be given, in which q_{glu} = the specific rate of glucose catabolism (g-moles glucose/g dry weight of organisms/hr),
q_{ac} = the specific rate of acetate production (g-moles acetate/g dry weight of organisms/hr),
q_{o2}, the specific rate of oxygen uptake (g-moles oxygen/g dry weight of organisms/hr),
P/O = the amount (moles) of inorganic phosphate esterified per g-atom of oxygen taken up. In the paper cited above it was concluded that at all growth rates q_{ATP} is carefully adjusted to meet the ATP requirement for cell formation and maintenance.

For substates which do not yield ATP by substrate phosphorylation in catabolism the relation:

$$q_{ATP} = \frac{\mu.P/O}{Y_O} = \frac{\mu}{Y_{ATP}^{MAX}} + m_e$$

may be given.

Therefore the molar growth yield is dependent on the following parameters: μ, Y_{ATP}^{MAX}, m_e and P/O. These parameters are constants for a given growth condition but may differ under different growth conditions. Influences of growth conditions on these parameters will be treated separately.

Influence of growth conditions on Y_{ATP}^{MAX}. In this communication it was shown on theoretical grounds that Y_{ATP}^{MAX} is dependent on the composition of the medium and can vary from 28.6 to 32.1 (Table 5) with glucose as carbon source and with various supplements.

Previously, Y_0 values have been determined for a large number of substrates (Stouthamer, 1969; Payne, 1970). It was thought that the P/O ratio could be determined by the equation Y_0/Y_{ATP} = P/O. It is now evident that Y_{ATP} is dependent on μ (Stouthamer and Bettenhaussen, 1973) and that Y_{ATP}^{MAX} is dependent on the growth substrate (Table 6). Consequently, previous determinations of P/O ratios from molar growth yields were wrong, because the influence of m_e and changes in Y_{ATP}^{MAX} were not taken into account (Stouthamer and Bettenhaussen, 1973). For citric acid cycle intermediates lower Y_0 values have been found than for sugars (Hadjipetrou et al., 1964; Stouthamer, 1969), which is in accordance with the higher Y_{ATP}^{MAX} values for the latter substrates. The influence of growth rate and growth condition on Y_0 values has been studied by Hernandez and Johnson (1967) for *Candida utilis*. Their results are reproduced in Table 7. Addition of amino acids little affects the Y_0 value with glucose, whereas addition of amino acids to cells growing with ethanol and acetate affects it greatly. These

results are in accordance with the calculations given in Table 5 and Table 6, which predict that the addition of amino acids will have no effect on the ATP requirement for cell formation from glucose and inorganic salts (Table 5) and that large amounts of ATP will be required to form amino acids from acetate (Table 6). Furthermore the low Y_0 values for acetate and ethanol are in accordance with the results of Hadjipetrou et al. (1964) and Stouthamer (1969).

Another example of the influence of the growth medium on Y_{ATP}^{MAX} is found with N_2-fixing microorganisms. With these organisms the growth yield is much less under conditions of N_2-fixation than under conditions of ammonia assimilation (Hill, Drozd and Postgate, 1972). This is due to the large amount of ATP required for N_2-fixation, which gives a strongly decreased Y_{ATP}^{MAX}.

The influence of growth rate and carbon source on molar growth yields has been studied by Kapralek (1972) in *Citrobacter freundii*. In contrast with the assumption of Kapralek (1972), it was calculated that the net ATP production in this organism is 3 moles/mole glucose or galactose and 1 mole/mole pyruvate. These figures agree with those found by Stouthamer and Bettenhaussen (1972) for *Proteus mirabilis* growing in complex medium. With the equations given earlier a m_e value of 0.017–0.018 g-moles ATP/g dry weight of organisms/hr can be calculated from the data for glucose ($\mu = 0.51$; $Y_{ATP} = 15.0$) and galactose ($\mu = 0.35$; $Y_{ATP} = 12.4$), assuming that $Y_{ATP}^{MAX} = 31.9$ (Table 5, last column). With this value for m_e it can be calculated that Y_{ATP}^{MAX} for pyruvate ($\mu = 0.42$; $Y_{ATP} = 10.8$) is about 20. This may be explained by more ATP being needed for polysaccharide biosynthesis, the synthesis of phosphoribosylpyrophosphate and synthesis of lipids during growth on pyruvate, amino acids and nucleic acid bases than during growth on glucose. Similarly it has been observed that Y_{ATP} for *Aerobacter aerogenes* with pyruvate and citrate (Stouthamer, unpublished results) is much smaller than Y_{ATP} for glucose (Hadjipetrou et al., 1964). In the former case large amounts of ATP are required for amino acid and nucleic acid synthesis, resulting in low Y_{ATP}^{MAX} and Y_{ATP} values.

Table 7. Influence of growth rate and growth conditions on Y_0 values reported for *Candida utilis*. Data from Hernandez and Johnson (1967).

Growth condition	Y_0 (g cells/g atom O)	μ (hr^{-1})
Glucose	21.1	0.30
Glucose + amino acids	21.6	0.69
Acetate	11.2	0.30
Acetate + amino acids	13.0	0.43
Ethanol	9.8	0.20
Ethanol + amino acids	14.4	0.38

For *Clostridium kluyveri* growing with ethanol and acetate a Y_{ATP}^{MAX} value of 15.4 has been calculated by Decker, Jungermann and Thauer (1970). This value too, is much lower than the figure given in Table 5 for growth with glucose. Therefore the influence of the composition of the growth medium on Y_{ATP}^{MAX} is well-documented in the literature.

Influence of growth conditions on maintenance coefficient. Previously it has been suggested that the maintenance coefficient is characteristic for an organism (Stouthamer and Bettenhaussen, 1973). The only factor detected so far to have a strong influence on the maintenance coefficient of *A. aerogenes* growing in minimal medium with glucose in excess, was the concentration of NH_4Cl (Stouthamer and Bettenhaussen, unpublished results). It was suggested that much of the maintenance energy was needed for the maintenance of the right ionic composition of the cells and the maintenance of the right intracellular pH (Stouthamer and Bettenhaussen, 1973) thus presumably for transport of ions. Furthermore a great difference was observed between the maintenance coefficients of carbon-limited and tryptophan-limited chemostat cultures of *A. aerogenes* (Pirt, 1965; Stouthamer and Bettenhaussen, 1973). More examples of such differences are reviewed by Stouthamer and Bettenhaussen (1973). Earlier it was reported that the maintenance coefficient of carbon-limited, nitrogen-fixing populations of *A. chroococcum* was dramatically different from that of comparable ammonia-utilizing organisms (Dalton and Postgate, 1969). In accordance with the high maintenance coefficient of nitrogen-fixing *Azotobacter* cultures only very low Y_0 values are found which strongly depend on the growth rate (Nagai, Nishizawa and Aiba, 1969).

Influence of growth conditions on the P/O ratio. In a previous communication it was pointed out that the P/O ratio may vary for the oxidation of different substrates (Stouthamer, 1969). In the respiratory chain 3 phosphorylation sites are present for the oxidation of NADH but only 2 for the oxidation of malate and succinate. During oxidation of glucose comparatively more NADH will be oxidized than during oxidation of citric acid cycle intermediates. Consequently the overall P/O ratio for the oxidation of glucose will be higher than for the oxidation of a citric acid cycle intermediate.

Furthermore the amount of ATP needed for the transport of a compound into the cell must be subtracted from the amount of ATP produced by oxidative phosphorylation. Moreover with acetate the amount of ATP needed to convert acetate into acetyl-CoA must be subtracted. From these considerations the total ATP production (substrate phosphorylation plus oxidative phosphorylation) assuming full energetic coupling at each phosphorylation site may be calculated. By dividing these values by the oxygen uptake the following P/O

ratios are found: glucose, 3.00; malate, 2.50; succinate, 2.43; acetate, 2.25. It is evident that for smaller substrates still lower overall P/O ratios will be obtained. Even if full energetic coupling at all phosphorylation sites does not exist the P/O ratios will depend on the substrate. McKechnie and Dawes (1969) have shown that the Y_0 values for growth of *Pseudomonas aeruginosa* on glucose, gluconate and 2-ketogluconate increase in that order, which is in agreement with the observation that glucose is partly converted into 2-ketogluconate by membrane-bound oxidases. In this case less phosphorylation sites are involved in the oxidation of glucose and gluconate than in the oxidation of 2-ketogluconate.

However there is another possible reason for variation in the P/O ratio in growing cultures. It is known that the respiratory chain in bacteria is branched (for review see White and Sinclair, 1971). In *Azotobacter vinelandii* one path to oxygen is associated with more oxidative phosphorylation than the other (Ackrell and Jones, 1971*a*). In the presence of excess oxygen, oxidative phosphorylation seems to be less efficient (Ackrell and Jones, 1971*b*). This is caused by absence of energetic coupling at phosphorylation site I and an increase in the concentration of cytochrome a_2, which is present in a branch of the respiratory chain in which no phosphorylation site is present (Jones et al., 1973).

Conclusions. From the study described in this paper it is clear that the growth parameters Y_{ATP}^{MAX}, m_e and P/O may vary strongly under different growth conditions. Therefore it is extremely difficult to determine these parameters. Continuous cultivation may yield a solution to this problem. The various parameters can be determined by the equations given above. However, as discussed earlier, all influences which might result in changes in Y_{ATP}^{MAX}, m_e and P/O must carefully be taken into account.

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