Growth Yields and Respiratory Efficiency of *Acinetobacter calcoaceticus*

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Acinetobacter calcoaceticus NCIB 8250 was grown in batch culture on 46 separate sources of carbon + energy and the molar growth yields were measured. The organism was also grown in continuous culture on L-mandelate, phenylglyoxylate and succinate and the results used to calculate maximum molar growth yields. The elemental composition of the bacteria was determined. Growth equations for each substrate were constructed so that the amount of oxygen consumed per mol of substrate could be calculated. Growth yields on oxygen (Y_{O_2}) were then estimated. The Y_{O_2} values were low [averaging about 19 g dry wt (mol O_2)⁻¹]. The most likely explanation for the low yields is that the effective P/O ratio is only about one, although there are probably at least two sites of oxidative phosphorylation.

INTRODUCTION

Acinetobacter calcoaceticus is an obligate aerobe and most strains are metabolically versatile (Baumann *et al.*, 1968). It is common in natural environments (Baumann, 1968) and has been identified as a member of a number of microbial communities (Wilkinson *et al.*, 1974; Slater & Bull, 1982). Several possible industrial applications of *A. calcoaceticus* have been investigated (Du Preez *et al.*, 1981).

Hardy & Dawes (1985) found rather low values for maximum molar growth yields on oxygen and succinate when *A. calcoaceticus* NCIB 8250 was grown in continuous culture under conditions of carbon or oxygen limitation. I have measured yields of the same strain on 46 substrates in batch cultures. Three of the compounds, including succinate, were also used as substrates in continuous cultures in order to compare the results with those of Hardy & Dawes (1985) and to check the calculations based on measurements of yields in batch cultures. The results show that the low yields found by Hardy & Dawes (1985) were not simply a consequence of some peculiarity of growth on succinate and indicate that their conclusions apply to growth of *A. calcoaceticus* on a wide range of compounds. In addition, the estimates of yields should serve as a basis for evaluating potential industrial uses of *A. calcoaceticus* as well as for quantifying the contribution made to the microbial communities of which it is a member.

METHODS

Organism. *Acinetobacter calcoaceticus* NCIB 8250 was obtained from the National Collection of Industrial Bacteria, Aberdeen, UK. Stock cultures were maintained in Oxoid cooked meat medium stored at 4 °C. Subcultures were made into Oxoid nutrient broth at intervals of approximately 2 months and were also kept at 4 °C. Inocula used in growth experiments were produced by a further subculture into nutrient broth and incubated at 30 °C for 17 to 24 h immediately before use.

Measurements of molar growth yields in batch cultures. Media contained an appropriate amount of carbon source, 1 g (NH₄)₂SO₄ and 2 g KH₂PO₄, adjusted to pH 7.0 and made to 1 litre. All substrates were tested at four concentrations with the highest concentration chosen to produce a yield equivalent to an optical density at 500 nm of between 0.4 and 0.7. Each experiment contained controls without added carbon source. Media were dispensed in 50 ml amounts in 250 ml Erlenmeyer conical flasks plugged with disposable foam bungs and autoclaved at 109 °C. Heat-labile compounds were adjusted to pH 7.0 and then sterilized by filtration through Millipore filters (0.22 µm pore size) before addition to sterile solutions of (NH₄)₂SO₄ and KH₂PO₄. Inocula (0.1 ml of a nutrient

broth culture) and 1.0 ml of sterile 2% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were added to the flasks, which were then placed on a rotary shaker (LH Engineering, Ltd) and shaken at 30 °C at about 180 oscillations min^{-1} . Growth was measured (usually at about 17, 24, 48 and 72 h) by taking samples aseptically and determining the optical density at 500 nm in a Spectronic 20 colorimeter using round tubes of internal diameter 11.7 mm. The same colorimeter was used throughout these experiments and the relationship between OD_{500} and bacterial concentration was linear up to about 0.4; suspensions which were more concentrated than this were diluted with basal medium before measurement of the OD_{500} . Experiments with bacteria grown on a range of substrates showed that a suspension with an OD_{500} equivalent to 1.0 contained 352 μg dry wt ml^{-1} (the standard deviation was 17 μg dry wt ml^{-1} for 19 batches of bacteria, each assayed in duplicate or triplicate). Molar growth yields [$Y_{\text{substrate}}$; g dry wt (mol substrate) $^{-1}$] were calculated as the maximum slopes of graphs of yield (μg dry wt ml^{-1} at whichever time of sampling gave the highest yield) against substrate concentration (mM). The number of independent experiments with each substrate is listed in Table 1.

Measurements of maximum molar growth yields in continuous cultures. Bacteria were grown in a chemostat similar to that described by Baker (1968). The medium was the same as that used for batch cultures except that it also contained a trace metal mixture (Beggs & Fewson, 1977). The culture volume was about 600 ml, the temperature 30 °C and the air flow rate 200 ml min^{-1} . Succinate (3.5 mM in the reservoir), L-mandelate (1.5 mM) or phenylglyoxylate (1.5 mM) were used as the limiting substrates. Yield was dependent on the concentration of carbon source but was not affected by doubling the concentration of $(\text{NH}_4)_2\text{SO}_4$ or KH_2PO_4 . At least 10 replacement volumes of medium were added before each sample was taken for determination of OD_{500} in a Pye Unicam SP800 spectrophotometer using 1 cm cuvettes. Samples were taken at growth rates between about 0.12 and 0.6 h^{-1} . Yields (μg dry wt ml^{-1}) were calculated by reference to standard curves of OD_{500} against bacterial dry weight ($\mu\text{g ml}^{-1}$). These curves varied from instrument to instrument but a bacterial suspension with an OD_{500} equivalent to 1.0 contained between 250 and 300 μg dry wt ml^{-1} . Maximum molar growth yields [$Y_{\text{substrate}}^{\text{max}}$; g dry wt (mol substrate) $^{-1}$] and maintenance coefficients [m ; mmol substrate (g dry wt) $^{-1} \text{h}^{-1}$] were derived from the intercepts and slopes respectively of graphs of reciprocal of yield [mol substrate (g dry wt) $^{-1}$] against reciprocal of growth rate (h) (Pirt, 1965). These graphs were all straight lines (correlation coefficients > 0.9). Values listed in Table 1 are means from independent duplicate chemostat experiments.

Measurements of growth rates. The procedure was the same as that used previously (Fewson, 1967) except that the optical density was measured at 500 nm.

Elemental composition. Washed and dried bacteria were analysed as follows: carbon, hydrogen and nitrogen by Dr G. Weiler and Dr F. B. Strauss (latterly Dr F. B. Strauss; Oxford), Butterworth Microanalytical Consultancy Ltd (Teddington) and the Microanalysis Laboratory, Department of Chemistry, University of Glasgow; phosphorus and sulphur by Dr G. Weiler and Dr F. B. Strauss; oxygen by Butterworth Microanalytical Consultancy Ltd; calcium, iron and magnesium by Dr M. R. Moore (Department of Materia Medica, Stobhill Hospital, Glasgow) using colorimetric methods; chlorine, copper, manganese, potassium, sodium and zinc by Dr F. C. Gillespie (Western Regional Hospital Board Department of Clinical Physics and Bio-Engineering, Glasgow) using neutron activation analysis.

RESULTS AND DISCUSSION

Elemental composition

Values for each element are listed as the mean percentage of dry weight $\pm$ SD with the number of different batches of bacteria analysed (mostly in duplicate) given in parentheses: C, 47.64 $\pm$ 1.40 (23); H, 6.64 $\pm$ 0.21 (23); N, 12.32 $\pm$ 1.47 (23); O (which in other studies has usually been estimated by difference), 28.93 $\pm$ 0.90 (10); P, 2.11 $\pm$ 0.35 (15); S, 1.75 $\pm$ 0.31 (14). These results were obtained for bacteria grown in batch cultures in which growth had been limited by the carbon + energy source (usually succinate) and there was no discernible trend of differences amongst bacteria grown on different carbon sources. Minor elements showed more variation, possibly as a consequence of different growth rates or the choice of washing procedure (see e.g. Tempest *et al.*, 1966). The ranges were (percentage of dry weight): K, 0.3 to 1.5; Mg, 0.1 to 0.4; Cl, 0.05 to 0.8; Na, 0.07 to 0.19; Ca, Fe, Mn, Cu and Zn (total), 0.021 to 0.042.

Growth in batch cultures

A. calcoaceticus NCIB 8250 can grow on over one hundred compounds as sole sources of carbon + energy (Fewson, 1967; Baumann *et al.*, 1968). The present work was restricted to 46 compounds (Table 1) which satisfied the following criteria: (a) they could be obtained in sufficiently pure form; (b) they were not unstable or too volatile; (c) they were soluble enough to

give adequate concentrations in the medium; (d) they supported fairly rapid growth (mean generation times generally 1 h or less, and not more than about 3 h).

In preliminary experiments the conditions were shown to be optimal with respect to: volume of medium in the growth flasks, speed of shaking, temperature, pH value.

If the nitrogen source in the medium was nitrate ($1 \text{ g KNO}_3 \text{ l}^{-1}$) rather than ammonia the carbon growth yield was lowered by about a quarter (19 to 34%) for all the substrates tested (acetate, azelaate, fumarate, 2-oxoglutarate, L-malate, phenylacetate, phenylglyoxylate and succinate). This is consistent with the theoretical estimates of Stouthamer (1979). The effect on growth rate was rather more varied; for instance growth on 2-oxoglutarate was hardly affected (mean generation times of 97 to 114 min on either nitrate or ammonia in several experiments) whereas growth on succinate/nitrate (mean generation time 92 to 99 min) was much slower than that on succinate/ammonia (50 to 56 min). These results confirm that supply of reductant limited growth in these experiments.

Growth in continuous cultures

The maximum growth yield of $41.1 \text{ g dry wt (mol succinate)}^{-1}$ (Table 1) was close to the values of 42.1 to $42.7 \text{ g dry wt mol}^{-1}$ found by Hardy & Dawes (1985) for various conditions of succinate or oxygen limitation.

Maintenance coefficients on L-mandelate, phenylglyoxylate and succinate were 0.34 , 0.66 and $0.67 \text{ mmol substrate (g dry wt)}^{-1} \text{ h}^{-1}$ respectively.

Comparison of batch and continuous cultures

Yields in batch cultures are often felt to be inadequate as the basis for calculations of respiratory efficiency and other theoretical aspects of growth yields. However, comparison of the values in Table 1 for mandelate or phenylglyoxylate (which might be suspected to give very different yields in batch and continuous cultures because they support non-exponential growth in batch cultures; Cook *et al.*, 1975) or succinate revealed a maximum discrepancy of about 13% between the batch and continuous cultures. This difference was presumably caused by maintenance requirements (Pirt, 1965, 1982; but see van Verseveld *et al.*, 1984). In addition, Jones *et al.* (1973) determined the maximum molar growth yield of strain NCIB 8250 growing on phenol in continuous culture and obtained a value very close to the yield found for batch culture in the present work (Table 1). These sorts of differences between molar growth yields and maximum molar growth yields are insufficient seriously to alter the final conclusions. More important, studies confined to continuous cultures are so time-consuming that they cannot easily be extended to a wide range of substrates and are therefore in danger of producing non-representative results.

Calculation of oxygen uptake during growth

The values for growth yields, together with the elemental composition of the bacteria, allowed equations to be calculated for growth on each of the substrates. Thus for the yield observed by Hardy & Dawes (1985) for continuous culture on succinate the equation was:

In the case of aromatic compounds an allowance was made for oxygenative reactions because presumably they cannot lead to oxidative phosphorylation (Dagley, 1978). For continuous culture on phenylglyoxylate the equation was therefore:

Ammonia would be produced, not consumed, when amino acids and related compounds were used as substrates. So for growth on glutamate, where aspartase appears to be responsible for ammonia formation (A. C. Borthwick, unpublished results):

Table 1. *Growth yields and calculated Y_{O_2} values for *A. calcoaceticus**

Molar growth yields of strain NCIB 8250 were measured for batch cultures as described in Methods and then the Y_{O_2} values calculated as explained in the text. Maximum molar growth yields (shown in **bold type**) were determined using continuous cultures; results for strain NCIB 8250 growing on L-mandelate, phenylglyoxylate and succinate were obtained in the present work and the other values are taken from the papers listed in the footnotes. Calculations were made using the maximum appropriate number of significant figures. Numbers of atoms of O taken up and values for Y_{O_2} were rounded off to two places of decimals for presentation.

Substrate	Growth yield			Calculated oxygen uptake [mol O ₂ (mol substrate) ⁻¹]	Oxygen required for oxygenation [mol O ₂ (mol substrate) ⁻¹]	Calculated Y_{O_2} [g dry wt (mol O ₂) ⁻¹]
	Mean [g dry wt (mol substrate) ⁻¹]	No. of experiments	SEM as % of mean			
Acetate	20.1	7	2.9	1.185		16.96
Acetate*	30.8			0.755		40.80
Adipate	70.4	3	2.4	3.65		19.28
L-Alanine	28.9	3	4.3	1.825		15.83
L-β-Alanine	28.9	3	0	1.825		15.83
L-Asparagine	29.9	3	0.5	2.035		14.70
L-Aspartate	30.2	5	5.1	1.78		16.96
Azelaate	102.1	4	2.1	6.87		14.86
Benzaldehyde	72.5	4	2.0	5.06	2	23.70
Benzoate	62.7	9	4.6	4.955	2	21.22
Benzyl alcohol	79.9	6	4.6	5.26	2	24.50
Butane 2,3-diol	53.9	3	1.4	3.31		16.28
Butyrate	48.9	7	2.0	3.02		16.20
Caproate	81.7	3	2.3	4.695		17.40
Crotonate	38.4	3	2.6	2.95		13.02
Ethanol	29.2	5	3.3	1.82		16.04
Ethanol*	38.9			1.425		27.30
Ethanol†	35.7			1.55		23.04
Fumarate	33.8	12	1.9	1.635		20.68
Glutaconate	45.6	5	1.6	2.65		17.20
L-Glutamate	52.1	6	2.2	2.39		21.80
L-Glutamine	50.3	2	0.2	2.71		18.56
Glutarate	51.7	3	3.3	2.90		17.82
Heptoate	104.9	3	3.1	5.255		19.96
2-Hexenoate	86.2	5	5.3	4.01		21.50
L-Histidine	44.0	5	1.9	3.215		13.68
4-Hydroxybenz-aldehyde	68.3	1		4.73	2	25.02
2-Hydroxybenzyl alcohol	69.3	1		5.19	2	21.72
4-Hydroxybenzyl alcohol	72.9	1		5.05	2	23.90
4-Hydroxybenzoate	57.7	3	4.0	4.665	2	21.65
DL-Lactate	31.3	5	1.6	1.74		17.98
DL-Lactate‡	35.8			1.555		23.02
L-Malate	31.7	17	2.5	1.715		18.48
L-Mandelate	64.8	44	1.0	5.875	2	16.72
L-Mandelate	70.6			5.645	2	19.36
3-Oxoadipate	62.3	3	9.1	2.985		20.88
2-Oxoglutarate	46.5	7	1.4	2.125		21.88
Phenol	56.0	3	3.8	4.73	2	20.52
Phenol§	55.5			4.755	2	20.14
Phenylacetate	93.6	4	1.9	5.21	2	29.16
Phenylglyoxylate	65.8	9	4.5	5.34	2	19.70
Phenylglyoxylate	66.5			5.30	2	20.15
Pimelate	75.0	3	1.7	4.97		15.09
L-Proline	53.2	4	1.2	3.35		15.88
Propionate	31.0	6	1.3	2.245		13.80
Salicylate	58.1	3	2.7	4.65	2	21.92
Sebacate	137.3	3	2.1	6.935		19.80

Table 1. *Continued.*

Substrate	Growth yield			Calculated oxygen uptake [mol O ₂ (mol substrate) ⁻¹]	Oxygen required for oxygenation [mol O ₂ (mol substrate) ⁻¹]	Calculated Y _{O₂} [g dry wt (mol O ₂) ⁻¹]
	Mean [g dry wt (mol substrate) ⁻¹]	No. of experiments	SEM as % of mean			
L-Serine	21.5	2	0	1.635		13.15
D-Serine	22.9	1		1.57		14.58
Succinate	35.9	18	2.0	2.055		17.46
Succinate	41.1			1.835		22.40
Succinate†	42.1			1.795		23.46
L-Tyrosine	90.5	3	1.2	5.84	2	23.56
Valerate	64.4	3	7.1	3.90		16.52
Vanillate	58.8	3	2.9	5.625	2	16.22

* A strain isolated from the sediment of a freshwater dam (Du Preez *et al.*, 1981).

† Strain 4736 (Abbott *et al.*, 1974).

‡ *A. lwoffii* (*calcoaceticus*) 4B (Jones *et al.*, 1977).

§ Strain NCIB 8250 (Jones *et al.*, 1973).

¶ Strain NCIB 8250 growing at dissolved O₂ tension of 80 mmHg (Hardy & Dawes, 1985).

Table 2. *Comparison of the calculated amounts of O₂ uptake with the values obtained by measuring respiration*

The amounts of O₂ uptake calculated from growth experiments were taken from Table 1. Experimental stoichiometries of O₂ uptake were derived from measurements of respiration in the Warburg apparatus in the experiments described by Kennedy & Fewson (1968). Values are given as means ± SD with the number of experiments in parentheses.

Substrate	O ₂ uptake calculated from growth experiments [mol O ₂ (mol substrate) ⁻¹]	O ₂ uptake measured in Warburg apparatus [mol O ₂ (mol substrate) ⁻¹]
Acetate	1.185	1.06 ± 0.09 (6)
Benzoate	4.955	4.92 ± 0.49 (9)
Fumarate	1.635	1.57 ± 0.17 (3)
4-Hydroxybenzoate	4.715	4.47 ± 0.12 (11)
L-Mandelate	5.875	5.84 ± 0.47 (7)
2-Oxoglutarate	2.125	2.05 ± 0.21 (2)
Phenylglyoxylate	5.34	5.48 ± 0.36 (4)
Salicylate	4.65	4.90 ± 0.41 (9)
Succinate	2.055	1.79 ± 0.21 (4)

The validity of these calculations was checked in two ways. First, the $Y_{O_2}^{\max}$ value calculated from the $Y_{\text{succinate}}^{\max}$ value of Hardy & Dawes (1985) was 23.46 (Table 1), in close agreement with the experimental value of 23.15 determined from measurements of O₂ uptake (Hardy & Dawes, 1985). Secondly, results of experiments on O₂ uptake by bacterial suspensions in growth media were used to measure total amounts of O₂ uptake per mol of substrate (Table 2). These experiments were done for quite different reasons and were used at the time only to measure rates, rather than amounts, of O₂ uptake. However, the experimental stoichiometries were in close agreement with the calculated stoichiometries and the largest discrepancy was about 15%.

The calculated amounts of O₂ used for respiration, together with the growth yields, were then used to calculate Y_{O₂} values (Table 1). For example in the case of continuous culture on phenylglyoxylate, Y_{O₂} = growth yield/calculated respiratory O₂ uptake = 66.5 g dry wt (mol phenylglyoxylate)⁻¹/3.30 mol O₂ respired (mol phenylglyoxylate)⁻¹ = 20.15 g dry wt (mol O₂)⁻¹.

Respiratory efficiency

The mean of the Y_{O_2} values listed in Table 1 for strain NCIB 8250 is about 19 g dry wt per mol of O_2 , which is equivalent to 9.5 g dry wt per atom of O. This is close to the Bauchop & Elsdén (1960) empirical yield constant of 10.5 g dry wt per mol of ATP. It is also a little less than the theoretical yields calculated by Stouthamer (1979) for growth on acetate/salts or malate/salts media (10.0 and 15.4 g dry wt per mol ATP respectively). These are probably fair values to assume for strain NCIB 8250 because acetate and malate are representative of the amphibolic products of metabolism of the substrates listed in Table 1. [This strain does not grow on carbohydrates (Fewson, 1967; Baumann *et al.*, 1968) which might be expected to support higher Y_{ATP}^{max} values (Stouthamer, 1979)]. It is therefore tempting to suggest that there is only a single site of oxidative phosphorylation in strain NCIB 8250. This conclusion appears to be supported by calculations (not shown) of theoretical maximum molar growth yields for continuous culture on L-mandelate assuming one, two or three sites of phosphorylation; the theoretical yields would then be about 69, 97 or 111 g dry wt mol⁻¹ respectively. These calculations (based on Table 6 of Stouthamer, 1979) lead to the same conclusions as that drawn by Hardy & Dawes (1985) for similar calculations based on growth on succinate: the observed yield (70.6 g dry wt mol⁻¹) corresponded closely to the assumption of a single site of phosphorylation. An independent calculation which produced a similar conclusion concerns those substrates which must be oxidized by NAD-dependent dehydrogenases before any of the molecule can enter the amphibolic pathways. Successive pairs of compounds of this sort include benzyl alcohol/benzaldehyde, benzaldehyde/benzoate, 4-hydroxybenzyl alcohol/4-hydroxybenzaldehyde, 4-hydroxybenzaldehyde/4-hydroxybenzoate, 2-hydroxybenzyl alcohol/salicylate (two steps). The average difference in yield for these pairs was 7.3 g dry weight per 2 H, again suggesting that a single site of oxidative phosphorylation is linked to NADH oxidation.

The Y_{O_2} values for succinate were close to the mean of all the Y_{O_2} values and so succinate seems to be a good representative substrate. This apparently validates the generality of the conclusions reached by Hardy & Dawes (1985). However, this tends to disguise the rather wide range of Y_{O_2} values (Table 1). There were several values of less than 16 or more than 23. This variation suggests that the explanation of a single site of phosphorylation may be too facile. Several reasons for the low and varied yields are possible. (a) The precise balance of amphibolic products probably varies from substrate to substrate and so there will not be a unique value for the theoretical Y_{ATP}^{max} (Stouthamer, 1979). (b) Products might accumulate. Some strains of *A. calcoaceticus* form compounds such as emulsifiers (Sar & Rosenberg, 1983) but probably not under the conditions used in the present study (Rubinovitz *et al.*, 1982). I did not measure C-recovery with all substrates; however, when strain NCIB 8250 was growing in continuous culture on limiting succinate there was no significant accumulation of products (Hardy & Dawes, 1985). Similarly, benzyl alcohol and benzaldehyde appeared transiently during batch culture on L-mandelate or phenylglyoxylate but they were then oxidized and the final recovery of carbon in CO_2 and cells was $100 \pm$ about 5% (Cook *et al.*, 1975). (c) Catabolism and biosynthesis might be uncoupled and slip reactions could dissipate ATP (Neijssel & Tempest, 1976; Stouthamer, 1979). (d) Even though the potential P/O ratio might be two or three, very rapid proton decay across the membrane might lead to an actual P/O ratio near one, as suggested for *Rhizobium leguminosarum* by Ratcliffe *et al.* (1983).

Failure to make use of all the potential synthesis of ATP would be consistent with the conclusions that the redox chain of *A. calcoaceticus* HO1-N is divided into two equivalent sites of energy conservation (Ensley *et al.*, 1981) and that proton translocating loops 0, 1 and 2 are active in *A. lwoffii* 4B (Meyer & Jones, 1973; Jones *et al.*, 1975). Results of experiments to determine $\rightarrow H^+/O$ quotients all seem to suggest that there are at least two sites of oxidative phosphorylation (Meyer & Jones, 1973; Jones *et al.*, 1975; Hardy & Dawes, 1985). This notion is also supported by a consideration of the differences in maximum molar growth yields on L-mandelate and phenylglyoxylate (Table 1). L-Mandelate gave 4.1 g more dry weight per mol than did phenylglyoxylate (70.6 – 66.5 g dry wt mol⁻¹). This is about half the value for the NAD-linked processes described above. L-Mandelate dehydrogenase is certainly not NAD-linked (Hills & Fewson, 1983) and may be flavin-dependent (N. Allison, unpublished results). This result

therefore suggests that (a) flavin oxidation is coupled to phosphorylation and (b) NADH oxidation is coupled to at least two sites. This in turn is consistent with $Y_{ATP}^{\max}$ values of around 10 and effective P/O ratios of one, even though several sites of phosphorylation may be involved.

Other strains of *A. calcoaceticus*

Bearing in mind the probable requirements for maintenance, the yields of strains 4B (Jones *et al.*, 1977) and NCIB 8250 growing on DL-lactate were fairly close, as were the yields of strains 4736 (Abbott *et al.*, 1974) and NCIB 8250 growing on ethanol (Table 1). By far the highest $Y_{O_2}^{\max}$ value for *A. calcoaceticus* appears to be the value of 40.8 g dry wt (mol O₂)⁻¹ for growth on acetate of the strain used by Du Preez *et al.* (1981) (Table 1). There is a great spectrum of strains in the species *A. calcoaceticus* (Baumann *et al.*, 1968) but the yield reported by Du Preez *et al.* (1981) for growth of their strain on ethanol was not proportionately so much greater than that for strains 4736 or NCIB 8250 (Table 1) so it is not possible to dismiss the acetate yield simply as a strain difference. However, the very high yield on acetate arose by extrapolation of data indicating an extremely high maintenance coefficient [7.45 mmol (g dry wt)⁻¹ h⁻¹; Du Preez *et al.*, 1981]. In reality the highest yield was 25.2 g dry wt mol⁻¹ at a growth rate as high as about 1 h⁻¹. This is not so different from the yield of strain NCIB 8250 on acetate in batch culture (Table 1); in addition, the yield of strain NCIB 8250 growing on acetate in continuous culture at 0.2 to 0.3 h⁻¹ was about 18.5 g dry wt mol⁻¹ (G. A. Hardy, private communication) which compares closely with a yield of about 18 g dry wt mol⁻¹ at 0.3 h⁻¹ interpolated from the results of Du Preez *et al.* (1981). It is very unlikely that the $Y_{ATP}^{\max}$ for growth on acetate would be over 20 g dry wt mol⁻¹ so the results of Du Preez *et al.* (1981) provide further evidence that at least under some conditions *A. calcoaceticus* has more than one site of phosphorylation. The experimental yields of *A. calcoaceticus* growing on acetate are not too different from those of *Escherichia coli*, which also has an extremely high maintenance coefficient for acetate (Hempfling & Mainzer, 1975).

Conclusions

Growth yields of *A. calcoaceticus* are low. There are various possible explanations for this. The most likely is that the effective P/O ratio is only about one under most conditions even though there may be two or more sites of oxidative phosphorylation. Perhaps it is disappointing that an organism which is so metabolically versatile is so inefficient in this respect. This is further evidence that the principal selective pressures operating on bacteria have not tended to maximize yield (Tempest, 1978; Westerhoff *et al.*, 1983).

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REFERENCES

- ABBOTT, B. J., LASKIN, A. I. & MCCOY, C. J. (1974). Effect of growth rate and nutrient limitation on the composition and biomass yield of *Acinetobacter calcoaceticus*. *Applied Microbiology* **28**, 58–63.
- BAKER, J. (1968). Low cost continuous culture apparatus. *Laboratory Practice* **17**, 817–824.
- BAUCHOP, T. & ELSDEN, S. C. (1960). The growth of microorganisms in relation to their energy supply. *Journal of General Microbiology* **23**, 457–469.
- BAUMANN, P. (1968). Isolation of *Acinetobacter* from soil and water. *Journal of Bacteriology* **96**, 39–42.
- BAUMANN, P., DOUDOROFF, M. & STANIER, R. Y. (1968). A study of the *Moraxella* group. II. Oxidase-negative species (Genus *Acinetobacter*). *Journal of Bacteriology* **95**, 1520–1541.
- BEGGS, J. D. & FEWSON, C. A. (1977). Regulation of synthesis of benzyl alcohol dehydrogenase in *Acinetobacter calcoaceticus* NCIB 8250. *Journal of General Microbiology* **103**, 127–140.
- COOK, A. M., BEGGS, J. D. & FEWSON, C. A. (1975). Regulation of growth of *Acinetobacter calcoaceticus* NCIB 8250 on L-mandelate in batch culture. *Journal of General Microbiology* **91**, 325–337.
- DAGLEY, S. (1978). Pathways for the utilization of organic growth substrates. In *The Bacteria*, vol. VI, pp. 305–388. Edited by L. N. Ornston & J. R. Sokatch. New York: Academic Press.
- DU PREEZ, J. C., TOERIEN, D. F. & LATEGAN, P. M. (1981). Growth parameters of *Acinetobacter calcoaceticus* on acetate and ethanol. *European Journal of Applied Microbiology and Biotechnology* **13**, 45–53.
- ENSLEY, B. D., IRWIN, R. M., CARREIRA, L. A., HOFFMAN, P. S., MORGAN, T. V. & FINNERTY, W. R. (1981). Effects of growth substrates and respiratory chain composition on bioenergetics in *Acinetobacter* sp. strain HO1-N. *Journal of Bacteriology* **148**, 508–513.

- FEWSON, C. A. (1967). The growth and metabolic versatility of the Gram-negative bacterium NCIB 8250 ('Vibrio 01'). *Journal of General Microbiology* **46**, 255–266.
- HARDY, G. A. & DAWES, E. A. (1985). Effect of oxygen concentration on the growth and respiratory efficiency of *Acinetobacter calcoaceticus*. *Journal of General Microbiology* **131**, 855–864.
- HEMPFLING, W. P. & MAINZER, S. E. (1975). Effects of varying the carbon source limiting growth on yield and maintenance characteristics of *Escherichia coli* in continuous culture. *Journal of Bacteriology* **123**, 1076–1087.
- HILLS, C. A. & FEWSON, C. A. (1983). Mutant strains of *Acinetobacter calcoaceticus* possessing additional mandelate dehydrogenases. Identification and preliminary characterization of the enzymes. *Biochemical Journal* **209**, 379–386.
- JONES, C. W., BRICE, J. M., DOWNS, A. J. & DROZD, J. W. (1975). Bacterial respiration-linked proton translocation and its relationship to respiratory-chain composition. *European Journal of Biochemistry* **52**, 265–271.
- JONES, C. W., BRICE, J. M. & EDWARDS, C. (1977). The effect of respiratory chain composition on the growth efficiencies of aerobic bacteria. *Archives of Microbiology* **115**, 85–93.
- JONES, G. L., JANSEN, F. & MCKAY, A. J. (1973). Substrate inhibition of the growth of bacterium NCIB 8250 by phenol. *Journal of General Microbiology* **74**, 139–148.
- KENNEDY, S. I. T. & FEWSON, C. A. (1968). Metabolism of mandelate and related compounds by bacterium NCIB 8250. *Journal of General Microbiology* **53**, 259–273.
- MEYER, D. J. & JONES, C. W. (1973). Oxidative phosphorylation in bacteria which contain different cytochrome oxidases. *European Journal of Biochemistry* **36**, 144–151.
- NEIJSSSEL, O. M. & TEMPEST, D. W. (1976). The role of energy-spilling reactions in the growth of *Klebsiella aerogenes* NCTC 418 in aerobic chemostat culture. *Archives of Microbiology* **110**, 305–311.
- PIRT, S. J. (1965). The maintenance energy of bacteria in growing cultures. *Proceedings of the Royal Society B163*, 224–231.
- PIRT, S. J. (1982). Maintenance energy: a general model for energy-limited and energy-sufficient growth. *Archives of Microbiology* **133**, 300–302.
- RATCLIFFE, H. D., DROZD, J. W. & BULL, A. T. (1983). Growth energetics of *Rhizobium leguminosarum* in chemostat culture. *Journal of General Microbiology* **129**, 1697–1706.
- RUBINOVITZ, C., GUTNICK, D. L. & ROSENBERG, E. (1982). Emulsan production by *Acinetobacter calcoaceticus* in the presence of chloramphenicol. *Journal of Bacteriology* **152**, 126–132.
- SAR, N. & ROSENBERG, E. (1983). Emulsifier production by *Acinetobacter calcoaceticus* strains. *Current Microbiology* **9**, 309–314.
- SLATER, J. H. & BULL, A. T. (1982). Environmental microbiology: biodegradation. *Philosophical Transactions of the Royal Society B297*, 575–597.
- STOUTHAMER, A. H. (1979). The search for correlation between theoretical and experimental growth yields. In *International Reviews of Biochemistry. Microbial Biochemistry*, vol. 21, pp. 1–47. Edited by J. R. Quayle. Baltimore: University Park Press.
- TEMPEST, D. W. (1978). The biochemical significance of microbial growth yields: a reassessment. *Trends in Biochemical Sciences* **3**, 180–184.
- TEMPEST, D. W., DICKS, J. W. & HUNTER, J. R. (1966). The interrelationships between potassium, magnesium and phosphorus in potassium-limited chemostat cultures of *Aerobacter aerogenes*. *Journal of General Microbiology* **45**, 135–146.
- VAN VERSEVELD, H. W., CHESBRO, W. R., BRASTER, M. & STOUTHAMER, A. H. (1984). Eubacteria have 3 growth modes keyed to nutrient flow. Consequences for the concept of maintenance and maximal growth yield. *Archives of Microbiology* **137**, 176–184.
- WESTERHOFF, H. V., HELLINWERF, K. J. & VAN DAM, K. (1983). Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 305–309.
- WILKINSON, T. G., TOPIWALA, H. H. & HAMER, G. (1974). Interactions in a mixed bacterial population growing on methane in continuous culture. *Biotechnology and Bioengineering* **16**, 41–59.