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ORIGINAL PAPER

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Changes in the growth and enzyme level of *Zymomonas mobilis* under oxygen-limited conditions at low glucose concentration

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Abstract *Zymomonas mobilis* growing aerobically with 20 g glucose^{-1} (carbon-limited) in a chemostat exhibited an increase in both the molar growth yield ($Y_{x/s}$) and the maximum molar growth yield ($Y_{x/s}^{max}$) and a decrease in both the specific substrate consumption rate (q_s) and the maintenance energy consumption rate (m_e). Stepwise increase in the input oxygen partial pressure showed that anaerobic-to-aerobic transitional adaptation occurred in four stages: anaerobic (0 mm HgO_2), oxygen-limited (7.6–230 mm HgO_2), intermediate (273 mm HgO_2), and oxygen excess (290 mm HgO_2). The steady-state biomass concentration, $Y_{x/s}$, and intracellular ATP content increased between oxygen partial pressures of 7.6 and 120 mm HgO_2, accompanied by a decrease in the q_s and the specific acid production rate. The membrane ATPase activity decreased with increasing oxygen partial pressure and reached its lowest levels at 273 mm HgO_2, which was the highest input oxygen partial pressure where steady-state conditions were possible. Glucokinase, glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and alcohol dehydrogenase activities also decreased when the oxygen partial pressure was increased above 15 mm Hg, whereas pyruvate decarboxylase was unaffected by aeration. Growth inhibition at 290 mm HgO_2 was characterised by a drastic reduction in the pyruvate kinase activity and a collapse in the intracellular ATP pool. The growth and enzyme data suggest that at low glucose concentrations and oxygen-limited conditions, the increase in biomass yields is a reflection of a redirection of ATP usage rather than a net increase in energy production.

Key words *Zymomonas mobilis* · Chemostat · Anaerobic-to-aerobic transition · Maintenance energy coefficient · Biomass yield · Metabolic rate · ATPase activity · Energetics

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Abbreviations *D* Dilution rate · *N* Oxygen consumption rate · μ Growth rate · SAPR Specific acid production rate · s_o · *Tg* Oxygen partial pressure · T_l Dissolved oxygen tension · q_p Specific product production rate · q_s Specific substrate consumption rate · *x* Biomass concentration · $Y_{x/s}$ Molar growth yield · $Y_{x/s}^{max}$ Maximum molar growth yield · Y_{ATP}^{max} Molar growth yield (ATP)/maximum growth yield per mole ATP

Introduction

The growth and metabolism of *Zymomonas mobilis* under oxic conditions has been the topic of several investigations. Aerobic batch cultures grown on 2% glucose have exhibited a 25–40% reduction in the growth rates (μ) but the molar growth yield ($Y_{x/s}$) was unchanged (Bringer et al. 1984), whereas increasing the glucose concentration (Bringer et al. 1984; Pankova et al. 1988) or high oxygen transfer rates (Ishikawa et al. 1990) inhibited growth and reduced the $Y_{x/s}$. Pankova et al. (1988) have reported that aeration also causes a decrease in the activities of glucose-6-phosphate dehydrogenase and alcohol dehydrogenase. Studying the effects of aeration on growth and metabolism at high glucose concentration is, however, complicated by the presence of inhibitory metabolites such as acetaldehyde and also by the effect of glucose concentration on the observed growth kinetics. Rogers et al. (1982) have reported that increasing the feed glucose concentration of anaerobic chemostat cultures causes a decrease in both the $Y_{x/s}$ and μ, accompanied by an increase in the maintenance energy consumption rate (m_e). Paradoxically, however, two groups (Kalnenieks et al.1993; Zikmanis et al.1995) have demonstrated that oxidative phosphorylation occurs in cell vesicles and starved *Z. mobilis* cells supplied with a source of reductant.

This paper investigates the adaptation process at low glucose concentrations by characterising transitional changes in the enzyme activities and growth kinetics using small stepwise increments in the oxygen partial pressure. The relationship between energetics and the observed growth kinetics is also discussed.

Materials and methods

Bacterial strain and cultivation conditions

The strain *Z. mobilis* UQM 2716 (Australian Collection of Microorganisms, Brisbane, Australia) was maintained in a liquid medium consisting of 90 g l^{-1} glucose and 2 g l^{-1} each of KH_2PO_4, $MgSO_4$, $(NH_4)SO_4$, yeast extract and casein hydrolysate at 30° C, and was transferred every 12 h. The chemostat was a 2-l Setric (France) fermentor with a culture volume of 850 ml stirred at 300 rpm and a temperature of 30° C. The pH was regulated at 6.5 by the automatic addition of 0.8 M KOH. The semidefined medium consisted of 24 g glucose l^{-1}, and the other components were present in concentrations as described above. The dilution rate (D) was set at 0.138 h^{-1}, and the culture was carbon-limited with an effluent glucose concentration of less than 0.1 g l^{-1}. At least five culture volume exchanges occurred before three samples were taken 12 h apart to confirm that steady state was reached. Initially, anoxic conditions were achieved by sparging with high purity nitrogen (CIG, Australia), and cultures were aerated by sparging with humidified air at 2.9 l min^{-1}. Dissolved oxygen levels were measured with a Clarke-type dissolved-oxygen electrode (Ingold, Switzerland). To study the effects of stepwise transition from anaerobiosis to aerobiosis, aeration was controlled according to the method of Harrison and Pirt (1967) using high-purity oxygen and nitrogen mixtures. Different input oxygen partial pressures (T_g) were obtained by varying the oxygen to nitrogen ratio by means of individual flowmeters at a constant total gas flow rate of 150 ml min^{-1}. The electrode was first calibrated in sterile medium according to the equation: $T_l = T_g - N/K$ (Harrison and Pirt 1967), where T_l = dissolved oxygen tension, where N = oxygen consumption rate, and K is a constant dependent on aeration conditions and the oxygen solubility constant. Thus, when $N/K > T_g$, the electrode will record a reading of 0% saturation. The digital readings indicated a linear relationship between T_g and % oxygen saturation. Atmospheric pressure was ascertained by means of a mercury barometer. T_g was calculated by the following equation:

$$T_g = \frac{\text{ml } O_2 \cdot \text{min}^{-1}}{150 \text{ ml} \cdot \text{min}^{-1}} \cdot \text{atmospheric pressure in mm Hg}$$

(Hollywood 1987)

The kinetic values μ, q_s (specific substrate consumption rate), $Y_{x/s}$, $Y^{max}_{x/s}$ (maximum molar growth yield) and m_e were calculated from chemostat data as described by Pirt (1985). The m_e component due to ATPase activity was calculated as follows:

$$\mu\text{mol min}^{-1} \text{ (mg dry wt.)}^{-1} \cdot 180.15 \text{ g mol}^{-1} \cdot 1{,}000 \cdot 60 = (m_e)\text{g g}^{-1} \text{ h}^{-1}$$

Analytical methods

Steady-state culture samples for enzyme assays were collected by diverting the outflow tube of the chemostat to a bottle immersed in a refrigerated waterbath set at 3° C. The cells were harvested and washed in distilled water by centrifugation at 4° C and 10,000 × g for 10 min. Biomass concentration was estimated from the dry weight of washed cell samples. Cell extracts were obtained by two passages of a cell suspension through a French pressure cell (SLM-Aminco) at 137.9 MPa. Metabolic end-product concentrations were analyzed by HPLC (Waters) fitted with a Biorad HPX-87H organic acid column, and glucose concentration was determined enzymatically using a YSI (Yellow Springs) glucose analyser. Intracellular ATP concentration was determined enzymatically using a chemiluminescence kit (Sigma/Aldrich). A known volume of culture was removed from the fermentor rapidly and asceptically such that the entire process from culture sampling to luminescence detection did not exceed 10 s. The specific acid production rate (SAPR) of the culture was calculated from the rate of consumption of 0.8 M KOH as described by Reichelt and Doelle (1971):

$$\text{SAPR} = \frac{\text{ml alkali} \cdot 0.8 \text{ M} \cdot \text{h}^{-1} \cdot (\text{ml culture})^{-1}}{\text{g biomass dry wet} \cdot (\text{ml culture})^{-1}}$$

All fermentative enzymes were assayed spectrophotometrically by measuring the rate of formation or consumption of NADH at 340 nm using a Hitachi U-3000 split-beam spectrophotometer. Glucokinase, glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase and alcohol dehydrogenase were assayed according to Algar and Scopes (1985). Pyruvate decarboxylase was assayed according to Diefenbach and Duggleby (1991). The membrane ATPase activity was assayed by measuring the rate of release of inorganic phosphate as described by Jones and Doelle (1991). Membranes were prepared by centrifuging cell-free extract at 100,000 × *g* for 1 h and washing in 25 mM phosphate buffer (pH 6.5). The oxygen consumption rates of the membrane fractions were assayed using a Rank (Cambridge) oxygen electrode linked to a variable-speed chart recorder. The temperature was maintained at 30° C by a circulating water bath, and the assay buffer consisted of 25 mM KH_2PO_4 and 0.5 mM $MgCl_2$ (pH 6.5) with a total assay volume of 3 ml. Reaction was initiated by the addition of 0.5 mM NADH, and the O_2 consumption rate was determined from the recorder tracings.The KCN sensitivity titration of *Z. mobilis* membrane respiratory activity was performed as described by Baccarini-Melandri et al. (1973).

Results

Kinetic characteristics of *Z. mobilis* UQM 2716

Anaerobic chemostat cultures of strain UQM 2716 [s_o = 20 g glucose l^{-1}, D (dilution rate) = 0.138 h^{-1}] exhibited characteristics (Table 1) similar to those reported for other strains (Lawford and Ruggiero 1990). Aerating the cultures at a constant dilution rate caused a slight in-

Table 1 Kinetic characteristics of *Zymomonas mobilis* UQM 2716 grown aerobically and anaerobically in chemostat ($Y_{x/s}$ molar growth yield, q_s specific substrate consumption rate, $Y^{max}_{x/s}$ maximum molar growth yield, Y^{max}_{ATP} molar growth yield (ATP)/max. growth yield per mole ATP, maintenance energy, *D* dilution rate, *μ* growth rate)

Glucose (g l^{-1})	pH	Growth	$Y_{x/s}$ (g g^{-1}) (D = 0.1 h^{-1})	q_s (g g^{-1} h^{-1}) (D = 0.1 h^{-1})	$Y^{max}_{x/s}$ (g g^{-1})[a]	Y^{max}_{ATP} (g mol^{-1})[b]	m_e (g g^{-1} h^{-1})	m_e (ATPase) (g g^{-1} h^{-1})[c]
20	4.4	Anaerobic	0.020	5.0	0.049	8.83	3.27	2.16
20	4.4	Aerobic	0.027	3.68	0.120	21.7	2.75	0.90
20	6.5	Anaerobic	0.025	3.92	0.050	9.0	2.20	1.35
20	6.5	Aerobic	0.035	2.86	0.127	22.8	1.90	0.65

[a] Calculated from the equation $Y^{max}_{x/s} = \mu/(q_s - m_e)$ (Pirt 1985)
[b] Calculated from the $Y^{max}_{x/s}$ values assuming that one glucose gives one ATP net
[c] ATPase activity expressed in terms of m_e

crease in the $Y_{x/s}$ (Table 1), and the $Y^{max}_{x/s}$ and Y^{max}_{ATP} of aerobic cultures increased to 2.5-fold the anaerobic value. The q_s and the m_e decreased by 28 and 15%, respectively. Aeration also caused a reduction in the cellular ATPase activity. Uncoupled growth in *Z. mobilis* due to low pH manifests itself as a rise in the m_e, q_s and ATPase activity, and as a reduction in the $Y_{x/s}$, while the $Y^{max}_{x/s}$ remain unaffected (Jones and Doelle 1991; Lawford and Ruggiero 1990). Therefore, uncoupled growth may not sufficiently explain the effects of aeration observed above. A further observation is that the m_e component due to ATPase activity under anoxic conditions represents a greater ratio of the cells' total m_e than it does under oxic conditions (Table 1). We decided to investigate in more detail the effect on enzyme activity of gradual transition from anaerobiosis to aerobiosis in order to identify the reasons for achieving higher $Y_{x/s}$ values at lower glucose utilisation rates in aerobic cutures. To exclude the possibility that the changes in the q_s and $Y_{x/s}$ might be an artifact caused by changing aeration rates, aeration conditions were carefully controlled as described above.

The effect of input oxygen partial pressure on growth parameters and glucose metabolism

At all input oxygen partial pressures (pO_2) up to 235 mm Hg, the culture was oxygen-limited and the dissolved oxygen electrode recorded 0% saturation (Table 2). Between 7.6 and 120 mm HgO_2, the steady-state biomass concentration (x) and the $Y_{x/s}$ increased 25% (Table 2). Thereafter, the $Y_{x/s}$ remained steady at approximately 0.037 g g^{-1} up to 273 mm HgO_2. The q_s decreased by 22% from 4.67 g g^{-1} h^{-1} at 0 mm HgO_2 to a steady value of 3.65 g g^{-1} h^{-1} (Table 2). The specific acid production rate (SAPR), which was an additional indicator of fermentation characteristics, decreased parallel to the q_s up to 120 mm HgO_2, above which the SAPR started to increase again, corresponding to an increase in the steady-state acetic acid concentration (Table 3). The intracellular ATP concentration increased from 3.04 nmol(mg dry wt.)$^{-1}$ at 0 mm Hg to 3.42 nmol(mg dry wt.)$^{-1}$ at 7.6 mm HgO_2. The ATP content between 7.6 and 235 mm HgO_2 was higher than at 0 mm HgO_2, reaching 5.07 nmol(mg dry wt.)$^{-1}$ at

Table 2 The effect of input oxygen partial pressure on various growth parameters of a *Z. mobilis* chemostat culture (D = 0.138 h^{-1}, pH 6.5) (*D* dilution rate, *pO_2* oxygen partial pressure, *DO_2* dissolved oxygen, *s_{in}* feed glucose concentration, *s_{out}* effluent glucose concentration, *x* biomass concentration, *$Y_{x/s}$* molar growth yield, *q_s* specific substrate consumption rate, *SAPR* specific acid production rate)

pO_2 (mm Hg)	DO_2 (%)	Glucose		Growth parameter				
		s_{in} (g l^{-1})	s_{out} (g l^{-1})	x (g l^{-1})	$Y_{x/s}$ (g g^{-1})	q_s (g g^{-1} h^{-1})	SAPR (mmol g^{-1} h^{-1})	$[ATP]_i$ (nmol mg^{-1})
0	0	24	< 0.1	0.69	0.028	4.67	7.61	3.04
7.6	0	24	< 0.1	0.747	0.031	4.35	5.0	3.42
15	0	24	< 0.1	0.783	0.033	4.09	4.80	4.18
41	0	24	< 0.1	0.750	0.031	4.35	4.59	3.90
66	0	24	< 0.1	0.855	0.036	3.75	4.16	–
96	0	24	< 0.1	0.883	0.037	3.65	4.26	3.64
120	0	24	< 0.1	0.924	0.038	3.50	3.63	–
141	0	24	< 0.1	0.90	0.037	3.60	3.65	–
189	0	24	< 0.1	0.882	0.037	3.65	3.92	3.60
235	0	24	< 0.1	0.885	0.037	3.65	3.99	3.47
273	0.2	24	< 0.1	0.89	0.037	3.65	4.38	5.07
290[a]	25	24	8	0.486	0.020	4.50	–	1.69

[a] Non-steady-state

Table 3 Fermentation products and carbon balance (s_o = 24 g glucose l^{-1}; D = 0.138 h^{-1}) (*s_o* feed glucose concentration, *D* dilution rate, pO_2 oxygen partial pressure)

pO_2 (mm Hg)	Ethanol (g l^{-1})	Acetaldehyde (g l^{-1})	Acetate (g l^{-1})	Acetoin (g l^{-1})	Lactate (g l^{-1})	Glycerol (g l^{-1})	Carbon balance[a]
0	11.85	0	0.14	0.14	0.20	0.22	1.0
15.2	11.8	0	0.3	0.23	0.16	0.2	1.01
41	11.8	0.05	0.4	0.25	0.17	0.17	1.02
66	11.7	0.11	0.56	0.30	0.15	0.14	1.03
96	11.6	0.12	0.6	0.35	0.1	0.14	1.03
113	11.5	0.15	0.68	0.4	0.15	0.15	1.03
120	11.3	0.2	0.7	0.42	0.2	0.18	1.03
141	11	0.3	0.9	0.51	0.21	0.18	1.03
189	10.5	0.39	0.9	0.8	0.22	0.18	1.01
235	9.5	0.44	0.67	0.9	0.19	0.13	0.93
273	8.73	0.8	0.62	1.4	0.22	0.14	0.94
290[b]	5.9	0.81	0.4	1.02	0.1	0	0.98

[a] CO_2 estimated from steady-state values of ethanol + acetaldehyde + acetate and acetoin
[b] Non-steady-state values

273 mm HgO_2. Low levels of dissolved oxygen was detected in the culture at 273 mm Hg O_2 (Table 2), which was the highest pO_2 at which steady-state conditions were attainable. At 290 mm HgO_2, growth was inhibited. The biomass concentration fluctuated and the residual glucose rose to 8 g l^{-1}, indicating that the specific growth rate had decreased below the dilution rate of 0.138 h^{-1}. This was reflected in a sharp fall in the intracellular ATP pool to 1.69 nmol(mg dry wt.)$^{-1}$ and a 46% reduction of both x and $Y_{x/s}$. The culture q_s increased again to 4.5 g g^{-1} h^{-1}. The high levels of dissolved oxygen (25% saturation) at 290 mm HgO_2 were the result of the reduction in the biomass concentration. The profile of fermentation products is summarised in Table 3.

The effect of oxygen on the steady state activities of selected fermentative enzymes

The activities of the three fermentative enzymes involved in redox balance (glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and alcohol dehydrogenase) decreased steadily between 7.6 and 273 mm HgO_2. The glucose-6-phosphate dehydrogenase activity decreased in two stages (Fig. 1B). Between 7.6 and 41 mm HgO_2, the activity decreased by 25% and remained constant up to 189 mm HgO_2. Thereafter, it decreased a further 50% to its lowest level at 290 mm HgO_2, or 25% of the activity at 0 mm HgO_2. The calculated theoretical q_s indicates that the glucose-6-phosphate dehydrogenase activity was still present at a level that was not rate-limiting. Glyceraldehyde-3-phosphate dehydrogenase activity was less sensitive to low oxygen partial pressures, decreasing by 8.9% between 0 and 66 mm HgO_2. Thereafter, the activity fell sharply to its lowest level at 273 mm HgO_2, corresponding to 27% of the anaerobic activity. At 290 mm HgO_2, however, the glyceraldehyde-3-phosphate dehydrogenase activity increased again to 59.2% of the anaerobic activity. Alcohol dehydrogenase activity was the most sensitive to aeration, decreasing by 91% to 478 U(g dry wt.)$^{-1}$ at 273 mm Hg. At 0 mm HgO_2, alcohol dehydrogenase acitivity activity could potentially sustain a specific ethanol production rate [q_p (ethanol)] of 14 g g^{-1} h^{-1}, which was vastly in excess of the real culture q_p (ethanol) 2.37 g g^{-1} h^{-1} Hence, a 38% reduction in the alcohol dehydrogenase activity at 41 mm HgO_2 produced only a 9% reduction in the culture q_p (ethanol) to 2.17 g g^{-1} h^{-1}. The increase in the alcohol dehydrogenase activity to 1,130 U(g dry wt.)$^{-1}$ at 290 mmHgO_2, together with the q_s and the glyceraldehyde-3-phosphate dehydrogenase activity, caused the culture q_p (ethanol) to increase slightly from 1.35 g g^{-1} h^{-1} at 273 mm HgO_2 to 1.68 g g^{-1} h^{-1}.

The activities of the enzymes involved in energy metabolism, glucokinase and pyruvate kinase, are shown in Fig. 1A. The change in activity of glucokinase with increasing oxygen partial pressure was similar to that of glucose-6-phosphate dehydrogenase and occurred in two stages. The activity was highest between 0 and 15 mm HgO_2, above which the glucokinase activity decreased by

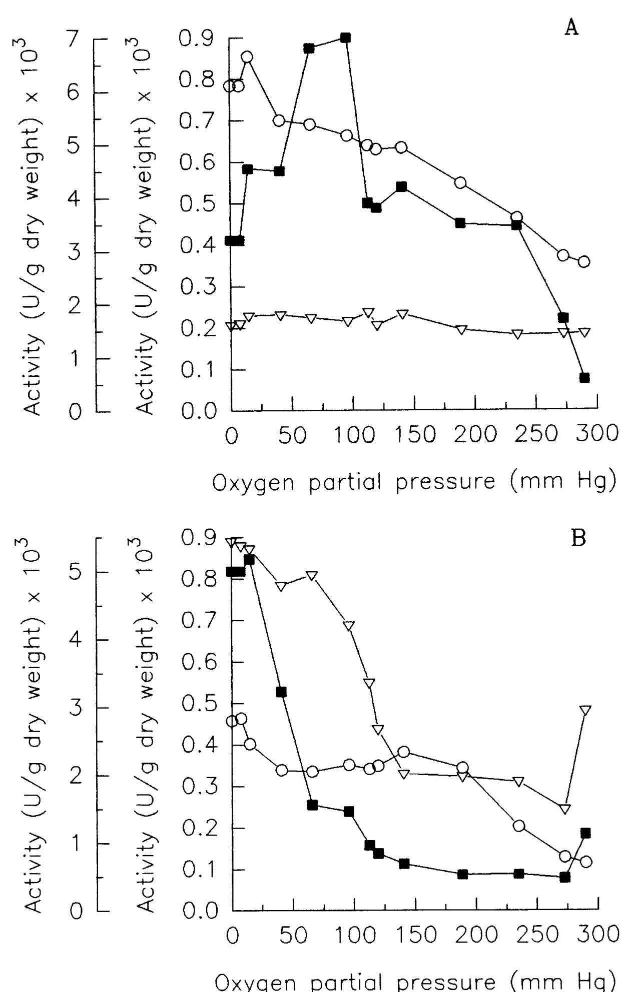

Fig. 1A, B The effect of increasing oxygen partial pressure on the levels of selected fermentative enzymes of *Zymomonas mobilis* (s = 24 g glucose l^{-1}; D = 0.138 h^{-1}). **A** ○ glucokinase, ▽ pyruvate decarboxylase, ■ pyruvate kinase; **B** ○ glucose-6-phosphate dehydrogenase, ▽ glyceraldehyde 3-phosphate dehydrogenase, ■ alcohol dehydrogenase (*s* glucose (substrate) concentration, *D* dilution rate)

20% and remained relatively constant up to 189 mm HgO_2. Thereafter, the activity decreased to a second plateau irrespective of whether the culture was in the oxygen-excess or oxygen-limited state (Fig. 1A), corresponding to a total decrease in activity of approximately 54%. Pyruvate kinase was present at high levels at all pO_2 up to 235 mm Hg, reaching a peak of 7,000 U(g dry wt.)$^{-1}$ at 96 mm HgO_2. The activity then decreased by about 50% at 273 mm HgO_2, and in the excess oxygen state the pyruvate kinase activity decreased sharply to about 570 U(g dry wt.)$^{-1}$ (or 16% of the activity in the oxygen-limited state). The drastic fall in the pyruvate kinase activity, in combination with the decline in the glucokinase and glucose-6-phosphate dehydrogenase activities to their lowest levels at 290 mm HgO_2, coincides with the observed collapse in the intracellular ATP pool and subsequent growth inhibition. Pyruvate decarboxylase was the only enzyme that was comparatively insensitive to oxygen partial pressure, remaining relatively constant at approximately 1,700

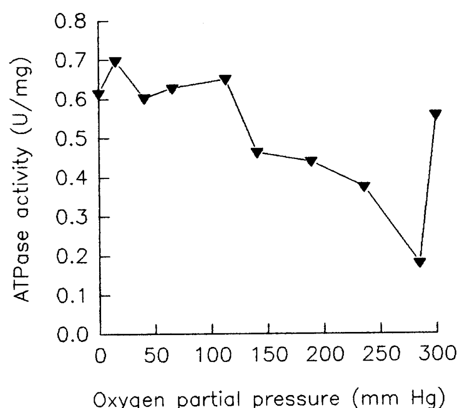

Fig. 2 The relationship between oxygen partial pressure and the membrane ATPase activity of *Z. mobilis* (s = 24 g glucose l^{-1}; D = 0.138 h^{-1})

U(g dry wt.)$^{-1}$ (Fig. 1A) in both the oxygen-limited and the oxygen-excess states.

Respiratory activity and the membrane-bound ATPase activity

The respiratory activity varied little with input oxygen partial pressures up to 235 mm Hg (Fig. 3), above which the activity increased to 0.48 U(mg protein)$^{-1}$ and further to 0.65 U(mg protein)$^{-1}$ at 290 mm HgO_2. The NADH dehydrogenase activity was approximately double the specific oxygen uptake rate. KCN titration indicated an apparent change in the sensitivity of the terminal oxidase to cyanide inhibition. The respiratory activity of membranes from the anoxic culture (0 mm HgO_2) was inhibited by 100–200 µM KCN. Membranes of aerobic cultures (40 and 100 mm HgO_2) exhibited a shift in the KCN sensitivity profile to lower KCN concentrations (approximately 20 µM) and the appearance of a plateau (Fig. 4) between 80 and 200 µM KCN, which lies in the concentration

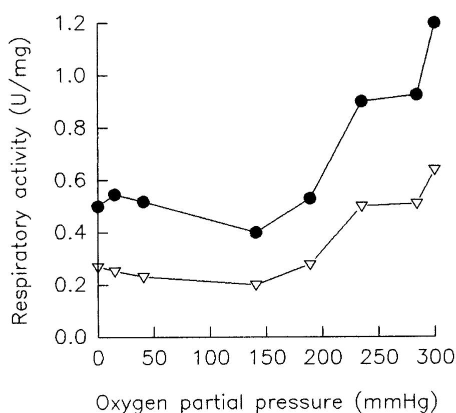

Fig. 3 The relationship between oxygen partial pressure and respiratory activity of *Z. mobilis* membrane preparation (● NADH oxidation, ▽ O_2 reduction)

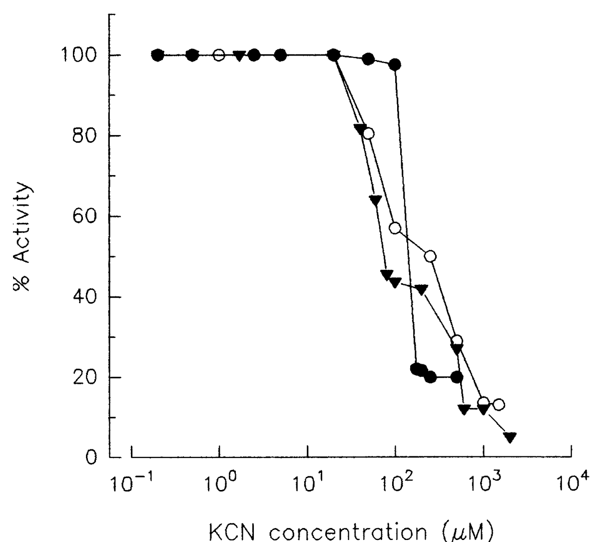

Fig. 4 KCN sensitivity titration profiles of Z. mobilis membrane respiratory activity (s = 24 g glucose l^{-1}; D = 0.138 h^{-1}). ● pO_2 = 0 mm Hg, ○ pO_2 = 40 mm Hg, ▼ pO_2 = 100 mm Hg (pO_2 oxygen partial pressure)

range at which the activity of the 0-mm HgO_2 membrane preparation was inhibited. Figure 4 suggests that under anoxic conditions, the activity is predominantly of the type that is inhibited by 100–200 µM KCN, whereas the cell membranes of aerobic cultures appeared to exhibit two types of activities. One was inhibited by about 20 µM KCN, representing 55% of the total activity; the remaining 45% was of the type inhibited by 100–200 µM KCN. However, detailed spectroscopic data is needed before a definitive statement can be made.

The membrane ATPase activity decreased rapidly from approximately 0.7 U(mg protein)$^{-1}$ to a value of 0.18 U(mg protein)$^{-1}$ between 120 and 273 mm HgO_2 (Fig. 2). At 290 mm HgO_2, when oxygen and glucose were in excess, the ATPase activity rose to 0.55 U(mg protein)$^{-1}$. The variation in the Michaelis-Menten constants of the membrane ATPase of steady-state chemostat cultures with oxygen partial pressure was less marked. The variation of K_m and V_{max} with oxygen partial pressure was approximately 9% in comparison with a 70% change in ATPase activity over a similar range of oxygen partial pressures. This would indicate that the reduction in ATPase activity of aerobic cultures is due to the reduction in the amount of ATPase rather than to a change in the properties of the enzyme.

Discussion

At low glucose concentration and oxygen-limited conditions, *Z. mobilis* adapts by decreasing the glucokinase, glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and ATPase activities and by reducing the q_s and m_e. Pankova et al. (1988) have also reported a decrease in the combined activities of 6-phos-

phogluconate dehydratase and 2KDPG aldolase. Growth was characterised by an increase in the$Y_{x/s}$ and $Y^{max}_{x/s}$. Curiously, however, when the rate of energy consumption for maintenance (m_e) is subtracted from the rate of energy supply (q_s) of Table 1, and assuming that the ratio of ATP:glucose is unchanged under oxic conditions (i.e. 1 mol ATP/mol glucose), there is a net ATP supply rate available for growth of 9.7 mmol ATP g^{-1} h^{-1} (pH 4.4) and 9.5 mmol ATP g^{-1} h^{-1} (pH 6.5) anaerobically, which decreases to 5.2 mmol ATP g^{-1} h^{-1} (pH 4.4) and 5.4 mmol ATP g^{-1} h^{-1} (pH 6.5) aerobically. When investigated in more detail at constant specific oxygen transfer rates, the anaerobic-to-aerobic transitional response of *Z. mobilis* could be characterised by four physiological stages: (1) anaerobic (control) growth (0 mm HgO_2); (2) the oxygen-limited state (7.6–235 mm HgO_2), where the increase in $Y_{x/s}$ and the cellular ATP concentration is accompanied by the reduction in the q_s and the activities of some enzymes involved in glucose metabolism; (3) the intermediate state (273 mm HgO_2), the highest oxygen partial pressure at which steady state was possible; and (4) the oxygen-excess state (290 mm HgO_2) was characterised by growth inhibition. The inhibitory effect of oxygen in *Z. mobilis* manifests itself as a drastic reduction in the glucokinase, glucose-6-phosphate dehydrogenase, and pyruvate kinase activities and, consequently, in a reduction in the intracellular ATP pool. The low levels of metabolic products and the low substrate concentration enabled us to conclude that the changes to growth and enzyme activities were due mainly to oxygen.

The effect of oxygen here appears to be different from pH energetic uncoupling because the $Y^{max}_{x/s}$ was also affected. In *Z. mobilis*, the respiratory activity is linked to an increase in the transmembrane pH gradient (Pankova et al. 1988) and can drive oxidative phosphorylation in cell vesicles and starved cells (Kalnenieks et al. 1993; Zikmanis et al. 1995). However, the increase in the $Y^{max}_{x/s}$ and the cellular ATP concentration observed in aerobic cultures may not necessarily prove oxidative phosphorylation is occurring. In this context, the investigations into the energetics of *E. coli* ATPase-negative mutants would be relevant here. The ATPase-negative *E. coli* mutants were unable to grow anaerobically or to carry out active uptake of amino acids without artificial electron acceptors (Yamamoto et al. 1973), but they were able to grow aerobically [Stouthamer and Bettenhaussen 1977; see also Ugurbil et al. (1982)] although oxidative phosphorylation was abolished because of the absence of functional ATPase. Therefore, only energy generated by substrate-level phosphorylation was available for growth. However, the replacement of ATP-dependent membrane energisation by respiratory activity in the ATPase-negative mutant led to a 107% increase in the Y^{max}_{ATP} over the wild-type strain grown anaerobically, and is indicative of the magnitude of the ATPase-mediated, growth-associated energy metabolism for membrane energisation and transport processes. It has been shown that amino acid uptake in anaerobic *Z. mobilis* cells is energy-dependent (Alexandri et al. 1990; Ruhrmann and Krämer 1992). *Z. mobilis* UQM 2716 exhibited an increase in the $Y^{max}_{x/s}$ (Y^{max}_{ATP}) of approximately 145% (pH 4.4) and 158% (pH 6.5) under oxic conditions. The results from the study of *E. coli* mutants would suggest that between two-thirds and three-quarters of the increase in the $Y^{max}_{x/s}$ (Y^{max}_{ATP}) observed here could be attributed to the replacement of ATPase-dependent membrane energisation anaerobically by respiratory activity aerobically. This is supported by the rapid decrease in the membrane ATPase activity of aerobic cultures, which is accompanied by a rise in the $Y_{x/s}$ and in the intracellular ATP pool at a lower q_s. Thus, it seems that in a growing *Z. mobilis* culture, if oxidative phosphorylation occurs under these conditions, then its contribution appears to be relatively small.

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