Journal of Microbiology and Biotechnology
Vol. 6. No. 4, 232-237, 1996

The Growth Yield of *Desulfovibrio desulfuricans* M6 on Different Substrates

PARK, DOO-HYUN[1], CHUL-SU SHIN[2], BYUNG-HONG KIM*, AND PYUNG-KYUN SHIN

Environmental Research Center, Korea Institute of Science and Technology
39-1 Hawolgok-Dong, Sungpook-Gu, Seoul 130-791, Korea
[1]Department of Biological Engineering, Seo Kyung University, 16-1 Jungneung-Dong, Sungbuk-Gu, Seoul, Korea, [2]Han Hyo Institute of Science

Growth yield of *Desulfovibrio desulfuricans* M6 was measured using different substrates. The cell yield of fermentative growth on pyruvate was 6.22 g cell mol^{-1} pyruvate. Since 1 ATP is available from substrate-level phosphorylation from the oxidation of pyruvate to acetate, Y_{ATP} of the bacterium should be the same as $Y_{pyruvate}$ (6.22 g cell mol^{-1} ATP). The cell yields of the bacterium on different electron donors were measured with sulfate as the electron acceptor. Cell yields on lactate, pyruvate and H_2 were 9.39, 13.76 and 8.45 g cell mol^{-1} substrate, respectively. From these figures ATP available from electron-transport phosphorylation (ETP) of the electron donors used was calculated. ATP produced by ETP of each electron donnor were 1.71 from pyruvate, 1.51 from lactate and 1.76 from H_2. These values show that electrons from the oxidation of lactate to pyruvate are consumed to reduce sulfate through a reverse electron transport mechanism requiring 0.2 ATP for each pair of electrons. Based on these results, discussions are made on the electron transport mechanism in the bacterium.

Sulfate-reducing bacteria are obligate anaerobes growing via anaerobic respiration using sulfate as their electron acceptor. They can be divided into incomplete oxidizers and complete oxidizers (6). Incomplete oxidizers oxidize electron donors such as lactate and pyruvate to acetate and use the electrons available from the oxidation to reduce sulfate to sulfide, while the complete oxidizers oxidize electron donors to CO_2 (7). Most species of *Desulfovibrio* are incomplete oxidizers (6).

The main energy metabolism in *Desulfovibrio* is the reduction of sulfate coupled to the oxidation of the electron donors. The reduction of sulfate is a multi-step reaction consuming 8 electrons (14). Sulfate is reduced to sulfite after being activated to adenosine-5'-phosphosulfate (APS), which requires two high energy phosphate bonds due to the thermodynamic reason (14). The reduction potential of the [HSO_4^-/HSO_3^-] half reaction is -0.516 volt (14).

Two molecules of lactate are oxidized to supply 8 electrons needed to reduce a molecule of sulfate to sulfide. Because 2 ATPs, produced by substrate-level phosphorylation (SLP) during the oxidation of 2 lactate to 2 acetate, are consumed to activate sulfate, the growth of the bacteria is dependent on the ATP available from electron-transport phosphorylation (ETP) (3). Obligate H_2 cycling model (12) and trace H_2 cycling model (10) have been put forward to explain ETP in *Desulfovibrio*. According to the obligate H_2 cycling model, all the electrons available from the oxidative processes are used to the reduce protons by cytoplasmic hydrogenase to produce H_2 which is then diffused to periplasmic region. Periplasmic hydrogenase, in turn, oxidizes H_2 to transfer electrons to cytochrome c_3. Electrons from the periplasmic cytochrome are transported back into the cytoplasm to reduce sulfate leaving protons behind in the periplasm. H_2 cycling results in the translocation of 8 protons from cytoplasm to periplasm. The trace H_2 cycling model is based on the fact that the oxidation of lactate cannot be coupled to the reduction of protons due to the high reduction potential of the pyruvate/lactate half reaction (-0.19 volts). Electrons from the oxidation of lactate, according to the model, are used directly in the reduction processes, while the oxidation of pyruvate is coupled to the reduction of protons as a part of the H_2 cycle.

Since it is thermodynamically unfavourable for the oxidation of lactate to be coupled to the reduction of protons, lactate cannot be used as substrate for the fer-

*Corresponding author
Key words: *Desulfovibrio desulfuricans* M6, bioenergetics, molar growth yield, Y_{ATP}, anaerobic respiration, sulfate-reduction

mentative growth of *Desulfovibrio* (4), but *Desulfovibrio* can grow fermentatively on pyruvate which is oxidized through phosphoroclastic reaction catalyzed by pyruvate: ferredoxin oxidoreductase (16). Hydrogen and sulfate support the growth of *Desulfovibrio* when acetate and carbon dioxide are present as the carbon sources (1, 2).

Hydrogenase activities are essential for the fermentative as well as respiratory growth of *Desulfovibrio*. Producing activity of hydrogenase is needed for the fermentative growth on pyruvate, and growth on H_2, acetate and CO_2 requires uptake activity of hydrogenase. Both hydrogenase activities play their parts for the respiratory growth on sulfate with lactate or pyruvate. *Desulfovibrio* is known to possess at least two different hydrogenases in different parts of the cell (9).

Studies were conducted to evaluate the property of energy metabolisms of *D. desulfuricans* M6 by measuring the growth yields of different modes of growth.

MATERIALS AND METHODS

Bacterial Strain and Its Cultivation

Desulfovibrio desulfuricans M6 was used throughout the study. The strain has been isolated and selected for its ability to reduce dibezothiophene in this laboratory (8). The basal medium used was Postgate medium C without ferrous ion (8). Combinations of electron donors and sulfate as the electron acceptor were employed as follows; lactate-sulfate (LS), pyruvate-sulfate (PS), pyruvate (P) and H_2-sulfate (HS). HS medium was added by acetate (30 mM) and $NaHCO_3$ (116 mM), and the headspace was pressurized to 15 psig with H_2/CO_2 mixture (4:1). The cultivation of bacterial cell was carried out using serum vials (Wheaton Scientific, NJ, USA). The medium was warmed to 30°C before inoculation with 5% inoculum and incubated at the same temperature.

Analyses

Acetate was quantified by a GC (Varian 3400, USA) equipped with a FI detector (5). The samples were separated in a glass column (3 mm × 2 m) packed with chromosorb WAW (Altech, IL, USA) using N_2 as a carrier gas at the flow rate of 30 ml per min. Injector, detector and oven temperatures were 220°C, 240°C and 180°C, respectively. Lactate and pyruvate were analyzed by HPLC (Youngin Scientific, Seoul) equipped with an Aminex HPX-87H ion exchange column (Bio-Rad) (5). A solution of 10% acetonitrile in 0.05 N H_2SO_4 was used as the mobile phase at the flow rate of 0.6 ml per min. A GC (Varian 3400) equipped with a TC detector was used to analyze H_2. A glass column (3 mm × 3 m) packed with Porapak-Q was used with helium as the carrier gas at the flow rate of 20 ml per min. Injector, detector and oven temperatures were 50°C, 110°C and 90°C, respectively. Dry cell weight was determined using a predetermined calibration curve in the function of the absorbance at 660 nm.

$$\text{Dry cell weight (g/l)} = 0.467 \times A_{660}$$

Growth Yield (Y_{sub}) Determination (11)

Y_{sub} was determined from Lineweaver-Burke plots based on equation (1).

$$\frac{r_s}{X} = \frac{1}{Y_{x/s}} * \frac{r_x}{X} + m_s \qquad (1)$$

where

r_s : substrate consumption rate (mol substrate h^{-1})
r_x : cell growth rate (g cell l^{-1} h^{-1})
$Y_{x/s}$: yield coefficient for biomass on substrate (g cell mol substrate^{-1})
m_s : maintenance constant (mol substrate g cell^{-1} hr^{-1})
X : cell mass (g cell)

RESULTS AND DISCUSSION

Growth Yields from Different Metabolisms

D. desulfuricans M6 was cultivated fermentatively and respiratorily using pyruvate, lactate or H_2 as an electron donor with or without sulfate, and the changes in absorbance and the concentrations of electron donor and product were monitored (Fig. 2 through Fig. 5). The growth of the bacterium was linear not exponential as observed in other sulfate reducers due to the inhibitory effect of hydrogen sulfide (15). Pyruvate was consumed

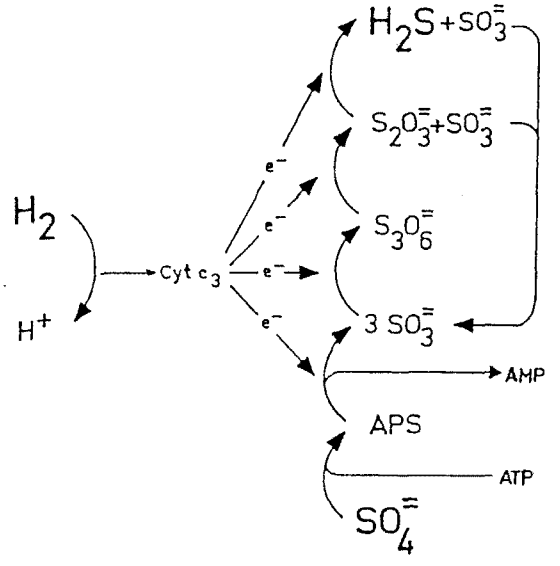


Fig. 1. Scheme of the electron transport system involved in sulfate reduction to sulfide in sulfate-reducing bacteria (17).
Abbreviations: cyt c_3, cytochrome c_3; APS, adenosine-5'-phosphosulfate; — e^- →, electron transport chain.

completely in the respiratory metabolism with sulfate as the electron acceptor (Fig. 4A), while consumption by fermentation was slow (Fig. 3A). The fermentative growth on pyruvate was also linear in the absence of hydrogen sulfide. These results show that H_2 accumulated during the fermentation inhibits the metabolism of pyruvate.

The growth curves (as shown in part A of the Fig. 2-5.) were used to determine the growth and substrate consumption rates, which were then plotted to obtain growth yields as shown in part B of the Fig. 2 through 5. Table 1 summarizes growth yield calculations. The higher molar growth yield (Y_{sub}) of 13.76 g cell mol^{-1} pyruvate was observed in medium PS, and the lowest yield of 6.22 g cell mol^{-1} pyruvate in medium P. Media LS and HS gave Y_{sub} of 9.39 and 8.46 g cell mol^{-1}, respectively. Assuming a constant Y_{ATP}, the differences in Y_{sub} show that the amount of ATP synthesized by the bacterium in each medium is different (18). Because the growth rates were similar in all growth experiments (as shown in part A of Fig. 2 through 5), it is assumed that the energy consumed for the maintenance in terms of mol ATP g^{-1} cell h^{-1} is similar throughout the experiments. Based on this assumption the Y_{ATP} of 6.22 g cell mol^{-1} ATP was used to calculate the amount of ATP synthesized from the respiration.

ATP Synthesized through ETP in Medium PS

Because only one ATP is produced by SLP during the fermentative metabolism of pyruvate, the molar growth yield in medium P can be used as Y_{ATP}. (Eq. ①). This figure is lower than the usual Y_{ATP} of 10.5 g cell mol^{-1} ATP. This is due to the high ATP demand to synthesize cell materials from pyruvate (13).

$$\text{pyruvate} + \text{ADP} \longrightarrow \text{acetate} + \text{ATP} + H_2 \qquad ①$$

For the reduction of each sulfate molecule 4 molecules of pyruvate are oxidized to acetate with a gain of 4 ATP through SLP. The activation of sulfate consumes 2 ATP. From the oxidation of pyruvate and the reduction of sulfate, a net gain of 0.5 ATP/pyruvate is achieved in addition to the ATP obtained through ETP (Eq. ②).

$$4\,\text{pyruvate} + 4\,\text{ADP} \longrightarrow 4\,\text{acetate} + 4\,\text{ATP} + 8\,\text{H}$$
$$HSO_4^- + 8\text{H} + 2\,\text{ATP} \longrightarrow HS^- + 2\text{ADP}$$
$$4\,\text{pyruvate} + HSO_4^- + 2\text{ADP} \rightarrow 4\,\text{acetate} + HS^- + 2\text{ATP} \qquad ②$$

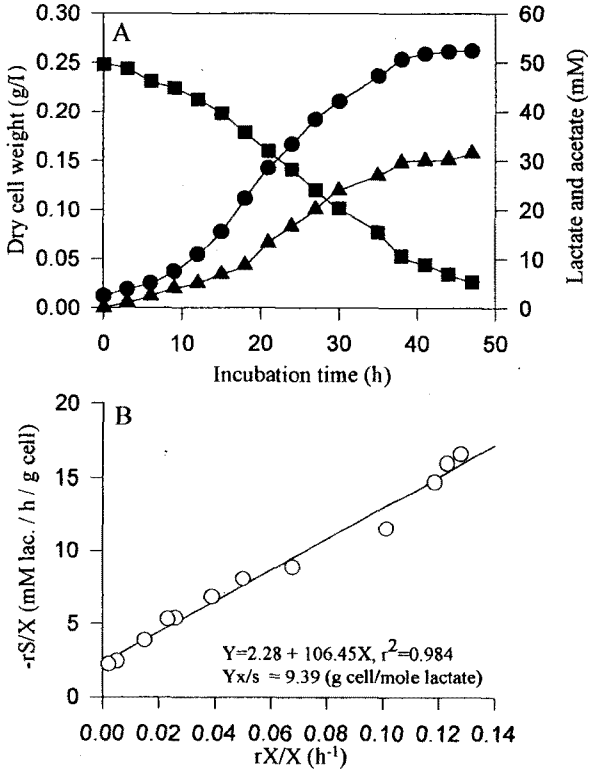


Fig. 2. Growth, substrate consumption and metabolite production of *D. desulfuricans* M6 on lactate-sulfate (A), plotting for Yx/s of *D. desulfuricans* M6 (B).
—●—, Dry cell weight; —■—, Lactate; —▲—, Acetate.

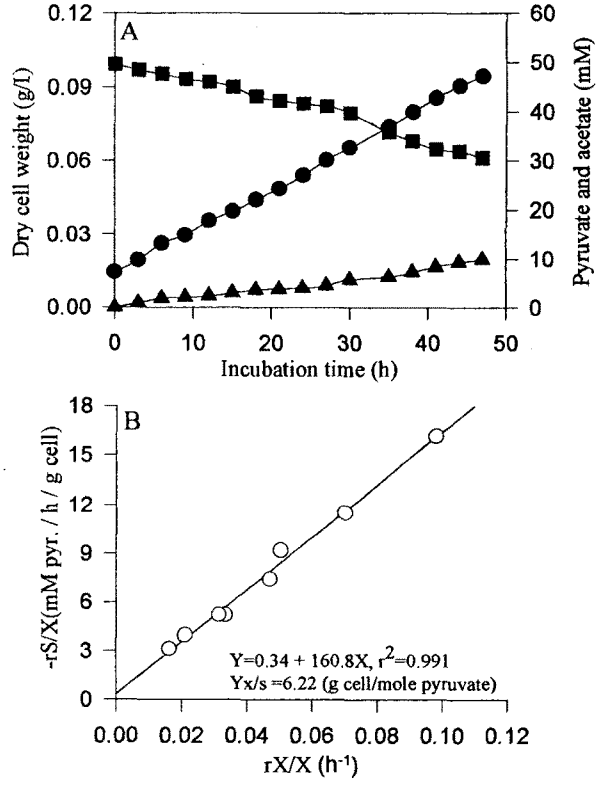


Fig. 3. Growth, substrate consumption and metabolite production of *D. desulfuricans* M6 on pyruvate (A), plotting for Yx/s of *D. desulfuricans* M6 (B).
—●—, Dry cell weight; —■—, Pyruvate; —▲—, Acetate.

Y_{sub}, 13.76 g cell mol^{-1} pyruvate, in PS is the sum of cells synthesized using ATP by SLP and ATP by ETP. Cell mass synthesized by energy available from ETP can be calculated by substracting ($Y_{ATP} \times$ ATP from SLP) from Y_{sub} 13.76. Cell mass synthesized by the energy available from SLP is 3.11 g/pyruvate$_{(SLP)}$ (6.22 g cell mol^{-1} ATP $\times$ 0.5 ATP). The amount of ATP synthesized by the energy available from ETP can be calculated by dividing cell mass synthesized by energy from ETP (13.76 − 3.11) by Y_{ATP} as 1.71 ATP (Eq. ③).

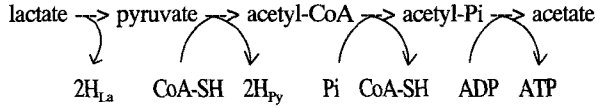
$$Y_{ATP} = 6.22 \text{ g cell mol}^{-1} \text{ ATP},$$
$$Y_{PYRUVATE(ETP)} = 13.76 - 3.11$$
$$= 10.65 \text{ g cell mol}^{-1} \text{ pyruvate}$$
$$ATP_{ETP} = 10.65/6.22 = 1.71 \text{ ATP/pyruvate} \quad ③$$

ATP Synthesized through ETP in Medium LS

The reduction of lactate to acetate yields 1 ATP/lactate, and the oxidation 2 lactate is coupled to the reduction of sulfate to sulfide. Because 2 ATP synthesized through SLP during the oxidation of lactate are consumed to activate sulfate, cell mass obtained in medium LS is solely from ATP of ETP (Eq. ④).

$$2 \text{ lactate} + 2 \text{ ADP} \longrightarrow 2 \text{ acetate} + 2 \text{ ATP} + 8 \text{ H}$$
$$HSO_4^- + 8 \text{ H} + 2 \text{ ATP} \longrightarrow HS^- + 2 \text{ ADP}$$
$$2 \text{ lactate} + HSO_4^- \longrightarrow 2 \text{ acetate} + HS^- \quad ④$$

The amount of ATP synthesized through ETP can be calculated by dividing the cell mass, 9.39 g cell mol^{-1} lactate, by Y_{ATP}, 6.22 g cell mol^{-1} ATP, as 1.51 ATP/lactate (ETP). Lactate is oxidized by the following reactions (15):



Two pairs of electrons are available from the oxidation. They are termed as $2H_{La}$ and $2H_{Py}$ in the reaction. To obtain 1.51 ATP/lactate (ETP) both pairs of the electrons are consumed to reduce sulfate. Assuming that the consumption of $2H_{Py}$ generates the same amount of ATP as the oxidation of pyruvate in PS, ATP synthesized from the consumption of $2H_{La}$ can be calculated as equation ⑤:

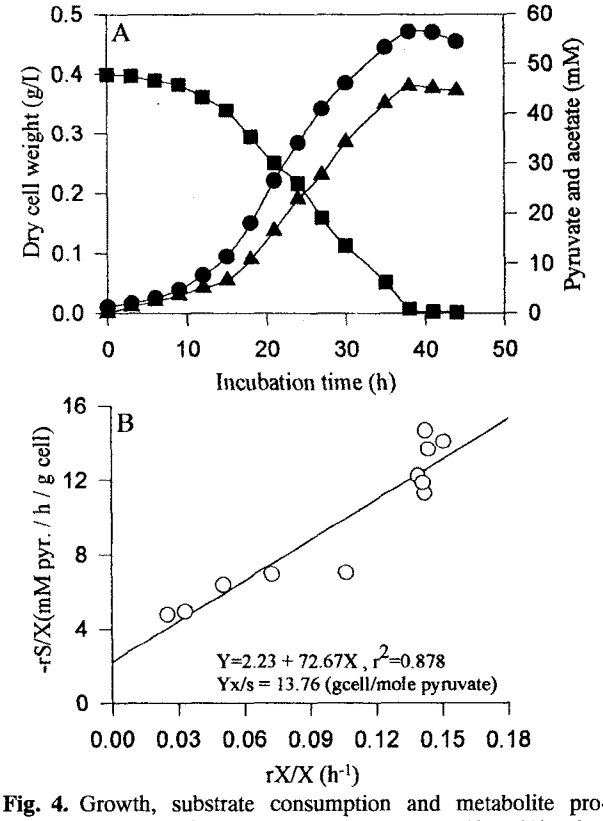


Fig. 4. Growth, substrate consumption and metabolite production of *D. desulfuricans* M6 on pyruvate-sulfate (A), plotting for Yx/s of *D. desulfuricans M6 (B).*
—●—, Dry cell weight; —■—, Pyruvate; —▲—, Acetate.

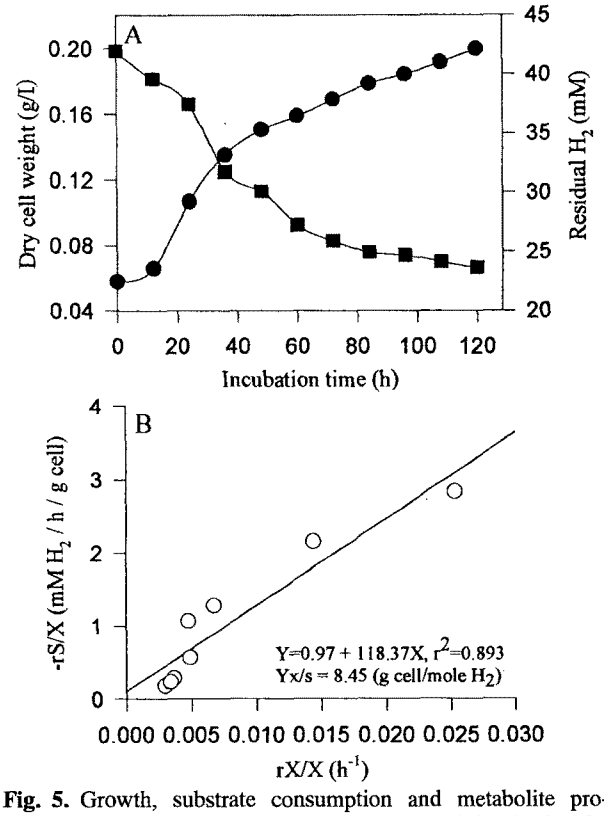


Fig. 5. Growth, substrate consumption and metabolite production of *D. desulfuricans M6* on H_2-sulfate (A), plotting for Yx/s of *D. desulfuricans M6* (B).
—●—, Dry cell weight; —■—, H_2.

Table 1. The yield coefficient of *D. desulfuricans* M6 grown on different electron donors using sulfate as an electron acceptor.

e^- donor	e^- acceptor	Y_{sub}	Y_{sub}/SLP^b	Y_{sub}/ETP^c	ATP for growth
Pyruvate	–[a]	6.22	6.22	0.0	1.0
Pyruvate	Sulfate	13.76	3.11	10.65	2.21
Lactate	Sulfate	9.39	0.0	9.39	1.51
H_2	Sulfate	8.45	0.0	8.45	1.26

Y_{sub}: growth yield (g cell mol^{-1} substrate). [a]Sulfate, which is an electron accepter, was not added in the culture. [b]Growth yield by substrate level phosphorylation (g cell mol^{-1} substrate), which is calculated from the metabolic pathway. The growth yield of fermentative growth on pyruvate, 6.22 was used as Y_{ATP}. [c]Growth yield by electron transport phosphorylation (g cell mol^{-1} substrate), which is calculated by substracting growth yield of SLP from Y_{sub}.

$$ATP(H_{La-ETP}) = ATP_{lactate-ETP} - ATP_{pyruvate-ETP}$$
$$= 1.51 - 1.71 = -0.2\ ATP \quad (5)$$

This value indicates that electrons from the oxidation of lactate to pyruvate are transferred to sulfate reduction through a reverse electron transport mechanism consuming 0.2 ATP per pair of electrons. Based on this observation it is concluded that the obligate H_2 cycling model is not appropriate to explain ETP in *Desulfovibrio*.

In biological energy transduction process there is a minimum free energy change which can be conserved as ATP. In the SLP process the minimum free energy change should be higher than the phosphorylation potential, and the potential energy of one proton in the proton gradient is the minimum energy to be conserved in the ETP process (17). The value of 0.2 ATP required for the reverse electron transport indicates that five protons are translocated to synthesize 1 ATP by the membrane-bound ATPase in *Desulfovibrio desulfuricans* M6.

ATP Synthesized through ETP in Medium HS

In medium HS, ATP is synthesized only through ETP and 2 ATP is consumed to activate sulfate. Since 4 molecules of H_2 are consumed to reduce a molecule of sulfate the number of ATP synthesized through ETP can be calculated as equation (6).

$$H_2 + xADP \longrightarrow 2H + xATP$$
$$0.25\,HSO_4^- + 2\,H + 0.5\,ATP \longrightarrow 0.25\,HS^- + 0.5\,ADP$$
$$0.25\,HSO_4^- + H_2(x-0.5)\,ADP \rightarrow 0.25\,HS^- + (x-0.5)\,ATP \quad (6)$$

The molar growth yield of *D. desulfuricans* M6 in medium HS was 8.45 g cell mol^{-1} H_2 (Table 1). The number of ATP used for the synthesis of cell mass can be calculated by dividing the Y_{sub} by Y_{ATP}, 6.22 as 1.26 ATP. The number of ATP synthesized through ETP in medium HS can be calculated as 1.76 by equation (7).

$$(X - 0.5) = 1.26, \quad \therefore X = 1.76 \quad (7)$$

The number of ATP synthesized through ETP was calculated as 1.71 in medium PS and 1.76 in medium HS. The electron donnors used in the two media have low reduction potentials; -0.42 volts of aceyl-CoA/pyruvate and -0.41 volts of H^+/H^2. In the previous section, evidence was presented showing 5 protons are needed to be translocated across the membrane for each molecule of ATP. According to the H_2 cycling model only 2 protons are translocated for every molecule of H_2 consumed. Results obtained in this study indicate that *D. desulfuricans* M6 has an electron transport chain which generates much higher proton gradient than predicted by the H_2 cycling model because the Y_{sub} on H_2 is much higher than expected.

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(Received February 5, 1996)