

Syntrophism among Prokaryotes

BERNHARD SCHINK AND ALFONS J. M. STAMS

Introduction: Concepts of Cooperation in Microbial Communities, Terminology

The study of pure cultures in the laboratory has provided an amazingly diverse diorama of metabolic capacities among microorganisms, and has established the basis for our understanding of key transformation processes in nature. Pure culture studies are also prerequisites for research in microbial biochemistry and molecular biology. However, desire to understand how microorganisms act in natural systems requires the realization that microorganisms don't usually occur as pure cultures out there, but that every single cell has to cooperate or compete with other micro- or macroorganisms. The pure culture is, with some exceptions such as certain microbes in direct cooperation with higher organisms, a laboratory artifact. Information gained from the study of pure cultures can be transferred only with great caution to an understanding of the behavior of microbes in natural communities. Rather, a detailed analysis of the abiotic and biotic life conditions at the microscale is needed for a correct assessment of the metabolic activities and requirements of a microbe in its natural habitat.

In many cases, relationships of bacteria with other organisms may be relatively unimportant, as appears to be the case with most aerobes: they can usually degrade even fairly complex substrates to water and carbon dioxide without any significant cooperation with other organisms. Nutritional cooperation may exist, but may be restricted to the transfer of minor growth factors, such as vitamins, from one organism to the other. However, we have to realize that this assumption is based on experience gained from pure cultures that were typically enriched and isolated in simple media, and the selection aimed at organisms that were easy to handle, independent of possible interactions with others. Estimations assume that we know only a small fraction of the microorganisms present in nature, perhaps 0.1–1.0%.

Thus, we cannot exclude that other bacteria out there might depend to a large extent on cooperation with partner microbes, and perhaps this is just one of the reasons why we failed so far to isolate them.

Anaerobic microorganisms, on the other hand, depend to a great extent on the cooperation of several metabolic types of bacteria in feeding chains. The complete conversion of complex organic matter, e.g., cellulose, to methane and carbon dioxide in a lake sediment is catalyzed by the concerted action of at least four different metabolic groups of bacteria, including primary fermenters, secondary fermenters, and at least two types of methanogenic archaeobacteria (Bryant, 1979; McInerney, 1988; Stams, 1994; Schink, 1991; Schink, 1997). The degree of mutual dependence among these different metabolic groups ("functional guilds") can vary considerably; whereas the latter members in the feeding line always depend on the former ones for substrate supply, they may also influence significantly the former chain members by removal of metabolic products. In an extreme case, this can mean that the fermenting bacterium depends entirely on cooperation with a methanogen to fulfill its function in, e.g., methanogenic fatty acid oxidation. This type of cooperation is called "syntrophic."

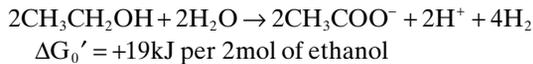
Mutual metabolic dependencies also can emerge from the cooperation of phototrophs with sulfur- or sulfate-reducing bacteria. Sulfur-reducing, acetate-oxidizing, chemotrophic bacteria such as *Desulfuromonas acetoxidans* and phototrophic green sulfide-oxidizing bacteria like *Chlorobium* sp. can cooperate closely in a phototrophic conversion of acetate plus CO₂ to bacterial cell mass, using a sulfide/sulfur cycle as an electron shuttle system between both. The two partners cooperate very closely also in this system for which the term "syntrophy" was originally coined (Biebl and Pfennig, 1978).

Syntrophy is a special case of symbiotic cooperation between two metabolically different types of bacteria which depend on each other for degradation of a certain substrate, typically

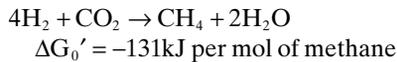
through transfer of one or more metabolic intermediate(s) between the partners. The pool size of the shuttling intermediate has to be kept low to allow efficient cooperation.

The term “syntrophy” should be restricted to those cooperations in which partners depend on each other to perform the metabolic activity observed and in which the mutual dependence cannot be overcome by simply adding a cosubstrate or any type of nutrient. A classical example is the “*Methanobacillus omelianskii*” culture (Barker, 1940), which was later shown to be a coculture of two partner organisms, the S strain and the strain M.o.H. (Bryant et al., 1967). Both strains cooperate in the conversion of ethanol to acetate and methane by interspecies hydrogen transfer, as follows:

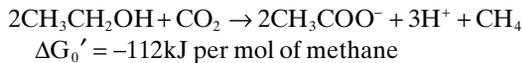
Strain S:



Strain M.o.H.



Coculture:



Thus, the fermenting bacterium cannot be grown with ethanol in the absence of the hydrogen-scavenging partner organism because it carries out a reaction that is endergonic under standard conditions. The first reaction can occur and provide energy for the first strain only if the hydrogen partial pressure is kept low enough ($<10^{-3}$ bar) by the methanogen. Therefore, neither partner can grow with ethanol alone, and the degradation of ethanol depends on the cooperating activities of both.

We avoid in this article the term “consortium” which is quite often used to describe any kind of enrichment cultures cooperating in whatever way. This term was originally coined for the structured phototrophic aggregates *Pelochromatium* and *Chlorochromatium* etc. and should be restricted to such spatially well-organized systems (Pfennig, 1980; Overmann, 2001).

Electron Flow in Methanogenic and Sulfate-Dependent Degradation

The degradation of complex organic matter to methane and CO_2 is a process widespread in anoxic environments which receive only a limited supply of oxygen, nitrate, sulfate, or oxidized iron or manganese species. Methanogenesis is the typical terminal electron-accepting process in freshwater sediments rich in organic matter, in

swamps or water-logged soils such as rice paddies, or in anaerobic wastewater and sewage treatment plants. It is also an important process in fermentations occurring in the intestinal tract of animals, especially of ruminants. Methanogenic degradation is the least exergonic process in comparison to aerobic degradation or the alternative anaerobic respiration. Conversion of hexose to methane and carbon dioxide releases only 15% of the energy that would be available in aerobic degradation, and this small energy yield of methanogenic degradation may be the reason why methanogenesis is the last one to occur, after the other electron acceptors have been reduced.

The carbon and electron flow in methanogenic degradation of complex organic matter follows a rather simple pattern. Polymers (polysaccharides, proteins, nucleic acids, and also lipids) are first converted to oligo- and monomers (sugars, amino acids, purines, pyrimidines, fatty acids, and glycerol), typically through the action of extracellular hydrolytic enzymes. These enzymes are produced by the “classical” primary fermenting bacteria which ferment the monomers further to fatty acids, branched-chain fatty acids, succinate, lactate, alcohols, aromatic acids, etc. (group 1; Fig. 1). Some of these fermentation products, such as acetate, H_2 , CO_2 and other one-carbon compounds, can be used directly by methanogens which convert them to methane and carbon dioxide (groups 2 and 3; Fig. 1). For methanogenic degradation of other fermentation products, e.g., fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, branched-chain and aromatic fatty acids, a further group of fermenting bacteria, the so-called “secondary fermenters” or “obligate proton reducers” (group 4; Fig. 1) is needed. These bacteria convert their substrates to acetate, carbon dioxide, hydrogen, perhaps also formate, which are subsequently used by the methanogens.

The situation is slightly different in sulfate-rich anoxic habitats such as marine sediments. There, the primary processes of polymer degradation are carried out by primary fermenting bacteria which form the classical fermentation products. Different from methanogens, sulfate-reducing bacteria are metabolically versatile, and a broad community of sulfate reducers can use all products of primary fermentations, and oxidize them to carbon dioxide, simultaneously reducing sulfate to sulfide (Widdel, 1988). As a consequence, the complete oxidation of complex organic matter to carbon dioxide with simultaneous sulfate reduction proceeds in a two-step process and does not depend on syntrophic fermentations. One might add that this two-step scheme might be augmented also by cooperative sidepaths taken by different types of sulfate-reducing bac-

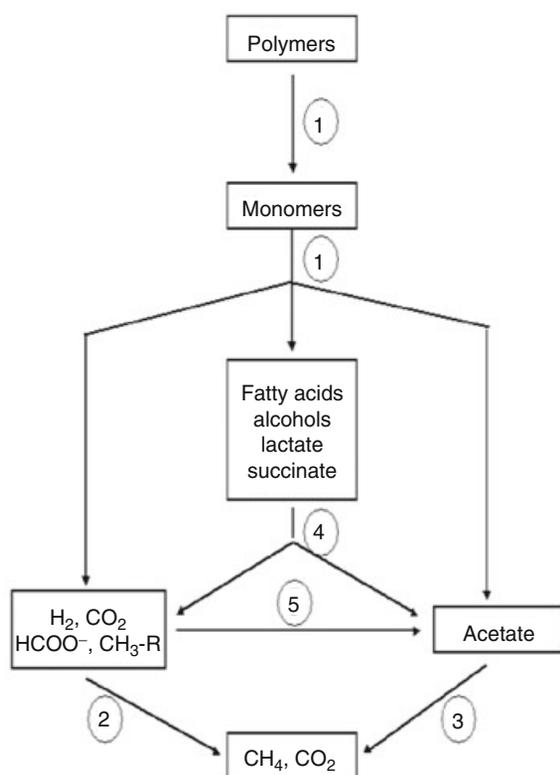


Fig. 1. Carbon and electron flow in the methanogenic degradation of complex organic matter. Groups of prokaryotes involved: 1) primary fermentative bacteria; 2) hydrogen-oxidizing methanogens; 3) acetate-cleaving methanogens; 4) secondary fermenting bacteria (syntrophs); and 5) homoacetogenic bacteria.

teria, e.g., completely and incompletely oxidizing ones (Küver and Widdel, 2001), but such cooperative activities are not required for complete sulfate-dependent oxidation of organic matter.

In methanogenic and sulfate-rich environments, the primary fermenting bacteria (group 1) profit from the activities of the hydrogen-oxidizing partners at the end of the degradation chain as well. A low hydrogen partial pressure ($<10^{-4}$ bar) allows electrons at the redox potential of NADH (-320 mV) to be released as molecular hydrogen, and fermentation patterns can shift to more acetate, CO_2 , and hydrogen production rather than to ethanol or butyrate formation, thus allowing additional ATP synthesis via substrate-level phosphorylation, as opposed to production of reduced fermentation products (ethanol, lactate and butyrate). Thus, such fermenting bacteria may profit from hydrogen-oxidizing partners, but they do not depend on such cooperation.

In a well-balanced anoxic sediment in which an active hydrogen-utilizing community maintains a low hydrogen partial pressure, the flux

of carbon and electrons goes nearly exclusively through the “outer” paths of the electron flow scheme (Fig. 1), and therefore reduced fermentation intermediates play only a minor role. Nonetheless, the flux through the “central” paths will never become zero because long-chain and branched-chain fatty acids and others are always produced in the fermentation of lipids, and amino acids as well. The reduced intermediates of the central path become more important if the hydrogen pool increases for any reason, e.g., excess supply of fermentable substrate, inhibition of hydrogenotrophic methanogens due to a drop in pH (<6.0), or to the presence of toxic compounds, etc. Under such conditions, the pools of fatty acids increase and might even shift the pH further downwards, thus inhibiting the hydrogenotrophic methanogens even further. The consequence may be that the whole system “turns over,” meaning that methanogenesis ceases entirely and the fermentation stops with accumulation of huge amounts of foul-smelling fatty acids, as this is encountered with ill-balanced anaerobic sewage digestors. Obviously, the hydrogen/formate-utilizing methanogens act as the primary regulators in the total methanogenic conversion process (Bryant, 1979; Zehnder, 1978; Zehnder et al., 1982), and the syntrophically fatty acid-oxidizing bacteria are affected most severely by a failure in methanogenic hydrogen or formate removal.

The function of homoacetogenic bacteria (group 5; Fig. 1) in the overall process is less well understood. They connect the pool of one-carbon compounds and hydrogen with that of acetate. Owing to their metabolic versatility, they can participate also in sugar fermentation and degradation of special substrates such as *N*-methyl compounds or methoxylated phenols (Schink, 1994). In certain environments, e.g., at lower pH or low temperature, they may even successfully compete with hydrogenotrophic methanogens and take over their function to a varying extent (see below).

Energetic Aspects

Anaerobes grow with small amounts of energy, and syntrophically cooperating anaerobes are extremely skilled in the exploitation of minimal energy spans. Synthesis of ATP as the general currency of metabolic energy in living cells requires $+32$ kJ per mol at equilibrium under standard conditions; under the conditions assumed to prevail in an actively growing cell ($[\text{ATP}] = 10$ mM; $[\text{ADP}] = 1$ mM; and $[\text{P}_i] = 10$ mM), $+49$ kJ per mol is required (Thauer et al., 1977). In addition, part of the total energy

budget is always lost in irreversible reaction steps as heat, thus rendering the overall metabolic process irreversible. This heat loss (on average about 20 kJ per mol ATP) has to be added to the above value, which gives a total of about 70 kJ per mol ATP synthesized irreversibly in the living cell. This is the minimum amount of energy required for the synthesis of one mol of ATP in all known metabolic systems (Schink, 1990). One may argue that (especially under conditions of energy limitation) an organism may waste less energy in heat production, or that it may operate at an energy charge considerably lower than that quoted above for well-growing *Escherichia coli* cells. Nonetheless, one cannot expect the energy requirement for irreversible ATP synthesis to go substantially below about +60 kJ per mol.

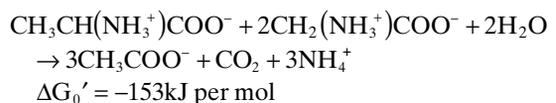
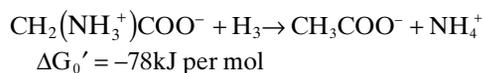
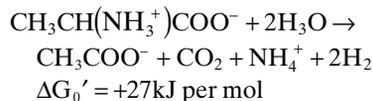
The key postulate of the Mitchell theory of respirative ATP synthesis is that ATP formation is coupled to a vectorial transport of charged groups, typically protons, across a semipermeable membrane (Mitchell, 1966). For several years, it was widely accepted that three protons cross the membrane (either of bacteria or mitochondria) per ATP hydrolyzed. As a consequence, the smallest quantum of metabolically convertible energy is that of an ion transported across the cytoplasmic membrane, equivalent to one third of an ATP unit. Combined with the calculations above, this means that a bacterium needs a minimum of about -20 kJ per mol reaction to exploit a reaction's free energy change (Schink and Thauer, 1988; Schink, 1990).

On the basis of studies on the structure and function of F_1 - F_0 ATPases in recent years, the stoichiometry of ATP synthesis versus proton translocation appears not to be as strictly fixed as suggested above. Rather, the system may operate like a sliding clutch, meaning that at very low energy input, the energy transfer into ATP synthesis may be substoichiometric. Moreover, the stoichiometry is not necessarily three protons per one ATP, but is governed by the number of subunits arranged in the F_0 versus the F_1 complex. This concept would allow also stoichiometries of 4 to 1, perhaps even 5 to 1 (Engelbrecht and Junge, 1997; Cherepanov et al., 1999; Stock et al., 1999; Dimroth, 2000; Seelert et al., 2000). As a consequence, the minimum energy increment that can still be used for ATP synthesis may be as low as -15 or -12 kJ per mol reaction. In some cases, to make their living, bacteria cooperating in syntrophic fermentations are limited to this range of energy; Hoehler et al. (2001) calculated from metabolite concentrations in natural habitats for the partner bacteria cooperating in syntrophic conversions minimum amounts of exploitable energy in the range of -10 to -19 kJ per mol reaction.

Degradation of Amino Acids

During protein hydrolysis, a complex mixture of amino acids and small peptides is produced. These amino acids and peptides can serve as energy substrates for anaerobic microorganisms. Detailed information on the anaerobic fermentation of amino acids can be found in Barker (1981) and McInerney (1988). Many anaerobic amino acid-degrading bacteria require complex mixtures of amino acids, perhaps because a one-sided diet causes a serious imbalance in the internal amino acid metabolism of these cells, but growth by fermentation of single amino acids is also common. Mixtures of amino acids are often degraded by coupled fermentation of pairs of amino acids via the Stickland reaction. Table 1 summarizes some oxidative conversions of amino acids. In the classical Stickland fermentation, these oxidation reactions are coupled within the same organism to reduction of other amino acids such as glycine or proline. Also phenylalanine, leucine, or compounds like sarcosine and betaine (Naumann et al., 1983; Gottschalk, 1986) can act as electron acceptors, and oxidation of leucine coupled to the reduction of acetate to butyrate has been described as well (Girbal et al., 1997).

These pure culture fermentations can also be catalyzed by cooperation of two different bacteria via interspecies hydrogen transfer, according to the following reactions with a mixture of alanine and glycine:



Thus, the electrons derived in amino acid degradation by a fermenting bacterium can be used in glycine reduction as shown, but can be transferred as well in the form of molecular hydrogen to sulfate-reducing, homoacetogenic or methanogenic partner bacteria, depending on the availability of such partner bacteria and their respective electron acceptors. That amino acid oxidation and glycine reduction can be uncoupled from each other has been shown in detail with *Eubacterium acidaminophilum* (Zindel et al., 1988). This bacterium can run either one of the first two reactions separately or combine them on its own, according to the third reaction, depending on the partner bacteria which act as hydrogen sources or sinks, and on the availability

Table 1. Changes of Gibbs free energies under standard conditions in hydrogen-releasing reactions during fermentation of amino acids.

Fermentation reaction	$\Delta G_0'$ (kJ per mol rct.)
Alanine + 2H ₂ O → acetate ⁻ + CO ₂ + NH ₄ ⁺ + 2H ₂	+2.7
Glycine + 2H ₂ O + H ⁺ → 2CO ₂ + NH ₄ ⁺ + 3H ₂	+17.8
Serine + H ₂ O → acetate ⁻ + CO ₂ + NH ₄ ⁺ + H ₂	-85.3 ^a
Threonine + H ₂ O → propionate ⁻ + CO ₂ + NH ₄ ⁺ + H ₂	-83.0 ^a
Histidine + 4H ₂ O + H ⁺ → glutamate ⁻ + CO ₂ + 2NH ₄ ⁺ + H ₂	^b
Proline + 2H ₂ O → glutamate ⁻ + H ⁺ + 2H ₂	^b
Glutamate ⁻ + 2H ₂ O + H ⁺ → propionate ⁻ + 2CO ₂ + NH ₄ ⁺ + 2H ₂	-16.6
Glutamate ⁻ + 2H ₂ O → 2 acetate ⁻ + CO ₂ + NH ₄ ⁺ + H ₂	-38.6 ^a
Aspartate ⁻ + 2H ₂ O + H ⁺ → acetate ⁻ + 2CO ₂ + NH ₄ ⁺ + 2H ₂	-24.1

^aThese fermentations may also allow growth in pure culture.

^bThese reactions are always coupled to further fermentation of glutamate.

All calculations are based on published tables (see Thauer et al., 1977; Dimroth, 1983). For H₂S and CO₂, values for the gaseous state were used.

of selenium in the medium which is required for expression of the active glycine reductase complex.

In methanogenic environments, methanogens can act as scavengers of reducing equivalents in the oxidation of amino acids, thus taking over the role of the reductive part of the Stickland reaction. Nagase and Matsuo (1982) observed that in mixed methanogenic communities, the degradation of alanine, valine and leucine was inhibited when methanogens were inhibited. Nanninga and Gottschal (1985) could stimulate the degradation of these amino acids by addition of hydrogen-scavenging sulfate-reducers. These early observations indicated a functional role for hydrogen consumption also in the degradation of amino acids.

Influence of Methanogens

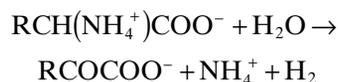
Methanogens can affect anaerobic oxidation in various ways. Fermentative oxidation of some amino acids can be coupled to hydrogen formation only if the hydrogen partial pressure is kept low, similar to the "*Methanobacillus omelianskii*" example described above. This results in an obligately syntrophic relationship between the fermenting microorganism and the methanogen. Some amino acids are degraded by the same fermenting bacterium either in pure culture or in mixed culture with methanogens; however, methanogens can cause a shift in metabolism resulting in a changed product formation pattern.

Clostridium sporogenes (Wildenauer and Winter, 1986; Winter et al., 1987), *Eubacterium acidaminophilum* (Zindel et al., 1988), *Acidaminobacter hydrogeniformans* (Stams and Hansen, 1984), *Aminomonas paucivorans* (Baena et al., 1999a), *Aminobacterium colombiense* and *Aminobacterium mobile* (Baena et al., 1998; Baena et al., 2000), as well as strain PA-1 (Barik et al., 1985) are mesophilic bacteria which degrade one or more amino acids in syntrophic

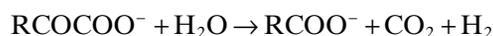
association with hydrogen-consuming anaerobes. Moderately thermophilic bacteria include *Caloramator coolhaasii* (Plugge et al., 2000), *Caloramator proteoclasticus* (Tarlera et al., 1997; Tarlera and Stams, 1999), *Thermanaerovibrio acidaminovorans* (previously named *Selenomonas acidaminovorans*; Cheng et al., 1992; Baena et al., 1999b), *Gelria glutamica* (Plugge et al., 2002) and *Clostridium P2* (Olygsson et al., 1993; Olygsson, 1994). There are probably many other fermenting bacteria with the ability to grow syntrophically with certain amino acids and to release reducing equivalents as molecular hydrogen; however, such capacities are only rarely checked.

Obligately Syntrophic Amino Acid Deamination

The initial step in the oxidative degradation of alanine, valine, leucine and isoleucine is an NAD(P)-dependent deamination to the corresponding α -keto acid, and the reaction, if coupled to reoxidation of NAD(P)H by proton reduction, would read as follows:



The $\Delta G_0'$ of this reaction is about +55 kJ per mol; thus, very efficient hydrogen consumption would be required to pull this reaction. The α -keto acid is converted further via oxidative decarboxylation to a fatty acid:



a reaction which is much more favorable ($\Delta G_0'$ about -52 kJ per mol). Therefore, it is not surprising that one does not observe bacteria catalyzing the first reaction step only, and that the organisms involved—as far as they have been tested—can grow in pure culture with α -keto

acids. Such α -keto acids were used successfully for the isolation of amino acid-degrading proton-reducing bacteria (Stams and Plugge, 1990). Conversion of the keto acids to the corresponding fatty acids likely proceeds through energy-rich CoA and phosphate derivatives, thus allowing ATP synthesis via substrate-level phosphorylation.

Eubacterium acidaminophilum degrades aspartate to acetate in syntrophic association with methanogens (Zindel et al., 1988). The degradation pathway is not known. Aspartate is either oxidized directly to oxaloacetate by means of an aspartate dehydrogenase or is degraded first to fumarate by aspartase activity. Fumarate is then converted to malate which is subsequently oxidized to oxaloacetate. In this pathway, the conversion of malate to oxaloacetate plus H_2 is most unfavorable; the $\Delta G_0'$ is +48 kJ per mol. Recently, a moderately thermophilic sugar-fermenting bacterium, *Gelria glutamica*, was isolated which can grow also by the analogous conversion of glutamate to propionate, provided that the hydrogen partial pressure is kept low by a methanogen (Plugge et al., 2002). In this bacterium, glutamate is first oxidatively deaminated to α -ketoglutarate, which is subsequently oxidatively decarboxylated to succinyl-CoA and further to propionate (Plugge et al., 2001). In coculture with methanogens, it is also able to oxidize proline to propionate. This is remarkable because proline is generally thought to be reductively degraded to aminovalerate. Similar to the above-mentioned amino acids, the aromatic amino acids phenylalanine, tryptophan and tyrosine might also support growth by oxidative deamination leading to phenylacetate, indolylacetate, *p*-hydroxyphenylacetate, respectively, as products. This, however, has not yet been demonstrated for syntrophic amino acid-degrading bacteria.

Syntrophic Arginine, Threonine and Lysine Fermentation

Acidaminobacter hydrogenoformans, *Aminomonas paucivorans* and *T. acidaminovorans* can grow with histidine, ornithine, arginine, lysine and threonine when cocultured with hydrogenotrophic bacteria (Stams and Hansen, 1984; Cheng et al., 1992; Baena et al., 1999a). Remarkably, *T. acidaminovorans* and *A. paucivorans* grow in pure culture with arginine, forming citrulline and/or ornithine as products. In this conversion, carbamyl phosphate is formed as an intermediate, which is further converted to yield ATP (Plugge and Stams, 2001). Ornithine and citrulline could be degraded when these bacteria were cocultured with a hydrogenotrophic methanogen. It is not clear which pathway is used for

ornithine degradation, and which metabolic step is hampering growth of these bacteria in pure culture.

Acidaminobacter hydrogenoformans degrades threonine and lysine in syntrophic association with a hydrogen-consuming anaerobe. Acetate is the main product of the degradation of these two amino acids, and large amounts of hydrogen are formed. In pure culture, these compounds do not support growth. The stoichiometry of the fermentation is not exactly known, and the pathways involved in these fermentations have not been resolved as yet.

Facultatively Syntrophic Growth with Amino Acids

The effect of methanogens on the metabolism of amino acid-fermenting anaerobes has been studied most extensively with glutamate. Glutamate fermentation is carried out by a variety of fastidious anaerobes, including a number of *Clostridium* species, *Peptostreptococcus asacharolyticus*, and *Acidaminococcus fermentans* (Gottschalk, 1986). These microorganisms ferment glutamate to acetate and butyrate by two different pathways, the β -methylaspartate or the hydroxyglutarate pathway (Buckel and Barker, 1974). In this fermentation, reducing equivalents formed in the oxidation of glutamate to acetate are disposed of either partly or completely by reductive formation of butyrate from acetate (acetyl-CoA).

Anaeromusa acidaminophila ("*Selenomonas acidaminophila*") ferments glutamate to acetate plus propionate (Nanninga et al., 1987; Baena et al., 1999b). In this bacterium, reducing equivalents are disposed of by reduction of pyruvate to propionate. However, in the last decade, several bacteria have been isolated which during growth on glutamate release reducing equivalents exclusively as hydrogen, both in the formation of acetate and the formation of propionate (Table 1). These microorganisms ferment glutamate to acetate only (*Caloramator coolhaasii* [Plugge et al., 2000] and *Caloramator proteoclasticus* [Tarlera and Stams, 1999]), propionate only (*Aminobacterium colombiense* [Baena et al. 1998] and *Gelria glutamica* [Plugge et al., 2002]) or acetate plus propionate (*A. hydrogenoformans* [Stams and Hansen, 1984; Meijer et al., 1999], *T. acidaminovorans* [Cheng et al., 1992; Baena et al., 1999b], and *Aminomonas paucivorans* [Baena et al., 1999a]), with CO_2 as coproduct (Table 1).

Acidaminobacter hydrogenoformans ferments glutamate to 2 acetate, 1 CO_2 , NH_3 and 1 H_2 , or to 1 propionate, 2 CO_2 , NH_3 and 2 H_2 (Table 1; Fig. 2). In pure culture, *A. hydrogenoformans* can also form formate. However, in the presence of the hydrogen-utilizing *Methanobrevibacter*

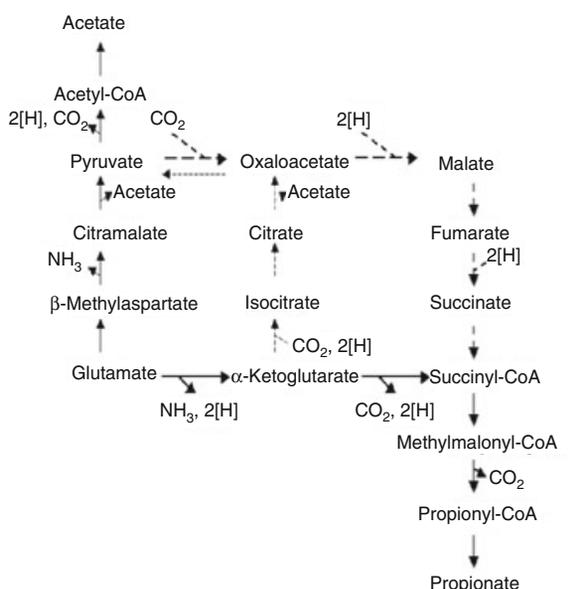


Fig. 2. Pathways involved in the fermentation of glutamate to propionate and acetate in anaerobic bacteria. Thin solid line (left part): acetate formation by the commonly used β -methylaspartate pathway. Thick solid line: oxidative formation of propionate as catalyzed by, e.g., *Gelria glutamica*. Dashed line: reductive formation of propionate as performed by *Anaeromusa acidaminophila*. Dotted line: acetate formation via enzymes of the reductive tricarboxylic acid (TCA) cycle as used by *Acidaminobacter hydrogenoformans* when cocultured with hydrogenotrophic methanogens.

arboriphilus, formate is not formed (Stams and Hansen, 1984). The ratio at which acetate and propionate are formed depends on the hydrogen partial pressure (Stams and Hansen, 1984). Propionate formation is favored at low hydrogen partial pressure, whereas at high hydrogen pressure mainly acetate is formed. Enzyme measurements revealed that the strain when grown in pure culture uses the β -methylaspartate pathway for acetate formation. However, if the bacterium is grown in coculture with a methanogen, both acetate and propionate appear to be formed via α -ketoglutarate (Stams et al., 1998). Reductive carboxylation of α -ketoglutarate leads via isocitrate to citrate which is cleaved to acetate and pyruvate, and pyruvate is converted further to acetate. Propionate formation occurs via oxidative decarboxylation of α -ketoglutarate to succinyl-CoA, which is further converted to propionate. Apparently, a crucial step that determines the degradation pathway is the oxidative deamination of glutamate to α -ketoglutarate which is highly endergonic if coupled to hydrogen formation. Under standard conditions, this conversion is energetically impossible ($\Delta G_0' = +60$ kJ per mol). However, at a hydrogen partial pressure of 10^{-5} atm, the $\Delta G_0'$ of this reaction would be +30 kJ per mol, and if the intracellular

NH_4^+ concentration would be 10 mM and the glutamate/ α -ketoglutarate ratio about 500, the $\Delta G_0'$ of this reaction would become about zero. A similar shift in product formation was described for *Aminomonas paucivorans* (Baena et al., 1999a).

Thermanaerovibrio acidaminovorans also forms acetate and propionate as products. Unlike the mesophilic organisms, this thermophile forms propionate already in pure culture. This bacterium uses the β -methylaspartate pathway for acetate formation both in pure culture and in coculture with a methanogen (Plugge et al., 2002) Some of the glutamate-fermenting bacteria also are able to grow with histidine, forming similar products and shifts in product formation as with glutamate. Histidine is supposed to be degraded through glutamate as an intermediate (Gottschalk, 1986).

Stickland Reaction versus Methanogenesis

Some of the bacteria which can degrade amino acids syntrophically, including *Clostridium sporogenes*, *Eubacterium acidaminophilum*, *Acidaminobacter hydrogenoformans* and *Caloramator proteoclasticus*, are able to perform a Stickland reaction. The environmental conditions under which the Stickland reaction is favored and conditions under which reducing equivalents are disposed of as molecular hydrogen remains to be determined. Energetically, the reductive conversion of glycine is more favorable than methanogenesis, homoacetogenesis, or sulfate reduction (Table 2). It is likely that in environments rich in amino acids, the Stickland reaction will dominate, whereas in environments with low amino acid supply and high methanogenic activity, reducing equivalents may be preferentially channeled to methanogenesis. Unfortunately, up to now little information was available on such comparative ecological aspects.

During growth of a coculture of *Caloramator proteoclasticus* and *Methanobacterium thermoautotrophium* Z245 in a medium with alanine plus glycine, about 60% of the reducing equivalents were channeled to methanogenesis when a dense culture of the methanogen was inoculated with 0.2% of a culture of *C. proteoclasticus*. However, if 1% inoculum was applied, all the reducing equivalents were used to reduce glycine.

Syntrophic Degradation of Fermentation Intermediates

Syntrophic Ethanol Oxidation

The case of "*Methanobacillus omelianskii*" is the classical example of interspecies hydrogen

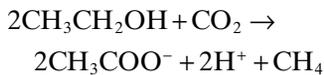
Table 2. Changes of Gibbs free energies under standard conditions in hydrogen-consuming reactions involved in interspecies hydrogen transfer.

	G ₀ ' (kJ per mol rct.)	G ₀ ' (kJ per electron pair)
4H ₂ + 2 CO ₂ → CH ₃ COO ⁻ + H ⁺ + 2H ₂ O	-94.9	-23.8
4H ₂ + CO ₂ → CH ₄ + 2H ₂ O	-131.0	-32.7
H ₂ + S ⁰ → H ₂ S	-33.9	-33.9
4H ₂ + SO ₄ ²⁻ + H ⁺ → HS ⁻ + 4H ₂ O	-151.0	-37.6
H ₂ C(NH ₃ ⁺)COO ⁻ + H ₂ → CH ₃ COO ⁻ + NH ₄ ⁺	-78.0	-78.0
Fumarate ²⁻ + H ₂ → succinate ²⁻	-86.0	-86.0

All calculations are based on published tables (see Thauer et al., 1977; Dimroth, 1983). For H₂S and CO₂, values for the gaseous state were used.

transfer. Both partners operate in an overall reaction process which becomes exergonic for the first partner only through maintenance of a low hydrogen partial pressure by the second partner. After description of the cooperative nature of this process, the original S-strain was lost, but other syntrophically ethanol-oxidizing bacteria have been isolated, such as *Thermoanaerobium brockii* (Ben-Bassat et al., 1981) and various *Pelobacter* strains (Schink, 1984; Schink, 1985a; Eichler and Schink, 1986). Also certain ethanol-oxidizing sulfate reducers such as *Desulfovibrio vulgaris* are able to oxidize ethanol in the absence of sulfate by hydrogen transfer to a hydrogen-oxidizing methanogenic partner.

Unfortunately, the energetics of this syntrophic cooperation are still unclear. The total reaction



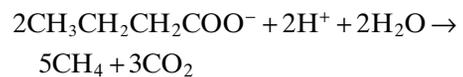
yields -112 kJ per 2 mol ethanol under standard conditions. On the side of the ethanol oxidizer, e.g., the "S-strain" of "*Methanobacillus omelianskii*" (Bryant et al., 1967) or other fermenting anaerobes with similar metabolic capacities such as *Pelobacter acetylenicus* (Schink, 1985a), ethanol dehydrogenase, acetaldehyde ferredoxin oxidoreductase (acetyl CoA-forming), phosphotransacetylase, and acetate kinase have been shown to be involved, forming one ATP per ethanol through substrate-level phosphorylation. Since the methanogenic hydrogen oxidizer requires at least one third of an ATP unit for growth (-20 kJ per reaction run, see above), only about -45 kJ is available to the ethanol oxidizer per mol ethanol oxidized, which is too little energy to form one full ATP. It has to be postulated, therefore, that part of the energy bound in ATP has to be reinvested somewhere to push the overall reaction and balance the energy budget, but this reverse electron transport system has not yet been identified. In syntrophically ethanol-oxidizing *Desulfovibrio* strains, the pathway leads from acetaldehyde directly to acetate, without a phosphorylation

step, which explains why *Desulfovibrio* cannot grow in this syntrophic association (Kremer et al., 1988).

Syntrophic Butyrate Oxidation

Similar cooperations have been described with syntrophic cultures degrading fatty acids. An overview of the reactions catalyzed is presented in Table 3; a list of described strains of syntrophically fermenting bacteria follows in Table 4. In general, degradation of fatty acids to acetate and hydrogen or, in the case of propionate, to acetate, hydrogen and CO₂, are reactions far more endergonic under standard conditions than ethanol oxidation. Consequently, for fatty acid degradation, the hydrogen partial pressure has to be decreased to substantially lower values (<10⁻⁴ bar) than with ethanol (<10⁻³ bar).

The energetic situation of the partner bacteria involved in butyrate conversion to methane and CO₂ has been discussed in detail in earlier publications (Wallrabenstein and Schink, 1994a; Schink, 1997). The overall reaction



yields under standard conditions a ΔG₀' of -177 kJ per 2 mol of butyrate. With concentrations better comparable to those prevailing in a natural habitat, e.g., a freshwater sediment or a sewage sludge digester (butyrate: 10 μM, CH₄: 0.7 bar, and CO₂: 0.3 bar), the free energy of this process changes to -140 kJ per 2 mol of butyrate. Since the overall process is shared by seven partial reactions (two are involved in the butyrate oxidation, one in CO₂ reduction to methane, and four in acetate cleavage) the free energy change is about -20 kJ per mol for every partial reaction, if the energy is shared by all reactions at equal rates. Measured partial pressures of hydrogen and acetate concentrations in active sewage sludge and various sediments (Zehnder et al., 1982) are in a range of 10⁻⁴-10⁻⁵ bar and 10-100 μM, respectively, which is in good agreement with the assumption of equal energy sharing in

Table 3. Changes of Gibbs free energies under standard conditions in hydrogen-releasing reactions during oxidation of fermentation intermediates.

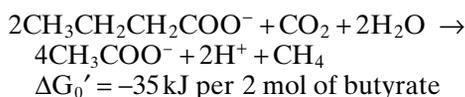
	G_0' (kJ per mol rct.)	No. of electron pairs
Primary alcohols		
$\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$	+9.6	2
Fatty acids		
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{H}^+ + 2\text{H}_2$	+48.3	2
$\text{CH}_3\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{CO}_2 + 3\text{H}_2$	+76.0	3
$\text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 4\text{H}_2$	+94.9	4
$\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{COO}^- + \text{CO}_2 + 2\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COO}^- + 2\text{H}^+ + \text{H}_2$	+25.2	1
Glycolic acid		
$\text{CH}_2\text{OHCOO}^- + \text{H}^+ + \text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 3\text{H}_2$	+19.3	3
Aromatic compounds		
$\text{C}_6\text{H}_5\text{COO}^- + 6\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COO}^- + 2\text{H}^+ + \text{CO}_2 + 3\text{H}_2$	+49.5	3
$\text{C}_6\text{H}_5\text{OH} + 5\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COO}^- + 3\text{H}^+ + 2\text{H}_2$	+10.2	2
Amino acids		
$\text{CH}_3\text{CH}(\text{NH}_3^+)\text{COO}^- + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{NH}_4^+ + \text{CO}_2 + 2\text{H}_2$	+2.7	2

All calculations are based on published tables (see Thauer et al., 1977; Dimroth 1983). For H_2S and CO_2 , values for the gaseous state were used.

such a cooperative community. The corresponding energy yields of about -20 kJ per mol reaction are confirmed by growth yield determinations with pure cultures of methanogens (Schönheit et al., 1980; Zehnder et al., 1982).

On the side of the syntrophic fermenting partner, 1 ATP is synthesized by substrate-level phosphorylation through thiolitic acetoacetyl-CoA cleavage (Wofford et al., 1986), but part of this energy has to be reinvested in reverse electron transport to allow proton reduction with electrons from the butyryl CoA dehydrogenase reaction at a hydrogen partial pressure of 10^{-4} – 10^{-5} bar (Thauer and Morris, 1984). Experimental evidence of a reverse electron transport system between the crotonyl-CoA/butyryl-CoA couple ($E^{\circ} = -125$ mV) and the H^+/H_2 couple has been provided with *Syntrophomonas wolfei* (Wallrabenstein and Schink, 1994a). If two protons are transferred in this reverse electron transport system, one third of the ATP synthesized by substrate-level phosphorylation (equivalent to -20 kJ per mol) would remain for growth and maintenance of the fatty acid-oxidizing bacterium, in accordance with the above assumptions.

The energetic situation of a binary mixed culture degrading butyrate to acetate and methane is considerably more difficult:



This overall reaction has to feed two organisms in three partial reactions, so each step has only -12 kJ available under standard conditions, and the total changes to -46 kJ at butyrate and acetate concentrations in the range of 10 mM as used in laboratory cultures. Under these condi-

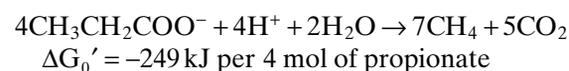
tions, the energetic situation for the partners gets tough (-15 kJ per mol reaction), especially at the end of the substrate conversion process. Very slow, often nonexponential growth and substrate turnover as usually observed with such binary mixed cultures (Dwyer et al., 1988) indicates that the energy supply is insufficient. We have often observed, as did other authors, that accumulating acetate (>10 mM) inhibits butyrate degradation in such cultures substantially.

The energetic difference between the ternary mixed culture and an artificial binary mixed culture demonstrates that the acetate-cleaving methanogens fill an important function in removal of acetate, and with this, "pull" the butyrate oxidation reaction. The above calculations also explain why addition of an acetate-cleaving methanogen to a defined binary mixed culture enhances growth and substrate turnover considerably (Ahiring and Westermann, 1988; Beaty and McInerney, 1989).

Syntrophic oxidation of long-chain fatty acids from lipid hydrolysis probably proceeds via β -oxidation with concomitant release of electrons as hydrogen via reverse electron transport, analogous to the process described above for butyrate oxidation. Long-chain dicarboxylic acids are degraded stepwise by β -oxidation, analogous to fatty acids, and decarboxylation occurs at the C-5 or C-4 state (glutarate or succinate; Matthies and Schink, 1993).

Syntrophic Propionate Oxidation

For syntrophic propionate oxidation according to the equation



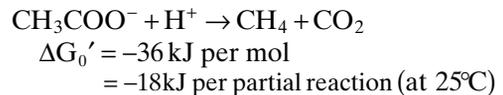
a metabolic flow scheme can be drawn, leaving a free energy change in the range of -22 to -23 kJ per mol reaction (11 partial reactions) to all partners involved (Stams et al., 1989; Schink, 1991). Studies in defined mixed cultures and in undefined communities in rice field soil have basically confirmed this assumption, although the amount of energy available to the propionate oxidizer may be sometimes substantially lower (Scholten and Conrad, 2000; Fey and Conrad, 2000). The pathway of propionate oxidation in such bacteria is basically a reversal of fermentative propionate formation, including methylmalonyl CoA, succinate, malate, pyruvate, and acetyl CoA as intermediates (Koch et al., 1983; Schink, 1985b; Schink, 1991; Houwen et al. 1987; Houwen et al., 1990). The initial substrate activation is accomplished by CoA transfer from acetyl CoA (Houwen et al., 1990; Plugge et al., 1993) or succinyl CoA. Of the redox reactions involved, succinate oxidation and malate oxidation are the most difficult ones to couple to proton reduction: hydrogen partial pressures of 10^{-15} or 10^{-8} bar would be required, respectively, which are far lower concentrations than a methanogen can maintain. The enzymes and electron transfer components involved in propionate oxidation were studied with *Syntrophobacter wolinii* (Houwen et al., 1990; Plugge et al., 1993) and *Syntrophobacter pfennigii* (Wallrabenstein et al., 1995b). Experiments on hydrogen formation in the presence and absence of protonophores (carbonyl cyanide *m*-chlorophenyl hydrazone [CCCP] or dicyclohexyl carbodiimide [DCCD]) indicated again that an intact proton or sodium motive force maintained by ATP hydrolysis is required for hydrogen release, probably in the first oxidation step (Dörner, 1992). The situation is complicated further by the fact that all syntrophically propionate-oxidizing bacteria known so far have turned out to be able also to reduce sulfate (Harmsen et al., 1993; Harmsen et al., 1995; Wallrabenstein et al., 1994b; Wallrabenstein et al., 1995b). Thus, electron transport components detected in these bacteria (even after growth in syntrophic mixed culture) do not necessarily refer to a function in syntrophic oxidation but may be involved in the sulfate-reducing apparatus.

Studies with *Syntrophobacter fumaroxidans* have shown that not only hydrogen but also formate could contribute to interspecies electron transport in this system. Syntrophic propionate oxidation was possible only in cooperation with formate- and hydrogen-oxidizing methanogens, not with *Methanobrevibacter* strains that are unable to oxidize formate (Dong et al., 1994b). This view was further supported by enzyme measurements: cells grown syntrophically with propionate contained tenfold higher formate

dehydrogenase activity in comparison with cells grown in pure culture with fumarate; the hydrogenase activity was unchanged (F. A. M. de Bok, unpublished observation). An alternative for syntrophic propionate degradation was discovered in the late 1990s. *Smithella propionica* (Liu et al., 1999) converts propionate to a mixture of acetate and butyrate in the presence of a hydrogen- and formate-oxidizing partner bacterium. The labelling patterns of products formed from specifically labelled propionate indicate that propionate degradation by this bacterium proceeds neither through methylmalonyl CoA nor through the acrylyl CoA pathway but through dimerization to a six-carbon intermediate that is subsequently cleaved to an acetyl and a butyryl moiety, with partial further oxidation (de Bok et al., 2001). This new pathway could also explain the results of labelling experiments with an enrichment culture which were first interpreted as indicative of a reductive carboxylation of propionate to a butyryl residue (Tholozan et al., 1988; Tholozan et al., 1990).

Syntrophic Acetate Oxidation

A special case is the syntrophic conversion of acetate to 2CO_2 and 4H_2 which was described first for a moderately thermophilic (58°C) bacterium, strain AOR (Zinder and Koch, 1984). This syntrophic acetate oxidizer could be grown in pure culture like a homoacetogen by hydrogen-dependent reduction of CO_2 to acetate, thus reversing syntrophic acetate oxidation (Lee and Zinder, 1988b; Lee and Zinder, 1988c). Biochemical studies revealed that it uses the carbon monoxide dehydrogenase pathway ("Wood pathway"), as do other homoacetogens (Lee and Zinder, 1988a). The small energy span available in acetate conversion to methane and CO_2 is hardly sufficient to feed two bacteria:



The free energy change is slightly higher at 58°C (-42 kJ per mol) than under standard conditions (25°C). However, a syntrophic acetate-oxidizing culture has been described as well which operates at 35°C (Schnürer et al., 1994; Schnürer et al., 1996) and thus proves that this free-energy change (-38°C) is sufficient for acetate degradation and (very little) growth at this temperature. Another interesting feature of this type of metabolism is that these bacteria can run acetate formation and acetate degradation, in both directions, with probably the same biochemical reaction apparatus, just depending on the prevailing concentrations of substrates and

products, and even can synthesize ATP both ways. This example shows how close to the thermodynamic equilibrium the energy metabolism of an anaerobic bacterium can operate.

The higher energy yield at elevated temperature may explain why at 35°C and lower this reaction is typically carried out by one single bacterium, e.g., *Methanosarcina barkeri* or *Methanosaeta soehngenii*, and why cooperations of syntrophic associations of the above-mentioned type are found nearly exclusively at elevated temperatures. Nonetheless, acetate conversion to methane at higher temperatures can as well be catalyzed by a single aceticlastic methanogen, e.g., *Methanosarcina thermophila*. Syntrophic acetate oxidation at lower temperatures is found only if further stress factors such as high ammonium concentrations inhibit aceticlastic methanogens (Schnürer et al., 1994).

Unfortunately, strain AOR was lost only few years after its description. Another thermophilic strain with similar properties, *Thermoacetogenium phaeum*, was isolated recently (Hattori et al., 2000), and was found to use the CO dehydrogenase pathway (the Wood pathway) for acetate oxidation, too (S. Hattori et al., unpublished observation), and the same is true for the mesophilic acetate-oxidizing *Clostridium ultunense* (Schnürer et al., 1997). However, we do not know yet to what extent and in which steps energy is conserved by these bacteria: either in the acetate synthesis or the acetate oxidation reaction chain.

Syntrophic acetate oxidation was observed also in a coculture of the iron-reducing bacterium *Geobacter sulfurreducens* together with the nitrate-reducing anaerobe *Wolinella succinogenes* (Cord-Ruwisch et al., 1998). In this coculture, the hydrogen partial pressures were far below the levels observed in the cultures mentioned above, and it was hypothesized that perhaps an extracellular cytochrome could act as electron shuttling vector in these cultures. We found out recently that not the cytochrome but cysteine added to the cultures as reducing agent actually mediates interspecies electron transfer in this artificially composed syntrophic coculture (J. Kaden and B. Schink, in preparation).

Syntrophic Glycolate Oxidation

Glycolate is not a fermentation intermediate but an important excretion product of algal cells. It is syntrophically oxidized by homoacetogenic and methanogenic cocultures to two molecules of CO₂, and hydrogen is the electron carrier between the fermenting bacterium (*Syntrophobotulus glycolicus*) and a hydrogen-oxidizing partner bacterium (Friedrich et al., 1991; Friedrich et al., 1996). The primary fermentation

is an endergonic process under standard conditions (Table 3) and needs coupling to, e.g., a methanogenic partner. The degradation pathway includes oxidation of glycolate to glyoxylate, condensation of glyoxylate with acetyl CoA to form malyl CoA, ATP formation in a malyl CoA synthetase reaction, malate oxidation and decarboxylation by the malic enzyme, and oxidative decarboxylation of pyruvate to acetyl CoA through pyruvate synthase, thus closing the chain for a new reaction cycle (Friedrich et al., 1991). In this cycle, the oxidation of glycolate to glyoxylate is the critical oxidation step ($E^{\circ} = -92$ mV) which, if coupled to proton reduction, requires a reverse electron transport which has to be fueled by partial hydrolysis of the ATP formed in substrate-level phosphorylation. Proof of proton gradient-dependent hydrogen release was provided in experiments with membrane vesicles: Such vesicles converted glycolate stoichiometrically to glyoxylate and hydrogen in the presence of ATP. This hydrogen formation was abolished entirely by addition of CCCP and other protonophores, as well as by DCCD. Monensin and other sodium ionophores had no specific effect (Friedrich and Schink, 1993). The process is even reversible: Membrane vesicles incubated in the presence of glyoxylate and hydrogen catalyzed a substrate-dependent net synthesis of ATP from ADP and P_i. The ratio of hydrogen-dependent glyoxylate reduction over ATP formation in isolated membrane vesicle preparations (0.2–0.5 mol per mol; Friedrich and Schink, 1995) indicates that probably two thirds of an ATP unit can be formed this way per reaction run. Thus, at least with this system, we have rather reliable data on the reaction stoichiometry.

Syntrophic Oxidation of Aromatic Compounds

So far, defined syntrophic cocultures for methanogenic degradation of aromatic substrates exist only for benzoate, gentisate, and hydroquinone (Table 3). The biochemistry of anaerobic degradation of aromatic compounds has been studied in most detail so far with benzoate, and a degradation pathway has been elaborated in the last years mainly with phototrophic and nitrate-reducing bacteria (for reviews of this subject, see Fuchs et al., 1994; Heider and Fuchs, 1997; Schink et al., 2000). The basic concept is an initial activation to benzoyl-CoA by an acyl CoA synthetase reaction requiring two ATP equivalents, followed by partial ring saturation and subsequent ring opening by a mechanism analogous to β -oxidation of fatty acids. The resulting C₇-dicarboxylic acid undergoes further β -oxidation to form three acetate residues and one CO₂.

The reductive dearomatization of benzoyl CoA in nitrate reducers requires two ATP equivalents and leads to cyclohexa-2,6-diene carboxyl CoA which undergoes further hydration and oxidation (Heider and Fuchs, 1997). This makes the initial steps in the total process rather ATP consuming (4 ATP equivalents consumed before ring cleavage). Nitrate reducers will gain these ATP investments back during subsequent oxidation of the acetyl residues. Syntrophically benzoate-oxidizing bacteria (Auburger and Winter, 1992; Schöcke and Schink, 1997; Elshahed et al., 2001) activate benzoate through a benzoyl-CoA ligase reaction, too, which consumes two ATP units and forms pyrophosphate as side product. Part of the energy invested can be regained through a membrane-bound, proton-translocating pyrophosphatase (Schöcke and Schink, 1998), and another fraction of an ATP can be conserved by the action of a membrane-bound, sodium ion-translocating glutacoyl-CoA decarboxylase which stores the energy of the C₅ dicarboxylic acid decarboxylation to crotonate in a transmembrane sodium ion gradient (Schöcke and Schink, 1999).

The overall energy budget of these fermenting bacteria can be balanced to a net gain of 1/3 to 2/3 ATP (about -45 kJ per reaction run in the ternary coculture) per benzoate oxidized, only if the ring dearomatization consumes significantly less ATP than observed with the nitrate reducers. Product patterns obtained from incubation experiments with labeled benzoate indicate that indeed a different primary reduction reaction is involved (Schöcke and Schink, 1999; Elshahed et al., 2001), but these results are not conclusive as yet. If degradation of benzoate and phthalates (carboxy-benzoates) by granular sludge was inhibited by bromoethane sulfonate, substantial amounts of cyclohexane carboxylate were formed (Kleerebezem et al., 2000), indicating again that such reduced derivatives may be side products of benzoyl CoA reduction in fermentative benzoate degraders.

Although the energetic situation of syntrophic benzoate oxidizers in methanogenic cocultures is substantially better than that of butyrate oxidizers, it appears that these bacteria depend on efficient acetate removal as well. Benzoate was nearly completely converted to acetate, methane and CO₂ in binary mixed cultures with *Methanospirillum hungatei* as hydrogen scavenger. Remnant benzoate concentrations at apparent equilibrium (in the range of 20–70 μM) increased in the presence of added acetate or propionate, and decreased in the presence of a more efficient hydrogen consumer, e.g., a sulfate-reducing partner. The corresponding hydrogen concentrations measured in such cultures were in the range of 0.5–5 × 10⁻⁵ bar, leaving a total ΔG of -30 to

-45 kJ per mol reaction for the benzoate degrader. Similar results were obtained during studies on the kinetics of benzoate degradation with *Syntrophus aciditrophicus* in the presence of a sulfate-reducing partner bacterium (Warikoo et al., 1996).

Syntrophic Oxidation of Branched-Chain Fatty Acids

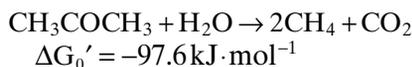
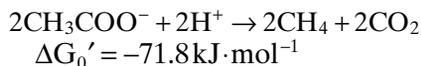
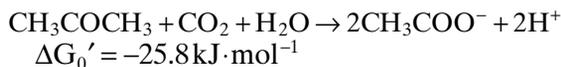
Branched-chain fatty acids are formed during fermentative degradation of the corresponding amino acids by oxidative deamination and decarboxylation, or reductive deamination (see above). The further degradation is not difficult with 2-methyl butyrate (neovalerate) because it can be β-oxidized, analogous to the degradation of butyrate to acetate and propionate by, e.g., *Syntrophospora bryantii* (Stieb and Schink, 1985). Methanogenic degradation of isobutyrate proceeds via isomerization to butyrate and further β-oxidation (Stieb and Schink, 1989); the isobutyrate/butyrate isomerization has been described in a defined culture as a coenzyme B₁₂-dependent rearrangement of the carbon skeleton (Matthies and Schink, 1992). A similar isomerization may also occur as a side reaction with valerate, leading to formation of 2-methylbutyrate (Wu et al., 1994a). Whether a similar isomerization occurs also during isobutyrate degradation by the thermophilic *Syntrophothermus lipocalidus* (Sekiguchi et al., 2000) still remains to be examined.

So far, only one defined culture has been described as capable of fermentative degradation of 3-methyl butyrate (isovalerate; Stieb and Schink, 1986), and this culture specializes in degradation of this substrate, converting it with CO₂ as cosubstrate to three molecules of acetate and one molecule of hydrogen (Tables 3 and 4). The pathway is rather complicated and involves a carboxylation, a dehydrogenation of a saturated fatty acid residue, and one substrate-level phosphorylation step (Stieb and Schink, 1986). The whole conversion is endergonic under standard conditions and depends on syntrophic hydrogen removal. Since three acetate residues are released per substrate molecule oxidized, acetate should have a far more pronounced influence on the total energetics. Details of the energetics of metabolism of this bacterium have been discussed earlier (Schink, 1991).

Fermentation of Acetone

A special situation is the fermentative conversion of acetone to methane and CO₂, which is catalyzed by syntrophically cooperating bacteria as well. In this case, acetate is the only interme-

diate between both partners, as illustrated by the following equations:



Although in this case all partial reactions are exergonic under standard conditions, the primary fermenting bacterium depends on the methanogenic partner, and acetone degradation in the mixed culture is substantially impaired in the presence of acetylene as an inhibitor of methanogens (Platen and Schink, 1987). Experiments with the primary acetone-fermenting bacterium in dialysis cultures revealed that acetate accumulation at concentrations higher than 10 mM inhibited growth and acetone degradation (Platen et al., 1994). Under these conditions, the free energy available to the acetone fermenter is still in the range of -40 kJ per mol. Since acetone metabolism by these bacteria starts with an endergonic carboxylation reaction, this might be the amount of energy that they need to invest into this primary substrate activation reaction, perhaps through a membrane-associated enzyme system (Dimroth, 1987). Unfortunately, the acetone-fermenting bacterium was never obtained in pure culture and hence detailed studies on its biochemistry and energetics were never performed.

Interspecies Metabolite Transfer

Although hydrogen due to its small size and fast diffusion appears to be an ideal carrier for electrons between bacteria of different metabolic types, formate also could act in a similar manner. A possible alternative involvement of formate in such electron transfer processes had been considered from the very beginning (Bryant et al., 1967; McInerney and Wofford, 1992) because the original partner bacteria used could oxidize both hydrogen and formate. The standard redox potential of the CO_2 /formate couple is nearly identical with that of H^+/H_2 at pH 7.0 (-420 versus -414 mV) and hence the energetic problems are the same with both. Because both electron carrier systems couple inside the cell with similar, if not the same electron transfer components, e.g., ferredoxins, most bacteria involved in interspecies electron transfer exchange hydrogen against formate and vice versa (e.g., Wu et al., 1993; Bleicher and Winter, 1994). This renders a differentiation between both electron transfer systems rather difficult, and in many cases,

both carriers may even be used simultaneously (Schink, 1991). Thus, a formate/ CO_2 shuttle could replace hydrogen transfer, and this idea has been brought up again on the basis of experiments with undefined floc cultures from methanogenic fermenters (Thiele and Zeikus, 1988) and with pure cultures (Thiele and Zeikus, 1988; Zindel et al., 1988), as well as on the basis of calculations of diffusion kinetics (Boone et al., 1989a; Boone et al., 1989b).

Exclusive action of hydrogen as electron carrier has been proven so far only for the butyrate-oxidizing coculture *Syntrophomonas wolfei* (Wofford et al., 1986), the glycolate-oxidizing *Syntrophobotulus glycolicus* (Friedrich and Schink, 1993) and the thermophilic, syntrophically acetate-oxidizing strain AOR (Lee and Zinder, 1988a; Lee and Zinder, 1988b; Lee and Zinder, 1988c); all these strains exhibit in coculture high hydrogenase and very little formate dehydrogenase activity. Syntrophic oxidation of propionate by *Syntrophobacter fumaroxidans* or of butyrate by *Syntrophospora bryantii* require partner bacteria that are able to use both hydrogen and formate, indicating that both carriers are involved in interspecies electron transfer (Dörner, 1992; Dong et al., 1994a; Dong et al., 1994b; Dong and Stams, 1995a; Dong and Stams, 1995b). Nonetheless, hydrogenase activities in these cultures exceeded formate dehydrogenase activities substantially indicating that hydrogen played a dominant role as well. Similar conclusions were drawn from hydrogen and formate transfer experiments with thermophilic granular sludge preparations and different partner bacteria (Schnürer et al., 1994). In isobutyrate-degrading cocultures, formate appeared to play a role besides hydrogen as an electron carrier (Wu et al., 1996). On the basis of calculations of diffusion kinetics (Boone et al., 1989a; Boone et al., 1989b), one can speculate that formate/ CO_2 would be the preferred electron transfer system in suspended cultures of single cells where the carrier molecule has to diffuse over long distances through an aqueous phase, whereas hydrogen would be more efficient in densely packed aggregates which dominate in anaerobic digestors and probably also in sediments.

A key problem in an assessment of the relative importance of formate as electron shuttle is a reliable measurement of formate at low concentrations: at 0.3 bar CO_2 as typical of e.g., sewage sludge or sediments, a hydrogen partial pressure of 10^{-4} – 10^{-5} bar (as required for the redox reactions discussed) is equivalent to formate concentrations of about 1–10 μM (Schink, 1994). Whereas hydrogen partial pressures can today be measured reliably down to 10^{-7} bar with mercury oxide based detectors, there is hardly an

efficient method available which allows measurement of formate in concentrations of few micromolar and less. Moreover, most indications of formate formation were obtained in the presence of inhibitors that caused accumulation of measurable amounts of formate, but it remains doubtful whether such experiments describe adequately the electron flow in the undisturbed system.

Beyond hydrogen and formate, acetate is also excreted by syntrophically fermenting bacteria and is further metabolized by methanogens. The model used above shows that also acetate removal can have a profound influence on the total energetics of syntrophic degradation of fatty acids or benzoate. Its importance may be even higher with, e.g., isovalerate degradation, since three molecules of acetate and only one hydrogen molecule are formed (Table 3). Indeed, inhibition by acetate accumulations or by addition of acetate has been reported for syntrophic degradation of fatty acids and for benzoate (Ahring and Westermann, 1988; Fukuzaki et al., 1990; van Lier et al., 1993; Warikoo et al., 1996; Schöcke and Schink, 1997).

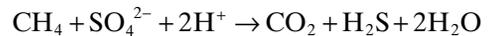
In the recently reported acetate-oxidizing coculture of *Geobacter sulfurreducens* with *Wolinella succinogenes*, we observed that cysteine acted as electron carrier between both partners (J. Kaden and B. Schink, unpublished observation). The use of this carrier system of higher redox potential ($E^{\circ} = -270$ mV) than the hydrogen or formate system makes sense in the coupling to the comparably positive electron acceptor used by this coculture. Perhaps the same carrier is active also in other syntrophic associations which exhibit fast electron transfer to an acceptor system of comparably high redox potential (Daniel et al., 1999; Meckenstock, 1999). In the natural environment, also exogenous electron carriers such as humic substances may play a role as interspecies electron carriers, as suggested for electron transfer from iron-reducing bacteria to insoluble iron minerals (Lovley et al., 1996). Cervantes et al. (2000) observed recently that the oxidation of phenolic compounds in methanogenic sewage sludge can be coupled to the reduction of the humic compound analogue anthraquinone-2,6-disulfonate, indicating that this acceptor could perhaps be reduced also by syntrophic phenol oxidizers.

Anaerobic Methane Oxidation

Anaerobic, sulfate-dependent methane oxidation is an important reaction in anoxic marine sediments, as documented on the basis of measurements of sulfate and methane gradients in such sediments (Reeburgh, 1980; Iversen and

Jørgensen, 1985). The process could also be demonstrated with radiolabelled methane in anoxic incubations of sediment samples. Zehnder and Brock (1979) showed by similar experiments that methanogenic bacteria were responsible for this oxidation of labelled methane and that methane oxidation and formation were simultaneous. However, in their assays, methane production always exceeded methane oxidation by two to three orders of magnitude, and thus this process could hardly explain the net methane oxidation observed in sediments. For a long time, no cultures were available to study this process in detail because all efforts to isolate anaerobic methane oxidizers failed, and all reports on putative methane-oxidizing anaerobes in the literature have turned out to be unsubstantiated due to lack of sufficient controls.

From a thermodynamic point of view, sulfate-dependent methane oxidation is an exergonic reaction



which yields under standard conditions a $\Delta G_0'$ of -18 kJ per mol. Concentrations of the reaction partners in situ in the active sediment layers are in the range of 10^{-2} bar methane, and 1–3 mM of both sulfate and free hydrogen sulfide. Thus, the overall energetics become only slightly more favorable if in situ conditions are taken into consideration. This amount of energy can feed only one bacterium, provided that the bacterium is able to exploit this biological minimum energy quantum. Based on the observation that methanogens can catalyze an oxygen-independent methane oxidation (Zehnder and Brock, 1979) and the description of a reversal of homoacetogenic fermentation by strain AOR and others (see above; Table 3), it was speculated that "reversed methanogenesis" may be the key to an understanding of this process (Hoehler et al., 1994; Schink, 1997). If the overall reaction is actually a syntrophic cooperation involving a methanogen running methane formation backwards and a sulfate-reducing bacterium, it is obvious that only one of the partners can gain metabolic energy from the reaction, and the other one has to run this process only as a cometabolic activity. This would explain at least why scientists have always failed to enrich for methane-oxidizing sulfate reducers in the past, simply because one cannot enrich for a bacterium on the basis of a cometabolic activity.

The last three years have advanced our view of anaerobic methane oxidation dramatically (Valentine and Reeburgh, 2000a). Analysis of lipids of marine archaea and sulfate-reducing bacteria in anoxic sediment layers indicated that these organisms fed on (^{13}C -depleted) methane

(Pancost et al., 2000), and similar findings, combined with molecular population analysis, were reported for archaeal/bacterial communities in marine sediments and close to submarine methane seeps and gas hydrates (Hinrichs et al., 1999; Orphan et al., 2001; Thomsen et al., 2001). In sediments overlying methane hydrates off the coast of Oregon, United States, active anaerobic methane oxidation was found to be associated with discrete, spherical microbial aggregates which consisted, according to fluorescent in-situ hybridization analysis (FISH), of *Methanosarcina*-like archaea in the center, surrounded by *Desulfosarcina*-related sulfate-reducing bacteria (Boetius et al., 2000). The energetics of sulfate-dependent methane oxidation at these gas hydrate sites (with methane pressures of about 80 bar) are considerably more favorable than in deeplying marine sediments, and the overall free energy change of the reaction in situ (-40 kJ per mol) may really allow energy conservation and growth for both partners in this cooperation. Thus, these aggregates represent a first model system to understand sulfate-dependent methane oxidation as a syntrophic cooperation phenomenon, but it still needs to be proven whether this model can also be applied to methane oxidation in deeplying, methane-poor marine sediments.

It is also still open whether methane activation proceeds through a reversal of the methyl-coenzyme M reductase reaction, and which metabolites are transferred between the two partners. Representatives of both genera, *Methanosarcina* sp. and *Desulfosarcina* sp., are metabolically rather versatile, and could metabolize either hydrogen, formate, methanol or acetate. Various cultures of methanogens failed to produce significant amounts of hydrogen from methane (Valentine et al., 2000b), indicating that "reverse methanogenesis" is not a widespread capacity among methanogens. From an energetic point of view, a transfer of acetate between both partners appears the easiest solution, but this is still a matter of speculation.

“Obligately Syntrophic” Bacteria: Cultivation and Biochemical Studies

The mutual dependence of partner bacteria in syntrophic associations has caused severe difficulties in the cultivation of such organisms, and defined cocultures have been obtained only recently. For isolation, pure cultures of known methanogenic or sulfate-reducing partner bacteria are usually provided in excess as a background "lawn" during the cultivation and

dilution process to isolate the syntrophically fermenting bacterium in defined binary or ternary mixed culture. Today, all well-described syntrophically fermenting bacteria can be cultivated also in pure culture with different substrate combinations (see below). Hence, we should no longer talk about "obligately syntrophic bacteria" (because they are not obligately syntrophic) but only about syntrophic relationships or syntrophic conversion processes.

Many efforts have been made to grow syntrophically fermenting bacteria in the absence of partner bacteria. Removal of hydrogen by nonbiological procedures (low pressure, and gas diffusion through thin membranes) had only little success with ethanol oxidation, and no success at all with fatty acid oxidation. In other cases, hydrogen removal by palladium catalysts spread on either charcoal or CaCO_3 surfaces, with alkenes or alkynes as oxidant have shown some success (Mountfort et al., 1986), as did efforts to couple hydrogen release to reoxidation by electrochemically controlled platinum electrodes. Whether a new cultivation apparatus which maintains the hydrogen partial pressure at subnanomolar concentrations (Valentine et al., 2000c) will hold more promise has still to be proven. More successful was the use of fumarate as external electron acceptor in cultivation of syntrophic propionate degraders (Stams et al., 1993). Today, pure cultures of syntrophically fermenting bacteria of all known metabolic types have been isolated. Typically, this has been accomplished with substrates that are more oxidized than the original one, and can be fermented by dismutation. As an example, ethanol-oxidizing syntrophs can be grown in pure culture with acetaldehyde analogues such as acetoin or acetylene (Schink, 1985a; Eichler and Schink, 1986), butyrate- or benzoate-degrading syntrophs with crotonate (Beatty and McInerney, 1987a; Zhao et al., 1989; Wallrabenstein et al., 1995a) or with pentanoate as external electron acceptor (Dong et al., 1994a), and syntrophically propionate-degrading bacteria with pyruvate (Wallrabenstein et al., 1994b) or propionate plus fumarate (Stams et al., 1993). Beyond that, all syntrophic propionate oxidizers (with the exception of *Smithella propionica*) have been shown to be able also to reduce sulfate, and can be isolated in pure culture with propionate plus sulfate, although they grow only very slowly with this substrate combination (Harmsen et al., 1993; Harmsen et al., 1995; Wallrabenstein et al., 1994b; Wallrabenstein et al., 1995b).

Biochemical studies with defined cocultures of syntrophically fermenting bacteria have been carried out successfully with cell-free extracts prepared by, e.g., lysozyme (Wofford et al., 1986) or mutanolysin (Wallrabenstein and Schink,

1994a) treatment, which opens selectively only the fermenting bacterium and leaves the methanogenic partner intact, owing to its archaeal cell wall chemistry. In another approach, the partner organisms were separated by centrifugation in Percoll gradients before cell disruption and enzyme assays (Beatty et al., 1987b).

Homoacetogenic Conversions and the Effect of Temperature

Under standard conditions, methanogenic hydrogen oxidation yields more energy than homoacetogenic hydrogen oxidation (Table 2), and one would therefore assume that homoacetogens have little chance to compete successfully against methanogens for hydrogen at limiting concentrations. The function of homoacetogens in the complex electron flow scheme depicted in Fig. 1 remains unclear, therefore, and the general assumption is that they take advantage of their metabolic versatility which allows them to compete with several partners of various metabolic types, and consume two or more substrates simultaneously (Schink, 1994). Such simultaneous utilization of more than one substrate may increase the effective affinity for every single substrate, as studies with *Escherichia coli* in continuous cultures with multiple substrate supply have proven (Egli, 1995; Lendenmann et al., 1996). However, there are exceptional situations in which homoacetogens may definitively out-compete methanogens in their function as hydrogen consumers in sulfate-poor anoxic environments. One such situation may be slightly acidic lake sediments such as that found in Knaack Lake, Wisconsin, United States, where at pH 6.1 the total electron flow goes through the acetate pool, and no methane is formed by direct CO₂ reduction (Phelps and Zeikus, 1984). Obviously, hydrogen-oxidizing methanogens do not perform sufficiently well under these conditions. Thus, homoacetogens take over their function, however, only at low acetate concentration (at 10⁻⁴ bar H₂ and 10 μM acetate), homoacetogenic hydrogen oxidation yields a ΔG of -26 kJ per mol of acetate and has to be maintained by acetoclastic methanogens.

Temperature is a further effector which improves the ability of homoacetogens to compete successfully against methanogens for hydrogen. At temperatures lower than 20°C, homoacetogens appear to take over significant parts of hydrogen oxidation in paddy soil and lake sediments (Conrad et al., 1989; Conrad and Wetter, 1990). The known species of hydrogen-oxidizing methanogens are not significantly active at such temperatures (Zeikus and Winfrey,

1976), and homoacetogens appear to be less restricted in this respect. Dominance of homoacetogenesis in the total electron flow is even more expressed in tundra wetland soils at temperatures lower than 10°C (Kotsyurbenko et al., 1996). This effect becomes understandable from a look at the temperature dependence of hydrogen-dependent methanogenesis and homoacetogenesis. At hydrogen partial pressures lower than 10⁻⁴ bar and acetate concentrations at 10 mM, homoacetogenesis reaches at 5°C the same energy gain as hydrogen-dependent methanogenesis does at 35°C. Thus, the general scheme of electron flow in methanogenic environments (Fig. 1) has to be modified for slightly acidic or for low temperature habitats (Fig. 3a). Under these conditions, there is no significant hydrogen-dependent methanogenesis (group 2), and the electrons flow nearly exclusively via acetogenesis and acetoclastic methanogenesis (groups 5 and 3).

The opposite situation emerges at high temperature habitats as discussed already above in the context of syntrophic acetate oxidation. Under these conditions, homoacetogenesis can operate in the opposite direction, and the electron flow goes from acetate through the C-1 pool and hydrogen towards methane (Fig. 3b). Thus, the general flow scheme in Fig. 1 represents kind of an intermediate situation that probably describes the situation of a sewage sludge digester correctly, but has to be modified for high and low temperature situations in the way indicated.

Structure and Growth Dynamics of Syntrophic Associations

The separation of metabolic functions and their distribution within metabolically different microorganisms, i.e., substrate oxidation and hydrogen formation in the one and hydrogen oxidation and CO₂ reduction in the other organism, is a rather unusual strategy that we do not observe, at least not to this extent, in oxic environments. It may have its advantages because it allows a high degree of metabolic specialization to the single organisms concerned, which requires very little effort into refined regulation of energy metabolism. However, the metabolic efficiency of such cooperating communities depends on an efficient metabolite transfer between the partners involved: the flux of, e.g., hydrogen between the hydrogen-forming fermenter and the hydrogen-consuming methanogen is inversely proportional to the distance between both (Schink and Thauer, 1988). Optimal metabolite transfer can be achieved

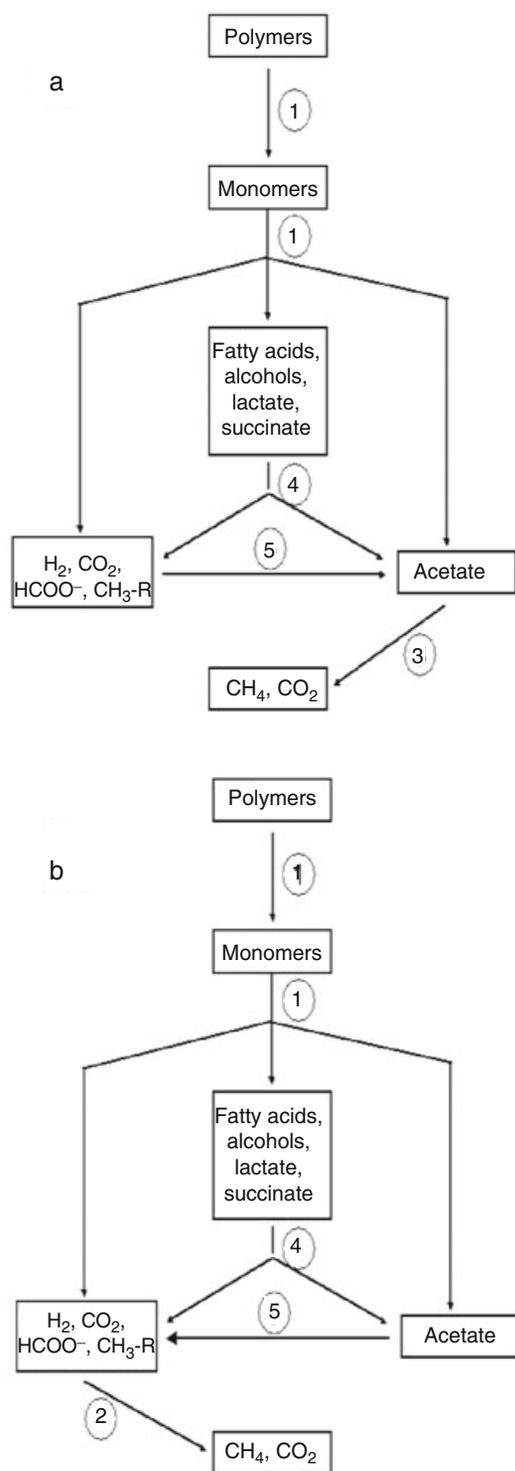


Fig. 3. Carbon and electron flow in methanogenic degradation of complex organic matter. Groups of prokaryotes involved: 1) primary fermentative bacteria; 2) hydrogen-oxidizing methanogens; 3) acetate-cleaving methanogens; 4) secondary fermenting bacteria (syntrophs); and 5) homo-acetogenic bacteria. Carbon and electron flow a) at low temperature; and b) at high temperature.

best when both partners are in close contact (i.e., directly attached to each other, forming an aggregate or floc). Such flocs form preferentially in anaerobic digestors in which fatty acids are degraded, although the establishment of stable floc formation may require substantial amounts of time, sometimes even several months after start-up (Lettinga et al., 1988). Indirect evidence of aggregate formation in sediments and sludges has been obtained on the basis of hydrogen exchange measurements (Conrad et al., 1985; Conrad et al., 1986), and the recently discovered aggregates involved in sulfate-dependent methane formation (Boetius et al., 2000) exhibit even a high degree of spatial organization.

Efforts to synthesize stable aggregates (granules) of microbiologically defined composition from pure cultures have been made, and the success depended very specifically on the type of partners used. Butyrate-degrading aggregates with only two partners were stable only with *Methanobacterium formicicum*, not with *Methanospirillum hungatei*, whereas propionate-degrading granules depended on the additional presence of an acetate-degrading partner (Wu et al., 1996). In suspended cultures, *M. hungatei* is often the dominant hydrogen utilizer, but it is obviously not the ideal partner for formation of efficient granules. This experiment demonstrates again that our enrichment and cultivation techniques influence to a high degree the results obtained, and may give a rather incorrect picture of the situation prevailing in the natural or semi-natural system. The composition and stability of the formed granules is further influenced by the fact that methanogenic granules in waste-degrading reactors have to deal with mixed substrate supplies that add further microbial constituents into the architecture of the resulting granule, including, e.g., sulfate-reducing bacteria (Wu et al., 1991). In any case, optimal cooperation will be secured in granules in which the partner organisms are randomly mixed to near homogeneity, rather than in situations in which the partners form "nests" of identical subpopulations.

A basic problem remains in that each partner bacterium multiplies and produces offspring only of its own kind. Thus, the situation given in Fig. 4a will change after several generations into one similar to that depicted in Fig. 4b, and the efficiency of metabolite transfer will decrease with age of the aggregates unless there are ways of internal mixing of the aggregates. Thin sections of methanogenic granules have shown examples of fully mixed as well as of nest structures (Dubourguier et al., 1988), and similar pictures were recently obtained with thin sections in which the partner bacteria were identified by specifically RNA-directed probes (Harmsen et

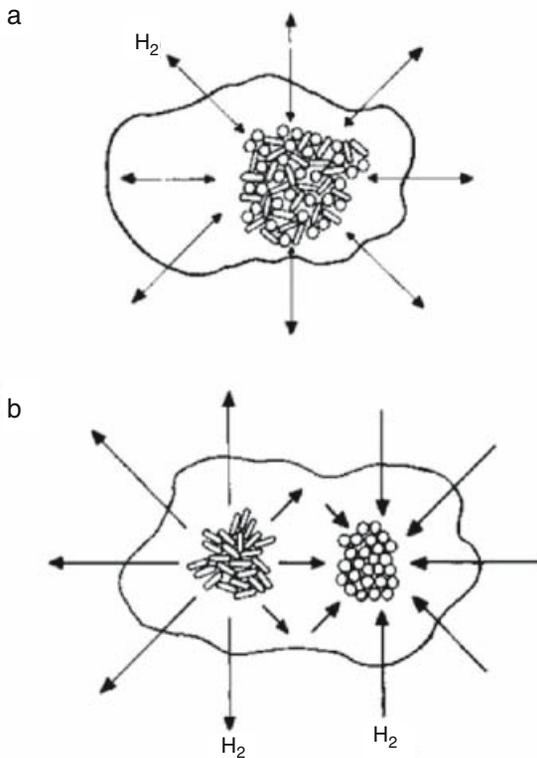


Fig. 4. Exchange of hydrogen (or other intermediates) in anaerobic bacterial flocs containing a) a homogeneously mixed community of hydrogen formers and hydrogen consumers or b) hydrogen producers and hydrogen consumers in spatially separated nests.

al., 1996). The largely segregated arrangement of the two partners in the spherical aggregates active in syntrophic sulfate-dependent methane oxidation (Boetius et al., 2000) probably represents a terminal stage in the growth of these partners, and the rather regular size of these aggregates indicates that it is limited by metabolite diffusion kinetics. The dynamics of growth and internal structure development in syntrophic aggregates appears to be an interesting object of research now that gene probes provide excellent tools for direct in situ identification of the various microbial components involved. First efforts in this respect have shown that there are clear functional separations between the subpopulations in granular sludge, especially if also some sulfate is available for fatty acid oxidation (Oude Elferink et al., 1998; Santegoeds et al., 1999). A similar study on syntrophic propionate oxidizers in thermophilic granular sludge revealed that these organisms were localized mainly in the centers of the granules, closely associated with hydrogen-scavenging methanogens (Imachi et al., 2000).

It may be worth mentioning in this context that the observed maximum specific growth rates of syntrophic amino acid-degrading associations are much lower than those of other amino acid-fermenting bacteria. For example, the μ_{\max} of *Clostridium* sp. growing on glutamate is 0.3–0.6 h⁻¹ (Laanbroek et al., 1979) and that of *S. acidaminophila* is 0.13 h⁻¹ (Nanninga et al., 1987). These bacteria use a butyric acid and propionic acid fermentation for the degradation of glutamate, respectively. However, the μ_{\max} of *A. hydrogenoformans* growing on glutamate in syntrophic association with a hydrogenotrophic anaerobe is only 0.10 h⁻¹. The μ_{\max} of a *Campylobacter* sp. growing on aspartate is about 0.17 h⁻¹ (Laanbroek et al., 1978), whereas the μ_{\max} of a coculture of *E. acidaminophilum* and a methanogen is below 0.1 h⁻¹ (Zindel et al., 1988). Nevertheless, in environments with a high methanogenic activity, e.g., granular sludge from anaerobic bioreactors, high numbers of bacteria can be counted which grow syntrophically with methanogens. Three explanations can be given for this: 1) the growth rates of the syntrophic associations at low substrate concentrations are higher than those of other amino acid-fermenting bacteria, i.e., the syntrophic associations have a higher affinity for the substrate; 2) the syntrophic associations grow on mixtures of substrates rather than on single substrates; and 3) the growth rates of the syntrophic associations are higher than the ones that have been measured. The reported growth rates of the cocultures refer to suspended growth, whereas in methanogenic biofilms and aggregates growth rates might be much higher because of the shorter interbacterial distances. These observations give further support to the assumption that the growth rates of syntrophic associations are limited by the rate of interspecies metabolite transfer.

Cooperation with Protozoa, Hydrogenosomes

The function of the primary fermenting bacteria (group 1) in conversion of complex organic matter to methane and CO₂ (Fig. 1) may be taken over also by eukaryotic organisms. Anaerobic fungi, ciliates and flagellates, are known which thrive in entirely anoxic environments under reducing conditions (Finlay and Fenchel, 1992; Fenchel and Finlay, 1995), and some of them are extremely oxygen-sensitive. Since aerobic respiration is not possible in such habitats, anaerobic protozoa do not contain mitochondria. Instead, intracellular organelles are present which release hydrogen and have been called

“hydrogenosomes.” The metabolism of these protozoa is fermentative; particles, especially bacterial cells, are ingested into food vacuoles and digested by hydrolysis and further fermentation, and acetate is probably the most important fermentation product.

Anaerobic protozoa can be associated with symbiotic methanogens, either extracellularly or intracellularly. Ciliates living in strictly anoxic, eutrophic sediments carry methanogenic partner bacteria inside the cell (van Bruggen et al., 1983; van Bruggen et al., 1985), often closely associated with the hydrogenosomes. The advantage of this cooperation with hydrogenotrophic methanogens for the protozoan host is obvious: removal of hydrogen and maintenance of a low hydrogen/formate concentration in the cell allows fermentation of complex organic matter mainly to acetate and CO₂. Thus, waste of organic precursors into reduced endproducts such as ethanol, fatty acids, etc. can be avoided, and the fermenting protozoon obtains a maximum ATP yield. The symbiotic methanogen takes over part of the function that mitochondria have in aerobic higher cells. Reducing equivalents are removed by the symbiotic partner, and the eukaryotic host cell runs a fermentative metabolism with maximum ATP yield.

It is assumed that the hydrogen released by hydrogenosomes stems mainly from pyruvate oxidation to acetyl CoA (pyruvate synthase reaction; Müller, 1988). Hydrogenosomes contain this enzyme, as well as ferredoxin and hydrogenase. In some cases, especially with the larger types of anaerobic protozoa, close associations of methanogenic endosymbionts with hydrogenosomes have been observed. Smaller protozoa may achieve the same effect of hydrogen release also with an extracellular partner bacteria because the diffusion distance to the surface may be short enough. The same applies to the rumen ciliates which in their comparably rich habitat cooperate only occasionally with symbiotic partners on their cell surface (Stumm et al., 1982).

Hydrogenosome and methanogenic endosymbiont together form a functional entity. In some cases, especially with the comparably big ciliates such as *Plagiopyla frontata*, hydrogenosomes and methanogens are organized in an alternating sandwich arrangement that allows optimal hydrogen transfer in highly refined structures (Finlay and Fenchel, 1992). It has been speculated that hydrogenosomes of strictly anaerobic protozoa have evolved from mitochondria of their aerobic predecessors; other speculations assume a relationship of hydrogenosomes to clostridia. The high structural development of hydrogenosomes in some protozoa may suggest that such arrangements could operate also in

transfer of hydrogen from less easily available electron donors than the pyruvate synthase system represents. Unfortunately, detailed studies on the cooperation of methanogenic endosymbionts with their protozoan hosts have been hampered so far by extreme difficulties in handling defined cultures of strictly anaerobic protozoa.

Taxonomy of Syntrophs

The survey of described bacteria active in syntrophic oxidation of alcohols, fatty acids, and aromatic compounds (Table 4) documents that these bacteria are found only in two groups within the taxonomic system based on sequence similarities of the 16S rRNA, namely, the Gram-positive bacteria with low G+C content, and the Δ -Proteobacteria. Some metabolic specializations are clustered in certain taxonomic groups, e.g., ethanol, propionate and benzoate oxidation in the Δ -Proteobacteria (with the exception of *Thermoanaerobium brockii*), or fatty acid β -oxidation and acetate oxidation in the Gram-positive bacteria with low G+C content. Nonetheless, these groups are not really homogeneous but encompass representatives of many other metabolic types, e.g., sulfate reducers that do not exhibit any tendency to transfer electrons to partner organisms. Finally, as pointed out above, the fermentative degradation of amino acids includes so many different expressions of obligate and facultative syntrophy within taxonomically extremely different groups of organisms that syntrophy can hardly be assumed to be associated with a single evolutionary trait. Rather, syntrophy appears to be kind of a lifestyle that is experienced and perfected by many different organisms to varying extents.

Conclusions

The energetics of syntrophic fatty acid and alcohol-oxidizing processes represent exciting examples of energy metabolism based on the smallest energy quantum that, to our present understanding, can be exploited by living cells. This minimum amount of energy which can be converted into ATP in the living cell is in the range of -20 kJ per reaction run or even lower, and this is the amount of energy available to the respective partners in most of the degradation processes discussed here. Models of metabolic cooperation and energy sharing between syntrophic partners can be based on this assumption, and experimental evidence of reverse electron transport systems to balance the energy

Table 4. Pure or defined mixed cultures of bacteria catalyzing syntrophic substrate oxidations via interspecies hydrogen transfer.

Isolate	Substrate range	Gram type	Phylogenetic position	Reference (s)
a) Oxidation of primary alcohols				
S-strain	Ethanol	-	Unknown	Bryant et al., 1967
<i>Desulfovibrio vulgaris</i>	Ethanol + sulfate	-	δ -Proteobacteria	Bryant et al., 1977
<i>Thermoanaerobacter brockii</i>	Ethanol, sugars etc.	+	Low G+C Gram positives	Ben-Bassat et al., 1981
<i>Pelobacter venetianus</i>	Ethanol, propanol	-	δ -Proteobacteria	Schink and Stieb, 1983
<i>Pelobacter acetylenicus</i>	Ethanol, acetylene	-	δ -Proteobacteria	Schink, 1985
<i>Pelobacter carbinolicus</i>	Ethanol, 2,3-butanediol	-	δ -Proteobacteria	Schink, 1984
b) Oxidation of butyrate and higher homologues				
<i>Syntrophomonas wolfei</i>	C ₄ -C ₈	-	Low G+C Gram positives	McInerney et al., 1979, 1981
<i>Syntrophomonas sapovorans</i>	C ₄ -C ₁₈	-	Low G+C Gram positives	Roy et al., 1986
<i>Syntrophospora bryantii</i>	C ₄ -C ₁₁ , 2-methylvalerate	+	Low G+C Gram positives	Stieb and Schink, 1985; Zhao et al., 1989
<i>Thermosyntrophus lipolytica</i>	C ₄ -C ₁₈ , crotonate, and betaine	+	Low G+C Gram positives	Svetlichnyi et al., 1996
<i>Syntrophothermus lipocalidus</i>	C ₄ -C ₁₀ , isobutyrate, and crotonate	+	Low G+C Gram positives	Sekiguchi et al., 2000
c) Oxidation of propionate				
<i>Syntrophobacter wolinii</i>	Propionate,	-	δ -Proteobacteria	Boone and Bryant, 1980
<i>Syntrophobacter pfennigii</i>	Pyruvate	-	δ -Proteobacteria	Wallraabenstein et al., 1995a
<i>Syntrophobacter fumaroxidans</i>	Propionate + fumarate	-	δ -Proteobacteria	Harmsen et al., 1998
<i>Smithella propionica</i>	Propionate	-	δ -Proteobacteria	Liu et al., 1999
d) Oxidation of acetate				
<i>Thermoacetogenium phaeum</i>	Acetate, pyruvate, glycine, cysteine, formate, and H ₂ /CO ₂	+	Low G+C Gram positives	Hattori et al., 2000
<i>Clostridium ultunense</i>	Acetate, formate, and cysteine	+	Low G+C Gram positives	Schnürer et al., 1996
e) Oxidation of isovalerate				
Strain Gralval	Isovalerate only	+	Unknown	Stieb and Schink, 1986
f) Oxidation of glycolate				
<i>Syntrophobotulus glycolicus</i>	Glycolate and glyoxylate	+	Low G+C Gram positives	Friedrich et al., 1991, 1996
g) Oxidation of aromatic compounds				
<i>Syntrophus buswellii</i>	Benzoate and crotonate	-	δ -Proteobacteria	Mountfort and Bryant, 1982
<i>Syntrophus gentianae</i>	Benzoate, gentisate, and hydroquinone	-	δ -Proteobacteria	Wallraabenstein et al., 1995b
<i>Syntrophus acidithiobacillus</i>	Benzoate and crotonate	-	δ -Proteobacteria	Jackson et al., 1999

requirements for hydrogen release have been obtained in several instances.

Recent studies on the biochemistry of syntrophic fatty acid oxidizers revealed that these bacteria are by no means "primitive" but actually admirable creatures from the point of view of energy conservation and efficient energy utilization. Most of these bacteria grow in plain mineral media and synthesize all their cellular components on the basis of only the minimum quantum of energy which can be exploited by living cells at all. They are spectacular examples of how diligently nature has organized the components of global energy flux down to those environments where very little energy is available to their living inhabitants.

Acknowledgments. This article is dedicated to the late Marvin Bryant who made fundamental contributions to our present understanding of cooperations in methanogenic microbial communities. The text is based on other reviews written earlier on the same or related subjects (Stams, 1994; Schink, 1991; Schink, 1994; Schink, 1997). The authors express their gratitude to Marvin P. Bryant, Ralph Wolfe, Norbert Pfennig, Rudolf Thauer, Fritz Widdel, Alex Zehnder, and Ralf Conrad for numerous fruitful discussions about the biology and energetics of syntrophic anaerobes, and to their former and present coworkers for their dedicated work on these extremely fastidious bacteria.

Literature Cited

- Ahring, B. K., and P. Westermann. 1988. Product inhibition of butyrate metabolism by acetate and hydrogen in a thermophilic coculture. *Appl. Environ. Microbiol.* 54:2393–2397.
- Auburger, G., and J. Winter. 1992. Purification and characterization of benzoyl CoA ligase from a syntrophic, benzoate-degrading, anaerobic mixed culture. *Appl. Microbiol. Biotechnol.* 37:789–95.
- Baena, S., M.-L. Fardeau, M. Labat, B. Ollivier, J.-L. Garcia, and B. K. C. Patel. 1998. *Aminobacterium colombiense*, gen.nov., sp.nov., an amino acid degrading anaerobe isolated from anaerobic sludge. *Anaerobe* 4:241–250.
- Baena, S., M.-L. Fardeau, B. Ollivier, M. Labat, P. Thomas, J.-L. Garcia, and B. K. C. Patel. 1999a. *Aminomonas paucivorans* gen.nov., sp.nov., a mesophilic, anaerobic, amino-acid-utilizing bacterium. *Int. J. Syst. Bacteriol.* 49:975–982.
- Baena, S., M.-L. Fardeau, T. H. S. Woo, B. Ollivier, M. Labat, and B. K. C. Patel. 1999b. Phylogenetic relationships of three amino-acid-utilizing anaerobes, *Selenomonas acidaminovorans*, "*Selenomonas acidaminophila*" and *Eubacterium acidaminophilum*, as inferred from partial 16S rDNA nucleotide sequences and proposal of *Thermanaerovibrio acidaminovorans* gen.nov., comb. nov. and *Anaeromusa acidaminophila* gen.nov., comb. nov. *Int. J. Syst. Bacteriol.* 49:969–974.
- Baena, S., M.-L. Fardeau, M. Labat, B. Ollivier, B. J.-L. Garcia, and B. K. C. Patel. 2000. *Aminobacterium mobile*, sp.nov., a new anaerobic amino-acid-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* 50:259–264.
- Barik, S., W. J. Brulla, and M. P. Bryant. 1985. PA-1, a versatile anaerobe obtained in pure culture, catabolizes benzenoids and other compounds in syntrophy with hydrogenotrophs, and P-2 plus *Wolinella* sp. degrades benzenoids. *Appl. Environ. Microbiol.* 50:304–310.
- Barker, H. A. 1940. Studies upon the methane fermentation. IV: The isolation and culture of *Methanobacterium omelienskii*. *Ant. v. Leeuwenhoek* 6:201–220.
- Barker, H. A. 1981. Amino acid degradation by anaerobic bacteria. *Ann. Rev. Biochem.* 50:23–40.
- Beaty, P. S., and M. J. McInerney. 1987a. Growth of *Syntrophomonas wolfei* in pure cultures on crotonate. *Arch. Microbiol.* 147:389–393.
- Beaty, P. S., N. Q. Wofford, and M. J. McInerney. 1987b. Separation of *Syntrophomonas wolfei* from *Methanospirillum hungatei* in syntrophic cocultures by using Percoll gradients. *Appl. Environ. Microbiol.* 53:1183–1185.
- Beaty, P. S., and M. J. McInerney. 1989. Effect of organic acid anions on the growth and metabolism of *Syntrophomonas wolfei* in pure culture and in defined consortia. *Appl. Environ. Microbiol.* 55:977–983.
- Ben-Bassat, A., R. Lamed, and J. G. Zeikus. 1981. Ethanol production by thermophilic bacteria: Metabolic control of end product formation in *Thermoanaerobium brockii*. *J. Bacteriol.* 146:192–199.
- Biebl, H., and N. Pfennig. 1978. Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. *Arch. Microbiol.* 117:9–16.
- Bleicher, K., and J. Winter. 1994. Formate production and utilization by methanogens and by sewage sludge consortia—interference with the concept of interspecies formate transfer. *Appl. Microbiol. Biotechnol.* 40:910–915.
- Boetius, A., K. Ravensschlag, C. J. Schubert, D. Rickert, F. Widdel, A. Giesecke, R. Amann, B. B. Jorgensen, U. Witte, and O. Pfannkuche, O. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–626.
- Boone, D. R., and M. P. Bryant. 1980. Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. *Appl. Environ. Microbiol.* 40:626–632.
- Boone, D. R., R. L. Johnson, and Y. Liu. 1989a. Diffusion of the interspecies electron carriers H₂ and formate in methanogenic ecosystems, and implications in the measurement of K_M for H₂ or formate uptake. *Appl. Environ. Microbiol.* 55:1735–1741.
- Boone, D. R., R. L. Johnson, and Y. Liu. 1989b. Microbial ecology of interspecies hydrogen and formate transfer in methanogenic ecosystems. *In: T. Hattori, Y. Ishida, Y. Maruyama, R. Y. Morita, and A. Uchida (Eds.) Recent Advances in Microbial Ecology.* Japan Scientific Society Press. Tokyo, Japan. 450–453.
- Bryant, M. P., E. A. Wolin, M. J. Wolin, and R. S. Wolfe. 1967. *Methanobacillus omelienskii*, a symbiotic association of two species of bacteria. *Arch. Mikrobiol.* 59:20–31.
- Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill. 1977. Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H₂-utilizing methanogenic bacteria. *Appl. Environ. Microbiol.* 33:1162–1169.

- Bryant, M. P. 1979. Microbial methane production—theoretical aspects. *J. Anim. Sci.* 48:193–201.
- Buckel, W., and H. A. Barker. 1974. Two pathways of glutamate fermentation by anaerobic bacteria. *J. Bacteriol.* 117:1248–1260.
- Cervantes, F. J., S. van der Velde, G. Lettinga, and J. A. Field. 2000. Quinones as terminal electron acceptors for anaerobic microbial oxidation of phenolic compounds. *Biodegradation* 11:313–321.
- Cheng, G., C. M. Plugge, W. Roelofsen, F. P. Houwen, and A. J. M. Stams. 1992. *Selenomonas acidaminovorans* sp. nov., a versatile thermophilic proton-reducing anaerobe able to grow by decarboxylation of succinate to propionate. *Arch. Microbiol.* 157:169–175.
- Cherepanov, D. A., A. Y. Mulkidjanian, and W. Junge. 1999. Transient accumulation of elastic energy in proton translocating ATP synthase. *FEBS Lett.* 449:1–6.
- Conrad, R., T. J. Phelps, and J. G. Zeikus. 1985. Gas metabolism evidence in support of the juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. *Appl. Environ. Microbiol.* 50:595–601.
- Conrad, R., B. Schink, and T. J. Phelps. 1986. Thermodynamics of H₂-consuming and H₂-producing metabolic reactions in diverse methanogenic environments under in situ conditions. *FEMS Microbiol. Ecol.* 38:353–360.
- Conrad, R., F. Bak, H. J. Seitz, B. Thebrath, H. P. Mayer, and H. Schütz. 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiol. Ecol.* 62:285–294.
- Conrad, R., and B. Wetter. 1990. Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other anaerobic bacteria. *Arch. Microbiol.* 155:94–98.
- Cord-Ruwisch, R., D. R. Lovley, and B. Schink. 1998. Growth of *Geobacter sulfurreducens* with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. *Appl. Environ. Microbiol.* 64:2232–2236.
- Daniel, R., F. Warnecke, J. S. Potekhina, and G. Gottschalk. 1999. Identification of the syntrophic partners in a coculture coupling anaerobic methanol oxidation to Fe(III) reduction. *FEMS Microbiol. Lett.* 180:197–203.
- De Bok, F. A., A. J. Stams, C. Dijkema, and D. R. Boone. 2001. Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. *Appl. Environ. Microbiol.* 67:1800–1804.
- Dimroth, K. 1983. Thermochemische Daten organischer Verbindungen. In: C. Synowietz, D'Ans-Lax Taschenbuch für Chemiker und Physiker, Bd. 2. Springer, Berlin, Germany. 2:997–1038.
- Dimroth, P. 1987. Sodium transport decarboxylases and other aspects of sodium ion cycling in bacteria. *Microbiol. Rev.* 51:320–340.
- Dimroth, P. 2000. Operation of the F₀ motor of the ATP synthase. *Biochem. Biophys. Acta* 1458:374–386.
- Dong, X., G. Cheng, and A. J. M. Stams. 1994a. Butyrate oxidation by *Syntrophospira bryantii* in coculture with different methanogens and in pure culture with pentanoate as electron acceptor. *Appl. Microbiol. Biotechnol.* 42:647–652.
- Dong, X., C. M. Plugge, and A. J. M. Stams. 1994b. Anaerobic degradation of propionate by a mesophilic acetogenic bacterium in co- and triculture with different methanogens. *Appl. Environ. Microbiol.* 60:2834–2838.
- Dong, X., and A. J. M. Stams. 1995a. Evidence for H₂ and formate formation during syntrophic butyrate and propionate degradation. *Anaerobe* 1:35–39.
- Dong, X., and A. J. M. Stams. 1995b. Localization of enzymes involved in H₂ and formate metabolism in *Syntrophospira bryantii*. *Ant. v. Leeuwenhoek* 67:345–350.
- Dörner, C. 1992. Biochemie und Energetik der Wasserstofffreisetzung in der syntrophen Vergärung von Fettsäuren und Benzoat (thesis). Universität Tübingen. Tübingen, Germany. 58–61.
- Dubourgier, H. C., G. Prensier, and G. Albagnac. 1988. Structure and microbial activities of granular anaerobic sludge. In: G. Lettinga, A. J. B. Zehnder, J. T. C. Grotenhuis, and L. W. Hulshoff (Eds.) *Granular Anaerobic Sludge: Microbiology and Technology*. Pudoc, Wageningen, The Netherlands. 18–33.
- Dwyer, D. F., E. Weeg-Aeressens, D. R. Shelton, and J. M. Tiedje. 1988. Bioenergetic conditions of butyrate metabolism by a syntrophic, anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. *Appl. Environ. Microbiol.* 54:1354–1359.
- Egli, T. 1995. The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates. In: J. G. Jones (Ed.) *Advances in Microbial Ecology*. Plenum Press, New York, NY. 14:305–386.
- Eichler, B., and B. Schink. 1986. Fermentation of primary alcohols and diols, and pure culture of syntrophically alcohol-oxidizing anaerobes. *Arch. Microbiol.* 143:60–66.
- Elshahed, M. S., V. K. Bhupathiraju, N. Q. Wofford, M. A. Nanny, and M. J. McInerney. 2001. Metabolism of benzoate, cyclohex-1-ene carboxylate, and cyclohexane carboxylate by “*Syntrophus aciditrophicus*” strain SB in syntrophic association with H₂-using microorganisms. *Appl. Environ. Microbiol.* 67:1728–1738.
- Engelbrecht, S., and W. Junge. 1997. ATP synthase: A tentative structural model. *FEBS Lett.* 414:485–491.
- Fenchel, T., and B. J. Finlay. 1995. *Ecology and Evolution in Anoxic Worlds*. Oxford University Press, Oxford, UK. 108–171.
- Fey, A., and R. Conrad. 2000. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl. Environ. Microbiol.* 66:4790–4797.
- Finlay, B. J., and T. Fenchel. 1992. Methanogens and other bacteria as symbionts of free-living anaerobic ciliates. *Symbiosis* 14:375–390.
- Friedrich, M., U. Laderer, and B. Schink. 1991. Fermentative degradation of glycolic acid by defined syntrophic cocultures. *Arch. Microbiol.* 156:398–404.
- Friedrich, M., and B. Schink. 1993. Hydrogen formation from glycolate driven by reversed electron transport in membrane vesicles of a syntrophic glycolate-oxidizing bacterium. *Eur. J. Biochem.* 217:233–240.
- Friedrich, M., and B. Schink. 1995. Electron transport phosphorylation driven by glyoxylate respiration with hydrogen as electron donor in membrane vesicles of a glyoxylate-fermenting bacterium. *Arch. Microbiol.* 163:268–275.
- Friedrich, M., N. Springer, W. Ludwig, and B. Schink. 1996. Phylogenetic position of *Desulfobacterium glycolicum* gen. nov. sp. nov. and *Syntrophobacterium glycolicum* gen. nov. sp. nov., two strict anaerobes growing with glycolic acid. *Int. J. Syst. Bacteriol.* 46:1065–1069.

- Fuchs, G. M., E. S. Mohamed, U. Altenschmidt, J. Roch, A. Lach, R. Brackmann, C. Lockmeyer, and B. Oswald. 1994. Biochemistry of anaerobic biodegradation of aromatic compounds. In: C. Ratledge (Ed.) *Biochemistry of Microbial Degradation*. Kluwer Academic Publishers, Dordrecht, The Netherlands. 513–553.
- Fukuzaki, S., N. Nishio, M. Shobayashi, and S. Nagai. 1990. Inhibition of the fermentation of propionate to methane by hydrogen, acetate, and propionate. *Appl. Environ. Microbiol.* 56:719–723.
- Girbal, L., J. Öygsson, B. J. Reinders, and J. C. Gottschal. 1997. Why does *Clostridium acetireducens* not use interspecies hydrogen transfer for growth on leucine?. *Curr. Microbiol.* 35:155–160.
- Gottschalk, G. 1986. *Bacterial Metabolism*, 2nd ed. Springer, New York, NY.
- Harmsen, H., B. Wullings, A. D. L. Akkermans, W. Ludwig, and A. J. M. Stams. 1993. Phylogenetic analysis of *Syntrophobacter wolinii* reveals a relationship with sulfate-reducing bacteria. *Arch. Microbiol.* 160:238–240.
- Harmsen, H. J. M., H. M. P. Kengen, A. D. L. Akkermans, and A. J. M. Stams. 1995. Phylogenetic analysis of two syntrophic propionate-oxidizing bacteria in enrichment cultures. *Syst. Appl. Microbiol.* 18:67–73.
- Harmsen, H. J. M., H. M. P. Kengen, A. D. L. Akkermans, A. J. M. Stams, and W. M. de Vos. 1996. Detection and localization of syntrophic propionate-oxidizing bacteria in granular sludge by in situ hybridization using 16S rRNA-based oligonucleotide probes. *Appl. Environ. Microbiol.* 62:1656–1663.
- Harmsen, H. J., B. L. Van Kwijk, C. M. Plugge, A. D. Akkermans, W. M. De Vos, and A. J. Stams. 1998. *Syntrophobacter fumaroxidans* sp. nov., a syntrophic propionate-degrading sulfate-reducing bacterium. *Int. J. Syst. Bacteriol.* 48:1383–1387.
- Hattori, S., Y. Kamagata, S. Hanada, and H. Shoun. 2000. *Thermaetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int. J. Syst. Evol. Microbiol.* 50:1601–1609.
- Heider, J., and G. Fuchs. 1997. Anaerobic metabolism of aromatic compounds. *Eur. J. Biochem.* 243:577–596.
- Hinrichs, K. U., J. M. Hayes, S. P. Sylva, P. G. Brewer, and E. F. DeLong. 1999. Methane-consuming archaeobacteria in marine sediments. *Nature* 398:802–805.
- Hoehler, T. M., M. J. Alperin, D. B. Albert, and C. S. Martens. 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen-sulfate reducer consortium. *Global Biogeochem. Cycl.* 8:451–463.
- Hoehler, T. M., M. J. Alperin, D. B. Albert, and C. S. Martens. 2001. Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. *FEMS Microbiol. Ecol.* 38:33–41.
- Houwen, F. P., C. Dijkema, C. H. H. Schoenmakers, A. J. M. Stams, and A. J. B. Zehnder. 1987. ¹³C-NMR study of propionate degradation by a methanogenic coculture. *FEMS Microbiol. Lett.* 41:269–274.
- Houwen, F. P., J. Plokker, A. J. M. Stams, and A. J. B. Zehnder. 1990. Enzymatic evidence for involvement of the methylmalonyl-CoA pathway in propionate oxidation by *Syntrophobacter wolinii*. *Arch. Microbiol.* 155:52–55.
- Imachi, H., Y. Sekiguchi, Y. Kamagata, A. Ohashi, and H. Harada. 2000. Cultivation and in situ detection of a thermophilic bacterium capable of oxidizing propionate in syntrophic association with hydrogenotrophic methanogens in a thermophilic methanogenic granular sludge. *Appl. Environ. Microbiol.* 66:3608–3615.
- Iversen, N., and B. B. Jørgensen. 1985. Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). *Limnol. Oceanogr.* 30:944–955.
- Jackson, B. E., V. K. Bhupathiraju, R. S. Tanner, C. R. Woese, and M. J. McInerney. 1999. *Syntrophus aciditrophicus* sp. nov., a new anaerobic bacterium that degrades fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms. *Arch. Microbiol.* 171:107–114.
- Kaden, J., A. S. Galushko, B. Schink. 2002. Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*. *Arch. Microbiol.* 178(1):53–58.
- Kleerebezem, R., and A. J. Stams. 2000. Kinetics of syntrophic cultures: A theoretical treatise on butyrate fermentation. *Biotechnol. Bioeng.* 67:529–543.
- Koch, M., J. Dolfing, K. Wuhrmann, and A. J. B. Zehnder. 1983. Pathway of propionate degradation by enriched methanogenic cultures. *Appl. Environ. Microbiol.* 45:1411–1414.
- Kotsyurbenko, O. R., A. N. Nozhevnikova, T. I. Soloviova, and G. A. Zavarzin. 1996. Methanogenesis at low temperatures by microflora of tundra wetland soil. *Ant. v. Leeuwenhoek* 69:75–86.
- Kremer, D. R., H. E. Nienhuis-Kuiper, and T. A. Hansen. 1988. Ethanol dissimilation in *Desulfovibrio*. *Arch. Microbiol.* 150:552–557.
- Laanbroek, H. J., L. J. Stal, and H. Veldkamp. 1978. Utilization of hydrogen and formate by *Campylobacter spec.* under aerobic and anaerobic conditions. *Arch. Microbiol.* 119:99–102.
- Laanbroek, H. J., A. J. Smit, G. Klein-Nulend, and H. Veldkamp. 1979. Competition for glutamate between specialized and versatile *Clostridium* species. *Arch. Microbiol.* 120:330–335.
- Lee, M. J., and S. H. Zinder. 1988a. Carbon monoxide pathway enzyme activities in a thermophilic anaerobic bacterium grown acetogenically and in a syntrophic acetate-oxidizing coculture. *Arch. Microbiol.* 150:513–518.
- Lee, M. J., and S. H. Zinder. 1988b. Hydrogen partial pressures in a thermophilic acetate-oxidizing methanogenic cocultures. *Appl. Environ. Microbiol.* 54:1457–1461.
- Lee, M. J., and S. H. Zinder. 1988c. Isolation and characterization of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H₂-CO₂. *Appl. Environ. Microbiol.* 54:124–129.
- Lendenmann, U., M. Snozzi, and T. Egli. 1996. Kinetics of the simultaneous utilization of sugar mixtures by *Escherichia coli* in continuous culture. *Appl. Environ. Microbiol.* 62:1493–1499.
- Lettinga, G., A. J. B. Zehnder, J. T. C. Grotenhuis, and L. W. Hulshoff (Eds.). 1988. *Granular Anaerobic Sludge: Microbiology and Technology*. Pudoc, Wageningen, The Netherlands.
- Liu, Y., D. L. Balkwill, H. C. Aldrich, G. R. Drake, and D. R. Boone. 1999. Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolinii*. *Int. J. Syst. Bacteriol.* 49:545–556.

- Lovley, D. R., J. D. Coates, E. L. Blunt-Harris, E. J. P. Phillips, and J. C. Woodward. 1996. Humic substances as electron acceptors for microbial respiration. *Nature* 382:445–448.
- Matthies, C., and B. Schink. 1992. Reciprocal isomerization of butyrate and isobutyrate by strain WoGl3, and methanogenic isobutyrate degradation by a defined triculture. *Appl. Environ. Microbiol.* 58:1435–1439.
- Matthies, C., and B. Schink. 1993. Anaerobic degradation of long-chain dicarboxylic acids by methanogenic enrichment cultures. *FEMS Microbiol. Lett.* 111:177–182.
- McInerney, M. J., M. P. Bryant, and N. Pfennig. 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. *Arch. Microbiol.* 122:129–135.
- McInerney, M. J., M. P. Bryant, R. B. Hespell, and J. W. Costerton. 1981. *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl. Environ. Microbiol.* 41:1029–1039.
- McInerney, M. J. 1988. Anaerobic hydrolysis and fermentation of fats and proteins. *In: A. J. B. Zehnder (Ed.) Biology of Anaerobic Microorganisms.* John Wiley and Sons. New York, NY. 373–415.
- McInerney, M. J., and N. Q. Wofford. 1992. Enzymes involved in crotonate metabolism in *Syntrophomonas wolfei*. *Arch. Microbiol.* 158:344–349.
- Meckenstock, R. U. 1999. Fermentative toluene degradation in anaerobic defined syntrophic cocultures. *FEMS Microbiol. Lett.* 177:67–73.
- Meijer, W. G., M. E. Nienhuis-Kuiper, and T. A. Hansen. 1999. Fermentative bacteria from estuarine mud: Phylogenetic position of *Acidaminobacter hydrogenoformans* and description of a new type of Gram-negative, propionigenic bacterium as *Propionibacter pelophilus* gen. nov., sp. nov. 49. *Int. J. Syst. Bacteriol.* 49:1039–1044.
- Mitchell, P. 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Cambridge Phil. Soc.* 41:445–502.
- Mountfort, D. O., and M. P. Bryant. 1982. Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. *Arch. Microbiol.* 133:249–256.
- Mountfort, D. O., and H. F. Kaspar. 1986. Palladium-mediated hydrogenation of unsaturated hydrocarbons with hydrogen gas released during anaerobic cellulose degradation. *Appl. Environ. Microbiol.* 52:744–750.
- Müller, M. 1988. Energy metabolism of protozoa without mitochondria. *Ann. Rev. Microbiol.* 42:465–488.
- Nagase, M., and T. Matsuo. 1982. Interaction between amino-acid degrading bacteria and methanogenic bacteria in anaerobic digestion. *Biotechnol. Bioeng.* 24:2227–2239.
- Nanninga, H. J., and J. C. Gottschal. 1985. Amino acid fermentation and hydrogen transfer in mixed cultures. *FEMS Microbiol. Ecol.* 31:261–269.
- Nanninga, H. J., W. J. Drent, and J. C. Gottschal. 1987. Fermentation of glutamate by *Selenomonas acidaminophila* sp. nov. *Arch. Microbiol.* 147:152–157.
- Naumann, E., H. Hippe, and G. Gottschalk. 1983. Betaine: New oxidant in the Stickland reaction and methanogenesis from betaine and L-alanine by a *Clostridium sporogenes*-*Methanosarcina barkeri* coculture. *Appl. Environ. Microbiol.* 45:474–483.
- Öygsson, J., F. P. Houwen, and B. H. Svensson. 1993. Anaerobic degradation of protein and the role of methane formation in steady state thermophilic enrichment cultures. *Swedish J. Agric. Res.* 23:45–54.
- Öygsson, J. 1994. The role of interspecies hydrogen transfer on thermophilic protein and amino acid metabolism (PhD thesis). Swedish University of Agricultural Sciences. Uppsala, Sweden. Chapter 4.
- Orphan, V. J., K.-U. Hinrichs, W. Ussler, C. K. Paull, L. T. Taylor, S. P. Sylva, J. M. Hayes, and E. F. DeLong. 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Appl. Environ. Microbiol.* 67:1922–1934.
- Oude Elferink, S. J. W. H., W. J. C. Vorstman, A. Sopjes, and A. J. M. Stams. 1998. Characterization of the sulfate-reducing and syntrophic population in granular sludge from a full-scale anaerobic reactor treating papermill wastewater. *FEMS Microbiol. Ecol.* 27:185–194.
- Overmann, J. 2002. Phototrophic consortia: A tight cooperation between non-related eubacteria. *In: J. Seckbach (Ed.) Symbiosis: Mechanisms and Model Systems.* Kluwer. Dordrecht, The Netherlands. 239–255.
- Pancost, R. D., J. S. S. Damsté, S. de Lint, M. J. E. C. van der Maarel, K. C. Gottschal, and the Medinaut Shipboard Scientific Party. 2000. Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean sediments by a consortium of methanogenic archaea and bacteria. *Appl. Environ. Microbiol.* 66:1126–1132.
- Pfennig, N. 1980. Syntrophic mixed cultures and symbiotic consortia with phototrophic bacteria: A review. *In: G. Gottschalk, N. Pfennig, H. Werner (Eds.) Anaerobes and Anaerobic Infections.* Fischer. Stuttgart, Germany. New York, NY. 127–131.
- Phelps, T. J., and J. G. Zeikus. 1984. Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. *Appl. Environ. Microbiol.* 48:1088–1095.
- Platen, H., and B. Schink. 1987. Methanogenic degradation of acetone by an enrichment culture. *Arch. Microbiol.* 149:136–141.
- Platen, H., P. H. Janssen, and B. Schink. 1994. Fermentative degradation of acetone by an enrichment culture in membrane-separated culture devices and in cell suspensions. *FEMS Microbiol. Lett.* 122:27–32.
- Plugge, C. M., C. Dijkema, and A. J. M. Stams. 1993. Acetyl-CoA cleavage pathway in a syntrophic propionate oxidizing bacterium growing on fumarate in the absence of methanogens. *FEMS Microbiol. Lett.* 110:71–76.
- Plugge, C. M., E. G. Zoetendal, and A. J. M. Stams. 2000. *Caloramator coolhaasii*, sp. nov. a glutamate-degrading, moderately thermophilic anaerobe. *Int. J. Syst. Bacteriol.* 50:1155–1162.
- Plugge, C. M., and A. J. M. Stams. 2001a. Arginine catabolism by *Thermanaerovibrio acidaminovorans*. *FEMS Microbiol. Lett.* 195:259–262.
- Plugge, C. M., M. Balk, E. G. Zoetendal, and A. J. M. Stams. 2002. *Gelria glutamica*, gen. nov., sp. nov., a thermophilic obligate syntrophic glutamate-degrading anaerobe. *Int. J. Syst. Evol. Microbiol.* 52(Pt 2):401–407.
- Plugge, C. M., J. M. van Leeuwen, T. Hummelen, M. Balk, and A. J. M. Stams. 2001b. Elucidation of the pathways of catabolic glutamate conversion in three thermophilic anaerobic bacteria. *Arch. Microbiol.* 176:29–36.
- Reeburgh, W. S. 1980. Anaerobic methane oxidation: Rate distributions in Skan Bay sediments. *Earth Plan. Sci. Lett.* 47:345–352.
- Roy, F., E. Samain, H. C. Dubourgier, and G. Albagnac. 1986. *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and

- unsaturated long chain fatty acids. *Arch. Microbiol.* 145:142–147.
- Santegoeds, C. M., L. R. Damgaard, G. Hesselink, J. Zopfi, P. Lens, G. Muyzer, and D. de Beer. 1999. Distribution of sulfate-reducing and methanogenic bacteria in anaerobic aggregates determined by microsensors and molecular analyses. *Appl. Environ. Microbiol.* 65:4618–4629.
- Schink, B. 1984. Fermentation of 2,3-butanediol by *Pelobacter carbinolicus* sp. nov., and *Pelobacter propionicus*, sp. nov., and evidence for propionate formation from C₂ compounds. *Arch. Microbiol.* 137:33–41.
- Schink, B. 1985a. Fermentation of acetylene by an obligate anaerobe, *Pelobacter acetylenicus* sp. nov. *Arch. Microbiol.* 142:295–301.
- Schink, B. 1985b. Mechanism and kinetics of succinate and propionate degradation in anoxic freshwater sediments and sewage sludge. *J. Gen. Microbiol.* 131:643–650.
- Schink, B., and R. K. Thauer. 1988. Energetics of syntrophic methane formation and the influence of aggregation. *In: G. Lettinga, A. J. B. Zehnder, J. T. C. Grotenhuis, and L. W. Hulshoff* (Eds.) *Granular Anaerobic Sludge: Microbiology and Technology*. Pudoc. Wageningen, The Netherlands. 5–17.
- Schink, B. 1990. Conservation of small amounts of energy in fermenting bacteria. *In: R. K. Finn and P. Prève* (Eds.) *Biotechnology: Focus 2*. Hanser Publishers. New York, NY. 63–89.
- Schink, B. 1991. Syntrophism among prokaryotes. *In: A. Balows, H. G. Trüper, M. Dworkin, and K. H. Schleifer* (Eds.) *The Prokaryotes*, 2nd ed. Springer. New York, NY. 276–299.
- Schink, B. 1994. Diversity, ecology, and isolation of acetogenic bacteria. *In: H. L. Drake* (Ed.) *Acetogenesis*. Chapman and Hall. New York, NY. 197–235.
- Schink, B., and M. Friedrich. 1994. Energetics of syntrophic fatty acid degradation. *FEMS Microbiol. Rev.* 15:85–94.
- Schink, B. 1997. Energetics of syntrophic cooperations in methanogenic degradation. *Microbiol. Molec. Biol. Rev.* 61:262–280.
- Schink, B., B. Philipp, and J. Müller. 2000. Anaerobic degradation of phenolic compounds. *Naturwissenschaften* 87:12–23.
- Schnürer, A., F. P. Houwen, and B. H. Svensson. 1994. Mesophilic syntrophic acetate oxidation during methane formation by a triculture at high ammonium concentration. *Arch. Microbiol.* 162:70–74.
- Schnürer, A., B. Schink, and B. H. Svensson. 1996. *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. *Int. J. Syst. Bacteriol.* 46:1145–1152.
- Schnürer, A., B. H. Svensson, and B. Schink. 1997. Enzyme activities in and energetics of acetate metabolism by the mesophilic syntrophically acetate-oxidizing anaerobe *Clostridium ultunense*. *FEMS Microbiol. Lett.* 154:331–336.
- Schöcke, L., and B. Schink. 1997. Energetics of methanogenic benzoate degradation by *Syntrophus gentianae* in syntrophic coculture. *Microbiology* 143:2345–2351.
- Schöcke, L., and B. Schink. 1998. Membrane-bound proton-translocating pyrophosphatase of *Syntrophus gentianae*, a syntrophically benzoate-degrading fermenting bacterium. *Eur. J. Biochem.* 256:589–594.
- Schöcke, L., and B. Schink. 1999. Biochemistry and energetics of fermentative benzoate degradation by *Syntrophus gentianae*. *Arch. Microbiol.* 171:331–337.
- Scholten, J. C. M., and R. Conrad. 2000. Energetics of syntrophic propionate oxidation in defined batch and chemostat cocultures. *Appl. Environ. Microbiol.* 66:2934–2942.
- Schönheit, P., J. Moll, and R. K. Thauer. 1980. Growth parameters (K_s, v_{max}, Y_s) of *Methanobacterium thermoautotrophicum*. *Arch. Microbiol.* 127:59–65.
- Seelert, H., A. Poetsch, N. A. Dencher, A. Engel, H. Stahlberg, and D. J. Müller. 2000. Proton-powered turbine of a plant motor. *Nature* 405:418–419.
- Sekiguchi, Y., Y. Kamagata, K. Nakamura, A. Ohashi, and H. Harada. 2000. *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. *Int. J. Syst. Evol. Microbiol.* 50:771–779.
- Stams, A. J. M., and T. A. Hansen. 1984. Fermentation of glutamate and other compounds by *Acidaminobacter hydrogeniformans* gen. nov., sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulfate-reducing and methanogenic bacteria. *Arch. Microbiol.* 137:329–337.
- Stams, A. J. M., J. T. C. Grotenhuis, and A. J. B. Zehnder. 1989. Structure-function relationship in granular sludge. *In: T. Hattori, Y. Ishida, Y. Maruyama, R. Y. Morita, and A. Uchida* (Eds.) *Recent Advances in Microbial Ecology*. Japan Scientific Society Press. Tokyo, Japan. 440–445.
- Stams, A. J. M., and C. M. Plugge. 1990. Isolation of syntrophic bacteria on metabolic intermediates. *In: J. P. Belaich, M. Bruschi, and J. L. Garcia* (Eds.) *Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer*. Plenum Publishing. New York, NY. 473–476.
- Stams, A. J. M., J. B. van Dijk, C. Dijkema, and Plugge, C. M. 1993. Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. *Appl. Environ. Microbiol.* 59:1114–1119.
- Stams, A. J. M. 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. *Ant. v. Leeuwenhoek* 66:271–294.
- Stams, A. J. M., C. Dijkema, C. M. Plugge, and P. Lens. 1998. Contribution of ¹³C-NMR spectroscopy to the elucidation of pathways of propionate formation and degradation in methanogenic environments. *Biodegradation* 9:463–473.
- Stieb, M., and B. Schink. 1985. Anaerobic oxidation of fatty acids by *Clostridium bryantii* sp. nov., a spore-forming, obligately syntrophic bacterium. *Arch. Microbiol.* 140:387–390.
- Stieb, M., and B. Schink. 1986. Anaerobic degradation of isovalerate by a defined methanogenic coculture. *Arch. Microbiol.* 144:291–295.
- Stieb, M., and B. Schink. 1989. Anaerobic degradation of isobutyrate by methanogenic enrichment cultures and by a *Desulfococcus multivorans* strain. *Arch. Microbiol.* 151:126–132.
- Stock, D., A. G. W. Leslie, and J. E. Walker. 1999. Molecular architecture of the rotary motor in ATP synthase. *Science* 286:1700–1705.
- Stumm, C. K., H. J. Gijzen, and G. D. Vogels. 1982. Association of methanogenic bacteria with ovine rumen ciliates. *Br. J. Nutr.* 47:95–99.

- Svetlitsnyi, V., F. Rainey, and J. Wiegel. 1996. *Thermosyntropha lipolytica* gen. nov., sp. nov., a lipolytic, anaerobic, alkalitolerant, thermophilic bacterium utilizing short- and long-chain fatty acids in syntrophic coculture with a methanogenic archaeum. *Int. J. Syst. Bacteriol.* 46:1131–1137.
- Tarlera, S., L. Muxi, M. Soubes, and A. J. M. Stams. 1997. *Caloramator proteoclasticus* sp. nov., a new moderately thermophilic anaerobic proteolytic bacterium. *Int. J. Syst. Bacteriol.* 47:651–656.
- Tarlera, S., and A. J. M. Stams. 1999. Degradation of proteins and amino acids by *Caloramator proteoclasticus* in pure culture and in coculture with *Methanobacterium thermoautotrophicum* Z245. *Appl. Microbiol. Biotechnol.* 53:133–138.
- Thauer, R. K., K. Jungermann, and K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* 41:100–180.
- Thauer, R. K., and J. G. Morris. 1984. Metabolism of chemotrophic anaerobes: Old views and new aspects. *In: D. P. Kelly and N. G. Carr (Eds.) The Microbe 1984. Part II: Prokaryotes and Eukaryotes.* Cambridge University Press. Cambridge, UK. 123–168.
- Thiele, J. H., and J. G. Zeikus. 1988. Control of interspecies electron flow during anaerobic digestion: Significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Appl. Environ. Microbiol.* 54:20–29.
- Tholozan, J. L., E. Samain, J. P. Grivet, R. Moletta, H. C. Dubourguier, and G. Albagnac. 1988. Reductive carboxylation of propionate to butyrate in methanogenic ecosystems. *Appl. Environ. Microbiol.* 54:441–445.
- Tholozan, J. L., E. Samain, J. P. Grivet, and G. Albagnac. 1990. Propionate metabolism in a methanogenic enrichment culture: Direct reductive carboxylation and acetogenesis pathways. *FEMS Microbiol. Ecol.* 73:291–298.
- Thomsen, T. R., K. Finster, and N. B. Ramsing. 2001. Biogeochemical and molecular signatures of anaerobic methane oxidation in a marine sediment. *Appl. Environ. Microbiol.* 67:1646–1656.
- Valentine, D. L., and W. S. Reebergh. 2000a. New perspectives on anaerobic methane oxidation. *Environ. Microbiol.* 2:477–484.
- Valentine, D. L., D. C. Blanton, and W. S. Reebergh. 2000b. Hydrogen production by methanogens under low-hydrogen conditions. *Arch. Microbiol.* 174:415–421.
- Valentine, D. L., W. S. Reebergh, and D. C. Blanton. 2000c. A culture apparatus for maintaining H₂ at sub-nanomolar concentrations. *J. Microbiol. Meth.* 39:243–251.
- van Bruggen, J. J. A., C. K. Stumm, and G. D. Vogels. 1983. Symbiosis of methanogenic bacteria and sapropelic protozoa. *Arch. Microbiol.* 136:89–95.
- van Bruggen, J. J. A., C. K. Stumm, K. B. Zwart, and G. D. Vogels. 1985. Endosymbiotic methanogenic bacteria of the sapropelic amoeba *Mastigella*. *FEMS Microbiol. Ecol.* 31:187–192.
- van Lier, J. B., K. C. Grolle, C. T. Frijters, A. J. M. Stams, and G. Lettinga. 1993. Effects of acetate, propionate, and butyrate on the thermophilic anaerobic degradation of propionate by methanogenic sludge and defined cultures. *Appl. Environ. Microbiol.* 59:1003–1011.
- Wallrabenstein, C., and B. Schink. 1994a. Evidence of reversed electron transport involved in syntrophic butyrate and benzoate oxidation by *Syntrophomonas wolfei* and *Syntrophus buswellii*. *Arch. Microbiol.* 162:136–142.
- Wallrabenstein, C., E. Hauschild, and B. Schink. 1994b. Pure culture and cytological properties of *Syntrophobacter wolinii*. *FEMS Microbiol. Lett.* 123:249–254.
- Wallrabenstein, C., N. Gorny, N. Springer, W. Ludwig, and B. Schink. 1995a. Pure culture of *Syntrophus buswellii*, definition of its phylogenetic status, and description of *Syntrophus gentianae* sp. nov. *Syst. Appl. Microbiol.* 18:62–66.
- Wallrabenstein, C., E. Hauschild, and B. Schink. 1995b. *Syntrophobacter pfennigii* sp. nov., a new syntrophically propionate-oxidizing anaerobe growing in pure culture with propionate and sulfate. *Arch. Microbiol.* 164:346–352.
- Warikoo, V., M. J. McNerney, J. A. Robinson, and J. M. Sufliita. 1996. Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. *Appl. Environ. Microbiol.* 62:26–32.
- Widdel, F. 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria. *In: A. J. B. Zehnder (Ed.) Biology of Anaerobic Microorganisms.* John Wiley and Sons. New York, NY. 469–585.
- Wildenauer, F. X., and J. Winter. 1986. Fermentation of isoleucine and arginine by pure and syntrophic cultures of *Clostridium sporogenes*. *FEMS Microbiol. Ecol.* 38:373–379.
- Winter, J., F. Schindler, and F. X. Wildenauer. 1987. Fermentation of alanine and glycine by pure and syntrophic cultures of *Clostridium sporogenes*. *FEMS Microbiol. Ecol.* 45:153–161.
- Wofford, N. Q., P. S. Beaty, and M. J. McNerney. 1986. Preparation of cell-free extracts and the enzymes involved in fatty acid metabolism in *Syntrophomonas wolfei*. *J. Bacteriol.* 167:179–185.
- Wu, W.-M., M. K. Jain, R. F. Hickey, and J. G. Zeikus. 1991. Characterization of metabolic performance of methanogenic granules treating brewery wastewater: Role of sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 57:3438–3449.
- Wu, W.-M., R. F. Hickey, M. Jain, and J. G. Zeikus. 1993. Energetics and regulations of formate and hydrogen metabolism by *Methanobacterium formicicum*. *Arch. Microbiol.* 159:57–65.
- Wu, W.-M., M. K. Jain, R. F. Hickey, and J. G. Zeikus. 1994. Anaerobic degradation of normal- and branched-chain fatty acids with four or more carbons to methane by a syntrophic methanogenic triculture. *Appl. Environ. Microbiol.* 57:2220–2226.
- Wu, W.-M., M. K. Jain, R. F. Hickey, and J. G. Zeikus. 1996. Perturbation of syntrophic isobutyrate and butyrate degradation with formate and hydrogen. *Biotechnol. Bioeng.* 52:404–411.
- Zehnder, A. J. B. 1978. Ecology of methane formation. *In: R. Mitchell (Ed.) Water Pollution Microbiology.* John Wiley and Sons. London, UK. 2:349–376.
- Zehnder, A. J. B., and T. D. Brock. 1979. Methane formation and methane oxidation by methanogenic bacteria. *J. Bacteriol.* 137:420–432.
- Zehnder, A. J. B., K. Ingvorsen, and T. Marti. 1982. Microbiology of methane bacteria. *In: D. E. Hughes, D. A. Stafford, B. I. Wheatley, W. Baader, G. Lettinga, E. J. Nyns, W. Verstraete (Eds.) Anaerobic Digestion.* Elsevier Biomedical Press. Amsterdam, The Netherlands. 45–68.
- Zeikus, J. G., and M. Winfrey. 1976. Temperature limitation of methanogenesis in aquatic sediments. *Appl. Environ. Microbiol.* 31:99–107.

- Zhao, H., D. Yang, C. R. Woese, and M. P. Bryant. 1989. Assignment of *Clostridium bryantii* to *Syntrophospora bryantii* gen. nov., nov. comb., based on 16S rRNA sequence analysis of its crotonate-grown pure culture. *Int. J. Syst. Bacteriol.* 40:40–44.
- Zinder, U., W. Freudenberg, M. Rieth, J. R. Andreesen, J. Schnell, and F. Widdel. 1988. *Eubacterium acidaminophilum* sp. nov., a versatile amino acid-degrading anaerobe producing or utilizing H₂ or formate: Description and enzymatic studies. *Arch. Microbiol.* 150:254–266.
- Zinder, S. H., and M. Koch. 1984. Non-aceticlastic methanogenesis from acetate: Acetate oxidation by a thermophilic syntrophic coculture. *Arch. Microbiol.* 138:263–272.