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Chapter 8
Syntrophic Degradation of Fatty Acids by Methanogenic Communities

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Abstract In methanogenic environments degradation of fatty acids is a key process in the conversion of organic matter to methane and carbon dioxide. For degradation of fatty acids with three or more carbon atoms syntrophic communities are required. This chapter describes the general features of syntrophic degradation in methanogenic environments and the properties of the microorganisms involved. Syntrophic fatty acid-degrading communities grow at the minimum of what is thermodynamically possible and they employ biochemical mechanisms to share the minimum amount of chemical energy that is available. Aggregation of the syntrophic fatty acid-degrading communities is required for high rate conversion.

Keywords Syntrophic • Methanogenesis • Fatty acid degradation • Metabolic interaction • Hydrogen flux • Propionate degradation • Butyrate degradation • Energetics

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8.1 Introduction

Methanogenesis under controlled conditions in bioreactors is a sustainable way to conserve chemical energy in organic waste component as biogas. Methanogenesis is a microbial process in which metabolic interactions between different physiological types of microorganisms play a crucial role. Methanogens are rather restricted in their physiological abilities. These microorganisms belong to the archaea and derive energy from the conversion of a few simple substrates, which include H_2/CO_2, formate, methanol, methylated amines, and acetate (Liu and Whitmann 2008; Thauer et al. 2008). More complex substrates are not used by methanogens. Consequently, other microorganisms are required to degrade complex organic molecules to compounds that are substrates for methanogens (Schink and Stams 2006; McInerney et al. 2008). This results in a food chain as depicted in Fig. 8.1. Polymers in complex organic matter are first hydrolyzed by extracellular enzymes. Polysaccharides yield monomeric and oligomeric C6 and C5 sugars, while proteins are converted to mixtures of amino acids and small peptides. RNA and DNA are transformed to C5 sugars and nucleic bases purines (adenine and guanine) and pyrimidines (cytosine, thymine, and uracil). Lipids are degraded to glycerol and long-chain fatty acids. The general pattern of further anaerobic mineralization of organic matter is that fermentative bacteria degrade easily degradable compounds like sugars, amino acids, purines, pyrimidines, and glycerol to a variety of fatty acids, carbon dioxide, formate, and hydrogen. Then, higher fatty acids are degraded by so called acetogenic bacteria, finally to the methanogenic substrates. All these processes, fermentation,

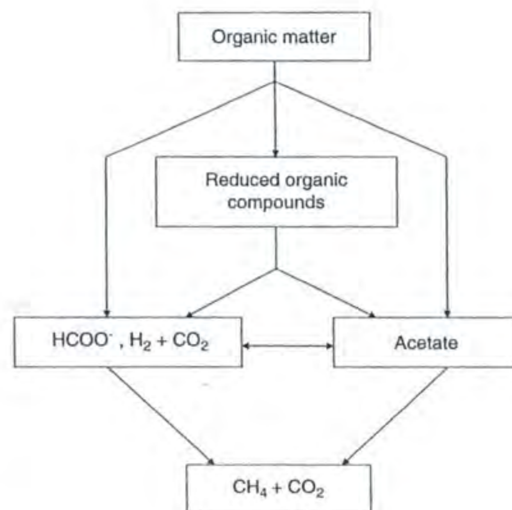


Fig. 8.1 Schematic representation of methanogenic degradation of complex organic matter

acetogenesis, and methanogenesis, take place simultaneously, but often, because of differences in growth rates and specific activities of the microorganisms involved, the processes are partially uncoupled, resulting in the accumulation of especially fatty acids. For complete mineralization of complex organic matter, acetogenic bacteria that degrade fatty acids play a crucial role. This chapter addresses the physiological properties of acetogenic bacteria that degrade propionate, butyrate, and higher fatty acids. Propionate and butyrate are important intermediates in the anaerobic fermentation of sugars and amino acids (Schink and Stams 2006; Stams 1994), while long-chain fatty acids are formed in the hydrolysis of lipids and fats (McInerney 1988; Sousa et al. 2009). To optimize methanogenesis in bioreactors especially the metabolic interactions of syntrophic communities need to be understood.

8.2 General Features of Syntrophic Fatty Acid Conversion

Bacteria that degrade and grow on fatty acids have to deal with the unfavorable energetics of the conversion processes. Table 8.1 illustrates the conversion of some fatty acids to the methanogenic substrates acetate and hydrogen. It is evident that bacteria can only derive energy for growth from these conversions when the product concentrations are kept low. This results in an obligate dependence of acetogenic

Table 8.1 Standard Gibbs free energy changes for some of the reactions involved in syntrophic fatty acid degradation metabolism

	$\Delta G^{0\prime}$ (kJ)
Fatty acid oxidation	
$\text{Propionate}^- + 3H_2O \rightarrow \text{acetate}^- + HCO_3^- + H^+ + 3H_2$	76
$\text{Butyrate}^- + 2H_2O \rightarrow 2\text{acetate}^- + H^+ + 2H_2$	48
$\text{Caproate}^- + 4H_2O \rightarrow 3\text{acetate}^- + 14H_2 + 2H^+$	96
$\text{Palmitate}^- + 14H_2O \rightarrow 8\text{acetate}^- + 14H_2 + 7H^+$	353
$\text{Stearate}^- + 16H_2O \rightarrow 9\text{acetate}^- + 16H_2 + 8H^+$	404
$\text{Oleate}^- + 16H_2O \rightarrow 9\text{acetate}^- + 15H_2 + 8H^+$	338
Hydrogen utilization by methanogens, homoacetogens, and sulfate reducers	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	136
$4H_2 + 2HCO_3^- + H^+ \rightarrow \text{acetate}^- + 4H_2O$	105
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	152
Acetate utilization by methanogens and sulfate reducers	
$\text{Acetate}^- + H_2O \rightarrow HCO_3^- + CH_4$	31
$\text{Acetate}^- + SO_4^{2-} + 2H_2O \rightarrow HS^- + 2HCO_3^-$	47
Intracellular redox conversions	
$2Fd_{(red)} + 2H^+ \rightarrow 2Fd_{(ox)} + H_2$	3
$NADH + H^+ \rightarrow NAD^+ + H_2$	18
$FADH_2 \rightarrow FAD + H_2$	37

Standard Gibbs energies of formation of obtained from Thauer et al. (1977); for palmitate, stearate, and oleate standard Gibbs energies of formation were calculated from the structures of the compounds using a group contribution method described by Mavrovouniotis (1991)

bacteria on methanogenic archaea for product removal. As the methanogens obtain their substrates for growth from the acetogens, syntrophic communities are enriched with propionate, butyrate, and higher fatty acids.

Obligate syntrophic communities of acetogenic bacteria and methanogenic archaea have several unique features: (1) they degrade fatty acids coupled to growth, while neither the archaeon nor the bacterium alone is able to degrade these compounds; (2) intermicrobial distances influence biodegradation rates and specific growth rates, which in nature results in the self-aggregation of bacteria and archaea to compact aggregates; (3) the syntrophic communities grow in conditions that are close to thermodynamical equilibrium; and (4) the communities have evolved biochemical mechanisms that allow sharing of chemical energy. There still is discussion whether hydrogen and formate are the primary compounds for interspecies electron transfer, and it is still unclear what their relative importance is. Here, mainly syntrophy in terms of interspecies hydrogen transfer is discussed, but where relevant interspecies formate transfer will be discussed as well.

In syntrophic fatty acid-degrading communities, hydrogen has to diffuse from the acetogen to the methanogen. Therefore, the specific flux of hydrogen can be derived from the Fick's diffusion law (8.1) (Ishii et al. 2006; Schink and Thauer 1988).

$$J = D_{H_2} \times \left\{ [H_2]_{acetogen} - [H_2]_{methanogen} \right\} / \text{day}. \tag{8.1}$$

J: specific interspecies hydrogen flux (μmol/μm²/s).

D_{H2}: diffusion constant for hydrogen (4.9×10^{-5} m²/s at 298 K).

$[H_2]_{acetogen}$: hydrogen concentration at the outside surface of the acetogen (μmol/μm³).

$[H_2]_{methanogen}$: hydrogen concentration at the outside surface of the methanogen cell (μmol/μm³).

D: average distance between the acetogen and the methanogen (μm).

The flux of hydrogen between the two species can be calculated by multiplying the J value by the total surface area of hydrogen-releasing acetogens.

The total hydrogen flux is directly dependent on the surface area of the acetogen, the diffusion constant of hydrogen, the concentration difference of hydrogen between the acetogens and the methanogens, and the distance between the two microorganisms The maximum difference in concentration between the acetogens and the methanogens is determined by the thermodynamical borders of the conversions carried out by these microorganisms. These thermodynamical borders refer to nongrowing conditions; if the microorganisms have to conserve metabolic energy for growth, the difference in concentration is smaller. The highest concentration that can be formed by the acetogen and the lowest concentration that can be reached by the methanogen can be calculated from data in Table 8.1, provided that the concentrations of other compounds are known.

Such calculations were made for the degradation of a number of compounds in different anoxic environments (Conrad et al. 1986). Cord-Ruwisch et al. (1988) showed that the measured threshold values for hydrogen of the different types of hydrogen-utilizing microorganisms correlated with the values expected from the ΔG°'s. The threshold value for hydrogen of growing methanogens and sulfate reducers are 3–10 and 1–2 Pa, respectively. These differences explain why the growth rate of acetogenic bacteria depends on the type of hydrogenotrophic microorganism. The maximum specific growth rates of propionate- and butyrate-degrading acetogenic bacteria in coculture with methanogens were 0.10, and 0.19/day, respectively, while in coculture with sulfate reducers these values were 0.19 and 0.31/day, respectively (Boone and Bryant 1980; McInerney et al. 1979; Mountfort and Bryant 1982).

The diffusion distance between the acetogens and methanogens is determined by the biomass density. Clustering of cells will lead to increased fluxes (Schink and Thauer 1988). Assuming that bacteria have a diameter of 2 μm, it can be calculated that in cultures containing 10^8, 10^9, 10^{10}, and 10^{11} cells/ml the average intermicrobial distances are about 25, 10, 4, and 0.5 μm, respectively. Therefore, in syntrophic conversion the activity per cell, and consequently the specific growth rate, will increase with decreasing intermicrobial distances. This effect may explain the apparent long lag-phases often observed during subcultivation of syntrophic cultures. In methanogenic granular sludge the cell densities are extremely high: up to 10^{12} CFU/ml wet sludge (Grotenhuis et al. 1991; Dolfing et al. 1985). Such high cell densities are favorable for interspecies hydrogen transfer, resulting in very high methanogenic activities with fatty acids as substrates. In propionate-adapted methanogenic granules, microbial structures were observed in which the distances between propionate-oxidizing bacteria and methanogens were in the micrometer range (Grotenhuis et al. 1991). Disrupture of the structure led to a reduction of the propionate degradation rate by 90%, while the acetate degradation rate by methanogens was largely unaffected. Moreover, shortening of intermicrobial distances by the creation of artificial precipitates (Stams et al. 1992) or by the addition of extra methanogens (Dwyer et al. 1988; Schmidt and Ahring 1993) led to increased methane formation rates with propionate and butyrate.

Using the van't Hoff equation, Gibbs free energy changes at different temperatures can be calculated. ΔG and ΔH values have been listed by Thauer et al. (1977) and Chang (1977), respectively. A change in temperature has an effect on the reaction-dependent part and on the concentration-dependent part of the $\Delta G'$ values. Calculations of $\Delta G'$ values at different temperatures show that hydrogen formation becomes energetically more favorable at higher temperatures, whereas hydrogen-consuming reactions become less favorable. Consequently, lower hydrogen partial pressures can be reached by the methanogens at lower temperatures. The opposite is true for the highest hydrogen concentrations that can be formed by the acetogens. These calculations fit with the lowest and highest hydrogen partial pressures which have been measured with mesophilic and thermophilic methanogens and acetogens. An increase in temperature affects the flux of hydrogen in two ways: the diffusion coefficient becomes higher and the concentration gradient between the methanogen and the acetogen becomes steeper.

8.3 Syntrophic Propionate-Degrading Bacteria

Boone and Bryant (1980) described *Syntrophobacter wolinii* a bacterium that grows in syntrophic association with methanogens or sulfate-reducing bacteria. Since then, several other mesophilic and thermophilic bacteria that grow in syntrophy with methanogens have been described (Table 8.2). These include Gram-negative bacteria (*Syntrophobacter* and *Smithella*) and Gram-positive bacteria (*Pelotomaculum* and *Desulfotomaculum*) (McInerney et al. 2008). Phylogenetically, both groups are related to sulfate-reducing bacteria and some indeed grow by sulfate reduction (Table 8.2). *Syntrophobacter* and *Desulfotomaculum* species are able to reduce sulfate, but *Smithella* and *Pelotomaculum* are not. Most syntrophic propionate-degrading bacteria are able to grow in pure culture by fermentation of fumarate or pyruvate. Fermentative growth or sulfate-dependent growth has been used to successfully obtain the bacteria in pure culture. The only exceptions are *Pelotomaculum schinkii* (de Bok et al. 2005) and *Pelotomaculum propionicicum* (Imachi et al. 2007), which seem to be true propionate-degrading syntrophs. Pure cultures of these strains are not available: *P. thermopropionicum* and *Desulfotomaculum thermobenzoicum* (subsp. *thermopropionicum*) are moderately thermophilic and grow in syntrophy with thermophilic methanogens (Imachi et al. 2002; Plugge et al. 2002). A marine propionate-degrading syntrophic community has been described, but the identity of the propionate-degrading bacterium is not known (Kendall et al. 2006).

Two pathways for propionate metabolism are known, the methylmalonyl-CoA pathway (Fig. 8.2b) and a dismutation pathway. In the latter pathway two propionate molecules are converted to acetate and butyrate, the butyrate being degraded to acetate and hydrogen as described below. Thus far, this pathway is only found in *Smithella propionica* (Liu et al. 1999; de Bok et al. 2001). The methylmalonyl-CoA pathway is found in the other syntrophic propionate-oxidizing bacteria (McInerney et al. 2008). In the methylmalonyl-CoA pathway, propionate is first activated to propionyl-CoA and then carboxylated to methylmalonyl-CoA. Methylmalonyl-CoA is rearranged to form succinyl-CoA, which is converted to succinate. Succinate is oxidized to fumarate, which is then hydrated to malate and oxidized to oxaloacetate. Pyruvate is formed by decarboxylation, and is further oxidized in a HS-CoA-dependent decarboxylation to acetyl-CoA and finally to acetate.

8.4 Syntrophic Butyrate- and LCFA-Degrading Bacteria

McInerney et al. (1981) enriched and characterized *Syntrophomonas wolfei*, a bacterium that degrades butyrate and some other short-chain fatty acids in syntrophic association with methanogens. Several other bacteria since then have been described that grow with butyrate or higher fatty acids in syntrophy with hydrogenotrophic methanogens or sulfate reducers (Table 8.3). Thus far, *Algorimarina butyrica* is the only psychrophilic bacterium that is known to degrade butyrate in syntrophy with

Table 8.2 Selected characteristics of propionate-oxidizing acetogenic bacteria: propionate use in pure culture (in the presence of an external electron acceptor) or in coculture with a hydrogen-utilizing partner is highlighted in gray

	Cell width (μm)	Cell length (μm)	Gram reaction	Motility	Spore formation	pH (range)[a]	Temperature range (°C)[a]	Propionate + sulfate	Propionate + nitrate	Propionate + methanogen	Propionate	References
Syntrophobacter fumaroxidans	1.1–1.6	1.8–2.5	–	–	–	6.0–8.0 (7)	20–40 (37)	+	–	+	+	Harmsen et al. (1998)
Syntrophobacter pfennigii	1.0–1.2	2.2–3.0	–	+	–	6.2–8.0 (7.0–7.3)	20–37 (37)	+	–	–	+	Wallrabenstein et al. (1995)
Syntrophobacter sulfatireducens	1.0–1.3	1.8–2.2	–	–	–	6.2–8.8 (7.0–7.6)	20–48 (37)	+	–	–	+	Chen et al. (2005)
Syntrophobacter wolinii	0.6–1.0	1.0–4.5	–	–	–	5.5–7.7 (6.9)	23–40 (35)	+	–	ND	+	Harmsen et al. (1998), Boone and Bryant (1980), Wallrabenstein et al. (1994), and Liu et al. (1999)
Pelotomaculum schinkii	1	2.0–2.5	+	–	+	ND	ND	–	–	–	+	de Bok et al. (2005)
Pelotomaculum thermopropionicum	0.7–0.8	1.7–2.8	–[b]	–	+	6.5–8.0 (7.0)	45–65 (55)	–	–	+	+	Imachi et al. (2002)
Pelotomaculum propionicicum	1.0	2.0–4.0	+	ND	+	6.5–7.5 (6.5–7.2)	25–45 (37)	–	–	–	+	Imachi et al. (2007)
Smithella propionica	0.5	3–10	–	+	–	6.3–7.8 (7)	23–40 (33)	ND	ND	ND	+	Liu et al. (1999)
Desulfotomaculum thermobenzoicum thermosyntrophicum	1	3–11	–[b]	+	+	6–8 (7.0–7.5)	45–62 (55)	+	–	–	+	Plugge et al. (2002)

Substrate utilization: +, utilized, –, not utilized. *ND* not determined or not reported

[a]Optimum in parentheses

[b]Cells stain Gram-negative but the organism has a Gram-positive cell wall ultrastructure

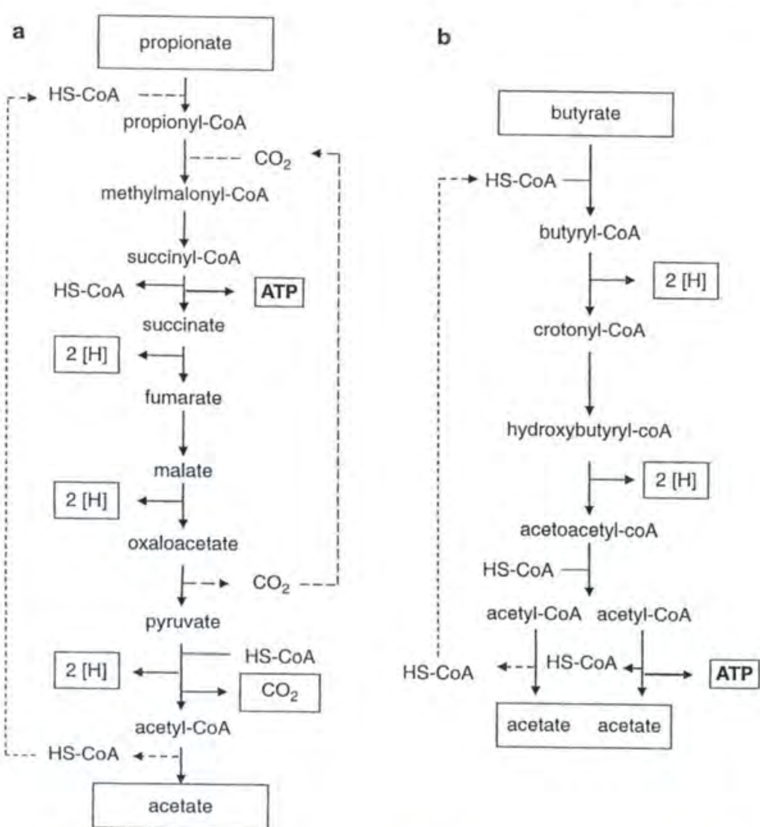


Fig. 8.2 Pathways of propionate (**a**) and butyrate (**b**) degradation by acetogenic bacteria growing in syntrophic association with methanogens. ATP production by substrate-level phosphorylation

methanogens (Kendall et al. 2006). This marine bacterium is not able to grow on medium-chain fatty acids like valerate or caproate or longer fatty acids such as palmitate. Mesophilic bacteria capable of syntrophic fatty acid metabolism are mainly species of *Syntrophomonas*, though *Syntrophomonas bryantii* was previously named *Syntrophospora bryantii* and *Clostridium bryantii* (McInerney et al. 2008; Sousa et al. 2009; Wu et al. 2006a). The only exception is *Syntrophus aciditrophicus*, a syntrophic benzoate-degrading bacteria that it is also able to degrade medium- and long-chain fatty acids in coculture with a methanogen (Jackson et al. 1999). Thermophilic syntrophic butyrate-degrading bacteria are *Thermosyntropha lipolytica* (Svetlitshnyi et al. 1996) and *Syntrophothermus lipocalidus* (Sekiguchi et al. 2000). None of the fatty acid-degrading bacteria that grow syntrophically with

Table 8.3 Selected characteristics of fatty acid-oxidizing acetogenic bacteria: fatty acids used in coculture with a hydrogen-utilizing partner are highlighted in gray; utilization of crotonate in pure culture is also indicated

	Cell width (μm)	Cell length (μm)	Gram reaction	Motility	Spore formation	pH (range)[a]	Temperature range (°C)[a]	Crotonate	Butyrate, C4:0	Caproate, C6:0	Caprylate, C8:0	Caprate, C10:0	Laurate, C12:0	Myristate, C14:0	Palmitate, C16:0	Stearate, C18:0	Oleate, C18:1	Linoleate, C18:2	References
Algorimarina butyrica	ND	ND	–	+	–	6.2–7.1	10–20 (15)	–	+	–	ND	ND	ND	ND	ND	ND	ND	ND	Kendall et al. (2006)
Syntrophomonas bryantii	0.4	4.5–6.0	±	–	+	6.5–7.5	20–40 (28–34)	+	+	+	+	+	–	–	–	–	ND	ND	Stieb and Schink (1985), Zhao et al. (1990) and Wu et al. (2006a)
Syntrophomonas cellicola	0.4–0.5	3.0–10.0	±	+	+	6.5–8.5 (7.0–7.5)	25–45 (37–40)	+	+	+	+	–	–	–	ND	ND	ND	ND	Wu et al. (2006a)
Syntrophomonas curvata	0.5–0.7	2.3–4.0	±	+	–	6.3–8.4 (7.5)	20–42 (35–37)	+	+	+	+	+	+	+	+	+	+	–	Zhang et al. (2004) Zhang et al. (2005)
Syntrophomonas erecta erecta	0.6–0.9	2.0–8.0	–	+	–	6.0–8.8 (7.8)	25–47 (37–40)	+	+	+	+	–	–	–	–	–	–	–	Zhang et al. (2005) and Wu et al. (2006b)
Syntrophomonas erecta sporosyntropha	0.5–0.7	4.0–14.0	–	+	+	5.5–8.4 (7.0)	20–48 (37–40)	+	+	+	+	–	–	–	–	–	–	–	Wu et al. (2006b)
Syntrophomonas palmitatica	0.4–0.6	1.5–4.0	–	–	–	6.5–8.0 (7.0)	30–50 (37)	+	+	+	+	+	+	+	+	+	–	–	Hatamoto et al. (2007)
Syntrophomonas saponavida	0.4–0.6	2.0–4.0	–	+	–	ND	ND	+	+	+	+	+	+	+	+	+	–	–	Lorowitz et al. (1989) and Wu et al. (2007)
Syntrophomonas sapovorans	0.5	2.5	–	+	–	6.3–8.1 (7.3)	25–45 (35)	–	+	+	+	+	+	+	+	+	+	+	Roy et al. (1986) and Zhang et al. (2005)
Syntrophomonas wolfei methylbutyratica	0.4–0.5	3.0–6.0	±		+	6.5–8.5 (7.0–7.6)	25–45 (37–40)	+	+	+	+	–	–	–	–	–	ND	ND	Wu et al. (2007)
Syntrophomonas wolfei wolfei	0.5–1.0	2.0–7.0	–	+	–	6.2–8.1 (7.0–7.5)	25–45 (35–37)	+	+	+	+	–	–	–	–	–	–	–	McInerney et al. (1979), McInerney et al. (1981) and Zhang et al. (2005)
Syntrophomonas zehnderi	0.4–0.7	2.0–4.0	±	+	+	ND	25–40 (37)	–	+	+	+	+	+	+	+	+	+	±	Sousa et al. (2007)
Syntrophus aciditrophicus	0.5–0.7	1.0–1.6	–	–	–	ND	25–42 (35)	+	+	+	+	ND	ND	ND	+	+	ND	ND	Jackson et al. (1999)
Syntrophothermus lipocalidus	0.4–0.5	2.0–4.0	–	+	–	5.8–7.5 (6.5–7.0)	45–60 (55)	+	+	+	+	+	–	–	–	–	–	–	Sekiguchi et al. (2000)
Thermosyntropha lipolytica	0.3–0.4	2.0–3.5	–[b]	–	–	7.1–9.5 (8.1–8.9)	52–70 (60–66)	+	+	+	+	+	+	+	+	+	+	+	Svetlitshnyi et al. (1996)

Substrate utilization: +, utilized, ±, poorly utilized, –, not utilized. *ND* not determined or not reported

[a]Optimum in parentheses

[b]Cells stain Gram-negative in both exponential and stationary phase, but the organism has a Gram-positive cell wall ultrastructure

methanogens has been described to be able to reduce sulfate. Most fatty acid degraders are able to ferment crotonate, which was used to obtain pure cultures. However, *Synthrophomonas sapovorans* and *Syntrophomonas zehnderi* are not able to ferment crotonate, and are only available in syntrophic methanogenic cocultures (Roy et al. 1986; Sousa et al. 2007).

Butyrate and longer chain fatty acids are degraded via so-called β-oxidation (McInerney et al. 2008; Schink and Stams 2006). In a series of reactions acetyl groups are cleaved off yielding acetate and hydrogen. To metabolize fatty acids, first activation to a HS-CoA derivative takes place. The HS-CoA-derivative is then dehydrogenated to form an enoyl-CoA. After water addition, a second dehydrogenation takes place to form a ketoacylacetyl-CoA. After hydrolysis acetyl-CoA and an acyl-CoA are formed, which enters another cycle of dehydrogenation and the cleaving off of acetyl-CoA. Figure 8.2b shows the pathway of syntrophic butyrate degradation.

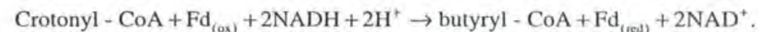
8.5 Energetics of Syntrophic Degradation

Based on the energy released by the hydrolysis of ATP and the concentrations of adenylate molecules in growing bacteria, it is estimated that a free energy change of −60 to −70 kJ is required for the synthesis of 1 mol of ATP under physiological conditions. This amount of energy does not need to be supplied in one single step, as is the case in substrate-level phosphorylation, but can be accomplished in smaller amounts, for example, by proton or sodium translocation across the cytoplasmic membrane. If a ratio of three protons translocated per ATP is assumed, about 20 kJ energy difference is the smallest energy quantum that may allow energy conservation and growth. It can be calculated that under in situ conditions about 20 kJ energy difference (the equivalent of 1/3 ATP) is available for bacteria that grow syntrophically on propionate or butyrate. Following the pathway of propionate and butyrate degradation (Fig. 8.2a, b), seemingly one ATP can be formed from the conversion of the energy rich HS-CoA esters. However, syntrophic bacteria encounter an energetic barrier in the formation of hydrogen from intracellular redox mediators. In the propionate oxidation pathway three oxidation steps are involved, succinate oxidation to fumarate, malate oxidation to oxaloacetate and pyruvate oxidation to acetyl-CoA. In these oxidation steps electrons are released at the energetic level of $FADH_2$, NADH, and reduced ferredoxin, respectively, and need to be coupled to hydrogen formation (Table 8.1). Similarly, in butyrate oxidation electrons are released at the energetic level of $FADH_2$ and NADH, in the oxidation of butyryl-CoA to crotonyl-CoA and of hydoxybutyryl-CoA to acetoacetyl-CoA, respectively.

Methanogens are able to bring the hydrogen level down to about 1 Pa under nongrowing conditions. At this hydrogen level, NADH oxidation and reduced ferredoxin oxidation coupled to hydrogen formation is energetically feasible for the fatty acid degraders. However, at a hydrogen partial pressure of 1 Pa $FADH_2$ oxidation

still is not feasible. Therefore, propionate- and butyrate-oxidizing bacteria have to invest metabolic energy to push this reaction. The biochemical mechanism that is used for this is not completely clear. The complete genome sequences of *S. aciditrophicus*, *S. wolfei*, *Syntrophobacter fumaroxidans*, and *P. thermopropionicum* have been reported. From the predicted localization of key enzymes involved a speculation of the biochemical mechanisms of reversed electron flow can be made.

The mechanism to drive succinate oxidation to fumarate ($E^{o\prime}$ = +33 mV) during syntrophic growth is possibly similar to the mechanism of energy conservation during fumarate respiration in *Wolinella succinogenes*, but operating in reversed mode (Müller et al. 2010). *Bacillus subtilis* also uses reversed electron transfer in succinate oxidation by coupling it to menaquinone reduction ($E^{o\prime}$ = −80 mV). Menaquinone binds cytochrome b close to the outside of the cell membrane. When reduced menaquinone is oxidized at the cytoplasmic side of the membrane and inward movement of protons is achieved (Schirawski and Unden 1998). In syntrophic propionate metabolism, menaquinone oxidation is linked to a membrane-bound hydrogenase or formate dehydrogenase. Genome and biochemical analysis of *S. fumaroxidans* revealed the presence of a membrane integrated succinate dehydogenase gene cluster containing menaquinone (Sfum_1998, 1999, and 2000) and several periplasmic and cytoplasmic hydrogenases and formate dehydrogenases (Table 8.4a) (Müller et al. 2010; Worm et al. 2010). A requirement of 2/3 ATP for reversed electron transport was determined. This is schematically presented in Fig. 8.3. A similar and phylogenetically related succinate dehydrogenase gene cluster was found in *P. thermopropionicum* (Kosaka et al. 2006, 2008). This indicates that succinate oxidation to fumarate requires a proton gradient over the membrane and the investment of ATP to form hydrogen at the outside of the cytoplasmic membrane. In syntrophic communities the methanogens directly scavenge the hydrogen formed. Menaquinone found in *S. wolfei* possibly functions as the electron carrier between a membrane-associated butyryl-CoA dehydrogenase and a cytoplasmically oriented hydrogenase (Müller et al. 2010). Inward movement of protons by the quinone loop is compensated by proton extrusion by a membrane-bound ATPase, similar to succinate oxidation in syntrophic propionate oxidation, though in *S. aciditrophicus* hydrogenases seem to be located at the inner aspect of the cell (Table 8.4). An alternative mechanism to produce H_2 from thermodynamically difficult substrates comes from recent studies with *Clostridium kluyveri* (Herrmann et al. 2008). *C. kluyveri* ferments ethanol and acetate to butyrate and some hydrogen. A soluble enzyme complex in *C. kluyveri* couples the energetically favorable reduction of crotonyl-CoA to butyryl-CoA by NADH with the unfavorable reduction of ferredoxin (Fd) by NADH:



$$\text{Crotonyl-CoA} + Fd_{(ox)} + 2NADH + 2H^+ \rightarrow \text{butyryl-CoA} + Fd_{(red)} + 2NAD^+.$$

The reverse mechanism may be involved in syntrophic butyrate degradation, though in that case it remains unclear how the bacteria form reduced ferredoxin needed for that conversion.

Table 8.4 Genes annotated as formate dehydrogenase and hydrogenase in the genomes of *Syntrophobacter fumaroxidans* (a) and *Syntrophus aciditrophicus* (b) (http://img.jgi.doe.gov). Periplasmic localization of these cofactor binding enzymes is indicated by the presence of a twin arginine translocation motif in the N-terminal amino acid sequence

Enzyme	Locus tag	Localization	Metal content
(a) *Genes annotated as formate dehydrogenase or hydrogenase in the genome of S. fumaroxidans*			
Formate dehydrogenase	Sfum_2706, Sfum_2705[a]	Cytoplasm	W, Se
Formate dehydrogenase	Sfum_1274, Sfum_1273[a]	Periplasm	W, Se
Formate dehydrogenase	Sfum_3509	Periplasm	W/Mo
Formate dehydrogenase	Sfum_0031, Sfum_0030[a]	Periplasm	W/Mo, Se
Hydrogenase	Sfum_0844	Periplasm	Fe
Hydrogenase	Sfum_2952	Periplasm	Ni, Fe
Hydrogenase	Sfum_2221	Cytoplasm	Ni, Fe
Hydrogenase	Sfum_2716	Cytoplasm	Ni, Fe
Hydrogenase	Sfum_3537[a]	Cytoplasm	Ni, Fe, Se
Hydrogenase	Sfum_3954[a]	Periplasm	Ni, Fe, Se
(b) *Genes annotated as formate dehydrogenase or hydrogenase in the genome of S. aciditrophicus*			
Formate dehydrogenase	Syn_00635, Sfum_00634[a]	Periplasm	W/Mo, Se
Formate dehydrogenase	Syn_00630, Syn_00629[a]	Cytoplasm	W/Mo, Se
Formate dehydrogenase	Syn_02137, Syn_002138[a]	Cytoplasm	W/Mo, Se
Formate dehydrogenase	Syn_00602, Sfum_00603[a]	Periplasm	W/Mo, Se
Hydrogenase	Syn_01370	Cytoplasm	Fe
Hydrogenase	Syn_02222	Cytoplasm	Ni, Fe

[a]Selenocysteine residues were predicted by recognition of a TGA with a downstream sequence containing a ribonuclear fold that comply with the consensus bSECIS structural model as described by Zhang and Gladyshev (2005)

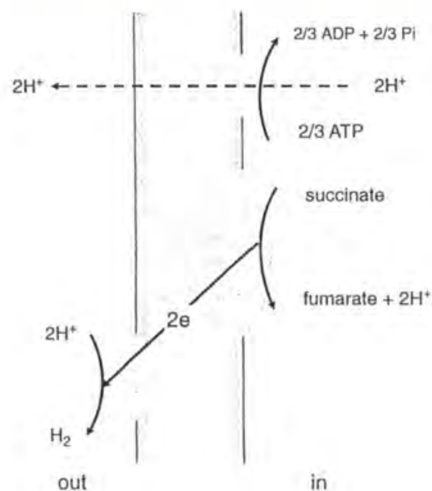


Fig. 8.3 Hypothetical mechanism of membrane integrated, ATP-dependent succinate oxidation in propionate-degrading acetogenic bacteria

A special mechanism of propionate and butyrate activation exists in syntrophic bacteria. In general, carboxylic acids are activated by kinases or thiokinases and require one ATP. *S. fumaroxidans* and *P. thermopropionicum* activate propionate through HS-CoA-transferase (Kosaka et al. 2006, 2008; Plugge et al. 1993). Sequence analysis of these genes reveals a very high homology between the HS-CoA transferase of both microorganisms (79% sequence identity, Sfum_1163 and Pth_1771). In butyrate-oxidizing syntrophs, the initial activation of butyrate also occurs by HS-CoA transferase (Wofford et al. 1986). However, no clear sequence similarity exists between the HS-CoA transferases of propionate oxidizers and butyrate oxidizers.

8.6 Conclusions and Perspectives

Syntrophic communities operate in conditions that are close to thermodynamical equilibrium. This implies that changes in environmental conditions affect conversion rates and growth of the syntrophic communities. In anaerobic waste and wastewater treatment disturbances in operation of the process results in the accumulation of fatty acids, coinciding with a pH decrease that may be detrimental for the whole process. On the other hand, the characteristics of syntrophic degradation may also be used to optimize degradation, as was done to develop a process for efficient anaerobic conversion of wastewaters that contain large amounts of lipids and long-chain fatty acids (Alves et al. 2009).

Several bacteria have been isolated and characterized that have the ability to degrade fatty acids in syntrophy with hydrogen-consuming methanogens. The biochemical pathways that these bacteria employ and the energetic barriers that these bacteria have to overcome are known. However, the exact biochemical mechanisms by which these bacteria overcome the energetic barriers are not yet fully understood. Genome sequence information can be used to unravel how these fatty acid-degrading bacteria regulate their metabolism in changing environmental conditions.

Degradation of organic waste to biogas, which consists mainly of methane and carbon dioxide, is a unique process. Irrespectively of the microorganisms involved and the pathways that they employ, a variety of organic compounds can be easily and completely degraded to biogas. The substrate conversion rates and growth rates of fatty acid-degrading communities determine the overall efficiency of the methanogenic process. However, to make anaerobic digestion unfailing and to increase the overall rates of methanogenesis, the metabolic interactions between bacteria and archaea deserve to be further explored.

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Chapter 9
Anaerobic Digestion as an Effective Biofuel Production Technology

Serge R. Guiot and Jean-Claude Frigon

Abstract The methane produced from the anaerobic digestion of organic wastes and energy crops represents an elegant and economical mean of generating renewable biofuel. Anaerobic digestion is a mature technology and is already used for the conversion of the organic fraction of municipal solid wastes and primary and secondary sludge from wastewater treatment plant. High methane yield up to 0.45 Nm^3 CH_4/kg volatile solids (VS) or 12,390 Nm^3 CH_4/ha can be achieved with sugar and starch crops, although these cultures are competing for high quality land with food and feed crops. The cultivation of lignocellulosic crops on marginal and set-aside lands is a more environmentally sound and sustainable option for renewable energy production. The methane yield obtained from these crops is lower, 0.17–0.39 Nm^3 CH_4/kg VS or 5,400 Nm^3 CH_4/ha, as its conversion into methane is facing the same initial barrier as for the production of ethanol, e.g., hydrolysis of the crops. Intensive research and development on efficient pretreatments is ongoing to optimize the net energy production, which is potentially greater than for liquid biofuels, since the whole substrate excepted lignin is convertible into methane. Algal biomass is another alternative to food and feed crops. Their relatively high methane potential (up to 0.45 Nm^3 CH_4/kg VS fed) combined with their higher areal biomass productivity make them particularly attractive as a feedstock for an anaerobic digestion-based biorefinery concept.

Keywords Anaerobic digestion • Waste treatment • Energy crops • OLR • HRT • Wet and dry technologies • Energy yields • Microalgae biomethane potential

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