Open access • Journal Article • DOI:10.1007/BF00693407

Fermentation of acetylene by an obligate anaerobe,Pelobacter acetylenicus sp. nov.

Bernhard Schink

Institutions: University of Konstanz

Published on: 01 Aug 1985 - Archives of Microbiology (Springer-Verlag)

Topics: Acetylene hydratase, Acetaldehyde, Pelobacter, Acetate kinase and Alcohol dehydrogenase

First publ. in: Archives of Microbiology, 142 (1985), 3, pp. 295-301

Fermentation of acetylene by an obligate anaerobe, *Pelobacter acetylenicus* sp. nov.*

Bernhard Schink

Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, Federal Republic of Germany

Abstract. Four strains of strictly anaerobic Gram-negative rod-shaped non-sporeforming bacteria were enriched and isolated from marine and freshwater sediments with acetylene (ethine) as sole source of carbon and energy. Acetylene, acetoin, ethanolamine, choline, 1,2-propanediol, and glycerol were the only substrates utilized for growth, the latter two only in the presence of small amounts of acetate. Substrates were fermented by disproportionation to acetate and ethanol or the respective higher acids and alcohols. No cytochromes were detectable; the guanine plus cytosine content of the DNA was 57.1 ± 0.2 mol%. Alcohol dehydrogenase, aldehyde dehydrogenase, phosphate acetyltransferase, and acetate kinase were found in high activities in cell-free extracts of acetylene-grown cells indicating that acetylene was metabolized via hydration to acetaldehyde. Ethanol was oxidized to acetate in syntrophic coculture with hydrogen-scavenging anaerobes. The new isolates are described as a new species in the genus *Pelobacter*, *P. acetylenicus*.

Key words: *Pelobacter acetylenicus* species description – Acetylene fermentation – Anaerobic hydrocarbon degradation – Acetylene hydratase – Syntrophic ethanol oxidation

Anaerobic degradation of hydrocarbons has repeatedly been reported in the elder literature (Novelli and ZoBell 1944; ZoBell 1946; Rosenfeld 1947; ZoBell and Prokop 1966; Davis and Yarbrough 1966) but until now no conclusive evidence for significant anaerobic degradation of saturated hydrocarbons has been provided. The general theory today is that microbial attack on saturated hydrocarbons depends on the action of oxygenases which need molecular oxygen as a reactant (Foster 1962; Gibson 1975; Perry 1979; Atlas 1981).

Unsaturated linear hydrocarbons on the other hand can be subject to microbial attack in the absence of oxygen. Complete anaerobic degradation of 1-hexadecene by methanogenic enrichment cultures was recently reported (Schink 1985a). Degradation appeared to proceed via hydration to 1-hexadecanol, oxidation to palmitic acid, and subsequent β-oxidation to acetate residues. Squalene, a branched unsaturated hydrocarbon, was only incompletely degraded, probably by means of hydration and carboxylation reactions (Schink 1985a). No significant anaerobic degradation could be observed with ethylene (ethene), the most simple unsaturated hydrocarbon (Schink 1985a, b).

It was reported recently that also acetylene can be metabolized in the absence of molecular oxygen (Watanabe and de Guzman 1980). Enrichment cultures with acetylene as sole carbon source were obtained in mineral media with sulfate as electron acceptor, and acetate could be identified as an intermediary metabolite (Culbertson et al. 1981). However, these enrichment cultures were difficult to maintain, and the acetylene-degrading bacteria could not be identified (C. W. Culbertson and R. S. Oremland, Abstr. 3rd Int. Symp. Microb. Ecol., East Lansing, Mi. 1983, A-4).

In the present study, pure cultures of acetylene-fermenting anaerobes are described which were obtained by enrichment with acetylene from freshwater and marine sources. The biochemistry of acetylene fermentation was elucidated, and the isolates were assigned to the genus *Pelobacter* as a new species, *P. acetylenicus*.

Materials and methods

Enrichment cultures were inoculated with sediment samples taken from Canal Grande and a channel in the Old Jewish Ghetto in the City of Venice, Italy, samples taken from polluted freshwater creeks near Konstanz, FRG, and with anaerobic sludge from the municipal sewage treatment plants in Konstanz and Göttingen, FRG.

Media and growth conditions

All procedures for cultivation and isolation as well as all procedures for analysis of metabolic products were essentially as described in earlier papers (Widdel and Pfennig 1981; Schink and Pfennig 1982; Schink 1984). The mineral medium for enrichment and isolation contained 30 mM sodium bicarbonate as buffer, sodium sulfide as reducing agent, and the trace element solution SL 10 (Widdel et al. 1983). The pH was 7.2–7.4. Growth experiments were carried out at 28°C. Mass cultivation of pure cultures was carried out in 10 l glass bottles containing the usual mineral medium under a 1 l headspace of N_2/CO_2 (80%/20%). The bottles were closed by a rubber stopper carrying three outlets for medium preparation and controlled sterile gas exchange (Fig. 1). After completion of the autoclaved, cooled medium as described (Widdel and Pfennig 1981), bottle cultures were inoculated with 500 ml of an acetoin-grown cell suspension. All outlets were closed and the bottles were brought to the incubator room. After adjustment to ambient temperature

* Dedicated to Professor Dr. Norbert Pfennig on occasion of his 60th birthday

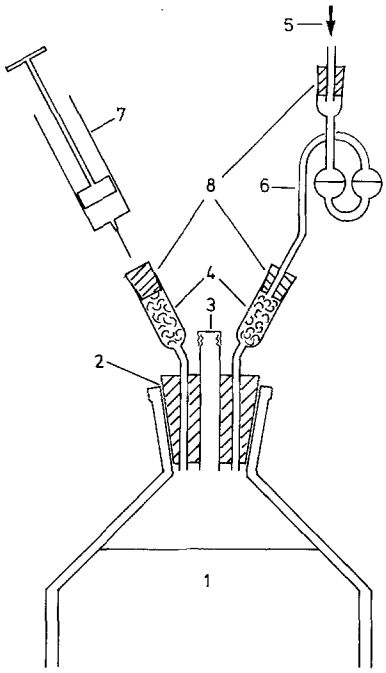

Fig. 1. Device for controlled acetylene feeding in mass cultures. *1* Stirred 10 l Pyrex bottle containing autoclaved complete mineral medium under N_2/CO_2 gas mixture (80%/20%), *2* rubber stopper with outlets, *3* screw cap-closed glass tube for addition of bicarbonate, vitamins, sulfide, pH adjustment etc., *4* sterile glass wool filters, one of them connected to a rubber baloon filled with 100% acetylene (*5*) via a bubbling tube (*6*). *7* 100 ml glass syringe with needle for removal of defined gas volumes through the rubber stopper (*8*)

(28° C), one of the glass wool filters was connected via a bubbling tube with a rubber balloon containing about 10 l of 100% acetylene which was washed free of water-soluble contaminants by bubbling through a small amount (50 ml) of water. After equilibration of the gas pressure, 200 ml of gas was removed from the culture headspace by syringe through the other gas filter. By this procedure, a nearly constant acetylene partial pressure of 0.2 bar was achieved. Bubbles in the bubbling tube indicated that part of the acetylene dissolved in the medium and that further acetylene was lateron consumed by the bacteria. When the cell suspension reached densities higher than $OD_{650} = 0.1$, the acetylene partial pressure in the headspace was increased by further gas removal to about 0.3 bar at maximum. The actual acetylene concentration in the headspace was checked by gas chromatography.

Isolation

Pure cultures of acetylene-fermenting bacteria were obtained by a modified agar shake culture technique (Pfennig 1978) in 120 ml Meplats bottles each containing 9 ml of mineral agar medium. Bottles were gassed with N_2/CO_2 (80%/20%) mixture before cooling, and the inoculum material was diluted transferring 0.2 ml of culture liquid by syringes through the butyl rubber stoppers from bottle to bottle in six steps each. The medium was cooled as a thin film on one flat side of the bottles, and 10 ml of acetylene gas was added to each bottle by syringe. Colonies were picked with bended sterile Pasteur pipettes. A further purification series was carried out with acetoin as substrate in usual agar shake tubes (Pfennig 1978).

Chemical and biochemical determinations

Sulfide was determined after Cline (1969), protein after Goa (1953) as modified by Kuenen and Veldkamp (1972). Cytochromes were quantified by absorption spectra taken in a Shimadzu UV 300 spectrophotometer. Acetylene, aldehydes, alcohols and fatty acids were measured by gas chromatography as described (Schink and Pfennig 1982). Solubility of acetylene was calculated after published data (Gordon and Ford 1972), and checked by dissolution experiments: Discrete amounts of acetylene were added to a certain amount of growth medium in rubber-sealed tubes, and the acetylene concentration remaining in the headspace was checked by gas chromatography. At 25° C, 1 bar of acetylene is in equilibrium with 1 l acetylene dissolved in 1 l of water which corresponds to a 40.8 mM concentration in the liquid. The equilibrium was usually reached within 5 min.

All enzyme assays were carried out with French pressure cell extracts prepared in 50 mM potassium phosphate buffer, pH 7.0, under a nitrogen gas atmosphere. All assays were performed under strictly anaerobic conditions in rubber-sealed nitrogen-flushed cuvettes using a Zeiss PM 4 spectrophotometer at 25° C.

Alcohol dehydrogenase and acetate kinase were assayed after Bergmeyer (1974), aldehyde dehydrogenase and pyruvate dehydrogenase after Odom and Peck (1981), acetoin dehydrogenase after Schink (1984) and phosphate acetyl transferase after Oberlies et al. (1980). Hydrogenase was assayed using a method modified after Schink and Schlegel (1979). The 1 ml-cuvette contained 100 mM potassium phosphate buffer, 5 mM benzyl viologen, 1 – 5 µl of crude cell extract, and a trace of sodium dithionite for initial reduction of the system. The reaction was started by flushing the cuvette with hydrogen gas. Numerous attempts were made to prove acetylene hydratase activity in cell-free extracts using the assay systems of DeBont and Peck (1980) and various modifications of methods used for detection of aldehyde dehydrogenase, diol dehydratases (Toraya et al. 1979; Schütz and Radler 1984), acetylene dicarboxylate dehydratase (Yamada and Jakoby 1958) or tartrate dehydratases (Bergmeyer 1974). No reaction was either found with acetylene monocarboxylate or acetylene dicarboxylate as substrates.

All chemicals were of analytical or reagent grade and were obtained from Merck, Darmstadt, Sigma, München, and Fluka, Neu-Ulm, FRG. Acetylene was a kind gift of Prof. P. Böger, Konstanz, and was obtained in high purity (for flame photometry) from Linde AG, München, FRG.

Results

Enrichment, isolation, and enumeration

50 ml-enrichment cultures with freshwater or saltwater mineral medium and 10 mM sulfate were inoculated with 5 ml each of sewage sludge, freshwater or marine sediment, respectively. The headspace of each bottle (70 ml) was flushed with N_2/CO_2 gas mixture (80%/20%), and 10 ml of washed acetylene gas was added through the butyl rubber stoppers by a syringe. Bottles were incubated at 28° C in the dark, and the disappearance of acetylene was followed by gas chromatography. In freshwater enrichment cultures, acety-

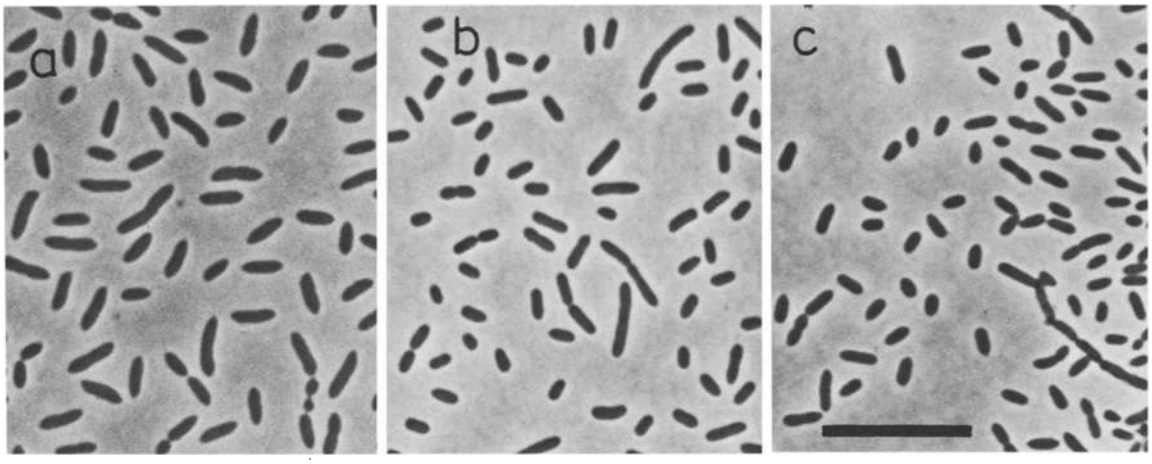

Fig. 2a–c. Phase contrast photomicrographs of acetylene-fermenting isolates. Bar equals 10 μm for all panels. **a** Strain WoAcy 1, **b** strain GhAcy 1, **c** strain GhAcy 3

lene disappeared only slowly after a lag phase of more than one week, whereas in the marine enrichments acetylene was used up immediately and completely within 3–5 days. Transfers were made after substrate consumption, and subcultures used up the acetylene added within 2–4 days. Acetate accumulated in the culture fluid, and the sulfide content increased to 3–4 mM concentration. After one further transfer, enrichment cultures were subjected to agar shake dilutions in Meplats bottles with acetylene as substrate and 2 mM acetate as further carbon source. Colonies developed within 3 weeks. They were either white and lens-shaped, white and fluffy, or yellow and lens-shaped. In shakes from Rio Marin, Venice, also some orange-red spherical colonies grew. All types of colonies were picked and transferred into liquid medium. Only the cultures derived from the white and the yellow lens-shaped colonies grew again with acetylene. In a first screening test, they both proved to grow well with acetoin, and a further agar shake dilution series was run with acetoin as substrate because contamination of acetylene cultures was difficult to prevent. Finally, four strains were chosen for further characterization.

Most-probable-number enumerations (American Public Health Association 1969) of acetylene-degrading anaerobes were carried out with the various sediment and sludge samples in mineral medium with sulfate added as electron acceptor. Less than 10 cells per ml were found in sewage sludge and freshwater creek sediment, and 100 to 500 cells per ml in the two marine sediment samples taken in the City of Venice, Italy.

Cytological properties

A freshwater strain isolated from creek sediment (WoAcy 1), a further freshwater strain from sewage sludge (KoAcy 23), and two strains from marine sediment from Venice (GhAcy 1, GhAcy 3) were taken for further characterization. They all were Gram-negative, non-sporeforming rods which were motile in young cultures but often lost motility with ageing. Cells of strains WoAcy 1, KoAcy 23 and GhAcy 3 looked nearly identical in the microscope (Fig. 2). They were $0.6-0.8 \times 1.5-4$ μm in size and had pointed ends. Often cells of different length formed chains, and strain GhAcy 3 had a thin slime capsule which was visible in Indian ink preparations (not shown). Cells of strain GhAcy 1 were slightly smaller and had rounded ends. Spores were never formed, neither in the usual mineral medium nor in specific sporulation media (Hollaus and Sleytr 1972; Duncan and Strong 1968). Acetylene was not degraded by pasteurized sediment samples. The guanine plus cytosine content of the DNA of strain WoAcy 1 was determined by thermal denaturation as described earlier (Schink 1984) and was found to be 57.1 ± 0.2 mol%. Ultrathin sections made with the same strain exhibited a typical Gram-negative cell wall architecture (not shown).

Physiological properties

All isolates were strictly anaerobic. Only acetylene, acetoin, ethanolamine, choline, 1,2-propanediol and glycerol were used as substrates for growth, the latter two only in the presence of acetate as additional carbon source. Slight growth was also found with strain WoAcy 1 with 2,3-butanediol and 1,2-butanediol plus acetate. No other substrate out of more than 40 substrates tested was used by any isolate. The complete list of substrates unable to support growth is given at the end of the discussion section. No growth was found either with acetaldehyde, acetylene carboxylate, acetylene dicarboxylate, ethylene, cyanide, acetonitril, or acetamide. Neither sulfate, sulfur, thiosulfate, sulfite, nor nitrate was reduced. Growth was optimal if acetylene was added to the headspace of a half-filled vial to 10–20% concentration which corresponds to a concentration of about 4–8 mM in the liquid medium. At higher concentrations, growth was delayed or completely inhibited. For mass cultures, a device was developed, therefore, which allowed to maintain a low but constant acetylene partial pressure over the medium to ensure growth and prevent inhibition (Fig. 1).

Acetylene and most other substrates were fermented to nearly equal amounts of acetate and ethanol (Table 1). Also traces of acetaldehyde (ethanol) were formed from acetylene. Growth yields with acetylene, ethanolamine, and choline were 3.5–4.4 g dry cell matter per mol substrate. Growth yields decreased with increasing amounts of acetylene provided. With acetoin, 9.0 g per mol were achieved. Choline was fermented to acetate, ethanol and trimethylamine which was identified by its characteristic smell. Glycerol was disproportionated to 1,3-propanediol and 3-hydroxypro-

Table 1. Stoichiometry of fermentation and growth yields of strain WoAcy 1. Experiments with dissolved substrates were carried out in 20 ml tubes which were filled completely. All figures are means of at least two independent assays. Growth with acetylene was measured in rubber-sealed 20 ml tubes containing 10 ml of inoculated mineral medium under a N_2/CO_2 gas atmosphere. Acetylene was added by a 2 ml plastic syringe with small dead volume in steps of 2 and 1 ml to a total amount of 2, 5, and 10 ml, respectively. The concentration of dissolved acetylene never exceeded 5 mM, therefore. More than 98% of the acetylene added was degraded as checked by gas chromatographic analysis of the headspace (see Materials and methods)

Substrate	Amount of substrate supplied (μmol)	Cell dry matter formed (mg)[a]	Substrate assimilated (μmol)[b]	Products formed (μmol)			Growth yield (g/mol)	Carbon recovery %
				acetate	ethanol	acetaldehyde		
Acetylene	82	0.3	3.9	51	26	3.2	3.6	102
Acetylene	205	0.71	9.23	106	73	4.2	3.5	94
Acetylene	410	0.86	11.2	205	138	6.1	2.1	88
Acetoin	100	0.90	11.7	126	84	0.5	9.0	111
Ethanolamine	200	0.88	11.4	118	70	0.5	4.4	99.7
Choline	200	0.86	11.2	118	72	0.5	4.3	100.6

[a] Cell dry weights were calculated by cell density using the conversion factor 0.1 $OD_{650} \triangleq 23.8$ mg dry matter per l, which was obtained by direct determination in 500 ml cultures grown with acetoin

[b] Substrate assimilated was calculated using the formula $C_4H_7O_3$ for cell material

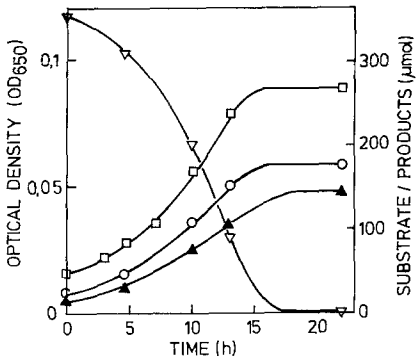

Fig. 3. Growth curve of strain WoAcy 1 growing with acetylene as sole source of carbon and energy. Experiments were carried out in triplicates in a 30°C water bath, each point is a mean of three independent determinations. Growth was followed in half-filled 100 ml culture bottles with rubber septa and tube-like side arms which allowed direct insertion into a Bausch and Lomb Spectronic 70 spectrophotometer. Samples for acetylene, acetate and ethanol determination were taken from the headspace and the culture fluid at times indicated. (□) Optical density (OD_{650}), (▽) acetylene, (○) acetate, (▲) ethanol

pionate. With 1,2-propanediol, the following fermentation balance was obtained:

200 μmol 1,2-propanediol + 100 μmol acetate → 38 μmol propionaldehyde + 136 μmol propionate + 41 μmol propanol + 83 μmol acetate + 22 μmol ethanol.

This balance indicates that i) propionaldehyde is formed as an intermediate of 1,2-propanediol fermentation and that ii) acetate added as a carbon source is also used as an electron sink to form ethanol. The propionaldehyde formed combined with the sulfide present as reducing agent to ill-smelling mercaptanoid compounds (Bayer 1954).

A growth curve of strain WoAcy 1 with acetylene as substrate is presented in Fig. 3. The doubling time was 5.0–5.5 h ($\mu = 0.132\ h^{-1}$) at 30–34°C which was the optimum temperature range. The same growth rates were found with acetoin. Growth limits with acetoin were at 15 and 45°C. The optimum pH was 6.5–7.5; no growth was found at pH 6.0 and pH 8.0. Vitamins, although present in the enrichment medium, were not required. Strain WoAcy 1 grew in freshwater medium as well as in saltwater medium, and the same was true for strain GhAcy 3. Strain WoAcy 1 formed a thin slime capsule in saltwater medium whereas strain GhAcy 3 lost its slime capsule in freshwater medium. Contrary, strain GhAcy 1 did not grow in freshwater medium and only weakly in brackish water medium with 1% sodium chloride.

Table 2. Activities of catabolic enzymes in strain WoAcy 1 after growth with acetylene or acetoin as substrates

Enzyme	EC	Specific activity (μmol · min^{-1} · mg protein^{-1})	
		Acetylene-grown	Acetoin-grown
Acetoin dehydrogenase	1.1.1.5	0.28	0.38
Aldehyde dehydrogenase[a]	1.2.1.3	0.83	0.95
Pyruvate dehydrogenase[a]	1.2.7.1	0.12	0.15
Phosphate acetyltransferase	2.3.1.8	19.05	35.8
Acetate kinase	2.7.2.1	3.36	4.3
Alcohol dehydrogenase	1.1.1.1	0.11	0.41
Hydrogenase[b]	1.8.3.1	5.6	7.8

[a] Benzyl viologen and coenzyme A-dependent

[b] Benzyl viologen-dependent

In coculture with hydrogen-scavenging anaerobes such as *Methanospirillum hungatei* or *Acetobacterium woodii*, strain WoAcy 1 grew with ethanol as substrate, and converted it completely to acetate and methane or acetate alone, respectively. Neither lactate, malate, fumarate, nor ethylene (ethene) was oxidized in similar mixed cultures.

Biochemical properties

Enzymes involved in acetylene and acetoin degradation were studied in crude extracts of cells grown with either substrate. The results are presented in Table 2. The activities of the enzymes tested did not differ significantly between both cell extracts. Acetoin dehydrogenase, aldehyde dehydrogenase, phosphate acetyltransferase, acetate kinase, and alcohol de-

hydrogenase were measured in high specific activities which make involvement in dissimilatory substrate degradation probable. Pyruvate dehydrogenase probably only operated in cell carbon assimilation. The presence of hydrogenase activity explains the ability of this strain to cooperate with hydrogen-scavenging anaerobes in syntrophic alcohol oxidation. In spite of numerous efforts, acetylene hydratase could not be found in cell extracts by the various methods applied although numerous modifications of published methods for demonstration of related enzymes were tried (see Methods section). Formation of acetaldehyde from acetylene was demonstrated in an experiment with unbroken cells. 5 ml of a dense suspension (OD_{650} about 1,2) of acetylene-grown cells was incubated in a 17 ml tube sealed with a butyl rubber septum under an atmosphere of 50% N_2 and 50% acetylene. About one third of the acetylene was consumed by the suspension within 3 h and gave rise to an accumulation of up to 6.5 mM acetaldehyde in the suspension fluid. At this acetaldehyde concentration, acetylene consumption stopped, and the accumulated acetaldehyde was not degraded any further either.

Cytochromes were not detected by redox difference spectroscopy of crude cell extracts of membrane preparations of acetylene- or acetoin-grown cells.

Discussion

Physiology

Degradation of acetylene was first observed with the aerobic bacterium *Mycobacterium lacticola* (Birch-Hirschfeld 1932) and was described lateron independently for a *Nocardia rhodochrous* isolate (Kanner and Bartha 1979) and a *Rhodococcus* strain A 1 (DeBont et al. 1980; DeBont and Peck 1980). All three described organisms are very similar if not identical, and the difference in generic designations are due only to differing taxonomic schemes (Kanner and Bartha 1982). Acetylene was degraded via hydration to acetaldehyde and further complete oxidation. Although acetylene hydratase activity could be proven undoubtedly only once (DeBont and Peck 1980) there is no doubt that acetaldehyde is the first intermediate in aerobic acetylene degradation (Kanner and Bartha 1982; R. Bartha, personal communication). Acetylene, unlike ethylene, appears to be attacked by a hydratase rather than an oxygenase enzyme; oxygen only acts as terminal electron acceptor and even inhibits the acetylene hydratase reaction at higher concentrations (DeBont and Peck 1980).

Anaerobic degradation of acetylene was demonstrated with estuarine sediment samples in the complete absence of oxygen (Culbertson et al. 1981). The reaction was inhibited by air and by antibiotics, and acetate was identified as an intermediate of sulfate-dependent acetylene oxidation. Enrichment cultures of anaerobic acetylene-oxidizing bacteria which formed acetaldehyde, acetate, and ethanol as intermediates were obtained, however, the bacteria involved could not be identified (C. W. Culbertson and R. S. Oremland, Abstr. 3rd Int. Symp. Microb. Ecol., East Lansing, Michigan, 1983, A-4). Anaerobic acetylene decomposition was also demonstrated with anoxic soil samples (Watanabe and De Guzman 1980).

Studies were initiated some years ago in the author's laboratory on the limitations of anaerobic biodegradation of hydrocarbons. Enrichment cultures with various sediment samples and acetylene as substrate did not produce significant amounts of methane because acetylene, just as ethylene, strictly inhibits methanogenic bacteria (Elleway et al. 1971; Oremland and Taylor 1975; Sprott et al. 1982; Schink 1985b). Enrichments were therefore started with sulfate as electron acceptor, but acetylene degradation did not depend on the presence of sulfate. The isolates which were finally obtained did not reduce sulfate but fermented acetylene to nearly equal amounts of acetate and ethanol. Thus, acetylene fermentation was not linked to syntrophic sulfate reduction as this was previously assumed (Culbertson et al. 1981). The isolated strains were strictly anaerobic and very similar to other Gram-negative rod-shaped bacteria isolated in the same laboratory with polyethylene glycol, 2,3-butanediol, acetoin, and 1,2-diols as substrates (Schink and Stieb 1983; Schink 1984; Eichler and Schink 1985). To my knowledge, these are the first pure cultures of strict anaerobes degrading a hydrocarbon in the absence of oxygen.

The pathway of acetylene degradation was studied with a freshwater isolate, strain WoAcy 1. Alcohol dehydrogenase, aldehyde dehydrogenase, phosphate acetyltransferase, and acetate kinase were found in high activities suggesting that acetylene was metabolized via hydration to acetaldehyde (Fig. 4). Unfortunately, acetylene hydratase activity could not be demonstrated in cell extracts, either because this enzyme was destroyed during cell disruption, or because it requires specific unknown cofactors. The acetylene hydratase of *Rhodococcus* sp. did not depend on cofactors (DeBont and Peck 1980), and this was true also for acetylene dicarboxylate hydratase (Yamada and Jakoby 1958). Additions of thiamine pyrophosphate or coenzyme B_{12} to the reaction mixture in the present study were unsuccessful, too. Involvement of acetaldehyde in anaerobic acetylene degradation is supported by the appearance of traces of acetaldehyde during acetylene degradation, by accumulation of acetaldehyde during acetylene degradation in dense cell suspensions, and by the fact that all substrates degraded by the new isolates can be metabolized via acetaldehyde as a central intermediate (Fig. 4). Glycerol and 1,2-propanediol take analogous pathways via 3-hydroxypropionaldehyde and propionaldehyde, respectively. In the latter case, the aldehyde accumulated to measurable concentrations, and it was further demonstrated that acetate provided as substrate for cell carbon assimilation could in part be reduced via acetaldehyde to ethanol. This finding again corroborates the hypothesis that acetaldehyde plays a central role in dissimilatory and assimilatory metabolism of these isolates. Uncontrolled accumulation of acetaldehyde could also be the reason for the observed inhibition of acetylene metabolism at enhanced acetylene concentrations.

If the suggested metabolic scheme (Fig. 4) is true, acetylene is so far the only hydrocarbon which is metabolized in the absence and presence of molecular oxygen in the same manner. The energetics of acetylene fermentation deserve some further discussion. Hydration to acetaldehyde is a highly exergonic reaction (calculations after Thauer et al. 1977):

$$C_2H_2 + H_2O \rightarrow CH_3CHO \qquad \Delta G'_0 = -111.9 \text{ kJ}.$$

The further disproportionation to acetate and ethanol is by far less exergonic:

$$2CH_3CHO + H_2O \rightarrow CH_3CH_2OH + CH_3COO^- + H^+ \qquad \Delta G'_0 = -17.3 \text{ kJ per mol aldehyde}.$$

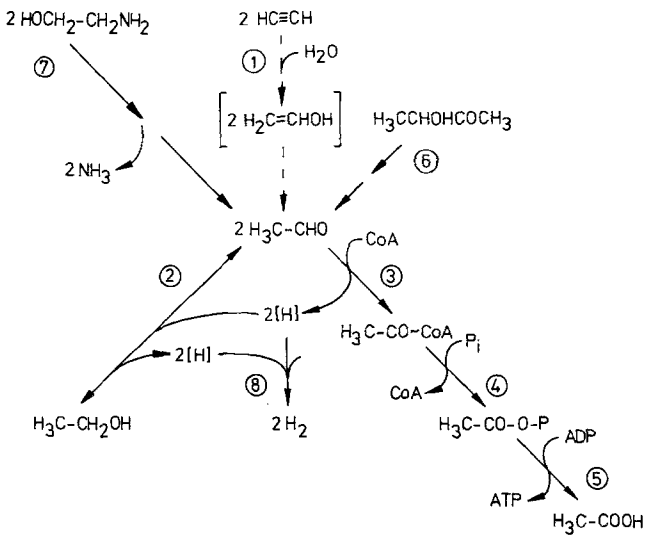

Fig. 4. Hypothetical scheme of energy metabolism of strain WoAcy 1. Numbers in circles stand for the following enzymes: (*1*) acetylene hydrating enzyme system, (*2*) alcohol dehydrogenase. (*3*) aldehyde dehydrogenase, (*4*) phosphate acetyltransferase, (*5*) acetate kinase, (*6*) acetoin dehydrogenase, (*7*) ethanolamine ammonia lyase, (*8*) Hydrogenase. Enzymes 2–6 and 8 were determined in crude cell extracts (see Table 2); the activity of enzyme system 1 could only be demonstrated with unbroken cell suspensions

These calculations clearly demonstrate that there is no need for a syntrophic cooperation of acetylene fermenters with sulfate reducers as this was previously assumed (Culbertson et al. 1981).

The cell yields obtained in the present study (about 3.5 g/mol acetylene) are low compared with the total change of Gibbs free energy of acetylene fermentation to acetate and ethanol (−129.2 kJ). If a growth yield of about 10 g dry cell matter per mol ATP is assumed – as this was observed with numerous anaerobes growing with simple C-2 and C-3 compounds (Stouthamer 1979) – it appears that the new isolates conserve only the free energy available in the acetate kinase reaction (0.5 mol ATP per mol acetylene) and not the amount of free energy available from acetylene hydration. This resembles again the situation of 1,2-diol-fermenting *Pelobacter* strains which do not conserve the free energy of diol dehydration either (Schink and Stieb 1983; Schink 1984). The high growth yield obtained with aerobic acetylene oxidizers (about 38 g per mol acetylene; Kanner and Bartha 1979) can easily be brought forth by complete aerobic oxidation of the acetaldehyde residue, and is not likely to include energy conservation in the acetylene hydrase reaction either.

Hydrations of nitriles and amides are reactions quite similar to acetylene hydration. *Rhodococcus* and *Nocardia rhodochrous* strains were described to grow aerobically with these compounds (Miller and Gray 1982; Collins and Knowles 1983) and again both these strains may be taxonomically identical. The anaerobic isolates described in this paper did not grow with cyanide, acetonitril or acetamide, but it cannot be excluded that they are able to hydrolyze these compounds as well. Since these substrates are rather unstable in the sulfide-reduced medium used, this problem needs further investigation in the future.

The ecological meaning of acetylene fermentation in anoxic environments is open to discussion. Certainly, utilization of other substrates such as ethanolamine, choline, glycerol, or syntrophic oxidation of ethanol play a more important role in energy metabolism of our isolates in nature than acetylene fermentation. It can be speculated that the observed hydration of acetylene to acetaldehyde is only a byfunction of a highly active, unspecific hydrase enzyme which mainly acts in the natural environment in detoxification of acetylenic compounds, nitriles, cyanides etc.

Taxonomy

The freshwater strains WoAcy 1, KoAcy 23, and the marine isolate GhAcy 3 appeared to be quite similar in all properties examined. The marine isolate GhAcy 1 was slightly different from the others in depending on saltwater medium, having rounded instead of pointed cell ends, and forming yellowish instead of white lens-shaped colonies in agar shake cultures. All isolates resemble morphologically and physiologically very much the recently isolated *Pelobacter* strains *P. venetianus* (Schink and Stieb 1983) and *P. carbinolicus* (Schink 1984), however, are unable to use polyethyleneglycol, ethylene glycol, or 2,3-butanediol. These two species, on the other hand, do not grow with acetylene. Since also the guanine plus cytosine content of strain WoAcy 1 (57.1%) differs considerably from those of the other two species (52.2% and 52.3%) it appears justified to establish a new species, *Pelobacter acetylenicus*.

P. acetylenicus sp. nov. a.ce.ty.le' ni.cus. M.L. adj. referring to acetylene utilization.

Rod-shaped cells, 0.6–0.8 × 1.5–4 µm in size, with slightly pointed ends, single, in pairs, or in chains. Motile in young cultures, Gram-negative, non-sporeforming.

Strictly anaerobic chemoorganotroph, acetylene, acetoin, ethanolamine, choline, 1,2-propanediol and glycerol used as substrate, the latter two in the presence of acetate. Substrates fermented to acetate and ethanol or the respective higher acids and alcohols. Ethanol oxidized to acetate in the presence of hydrogen-scavenging anaerobes. Grows in freshwater medium as well as in the presence of 2% (w/v) sodium chloride. Sulfate, sulfur, thiosulfate, sulfite, or nitrate not reduced. Indole not formed, gelatine or urea not hydrolyzed. No catalase activity, no cytochromes.

Substrates that did not support growth included ethylene, acetaldehyde (1–5 mM), potassium cyanide, acetonitril, acetamide, ethylene glycol, di-ethylene glycol, polyethyleneglycol, glycolate, glyoxylate, glycolaldehyde, betain, methanol (5 mM), trimethoxybenzoate, formate, malate, fumarate, lactate, pyruvate, tartrate, citrate, aspartate, glutamate, casamino acids, yeast extract, xylose, arabinose, fructose, glucose, mannose, lactose, sucrose, maltose, salicin, mannitol, melezitose, raffinose, sorbose, rhamnose, trehalose. All substrates were given 10 mM or 0.1% (w/v) unless stated otherwise. Selective enrichment from anoxic sediments in mineral medium with acetylene (10–20% in the gas phase) as sole substrate. pH range: 6.5–7.5. Temperature range: 15–40° C, optimum at 28–34° C.

DNA base ratio: 57.1 ± 0.2 mol% G + C (thermal denaturation).

Habitats: anoxic muds of freshwater and marine origin.

Type strain: WoAcy 1, DSM 2348, deposited in Deutsche Sammlung von Mikroorganismen, Göttingen.

Acknowledgement. The author is indebted to Prof. Dr. N. Pfennig for generous support and valuable discussions, to Waltraud Dilling and Elisabeth Kayser for technical help, and to R. S. Oremland and R. von Bartha for discussions and helpful hints. This study was in part financed by the Deutsche Forschungsgemeinschaft.

References

American Public Health Association Inc (ed) (1969) Standard methods for the examination of water and wastewater including bottom sediments and sludge. New York, pp 604–609

Atlas RM (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol Rev 45:180–209

Bayer O (1954) Sauerstoffverbindungen. II. Teil 1. Aldehyde. In: Müller E (ed) Methoden der organischen Chemie, Bd. 7, 1; (Houben-Weyl). Thieme, Stuttgart, pp 449–451

Bergmeyer HU (1974) Methoden der enzymatischen Analyse, 3rd edn. Verlag Chemie, Weinheim

Birch-Hirschfeld L (1932) Die Umsetzung von Acetylen durch *Mycobacterium lacticola*. Zentbl Bakteriol Parasitenkd Infektionskr Hyg Abt 2, 86:113–130

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Collins PA, Knowles CJ (1983) The utilization of nitriles and other aliphatic and aromatic nitriles and amides. J Gen Microbiol 129:711–718

Culbertson CW, Zehnder AJB, Oremland RS (1981) Anaerobic oxidation of acetylene by estuarine sediments and enrichment cultures. Appl Environ Microbiol 41:396–403

Davis JB, Yarbrough HF (1966) Anaerobic oxidation of hydrocarbons by *Desulfovibrio desulfuricans*. Chem Geol 1:137–144

DeBont JAM, Peck MW (1980) Metabolism of acetylene by *Rhodococcus* A 1. Arch Microbiol 127:99–104

DeBont JAM, Primrose SB, Collins MD, Jones D (1980) Chemical studies on some bacteria which utilize lower unsaturated hydrocarbons. J Gen Microbiol 117:97–102

Duncan CL, Strong DH (1968) Improved medium for sporulation of *Clostridium perfringens*. Appl Microbiol 16:82–89

Eichler B, Schink B (1985) Fermentation of primary alcohols and diols and pure culture of syntrophically alcohol-oxidizing anaerobes. Arch Microbiol (submitted)

Elleway RF, Sabine JR, Nicholas DJD (1971) Acetylene reduction by rumen microflora. Arch Mikrobiol 76:277–291

Foster JW (1962) Hydrocarbons as substrates for microorganisms. Antonie van Leeuwenhoek 7 Microbiol Serol 28:241–274

Gibson DT (1975) Microbial degradation of hydrocarbons. In: Goldberg ED (ed) The nature of seawater. Dahlem Konferenzen, Berlin. Abakon Verlagsgesellschaft, Berlin, pp 667–696

Goa J (1953) A microbiuret method for protein determination; determination of total protein in cerebrospinal fluid. Scand J Clin Lab Invest 5:218–222

Gordon AJ, Ford RA (1972) The chemist's companion. A handbook of practical data, techniques, and references. Wiley, New York London

Hollaus F, Sleytr U (1972) On the taxonomy and fine structure of some hyperthermophilic saccharolytic clostridia. Arch Mikrobiol 86:129–146

Kanner D, Bartha R (1979) Growth of *Nocardia rhodochrous* on acetylene gas. J Bacteriol 139:225–230

Kanner D, Bartha R (1982) Metabolism of acetylene by *Nocardia rhodochrous*. J Bacteriol 150:989–992

Kuenen JG, Veldkamp H (1972) *Thiomicrospira pelophila* nov. gen., nov. sp., a new obligately chemolithotrophic colourless sulfur bacterium. Antonie van Leeuwenhoek 7 Microbiol Serol 38:241–256

Miller JM, Gray DO (1982) The utilization of nitriles and amides by a *Rhodococcus* species. J Gen Microbiol 128:1803–1809

Novelli GD, ZoBell CE (1944) Assimilation of petroleum hydrocarbons by sulfate-reducing bacteria. J Bacteriol 47:447–448

Oberlies G, Fuchs G, Thauer RK (1980) Acetate thiokinase and the assimilation of acetate in *Methanobacterium thermoautotrophicum*. Arch Microbiol 128:248–252

Odom JM, Peck HD (1981) Localization of dehydrogenases, reductases and electron transfer components in the sulfate-reducing bacterium *Desulfovibrio gigas*. J Bacteriol 147:161–169

Oremland RS, Taylor BF (1975) Inhibition of methanogenesis in marine sediments by acetylene and ethylene: validity of the acetylene reduction assay for anaerobic microcosms. Appl Microbiol 30:707–709

Perry JJ (1979) Microbial cooxidations involving hydrocarbons. Microbiol Rev 43:59–79

Pfennig N (1978) *Rhodocyclus purpureus* gen. nov. and sp. nov., a ring-shaped, vitamin B_{12}-requiring member of the family Rhodospirillaceae. Int J Syst Bacteriol 28:283–288

Rosenfeld WD (1947) Anaerobic oxidation of hydrocarbons by sulfate reducing bacteria. J Bacteriol 54:664–665

Schink B (1984) Fermentation of 2,3-butanediol by *Pelobacter carbinolicus* sp. nov. and *Pelobacter propionicus*, sp. nov., and evidence for propionate formation from C_2 compounds. Arch Microbiol 137:33–41

Schink B (1985a) Degradation of unsaturated hydrocarbons by methanogenic enrichment cultures. FEMS Microbiol Ecol 31 (in press)

Schink B (1985b) Inhibition of methanogenesis by ethylene and other unsaturated hydrocarbons. FEMS Microbiol Ecol 31 (in press)

Schink B, Pfennig N (1982) Fermentation of trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a new strictly anaerobic, non-sporeforming bacterium. Arch Microbiol 133: 195–201

Schink B, Schlegel HG (1979) The membrane-bound hydrogenase of *Alcaligenes eutrophus*. I. Solubilization, purification, and biochemical properties. Biochim Biophys Acta 567:315–324

Schink B, Stieb M (1983) Fermentative degradation of polyethylene glycol by a new, strictly anaerobic, Gram-negative, non-sporeforming bacterium, *Pelobacter venetianus* sp. nov. Appl Environ Microbiol 45:1905–1913

Schütz H, Radler F (1984) Propanediol-1,2-dehydratase and metabolism of glycerol of *Lactobacillus brevis*. Arch Microbiol 139:366–370

Sprott GD, Jarrell KF, Shaw KM, Knowles R (1982) Acetylene as an inhibitor of methanogenic bacteria. J Gen Microbiol 128:2453–2462

Stouthamer AH (1979) The search for correlation between theoretical and experimental growth yields. In: Quayle JR (ed) International review of biochemistry, microbial biochemistry, vol 21. University Park Press, Baltimore, pp 1–47

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

Toraya T, Honda S, Fukui S (1979) Fermentation of 1,2-propanediol and 1,2-ethanediol by some genera of Enterobacteriaceae, involving coenzyme B_{12}-dependent diol dehydratase. J Bacteriol 139:39–47

Watanabe I, De Guzman MR (1980) Effect of nitrate on acetylene disappearance from anaerobic soil. Soil Biol Biochem 12:193–194

Widdel F, Pfennig N (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. Arch Microbiol 129:395–400

Widdel F, Kohring G-W, Mayer F (1983) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. Arch Microbiol 134:286–294

Yamada EW, Jakoby WB (1958) Enzymatic utilization of acetylenic compounds. I. An enzyme converting acetylenedicarboxylic acid to pyruvate. J Biol Chem 233:706–711

ZoBell CE (1946) Action of microorganisms on hydrocarbons. Bacteriol Rev 10:1–49

ZoBell CE, Prokop JF (1966) Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Z Allg Mikrobiol 6:143–162

Received February 8, 1985/Accepted May 8, 1985