GROWTH AND HYDROGENASE ACTIVITY OF A NEW BACTERIUM, HYDROGENOMONAS FACILIS

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The elucidation in nonsulfur purple bacteria of the parallelism between the hydrogen donors for photosynthesis and for heterotrophic growth helped clarify bacterial photosynthesis and photosynthesis in general (van Niel, 1949). Further comparative studies on the fixation of CO_2 by autotrophic bacteria and here, in particular, by a hydrogen bacterium should further the understanding of how CO_2 is assimilated in photosynthesis. Two ways of posing the problem of CO_2 fixation in hydrogen bacteria are: how is hydrogen utilized? and what is the connection between hydrogen assimilation and heterotrophic growth?

This paper reports some cultural characteristics of a new autotrophic hydrogen bacterium and describes its highly active hydrogenase.

EXPERIMENTAL METHODS

Cultures were isolated, grown, and maintained on the following basal medium adjusted to pH 6.8 to 7.2: KH_2PO_4 0.1 g, NH_4NO_3 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.02 g, $FeSO_4 \cdot 7H_2O$ 0.001 g, and $CaCl_2 \cdot 2H_2O$ 0.001 g, distilled water to 100 ml. Where desired, 1.5 per cent washed agar was incorporated. For autotrophic growth, the base was supplemented with 0.05 per cent $NaHCO_3$. Stock solutions of the bicarbonate were autoclaved separately, flushed with CO_2, and added to the sterile medium prior to inoculation. Incubation was at 25 C in desiccators with the atmosphere initially adjusted to 10 per cent CO_2, 30 per cent air, and 60 per cent H_2.

Since hydrogenase activity decreased in older cells, 2- to 3-day autotrophic cultures on heavily inoculated plates were harvested in M/30 Sørensen's phosphate buffer at pH 7.17. These were centrifuged down once, resuspended in buffer, and refrigerated at 6 C. Liquid cultures also yielded satisfactory cells. In Warburg experiments, the oxidation of H_2 and various organic substrates, the reduction of nitrate, and the fixation of CO_2 (Schatz, to be published) were readily demonstrated with cells so prepared. Over at least a 6-week period at 6 C, hydrogenase and the CO_2-fixing capacity showed slight if any decrease.

The heterotrophic agar medium employed consisted of KH_2PO_4 0.02 g, $MgSO_4 \cdot 7H_2O$ 0.01 g, Na_3 citrate $\cdot 2H_2O$ 0.05 g, Na acetate $\cdot 3H_2O$ 0.03 g, Na_2 succinate $\cdot 6H_2O$ 0.2 g, NaH glutamate 0.1 g, Difco tryptose 0.5 g, yeast extract (Difco) 0.1 g, corn starch 0.2 g, and agar 1.5 g, distilled water to 100 ml; pH 6.8 to 7.2.

For manometric work, ordinary 18.0 ml ($\pm$2.0 ml), center well, 2 side arm Warburg vessels were employed in a 30 C bath. Unless otherwise indicated, ex-

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periments were conducted with M/30 phosphate buffer at pH 7.17 and under 25 per cent air plus 75 per cent H_2. Autorespiration was determined in 25 per cent air plus 75 per cent N_2. The center well routinely contained KOH. Anaerobic experiments were conducted under N_2 or H_2. The manometric systems and all reagents, including cell suspensions, were rendered O_2-free by flushing with the appropriate vessel gas passed through heated copper gauze.

To determine uptake of O_2 and H_2, one vessel side arm was initially filled with 0.1 ml 40.0 per cent pyrogallol and the other with 0.1 ml 100.0 per cent KOH. When both side arms were tipped, metabolism was halted and uptake of O_2 by alkaline pyrogallol occurred. Since the solubilities of O_2 and H_2 are small and numerically close

$$(\alpha_{H_2}^{30\,C} = 0.02; \qquad \alpha_{O_2}^{30\,C} = 0.03)$$

K_{O_2} was employed for both O_2 and H_2, as was done by Gaffron (1942). The uptake of these gasses was calculated in the following manner.

Let (1) a = manometric reading at zero time
(2) b = manometric reading before tipping side arms
(3) c = manometric reading after tipping side arms.

For O_2 consumption,

(4) $K_{O_2}(b - c)$ = μL unassimilated O_2 absorbed by the alkaline pyrogallol.

If (5) x = total μL O_2 originally present, computed according to equation (4) from the control flask with its side arms tipped at zero time,

then (6) $x - K_{O_2}(b - c)$ = μL O_2 assimilated by cells.

For calculating H_2 consumption,

(7) $K_{O_2}(a - c)$ = μL H_2 assimilated plus all O_2 consumed by cells and absorbed by alkaline pyrogallol.

Therefore (8) $K_{O_2}(a - c) - x$ = μL H_2 assimilated by cells.

EXPERIMENTAL RESULTS

Cultures isolated. By the enrichment technic mentioned earlier, 2 strains of facultatively autotrophic hydrogen bacteria were isolated from a lawn soil. One was a nonmotile, obligately aerobic, gram-positive rod. (Its nucleic acid composition has since been studied by Chargaff *et al.*, 1950.) The other bacterium, a new organism whose ability to fix CO_2 has already been briefly reported in an abstract (Schatz, 1950), is now designated *Hydrogenomonas facilis* because of the ease with which it lent itself to experimentation. Of the 2 isolates, *H. facilis* was chosen for the present work because of its closer relationship to certain already much-studied *Nitrobacteriaceae*.

Characteristics of H. facilis. The morphological and cultural properties of *H. facilis* may be summarized as follows:

Gram-negative rods with rounded ends.

Most cells uniformly stained; some with cross-bands.

Cells 0.3 by 2.0 μ in autotrophic and 0.4 by 2.5 μ in heterotrophic cultures.

Rods single, in pairs, or in short chains.

Motile by 1 or 2 polar flagella.

Pellicle and turbidity in liquid media.

Agar colonies round, raised, glistening, translucent, nonfluorescent, nonmucoid, entire, no distinctive odor. Colonies on heterotrophic agar convex, 3 mm in diameter after 8 days at 30 C, with raised knob in center, concentric zonation, five radial surface striations, a finely serrate edge, a somewhat darker central area, light buff in color. Autotrophic colonies gray, up to 1 mm in diameter after 5 days at 30 C.

Gelatin liquefied.

Milk slowly digested with alkalinization.

Growth on potato without pigmentation.

Nonhemolytic.

H_2S, indole, and acetoin not produced.

Growth experiments. A number of organic substrates including glucose, tyrosine, and succinate permitted good growth in 100 per cent O_2. Autotrophic development with CO_2 as the sole C-source took place at 30 per cent but not at 40 per cent O_2. Like *Bacillus pycnoticus* (Ruhland, 1924), *H. facilis* grew on the traces of O_2 in commercial tank H_2 and CO_2. To eliminate O_2 in anaerobic experiments, which were conducted in desiccators, plates of *H. facilis* with heavy growth served as O_2-removers.

Glucose or CO_2 as C-source allowed good growth with ammonia or nitrate as N-source. In an atmosphere of 10 per cent CO_2 plus 90 per cent H_2, autotrophic growth did not occur with nitrate or sulfate as H-acceptor. Under these same conditions, the addition of glucose, acetate, or succinate, alone or combined, did not permit anaerobic growth. *H. facilis* developed on the complex heterotrophic agar only in the presence of O_2. Under 10 per cent CO_2 plus 90 per cent air, no growth took place in the mineral base with ammonia, nitrite, thiosulfate, or elementary sulfur. With CO_2 or glucose as C-source, nitrite was produced from nitrate but not from ammonia. Nitrite was demonstrated by the usual diazotization with sulfanilate and subsequent conversion to the distinctive red dye.

Miscellaneous growth experiments revealed that (1) auto- and heterotrophic agar cultures survived equally well under their respective atmospheres at both 6 C and 25 C, (2) aging the slants at both these temperatures did not cause a differential loss of capacity for growth on CO_2 or organic substrates, and (3) serial transfers of heterotrophic cultures retained the ability to develop autotrophically irrespective of the composition of the medium, incubation temperature, O_2 tension, and number of transfers. The experimental details and results are presented in table 1. *H. facilis*, therefore, exhibits a well-sustained capacity for autotrophic growth.

Approximately 2 months after isolation, *H. facilis* exhibited plaques suggestive of bacteriophage. In an effort to secure a culture free of bacteriophage, the organism was repeatedly streaked on mineral agar, and single autotrophic colonies were picked. But about 6 months and again a year after this purification, plaques were observed on mineral agar plates. During this interval the organism had been subcultured many times. The plaques were 3 to 4 mm in diameter and contained

small numerous secondary colonies. The abundance and rapid growth of these resistant strains probably explain why liquid cultures never cleared. Evidence of phage was never observed with heterotrophic cultures. This appears to be the first recorded instance of a bacteriophage for an autotroph, and it may prove useful in obtaining cell-free preparations for enzyme studies.

From the literature and personal communications of colleagues, the impression was gained that some hydrogen bacteria are fastidious and prone to lose autotrophy or viability. Grohmann (1924) isolated a micrococcus that died out when

TABLE 1

Survival of Hydrogenomonas facilis and stability of its autotrophism

MEDIA	INCUBATION		AGE OF CULTURES, DAYS	NUMBER OF DAILY TRANSFERS	GROWTH ON TRANSFER TO	
	Atmosphere	Degrees C			Autotrophic agar	Heterotrophic agar
Survival						
Heterotrophic and mineral agar slants	Air	6	47		+	+
		6	72		+	+
		25	72		0	0
Mineral agar slants	10% CO_2, 25% air, 65% H_2	25	72		+	+
Mineral solution	10% CO_2, 25% air, 65% H_2	25	72		+	+
Stability of autotrophism						
Heterotrophic agar slants	Air	25		89	+	+
		35		19	+	+
	100% O_2	25		10	+	+
Mineral agar slants supplemented with:						
Glucose—1.0%	Air	25		39	+	+
Ethanol—0.2%	Air	25		39	+	+
Tyrosine—0.1%	Air	25		39	+	+
Na_2 succinate·$6H_2O$—0.2%	Air	25		39	+	+

repeatedly transferred on organic substrates. Frequent subculture on potato yielded diminishing growth and finally loss of 10 other hydrogen bacteria. The *Hydrogenomonas flava* of Kluyver and Manten (1942) lost its capacity for autotrophic development when maintained on organic media. In the culture collection of the Hopkins Marine Station, all 9 isolates of *Hydrogenomonas* sp. died out. On the contrary, *Pseudomonas saccharophila* was still capable of autotrophic development after heterotrophic maintenance for over 10 years (Whelton and Doudoroff, 1945).

When maintained aerobically as ordinary heterotrophs on agar media, hydro-

gen bacteria are subjected to approximately 20 per cent O_2. This is considerably greater than the O_2 tension which they commonly encounter in natural habitats such as soil and water. This pO_2 therefore may be injurious and may account for the following phenomena: (1) the loss of autotrophy, (2) the dying out of cultures, (3) the growth inhibition of some hydrogen bacteria by O_2 tensions above 8 per cent (Niklewski, 1910; Kluyver and Manten, 1942), and (4) the harmful effect of high O_2 concentration, especially above 20 per cent, on the hydrogenase of algae and of many autotrophic and heterotrophic bacteria (Stephenson and Stickland, 1931; Hoogerheide and Kocholaty, 1938; Gaffron, 1942; Kluyver and Manten, 1942; Wilson *et al.*, 1942; Wilson and Wilson, 1942; Hoberman and Rittenberg, 1943; and Lascelles and Still, 1946).

If autotrophy in *H. facilis* were labile, then repeated heterotrophic subculture in 100 per cent O_2 might eventuate in the disappearance of its hydrogenase. But after 10 daily transfers on heterotrophic agar slants under 100 per cent O_2, and in another experiment after 39 repeated subcultures on glucose, ethanol, succinate, and tyrosine agar media in air, *H. facilis* retained its ability to develop as an autotroph (table 1).

Manometric experiments. Autotrophically-grown cells of *H. facilis* showed remarkable hydrogenase activity. Most preparations gave a measurable uptake of O_2 and H_2 with as little as 0.04 mg dry weight of bacteria per vessel. This amount represents approximately 5.0 μg of cell nitrogen generally equivalent to about 10^9 viable organisms. This high activity made it unnecessary to starve cells to reduce autorespiration. Since endogenous respiration accounted for little of the gas exchange, it was not considered in the interpretation of experimental results from studies on hydrogenase.

For autotrophically cultured *H. facilis*, the values of Q_{H_2}(mg) with O_2 as H-acceptor ranged from 400 to 1,100. The hydrogenase activity varied for different preparations but was stable for any single lot of cells refrigerated at 6 C. An average rate of hydrogenase activity for 0.4 mg dry weight of *H. facilis* representing approximately 10^{10} viable cells per vessel was Q_{H_2}(mg) = 720. On the basis of this datum, *H. facilis* consumed each hour 51 per cent and 6 per cent of its own dry weight equivalent of O_2 and H_2, respectively. This is an oxidation of approximately 3×10^7 molecules of H_2 by each viable cell per minute.

A condition in which some essential soluble constituent of an enzyme system is diluted below a critical concentration for activity has been reported for hydrogenase in intact cells of *Escherichia coli* (Lascelles and Still, 1946) and *Azotobacter vinelandii* (Wilson *et al.*, 1942). Such a dilution effect was not observed with *H. facilis*. With O_2 as H-acceptor, the Q_{H_2}(mg) of *H. facilis* was directly proportional to cell concentration between 0.04 and 0.5 mg dry weight of bacteria per vessel. Above this range, gas diffusion limited the rate of reaction for the given rate of agitation. With nitrate and methylene blue as H-acceptors, the H_2 uptake varied directly with cell concentration down to the lower limit for the demonstration of hydrogenase activity. The same linear relationship existed for the respiration of pyruvate and α-ketoglutarate.

In an atmosphere of H_2, the reduction of nitrate by *H. facilis* required no adaptation and proceeded rapidly (figure 1). This reduction may be formulated in accordance with one or more of the three reactions in table 2. The results on the reduction of nitrate in the presence of H_2 can be interpreted to mean that *H. facilis* either quantitatively effected reaction (1) in table 2, or carried out a combination of the 3 reactions leading to the same net gas exchange. That the stoichiometric relationship between added nitrate and H_2 uptake (figure 1) indeed represented complete reduction to nitrite is suggested by the intense test for nitrite after the reaction had gone to completion and by the inability of the cells to reduce added nitrite. As will be reported in another communication dealing with the fixation of CO_2 by *H. facilis*, the total gas uptake in an atmosphere of H_2 containing 1 per cent CO_2 was the same as in H_2 alone. Reactions (2) and (3)

TABLE 2

*Anaerobic oxidation of hydrogen with nitrate as H-acceptor**

	μM KNO_3 per vessel					
	10.0	10.0	10.0	0	5.0	10.0
	μL Gas exchange					
	Theoretical			Observed		
	H_2 uptake	N_2 evolution	Net gas uptake	Net gas uptake		
(1) $KNO_3 + H_2 \rightarrow KNO_2 + H_2O$	224	0	224	0	117	215
(2) $KNO_3 + 5/2H_2 \rightarrow KOH + \frac{1}{2}N_2 + 2H_2O$	560	112	448			
(3) $KNO_3 + 4H_2 \rightarrow KOH + NH_3 + 2H_2O$	896	0	896			
Q_{H_2}(mg)				70		
Q_{KNO_3}(mg)				330 μg (= 3.3 μM)		

* 4.6 mg dry cells per vessel. Q values calculated from the 45-min interval during which 10.0 μM of nitrate were actually being reduced (figure 1).

in table 2 are ruled out for the additional reason that gas consumption in the presence of CO_2 would exceed the theoretical values since CO_2 would be absorbed by the NH_3 or KOH formed in reactions (2) and (3) and would result in an additional decrease in pressure, which was not observed.

The failure of *H. facilis* to reduce nitrate below the level of nitrite may reflect the inactivation of the nitrite-reducing enzyme by O_2. This interpretation is suggested by the finding that in *Pseudomonas denitrificans* the reduction of nitrate was much less sensitive to O_2 inhibition than was nitrite reduction (Sachs and Barker, 1949). It remains to be determined how far nitrate is reduced by *H. facilis* during both autotrophic and heterotrophic growth.

In the presence of molecular H_2, methylene blue also served as H-acceptor with Q_{H_2}(mg) values up to 2,200. In an atmosphere of H_2, cell suspensions exhibited no significant gas uptake when supplied with acetate, pyruvate, fumarate,

malate, oxalacetate, α-ketoglutarate, formate, acetaldehyde, acetone, sulfate, nitrite, 1 per cent CO_2 in N_2, and elementary sulfur. These negative results contrast with the behavior of other organisms for which certain of these compounds served as H-acceptors (Stephenson and Stickland, 1931; Lascelles and Still, 1946).

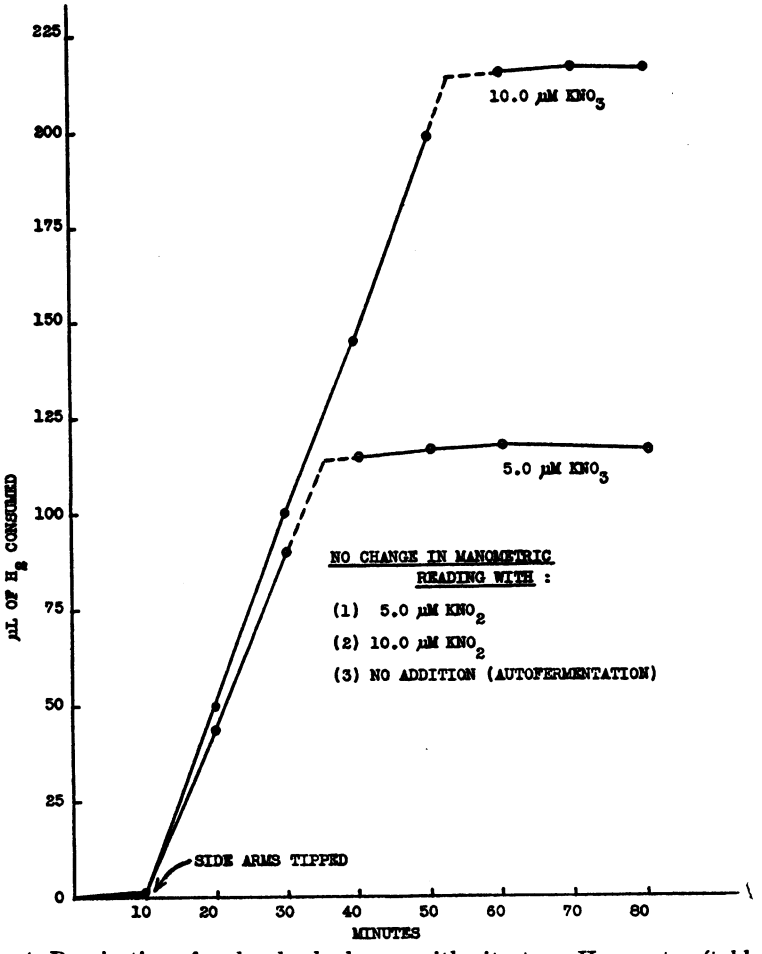

Figure 1. Respiration of molecular hydrogen with nitrate as H-acceptor (table 2).

In air, autotrophically grown *H. facilis* oxidatively assimilated acetate, lactate, pyruvate, succinate, fumarate, malate, oxalacetate, and α-ketoglutarate at approximately the same rate and without an adaptation period. Glucose was oxidized more slowly; the rate increased in the range from 2.0 to 30.0 μM of substrate per flask. With formate and oxalate, O_2 uptake was but slightly above the endogenous level. Citrate, acetone, nitrite, thiosulfate, and elementary sulfur were not respired.

The oxyhydrogen reaction occurred most rapidly between pH 6.0 and 7.0 and

fell off more abruptly on the acid than on the alkaline side (table 3). This coincides with the pH for maximum activity of hydrogenase in *E. coli* (Lascelles and Still, 1946), *A. vinelandii* (Wilson *et al.*, 1942), *Clostridium sporogenes* (Hoogerheide and Kocholaty, 1938), and a sulfate-reducer (Stephenson and Stickland, 1931). At pH 7.17, the Q_{H_2}(mg) increased with increasing concentration of phosphate over the range tested (table 3). Consumption of H_2 and O_2 at M/110 phosphate was about 84 per cent of the uptake at M/30 phosphate.

Kluyver and Manten's *H. flava*, when heterotrophically grown, failed to oxidize H_2. In contrast, the combustion of H_2 by *H. facilis* was easily demonstrated with

TABLE 3

*Influence of pH and phosphate buffer concentration on the rate of the oxyhydrogen reaction**

pH (M/30 PHOSPHATE)	Q_{H_2} (MG)	PHOSPHATE BUFFER (pH 7.17)	Q_{H_2} (MG)
3.9	76	M/30	370
4.9	270	M/45	350
6.0	400	M/75	340
7.0	360	M/90	330
7.8	300	M/110	310
8.1	290		
		Tap H_2O (pH 7.20)	220

* Q_{H_2} (mg) = 7 for autorespiration in M/30 phosphate at pH 7.17. The rather low Q_{H_2} (mg) values are probably due to the physiological state of these particular cells which had been incubated for 5 days before harvest.

TABLE 4

Aerobic oxidation of molecular hydrogen by heterotrophically grown Hydrogenomonas facilis

CELLS GROWN* IN AIR ON BASAL MINERAL AGAR PLUS	HOURS INCUBATION BEFORE HARVEST	Q_{H_2} (MG)	Q_{O_2} (MG) FOR AUTORESPIRATION
Na lactate—0.2%	48	130	9
Na_2 succinate·$6H_2O$—0.2%	21	15	4
Glucose—1.0%	72	32	11
Tryptose—0.5 %	48	85	10

* Plates for cell production inoculated with suspension from agar slants of the same composition. Cells tested within a day or two following harvest.

cells grown on organic substrates (table 4). For this reason, hydrogenase appears to be constitutive in *H. facilis*. Ordal and Halvorson (1939) demonstrated hydrogenase in *E. coli* grown on complex media. Billen and Lichtstein (1950) reported that hydrogenase was absent in *E. coli* cultured in a synthetic glucose-mineral medium. The addition of certain amino acids, most effectively glutamate and methionine, led to the production of the enzyme. For *H. facilis* grown autotrophically and on lactate, the Q_{H_2}(mg) values were about 570 and 130, respectively, (tables 4 and 5). Despite the lower activity of cells grown heterotrophically,

H. facilis produced hydrogenase when the organism was cultured in synthetic medium on certain organic carbon sources in the absence of any amino acids.

With molecular O_2 as H-acceptor, the oxidation of H_2 by *H. facilis* is complete (table 5). Similar findings have been reported for *B. pycnoticus* (Ruhland, 1924), *H. flava* (Kluyver and Manten, 1942), *E. coli* (Lascelles and Still, 1946), and *A. vinelandii* (Lee *et al.*, 1942). In contrast to this behavior of bacteria effecting the oxyhydrogen reaction, anaerobically adapted green algae carry the oxyhydrogen reaction to completion only when CO_2 is present (Gaffron, 1942).

In 20 per cent O_2, *H. flava* burned H_2 only for 30 to 90 min. At the end of this period, the cells were dead. Only under 8 per cent O_2 or less was gas consumption constant for more than 90 min (Niklewski, 1910; Kluyver and Manten, 1942).

TABLE 5

*Completeness of the oxyhydrogen reaction effected by Hydrogenomonas facilis**

	ATMOSPHERE	μL GAS CONSUMED AFTER			
		20 min	40 min	60 min	90 min
Autorespiration	25% Air + 75% N_2				
O_2		0	2	4	6
Q_{O_2} (mg)		15			
H_2 Oxidation	25% Air + 75% H_2				
$H_2 + O_2$		82	164	214	289
$H_2 = \frac{2}{3}(H_2 + O_2)$		55	109	143	193
Q_{H_2} (mg)		571			
$\frac{1}{3}(H_2 + O_2)$ = calculated O_2		27	55	71	96
Determined O_2		24	61	76	108
Q_{O_2} (mg)		305			
H_2/determined O_2		2.2	1.8	1.9	1.8
Mean H_2/determined O_2		1.9			

* 0.26 mg dry weight equivalent of cells per vessel. Q values calculated from the 60 min interval data.

The hydrogenase activity of *H. facilis* was greater at 5 per cent O_2 than at O_2 tensions above or below this level (figure 2). This 5 per cent O_2 concentration also permitted the highest activity for the hydrogenase of *E. coli* (Lascelles and Still, 1946). It is likely that diffusion of O_2 limited the oxyhydrogen reaction by *H. facilis* at concentrations below 5 per cent; however, experiments with more vigorous shaking were not conducted. Concentrations of O_2 above 5 per cent are also known to inactivate the hydrogenase of *E. coli* (Lascelles and Still, 1946).

The rate of the oxyhydrogen reaction effected by *H. facilis* decreased more rapidly in the original atmospheres (figure 2) with the higher concentrations of

O_2. The recovery of hydrogenase activity in the replaced atmospheres was greater for the cells exposed to the lower original concentrations of O_2. These observations suggest that the decreasing gas uptake with increasing O_2 in the original atmospheres is due to inactivation of hydrogenase rather than to a limiting supply of O_2. Hoberman and Rittenberg (1943) demonstrated that O_2 inactivated the hydrogenase of *Proteus vulgaris* and that this effect was largely reversible by H_2.

H. facilis developed autotrophically in the mineral base containing 0.4 per cent NH_4Cl as N-source. In figure 2, the greatest hydrogenase activity took place at the highest pN_2. These observations indicated that the hydrogenase of *H.*

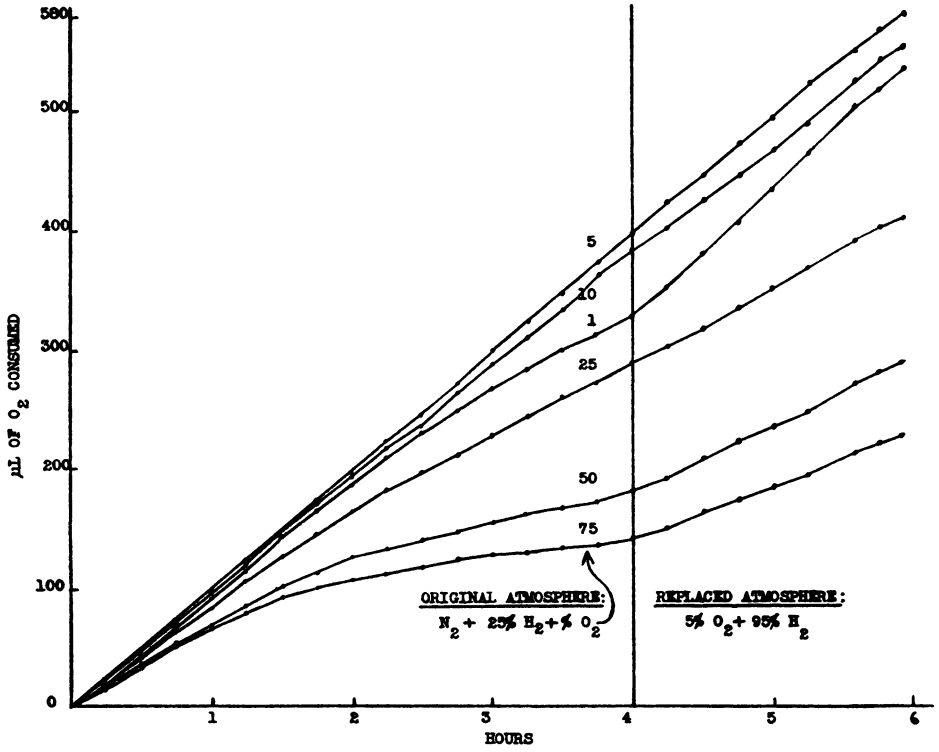

Figure 2. Influence of oxygen concentration on the hydrogenase activity of *Hydrogenomonas facilis*.

facilis was not inhibited by N_2 and NH_3, both of which injure hydrogenase in *Rhodospirillum rubrum* (Gest, Kamen, and Bregoff, 1950). Hydrogenase in *A. vinelandii* was more active in cells compelled to fix N_2 for growth than when NH_4NO_3 was supplied as N-source. However, N_2 did not inhibit the hydrogenase of this organism (Lee *et al.*, 1942).

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SUMMARY

Hydrogenomonas facilis, a new bacterium capable of utilizing hydrogen, is an obligate aerobe that develops heterotrophically on many substrates. It grows autotrophically in traces up to 30 per cent but not 40 per cent O_2. In manometric experiments, autotrophically-grown cells reduce nitrate to nitrite and oxidize H_2 to H_2O. For these reactions with O_2 and nitrate as H-acceptors, average Q_{H_2}(mg) values of about 720 and 70, respectively, were obtained.

In Warburg experiments, the hydrogenase of *H. facilis* was most active near pH 6.5, 5 per cent O_2, and M/30 phosphate buffer. In addition to nitrate and O_2, methylene blue was an effective H-acceptor. Under the same conditions, acetate, pyruvate, fumarate, malate, oxalacetate, α-ketoglutarate, formate, acetaldehyde, acetone, nitrite, 1 per cent CO_2 in N_2, and elementary sulfur were not reduced. Of these substances, the Krebs cycle intermediates were respired by autotrophically-grown cells of *H. facilis* rapidly, without adaptation, and at approximately the same rate. Citrate, acetone, nitrite, thiosulfate and elementary sulfur were not oxidized.

Hydrogenase was present in *H. facilis* grown on organic media. This enzyme was reversibly inactivated by pO_2 above 5 per cent and was not inhibited by N_2 or NH_3.

The physiological stability and hardiness of *H. facilis* recommend it for studies of H_2 utilization.

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