Methanogenesis: Syntrophic Metabolism

Jessica R. Sieber, Michael J. McInerney, Nicolai Müller, Bernhard Schink[3], Rob P. Gunsalus and Caroline M. Plugge[5*]

J.R. Sieber
Department of Biology, University of Minnesota-Duluth, Duluth, MN, USA
jrsieber@d.umn.edu
M.J. Mc Inerney
Department of Botany and Microbiology, University of Oklahoma, Norman, OK, USA
mcinerney@ou.edu
N. Müller • B. Schink
Department of Biology, Universität Konstanz, D-78457 Konstanz, Germany
nicolai.mueller@uni-konstanz.de; Bernhard.Schink@uni-konstanz.de
R.P. Gunsalus
Department of Microbiology and Molecular Genetics, UCLA, Los Angeles, CA, USA
robg@microbio.ucla.edu
C.M. Plugge (✉)
Laboratory of Microbiology, Wageningen University and Research, 6708 WE Wageningen, The Netherlands
caroline.plugge@wur.nl

Contents

Abstract

Syntrophy is a mutualistic interaction in which two metabolically different types of microorganisms are linked by the need to keep metabolites exchanged between the two partners at low concentrations to make the overall metabolism of both organisms feasible. In most cases, the cooperation is based on the transfer of hydrogen, formate, or acetate from fermentative bacteria to methanogens to make the degradation of electron-rich substrates thermodynamically favorable. Syntrophic metabolism proceeds at very low Gibbs' free energy changes, close to the minimum free energy change needed to conserve energy biologically, which is the energy needed to transport one proton across the cytoplasmic membrane. Pathways for syntrophic degradation of fatty acids predict the net synthesis of about one-third of an ATP per round of catabolism. Syntrophic metabolism entails critical oxidation-reduction reactions in which H_2 or formate production would be thermodynamically unfavorable unless energy is invested. Molecular insights into the membrane processes involved in ion translocation and reverse electron transport revealed that syntrophs harbour multiple systems for reverse electron transfer. While much evidence supports the interspecies transfer of H_2 and formate, other mechanisms of interspecies electron transfer exist including cysteine cycling and possibly direct interspecies electron transfer as electric current via conductive pili or (semi)conductive minerals.

1 Introduction

Syntrophy is an energetically limited interaction between cells of different species, e.g., the fatty acid degrader and the methanogen (Table 1) (McInerney et al., 2008; Schink, 1997; Schink and Stams, 2013). The mutual dependence between the two metabolic types of organisms is so extreme that neither one functions without the activity of its partner. Together, the partners perform functions that neither one can do alone. The degradation of the respective substrate, in this case, a fatty or aromatic acid (Table 1), is thermodynamically unfavorable if the product concentrations are at standard conditions (1 M concentration, or 1 atm for gasses). The function of methanogens is to consume hydrogen, for example, to low steady-state pressure (10^{-4}–10^{-5} atm) to make fatty and aromatic acid oxidation thermodynamically favorable (Table 1). This chapter focuses on obligate syntrophy where reverse electron transport is a key requirement in the energy budget.

Table 1. Reactions involved in syntrophic metabolism.

Reactions	$\Delta G^{o\prime}$ [a] (kJ/mol)	$\Delta G'$ [b] (kJ/mol)
Methanogenic reactions		
$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	-135.6	-15.8
$4\ HCOO^- + H_2O + H^+ \rightarrow CH_4 + 3\ HCO_3^-$	-130.4	-11.8
Syntrophic Oxidations		
$Acetate^- + 4\ H_2O \rightarrow 2\ HCO_3^- + H^+ + 4\ H_2$	+104.6	-1.5
$Propionate^- + 3\ H_2O \rightarrow Acetate^- + HCO_3^- + H^+ + 3\ H_2$	+76.1	-16.9
$Butyrate^- + 2\ H_2O \rightarrow 2\ Acetate^- + H^+ + 2\ H_2$	+48.6	-39.2
$Benzoate^- + 7\ H_2O \rightarrow 3\ Acetate^- + HCO_3^- + 3\ H^+ + 3\ H_2$	+70.1	-68.5

[a] Calculated from the data in Thauer et al. (1977) with the free energy of formation for benzoate given in Kaiser and Hanselmann (1982).

[b] Calculated on the basis of the following conditions observed in methanogenic ecosystems: partial pressures of H_2 of 1 Pa and of CH_4 of 50 kPa, 50 mM bicarbonate, and the concentrations of the substrates and acetate at 0.1 mM each.

2 Importance of Syntrophy

Syntrophic metabolism is an essential, but the least energetically favorable step in the conversion of organic matter to methane and carbon dioxide in anoxic environments. Biological methane production, also termed methanogenesis, is an important process in the global carbon cycle, accounting for about 1 to 2% of the carbon fixed annually by photosynthesis (Hedderich and Whitman 2006). Annual global methane emissions into the atmosphere are large, about 550 to 650 Teragram (Tg) (1 Tg equals 10^{12} g), and more than 70% (400 to 450 Tg) of these emissions are due to microbial activity (Ehhalt et al. 2001; IPCC 2014). Syntrophic metabolism is often the rate-limiting step in methanogenesis (McCarty 1971; McInerney et al. 1981) and, thus, is an important process controlling the global carbon flux.

The degradation of natural polymers such as polysaccharides, proteins, nucleic acids, and lipids to CO_2 and CH_4 involves a complex microbial community (McInerney et al. 1981; Schink and Friedrich 1994). Fermentative bacteria hydrolyze the polymeric substrates such as polysaccharides, proteins, and lipids, and ferment the hydrolysis products to acetate and longer-chain fatty acids, CO_2, formate, H_2. Propionate and

longer-chain fatty acids, alcohols, and some amino acids and aromatic compounds are syntrophically metabolized to the methanogenic substrates: H_2, formate, and acetate (Schink 1997; Schink and Stams 2013). Lastly, two different groups of methanogens, the hydrogenotrophic methanogens and the acetotrophic methanogens, complete the process, converting acetate, formate and H_2 produced by other microorganisms to methane and carbon dioxide.

The syntrophic degradation of fatty and aromatic acids accounts for much of the carbon flux in methanogenic environments (McCarty 1971; Pavlostathis and Giraldo-Gomez 1991). Initial anaerobic transformations of aromatic compounds (Heider and Fuchs 1997a; Heider and Fuchs 1997b; Schink et al. 2000) generally lead to the conversion of diverse aromatic compounds into benzoyl-coenzyme A (CoA) (Merkel et al. 1989; Gallert and Winter 1994; Gibson et al. 1994; Gibson et al. 1997; Breese and Fuchs 1998; Hirsch et al. 1998). In methanogenic environments, the reduction and cleavage of the aromatic ring are catalyzed by syntrophic associations of benzoate-degrading microorganisms and hydrogen- and/or formate-using methanogens (Ferry and Wolfe 1976; Mountfort and Bryant 1982; Szewzyk and Schink 1989).

3 Bioenergetic Considerations

Syntrophy is a fascinating process, especially from a bioenergetic perspective. Syntrophic metabolism releases very little free energy, which must be shared among the partner organisms involved (Schink 1997). Organisms capable of syntrophic metabolism operate at free energy changes very close to the minimum increment of energy required for ATP synthesis (Schink 1997; Hoehler 2004). This minimum amount of energy needed for ATP synthesis has been predicted to be about -20 kJ mol^{-1} (Schink 1997) but, depending on the H^+/ATP stoichiometry of the ATPases involved, may be as low as -10 to -15 kJ mol^{-1} (Spahn et al. 2015; Lever et al. 2015). Most of the free energy changes observed during syntrophic metabolism are in the range of -20 kJ mol^{-1} (Schink 1997) although some studies have found free energy changes less than -10 kJ mol^{-1} (Dwyer et al. 1988; Scholten and Conrad 2000). Coupling energy-transforming reactions at the cytoplasmic membrane with translocation of different ions, e. g., protons and Na^+ ions, of different energetic values may allow to operate an energy metabolism at such a low energy yield. The

recently discovered phenomenon of electron bifurcation and electron confurcation (Li et al. 2008; Buckel and Thauer, 2013) may provide a further option for ATP synthesis at extremely low energy yields. According to this concept, electrons at an intermediate redox potential can be shifted through a flavin carrier to a lower potential at the expense of a simultaneous transport of other electrons of the same potential to a higher one. Thus, a certain (minimal) potential difference can be achieved for a single electron by running two electrons over only half that potential difference. Electron bifurcation and confurcation reactions have been found repeatedly in the recent past as essential means to understand energy coupling in the metabolism of methanogens, sulfate reducers, and fermenting bacteria, including syntrophically fermenting ones (section 6).

The second fascinating feature of syntrophic metabolism is the necessity for reverse electron transport. In syntrophic metabolism, there are critical oxidation-reduction reactions that are thermodynamically unfavorable. For example, the production of H_2 (E' of -261 mV at 1 Pa H_2) or formate (E' of -258 mV at 1 µM formate) (Schink 1997) from electrons generated from the oxidation of acyl-CoA intermediates to their respective enoyl-CoA intermediates (E' of -10 mV) (Sato et al. 1999) has a ΔE’ of about -250 mV. A H_2 partial pressure of about 10^{-5} Pa would make this reaction thermodynamically favorable (Schink 1997). The syntrophic oxidation of propionate by *Syntrophobacter wolinii* through the methylmalonyl-CoA pathway (Houwen et al. 1990) involves the oxidation of succinate to fumarate ($E^{o'}$ of + 33 mV) (Thauer et al. 1977). Here again, a very low H_2 partial pressure (10^{-6} Pa) is needed for H_2 production to be thermodynamically favorable (Schink 1997). Methanogens cannot generate such low H_2 partial pressures because hydrogenotrophic methanogenesis reaches thermodynamic equilibrium at 0.2 Pa H_2. H_2 or formate production up to concentrations that support the energy metabolism of methanogens requires an input of energy, a process called reversed electron transport. The most likely energy source for this energy input is an ion gradient that is provided by a membrane-bound ATPase. Consistent with the requirement for an ion gradient for H_2 production, the protonophore (CCCP) and the ATP synthase inhibitor (DCCD) inhibited H_2 production from butyrate by *Syntrophomonas wolfei* and from benzoate by *Syntrophus buswellii* (Wallrabenstein and Schink 1994). Similarly, H_2 formation from glycolate by membrane vesicles of *Syntrophobotulus glycolicus* (Friedrich et al. 1996) required ATP or a proton gradient (Friedrich and Schink 1993; Friedrich and Schink 1995).

While it is clear that reversed electron transport is needed for syntrophic metabolism, the nature of such a system has been elucidated only in few cases so far (section 6).

How do syntrophic microbial associations operate at these low-energy conditions? Do they have novel mechanisms for energy conservation or are they more efficient at conserving energy than other microorganisms? We will analyze what is known about syntrophic metabolism in an attempt to answer these questions. Further details on the physiology of the organisms capable of syntrophic metabolism are available in several comprehensive reviews (Schink 1997; Schink and Stams 2013; McInerney et al. 2008; Sieber et al. 2012; Schink et al. 2017).

4 Interspecies Electron Transfer

Above, we defined syntrophy based on the exchange of H_2 between the syntrophic partners. However, there are also other mechanisms to transfer electrons. Most hydrogenotrophic methanogens use either formate or H_2, or both simultaneously (Hedderich and Whitman 2006; Liu and Whitman 2008). There is very little difference in free energy change for methane production when H_2 versus formate serves as the electron donor (Table 1). The conclusion of many studies is that syntrophic metabolism can involve either interspecies transfer of H_2 and/or formate. Syntrophic metabolism by H_2 transfer was shown for glycolate metabolism by *Syntrophobotulus glycolicus* (Friedrich et al. 1996), sugar metabolism by *Syntrophococcus sucromutans* (Krumholz and Bryant 1986), acetate metabolism by a thermophilic, syntrophic acetate-oxidizing strain AOR (Lee and Zinder 1988b) and ethanol metabolism by the S-organism (Bryant et al. 1967), by culturing these organisms with a methanogen that uses only H_2. In a similar fashion, syntrophic formate transfer was shown for an amino acid degrader with a sulfate-reducing partner that uses formate but not H_2 (Zindel et al. 1988). Syntrophic propionate degradation by *Syntrophobacter fumaroxidans* (Dong et al. 1994b; Dong and Stams 1995) and syntrophic butyrate degradation by *Syntrophomonas* (*Syntrophospora) bryantii* (Dong et al. 1994a) occurred only with a methanogen that used both H_2 and formate, and not with a methanogen that used only H_2, implicating the need for formate metabolism. Proteomic and enzymatic analyses showed high levels of formate dehydrogenase in both *S. fumaroxidans* and its methanogenic partner,

arguing for formate as an important electron carrier (de Bok et al. 2002a; de Bok et al. 2002b; de Bok et al. 2003). Transcriptomics of genes coding for formate dehydrogenases (fdh) and hydrogenases (hyd) in a coculture of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei* revealed that all *fdh* and *hyd* genes were transcribed and transcription levels of the individual genes varied significantly depending on the substrate and growth conditions (Worm et al. 2011). This shows that both syntrophic partners tightly regulate their interspecies metabolism to grow together. Flux analysis of this coculture (de Bok et al. 2002a) and of a butyrate-degrading coculture (Boone et al. 1989) indicated that H_2 diffusion was too slow to account for the observed rates of syntrophic propionate oxidation. Also, syntrophic acetate oxidation by *Thermacetogenium phaeum* appears to use both formate and H_2 as electron carriers (Hattori et al. 2001). The use of H_2 and/or formate as the interspecies electron carrier provides an explanation why so many methanogens use both H_2 and formate. Genomic analyses support the involvement of both compounds, as the genomes of *Syntrophus aciditrophicus, Syntrophomonas wolfei* and *Methanospirillum hungatei*, the methanogenic partner most often observed in syntrophic associations, have multiple formate dehydrogenase and hydrogenase genes (McInerney et al. 2007; Sieber et al. 2008; Sieber et al. 2012). More recent evidence has revealed that while hydrogenase and formate dehydrogenase activity is present during syntrophic growth in whole cell assays of both *S. wolfei* and *S. aciditrophicus*, *S. wolfei* relies on hydrogen transfer predominantly while *S. aciditrophicus* can use both hydrogen and formate at the same time when growing with *M. hungatei* (Sieber et al. 2014).

Molecules other than H_2 or formate may be involved in interspecies electron transfer, such as humic compounds with anthraquinone disulfonate as a defined representative (Lovley et al. 1998), activated carbon or biochar (Liu et al. 2012; Chen et al. 2014) or various iron oxides (Kato et al. 2012a; Kato et al., 2012b; Viggi et al. 2014; Zhou et al. 2014). An acetate-oxidizing coculture of *Geobacter sulfurreducens* and *Wolinella succinogenes* used cysteine as the interspecies electron carrier (Kaden et al. 2002). Nonetheless, one has to keep in mind that the transferred electrons have to reach the methanogenic partner at a redox potential low enough to provide it with a minimum amount of energy, i. e., at E_0' = ca. -270 mV (Schink et al. 2017). Certain iron oxides such as goethite, hematite, or magnetite have redox potentials low enough to accomplish this (Straub et al. 2001);

others such as ferrihydrite or humic compounds can act as efficient electron carriers only in reduction of more positive acceptors such as ferric iron, fumarate, nitrate and the corresponding acceptor-reducing bacteria (Schink et al. 2017).
Syntrophic methanogenesis has been shown to be promoted by the presence of (semi)conductive mineral particles (Kato et al., 2012a; Kato et al., 2012b; Kouzouma et al., 2015). Interspecies electron transfer via electric currents through magnetite particles was calculated to be an intrinsically faster mechanism compared to interspecies H_2 transfer (Viggi et al., 2014). It remains to be revealed what the underlying mechanisms are and what this means for syntrophic methanogenesis in nature where (semi)conductive minerals are abundant.

Another option is direct electron transfer between syntrophic partners by electron-conductive pili or nanowires (Reguera et al. 2005; Gorby et al. 2006) or by direct cell-to-cell transfer (Shresta et al. 2013; Shresta and Rotaru 2014; Li et al. 2015). Interspecies electron transfer by nanowires is difficult to prove in syntrophic associations because we cannot mutate the pilus genes in either of the syntrophic partners at present. Nanowire-like structures connecting the syntrophic propionate degrader *Pelotomaculum thermopropionicum* with its methanogenic partner have been observed by electron microscopy (Ishii et al. 2005; Gorby et al. 2006), and scanning tunneling microscopy showed that these structures were electron transmissive (Gorby et al. 2006). Electron transfer via direct cell contact or nanowires would require close spatial associations between the cooperating partners. Some researchers point to aggregation of cells in cocultures as proof of direct electron transfer (Logan and Regan 2006), but aggregation also reduces the distance between the syntrophic partners and would increase the rate of H_2 or formate transfer as well (Conrad and Zeikus 1985; Thiele and Zeikus 1988; Ishii et al. 2005). Whether adhesins produced by certain methanogens may help to establish interactions with fermenting partners specifically for interspecies electron transfer (Ng et al., 2016) remains to be examined.

5 Biochemical Pathways for Syntrophic Metabolism

The pathways for several syntrophic metabolisms are known and an analysis of the bioenergetics of these pathways illustrates how small amounts of energy are conserved during syntrophic metabolism (Schink 1997).

5.1 Acetate Metabolism

Syntrophic acetate metabolism is a remarkable process that supports the concept that syntrophic metabolism is very energy efficient. A thermophilic organism, strain AOR, was found to produce acetate when grown axenically with H_2 and CO_2, and oxidizes acetate when grown syntrophically (Lee and Zinder 1988a; Hattori et al. 2000). Since then two thermophilic, *Pseudothermotoga lettingae* and *Thermacetogenium phaeum* (Hattori et al. 2000, Balk et al. 2002), and three mesophilic, *Tepidanaerobacter acetatoxydans*, *Clostridium ultunense*, and *Syntrophaceticus schinkii*, syntrophic acetate-oxidizing bacteria have been characterized (Schnurer et al., 1996; Westerholm et al., 2010; Westerholm et al., 2011). The known species are phylogenetically diverse but poorly understood as strict cultivation requirements, slow growth, and difficulties in reconstituting the thermodynamically unfavorable syntrophic acetate metabolism under laboratory conditions prevent the thorough investigation of their metabolism. Enzyme activity studies using crude cell extract and genome analysis indicate the involvement of the reversed Wood Ljungdahl (WL) pathway in syntrophic acetate oxidation for *T. phaeum*, *C. ultunense* and *S. schinkii* (Oehler et al. 2012, Manzoor et al, 2016) (Figure 1). The genome of *T. phaeum* encodes all enzymes of the WL pathway and most of the WL enzymes were encoded only once, indicative of bidirectional catalysis. Although electron transfer mechanisms involved in acetate oxidation by *T. phaeum* still remain unknown, they might be similar, or even the same as those used for acetate synthesis. In this context, the enzymes formate dehydrogenase, CO dehydrogenase, methylene-THF reductase and hydrogenase are of special relevance as these may contribute to energy conservation in both directions.

The working draft genome of *Syntrophaceticus schinkii* indicates limited metabolic capacities with the lack of organic nutrient uptake systems, chemotactic machineries, carbon catabolite repression, and incomplete biosynthesis pathways (Manzoor et al. 2016). During syntrophic growth, Ech hydrogenase, [FeFe] hydrogenases, [NiFe] hydrogenases, F_1F_0-ATP synthase and membrane-bound and cytoplasmic formate

dehydrogenases were highly expressed, whereas Rnf and a predicted oxidoreductase/heterodisulfide reductase complex, both encoded in the genome, were not expressed. Remarkably, a transporter sharing similarities to the high-affinity acetate transporters of acetotrophic methanogens was also found to be expressed, suggesting that *S. schinkii* can potentially compete with methanogens for acetate. *S. schinkii* appears to be a niche-adapted microorganism specialized in, and consequently reliant on, syntrophic acetate oxidation. Its large set of respiratory complexes might contribute to overcome limiting bioenergetic barriers, and drives efficient energy conservation from reactions operating close to the thermodynamic equilibrium, which might enable *S*. *schinkii* to occupy the same niche as the acetotrophic methanogens.

The genome of *T. acetatoxydans* lacks genes encoding formate dehydrogenase and the F_1F_0 ATP synthase. The WL pathway is organized into one operon but lacks formate dehydrogenase. The lack of an F_1F_0 ATP synthase may have implications for harvesting the ATP coming from substrate-level phosphorylation during formyl-THF synthase activity and using it for acetate activation (Müller et al. 2015) As the genome encodes for all genes to perform an oxidative TCA cycle, in analogy with *Desulfobacter postgatei,* this pathway is postulated for acetate oxidation in *T. acetatoxydans* rather than the WL pathway (Möller et al., 1987). An alternative pathway, which bypasses the carbonyl branch of the WL pathway by combining the glycine cleavage system with the methyl branch of the WL pathway, has been hypothesized for a terephthalate-degrading *Mesotoga* community, which is dominated by the thermophilic syntrophic acetate oxidizer, *Pseudothermotoga lettingae* (Nobu et al., 2015). As *P. lettingae* does not contain genes for acetyl-CoA synthase/carbon monoxide dehydrogenase, the WL pathway is not encoded with a complete set of genes (Hattori 2008) and such a glycine shunt may be operational in *P. lettingae*.

Other organisms capable of syntrophic acetate metabolism include *Geobacter sulfurreducens* (Cord-Ruwisch et al. 1998), the haloalkaline "*Candidatus* Contubernalis alkalaceticum" and 'Ca. Syntrophonatronum acetioxidans', (Zhilina et al. 2005, Sorokin et al. 2014). *G. sulfurreducens* metabolizes acetate through the citric acid cycle (Galushko and Schink 2000).

5.2 Propionate Metabolism

Two pathways for propionate metabolism are known, the methylmalonyl-CoA pathway and a dismutation pathway (Figure 2). The methylmalonyl-CoA pathway is found in many syntrophic propionate oxidizers including *Syntrophobacter* species (Boone and Bryant 1980; Wallrabenstein et al. 1995; Harmsen et al. 1998; Chen et al. 2005), *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* (Plugge et al. 2002), *Pelotomaculum thermopropionicum* (Imachi et al. 2002), and *Pelotomaculum schinkii* (de Bok et al. 2005). The dismutation pathway has been detected only in *Smithella propionica* (Liu et al. 1999; de Bok et al. 2001). *S. propionica* produces acetate and butyrate from propionate (de Bok et al. 2001). To explain the unusual labeling patterns observed in acetate and butyrate when different position-labeled propionate compounds were used, de Bok et al. (2001) concluded that two propionate molecules must condense to form a six-carbon intermediate, which is then rearranged to a 3-ketohexanoic acid intermediate before it is cleaved to form butyrate and acetate (Figure 2A). The enzymes involved in these reactions are not yet known.

The methylmalonyl-CoA pathway (Figure 2B), also called the randomizing pathway, involves the activation of propionate to propionyl-CoA by transfer of a CoA group from acetyl-CoA and the synthesis of methylmalonyl-CoA by transfer of a carboxyl group from oxaloacetate by a transcarboxylase (Houwen et al. 1990, Plugge et al., 2012). Methylmalonyl-CoA is then rearranged to form succinyl-CoA, which is oxidized via fumarate, oxaloacetate and pyruvate to acetate. The pathway predicts that one ATP is generated by substrate-level phosphorylation per propionate degraded and three electron pairs are released. Genomic and proteomic analyses show that the methylmalonyl-CoA pathway is operative in *Syntrophobacter fumaroxidans* and *P. thermopropionicum* (Plugge et al., 2012; Kosaka et al. 2006). The production of H_2 or formate from electrons derived from the oxidation of succinate is energetically unfavorable. Succinate reduced cytochrome *b* in membranes of *S. fumaroxidans,* and 2-(heptyl)-4-hydroxyquinoline-*N*-oxide inhibited succinate oxidation, suggesting the involvement of a reversed electron transport (Van Kuijk et al. 1998) (Figure 5C). Both *S. fumaroxidans* and *P. thermopropionicum* have a membrane-bound succinate dehydrogenase/fumarate reductase (Sdh1ABC) with Sdh1C as the heme-containing,

transmembrane protein that interacts with menaquinone (Kosaka et al. 2006; Kosaka et al. 2008; Plugge et al. 2012). The binding of menaquinone to Sdh1C close to the outside of the cell membrane allows an inward movement of protons when menaquinone is oxidized on the cytoplasmic side of the membrane by membrane-bound formate dehydrogenases or hydrogenases (Müller et al. 2010; Plugge et al. 2012; Worm et al. 2014).

Electron confurcation, coupling the oxidation of reduced ferredoxin and NADH to make either H_2 or formate, has been proposed to explain H_2 or formate production from NADH (Müller et al. 2010; Sieber et al. 2012; Worm et al. 2014). This electron confurcation would allow continued substrate metabolism when H_2 and formate levels increase to a point where their production from NADH becomes unfavorable without energy input. All the genes for the major subunits of the hydrogenases and FDHs in *Syntrophobacter fumaroxidans* were expressed during growth in coculture and in pure culture, irrespective of the substrate (Worm et al., 2011). Significantly higher expression of the confurcating hydrogenase, a periplasmic FDH, and the hydrogen-formate lyase was observed during syntrophic growth versus axenic growth (details in section 6).

Reducing equivalents generated in cytosolic reactions, such as the oxidation of malate to oxaloacetate and pyruvate to acetyl-CoA and CO_2, probably produce NAD(P)H and reduced ferredoxin, respectively. Several soluble cytosolic hydrogenases (Sfum_0844-46) and formate dehydrogenases (Sfum_2703-07) then probably catalyze H_2 or formate production with the above-reduced electron carriers via confurcation. The energetically favorable production of H_2 or formate with reduced ferredoxin can presumably provide the energetic input to enable the energetically unfavorable formation of H_2 from NADH. Malate oxidation to oxaloacetate ($E°' = -176$ mV) is coupled to NAD^+ reduction ($E°' = -320$ mV) (Van Kuijk and Stams, 1996).

Molar growth yields indicate that *S. fumaroxidans* synthesizes two-thirds of an ATP per fumarate when H_2 is the electron donor (Van Kuijk et al. 1998). This observation suggests that *S. fumaroxidans* consumes two-thirds of an ATP to drive H_2 production from succinate when grown syntrophically with propionate; this leaves about one-third of an ATP available to support growth. The free energy change needed for

irreversible ATP synthesis is estimated to be about -70 kJ mol^{-1} (Schink 1997). If 3 to 5 protons are used to make ATP by the ATP synthase, then the minimum free energy change needed to form ATP in increments is -23 to -14 kJ mol^{-1} (Schink 1997). This analysis predicts that syntrophic propionate metabolism should have a free energy change of about -20 kJ mol^{-1} to allow for the net synthesis of one-third of an ATP. Measured free energy changes during syntrophic propionate metabolism by *S. fumaroxidans* lower than -30 kJ mol^{-1} have been observed (Scholten and Conrad 2000), which is in agreement with the energetic model. However, under some growth conditions, the free energy available from syntrophic propionate metabolism was < -10 kJ mol^{-1}. Thus, we do not yet fully understand the bioenergetics of syntrophic propionate metabolism.

5.3 Butyrate Metabolism

Organisms capable of syntrophic butyrate metabolism include all species of *Syntrophomonas* (McInerney et al. 1981; Lorowitz et al. 1989; Zhang et al. 2004; Zhang et al. 2005; Sobieraj and Boone 2006; Wu et al. 2006b; Wu et al. 2006a; Sousa et al. 2007; Wu et al. 2007a; Wu et al. 2007b), *Syntrophus aciditrophicus* (Jackson et al, 1999), *Thermosyntropha lipolytica* (Svetlitshnyi et al. 1996), and *Syntrophothermus lipocalidus* (Sekiguchi et al. 2000). The most intensively studied model organism representing butyrate oxidizing bacteria is *Syntrophomonas wolfei*, which is specialized on syntrophic degradation of four to eight carbon fatty acids but can also grow axenically with several unsaturated fatty acids, especially crotonate (McInerney et al. 1981, Sieber et al. 2010). No other growth-supporting substrates are known. Syntrophic butyrate metabolism proceeds via the β-oxidation pathway (Figure 3) (Wofford et al. 1986). Similar to syntrophic propionate metabolism, butyrate is activated to butyryl-CoA by the transfer of the CoA group from acetyl-CoA; butyryl-CoA is then β-oxidized to two acetyl-CoA molecules (Wofford et al. 1986). One of the acetyl-CoA molecules is used to activate butyrate and the other one is used for ATP synthesis. The oxidation of butyryl-CoA to crotonyl-CoA produces reduced electron transfer flavoprotein ($E^{o'}$ of -10 mV) (Sato et al. 1999) (Figure 3) and the oxidation of L-3-hydroxylbutyryl-CoA to 3-oxobutyryl-CoA produces NADH. H_2 production (E' of -292 mV at 10 Pa H_2) from electrons derived from NADH ($E^{o'}$ of -320 mV) (Thauer et al. 1977) is favorable at the partial pressures maintained by methanogens (about 1-10 Pa) and occurs through an NADH-dependent hydrogenase

or NADH dependent formate dehydrogenase, depending on the cultivation conditions (Sieber et al 2010, Schmidt et al. 2013, Sieber et al, 2014) (Figure 3). However, H_2 production with electrons derived from the oxidation of butyryl-CoA to crotonyl-CoA requires reversed electron transport (Wallrabenstein and Schink 1994). Reversed electron transport is fueled by a transmembrane proton potential generated by ATPase. A membrane-bound iron-sulfur oxidoreductase redox-linked to a membrane-bound hydrogenase or formate dehydrogenase form a redox loop system via menaquinone (Crable et al 2016; Schmidt et al. 2013). First, butyryl-CoA is oxidized to crotonyl-CoA and two electrons are transferred to an electron-transferring flavoprotein (ETF), while two protons are being released into the cytoplasm. Reduced ETF is reoxidized at the membrane by an iron-sulfur oxidoreductase. Two protons from the periplasmic space drive the reduction of menaquinone to menaquinol with two electrons from butyryl-CoA oxidation located on the membrane-bound iron-sulfur oxidoreductase (Figure 3). Menaquinol can be oxidized by either a membrane-bound hydrogenase or formate dehydrogenase to release H_2 or formate, most likely depending on the cultivation conditions (Müller et al. 2009, Schmidt et al. 2013, Sieber et al. 2014, Sieber et al. 2015, Crable et al. 2016) or the available partner organism. Production of H_2 or formate consumes two protons from the exoplasmic space, respectively, which counterbalances the two protons released in the cytoplasm during oxidation of butyryl-CoA (Figure 3). During reoxidation of menaquinol, two protons are released into the cytoplasm, thus the net amount of protons that need to be transported across the membrane per mol of butyryl-CoA oxidized amounts to two protons. This corresponds to about two-thirds of an ATP equivalent, which is needed to overcome this energy barrier, therefore leaving about one-third of an ATP available to support growth. The measured free energy changes available during syntrophic butyrate metabolism ranged from -5 to -17 kJ mol^{-1} (Dwyer et al. 1988; Jackson and McInerney 2002), somewhat lower than that predicted to be needed for ATP synthesis (see section 3).

5.4 Benzoate Metabolism

Syntrophic benzoate degraders include three species of *Syntrophus: S. buswellii*, *S. gentianae* and *S. aciditrophicus*, as well as *Sporotomaculum syntrophicum*, *Pelotomaculum terephthalicicum*, *Pelotomaculum isophthalicicum and Syntrophorhabdus aromaticivorans* (McInerney et al. 2008, Nobu et al. 2014). The

reduction of benzoyl-CoA represents a considerable energy barrier for anaerobic microorganisms because the mid-point potential of the first electron transfer is about -1.8 V (Heider and Fuchs 1997a; Boll and Fuchs 1998; Boll et al. 2000), which is well below that of any physiological electron donors (-0.4 V) (Boll and Fuchs 1998). In *Thauera aromatica*, benzoyl-CoA reduction requires the hydrolysis of two ATP molecules per electron pair to overcome this barrier (Boll et al. 1997). This ATP dependent enzyme system is not found in the genomes of *S. aciditrophicus* (McInerney et al. 2007) or *S. aromaticivorans* (Nobu et al. 2014). Both appear to employ an ATP-independent, type II benzoyl-CoA reductase similar to the tungsten-dependent benzoyl-CoA reductase, BamBC, found in *Geobacter metallireducens* (Kung et al. 2009; Wischgoll et al. 2005).

Previous studies detected 2-hydroxycyclohexane carboxylate, cyclohex-1-ene carboxylate and pimelate in culture fluids of *S. aciditrophicus* grown with benzoate and the enzyme activities needed to convert cyclohex-1-ene carboxyl-CoA to pimelyl-CoA in cell-free extracts of *S. aciditrophicus* (Elshahed et al. 2001). The intermediates and enzyme activities detected were consistent with the metabolism of cyclohex-1-ene carboxyl-CoA to pimelyl-CoA by the pathway found in *Rhodopseudomonas palustris* (Harwood et al. 1998). However, genes homologous to those involved in benzoate metabolism in *R. palustris* were not detected in the *S. aciditrophicus* genome (McInerney et al. 2007). Interestingly, the genome of *S. aciditrophicus* contains genes with homology to those of the benzoyl-CoA degradation pathway found in *G. metallireducens* (Figure 4) (McInerney et al. 2007). The genes for the cyclohex-1,5-diene carboxyl-CoA hydratase and the 6-oxocyclohex-1-ene carboxyl-CoA hydrolase of *S. aciditrophicus* have been cloned and expressed in *Escherichia coli* (Peters et al. 2007; Kuntze et al. 2008). Enzymatic analysis showed that the S*. aciditrophicus* cyclohex-1,5-diene carboxyl-CoA hydratase converts cyclohex-1,5-diene carboxyl-CoA to 6-hydroxycyclohex-1-ene carboxyl-CoA, and that the *S. aciditrophicus* 6-oxocyclohex-1-ene carboxyl-CoA hydrolase makes 3-hydroxypimelyl-CoA from 6-oxocyclohex-1-ene carboxyl-CoA. 3-Fluorobenzoate-degrading cultures of *S. aciditrophicus* produced a metabolite with two double bonds, either 1-carboxyl-3-fluoro-2,6-cyclohexadiene or 1-carboxyl-3-fluoro-3,6-cyclohexadiene, consistent with an initial two-electron reduction of the benzoyl-CoA ring (Mouttaki et al. 2009). Thus, it appears that *S. aciditrophicus* uses a two-electron reduction reaction to convert benzoyl-CoA to cyclohex-1,5-diene

carboxyl-CoA and the benzoyl-CoA degradation pathway as found in *G. metallireducens* (Wischgoll et al. 2005) to degrade benzoyl-CoA to acetyl-CoA (Figure 4).

Cyclohexane carboxylate accumulates during syntrophic benzoate metabolism (Elshahed et al. 2001). Cyclohexane carboxylate and benzoate formation were observed when *S. aciditrophicus* was grown with crotonate (Mouttaki et al. 2007). Intermediates detected during crotonate metabolism were the same as those detected during syntrophic benzoate metabolism, which suggests that the pathway for benzoate metabolism is reversible. Cyclohexane carboxylate can be both utilized as a substrate and produced as a fermentation end-product of crotonate or benzoate metabolism by *S. aciditrophicus* (Kung et al. 2013, Kung et al. 2014, Mouttaki et al. 2008). Cyclohexane carboxyl-CoA is converted to cyclohex-1,5-diene carboxyl-CoA by two consecutive redox reactions catalyzed by two different acyl-CoA dehydrogenases (Kung et al. 2013) (Figure 4).

S. aciditrophicus utilizes a unique mechanism for substrate-level phosphorylation, an AMP-forming, acetyl-CoA synthetase (Acs), to form ATP from acetyl-CoA, AMP and pyrophosphate rather than by phosphotransacetylase and acetate kinase that is used by almost all bacteria (James et al. 2016). Pyrophosphate needed for the Acs reaction can be made by ligase reactions involved in substrate activation (Elshahed et al. 2001; Schöcke and Schink 1998) and by membrane-bound pyrophosphatases (Schöcke and Schink 1998). The decarboxylation of glutaconyl-CoA by a sodium-linked membrane-bound decarboxylase (Beatrix et al. 1990; Schöcke and Schink 1998) would provide chemiosmotic energy needed for pyrophosphate synthesis. The reduction of benzoyl-CoA reduction requires a low-potential electron donor such as reduced ferredoxin. One mechanism for the production of reduced ferredoxin in *S. aciditrophicus* is by the membrane-bound ion pump called Rnf, which uses the chemiosmotic gradient to drive the unfavorable reduction of ferredoxin with electrons derived from NADH oxidation (Figure 5) (McInerney et al. 2007). Further work is needed on the energetics of ion translocation, pyrophosphate synthesis, H_2 and formate production, and benzoyl-CoA reduction to understand how net ATP synthesis occurs during syntrophic benzoate metabolism. The measured free energy changes during syntrophic benzoate metabolism range from about -30 to -45 kJ of energy

(Warikoo et al. 1996; Schöcke and Schink 1997), which suggest that about one-third of an ATP or more could be formed per benzoate.

6 Mechanisms for Reverse Electron Transport

The oxidation of acyl-CoA intermediates (E^{o}' of -10 mV) derived from syntrophic fatty acid and aromatic compound degradation coupled to H_2 or formate production (E' of about -261 to -260 mV at 1 Pa H_2 and -258 at 1 μM formate) is unfavorable (ΔE' of about -250 mV) (Schink 1997). Energy input in the form of reversed electron transport is needed to make this reaction favorable (Sieber et al. 2012; Schink 1997). The current model to explain reversed electron transport during syntrophic butyrate degradation is a quinone loop (Schink and Friedrich 1994) (Figure 5A). Genomic analysis detected a gene for a membrane-bound iron-sulfur oxidoreductase with a DUF224 domain adjacent to genes for electron transfer flavoprotein (*etfAB*) (Sieber et al. 2012). During beta-oxidation, EtfAB transfers electrons from acyl-CoA dehydrogenases to a membrane-bound electron transfer flavoprotein:menaquinone oxidoreductase. The co-localization of *etfA* and *etfB* with the gene for the membrane-bound iron-sulfur oxidoreductase suggests that the membrane oxidoreductase may serve as an EtfAB:menaquinone oxidoreductase to receive electrons from acyl-CoA dehydrogenases via EtfAB and subsequently reduce menaquinone to menaquinol (Crable et al. 2016; Müller et al. 2010; Narihiro et al. 2016; Schmidt et al. 2013; Sieber et al 2010; Worm et al. 2014). Menaquinol can be reoxidized by either a membrane-bound hydrogenase (Crable et al. 2016; Sieber et al. 2014) or a membrane-bound formate dehydrogenase (Schmidt, et al. 2013) depending upon the growth condition. The inward movement of protons by the quinone loop along with the consumption of protons on the outside of the membrane during H_2 or formate production would supply the chemiosmotic energy needed for reversed electron transport (Crable et al. 2016; Schmidt, et al. 2013; Sieber et al 2010).

In support of this model, electron transfer flavoprotein (EftAB2) and the membrane-bound iron-sulfur oxidoreductase (SWOL_RS03525 gene product) with a DUF224 domain were highly abundant in the *S. wolfei* proteome, suggesting that these enzymes provide a conduit for electron flow between acyl-CoA dehydrogenases and membrane redox carriers (Crable et al., 2016; Schmidt, et al. 2013; Sieber et al. 2015). The SWOL_RS03525 gene product was detected in highly purified

preparations of butyryl-CoA dehydrogenase (Bcd) (Müller et al. 2009), consistent with a close interaction between the SWOL_RS03525 gene product and Bcd. Peptides of a membrane-bound formate dehydrogenase (Fdh2) (Schmidt, et al. 2013) and transcripts of genes for a membrane-bound hydrogenase (*hyd2A*) (Sieber et al. 2014) were high in syntrophically-grown *S. wolfei* cells.

The oxidation of succinate to fumarate (E°' of +33 mV) coupled to H_2 or formate production during syntrophic propionate degradation is also an unfavorable reaction that involves reversed electron transport (Müller et al. 2010; Sieber et al. 2012) (Figure 5B). *S. fumaroxidans* and *P. thermopropionicum* both have a membrane-bound succinate dehydrogenase/fumarate reductase (Sdh1ABC) with Sdh1C being the heme-containing, transmembrane protein that interacts with menaquinone (Kosaka et al. 2006; Kosaka et al. 2008; Plugge et al. 2012). The binding of menaquinone to Sdh1C close to the outside of the cell membrane would allow the inward movement of protons when menaquinone is oxidized on the cytoplasmic side of the membrane by membrane-bound formate dehydrogenases or hydrogenases (Müller et al. 2010; Plugge et al. 2012; Worm et al. 2014). The genes for Sdh1 were highly expressed in propionate-grown cells of *P. thermopropionicum* (Kato et al. 2009) and Sdh1 was more abundant when *P. thermopropionicum* was grown in coculture on propionate than after growth on butanol (Kosaka et al. 2006). The genes for a periplasmic formate dehydrogenase (*fdh2*) were upregulated during syntrophic propionate growth of *P. thermopropionicum* and of *S. fumaroxidans* (Kato et al. 2009; Worm et al. 2011), consistent with the importance of interspecies formate transfer during syntrophic propionate growth (de Bok et al. 2002a).

The unfavorable production of H_2 or formate from electrons derived from syntrophic lactate oxidation ($E^{o'}$ of -190 mV) is also driven by a quinone loop mechanism (Figure 5C) (Sieber et al. 2012). Transposon mutagenesis showed that a quinone-reducing complex (Qrc) and a periplasmic, tetraheme cytochrome c_3 were required for syntrophic growth of *Desulfovibrio desulfuricans* G20 on lactate (Li et al. 2011). A mutation in a periplasmic hydrogenase, *hydA*, impaired syntrophic growth of *D. desulfuricans* G20 on lactate.

Electron confurcation, which couples the oxidation of reduced ferredoxin and NADH to produce either H_2 or formate, has been proposed to explain H_2 or formate

production from NADH (Müller et al. 2010; Sieber et al. 2012; Worm et al. 2014) (Figure 5 D). However, it is not clear that electron confurcation is needed for H_2 and formate production during syntrophic metabolism. The redox potentials for H_2 and formate production during syntrophic metabolism (E' of about -260 mV at 1 Pa H_2 and -258 at 1 μM formate) are close to the physiological redox potential of NADH oxidation (E' of -280 mV) (Buckel and Thauer 2013). However, electron confurcation would allow continued substrate metabolism when H_2 and formate levels increase to a point where their production from NADH becomes unfavorable. The genomes of a number of syntrophic metabolizers have NADH-linked hydrogenases and formate dehydrogenases (Sieber et al., 2012; Narihiro et al. 2016; Worm et al. 2014) that have high homology to known confurcating hydrogenases (Schut and Adams 2009) and formate dehydrogenases (Wang et al. 2013). A gene for NADH-linked confurcating hydrogenase was highly expressed and the hydrogenase was abundant in the proteome when *S. wolfei* was grown syntrophically on butyrate (Sieber et al. 2014; Sieber et al., 2015). Another study (Schmidt et al. 2013) found both a NADH-linked formate dehydrogenase and a NADH-linked hydrogenase abundant in *S. wolfei*. Both *S. fumaroxidans* and *P. thermopropionicum* expressed genes for NADH-linked hydrogenases and formate dehydrogenases during syntrophic growth on propionate (Kato et al. 2009; Worm et al. 2011), and the genes for one NADH-linked formate dehydrogenase were upregulated in *P. thermopropionicum* during syntrophic growth on propionate (Kato et al. 2009).

Reduced ferredoxin needed to drive confurcation at high H_2 or formate concentrations can be made during syntrophic propionate and lactate metabolism by oxidizing pyruvate arising during the degradation of the growth substrate. It is not clear how other syntrophic fatty and aromatic acid degraders make reduced ferredoxin as the benzoyl-CoA degradation and beta-oxidation pathways form NADH and $EtfABH_2$ rather than reduced ferredoxin. One possibility is the use of ion pumps to produce reduced ferredoxin from electrons derived from NADH oxidation (Figure 5E). Rnf is found in the genomes of many syntrophic metabolizers (Sieber et al. 2012; Worm et al. 2014) and uses the inward movement of sodium ions or protons to produce reduced ferredoxin from electrons derived from NADH. Another ion pump, Ifo, is believed to function in a similar manner (Nobu et al. 2014). Fix is another membrane complex that catalyzes reversed electron transport and is believed to use the chemiosmotic gradient to drive the unfavorable reduction of menaquinone with

electrons derived from the oxidation of $EtfABH_2$ (Sieber et al. 2010; Sieber et al. 2012). Peptides of the Fix system were in low abundance in the *S. wolfei* proteome, suggesting a biosynthetic role for Fix rather than serving as the main conduit of electrons derived from acyl-CoA oxidation (Sieber et al. 2015). Reduced ferredoxin made by ion pumps or by pyruvate metabolism could be used to drive H_2 and formate production from NADH when H_2 and formate concentrations increase. In addition, reduced ferredoxin could be used to drive the unfavorable reduction of NAD^+ (E' of – 280 mV) with electrons derived from the oxidation of acyl-CoA intermediates (E_o' of -10 mV) or lactate (E^o' of -190 mV) by confurcating butyryl-CoA dehydrogenases (Li et al. 2008) or lactate dehydrogenases (Weghoff et al. 2015) (Figure 5F). However, experimental evidence suggests that the butyryl-CoA dehydrogenases in *S. wolfei* may not be confurcating (Müller et al. 2009). Whether other syntrophic metabolizers contain confurcating dehydrogenases remains to be shown.

7 Research Needs

The concept of a minimum free energy change for energy conservation provides the framework to understand how bacteria exploit small free energy changes. Pathways for syntrophic metabolism of fatty acids predict that ATP can be synthesized at increments of about one-third of an ATP, which is consistent with the measured free energy changes observed for the syntrophic metabolism of these compounds. However, there is still much that we do not understand about how microorganisms exploit small free energy changes. The value for the minimum energy quantum depends on the proton or sodium stoichiometry of the ATP synthase, the membrane potential, and the change in the free energy needed to make ATP. Our current understanding of the minimum energy quantum was developed with information from bacteria that use very exergonic catabolic reactions. Syntrophic metabolizers may have ATP synthases with different ion-to-ATP stoichiometries and maintain different membrane potentials and free energies of phosphorylation than other bacteria. Also, we need to understand the metabolome of syntrophic metabolizers to determine how the concentrations of reactants and products affect the equilibrium of key reactions involved in syntrophy. For example, the necessity of confurcation reactions in syntrophic metabolism could be determined by comparison of $NADH/NAD^+$ ratios relative to H_2 and formate levels. The ratios of enoyl-CoA to acyl-

CoA intermediates and 3-hydroxyacyl-CoA to 3-oxoacyl-CoA intermediates may influence the equilibrium of key redox reactions during syntrophic fatty and aromatic acid metabolism. ATP synthesis is dependent on the presence of energy-rich compounds such as acetyl phosphate. However, ATP could be synthesized using an acyl-CoA metabolite, AMP, and pyrophosphate, depending on the internal concentrations of the reactants and products. We know very little about the enzyme systems involved in electron flow during direct electron transfer, which makes it difficult to determine from metatranscriptomic or metaproteomic data whether electrons are transferred directly or via interspecies H_2 or formate transfer.

Single-cell microbiology is an attractive approach as even isogenic populations of microorganisms have substantial cell-to-cell heterogeneity at cellular and gene levels. Until recently we have not been able to identify microbes and note their mostly invisible activities, such as nutrient consumption, at the level of the single cell, not even in the laboratory. This is currently changing with the rapid increase of new technologies for single-cell microbiology (Musat et al., 2012; Wessel et al., 2013), that enable to observe "who does what, where, when, and together with whom". Single cells taken from the environment can be identified and their genomes sequenced. Individual microbes can be observed *in situ* with a range of innovative microscopic and spectroscopic methods, enabling localization, identification, or functional characterization of cells in an environmental sample, combined with the detection of the uptake of labeled compounds. They can be placed into fabricated microfluidic environments, to study their interactions. These novel methods hold potential for testing under well-controlled conditions.

While the physiology of the syntrophic communities has been studied for several decades (Stams and Plugge, 2009), relatively little is known about the genes and their expression dynamics associated with the syntrophic interactions, partially due to the lack of suitable methodologies for measurements of biological properties within mixed-culture systems. Applying single cell methods enables the detailed study of mechanisms underlying syntrophy. These mechanisms include choice of hydrogen and formate as interspecies electron compounds, localization of hydrogenases and formate dehydrogenases.

Syntrophic partners regulate their metabolism to grow together at the limits of what is thermodynamically feasible (Stams and Plugge, 2009). The methanogens are favored if H_2 and formate concentrations are high, while the syntroph requires low H_2 and formate concentrations. Metabolic flexibility to cope with these fluctuations in H_2 and formate levels is essential and does occur. This suggests that syntrophic populations have numerous possibilities for interspecies electron transfer. In triplicate cocultures the ribosomal activity of the methanogen varied up to 10 times reflects this flexibility, though the trigger for it is not yet clear (Worm et al., 2011). To assess the level of metabolic flexibility of individual celss in a community, the specific environmental adaptation of syntrophic communities should be investigated further. To assess the level of metabolic flexibility of each individual in a syntrophic coculture, Qi et al. (2014) demonstrated gene expression heterogeneity. A dual-culture of *Desulfovibrio vulgaris* with *Methanosarcina barkeri* demonstrated very significant cell-to-cell gene-expression heterogeneity for selected *D. vulgaris* genes in both the monoculture and the syntrophic coculture.

Many syntrophic associations are highly organized, multicellular structures with the partners in close physical proximity to each other. We know very little about the molecular mechanisms involved in the formation and maintenance of these catalytic units. Regulatory mechanisms that control the development of attached consortia most likely are similar to those involved in biofilm formation. Transcriptomic analyses of each syntrophic partner would identify gene systems that respond to the syntrophic lifestyle and may provide clues as to the chemical signals that each organism uses to communicate with its partners. In addition, we do not understand the extent to which syntrophic metabolizers regulate their metabolisms in response to environmental stimuli such as H_2 concentration versus the global cellular status such as energy charge. The combination of metagenomics and metatranscriptomic analyses will allow us to interrogate the regulatory mechanisms involved in establishing and maintaining multispecies associations in order to quantify and predict the behavior of microorganisms and microbial communities in natural ecosystems. A thorough understanding of the formation and structure of dense microbial aggregates is essential for application of methanogenesis.

Many syntrophic associations still need to be discovered. Besides syntrophic oxidations of the compounds discussed here, syntrophic interactions may also play

an important role in the degradation of compounds that are considered to be easily fermentable, e.g, sugars as shown by Krumholz and Bryant (1986) and Müller et al. (2008). Besides freshwater environments, sulfate-depleted marine sediments are also important methanogenic environments (Colwell et al. 2008). Syntrophic interactions in these marine methanogenic environments have not been studied thoroughly (Kendall et al. 2006). Meta –omics studies have discovered a large number of new phyla but only speculate about their metabolism. To identify still unknown syntrophic interactions, a holistic approach integrating physiology, ecology and genomics can create a step stone in understanding microorganisms, microbial communities and their potential application.

Acknowledgements

The work on syntrophic benzoate metabolism was supported by contract DE-FG02-96ER20214 from Physical Biosciences Division, Office of Science, U. S. Department of Energy and the work on reverse electron transfer was supported by National Science Foundation grant 1515843 to M.J.M. B. S. and N. M. are indebted to the German Research Foundation (DFG) for funding work on syntrophic butyrate oxidation and interspecies electron transfer.

8 References

Balk M, Weijma J, Stams AJM (2002) *Thermotoga lettingae* sp. nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor. Int J Syst Evol Microbiol 52:1361-1368.

Beatrix B, Bendrat K, Rospert S, Buckel W (1990) The biotin-dependent sodium ion pump glutaconyl-CoA decarboxylase from *Fusobacterium nucleatum* (subsp. nucleatum). Comparison with the glutaconyl-CoA decarboxylases from gram-positive bacteria. Arch Microbiol 154:362-369.

Boll M, Albracht SS, Fuchs G (1997) Benzoyl-CoA reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. A study of adenosine triphosphatase activity, ATP stoichiometry of the reaction and EPR properties of the enzyme. Eur J Biochem 244:840-851.

Boll M, Fuchs G (1998) Identification and characterization of the natural electron donor ferredoxin and of FAD as a possible prosthetic group of benzoyl-CoA reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. Eur J Biochem 251: 946-954

Boll M, Fuchs G, Tilley G, Armstrong FA, Lowe DJ (2000) Unusual spectroscopic and electrochemical properties of the 2[4Fe-4S] ferredoxin of *Thauera aromatica*. Biochem 39:4929-4938.

Boone DR, Bryant MP (1980) Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. Appl Environ Microbiol 40: 626-632

Boone DR, Johnson RL, Liu Y (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of K_m for H_2 or formate uptake. Appl Environ Microbiol 55:1735-1741.

Breese K, Fuchs G (1998) 4-Hydroxybenzoyl-CoA reductase (dehydroxylating) from the denitrifying bacterium *Thauera aromatica*--prosthetic groups, electron donor, and genes of a member of the molybdenum-flavin-iron-sulfur proteins. Eur J Biochem 251:916-923.

Bryant MP, Wolin EA, Wolin MJ, Wolfe RS (1967) *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch Microbiol 59:20-31.

Buckel W, Thauer RK (2013) Energy conservation via electron bifurcating ferredoxin reduction and proton/Na^+ translocating ferredoxin oxidation. Biochim Biophys Acta 1827:94-113.

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.

Chen S, Rotaru A-E, Shrestha PM (2014) Promoting interspecies electron transfer with biochar. Sci Rep 4:5019.

Colwell FS, Boyd S, Delwiche ME, Reed DW, Phelps TJ, Newby DT (2008) Estimates of biogenic methane production rates in deep marine sediments at Hydrate Ridge, Cascadia margin. Appl Environ Microbiol 74:3444-3452.

Conrad R, Phelps TJ, Zeikus JG (1985) Gas metabolism evidence in support of the juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. Appl Environ Microbiol 50:595-601.

Cord-Ruwisch R, Lovley DR, Schink B (1998) Growth of *Geobacter sulfurreducens* with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. Appl Environ Microbiol 64:2232-2236.

Crable BR, Sieber JR, Mao X, Alvarez-Cohen L, Gunsalus RP, Ogorzalek Loo RR, Nguyen H, McInerney MJ (2016) Membrane complexes of *Syntrophomonas wolfei* involved in syntrophic butyrate degradation and hydrogen formation. Front Microbiol 7:1795.doi 10.3389/fmicb.2016.01795.

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 (2002a) 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, Roze EH, Stams AJM (2002b) Hydrogenases and formate dehydrogenases of *Syntrophobacter fumaroxidans*. Antonie van Leeuwenhoek 81:283-291.

de Bok FAM, Hagedoorn PL, Silva PJ, Hagen WR, Schiltz E, Fritsche K, Stams AJM (2003) Two W-containing formate dehydrogenases (CO_2-reductases) involved in syntrophic propionate oxidation by *Syntrophobacter fumaroxidans*. Eur J Biochem 270:2476-2485.

de Bok FAM, Harmsen HJM, 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., co-cultured with *Methanospirillum hungatei*, and emended description of the genus *Pelotomaculum*. Int J Syst Evol Microbiol 55:1697-1703.

Dong X, Cheng G, Stams AJM (1994a) Butyrate oxidation by *Syntrophospora bryantii* in co-culture with different methanogens and in pure culture with pentenoate as electron acceptor. Appl Microbiol Biotechnol 42:647-652.

Dong X, Plugge CM, Stams AJM (1994b) Anaerobic degradation of propionate by a mesophilic acetogenic bacterium in coculture and triculture with different methanogens. Appl Environ Microbiol 60:2834-2838.

Dong X, Stams AJM (1995) Evidence for H_2 and formate formation during syntrophic butyrate and propionate degradation. Anaerobe 1:35-39.

Dwyer DF, Weeg-Aerssens E, Shelton DR, Tiedje JM (1988) Bioenergetic conditions of butyrate metabolism by a syntrophic, anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. Appl Environ Microbiol 54:1354-1359.

Ehhalt D, Prather M, Dentener F, Derwent R, Dlugokencky E, Holland E, Isaksen I, Katima J, Kirchhoff V, Matson P (2001) Atmospheric chemistry and greenhouse gases. Climate Change 2001: The Scientific Basis: 239-287.

Elshahed MS, Bhupathiraju VK, Wofford NQ, Nanny MA, McInerney MJ (2001) Metabolism of benzoate, cyclohex-1-ene carboxylate, and cyclohexane carboxylate by *Syntrophus aciditrophicus* strain SB in syntrophic association with H_2-using microorganisms. Appl Environ Microbiol 67:1728-1738.

Ferry JG, Wolfe RS (1976) Anaerobic degradation of benzoate to methane by a microbial consortium. Arch Microbiol 107:33-40.

Friedrich M, Schink B (1993) Hydrogen formation from glycolate driven by reversed electron transport in membrane vesicles of a syntrophic glycolate-oxidizing bacterium. Eur J Biochem 217:233-240.

Friedrich M, Schink B (1995) Electron transport phosphorylation driven by glyoxylate respiration with hydrogen as electron donor in membrane vesicles of a glyoxylate-fermenting bacterium. Arch Microbiol 163:268-275.

Friedrich M, Springer N, Ludwig W, Schink B (1996) Phylogenetic positions of *Desulfofustis glycolicus* gen. nov., sp. nov., and *Syntrophobotulus glycolicus* gen. nov., sp. nov., two new strict anaerobes growing with glycolic acid. Int J Syst Bacteriol 46:1065-1069.

Gallert C, Winter J (1994) Anaerobic degradation of 4-hydroxybenzoate: Reductive dehydroxylation of 4-hydroxybenzoyl-CoA and ATP formation during 4-hydroxybenzoate decarboxylation by the phenol-metabolizing bacteria of a stable, strictly anaerobic consortium. Appl Microbiol Biotechnol 42:408-414.

Galushko AS, Schink B (2000) Oxidation of acetate through reactions of the citric acid cycle by *Geobacter sulfurreducens* in pure culture and in syntrophic coculture. Arch Microbiol 174:314-321.

Gibson J, Dispensa M, Fogg GC, Evans DT, Harwood CS (1994) 4-Hydroxybenzoate-coenzyme A ligase from *Rhodopseudomonas palustris*: purification, gene sequence, and role in anaerobic degradation. J Bacteriol 176: 634-641

Gibson J, Dispensa M, Harwood CS (1997) 4-hydroxybenzoyl coenzyme A reductase (dehydroxylating) is required for anaerobic degradation of 4-hydroxybenzoate by *Rhodopseudomonas palustri*s and shares features with molybdenum-containing hydroxylases. J Bacteriol 179:634-642.

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS (2006) Electrically conductive bacterial nanowires produced by

Shewanella oneidensis strain MR-1 and other microorganisms. Proc Natl Acad Sci USA 103:11358-11363 .

Harmsen HJM, Van Kuijk BLM, Plugge CM, Akkermans ADL, De Vos WM, Stams AJM (1998) *Syntrophobacter fumaroxidans* sp. nov., a syntrophic propionate-degrading sulfate-reducing bacterium. Int J Syst Bacteriol 48:1383-1387.

Harwood CS, Burchhardt G, Herrmann H, Fuchs G (1998) Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. FEMS Microbiol Rev 22:439-458.

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, Luo H, Shoun H, Kamagata Y (2001) Involvement of formate as an interspecies electron carrier in a syntrophic acetate-oxidizing anaerobic microorganism in coculture with methanogens. J Biosci Bioeng 91:294-298.

Hattori S (2008) Syntrophic acetate-oxidizing microbes in methanogenic environments. Microbes and Environments 23:118-127.

Hedderich R, Whitman WB (2006) Physiology and biochemistry of the methane-producing Archaea, In Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds), The Prokaryotes: an evolving electronic resource for the microbiological community, Ed 3rd, Vol 2. Springer-Verlag, New York pp 1050–1079

Heider J, Fuchs G (1997a) Anaerobic metabolism of aromatic compounds. Eur J Biochem 243:577-596.

Heider J, Fuchs G (1997b) Microbial anaerobic aromatic metabolism. Anaerobe 3:1-22.

Hirsch W, Schagger H, Fuchs G (1998) Phenylglyoxylate:NAD^+ oxidoreductase (CoA benzoylating), a new enzyme of anaerobic phenylalanine metabolism in the denitrifying bacterium *Azoarcus evansii*. Eur J Biochem 251:907-915.

Hoehler T (2004) Biological energy requirements as quantitative boundary conditions for life in the subsurface. Geobiology 2:205-215.

Houwen FP, Plokker J, Stams AJM, Zehnder AJB (1990) Enzymatic evidence for involvement of the methylmalonyl-CoA pathway in propionate oxidation by *Syntrophobacter wolinii*. Arch Microbiol 155:52-55.

Imachi H, Sekiguchi Y, Kamagata Y, Hanada S, Ohashi A, Harada H (2002) *Pelotomaculum thermopropionicum* gen. nov., sp. nov., an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. Int J Syst Evol Microbiol 52:1729-1735.

IPCC Climate change 2014: mitigation of climate change (2014) Contribution of working group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Ishii S, Kosaka T, Hori K, Hotta Y, Watanabe K (2005) Coaggregation facilitates interspecies hydrogen transfer between *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus*. Appl Environ Microbiol 71:7838-7845.

Jackson, BE, Bhupathiraju, VK, Tanner, RS, Woese, CR, and 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.

Jackson BE, McInerney MJ (2002) Anaerobic microbial metabolism can proceed close to thermodynamic limits. Nature 415:454-456.

James KL, Ríos-Hernández LA, Wofford NQ, Mouttaki H, Sieber JR, Sheik CS, Nguyen HH, Yang Y, Xie Y, Erde J, Rohlin L, Karr EA, Loo JA, Ogorzalek Loo RR, Hurst GB, Gunsalus RP, Szweda LI, McInerney MJ (2016) Pyrophosphate-dependent ATP formation from acetyl coenzyme A in *Syntrophus aciditrophicus,* a new twist on ATP formation. MBio 7:e01208-16.

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.

Kaiser JP, Hanselmann KW (1982) Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. Arch Microbiol 133:185-194.

Kato S, Kosaka T, Watanabe K (2009) Substrate-dependent transcriptomic shifts in *Pelotomaculum thermopropionicum* grown in syntrophic co-culture with *Methanothermobacter thermautotrophicus*. Microbial Biotechnol 2:575–584.

Kato S, Hashimoto K, Watanabe K (2012a). Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals. Environ Microbiol 14:1646–1654 .

Kato S, Hashimoto K, Watanabe K (2012b). Microbial interspecies electron transfer via electric currents through conductive minerals. Proc Natl Acad Sci 109:10042–10046.

Kendall MM, Liu Y and Boone DR (2006) Butyrate- and propionate-degrading syntrophs from permanently cold marine sediments in Skan Bay, Alaska, and description of *Algorimarina butyrica* gen. nov., sp. nov. FEMS Microbiol Lett 262:107-114.

Kosaka T, Uchiyama T, Ishii S, Enoki M, Imachi H, Kamagata Y, Ohashi A, Harada H, Ikenaga H, Watanabe K (2006) Reconstruction and regulation of the central catabolic pathway in the thermophilic propionate-oxidizing syntroph *Pelotomaculum thermopropionicum*. J Bacteriol 188:202-210.

Kosaka T, Kato S, Shimoyama T, Ishii S, Abe T, Watanabe K (2008) The genome of *Pelotomaculum thermopropionicum* reveals niche-associated evolution in anaerobic microbiota. Genome Research 18: 442-448.

Kouzuma A, Kato S, Watanabe K (2015) Microbial interspecies interactions: recent findings in syntrophic consortia. Front Microbiol 6:477.

Krumholz LR, Bryant MP (1986) *Syntrophococcus sucromutans* sp. nov. gen. nov. uses carbohydrates as electron donors and formate, methoxymonobenzenoids or *Methanobrevibacter* as electron acceptor systems. Arch Microbiol 143:313-318.

Kung JW, Löffler C, Dörner K, Heintz D, Gallien S, Dorsselaer AV, Friedrich A, Boll M (2009) Identification and characterization of the tungsten-containing class of benzoyl-coenzyme A reductases. Proc Natl Acad Sci USA 106:17687-17692.

Kung JW, Seifert J, von Bergen M, Boll M (2013) Cyclohexanecarboxyl-coenzyme A (CoA) and cyclohex-1-ene-1-carboxyl-CoA dehydrogenases, two enzymes involved in the fermentation of benzoate and crotonate in *Syntrophus aciditrophicus.* J Bacteriol 195:3193-200.

Kung JW, Meier AK, Mergelsberg M, Boll M (2014) Enzymes involved in a novel anaerobic cyclohexane carboxylic acid degradation pathway. J Bacteriol 196:3667-3674.

Kuntze K, Shinoda Y, Moutakki H, McInerney MJ, Vogt C, Richnow HH, Boll M (2008) 6-Oxocyclohex-1-ene-1-carbonyl-coenzyme A hydrolases from obligately anaerobic bacteria: characterization and identification of its gene as a functional marker for aromatic compounds degrading anaerobes. Environ Microbiol 10:1547-1556.

Lee MJ, Zinder SH (1988a) Carbon monoxide pathway enzyme activities in a thermophilic anaerobic bacterium grown acetogenically and in a syntrophic acetate-oxidizing coculture. Arch Microbiol 150:513-518.

Lee MJ, Zinder SH (1988b) Isolation and characterization of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H_2-CO_2. Appl Environ Microbiol 54:124-129.

Lever MA, Rogers KL, Lloyd KG, Overmann J, Schink B, Thauer RK, Hoehler TM, Jorgensen BB (2015) Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. FEMS Microbiol Rev 39:688-728.

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

Li H, Chang J, Liu P, Fu L, Ding D, Lu Y (2015) Direct interspecies electron transfer accelerates syntrophic oxidation of butyrate in paddy soil enrichments. Environ Microbiol 17:1533-1547.

Li X, McInerney MJ, Stahl DA, Krumholz LR. (2011) Metabolism of H_2 by *Desulfovibrio alaskensis* G20 during syntrophic growth on lactate. Microbiol 151:2912-2921.

Liu FH, Rotaru A-E, Shrestha PM, Malvankar N S, Nevin KP, Lovley DR (2012) Promoting direct interspecies electron transfer with activated carbon. Energ Environ Sci 5:8982-8989.

Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR (1999) Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolinii*. Int J Syst Bacteriol 49:545-556.

Liu Y, Whitman WB (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea, In Wiegel J, Maier RJ, Adams MW (eds), Incredible Anaerobes From Physiology to Genomics to Fuels Ed 1, Vol 1125. Ann N Y Acad Sci pp 171–189.

Logan BE, Regan JM (2006) Electricity-producing bacterial communities in microbial fuel cells. Trends in Microbiology 14:512-518.

Lorowitz WH, Zhao H, Bryant MP (1989) *Syntrophomonas wolfei* subsp. *saponavida* subsp. nov., a long-chain fatty-acid-degrading, anaerobic, syntrophic bacterium. Int J Syst Bacteriol 39:122–126.

Lovley DR, Fraga JL, Blunt-Harris EL, Hayes L A, Phillips EJP, Coates JD (1998) Humic substances as a mediator for microbially catalyzed metal reduction. Acta Hydrochim Hydrobiol 26(3):152-157.

Manzoor S, Bongcam-Rudloff E, Schnurer A, Muller B (2016) Genome-guided analysis and whole transcriptome profiling of the mesophilic syntrophic acetate oxidising bacterium *Syntrophaceticus schinkii*. PLoS ONE 11:e0166520.

McCarty PL (1971) Energetics and kinetics of anaerobic treatment. Anaerobic biological treatment processes: 91-107

McInerney MJ, Bryant MP, Hespell RB, Costerton JW (1981) *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. Appl Environ Microbiol 41:1029-1039

McInerney MJ, Rohlin L, Mouttaki H, Kim U, Krupp RS, Rios-Hernandez L, Sieber J, Struchtemeyer CG, Bhattacharyya A, Campbell JW, Gunsalus RP (2007) The genome of *Syntrophus aciditrophicus*: life at the thermodynamic limit of microbial growth. Proc Natl Acad Sci USA 104:7600-7605.

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 RJ, Adams MW (eds), Incredible Anaerobes From Physiology to Genomics to Fuels Ed 1, Vol 1125. Ann N Y Acad Sci pp 58-72

Merkel SM, Eberhard AE, Gibson J, Harwood CS (1989) Involvement of coenzyme A thioesters in anaerobic metabolism of 4-hydroxybenzoate by *Rhodopseudomonas palustris*. J Bacteriol 171:1-7.

Möller D, Schauder R, Fuchs G, Thauer RK (1987) Acetate oxidation to CO_2 via a citric acid cycle involving an ATP-citrate lyase: a mechanism for the synthesis of ATP via substrate level phosphorylation in *Desulfobacter postgatei* growing on acetate and sulfate. Arch Microbiol 148:202–207.

Mountfort DO, Bryant MP (1982) Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch Microbiol 133:249-256.

Mouttaki H, Nanny MA, McInerney MJ (2007) Cyclohexane carboxylate and benzoate formation from crotonate in *Syntrophus aciditrophicus*. Appl Environ Microbiol 73:930-938.

Müller B, Manzoor S, Niazi A, Bongcam-Rudloff E, Schnürer A (2015) Genome-guided analysis of physiological capacities of *Tepidanaerobacter acetatoxydans* provides insights into environmental adaptations and syntrophic acetate oxidation. PLoS ONE 10: e0121237.

Müller N, Griffin BM, Stingl U, 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 coenzyme A dehydrogenase in reversed electron transport during syntrophic butyrate oxidation by *Syntrophomonas wolfei*. J Bacteriol 191:6167-6177.

Müller N, Worm P, Schink B, Stams AJM, Plugge CM (2010) Syntrophic butyrate and propionate oxidation processes: from genomes to reaction mechanisms. Environ Microbiol Rep 2:489–499.

Musat N, R Foster, T Vagner, B Adam, MMM Kuypers (2012). Detecting metabolic activities in single cells, with emphasis on nanoSIMS. FEMS Microbiol Rev 36:486–511.

Narihiro T, Nobu MK, Tamaki H, Kamagata Y, Sekiguchi Y, Liu W-T (2016) Comparative genomics of syntrophic branched-chain fatty acid degrading bacteria. Microbes Environ 31:288-292.

Ng F, Kittelmann S, Patchett ML, Attwood GT, Janssen PH, Rakonjac J, Gagic D (2016) An adhesin from hydrogen-utilizing rumen methanogen *Methanobrevibacter ruminantium* M1 binds a broad range of hydrogen-producing microorganisms. Environ Microbiol 18(9): 3010–3021.

Nobu MK, Narihiro T, Tamaki H, Qiu Y-L, Sekiguchi Y, Woyke T, Goodwin L, Davenport KW, Kamagata Y, Liu W-T (2014) The genome of *Syntrophorhabdus aromaticivorans* strain UI provides new insights for syntrophic aromatic compound metabolism and electron flow. Environ Microbiol 17:4861-4872.

Nobu MK, Narihiro T, Rinke C, Kamagata Y, Tringe SG, Woyke T, Liu W-T (2015) Microbial dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor. ISME J 9:1710 - 1722.

Oehler D, Poehlein A, Leimbach A, Muller N, Daniel R, Gottschalk G, Schink B (2012) Genome-guided analysis of physiological and morphological traits of the fermentative acetate oxidizer *Thermacetogenium phaeum*. BMC Genomics 13:723.

Pavlostathis SG, Giraldo-Gomez E (1991) Kinetics of anaerobic treatment: A critical review. Crit Rev Environ Control 21:411-490.

Peters F, Shinoda Y, McInerney MJ, Boll M (2007) Cyclohexa-1,5-diene-1-carbonyl-coenzyme A (CoA) hydratases of *Geobacter metallireducens* and *Syntrophus aciditrophicus*: Evidence for a common benzoyl-CoA degradation pathway in facultative and strict anaerobes. J Bacteriol 189:1055-1060.

Plugge CM, Balk M, Stams AJM (2002) *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* subsp. nov., a thermophilic, syntrophic, propionate-oxidizing, spore-forming bacterium. Int J Syst Evol Microbiol 52:391-399.

Plugge CM, Henstra AM, Worm P, Paulitsch AH, Scholten JCM, Lykidis A, Lapidus AL, Goltsman, Kim E, McDonald E, Rohlin L, Crable BR, Gunsalus RP, Stams AJM, McInerney MJ (2012) Complete genome sequence of *Syntrophobacter fumaroxidans* strain (MPOB^T) Stand Genomic Sci 7**:**91-106

Qi Z, Pei G, Chen L, Zhang W (2014) Single-cell analysis reveals gene-expression heterogeneity in syntrophic dual-culture of *Desulfovibrio vulgaris* with *Methanosarcina barkeri*. Scientific Rep 4:7478.

Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. Nature 435:1098-1101.

Sato K, Nishina Y, Setoyama C, Miura R, Shiga K (1999) Unusually high standard redox potential of acrylyl-CoA/propionyl-CoA couple among enoyl-CoA/acyl-CoA couples: a reason for the distinct metabolic pathway of propionyl-CoA from longer acyl-CoAs. J Biochem 126:668-675.

Schink B, Friedrich M (1994) Energetics of syntrophic fatty acid oxidation. FEMS Microbiol Rev 15:85-94.

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262-280.

Schink B, Philipp B, Müller J (2000) Anaerobic degradation of phenolic compounds. Naturwissenschaften 87:12-23.

Schink B, Stams AJM (2013).Syntrophism among prokaryotes. In: Rosenberg E, Delong E, Lory S, Stackebrandt E, Thompson F (Eds) The Prokaryotes, NewYork: Springer Berlin Heidelberg , pp 471–493.

Schink B, Montag D, Keller A, Müller N (2017) Hydrogen or formate – alternative key players in methanogenic degradation. Environ Microbiol Rep DOI: 10.1111/1758-2229.12524

Schmidt A, Müller N, Schink B, Schleheck D (2013) A proteomic view at the biochemistry of syntrophic butyrate oxidation in *Syntrophomonas wolfei.* PloS ONE 8, e56905.

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 (1998) Membrane-bound proton-translocating pyrophosphatase of *Syntrophus gentianae*, a syntrophically benzoate-degrading fermenting bacterium. Eur J Biochem 256:589-594.

Scholten JCM, Conrad R (2000) Energetics of syntrophic propionate oxidation in defined batch and chemostat cocultures. Appl Environ Microbiol 66:2934-2942.

Schut G, Adams M (2009) The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. J Bacteriol 191:4451-4417.

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.

Shrestha PM, Rotaru A-E, Aklujkar M, Liu F, Shrestha M, Summers ZM, Malvankar N, Flores D C, Lovley DR (2013) Syntrophic growth with direct interspecies electron transfer as the primary mechanism for energy exchange. Environ Microbiol Rep 5(6):904-910.

Shresta PM, Rotaru A-E (2014) Plugging in or going wireless: strategies for interspecies electron transfer. Front Microbiol Doi: 103389/fmicb.2014.00237.

Sieber JR, Gunsalus RP, Rohlin L, McInerney MJ, Sims DR, Han C, Kim E, Lykidis A, Lapidus AL (2008) Genomic insights into syntrophic fatty acid metabolism: electron transfer processes of *Syntrophomonas wolfei*. American Society of Microbiology 108th General Meeting. Boston, MA, Abst. I-002 p. 071

Sieber JR, Sims DR, Han C, Kim E, Lykidis A, Lapidus AL, McDonnald E, Rohlin L, Culley DE, Gunsalus RP, McInerney MJ (2010) The genome of *Syntrophomonas wolfei*: new insights into syntrophic metabolism and biohydrogen production. Environ Microbiol 12:2289-2301.

Sieber JR, McInerney MJ, Gunsalus RP (2012) Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. Ann Rev Microbiol 66:429–452.

Sieber JR, Le H, McInerney MJ (2014) The importance of hydrogen and formate transfer for syntrophic fatty, aromatic and alicyclic metabolism. Environ Microbiol 16:177-188.

Sieber JR, Crable BR, Sheik CS, Hurst GB, Rohlin L, Gunsalus RP, McInerney MJ (2015) Proteomic analysis reveals metabolic and regulatory systems involved the syntrophic and axenic lifestyle of *Syntrophomonas wolfei*. Front Microbiol 6:115.

Sobieraj M, Boone DR (2006) *Syntrophomonadaceae*, In Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds), The Prokaryotes: an evolving electronic resource for the microbiological community, Ed 3, Vol 4. Springer-Verlag, New York pp 1041-1046.

Sorokin DY, Abbas B, Tourova TP, Bumazhkin BK, Kolganova TV, Muyzer G (2014) Sulfate-dependent acetate oxidation under extremely natron-alkaline conditions by syntrophic associations from hypersaline soda lakes. Microbiol 160:723-732.

Sousa DZ, Smidt H, Alves MM, Stams AJM (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.

Spahn S, Brandt K, and Müller V (2015) A low phosphorylation potential in the acetogen *Acetobacterium woodii* reflects its lifestyle at the thermodynamic edge of life. Arch Microbiol 197:745–751.

Stams AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nature Microbiol Rev 7:568-577.

Straub KL, Benz M, Schink B (2001) Iron metabolism in anoxic environments at near neutral pH. FEMS Microbiol Ecol 34(3):181-186.

Svetlitshnyi 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

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

Thiele JH, Zeikus JG (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl Environ Microbiol 54:20-29.

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

Viggi CC, Rosetti S, Fazi S, Paiano P, Majone M, Aulenta F (2014) Magnetite particles triggering a faster and more robust syntrophic pathway of methanogenic propionate degradation. Environ Sci Technol 48(13):7536-7543.

Wallrabenstein C, Schink B (1994) Evidence of reversed electron transport in syntrophic butyrate or 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

Warikoo V, McInerney MJ, Robinson JA, Suflita JM (1996) Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. Appl Environ Microbiol 62:26-32.

Wang S, Huang H, Kahnt J, Thauer RK (2013) *Clostridium acidurici* electron-bifurcating formate dehydrogenase. Appl Environ Microbiol 79:6176-6179.

Weghoff MC, Bertsch J, Müller V (2015) A novel mode of lactate metabolism in strictly anaerobic bacteria. Environ Microbiol 17:670-677.

Wessel AK, L Hmelo, MR Parsek, M Whiteley (2013) Going local: Technologies for exploring bacterial microenvironments. Nat Rev Microbiol 11(5):337–348.

Westerholm M, Roos S, Schnurer A (2010) *Syntrophaceticus schinkii* gen. nov., sp nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter. FEMS Microbiol Lett 309:100–104.

Westerholm M, Roos S, Schnurer A (2011) *Tepidanaerobacter acetatoxydans* sp. nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from two ammonium-enriched mesophilic methanogenic processes. Syst Appl Microbiol 34:260–266.

Wischgoll S, Heintz D, Peters F, Erxleben A, Sarnighausen E, Reski R, Van Dorsselaer A, Boll M (2005) Gene clusters involved in anaerobic benzoate degradation of *Geobacter metallireducens*. Mol Microbiol 58:1238-1252.

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.

Worm P, Stams AJM, Cheng X, Plugge CM (2011) Growth- and substrate-dependent transcription of formate dehydrogenase and hydrogenase coding genes in *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. Microbiol 157:280-289.

Worm P, Koehorst JJ, Visser M, Sedano-Núñez VT, Schaap PJ, Plugge CM, Sousa DZ, Stams AJM (2014) A genomic view on syntrophic versus non-syntrophic lifestyle in anaerobic fatty acid degrading communities. Biochim Biophys Acta 1837:2004-2016.

Wu C, Liu X, Dong X (2006a) *Syntrophomonas erecta* subsp. *sporosyntropha* subsp. nov., a spore-forming bacterium that degrades short chain fatty acids in co-culture with methanogens. Syst Appl Microbiol 29:457-462.

Wu C, Liu X, Dong X (2006b) *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.

Wu C, Dong X, Liu X (2007a) *Syntrophomonas wolfei* subsp. *methylbutyratica* subsp. nov., and assignment of *Syntrophomonas wolfei* subsp. *saponavida* to *Syntrophomonas saponavida* sp. nov. comb. nov. Syst Appl Microbiol 30:376-380.

Wu C, Dong X, Liu X (2007b) *Syntrophomonas wolfei* subsp. *methylbutyratica* subsp. nov., and assignment of *Syntrophomonas wolfei* subsp. *saponavida* to *Syntrophomonas saponavida* sp. nov. comb. nov. Syst Appl Microbiol 30:376–380.

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.

Zhilina TN, Zavarzina DG, Kolganova TV, Turova TP, Zavarzin GA (2005) "*Candidatus* Contubernalis alkalaceticum," an obligately syntrophic alkaliphilic bacterium capable of anaerobic acetate oxidation in a coculture with *Desulfonatronum cooperativum*. Microbiology 74:800-809.

Zhou S, Xu J, Yang G, Zhuang L (2014) Methanogenesis affected by the co-occurrence of iron(III)oxides and humic substances. FEMS Microbial Ecol 88:107-120.

Zindel U, Freudenberg W, Rieth M, Andreesen JR, Schnell J, Widdel F (1988) *Eubacterium acidaminophilum* sp. nov., a versatile amino acid-degrading anaerobe producing or utilizing H_2 or formate. Arch Microbiol 150: 254-266

Figure 1. Pathway for acetate oxidation and synthesis in syntrophic acetate oxidizers, adapted from Hattori (2008). The enzymes involved are as follows: 1, acetate kinase ; 2, phosphotransacetylase; 3, carbon monoxide dehydrogenase; 4, methyltransferase; 5, methylene-THF reductase; 6, methylene-THF dehydrogenase; 7, methenyl-THF cyclohydrolase; 8, formyl-THF synthetase; 9, formate dehydrogenase. THF is tetrahydrofolate and [H] is reducing equivalents.

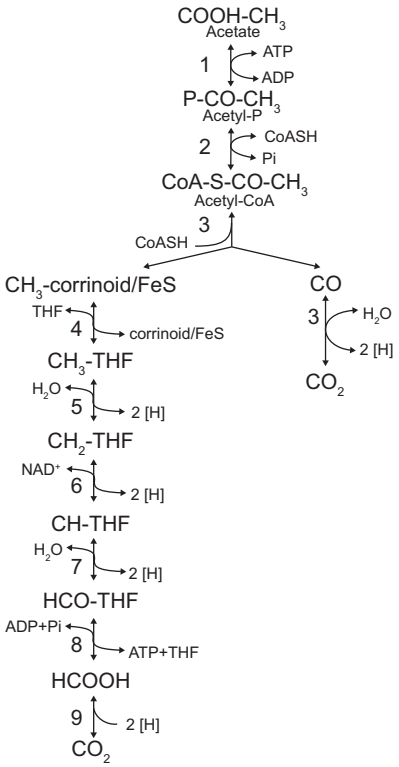
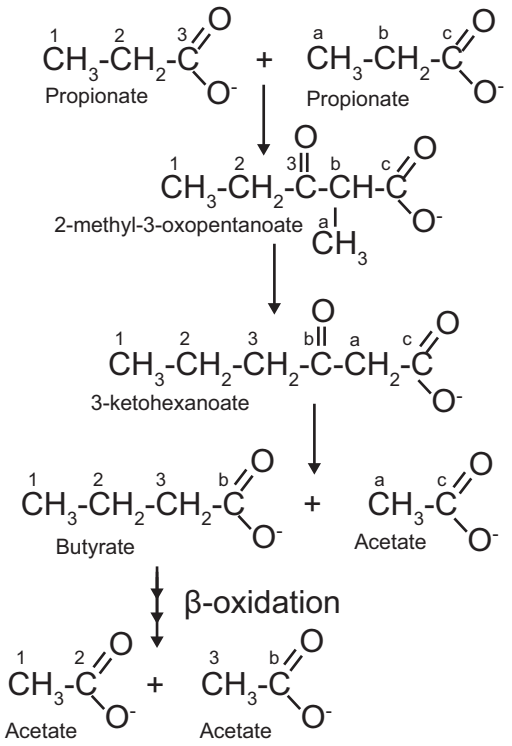


Figure 2. Two pathways for syntrophic propionate metabolism. A, The pathway for the metabolism of propionate by *Smithella propionica*. The carbons in each original propionate are labeled. The enzymes involved in this pathway have yet to be described and CoA esters of the compounds shown may be involved. This figure was adapted from de Bok et al. (2001). B, The methylmalonyl-CoA pathway for propionate metabolism, found in *P. thermopropionicum,* adapted from Kosaka et al. (2006). The enzymes involved are as follows: 1, propionate CoA transferase; 2, propionyl-CoA: oxaloacetate transcarboxylase; 3, methylmalonyl-CoA epimerase; 4, methylmalonyl-CoA mutase; 5, succinyl-CoA synthetase; 6, succinate dehydrogenase/fumarate reductase; 7, fumarate hydratase; 8, malate dehydrogenase; 9, pyruvate dehydrogenase; 10, pyruvate: formate lyase; 11, acetyl-CoA synthase; and 12, acetate kinase. Fd is ferredoxin and [H] is reducing equivalents.

2a.


Propionate
Propionate
2-methyl-3-oxopentanoate
3-ketohexanoate
Butyrate
Acetate
β-oxidation
Acetate
Acetate

2b.

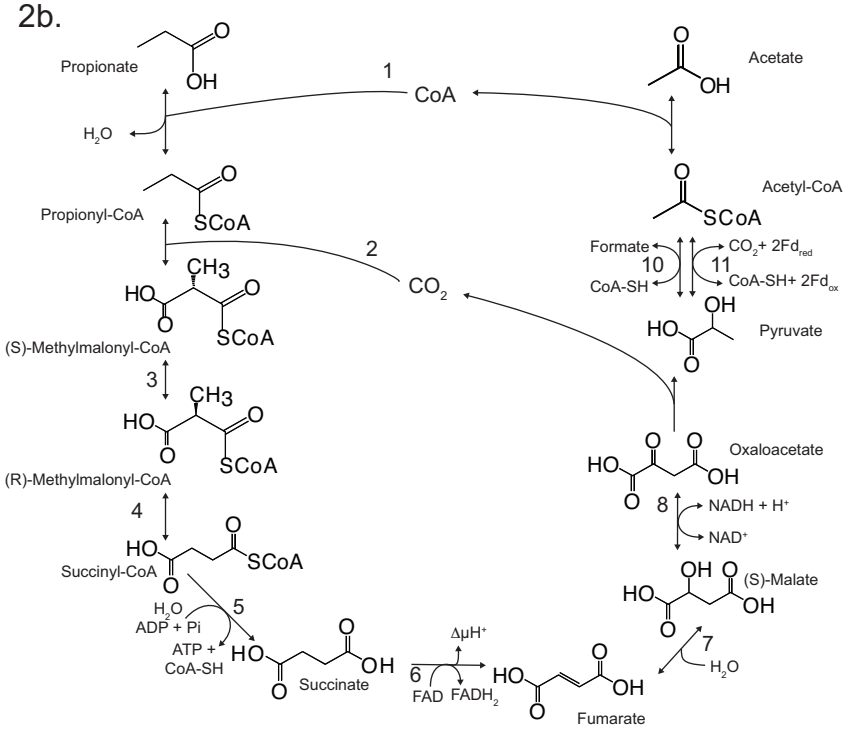

Propionate
Acetate
CoA
H2O
Propionyl-CoA
Acetyl-CoA
SCoA
Formate
CO2+ 2Fdred
10
11
CoA-SH
CoA-SH+ 2Fdox
2
CO2
(S)-Methylmalonyl-CoA
Pyruvate
3
(R)-Methylmalonyl-CoA
Oxaloacetate
4
8
NADH + H+
NAD+
Succinyl-CoA
(S)-Malate
H2O
ADP + Pi
5
ATP +
CoA-SH
Succinate
ΔμH+
6
FAD
FADH2
7
H2O
Fumarate

Figure 3. The β-oxidation pathway for butyrate metabolism in *Syntrophomonas wolfei*, adapted from Wofford et al. (1986), Sieber et al. (2010), Schmidt et al. (2013), Crable et al. (2016). The enzymes involved are: 1, CoA transferase; 2, acyl-CoA dehydrogenase; 3, enoyl-CoA hydratase; 4, L-(+)-3-hydroxybutyryl-CoA dehydrogenase; 5, 3-ketoacyl-CoA thiolase; 6, phosphotransacetylase; 7, acetate kinase;, ETF, electron transport flavoprotein; ETF^{2-}, reduced form of ETF. The grey inset shows the membrane bound reverse electron transport system: 8, iron-sulfur oxidoreductase; 9, hydrogenase; 10, formate dehydrogenase; MK, menaquinone, MKH_2, menaquinol. NADH reoxidation systems: 11, NADH-dependent hydrogenase; 12, NADH-dependent formate dehydrogenase.

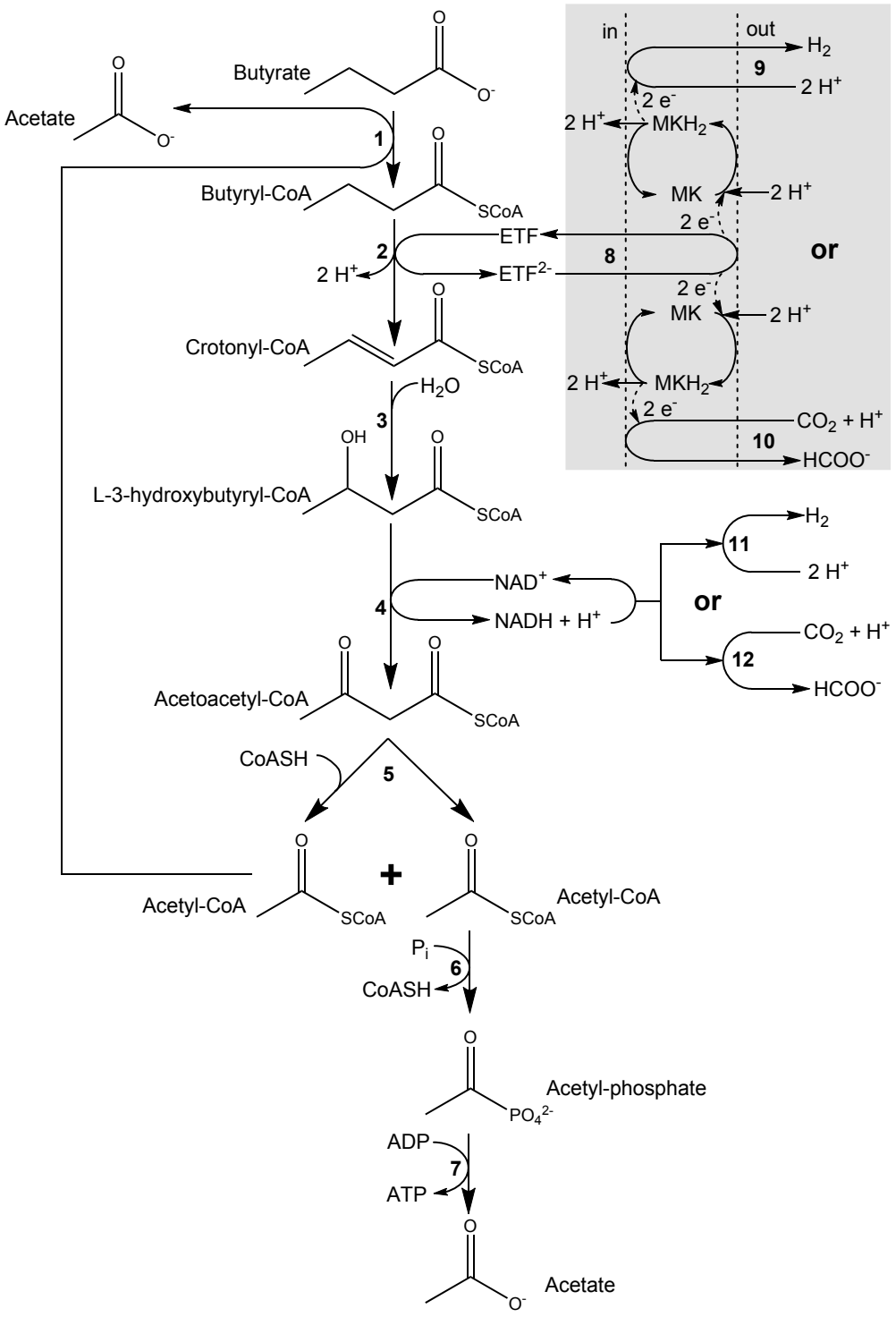

Butyrate
Acetate
1
Butyryl-CoA
SCoA
2
2 H+
ETF
ETF2-
Crotonyl-CoA
H2O
3
OH
L-3-hydroxybutyryl-CoA
4
NAD+
NADH + H+
Acetoacetyl-CoA
CoASH
5
Acetyl-CoA
Acetyl-CoA
Pi
6
CoASH
Acetyl-phosphate
PO4 2-
ADP
7
ATP
Acetate
in
out
H2
9
2 H+
2 e-
MKH2
MK
or
8
10
CO2 + H+
HCOO-
11
12

Figure 4. Pathway for syntrophic benzoate metabolism adapted from McInerney et al. (2007). The enzymes involved are: 1, benzoyl-CoA ligase; 2, benzoyl-CoA reductase; 3, cyclohex-1,5-dienoyl-CoA hydratase; 4, 6-hydrocyclohex-1-ene-carboxyl-CoA dehydrogenase; 5, 6-oxocyclohex-1-ene-carboxyl-CoA hydrolase; 6, β-oxidation enzymes; 7, glutaconyl-CoA decarboxylase; 8, β-oxidation enzymes (see Figure 3 for more detail); 9, AMP-forming, acetyl-CoA synthetase. [H] are reducing equivalents.

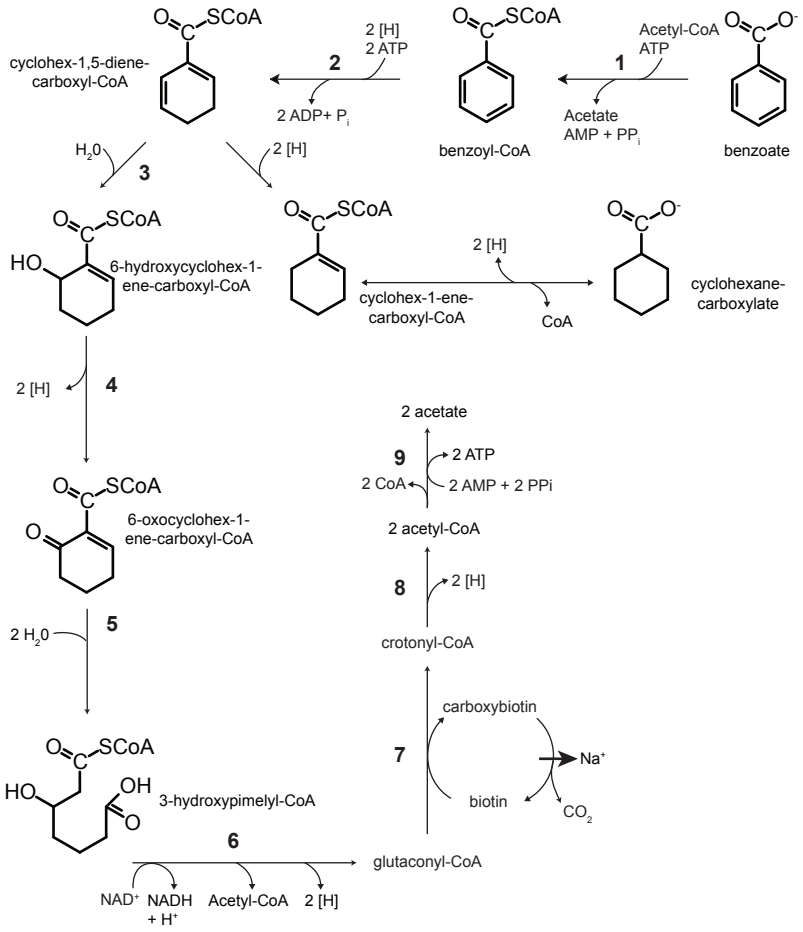
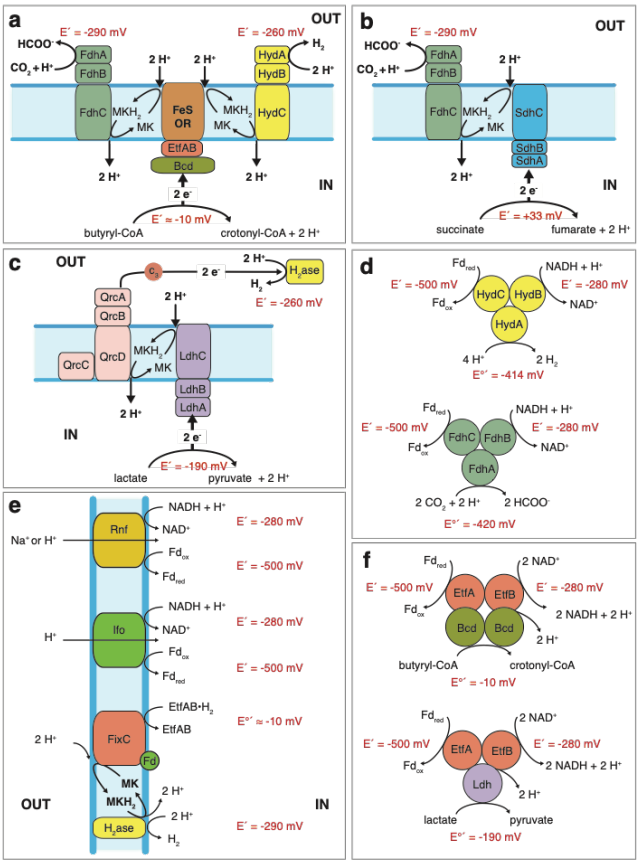


Figure 5. Proposed models for reversed electron transfer. A, syntrophic butyrate metabolism; B, syntrophic propionate metabolism; C, syntrophic lactate metabolism; D, confurcating hydrogenases and formate dehydrogenases; E, ion pumps; and F, confurcating dehydrogenases. Redox values under physiological conditions are from Buckel and Thauer (2013) with H_2 at 10 Pa and formate at 10 μM. Abbreviations: Bcd, butyryl-CoA dehydrogenase; Etf, electron transfer flavoprotein; FeS ox, iron-sulfur oxidoreductase with a DUF224 domain; Fd, ferredoxin; Fdh, formate dehydrogenase; Hyd, hydrogenase; Ifo, ion-translocating ferredoxin oxidoreductase; Ldh, lactate dehydrogenase; Hase, hydrogenase; MK, menaquinone; Rnf, ion-translocating NADH:ferredoxin oxidoreductase; Sdh, succinate dehydrogenase; in, cytoplasm; out, outside the cytoplasmic membrane.


a
OUT
E´ = -290 mV
E´ = -260 mV
HCOO⁻
CO2 + H+
FdhA
FdhB
2 H+
2 H+
HydA
HydB
H2
2 H+
FdhC
MKH2
MK
FeS OR
MKH2
MK
HydC
EtfAB
Bcd
2 H+
2 H+
IN
2 e⁻
E´ = -10 mV
butyryl-CoA
crotonyl-CoA + 2 H+
b
OUT
E´ = -290 mV
HCOO⁻
CO2 + H+
FdhA
FdhB
2 H+
FdhC
MKH2
MK
SdhC
SdhB
SdhA
2 H+
IN
2 e⁻
E´ = +33 mV
succinate
fumarate + 2 H+
c
OUT
O2
2 e⁻
2 H+
H2ase
H2
E´ = -260 mV
QrcA
QrcB
2 H+
QrcC
QrcD
MKH2
MK
LdhC
LdhB
LdhA
2 H+
IN
2 e⁻
E´ = -190 mV
lactate
pyruvate + 2 H+
d
Fdred
NADH + H+
E´ = -500 mV
E´ = -280 mV
Fdox
HydC
HydB
NAD+
HydA
4 H+
2 H2
E°´ = -414 mV
Fdred
NADH + H+
E´ = -500 mV
FdhC
FdhB
E´ = -280 mV
Fdox
NAD+
FdhA
2 CO2 + 2 H+
2 HCOO⁻
E°´ = -420 mV
e
NADH + H+
Rnf
NAD+
E´ = -280 mV
Na+ or H+
Fdox
Fdred
E´ = -500 mV
NADH + H+
Ifo
NAD+
E´ = -280 mV
H+
Fdox
Fdred
E´ = -500 mV
EtfAB+H2
EtfAB
E°´ ≈ -10 mV
2 H+
FixC
Fd
MK
MKH2
2 H+
OUT
2 H+
IN
H2ase
H2
E´ = -290 mV
f
Fdred
2 NAD+
E´ = -500 mV
EtfA
EtfB
E´ = -280 mV
Fdox
2 NADH + 2 H+
Bcd
Bcd
2 H+
butyryl-CoA
crotonyl-CoA
E°´ = -10 mV
Fdred
2 NAD+
E´ = -500 mV
EtfA
EtfB
E´ = -280 mV
Fdox
2 NADH + 2 H+
Ldh
2 H+
lactate
pyruvate
E°´ = -190 mV