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Metabolic and energetic aspects of the growth of *Bacillus stearothermophilus* in glucose-limited and glucose-sufficient chemostat culture

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Abstract. With a glucose-limited chemostat culture of *Bacillus stearothermophilus*, increasing the incubation temperature progressively from 45° C to 63° C led to a progressive marked increase in the maintenance rates of glucose and oxygen consumption. Hence, at a fixed low dilution rate the yield values with respect to glucose and oxygen decreased substantially with increased temperature. However, the apparent $Y^{max}_{glucose}$ and $Y^{max}_{O_2}$ values did not decrease but actually increased with temperature, being highest at 63° C (i.e., close to the maximum growth temperature). With glucose-sufficient cultures growing at a fixed low dilution rate (0.2 h^{-1}) and at their optimum temperature (55° C), glucose and oxygen consumption rates invariably were higher than that of a corresponding glucose-limited culture. Cation (K^+ or Mg^{2+})-limited cultures expressed the highest metabolic rates and with the K^+ limited culture this rate was found to be very markedly temperature dependent. As the temperature was increased from 45° C to 63° C the rate of glucose consumption increased 1.8-fold, and that of oxygen consumption by 3.7-fold. The culture pH value also exerted a noticeable effect on the metabolic rate of a glucose-limited culture, particularly at the extremes of pH tolerance (5.5 and 8.5, respectively). A K^+-limited culture was less affected with respect to metabolic rate by the culture pH value though the steady state bacterial concentration, and thus the cellular K^+ content, changed substantially. These results are discussed in relation to previous findings of the behaviour of this organism in batch culture, and to the behaviour of other thermophilic *Bacillus* species in chemostat culture.

Key words: *Bacillus stearothermophilus* – Chemostat culture – Growth energetics – Glucose limitation – Cation limitation – Metabolic rate – Maintenance rate – Yield values – pH – Temperature

From a detailed quantitative study of the growth of *Bacillus stearothermophilus* var. *non-diastaticus* in batch culture at several different incubation temperatures, Coultate and Sundaram (1975) concluded that the optimum temperature for growth rate was substantially different from that for growth yield on either glucose or succinate. At the higher growth temperatures (i.e., at or above the optimum for growth rate), carbon substrate was used inefficiently and incompletely which suggested a partial dissociation of catabolism from anabolism as well as, possibly, a partial uncoupling of energy production from respiration. However, with these batch cultures there invariably is present an excess of carbon substrate throughout the exponential growth phase thereby providing the potential for metabolic uncoupling; hence it does not necessarily follow that similar findings will be manifest when organisms are growing in the presence of low, growth-constraining concentrations of carbon substrate such as can be effected in chemostat culture. Therefore, we initiated a study of the behaviour of *B. stearothermophilus* in glucose-limited and glucose-sufficient chemostat culture. The strain of organism which we used is probably identical with that used by Coultate and Sundaram (1975), and whereas glucose-sufficient cultures indeed showed a marked temperature-dependent propensity to extensively dissociate catabolism from anabolism, glucose-limited cultures gave results that did not entirely accord with those reportedly observed with batch cultures, as detailed in this paper.

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Materials and methods

Organism

The organism used in this study was obtained from the Laboratory of Microbiology, University of Amsterdam, The Netherlands, as a prototrophic strain of *Bacillus stearothermophilus*. It was found to grow readily, aerobically, at 55° C in a defined simple salts medium (Evans et al. 1970) with glucose as the sole carbon substrate, though growth rate was markedly stimulated by low levels of biotin (0.5 μM, end concentration). Besides glucose, the organism was found to utilize fructose, α-methyl glucoside, mannitol, glycerol, lactate, ethanol and succinate. No growth was evident on starch, or when the organisms were cultured anaerobically on glucose, either in the absence or presence of nitrate. The organism therefore appears to be closely similar to, or identical with, that isolated by Epstein and Grossowicz (1969) and named *B. stearothermophilus* var. *non-diastaticus*.

In batch culture, when growing in a simple salts medium plus 55 mM glucose (pH 7.0), a maximum growth rate was evident at 55° C and was 1.53 h^{-1}. Growth rate was perceptibly slower at 58° C (1.40 h^{-1}) and at 50° C (1.20 h^{-1}). No growth was observed after 2 days incubation at 37° C, but at 45° C the specific growth rate was 0.65 h^{-1}. The organism grew at a rate of 0.97 h^{-1} at 63° C but not at all at 68° C.

The pH optimum varied with the nature of the growth environment but appeared to fall within the range of 6.0 to 7.0. The extremes at which growth at a rate of 0.2 h^{-1} could be sustained in chemostat culture were, respectively, 5.5 and 9.0.

Culture conditions

Organisms were grown routinely in chemostats (LH Fermentation Ltd., 500 series, 1 l growth vessel) using a working volume of 700 ml and with automatic pH, temperature and foam controls. Dissolved oxygen levels were monitored using a polarographic oxygen electrode (Ingold GmbH & Co KG) and the stirring speed adjusted to maintain a value greater than 20% saturation. The medium, as described by Evans et al. (1970), was sterilized in 20 l batches by autoclaving at 121°C for 30 min, the glucose (concentrated solution, slightly acidified) being sterilized separately and then added to the bulk medium to give the required end concentration (usually 26 mM for a glucose-limited culture medium and 150 mM for all glucose-sufficient media). The concentrations of other growth limiting nutrients were adjusted, where appropriate, such as to support a steady state concentration of bacteria equivalent to 1–2 mg dry weight cells · ml^{-1}.

Metabolic rates

The rate of glucose consumption was determined using the formula:

$$q_{\text{glucose}} = \frac{fS - f's}{x \cdot V}$$

where f is the medium flow rate, f' the output flow rate (i.e., medium plus titrant and antifoam), S the input glucose concentration, s the residual glucose concentration (usually immeasurably small with glucose-limited cultures), x the steady state bacterial equivalent dry weight concentration and V the culture volume.

Oxygen consumption rate and CO_2 production rate were determined from their concentrations in the effluent gas as measured using, respectively, a Servomex Model 570A paramagnetic oxygen analyser and a Model PA404 infrared CO_2 analyser (Servomex Ltd., Crowborough, Sussex, England). With a controlled air flow rate of 1.0 l · min^{-1}, metabolic rates [as mmol · h^{-1} (g dry wt cells)$^{-1}$] were calculated according to the following equation:

$$q_{O_2}\,(\text{or } CO_2) = \frac{\%\text{ gas used (or produced)}}{x \cdot V} \cdot 25{,}000\,.$$

Dry air, at 20°C, was taken to contain 20.9% oxygen and 1 mol gas was assumed to occupy 24.0 l at the monitoring temperature.

The rate of acetate poduction was determined from its concentration in the culture fluid according to the equation:

$$q_{\text{acetate}} = \frac{\text{culture concentration (mM)}}{x} \cdot D$$

where D is the dilution rate (f'/V).

Carbon balances were constructed from the rates of glucose consumption and of products formation as follows:

$$\text{Carbon balance (\%)} = \frac{[(41.7) \cdot D + q_{CO_2} + 2q_{\text{acetate}}] \cdot 100}{6 \cdot q_{\text{glucose}}}\,.$$

In the construction of this equation, biomass was taken to contain 50%, w/w, carbon (see Herbert 1976). The factor 41.7 derives from 1000/24 mmol C · (g dry wt cells)$^{-1}$.

An evaluation of the rate of ATP generation (q_{ATP}) was made from the rates of oxygen consumption and of acetate production as outlined below.

The complete oxidation of 1 mol of glucose to 6 mol of CO_2 by way of the glycolytic pathway and tricarboxylic acid cycle generates 10 mol NAD(P)H, 2 mol reduced flavoprotein and 4 mol A(G)TP. 6 mol of oxygen are consumed in the process of re-oxidizing the reduced carriers and hence the ATP production rate will be related to the oxygen consumption rate by the generalized formula:

$$q_{ATP} = \frac{10n + 2m + 4}{6}\,\text{mol ATP} \cdot \text{mol } O_2{}^{-1},$$

where n and m are P/O ratios for the oxidation of NAD(P)H and reduced flavoprotein, respectively. Most frequently, $m = n - 1$, and hence the above expression reduces to:

$$q_{ATP} = \frac{10n + 2(n-1) + 4}{6} = \frac{12n + 2}{6}\,\text{mol ATP} \cdot \text{mol } O_2{}^{-1}\,.$$

In the oxidation of glucose to acetate, by way of glycolysis, 1 mol of glucose gives rise to 2 mol acetate, 2 mol CO_2, 4 mol NADH and 4 mol ATP. Hence the ATP production rate will be related to the acetate production rate by the formula:

$$q_{ATP} = (2n + 2) \cdot q_{HAc}\,.$$

Therefore, when organisms are growing on glucose and producing as sole products cells, CO_2 and acetate, the rate of ATP production will be related to both the rates of oxygen consumption and acetate production by the formula:

$$q_{ATP} = (q_{O_2} - q_{HAc})\,(2n + 0.33) + (2n + 2) \cdot q_{HAc}\,,$$

which reduces to:

$$q_{ATP} = (2n + 0.33) \cdot q_{O_2} + 1.67\, q_{HAc}\,.$$

Hence, when $n = 2$, $q_{ATP} = 4.33\, q_{O_2} + 1.67\, q_{HAc}$: and when $n = 3$, $q_{ATP} = 6.33\, q_{O_2} + 1.67\, q_{HAc}$.

Analyses

The culture bacterial dry weight concentration was determined using the procedure of Herbert et al. (1971). Glucose present in the medium and in the culture supernatant fluids was determined enzymatically with glucose oxidase following the procedure of Bergmeyer and Bernt (1974). Acetate was determined using a commercial test kit supplied by Boehringer Mannheim GmbH. This method was based on that described by Bergmeyer and Mollering (1974).

Results

That incubation temperature may affect metabolic rate and growth rate differentially such that the optimum temperature for growth yield may be different from that for growth rate (Coultate and Sundaram 1975) was indeed found to be

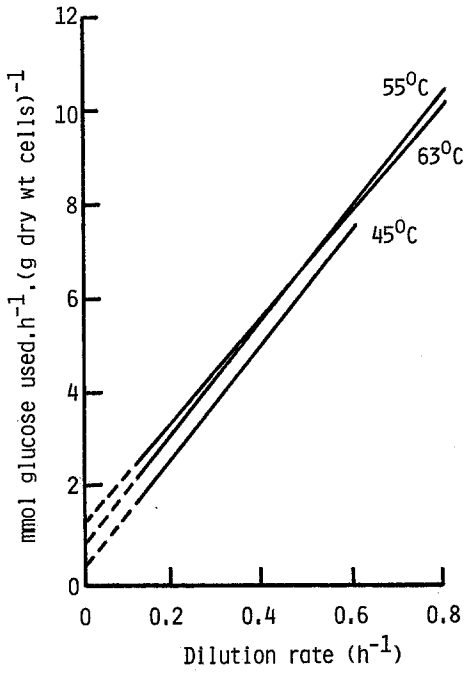

Fig. 1. Relationship between growth rate and the specific rate of glucose consumption observed with a glucose-limited chemostat culture of *Bacillus stearothermophilus* growing at, respectively, 45° C, 55° C and 63° C with the pH value controlled at 7.0 ± 0.1. The datum points have been omitted for clarity and the lines drawn using the data contained in Table 1

Table 1. Influence of incubation temperature on the growth parameters of a glucose-limited chemostat culture of *Bacillus stearothermophilus* growing at different dilution rates and at a pH value of 7.0

Parameter	Incubation temperature (° C)		
	45	55	63
m (glucose)[a]	0.10	0.75	1.05
m (oxygen)	1.02	4.72	6.32
$Y^{max}_{glucose}$ [a]	80.0	82.5	87.4
Y^{max}_{oxygen}	29.2	29.4	35.5
Correlation coefficients with respect to growth rate:			
$q_{glucose}$ [a]	0.999	0.992	0.999
q_{oxygen}	0.980	0.995	0.995
No. datum points:	17	47	25

[a] $m_{glucose}$ and m_{oxygen} are the calculated rates of glucose and oxygen consumption at $D = 0$: $Y^{max}_{glucose}$ and Y^{max}_{oxygen} are the calculated maximum yield values with respect to glucose and oxygen; and the q values are the specific rates of glucose and oxygen consumption, respectively. The q values relevant to any particular growth rate [expressed as mmol · h^{-1} · (g dry wt cells)$^{-1}$] can be obtained from the expression: $q = 1000 \cdot D/Y^{max} + m$

the case with a glucose-limited chemostat culture of *Bacillus stearothermophilus* when growing at a low dilution rate (0.1 h^{-1}) where progressively increasing the incubation temperature from 45° C to 63° C caused a progressive lowering of the steady state bacterial concentration with a concomitant increase in the rate of glucose consumption. Hence the yield value with respect to glucose (and also to oxygen) declined progressively as the temperature was increased. However, with the same culture growing at a higher dilution rate (0.2 h^{-1}) the influence of temperature was less marked and this prompted a detailed study of the influence of both temperature and growth rate on the metabolic rates expressed by glucose-limited cultures. The results obtained (Fig. 1; Table 1) confirmed previous findings in that whereas incubation temperature had a marked influence on metabolic rates at low dilution rates, this was less evident at higher dilution rates and, indeed, at dilution rates above 0.4 h^{-1} cultures grew with a higher efficiency (i.e., higher yield) at 63° C than at 55° C. Moreover, extrapolation of these data suggest that had cultures incubated at 45° C been able to grow at a rate higher than 0.65 h^{-1} (their actual μ_{max} value) then their metabolic rate and yield value would have converged with that of the culture growing at 63° C at a dilution rate of 0.9 h^{-1}.

In the above connection, analyses of these data according to the equation of Pirt (1965) — that is, $q = D/Y^{max} + m$ — shows that, whereas temperature clearly affects the so-called "maintenance" rate of glucose consumption (m), the derived maximum yield value for glucose (and for oxygen) is inverted: that is, the highest apparent maximum yield value was manifest at 63° C and the lowest value at 45° C (Table 1).

As found with glucose-limited chemostat cultures of *Klebsiella aerogenes* (Teixeira de Mattos et al. 1984) and *Clostridium butyricum* (Crabbendam et al. 1985), glucose-limited cultures of *B. stearothermophilus* seemingly are able to accelerate their rate of glucose consumption substantially and instantaneously upon relief of the growth limitation — a phenomenon that has been termed "reactivity" (Leegwater 1983). Thus, at dilution rates of between 0.1 h^{-1} and 0.4 h^{-1}, addition of a cell-saturating pulse of glucose to a steady state glucose-limited chemostat culture led to increases in glucose consumption rate of between 3-fold (at $D = 0.1$ h^{-1}) and 2-fold (at $D = 0.4$ h^{-1}) without any concomitant instantaneous change in growth rate (data not shown). Hence, *B. stearothermophilus* is able to extensively dissociate glucose catabolism from anabolism (at least transiently) and this raised the question of how these organisms might respond to environments in which the rate of anabolism is severely constrained by the availability of some anabolic substrate whilst the availability of catabolic substrate (glucose) is unconstrained.

When growing at a fixed dilution rate of 0.2 h^{-1} (55° C; pH 7.0), the rates of glucose and oxygen consumption varied markedly with the nature of the growth limitation (Table 2). As expected, glucose-limited cultures expressed the lowest metabolic rate, the rates expressed by all the glucose-sufficient cultures being some 1.2- to 3.1-fold higher. The highest rate of metabolism (and lowest yield value) was observed with the potassium-limited culture, a situation closely similar to that observed with potassium-limited cultures of *K. aerogenes* when growing on glucose (Hueting et al. 1979). In this latter case, however, much of the excess glucose consumed was found to have been converted to gluconic acid and 2-ketogluconic acid by way of membrane-bound glucose- and gluconate dehydrogenases (Neijssel et al. 1983). Hence we sought to determine whether the excess glucose metabolized by potassium-limited cultures of *B. stearothermophilus* was converted to, and excreted as, overflow metabolites and, if so, what they were.

From the data presented in Table 2, it is clear that carbon recoveries based solely on cell carbon and CO_2-carbon produced (divided by the glucose carbon consumed) ranged from

Table 2. Glucose utilization rates, and rates of products formation, expressed by variously limited chemostat cultures of *Bacillus stearothermophilus* when growing at a fixed dilution rate (0.2 h^{-1}), temperature (55° C) and pH value (7.0)[a]

Limitation	Glucose	Ammonia	S(methionine)	Phosphate	Magnesium	Potassium
Glucose used:	46.1	53.6	74.9	85.0	109.5	123.8
Products formed:						
Cells	20	20	20	20	20	20
Carbon dioxide	25.0	29.5	39.2	48.2	75.8	55.4
Acetate	–	1.0	14.5	17.5	13.1	42.7
$Y_{glucose}$	62.5	54.1	39.0	33.8	26.3	23.8
Y_{oxygen}	19.0	16.1	12.2	9.9	6.3	8.9
Carbon recovery	98	94	98	101	99	95
Carbon recovery (excluding acetate)	98	92	79	80	87	61

[a] All values (except yields and carbon recoveries) are expressed as matom carbon · h^{-1}, and normalized to a cell production rate of 20 matom carbon · h^{-1}

Table 3. Influence of dilution rate on the rate of glucose consumption and of products formation of an S(methionine)-limited chemostat culture of *B. stearothermophilus* growing at a fixed temperature (55° C) and pH value (7.0)

Dilution rate (h^{-1})	Specific rates [mmol · h^{-1} · (g dry weight cells)$^{-1}$]					Carbon recovery (%)
	$q_{glucose}$	$q_{carbon\ dioxide}$	$q_{acetate}$	$^*q_{ATP(2)}$	$^*q_{ATP(3)}$	
0.2	5.15 ± 0.27	16.33 ± 0.24	3.02 ± 0.43	76	105	99
0.4	7.99 ± 0.34	17.78 ± 0.38	6.84 ± 0.07	88	124	100
0.6	9.31 ± 0.23	20.83 ± 0.55	5.05 ± 0.32	99	140	100
0.8	9.90 ± 0.16	25.65 ± 0.46	0.40 ± 0.17	112	163	101
1.0	11.85 ± 0.33	29.35 ± 0.90	nil	127	186	100
Correlation coefficients (versus dilution rate)						
	0.973	0.984	–	0.998	0.997	

N.B. Each mean and standard deviation value is derived from four separate determinations made at each dilution rate. $^*q_{ATP(2)}$ and $q_{ATP(3)}$ refer to the calculated rates of ATP generation assuming *P/O* quotients of, respectively, two and three

0.92 (or 92%) in the case of the ammonia-limited culture to 0.61 in the case of the potassium-limited culture. Clearly, with this latter culture a substantial proportion of the glucose consumed was converted to overflow metabolites, and these were found to comprise mainly, if not exclusively, acetate (Table 2). Thus, taking into account acetate production, carbon recoveries for all cultures were better than 94% and, except for the potassium- and ammonia-limited cultures, better than 98%.

When growing at 55° C, all the glucose-sufficient cultures of *B. stearothermophilus* showed a higher degree of metabolic instability than had been encountered previously with mesophilic species (including *B. subtilis*) when growing at their optimum temperatures. However, a sulphate-limited culture of the thermophile was particularly unstable and, when growing at a dilution rate of 0.2 h^{-1}, consumed glucose and oxygen at rates that varied randomly between, respectively, 5 and 13, and 17 and 26 mmol · h^{-1} · (g dry wt cells)$^{-1}$. Changing the sulphur source from sulphate to methionine led to the culture being much more stable (Table 3). Progressively increasing the dilution rate led to concomitant increases in the glucose and oxygen consumption rates but to a variable rate of acetate excretion. Interestingly, whereas the correlation between growth rate and either glucose or oxygen consumption rate was not particularly impressive, that between growth rate and apparent ATP generation rate was quite good. In this latter connection, the calculated Y_{ATP}^{max} and m_{ATP} values were, respectively, 15.9 g · mol^{-1} and 62.6 mmol · h^{-1} · (g dry wt cells)$^{-1}$ if a *P/O* quotient of 2 is assumed, and 10.0 g · mol^{-1} and 83.3 mmol · h^{-1} · (g dry wt cells)$^{-1}$ if a *P/O* quotient of 3 is assumed. This S(methionine)-limited culture proved to be rather exceptional in that with all the other glucose-sufficient cultures the correlation between growth rate and metabolic rate was markedly non-linear (data not shown).

Because the highest rate of glucose catabolism was evident with the potassium-limited culture, this condition was singled out for more detailed study – particularly a study of the influence of incubation temperature and culture pH value.

Whereas only at low dilution rates did the incubation temperature markedly affect the rate of glucose metabolism and yield value of a glucose-limited culture, with a potassium-limited culture the effect was dramatic (Fig. 2). Thus, at a dilution rate of 0.2 h^{-1}, increasing the incubation temperature from 45° C to 63° C led to a 70% increase in the rate of glucose consumption (from 6.4 to 10.9 mmol · h^{-1} · (g dry wt cells)$^{-1}$ and to more than doubling of the respiration rate [from 17.8 to 40.1 mmol O_2 · h^{-1} · (g dry wt cells)$^{-1}$]. There was also a progressive near-linear increase in the assessed rate of ATP generation. In this latter calculation a *P/O* quotient of 2 was assumed, but a qualitatively similar pattern would be manifest had a *P/O* quotient of 3 been selected. Clearly, bearing in mind the marked increases in both respiration rate and acetate excretion rate, there was a marked increase in energy flux

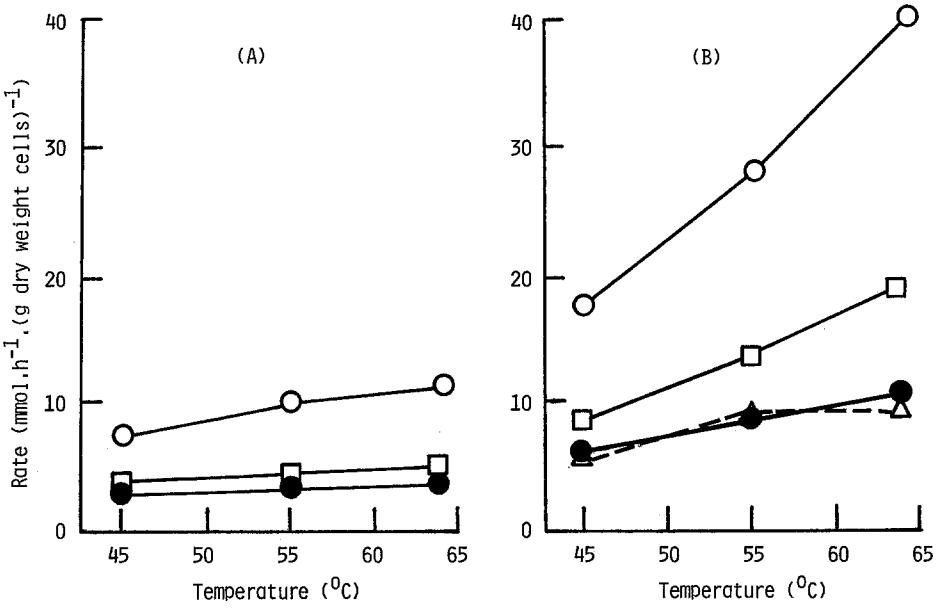

Fig. 2A, B
Influence of incubation temperature on the metabolic rates of (**A**) a glucose-limited, and (**B**) a potassium-limited chemostat culture of *Bacillus stearothermophilus* when growing at a fixed dilution rate of 0.2 h^{-1} and a pH value of 7.0 ± 0.1. (○) specific rate of oxygen consumption, (●) specific rate of glucose consumption, (△) specific rate of acetate production, and (□) apparent specific rate of ATP generation ($\times 10^1$) assuming a *P/O* quotient of 2

Table 4. Influence of the culture pH value on the metabolic rate and growth yield of *Bacillus stearothermophilus* when growing in a glucose-limited chemostat culture (D = 0.2 h^{-1}; 55°C; glucose input concentration = 26 mM)

pH value	mmol · h^{-1} · g^{-1}		g · mol^{-1}	g · l^{-1}	Carbon recovery (%)
	$q_{glucose}$	q_{CO_2}	$Y_{glucose}$	Cell dry wt	
5.5	4.95	18.5	40.5	1.05	91
6.0	2.92	9.3	68.4	1.78	100
6.5	2.94	9.4	68.0	1.77	101
7.0	3.05	9.9	65.6	1.71	103
7.5	3.16	10.3	63.3	1.65	98
8.0	3.78	13.1	52.9	1.38	95
8.5	4.13	15.4	48.4	1.26	96

associated with an increase in the incubation temperature. Extrapolation of these flux rate data suggests that, at temperatures below 30°C, the organisms would be metabolically inert.

To the best of our knowledge, no systematic study has been made of the influence of culture pH value on the kinetics of growth of *B. stearothermophilus*. Most work with this organism has involved batch cultures in which media were adjusted to an initial pH value of 7.0–7.5 and, indeed, the chemostat culture data presented above were derived from cultures in which the pH value was maintained automatically at 7.0 ± 0.1. Hence we sought to determine the influence of pH on the metabolic rate and yield value of steady state cultures of *B. stearothermophilus* when growing at 55°C and at a fixed dilution rate of 0.2 h^{-1}. Two culture conditions were selected for comparison, a glucose limitation and a potassium limitation (with a maintained excess of glucose). With the glucose-limited culture, steady state conditions could be realized at pH values from 5.5 to 8.5, the cultures washing out at pH values of, respectively, 5.0 and 9.0. Over the pH range 6.0 to 7.5 there was a small but progressive increase in metabolic rate with a minimum being expressed at pH 6.0 (Table 4). Hence the optimum pH value with respect to growth yield was actually 6.0 though the yield value expressed at pH 7.0 was only marginally lower. When the pH value was lowered from 6.0 to 5.5, or raised from 7.5 to 8.5, there was a marked rise in metabolic rate; presumably maintenance of cytoplasmic pH at these extremes of environmental pH was energetically expensive.

In contrast to the glucose-limited culture, the potassium-limited culture expressed a uniformly higher metabolic rate that was not markedly affected by the medium pH value (Table 5). There was an evident increase in the specific rate of glucose consumption and of acetate excretion as the pH was raised from 6.5 to 8.0 but the assessed rate of energy generation (q_{ATP}) remained relatively constant at 130 ± 12 mmol ATP · h^{-1} · (g dry wt cells)$^{-1}$. Significantly, the culture washed out when the pH value was lowered from 6.5 to 6.0 which well may have been provoked by the presence of much acetic acid in its undissociated form acting as a respiratory uncoupler (see Hueting and Tempest 1977). Somewhat inexplicably, the steady state bacterial dry weight concentration increased progressively as the culture pH value was raised progressively from 6.0 to 8.5 (Table 5). This suggested a marked progressive decrease in the bacterial potassium content which seemingly was not due to the intracellular deposition of storage-type polymers since, with the culture growing at pH 8.0, almost 70% of the cellular dry weight could be accounted for as protein (data not shown).

Table 5. Influence of culture pH value on the kinetics of growth of *Bacillus stearothermophilus* in potassium-limited chemostat culture (D = 0.2 h^{-1}; 55° C)

Medium pH value	Culture bacterial dry weight (mg · ml^{-1})	Cellular K^+ content[a] (%, w/w)	Rates: mmol · h^{-1} · (g dry wt cell)$^{-1}$					Carbon recovery (%)
			$q_{glucose}$	q_{CO_2}	q_{O_2}	$q_{acetate}$	q_{ATP}	
6.0	washout							
6.5	1.37	1.46	8.7	25.6	24.6	9.4	122	101
7.0	2.48	0.81	8.6	23.1	24.1	8.9	119	95
7.5	2.77	0.72	11.0	25.8	26.5	13.9	138	94
8.0	3.47	0.57	12.4	25.0	25.9	18.1	142	93
8.5	3.92	0.51	10.2	22.0	23.9	13.8	127	95

[a] Based on the assumption that all the culture K^+ is contained within the cells

Discussion

When growing in chemostat culture under conditions of carbon-substrate limitation, heterotrophic aerobes generally metabolize the carbon substrate solely to biomass and CO_2. However, the proportionation of substrate carbon between biomass synthesis (anabolism) and CO_2 formation (catabolism) often is markedly growth rate-dependent, which has been ascribed to the cells' need to expend energy to fulfil growth rate-independent maintenance functions (Pirt 1965). Thus, at low growth rates, as compared with high growth rates, proportionately more of the carbon substrate is oxidized to CO_2 and less assimilated into biomass. In plots of metabolic rate versus growth rate, this is manifest by the line of regression intercepting the y-axis at some finite value of metabolic rate (the so-called maintenance rate). Hence, because yield value is, by definition, the ratio of the growth rate to the metabolic rate, the yield declines to zero as the growth rate is lowered towards zero.

Plots of metabolic rate versus growth rate for glucose-limited cultures of *Bacillus stearothermophilus* (Fig. 1) were linear, as expected (see Table 1 for statistical analysis of the actual data), but were influenced by the incubation temperature in a complex manner. Thus, as the incubation temperature was increased from 45° C to 55° C, and then to 63° C, the intercept on the y-axis increased suggesting a marked temperature-dependent increase in the maintenance energy requirement. However, the slopes of the lines were not parallel indicating that temperature also influenced the apparent maximum yield value ($Y^{max}_{glucose} = \Delta\mu/\Delta q$).

Surprisingly, the maximum yield value for glucose, and for oxygen (Table 1), increased with increasing temperature and, in fact at growth rates above 0.4 h^{-1}, cultures incubated at 63° C expressed a higher actual yield value than those incubated at 55° C. The reason for this is entirely unclear, but the data were carefully checked and validated. Thus, in contrast to the findings of Coultate and Sundaram (1975) made with batch cultures, glucose-limited chemostat cultures expressed an increased yield value at increased temperatures, as was also found by Kuhn et al. (1980) with glucose-limited chemostat cultures of *Bacillus caldotenax*.

The relatively high apparent maintenance rates of glucose and oxygen consumption, and the marked effects of temperature upon these parameters (Fig. 1, Table 7), accord with observations made on other thermophilic organisms such as *B. caldotenax* (Kuhn et al. 1980), *Thermus thermophilus* (Sonnleitner et al. 1982), *T. aquaticus* (Cometta et al. 1982) and *B. acidocaldarius* (Farrand et al. 1983), but not with the thermophilic anaerobe *Thermoanaerobacter ethanolicus* (Lacis and Lawford 1985). With the thermoacidophilic bacterium, *B. acidocaldarium*, increases in maintenance rate correlated with increases in the permeability of the cytoplasmic membrane to protons and hence to the cells' need to catalyse enhanced rates of substrate oxidation in order to avoid a potentially lethal acidification of the cytoplasm (Farrand et al. 1983). However, an earlier study of a thermophilic neutrophile *B. stearothermophilus* by Chicken et al. (1981) showed that, when assayed at the growth temperature, the rate of decay of the transmembrane pH gradient was fairly constant, though quite fast. This indicated that though the cytoplasmic membrane of this organism was relatively leaky to protons (as compared with mesophilic species) it nevertheless did not become increasingly leaky as the growth temperature was increased from 37° C to 65° C. Because the organism used by Chicken et al. (1981) is probably closely similar to that used in our studies, their results would imply that the changes in apparent maintenance rate (Fig. 1, Table 1) were not a consequence of changes in membrane permeability to protons.

Notwithstanding the above conclusion, it has been argued that the high respiration rate (and high maintenance rate) manifest by K^+-limited cultures of some bacteria (*Klebsiella aerogenes*, *Escherichia coli*) are a consequence of a K^+ leakage current induced by the large transmembrane K^+ gradient extant across the cytoplasmic membrane of organisms growing under these specific nutrient-limited conditions (Hueting et al. 1979; Mulder et al. 1986). And with K^+-limited chemostat cultures of *B. stearothermophilus* the metabolic rate and respiration rate were very markedly influenced by the growth temperature (Fig. 2). This observation is compatible with the hypothesis that increasing incubation temperatures led to increasing rates of K^+ leakage from the cytoplasm and hence increasing rates of energy generation (and dissipation) associated with "pulling" the K^+ back into the cell. In this connection, it has been clearly demonstrated with *E. coli* that the high affinity K^+ uptake system is coupled to, and driven by, ATP hydrolysis (Rhoads and Epstein 1977; Epstein and Laimins 1980). It may be significant, therefore, that the apparent rate of ATP generation evident in a K^+-limited chemostat culture of *B. stearothermophilus*, growing at a fixed rate, was found to be a near-linear function of the incubation temperature (Fig. 2).

The capacity of many, if not all, microorganisms to extensively dissociate catabolism from anabolism has been recognized for decades and is most evident in the case of

(non-growing) washed suspensions of organisms when incubated with suitable carbon substrates. But it has been similarly evident for decades that actively-growing organisms also can dissociate catabolism from anabolism, albeit only partially (see, for example, Rosenberger and Elsden 1960; Gunsalus and Shuster 1961); and important industrial processes such as the production of citric acid and gluconic acid from glucose depend upon this fact. With organisms growing in chemostat culture, partial dissociation of catabolism from anabolism generally is manifest when growth is constrained by the availability of an essential anabolic substrate such as ammonia, sulphate or phosphate, and carbon substrate is present in substantial excess. Often the excess carbon substrate consumed is not fully oxidized to CO_2 and so-called overflow products are excreted into the medium (Neijssel and Tempest 1979, 1986). This pattern of behaviour was apparent with glucose sufficient chemostat cultures of *B. stearothermophilus* when growing at a relatively low dilution rate (Table 2). Here the rate of glucose consumption was markedly influenced by the nature of the growth limitation, being only marginally greater than that of a glucose-limited culture in the case of ammonia limitation, and more than twofold higher in the case of cation-limited cultures. In this latter connection, there was an interesting difference between the magnesium- and the potassium-limited culture in that the former expressed a higher respiration rate and produced only a modest amount of acetate whereas the latter expressed a lower respiration rate and a much higher rate of acetate production. This suggested possible differences in the activities of TCA cycle enzymes and in acetate kinase and, indeed, citrate synthase was four times more active in the magnesium limited cell extracts, as compared with extracts from potassium-limited cells, and the acetate kinase was only one-third as active (unpublished data). The reason for these apparent differences in the levels of enzyme expression is not immediately apparent; acetate kinase is, of course, a Mg^{2+}-requiring enzyme, but it does not follow that magnesium availability should influence the synthesis of this kinase.

So far as we are aware, the effects of medium pH value on the energetics of growth of thermophilic bacteria has been studied only with respect to the acidophilic organism *B. acidocaldarius* (Farrand et al. 1983). These authors reported a lowered yield value and increased maintenance rate when the medium pH value was decreased below 4.3. A similar effect was noticed with the neutrophilic *B. stearothermophilus* when the pH value of a glucose-limited culture was lowered from 6.0 to 5.5 (Table 4). However, the yield value expressed by this culture also decreased when the pH value was increased above 7.5, though, surprisingly, a similar pattern of response was not evident with a potassium-limited culture (Table 5). Here, however, it must be borne in mind that the metabolic rate of the latter culture was some 2- to 3-fold higher than that of the glucose-limited culture which could, perhaps, mask any additional energetic requirement for maintenance of cytoplasmic pH when the organisms were growing at alkaline pH values.

A puzzling feature of the potassium-limited culture was the change in biomass concentration that accompanied changes in culture pH value. One might expect that potassium-limited cells would contain the minimum amount of K^+ needed for them to function and grow at the imposed rate in the prescribed environment. If this is the case, then either the cells' minimum K^+ requirement is decreased at alkaline pH values or additional cell components such as storage polymers are being synthesized that add to the culture bacterial dry weight but not to the cells' K^+ requirement. The latter possibility can be discounted since 70% of the bacterial dry weight could be accounted for by protein. Clearly this is an important observation that needs to be studied further, and this we are in the process of doing.

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