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Heterotrophic growth of *Thiobacillus acidophilus* in batch and chemostat cultures

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Abstract. Heterotrophic growth of the facultatively chemolithoautotrophic acidophile *Thiobacillus acidophilus* was studied in batch cultures and in carbon-limited chemostat cultures. The spectrum of carbon sources supporting heterotrophic growth in batch cultures was limited to a number of sugars and some other simple organic compounds. In addition to ammonium salts and urea, a number of amino acids could be used as nitrogen sources. Pyruvate served as a sole source of carbon and energy in chemostat cultures, but not in batch cultures. Apparently the low residual concentrations in the steady-state chemostat cultures prevented substrate inhibition that already was observed at 150 μM pyruvate. Molar growth yields of *T. acidophilus* in heterotrophic chemostat cultures were low. The Y_{max} and maintenance coefficient of *T. acidophilus* grown under glucose limitation were 69 g biomass · mol^{-1} and 0.10 mmol · g^{-1} · h^{-1}, respectively. Neither the Y_{max} nor the maintenance coefficient of glucose-limited chemostat cultures changed when the culture pH was increased from 3.0 to 4.3. This indicates that in *T. acidophilus* the maintenance of a large pH gradient is not a major energy-requiring process. Significant activities of ribulose-1,5-bisphosphate carboxylase were retained during heterotrophic growth on a variety of carbon sources, even under conditions of substrate excess. Also thiosulphate- and tetrathionate-oxidising activities were expressed under heterotrophic growth conditions.

Key words: *Thiobacillus acidophilus* – Acidophiles – Maintenance – Growth yields – Organic acids

Acidophilic, sulphur-oxidising bacteria play an important role in the biological leaching of metal ores (Norris and Kelly, 1988). Furthermore, these organisms may be applicable for the biological desulphurisation of coal (Bos et al. 1988; Klein et al. 1988).

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The obligate chemolithoautotroph *Thiobacillus ferrooxidans* has been used frequently as a model organism to study the physiology and bioenergetics of growth in acidic environments (for a review see Ingledew 1982). Due to its autotrophic lifestyle, biomass yields of *T. ferrooxidans* grown on ferrous iron or reduced sulphur compounds are low. Furthermore, the nature of the substrates supporting autotrophic growth prevents the use of high substrate concentrations for obtaining high biomass concentrations. During biochemical studies large amounts of biomass are frequently required. In these cases, facultatively autotrophic acidophiles may provide an attractive alternative model system.

The facultative autotroph *Thiobacillus acidophilus* was first isolated as a contaminant of a ferrous-iron grown *T. ferrooxidans* culture (Guay and Silver 1975). Growth substrates for its autotrophic growth include elemental sulphur (Guay and Silver 1975), tetrathionate (Norris et al. 1986), thiosulphate and trithionate (Mason et al. 1987). Recently, mixotrophic growth of *T. acidophilus* on glucose and tetrathionate has been reported (Mason and Kelly 1988). The organism can not grow autotrophically on ferrous iron but is capable of maintaining itself in ferrous iron-grown cultures of *T. ferrooxidans* (Guay and Silver 1975; Arkesteyn and de Bont 1980). Apparently *T. acidophilus* can grow on an hitherto unidentified excretion product of *T. ferrooxidans*.

T. acidophilus is an attractive model organism to study the bioenergetics of acidophilic growth (Matin et al. 1982; Matin and Matin 1982; Zychlinsky and Matin 1983a, b), but little attention has so far been paid to its heterotrophic potential.

Carbon sources supporting heterotrophic growth of *T. acidophilus* include a number of monosaccharides, TCA-cycle intermediates and amino acids (Guay and Silver 1975). With two exceptions (Arkesteyn and de Bont 1980; Mason and Kelly 1988) studies on the heterotrophic growth of *T. acidophilus* have been performed with batch cultures.

T. acidophilus and similar acidophilic heterotrophs have been isolated from bioleaching populations. The

presence of heterotrophs has been demonstrated to stimulate growth and leaching of metal ores by autotrophic cultures (Tsuchya et al. 1974; Nerkar et al. 1977). It has been suggested that in this respect the scavenging of toxic organic compounds is of key importance.

In the present study, the heterotrophic growth of *T. acidophilus* was investigated in carbon-limited chemostat cultures. The aim of this study was twofold. First, to further characterise *T. acidophilus* in view of its potential usefulness as a model acidophile. Secondly, to obtain more insight in the ecological role of *T. acidophilus* and related organisms in mixed bioleaching populations.

Material and methods

Organism and growth conditions

Thiobacillus acidophilus DSM 700 was obtained from the Deutsche Sammlung von Mikroorganismen as a liquid culture on glucose. A sample was plated on mineral medium supplemented with glucose and solidified with 0.8% agarose. A single colony was inoculated in 200 ml mineral medium plus glucose (20 mM). The resulting culture was made 10% (v/v) with dimethylsulphoxide and stored at −70° C in 1 ml aliquots. These frozen samples were used as inocula for continuous culture studies.

Thiobacillus acidophilus ATCC 27807 was obtained from the American Type Culture Collection as a freeze-dried culture and used directly as an inoculum for a glucose-limited chemostat culture in a control experiment (see Discussion).

Mineral medium

T. acidophilus was grown in a mineral medium containing per litre of demineralised water: $(NH_4)_2SO_4$, 3.0 g; KH_2PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Na_2SO_4, 1.4 g; $CaCl_2 \cdot 2H_2O$, 0.26 g; $FeSO_4 \cdot 7H_2O$, 11 mg; $ZnSO_4 \cdot 7H_2O$, 0.9 mg; $MnCl_2 \cdot 2H_2O$, 2.0 mg; $CoCl_2 \cdot 6H_2O$, 0.6 mg; $CuSO_4 \cdot 5H_2O$, 0.6 mg; $NaMoO_4$, 0.8 mg; H_3BO_3, 2.0 mg; KI, 0.2 mg; EDTA, 30 mg; nitrilo-triacetic acid, 5 mg and silicon antifoaming agent (BDH Chemicals, Poole, Dorset, UK), 25 µl. The medium was adjusted to pH 3.0 with 1 M H_2SO_4 and autoclaved at 120°C. Carbon sources were either autoclaved at 110° C or filter-sterilised before addition to the autoclaved mineral medium.

Growth conditions

Batch cultures for the screening of carbon sources were grown in 300 ml Erlenmeyer flasks containing 100 ml of mineral medium, adjusted to pH 3.5. Organic compounds were added to a final concentration of 10 mM. The cultures were inoculated with 1 ml samples from glucose-limited chemostat cultures and incubated on a rotatory shaker for 10 days at 30° C.

Batch cultures for screening of nitrogen sources were performed in 300 ml Erlenmeyer flasks containing 100 ml ammonium-free mineral medium adjusted to pH 3.5 and supplemented with 20 mM glucose. Nitrogen-containing compounds were added to a final concentration of 10 mM. The cultures were inoculated with 1 ml of a cell suspension obtained from a nitrogen-limited chemostat culture of *T. acidophilus* and incubated on a rotatory shaker for 10 days at 30°C.

Continuous cultivation was performed in Applikon laboratory fermenters with a working volume of 1 l. The pH was automatically titrated with either 1 M KOH or 0.5 M H_2SO_4. The cultures were continuously gassed with water-saturated air (1 l · min^{-1}) and stirred at 600 rpm. The dissolved oxygen concentration in the cultures was monitored with a steam-sterilisable Clark-type electrode. Unless otherwise indicated, chemostat cultures were grown at a dissolved oxygen concentration of above 75% of air saturation, at 30° C, at pH 3.0 and at a dilution rate of 0.05 h^{-1}.

Control of culture purity

The purity of the chemostat cultures was routinely checked by phase-contrast microscopy and by plating on mineral medium plus glucose, solidified with 0.8% (w/v) agarose. Additionally, immunofluorescence assays were performed. Antisera against *T. acidophilus* were obtained as described previously (Muyzer et al. 1987).

Analytical procedures

Dry weight

The dry weight of cell suspensions was determined by filtrating culture samples over nitrocellulose filters (pore diameter 0.45 µm, Schleicher and Schüll, Dassel, FRG). The cells were washed three times with demineralised water and dried to constant weight at 70° C.

Protein determination

The protein content of whole cells was assayed with a modified biuret method: cells were harvested from continuous cultures, washed with demineralised water and resuspended to a concentration of approximately 2.5 mg dry weight · ml^{-1}. The concentrate was boiled in 1 M KOH for 10 min and subsequently cooled on ice. $CuSO_4 \cdot 5H_2O$ was then added to a final concentration of 25 mM. After 5 min, the mixture was centrifuged in an Eppendorf benchtop centrifuge (13,000 × *g*) for 2 min. The absorbance of the supernatant was measured at 550 nm. The protein content of cell-free extracts was determined by the method of Bradford (1976). In both assays, bovine serum albumin (fatty acid free, Sigma, St. Louis, USA) was used as a standard.

Organic carbon content

A Beckman model 915 B Tocamaster total organic carbon analyser was used to determine the carbon content of whole cultures and culture supernatants, the carbon content of the bacteria being obtained from the difference. Cell suspensions were acidified with H_3PO_4 prior to analysis in order to expel carbon dioxide accumulated inside the cells.

Substrate determinations

Glucose concentrations in media and culture supernatants were measured with the GOD-PAP method (Boehringer, Mannheim, FRG). Glycerol and fructose were assayed with Boehringer testkits 148270 and 139106, respectively. Pyruvate and malate were determined by HPLC on a HPX-87H column (300 × 7.8 mm, Bio-Rad, USA) at room temperature. Sample were eluted with 0.01 N H_2SO_4 at a flow rate of 0.6 ml · min^{-1}. Detection was by means of a Waters 441 UV-meter at 210 nm, coupled to a Waters 741 data module. Peak areas were proportional to concentrations.

Measurements of substrate-dependent oxygen consumption

Respiration rates of cells were assayed polarographically with a Clark-type oxygen electrode (Yellow Springs Instruments Inc.,

Yellow Springs, Ohio, USA) at 30°C. Cells from carbon-limited chemostat cultures were assayed directly, without dilution, in the culture fluid. The determinations were carried out at the temperature at which the cells had been grown. Calculations were made on the basis of an oxygen concentration of 236 μM in air-saturated water at 30°C. The values presented here have been corrected for the (low) endogenous respiration rates.

Ribulose-1,5-bisphosphate carboxylase (RuBPCase)

Cells were harvested from chemostat cultures by centrifugation (10,000 × *g*, 10 min), washed with a buffer containing 100 mM Tris-HCl, 20 mM $MgSO_4$, 2.0 mM $NaHCO_3$ and 5 mM dithiothreitol (pH 8.2). Cells were resuspended in the same buffer to a concentration of approximately 10 mg dry weight · ml^{-1} and were disrupted by sonication at 4°C in an MSE 150 W sonifier (6 bursts of 30 s with intermittent cooling). Intact cells and debris were removed by centrifugation (40,000 × *g*, 20 min). RuBPcase was assayed as described by Beudeker et al. (1980). The rate of ^{14}C incorporation was proportional to the amount of cell-free extract added. Incorporation of ^{14}C was dependent on addition of ribulose-1,5-bisphosphate.

Chemicals

Ribulose-1,5-bisphosphate was obtained from Sigma Chemical Co. (Boston, Missouri, USA). [^{14}C]-$NaHCO_3$ (2.11 TBq · mol^{-1}) was obtained from Amersham International PLC. D-xylulose was prepared as described by Pronk et al. (1988). All other chemicals were reagent grade and obtained from commercial sources.

Results

Carbon and energy sources for heterotrophic growth in bath cultures

In the original description of the organism, a small number of organic compounds were reported to support heterotrophic growth of *Thiobacillus acidophilus* (Guay and Silver 1975). In order to obtain more insight into the metabolic versatility of this facultative chemolithoautotroph, a wide variety of organic compounds was screened as possible carbon sources for heterotrophic growth in batch cultures.

The organism was able to grow on: L-arabinose, L-aspartate, citrate, D-fructose, D-galactose, D-glucose, L-glutamate, L-malate, mannitol, D-ribose and D-xylose, in accordance with the results of Guay and Silver (1975). Growth also occurred in batch cultures containing L-fucose, D-gluconate, glycerol or D-xylulose as sole carbon sources.

T. acidophilus failed to grow in mineral medium supplemented with acetate, ascorbate, benzoate, D-cellobiose, glyoxylate, DL-lactate, D-lactose, D-maltose, DL-mandelate, D-mannose, D-melibiose, phenol, phenylalanine, L-proline, pyruvate, D-raffinose, rhamnose, sorbose, succinate, trehalose, tryptophane or L-tyrosine, in accordance with Guay and Silver (1975). Other substrates which did not support growth of *T. acidophilus* in batch cultures were acetone, acetoin, adipate, adonitol, L-alanine, *p*-aminobenzoate, L-arginine, L-asparagine, butanol, butyrate, L-citrulline, creatine, cyclohexane carboxylate, cyclohexanol, L-cysteine, 2-deoxy-D-glucose, dulcitol, ethanol, ethylacetate, ethylamine, formaldehyde, formate, fumarate, gentiobiose, glucuronate, L-glutamine, glutarate, glycine, glycollate, *n*-hexadecane, L-histidine, *o*-hydroxybenzoate, *p*-hydroxy-benzoate, L-isoleucine, 2-ketogluconate, 5-ketogluconate, α-oxoglutarate, L-leucine, L-lysine, D-melezitose, methanol, methylacetate, methylamine, L-methionine, α-methylglucoside, L-ornithine, oxalate, palatinose, phenylethylamine, pimelate, propionate, propylene glycol, L-serine, sucrose, L-threonine and xylitol.

According to Guay and Silver (1975), *T. acidophilus* can use D-sucrose as a carbon source for heterotrophic growth. In our hands, growth on D-sucrose was dependent on non-biological acid hydrolysis of this disaccharide.

Nitrogen sources for heterotrophic growth

T. acidophilus can use ammonium salts and urea, but not nitrate as a sole source of cell nitrogen (Guay and Silver, 1975). We screened a number of nitrogen-containing compounds as possible nitrogen sources for glucose-grown *T. acidophilus*. The following compounds could be used as nitrogen sources: L-alanine, L-asparagine, L-aspartate, L-citrulline, creatine, L-cysteine, L-cystine, L-glutamate, L-glutamine, glycine, L-lysine, L-methionine, L-ornithine, L-serine and L-tyrosine. Growth did not occur when nitrogen-free mineral medium was supplemented with glucose and either one of the following compounds: L-arginine, glucosamine, L-histidine, L-isoleucine, L-leucine, methylamine, L-phenylalanine, L-threonine or L-valine. Weak growth was observed with L-proline and L-tryptophane.

Growth yields and cellular composition in carbon-limited chemostat cultures

When grown on glucose in batch cultures at pH 3.0 and at 30°C, *T. acidophilus* exhibits a specific growth rate of 0.084 h^{-1} (Guay and Silver 1975; Pronk, unpublished). Growth in glucose-limited chemostat cultures was initially studied at a dilution rate of 0.05 h^{-1}. The mineral medium used in this study is a modification of the medium described by Bounds and Colmer (1972). Apart from the addition of sodium sulphate and trace elements, the medium differs from the Bounds and Colmer medium by a tenfold lower potassium phosphate concentration. With the original phosphate concentration, *T. acidophilus* accumulated significant amounts of polyphosphate as judged by electron microscopy (data not shown). Polyphosphate accumulation did not occur with the modified medium. A similar observation has been made by Gommers and Kuenen (1988) with *Thiobacillus* strain Q.

To check whether the mineral medium and growth conditions used throughout this study could support carbon-limited growth, several parameters were studied in chemostat cultures grown on mineral medium supplemented with increasing concentrations of glucose. The

Table 1. Growth of *Thiobacillus acidophilus* in chemostat cultures ($D = 0.05\ h^{-1}$, pH = 3.0, T = 30° C) under various growth limitations. RuBPCase activities and oxidation rates represent the means of two independent assays

Growth-limiting substrate	Molar growth yield g dry weight · mol^{-1}	RuBPCase nmol · [min · mg protein]$^{-1}$	Oxidation rate nmol O_2 · [min · mg dry weight]$^{-1}$	
			Thiosulphate	Tetrathionate
Glucose	60	5.1	48	39
Fructose	60	4.7	46	31
L-Malate	25	4.6	80	44
Glycerol	41	5.0	48	40
Pyruvate	24	n.d.[a]	45	30
Nitrogen[b]	n.d.[a]	2.2	57	9
Thiosulphate[c]	5.5	40	350	130

[a] n.d. = not determined

[b] Ammonium-limited chemostat cultures were grown with glucose as a carbon source. The residual glucose concentration in the culture was 3.8 mM. Enzyme activities were measured at least 10 volume exchanges after a shift to ammonium limitation

[c] Thiosulphate-limited cultures were grown at a dilution rate of 0.03 h^{-1}

carbon and protein contents of the biomass were $(48 \pm 1)\%$ and $(67 \pm 2)\%$, respectively. Electron microscopy of thin sections did not reveal any significant accumulation of storage polymers. The dry weight of the cultures increased linearly with the glucose concentration in the influent medium and residual glucose could not be detected in the growth medium with the analysis methods used. These results demonstrate that the cultures were indeed glucose-limited.

At a dilution rate of 0.05 h^{-1}, the molar growth yield of *T. acidophilus* was 60 ± 1 g biomass (mol glucose)$^{-1}$. This growth yield is low as compared to the growth yields observed with many other heterotrophic microorganisms on this substrate (Linton and Stephenson 1978). Low growth yields were also observed with carbon-limited chemostat cultures grown on fructose, glycerol and malate (Table 1).

Maintenance energy requirement

To check whether the low growth yields observed in carbon-limited chemostat cultures were influenced by maintenance requirements, molar growth yields of glucose-limited chemostat cultures (pH 3, 30° C) were determined at various dilution rates. The specific glucose consumption rate plotted against the dilution rate yielded a straight line (Fig. 1). From this plot, a theoretical maximum growth yield of 69 g dry weight · (mol glucose)$^{-1}$ was calculated. The maintenance coefficient derived from this plot was 0.10 mmol glucose · (g dry weight · h)$^{-1}$. This value is lower than the maintenance coefficients observed for various neutrophilic bacteria grown on glucose (Table 2), suggesting that growth of *T. acidophilus* at low pH values does not require excessive proton-pumping activity.

In acidophilic microorganisms, the pH of the cytoplasm is poised at near-neutral values (Matin et al. 1982). Therefore, growth at low pH values requires the maintenance of a large pH gradient over the cytoplasmic membrane. To investigate whether the maintenance of this gradient imposes a major energy requirement on growing

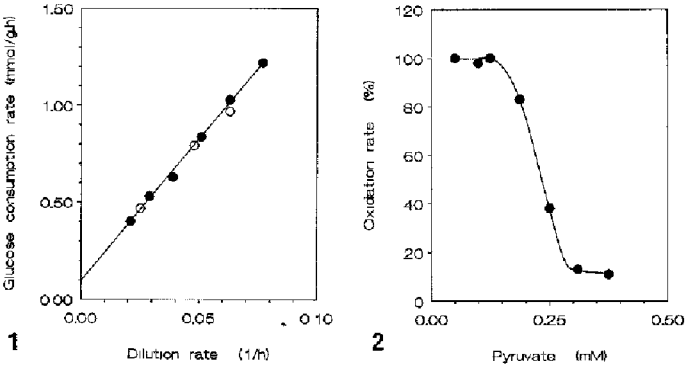

Fig. 1. Specific rates of glucose consumption by glucose-limited chemostat cultures of *Thiobacillus acidophilus* grown at various dilution rates. ●: cultures grown at pH 3.0; ○: cultures grown at pH 4.3. **Fig. 2.** Oxidation of pyruvate by intact cells of *Thiobacillus acidophilus*: effect of substrate concentration. Cells were pregrown in a glucose-limited chemostat culture ($D = 0.05\ h^{-1}$, pH = 3.0, T = 30° C, $S_R = 10$ mM). Oxygen uptake rates were measured with a Clark-type oxygen electrode. 100% Activity corresponds with an oxygen uptake rate of 25 nmol $O_2 \cdot min^{-1} \cdot$ (mg dry weight)$^{-1}$

cells, glucose-limited cultures were grown at pH 4.3. This increase of the culture pH affected neither the maximum growth yield nor the maintenance coefficient (Fig. 1). This also indicates that the maintenance of a large pH gradient does not impose a major energy requirement on growing *T. acidophilus* cells.

Growth of T. acidophilus on pyruvate

When studying the heterotrophic growth of *T. acidophilus*, we were particularly interested in growth of the organism of pyruvate. Pyruvate can be excreted in significant amounts by *Thiobacillus ferrooxidans*. Concentrations of up to 100 µM have been reported in iron-grown batch cultures of a strain of this organism (Schnaitman and Lundgren 1965). In our hands, pyruvate concentrations of approximately 10 µM could be reproducibly detected in thiosulphate-limited chemostat cul-

Table 2. Maintenance coefficient of *Thiobacillus acidophilus* and of a number of neutrophilic bacteria. All data were obtained from glucose-limited chemostat cultures with ammonium as a nitrogen source

Organism	m_s mmol glucose · [g biomass · h]$^{-1}$	Reference
Beneckea natriegens	0.35	Linton et al., 1977
Escherichia coli	0.31	Schulze and Lipe, 1964
Rhodobacter sphaeroides	0.19	Nishizawa et al., 1974
Bacillus licheniformis	0.24	Frankena et al., 1985
T. acidophilus	0.10	this study

tures of *T. ferrooxidans* ATCC 19859 (J. T. Pronk and W. Hazeu, unpublished results). Growth of *T. acidophilus* on pyruvate might offer an explanation for the presence of this organism in ferrous iron-grown *T. ferrooxidans* cultures (Guay and Silver 1975; Arkesteyn and de Bont, 1980).

As reported above, *T. acidophilus* did not grow in batch cultures supplemented with 10 mM pyruvate. However, when added at low concentrations pyruvate was readily oxidized by cells from glucose-limited chemostat cultures. Pyruvate oxidation was strongly inhibited at concentrations above 200 µM (Fig. 2). To investigate whether the organism was capable of growth on pyruvate under substrate-limited growth conditions, the influent medium of a glucose-limited chemostat culture ($D = 0.05\ h^{-1}$, pH = 3.0) was exchanged for mineral medium supplemented with pyruvate as a sole carbon source. Indeed, under such conditions *T. acidophilus* exhibited pyruvate-limited growth, with a molar growth yield of 24 g biomass · (mol pyruvate)$^{-1}$ (Table 1). The residual substrate concentration in pyruvate-limited chemostat cultures was below the detection level, 5 µM. The substrate inhibition kinetics of cells grown in pyruvate-limited chemostat cultures were similar to those of glucose-grown cells (data not shown).

As mentioned above, α-ketoglutarate and succinate did not support heterotrophic growth in batch cultures. However, when added at low concentrations, these substrates could be used for respiration of *T. acidophilus* cells pregrown on glucose. It remains to be investigated whether the organism can grow on these and other organic acids in carbon-limited chemostat cultures.

Regulation of thiosulphate- and tetrathionate-oxidizing capacity

Cells from carbon-limited chemostat cultures were capable of oxidizing reduced sulphur compounds, including thiosulphate and tetrathionate. The oxidation rates observed with these inorganic electron donors did not depend upon the carbon source used (Table 1). Thiosulphate and tetrathionate oxidation rates were three- to eightfold lower than the activities measured with cells grown in autotrophic, thiosulphate-limited chemostat cultures. In all carbon-limited chemostat cultures, the residual substrate concentrations were below the detection limits of the analysis methods used. Therefore, these results do not exclude the possibility that thiosulphate- and tetrathionate-oxidizing activities might be repressed in the presence of excess organic carbon in the growth medium. To examine this possibility, oxidation of thiosulphate and tetrathionate was studied in cells grown on glucose in nitrogen-limited chemostat cultures. Oxygen consumption rates with thiosulphate were not reduced under nitrogen-limited growth conditions. However, the ability to oxidize tetrathionate was significantly lower than in glucose-limited chemostat cultures (Table 1).

Regulation of Ribulose-1,5-bisphosphate carboxylase

Cell-free extracts from cells grown in carbon-limited chemostat cultures exhibited significant activities of ribulose-1,5-bisphosphate carboxylase (RuBPCase; EC 4.1.1.39), the key enzyme of the Calvin cycle for carbon dioxide fixation. The RuBPCase activities found in carbon-limited heterotrophic cultures were approximately eightfold lower than the activities found in extracts prepared from cells grown autotrophically under thiosulphate limitation (Table 1). The RuBPCase activities observed in cell-free extracts from autotrophic cultures grown at a dilution rate of $0.03\ h^{-1}$ were sufficient to account for the biomass production observed in these cultures.

RuBPCase activities in cell-free extracts prepared from cells grown in nitrogen-limited chemostat cultures were significantly lower than those found in carbon-limited cultures (Table 1). However, even after prolonged cultivation under nitrogen-limited growth conditions (over 15 volume exchanges in the chemostat), significant RuBPCase activities were detectable in cell-free extracts. Apparently, the regulation of inorganic carbon fixation and inorganic sulphur oxidation in *T. acidophilus* is less strict than in the neutrophilic facultative chemolithoautotroph *T. versutus* (Gottschal et al. 1981).

Discussion

Ecology of Thiobacillus acidophilus

Although *T. acidophilus* is capable of heterotrophic growth, its metabolic versatility is considerably less developed than that of the neutrophilic chemolithoautotrophs *T. novellus* and *T. versutus* (Taylor and Hoare 1969). The spectrum of carbon sources supporting heterotrophic growth is restricted to a small number of sugars and some simple intermediates of central metabolic path-

ways. Our results confirm and extend the original observations of Guay and Silver (1975) and may be useful for the identification of new acidophilic isolates.

The results obtained with pyruvate as a carbon source indicate that growth data from batch cultures should be interpreted cautiously. Toxicity of organic acids like pyruvate to acidophilic bacteria is a well-known phenomenon. The effects of these compounds have been explained from their accumulation in the cytoplasm, leading to a decrease of the internal pH (Ingledew 1982; Alexander et al. 1987).

Kingma and Silver (1981) reported that pyruvate, when added at low concentrations, could be cometabolised by glucose- and elemental sulphur-growing *T. acidophilus*. However, in their batch experiments no growth was observed on pyruvate as the sole carbon source. Growth of *T. acidophilus* in pyruvate-limited chemostat cultures (Table 1) sheds new light on the possible role of this organism in natural bioleaching populations and on its occurrence in ferrous iron-grown *T. ferrooxidans* cultures (Guay and Silver 1975; Arkesteyn and de Bont 1980). Pyruvate inhibits growth and respiration of the obligate autotrophs *T. ferrooxidans* and *T. thiooxidans* (Schnaitman and Lundgren 1965; Alexander et al. 1987; Rao and Berger 1970). By scavenging pyruvate that is excreted by *T. ferrooxidans*, *T. acidophilus* may avoid inhibition of the autotrophic, "catalytic" population and thereby increase the stability and performance of bioleaching populations.

Energetics of growth

The growth yields of *T. acidophilus* on glucose reported here (Table 1, Fig. 1) are much lower than the yield figure reported by Mason and Kelly (1988). We have checked both the growth medium and the *T. acidophilus* strain used in our studies. However, neither the use of the mineral medium of Mason and Kelly (1988) nor the use of *T. acidophilus* ATCC 27807 resulted in the high growth yield (112.5 g dry weight · [mol glucose]$^{-1}$ at a dilution rate of 0.03 h^{-1}) reported in the latter paper. Our yield data are in good agreement with those observed by Arkesteyn and de Bont (1980). The reason for the discrepancy between our yield data and those of Mason and Kelly (1988) remains unclear.

Neither the maximum growth yield nor the maintenance coefficient of *T. acidophilus* changed significantly when the culture pH was increased from pH 3.0 to pH 4.3, a step corresponding with a twenty-fold decrease of the external free proton concentration (Fig. 1). This observation indicates that the maintenance of a large ΔpH by this acidophilic bacterium is not a major energy-requiring process. From experiments done with non-growing cells and spheroplasts of *T. acidophilus*, Matin and coworkers concluded that growth at low external pH values influenced the magnitude of the electrical component of the proton motive force ($\Delta\Psi$), but not the magnitude of the total proton motive force (Matin et al. 1982; Matin and Matin 1982; Zychlinsky and Matin 1983a and b). These authors therefore concluded that the maintenance of a near-neutral intracellular pH is not an energy-requiring process. Our results obtained with growing cultures of *T. acidophilus* are in full agreement with this conclusion.

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