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Energetics of Growth of a Defined Mixed Culture of *Desulfovibrio vulgaris* and *Methanosarcina barkeri*: Maintenance Energy Coefficient of the Sulfate-Reducing Organism in the Absence and Presence of Its Partner

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The maintenance energy coefficient of *Desulfovibrio vulgaris* was studied by using a chemostat, with *Methanosarcina barkeri* or sulfate as the electron acceptor; lithium lactate or sodium pyruvate served as the electron donor. The experiments showed that the growth energetics of *D. vulgaris* or *M. barkeri* were greatly affected by maintenance energy coefficients. When *D. vulgaris* grew on lactate or pyruvate medium with sulfate, these coefficients reached 4.40 and 2.80 mM g^{-1} h^{-1}, respectively; on lactate medium in the presence of *M. barkeri* the same coefficient reached a value of 2.90 mM g^{-1} h^{-1}. Results also showed that the increase of the value of the maintenance energy coefficient corresponded to a decrease of the biomass produced. *D. vulgaris* maximal growth yield values calculated by use of the Pirt equation were slightly higher with *M. barkeri* (maximal growth yield, 10 g/mol) than with sulfate (maximal growth yield, 7.5 g/mol). This finding could be interpreted by reference to the ATP-generating reactions involved in *D. vulgaris* growth in the presence of sulfate or *M. barkeri*.

Maintenance energy is that part of substrate metabolized which is not used for biosynthesis purposes. According to many workers (5, 11, 16, 17), this energy can be used by cells, for example, for their motility requirements or for the regulation of their internal osmotic pressure. In continuous culture limited by the energy source, the growth yield of some microorganisms decreases significantly when very low growth rates are set (11, 16). Variation of the yields in relation to growth rates has been described by Pirt (11) by the following equation:

$$\frac{1}{Y_{app}} = \frac{1}{Y_{max}} + \frac{m_s}{\mu_{app}} \quad (1)$$

In this relation, Y_{app} is the observed growth yield, Y_{max} is the maximum theoretical yield, μ_{app} is the observed growth rate, and m_s is the maintenance energy coefficient, suppposed to be independent of the growth rate. Other workers have reported that this coefficient could in fact be composed of two terms, the first one depending on growth rate and the second one not depending on growth rate (4, 15).

The characterization of the maintenance energy coefficient has often been done by using organisms considered more recent than sulfate-reducing or methane-producing bacteria (4, 9, 16). The present communication is a study of the maintenance energy coefficients of *Desulfovibrio vulgaris* and *Methanosarcina barkeri* in pure cultures or cocultures.

MATERIALS AND METHODS

Microorganisms. *D. vulgaris* Hildenborough (NCIB 8303) and *M. barkeri* DSM 800 were used for this study.

Media and growth conditions. *D. vulgaris* was routinely transferred every 2 days on media previously described (18). The medium for growth of *M. barkeri* was composed of two parts. The composition of part A was (per liter): K_2HPO_4, 0.35 g; KH_2PO_4, 0.25 g; NH_4Cl, 0.5 g; NaCl, 0.5 g; Casitone, 2.0 g; yeast extract (Difco Laboratories), 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; trace elements mixture, 1 ml; H_2O, 999 ml. One liter of trace elements mixture included: MgO, 10.6 g; $CaCO_3$, 2.0 g; $FeSO_4 \cdot 7H_2O$, 6.20 g; $ZnSO_4 \cdot 7H_2O$, 1.44 g; $MnSO_4 \cdot 4H_2O$, 1.12 g; $CuSO_4 \cdot 5H_20$, 0.25 g; $CoSO_4 \cdot 7H_2O$, 0.9 g; BO_3H_3, 0.06 g; $(Mo)_7(NH_4)_6O_{24} \cdot 4H_2O$, 1.0 g; $Ni(NO_3)_2 \cdot 6H_2O$, 0.06 g; $NaSeO_3$, 0.02 g; 36 N HCl, 51.4 ml.

The composition of part B was (per 100 ml):

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$Na_2S \cdot 9H_2O$, 2.5 g; cysteine-hydrochloride, 1.25 g; $NaHCO_3$, 4.0 g; H_2O, 100 ml. This solution was kept under anaerobic conditions by using nitrogen gas.

Growth medium C was obtained by mixing in Hungate-type culture tubes parts A and B in a ratio of 98 and 2% (vol/vol), respectively. The initial pH was adjusted to 7. Anaerobiosis was produced in culture vessels by evacuation and replacement with nitrogen gas. Methanol or H_2-CO_2 was the growth substrate for *M. barkeri*. Media were sterilized by autoclaving at 110°C for 30 min.

For control experiments continuous growth of *D. vulgaris* with sulfate was performed in medium C with some variations: NaCl was replaced by Na_2SO_4, 4 g/liter, and $MgSO_4 \cdot 7H_2O$ was increased to 2 g/liter.

For mixed culture experiments medium C was used which contained $MgCl_2 \cdot 6H_2O$ in lieu of $MgSO_4 \cdot 7H_2O$. Lithium lactate or sodium pyruvate (Merck & Co., Inc.) was used as the energy source. Well-adapted mixed inocula of both organisms were constituted after three to four successive subcultures in the same medium; 10 to 20% inoculum (vol/vol) was added to the growth medium. All the experiments were performed at 30°C. Continuous cultures were conducted in a Pyrex glass fermentor (LKB 1601-012; LKB Instruments Inc.). It consisted of a 4-liter culture chamber connected to a 20-liter new media carboy and to a 20-liter effluent reservoir. Rubber connections and pipes for liquid pumping were of thick-walled silicone. The fermentor was filled or emptied with a reversible peristaltic pump.

Analytical methods. For biomass assessment, mixed bacteria cultures were identified in a counting cell chamber equipped with a 40-μl cell. Then they were separated by differential continuous centrifugation in a model T1 Sharples apparatus at 40,000 × *g*. Methane-producing and sulfate-reducing bacteria were collected in the lower and upper part of the rotor, respectively. Molar growth yields were determined from the weight of washed and dried cells. The biomass of *D. vulgaris* grown under pure culture conditions was assessed from a calibration curve.

Lactate was determined enzymatically by the Boehringer technique (Food Analysis; Boehringer Mannheim Corp.). Pyruvate was determined by the chemical method of Friedemann (6).

RESULTS

Maintenance energy coefficients assessment. *D. vulgaris* was studied under lactate-limited chemostat growth conditions; sulfate or *M. barkeri* was the electron sink. Growth yields were measured after at least three cycles of stationary state and are correlated to dilution rate in Table 1. A marked and gradual decrease of the yields was noticeable at low dilution rates for both organisms. These results thus reflected the influence of a significant maintenance energy coefficient. Pyruvate was also used as an energy source. Figure 1 presents the yields plotted as a reciprocal function against dilution rates. Maintenance energy coefficients were determined from slopes of three linear curves obtained. As indicated in Table 2, a high maintenance coefficient characterized the growth of *D. vulgaris*. Although this parameter was not defined in *M. barkeri* numerically, owing to simultaneous uptake of H_2-CO_2 and acetate as growth substrates (20), it proved to be important in regard to growth yields measured at various dilution rates.

Effect of maintenance energy coefficient on biomass yields. Assuming that all bacterial cells contained in the chemostat are viable, one could theoretically determine the biomass (M) of a microorganism at any dilution rate by using a combination of equation 1 and the equation of Monod (8):

$$\mu_0 = \frac{\mu_{max}\,(S)}{K_m + (S)} \qquad (2)$$

where, according to Sinclair and Topiwala (14) and Van Uden (19),

$$\mu_0 = \mu_{app} + a$$

and

$$a = m_s\, Y_{max}$$

In equation 1, $\mu_{app} = D$ (dilution rate) at steady state. In equation 2, (S) is the molar concentration of energy substrate of the growth medium, K_m is the affinity reciprocal function of whole cells for the energy substrate, μ_0 is the theoretical specific growth rate including the maintenance rate (a), and μ_{max} is the maximal specific growth rate when cells are grown under excess substrate conditions. The biomass (M) can be described in the following relationships, which are derived from the equations cited above:

$$(M) = [(S)_i - (S)]\, Y_{app}$$

and finally,

$$(M) = \frac{Y_{max}\,\mu_{app}}{\mu_{app} + m_s\, Y_{max}} \left[(S)_i - \frac{(\mu_{app} + m_s\, Y_{max})K_m}{\mu_{max} - (\mu_{app} + m_s\, Y_{max})}\right] \qquad (3)$$

TABLE 1. Determination of growth yields in a chemostat as a function of dilution rate with lactate as the energy source[a]

$D = \mu_{app}$ (h^{-1})	Growth yield (g/mol)		
	D. vulgaris ($y_{lactate}$)		*M. barkeri*[b] (y_{CH_4})
	Sulfate[a]	*M. barkeri*[a]	
0.0125	2.06	2.93	2.52
0.025	3.28	4.45	4.50
0.050	4.33	6.24	10.90
0.120	5.77		
0.150	7.23		
0.206	6.65		

[a] Hydrogen acceptor.
[b] In the presence of *D. vulgaris*.

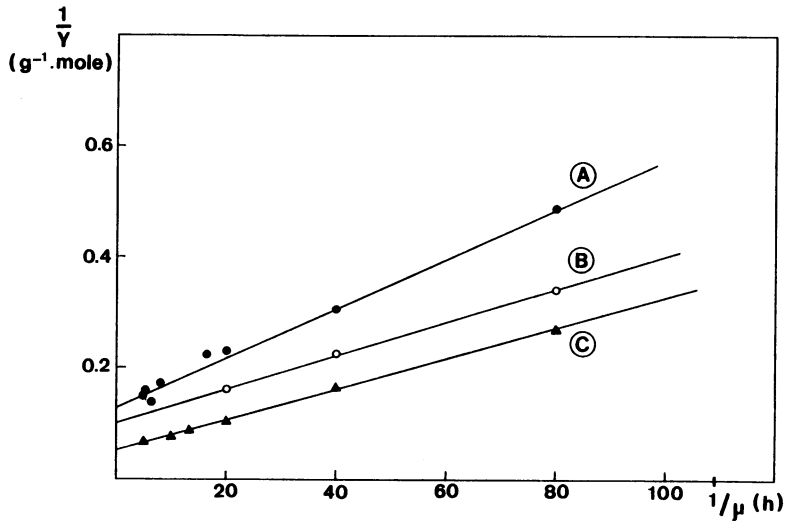

FIG. 1. Assessment of the maintenance energy coefficient of *D. vulgaris* Hildenborough (NCIB 8303) by using the relation of Pirt (11). Curves A, B, and C, were obtained when *D. vulgaris* was grown on lactate-sulfate, lactate-*M. barkeri* DSM 800, and pyruvate-sulfate media, respectively.

A similar equation has already been proposed by Van Uden (19) and Sinclair and Topiwala (14).

$(S)_i$ is the initial concentration of growth substrate in the medium. Equation 3 was fed into a computer, which drew the theoretical curves in Fig. 2. Constant parameters were programmed in accordance with *D. vulgaris* growth characteristics. These curves indicate theoretical biomass yields of *D. vulgaris* in correlation with variable values for the maintenance energy coefficient.

For these experiments 14.6 mM lactate and 36.3 mM sulfate were used. At each dilution rate, the biomass of *D. vulgaris* was assessed on a calibrated curve and then reported on the theoretical curves in Fig. 2. Experimental values described curve 5 (Fig. 2) obtained for the true value of m_s equal to 4.40 mM g^{-1} h^{-1} when *D. vulgaris* was metabolizing lactate-sulfate medium. So thus, there was good agreement between theoretical considerations and the experiment results. As also seen in Fig. 2, a decrease in the dilution rate caused a decline in the steady-state biomass concentrations because of the control exerted by the maintenance energy coefficient. For example, when dilution rates reached values of less than 0.05 h^{-1}, a marked shift in biomass production was observed. For high dilution rates, an increase in the maintenance coefficient results in a decrease in the critical dilution rate. This critical dilution rate corresponds to the apparent μ_{max} ($\mu_{max\ app}$), which is the value obtained in batch experiments, differing from the actual μ_{max} by the relation:

$$\mu_{max\ app} = \mu_{max} - m_s\ Y_{max}$$

For example, curve 5 (Fig. 2), which describes our experiment, gives an apparent μ_{max} of 0.24 h^{-1}, very close to the apparent μ_{max} measured in batch experiments. However, for calculation of this curve, a theoretical μ_{max} of 0.3 h^{-1} was necessary.

DISCUSSION

Michaelis kinetics of *D. vulgaris* metabolism on lactate-sulfate medium allowed us to show theoretically and experimentally that cell material formation was greatly affected by the maintenance energy coefficient. The decline in biomass yields became more pronounced as the maintenance energy increased and growth rates decreased. The maintenance energy coefficient (m_s) and cellular rate of catabolic activity (A_c) could be easily compared with each other, for example, in a chemostat, at the low dilution rate (D = 0.0125 h^{-1}), which is more convenient for the study of association between *D. vulgaris* and

TABLE 2. Chemostat growth parameters of *D. vulgaris* Hildenborough (NCIB 8303)

Growth condition	m_s (mM g^{-1} h^{-1})	Y_{max} (g/mol)
Lactate-sulfate	4.40	7.50
Lactate-*M. barkeri*	2.90	10.0
Pyruvate-sulfate	2.80	20.60

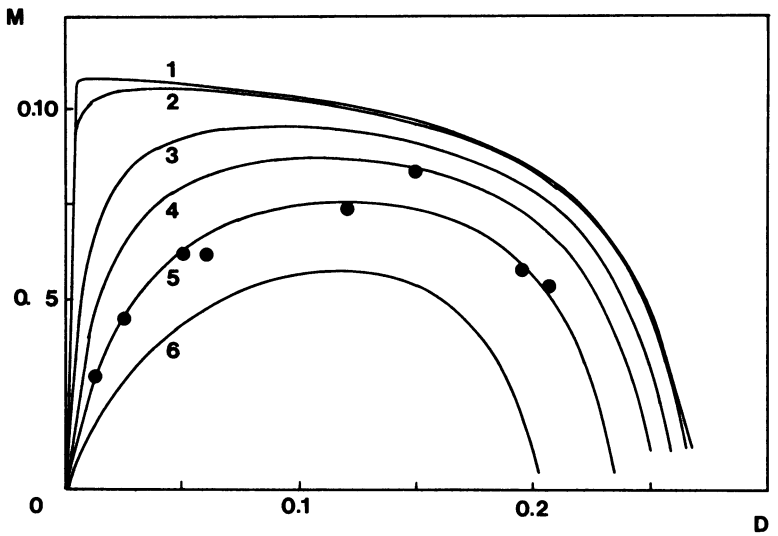

FIG. 2. Simulation curves representing biomass formation by *D. vulgaris* Hildenborough (NCIB 8303) as a function of maintenance energy coefficient (m_s). Growth was conducted in a chemostat with 14.60 mM lactate as the energy source in the presence of 36 mM sulfate. Points are experimental results. Curves were drawn with a computer according to equation 3 in the text. Constant parameters for all of the curves were Y_{max} = 7.5 g/mol, μ_{max} = 0.30 h^{-1}, and K_m = 1.65 mM. Curve 1, m_s = 0.01 mM g^{-1} h^{-1}; curve 2, m_s = 0.1 mM g^{-1} h^{-1}; curve 3, m_s = 1 mM g^{-1} h^{-1}; curve 4, m_s = 2.2 mM g^{-1} h^{-1}; curve 5, m_s = 4.4 mM g^{-1} h^{-1}; curve 6, m_s = 8.8 mM g^{-1} h^{-1}. M, Dry weight of bacteria (grams per liter); D, dilution rate (h^{-1}). Y_{max} and μ_{max} values were chosen somewhat higher than those observed in batch experiments (18) to take into account the maintenance influence on apparent values. K_m was calculated from a reciprocal plot of A_c (D/Y) versus residual lactate concentration measured for various values of D in the chemostat experiment.

M. barkeri because of the relatively low growth rate of the latter organism. Under these conditions, the amount of energy devoted to maintenance requirements reaches 68% (m_s/A_c = 2.9/4.3) of the total energy available during the metabolism of lactate by *D. vulgaris*. Maintenance energy coefficients measured for *D. vulgaris* were of the same order of magnitude as that calculated by Badziong and Thauer (1), who studied *D. vulgaris* Marburg under batch culture growth conditions. Maintenance energy coefficients in sulfate-reducing bacteria are among the highest in respect to those obtained for other microorganisms (4, 9, 16).

This parameter might play a key role in energy uncoupling occurring in sulfate-reducing bacteria (7, 13) and in other kinds of microorganisms (2, 3, 12). Maintenance energy coefficients could play a role in wastage of energy excess, produced in bacteria because of the large excess of catabolic reaction rate versus the anabolic reaction rate.

In lactate medium, the growth yield of *D. vulgaris* was higher in the presence of *M. barkeri* than in presence of sulfate as the electron sink (Tables 1 and 2). These results could be explained on the basis of metabolic reactions. When the methane-producing bacterium served as the hydrogen acceptor, *D. vulgaris* did not reduce sulfate and saved energy required for sulfate activation to form adenosine phosphosulfate (10). Despite a loss of ATP originating from oxidative phosphorylation linked to sulfate reduction (10), the presence of *M. barkeri* involved a situation more favorable to energy metabolism in *D. vulgaris* which cannot be easily understood. It would be interesting to determine whether the presence of *M. barkeri* increases the efficiency of energy utilization in *D. vulgaris* by decreasing a possible uncoupling of energy; another possibility would be an increase of the available ATP for the growth of *D. vulgaris* in the presence of *M. barkeri* through some unknown mechanism.

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