Syntrophic Associations in Methanogenic Degradation

Bernhard Schink

1 Introduction

For many decades, microbiology has tried to understand the activities of microorganisms in nature on the basis of pure culture studies which allow reliable identification of the actors in play and reproducible assessments of their activities under defined conditions. This approach has undoubtedly been successful, but it has overlooked that microbes in nature interact with each other and may depend on these interactions to a various extent. The mutual relationship of partner organisms to each other may vary from only marginal interaction to absolute mutual dependence on each other. Some microorganisms excrete metabolites, e.g., precursors of vitamins or certain amino acids, which are used by a partner organism that lacks specific synthesis pathways and profits from this support, even if it could synthesize the respective compound on its own and this way only saves biosynthetic energy. Types of more intense cooperation and mutual interdependence are found preferentially among anaerobic bacteria, although we have to admit that our view is probably constrained by the cultures we know: Since especially aerobic bacteria are usually isolated with simple media that select for easy-to-cultivate organisms degrading a simple cocktail of substrates on their own, we may overlook other bacteria that are outcompeted under such conditions, and may display more-refined types of interaction with others. Since we know of only a small fraction of all the microorganisms present in the environment, we cannot exclude that other bacteria in the natural environment might depend to a large extent upon cooperations with partners, and perhaps this is just one of the reasons why we so far have failed to cultivate them.

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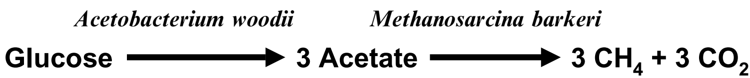
2
Types of Cooperation Among Anaerobic Microorganisms

Whereas aerobic bacteria are usually considered to be able to degrade complex organic matter completely to CO_2 and H_2O, this is true in the anaerobic world only in some exceptional cases. Complex biomass is typically degraded in several steps, including classical (primary) fermentations, with subsequent further oxidation by sulfate reduction or iron reduction, or by coupling primary fermentations with secondary fermentations to methanogenesis at the very end (Bryant 1979; McInerney 1988; Stams 1994; Schink 1997). This kind of job-sharing among anaerobic microorganisms makes the whole process more complicated at first sight, but ascribes to every single organism only a limited task it has to fulfill and, with this, far less effort is needed for regulation of its metabolism.

A



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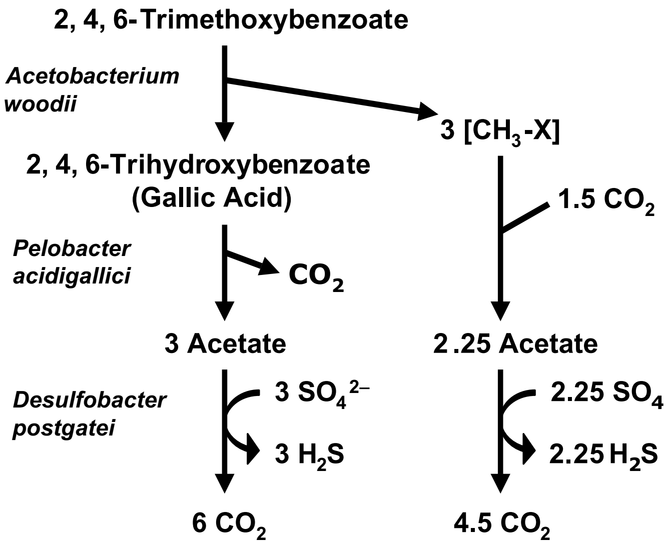


Fig. 1. Metabiotic cooperations in defined cocultures degrading glucose (**A**) and trimethoxybenzoate to methane and CO_2 (**B**)

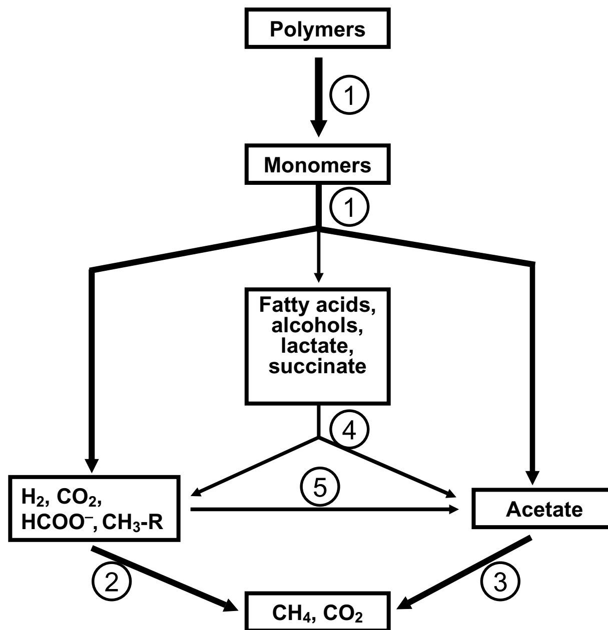


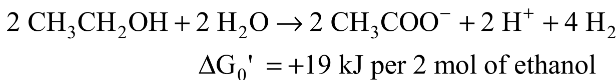
Fig. 2. Carbon and electron flow in the methanogenic degradation of complex organic matter. Groups of prokaryotes involved: *1* primary fermentative bacteria; *2* hydrogen-oxidizing methanogens; *3* acetate-cleaving methanogens; *4* secondary fermenting bacteria (syntrophs); and *5* homoacetogenic bacteria

The interdependence among these partners may vary from an "assembly line"-type of cooperation called metabiosis in which only the later partner in the line profits from the former one but the advantage to the former members in the line by the later partners is negligible. Examples of this kind are degradation of glucose via acetate to methane by cooperation of *Acetobacterium woodii* and *Methanosarcina barkeri* (Fig. 1a; Winter and Wolfe 1979), or complete oxidation of trimethoxybenzoate via gallic acid and acetate by a triculture consisting of *A. woodii*, *Pelobacter acidigallici*, and *Desulfobacter postgatei* (Fig. 1b; Kreikenbohm and Pfennig 1985). Degradation of sugars and polysaccharides by clostridia is influenced positively by cooperation with hydrogen-consuming methanogens that shift the fermentation pattern to more acetate formation and, with this, to higher ATP yields (Schink 1997). Degradation of such compounds in sediments or in well-balanced sludge digestors may proceed nearly exclusively through acetate plus hydrogen, i.e., through the bold arrows in Fig. 2, with very little production of reduced side products such as butyrate and other fatty acids. Excessive production of these reduced side products is typically found in pure cultures or in unbalanced reactors receiving easily fermentable substrates at high rates that cannot be counterbal-

anced by sufficient growth of methanogenic partners. Cooperative interactions between fermenting bacteria and methanogenic partners have been found to be involved also in the anaerobic degradation of amino acids (Wildenauer and Winter 1986; Winter et al. 1987). The extent of cooperation between the partners degrading amino acids varies dramatically depending on the degradation pathways, from total independence of each other to obligately syntrophic relationships. Quite often, different degradation pathways are used for one specific amino acid in the presence or absence of hydrogen-scavenging partner organisms (Schink and Stams 2001). Finally, there are the strictly syntrophic relationships in which both partners depend on each other for energetic reasons and together perform a fermentation process that neither could run on its own, as is typical of syntrophic associations.

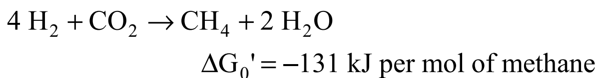
Syntrophy is a special case of symbiotic cooperation between two metabolically different types of bacteria that depend on each other for degradation of a certain substrate, typically through transfer of one or more metabolic intermediate(s) between the partners. The pool size of the intermediate shuttled between the partners has to be kept low to allow efficient cooperation. The term "syntrophy" should be restricted to those cooperations in which both 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, 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:

S Strain:



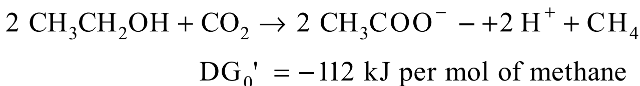
$$2\ CH_3CH_2OH + 2\ H_2O \rightarrow 2\ CH_3COO^- + 2\ H^+ + 4\ H_2$$
$$\Delta G_0' = +19 \text{ kJ per 2 mol of ethanol}$$

Strain M.o.H.:



$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$$
$$\Delta G_0' = -131 \text{ kJ per mol of methane}$$

Coculture:



$$2\ CH_3CH_2OH + CO_2 \rightarrow 2\ CH_3COO^- - +2\ H^+ + CH_4$$
$$DG_0' = -112 \text{ kJ per mol of methane}$$

Thus, the fermenting bacterium cannot grow with ethanol in the absence of the hydrogen-scavenging partner because it carries out a reaction that is endergonic under standard conditions. The first reaction can 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 ethanol degradation depends on the cooperating activities of both.

In this article, we avoid the term “consortium”, which is often used to describe any kind of enrichment cultures cooperating in whatever way. This term was originally coined for the structured phototrophic aggregates like *“Pelochromatium”* and *“Chlorochromatium”* and should be restricted to such spatially well-organized systems (see Chap. 2).

3
Energetical 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 under the conditions prevailing in an actively growing cell requires +49 kJ per mol (Thauer et al. 1977). Since part of the total energy is always lost in irreversible reaction steps as heat (on average about 20 kJ per mol ATP) a total of about 70 kJ per mol ATP synthesized has to be calculated for ATP synthesis in a living cell (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 for well-growing cells. Nonetheless, one cannot expect the energy requirement for irreversible ATP synthesis to go substantially below about +60 kJ per mol.

According to the Mitchell theory of respirative ATP synthesis, ATP formation is coupled to a vectorial transport of charged groups, typically protons, across a semipermeable membrane. If the ratio of proton translocation over ATP synthesized is 3, the smallest quantum of metabolically convertible energy, equivalent to the transport of a monovalent cation across the charged cytoplasmic membrane, is equivalent to one-third of an ATP unit. 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 and Stams 2001).

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 assumed so far. 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 determined 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.

The postulate that there is a minimum amount of approximately –12 to –15 kJ per reaction needed to drive ATP synthesis has been questioned recently in a paper where the remnant energy in starving syntrophically fermenting bacteria had been determined to be as low as –4 kJ per mol (Jackson and McInerney 2002). However, the authors showed only that the "battery" can burn down to such low values at low energy supply; they did not prove that the system can produce ATP under these conditions and thus they did not disprove the concept of a minimum amount of energy for ATP synthesis in the range discussed above.

4 Concept of Syntrophic Energy Metabolism

The effect of hydrogen- and acetate-scavenging partner organisms on syntrophic fermentations becomes obvious if one compares such fermentations under standard conditions (which are roughly comparable to those prevailing in pure cultures) and under conditions that are similar to those prevailing in natural or semi-natural environments. As shown in Table 1, the endergonic fermentations of ethanol, alanine and butyrate turn into exergonic reactions if conditions are assumed that are comparable to those we find in a lake sediment or a sewage sludge digestor; for these calculations, I used hydrogen and acetate concentrations that are at the energetical limit to provide an energy minimum to the methanogens consuming these intermediates. It is obvious that alanine fermentation even allows synthesizing a full ATP unit under these conditions and is, with this, a little atypical.

In most other instances of syntrophic cooperations, the partner organisms have to live with only fractions of an ATP equivalent per reaction run. This can be accomplished by combinations of substrate level phosphorylation steps with a reinvestment of ATP fractions, typically in one or more reversed electron transport processes. The situation is most delicate with syntrophic associations degrading fatty acids such as butyrate, long-chain fatty acids, propionate, or acetate which leave the absolute minimum of 1/3 – 1/4 ATP equivalent (corresponding to 15 – 20 kJ per reaction run) to every partner.

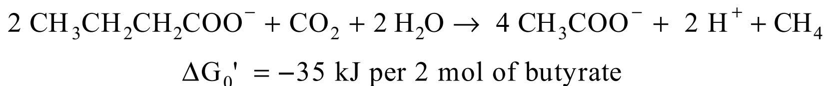
Syntrophic acetate conversion to methane and CO_2 can yield this minimum amount of energy only at enhanced temperature: the reaction operates at its lower temperature limit at 37°C (Schink and Stams 2001) but runs far better at 55–60°C (Zinder and Koch 1984; Hattori et al. 2000). The energetical situation of syntrophic ethanol conversion to methane plus CO_2 is considerably easier, but so far we do not have a convincing concept how energy sharing between the partners is accomplished at the biochemical level.

5 Energy Metabolism in Syntrophically Fermenting Bacteria

5.1 Butyrate Oxidation

In syntrophically butyrate-oxidizing bacteria (McInerney et al. 1979), 1 ATP is synthesized by substrate-level phosphorylation through thiolytic 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} to 10^{-5} bar (Thauer and Morris 1984). Experimental evidence of a reverse electron transport system between the crotonyl-CoA/butyryl-CoA couple ($E°'= -125$ mV) and the H^+/H_2 couple has been provided with Syntrophomonas wolfei (Wallrabenstein and Schink 1994). 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:



$$2\ CH_3CH_2CH_2COO^- + CO_2 + 2\ H_2O \rightarrow\ 4\ CH_3COO^- +\ 2\ H^+ + CH_4$$

$$\Delta G_0' = -35\ \text{kJ per 2 mol of butyrate}$$

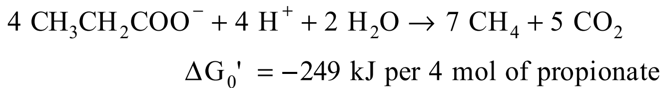
The energy gain increases to –46 kJ at butyrate and acetate concentrations in the range of 10 mM as typically used in laboratory cultures. Under these conditions, the energetic situation for the partners gets tough (–15 kJ per mol partial reaction), especially at the end of the substrate conversion process. Very slow, often non-exponential 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, either thermodynamically via product accumulation or by uncoupling caused by accumulating acid residues.

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

5.2 Syntrophic Propionate Oxidation

For syntrophic propionate oxidation according to the equation



$$4\ CH_3CH_2COO^- + 4\ H^+ + 2\ H_2O \rightarrow 7\ CH_4 + 5\ CO_2$$

$$\Delta G_0' = -249 \text{ kJ per 4 mol of propionate}$$

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 under standard conditions (Stams et al. 1989; Schink 1991), and this values decreases to –19 kJ in laboratory cultures and to –12 kJ at propionate concentrations prevailing in sediments or sludge digestors (Table 1). Studies in defined mixed cultures and in undefined communities in rice field soil have basically confirmed these calculations (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 1985, 1991; Houwen et al. 1987, 1990). Propionate is activated 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. The enzymes and electron transfer components involved in propionate oxidation were studied with Syntrophobacter wolinii (Boone and Bryant 1980; Houwen et al. 1990; Plugge et al. 1993) and Syntrophobacter pfennigii (Wallrabenstein et al. 1995). Experiments on hydrogen formation in the presence and absence of protonophores or the ATPase inhibitor DCCD indicated that an intact proton motive force maintained by ATP hydrolysis is required for hydrogen release, probably in the first oxidation step (Dörner 1992).

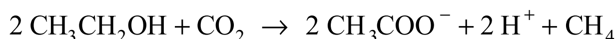
Studies with *Syntrophobacter fumaroxidans* have shown that not only hydrogen but also formate contributes to interspecies electron transport in this system: Syntrophic propionate oxidation was possible only in cooperation with formate- and hydrogen-oxidizing methanogens, not with *Methanobrevibacter* strains that are unable to oxidize formate (Dong et al. 1994). Enzyme

measurements in 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 (de Bok et al. 2002a,b, 2004).

5.3 Syntrophic Ethanol Oxidation

Although the case of "*Methanobacillus omelianskii*" is the classical example of interspecies hydrogen transfer, numerous further syntrophically ethanol-oxidizing bacteria have been isolated, such as *Thermoanaerobium brockii* (Ben-Bassat et al. 1981) and various *Pelobacter* strains (Eichler and Schink 1986). Also certain ethanol-oxidizing sulfate reducers such as *Desulfovibrio vulgaris* oxidize ethanol in the absence of sulfate by hydrogen transfer to a hydrogen-oxidizing methanogenic partner.

Unfortunately, the biochemistry of this syntrophic cooperation is still unclear. The total reaction

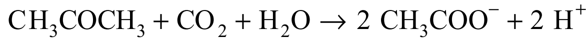


$$2\,CH_3CH_2OH + CO_2 \rightarrow 2\,CH_3COO^- + 2\,H^+ + CH_4$$

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*" or *Pelobacter acetylenicus*, ethanol dehydrogenase, acetaldehyde acceptor oxidoreductase (acetyl CoA-forming), phosphotransacetylase, and acetate kinase have been detected, 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–45 kJ is available to the ethanol oxidizer per mol ethanol oxidized which is too little energy to form one full ATP. Therefore, part of the energy bound in ATP has to be reinvested to push the overall reaction and balance the energy budget, but this reverse electron transport system has not yet been identified. It could be the ethanol dehydrogenase enzyme itself which couples ethanol oxidation to acetaldehyde ($E°'$= –195 mV) with NAD+ reduction ($E°'$= –320 mV), electron transfer from NAD+ to a ferredoxin-like electron carrier, or the hydrogen-releasing hydrogenase itself of which some have recently been reported to couple to proton translocations (Sapra et al. 2003; Hedderich 2004).

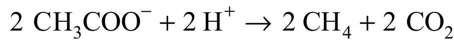
5.4 Fermentation of Acetone

A special situation is the fermentative conversion of acetone to methane and CO2, which is catalyzed by syntrophically cooperating bacteria as well, with acetate as the only intermediate transferred between both partners:



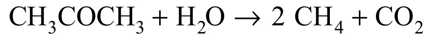
$$CH_3COCH_3 + CO_2 + H_2O \rightarrow 2\ CH_3COO^- + 2\ H^+$$

$$\Delta G_0' = -25.8\ kJ \cdot mol^{-1}$$



$$2\ CH_3COO^- + 2\ H^+ \rightarrow 2\ CH_4 + 2\ CO_2$$

$$\Delta G_0' = -71.8\ kJ \cdot mol^{-1}$$



$$CH_3COCH_3 + H_2O \rightarrow 2\ CH_4 + CO_2$$

$$\Delta G_0' = -97.6\ kJ \cdot mol^{-1}$$

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 about –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). Another reason could be that these bacteria cannot operate with energy fractions smaller than the equivalent of one ATP unit. Unfortunately, the acetone-fermenting bacterium was never obtained in pure culture or in defined coculture, and hence detailed studies on its biochemistry and energetics could never be performed.

5.5
Syntrophic Oxidation of Hexoses

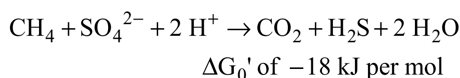
Fermentation of hexose to 2 acetate, 2 CO_2 and 4 H_2 as sole products is exergonic (–216 kJ per mol) but does not yield sufficient energy to synthesize 4 ATP by substrate level phosphorylation that are directly linked with this fermentation via glycolysis. Hydrogen removal to a low concentration makes this reaction exergonic enough to allow fermentation according to this pattern. We could recently show that there is a considerable number of primary fermenting bacteria in a lake sediment that can ferment sugars only to acetate, CO_2 and H_2, but they depend on a hydrogen-scavenging partner (Schink and Stingl, unpubl.) and have been overlooked because our usual isolation strategies select those organisms that can switch to a different fermentation pattern in pure culture. The biochemistry of this metabolism has still to be worked

out, but the evidence at hand suggests that these organisms, similar to the acetone fermenters mentioned above, may be unable to subfractionate the ATP unit by reversed electron transport systems.

5.6 Anaerobic Oxidation of Methane

Anaerobic, sulfate-dependent methane oxidation is an important process in anoxic marine sediments which involves again two different organisms cooperating in a syntrophic way, at least in most systems studied so far.

Sulfate-dependent methane oxidation is exergonic under standard conditions



$$CH_4 + SO_4^{2-} + 2\,H^+ \rightarrow CO_2 + H_2S + 2\,H_2O$$
$$\Delta G_0' \text{ of } -18 \text{ kJ per mol}$$

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 it 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 (Zinder and Koch 1984; see Table 1), it was speculated that “reversed methanogenesis” may be the key to 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 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.

Evidence of such syntrophic cooperation in sulfate-dependent methane oxidation was recently obtained through analysis of lipids of marine archaea and sulfate-reducing bacteria in anoxic sediment layers feeding 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).

Table 1. Energetics of some key reactions in methanogenic degradation

Reaction	G_0' kJ per mol rct.	G' in situ[a] kJ per mol rct.
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$	+ 9.6	–42
Alanine + $2\ H_2O \rightarrow CH_3COO^- + CO_2 + NH_4^+ + 2\ H_2$	+2.7	–77
$CH_3CH_2CH_2COO^- + 2\ H_2O \rightarrow 2\ CH_3COO^- + 2\ H^+ + 2\ H_2$	+48.3	–26
$CH_3CH_2COO^- + 2\ H_2O \rightarrow CH_3COO^- + CO_2 + 3\ H_2$	+76	–12
$CH_3COO^- + H^+ + 2\ H_2O \rightarrow 2\ CO_2 + 4\ H_2$	+94.9	–8

All calculations are based on published tables (Thauer et al. 1977).

[a]For in situ conditions, the following concentrations were assumed: substrate and acetate 10^{-4} M, H_2 $10^{-4.5}$ atm., CO_2 10^{-2} atm

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 deep-lying 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 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 deep-lying, methane-poor marine sediments. It is also still open which metabolites are transferred between the partners; preliminary evidence indicates that it it neither hydrogen nor formate, methanol or acetate (Nauhaus et al. 2002).

6
Types of Interspecies Metabolite Transfer

In most syntrophic methanogenic associations, hydrogen is the electron carrier between oxidative and reductive metabolic processes. Its small size and ease of diffusibility make it an excellent candidate for such interspecies electron transfer reactions. Nonetheless, in several cases, formate has also been shown to act as an electron carrier through a formate/CO_2 cycle. Theoretical considerations indicate that the formate system has certain advantages in an aqueous phase, whereas hydrogen might be better suited as carrier in densely packed microbial aggregates (Boone et al. 1989). Both carrier systems might also operate simultaneously in one degradative process, as observed in syntrophic propionate oxidation (see above), or the bacteria may switch between both electron transfer channels depending on the prevailing environmental conditions. Syntrophic oxidation of long chain fatty acids profits as well by efficient removal of the coproduct acetate through the activity of acetotrophic methanogens, and the same appears to be true for all fatty acid degrading systems examined so far. Methanogenic acetone degradation gives an example of interspecies transfer of acetate only (see above).

An artificially combined syntrophically acetate-degrading culture described recently consisting of the iron(III) reducing *Geobacter sulfurreducens* and the fumarate- or nitrate-reducing bacterium *Wolinella succinogenes* (Cord-Ruwisch et al. 1998) oxidizes acetate with nitrate as electron acceptor at high rate, obviously independent of interspecies hydrogen

transfer. The interspecies electron transfer in this coculture is accomplished by cysteine, which establishes a cysteine/cystine cycle for electron transfer between both partners or, alternatively, through a H_2S/S^0 cycle (Kaden et al. 2002). This electron transfer through sulfur compounds is similar to interspecies electron transfer between a green phototroph and a chemotrophic sulfur-reducing bacterium in the association "*Chloropseudomonas ethylica*" (Biebl and Pfennig 1978), which also cooperates through an H_2S/S_0 cycle. Interspecies electron transfer has gained new interest through the discovery that microbial iron(III) reduction in natural environments can be mediated by humic compounds (Lovley et al. 1996); in the laboratory, usually anthraquinone-2,6-disulfonate is used as a model substrate. Several fermenting bacteria, e.g., *Propionibacterium* sp. can reduce such external electron carriers (Benz et al. 1998; Emde and Schink 1990) and can deliver electrons this way indirectly to Fe(III) minerals as well although they have never been regarded as iron-reducing bacteria. Electrons from quinoid carriers can as well be taken up by, e.g., nitrate-reducing bacteria or others (Lovley et al. 1999), and humic compounds can thus mediate electron transfer systems between rather different types of bacteria that would usually not be thought of as cooperation partners.

7
Outlook

The few examples mentioned here should illustrate that there are many different types of cooperation between prokaryotes, especially among anaerobic bacteria. Especially in syntrophic associations of fermenting bacteria and methanogenic partners, which catalyze the last steps in methanogenic degradation of organic matter, the partner organisms are forced to very close cooperation in order to exploit the very small amounts of energy available to these steps, which range at the lowermost limit of energy that can be converted into ATP at all. New types of syntrophic associations have also shown that beyond hydrogen, other metabolites may also be exchanged between the partners involved, and the concept of extracellular electron transfer between different bacterial species resembles other types of extracellular electron transfer such as reduction of insoluble ferric iron oxides, be they catalyzed via intermediate dissolved carrier systems or not. The recently discovered cases of syntrophic anaerobic oxidation of methane and of sugars are only two more examples of this kind. Thus, interspecies interactions as we studied them with our syntrophic associations in the past may be good model systems to open our eyes also for unexpected types of interactions that we may not have considered before.

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