Growth Kinetics of *Thiobacillus denitrificans* in Anaerobic and Aerobic Chemostat Culture

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Thiobacillus denitrificans was cultured chemolithotrophically under aerobic and anaerobic conditions in a chemostat with thiosulphate, nitrate or nitrite as limiting nutrient. Estimations of growth yields and maintenance coefficients showed that *T. denitrificans* grew more efficiently than other thiobacilli both aerobically and anaerobically. Relative growth yield data enabled the probable amounts of ATP generated during thiosulphate-limited aerobic growth and nitrate-limited anaerobic growth on thiosulphate to be calculated as, respectively, 6 to 7 and 4 to 5 mol ATP formed per mol thiosulphate oxidized. The energy available from tetrathionate oxidation was almost twice that from thiosulphate.

INTRODUCTION

Thiobacillus denitrificans is an obligate chemolithotroph which uses the oxidation of reduced inorganic sulphur compounds for the respiratory reduction of oxygen or, under anaerobic conditions, of nitrate to dinitrogen gas. It is capable of wholly autotrophic growth, effecting reductive fixation of carbon dioxide by the Calvin cycle under both aerobic and anaerobic conditions. It is the only well established example of a facultatively anaerobic thiobacillus and has been subject to considerable physiological and biochemical study largely concerned with batch cultures and the mechanisms of sulphur compound oxidation and nitrate reduction (Beijerinck, 1904; Lieske, 1912; Baalsrud & Baalsrud, 1952, 1954; Aubert, Millet & Milhaud, 1959; Woolley, Jones & Happold, 1962; Bowen, Butler & Happold, 1965; Bowen, Happold & Taylor, 1966; Sargeant *et al.*, 1966; Adams, Warnes & Nicholas, 1971; Taylor, Hoare & Hoare, 1971; Aminuddin & Nicholas, 1973, 1974*a, b*; Schedel, Legall & Baldensperger, 1975; Sawhney & Nicholas, 1977). Earlier claims that it lost its capacity for anaerobic growth following culture aerobically (Vishniac & Santer, 1957; Woolley *et al.*, 1962) have never been substantiated and only recently has any attempt been made to study the bioenergetics of this organism by means of continuous chemostat culture (Justin & Kelly, 1976; Timmer-ten-Hoor, 1976).

This study was performed to seek information on the behaviour of continuous cultures of *T. denitrificans* subject to substrate limitation under aerobic and anaerobic conditions and to determine the ease with which adaptation between aerobiosis and anaerobiosis could occur. The results enable calculation of comparative growth yields and 'maintenance energy' requirements under a variety of physiological steady state conditions.

METHODS

Organism and culture conditions. *Thiobacillus denitrificans* NCIB 9548 was maintained anaerobically in completely filled bottles or aerobically in flasks shaken at 30 °C. Anaerobic nitrate-limited cultures and aerobic thiosulphate-limited cultures were grown in a medium containing (g l⁻¹ in distilled water): Na₂S₂O₃ · 5H₂O, 5; KNO₃, 2; KH₂PO₄, 2; NH₄Cl, 1; MgSO₄ · 7H₂O, 0.4; FeSO₄ · 7H₂O, 1 ml of 2% (w/v) solution in

1 M-HCl; trace metal solution (Tuovinen & Kelly, 1973), 1 ml; adjusted to pH 7.0. For anaerobic growth on nitrite-limited medium, KNO_3 was omitted from the medium and a solution of NaNO_2 was separately pumped into the chemostat apparatus to avoid chemical reaction in the bulk medium between nitrite and thiosulphate. Similarly to obtain cultures growing on tetrathionate, a thiosulphate-free basal medium feed at pH 9.2 was employed together with a separate supply of $\text{K}_2\text{S}_4\text{O}_8$ solution at pH 3.5, the two mixing in the chemostat vessel and being automatically maintained at pH 7.0. For anaerobic thiosulphate-limitation, KNO_3 was supplied at 3 g l⁻¹.

Two types of chemostat were used. Most of the studies were carried out in an LH modular type series 500 fermenter (LH Engineering, Slough, Buckinghamshire) with a culture volume of 750 ml, provided with agitation (750 rev. min⁻¹), temperature control (30 °C), pH control, aeration and dissolved oxygen monitoring modules. Some aerobic cultures were grown in an LH CC 1500 fermenter with a working volume of 3 l and control of temperature, pH, stirring and aeration. Culture pH was maintained at pH 7.0 by automatic titration with 0.5 M-NaOH. Anaerobic cultures were continuously flushed with 5% (v/v) CO_2 in N_2 at 15 ml min⁻¹ for a 750 ml culture and the medium reservoir vessels were held under N_2 . The effluent gas from the culture was passed through a Drechsel bottle trap containing alkaline pyrogallol. Medium solutions were pumped into the vessel through silicone or black butyl rubber tubing (for aerobic and anaerobic cultures, respectively) by means of Watson Marlow MHRE7 flow inducers (Watson Marlow, Falmouth, Cornwall). Aerobic cultures were poised at selected concentrations of dissolved oxygen by controlling the flow rate into the culture of air containing 5% (v/v) CO_2 . Possible wall growth in the culture vessels was minimized by coating them with dichlorosilane, applied as a 5% solution in chloroform.

Collection of gases from chemostat. The effluent gas from a thiosulphate-limited anaerobic culture was passed through a U-tube packed with molecular sieve beads and cooled in liquid nitrogen. The condensed gases were revaporized and analysed for NO and N_2O by gas chromatography.

Analysis of steady state cultures. Samples were removed from steady state cultures at regular intervals for chemical and microbiological analysis. Biomass was measured as A_{460} using appropriate dry weight-absorbance calibration curves. (Direct determination of dry weights of organisms in cultures at different dilution rates confirmed that this was a reliable method for monitoring biomass concentration, but showed that a different relationship existed for aerobic and anaerobic cultures.) $A = 0.5$ was equivalent to 130 mg dry wt l⁻¹ in an anaerobic culture but to 175 mg dry wt l⁻¹ in an aerobic culture: this relationship was constant up to $A = 1.0$. For estimation of protein, organisms from 4 ml samples were centrifuged, washed with distilled water, recentrifuged and finally heated in a boiling water bath for 10 min after adding 2.5 ml 0.5 M-NaOH. Solubilized protein was then determined (Lowry *et al.*, 1951). Microbiological purity of steady state cultures was checked by plating 10^{-6} dilutions of cultures on (i) agar medium of the same composition as the liquid culture; (ii) agar medium as in (i) supplemented with 0.1% (w/v) glucose and 0.65% (w/v) nutrient broth; or (iii) agar with glucose and nutrient broth only.

Supernatant liquids from culture samples were analysed for their content of thiosulphate, trithionate and tetrathionate (Kelly, Chambers & Trudinger, 1969). Nitrate (0.03 to 3 mg) was determined by titration with 1.67 mM- $\text{K}_2\text{Cr}_2\text{O}_7$ using 0.025 M-ferroin as indicator (Kolthoff & Belcher, 1957). Nitrite was determined by the Griess-Ilosvay method: samples containing up to 0.3 $\mu\text{mol NO}_2^-$ were mixed with 1 ml 0.3 M- CdSO_4 and 1 ml reagent [equal volumes of 0.7% (w/v) sulphanic acid in 30% (v/v) acetic acid and of 0.1 g α -naphthylamine boiled in 20 ml water then supplemented with 150 ml 30% acetic acid], and made up to 10 ml with water; absorbance at 530 nm was read after 25 min. To overcome interference by thiosulphate with the assays of both nitrate and nitrite, all samples were supplemented with $\text{Na}_2\text{S}_2\text{O}_3$ to a total concentration of 12 mM and all calibration curves were prepared with standard nitrate and nitrite containing 12 mM-thiosulphate. Any sulphur in the centrifuged pellet samples was redissolved in acetone and estimated colorimetrically (Bartlett & Skoog, 1954).

Elemental analyses. Organisms were harvested by centrifuging, washed and dried at 105 °C before analysis for carbon, hydrogen and nitrogen using a Perkin-Elmer elemental analyser.

RESULTS

Anaerobic nitrate-limited chemostat culture

Commencing with a late exponential phase batch culture continually flushed with N_2/CO_2 , a continuous culture was established without difficulty using a medium containing approximately 20 mM-thiosulphate and 20 mM-nitrate. Nitrate was the limiting nutrient in this medium because the stoichiometry for anaerobic thiosulphate respiration by *T. denitrificans* is given by:

Fig. 1. Steady state biomass and yield of *Thiobacillus denitrificans* grown in anaerobic chemostat culture on thiosulphate (20 mM) with nitrate (23 mM) limitation. (a) Biomass (mg l⁻¹) as dry wt (▲) and protein (△); yield [g (mol thiosulphate oxidized)⁻¹] as dry wt (●) and protein (○); unoxidized thiosulphate (□). (b) Double reciprocal plots of dilution rates and yields: 1/[g dry wt (mol thiosulphate oxidized)⁻¹] (●) and 1/[g protein (mol thiosulphate oxidized)⁻¹] (○).

Steady states were maintained at seven dilution rates (D) between 0.02 and 0.08 h⁻¹. Washout occurred at $D = 0.09$ h⁻¹. Precise determination of input concentrations of thiosulphate and nitrate and steady state residual concentrations of these and of biomass enabled estimation of steady state yields in terms of g dry wt (or g protein) per mol thiosulphate (or nitrate) consumed. Residual nitrate was almost undetectable and was no more than 5 to 7% of the input concentration. Nitrite, sulphur and polythionates were not accumulated. About 3 mM-thiosulphate remained at low D values, decreasing to 1 mM at $D = 0.08$ h⁻¹ (Table 1). Biomass and yield increased with increased dilution rate (Fig. 1a). The yield increased from 7.03 g dry wt per mol thiosulphate oxidized at $D = 0.02$ h⁻¹ to 9.7 at $D = 0.08$ h⁻¹. The mean protein content (% w/w, of dry wt) did not vary with dilution rate and was $75.8 \pm 3.7\%$ (S.E.M. of seven dilution rates). Plotting reciprocals of yields against reciprocals of D produced linear graphs (Fig. 1b) from which the true growth yield (Y_g) and apparent maintenance coefficient (m) could be calculated (Pirt, 1965). Y_g was 11.63 g dry wt (mol thiosulphate)⁻¹ or 8.51 g protein mol⁻¹, and m was 1.4 mmol thiosulphate h⁻¹ (g dry wt)⁻¹.

The specific rate of thiosulphate oxidation [q , in mmol h⁻¹ (g dry wt)⁻¹] in the steady state chemostat cultures was calculated for each dilution rate from the thiosulphate consumption rate and the steady state biomass. The value of q increased from 2.85 at $D = 0.02$ h⁻¹ to 8.24 at $D = 0.08$ h⁻¹ and gave a linear graph (fitted by regression analysis) when plotted against D . This plot gave an alternative means of calculating m (the q intercept of the q v. D plot) as 1.17 and Y_g (the reciprocal of the slope of the q v. D plot) as 11.1. Mean values from the two procedures for determining Y_g and m were thus 11.37 g dry wt (mol thiosulphate)⁻¹ and 1.29 mmol thiosulphate h⁻¹ (g dry wt)⁻¹.

A typical substrate and product balance for these steady states was given at $D = 0.06$ h⁻¹ at which the oxidation of 17.07 mM-thiosulphate was accompanied by the disappearance of 22.75 mM-nitrate and the production of 155 mg dry wt bacteria, giving a yield of 9.08 g (mol thiosulphate)⁻¹. This corresponds to a nitrate:thiosulphate ratio of 1.33, lower than the theoretical ratio of 1.6 given by the oxidation equation. The production of 155 mg biomass is, however, equivalent to 73.5 mg C (6 mmol CO₂) fixed and consequently to the use

of 3 mmol thiosulphate to provide reducing equivalents for fixation (see Discussion). Thus the thiosulphate consumed for energetic purposes (i.e. nitrate reduction) was only 17–3 i.e. 14 mmol, giving a true nitrate:thiosulphate ratio of 1.62, in accord with the equation of thiosulphate oxidation.

Anaerobic nitrate-limited chemostat culture growing on tetrathionate instead of thiosulphate

Replacing thiosulphate by 11.4 mM- $K_2S_4O_6$ under the conditions described in the preceding section resulted in easy transition to steady states in which more than 95% of the tetrathionate was consumed and yield values at $D = 0.025$, 0.05 and 0.076 h^{-1} were, respectively, 18.06, 19.56 and 20.70 g dry wt (mol tetrathionate) $^{-1}$, indicating a Y_G of 21.5 g dry wt (mol tetrathionate) $^{-1}$.

Anaerobic nitrite-limited chemostat culture

A nitrate-limited culture was switched to a supply of 21 mM-thiosulphate with 20 mM-nitrite and steady states were sustained at dilution rates of 0.07 and 0.08 h^{-1} . Washout occurred at about $D = 0.08 \text{ h}^{-1}$. Nitrite was completely consumed, but small amounts (0.87 mM) of trithionate were detected along with 7.51 mM unused thiosulphate. The steady state biomass at $D = 0.07$ was 149 mg dry wt l^{-1} , indicating a yield of 12.23 g (mol thiosulphate) $^{-1}$.

Anaerobic thiosulphate-limited chemostat culture

With thiosulphate (10 to 20 mM) as the limiting nutrient and nitrate supplied in excess at 30 mM, virtually all the input thiosulphate was completely oxidized with no polythionate or sulphur formation (Tables 1 and 2). Nitrate consumption frequently exceeded the theoretical requirement, while nitrite formation and disappearance showed a harmonic oscillation at fixed dilution rates, with little dampening at higher D values. The effluent gases from the culture contained small amounts of N_2O and NO (detected as NO_2). Eight steady states were maintained between $D = 0.021$ and 0.083 h^{-1} . Washout occurred at $D = 0.09 \text{ h}^{-1}$. Biomass and yield tended to increase at higher D values in a similar manner to the nitrate-limited cultures (Table 1). The biomass values indicated growth efficiency similar to that of nitrate-limited cultures, as was previously shown at a single dilution rate (0.03 h^{-1}) by Timmer-ten-Hoor (1976). Greater variability in yield (and apparent protein content of cultures) was found with thiosulphate-limited anaerobic cultures than with those limited by nitrate, possibly due to variable nitrite accumulation. Consequently determination of Y_G was somewhat imprecise and values between 9.2 and 14.2 g dry wt (mol thiosulphate) $^{-1}$ could be deduced from the data (mean 11.7). A plot of the q v. D data of Table 1 indicated $Y_G = 9.22$ g dry wt (mol thiosulphate) $^{-1}$ and $m = 1.20$ mmol thiosulphate h^{-1} (g dry wt) $^{-1}$, calculated by linear regression analysis.

Aerobic thiosulphate-limited chemostat culture

In two chemostat runs, eight steady states were established at $D = 0.02$ to 0.13 h^{-1} with culture washout between 0.13 and 0.14 h^{-1} . Thiosulphate was completely consumed in all steady states and was the growth-limiting nutrient (Table 1). However, aeration with air alone [at $250 \text{ ml (l culture volume)}^{-1} \text{ min}^{-1}$] was also CO_2 limiting as the yield at $D = 0.02 \text{ h}^{-1}$ was increased from 7.5 g dry wt mol^{-1} with air to 11.8 when the gas flow was supplemented with 5% (v/v) CO_2 . A similar phenomenon was seen with *T. ferrooxidans* grown on tetrathionate (Eccleston & Kelly, 1978) and presumably indicates dependence on CO_2 concentration of the efficiency of energy coupling during thiosulphate oxidation. All subsequent steady states were attained with excess CO_2 supply and showed an increase in steady state yield with increasing dilution rate from 10.7 g dry wt (mol thiosulphate) $^{-1}$ at $D = 0.02$ to 12.8 at $D = 0.08 \text{ h}^{-1}$. Y_G was 14.7 g dry wt (mol thiosulphate) $^{-1}$ and m was 0.57 mmol thiosulphate h^{-1} (g dry wt) $^{-1}$, as calculated from a plot of q v. D .

Table 1. Comparison of steady state biomass and thiosulphate consumption by *Thiobacillus denitrificans* in (A) nitrate-limited anaerobic, (B) thiosulphate-limited anaerobic and (C) thiosulphate-limited aerobic cultures

Input thiosulphate concentrations were 20 mM for A and C, and 17 mM for B.

Dilution rate* (h ⁻¹)	Steady state biomass (mg dry wt l ⁻¹)			Steady state thiosulphate consumption (mmol l ⁻¹)			$q_{s_2O_3}$ [mmol h ⁻¹ (g dry wt) ⁻¹]		
	A	B†	C†	A	B	C	A	B†	C†
0.02	115	119	211	16.4	15.4	19.7	2.85	2.82	1.88
0.03	125	117	ND	16.4	17.0	—	3.95	4.37	—
0.04	135	100	256	16.3	17.0	19.7	4.83	6.80	3.08
0.05	145	123	ND	16.0	17.0	—	5.52	6.92	—
0.06	155	142	200	17.1	17.0	19.5	6.61	7.18	5.85
0.07	160	147	ND	17.2	17.0	—	7.51	8.10	—
0.08	180	132	250	18.5	17.0	19.5	8.24	10.30	6.24
0.10	w	w	340	—	—	19.5	—	—	5.74

w, Culture washout; ND, not determined.

* Approximate dilution rates given for ease of comparison: actual values employed were up to 5 % greater than indicated.

† Mean values from more than one steady state determination.

Table 2. Effect of input nutrient concentration on steady state conditions and yield of anaerobic cultures of *Thiobacillus denitrificans* at constant dilution rate (0.08 h⁻¹)

Between six and eight volume changes of each medium were passed through the culture to establish true steady states before changing to the next medium mixture. Growth yields are expressed as g dry wt (mol thiosulphate)⁻¹.

Input concn (mM)			Steady state concn (mM)			Biomass (mg l ⁻¹)	Yield
Thiosulphate	Nitrate	Nitrite	Thiosulphate	Nitrate	Nitrite		
20.14	19.78	0	1.61	0.89	0.007	180	9.71
20.14	29.67	0	1.25	2.47	0	180	9.53
10.07	14.84	0	0.63	0.92	0	90	9.53
10.07	29.67	0	0	8.78	0.025	85	8.44
20.14	29.67	0	1.25	2.97	0	180	9.53
40.28	29.67	0	21.25	0.59	0.023	c. 45	c. 2.37
40.28	29.67	5.0	16.25	0.69	0.015	110	4.58

Specific rates of thiosulphate consumption

As described, $q_{s_2O_3}$ was used as a means of calculating Y_G and m . Values of $q_{s_2O_3}$ increased with D for all three conditions studied (Table 1). Over the range 0.02 to 0.08 h⁻¹ these values ranged from 1.9 to 10.3 mmol h⁻¹ (g dry wt)⁻¹ for the three growth conditions. Linear regression analysis fits of seven or eight values for each growth state indicated m values between 0.6 (aerobic) and 1.2 (anaerobic, nitrate- or thiosulphate-limited) mmol thiosulphate h⁻¹ (g dry wt)⁻¹.

Responses in anaerobic chemostat cultures to variation of nutrient supplies

Alteration of the input concentration of thiosulphate or nitrate demonstrated that each could be made growth-limiting (Table 2). When a large excess of thiosulphate was supplied under nitrate limitation, culture lysis was observed with a low steady state biomass. Supplementation with a small amount of nitrite, allowing more thiosulphate metabolism, partially alleviated this effect (Table 2).

Elemental composition of Thiobacillus denitrificans

Organisms from chemostats operating at five different dilution rates (between 0.02 and 0.08 h⁻¹) grown anaerobically or aerobically with thiosulphate-limitation or anaerobically with nitrate-limitation all had essentially the same content of carbon, hydrogen and nitrogen as a percentage of the dry wt. From 14 separate analyses, the composition [% (w/w) $\pm$ s.e.m.] was: carbon, 47.40 $\pm$ 1.02; hydrogen, 6.88 $\pm$ 0.23; nitrogen, 12.70 $\pm$ 0.79. The C/N ratios for the three culture conditions (A, B and C) were 3.52, 3.87 and 3.63, respectively. We conclude that growth rate and condition did not significantly affect the gross composition of the organism.

DISCUSSION

Our observations demonstrate that *Thiobacillus denitrificans* can grow efficiently in chemostat culture under several conditions of nutrient limitation both anaerobically and aerobically. Our data for growth yield and maintenance enable us to estimate the relative growth efficiencies and the amounts of ATP generated by anaerobic and aerobic thiosulphate oxidation. The true growth yield of 11.37 g dry wt (mol thiosulphate)⁻¹ for anaerobic nitrate-limited culture can be compared with the observed maximum yield (at $D = 0.08$, uncorrected for m) of 9.7 and the average yields of 9.3 and 5.7 that can be calculated from the data for batch cultures reported by Taylor *et al.* (1971) and Lieske (1912). Timmer-ten-Hoor (1976), using a continuous culture at $D = 0.03$ h⁻¹, obtained 9.26 under similar conditions.

In anaerobic cultures, growth yields (in terms of g protein or dry wt per mol thiosulphate consumed) were comparable with either nitrate- or thiosulphate-limitation (as reported for a single fixed dilution rate by Timmer-ten-Hoor, 1976), whereas the yield was higher in aerobic thiosulphate-limited culture, giving Y_g values [g dry wt (mol thiosulphate)⁻¹] of 11.37 for anaerobic (nitrate-limited) and 14.69 for aerobic cultures, as expected from the greater energy available from the aerobic oxidation of thiosulphate (Timmer-ten-Hoor, 1976; Kelly, 1978). These Y_g values are comparable with the higher of two values reported by Hemphling & Vishniac (1967), but are considerably higher than more recently determined values for aerobic *T. neapolitanus* of 5.27 (Kelly, unpublished) and 6.5 (J. G. Kuenen, personal communication); 7.48 for *T. ferrooxidans* (Kelly, Eccleston & Jones, 1977; Eccleston & Kelly, 1976, 1978); 5.2 for *Thiomicrospira pelophila* and about 7 for *Thiobacillus* A2 (J. G. Kuenen, personal communication). The values for maintenance coefficient (m) are comparable with others reported recently (Justin & Kelly, 1976) and from our subsequent calculations are equivalent to m_{ATP} values in the range 4 to 11 mmol h⁻¹ (g dry wt)⁻¹. There was indication from some of our data that m might decrease at lower dilution rates.

Using Y_g values around 7 for *T. ferrooxidans* growing on thiosulphate, we calculated (Kelly *et al.*, 1977; Eccleston & Kelly, 1978) that aerobic thiosulphate oxidation supported synthesis of only 3 mol ATP per mol, and that, while 2 mol ATP could be produced by electron transport phosphorylation, it was possible that the oxidation of the sulphane-sulphur of thiosulphate was not energy-conserving. This would be consistent with the operation of a non-energy-linked oxygenase system in aerobic thiobacilli for the conversion of sulphur to sulphite (Suzuki & Silver, 1966; Silver & Lundgren, 1968; Taylor, 1968). The considerably higher values reliably reported now for *T. denitrificans* in this paper and previously (Justin & Kelly, 1976; Timmer-ten-Hoor, 1976) indicate that more efficient energy coupling systems may exist in this facultatively anaerobic thiobacillus.

At a carbon content of 47.4 % (w/w) of the dry wt, the anaerobic Y_g of 11.37 indicates the fixation of 5.39 g carbon or 0.45 mol CO₂ per mol thiosulphate. By the Calvin cycle this requires 0.9 mol NADH and 1.35 mol ATP. Since the oxidation of 1 mol thiosulphate generates 8 reducing equivalents (H), and 0.9 NADH requires 1.8 (H) for its formation, only 6.2 (H) are available for energy coupling by electron transport phosphorylation. Conse-

quently, the observed Y_G and fixation of 0.45 mol CO_2 per mol thiosulphate means that this is supported energetically by the oxidation of 0.775 mol thiosulphate. The fixation and conversion of 0.45 mol CO_2 to the level of cell constituents also requires 0.39 mol ATP for biosynthesis from the hexose level (Stouthamer, 1973), and the reduction of 0.9 mol NAD probably requires 1.8 mol ATP to effect electron transport from the level of cytochrome *c* to NAD^+ (assuming 2 mol ATP required per mol NAD^+ reduced). Consequently, total ATP indicated to be available from the oxidation of 0.775 mol thiosulphate was 3.54 mol, i.e. 4.57 mol ATP per mol thiosulphate oxidized for energetic purposes with nitrate as the terminal electron acceptor. Aerobically, the Y_G of 14.69 can be calculated to indicate 6.43 mol ATP per mol thiosulphate oxidized. In both these calculations, if NAD^+ reduction requires only one mol ATP per mol, the ATP yields are reduced to 3.41 and 5.27 for anaerobic and aerobic growth, respectively. Taking the higher values and assuming that they represent 4 to 5 and 6 to 7 mol ATP formed per mol thiosulphate, respectively, and that 1 to 2 mol ATP are formed in each case by substrate level phosphorylation (Peck, 1968), at least 3 and 5 mol ATP are formed by oxidative phosphorylation anaerobically and aerobically, respectively. This must indicate that the oxidation of the sulphane-sulphur of thiosulphate supports phosphorylation (Kelly, 1978) and hence is not effected by the sulphur oxygenase: this could in any case not be significant during anaerobic growth.

These calculations indicate that for each mol thiosulphate *used for energetic purposes* [i.e. corrected for (H) requirement for CO_2 fixation] the amount of growth supported is 14.66 g dry wt under anaerobic nitrate-limitation and 20.69 g dry wt under aerobic thiosulphate-limitation. Anaerobically, available energy would seem to be 71 % of that available aerobically.

Thermodynamically, the theoretically available free energy for nitrate-linked and oxygen-linked thiosulphate oxidation is 741 and 936 kJ mol⁻¹, respectively (Kelly, 1978), indicating that no better than 79.2 % of the aerobic growth yield would be expected anaerobically, in moderate agreement with the observed result.

The anaerobic nitrate-limited Y_G values for thiosulphate (11.37) and tetrathionate (21.5) are equivalent to, respectively, 14.66 and 28.39 g dry wt per mol thiosulphate or tetrathionate oxidized for energetic purposes, indicating that the energy available from thiosulphate oxidation is 51.7 % of that available from tetrathionate oxidation, in reasonable agreement with the relative free energy available from their oxidation of 54 to 56 % (Kelly, 1978).

The detailed response of *T. denitrificans* to progressive transition from aerobic to anaerobic continuous culture is described in the following paper (Justin & Kelly, 1978).

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