CHEMOSYNTHETIC FIXATION OF CARBON DIOXIDE AND CHARACTERISTICS OF HYDROGENASE IN RESTING CELL SUSPENSIONS OF HYDROGENOMONAS RUHLANDII NOV. SPEC.[1, 2]

LESTER PACKER AND WOLF VISHNIAC

Department of Microbiology, Yale University, New Haven, Connecticut

Received for publication January 28, 1955

The use of molecular hydrogen by "Knallgas" or hydrogen-oxidizing bacteria was first described by Kaserer (1905, 1906). Subsequently, other investigators such as Nabokitch and Lebedeff (1907), Lebedeff (1908), Niklewski (1910, 1914), and especially Ruhland (1924) have characterized this group of facultative autotrophs primarily with regard to their distribution, growth requirements, and quantitative consumption of carbon dioxide, oxygen, and hydrogen by growing cultures. Some of the results of the earlier work have been recently reviewed by Gest (1954) and Schlegel (1954). Surprisingly little is known of the mechanism by which hydrogen is utilized by these organisms, which when grown autotrophically depend entirely on hydrogen oxidation for energy. To study the mechanism of hydrogen oxidation a Knallgas bacterium, later named *Hydrogenomonas ruhlandii*, was isolated from soil. This organism was found to be amenable to an enzymatic study of the reactions involved in hydrogen activation and transfer (Packer and Vishniac, 1954, 1955). It is hoped that the information obtained on the hydrogen metabolism of this species may be of general significance for the physiological type it represents. This paper reports on the isolation, cultivation, and some physiological characteristics of the new species herein described.

EXPERIMENTAL METHODS

Several strains of hydrogen-oxidizing bacteria were isolated from soil inoculated enrichment cultures. The medium used for isolation and subsequent maintenance contained KH_2PO_4, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; $CaCl_2 \cdot 2H_2O$, 0.001 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; NH_4Cl, 0.1 g; $NaHCO_3$, 0.05 g; distilled H_2O, 100 ml. Freshly prepared media without the KH_2PO_4 and $NaHCO_3$ were distributed into flasks and sterilized. The $NaHCO_3$ (saturated with carbon dioxide) and KH_2PO_4 (previously neutralized with KOH) were added aseptically to the medium. The final pH was 7.2. The cultures were incubated in desiccators at atmospheric pressure with a gas mixture containing 63 per cent H_2, 13 per cent CO_2, 10 per cent O_2, and 14 per cent N_2 at 25 C. One pure culture, later designated *H. ruhlandii*, was selected as the experimental organism. Liquid stock cultures were transferred weekly and served as the inoculum for mass cultures and growth experiments. Mass cultures were prepared by spreading 3–4 drops of a stock culture over the surface of agar plates of the mineral composition described above. After 4–5 days of incubation under the usual gas mixture, the cells were harvested from the plate surface with the aid of 0.02 M phosphate buffer at pH 7.5. The cell suspension was then filtered through cheesecloth to remove small bits of agar that may have been carried into the suspension by the harvest procedure. The filtered suspension was centrifuged for 30 minutes at 3,500 ×G at 0 C, the supernatant fluid discarded, and the cells washed by resuspending them in phosphate buffer. They were then washed a second time by the same procedure and finally taken up in phosphate buffer and stored at 2 C until used. One hundred and fifty smear plates generally yielded about 2.5 g dry weight of cells. Resting cell suspensions prepared in this manner were used for all manometric experiments. Usual manometric techniques were employed; the manometric method used for determining the simultaneous uptake of CO_2, O_2, and H_2 has been described by Schatz (1952). The routine descriptive tests were carried out by procedures outlined in the *Manual of Pure Culture Study for Bacteria*.

[1] From a thesis to be submitted by Lester Packer to the Department of Microbiology, Yale University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] This research was supported in part by a grant from the National Science Foundation.

RESULTS

Description. H. ruhlandii nov. spec. is a gram negative, motile rod with rounded ends. The cells occur singly and occasionally in small groups, and their dimensions range from 0.4 μ to 0.75 μ in width and from 0.75 μ to 2.0 μ in length. The mean length and width are 1.1 μ and 0.5 μ, respectively. Longer cells frequently showed bipolar staining. Colonies on mineral agar plates are 1–2 mm in diameter, convex, glistening, and gray-white in color. Streak inoculation on nutrient agar slants showed a filiform zone of growth. Growth on potato slants was heavy and moist with an orange-brown coloration. Nutrient agar stab cultures showed a predominant surface growth while gelatin stabs showed only surface growth. Growth on gelatin was without liquefaction. Growth on Kliegler's iron agar slants indicated that hydrogen sulfide was not produced, and that acid was not produced from glucose or lactose. The organism grew diffusely in broths containing sucrose, galactose, glucose, raffinose, and mannose without any acid or gas production. *H. ruhlandii* also grew well on tryptone, nitrate, and nutrient broth, and Koser's citrate medium but slowly on litmus milk showing reduction after 3–4 weeks.

Growth experiments. H. ruhlandii is a member of the facultatively autotrophic hydrogen (or Knallgas) bacteria. It can grow as a strict autotroph or heterotrophically on a variety of substrates such as the various carbohydrates previously mentioned. Pyruvate, acetate, citrate, succinate, fumarate, malate, and α-ketoglutarate can also serve as good substrates for heterotrophic growth, when tested on the basal mineral medium plus the substrate in air. Under these conditions formate did not serve as a substrate. Heterotrophically-grown cells were found to lose their ability to grow autotrophically. This fact seems to be a characteristic feature of facultative autotrophs and has been previously reported by Kluyver and Manten (1942). The loss of autotrophy appears to be influenced by the oxygen tension. Autotrophic cultures grew best under 5 per cent O_2, almost as well under 10 per cent O_2, but very poorly under 20 per cent O_2. If nutrient broth-grown heterotrophic cultures (20 per cent O_2) were transferred to the basal mineral medium under autotrophic conditions, growth did not occur. However, if the nutrient broth-grown heterotrophic cultures were grown under an atmosphere containing 90 per cent N_2 + 10 per cent O_2 and then transferred to the basal mineral medium under autotrophic conditions, growth occurred rapidly. Thus heterotrophically-grown cultures under reduced O_2 tension retained their ability to grow autotrophically. The effect of oxygen tension on autotrophy was first observed in *Hydrogenomonas facilis* (H. Koffler, personal communication). Evidence will be presented later to show that loss of autotrophy is related to the inability to activate molecular hydrogen.

H. ruhlandii cannot utilize molecular nitrogen when tested for its ability to grow in a nitrogen-free basal mineral medium under the usual atmosphere (14 per cent N_2). If oxygen is omitted from the gas atmosphere, growth also does not occur. Thus an anaerobic reduction of CO_2 cannot be carried out with CO_2 and H_2 only as occurs in *Clostridium aceticum* (reaction 1) (Wieringa, 1940) or methane bacteria (reaction 2) (Söhngen, 1906). Both these anaerobic processes are exergonic.[3]

(1) $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad \Delta F = -6.4$ kc

(2) $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta F = -13.6$ kc

Mineral nutrition experiments were carried out under autotrophic conditions and showed a requirement for calcium and iron. The apparent high requirement for iron ($FeSO_4 \cdot 7H_2O$, 0.01 per cent) is presumably due in part to the precipitation of iron (as ferric hydroxide) during heat sterilization of the medium.

Physiological experiments on the oxy-hydrogen reaction. The uptake of molecular H_2 by resting cell suspensions of *H. ruhlandii* was easily demonstrated manometrically when resting cells incubated at 30 C under H_2 were given oxygen or methylene blue as hydrogen acceptors. CO_2, potassium ferricyanide, pyruvate, malate, fumarate, oxidized glutathione, tetrathionate, citrate, dehydroascorbic acid, acetate, lactate, α-ketoglutarate, fructose, and formate did not serve as hydrogen acceptors when tested under 100 per cent H_2. Some of the properties of the oxy-hydrogen reaction were studied by following the rate of H_2 and O_2 uptake with time under varying conditions. The oxy-hydrogen reaction occurs

[3] The changes in free energy have been estimated from standard free energy tables.

according to the equation,

$$(3) \qquad H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O$$

as shown by the quantitative determination of H_2 and O_2 uptake in table 1. Table 1 also shows that the endogenous oxygen uptake is very low, being only 9 μL in 2 hours with 0.5 mg cells dry weight. It can also be seen that the rate of the oxy-hydrogen reaction is linear with respect to cell concentration. At higher cell concentrations (1 mg dry wt/vessel) the rate is no longer linear, possibly due to a limited gas diffusion.

The rate of the oxy-hydrogen reaction is influenced by the concentration of inorganic orthophosphate. At pH 7.2 the reaction proceeds best between 0.033–0.067 M orthophosphate. Higher or lower concentrations of phosphate sharply decreased the rate of the oxy-hydrogen reaction.

Using resting cells in 0.033 M orthophosphate at varying hydrogen ion concentrations, the influence of pH on the oxy-hydrogen reaction was studied. Figure 1 summarizes the results of these experiments. The optimum pH was 7.2 with the rate of the reaction falling off a higher and lower pH. The rate fell off more rapidly in the acid region.

TABLE 1

Ratio of H_2:O_2 uptake with resting cell suspensions of Hydrogenomonas ruhlandii

	Dry Weight of Cells		0.5 mg
	0.25 mg	0.5 mg	
Total gas uptake/ 120 minutes	111 μL	211 μL	9 μL
O_2 uptake	34 μL	70 μL	9 μL
H_2 uptake	77 μL	141 μL	—
H_2:O_2 uptake	1:2.29	1:2.01	—
	99% H_2 + 1% O_2		100% air

Manometric experiment at 30 C. The Warburg vessels contained: Atmosphere—100% air in control, 99% H_2 + 1% O_2 in experimentals; main vessel chamber—cells in 0.033 M phosphate buffer at pH 7.2; vent arm—50% KOH 0.2 ml and strip of filter-paper; side arm—40% pyrogallol 0.1 ml + 3 N H_2SO_4 0.1 ml; procedure—Gas uptake occurred for 2 hours. At that time the contents of the side arm and vent arm were mixed. The alkaline pyrogallol absorbed the remaining oxygen in the vessels and stopped metabolism. The oxygen uptake was calculated by subtracting the value obtained for residual oxygen from the total initial oxygen present in a zero time control. Subtracting the O_2 uptake from the total gas uptake gave the H_2 uptake.

Under similar conditions the oxygen concentration was shown to influence the rate of the oxy-hydrogen reaction. The best rate was obtained between 5–9 per cent oxygen when the remainder of the atmosphere was hydrogen. Above 10 per cent oxygen the rate fell off markedly, whereas from 1–3 per cent oxygen the rate was only 10 per cent lower than the optimum. After 1 hour, oxygen tensions above 10 per cent progressively decrease the rate of the reaction.

The hydrogenase activity as measured by the rate of the oxy-hydrogen reaction was stable on storage. If washed cell suspensions are stored at 20 C, they show a slight loss of activity in two weeks. The greatest loss of activity found over a two week period was 40 per cent. Freezing the cell suspension resulted in a small loss of activity after 7 weeks. However, frozen preparations were not used since some destruction of the cells might occur during freezing and thawing.

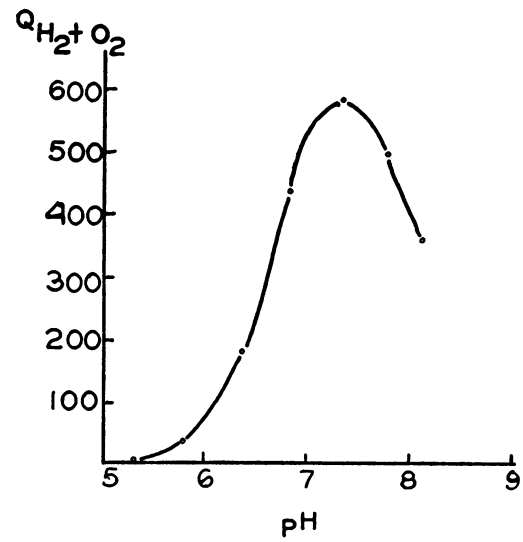

Figure 1. The influence of pH on the rate of the oxy-hydrogen reaction.

A manometric experiment carried out at 30 C under an atmosphere of 97 per cent H_2 + 3 per cent O_2. Each vessel contained 0.5 mg cells dry weight suspended in 2 ml of 0.033 M orthophosphate buffers at varying pH. The cell suspensions were washed 3 times in buffer of the appropriate pH before use. The Q values (per mg cells dry wt) were calculated from 60 minutes of gas uptake. The center wells contained 0.2 ml 15% KOH.

As mentioned earlier, the conditions under which the cells were grown strongly influenced their subsequent ability to develop as autotrophs. This loss of autotrophy must have involved an inability to assimilate CO_2 or an inability to use hydrogen. This lack of autotrophy appears to be a decrease in hydrogenase, the enzyme responsible for the activation of molecular hydrogen. A comparison of autotrophically and heterotrophically grown cells of *H. ruhlandii* showed that the ability to activate hydrogen is lost by heterotrophically grown cells. Table 2 summarizes these results and indicates that hydrogenase is not constitutive for this organism. It can also be seen from table 2 that only pyruvate-grown cells have some residual hydrogenase activity. This same effect with pyruvate-grown cells has been reported for several other strains of Knallgas bacteria (Bovell and Marr, *personal communication*). The metabolism of pyruvate frequently involves hydrogen evolution (Woods and Clifton, 1938; Koepsell and Johnson, 1942). A similar reaction in *H. ruhlandii* may account for the presence of hydrogenase. That loss of autotrophy actually involves hydrogenase and not some other step in the oxy-hydrogen reaction is borne out by the parallel data obtained with oxygen and methylene blue. The formation of hydrogenase is dependent not only on the presence of hydrogen, but also on the partial pressure of oxygen. Lower oxygen tensions favor the production of hydrogenase. The sensitivity of hydrogenase to oxygen has been reported by Wieland and Pistor (1938), Lascelles and Still (1946), Joklik (1950), Fisher, Krasna, and Rittenberg (1954), and others.

Although heterotrophically grown cells lose autotrophy, the reverse situation does not obtain. Autotrophically grown washed cell suspensions can oxidize a variety of organic substrates as shown in table 3. Kluyver and Manten (1942) and Wilson *et al.* (1953) have shown with other species of Knallgas bacteria that autotrophically grown cells can oxidize an organic substrate (sodium lactate) and hydrogen, simultaneously. It is likely that *H. ruhlandii* behaves similarly since sodium lactate is oxidized at a faster rate than any of the other substrates tested.

Physiological experiments on carbon dioxide assimilation. Since carbon dioxide cannot serve as a direct hydrogen acceptor anaerobically, it follows that the energy for the chemosynthetic

TABLE 2

Hydrogenase activity of resting cell suspensions of Hydrogenomonas ruhlandii grown under different conditions

Cells Grown on	97% H_2 + 3% O_2	100% H_2 + 10 μM Methylene Blue
	O_2 + H_2 uptake/hr	H_2 uptake/hr
	μL	
Glucose	0	0
Lactate	0	0
Pyruvate	10	22
α-Ketoglutarate	0	0
Fumarate	0	0
Autotrophically	267	95

Liquid cultures of *H. ruhlandii* were grown for 4 days on the basal mineral medium under H_2, O_2, and CO_2 (autotrophically) or under air plus an organic substrate (heterotrophically). The cultures were centrifuged and washed 2 times with 0.033 M phosphate buffer at pH 7.2, resuspended in the buffer so that there was 0.5 mg cells dry weight per 2 ml suspension. Each Warburg vessel contained 0.5 mg cells dry weight in phosphate buffer, 0.2 15% KOH in center well, and the additions indicated above.

TABLE 3

Oxidation of organic substrates by autotrophically grown resting cell suspensions of Hydrogenomonas ruhlandii

Substrate	Q_{O_2} (per mg cells)
Sodium pyruvate	19
Sodium acetate	3
Sodium citrate	2
Sodium succinate	26
Sodium fumarate	32
Sodium malate	31
Sodium α-ketoglutarate	18
Sodium formate	2
Sodium lactate	44
Fructose	6
Glucose	1

Warburg flasks contained 0.5 mg cells dry weight in 2.0 ml phosphate buffer (0.033 M at pH 7.2), 10 μM of the substrate as listed above in the side arm, 100% air atmosphere at 30 C, and 0.20 ml 15% KOH in center well. The substrate was tipped in from the side arm to start the reaction. The values in the table were corrected for endogenous oxygen uptake. Q values were calculated from O_2 uptake over a 5 hour period.

TABLE 4

Carbon dioxide fixation in resting cell suspensions of Hydrogenomonas ruhlandii under an atmosphere containing 2% oxygen

	Mg Dry Weight of Cells per Vessel					
	0.05	0.1	0.2	0.4	0.5	0.6
μL of total gas uptake	12	37	69	144	197	227
μL CO_2 fixed	0	1	5	13	17	20
μL O_2 fixed	2	10	16	40	48	61
μL H_2 fixed	10	26	48	91	132	146
CO_2:O_2:H_2	—	1:9.3:31.3	1:3.1:9.5	1:2.7:6.2	1:2.6:6.8	1:2.7:6.7
Number of times oxy-hydrogen reaction is occurring per mole of CO fixed	0 ×	18 ×	6 ×	4 ×	4 ×	4 ×

The experiment was carried out in Warburg flasks gassed with an atmosphere containing 1% CO_2, 2% O_2, and 97% H_2 at 30 C. The main compartment contained varying cell densities in 0.033 M phosphate buffer at pH 7.2. The side arm contained 0.1 ml 3 N H_2SO_4 and 0.1 ml 40% pyrogallol. The vent arm contained filter paper.

Procedure: The contents of the side arm were tipped into the main vessel chamber at 0 time for the controls and at the end of the experiment in the other vessels. The controls were used to determine the initial composition of the gases. The H_2SO_4 served to stop metabolism and liberate the CO_2 present as bicarbonate in the aqueous phase. Following this a measured amount of potassium hydroxide was added through the vent arm; this served to absorb the CO_2 in the gas phase. The alkaline contents of the vent arm were then tipped into the main chamber and served to make the pyrogallol alkaline, resulting in the removal of oxygen from the gas phase. The hydrogen uptake was determined by difference from the total gas uptake and the CO_2 and O_2 uptakes. The time of the experiment was 60 minutes.

fixation of carbon dioxide must come from the oxy-hydrogen reaction. The manner in which *H. ruhlandii* couples the energy derived from hydrogen oxidation to carbon dioxide fixation was studied by following the uptake of H_2, O_2, and CO_2 by resting cells, simultaneously. Using this method, CO_2 fixation was readily demonstrated even with cell suspensions that were several weeks old. With age, however, the amount as well as the efficiency of the assimilation decreased; that is to say, a greater amount of hydrogen and oxygen was taken up for every mole of carbon dioxide. Table 4 summarizes data on the quantitative uptake of H_2, O_2, and CO_2 obtained with fresh cell suspensions under an atmosphere containing 2 per cent O_2. From the ratio of the gas uptakes the equations effected by *H. ruhlandii* under these conditions can best be formulated as:

$$(4) \qquad 4H_2 + 2O_2 \rightarrow 4H_2O$$

$$(5) \qquad CO_2 + 4(H) \rightarrow (CH_2O) + H_2O$$

$$(6) \quad \text{Sum: } CO_2 + 2O_2 + 6H_2 \rightarrow (CH_2O) + 5H_2O$$

The basis for this formulation is that since CO_2 is not directly reduced by H_2, the H_2 and O_2 uptake must take place as in reaction (4). The H_2 still unaccounted for by reaction (4) was 2 moles and must correspond to the reduction of one mole of CO_2 as written in reaction (5). Table 4 also shows that the results with low cell concentrations are variable. This is undoubtedly due to the small total gas uptake which made the method unreliable. These data for the assimilation of H_2, O_2, and CO_2 are in agreement with those reported by Schatz (1952) for *H. facilis*. These results are probably more significant than gas analyses carried out with growing cultures.

Thermodynamic considerations (see discussion) indicate that it should be possible to achieve greater efficiencies for CO_2 fixation. That is to say, for every time reaction (5) occurs, reaction (4) need occur less frequently than the data indicate. However, it is difficult to determine the minimum number of times reaction (4) is required to occur per reaction (5) since reaction (4) can occur independently of reaction (5). Better data might be obtained if some method were available to limit strictly the occurrence of the oxy-hydrogen reaction in intact cells. In an attempt to improve the conditions for carbon dioxide fixation, the experiment described in

table 4 was repeated under an atmosphere containing 8 per cent O_2. It was found that both the O_2 and cell concentration influenced the efficiency of the carbon dioxide fixation. The following formulation can be made from the data in table 5.

(7) with 0.2 mg cells dry weight, $2H_2 + O_2 \rightarrow 2H_2O$

(8) with 0.4 mg cells dry weight, $2.7H_2 + 1.3O_2 \rightarrow 2.7H_2O$

(9) with 0.6 mg cells dry weight, $3.5H_2 + 1.7O_2 \rightarrow 3.5H_2O$ occurs for every time one mole of CO_2 is assimilated according to the equation,

(10) $CO_2 + 2H_2 \rightarrow (CH_2O) + H_2O$

Thus, at 8 per cent O_2, the optimum concentration for the occurrence of the oxy-hydrogen reaction, the efficiency of the carbon dioxide fixation, becomes an inverse function of the cell density. The gas ratios shown in reactions (7) and (10) represent the best efficiency thus far reported for hydrogen-oxidizing bacteria.

TABLE 5

Carbon dioxide fixation in resting cell suspension of Hydrogenomonas ruhlandii under an atmosphere containing 8% oxygen

	Mg. Dry Weight of Cells per Vessel		
	0.2	0.4	0.6
μL total gas uptake	71	127	213
μL CO_2 uptake	12	17	24
μL H_2 uptake corresponding to CO_2 uptake	24	34	48
μL H_2 and O_2 uptake corresponding to oxy-hydrogen reaction	35	76	141
μL H_2 uptake corresponding to oxy-hydrogen reaction	24	50	94
μL O_2 uptake corresponding to oxy-hydrogen reaction	12	25	47
CO_2:O_2:H_2	1:1:2	1:1.3:3.7	1:1.7:5.5

Experimental conditions and procedure were the same as described in table 4, with the exception that the atmosphere was 1% CO_2, 8% O_2, and 91% H_2, and the pyrogallol was omitted since the oxygen tension was too high to permit a quantitative absorption of O_2. The O_2 uptake was calculated from the data on total gas uptake and CO_2 uptake as shown in the table. The time of the experiment was 60 minutes.

DISCUSSION

H. ruhlandii is placed in the genus *Hydrogenomonas* since it is an obligately aerobic organism capable of growing autotrophically with carbon dioxide as the sole carbon source and molecular hydrogen as the sole hydrogen donor. Members of this genus are further characterized by being gram negative rods having the ability to grow on organic substrates in the absence of H_2 (see Breed *et al.*, 1948). *Bergey's Manual* states: "Bacteria with similar physiological characteristics but differing in morphology are placed in the genera, *Bacterium*, *Bacillus*, and *Clostridium*." Only five species have so far been adequately described as *Hydrogenomonas* species to date. Three of them, *H. pantotropha*, *H. vitrea*, and *H. flava*, are described in *Bergey's Manual*; the others are *H. facilis* (Schatz and Bovell, 1952) and *H. carboxydovorans* (Kistner 1953). Two incompletely described species are also known: *H. agilis* and *H. minor* (Niklewski, 1914). *Pseudomonas saccharophila* (Doudoroff, 1940) has been isolated as an autotrophic hydrogen bacterium. Table 6 summarizes some of the distinguishing features of the well described hydrogen oxidizers.

Although data have been obtained on the ratio of CO_2:O_2:H_2 uptake which indicate a better efficiency than earlier reported for the chemosynthetic assimilation of CO_2, even better efficiencies seem possible on thermodynamic grounds. The chemosynthetic fixation of CO_2 effected by *H. ruhlandii* can be written as,

(11) $CO_2 + 2H_2 \rightarrow (CH_2O) + H_2O\ \Delta F = +8.2$ kc

It is an endergonic reaction and must therefore be coupled to the strongly exergonic oxy-hydrogen reaction.

(12) $H_2 + \frac{1}{2}O_2 \rightarrow H_2O\ \Delta F = -56.5$ kc

If the process occurred with 100 per cent efficiency, for every single time reaction (12) occurred, reaction (11) could occur about 7 times. Therefore, using our best data obtained thus far as in reaction (7), the efficiency of the process can be calculated,

$$\frac{8.2}{2 \times 56.5} \times 100 = 7.25 \text{ per cent efficiency}$$

TABLE 6

Summary of some of the distinguishing features of species in the genus Hydrogenomonas, when grown autotrophically

Species	Growth in Liquid Media	Influence of O_2 on Growth	Characteristics	Known Hydrogen Acceptors
H. pantotropha	diffuse, no pellicle formation	insensitive to high O_2 tensions	yellow and slimy	O_2
H. vitrea	pellicle formed	grows only between 2–8% O_2	yellow center transparent, and fluorescent	O_2
H. flava	no pellicle formed	grows only between 2–8% O_2	yellow, and adhere to medium	O_2
H. facilis	pellicle and turbidity	insensitive to high O_2 tensions	gray	O_2, KNO_3
H. ruhlandii	diffuse, no pellicle formation	grows best below 10% O_2	gray	O_2, KNO_3 not utilized
H. carboxydovorans	—	insensitive to high O_2 tensions	yellow to light brown	O_2

In view of these considerations, the earlier basis (used by Baas-Becking and Parks (1927), Burk (1931), and others since then) for describing the efficiency of this chemosynthetic process is unsatisfactory. These early calculations defined the efficiency of the process as the free energy of the product of CO_2 fixation, (CH_2O), divided by the free energy of the energy yielding reaction. Using the data obtained in reaction (7) and this definition of efficiency (after Baas-Becking and Parks, 1927), we obtain,

$$\frac{108}{2 \times 56.5} \times 100 = 96 \text{ per cent efficiency.}$$

Although this result might have some usefulness when comparing the present process to similar processes occurring elsewhere, it does not correctly describe the efficiency of hydrogenomonads.

SUMMARY

Hydrogenomonas ruhlandii nov. spec., a facultatively autotrophic hydrogen-oxidizing (Knallgas) bacterium, has been described. The morphological characteristics and cultural behavior of this organism under autotrophic and heterotrophic conditions have been presented.

The properties of its hydrogenase activity using resting cell suspensions have been studied manometrically. Such cell suspensions could carry out the oxy-hydrogen reaction optimally at pH 7.2 in 0.033 M orthophosphate buffer under an atmosphere of 5–9 per cent O_2 when the remainder of the gas phase was 91–95 per cent H_2. It can couple the energy yielding oxy-hydrogen reaction to the reduction of carbon dioxide according to the equations

$$2H_2 + O_2 \rightarrow 2H_2O$$
$$CO_2 + 2H_2 \rightarrow (CH_2O) + H_2O$$
$$\text{Sum: } CO_2 + O_2 + 4H_2 \rightarrow (CH_2O) + 3H_2O$$

When conditions were not optimal, as for example with old cell suspensions, improper cell densities, or limiting O_2 tension, the process was less efficient. Namely, the oxy-hydrogen reaction occurred more than twice, for every time one mole of CO_2 was assimilated. The free energy relationships of the chemosynthetic utilization of CO_2 by this organism were discussed, and the efficiency with which the chemosynthetic process occurs was described. The equations given above represent our best ratios for the simultaneous uptake of H_2, O_2, and CO_2 and represent an efficiency of 7.25 per cent for the utilization of the energy of the oxy-hydrogen reaction for the assimilation of CO_2.

REFERENCES

BAAS-BECKING, L. G. M., AND PARKS, G. S. 1927 Energy relations in the metabolism of autotrophic bacteria. Physiol. Revs., **7**, 85–106.

BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1948 *Bergey's manual of determinative bacteriology*. 6th ed. The Williams and Wilkins Co., Baltimore, Md.

BURK, D. 1931 The reversibility of coupled reactions in biological systems and the second law of thermodynamics. J. Physiol. Chem., **35**, 432–455.

DOUDOROFF, M. 1940 The oxidative assimilation of sugars and related substances by *Pseudomonas saccharophila* with a contribution to the problem of the direct respiration of di- and polysaccharides. Enzymologia, **9**, 59–72.

FISHER, H. F., KRASNA, A. I., AND RITTENBERG, D. 1954 The interaction of hydrogenase with oxygen. J. Biol. Chem., **209**, 569–578.

GEST, H. 1954 Oxidation and evolution of molecular hydrogen by microorganisms. Bacteriol. Revs., **18**, 43–73.

JOKLIK, W. K. 1950 The hydrogenase of *E. coli* in the cell-free state. I. Concentration, properties, and activation. Australian J. Exptl. Biol. Med. Sci., **28**, 321–329.

KASERER, H. 1905 Ueber die Oxydation des Wasserstoffes und des Methans durch Mikroorganismen. Z. landwirtsch. Versuchsw. Deut. Oesterr., **8**, 789–794.

KASERER, H. 1906 Die Oxydation des Wasserstoffes durch Mikroorganismen. Centr. Bakteriol. Parasitenk., **16**, 681–696.

KISTNER, A. 1953 On a bacterium oxidizing carbon monoxide. Proc. Koninkl. Ned. Akad. Wetensch., **56**, 443–450.

KLUYVER, A. J., and MANTEN, A. 1942 Some observations on the metabolism of bacteria oxidizing molecular hydrogen. Antonie van Leeuwenhoek. J. Microbiol. Serol., **8**, 71–85.

KOEPSELL, H. J., AND JOHNSON, M. J. 1942 Dissimilation of pyruvic acid by cell-free preparations of *Clostridium butylicum*. J. Biol. Chem., **145**, 379–386.

LASCELLES, J., AND STILL, J. L. 1946 Utilization of molecular hydrogen by bacteria. Australian J. Exptl. Biol. Med. Sci., **24**, 37–48.

LEBEDEFF, A. F. 1908 Über die Assimilation des Kohlenstoffes bei Wasserstoffoxydierenden Bakterien. Biochem. Z., **7**, 1–10.

NABOKITCH, A. J., AND LEBEDEFF, A. F. 1907 Ueber die Oxydation des Wasserstoffes durch Bakterien. Centr. Bakteriol. Parasitenk. **17**, 350–355.

NIKLEWSKI, B. 1910 Über die Wasserstoffoxydation durch Mikroorganismen. Jahrb. wiss. Botan., **48**, 113–142.

NIKLEWSKI, B. 1914 Über die Wasserstoffaktivierung durch Bakterien unter besonderer Berücksichtigung der neuen Gattung *Hydrogenomonas agilis*. Centr. Bakteriol. Parasitenk., **40**, 430–433.

PACKER, L., AND VISHNIAC, W. 1954 Hydrogen metabolism in a *Hydrogenomonas sp.* Bacteriol. Proc., **1954**, 111–112.

PACKER, L., AND VISHNIAC, W. 1955 Studies on the mechanism of the enzymatic reduction of diphosphopyridine nucleotide by hydrogen. Bacteriol. Proc., **1955**, 116–117.

RUHLAND, W. 1924 Beiträge zur Physiologie der Knallgasbakterien. Jahrb. wiss. Botan., **63**, 321–389.

SCHATZ, A. 1952 Uptake of carbon dioxide, hydrogen and oxygen by *Hydrogenomonas facilis*. J. Gen. Microbiol., **6**, 329–335.

SCHATZ, A., AND BOVELL, C. 1952 Growth and hydrogenase activity of a new bacterium, *Hydrogenomonas facilis*. J. Bacteriol., **63**, 87–98.

SCHLEGEL, H. G. 1954 Die Rolle des molekularen Wasserstoffes im Stoffwechsel der Mikroorganismen. Arch. Mikrobiol., **20**, 293–322.

SÖHNGEN, N. L. 1906 Sur le role du Méthane dans la vie organique. Rec. trav. chim. biol., **29**, 238–274.

WIELAND, H., AND PISTOR, H. J. 1938 Mechanism of the oxidation process. XLIX. The dehydrogenating enzyme system of *Acetobacter peroxydans*. Ann. Chem. Justus Liebigs, **535**, 205–219.

WIERINGA, K. T. 1940 The formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. Antonie van Leeuwenhoek. J. Microbiol. Serol., **6**, 251–262.

WILSON, E., STOUT, H. A., POWELSON, D., AND KOFFLER, H. 1953 Comparative biochemistry of the hydrogen bacteria. I. The simultaneous oxidation of hydrogen and lactate. J. Bacteriol., **65**, 283–287.

WOODS, D. D., AND CLIFTON, C. E. 1938 Studies on the metabolism of the strict anaerobes (genus *Clostridium*). VII. The decomposition of pyruvate and l(−) glutamic acid by *Clostridium tetanomorphum*. Biochem. J., **32**, 345–356.