

# Syntrophy in Methanogenic Degradation

Petra Worm, Nicolai Müller, Caroline M. Plugge, Alfons J.M. Stams,  
and Bernhard Schink

## Contents

1	Introduction .....	144
2	Syntrophic Culture Systems, Microbiology, Biochemistry, and Molecular Biology .....	148
2.1	Ethanol .....	148
2.2	Butyrate .....	150
2.3	Propionate .....	152
2.4	Acetate .....	156
2.5	Branched Fatty Acids .....	157
2.6	Benzoate .....	157
2.7	Sugars .....	158
2.8	Amino Acids .....	159
2.9	Carrier Systems .....	161
2.10	Alternative Substrates for Pure Cultures and Technical Systems to Replace Methanogens .....	162
2.11	Anaerobic Methane Oxidation .....	164
3	Spatial Organization of Syntrophic Communities .....	164
4	Concluding Remarks .....	165
	References .....	166

**Abstract** This chapter deals with microbial communities of bacteria and archaea that closely cooperate in methanogenic degradation and perform metabolic functions in this community that neither one of them could carry out alone. The methanogenic degradation of fatty acids, alcohols, most aromatic compounds, amino acids, and others is performed in partnership between fermenting bacteria and methanogenic archaea. The energy available in these processes is very small,

---

P. Worm, C.M. Plugge, and A.J. Stams

Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands

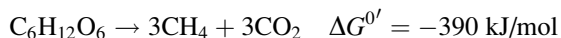
N. Müller, and B. Schink (✉)

Microbial Ecology, Department of Biology, University of Konstanz, 78457 Konstanz, Germany  
e-mail: Bernhard.Schink@uni-konstanz.de

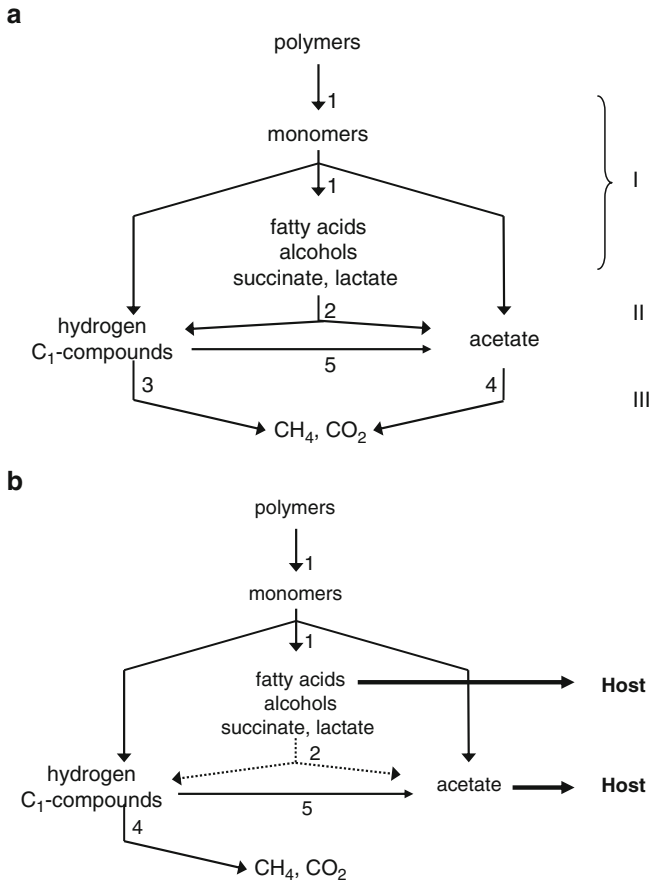
attributing only fractions of an ATP unit per reaction run to every partner. The biochemical strategies taken include in most cases reactions of substrate-level phosphorylation combined with various kinds of reversed electron transport systems in which part of the gained ATP is reinvested into thermodynamically unfavourable electron transport processes. Altogether, these systems represent fascinating examples of energy efficiency at the lowermost energy level that allows microbial life.

## 1 Introduction

In oxygen-limited environments, such as lake sediments or the lower layers of eutrophic lakes in summertime, biomass oxidation has to be coupled to alternative electron acceptors such as nitrate, Mn(IV), Fe(III), sulfate, or CO<sub>2</sub> (which is reduced to methane) (Zehnder 1978; Schink 1989). The relative importance of these alternative electron acceptors depends on their availability in the respective habitat; most freshwater sediments are rich in iron oxides, and marine sediments are well supplied with sulfate due to the high sulfate content of seawater (28 mM). Only methanogenesis is independent of external electron acceptors because the methanogenic degradation of biomass is actually a dismutation of organic carbon:



Whereas aerobic, nitrate-reducing or manganese-reducing bacteria typically are able to degrade polymeric organic compounds via the respective monomers to CO<sub>2</sub> and other inorganic products in one single cell, the conversion of complex organic matter by iron reducers or sulfate reducers requires a cooperation with fermenting bacteria, which feed the respective terminal oxidizers with classical fermentation products such as fatty acids, alcohols, and others. Methanogenic degradation of organic matter is even more complex and requires cooperation of three different metabolic groups (guilds) of bacteria, including primary fermenters, secondary fermenters, and methanogens (Bryant 1979; Fig. 1a). Primary fermenting bacteria are known for long times and have been isolated by classical procedures with all kinds of polymeric or monomeric substrates. Also anaerobic protozoa, including flagellates and ciliates, can operate in this manner (see Fenchel and Finlay 2010). Different from iron reducers or sulfate reducers, methanogenic archaea use only very few substrates, including hydrogen, CO<sub>2</sub>, other C<sub>1</sub>-compounds, and acetate. In one exceptional case, a methanogen can also oxidize isopropanol and ethanol (Widdel et al. 1988). Thus, the majority of classical fermentation products such as alcohols, fatty acids, branched-chain fatty acids and aromatic fatty acid residues from partial degradation of amino acids, long-chain fatty acids from lipid hydrolysis, and heterocyclic aromatic compounds deriving from nucleic acids all need to be fermented further to those substrates that methanogens can use (Bryant 1979; Schink 1997; Schink and Stams 2002; McInerney et al. 2008; Stams and Plugge 2009).



**Fig. 1** Methanogenic degradation of complex organic matter by cooperation of different metabolic groups. **(a)** Electron flow in a freshwater sediment or biogas reactor. **(b)** Electron flow in plant digestion in the rumen. Metabolic groups of organisms involved: primary fermenters (1), secondary fermenters (2), hydrogen and C<sub>1</sub>-compounds-using methanogens (3), acetoclastic methanogens (4), and homoacetogenic bacteria (5) (modified after Schink 1997)

This is the function of the secondary fermenting bacteria that depend on close cooperation with methanogenic partners and are the subject of this chapter.

Methanogenic environments are widely distributed in nature. Wetlands, freshwater sediments, swamps, and digestive tracts of ruminants and insects are environments that produce high amounts of methane. Man-made systems, such as rice paddies and anaerobic bioreactors and landfills, are other important sources of methane production.

Methanogenic archaea catalyze the final step in the overall anaerobic degradation of organic material to methane and CO<sub>2</sub>. One metabolic group of methanogenic archaea converts CO<sub>2</sub> plus hydrogen or formate to methane, whereas others use acetate or methanol. Acetate, the most important intermediate in anaerobic

digestion, accounts for approximately two-thirds of all methane produced, while the last third is produced from the reduction of CO<sub>2</sub> with electrons derived from the oxidation of hydrogen or formate (Ferry 1992; Liu and Whitman 2008). Currently, only two types of acetoclastic methanogens have been identified: *Methanosaeta* sp. and *Methanosarcina* sp. *Methanosarcina* sp. is a genus of versatile methanogens, including species capable of growing with different substrates including acetate, methanol, methylamines, and H<sub>2</sub>/CO<sub>2</sub>, whereas *Methanosaeta* sp. uses only acetate. *Methanosaeta* sp. is widely distributed in nature and, because of its high affinity for acetate, it outcompetes *Methanosarcina* sp. in low-acetate environments (Conklin et al. 2006). In rumen and other animal gastrointestinal tracts, however, *Methanosarcina* sp. are typically present, due to the high acetate concentrations occurring in these environments (see Conway de Macario and Macario 2010). Both acetoclastic archaea grow very slowly, with doubling times of 1–12 (*Methanosaeta*) and 0.5–2 (*Methanosarcina*) days (Jetten et al. 1992). Despite their restricted substrate range (H<sub>2</sub>/CO<sub>2</sub>, formate, and methylated C<sub>1</sub>-compounds), methanogenic archaea are phylogenetically very diverse. They are classified into five orders (Whitman et al. 2006). Representatives of the orders Methanobacteriales and Methanomicrobiales are commonly present in animal gastrointestinal tracts.

The importance of the secondary fermenting bacteria varies with the kind of substrate utilized and the efficiency of the methanogenic partners at the end of the anaerobic feeding chain. If the methanogens maintain a low concentration of hydrogen and acetate, numerous classical primary fermentations are shifted to the formation of hydrogen, CO<sub>2</sub>, and acetate and produce much less reduced side products such as fatty acids than they do in pure culture (Iannotti et al. 1973; Tewes and Thauer 1980; Schink and Zeikus 1982). Thus, the majority of electrons from substrate degradation will flow through the outer lines of the scheme depicted in Fig. 1a, and the electron flow through the central part may be only of minor importance to ensure complete degradation of biopolymers as this is typical for freshwater lake sediments, swamps, or sewage sludge digesters.

The situation is basically different in the fermentations proceeding inside the gastrointestinal tracts of animals. There, the host organism is not favoured by complete degradation of biopolymers inside the gut to methane and CO<sub>2</sub> but uses a substantial part of the overall electron input for its own support, e.g., in the form of fatty acids. Since the retention time of the feed inside the guts is limited to a few hours or 2 days at maximum, neither the slow-growing acetate-utilizing methanogens nor the fatty acid-degrading syntrophic associations will establish. Therefore, these fatty acids accumulate in the gut and are taken up by the host. In ruminants, this acid transfer proceeds at concentrations of 60 mM acetate, 20 mM propionate, and 10 mM butyrate (Bryant 1977); in termite guts, only acetate is produced to major amounts (Breznak and Kane 1990; Brune 2007). Methanogenesis in the intestinal tracts is restricted to hydrogen utilization, in order to shift the overall electron flow mainly towards fatty acids production and to minimize unwanted side fermentations such as alcohol formation (Fig. 1b).

The ability to transfer electrons to a partner organism is an important metabolic feature associated with many physiologically diverse microorganisms. This trait is

usually referred to as syntrophism. Syntrophism is a special type of symbiosis between two microorganisms in which growth of one organism depends on supply of growth factors or nutrients or removal of products by a partner organism. Especially among anaerobic microorganisms, cooperation of several metabolic types of bacteria in the feeding chain is a common feature. The mutual dependence can be explained calculating the changes in Gibbs' free energy ( $\Delta G^{0'}$ ) for the oxidation of, e.g., ethanol to hydrogen,  $\text{CO}_2$ , and acetate (Bryant et al. 1967). Under defined standard conditions with gases at  $10^5$  Pa pressure, 1 M concentration of products/substrates at pH 7.0 and 298 K, the Gibbs' free energy value for ethanol oxidation is positive with +9.6 kJ/reaction (Table 1). This indicates that the reaction

**Table 1** Equations and standard free energy changes for relevant reactions described in the chapter (Gibbs' free energy changes are taken from Thauer et al. 1977)

Reaction	$\Delta G^{0'}$ (kJ/reaction)
<i>For secondary fermentation reactions</i>	
Glucose $\rightarrow$ 2 Acetate <sup>-</sup> + 2H <sup>+</sup> + 2CO <sub>2</sub> + 4H <sub>2</sub>	-216
Ethanol + H <sub>2</sub> O $\rightarrow$ Acetate <sup>-</sup> + H <sup>+</sup> + 2H <sub>2</sub>	+9.6
Propionate <sup>-</sup> + 2H <sub>2</sub> O $\rightarrow$ Acetate <sup>-</sup> + CO <sub>2</sub> + 3H <sub>2</sub>	+76
Butyrate <sup>-</sup> + 2H <sub>2</sub> O $\rightarrow$ 2 Acetate <sup>-</sup> + H <sup>+</sup> + 2H <sub>2</sub>	+48
Crotonate <sup>-</sup> + 2H <sub>2</sub> O $\rightarrow$ 2 Acetate <sup>-</sup> + H <sup>+</sup> + H <sub>2</sub>	-6.2
Acetate <sup>-</sup> + 2H <sub>2</sub> O $\rightarrow$ 2CO <sub>2</sub> + 4H <sub>2</sub>	+96
Benzoate <sup>-</sup> + 6H <sub>2</sub> O $\rightarrow$ 3 Acetate <sup>-</sup> + CO <sub>2</sub> + 2H <sup>+</sup> + 3H <sub>2</sub>	+49.5
Phenol + 5H <sub>2</sub> O $\rightarrow$ 3 Acetate <sup>-</sup> + 3H <sup>+</sup> + 2H <sub>2</sub>	+5.7
Acetone + CO <sub>2</sub> $\rightarrow$ 2 Acetate <sup>-</sup> + 2H <sup>+</sup>	-34
Alanine + 3H <sub>2</sub> O $\rightarrow$ Acetate <sup>-</sup> + H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + 2H <sub>2</sub>	+7.5
Isoleucine + 3H <sub>2</sub> O $\rightarrow$ 2-Methylbutyrate <sup>-</sup> + H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + 2H <sub>2</sub>	+7.5
Valine + 3H <sub>2</sub> O $\rightarrow$ Isobutyrate <sup>-</sup> + H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + 2H <sub>2</sub>	+9.7
Leucine + 3H <sub>2</sub> O $\rightarrow$ Isovalerate <sup>-</sup> + H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + 2H <sub>2</sub>	+4.2
Leucine + 3H <sub>2</sub> O $\rightarrow$ $\alpha$ -Ketoisocaproate <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + H <sub>2</sub>	+51
$\alpha$ -Ketoisocaproate <sup>-</sup> $\rightarrow$ Isovalerate <sup>-</sup> + H <sub>2</sub>	-56
Glutamate <sup>-</sup> + 2H <sub>2</sub> O $\rightarrow$ Acetate <sup>-</sup> + 0.5H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + 0.5 Butyrate <sup>-</sup>	-58
Glutamate <sup>-</sup> + 4H <sub>2</sub> O $\rightarrow$ Propionate <sup>-</sup> + 2HCO <sub>3</sub> <sup>-</sup> + H <sup>+</sup> + NH <sub>4</sub> <sup>+</sup> + 2H <sub>2</sub>	-5.8
Glutamate <sup>-</sup> + 3H <sub>2</sub> O $\rightarrow$ 2 Acetate <sup>-</sup> + H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + H <sub>2</sub>	-34
Aspartate <sup>-</sup> + 3H <sub>2</sub> O $\rightarrow$ Acetate <sup>-</sup> + H <sup>+</sup> + 2HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + 2H <sub>2</sub>	-14
Serine + 2H <sub>2</sub> O $\rightarrow$ Acetate <sup>-</sup> + H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup> + H <sub>2</sub>	-90
<i>For reactions of methanogenic archaea</i>	
4H <sub>2</sub> + CO <sub>2</sub> $\rightarrow$ CH <sub>4</sub> + 2H <sub>2</sub> O	-131
4 Formate <sup>-</sup> + 4H <sup>+</sup> $\rightarrow$ CH <sub>4</sub> + 3CO <sub>2</sub> + 2H <sub>2</sub> O	-145
4CO + 2H <sub>2</sub> O $\rightarrow$ CH <sub>4</sub> + 3CO <sub>2</sub>	-211
Acetate <sup>-</sup> + H <sup>+</sup> $\rightarrow$ CH <sub>4</sub> + CO <sub>2</sub>	-35
4 Methanol $\rightarrow$ 3CH <sub>4</sub> + CO <sub>2</sub> + 2H <sub>2</sub> O	-106
H <sub>2</sub> + Methanol $\rightarrow$ CH <sub>4</sub> + H <sub>2</sub> O	-113
CO <sub>2</sub> + H <sub>2</sub> O $\rightarrow$ H <sup>+</sup> + HCO <sub>3</sub> <sup>-</sup>	+4.8
CO <sub>2</sub> + H <sub>2</sub> $\rightarrow$ Formate <sup>-</sup> + H <sup>+</sup>	-4.5
<i>For hydrogen-consuming reactions</i>	
Crotonate + H <sub>2</sub> $\rightarrow$ Butyrate <sup>-</sup>	-75
Pentenoate + H <sub>2</sub> $\rightarrow$ Valerate <sup>-</sup>	-75
Glycine + H <sub>2</sub> $\rightarrow$ Acetate <sup>-</sup> + NH <sub>4</sub> <sup>+</sup>	-78
CO <sub>2</sub> + H <sub>2</sub> $\rightarrow$ Formate <sup>-</sup> + H <sup>+</sup>	-4.5

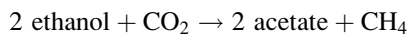
cannot take place nor can any microbe gain energy from this oxidation. However, the Gibbs' free energy becomes negative when the hydrogen partial pressure ( $p_{H_2}$ ) decreases. This example of interspecies hydrogen transfer is characteristic for the way how organic matter is degraded in methanogenic habitats.

## 2 Syntrophic Culture Systems, Microbiology, Biochemistry, and Molecular Biology

Life depends on the availability of energy, which is stored inside the cell in the form of ATP. Under physiological conditions, including heat losses, the synthesis of ATP requires 60–70 kJ/mol (Thauer et al. 1977). Membrane-bound ATPases couple the hydrolysis or synthesis of ATP to the transport of protons (in some cases also  $Na^+$  ions) across the cytoplasmic membrane. Depending on the stoichiometry of the ATPase system in question, the ratio of ions translocated versus ATP synthesized or hydrolyzed may vary between 3 and 5; in most cases, a ratio of 3–4 appears to be justified (Engelbrecht and Junge 1997; Cherepanov et al. 1999). As a consequence, the smallest amount of energy that can still be converted to ATP – and with this into metabolic activity and growth – is equivalent to one-third or one-fourth of an ATP unit, i.e., in the range of –15 to –20 kJ/mol reaction (Schink 1997; Schink and Stams 2002). It is this minimum increment of energy with which syntrophically fermenting methanogenic communities have to operate.

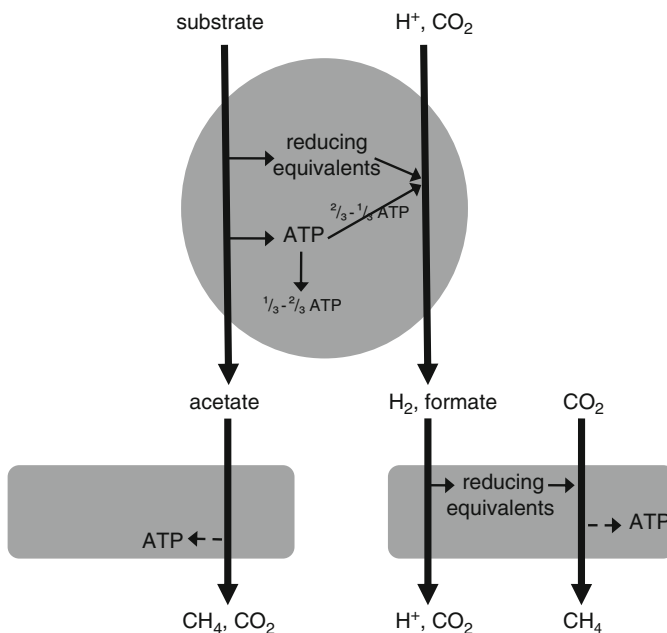
### 2.1 Ethanol

The biochemistry of syntrophic oxidation of ethanol, although the oldest syntrophic system is known, has still not been elucidated in detail. Early work on the so-called S-organism indicated that ethanol is oxidized via acetaldehyde to acetyl-CoA and further to acetate, including ATP synthesis by acetate kinase (Reddy et al. 1972a, b, c). This concept was confirmed by similar studies on the ethanol-oxidizing bacteria *Pelobacter acetylenicus* and *Pelobacter carbinolicus* (Schink 1985, Eichler and Schink 1986). Nonetheless, the energetics of this reaction chain is still unclear. The overall reaction



yields  $\Delta G^{0r} = -112$  kJ/mol, which leaves a total of about –40 kJ per ethanol oxidation reaction for the syntrophic ethanol oxidizer, indicating that part of the ATP formed by substrate-level phosphorylation has to be invested into reversed electron transport. Oxidation of acetaldehyde to acetyl-CoA ( $E^{0r} = -370$  mV) can be coupled to hydrogen formation via ferredoxin at a sufficiently low hydrogen

pressure. A ferredoxin-like electron carrier has been purified from *P. acetylenicus* (Kowalski and Schink, unpublished). The energetically difficult reaction is the transfer of electrons from the acetaldehyde/ethanol coupled ( $-196\text{ mV}$ ) to hydrogen formation. Such a reaction requires energy input in the form of, e.g., a reversed electron transport, a feature that is common to all syntrophically fermenting bacteria studied so far (Fig. 2). Hydrogen formation from ethanol in crude extracts of *P. acetylenicus* is stimulated by ATP (Hauschild 1997). Since *P. acetylenicus* also contains a menaquinone-like electron carrier (Strohm and Schink, unpublished), a basically similar reversed electron transport system as suggested for *Syntrophomonas wolfei* (discussed in Sect. 2.2) can be anticipated, but experimental evidence has not been provided yet. A comproportionating [FeFe] hydrogenase as described for *Thermotoga maritima* (Schut and Adams 2009) could finally release the electrons from NADH and ferredoxin towards proton reduction. *T. maritima* ferments glucose to acetate,  $\text{CO}_2$ , and  $\text{H}_2$  via the Embden–Meyerhof pathway, generating two NADH and four reduced ferredoxins per molecule of glucose. In order to re-oxidize these carriers, a proposed bifurcating [FeFe] hydrogenase uses electrons from NADH and reduced ferredoxin in a 1:1 ratio to produce  $\text{H}_2$ . Schut and Adams (2009) found genes with sequence similarity to this [FeFe] hydrogenase in several other microorganisms, including the ethanol-degrading *P. carbinolicus*, the butyrate-degrading *S. wolfei* (discussed in Sect. 2.2), and the propionate-degrading *Syntrophobacter fumaroxidans* (discussed in Sect. 2.3).



**Fig. 2** Conversions performed by a secondary fermenting bacterium (*top*), a hydrogen- and formate-using methanogen (*bottom right*), and an acetoclastic methanogen (*bottom left*)

Also sulfate reducers like *Desulfovibrio vulgaris* have been shown to couple ethanol oxidation to acetate with electron transfer to methanogenic partners (Bryant et al. 1977). However, this activity appears not to be coupled to ATP synthesis by the sulfate reducer because *D. vulgaris* does not grow in such co-cultures (Kremer et al. 1988). The classical sulfate reducers oxidize ethanol via acetaldehyde directly to acetate without intermediate formation of an activated acetyl residue; thus, no ATP is formed by substrate-level phosphorylation and the cultures do not grow (Kremer et al. 1988). Nonetheless, syntrophic growth of an ethanol-degrading sulfate reducer in the absence of sulfate with a methanogen as electron scavenger has been documented (Walker et al. 2009). Ethanol-degrading sulfate reducers that grow in the absence of sulfate in co-culture with methanogens have to form ATP through substrate-level phosphorylation via acetyl-CoA or acetyl phosphate as intermediates or the electron transfer from the intermediate carriers to hydrogen or formate as extracellular electron carriers has to be coupled to some kind of net ion translocation, which is coupled to ATP synthesis.

## 2.2 Butyrate

Anaerobic butyrate degraders known to date belong to only two groups of bacteria: the genus *Syntrophomonas* within the phylum Firmicutes and the genus *Syntrophus* within the order Syntrophobacterales of the phylum Proteobacteria (Table 2). Fermentation of butyrate to acetate and hydrogen is endergonic (Table 1) and occurs only at very low hydrogen partial pressures, e.g., in the presence of methanogenic archaea (Schink 1997). Syntrophic butyrate oxidizers use only very few substrates. Beyond oxidation of saturated fatty acids in co-culture with methanogens, axenic growth is possible only with unsaturated fatty acids such as crotonate (Schink 1997; McInerney et al. 2008). They cannot use external electron acceptors for growth, thus reflecting the high degree of specialization of these bacteria for syntrophic cooperation (Schink 1997).

Butyrate is oxidized via  $\beta$ -oxidation to acetate yielding one mole ATP per mole of butyrate. The reducing equivalents are transferred to flavoenzymes and  $\text{NAD}^+$  (Wofford et al. 1986). Re-oxidation of these electron carriers of a relatively positive redox potential with protons to form hydrogen is energetically difficult. Of course, a low hydrogen partial pressure helps to facilitate those reactions, but no known methanogen is able to maintain a hydrogen partial pressure low enough ( $10^{-10}$  atm) to allow direct proton reduction with these electrons (Thauer and Morris 1984; Schink 1997). Therefore, it was postulated that syntrophic butyrate degraders have to invest energy into a reversed electron transport, thus leaving only a fraction of an ATP for growth of the bacterium (Thauer and Morris 1984).

Recently, Müller et al. (2009) showed that an enzyme system similar to the comproportionating [FeFe] hydrogenase of *T. maritima* is essential in butyrate oxidation by *S. wolfei*. The comproportionating [FeFe] hydrogenase of *T. maritima* drives the endergonic reduction of protons to hydrogen with NADH by exergonic



**Table 2** Propionate-degrading syntrophic bacteria (modified after McInerney et al. 2008)

Organism	Growth possible with		Propionate + syntrophic partner	Phylogenetic position	Reference
	Propionate + sulfate	Propionate + fumarate			
<i>Syntrophobacter fumaroxidans</i>	+	+	+	δ-Proteobacteria	Harmsen et al. (1998)
<i>Syntrophobacter pfennigii</i>	+	-	+	δ-Proteobacteria	Wallraabenstein et al. (1995)
<i>Syntrophobacter sulfatireducens</i>	+	-	+	δ-Proteobacteria	Chen et al. (2005)
<i>Syntrophobacter wolnini</i>	+	ND	+	δ-Proteobacteria	Boone and Bryant (1980)
<i>Pelotomaculum schinkii</i>	-	-	+	Low G + C Gram positives	De Bok et al. (2005)
<i>Pelotomaculum thermopropionicum</i>	-	+	+	Low G + C Gram positives	Imachi et al. (2002)
<i>Pelotomaculum propionicum</i>	-	-	+	Low G + C Gram positives	Imachi et al. (2007)
<i>Smithella propionica</i>	ND	ND	+		Liu et al. (1999)
<i>Desulfotomaculum thermobenzoicum</i>	+	-	+	Low G + C Gram positives	Plugge et al. (2002b)
<i>thermosyntrophicum</i>					

reduction of another couple of protons with reduced ferredoxin, which is produced in pyruvate oxidation during growth on glucose (Schut and Adams 2009). In butyrate oxidation by *S. wolfei*, no such ferredoxin-reducing reaction is involved. Nonetheless, hydrogen formation from NADH is likely catalyzed by a [FeFe] hydrogenase homologue in *S. wolfei*. This reaction is possible already at a hydrogen partial pressure of  $10^{-3}$  atm (Schink 1997; Müller et al. 2009). Since the enzyme found in *S. wolfei* is associated with a formate dehydrogenase-like protein analogous to its homologue in *Eubacterium acidaminophilum*, interspecies electron transfer may occur via hydrogen and/or formate, depending on the environmental conditions (Graentzdoerffer et al. 2003; Müller et al. 2009).

The thermodynamically most difficult step in butyrate oxidation is the transfer of electrons derived from butyryl-CoA oxidation to  $\text{NAD}^+$ , for which a redox potential difference of at least +200 mV has to be overcome (Schink 1997). It was hypothesized that electrons from butyrate oxidation are transferred to quinones in the membrane, and that the reduced quinones are re-oxidized with  $\text{NAD}^+$  (Wallrabenstein and Schink 1994). Such a reaction would require energetization by, e.g., a proton gradient, which was found to be essential for hydrogen formation from butyrate by *S. wolfei* (Wallrabenstein and Schink 1994).

The *S. wolfei* [FeFe] hydrogenase catalyzes the reduction of quinones with NADH, indicating that, besides forming hydrogen from NADH oxidation, this enzyme also catalyzes the proton gradient-driven endergonic oxidation of quinones with  $\text{NAD}^+$  (Müller et al. 2009). However, a direct linkage between quinol oxidation and proton translocation has not been demonstrated so far.

Another possible mechanism for reversed electron transport during butyrate oxidation was postulated for *Syntrophus aciditrophicus* based on genome data. Here, an Rnf complex could oxidize NADH and transfer electrons to ferredoxin, driven by influx of protons or sodium ions into the cell (McInerney et al. 2008). Electrons that arise during butyryl-CoA oxidation could be transferred to components of the membrane, where  $\text{NAD}^+$  is reduced in a similar manner as postulated for *S. wolfei* (McInerney et al. 2008). With the Rnf complex, *S. aciditrophicus* has the potential prerequisites for producing reduced ferredoxin during butyrate degradation, which may drive comproportionating reactions such as NADH oxidation by [FeFe] hydrogenases or bifurcating reactions such as butyryl-CoA oxidation by the Bcd/EtfAB complex (Li et al. 2008; Herrmann et al. 2008). In contrast, genes that encode for the Rnf complex are not present in the genome of *S. wolfei*, indicating that the pathway of butyrate degradation is different in both organisms and does not include reduced ferredoxin in *S. wolfei* (Müller et al. 2009).

### 2.3 Propionate

All currently identified syntrophic propionate-oxidizing bacteria are affiliated with the class Deltaproteobacteria within the phylum of Proteobacteria (McInerney et al. 2005), or the low G + C Gram-positive bacteria in the class Clostridia within

the phylum Firmicutes (Imachi et al. 2002; Plugge et al. 2002b; de Bok et al. 2005) (Table 3). Some of the *Syntrophobacter* sp. are able to use sulfate as the electron acceptor for propionate oxidation (McInerney et al. 2005) and can grow in pure culture by propionate oxidation with sulfate. In addition, they can grow by fermentation of pyruvate or fumarate. *Smithella propionica* is phylogenetically related to the genus *Syntrophus* (Liu et al. 1999) and lacks the ability to reduce sulfate. *S. propionica* does not oxidize propionate but ferments it to acetate plus butyrate and grows in pure culture on crotonate (de Bok et al. 2001; Liu et al. 1999). These substrates or substrate combinations have been used to obtain axenic cultures of the syntrophs since they bypass the energetically unfavourable steps in propionate oxidation. *Pelotomaculum schinkii*, however, could not be obtained in pure culture until today nor could it grow on any other compound but propionate. As such, this organism remains a true obligately syntrophic bacterium (de Bok et al. 2005).

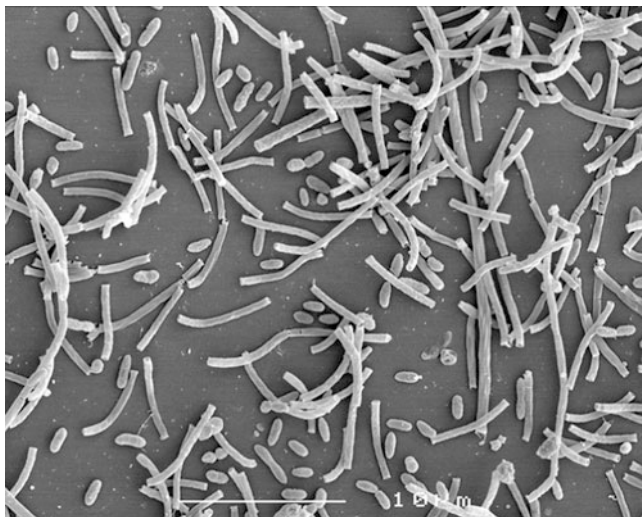
All species described to date were isolated from anoxic reactors, indicating the importance of these organisms in those types of reactors. The significance of these

**Table 3** Fatty acid-degrading syntrophic bacteria (modified after McInerney et al. 2008)

Organism	Substrate range	Phylogenetic position	Reference
<i>Syntrophomonas wolfei</i> subsp. <i>wolfei</i>	C <sub>4:1</sub> –C <sub>6:1</sub> , C <sub>4</sub> –C <sub>8</sub>	Low G + C Gram positives	McInerney et al. (1979, 1981)
<i>Syntrophomonas sapovorans</i>	C <sub>16:1</sub> , C <sub>18:1</sub> , C <sub>18:2</sub> , C <sub>4</sub> –C <sub>18</sub>	Low G + C Gram positives	Roy et al. (1986)
<i>Syntrophomonas bryantii</i>	C <sub>4:1</sub> , C <sub>4</sub> –C <sub>11</sub> , 2-methylvalerate	Low G + C Gram positives	Stieb and Schink (1985)
<i>Syntrophomonas curvata</i>	C <sub>4:1</sub> , C <sub>18:1</sub> , C <sub>4</sub> –C <sub>18</sub>	Low G + C Gram positives	Zhang et al. (2004)
<i>Syntrophomonas erecta</i> subsp. <i>erecta</i>	C <sub>4:1</sub> , C <sub>4</sub> + C <sub>5:1</sub> , C <sub>4</sub> –C <sub>8</sub>	Low G + C Gram positives	Zhang et al. (2005)
<i>Syntrophomonas zehnderi</i>	C <sub>16:1</sub> , C <sub>18:1</sub> , C <sub>18:2</sub> , C <sub>4</sub> –C <sub>18</sub>	Low G + C Gram positives	Sousa et al. (2007)
<i>Syntrophomonas cellicola</i>	C <sub>4:1</sub> , C <sub>4</sub> –C <sub>8</sub> , C <sub>10</sub>	Low G + C Gram positives	Wu et al. (2006)
<i>Thermosyntropha lipolytica</i>	C <sub>4:1</sub> , C <sub>4</sub> –C <sub>18</sub> , C <sub>18:1</sub> , C <sub>18:2</sub> , triglycerides, yeast extract, tryptone, casamino acids, betaine, pyruvate, ribose, xylose	Low G + C Gram positives	Svetlitschnyi et al. (1996)
<i>Syntrophothermus lipocalidus</i>	C <sub>4:1</sub> , C <sub>4</sub> –C <sub>10</sub> , isobutyrate	Low G + C Gram positives	Sekiguchi et al. (2000)
<i>Syntrophus aciditrophicus</i>	C <sub>4:1</sub> , fatty acids	δ-Proteobacteria	Jackson et al. (1999)
<i>Syntrophus gentianae</i>	C <sub>4:1</sub>	δ-Proteobacteria	Szewzyk and Schink (1989)
<i>Syntrophus buswellii</i>	C <sub>4:1</sub>	δ-Proteobacteria	Mountfort and Bryant (1982)

bacteria in rumen or animal gastrointestinal tracts maybe very limited because they compete with the feeding interests of the host (see Sect. 1).

The question whether hydrogen or formate is transferred in syntrophic co-cultures has been studied in detail in propionate-degrading *S. fumaroxidans* co-cultures (Fig. 3). Thermodynamic calculations, flux measurements in defined co-cultures, and enzyme measurements all confirmed that interspecies formate transfer is an essential mechanism in syntrophic propionate degradation in suspended cultures (Dong et al. 1994a; Dong and Stams 1995). The terminal reductases were studied in detail and biochemical evidence for formate transfer was found (De Bok et al. 2002). Two formate dehydrogenases were isolated and characterized. In contrast to most formate dehydrogenases that contain molybdenum, one formate dehydrogenase (CO<sub>2</sub>-reductase) of *S. fumaroxidans* contains tungsten and has an unusually high specific activity both in the formate oxidation and in the CO<sub>2</sub> reduction assay (Reda et al. 2008). When syntrophic co-cultures of *S. fumaroxidans* and *Methanospirillum hungatei* were grown with limiting amounts of tungsten, the propionate degradation activity decreased. This decrease coincided adequately with decreased formate dehydrogenase activity while the hydrogenase activities remained almost unchanged (Plugge et al. 2009). In their natural habitats, syntrophically propionate-degrading bacteria form mixed microcolonies with methanogens in which interspecies distances are much shorter. Under these conditions, interspecies hydrogen transfer may become more important than interspecies formate transfer. In syntrophic propionate-degrading microcolonies, *Syntrophobacter*-like bacteria are often surrounded by *Methanobrevibacter* sp., methanogens that can use only hydrogen but not formate (Grotenhuis et al. 1991). Also in thermophilic sludge, interspecies hydrogen transfer appears to be the preferred path of electron transfer (Schmidt and Ahring 1993).



**Fig. 3** Scanning electron micrograph of a syntrophic propionate-degrading coculture of *S. fumaroxidans* (lemon- or oval shaped) and *M. hungatei* (rod shaped)

In addition, slow propionate degradation was observed, also in very concentrated cell suspensions of *S. fumaroxidans* and the hydrogen-oxidizing *Methanobrevibacter arboriphilus* (Dong et al. 1994a). See also Sect. 3.

The organisms involved in propionate degradation are genuine specialists in obtaining metabolic energy for growth, since they have to grow under thermodynamically very unfavourable conditions. The standard Gibbs' free energy change of the complete degradation of propionate to methane and CO<sub>2</sub> is about -60 kJ, which is approximately equivalent to the amount of energy needed to produce 1 mol of ATP. A community of three microorganisms brings about this conversion: one bacterium that degrades propionate to acetate, CO<sub>2</sub>, and hydrogen, and two methanogenic archaea: one that cleaves the acetate and another one that uses hydrogen to reduce CO<sub>2</sub> to methane. The actual energy that is available for each member of the community depends on the in situ concentrations of substrate, intermediates, and products and will vary during growth. Moreover, it depends also on the enzyme repertoire the microbes have.

Our model organism *S. fumaroxidans* degrades propionate via the methylmalonyl-CoA pathway. One ATP is harvested in the conversion of pyruvate to acetate via substrate-level phosphorylation. Reducing equivalents are released at three different redox potentials. Reduced ferredoxin is formed in the conversion of pyruvate to acetate, whereas NADH and FADH<sub>2</sub> are formed in the oxidation of malate and succinate, respectively. These intracellular redox mediators need to be re-oxidized by reduction of protons or CO<sub>2</sub>. The oxidation of reduced ferredoxin ( $E^{0'}$  Fd(ox)/Fd(red) = -398 mV) and NADH ( $E^{0'}$  NAD<sup>+</sup>/NADH = -320 mV) can be coupled to reduction of protons ( $E^{0'}$  = -414 mV) or CO<sub>2</sub> (-432 mV) only if the hydrogen or formate concentration is kept low by methanogens.

The disposal of reducing equivalents generated in pyruvate oxidation to acetyl-CoA is rather straightforward because most strictly anaerobic bacteria contain pyruvate: ferredoxin oxidoreductases (Chabrière et al. 1999). Here, electrons travel via ferredoxin to hydrogenases or formate dehydrogenases to produce hydrogen or formate, respectively. Hydrogen and formate are scavenged by the methanogens, thus enabling an efficient re-oxidation of the ferredoxin.

The oxidations of succinate and malate with protons are endergonic even at a hydrogen partial pressure as low as 1 Pa (the minimum level that can be achieved by methanogens). To drive these reactions, input of metabolic energy via reverse electron transport is required. The mechanism that drives succinate oxidation to fumarate ( $E^{0'}$  = +33 mV) during syntrophic growth may be similar to the mechanism of energy conservation in fumarate respiration by *Wolinella succinogenes* (Kröger et al. 2002), but operating in reverse. Experimental evidence was obtained that 2/3 ATP is needed to drive this conversion (van Kuijk et al. 1998). As such, the net ATP gain for the bacterium is 1/3 mol ATP per mole of propionate converted. However, until present, the molecular mechanisms involved in *S. fumaroxidans* and other syntrophic propionate oxidizers using the methylmalonyl-CoA pathway are not fully understood.

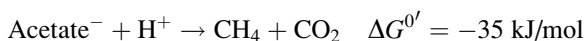
Also the oxidation of malate to oxaloacetate with NAD<sup>+</sup> is an endergonic reaction. Nonetheless, the purified malate dehydrogenase of *S. fumaroxidans*

exhibits a very high  $K_m$  value towards oxaloacetate and NADH and as such the organism may be able to efficiently perform this conversion (van Kuijk and Stams 1996). Still, the exact mechanism of NADH re-oxidation remains unclear.

*S. fumaroxidans* and *Pelotomaculum thermopropionicum* contain [FeFe] hydrogenases that are homologues to the comproportionating [FeFe] hydrogenase of *T. maritima*. This suggests that NADH and ferredoxin that are generated in the methylmalonyl-CoA pathway are simultaneously re-oxidized with the reduction of protons. These novel bifurcating enzyme complexes may be essential in these syntrophic fermentations. Additionally, the Rnf complex in *S. fumaroxidans* might use the membrane potential to reduce  $\text{NAD}^+$  with ferredoxin re-oxidation in order to stimulate NADH re-oxidation of the comproportionating [FeFe] hydrogenase.

## 2.4 Acetate

Although acetate can be used directly by certain methanogens such as *Methanosarcina* spp. and *Methanosaeta* spp. and is converted by these organisms to methane and  $\text{CO}_2$ , this situation is typical only for systems at moderate temperature and low salt content. At enhanced temperature, acetate can be oxidized to  $2 \text{CO}_2 + 4$  pairs of reducing equivalents ( $\text{H}_2$  or formate) in a reaction analogous to a reversal of homoacetate fermentation (see Table 1), and the electrons thus released are used by a methanogenic partner. This phenomenon has been observed first in a thermophilic reactor system (Zinder and Koch 1984), later also at lower temperature in sludge of enhanced ammonia content (Schnürer et al. 1996). The overall reaction



can hardly feed two organisms. The energy yield increases with rising temperature (Schink 1997); at  $60^\circ\text{C}$ ,  $\Delta G'$  is  $-42 \text{ kJ/mol}$ , which is just sufficient to allow two organisms to grow with this process. At lower temperatures, the energy supply becomes a serious problem, and doubling times increase to the range of several weeks (Schnürer et al. 1996). Indications were reported recently that also at slightly acidic conditions, e.g., in bogs, acetate is degraded in a syntrophic cooperation (Metje and Frenzel 2007); at pH 5.0, the  $\Delta G = -46 \text{ kJ/mol}$ .

The biochemistry of syntrophic acetate oxidation appears to be basically a reversal of the homoacetogenic acetate formation pathway (so-called Wood–Ljungdahl pathway or CO-dehydrogenase pathway). Acetate is activated to acetyl-CoA and cleaved by a CO-dehydrogenase/acetate synthase to a methyl and a carbonyl residue, which are oxidized separately through well-described pathways (Schnürer et al. 1997; Hattori et al. 2000, 2005). The question remains how the bacterium couples this pathway to ATP synthesis, especially since it can run the reaction chain also backwards to form acetate and to grow this way, at least to a minor extent.

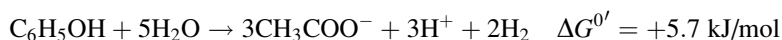
## 2.5 Branched Fatty Acids

Branched-chain fatty acids are formed during degradation of amino acids. Oxidative decarboxylation of valine leads to 2-methylpropionate (isobutyrate), leucine forms 3-methylvalerate (isovalerate), and isoleucine 2-methylbutyrate (neovalerate). Whereas neovalerate can be degraded easily by beta-oxidation to an acetyl and a propionyl residue, the other two acids pose some mechanistic difficulties. While isobutyrate is isomerized to butyrate in a B<sub>12</sub>-dependent reaction and subsequently cleaved to two acetyl residues (Stieb and Schink 1989), isovalerate degradation includes a carboxylation and subsequent formation of three acetyl residues (Stieb and Schink 1986). In all cases, the degradation of the branched carbon skeletons is slow and these branched fatty acids, similar to the corresponding residues of aromatic amino acids, accumulate in anoxic environments to a certain extent and may be taken up again by other anaerobic bacteria for reductive synthesis of amino acids (Allison and Bryant 1963), thus saving a lot of biosynthetic effort into amino acid synthesis.

## 2.6 Benzoate

Aromatic compounds were considered for a long time not to be degradable in the absence of oxygen, and reliable reports on their degradation in methanogenic ecosystems date back only into the late 1970s (Healy and Young 1978). The best-studied system is the syntrophic oxidation of benzoate by species of the genus *Syntrophus*, i.e., *S. buswellii*, *S. aciditrophicus*, and *S. gentianae*. Benzoate degradation in these bacteria proceeds via an initial activation to benzoyl-CoA by a ligase reaction, partial reduction to a cyclohexene derivative, addition of water to form a 2-hydroxylated cyclohexane carboxyl-CoA, and subsequent beta-oxidative ring cleavage and degradation to three acetyl moieties plus CO<sub>2</sub> (Schöcke and Schink 1997). Preliminary evidence indicates that the primary product of benzoyl-CoA reduction in syntrophically fermenting bacteria is cyclohexene carboxyl-CoA, different from the corresponding reaction observed in nitrate-reducing bacteria which forms a cyclohexadiene derivative (Fuchs 2008), and also the biochemistry of the reduction reaction appears to be different (Boll 2005). The overall ATP yield of the entire reaction chain has been calculated for *S. gentianae* to be 1/3 to 2/3 ATP equivalents, in accordance with the calculated energy yields (Schöcke and Schink 1999).

Phenol is another important aromatic compound that is degraded anaerobically through carboxylation to a 4-hydroxybenzoyl derivative and subsequent dehydroxylation to benzoyl-CoA. In nitrate-reducing bacteria, the initial carboxylation consumes the equivalent of two ATP units. The energetic situation of fermentative phenol degradation is very tight:





Even in syntrophic cooperation with a hydrogen-oxidizing partner, the phenol degrader obtains only little energy (approximately  $-40$  kJ/mol phenol; Schink 1997), thus keeping the overall energy budget small and hardly allowing to spend two ATP into the initial carboxylation reaction. So far, the details of the biochemistry of syntrophic phenol degradation have not been studied in detail; only recently, a defined co-culture of a syntrophically phenol-degrading bacterium has been isolated (Qiu et al. 2008).

## 2.7 Sugars

Sugars can be fermented by numerous groups of bacteria and archaea. The biochemical pathways of sugar oxidation are diverse but in most cases end up with pyruvate as a key metabolite. Most bacteria degrade sugars by converting mono- or disaccharides from polysaccharide cleavage into fructose or glucose, which are oxidized to pyruvate through the Embden–Meyerhof–Parnas pathway. Pyruvate can be further oxidized to acetate or  $\text{CO}_2$  by anaerobic respiration or be used as internal electron acceptor for fermentative production of a variety of acids or solvents.

Fermentation of sugars via the Embden–Meyerhof–Parnas pathway with subsequent acetyl-CoA phosphorylation usually yields acetate,  $\text{CO}_2$ , and hydrogen. Formation of only acetate,  $\text{CO}_2$ , and hydrogen would require formation of 4 mol ATP per mole of glucose: two in glycolysis and two in the acetate kinase reaction. However, the reaction provides a negative-free reaction enthalpy of only  $-216$  kJ/mol (Table 1), which is not sufficient for the formation of 4 ATP. This fermentation would need to re-oxidize the glycolysis-derived NADH with protons, which is endergonic under standard conditions. Most mesophilic sugar fermenters cope with this problem by releasing various reduced side products such as organic acids or alcohols. Thus, NADH can be re-oxidized without hydrogen formation, but on the other hand, only 2–3 mol of ATP per mole of glucose can be gained. In the presence of hydrogen-scavenging methanogenic partners, the formation of only acetate,  $\text{CO}_2$ , and hydrogen is favoured (Schink 1997). For example, the glucose-fermenting *Ruminococcus albus* shifts its fermentation pattern from acetate plus ethanol under axenic growth conditions to acetate,  $\text{CO}_2$ , and hydrogen in syntrophic co-culture (Iannotti et al. 1973). Obviously, the bacterium optimizes its ATP gain that is maximal if the hydrogen partial pressure is low enough to shift the thermodynamic equilibrium of glucose oxidation towards a more negative-free reaction enthalpy, thus allowing the formation of 4 ATP.

The facultatively anaerobic *Bacillus* sp. BoGlc83 grows anaerobically only in the presence of a methanogenic partner (Müller et al. 2008). At first, this organism did not release reduced side products and appeared to be forced to cooperate with methanogens, therefore. Later, it was shown that in co-cultures with *M. hungatei*, traces of lactate and succinate are formed besides acetate and methane at temperatures higher than  $20^\circ\text{C}$  and at glucose concentrations higher than 2 mM.



Nonetheless, no growth occurs in the absence of methanogens (Müller et al. 2008). Regarding that the natural habitat of *Bacillus* sp. BoGlc83 are cold profundal sediments, e.g., of Lake Constance, it seems likely that production of lactate and succinate are stress responses to unusual heat and high substrate concentrations much different from the cold and nutrient-poor natural environment of this organism.

## 2.8 Amino Acids

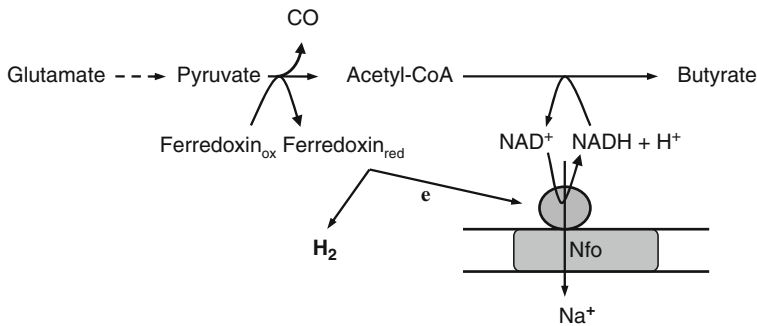
Much of our knowledge on anaerobic protein and amino acid degradation has been obtained through studies on ruminants, since protein is an important dietary product for ruminants (Allison 1970; Bryant 1977; Hobson and Wallace 1982). Proteins in the rumen are hydrolyzed by extracellular proteases and intracellular peptidases (Hazlewood and Nugent 1978) to single amino acids, peptides, and ammonia. Also in anaerobic digesters, the input of proteins coming from different sources of waste (e.g., slaughterhouses, beer breweries, and dairy industries) can be large. Proteins are composed of about 20 structurally different amino acids which require distinct biochemical pathways for degradation. As such, anaerobic degradation of amino acids by mixed methanogenic consortia is very complex and is performed by many fermentative microorganisms. Degradation involves oxidation and reduction reactions of one or more amino acids. Some amino acids are degraded preferentially via oxidation and others can also serve as electron acceptors. The combined oxidation and reduction of pairs of amino acids (Stickland reaction) is a well-known mechanism by which proteolytic clostridia degrade amino acids (Stickland 1934; Barker 1981; Stams 1994). In the Stickland reaction, the oxidation of one amino acid is coupled to the reduction of another one. In the oxidative branch, alanine and many other amino acids can be partly degraded; glycine is a classical electron acceptor in the reductive branch via a selenium-dependent glycine reductase (Andreesen 1994, 2004) (Table 1). Other couples have been described in the past (Barker 1981). Amino acid degradation is significantly affected by the presence of methanogens. Methanogens can act as scavengers of reducing equivalents in the oxidation of amino acids, taking over the role of the reductive branch of the Stickland reaction. Nagase and Matsuo (1982) observed that in mixed methanogenic communities, the degradation of alanine, valine, and leucine was inhibited by inhibition of methanogens, and Nanninga and Gottschal (1985) could stimulate the degradation of these amino acids by the addition of hydrogen-utilizing anaerobes. Several anaerobic bacteria have been described that grow syntrophically on amino acids in co-culture with methanogens (McInerney 1988; Stams 1994; Plugge and Stams 2005).

Usually, the first step in the degradation of amino acids is a deamination (Barker 1981; McInerney 1988; Andreesen et al. 1989). Deamination can be performed by anaerobic bacteria in three ways. Oxidative NAD(P)-dependent deamination of alanine, valine, leucine, or isoleucine leads to the corresponding keto acid. The  $\Delta G^{0'}$  of the deamination of alanine, valine, leucine, and isoleucine to the corresponding keto

acids when coupled to hydrogen formation is around +60 kJ/mol (Table 1). As a consequence, methanogens are needed to pull the reaction in a similar fashion as described for other syntrophic oxidations above. The keto acid is then converted via oxidative decarboxylation to a fatty acid releasing electrons at  $E^{0'} = -470$  mV, which can easily be transferred via ferredoxin to protons. Overall, the oxidative deamination of the four mentioned amino acids to fatty acids, ammonia, and hydrogen is slightly endergonic. The second mechanism is a reductive deamination and is found only in anaerobes (McInerney 1988; Andreesen et al. 1989; Andreesen 1994). Reducing equivalents are used to convert the amino acid to its corresponding fatty acid, with concomitant production of ammonia. An example is the reduction of glycine to acetate via the selenium-dependent glycine reductase (Stickland 1934; Andreesen 2004). The third mechanism, a redox-neutral reaction, results in the production of the corresponding keto acid. An example is the conversion of serine to pyruvate plus ammonia by the action of serine ammonia lyase or the C–C rearrangement of glutamate to 3-methylaspartate (Buckel and Barker 1974).

Glutamate is an abundant amino acid in proteins (McInerney 1988). In methanogenic habitats, glutamate can be metabolized in several different ways, leading to different growth yields. The effect of hydrogen removal by methanogenic partners on the metabolism of amino acid-fermenting anaerobes has been studied best with glutamate. Glutamate fermentation is carried out by a variety of anaerobes, including a number of *Clostridium* species, *Peptostreptococcus asaccharolyticus*, and *Acidaminococcus fermentans* (Gottschalk 1986; Boiangiu et al., 2005). These microorganisms ferment glutamate to acetate and butyrate by either the  $\beta$ -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 acetyl residues. *Anaeromusa acidaminophila* ferments glutamate to acetate plus propionate (Nanninga et al. 1987). In this bacterium, reducing equivalents are disposed of by reduction of pyruvate to propionate. Besides acetate, butyrate, and propionate also traces of hydrogen (up to 20 kPa) are formed during glutamate fermentation via the  $\beta$ -methylaspartase and the hydroxyglutarate pathway. Work in the laboratory of W. Buckel has recently unravelled the mechanisms underlying this hydrogen production (Buckel 2001a, b; Boiangiu et al. 2005). Pyruvate is oxidatively decarboxylated to acetyl-CoA by pyruvate:ferredoxin oxidoreductase. Re-oxidation of reduced ferredoxin proceeds in two ways: the majority (up to 80%) is re-oxidized during the synthesis of butyrate from two acetyl-CoA and the remaining 20% is used to reduce protons to hydrogen. This reaction is catalysed by an iron-only hydrogenase. For butyrate synthesis from two acetyl-CoA, however, reduced NADH is necessary. For *Clostridium tetanomorphum*, it was postulated that  $\text{NAD}^+$  is reduced by a membrane-bound NADH-ferredoxin oxidoreductase (Fig. 4) (Buckel 2001b; Boiangiu et al. 2005).

Several Bacteria have been isolated that during growth on glutamate release reducing equivalents exclusively as hydrogen, in the formation of acetate, propionate, or both. Microorganisms that ferment glutamate to acetate only include *Caloramator coolhaasii* (Plugge et al. 2000) and *Caloramator proteoclasticus*



**Fig. 4** Model of NADH-ferredoxin oxidoreductase (after Boiangiu et al. 2005)

(Tarlera et al. 1997). Propionate as the only product is formed from glutamate by *Aminobacterium colombiense* (Baena et al. 1998) and *Gelria glutamica* (Plugge et al. 2002a). *Acidaminobacter hydrogeniformans* (Stams and Hansen 1984; Meijer et al. 1999), *Thermanaerovibrio acidaminovorans* (Cheng et al. 1992; Baena et al. 1999a), and *Aminomonas paucivorans* (Baena et al. 1999b) form both acetate and propionate from glutamate. In syntrophy with methanogens, the hydrogen pressure can be lowered to 1 Pa and glutamate degradation to CO<sub>2</sub>, acetate, or propionate, and hydrogen becomes feasible (Plugge et al. 2002a). Hence, both under these conditions in the acetate and in the propionate-forming pathway, energy conservation to the extent of 1 ATP per mole glutamate is thermodynamically possible.

According to <sup>13</sup>C-labelling studies with 1-<sup>13</sup>C- and 3-<sup>13</sup>C-glutamate, the pathway of glutamate fermentation to acetate in *C. coolhaasii* proceeds via 3-methylaspartate and pyruvate. *T. acidaminovorans* forms propionate by oxidation of glutamate followed by decarboxylation of succinyl-CoA via methylmalonyl-CoA to propionyl-CoA (Plugge et al. 2001). Operation of the citric acid cycle can be excluded since no 2,3-double labelled propionate could be detected; obviously, neither fumarate nor free succinate was formed as intermediates. The formation of [2,3-<sup>13</sup>C] succinate indicated that glutamate is directly oxidized to succinyl-CoA, in which part of the succinyl-CoA is converted to succinate and excreted, and the majority is further converted to propionate.

## 2.9 Carrier Systems

Depending on the type of syntrophic conversion, the carrier system that transfers electrons from the producer to the consumer may vary. The best-studied and best-accepted electron carrier is hydrogen. However, formate is considered to be an important agent in interspecies electron transfer during propionate conversion as already discussed in Sect. 2.3. Formate can also act as electron carrier in syntrophic butyrate conversion by *S. wolfei* since this bacterium contains a formate

dehydrogenase with high homology to a formate dehydrogenase of *E. acidaminophilum* (FdhA-II) that was suggested to play a role also in interspecies formate transfer (Müller et al. 2009).

In syntrophic acetone-degrading methanogenic cultures, acetate was identified as the only interspecies carrier compound (Platen and Schink 1987; Platen et al. 1994). In this syntrophic culture, growth and conversion of acetone to acetate proceeded until acetate had accumulated to ~10 mM. Addition of an active acetoclastic methanogen (*Methanosaeta* sp.) greatly enhanced the acetone degradation rate. In addition, experiments with <sup>14</sup>C-labelled CO<sub>2</sub> showed that CO<sub>2</sub> is stoichiometrically incorporated into the formed acetate (Platen and Schink 1987).

Interspecies electron cycling through sulfur and sulfide has been described for *Desulfuromonas acetoxidans* in syntrophic cultures with *Chlorobium limicola*, a phototrophic green sulfur bacterium (Pfennig and Biebl 1976; Biebl and Pfennig 1978). Acetate oxidation by *D. acetoxidans* and electron transfer to the phototrophic green sulfur bacterium *C. limicola* (Biebl and Pfennig 1978) occurred in the presence of small amounts of sulfide (53–92 µM) in the light (Biebl and Pfennig 1978). A similar sulfur cycle mediated electron transfer was described in an artificial co-culture, which syntrophically oxidized acetate to CO<sub>2</sub> with concomitant reduction of nitrate (Kaden et al. 2002).

The discovery of bacterial nanowires and identification of presumed electron transfer components required for electrical conductivity in these pili-like structures provided a novel view on mechanisms involved in interspecies electron transfer (Gorby et al. 2006; Reguera et al. 2005). Pili-like structures have been identified in a number of pure and mixed cultures, and also syntrophic co-cultures of propionate-oxidizing *P. thermopropionicum* and *Methanothermobacter thermoautotrophicus* produced these pili-like structures. Analysis of the conductive properties of pili indicated that they could transfer electrons between cells of *Geobacter sulfurreducens* and the surface of Fe(III) oxides (Reguera et al. 2005). These pili were not required for attachment to the insoluble electron acceptor; rather they are interpreted to function as channels for electron transfer to the Fe(III) oxides, extending the electron transfer capabilities of the cells well beyond their outer surface (Reguera et al. 2005). Pili “nanowires” also served as electric conduits to mediate long-range electron transfer across biofilms formed on anode electrodes in microbial fuel cells, which could maximize current production per unit of anode surface area (Reguera et al. 2005).

## **2.10 Alternative Substrates for Pure Cultures and Technical Systems to Replace Methanogens**

Outside the laboratory, bacterial communities are nearly always communities composed of a wide variety of species. It is appropriate to consider the relevance of these interspecies interactions to the outcome of activity assays and the cultivability in the laboratory. Defined cultures of syntrophically fermenting bacteria are

required for detailed physiological and molecular studies and to understand their significant role in nature. To obtain such cultures, technical systems can be used to replace the methanogenic partner or alternative substrates can be supplied to bypass the energetically unfavourable steps occurring in syntrophic conversions.

The first axenic culture of an obligatory syntrophic bacterium was *S. wolfei* (Beaty et al. 1987). Studies on the butyrate metabolism of syntrophic co-cultures of *S. wolfei* and *M. hungatei* revealed a high activity of  $\beta$ -oxidation enzymes (Wofford et al. 1986). With this knowledge, Beaty and co-workers grew *S. wolfei* on agar plates containing crotonate as the sole source of carbon and energy. The pure culture obtained dismutated crotonate to butyrate and acetate, but exhibited butyrate oxidation only after re-association with a syntrophic partner. Later, it was shown that *S. wolfei* and *Syntrophospora bryantii* could grow in pure culture on butyrate plus 3-pentenoate (Amos and McInerney 1990; Dong et al. 1994b). Butyrate plus 3-pentenoate were converted to valerate, acetate, and propionate.

The first successful axenic culture of a syntrophically propionate-degrading bacterium was obtained from an enrichment culture by inhibiting the methanogens with bromoethanesulfonic acid (an analogue of coenzyme M) and subsequently adding fumarate as external electron acceptor. This allowed to isolate *S. fumaroxidans* (Stams et al. 1993; Harmsen et al. 1998) and to study the pathway of propionate oxidation (Plugge et al. 1993). Phylogenetically, *S. fumaroxidans* is very closely related to sulfate-reducing bacteria.

Some sulfate-reducing bacteria can alter their metabolism and act as syntrophically fermenting partners if sulfate becomes depleted (see above; Bryant et al. 1977; Scholten et al. 2007; Walker et al. 2009). Although this metabolic flexibility may be helpful for the enrichment and isolation of syntrophic bacteria, it can be applied only to already highly enriched syntrophic cultures. A strategy for isolation of syntrophs could be stepwise: from enrichment culture via molecular characterization (16S rRNA based) to a strategic choice of substrate, electron acceptor, or unsaturated compound for the isolation of the microorganism. Examples of unsaturated compounds used are fumarate, crotonate, pentenoate, and benzoate.

A cultivation apparatus capable of maintaining very low  $H_2$  (<0.01 Pa) pressures by mechanical means was developed by Valentine et al. (2000). This apparatus provided a method to study interspecies hydrogen transfer by externally providing the thermodynamic requirement for very low hydrogen concentrations, thus preventing the need for use of co-cultures to study the metabolic pathways. The culture vessel is constructed of glass and operates by sparging a liquid culture with purified gases, which remove hydrogen directly as it is produced. The culture device was constructed to decouple the syntrophic relationship in an ethanol-oxidizing methanogenic enrichment culture, allowing ethanol oxidation to dominate the methane production. Moreover, the culture apparatus was successfully used to grow pure cultures of the ethanol-oxidizing, proton-reducing *P. acetylenicus* (Valentine et al. 2000). This culture apparatus may have a potential to study also other forms of syntrophic metabolism; however, we have to realize that fatty acid oxidation requires hydrogen pressures substantially lower than ethanol oxidation.

## 2.11 Anaerobic Methane Oxidation

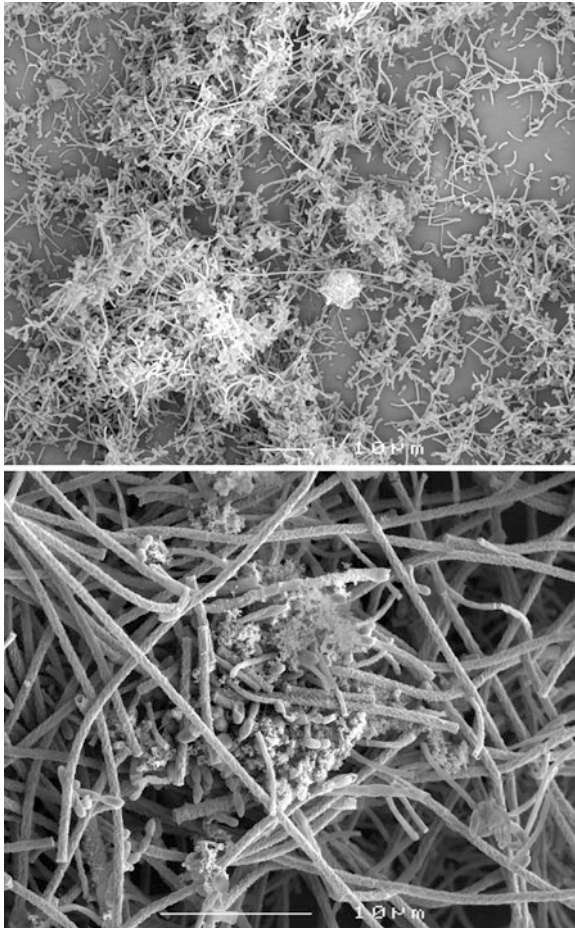
Although not a part of the methanogenic feeding chains discussed here, it is worth mentioning that also anaerobic oxidation of methane with sulfate as electron acceptor is, according to our present understanding, catalyzed by a syntrophic association of two organisms. One of the partners appears to be similar to methanogens but operates in reverse, i.e., it oxidizes methane by a reversal of the methane formation reaction (methyl-CoM reductase). The partner is a sulfate reducer that uses the intermediates released by its partner to reduce sulfate to sulfide. The overall reaction releases only little energy:



The reaction has been observed mainly in coastal shelf areas of the oceans at water depths between 800 and 1,000 m, at methane pressures of 80–100 atm over gas hydrate deposits (Boetius et al. 2000). Under these conditions, the reaction energetics are slightly more favourable (up to  $-40$  kJ/mol), thus barely feeding the two organisms involved with a minimum energy supply. Although this process is probably the most important reaction mitigating methane emissions to the atmosphere worldwide, it is still only barely understood. Especially, the identity of the electron carrier between the two partner organisms is still entirely enigmatic; from feeding experiments, we know that it is none of the “usual” carriers to be considered such as hydrogen, formate, methanol, or acetate (Nauhaus et al. 2002).

## 3 Spatial Organization of Syntrophic Communities

The close cooperation of two metabolically different organisms during syntrophic degradation requires short transport paths between the partners to optimize metabolite transfer, especially at low overall energy yields. The metabolite flux from one organism to the other is an inverse linear function of the diffusion distance (Schink and Thauer 1988). One should assume, therefore, that optimal transfer is ensured in mixed communities in which the partners are homogeneously mixed. Syntrophic co-cultures show a defined tendency to form mixed aggregates also in defined laboratory cultures (Fig. 5). However, since the respective partners are different organisms, they multiply separately and will form sooner or later nests of genetically identical organisms that compete with each other within the nests and have only limited exchange to the partner nests outside. One has to assume that such communities should mix through each other to maintain optimal metabolite transfer at short distances. Microscopic pictures of methanogenic communities in biogas reactors have shown that nests, as described, really do exist within such structures, but that in other areas, the partners appear to be fairly well mixed (Grotenhuis et al. 1991; Fang et al. 1995; Harmsen et al. 1996). It is still an open question how such mixing can be accomplished by organisms that appear to be basically immotile and do not show any means of gliding motility.



**Fig. 5** Scanning electron micrographs of a propionate-converting (a) coculture of *S. fumaroxidans* (oval shaped) and *M. hungatei* (rod shaped) and (b) triculture of *S. fumaroxidans* (oval shaped), *M. hungatei* (rod shaped), and *M. concilii* (long filaments) showing the close proximity of the syntrophic partners

#### 4 Concluding Remarks

The interrelationship of different trophic groups (guilds) within methanogenic microbial communities is a fascinating object to study, with perspectives to ecology, physiology, biochemistry, and energetics. These organisms cooperate in a very complex process, and they do so with minimum increments of energy for sustenance of life. These energy increments are at the lowermost range of energy that can be converted into ATP at all, and with this, these organisms are interesting model subjects to study energy starvation on a broader basis.



The question arises why nature designed methanogenic degradation in such a modular structure instead of having few types of organisms, which could convert polymeric substrates all the way down to methane plus CO<sub>2</sub>. Theoretical considerations suggested that metabolic pathways can be efficient only up to a limited length of reaction chains (Costa et al. 2006) and this may apply as well to, e.g., cellulose degradation to methane. One can argue as well that the strategy taken in these anaerobic communities is simply to establish a complex network of functions by independent modular units. This makes regulation easy for every single unit that acts only in a single function rather than combining many different metabolic tasks into one.

Methanogenesis in bioreactors is a sustainable technology to produce biogas from organic waste. More than 80% of the chemical energy in organic waste components is conserved as methane, which in aerobic conversion would have been lost. Presently, much research is done to replace fossil fuels to alternative sustainable (CO<sub>2</sub>-neutral) energy sources. Microbial methane formation from waste and wastewater will contribute to this development. From the technological viewpoint, it will be important to produce methane at a high rate and to convert all organic compounds to biomass. The proper functioning and structuring of syntrophic communities of anaerobic bacteria and archaea will be important in this respect. Further research is needed to get insight into the factors that regulate methane formation by syntrophic communities.

## References

- Allison MJ (1970) Nitrogen metabolism in rumen microorganisms. In: Phillipson AT (ed) Physiology and digestion and metabolism in the ruminant. Oriel, Stockfield, Norththumberland, UK, pp 456–473
- Allison MJ, Bryant MP (1963) Biosynthesis of branched-chain amino acids from branched-chain fatty acids by rumen bacteria. Arch Biochem Biophys 101:269–277
- Amos DA, McInerney MJ (1990) Growth of *Syntrophomonas wolfei* on unsaturated short chain fatty acids. Arch Microbiol 154:31–36
- Andreesen JR (1994) Glycine metabolism in anaerobes. Antonie Van Leeuwenhoek 66:223–237
- Andreesen JR (2004) Glycine reductase mechanism. Curr Opin Chem Biol 8:454–461
- Andreesen JR, Bahl H, Gottschalk G (1989) Introduction to the physiology and biochemistry of the genus *Clostridium*. In: Minton NP, Clarke DC (eds) Clostridia. Plenum, New York, pp 27–62
- Baena S, Fardeau M-L, Labat M, Ollivier B, Garcia J-L, Patel BKC (1998) *Aminobacterium colombiense*, gen. nov., sp. nov., an amino-acid degrading anaerobe isolated from anaerobic sludge. Anaerobe 4:241–250
- Baena S, Fardeau M-L, Ollivier B, Labat M, Thomas P, Garcia J-L, Patel BKC (1999a) *Aminomonas paucivorans* gen. nov., sp. nov., a mesophilic, anaerobic, amino-acid-utilizing bacterium. Int J Syst Bacteriol 49:975–982
- Baena S, Fardeau M-L, Woo THS, Ollivier B, Labat M, Patel BKC (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., sp. nov., comb. nov. Int J Syst Bacteriol 49:969–974



- Barker HA (1981) Amino acid degradation by anaerobic bacteria. *Annu Rev Biochem* 50:23–40
- Beaty PS, Wofford NQ, McInerney MJ (1987) Separation of *Syntrophomonas wolfei* from *Methanospirillum hungatei* in syntrophic cocultures by using percoll gradients. *Appl Environ Microbiol* 53:1183–1185
- Biebl H, Pfennig N (1978) Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. *Arch Microbiol* 117:9–16
- Boetius A, Ravensschlag K, Schubert CJ, Rickert D, Widdel F, Giesecke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–626
- Boiangiu CD, Jayamani E, Brügel D, Herrmann G, Kim J, Forzi L, Hedderich R, Vgenopoulou I, Pierik AJ, Steuber J, Buckel W (2005) Sodium ion pumps and hydrogen production in glutamate fermenting anaerobic bacteria. *J Mol Microbiol Biotechnol* 10:105–119
- Boll M (2005) Dearomatizing benzene ring reductases. *J Mol Microbiol Biotechnol* 10 (2–4):132–142
- Boone DR, Bryant MP (1980) Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. *Appl Environ Microbiol* 40:626–632
- Breznak JA, Kane MD (1990) Microbial H<sub>2</sub>/CO<sub>2</sub> acetogenesis in animal guts: nature and nutritional significance. *FEMS Microbiol Rev* 7(3–4):309–313
- Brune A (2007) Microbiology: woodworker's digest. *Nature* 450:487–488
- Bryant MP (1977) Microbiology of the rumen. In: Stevenson MJ (ed) *Duke's physiology of domestic animals*, 9th edn. Cornell University Press, Itaca, NY, pp 287–304
- Bryant MP (1979) Microbial methane production – theoretical aspects. *J Anim Sci* 48:193–201
- Bryant MP, Wolin EA, Wolin MJ, Wolfe RS (1967) *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. *Arch Mikrobiol* 59:20–31
- Bryant MP, Campbell LL, Reddy CA, Crabill MR (1977) Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H<sub>2</sub>-utilizing methanogenic bacteria. *Appl Environ Microbiol* 33:1162–1169
- Buckel W (2001a) Unusual enzymes involved in five ways of glutamate fermentation. *Appl Microbiol Biotechnol* 57:263–273
- Buckel W (2001b) Sodium ion-translocating decarboxylases. *Biochim Biophys Acta* 1505:15–27
- Buckel W, Barker HA (1974) Two pathways of glutamate fermentation by anaerobic bacteria. *J Bacteriol* 117:1248–1260
- Chabrière E, Charon MH, Volbeda A, Pieulle L, Hatchikian EC, Fontecilla-Camps JC (1999) Crystal structures of the key anaerobic enzyme pyruvate: ferredoxin oxidoreductase, free and in complex with pyruvate. *Nat Struct Biol* 6:182–190
- Chen S, Liu X, Dong X (2005) *Syntrophobacter sulfatireducens* sp. nov., a novel syntrophic, propionate-oxidizing bacterium isolated from UASB reactors. *Int J Syst Evol Microbiol* 55:1319–1324
- Cheng G, Plugge CM, Roelofsen W, Houwen FP, Stams AJM (1992) *Selenomonas acidaminovorans* sp. nov., a versatile thermophilic proton-reducing anaerobe able to grow by the decarboxylation of succinate to propionate. *Arch Microbiol* 157:169–175
- Cherepanov DA, Mulkidjanian AY, Junge W (1999) Transient accumulation of elastic energy in proton translocating ATP synthase. *FEBS Lett* 449:1–6
- Conklin A, Stensel HD, Ferguson J (2006) Growth kinetics and competition between *Methanosarcina* and *Methanosaeta* in mesophilic anaerobic digestion. *Water Environ Res* 78:486–496
- Conway de Macario E, Macario AJL (2010) Methanogenic archaea in humans and other vertebrates. In: Hackstein JHP (ed) *(Endo)symbiotic methanogens*. Springer, Heidelberg
- Costa E, Perez J, Kreft JU (2006) Why is metabolic labour divided in nitrification? *Trends Microbiol* 14:213–219
- De Bok FAM, Stams AJM, Dijkema C, Boone DR (2001) Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. *Appl Environ Microbiol* 67:1800–1804

- De Bok FAM, Luijten MLGC, Stams AJM (2002) Biochemical evidence for formate transfer in syntrophic propionate oxidizing cocultures of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. *Appl Environ Microbiol* 68:4247–4252
- De Bok FAM, Harmsen JM, Plugge CM, de Vries MC, Akkermans ADL, de Vos WM, Stams AJM (2005) The first true obligately syntrophic propionate-oxidizing bacterium, *Pelotomaculum schinkii* sp. nov., cocultured with *Methanospirillum hungatei*, and emended description of the genus *Pelotomaculum*. *Int J Syst Evol Microbiol* 55:1697–1703
- Dong X, Stams AJM (1995) Evidence for H<sub>2</sub> and formate formation during syntrophic butyrate and propionate degradation. *Anaerobe* 1:35–39
- Dong X, Plugge CM, Stams AJM (1994a) Anaerobic degradation of propionate by a mesophilic acetogenic bacterium in co- and triculture with different methanogens. *Appl Environ Microbiol* 60:2834–2838
- Dong X, Cheng G, Stams AJM (1994b) Butyrate oxidation by *Syntrophospora bryantii* in coculture with different methanogens and in pure culture with pentanoate as electron acceptor. *Appl Microbiol Biotechnol* 42:647–652
- Eichler B, Schink B (1986) Fermentation of primary alcohols and diols, and pure culture of syntrophically alcohol-oxidizing anaerobes. *Arch Microbiol* 143:60–66
- Engelbrecht S, Junge W (1997) ATP synthase: a tentative structural model. *FEBS Lett* 414:485–491
- Fang HHP, Chui HK, Li YY (1995) Microstructural analysis of UASB granules treating brewery wastewater. *Water Sci Technol* 31:129–135
- Fenchel T, Finlay BJ (2010) Free-living protozoa with endosymbiotic methanogens. In: Hackstein JHP (ed) (Endo)symbiotic methanogens. Springer, Heidelberg
- Ferry JG (1992) Methanogenesis: ecology, physiology, biochemistry & genetics. Chapman & Hall, New York
- Fuchs G (2008) Anaerobic metabolism of aromatic compounds. *Ann N Y Acad Sci* 1125:82–99
- Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealon KH, Fredrickson JK (2006) Electrically conductive nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc Natl Acad Sci USA* 103:11358–11363
- Gottschalk G (1986) Bacterial metabolism, 2nd edn. Springer, New York
- Graentzdoerffer A, Rauh D, Pich A, Andreesen JR (2003) Molecular and biochemical characterization of two tungsten- and selenium-containing formate dehydrogenases from *Eubacterium acidaminophilum* that are associated with components of an iron-only hydrogenase. *Arch Microbiol* 179:116–130
- Grotenhuis JTC, Smit M, Plugge CM, Xu YS, Van Lammeren AAM, Stams AJM, Zehnder AJB (1991) Bacteriological composition and structure of granular sludge adapted to different substrates. *Appl Environ Microbiol* 57:1942–1949
- Harmsen HJM, Akkermans ADL, Stams AJM, de Vos WM (1996) Population dynamics of propionate-oxidizing bacteria under methanogenic and sulfidogenic conditions in anaerobic granular sludge. *Appl Environ Microbiol* 62:2163–2168
- Harmsen HJM, Van Kuijk BLM, Plugge CM, Akkermans ADL, de Vos WM, Stams AJM (1998) Description of *Syntrophobacter fumaroxidans* sp.nov., a syntrophic propionate-degrading sulfate-reducing bacterium. *Int J Syst Bacteriol* 48:1383–1387
- Hattori S, Kamagata Y, Hanada S, Shoun H (2000) *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int J Syst Evol Microbiol* 50:1601–1609
- Hattori S, Galushko AS, Kamagata Y, Schink B (2005) Operation of the CO dehydrogenase/ acetyl-CoA pathway in both acetate oxidation and acetate formation by the syntrophically acetate-oxidizing bacterium *Thermacetogenium phaeum*. *J Bacteriol* 187:3471–3476

- Hauschild E (1997) Biochemie und Energetik der syntrophen Ethanol-Oxidation bei *Pelobacter acetylenicus* WoAcy1. Hinweise für die Beteiligung eines revertierten Elektronentransports. Thesis Konstanz
- Hazlewood GP, Nugent JHA (1978) Leaf fraction 1 protein as a nitrogen source for the growth of a proteolytic rumen bacterium. *J Gen Microbiol* 106:369–371
- Healy JB, Young LY (1978) Catechol and phenol degradation by a methanogenic population of bacteria. *Appl Environ Microbiol* 35(1):216–218
- Herrmann G, Jayamani E, Mai G, Buckel W (2008) Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. *J Bacteriol* 190:784–791
- Hobson PN, Wallace RJ (1982) Microbial ecology and activities in the rumen. *Crit Rev Microbiol* 9:253–320
- Iannotti EL, Kafkewitz D, Wolin MJ, Bryant MP (1973) Glucose fermentation products of *Ruminococcus albus* grown in continuous culture with *Vibrio succinogenes*: changes caused by interspecies transfer of H<sub>2</sub>. *J Bacteriol* 114:1231–1240
- Imachi H, Sekiguchi Y, Kamagata Y, Hanada S, Ohashi A, Harada H (2002) *Pelotomaculum thermopropionicum* gen. nov., an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. *Int J Syst Evol Microbiol* 52:1729–1735
- Imachi H, Sakai S, Ohashi A, Harada H, Hanada S, Kamagata Y, Sekiguchi Y (2007) *Pelotomaculum propionicum* sp. nov., an anaerobic, mesophilic, obligately syntrophic propionate-oxidizing bacterium. *Int J Syst Evol Microbiol* 57:1487–1492
- Jackson BE, Bhupathiraju VK, Tanner RS, Woese CR, McInerney MJ (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
- Jetten MSM, Stams AJM, Zehnder AJB (1992) Methanogenesis from acetate: a comparison of the acetate metabolism of *Methanotrix soehngenii* and *Methanosarcina* spp. *FEMS Microbiol Rev* 88:181–198
- Kaden J, Galushko AS, Schink B (2002) Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*. *Arch Microbiol* 178:53–58
- Kremer DR, Nienhuis-Kuiper HE, Hansen TA (1988) Ethanol dissimilation in *Desulfovibrio*. *Arch Microbiol* 150:552–557
- Kröger A, Biel S, Simon J, Gross R, Uden G, Lancaster CRD (2002) Fumarate respiration of *Wolinella succinogenes*: enzymology, energetics and coupling mechanism. *Biochim Biophys Acta* 1553:23–38
- Li F, Hinderberger J, Seedorf H, Zhang J, Buckel W, Thauer RK (2008) Coupled ferredoxin and crotonyl coenzyme a (CoA) reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Etf complex from *Clostridium kluyveri*. *J Bacteriol* 190:843–850
- Liu Y, Whitman WB (2008) Metabolic, phylogenetic and ecological diversity of methanogenic archaea. In: Wiegand J, Maier MJ, Adams MW (eds) *Incredible anaerobes: from physiology to genomics to fuels*, vol 1125. *Annals of the New York Academy of Sciences*, New York, pp 171–189
- Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR (1999) Characterisation of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov, sp. nov. and *Syntrophobacter wolinii*. *Int J Syst Bacteriol* 49:545–556
- McInerney MJ (1988) Anaerobic hydrolysis and fermentation of fats and proteins. In: Zehnder AJB (ed) *Biology of anaerobic microorganisms*. Wiley, New York, pp 373–415
- McInerney MJ, Bryant MP, Pfennig N (1979) Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. *Arch Microbiol* 122:129–135
- McInerney MJ, Bryant MP, Pfennig N (1981) *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl Environ Microbiol* 41:1029–1039
- McInerney MJ, Stams AJM, Boone DR (2005) Genus *Syntrophobacter*. In: Staley JT, Boone DR, Brenner DJ, de Vos P, Garrity GM, Goodfellow M, Krieg NR, Rainey FA, Schleifer KH (eds) *Bergey's manual of systematic bacteriology*, vol 2, 2nd edn. Springer, New York, pp 1021–1027

- McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJM, Schink B, Rohlin L, Gunsalus RP (2008) Physiology, ecology, phylogeny and genomics of microorganisms capable of syntrophic metabolism. In: Wiegel J, Maier MJ, Adams MW (eds) *Incredible anaerobes: from physiology to genomics to fuels*, vol 1125. *Annals of the New York Academy of Sciences*, New York, pp 58–72
- Meijer WG, Nienhuis-Kuiper ME, Hansen TA (1999) Fermentative bacteria from estuarine mud: Phylogenetic position of *Acidaminobacter hydrogeniformans* and description of a new type of Gram-negative, propionigenic bacterium as *Propionibacter pelophilus* gen. nov., sp. nov. *Int J Syst Bacteriol* 49:1039–1044
- Metje M, Frenzel P (2007) Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environ Microbiol* 9:954–964
- Mountfort DO, Bryant MP (1982) Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. *Arch Microbiol* 133:249–256
- Müller N, Stingl U, Griffin BM, Schink B (2008) Dominant sugar utilizers in sediment of Lake Constance depend on syntrophic cooperation with methanogenic partner organisms. *Environ Microbiol* 10:1501–1511
- Müller N, Schleheck D, Schink B (2009) Involvement of NADH:acceptor oxidoreductase and butyryl-CoA dehydrogenase in reversed electron transport during syntrophic butyrate oxidation by *Syntrophomonas wolfei*. *J Bacteriol* 191:6167–6177
- Nagase M, Matsuo T (1982) Interaction between amino-acid degrading bacteria and methanogenic bacteria in anaerobic digestion. *Biotechnol Bioeng* 24:2227–2239
- Nanninga HJ, Gottschal JC (1985) Amino acid fermentation and hydrogen transfer in mixed cultures. *FEMS Microbiol Ecol* 31:261–269
- Nanninga HJ, Drenth WJ, Gottschal JC (1987) Fermentation of glutamate by *Selenomonas acidaminophila*, sp. nov. *Arch Microbiol* 147:152–157
- Nauhaus K, Boetius A, Krüger M, Widdel F (2002) In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. *Environ Microbiol* 4:296–305
- Pfennig N, Biebl H (1976) *Desulfuromonas acetoxidans* gen. nov. sp. nov., a new anaerobic, sulfur-reducing acetate-oxidizing bacterium. *Arch Microbiol* 110:3–12
- Platen H, Schink B (1987) Methanogenic degradation of acetone by an enrichment culture. *Arch Microbiol* 149:136–141
- Platen H, Janssen PH, Schink B (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 CM, Stams AJM (2005) Syntrophism among Clostridiales. In: Duerre P (ed) *Handbook on Clostridia*. CRC, Boca Raton, pp 769–784
- Plugge CM, Dijkema C, Stams AJM (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 CM, Zoetendal EG, Stams AJM (2000) *Caloramator coolhaasii*, sp. nov. a glutamate-degrading, moderately thermophilic anaerobe. *Int J Syst Bacteriol* 50:1155–1161
- Plugge CM, van Leeuwen JM, Hummelen T, Balk M, Stams AJM (2001) Elucidation of the pathways of catabolic glutamate conversion in three thermophilic anaerobic bacteria. *Arch Microbiol* 176:29–36
- Plugge CM, Balk M, Zoetendal EG, Stams AJM (2002a) *Gelria glutamica*, gen. nov., sp. nov., a thermophilic obligate syntrophic glutamate-degrading anaerobe. *Int J Syst Evol Microbiol* 52:401–406
- Plugge CM, Balk M, Stams AJM (2002b) *Desulfotomaculum thermobenzoicum* subsp. *thermo-syntrophicum* subsp. nov., a thermophilic syntrophic propionate-oxidizing spore-forming bacterium. *Int J Syst Evol Microbiol* 52:391–399
- Plugge CM, Jiang B, de Bok FAM, Tsai C, Stams AJM (2009) Effect of tungsten and molybdenum on growth of a syntrophic coculture of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. *Arch Microbiol* 191:55–61

- Qiu YL, Hanada S, Ohashi A, Harada H, Kamagata Y, Sekiguchi Y (2008) *Syntrophorhabdus aromaticivorans* gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. *Appl Environ Microbiol* 74:2051–2058
- Reda T, Plugge CM, Abram NJ, Hirst J (2008) Reversible interconversion of carbon dioxide and formate by an electroactive enzyme. *Proc Natl Acad Sci USA* 105:10654–10658
- Reddy CA, Bryant MP, Wolin MJ (1972a) Characteristics of S organism isolated from *Methanobacillus omelianskii*. *J Bacteriol* 109:539–545
- Reddy CA, Bryant M, Wolin MJ (1972b) Ferredoxin- and nicotinamide adenine dinucleotide-dependent H<sub>2</sub> production from ethanol and formate in extracts of S organism isolated from *Methanobacillus omelianskii*. *J Bacteriol* 110:126–132
- Reddy CA, Bryant MP, Wolin MJ (1972c) Ferredoxin-dependent conversion of acetaldehyde to acetate and H<sub>2</sub> in extracts of S organism. *J Bacteriol* 110:133–138
- Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. *Nature* 435:1098–1101
- Roy F, Samain E, Dubourgier HC, Albagnac G (1986) *Synthrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. *Arch Microbiol* 145:142–147
- Schink B (1985) Fermentation of acetylene by an obligate anaerobe, *Pelobacter acetylenicus* sp. nov. *Arch Microbiol* 142:295–301
- Schink B (1989) Mikrobielle Lebensgemeinschaften in Gewässersedimenten. *Naturwissenschaften* 76:364–372
- Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* 61:262–280
- Schink B, Stams AJM (2002) Syntrophism among prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) *The prokaryotes: an evolving electronic resource for the microbiological community*, 3rd edn. Springer, New York, p 25
- Schink B, Thauer RK (1988) Energetics of syntrophic methane formation and the influence of aggregation. In: Lettinga G et al (eds) *Granular anaerobic sludge; microbiology and technology*. Pudoc, Wageningen, pp 5–17
- Schink B, Zeikus JG (1982) Microbial ecology of pectin decomposition in anoxic lake sediments. *J Gen Microbiol* 128:393–404
- Schmidt J, Ahring BK (1993) Effects of hydrogen and formate on the degradation of propionate and butyrate in thermophilic granules from an upflow anaerobic sludge blanket reactor. *Appl Environ Microbiol* 59:2546–2551
- Schnürer A, Schink B, Svensson BH (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, Svensson BH, Schink B (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, Schink B (1997) Energetics of methanogenic benzoate degradation by *Syntrophus gentianae* in syntrophic coculture. *Microbiology* 143:2345–2351
- Schöcke L, Schink B (1999) Energetics and biochemistry of fermentative benzoate degradation by *Syntrophus gentianae*. *Arch Microbiol* 171:331–337
- Scholten JC, Culley DE, Brockman FJ, Wu G, Zhang W (2007) Evolution of the syntrophic interaction between *Desulfovibrio vulgaris* and *Methanosarcina barkeri*: involvement of an ancient horizontal gene transfer. *Biochem Biophys Res Commun* 5:48–54
- Schut GJ, Adams MW (2009) The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. *J Bacteriol* 191:4451–4457
- Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (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

- Sousa DZ, Smidt H, Alves MM, Stams AJ (2007) *Syntrophomonas zehnderi* sp. nov., an anaerobe that degrades long-chain fatty acids in co-culture with *Methanobacterium formicicum*. *Int J Syst Evol Microbiol* 57:609–615
- Stams AJM (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie Van Leeuwenhoek* 66:271–294
- Stams AJM, Hansen TA (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 AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Microbiol* 7:568–577
- Stams AJM, van Dijk J, Dijkema C, Plugge CM (1993) Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. *Appl Environ Microbiol* 59:1114–1119
- Stickland LH (1934) The chemical reaction by which *Cl. sporogenes* obtains its energy. *Biochem J* 28:1746–1759
- Stieb M, Schink B (1985) Anaerobic oxidation of fatty acids by *Clostridium bryantii* sp. nov., a sporeforming, obligately syntrophic bacterium. *Arch Microbiol* 140:387–390
- Stieb M, Schink B (1986) Anaerobic degradation of isovalerate by a defined methanogenic coculture. *Arch Microbiol* 144:291–295
- Stieb M, Schink B (1989) Anaerobic degradation of isobutyrate by methanogenic enrichment cultures and by a *Desulfococcus multivorans* strain. *Arch Microbiol* 151:126–132
- Svetlitsnyi V, Rainey F, Wiegel J (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
- Szewzyk U, Schink B (1989) Degradation of hydroquinone, gentisate, and benzoate by a fermenting bacterium in pure or defined mixed culture. *Arch Microbiol* 151:541–545
- Tarlera S, Muxi L, Soubes M, Stams AJM (1997) *Caloramator proteoclasticus* sp. nov., a moderately thermophilic anaerobic proteolytic bacterium. *Int J Syst Bacteriol* 47:651–656
- Tewes FJ, Thauer RK (1980) Regulation of ATP synthesis in glucose fermenting bacteria involved in interspecies hydrogen transfer. In: Gottschalk G, Pfennig N, Werner H (eds) *Anaerobes and anaerobic infections*. G Fischer, Stuttgart, New York, pp 97–104
- Thauer RK, Morris JG (1984) Metabolism of chemotrophic anaerobes: old views and new aspects. In: Kelly DP, Carr NG (eds) *The microbe 1984: prokaryotes and eukaryotes part II*. Cambridge University Press, Cambridge, pp 123–168
- Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* 41:100–180
- Valentine DL, Reeburgh VS, Blanton DC (2000) A culture apparatus for maintaining H<sub>2</sub> at sub-nanomolar concentrations. *J Microbiol Methods* 39:243–251
- Van Kuijk BLM, Stams AJM (1996) Purification and characterization of malate dehydrogenase from the syntrophic propionate-oxidizing bacterium strain MPOB. *FEMS Microbiol Lett* 144:141–144
- Van Kuijk BLM, Schlösser E, Stams AJM (1998) Investigation of the fumarate metabolism of the syntrophic propionate-oxidizing bacterium strain MPOB. *Arch Microbiol* 169:346–352
- Walker CB, He Z, Yang ZK, Ringbauer JA Jr, Zhou J, Voordouw G, Wall JD, Hazen TC, Arkin AP, Stahl DA (2009) The electron transfer system of syntrophically grown *Desulfovibrio vulgaris*. *J Bacteriol* 191:5793–5801
- Wallrabenstein C, Schink B (1994) 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, Hauschild E, Schink B (1995) *Syntrophobacter pfennigii* sp. nov., new syntrophically propionate-oxidizing anaerobe growing in pure culture with propionate and sulfate. *Arch Microbiol* 164:346–352

- Whitman W, Bowen T, Boone D (2006) The methanogenic bacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) *The Prokaryotes*, vol 3, 3rd edn, Springer, New York, pp 165–207
- Widdel F, Rouviere PE, Wolfe RS (1988) Classification of secondary alcohol-utilizing methanogens including a new thermophilic isolate. *Arch Microbiol* 150:477–481
- Wofford NQ, Beaty PS, McInerney MJ (1986) Preparation of cell-free extracts and the enzymes involved in fatty acid metabolism in *Syntrophomonas wolfei*. *J Bacteriol* 167:179–185
- Wu C, Liu X, Dong X (2006) *Syntrophomonas cellicola* sp. nov., a spore-forming syntrophic bacterium isolated from a distilled-spirit-fermenting cellar, and assignment of *Syntrophospora bryantii* to *Syntrophomonas bryantii* comb. nov. *Int J Syst Evol Microbiol* 56:2331–2335
- Zehnder AJB (1978) Ecology of methane formation. In: Mitchell R (ed) *Water pollution microbiology*, vol 2. Wiley, London, pp 349–376
- Zhang C, Liu X, Dong X (2004) *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens. *Int J Syst Evol Microbiol* 54:969–973
- Zhang C, Liu X, Dong X (2005) *Syntrophomonas erecta* sp. nov., a novel anaerobe that syntrophically degrades short-chain fatty acids. *Int J Syst Evol Microbiol* 55:799–803
- Zinder SH, Koch M (1984) Non-aceticlastic methanogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. *Arch Microbiol* 138:263–272