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Growth of *Thiobacillus ferrooxidans* on Formic Acid

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A variety of acidophilic microorganisms were shown to be capable of oxidizing formate. These included *Thiobacillus ferrooxidans* ATCC 21834, which, however, could not grow on formate in normal batch cultures. However, the organism could be grown on formate when the substrate supply was growth limiting, e.g., in formate-limited chemostat cultures. The cell densities achieved by the use of the latter cultivation method were higher than cell densities reported for growth of *T. ferrooxidans* on ferrous iron or reduced sulfur compounds. Inhibition of formate oxidation by cell suspensions, but not cell extracts, of formate-grown *T. ferrooxidans* occurred at formate concentrations above 100 μM. This observation explains the inability of the organism to grow on formate in batch cultures. Cells grown in formate-limited chemostat cultures retained the ability to oxidize ferrous iron at high rates. Ribulose 1,5-bisphosphate carboxylase activities in cell extracts indicated that *T. ferrooxidans* employs the Calvin cycle for carbon assimilation during growth on formate. Oxidation of formate by cell extracts was NAD(P) independent.

The toxicity of low-molecular-weight organic acids for acidophilic microorganisms is a well-known phenomenon. The toxicity of organic acids is due to the fact that at low pH values of the growth medium, most of the organic acid is present in the undissociated form, which can diffuse easily over the cytoplasmic membrane. Since the internal pH of the acidophiles is nearly neutral (7), the acid molecules will dissociate upon entering the cytoplasm. In this way, influx of the undissociated acid down its concentration gradient will drive protons into the cell, leading to dissipation of the transmembrane pH gradient essential for growth in acidic environments (1, 13).

Batch cultures are ill-suited for growth studies on the metabolism of organic acids by acidophilic bacteria. For example, the acidophile *Thiobacillus acidophilus* is unable to grow on pyruvate in batch cultures. However, growth on this substrate was possible under substrate-limited growth conditions in chemostat cultures (24). Apparently, the toxic effects of this organic acid can be prevented by keeping the residual substrate concentration low, for example, by carbon-limited growth. This result prompted us to study the metabolism of organic acids by acidophilic microorganisms in more detail.

Formate can be used by a wide range of neutrophilic microorganisms as an energy source for both heterotrophic and autotrophic growth (25). In autotrophic organisms, carbon dioxide generated during the oxidation of formate can subsequently be used as a carbon source (26). According to the literature, the acidophilic thiobacilli are unable to grow on formate (16). In taxonomic studies, however, growth on formate is usually tested in batch cultures. At low pH values, formate (pK_a = 3.8) will occur mainly in the undissociated form. Therefore, the high substrate concentrations used in such experiments are likely to inhibit growth because of substrate toxicity. Earlier studies in our laboratory revealed that the facultatively autotrophic acidophile *T. acidophilus* could utilize formate as an energy source when this substrate was provided at low concentrations (23). It therefore seemed of interest to reexamine the ability of acidophilic bacteria to utilize formate.

The obligate chemolithoautotroph *Thiobacillus ferrooxidans* is of great economical importance because of its role in the microbial leaching of metal ores (22). Laboratory cultures of *T. ferrooxidans* are routinely grown on ferrous iron or reduced sulfur compounds (13). The low growth yields on these substrates require the use of high substrate concentrations for the production of biomass. The accumulation of mineral salts in the growth medium limits the attainable biomass densities. In addition to this, growth on ferrous iron leads to the accumulation of ferric ions in the growth medium. The ferric ions easily form precipitates, in particular at pH values above 2. Since oxidation of formic acid leads to the stoichiometric formation of water and carbon dioxide, autotrophic growth of microorganisms on this substrate does not cause accumulation of mineral salts in the growth medium.

The aim of the present study was to investigate the abilities of various acidophiles to utilize formate and in particular the possibility of formate-limited growth of *T. ferrooxidans*. Attention was focused on *T. ferrooxidans* since growth of dense cultures of this organism may be relevant both for its industrial applications and for its use as a model acidophile in fundamental studies.

MATERIALS AND METHODS

Organisms and culture conditions. Pure cultures of the microorganisms used in this study were obtained from the culture collection of the Laboratory of Microbiology and Enzymology, Delft, The Netherlands. Batch cultivation methods and growth media were as described by Kuenen and Tuovinen (18).

For growth of *T. ferrooxidans* on evaporated formate in "fed batch" cultures, modified 500-ml Erlenmeyer shake flasks were used. A glass tube (5-cm length by 1-cm diameter) was fitted on the bottom of each flask. Each flask was filled with 100 ml of mineral medium (see below) without a carbon source and inoculated with a *T. ferrooxidans* cell suspension (either cells harvested from ferrous iron-grown batch cultures or a cell suspension from a formate-limited

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chemostat culture) to a density of approximately 15 mg (dry weight) per liter. Subsequently, 2 ml of a concentrated formic acid solution (5 to 50% [vol/vol]) was added to the central reservoir tube, from which it could only enter the medium via evaporation. The cultures were incubated at 30°C on a rotatory shaker at 200 rpm.

Chemostat cultures were started by batch cultivation at pH 1.6 in mineral medium (20) supplemented with 180 mM $FeSO_4 \cdot 7H_2O$. The mineral medium used for continuous cultivation of *T. ferrooxidans* on formic acid contained the following per liter of demineralized water: $(NH_4)_2SO_4$, 3.0 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; Na_2SO_4, 1.0 g; $FeSO_4 \cdot 7H_2O$, 5 mg; $ZnSO_4 \cdot 7H_2O$, 1 mg; $CuSO_4 \cdot 5H_2O$, 2 mg; $MnSO_4 \cdot H_2O$, 1 mg; $NaMoO_4 \cdot 2H_2O$, 0.5 mg; $CoCl_2 \cdot 6H_2O$, 0.5 mg; $Na_2SeO_4 \cdot 10H_2O$, 1 mg; $NiCl_2 \cdot 6H_2O$, 1 mg. The medium was adjusted to pH 1.8 with H_2SO_4 and autoclaved at 120°C. Formic acid solutions were sterilized separately by autoclaving at 110°C.

Chemostat experiments were performed at 30°C in a 20-liter laboratory fermentor with a working volume of 18 liters. Cultures were aerated (2.5 liters $\cdot$ min^{-1}) and stirred at 500 rpm. During batch growth on ferrous iron, the pH of the culture was maintained at 1.6 by automatic titration of 1 M H_2SO_4. Continuous cultivation on formic acid did not require titration of the cultures, which remained at pH 1.8.

Gas analysis. Measurements of CO_2 production by the cultures and calculation of specific CO_2 production rates were performed as described previously (27).

Control of culture purity. The purity of formate-grown chemostat cultures of *T. ferrooxidans* was regularly tested by immunofluorescence microscopy with specific antisera raised against pure cultures of this organism (21).

Measurement of substrate-dependent oxygen uptake. Cells from batch cultures were harvested either by centrifugation (15,000 $\times$ *g*, 10 min) or by filtration over polycarbonate membrane filters (0.2-μm pore diameter; Nuclepore, Pleasanton, Calif.). Cells were washed and resuspended in mineral medium (pH 1.8 for *T. ferrooxidans* cultures and pH 3.0 for the other acidophilic bacteria). Oxygen uptake was measured at 30°C with a Clark-type oxygen electrode cell (Yellow Springs Instruments Inc., Yellow Springs, Ohio). Oxygen uptake rates were calculated assuming an oxygen concentration in air-saturated water of 0.24 mM and were corrected for endogenous respiration.

Cells from formate-limited chemostat cultures were assayed either directly in the culture fluid or after appropriate dilution with mineral medium. Endogenous respiration rates of chemostat-grown cells were negligible.

Preparation of cell extracts. The effluent of formate-limited chemostat cultures was collected at 4°C. Cells were harvested by centrifugation (15,000 $\times$ *g*, 15 min) and washed with a freshly prepared buffer (pH 7.0) containing 50 mM MOPSO (3-[*N*-morpholino]-2-hydroxypropanesulfonic acid), 2 mM L-cysteine, and 0.2 mM $Fe(NH_4)_2(SO_4)_2$ (10). Cells were resuspended in the same buffer to a final concentration of approximately 10 mg (dry weight) $\cdot$ ml^{-1} and disrupted by sonication at 0°C with an MSE 150 W Sonifier (10 30-s bursts, with intermittent cooling). Whole cells and debris were removed by centrifugation (45,000 $\times$ *g*, 20 min). The clear supernatants, containing 2 to 5 mg of protein ml^{-1}, were used as cell extracts. Cell extracts for ribulose 1,5-bisphosphate carboxylase (RuBPCase, EC 4.1.1.39) assays were prepared as described previously (24).

Enzyme assays. Dye-linked formate dehydrogenase was assayed spectrophotometrically at 30°C in a 1-ml assay mixture containing 100 mM potassium phosphate buffer, 10 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM 2,6-dichlorophenol-indophenol (DCPIP), and cell extract. The reaction was started by the addition of potassium formate to a final concentration of 40 mM. Enzyme activity was calculated from the decrease of absorbance at 522 nm by using a molar extinction coefficient for DCPIP of 8.6 mM^{-1} cm^{-1} (2). Enzyme activities were proportional to the amount of enzyme added.

NAD(P)-linked formate dehydrogenase was assayed in the same assay system, with 1 mM NAD or NADP instead of DCPIP. Reduction of NAD and NADP was monitored spectrophotometrically at 340 nm. Rates of NADH and NADPH oxidation by the extracts were negligible.

RuBPCase was assayed as described by Beudeker et al. (4).

Analytical procedures. Bacterial dry weight was determined as described previously (24). A Beckman 915B total organic carbon analyzer was used to determine the carbon contents of whole cultures and culture supernatants. The carbon content of the bacteria was calculated from the difference. Concentrations of formate in reservoir media were measured by the method of Lang and Lang (19). The protein contents of cell suspensions were estimated as described previously (24). Protein concentrations in cell extracts were determined by the method of Bradford (6), using bovine serum albumin as a standard.

Chemicals. Ribulose 1,5-bisphosphate was obtained from Sigma Chemical Co., and $[^{14}C]NaHCO_3$ (2.11 TBq $\cdot$ mol^{-1}) was obtained from Amersham International PLC. Formic acid (analytical grade) was obtained from Merck, Darmstadt, Federal Republic of Germany. All other chemicals were reagent grade and obtained from commercial sources.

RESULTS

Oxidation of formate by acidophilic bacteria. A variety of acidophilic bacteria, both heterotrophs and (facultative) autotrophs, were studied for their ability to oxidize formate. In view of the known toxicity of organic acids for acidophilic bacteria (1, 13), formate-dependent oxygen uptake was studied at a low substrate concentration (100 μM).

The ability to oxidize formate was widespread among acidophilic bacteria (Table 1). In all cases, the stoichiometry of formate and oxygen consumption was as expected (2:1).

The highest rate of formate oxidation was observed with the facultative autotroph *T. acidophilus* (Table 1). Significant rates of formate oxidation (30 to 63 nmol of O_2 $\cdot$ min^{-1} $\cdot$ mg (dry weight)$^{-1}$ were also observed in all acidophilic heterotrophs studied. The formate oxidation rates observed with obligately autotrophic bacteria were generally lower (Table 1). No oxidation of formate was observed with cells of *Thiobacillus thiooxidans* and *Thiobacillus concretivorus* (= *T. thiooxidans*) grown on elemental sulfur. Formate oxidation rates of ferrous iron-grown cells of *T. ferrooxidans* varied with the strain used (Table 1). The highest rates of formate-dependent oxygen uptake were observed with *T. ferrooxidans* ATCC 21834.

It can be expected that autotrophic organisms capable of oxidizing formate can also use this substrate as an energy source for growth. We therefore investigated whether *T. ferrooxidans* ATCC 21834 was capable of growth on formate as a sole source of energy.

Growth of *T. ferrooxidans* on evaporated formate. Attempts to grow *T. ferrooxidans* ATCC 21834 in batch cultures with 20 mM formate as a sole source of energy were unsuccessful. This was not surprising in view of the known toxicity of organic acids for this acidophile (1). To prevent toxic effects

TABLE 1. Oxidation of formate (100 μM) by washed cell suspensions of acidophilic microorganisms pregrown in shake flask cultures[a]

Organism	Growth substrate	Formate oxidation rate (nmol of O_2 $min^{-1} \cdot mg^{-1}$)
Heterotrophs		
Acetobacter diazotrophicus ATCC 49037	Glucose	56
Acidiphilium cryptum ATCC 33463	Glucose	63
Acidiphilium angustum ATCC 35903	Glucose	45
Acidiphilium organovorum ATCC 43141	Glucose	38
Acidiphilium sp. ATCC 35904	Glucose	30
Facultative autotroph *T. acidophilus* ATCC 27807	Glucose	210
Obligate autotrophs		
T. thiooxidans ATCC 19377	Elemental sulfur	0
T. thiooxidans ATCC 8085	Elemental sulfur	0
T. concretivorus ATCC 19703	Elemental sulfur	0
T. ferrooxidans ATCC 23270	Ferrous sulfate	12
T. ferrooxidans ATCC 19859	Ferrous sulfate	12
T. ferrooxidans ATCC 13728	Ferrous sulfate	15
T. ferrooxidans ATCC 13661	Ferrous sulfate	12
T. ferrooxidans ATCC 14119	Ferrous sulfate	0
T. ferrooxidans ATCC 19859	Ferrous sulfate	12
T. ferrooxidans ATCC 21834	Ferrous sulfate	55

[a] Oxygen uptake rates were determined with a Clark-type oxygen electrode at 30°C.

of formate, its accumulation in the cultures had to be prevented. This was achieved by using shake flasks equipped with a central formic acid reservoir (see Materials and Methods) from which the substrate could only enter the growth medium via evaporation. By using this system, *T. ferrooxidans* could be grown on formic acid as a sole source of energy. In contrast to "normal" growth curves, the increase of the biomass density in these cultures was linear with time. This can be explained by the fact that the (constant) rate of formic acid evaporation from the reservoir was growth limiting. The growth rate in the cultures was linearly dependent on the formic acid concentration in the reservoir (Fig. 1). This was to be expected, since the rate of formate evaporation will also increase linearly with the formic acid concentration in the reservoir. Under the experimental conditions, growth rates increased up to a formic acid concentration in the reservoir of 35% (vol/vol). At higher concentrations, growth did not occur. Apparently, the rate of formic acid diffusion into the latter cultures exceeded the rate of consumption by the bacteria. Obviously, the maximum concentration of formic acid that will allow growth depends on the initial cell density and the geometry of the culture flasks. Although this system may prove to be useful for screening the ability of acidophiles to utilize formate as an energy source, it is clearly not suited for more detailed physiological studies. Therefore, further studies of formate utilization were performed in formate-limited chemostat cultures.

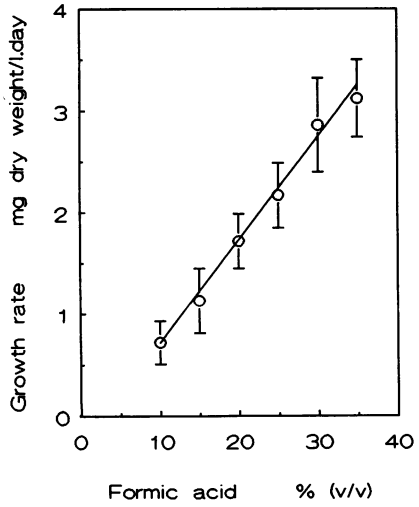

FIG. 1. Growth of *T. ferrooxidans* on evaporated formate in adapted Erlenmeyer flasks (pH 2.0, 30°C): effect of formate concentration in the reservoir on the growth rate. Independent, triplicate cultures were inoculated with cells from formate-limited chemostat cultures (D = 0.01 h^{-1}, pH 2.0, 30°C). Dry weights were estimated from the optical densities of the cultures by using calibration curves made with formate-limited chemostat cultures.

Growth of *T. ferrooxidans* in formate-limited chemostat cultures. *T. ferrooxidans* was pregrown as a batch culture on 180 mM ferrous iron. At the end of batch growth, the culture was switched to continuous cultivation at a dilution rate of 0.01 h^{-1} and the pH, which was maintained at 1.6 to prevent ferric iron precipitation, was raised to 1.8 (see below). The reservoir medium initially contained 20 mM formic acid as a sole energy source. This concentration was chosen to ensure that the formate-oxidizing capacity of the culture would be sufficient to prevent accumulation of formate in the culture. The formic acid concentration in the reservoir medium was increased stepwise. After every 20% increase, the formate concentration was kept the same until the increase of the biomass concentration in the cultures leveled off (usually after 2 to 4 days). Increase of the influent formic acid concentration by more than 30% at a time resulted in washout of the culture (data not shown).

The apparent molar growth yield of *T. ferrooxidans* grown under formate limitation at D = 0.01 h^{-1} was 1.36 g (dry weight) · mol of $formate^{-1}$ or 0.68 g (dry weight) · mol of $electrons^{-1}$. This growth yield is higher than the growth yield of the organism on ferrous iron (0.23 g [dry weight] per mol of Fe^{2+} [12]), but lower than the maximum growth yields reported for tetrathionate-limited chemostat cultures of this organism (0.92 g [dry weight] per mol of electrons [12]). The growth yield of *T. ferrooxidans* found in this study is lower than the growth yields observed with a variety of other bacteria grown autotrophically on formate (Table 2).

TABLE 2. Growth yields of aerobic bacteria grown autotrophically on formate[a]

Organism	Growth rate (h^{-1})	Growth yield (g [dry weight] · mol^{-1})	Reference
Pseudomonas oxalaticus	0.025–0.20	3.2[b]	8
Paracoccus denitrificans		2.9[b,c]	28
Thiobacillus versutus	0.05	3.0	10a
	0.011	1.4[c]	17
Xanthobacter autotrophicus	0.10	4.5	7a
Thiobacillus acidophilus	0.05	2.5	23
Thiobacillus ferrooxidans	0.01	1.3	This study

[a] Data were obtained from formate-limited chemostat cultures with ammonium salts as a nitrogen source.
[b] Maximum growth yield, corrected for maintenance.
[c] Data obtained from extended exponential culture.

Carbon balances gave a complete recovery of substrate carbon as biomass and carbon dioxide, with 5.44% of the substrate carbon being converted into biomass. The carbon and protein contents of formate-grown *T. ferrooxidans* were 48 and 65%, respectively.

Formate oxidation by intact cells of *T. ferrooxidans* was strongly inhibited at substrate concentrations above 100 μM (Fig. 2). This observation explains the inability of *T. ferrooxidans* strains to grow on formate in batch cultures, which usually contain more than 5 mM substrate. The optimum pH for formate oxidation was approximately 3.0 (Fig. 3). However, to reduce the risk of contamination, the culture pH was maintained at 1.8. The formate-dependent oxygen uptake rate measured at this pH (53 nmol of $O_2 \cdot min^{-1} \cdot mg^{-1}$) was virtually the same as the rate observed with cells from ferrous iron-grown batch cultures (Table 1). With the observed biomass yields (see below), the formate oxidation rate required for formate-limited growth at a dilution rate of 0.01 h^{-1} is 60 nmol of $O_2 \cdot min^{-1} \cdot mg^{-1}$. This rate was in good agreement with the oxidation rates observed in oxygen uptake experiments.

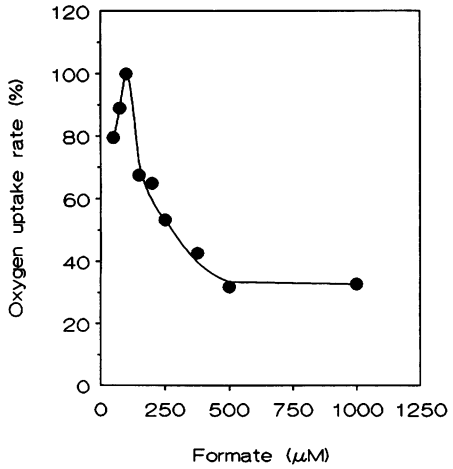

FIG. 2. Oxidation of formate by *T. ferrooxidans* cells: effect of substrate concentration. Formate-dependent oxygen uptake rates were measured at 30°C with cell suspensions taken directly from a formate-limited chemostat culture (D = 0.01 h^{-1}, pH 1.8, 30°C). An activity of 100% corresponds to an oxygen uptake rate of 55 nmol of $O_2 \cdot min^{-1} \cdot mg^{-1}$.

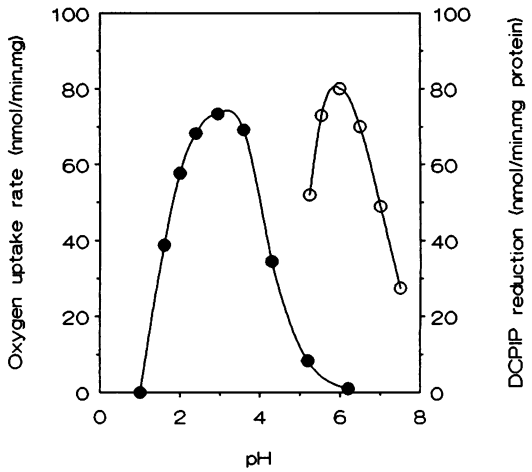

FIG. 3. Oxidation of formate by cell suspensions (●) and cell extracts (○) of *T. ferrooxidans*: effect of pH. Formate-dependent oxygen consumption rates were measured with cell suspensions from a formate-limited chemostat culture (D = 0.01 h^{-1}, pH 1.8, 30°C). Suspensions were adjusted to the appropriate pH by the addition of dilute H_2SO_4 or KOH. Oxidation of formate by cell extracts was assayed as formate-dependent reduction of DCPIP (see Materials and Methods).

Interestingly, cells from formate-limited chemostat cultures retained the ability to oxidize ferrous iron at high rates (Table 3). Specific iron oxidation rates remained constant after prolonged cultivation (over 3 months) in formate-limited chemostat cultures and were independent of the culture density. This is in contrast to earlier observations with *T. ferrooxidans* grown on reduced sulfur compounds, where the ability to oxidize ferrous iron was lost (11). *T. ferrooxidans* grown under formate limitation also oxidized sulfide and elemental sulfur. Observed oxygen uptake rates of formate- and ferrous iron-grown cells with ferrous iron, sulfide, and elemental sulfur were comparable (Table 3). Oxygen uptake by formate-grown cells with thiosulfate, tetrathionate, methanol, formaldehyde, and molecular hydrogen was not observed.

Cell extracts prepared from formate-grown chemostat cultures contained RuBPCase activities of 10 nmol · min^{-1} · mg of protein^{-1}. This activity is identical to the rate of carbon assimilation calculated from the observed growth yields and cellular composition (calculation not shown).

Oxidation of formate by cell extracts of *T. ferrooxidans*. Cell

TABLE 3. Oxidation of various substrates by formate- and ferrous iron-grown *T. ferrooxidans*[a]

Substrate	Concn (mM)	Oxygen uptake rate (nmol of O_2 min^{-1} mg^{-1})	
		Fe-grown cells	Formate-grown cells
Formate	0.1	55	53
Ferrous sulfate	4.5	630	750
Sodium sulfide	0.1	46	67
Elemental sulfur	0.1	19	7

[a] Cell suspensions from formate-limited chemostat cultures were used directly after sampling. Cells from ferrous iron-grown batch cultures (180 mM $FeSO_4$, pH 1.6) were harvested by filtration, washed twice, and resuspended in mineral medium (pH 1.8). Oxygen uptake rates were measured with a Clark-type electrode at 30°C. Elemental sulfur was added as a 10 mM stock solution in acetone.

extracts of formate-grown *T. ferrooxidans* LMD 81.69 catalyzed the oxidation of formate with DCPIP as an artificial electron acceptor. The optimum pH of the formate dehydrogenase was close to 6 (Fig. 3), suggesting a cytoplasmic localization. At this pH, the apparent K_m for formate was approximately 0.1 mM, with a V_{max} of 80 nmol of DCPIP · min^{-1} · mg of $protein^{-1}$. The formate-dependent DCPIP reduction rates were not enhanced by the addition of 0.2 mM phenazine methosulfate. In contrast to the observations made with intact cells (Fig. 2), substrate inhibition by formate did not occur up to a concentration of 80 mM. Formate dehydrogenase activities in cell extracts were instable at 4°C, with a half-life of approximately 4 h. No activity was observed with the electron acceptors NAD and NADP. The maximum rate of in vitro formate oxidation can account for approximately 40% of the in vivo rate of oxidation in the formate-limited chemostat cultures. This is substantial in view of the instability of the enzyme activity and the fact that an artificial electron acceptor was used.

DISCUSSION

The present study indicates that the ability to oxidize formate is expressed in many acidophilic bacteria, even in the absence of added formate. This suggests that formate oxidation may be of physiological and/or ecological significance for the organisms studied. Formate may occur in the natural environment of the acidophiles studied or, alternatively, may be an intermediate or side product of their metabolism. In both cases, detoxification of formate is necessary to prevent effects like those shown in Fig. 2.

When the formate concentration in the cultures was very low, *T. ferrooxidans* could use formate as a sole source of energy for autotrophic growth. Until recently, the spectrum of substrates supporting autotrophic growth of this organism was limited to ferrous iron and a number of reduced sulfur compounds (16). In a recent publication (9), it has been reported that *T. ferrooxidans* can also grow autotrophically on molecular hydrogen.

The growth yield of *T. ferrooxidans* in formate-limited chemostat cultures grown at a dilution rate of 0.01 h^{-1} was low compared with the growth yields of other autotrophic bacteria grown on formate (Table 2). At this low growth rate, however, growth yields may be significantly influenced by maintenance requirements. For the calculation of the theoretical maximum growth yield (Y_{max}) of *T. ferrooxidans*, the maintenance coefficient (m_s) for formate-limited growth is needed. If its maintenance coefficient is assumed to be on the same order as that of *Pseudomonas oxalaticus* (8), the Y_{max} of *T. ferrooxidans* would be approximately 2.5 g · mol^{-1}. This value is similar to the growth yields on formate of other autotrophic bacteria (Table 2). Also, the RuBPCase activities detected in cell extracts suggest that *T. ferrooxidans* employs the Calvin cycle for carbon assimilation during growth on formic acid.

Growth of *T. ferrooxidans* on formic acid may be advantageous for laboratory experiments since cells can be grown at high biomass densities. We have obtained biomass concentrations exceeding 0.7 g · $liter^{-1}$ in formate-limited chemostat cultures. These biomass densities are substantially higher than those attained with conventional cultivation methods for *T. ferrooxidans* or with a method based on the electrolytic reduction of ferric iron (5). Cells grown under formate limitation retain the ability to oxidize ferrous iron at high rates. However, growth on formate does not involve the precipitation problems associated with the use of ferrous iron-grown cultures. This may be particularly useful for the preparation of cell extracts and for enzyme purification procedures. An additional advantage is that the high concentrations of formic acid in the reservoir media make sterilization of these media unnecessary (data not shown). Growth of *T. ferrooxidans* on mixtures of formate and reduced sulfur compounds may facilitate studies into the enzymology and bioenergetics of sulfur oxidation by this organism.

Growth of *T. ferrooxidans* at high biomass densities may also be useful for some of its industrial applications. In this respect, biomass density has been implied as a potentially critical process parameter in both the bacterial leaching of metal ores and the microbial desulfuration of coal (3, 15). Dense inocula may also be useful to confer an advantage to selected *T. ferrooxidans* strains over naturally occurring varieties, for example, in heap leaching operations.

As was observed with a recently described formate dehydrogenase from the methylotrophic bacterium *Mycobacterium gastri* (14), formate-dependent reduction of DCPIP by cell extracts of formate-grown *T. ferrooxidans* was not stimulated by the addition of phenazine methosulfate. However, in contrast to the *M. gastri* formate dehydrogenase, the *T. ferrooxidans* enzyme appeared to be located in the soluble fraction of cell extracts. The *T. ferrooxidans* enzyme activity differs markedly from the *T. acidophilus* formate dehydrogenase activity, which can use both NAD and artificial electron acceptors (23).

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