JOURNAL OF BACTERIOLOGY, Jan. 1981, p. 191-199
0021-9193/81/010191-09$02.00/0

Vol. 145, No. 1

Microcalorimetric Studies of the Growth of Sulfate-Reducing Bacteria: Energetics of *Desulfovibrio vulgaris* Growth

ALFRED S. TRAORE,* CLAUDE E. HATCHIKIAN, JEAN-PIERRE BELAICH, AND JEAN LE GALL

Centre de la Recherche Scientifique, 13274 Marseille Cedex 2, France

The metabolism of *Desulfovibrio vulgaris* Hildenborough grown on medium containing lactate or pyruvate plus a high concentration of sulfate (36 mM) was studied. Molecular growth yields were 6.7 ± 1.3 and 10.1 ± 1.7 g/mol for lactate and pyruvate, respectively. Under conditions in which the energy source was the sole growth-limiting factor, we observed the formation of 0.5 mol of hydrogen per mol of lactate and 0.1 mol of hydrogen per mol of pyruvate. The determination of metabolic end products revealed that *D. vulgaris* produced, in addition to normal end products (acetic acid, carbon dioxide, hydrogen sulfide) and molecular hydrogen, 2 and 5% of ethanol per mol of lactate and pyruvate, respectively. Power-time curves of growth of *D. vulgaris* on lactate and pyruvate were obtained, by the microcalorimetric Tian-Calvet apparatus. The enthalpies (ΔH_{met}) associated with the oxidation of these substrates and calculated from growth thermograms were −36.36 ± 5 and −70.22 ± 3 kJ/mol of lactate and pyruvate, respectively. These experimental values were in agreement with the homologous values assessed from the theoretical equations of *D. vulgaris* metabolism of both lactate and pyruvate. The hydrogen production by this sulfate reducer constitutes an efficient regulatory system of electrons, from energy source through the pathway of sulfate reduction. This hydrogen value may thus facilitate interactions between this strain and other environmental microflora, especially metagenic bacteria.

Sulfate-reducing bacteria exhibit a strictly anaerobic mode of growth based on the use of sulfate as the terminal electron acceptor (23). This dissimilatory reduction of sulfate to sulfide is linked to the oxidation of organic electron donors, such as lactate, pyruvate, or ethanol. In addition to substrate level phosphorylation, oxidative phosphorylation coupled with the electron transfer has been clearly demonstrated (20).

These bacteria, which contain a high activity of hydrogenase (hydrogen:ferricytochrome c_3 oxidoreductase, EC 1.12.2.1) and cytochrome c_3, can either produce or consume molecular hydrogen. Growth by anaerobic oxidation of hydrogen with concomitant reduction of sulfate has been repeatedly reported (1, 17, 28, 29, 30). On the other hand, hydrogen is produced by some species of *Desulfovibrio* during growth on pyruvate medium lacking sulfate (21, 34).

Furthermore, production of hydrogen by pure cultures of *Desulfovibrio vulgaris* Hildenborough on lactate media high in sulfate has been described previously (14; S. A. Traoré, Ph.D. thesis, Université d'Aix Marseille, France, 1978). Production of hydrogen when sulfate is not a limiting factor is not easily understandable because of energetic considerations. This hydrogen production might play an important role in the interactions between sulfate-reducing bacteria and H_2-oxidizing bacteria, as methanogenic bacteria. Through interspecies hydrogen transfer, as defined by Bryant et al. (10), significant amounts of hydrogen are produced by *Desulfovibrio desulfuricans* and *D. vulgaris* and used for carbon dioxide reduction by methane bacteria.

The growth energetics of sulfate-reducing bacteria have already been studied by assessment of molecular growth yields (15, 16, 25); the purpose of this work was to study the growth of *D. vulgaris* by using microcalorimetric techniques. These techniques have been developed by Belaich et al. (5, 7) and are very convenient for this study of anaerobic growth (3, 4). The shape of the power-time curves of bacterial growth are dependent on physiological growth conditions, and heat quantities evolved during growth are correlated to fermentation products. Another interest of microcalorimetry is that it allows determination of all growth parameters calculable by the standard technique of optical density measurement.

MATERIALS AND METHODS

Strain and media. *D. vulgaris* Hildenborough, NCIB 8303 was used for this study. This strain was routinely cultivated on Starkey medium which contained (in grams per liter) the following: NH_4Cl, 2;

$MgSO_4 \cdot 7H_2O$, 2; Na_2SO_4, 4; K_2HPO_4, 0.5; and yeast extract (Difco Laboratories), 1. This medium was supplemented by the trace elements solution, prepared by the method of Bauchop and Elsden (2) and the recommendations of Vosjan (34); it contained (per liter) the following: 36 N HCl, 51.3 ml; MgO, 10.75 g; $CaCO_3$, 2 g; ferric citrate, 6 g; $ZnSO_4 \cdot 7H_2O$, 1.44 g; $MnSO_4 \cdot 4H_2O$, 1.12 g; $CuSO_4 \cdot 5H_2O$, 0.25 g; BO_3H_3, 0.06 g; $(Mo)7NH_4O_{24} \cdot 4H_2O$, 1 g. A 1-ml portion of this solution was added to 999 ml of Starkey medium. The pH was adjusted to 7.2 with 1 N NaOH.

Lithium lactate or sodium pyruvate (Merck & Co., Inc.) was used as the energy source; both lactate and pyruvate media were sterilized by autoclaving at 110°C for 30 min.

Analytical methods. Anaerobiosis was obtained with a vacuum pump. Air in the culture tubes was removed and replaced by argon gas.

Growth kinetics were followed with an automatic biophotometer (Bonet Maury and Jouan) at 30°C. All experiments were performed at 30°C; optical densities were read at 450 nm on a Zeiss (type MQ III) spectrophotometer.

Molecular growth yields were determined from the weight of the washed and dried cells; they were also estimated by determining the total nitrogen content of the cells by microkjeldahl techniques and applying the bacterial formula established in the chemical analysis laboratory of the Service Central de Microanalyse of the Centre National de la Recherche Scientifique. For this purpose, the bacterial cells were carefully washed and lyophilized. After lyophilization, the water content of the cells was measured with a thermogravimetric balance.

The hydrogen, carbon dioxide, and liquid end products were determined on a Girdel 30 gas chromatograph equipped with a flame ionization detector. Hydrogen sulfide was titrated by the method of Fogo and Popowsky (11).

Thermochemical methods. The heat of combustion of the bacteria was obtained by using a calorimetric bomb in the Laboratoire de l'Ecole Nationale Supérieure des Techniques Avancées (ENSTA, France).

To obtain power-time curves, we used a differential Calvet microcalorimeter, the properties of which were discussed in the monograph of Calvet and Prat (24); the experiments were conducted as previously described (7).

RESULTS

Figure 1 shows the final optical densities of bacteria obtained with different concentrations of lactate or pyruvate when the culture medium contained 36.3 mM sulfate. For both substrates, it can be seen that optical densities are proportional to the concentration of the substrate up to 15 mM. For higher concentrations, the optical densities increased slowly on pyruvate medium but remained constant on lactate medium. It can therefore be concluded that the energy source (pyruvate or lactate) will be the growth-limiting factor if its initial concentration does not exceed 15 mM.

Molecular growth yields (Y_{lact} and Y_{pyr}) (Table 1) were determined by the dry weight and total nitrogen content measurement methods. Although this last method regularly gave lower growth yield values, both techniques were in good agreement. Thus, Y_{lact} and Y_{pyr} values were 6.7 ± 1.3 and 10.1 ± 1.7 g/mol, respectively. These results show that lactate yielded less energy than pyruvate. This finding is extensively discussed below.

Growth kinetics patterns on pyruvate are shown in Fig. 2A and B; analogous patterns were recorded with lactate. In both cases, a substrate concentration of lower than 15 mM was the growth-limiting factor. When the substrate was exhausted, the increase of optical density

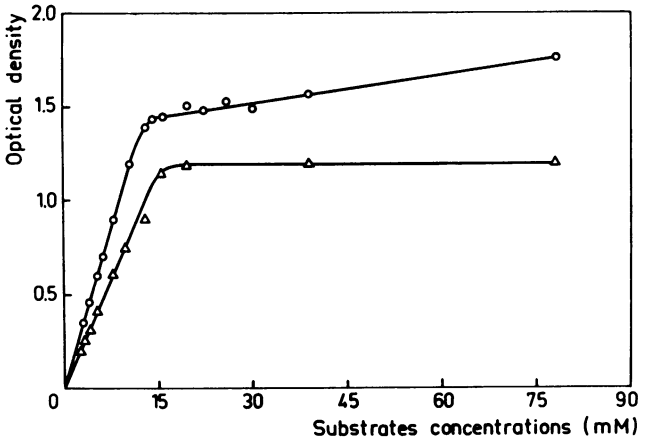

FIG. 1. *Comparative study of D. vulgaris Hildenborough growth on equimolar quantities of lactate* (△) *and pyruvate* (○).

TABLE 1. *Y_{lac} and Y_{pyr} values of D. vulgaris Hildenborough*[a]

Expt no.	Y_{lac} (g/mol)		Y_{pyr} (g/mol)	
	Dry wt	Nitrogen determination	Dry wt	Nitrogen determination
1	7.90	5.20	8.64	11.70
2	8.50	5.80	12.40	9.20
3			10.03	7.50
4	6.90	5.90	12.80	8.33
Mean	7.76	5.63	11.00	9.20

[a] Average means of Y_{lac} and Y_{pyr} were 6.70 ± 1.30 and 10.10 ± 1.70 g/mol, respectively.

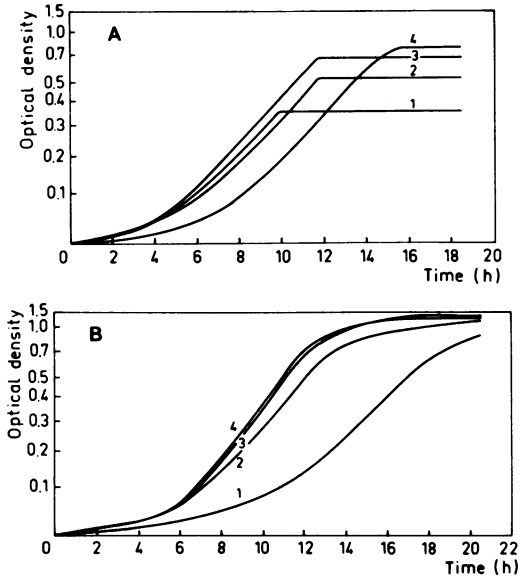

FIG. 2. *Growth of D. vulgaris Hildenborough with limiting energy source. Curves 1, 2, 3, and 4 have been obtained with 5.2, 7.8, 10.4, and 13 mM pyruvate, respectively, in the presence of 36.3 mM sulfate. B. Growth of D. vulgaris Hildenborough with excess energy source. Curves 1, 2, 3, and 4 have been obtained with a recorder biophotometer on 156, 52, 26, and 17.3 mM pyruvate, respectively, in the presence of 36.3 mM sulfate.*

stopped abruptly (Fig. 2A), whereas when the carbon source was not limiting growth, growth stopped only after a long slow-down period (Fig. 2B). Growth rates of *D. vulgaris* with lactate or pyruvate as the energy source are given in Table 2.

One can see that growth was more rapid on pyruvate than on lactate. The cellular rate of catabolic activities was calculated from molecular growth yields and growth rates (Table 2). Catabolic activity values were similar for both carbon substrates.

Power-time curves when pyruvate or lactate was the growth-limiting factor (Fig. 3) were typical of power-time curves found when growth is

TABLE 2. *Growth parameters of D. vulgaris Hildenborough*[a]

Substrate and expt no.	$t_{1/2}$ (h)[b]	$\mu^c = 0.69/t_{1/2}$	$Ac = \frac{\mu}{Ys}$[d] (mmol/g per h)
Lactate			
1	3.4	0.202	30.2
2	2.8	0.246	36.8
3	3	0.230	34.4
4	3.1	0.222	33.2
5	2.8	0.246	36.8
6	4.3	0.160	23.9
7	3	0.230	34.4
8	2.8	0.246	36.8
9	2.66	0.259	38.8
Mean	3.1 ± 0.5	0.222 ± 0.03	33.9 ± 4.5
Pyruvate			
1	2.6	0.265	26.5
2	2.3	0.300	29.8
3	2	0.345	34.2
4	2	0.345	34.2
5	2.3	0.300	29.8
6	2.4	0.287	28.5
7	2.6	0.265	26.3
8	2.4	0.287	28.5
9	2.08	0.332	33.0
Mean	2.3 ± 0.23	0.302	30.1 ± 3.0

[a] These experiments were done at 30°C on 7.8 mM of lactate or pyruvate.
[b] $t_{1/2}$, ——, Generation time
[c] μ, Growth rate.
[d] Ac, Catabolic activities; Ys, ——, Bacterial dry weight per mole of substrate metabolized.

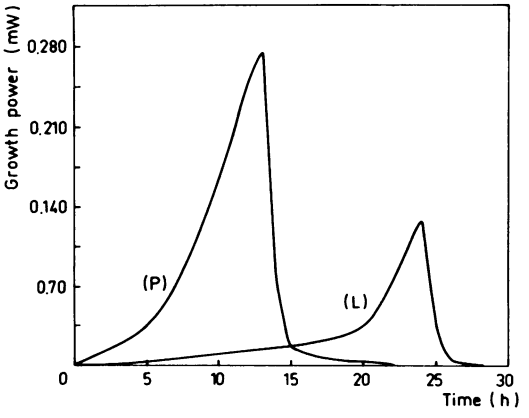

FIG. 3. *Comparison of growth thermograms of D. vulgaris Hildenborough growing on 10 ml of Starkey medium containing 7.8 mM lactate (L) and pyruvate (P). The experiments have been done at 30°C in a Tian-Calvet calorimeter.*

limited by the sole energy source (5). They show an exponential phase followed by a return to the baseline when the energy source becomes limiting. The integrations of power-time curves allowed the assessment of the enthalpies associated with the metabolism of the energetic substrate (ΔH_{met}). The experimental values for the two studied substrates are reported in Table 3. Mean values for lactate and pyruvate were -36.36 ± 5 and -70.22 ± 3 kJ/mol, respectively. It is noteworthy that the enthalpy corresponding to the more reduced substrate metabolism, i.e. lactate, was twice as low as that linked to the pyruvate metabolism. This result is not in agreement with the classical stoichiometry reactions proposed by Peck (19) for lactate oxidation:

$$CH_3CHOH\ COO^- + 0.5\ SO_4^{2-} + 0.71\ H^+ \rightarrow CO_2 + CH_3COO^- + 0.218\ H_2S + 0.282\ HS^- + H_2O \quad (1)$$

and for pyruvate oxidation:

$$CH_3CO\ COO^- + 0.25\ SO_4^{2-} + 0.359\ H^+ \rightarrow CO_2 + CH_3COO^- + 0.108\ H_2S + 0.142\ HS^- \quad (2)$$

From equations 1 and 2 it is possible to calculate the enthalpies associated with lactate or pyruvate metabolism; these values, -70.18 and -88.74 kJ/mol, respectively, for lactate and pyruvate, are in the same order of magnitude.

To explain the thermochemical results, the balance of fermentation products corresponding to the metabolism of both substrates has been performed. For this purpose all fermentation products formed in the calorimetric vessel are estimated at the end of growth, when the power-time curve returned to the baseline. (Table 4). The most striking features were that molecular hydrogen was produced in both cases and in a relatively high proportion when lactate was the energy source. In addition to hydrogen, sulfide, carbon dioxide, and acetate end products, the chemical balance of lactate or pyruvate metabolism indicated that small amounts of ethanol were also produced, accounting for, respectively, 2 and 5% of the oxidized substrates. The stoichiometric reaction of the oxidation of the two substrates by *D. vulgaris*, drawn from Table 4, can be written as follows for lactate oxidation:

$$CH_3CHOH\ COO^- + 0.37\ SO_4^{2-} + 0.56\ H^+ \rightarrow CO_2 + 0.98\ CH_3COO^- + 0.02\ CH_3CH_2OH + 0.16\ H_2S + 0.215\ HS^- + 0.5\ H_2O + 048\ H_2 \quad (3)$$

and for pyruvate oxidation:

$$CH_3CO\ COO^- + 0.2\ SO_4^{2-} + 0.15\ H_2O + 0.33\ H^+ \rightarrow CO_2 + 0.95\ CH_3COO^- + 0.05\ CH_3CH_2OH + 0.087\ H_2S + 0.113\ HS^- + 0.1\ H_2 \quad (4)$$

Equations 3 and 4 are very different from 1

TABLE 3. *Catabolism enthalpies of lactate (ΔHc_l) and pyruvate (ΔHc_p) by D. vulgaris Hildenborough*[a]

Expt no.	ΔHc_l		ΔHc_p	
	kcal/mol	kJ/mol	kcal/mol	kJ/mol
1	−8.28	−34.6	−17.25	−72.1
2	−10.60	−44.3	−17.38	−72.65
3	−9.29	−38.83	−17.1	−71.47
4	−9.26	−38.7	−17.63	−73.69
5	−8.66	−36.29	−17.33	−72.44
6	−7.8	−32.60	−16.4	−68.59
7	−7	−29.26	−15	−62.7
8			−16.3	−68.13
Mean	−8.6 ± 1.07	−36.36 ± 5	−16.8 ± 0.72	−70.22 ± 3

[a] All the results are given with their confidence interval of the mean ($P = 0.05$); 1 kcal/mol = 4.18 kJ/mol.

TABLE 4. *Fermentation products produced from 100 μmol of lactate or pyruvate by D. vulgaris Hildenborough*

Energy source	Metabolite amt (μmol)[a]				
	Acetate	Ethanol	Carbon dioxide	Hydrogen sulfide	Gaseous hydrogen
Lactate	96.80 ± 3.2	2.30 ± 0.8	99.02 ± 0.9	37.98 ± 4.1	47.90 ± 2.5
Pyruvate	94.50 ± 1.8	4.88 ± 1	99.07 ± 1.2	19.20 ± 1.5	7.20 ± 0.6

[a] The amounts represent mean values established from nine experiments.

and 2 proposed earlier (19). From our fermentation balances, it can be calculated that the enthalpies corresponding to the catabolism of lactate and pyruvate were, respectively, −30.26 and −78.96 kJ/mol. These data were in agreement with the experimental heat quantities accompanying the growth limited by lactate or pyruvate and especially the heat of lactate metabolism which is about half that of pyruvate.

H_2 production kinetics with lactate or pyruvate as electron donors. (i) Growth on lactate medium (equation 3). On lactate medium, H_2 formation was observed throughout growth (Fig. 4). It stopped only at the end when all of the lactate had been oxidized, and the formation rate was well correlated with the increase in optical density (Fig. 4). H_2 production seemed to precede sulfate reduction since a significant amount of H_2 was accumulated in the culture medium before significant amounts of hydrogen sulfide could be detected.

(ii) Growth on pyruvate medium (equation 4). In this case, H_2 evolution stopped before the exponential growth phase took place (Fig. 5) and the amounts of H_2 accumulated were rather low.

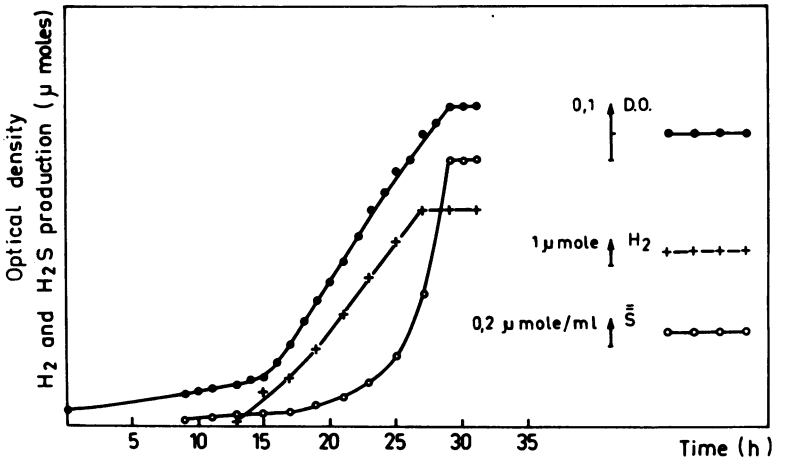

FIG. 4. *Kinetics of H_2 or H_2S formation during growth of D. vulgaris Hildenborough on 7.8 mM lactate in the presence of 36.3 mM sulfate.*

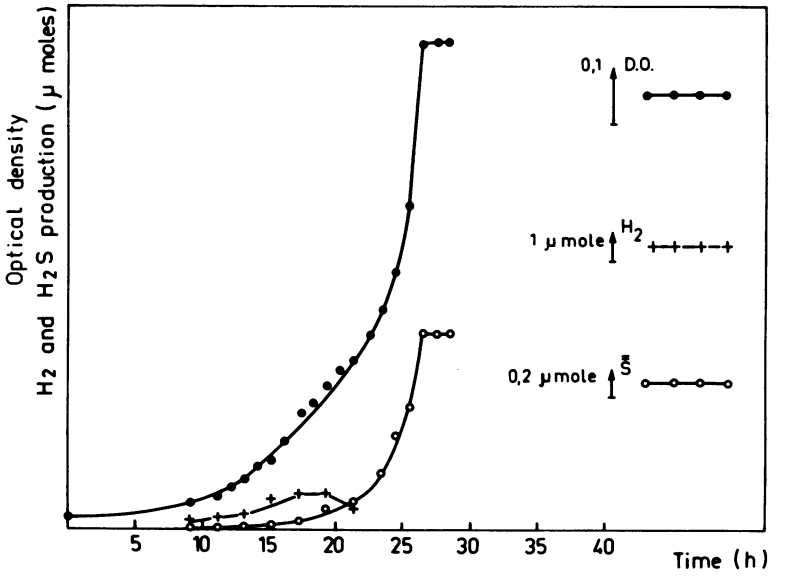

FIG. 5. *Kinetics of H_2 or H_2S formation during growth of D. vulgaris (Hildenborough) on 7.8 mM pyruvate in the presence of 36.3 mM sulfate.*

DISCUSSION

Table 6 shows all the enthalpy values which must be taken into consideration. Normally, the heat associated with the metabolism of the substrates (ΔH_{met}) and the heat corresponding to their catabolism (ΔH_{cat}) are two quite different sets of data which must be used carefully. Belaich (6) recently proposed that they are related by the equation:

$$\Delta H_{met} = (1 - \alpha)\Delta H_{cat} + Y_s \Delta h_{an} \quad (5)$$

In this relation, α represents the fraction of the energy source which is incorporated into the cellular material, Y_s is the molecular growth yield, and Δh_{an} is the enthalpy variation linked to the synthesis of 1 g (dry weight) of bacteria.

The quantity of the energy source incorporated as cellular carbon is always low in anaerobic growth on complex media. Belaich has shown (thesis, University d'Aix Marseille, France, 1967) that 50% of the cellular carbon of the anaerobic bacterium *Zymomonas mobilis*, when grown on complex medium with glucose as the carbon source, was provided by glucose. In the case of *D. desulfuricans* grown on complex medium supplemented with lactate, only 30% of the cellular carbon was derived from lactate (30). Therefore, taking the carbon content of the bacteria (Table 5) and 50% as the value of cellular carbon coming from the energy source, the share of cellular carbon (α) corresponding to lactate metabolism is:

$$\begin{aligned} \alpha_{lact} &= Y_{lact} \times 0.4892 \times \frac{1}{12} \times \frac{1}{3} \times \frac{50}{100} \\ &= 6.7 \times 0.4892 \times \frac{1}{12} \times \frac{1}{3} \times \frac{50}{100} \\ &= 0.045 \text{ mol} \end{aligned} \quad (6)$$

and to pyruvate metabolism is:

$$\begin{aligned} \alpha_{pyr} &= Y_{pyr} \times 0.4839 \times \frac{1}{12} \times \frac{1}{3} \times \frac{50}{100} \\ &= 10.1 \times 0.4839 \times \frac{1}{12} \times \frac{1}{3} \times \frac{50}{100} \\ &= 0.067 \text{ mol} \end{aligned} \quad (7)$$

In these relationships, 12 is the atomic weight of carbon, and 3 is the number of carbon atoms contained in the energy source (6).

As shown by Belaich (6), the Δh_{an} corresponding to the growth on complex medium is low and the order of magnitude of this enthalpy variation is 0.011 kcal/g (0.05 kJ/g). So, from equation 5, and values for ΔH_{cat}, α, and Y_s, the ΔH_{met} values of growth on lactate and pyruvate were −28.56 kJ and −70.16 kJ/mol, respectively; thus, the ΔH_{met} and ΔH_{cat} were due to low molecular growth yields and Δh_{an}. Comparison of the ΔH_{met} values obtained with the two stoichiometries (Table 6; A, C, and E; Table 7) shows clearly that the stoichiometry we propose for lactate metabolism explains its low experimental ΔH_{met} value; there is marked hydrogen production accompanying this metabolism, whereas a very low rate of hydrogen production occurs during pyruvate metabolism. Therefore, the experimental and theoretical ΔH_{met} values are comparable (Table 6A and C). Finally, the results obtained by classical chemical analysis techniques unambiguously show that *D. vulgaris* Hildenborough accumulates about 0.5 mol of H_2 per mol of lactate, even in the presence of high sulfate

TABLE 5. *Elemental analysis of molecular formula of D. vulgaris Hildenborough grown on lactate or pyruvate*

Energy source	Bacterial cell formula	Cell carbon content (%)
Lactate	$CH_{1.64}O_{0.33}N_{0.23}S_{0.01}P_{0.014}$	48.92
Pyruvate	$CH_{1.64}O_{0.31}N_{0.25}S_{0.07}P_{0.012}$	48.39

TABLE 6. *Comparison of experimental and theoretical metabolic enthalpies taking into account the anabolism enthalpies of bacteria grown on lactate or pyruvate*

Enthalpy variation (ΔH)	Substrate metabolized (kJ/mol)	
	Lactate	Pyruvate
A. ΔH_{met} experimental values	−36.36	−70.22
B. ΔH_{cat} by classical stoichiometries (equations 1 and 2 in text)	−70.18	−88.74
C. ΔH_{met} theoretical values (from B above and equation 5 in text)	−66.68	−80.29
D. ΔH_{cat} by our stoichiometries (equations 3 and 4 in text)	−30.26	−78.96
E. ΔH_{met} theoretical values (from B above and equation 5 in text)	−28.56	−70.16

TABLE 7. *Values of the standard enthalpies*[a]

Compound	$\Delta H°f$ (25°C, kcal/mol)	Reference
$CH_3CHOHCOO^-$	−164.11	(9)
CO_2	−98.9	(9)
CH_3COO^-	−115.98	(9)
CH_3CH_2OH	−68.60	(9)
H_2O	−68.31	(9)
$CH_3CO\ COO^-$	−142.5	(9)
H_2S	−9.56	(27)
HS^-	−6.81	(27)
SO_4^-	−213.55	(18)

[a] Heat of H_2PO_4 ionization at 30°C = +0.714 (2); $H_2S \rightarrow HS^- + H^+$ pK = 6.89 (11).

concentrations. It must be kept in mind that the growth was actually limited by lactate concentration (Fig. 1).

The significant amount of hydrogen production by the sulfate reducer *D. vulgaris* Hildenborough in lactate-sulfate media high in sulfate deserves further comment. Indeed, the lack of sulfate reduction due to hydrogen evolution corresponds to a loss of energy for bacterial growth, since it has been demonstrated that growth of these bacteria on lactate is quite dependent on sulfate respiration. The loss of energy related to hydrogen production can be estimated from the energy balance of redox reactions occurring during the reduction of sulfate and the intermediary compounds (sulfite, trithionate, and thiosulfate) as proposed by Siegel (26). As Fig. 6 indicates, the electrons released during the oxidation of lactate into pyruvate (E'_0 = −190 mV) could reduce adenosine phosphosulfate into sulfite and trithionate into thiosulfate, whereas the electrons coming from the last step of lactate metabolism (pyruvate → acetate, E'_0 = −690 mV) cold reduce sulfite into trithionate and thiosulfate into sulfide.

The amount of ATP available from the different redox reactions (Fig. 6) has been established assuming that for every two electrons transferred, a potential difference (ΔE) of about 300 mV is required to allow the synthesis of one molecule of ATP. It can be noted that the amount of ATP consumed for activation of sulfate into adenosine phosphosulfate is balanced by the ATP (20; Fig. 6) furnished by substrate level phosphorylation. Thus, the reduction of one molecule of sulfate by the eight electrons of two molecules of lactate could provide four ATP molecules per every two molecules or two ATP molecules per mol of lactate. Taking account of the fact that the production of H_2 from lactate constitutes an energy loss of 25%, the ATP gain would be only 1.5 ATP per mol of lactate.

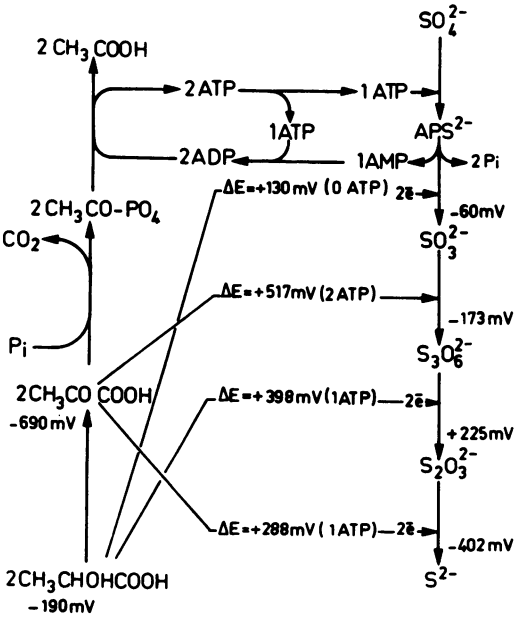

FIG. 6. *Electrons flow from lactate through sulfate reduction by the Siegel hypothesis (26).*

Such a scheme can be drawn when pyruvate serves as the energy source. Then the reduction of one molecule of sulfate requires the oxidation of four molecules of pyruvate. Since electrons from pyruvate are at a very low potential (E'_0 = −690 nmV), the amount of ATP synthesized is higher: 10 ATP molecules per 4 molecules of pyruvate (8 ATP molecules from oxidative phosphorylation plus 2 of 4 ATP molecules from substrate level phosphorylation). In this case, the loss of 10%, of total electrons into molecular hydrogen lowers the gain to 9 ATP molecules per 4 molecules or 2.25 ATP molecules per molecule of pyruvate.

Y_{lact} and Y_{pyr} values of 6.7 ATP and 10.1 g/mol, respectively, were in agreement with these ATP values, since the ratio of Y_{pyr}/Y_{lact} = 1.5 is quite identical to the ATP gain ratio:

$$\frac{\text{ATP gain on pyruvate medium}}{\text{ATP gain on lactate medium}} = \frac{2.25}{1.5} = 1.5$$

The greater lack of energy observed during growth on lactate as compared with that on pyruvate could be attributed to the greater amount of H_2 produced with the former substrate.

It is to be noted that Vosjan reported that hydrogen production can be attributed to iron deficiency in the medium (34). This reason does not apply in our growth conditions, since an excess of iron was added in a chelated form (Fe-citrate) which is readily usable by the cells (22); furthermore, hydrogen production occurred at the very early stage of growth. The inhibition of sulfur reductase activity of cytochrome c_3 of *D. vulgaris* Hildenborough by sulfide has been observed in our laboratory (12); in the same conditions, sulfide had no effect on the reductase activity of the homologous cytochromes from *D. desulfuricans* (strain Norway 4) or *D. gigas* (12). The present data show that *D. vulgaris* liberates 25% of the electrons from lactate into molecular hydrogen, thus lowering the sulfide concentration in the growth culture; low sulfide concentrations could prevent inhibition of the cytochrome by hydrogen sulfide poisoning. In con-

trast to the hypothesis of Vosjan (34), the production of hydrogen throughout growth in our experimental conditions could be considered as efficient regulation of electron flow by cytochrome c_3 during growth instea of cytochrome inefficacy in cells.

Recently, Tsuji and Yagi (31) have reported hydrogen production by *D. vulgaris* strain Miyazaki in lactate-sulfate media. A comparison of both their experimental conditions and results with ours reveals the following significant differences: (i) growth was limited by sulfate and not by lactate concentration (16.1 mM sulfate for 55.6 mM of lactic acid); (ii) hydrogen was only produced at the early stage of growth; and (iii) only trace amounts of hydrogen (0.014 mol of H_2 per mol of lactate) were produced as compared with the high quantities of hydrogen evolved in our experimental growth conditions. Nevertheless, it is clear that a comparison of the two strains would require the use of identical growth conditions.

The data on physiology, biochemistry, and metabolism of *D. vulgaris* strain Hildenborough indicate that this microorganism is especially well adapted among the sulfate reducers to perform interspecies hydrogen transfer. Indeed, one has to emphasize that in this strain, the hydrogenase is located in the periplasmic space (8, 32) and exhibits high specific activity (32, 33). In addition, as reported here, high amounts of hydrogen appear to be produced in lactate media high in sulfate. All of these features facilitate hydrogen transfer with H_2-utilizing microorganisms, such as methanogenic bacteria.

It could well be that, in natural conditions, the loss of energy induced by hydrogen production which is seen in pure culture, is counterbalanced by a facilitated utilization of high polymers, such as cellulose, by the complex microbial flora of which sulfate-reducing bacteria are very active members. This facilitation would be due to (i) accumulation of less sulfide, (ii) utilization of both H_2 and CO_2 from sulfate-reducing bacteria by methanogenic bacteria (hydrogen accumulation is inhibitory because of thermodynamic reasons [9]), and (iii) lowering of the acetate concentration and its possible inhibitory effect on several members of the complex flora.

The questions which remain to be answered deal with the enzymatic reactions implicated in this hydrogen production. It should be pointed out that in our growth conditions, the phosphoroclastic reaction might be the main method of hydrogen production. However, hydrogen concentration is greater on lactate than on pyruvate, which suggests that an additional reaction such as NADPH ferredoxine oxidoreductase activity could be implicated in hydrogen evolution from lactate as source of energy. A very interesting problem is that no lactate dehydrogenase has yet been purified from sulfate-reducing bacteria, and thus, the nature of its electron acceptor remains to be determined.

ACKNOWLEDGMENTS

This work was graciously supported by grants from PIRDES and COMES.

LITERATURE CITED

1. **Badziong, W., and R. K. Thauer.** 1978. Growth yields and growth rates of *Desulfovibrio vulgaris* (Marburg) growing on hydrogen plus sulfate and hydrogen plus thiosulfate as the sole energy sources. Arch. Microbiol. **117**:209–214.
2. **Bauchop, J. K., and S. R. Elsden.** 1960. The growth of microorganisms in relation to their energy supply. J. Gen. Microbiol. **23**:457.
3. **Belaich, A., and J. P. Belaich.** 1976. Microcalorimetric study of the anaerobic growth of *Escherichia coli*: growth thermograms in a synthetic medium. J. Bacteriol. **125**:14–18.
4. **Belaich, A., and J. P. Belaich.** 1976. Microcalorimetric study of the anaerobic growth of *Escherichia coli*: measurement of the affinity of whole cells for various energy substrates. J. Bacteriol. **125**:19–24.
5. **Belaich, J. P.** 1963. Thermogénèse et croissance de *Pseudomonas lindneri* en glucose limitant. C.R. Soc. Biol. **157**:316.
6. **Belaich, J. P.** 1979. Biological calorimetry, p. 1–42. *In* A. E. Beezer (ed.). Academic Press, Inc., London.
7. **Belaich, J. P., J. C. Senez, and M. Murgier.** 1968. Microcalorimetric study of glucose permeation in microbial cells. J. Bacteriol. **95**:1750–1757.
8. **Bell, G. R., J. LeGall, and H. D. Peck, Jr.** 1974. Evidence for the periplasmic location of hydrogenase in *Desulfovibrio gigas*. J. Bacteriol. **120**:994–997.
9. **Brown, H. D. (ed.).** 1969. Biochemical microcalorimetry, p. 305–316. Academic Press, Inc., New York.
10. **Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill.** 1977. Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. **33**:1162–1169.
11. **Dean, J. A. (ed).** 1973. Lange's handbook of biochemistry, 11th ed., p. 5–9. McGraw-Hill Book Co., New York.
12. **Fauque, G., D. Hervé, and J. Le Gall.** 1979. Structure-function relationship in hemoproteins: the role of cytochrome c in the reduction of colloidal sulfur by sulfate-reducing bacteria. Arch. Microbiol. **121**:261–264.
13. **Fogo, J. K., and M. Popowsky.** 1949. Spectrophotometric determination of hydrogen sulfide. Anal. Chem. **21**: 732–734.
14. **Hatchikian, E. C., M. Chaigneau, and J. Le Gall.** 1976. Analysis of gas production by growing cultures of three species of sulfate-reducing bacteria, p. 109–118. *In* H. G. Schlege, G. Gottschalk, and N. Pfennig (ed.), Microbial production and utilization of phases. Göttingen. Goltze.
15. **Khorosvi, B., and J. D. A. Miller.** 1975. A comparison of the growth of *Desulfovibrio vulgaris* under a hydrogen and an inert atmosphere. Plant Soil **43**:171.
16. **Magee, E. L. Jr., B. D. Ensley and L. L. Barton.** 1978. An assessment of growth yields and energy coupling in *Desulfovibrio*. Arch. Microbiol. **117**:21.
17. **Mechalas, B. J., and S. C. Rittenberg.** 1960. Energy coupling in *Desulfovibrio desulfuricans*. J. Bacteriol. **80**:501–507.

18. **National Bureau of Standards.** 1968. Chemical thermodynamics properties, table 14. U.S. National Bureau of Standards technical note 270-3. U.S. National Bureau of Standards, Washington, DC.
19. **Peck, H. D., Jr.** 1962. Symposium on metabolism of inorganic compounds. V. Comparative metabolism of inorganic sulfur compounds in microorganisms. Bacteriol. Rev. **26**:67-94.
20. **Peck, H. D., Jr.** 1966. Phosphorylation coupled with electron transport in extracts of sulfate-reducing bacterium *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. **22**:112-118.
21. **Postgate, J. R.** 1952. Growth of sulphate-reducing bacteria in sulphate free media. Research **5**:189-190.
22. **Postgate, J. R.** 1956. Iron and the synthesis of cytochrome c_3. J. Gen. Microbiol. **15**:186-193.
23. **Postgate, J. R., and L. L. Campbell.** 1966. Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. Bacteriol. Rev. **30**:732-738.
24. **Prat, H., and E. Calvet.** 1963. Recent progress in microcalorimetry. Pergamon Press, Inc., London.
25. **Senez, J. C.** 1962. Some considerations on the energetics of bacterial growth. Bacteriol Rev. **26**:95-107.
26. **Siegel, L. M.** 1975. Biochemistry of sulfur cycle, p. 217-286. *In* D. M. Greenberg, (ed.), Metabolic pathways, vol. 7. Academic Press, Inc., New York.
27. **Sober, H. A.** 1968. Handbook of biochemistry. CRC Press, Boca Raton, Fla.
28. **Sorokin Yu, I.** 1966. Sources of energy and carbon for biosynthesis in sulfate-reducing bacteria. Microbiology (USSR) **35**:643-647. (English translation.)
29. **Sorokin Yu, I.** 1966. Investigations of the structural metabolism of sulfate-reducing bacteria with ^{14}C. Microbiology (USSR) **35**:806-814. (English translation.)
30. **Sorokin Yu, I.** 1966. Role of carbon dioxide and acetate in the biosynthesis by sulfate-reducing bacteria. Nature (London) **210**:551-552.
31. **Tsuji, K., and T. Yagi.** 1980. Significance of hydrogen burst from growing cultures of *Desulfovibrio vulgaris* Miyazaki and the role of hydrogenase and cytochrome c_3 in energy production system. Arch. Microbiol. **125**: 35-42.
32. **Van der Western, H. M., S. G. Mayhew, and C. Veeger.** 1978. Separation of hydrogenase from intact cell of *Desulfovibrio vulgaris*. FEBS Lett. **86**:122-126.
33. **Van der Western, H. M., S. G. Mayhew, and C. Veeger.** 1980. Effect of growth conditions on the content and O_2^- stability of hydrogenase in the anaerobic bacterium *Desulfovibrio vulgaris* (Hildenborough). FEMS Microbiol. Lett. **7**:35-39.
34. **Vosjan, J. H.** 1975. Respiration and fermentation of the sulphate-reducing bacterium *Desulfovibrio desulfuricans* in continuous culture. Plant Soil. **43**:141.