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Are Growth Rates of *Escherichia coli* in Batch Cultures Limited by Respiration?

KLAUS B. ANDERSEN†* AND KASPAR VON MEYENBURG‡

University Institute of Microbiology, Øster Farimagsgade 2A, DK-1353 Copenhagen K, Denmark

Batch cultures of *Escherichia coli* were grown in minimal media supplemented with various carbon sources which supported growth at specific growth rates from 0.2 to 1.3/h. The respiration rates of the cultures were measured continuously. With few exceptions, the specific rate of oxygen consumption was about 20 mmol of O_2/h per g (dry weight), suggesting that the respiratory capacity was limited at this value. The adenosine triphosphate (ATP) required for the production of cell material from the different carbon sources was calculated on the basis of known ATP requirements in the biochemical pathways and routes of macromolecular synthesis. The calculated ATP requirements, together with the measured growth rates and growth yields on the different carbon sources, were used to calculate the rate of ATP synthesis by oxidative phosphorylation. This rate was closely related to the respiration rate. We suggest that aerobic growth of *E. coli* in batch cultures is limited by the rate of respiration and the concomitant rate of ATP generation through oxidative phosphorylation.

Escherichia coli, like other bacteria, can grow on a variety of carbon substrates. When the bacteria are grown in batch cultures on the different substrates, exponential growth is observed with rates characteristic for the individual substrates (34). Since there is no external limitation on the rate of exponential growth, the resultant growth rates must be inherent to the bacterial metabolism.

In chemostat cultures, however, the experimenter controls the growth rate by the setting the dilution rate of the chemostat (17). Many parameters have been correlated with the growth rate, both in chemostat cultures and in batch cultures (24). However, the fundamental question of why a characteristic growth rate is established in a batch culture has not yet been fully answered.

In this study, the specific respiration rate was measured for *E. coli* growing aerobically on different carbon substrates in batch cultures. Furthermore, the energy balance for the various cultures was calculated. The parameters used in the calculations are given in Table 1.

MATERIALS AND METHODS

Organism. *E. coli* B/r strain NF790, a *tonA* (phage T1-resistant) derivative of wild-type CP14, was used. All cultures were grown at 37°C in minimal salt medium (136 mM Na^+, 30 mM NH_4^+, 22 mM K^+, 2.1 mM Mg^{2+}, 90 μM Ca^{2+}, 1.7 μM Fe^{3+}, 15 mM SO_4^{2-}, 42 mM $H_2PO_4^-$, 22 mM HPO_4^{2-}, and 56 mM Cl^-, pH 7.0) (5). The carbon source was added to 2 g/liter unless otherwise noted. The cultures were pregrown in the experimental substrate for at least 10 generations in flasks before the experiments were started by inoculating 10 liters of fresh media.

Cell density and specific growth rate. The cell density (X) was measured as the absorbance at 450 nm (A_{450}). Dense cultures were diluted to give readings below 0.4. The values were converted to bacterial dry weight (dw), using the factor 190 mg/liter A_{450}. This conversion factor was obtained in the following way. Culture samples were filtered onto preweighed filters (Sartorius membrane filter; 0.45 μm). The filters were then washed with 1 M HCOOH, dried at 105°C, and reweighed. The value showed only a slight variation between culture conditions. The specific growth rate, k, was defined according to the formula $X(t) = X_0e^{kt}$ and was calculated from semilogarithmic plots of cell density.

Culture vessels. For respiration, yield, and growth rate measurements, the cultures were grown in a 15-liter Microferm fermentor (New Brunswick Scientific Co., New Brunswick, N.J.) with 10 liters of medium. The fermentor was equipped with automatic pH control (Titrator TTT 2/PHA 942; Radiometer, Copenhagen, Denmark). The cultures were aerated with 0.4 liters of atmospheric air per liter of medium per min and rapidly agitated with propellers (500 rpm). The air flow was measured by a soap bubble flow meter, accurate to ±0.5%. The fermentor was inoculated with approximately 100 ml of culture having an optical density of 0.4.

Respiration measurements. The inlet and outlet air from the fermentor was, after passing through a drying column with $CaSO_4$, monitored for the content of O_2 and CO_2 with continuously recording gas ana-

† Present address: The Fibiger-Laboratory, Nordre, Frihavnsgade 70, DK-2000 Copenhagen Ø, Denmark.

‡ Present address: Department of Microbiology, The Technical University of Denmark, DK-2800 Lyngby, Denmark.

TABLE 1. *List of parameters*[a]

Symbol	Description	Unit
X	Cell density	Milligrams (dry wt)/liter of medium
k	Specific rate of exponential growth ($X = X_0e^{kt}$)	1/hour
Y_S	Growth yield on substrate	Grams (dry wt)/mole of substrate
Y_{SC}	Growth yield on substrate carbon	Grams (dry wt)/mole of substrate carbon
V_{O_2}	Rate of O_2 exchange in the fermentor	Millimoles of O_2/hour per liter of medium
V_{CO_2}	Rate of CO_2 exchange in the fermentor	Millimoles of CO_2/hour per liter of medium
Q_{O_2}	Specific rate of O_2 consumption	Millimoles of O_2/hour per gram (dry wt)
Q_{CO_2}	Specific rate of CO_2 production	Millimoles of CO_2/hour per gram (dry wt)
Q_S	Specific rate of substrate consumption	Millimoles of substrate/hour per gram (dry wt)
Q_{SC}	Specific rate of substrate carbon consumption	Millimoles of substrate carbon/hour per gram (dry wt)
Q_{CC}	Specific rate of carbon incorporation into cell material	Millimoles of carbon/hour per gram (dry wt)
Q_{BC}	Specific rate of by-product carbon production	Millimoles of by-product carbon/hour per gram (dry wt)
$Q_{acetate}$	Specific rate of acetate production	Millimoles of acetate/hour per gram (dry wt)
RQ	Respiratory quotient	Moles of CO_2/mole of O_2
BQ	By-product quotient	Moles of by-product carbon/mole of substrate carbon
CC	Carbon content of cells	Millimoles of carbon/gram (dry wt)
T	ATP utilization in transport	Moles of ATP/mole of substrate
C	ATP utilization in catabolism	Moles of ATP/mole of substrate
S	Substrate utilization in anabolism	Moles of substrate/gram (dry wt)
K	ATP utilization in the synthesis of key metabolites	Millimoles of ATP/gram (dry wt)
M	ATP utilization in the production of cell material from key metabolites	Millimoles of ATP/gram (dry wt)
Y_{ATP}(ox.phosp.)	Growth yield on ATP formed in the oxidative phosphorylation	Grams (dry wt)/mole of ATP
Q_{ATP}(ox.phosp.)	Specific rate of ATP production in the oxidative phosphorylation	Millimoles of ATP/hour per gram (dry wt)
P/O′	Apparent P/O ratio	Moles of ATP/mole of O

[a] For exponential balanced growth, the following relationship between a specific rate of metabolism of a compound i and the growth yield on the same compound exists: $Q_i = k/Y_i$.

lyzers as described by Fiechter and Meyenburg (10). Essentially similar systems have been described by others (7, 12, 16, 31). The instruments were a Magnos-2 oxygen analyzer and a Uras-2 carbon dioxide analyzer from Hartmann und Braun A. G., Frankfurt am Main, Germany.

The CO_2 analyzer measured the extinction of infrared light of the gas (21). N_2 and O_2 did not interfere with the CO_2 readings. The instrument had a reading span of 0 to 2 volume percent of CO_2, within which the measurements were obtained.

The O_2 analyzer measured the heat transfer of a wind created by a magnetic field (21). This wind was proportional to the content of O_2 in the gas (O_2 is paramagnetic); however, the heat transfer also depends upon the heat capacity of the gas. The heat capacities of N_2 and O_2 are almost identical, but that of CO_2 is higher (39). Accordingly, the O_2 analyzer was found to be sensitive to CO_2. The instrument had a reading span of 19 to 21 volume percent of O_2 in O_2-N_2 mixtures, within which the response was linear. In ternary mixtures of O_2, CO_2, and N_2, obtained with two gas-mixing pumps, the O_2 reading was 0.16 volume percent higher for each volume percent of CO_2 in the mixture. The oxygen readings were thus corrected for the influence of CO_2. For each experiment, the instruments were calibrated with air from a standard gas mixture (20.68 volume percent O_2 and 0.91 volume percent CO_2 in N_2; Messer Griesheim, Duisburg, Germany). To ensure the base line of the instruments, atmospheric air was let into the instruments at short periods every hour. Furthermore, the standard gas was let through the system after measurement of the cultures, and its readings differed less than 0.02 volume percent from the calibrated values.

Since the experimental design involves a rapid flow of gas through the fermentor, the recordings of the changes in the gas composition are a continuous mea-

sure of the rates of the gas exchange in the fermentor (10).

The consumption rate of O_2 (V_{O_2}) and the production rate of CO_2 (V_{CO_2}) were calculated as the molar differences between the flow of O_2 and of CO_2 into and out of the fermentor, expressed per liter of medium. The flow of nitrogen was assumed to be constant since *E. coli* does not metabolize N_2 (10, 31).

The calculated rates V_{O_2} and V_{CO_2} are a measure of the gas exchange of the fermentor (cells, medium, and air volume above the medium). We have tested the response time of changes in the composition of the gas let through the fermentor. The response was monitored 1 min after the change, whereafter the readings exponentially equilibrated to the new values of gas composition. The half-times for these equilibrations were 2 min for O_2 and 7 min for CO_2. Similar equilibration to new rates of gas exchange of the cultures can be seen in Fig. 1 after the different growth phases.

The response time was due to the travel time of the gas through the tubes of the system. A correction was made for the response time by moving the curves of the gas measurements 1 min ahead.

The equilibrations to new levels of V_{O_2} and V_{CO_2} were due to the volume of both the gas above the medium and the gas dissolved in the medium. Since a larger amount of CO_2 than O_2 can be dissolved in the medium (39), a larger equilibration time for CO_2 was observed.

A correction for the equilibration of the gas response was calculated and can be expressed by the following formulas:

$$V_{O_2}(\text{corrected}) = V_{O_2}(\text{observed}) + \frac{2 \text{ min}}{\ln 2} dV_{O_2}(\text{observed})/dt \quad (1)$$

$$V_{CO_2}(\text{corrected}) = V_{CO_2}(\text{observed}) + \frac{7 \text{ min}}{\ln 2} dV_{CO_2}(\text{observed})/dt \quad (2)$$

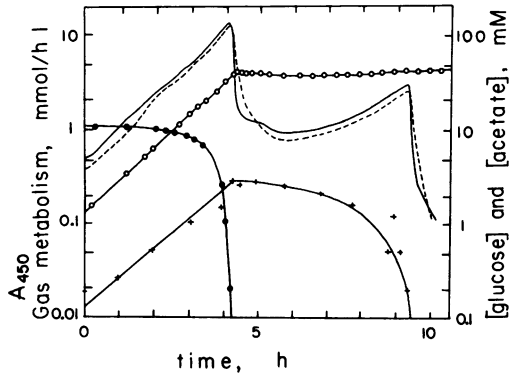

FIG. 1. *Semilogarithmic plot of the data obtained from a glucose batch culture of E. coli NF790. Symbols:* (——) *rate of oxygen consumption;* (- - -) *rate of carbon dioxide production;* (○) *optical density at 450 nm. The glucose concentration* (●) *was determined by a glucose oxidase assay (3), and the acetate concentration* (+) *was determined by an acetate kinase assay (4).*

If the rates of gas exchange are exponentially growing, i.e., ($V = V_0 e^{kt}$), the correction formula becomes:

$$V_{O_2}(\text{corrected}) = V_{O_2}(\text{observed}) \cdot \left(1 + \frac{k \cdot 2 \text{ min}}{\ln 2}\right) \quad (3)$$

$$V_{CO_2}(\text{corrected}) = V_{CO_2}(\text{observed}) \cdot \left(1 + \frac{k \cdot 7 \text{ min}}{\ln 2}\right) \quad (4)$$

For a glucose culture (k = 0.9/h), the correction factor thus becomes 1.04 for V_{O_2} and 1.15 for V_{CO_2}.

RESULTS

Measurements of growth, respiration, and by-product formation and utilization. A characteristic growth curve for a glucose batch culture is shown in Fig. 1. The curve exhibited three phases: (i) an exponential phase with respect to growth, O_2 utilization, CO_2 production, glucose utilization, and acetate production; (ii) a phase with very little growth during which acetate was utilized with concomitant O_2 consumption and CO_2 production; (iii) a phase with no growth and very low gas metabolism. This pattern of growth on glucose in batch culture agrees with previous reports (19). Not all cultures showed by-product formation and utilization. As an example, cultures growing on succinate showed an exponential phase followed directly by a phase with no growth and no gas metabolism.

As a control of the measurements, the total carbon balance for the cultures was calculated at the end of the by-product-utilizing phase (or at the end of the exponential phase if no by-product-utilizing phase was observed). Of the initially added substrate carbon, 90 to 100% could be accounted for as carbon in cell dry weight or as CO_2 formed.

During the exponential phase of growth, the cell density, rate of O_2 consumption, and rate of CO_2 production were balanced with respect to each other, as evidenced by the parallel course of the respective data in the semilogarithmic plot (Fig. 1). Also, the accumulation of acetate was balanced with the cell density (Fig. 1). The growth parameters of the different batch cultures are shown in Table 2.

Calculation of Q_{O_2} and Q_{CO_2}. The specific rates of oxygen consumption (Q_{O_2}) and CO_2 production (Q_{CO_2}) were calculated as the ratios of V_{O_2} and V_{CO_2}, respectively, and the cell density of the culture. The values were corrected according to equations 3 and 4. Q_{O_2} and Q_{CO_2} were found to be constant throughout the exponential phase of the various batch cultures analyzed.

Calculation of RQ. The respiratory quotient (RQ) was calculated as the ratio of the Q_{CO_2}

value to the Q_{O_2} value.

By-product formation. Cultures growing on glucose, galactose, glycerol, pyruvate, and lactate appeared to excrete by-products. This was indicated by a second growth phase during which the by-product(s) was utilized (see Fig. 1). We define the by-product quotient (BQ) as the molar amount of by-product carbon formed at the end of the exponential growth phase per mole of substrate carbon initially added to the culture. The amount of by-product carbon formed at the end of the exponential growth phase was calculated as the sum of the amount of CO_2 released and cell carbon formed in the by-product-utilizing phase.

In all cases, the by-product appeared to be acetate, as has previously been reported for growth on glucose (19). The concentration of acetate in the media was measured either by an acetate kinase assay (4) or by gas chromatography (8) and corresponded closely to the amount of CO_2 produced in the by-product-utilizing phase. During this phase the RQ was close to 1.0, a value typical for substrates such as acetate with an oxidation-reduction state of 0.0. Furthermore, several potential by-products (glycerol, lactate, pyruvate, ethanol, and acetate) were added to cultures in which respiration had ceased (Fig. 1). Of these, only acetate produced a Q_{O_2} value identical to the one measured during actual by-product utilization.

Specific growth rates. Table 2 shows the specific growth rates of batch cultures growing on a variety of carbon substrates. Cultures growing on sugars and sugar alcohols, which are believed to be degraded through glycolysis, had specific growth rates in the range of 0.7 to 1.0/h. An exception was the mannose culture, which had a specific growth rate of 0.4/h. Pyruvate and lactate enter glycolysis at a later step; these two substrates supported growth at a specific growth rates of about 0.4/h. Acetate, succinate, fumarate, and glycolate, which are degraded through the citric acid cycle, supported growth at low specific rates ranging from 0.2 to 0.4/h.

Specific respiration rates. Figure 2 shows the Q_{O_2} values for the various cultures in relation to the specific growth rate, k. Most cultures had Q_{O_2} values of approximately 20 mmol of O_2/h per g dw. Exceptions were the cultures growing on pyruvate or lactate, which had Q_{O_2} values around 30 mmol of O_2/h per g dw, and the culture growing on mannose, which had a low value of 7 mmol of O_2/h per g dw. No detectable by-products were produced from mannose in contrast to the other hexoses tested (Table 2).

Growth yields Y_S and Y_{SC}. Y_S and Y_{SC} were defined as the ratios of the cell mass formed at the end of the exponential growth phase to the molar amount of substrate added to the culture (35) and to the molar amount of substrate carbon added to the culture, respectively. The values from the fermentor cultures are shown in Table 2.

TABLE 2. *Growth parameters of E. coli growing on different carbon sources*[a]

Carbon source	No. of expt	k (1/h)	Y_{SC} (g [dw]/mol of SC)	Q_{O_2} (mmol of O_2/h per g [dw])	Q_{CO_2} (mmol of CO_2/h per g [dw])	RQ (mol of CO_2/mol of O_2)	BQ (mol of BC/mol of SC)
Single							
Glucose	6	0.89 ± 0.08	11.8 ± 1.5	19.8 ± 1.7	19.8 ± 1.8	1.01 ± 0.10	0.18 ± 0.04
Galactose	1	0.69	9.5	17.0	18.0	1.06	0.24
Mannose	1	0.38	12.0	6.8	7.1	1.05	0
Glycerol	2	0.87 ± 0.00	16.6	21.2 ± 2.5	14.3 ± 2.1	0.68 ± 0.02	0.02
Pyruvate	1	0.48	4.9	29.9	45.1	1.51	0.24
Lactate	1	0.52	7.8	32.3	31.9	0.99	0.12
Acetate	4	0.30 ± 0.09	11.7 ± 2.9	18.0 ± 2.9	18.2 ± 2.2	1.01 ± 0.04	0
Succinate	1	0.36	9.5	23.1	26.2	1.23	0
Fumarate	1	0.36	7.2	17.1	26.8	1.56	0
Glycolate	1	0.21	5.6	22.1	32.8	1.48	0
Mixed							
Glucose/casein hydrolysate	1	1.26	10.2 (glucose)	21.1	17.9	0.84	
Casein hydrolysate	1						
Phase I		0.92		24.3	34.7	1.42	
Phase II		0.92		24.0	28.4	1.18	
Glucose/mannose	1	0.76	11.0 (glucose)	18.3	17.8	0.97	
Glucose/acetate	1	0.92	15.7 (glucose)	21.0	21.0	1.00	
Glucose/lactate	1	0.87	15.0 (glycerol)	18.5	12.2	0.66	

[a] Parameters are defined in the text. Measurements of cultures growing on mixed carbon sources were performed in the phase where both carbon sources were present, and the growth yields for these cultures were determined for the carbon source which was exhausted first. The casein hydrolysate culture showed several phases of growth corresponding to exhaustion of the individual amino acids; the first two phases exhibited balanced exponential growth, and are shown in the table. SC, carbon source carbon; BC, by-product carbon.

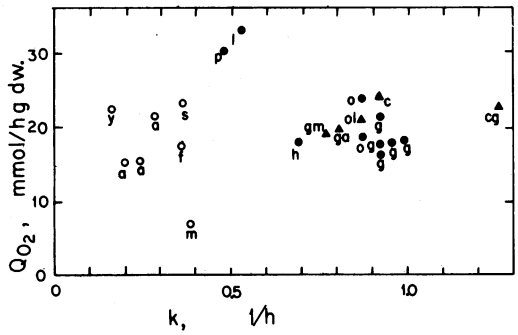

FIG. 2. *Specific oxygen consumption rate for batch cultures growing exponentially on various carbon sources, plotted against the specific growth rates. Symbols: (●) cultures in which a late phase of by-product utilization was observed; (○) cultures in which respiration stopped when the primary carbon source was used up; (▲) cultures in which by-product utilization could not be observed. Letters indicate carbon sources as follows: a, acetate; c, casein hydrolysate; f, fumarate; g, glucose; h, galactose; l, lactate; m, mannose; o, glycerol; p, pyruvate; s, succinate; y, glycolate. All compounds were added at 2 g/liter except casein hydrolysate, which was added at 4 g/liter. Double lettering shows that two different carbon sources were added together.*

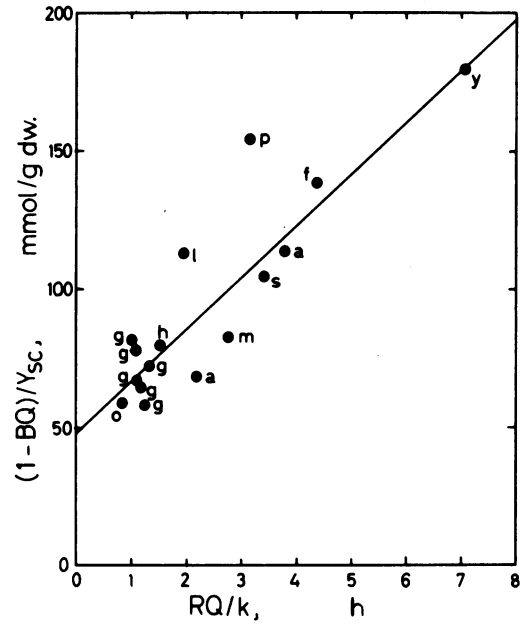

FIG. 3. *Corrected reciprocal growth yield (1 − BQ/Y_{SC}) plotted against RQ/k values for batch cultures growing on various carbon sources. The relationship is explained in equation 7 in the text, and the carbon sources are lettered as in Fig. 2.*

In general, Y_{SC} values were lower for cultures with low specific rates of growth (Table 2). This initially surprising relationship turned out to be consistent with the finding that Q_{O_2} was almost identical for these batch cultures growing with quite different specific growth rates (Table 2), as is explained below.

The carbon balance of the cultures can be expressed as

$$Q_{SC} = Q_{BC} + Q_{CC} + Q_{CO_2} \quad (5)$$

where Q_{SC} is the specific rate of substrate carbon consumption, Q_{BC} is the specific rate of by-product carbon production, which is equal to $BQ \cdot Q_{SC}$, and Q_{CC} is the specific rate of carbon incorporation into cell material. (All Q values are expressed as millimoles per hour per gram [dw].)

Since $Q_{BC} = BQ \cdot Q_{SC}$ and $Q_{CO_2} = RQ \cdot Q_{O_2}$, the equation can be rewritten as

$$Q_{SC} - BQ \cdot Q_{SC} = Q_{CC} + RQ \cdot Q_{O_2} \quad (6)$$

Furthermore, for balanced exponential growth, Q_{CC} is equal to $k \cdot CC$, where CC is the carbon content of cell material, i.e., 40 mmol of C/g (dw), and Q_{SC} is equal to k/Y_{SC}. Thus we arrive at the following equation:

$$(1 - BQ)/Y_{SC} = CC + Q_{O_2} \cdot RQ/k \quad (7)$$

Figure 3 shows a plot of the cultures of (1 − BQ)/Y_{SC} against RQ/k for the different cultures in Table 2. The line drawn in the figure was obtained by linear regression analysis, excluding the values from the lactate, pyruvate, and mannose cultures. The slope of the regression line was 19 mmol/h per g (dw), with an intercept at 47 mmol/g (dw) (the correlation coefficient [23] was 0.97).

The varying growth yields thus agree with the observation that Q_{O_2} is, for most cultures, approximately 20 mmol/h per g (dw), independent of growth rate. The oxidation-reduction state of the substrate influenced the growth yield. In other words, reduced substrates gave higher yields and oxidized substrates gave lower yields. In comparison with the relationship described above, the values of (1 − BQ)/Y_{SC} were higher than expected for the lactate and pyruvate cultures and lower than expected for the mannose culture. This reflects the high Q_{O_2} values of the lactate and pyruvate cultures and the low Q_{O_2} value for the mannose culture.

Rate of ATP production by oxidative phosphorylation. The ATP production rate was calculated from the known biochemical pathways of substrate degradation and of cell material production (Tables 3 and 4).

The ATP requirement for transport (T in Table 3) of glucose and mannose was assumed to be zero, since the transport of these two

TABLE 3. *Calculated parameters of ATP and carbon source utilization*[a]

Carbon source	T (mol of ATP/mol of CS)	C (mol of ATP/mol of CS)	S (mmol of CS/g [dw])	K (mmol of ATP/g [dw])	M (mmol of ATP/g [dw])
Glucose, mannose	0	−2	6.07	−2.11	36.8
Galactose	1	−2	6.07	−2.11	36.8
Glycerol	1	−1	12.14	−0.55	36.8
Pyruvate lactate	1	0	13.03	16.74	36.8
Acetate	1	1	22.44	30.38	36.8
Succinate, fumarate	1	0	12.14	8.85	36.8
Glycolate	1	0	23.12	8.85	36.8

[a] Parameters are defined in the text. Negative numbers in columns 2 and 4 denote production of ATP. CS, carbon source.

TABLE 4. *ATP metabolism of E. coli growing on different carbon sources*[a]

Substrate	No. of exp	Need of ATP for: Transport T/Y_S (mmol of ATP/g [dw])	Catabolism $C\ (1/Y_S - S)$ (mmol of ATP/g [dw])	Anabolism K (mmol of ATP/g [dw])	Anabolism M (mmol of ATP/g [dw])	$1/Y_{ATP}$ (ox.phosp.) (mmol of ATP/g [dw])	Q_{ATP} (ox.phosp.) (mmol of ATP/h per g [dw])	P/O' (mol of ATP/mol of O)
Glucose	6	0	−16.7 ± 3.7	−2.1	36.8	18.0 ± 3.7	16.6 ± 3.6	0.43 ± 0.09
Mannose	1	0	−15.6	−2.1	36.8	19.1	7.3	0.54
Galactose	1	17.5	−22.8	−2.1	36.8	29.4	20.4	0.60
Glycerol	1	20.1	−8.0	−0.6	36.8	48.3	42.1	0.83
Pyruvate	1	68.0	0	16.7	36.8	121.5	58.3	0.98
Lactate	1	42.7	0	16.7	36.8	96.2	50.1	0.77
Acetate	1	56.8	34.4	30.4	36.8	158.4	44.4	1.00
Succinate	1	26.3	0	8.9	36.8	72.0	25.9	0.56
Fumarate	1	34.7	0	8.9	36.8	80.4	29.0	0.85
Glycolate	1	89.3	0	8.9	36.8	135.0	28.4	0.64
Avg.								0.72

[a] ATP metabolism is calculated on basis of the values given in Tables 2 and 3. Positive numbers denote use of ATP, and negative numbers denote production of ATP in the pathway.

substrates is coupled to their phosphorylation (33), which was accounted for below. T was taken as 1 mol of ATP per mol of substrate for the other substrates, although transport for some substrates is directly coupled to the proton gradient of the membrane (33). The amount of proton gradient energy used in transport was lost for ATP generation, and we arbitrarily assumed that it corresponded to 1 mol of ATP per mol of substrate transported.

The ATP used or gained in catabolism per substrate molecule (C in Table 3) was calculated from data given in biochemistry textbooks (25). The general pathway was assumed to be glycolysis, which produces acetyl-coenzyme A. This metabolite can either be used in the citric acid cycle or hydrolyzed to acetate.

The ATP requirement in anabolism was calculated in two steps: (i) the ATP requirement or gain in the pathways converting the substrates to key metabolites (K in Table 3) and (ii) the ATP required for converting the key metabolites to cell material (M in Table 3). The key metabolites were: hexose phosphate, pentose phosphate, triose phosphate, glycerate phosphate, acetyl-coenzyme A, and citric acid cycle intermediates.

K was calculated on the basis of: (i) the amount of the different key metabolites required in the synthesis of 1 g (dw) (36) and (ii) the number of ATP molecules required to form each key metabolite from the substrate (25).

M is, by definition, identical for each substrate. Stouthamer has calculated the amount of ATP needed for the production of cell material (including the necessary mRNA) from glucose, based on known biochemical pathways (36). He found a value of 34.7 mmol of ATP/g (dw). Since the production of key metabolites from glucose has an ATP gain of 2.1 mmol of ATP/g (dw) (Table 3), M is 36.8 mmol of ATP/g (dw).

Table 3 also shows the total amount of substrate required in the synthesis of 1 g (dw) (S). This value was calculated on the basis of: (i) the amount of the different key metabolites required in the synthesis of 1 g (dw) (36) and (ii) the

number of substrate molecules required to form each key metabolite (25).

Table 4 gives the calculated minimal amount of ATP that must be produced by oxidative phosphorylation to form 1 g (dw) for each culture. This value, denoted as $1/Y_{ATP}$(ox.phosp.), is the sum of the amount of ATP needed for substrate transport, for catabolism, and for anabolism in the production of 1 g (dw). The amount of ATP needed for substrate transport is T times the total substrate uptake, $1/Y_S$ (Table 2). $1/Y_S$ minus the amount of substrate used in the anabolism (S in Table 3) is degraded in the catabolism; consequently $C(1/Y_S - S)$ is the amount of ATP needed (positive numbers) or produced (negative numbers) by catabolism. The amount of ATP needed in anabolism is the sum of K and M (Table 3).

Table 4 shows that $1/Y_{ATP}$(ox.phosp.) was very different for different substrates. Thus, growth on glucose required the formation of 18 mmol of ATP/g (dw) in oxidative phosphorylation, whereas growth on acetate required 158 mmol of ATP/g (dw). The value for growth on glucose or mannose was low because ATP was generated by glycolysis and because no ATP was needed for transport. The high value for glycolate cultures was mainly due to the expenditure for transport. In the case of acetate, the value was high because ATP was required not only for the transport but also for the activation of acetate. The specific rate at which ATP was produced by oxidative phosphorylation, Q_{ATP}(ox.phosp.), was calculated as k/Y_{ATP}(ox.phosp.) and is also shown in Table 4. With the exception of the mannose, pyruvate, and lactate cultures, these rates were within the range of 17 to 44 mmol of ATP/h g (dw).

The apparent efficiency of the oxidative phosphorylation (P/O′) was calculated as $Q_{ATP}(ox.phosp.)/2Q_{O_2}$ (Tables 2 and 4). The values of P/O′ ranged from 0.5 to 1.0 mol/mol, with an average value of 0.7 (Table 4). It is not clear whether the observed differences in P/O′ were real, substrate-induced differences or whether they reflected uncertainties of the basic measurements or assumptions of the calculation. However, the P/O′ ratio as well as Q_{O_2} varied little compared with the specific growth rate.

The equations $Q_{ATP}(ox.phosp.) = P/O \cdot 2Q_{O_2}$ and $Q_{ATP}(ox.phosp.) = k/Y_{ATP}(ox.phosp.)$ yield an expression for the specific growth rate,

$$k = Y_{ATP}(ox.phosp.) \cdot P/O \cdot 2Q_{O_2} \quad (8)$$

Since Q_{O_2} and P/O′ were found to be relatively constant for growth on the different carbon sources, we conclude that, to a first approximation, the specific growth rate is proportional to Y_{ATP}(ox.phosp.).

The calculations of the ATP metabolism need some comments.

(i) Our calculation of Y_{ATP}(ox.phosp.) may not be an accurate representation of the ATP metabolism in the cell, since it is based on the knowledge of the ATP stoichiometry of various reactions obtained from in vitro experiments, and since not all reactions in which ATP participates are necessarily known. The calculated values of the requirement for ATP thus represent minimum values. The maximum Y_{ATP} values for fermentative growth have been estimated experimentally, and there is a large discrepancy, which is not fully understood, between the experimental and theoretical values (6, 11, 36).

(ii) The value of P/O in vivo is also in dispute; different methods have yielded values ranging from 0.5 to 3 (14, 15, 27, 28).

(iii) Maintenance energy requirement was not accounted for in the calculations above. However, the maintenance oxygen requirement is generally found to be small compared with the Q_{O_2} values of batch cultures (9, 26, 27).

Therefore, our P/O′ values do not necessarily represent the true in vivo P/O ratio. However, values for P/O similar to ours have been obtained by analysis of the energetics of various microorganisms (2, 18, 31, 38).

Regulation of by-product formation. Formation of acetate during aerobic growth was observed only in cultures growing on glucose, glycerol, galactose, pyruvate, or lactate, all of which had Q_{O_2} values of 17 mmol of O_2/h per g (dw) or higher. In contrast, the mannose culture, which exhibited a lower Q_{O_2} value (7 mmol/h per g [dw]), did not accumulate acetate.

The regulation of acetate formation has previously been studied in chemostat cultures with substrate limitation (37). We have used α-methylglucoside as a regulator of glucose uptake (13, 22) in order to study acetate formation. α-Methylglucoside decreased the specific growth rate of glucose cultures (Fig. 4). The effect of α-methylglucoside was dual: first, the specific growth rate was slightly decreased from 0.97/h to 0.86/h at an α-methylglucoside/glucose ratio of 3.0; second, above this ratio the specific growth rate was markedly decreased. Thus, at a ratio of 5.0, the specific growth rate was decreased to 0.67/h.

Acetate accumulation was measured in the cultures where glucose uptake was inhibited to different degrees by α-methylglucoside (Fig. 4), and the specific rate of acetate production, $Q_{acetate}$, was calculated. Acetate production during growth on glucose was progressively inhibited by α-methylglucoside addition, and acetate production was no longer observed at inhibitor/substrate ratios above 3.0 (Fig. 4). Q_{O_2} was

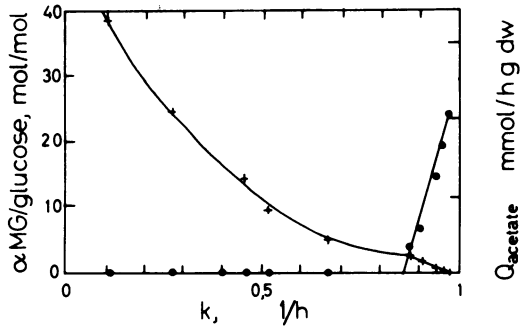

FIG. 4. *Effect of α-methylglucoside on growth rate and acetate production in glucose batch cultures. Cultures were grown in flasks in media supplemented with 2 mg of glucose per liter and various amounts of α-methylglucoside, and the acetate concentration was measured by gas chromatography (8). The molar ratio of α-methylglucoside to glucose (+) and the specific rate of acetate production (●) are plotted against the specific growth rate of the cultures.*

measured in parallel and found to be constant at approximately 22 mmol/h per g (dw) up to an inhibitor/substrate ratio of 3.0; at higher ratios, Q_{O_2} decreased in proportion to the decrease in growth rate (not shown).

The catabolism of glucose thus yields two products, acetate and CO_2. When glucose uptake was limited, the glycolytic pathway and the accompanying accumulation of acetate were quickly diminished. This pathway generates little ATP. The subsequent pathways leading to respiration and high yields of ATP begin to be restricted only after complete elimination of acetate formation. The difference in ATP yield per substrate molecule consumed in the respiratory and in the acetate-forming glucose catabolism offers a simple explanation of the observed bend in the curve of the specific growth rate versus the α-methylglucoside/glucose ratio (Fig. 4).

Cultures growing on mixed substrates. The addition of casein hydrolysate (mixture of amino acids) to a glucose culture did not change the Q_{O_2} value, but increased the specific growth rate (Fig. 2 and Table 2). Cultures growing in media supplemented with two substrates reached specific growth rates and Q_{O_2} values that were typical for the cultures growing on that substrate which alone supported the fastest growth (Table 2 and Fig. 2).

DISCUSSION

The Q_{O_2} value was measured for *E. coli* growing in batch cultures on a variety of substrates that supported growth at specific growth rates from 0.2 to 1.3/h. With few exceptions, the different cultures generated almost identical Q_{O_2} values of approximately 20 mmol of O_2/h per g (dw). The exceptions were the cultures growing on pyruvate or lactate, which had values around 30, and the culture growing on mannose, which had a value of 7 mmol of O_2/h per g (dw).

Limitation of respiration. The fact that the specific oxygen consumption rate for most cultures reached the same value may reflect a limitation in the respiration itself, or in the pathways connecting to it, which are common for all substrates tested (such as the citric acid cycle). However, a limitation in the capacity of the citric acid cycle would not be expected to lead to identical respiration rates, because varying amounts of NADH are produced outside this cycle on the different substrates. Thus, on acetate 100% and on glycerol only 50% of all NADH is generated in the citric cycle (25). We therefore believe that the limitation is in the respiratory system itself, i.e., in the respiratory chain or in the ATP synthetase, or both.

The higher respiration rates for cultures growing on lactate or pyruvate seem to contradict such a hypothesis. But these rates can possibly be explained by a partial oxidation through the membrane-bound pyruvate oxidase system, which is directly coupled to cytochrome b_1 in the respiration chain (29).

The mannose culture showed both a reduced respiration rate and a reduced specific growth rate compared with cultures growing on other hexoses. Furthermore, the mannose cultures did not accumulate acetate. In all these respects, the mannose culture resembled glucose cultures in which glucose uptake was limited by a α-methylglucoside. The exceptional behavior of mannose may, therefore, be explained by rate limitation on mannose uptake or on epimerase activity.

The following results appear to support the notion that respiration itself limits the growth rate.

(i) Coupling between growth and respiration. When the oxygen is removed from a culture growing on succinate (or another substrate which can only be utilized through respiration), the growth stops immediately (1). This indicates that the growth is very tightly coupled to the respiration.

On the other hand, respiration is not tightly coupled to anabolism as evidenced by the following observations. Addition of chloramphenicol (20) (which stops protein synthesis) or rifampin (30) (which stops RNA synthesis) stopped growth totally within 10 min, whereas the rate of respiration decreased slowly after inhibitor addition. Thus, half of the respiratory activity was remaining at 120 and 45 min after chloramphenicol and rifampin addition, respectively (1).

(ii) Production of by-products. The pathways leading to by-products are energetically unfavorable compared with complete oxidation of the substrate. If, however, respiration is limited, by-product formation can lead to extra ATP production and to faster growth, provided the by-product can be generated with a net gain of ATP. The results presented in Fig. 4 suggest that a culture with a capacity for degrading its substrate that cannot be matched by the rate of respiration will produce and excrete a by-product, from which pathway additional ATP may eventually be produced. An extreme case of aerobic growth with by-product formation has been observed in glucose cultures of *Saccharomyces cerevisiae* (2), where glycolytic ATP production, concomitant with ethanol production, becomes the predominant route of ATP generation at high rates of glucose catabolism and growth.

(iii) Growth in media with two different substrates. Cultures were grown on the following mixtures: glucose-mannose, glucose-acetate, and glycerol-lactate. The specific growth rate of these cultures was close to that obtained with the substrate, which by itself gave the higher specific growth rate. In all cases, the specific respiration rates were approximately 20 mmol of O_2/h per g (dw). Growth on two substrates thus does not increase respiration rates. An increase in Q_{O_2} might be expected if the pathways degrading the substrates were limiting and two substrates with different routes of degradation were added.

(iv) Growth in media with glucose and casein hydrolysate. Addition of casein hydrolysate to a glucose culture increased the specific growth rate from 0.9 to 1.4/h, whereas Q_{O_2} was unchanged. As pointed out by Stouthamer (36), ATP is not saved by adding amino acids to a glucose culture. The high growth rate must, therefore, be due to the ATP gained by glycolysis of the quantity of glucose which now is not needed as a substrate for amino acid biosynthesis.

Are the growth rates of *E. coli* limited by respiration? The data above show that respiration very likely limits the rate of metabolism. To estimate whether a limited capacity for respiration can account for the widely different growth rates obtained in cultures growing on different carbon substrates, the ATP metabolism of the cultures was calculated.

Calculation of the ATP metabolism revealed the existence of a tight relationship between the specific growth rate, the specific respiration rate, the efficiency of the oxidative phosphorylation, and the growth yield made possible by 1 mol of ATP generated by oxidative phosphorylation (equation 8).

Thus, this relationship supports the notion that the specific growth rate of batch cultures is limited by the specific respiration rate and the concomitant rate of ATP generation by oxidative phosphorylation.

The hypothesis that the specific growth rate of *E. coli* is, in most cases, limited by its respiration rate does not imply that the capacity of the respiratory system is low compared with other pathways, but rather that it tends to be developed to a maximal extent in the cell. It seems reasonable that in a well-organized system, different pathways are adjusted to one another. For example, a system with a pathway whose capacity is higher than its rate of functioning would be better adapted if the synthesis of the enzymes functioning in that pathway were decreased. The gain in synthetic capacity could then be directed toward faster growth (24). In a well-adapted system, the overall growth rate must, in the last analysis, be determined by the physical limit to which one or more pathways can be developed and to which other pathways adjust. Such physical limits may be of the following types: (i) diffusion rate of nutrients, oxygen, etc., into the cell and (ii) maximal content in the cell of an essential pathway. We propose that *E. coli* has evolved to a state of maximal respiratory capacity for aerobic balanced growth. Further emphasis on respiration would reduce the growth rate because other essential functions would suffer. Most of the enzymes involved in respiration and in oxidative phosphorylation are lodged in the cytoplasmic membrane. Therefore, our proposal concerning the respiration capacity states that, in balanced growth, a maximal fraction of the total membrane area is allotted to the respiratory and phosphorylating enzyme systems. Considerations of this kind have been discussed extensively by Harrison (14).

We know that the cells are larger in cultures with higher specific growth rates, implying a decrease in the surface-to-volume ratio (34). At a truly constant Q_{O_2} value per gram (dw) or volume unit, this would cause the relative membrane area required to lodge the respiratory and phosphorylating systems to increase slightly with growth rate.

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