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Growth Yields and Growth Rates of *Desulfovibrio vulgaris* (Marburg) Growing on Hydrogen plus Sulfate and Hydrogen plus Thiosulfate as the Sole Energy Sources

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Abstract. *Desulfovibrio vulgaris* (Marburg) was grown on H_2 plus sulfate and H_2 plus thiosulfate as the sole energy sources and acetate plus CO_2 as the sole carbon sources. Conditions are described under which the bacteria grew exponentially. Specific growth rates (μ) and molar growth yields (Y) at different pH were determined.

μ and Y were found to be strongly dependent on the pH. Highest growth rates and molar growth yields were observed for growth on H_2 plus sulfate at pH 6.5 ($\mu = 0.15\,h^{-1}$; $Y_{SO_4^{2-}} = 8.3\,g \cdot mol^{-1}$) and for growth on H_2 plus thiosulfate at pH 6.8 ($\mu = 0.21\,h^{-1}$; $Y_{S_2O_3^{2-}} = 16.9\,g \cdot mol^{-1}$).

The growth yields were found to increase with increasing growth rates: plots of $1/Y$ versus $1/\mu$ were linear. Via extrapolation to infinite growth rates a $Y^{max}_{SO_4^{2-}}$ of $12.2\,g \cdot mol^{-1}$ and a $Y^{max}_{S_2O_3^{2-}}$ of $33.5\,g \cdot mol^{-1}$ was obtained.

The growth yield data are interpreted to indicate that dissimilatory sulfate reduction to sulfide is associated with a net synthesis of 1 mol of ATP and that near to 3 mol of ATP are formed during dissimilatory sulfite reduction to sulfide.

Key words: *Desulfovibrio* – Chemolithothrophic growth – H_2 oxidation – Sulfate reduction – Thiosulfate reduction – Growth rates – Growth yields – Maintenance coefficients – Y^{max}_{ATP}.

Recently two sulfate-reducing bacteria were isolated that can grow on H_2 plus sulfate as the sole energy source and acetate plus CO_2 as the sole carbon sources (Badziong et al., 1978). One of the organisms was enriched from sewage digester sludge in Marburg (FRG), the other from eutrophic pond sediments in Madison (U.S.A.). The isolates were identified as *Desulfovibrio* species related to *D. vulgaris* and were considered as chemolithotrophic subspecies of this organisms. The two sulfate-reducers differed in their ability to use formate, ethanol and malate as electron donors and fumarate as electron acceptor and are therefore referred to as the Marburg strain and the Madison strain of *D. vulgaris*.

The finding that sulfate-reducing bacteria can grow on H_2 plus sulfate as the sole energy source clearly established that the reduction of sulfate to sulfide is coupled with a net synthesis of ATP. Growth yield studies performed at pH 7.2 revealed that 4–5 g cells (dry weight) are formed per mol of sulfate reduced with H_2. The growth yield data were interpreted to indicate that per mol of sulfate reduced to sulfide 1 mol of ATP is formed (Badziong et al., 1978).

The reduction of sulfate to sulfide is known to proceed via adenylylsulfate (APS) and sulfite (Ishimoto and Fujimoto, 1959; Peck, 1959, 1962; for reviews see: LeGall and Postgate, 1973; Siegel, 1975; Thauer et al., 1977):

$$SO_4^{2-} + 2H^+ + ATP \rightleftharpoons APS + PP_i \quad \Delta G^{o\prime} = +46 \text{ kJ/mol} \tag{1}$$

$$PP_i + H_2O \rightarrow 2P_i \quad \Delta G^{o\prime} = -21.9 \text{ kJ/mol} \tag{2}$$

$$APS + H_2 \rightarrow HSO_3^- + AMP + H^+ \quad \Delta G^{o\prime} = -68.6 \text{ kJ/mol} \tag{3}$$

$$HSO_3^- + 3\,H_2 \rightarrow SH^- + 3\,H_2O \quad \Delta G^{o\prime} = -171.7 \text{ kJ/mol} \tag{4}$$

(The $\Delta G^{o\prime}$-values given are from Thauer et al., 1977.)

Two mol of ATP are required for the activation of sulfate. This inference is made on the premise that the pyrophosphate produced in the ATP-sulfurylase reaction is completely hydrolyzed by pyrophosphatase present in sulfate-reducing bacteria (Ware and Postgate, 1971). But the possibility exists that all or a part of the pyrophosphate formed could be utilized as

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ATP equivalents (Barton et al., 1972; Reeves, 1976). If 1 mol of ATP is formed per mol of sulfate reduced to sulfide, and 2 mol of ATP are required for the activation of sulfate, then the reduction of sulfite to sulfide must be coupled with the synthesis of 3 mol of ATP.

The present work was undertaken to determine via growth yield studies the amount of ATP formed during the reduction of sulfite to sulfide. *D. vulgaris* (Marburg) was grown on thiosulfate plus H_2 rather than on sulfite plus H_2 as sulfite was found to be toxic at higher concentrations. The reduction of thiosulfate to sulfite proceeds via sulfite, which is generated in a reaction neither requireing nor forming ATP (Haschke and Campbell 1971; Hatchikian, 1975):

$$S_2O_3^{2-} + H_2 \rightarrow HS^- + HSO_3^- \qquad \Delta G^{o\prime} = -2.1 \text{ kJ/mol} \qquad (5)$$

Materials and Methods

A hydrogen-carbon dioxide mixture (80% H_2/20% CO_2) was obtained from Messer Griesheim GmbH (Düsseldorf). The gas contained less than 5 ppm O_2, which was removed by passing the gas over copper files heated to 370° C. U-^{14}C acetic acid, sodium salt (57 mCi/mmol) was purchased from Amersham Buchler GmbH & Co. KG (Braunschweig).

Source of the Organism. Desulfovibrio vulgaris (Marburg) was isolated from anaerobic sewage sludge of the Marburg sewage treatment plant (Badziong et al., 1978).

Media. Preparation and use of the media was performed under strictly anaerobic conditions using the methods described by Hungate (1969). The media had the following composition (per liter distilled water): 6.6 g $(NH_4)_2SO_4$ or 7.4 g $(NH_4)_2S_2O_3$; 0.575 g sodium acetate; 0.5 g KH_2PO_4; 1 g NaCl; 0.2 g $MgCl_2 \cdot 6\ H_2O$; 0.1 g $CaCl_2 \cdot H_2O$; 1 ml resazurin solution (0.2% in H_2O); 10 ml trace element solution. The trace element solution contained per liter distilled H_2O: 12.8 g nitrilotriacetic acid neutralized to pH 6.5 with NaOH; 300 mg $FeCl_2 \cdot 4\ H_2O$; 100 mg $MnCl_2 \cdot 4\ H_2O$; 170 mg $CoCl_2 \cdot 6\ H_2O$; 100 mg $ZnCl_2$; 20 mg $CuCl_2$; 10 mg H_3BO_3; and $Na_2MoO_4 \cdot 2\ H_2O$. After autoclaving the medium was cooled and the following components were added from sterile stock solutions: 50 ml of 8% Na_2CO_3 and about 1 ml NaS_2O_4 solution (0.5 M in H_2O). The media were adjusted to the desired pH with 5 M HCl.

Conditions of Cultivation. The bacteria were grown anaerobically in a 500 ml fermenter containing 400 ml media. After inoculation (5% inoculum) the culture was continuously stirred at 700 rpm and gassed with 80% H_2/20% CO_2 at a rate of 80 ml/min. The pH of the culture was kept constant with an automatic pH-stat using 5 M HCl.

Determination of Growth. Growth was followed by measuring the incorporation of ^{14}C from ^{14}C-acetate (approximately 19000 dpm/μmol) as described previously (Badziong et al., 1978). 1 ml samples of the culture were centrifuged to remove the cells and 0.1 ml aliquots of the supernatant were counted in Brays solution (Bray, 1960) using a Packard Tricarb Liquid Scintillation Spectrometer Model 2425. The counting efficiently was determined with ^{14}C-toluene as internal standard. From the amount of ^{14}C-acetate removed from the medium the amount of acetate incorporated into cells was calculated. It was secured, that during growth the specific radioactivity of acetate remained constant and that acetate was neither oxidized to CO_2 (Widdel and Pfennig, 1977) nor reduced to methan (Hatchikian et al., 1976). The amount of cells formed was calculated from (i) the amount of acetate incorporated, (ii) from the carbon content of the cells, and (iii) from the percentage of cell carbon derived from acetate. The dried cells contained 42.4% carbon of which 71.2% was derived from acetate (Badziong et al., 1978).

Determination of Sulfate and Thiosulfate Consumption. The amount of sulfate and thiosulfate consumed during growth was followed by measuring the amount of hydrogen sulfide formed. The hydrogen sulfide was trapped in 400 ml 1.4 M zinc acetate. Sulfide was determined using the methylene blue method described by King and Morris (1967). From the amount of hydrogen sulfide formed during growth the amount of sulfate or thiosulfate used was calculated. It was secured that per mol of sulfate 1 mol of sulfide and that per mol of thiosulfate 2 mol of sulfide were generated (Badziong et al., 1978).

Results

Logarithmic Growth

D. vulgaris (Marburg) was grown in 500 ml fermenters containing 400 ml medium rapidly gassed with 80% H_2/20% CO_2. Growth on H_2 plus sulfate and H_2 plus thiosulfate was found to be exponential (Fig. 1), when the gassing rate was 80 ml/min and the pH was kept constant. At lower gassing rates growth was linear rather than logarithmic probably due to limitation of hydrogen supply and to the accumulation of toxic hydrogen sulfide (Postgate, 1966, 1969; Badziong et al., 1978). The pH had to be kept constant as it was found that the growth rate (μ) strongly varied with the pH of the medium (Fig. 2). Without the use of a pH-stat the pH of the culture increased due to the formation of OH^--ions during sulfate and thiosulfate reduction.

$$SO_4^{2-} + 4\ H_2 \rightarrow H_2S + 2\ H_2O + 2\ OH^- \qquad (6)$$

$$S_2O_3^{2-} + 4\ H_2 \rightarrow 2\ H_2S + H_2O + 2\ OH^-. \qquad (7)$$

Specific Growth Rates

The dependence of μ on the pH of the culture is given in Figure 2. Highest growth rates were observed for growth on H_2 plus sulfate at pH 6.5 ($\mu = 0.15\ h^{-1}$) and for growth on H_2 plus thiosulfate at pH 6.8 ($\mu = 0.21\ h^{-1}$), equal to a mean doubling time of 4.7 h and 3.3 h, respectively. μ was found to decrease, when the culture was grown at higher and lower pH values.

Molar Growth Yields (Y)

$Y_{SO_4^{2-}}$ and $Y_{S_2O_3^{2-}}$ were determined at different times of logarithmic growth. The Y data given in Figure 3 are mean values of at least five determinations. $Y_{SO_4^{2-}}$ and $Y_{S_2O_3^{2-}}$ were found to be strongly dependent on the pH (Fig. 3). Highest yields were obtained at the pH at which μ was highest: at pH 6.5 $Y_{SO_4^{2-}}$ was 8.3 g · mol^{-1} and at pH 6.8 $Y_{S_2O_3^{2-}}$ was 16.9 g · mol^{-1}.

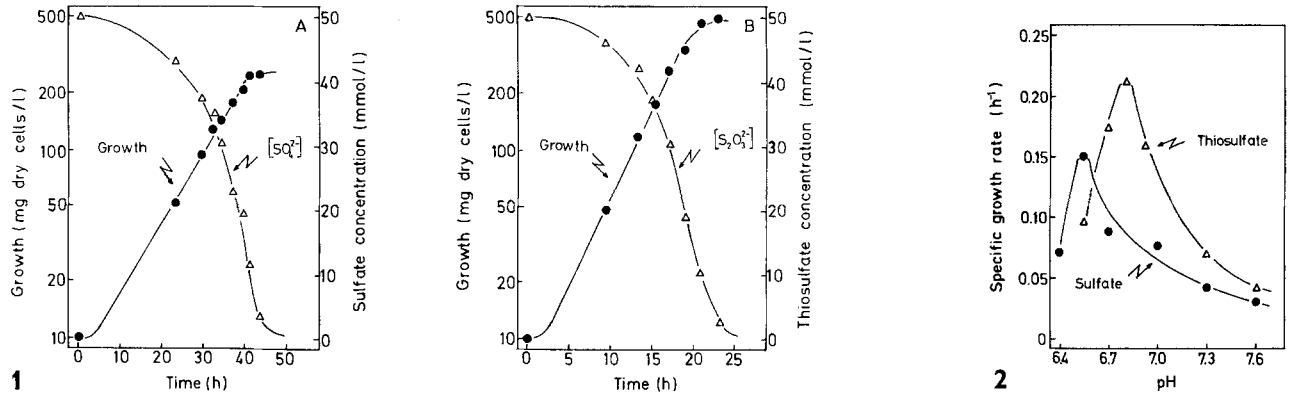

Fig. 1 A and B. Growth of *Desulfovibrio vulgaris* (Marburg) on H_2 plus sulfate (**A**) and H_2 plus thiosulfate (**B**) as the sole energy sources. The pH in both cultures was kept constant at pH 6.7

Fig. 2. Growth rate dependence on the pH during growth of *Desulfovibrio vulgaris* (Marburg) on H_2 plus sulfate and H_2 plus thiosulfate as the sole energy sources

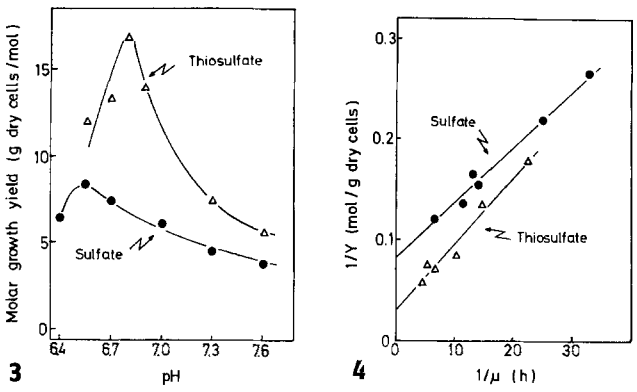

Fig. 3. Molar growth yield dependence on the pH during growth of *Desulfovibrio vulgaris* (Marburg) on H_2 plus sulfate and H_2 plus thiosulfate as the sole energy sources. Y data given are mean values of at least 5 determinations

Fig. 4. Double reciprocal plots of the molar growth yield (Y) versus the specific growth rate (μ). The Y and μ values were taken from Figures 2 and 3, respectively. Linear regression analysis was applied to fit the straight-line to the experimental data

A double reciprocal plot of $Y_{SO_4^{2-}}$ and $Y_{S_2O_3^{2-}}$ versus μ is shown in Figure 4. This plot is linear in agreement with the observation that at different pH the energy metabolism of the sulfate-reducing bacterium was not qualitatively altered. Via extrapolation to infinite growth rates a $Y^{max}_{SO_4^{2-}}$ of 12.2g mol^{-1} and $Y^{max}_{S_2O_3^{2-}}$ of 33.5g mol^{-1} was obtained.

Discussion

Growth yields (Y) are known to be dependent on the specific growth rate (μ), the maintenance coefficient (m), and the molar growth yield corrected for energy of maintenance (Y^{max}) (Pirt, 1965; van Uden 1969; Stouthamer and Bettenhaussen, 1973; Stouthamer, 1973, 1976). A list of symbols is given in Table 1.

$$1/Y = m/\mu + {}^1/y^{max}. \quad (8)$$

If m and Y^{max} are constants the plot of $1/Y$ against $1/\mu$ will be a straight-line with slope $= m$ and intersept on the ordinate $= 1/Y^{max}$.

In the present investigation $Y_{SO_4^{2-}}$ and $Y_{S_2O_3^{2-}}$ were determined in dependence of μ for *D. vulgaris* (Marburg) growing on H_2 plus sulfate and H_2 plus thiosulfate as the sole energy sources. Double reciprocal plots of Y versus μ were linear (Fig. 4). The data show good straight line fit over a wide range of specific growth rates, from 0.03 h^{-1} to 0.21 h^{-1} as predicted by Equation (8). This indicates that m and Y^{max} were constant under the experimental conditions (Pirt, 1965). From the intercept on the ordinate and the slope the following apparent Y^{max} and m values were determined: $Y^{max}_{SO_4^{2-}} = 12.2\,g \cdot mol^{-1}$; $m_{SO_4^{2-}} = 5.5$ $mmol \cdot g^{-1} \cdot h^{-1}$; $Y^{max}_{S_2O_3^{2-}} = 33.5\,g \cdot mol^{-1}$; $m_{S_2O_3^{2-}} = 6.6$ $mmol \cdot g^{-1} \cdot h^{-1}$.

The amount of ATP formed per mol of energy substrate consumed (γ) can be estimated from Y^{max} and the molar growth yield per mol ATP corrected for energy of maintenance (Y^{max}_{ATP}).

$$\gamma = \frac{Y^{max}}{Y^{max}_{ATP}}. \quad (9)$$

$1/Y^{max}_{ATP}$ is equal to the amount of ATP required for the synthesis of 1 g dry cells from the carbon and nitrogen sources at infinite growth rates where the amount of ATP required for maintenance ($m_{ATP}/\mu = 0$) becomes

Table 1. List of symbols (according to Stouthamer and Bettenhausen, 1973)

Symbol	Specification	Dimension
Y	Molar growth yield	g dry cells/mol substrate
$Y_{SO_4^{2-}}$	Molar growth yield for sulfate	g dry cells/mol sulfate
$Y_{S_2O_3^{2-}}$	Molar growth yield for thiosulfate	g dry cells/mol thiosulfate
Y^{max}	Molar growth yield corrected for energy of maintenance	g dry cells/mol substrate
$Y^{max}_{SO_4^{2-}}$	Molar growth yield for sulfate corrected for energy of maintenance	g dry cells/mol sulfate
$Y^{max}_{S_2O_3^{2-}}$	Molar growth yield for thiosulfate corrected for energy of maintenance	g dry cells/mol thiosulfate
Y^{max}_{ATP}	Molar growth yield per mol ATP corrected for energy of maintenance	g dry cells/mol ATP
μ	Specific growth rate	h^{-1}
m	Maintenance coefficient	mol substrate/g dry cells · h
$m_{SO_4^{2-}}$	Maintenance coefficient for sulfate	mol sulfate/g dry cells · h
$m_{S_2O_3^{2-}}$	Maintenance coefficient for thiosulfate	mol thiosulfate/g dry cells · h
m_{ATP}	Maintenance coefficient for ATP	mol ATP/g dry cells · h

zero. $1/Y^{max}_{ATP}$ can be calculated, if the composition of the cells and the ATP requirement for the synthesis of most of the cell components are known. *D. vulgaris* (Marburg) was grown on acetate plus CO_2 as sole carbon sources and NH_4^+ as nitrogen source. 70% of the cell carbon was found to be derived from acetate, 30% from CO_2 (Badziong et al., 1978; Sorokin, 1966a–c), indicating that, as in *Clostridium kluyveri*, acetate is assimilated via acetate activation to acetyl-CoA, reductive carboxylation of acetyl-CoA to pyruvate (Tomlinson and Barker, 1954; Tomlinson, 1954; Andrew and Morris, 1965) and then synthesis of cell components from pyruvate via known biosynthetic routes. The elementary composition of the cells was found to be 42% carbon and 12.6% nitrogen. The cells are assumed to contain 60% protein, 10% carbohydrates, 10% lipids, and 15% nucleic acids (Luria, 1960; Gunsalus and Shuster, 1961; Decker et al., 1970; Stouthamer, 1973). Acetate is most probably activated to acetyl-CoA via thiokinase at the expenditure of 2 equivalents of ATP per mol of acetate (Decker et al., 1970) and NH_4^+ assimilated into amino acids via glutamate dehydrogenase as the NH_4^+ concentration in the growth medium was high (Brenchley et al., 1973). Based on these assumptions it is calculated that approximately 80 mmol of ATP are required for the synthesis of 1 g dry cells from acetate and CO_2, equal to a Y^{max}_{ATP} of 12.5 g · mol^{-1}. If acetate is activated to acetyl-CoA at the expenditure of only 1 ATP then only 68 mmol ATP per 1 g dry cells would be required resulting in a Y^{max}_{ATP} of 14.6 g · mol^{-1}. If NH_4^+ is assimilated into amino acids via glutamine synthetase plus glutamate synthase rather than via glutamate dehydrogenase then 88 mmol of ATP per 1 g dry cells would be required and Y^{max}_{ATP} would be 11.4 g · mol^{-1}.

A comparison of $Y^{max}_{SO_4^{2-}}$ (12.2 g · mol^{-1}) with Y^{max}_{ATP} (11.4–14.6 g · mol^{-1}) indicates that during growth of *D. vulgaris* (Marburg) on H_2 plus sulfate approximately 1 mol of ATP is formed per mol of sulfate reduced to sulfide [see Eq. (9)]. A 1:1 stoichiometry has recently been proposed on the basis of more indirect data (Badziong et al., 1978). $Y^{max}_{S_2O_3^{2-}}$ (33.5 g · mol^{-1}) was found to be 2.7 times higher than $Y^{max}_{SO_4^{2-}}$ (12.2 g · mol^{-1}) suggesting that during growth of *D. vulgaris* (Marburg) on H_2 plus thiosulfate near to 3 mol of ATP were formed per mol of thiosulfate reduced to sulfide.

The free energy changes associated with the reduction of thiosulfate to sulfide is sufficient exergonic to allow the synthesis of 3 mol of ATP [Eqs. (11) and (12)]:

$$SO_4^{2-} + 4\,H_2 + H^+ \rightarrow HS^- + 4\,H_2O$$
$$\Delta G^{o\prime} = -152.2\ \text{kJ/mol} \qquad (10)$$

$$S_2O_3^{2-} + 4\,H_2 \rightarrow 2\,HS^- + 3\,H_2O$$
$$\Delta G^{o\prime} = -173.8\ \text{kJ/mol} \qquad (11)$$

$$ADP + P_i \rightleftharpoons ATP + H_2O$$
$$\Delta G^{o\prime} = +\ 31.8\ \text{kJ/mol}. \qquad (12)$$

As the reduction of thiosulfate to sulfide proceeds via sulfite (Nakatsukasa and Akagi, 1969; Haschke and Campbell, 1971; Hatchikian, 1975), which is generated in a reaction neither requireing nor forming ATP [see Eq. (5)], it is concluded that the reduction of sulfite to sulfide is also coupled with the synthesis of 3 mol of ATP.

It has been proposed, that sulfite is reduced to sulfide via three enzymes – bisulfite reductase, trithionate reductase and thiosulfite reductase – with trithionate and thiosulfate as free intermediates (for

reviews see: LeGall and Postgate, 1973; Siegel, 1975; Thauer et al., 1977):

$$3\,HSO_3^- + H_2 + H^+ \rightarrow S_3O_6^{2-} + 3\,H_2O$$
$$\Delta G^{o\prime} = -\,46.3\ \text{kJ/mol} \quad (13)$$

$$S_3O_6^{2-} + H_2 \rightarrow S_2O_3^{2-} + HSO_3^- + H^+$$
$$\Delta G^{o\prime} = -123\ \text{kJ/mol} \quad (14)$$

$$S_2O_3^{2-} + H_2 \rightarrow HS^- + HSO_3^-$$
$$\Delta G^{o\prime} = -\,2.1\ \text{kJ/mol}. \quad (15)$$

Reaction (13) is exergonic enough to be coupled with the synthesis of 1 mol of ATP, reaction (14) sufficient exergonic to drive the synthesis of 2 mol of ATP. The finding that near to 3 mol of ATP are formed per mol of thiosulfate reduced to sulfide neither supports nor excludes the proposed "recycling sulfite pool" mechanism.

The maintenance coefficient m_{ATP} can be calculated from m and γ:

$$m_{ATP} = m \cdot \gamma \quad (15)$$

m_{ATP} for growth on H_2 plus sulfate was $5.5\,\text{mmol} \cdot g^{-1} \cdot h^{-1}$, m_{ATP} for growth on H_2 plus thiosulfate was $17.8\,\text{mmol} \cdot g^{-1} \cdot h^{-1}$. The finding that m_{ATP} during growth on H_2 plus thiosulfate was considerably larger than during growth on H_2 plus sulfate is surprising and not understood. An explanation might be that the synthesis of 3 mol of ATP per mol of thiosulfate proceeds less irreversibly than the synthesis of 1 mol of ATP per mol of sulfate [see $\Delta G^{o\prime}$-values given in Eq. (10–12)], thus necessitating that part of the ATP formed during thiosulfate reduction is irreversibly hydrolyzed to ADP and P_i (Thauer et al., 1977).

Highest growth rates for *D. vulgaris* (Marburg) were observed at pH values between 6.5 and 6.8. This is an important observation. The media published for the cultivation of sulfate-reducing bacteria generally are adjusted to a pH 7.2–7.6 (Postgate, 1966, 1969; Pankhurst, 1971). At this pH the bacteria only grow slowly and the growth yields are low. This might be the explanation why growth yield studies so far failed to predict the amount of ATP formed during dissimilatory sulfate and sulfite reduction (Senez, 1962; Vosjan, 1970, 1975; Khosrovi et al., 1974).

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References

Andrew, I. G., Morris, J. G.: The biosynthesis of alanin by *Clostridium kluyveri*. Biochim. Biophys. Acta **97**, 176–179 (1965)

Badziong, W., Thauer, R. K., Zeikus, J. G.: Isolation and characterization of *Desulfovibrio* growing on hydrogen plus sulfate as the sole energy source. Arch. Microbiol. **116**, 41–49 (1978)

Barton, L. L., LeGall, J., Peck, H. D., Jr.: Oxidative phosphorylation in the obligate anaerobe, *Desulfovibrio gigas*. In: Horizons of bioenergetics (A. San Pietro, H. Gest, eds.), pp. 33–51. New York: Academic Press 1972

Bray, G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Analyt. Biochem. **1**, 279–285 (1960)

Brenchley, J. E., Prival, M., Magasanik, B.: Regulation of the synthesis of enzymes responsible for glutamate formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**, 6122–6128 (1973)

Decker, K., Jungermann, K., Thauer, R. K.: Energy production in anaerobic organisms. Angew. Chem. Int. Ed. Engl. **9**, 138–158 (1970)

Gunsalus, I. C., Shuster, C. W.: Energy-yielding metabolism in bacteria. In: The bacteria (I. C. Gunsalus, R. Y. Stanier, eds.), pp. 1–58. New York: Academic Press 1961

Haschke, R. H., Campbell, L. L.: Thiosulfate reductase of *Desulfovibrio vulgaris*. J. Bacteriol. **106**, 603–607 (1971)

Hatchikian, E. C.: Purification and properties of thiosulfate reductase from *Desulfovibrio gigas*. Arch. Microbiol. **105**, 249–256 (1975)

Hatchikian, E. C., Chaigneau, M., LeGall, J.: Analysis of gas production by growing cultures of three species of sulfate-reducing bacteria. In: Proc. Symp. Microbial Production and Utilization of Gases (H_2, CH_4, CO) (H. G. Schlegel, G. Gottschalk, N. Pfennig, eds.), pp. 109–118. Göttingen: Goltze 1976

Hungate, R. E.: A roll tube method for cultivation of strict anaerobes. In: Methods in microbiology, Vol. 3 B (J. R. Norris, D. W. Ribbons, eds.), pp. 117–132. London-New York: Academic Press 1969

Ishimoto, M., Fujimoto, D.: Adenosine-5′ phosphosulfonate as an intermediate in the reduction of sulfate by a sulfate-reducing bacterium. Proc. Jpn. Acad. Sci. **35**, 243–245 (1959)

Khosrovi, B., Mac Pherson, R., Miller, J. D. A.: Some observations on growth and hydrogen uptake by *Desulfovibrio vulgaris*. Arch. Microbiol. **80**, 324–337 (1974)

King, T. E., Morris, R. O.: Determination of acid-labile sulfide and sulfhydryl groups. In: Methods in enzymology, Vol. 10 (S. P. Colowik, N. O. Kaplan, eds.), pp. 634–641. New York: Academic Press 1967

Le Gall, J., Postgate, J. R.: The physiology of sulfate-reducing bacteria. Adv. Microbiol. Physiol. **10**, 81–133 (1973)

Luria, S. E.: The bacterial protoplasm: composition and organization. In: The bacteria, Vol. 1 (I. C. Gunsalus, R. Y. Stanier, eds.), pp. 1–34. New York: Academic Press 1960

Nakatsukasa, W., Akagi, J. M.: Thiosulfate reductase isolated from *Desulfotomaculum nigrificans*. J. Bacteriol. **98**, 429–433 (1969)

Pankhurst, E. S.: The isolation and enumeration of sulphate-reducing bacteria. In: Isolation of anaerobes (D. A. Shapton, R. G. Board, eds.), pp. 223–240. New York: Academic Press 1971

Peck, H. D.: The ATP-dependent reduction of sulfate with hydrogen in extracts of *Desulfovibrio desulfuricans*. Proc. Natl. Acad. Sci. U.S.A. **45**, 701–708 (1959)

Peck, H. D., Jr.: The role of adenosine-5′-phosphosulfate in the reduction of sulfate to sulfite by *Desulfovibrio desulfuricans*. J. Biol. Chem. **237**, 198–203 (1962)

Pirt, S. J.: The maintenance energy of bacteria in growing cultures. Proc. Roy. Soc. London **163 B**, 224–231 (1965)

Postgate, J. R.: Media for sulfur bacteria. Lab. Practice **15**, 1239–1244 (1966)

Postgate, J. R.: Media for sulfur bacteria: Some amendments. Lab. Practice **18**, 286 (1969)

Reeves, R. E.: How useful is the energy in inorganic pyrophosphate? TIBS **1**, 53–55 (1976)

Senez, J. C.: Some considerations on the energetics of bacterial growth. Bacteriol. Rev. **26**, 95–107 (1962)

Siegel, L. M.: Biochemistry of the sulfur cycle. In: Metabolic pathways, Vol. 7 (D. M. Greenberg, ed.), pp. 217–286. New York: Academic Press 1975

Sorokin, Yu. I.: Sources of energy and carbon for biosynthesis in sulfate-reducing bacteria [Engl. Transl.]. Microbiology (USSR) **35**, 643–647 (1966a)

Sorokin, Yu. I.: Investigations of the structural metabolism of sulfate-reducing bacteria with ^{14}C [Engl. Transl.]. Microbiology (USSR) **35**, 806–814 (1966b)

Sorokin, Yu. I.: Role of carbon dioxide and acetate in the biosynthesis by sulphate-reducing bacteria. Nature **210**, 551–552 (1966c)

Stouthamer, A. H.: A theoretical study on the amount of ATP required for synthesis of microbial cell material. Antonie van Leeuwenhoek. J. Microbiol. Serol. **39**, 545–565 (1973)

Stouthamer, A. H.: Yield studies in microorganisms. Durham, England: Meadowfield Press 1976

Stouthamer, A. H., Bettenhaussen, C.: Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. Biochim. Biophys. Acta **301**, 53–70 (1973)

Thauer, R. K., Jungermann, K., Decker, K.: Energy conservation in chemothrophic anaerobic bacteria. Bacteriol. Rev. **41**, 100–180 (1977)

Tomlinson, N.: Carbon dioxide and acetate utilization by *Clostridium kluyveri*. II. Synthesis of amino acids. J. Biol. Chem. **209**, 597–603 (1954)

Tomlinson, N., Barker, H. A.: Carbon dioxide and acetate utilisation by *Clostridium kluyveri*. I. Influence of nutritional conditions on utilisation patterns. J. Biol. Chem. **209**, 585–595 (1954)

van Uden, N.: Kinetics of nutrient-limited growth. Annu. Rev. Microbiol. **23**, 473–486 (1969)

Vosjan, J. H.: ATP generation by electron transport in *Desulfovibrio desulfuricans*. Antonie van Leeuwenhoek J. Microbiol. Serol. **36**, 584–586 (1970)

Vosjan, J. H.: Respiration and fermentation of the sulphate-reducing bacterium *Desulfovibrio desulfuricans* in a continuous culture. Plant and Soil **43**, 141–152 (1975)

Ware, D. A., Postgate, J. R.: Physiological and chemical properties of a reductant-activated inorganic pyrophosphatase from *Desulfovibrio desulfuricans*. J. Gen. Microbiol. **67**, 145–160 (1971)

Widdel, F., Pfennig, N.: A new anaerobic, sporing, acetate-oxidizing sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. Arch. Microbiol. **112**, 119–122 (1977)

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