

An in vivo analysis of the energetics of aldose oxidation by *Acinetobacter calcoaceticus*

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Summary. A continuous culture study was made of the energetics of oxidation of various aldose sugars by *Acinetobacter calcoaceticus* LMD 79.41. The consumption of aldoses during carbon- and energy-limited growth of the organism on mixtures of acetate and an aldose was independent of the pH of the culture. Acid production, however, was strongly dependent on this parameter. It is shown that aldose consumption without concurrent acid production is due to formation of the corresponding lactone, the hydrolysis of which is pH-dependent.

The cell yield of *A. calcoaceticus* on mixtures of acetate and glucose or xylose was much higher than during growth on acetate alone. This increase in cell yield was, however, dependent on the pH of the culture. Only at pH values which permitted a high rate of lactone hydrolysis an enhancement of the cell yield was observed. These results suggest that lactone hydrolysis has an important bearing on the efficiency of periplasmic oxidation of aldoses in bacteria.

Introduction

Various aerobic heterotrophic organisms may respire their substrates incompletely. These oxidized products, usually organic acids, are excreted into the medium. Members of the genus *Gluconobact-*

er, for example, are well known for their rapid and almost quantitative incomplete oxidation of a wide range of organic compounds. In these bacteria the quinoprotein glucose dehydrogenase (GDH, EC 1.1.99.17) is involved in the periplasmic oxidation of glucose to gluconic acid. This membrane-bound enzyme is found in many organisms, particularly in *Pseudomonas* spp. and acetic acid bacteria (Duine et al. 1986). The physiological meaning of such oxidative transformations is unclear. It has been suggested that the incomplete oxidation of glucose confers the ability of *Pseudomonas* to sequester the sugar as gluconate, a compound supposed to be not readily used by various other organisms (Dawes 1981). Another function of the glucose dehydrogenase may be in energy metabolism. However, several authors have concluded that the periplasmic conversion of glucose to gluconic acid yields no energy to the organism (Campbell et al. 1956; Mackechnie and Dawes 1969; Uspenshaya and Loitsyanska 1979). Their conclusion was based on investigations with organisms possessing several initial pathways of glucose metabolism (Lessie and Phibbs 1984), which severely complicates the evaluation of the energetics of the oxidation of glucose to gluconic acid.

Acinetobacter calcoaceticus seems to be an ideal model organism for an evaluation of the function of GDH. This organism is unable to use glucose or gluconate as a carbon source but a membrane-bound glucose dehydrogenase is synthesized constitutively (Visser et al. 1986). Electrons derived from glucose are fed into the electron transport chain at the level of cytochrome b (Beardmore-Gray and Anthony 1986). Thus, if the electron transport to oxygen is accompanied with proton translocation, glucose oxidation can be energy yielding. It is therefore to be expected that

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under conditions of energy limitation glucose oxidation will contribute to the energy budget of the cell. Indeed, preliminary results showed that addition of glucose to carbon-limited chemostat cultures of *A. calcoaceticus*, growing on acetate, resulted in a significant increase in cell yields (de Bont et al. 1984). Also *in vitro* studies with membrane vesicles of *A. calcoaceticus*, *Pseudomonas aeruginosa* and *E. coli* have shown that glucose oxidation can generate a proton motive force (van Schie et al. 1985; Pronk et al. 1985).

This paper presents a more detailed study on the *in vivo* energetics of oxidation of aldose sugars in *A. calcoaceticus*. Our data and those of Müller and Babel (1986) support the hypothesis that these reactions can be energy yielding.

Materials and methods

Organism and growth conditions. *A. calcoaceticus* LMD 79.41 was obtained from the culture collection of the Laboratory of Microbiology Delft, The Netherlands. The organism was grown under acetate limitation in a chemostat at a dilution rate of 0.15 h^{-1} at 30°C . An Applicon laboratory fermentor with a working volume of 1 litre was used. The dissolved oxygen tension was recorded with a steam-sterilizable Clark-type oxygen electrode and was kept constant at 40% of air saturation. In order to prevent foaming air was not sparged through, but blown over the culture. The pH was controlled at the desired value by automatic addition of 1 M KOH or 0.5 M H_2SO_4 .

The mineral medium was prepared according to van Schie et al. (1984). It contained a fixed sodium acetate concentration of 30 mM and varying concentrations of aldose sugars (glucose, galactose, xylose, 2-deoxyglucose) or potassium gluconate. When the culture had reached a steady state on acetate, glucose was added to the reservoir medium. When a new steady state on the mixture was obtained, the parameters of interest were measured and thereafter the culture was again switched to a medium containing 30 mM acetate for at least 10 volume changes before a new mixture of acetate and an aldose was tested. This procedure was adopted to prevent selection of glucose-utilizing variants which arise after prolonged exposure of *A. calcoaceticus* LMD 79.41 to glucose (van Schie, unpublished results).

Radiorespirometry. Radiorespirometric studies in chemostat cultures were performed by switching a steady state culture on acetate to a mixed substrate feed of 30 mM acetate and 10 mM D-[U- ^{14}C] glucose or 15 mM D-[U- ^{14}C] xylose with a specific activity of $0.5\text{--}1 \mu\text{Ci}/\text{mmol}$.

Dissolved $^{14}\text{CO}_2$ in the cell suspension was measured after centrifugation (15 min, 35,000 g) by counting the alkalinized (pH 12) and acidified (pH 2) supernatants. The difference between those two measurements accounted for the $^{14}\text{CO}_2$ dissolved into the culture fluid. The amount of label present in the acidified supernatant accounted for ^{14}C -gluconate or ^{14}C -xylonate. Samples of 1 ml were withdrawn from the supernatant and counted in 12 ml Aquasol scintillator fluid. For the measurement of label incorporated in biomass, cells (20 ml) were collected by centrifugation (15 min, 35,000 g), washed

twice with 50 mM phosphate buffer pH 6.6 and concentrated to 5 ml in the same buffer. From this concentrated cell suspension samples of 1 ml were counted in 12 ml Aquasol scintillator fluid.

Measurements of $^{14}\text{CO}_2$ in the gas flow were performed as described by Bruinenberg et al. (1986).

Hydrolysis of lactones. The rate of lactone hydrolysis was measured at fixed pH values with a Metrohm Ltd. automatic titrator type E 300R. A thermostated vessel (30°C) contained 15 ml demineralized water with 225 μmol of the desired lactone. The pH of the solution was controlled at a fixed value by titration with 50 mM NaOH. The rate of hydrolysis of the various lactones was calculated from recorder tracings of alkali consumption.

Total organic carbon measurements. A Beckman Model 915B Tocamaster Total Organic Carbon Analyser was used to determine the carbon content of whole cultures or culture supernatants, the carbon content of bacteria being obtained from the difference. Bacterial dry weight was calculated assuming a carbon content of 50%.

Electron microscopy. Electron microscopy of thin sections was performed as described by Bruinenberg et al. (1985).

Analytical assays. Glucose was measured with the GOD-PAP method, gluconate with gluconate kinase and 6-P-gluconate dehydrogenase and acetate with acetyl-CoA-synthetase, citrate synthase and malate dehydrogenase (test-combinations Boehringer, Mannheim). Protein was measured by the Bradford method (Bio-Rad Laboratories) with bovine serum albumin as a standard. Xylose was measured by HPLC using a HPX-81C Carbohydrate Column (Bio-Rad Laboratories) and a refractive index detector ERC-7500 according to the instructions of the manufacturer (Erma optical works LTD, Tokyo, Japan).

Chemicals. Radiochemicals were obtained from Amersham (UK); Aquasol scintillator fluid from NEN Chemicals (Boston, USA), xylose was from Merck (Darmstadt, FRG) and contained less than 0.02% glucose, D-glucono- δ -lactone was from Merck and D-galactono- γ -lactone from Pfanstiehl (Illinois), xylonolactone was prepared as a 20 mM solution with *A. calcoaceticus* LMD 79.41 as follows: A washed cell suspension was resuspended in demineralized water to a final concentration of 0.75 g/L, D-xylose was added (20 mM) and the pH of the aerated suspension was set at 5.7. When all xylose was consumed, cells were removed by centrifugation and the supernatant was acidified with 5 M HCl to a final pH of 3.5 to prevent hydrolysis of the xylonolactone formed.

Results

Most strains of *Acinetobacter calcoaceticus* can oxidize glucose to gluconic acid but do not grow on either of the two compounds (Juni 1978). When *A. calcoaceticus* LMD 79.41 was grown in batch cultures on mixtures of acetate and glucose both substrates were oxidized simultaneously. Linear proportionality was found between acetate concentration and amount of biomass produced (Fig. 1). In the presence of 5 mM glucose, which was oxidized completely to gluconic acid, the same growth

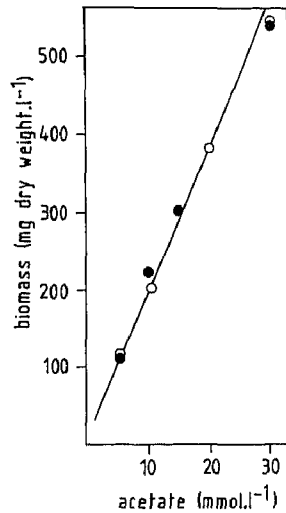


Fig. 1. Effect of increasing acetate concentrations on the growth yield of *A. calcoaceticus* grown in batch cultures, with (●) and without (○) additional 5 mM glucose

yields as with acetate alone were obtained (Fig. 1).

When *A. calcoaceticus* was grown in carbon-limited chemostat cultures on acetate the steady state biomass concentration was independent of the culture pH between 5.0–8.2. The effect of in-

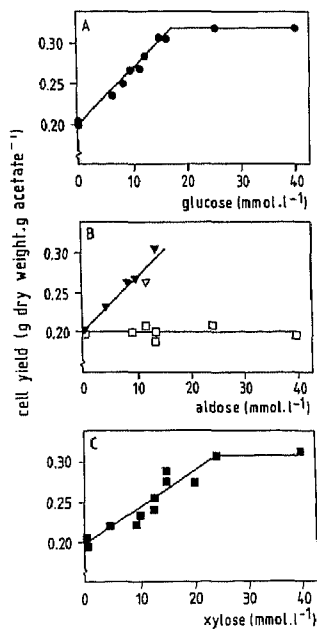


Fig. 2. Effect of increasing concentrations of aldose sugars in the reservoir of an acetate-limited ($S_r = 30$ mM) continuous culture of *Acinetobacter calcoaceticus* LMD 79.41 at $D = 0.15$ and $pH = 7.0$ on the biomass concentration. A, effect of glucose. B, effect of galactose (▼); 2-deoxy-glucose (▽); xylose (□). C, effect of xylose at $pH 8.2$

creasing concentrations of glucose in the medium reservoir of an acetate-limited continuous culture is shown in Fig. 2a. Acetate or glucose were not detectable in the culture fluid of steady state cultures and gluconic acid recovery was almost quantitatively (between 90 and 95%). In contrast to batch cultures, the presence of glucose resulted in an enhancement of the cell yield on acetate. Growth yields increased from 0.20 up to 0.32 g bacterial dry weight per g acetic acid. This enhancement was found to be linear up to 15 mM glucose, above this concentration a plateau level of the biomass concentration was reached. A further increase of the amount of glucose did not alter the cell yield on acetate (Fig. 2a). Protein content of cells was between 60% and 70% of biomass dry weight, depending on the analytical procedure, and remained constant when *A. calcoaceticus* was grown on the mixtures. The possible presence of storage products was investigated via electron microscopy of thin sections. No high density compounds were found which could account for polyhydroxybutyrate (PHB) or polyphosphates. Total carbohydrate content of the cells, as measured with the Anthrone reaction, showed no change in the content of reducing sugars. Therefore, the possibility that the increase in biomass was caused by accumulation of storage products or a change in cell composition could be excluded. Growth of the organism on mixtures of acetate and gluconate gave a similar biomass yield as compared to growth on acetate alone (Table 1), which proved that gluconate did not serve as a carbon or energy source.

Since the quinoprotein glucose dehydrogenase has a broad substrate specificity (Kleber et al. 1984) also other aldose sugars, which were oxidized at a high rate by whole cells, were tested. Galactose and 2-deoxyglucose could also serve as an additional energy source (Fig. 2b). Compara-

Table 1. Effect of glucose and gluconate on biomass yield of *A. calcoaceticus* in acetate-limited chemostat cultures at a dilution rate of 0.15 h^{-1} as a function of culture pH. The reservoir acetate concentration was 30 mM

Substrate [mM]	pH	Biomass (mg cells l ⁻¹)
None	—	*
Gluconate	60	7.0
Glucose	15	8.2
Glucose	15	6.0
Glucose	15	5.4
Glucose	15	5.0

* The biomass yield on acetate alone was independent of the culture pH between 5.0 and 8.2

Table 2. Acid or base titration during growth of *A. calcoaceticus* in acetate-limited continuous cultures ($D=0.15\text{ h}^{-1}$) in the presence or absence of aldoses at different pH values. The culture volume was 1 l

Substrate[s]	pH	H ₂ SO ₄ mmol H ⁺ · h ⁻¹	KOH mmol OH ⁻ · h ⁻¹	Expected H ⁺ production mmol · h ⁻¹
Acetate	7.0	0.56	0.0	—
Acetate + 15mM glucose	7.0	0.00	1.46	2.25
Acetate + 11 mM xylose	7.0	0.52	0.02	1.65
Acetate	8.2	0.62	0.00	—
Acetate + 15 mM xylose	8.2	0.00	1.62	2.25
Acetate	5.0	0.56	0.00	—
Acetate + 15 mM glucose	5.0	0.39	0.00	2.25

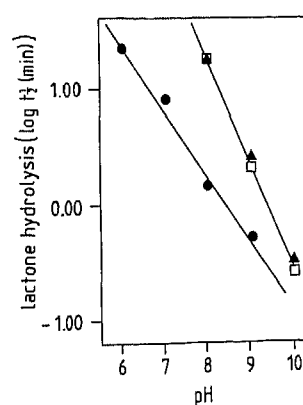
ble yields were obtained as with glucose/acetate mixtures. Surprisingly, addition of xylose to the medium reservoir of an acetate-limited culture did not affect the growth yield (Fig. 2b), although no residual xylose could be detected in these cultures.

When cultures were growing on acetate, titration with H₂SO₄ was necessary to maintain a pH of 7.0. During growth on mixtures of acetate and glucose, 2-deoxyglucose or galactose the pH had to be controlled with KOH due to the production of the corresponding aldonic acids. However, growth on a mixture of acetate and xylose was not accompanied with the expected acid production although all xylose was consumed (Table 2).

It is well established that the reaction product of aldose oxidation by GDH is a lactone which is hydrolyzed non-enzymically to the corresponding aldonic acid. The rate of lactone hydrolysis is determined by several factors such as temperature, concentration and especially pH. High pH values being favourable for the hydrolysis of lactones (Takahashi et al. 1963). It was therefore anticipated that in cultures growing on mixtures of acetate and xylose at pH 7.0, xylonolactone rather than xylonic acid was produced. Indeed, when the culture pH was abruptly increased from 7.0 to 8.5 an immediate subsequent fall of the pH was observed probably due to hydrolysis of xylonolactone to xylonic acid. In order to further investigate the difference in acid production from xylose and the other aldose sugars by cultures of *A. calcoaceticus*, a study was made of the kinetics of hydrolysis of various lactones as a function of pH. The results (Fig. 3) show that at all pH values the xylonolactone produced by *A. calcoaceticus* was more stable than δ -gluconolactone, which is known to be the product of glucose oxidation by microorganisms (Jermyn 1960). Xylonolactone hydrolysis was negligible at pH 7.0 whereas at this pH the half-life of δ -gluconolactone was only 8 min. Commercial γ -galactonolactone, however,

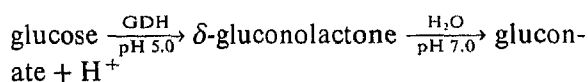
displayed the same hydrolysis kinetics as the xylonolactone collected from *A. calcoaceticus* cultures. Both lactones were rather stable at neutral pH but had half lives of less than 10 min only at pH 8.2 and above. It therefore seems likely that *A. calcoaceticus* produces the γ -lactone from xylose, which is stable at neutral pH, and the δ -lactone from hexoses which rapidly hydrolyze at pH 7.0.

Since an increase in cell yield was only observed when acid was produced from the aldose it was suspected that hydrolysis of the reaction product rather than the oxidation of the sugar itself was the main determinant for the yield enhancement. In order to verify this hypothesis *A. calcoaceticus* was cultivated at a pH which allowed rapid hydrolysis of xylonolactone. When a culture was grown acetate-limited at pH 8.2, and xylose was added to the reservoir medium, xylonic acid was formed as became clear from the KOH titration data (Table 2). A yield enhancement occurred proportional with increasing xylose concentrations up to 23 mM (Fig. 2c). A maximal yield of 0.31 g dry cells/g acetic acid was found which showed no further increase with in-

**Fig. 3.** Half life of various lactones as a function of the pH. D-glucono- δ -lactone (●); D-galactono- γ -lactone (▲) and xylonolactone (□) prepared with *A. calcoaceticus* (see Methods)

creasing xylose concentrations in the influent. These results clearly show that, at least with xylose, the increase in cell yield is dependent on the pH of the culture.

For a further testing of the hypothesis that acid production (i.e. lactone hydrolysis) is a prerequisite for in vivo energy generation from aldose sugars by GDH also the effect of pH on glucose utilization by *A. calcoaceticus* was examined. To this end the organism was grown at pH 5.0. At this pH value the hydrolysis of δ -gluconolactone is very slow and comparable with the rate of xylonolactone hydrolysis at pH 7.0 (Fig. 3). Also during growth on a mixture of acetate (30 mM) and glucose (15 mM) at this low pH value no residual glucose could be detected in the culture but, only a minor increase of the yield was observed (Table 1) probably due to the partial hydrolysis of gluconolactone as became clear from the acid titration data (Table 2). As with cultures growing in the presence of xylose at pH 7.0 a shift up in pH of a culture growing on acetate + glucose at pH 5.0 resulted in an immediate subsequent acid production. This phenomenon was neither observed with cultures growing on acetate alone at pH 5.0 nor with cultures growing on acetate + glucose at pH 7.0. Furthermore, it was not due to an activity of the organism as such. When a shift up in pH was made of a supernatant of the culture growing at pH 5.0 on glucose + acetate the same subsequent rapid acid production was observed. These observations can be summarized in the following reaction sequence:

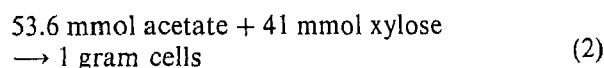
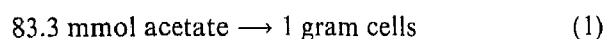


Various explanations may be given for the observed correlation between the cell yield and the hydrolysis of the lactone in steady state cultures. Firstly, titration with KOH required to sustain a constant pH when gluconic acid is produced from glucose results in an increase in the potassium concentration of the culture. This in turn could have a positive effect on the efficiency of cultures growing at pH 7.0 which would be absent in cultures growing at pH 5.0 owing to the high stability of gluconolactone at low pH values. However, when cultures growing on acetate + glucose at pH 5.0 were supplied with extra potassium in the medium feed this did not affect the cell yield. Secondly it can be argued that gluconate but not gluconolactone is partly metabolized by the organism which would lead to a pH dependent increase

in cell yield. A similar explanation could hold for the observed pH dependent increase in cell yield with xylose as the substrate.

In order to investigate the possible utilization of aldonic acids by *A. calcoaceticus*, radiorespirometric studies were performed with cultures growing on acetate + ^{14}C -glucose or on acetate + ^{14}C -xylose. The organism was grown at pH 8.2 since at this pH both glucose and xylose utilization resulted in an increase in cell yield. A mass balance of ^{14}C was constructed by measuring ^{14}C in the supernatant and in the biomass and the ^{14}C - CO_2 in the effluent gasflow. The results (Table 3) show that glucose is indeed partially metabolized to ^{14}C - CO_2 . This accounted for 9% of the amount of label added. A minor part of the label (2%) was incorporated into cell material (Table 3). With xylose as an additional energy donor much lower amounts of the label were recovered as $^{14}\text{CO}_2$ or incorporated into biomass. These processes accounted for 2.5% and 0.2% respectively of the total amount of label added (Table 3).

Although aldose metabolism beyond the level of corresponding aldonic acid was significant it can not explain the extreme increase in cell yield of cultures growing in the presence of aldose sugars compared to those growing on acetate alone. For simplicity only the case of xylose utilization is considered here since incorporation of xylose carbon into cell material was negligible. From the data presented in Fig. 2c the following Eq. (1) and (2) are derived:



Thus, 41 mmol xylose reduction equivalents obtained in the oxidation of xylose to xylonic acid, can replace $(83.3 - 53.6) \cdot 4 = 118.8$ acetate reduction equivalents. This unexpectedly high energetic value of the xylose reduction equivalents still holds when the amount of xylose oxidized to CO_2 (2%, Table 3) is taken in consideration. In this

Table 3. Fate of labelled aldoses in chemostat cultures of *A. calcoaceticus* growing on mixtures of acetate and aldoses at pH 8.2

Additional energy source	^{14}C - CO_2	^{14}C -biomass	^{14}C -supernatant	^{14}C -recovery
15 mM ^{14}C -xylose	2.6%	0.2%	97.1%	99.9%
10 mM ^{14}C -glucose	9.2%	2.9%	88.5%	100.6%

case $41 + (2:100) * 41 * 10 = 49$ xylose reduction equivalents would be produced.

Discussion

The role of membrane-bound GDH in energy metabolism of Gram-negative organisms has been puzzling for a long time. From yield studies with *Pseudomonas aeruginosa* grown on glucose and gluconate it was concluded that the first oxidative step in glucose metabolism does not yield energy (Campbell et al. 1956; Mackechnie and Dawes 1969). Also from studies with *Gluconobacter oxydans* it was concluded that production of gluconic acid does not provide biological useful energy (Uspenskaya and Loitsyanskaya 1979).

Our results with *A. calcoaceticus* grown in batch cultures (Fig. 1) support the above conclusion that oxidation of glucose to gluconate may be an energy spilling reaction (Fig. 1). Similar results have been obtained for *Thiobacillus* Q grown in batch cultures on mixtures of acetate/thiosulfate (Gommers and Kuenen 1986) and for batch cultures of *Hyphomicrobium* EG, on thiosulfate and methylamine (Suylen et al. 1986). Also in these cases both substrates were oxidized simultaneously but growth yields in batch cultures on acetate or methylamine did not increase in the presence of the additional energy source.

Batch cultivation of bacteria implies a high substrate concentration during exponential growth and therefore under these conditions growth may not be energy-limited. As a result energy derived from the auxiliary energy source may be spilled during these growth conditions. When however an organism is grown under carbon and energy limitation the presence of an additional energy source should alter the growth yield. Indeed when glucose was added to acetate-limited continuous cultures of *A. calcoaceticus* the biomass concentration increased (Fig. 2). Not only glucose but also other aldose sugars, which are oxidized by GDH, enhanced the growth yield on acetate in the same order of magnitude as glucose (Fig. 2). Comparable results have been obtained for *Thiobacillus* Q, addition of thiosulfate to an acetate-limited culture resulted in an increase in the growth yield on acetate (Gommers and Kuenen 1987). Also with *Hyphomicrobium* EG, addition of thiosulfate or sulphide to a methylamine-limited culture resulted in an increase in the growth yield on methylamine (Suylen et al. 1986). From these results it is clear that the contribution of additional electron donors to the bacte-

rial energy metabolism can only be studied with cultures growing under carbon and energy limitation.

An important finding concerning aldose oxidation by *A. calcoaceticus* was the effect of culture pH on the increase in cell yield. As became evident from our results, lactone hydrolysis is an important parameter in this respect. At a pH at which lactone hydrolysis is very slow (e.g. for xylose at pH 7.0 and for glucose at pH 5.0) it was found that although all sugar was oxidized, this did not result in an increase in the cell yield. From this it can be concluded that the formation of acid from the lactones is essential in the beneficial effect of sugars on the growth yield. Peculiarly, however, xylose can energize active transport at pH values (7.0) which prevent lactone hydrolysis (van Schie et al. 1985).

From experiments with labelled glucose and xylose it became evident that glucose is partly further oxidized to CO₂ by our strain of *A. calcoaceticus*. Kleber et al. (1984) showed that *A. calcoaceticus* 69-V, which could not use glucose as a sole carbon source, was able to accumulate glucose (in fact gluconic acid) at a low rate and to produce some CO₂ from glucose. Müller and Babel (1986) showed that glucose could serve as an additional energy source in acetate growing *A. calcoaceticus* 69-V. Their experiments were performed with a transient-state analysis by which a linear increasing glucose concentration gradient is supplied to the culture. Their results clearly showed an improvement of the yield on acetate after addition of glucose. However, it is difficult to interpret the yield data obtained with this technique since it does not involve steady state cultures. Furthermore, since their strain of *A. calcoaceticus* also incorporates glucose and produces CO₂ from glucose (Kleber et al. 1984) and the fact that very high glucose concentrations (up to 160 mM) were used, the conclusion that the increase of biomass approximates the limit of acetate carbon conversion may not be valid. From our data on xylose utilization by *A. calcoaceticus* LMD 79.41 it can be calculated that a maximal carbon conversion of only 50% is reached when the sugar is present in excess.

In contrast to those of Müller and Babel (1986) our results and calculations show that aldose oxidation by *A. calcoaceticus* LMD 79.41 is far more efficient than can be anticipated on the basis of the efficiency of acetate dissimilation. In cultures growing at pH 8.2 the energetic value of 1 xylose reduction equivalent (PQQH₂) equalled 3 reduction equivalents from acetate (NADH). It is

hardly conceivable that oxidation of PQQH₂ yields 3 times more ATP than oxidation of NADH particularly since electrons from PQQH₂ enter the electron transport chain at the level of cytochrome b (Beardmore-Gray and Anthony 1986). Even when it is taken into consideration that periplasmic oxidation of substrates may be extra efficient in terms of proton motive generation (Hooper and DiSpirito 1985), the increase in cell yield is unexpectedly high. Therefore the high efficiency of energy conservation by *A. calcoaceticus* must probably be explained by the assumption that as a result of the oxidation of aldoses to the corresponding aldonic acids also the efficiency of energy generation from acetate reduction equivalents is increased.

The finding that the utilization of an auxiliary energy substrate may lead to extreme changes in the efficiency of dissimilatory processes is not restricted to *A. calcoaceticus*. This was also demonstrated for *Pseudomonas oxalaticus*, *Thiobacillus versutus* and *Candida utilis* (Gommers et al. 1987). An increase in efficiency of energy generation may be accomplished in several ways. For instance by the synthesis of new cytochromes associated with an extra proton translocating loop. Such a mechanism was suggested for *Paracoccus denitrificans* during growth on a mixture of mannitol and methanol. Van Verseveld et al. (1979) showed that, under the cultivation conditions employed for growth on the mixture, the synthesis of cytochrome c was induced. However, in the case of *A. calcoaceticus* the possible introduction of extra proton translocating loops does not involve cytochrome c. In accordance with published data (Ensley and Finnerty 1980) cytochrome c was absent in our strain of *A. calcoaceticus* under all growth conditions (van Schie, unpublished results). Instead of *de novo* cytochrome synthesis a rechanneling of electron flow, via a more efficient branch of the electron transport chain, could be a reason for the increase in efficiency of energy conservation during utilization of acetate/aldose mixtures. Readjustment of the electron flows via cytochrome o and cytochrome d which are both present in *A. calcoaceticus* (Beardmore-Gray and Anthony 1986) could change the efficiency of dissimilation. However, proton translocations in the cytochrome oxidase d complex is still a matter of debate (Jones and Poole 1985).

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