Role of syntrophic microbial communities in high-rate methanogenic bioreactors

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ABSTRACT

Anaerobic purification is a cost-effective way to treat high strength industrial wastewater. Through anaerobic treatment of wastewaters energy is conserved as methane, and less sludge is produced. For high-rate methanogenesis compact syntrophic communities of fatty acid-degrading bacteria and methanogenic archaea are essential. Here, we describe the microbiology of syntrophic communities in methanogenic reactor sludges and provide information on which microbiological factors are essential to obtain high volumetric methane production rates. Fatty-acid degrading bacteria have been isolated from bioreactor sludges, but also from other sources such as freshwater sediments. Despite the important role that fatty acid-degrading bacteria play in high-rate methanogenic bioreactors, their relative numbers are generally low. This finding indicates that the microbial community composition can be further optimized to achieve even higher rates.

Key words | anaerobic treatment, granulation, methanogenesis, syntrophic communities, UASB reactor

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ANAEROBIC WASTEWATER TREATMENT

Anaerobic treatment is one of the most cost-effective ways to treat industrial wastewater with high organic matter content (Kosaric & Blaszczyk 1990; Lettinga 1995; van Lier *et al.* 2001). In particular, the introduction of the Upflow Anaerobic Sludge Blanket (UASB) reactor, over three decades ago, has resulted in improved treatment of industrial wastewaters. In a UASB reactor the wastewater is pumped from the bottom into the reactor, and is purified while passing a bed of compact biomass (granular sludge), which is formed by self-immobilization of anaerobic bacteria and methanogenic archaea (Lettinga *et al.* 1980; van Lier *et al.* 2001). The high density of these granules prevents microorganisms from being washed out, while the short intermicrobial distances are favourable for the transfer of metabolites from the bacteria to the methanogens. A variety of additional anaerobic bioreactor designs has been developed. Expanded Granular Sludge Blanket (EGSB) and Internal Circulation (IC) reactors have replaced the more conventional UASB systems (Franklin 2001). EGSB systems have a comparable design to UASB reactors, but contain an expanded granular sludge bed minimizing external mass transfer limitations around the biofilm. Anaerobic treatment systems can operate at different temperatures and are suited for different types of wastewater. Currently, anaerobic treatment technologies are widely applied to treat wastewaters from food processing industries, pulp and paper industries and chemical and petrochemical industries (Lettinga 1995; Macarie 2000).

METHANOGENESIS

Physiologically and phylogenetically different microbial groups are involved in the mineralization of complex organic matter to methane and carbon dioxide (Gujer & Zehnder 1983; de Bok *et al.* 2004; Stams *et al.* 2006). In general, biopolymers including proteins, carbohydrates, nucleic acids and fats are first hydrolyzed to mono- and oligomers, and these are then fermented to products that can be used by methanogens directly (acetate, hydrogen, formate) and to other fatty acids such as propionate, butyrate, branched-chain fatty acids and long-chain fatty acids (Gujer &

doi: 10.2166/wst.2012.192

Zehnder 1983). Acetogenic bacteria oxidize these fatty acids anaerobically to acetate, CO_2, H_2 and formate (Gujer & Zehnder 1983; Stams & Plugge 2009). Generally, about 70% of the methane is formed from acetate, while the remainder is formed from H_2/CO_2 and formate (Gujer & Zehnder 1983; Conrad 1999). However, acetate can also be degraded by syntrophic communities of bacteria and archaea, resulting in a more prominent role of H_2/CO_2 and formate as methanogenic substrates (Hattori 2008). In mesophilic digesters, high ammonia levels can cause syntrophic acetate oxidation (Westerholm *et al.* 2010). The ammonia tolerance of these syntrophic acetate oxidizers gives them a competitive advantage in ammonia-stressed systems. These bacteria, in association with ammonia-tolerant hydrogenotrophic methanogens, may consequently adopt the role of dominant acetate consumers in environments in which ammonia restrains aceticlastic methanogenic activity (Westerholm *et al.* 2011).

Propionate and butyrate are key intermediates in the mineralization of complex organic matter. The complete oxidation of propionate and butyrate can account for 20–43% of the total methane formation, depending on the type of digester and the nature of the organic compounds (Mackie & Bryant 1981).

SYNTROPHIC FATTY ACID DEGRADATION

Easily degradable compounds (soluble polysaccharides, proteins, sugars and amino acids) in industrial wastewater are rapidly degraded by primary fermenters during storage and the passage to the anaerobic bioreactor. Therefore, the main substrates that enter the anaerobic bioreactor are mixtures of fatty acids, mainly acetate, propionate and butyrate. Propionate and butyrate are important intermediates in methanogenesis. They are formed in the anaerobic fermentation of polysaccharides and proteins (Stams 1994; Schink & Stams 2006). Typically these fatty acids are further degraded by syntrophic associations of anaerobic bacteria and methanogenic archaea, which are dependent on each other for energy conservation and growth (McInerney *et al.* 2008; Sousa *et al.* 2009; Stams & Plugge 2009). For complete and high-rate methanogenesis such syntrophic communities are indispensable.

Bacteria that degrade and grow on fatty acids have to cope with the unfavourable energetics of the conversion processes. Table 1 illustrates the conversion of propionate and butyrate to the methanogenic substrates acetate, hydrogen and formate. It is evident that bacteria can only derive energy for growth from these conversions when the concentration of the products (especially hydrogen and formate) is kept low. This results in an obligate dependence of acetogenic bacteria on methanogenic archaea. Obligately syntrophic communities of propionate- and butyrate-degrading acetogenic bacteria and methanogenic archaea have several unique features: they degrade fatty acids coupled to growth, while neither the bacterium nor the methanogen alone is able to degrade these compounds; intermicrobial distances influence biodegradation rates and specific growth rates, which in methanogenic bioreactors results in the formation of aggregates of bacteria and archaea (granular sludge); the syntrophic communities grow in conditions that are close to thermodynamical equilibrium, and the communities have evolved biochemical mechanisms that allow sharing of chemical energy (Stams & Plugge 2009). There is still discussion on whether hydrogen and formate are the primary compounds for interspecies electron transfer, and it is still unclear what their relative importance is (Stams & Plugge 2009; Müller *et al.* 2010; Worm *et al.* 2011).

Table 1 | Standard Gibbs free energy changes of reactions involved in syntrophic propionate and butyrate degradation. Data from Thauer *et al.* (1977)

Reaction	$\Delta G^{0'}$
Fatty-acid oxidation by acetogens	
$Propionate^- + 3H_2O \Rightarrow acetate^- + HCO_3^- + H^+ + 3H_2$	$\Delta G^{0'} = +76$ kJ
$Propionate^- + 2HCO_3^- \Rightarrow acetate^- + H^+ + 3$ formate	$\Delta G^{0'} = +72$ kJ
$Butyrate^- + 2H_2O \Rightarrow 2acetate^- + H^+ + 2H_2$	$\Delta G^{0'} = +48$ kJ
$Butyrate^- + 2HCO_3^- \Rightarrow 2\ acetate^- + H^+ + 2\ formate^- + 3H_2O$	$\Delta G^{0'} = +46$ kJ
Hydrogen and formate utilization by methanogens	
$4H_2 + HCO_3^- + H^+ \Rightarrow CH_4 + 3H_2O$	$\Delta G^{0'} = -136$ kJ
$4\ Formate^- + H^+ \Rightarrow 3HCO_3^- + 2H_2O + CH_4$	$\Delta G^{0'} = -130$ kJ
Acetate utilization by methanogens	
$Acetate^- + H_2O \Rightarrow HCO_3^- + CH_4$	$\Delta G^{0'} = -31$ kJ

The reported maximum specific growth rates of propionate- and butyrate-degrading acetogenic bacteria in suspended co-culture with methanogens were 0.10 and 0.19 day^{-1}, respectively (McInerney *et al.* 1979; Boone & Bryant 1980; Mountfort & Bryant 1982). It is evident that fatty acid-degrading bacteria grow slowly, but it is not clear fully how fast they can grow in tight physical aggregation with methanogens. In fact, specific growth rates of syntrophic communities are difficult to determine as the conversion rate per cell is not constant, but is dependent on cell density of the partner microorganism.

Syntrophic propionate-degrading bacteria

Boone & Bryant (1980) described *Syntrophobacter wolinii*, a propionate-degrading bacterium that grows in syntrophic association with methanogens or sulfate-reducing bacteria (Table 2). Several other mesophilic and thermophilic bacteria that grow in syntrophy with methanogens have been described since then as reviewed by McInerney *et al.* (2008). These include Gram-negative bacteria (*Syntrophobacter* and *Smithella*) and Gram-positive bacteria (*Pelotomaculum* and *Desulfotomaculum*). Both groups are phylogenetically related to sulfate-reducing bacteria and some species are also able to use sulfate as a terminal electron acceptor for growth. *Syntrophobacter* and *Desulfotomaculum* species reduce sulfate, but *Smithella* and *Pelotomaculum* cannot reduce sulfate. Most syntrophic propionate-degrading bacteria are able to grow in pure culture by fermentation of fumarate or pyruvate. Fermentative growth or sulfate-dependent growth was used to obtain the bacteria in pure culture. The only exceptions are *Pelotomaculum schinkii* (de Bok *et al.* 2005) and *P. propionicicum* (Imachi *et al.* 2007), which seem to be true propionate-degrading syntrophs. *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). In sulfate-rich environments sulfate reduction is the main terminal process and several sulfate reducers that degrade propionate have been described. Therefore, in marine habitats syntrophic growth on propionate seems to be restricted to sulfate-depleted zones.

Two pathways for propionate metabolism are known, the methylmalonyl-CoA pathway 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.

Syntrophic butyrate- and LCFA-degrading bacteria

McInerney *et al.* (1981) enriched and characterized *Syntrophomonas wolfei*, a Gram-positive bacterium that degrades butyrate and some other short-chain fatty acids in syntrophic association with methanogens (Table 2). Several other bacteria have been described that grow with butyrate or higher fatty acids in syntrophy with methanogens or sulfate reducers as hydrogen scavengers (McInerney *et al.* 2008; Sousa *et al.* 2009). Thus far, *Algorimarina butyrica* is the only psychrophilic and marine bacterium that is known to degrade butyrate in syntrophy with methanogens (Kendall *et al.* 2006). This bacterium is not able to grow with other short-chain fatty acids like valerate or caproate or longer chain fatty acids such as palmitate. Mesophilic bacteria capable of syntrophic fatty acid metabolism are mainly species of *Syntrophomonas*, though *S. bryantii* was previously named *Syntrophospora bryantii* and *Clostridium bryantii* (Stieb & Schink 1985; Zhao *et al.* 1990; Wu *et al.* 2006a; Sousa *et al.* 2009). The only exception is

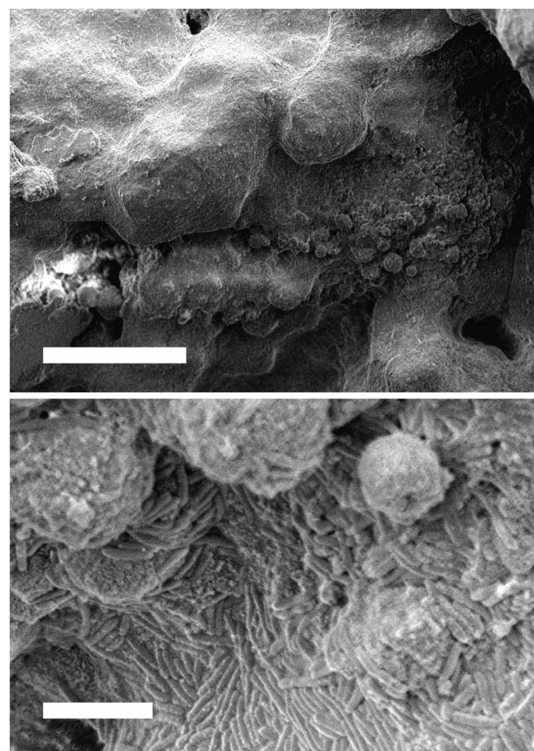

Figure 1 | Scanning electron microscopic (SEM) images that show embedded micro-colonies in an anaerobic methanogenic granule. The bar represents 50 μM (upper panel) and 5 μM (lower panel).

Table 2 | Isolation source of fatty-acid degrading bacteria (in grey: bacteria isolated from anaerobic reactor sludge) and the respective methanogenic partner

Syntrophic bacterium	Syntrophic partner	Substrates used in co-culture[a]	Isolated from	References
Syntrophobacter fumaroxidans	*Methanospirillum hungatei*	C_3	Granular sludge from a full-scale UASB reactor treating sugar beet waste, Breda, The Netherlands	Harmsen *et al.* (1998)
Syntrophobacter pfennigii	*Methanospirillum hungatei*	C_3	Anoxic sludge of a municipal sewage plant in Konstanz, Germany	Wallrabenstein *et al.* (1995)
Syntrophobacter sulfatireducens	*Methanospirillum hungatei*	C_3	Granular sludge from a UASB reactor treating brewery wastewater in Beijing, China	Chen *et al.* (2005)
Syntrophobacter wolinii	*Methanospirillum hungatei* *Desulfovibrio* sp.	C_3	Anaerobic sewage digester sludge in Illinois, USA	Boone & Bryant (1980)
Pelotomaculum schinkii	*Methanospirillum hungatei*	C_3	Granular sludge from a full-scale UASB reactor treating sugar beet waste, Breda, The Netherlands	de Bok *et al.* (2005)
Pelotomaculum thermopropionicum	*Methanothermobacter themautotrophicus*	C_3	Granular sludge from a thermophilic laboratory-scale UASB reactor, Japan	Imachi *et al.* (2002)
Pelotomaculum propionicicum	*Methanospirillum hungatei*	C_3	Granular sludge from a mesophilic laboratory-scale UASB reactor, Japan	Imachi *et al.* (2007)
Smithella propionica	*Methanospirillum hungatei* *Methanogenium* sp.	C_3, C_4	Granular sludge from a mesophilic laboratory-scale UASB reactor, USA	Liu *et al.* (1999)
Desulfotomaculum thermobenzoicum thermosyntrophicum	*Methanothermobacter thermautotrophicus*	C_3, C_4	Granular sludge from a thermophilic laboratory-scale UASB reactor, The Netherlands	Plugge *et al.* (2002)
Desulfotomaculum thermocisternum	*Methanococcus thermolithotrophicus*	C_3, C_4	North Sea oil reservoir formation water, Norway	Nilsen *et al.* (1996)
Algorimarina butyrica	*Methanogenium* sp.	C_4	Permanently cold, shallow anoxic marine sediments in Skan Bay, Alaska	Kendall *et al.* (2006)
Syntrophomonas bryantii	*Methanospirillum hungatei* *Desulfovibrio* sp.	C_4–C_{11}	Marine anoxic mud taken near Cuxhaven, mud of a creek near Konstanz, anaerobic digester sludge, Germany	Stieb & Schink (1985); Zhao *et al.* (1990); Wu *et al.* (2006a)
Syntrophomonas cellicola	*Methanobacterium formicicum* *Desulfovibrio* sp.	C_4–C_8, C_{10}	Sample from the walls of a distilled-spirit fermenting cellar in Hebei province, China	Wu *et al.* (2006a)
Syntrophomonas curvata	*Methanobacterium formicicum*	C_4–C_{18}, $C_{18:1}$	Granular sludge from a UASB reactor treating brewery wastewater in Beijing, China	Zhang *et al.* (2004; 2005)
Syntrophomonas erecta erecta	*Methanospirillum hungatei*	C_4–C_8	Granular sludge of a UASB reactor treating bean-curd farm wastewater in Beijing, China	Zhang *et al.* (2005); Wu *et al.* (2006b)
Syntrophomonas erecta sporosyntropha	*Methanobacterium formicicum*	C_4–C_8	River sediment in Beijing and rice field mud in Yunnan Province, China	Wu *et al.* (2006b)

(*continued*)

Table 2 | continued

Syntrophic bacterium	Syntrophic partner	Substrates used in co-culture[a]	Isolated from	References
Syntrophomonas palmitatica	*Methanospirillum hungatei*	C_4–C_{18}	Granular sludge from a laboratory-scale UASB reactor treating palm oil mill effluent, Japan	Hatamoto *et al.* (2007)
Syntrophomonas saponavida	*Methanospirillum hungatei* *Desulfovibrio* sp.	C_4–C_{18}	Anaerobic sewage digester sludge in Illinois, USA	Lorowitz *et al.* (1989); Wu *et al.* (2007)
Syntrophomonas sapovorans	*Methanospirillum hungatei*	C_4–C_{18}, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$	Sewage digester sludge in Marquette-Lez-Lille, France	Roy *et al.* (1986); Zhang *et al.* (2005)
Syntrophomonas wolfei methylbutyratica	*Methanobacterium formicicum*	C_4–C_8	Rice field mud in Jangxi province, China	Wu *et al.* (2007)
Syntrophomonas wolfei wolfei	*Methanospirillum hungatei* *Desulfovibrio* sp.	C_4–C_8	Sewage digester sludge and rumen digester, USA	McInerney *et al.* (1979; 1981); Zhang *et al.* (2005)
Syntrophomonas zehnderi	*Methanobacterium formicicum*	C_4–C_{18}, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$	Anaerobic sludge from an EGSB treating oleate-based effluent, Braga, Portugal	Sousa *et al.* (2007)
Syntrophus aciditrophicus	*Methanospirillum hungatei* *Desulfovibrio* sp.	C_4–C_8, C_{16}, C_{18}	Sewage sludge from a municipal sewage treatment plant in Norman, Oklahoma, USA	Jackson *et al.* (1999)
Syntrophothermus lipocalidus	*Methanothermobacter thermoautotrophicus*	C_4–C_{10}	Granular sludge from a laboratory-scale thermophilic UASB reactor, Japan	Sekiguchi *et al.* (2000)
Thermosyntropha lipolytica	*Methanobacterium* sp.	C_4–C_{18}, $C_{18:1}$, $C_{18:2}$	Alkaline hot springs of Lake Bogoria, Kenya	Svetlitshnyi *et al.* (1996)
Thermosyntropha tengcongensis	*Methanothermobacter thermautotrophicus*	C_4–C_{18}, $C_{18:1}$, $C_{18:2}$	Hot spring in Yunnan province, China	Zhang *et al.* (2012)

[a]The number of carbon atoms of the fatty acids and the position of the double bond are indicated.

Syntrophus aciditrophicus, a Gram-negative benzoate-degrading bacterium that it is also able to degrade medium- and long-chain fatty acids in co-culture 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 methanogens has been described to reduce sulfate. Most fatty acid degraders are able to ferment crotonate, which was used to obtain pure cultures. However, *Syntrophomonas sapovorans* and *S. zehnderi* are not able to ferment crotonate, and are only available in syntrophic methanogenic co-cultures (Roy *et al.* 1986; Sousa *et al.* 2007).

Butyrate and longer chain fatty acids are degraded via so-called β-oxidation (Schink & Stams 2006; McInerney *et al.* 2008). In a series of reactions acetyl groups are cleaved off yielding acetate, hydrogen and formate. 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.

EFFECT OF GRANULATION

The formation of granular sludge is one of the reasons for the success of anaerobic treatment of wastewaters (Kosaric & Blaszczyk 1990; Lettinga 1995). In particular, aggregation of syntrophic communities is essential to achieve high rates of methanogenesis with propionate and butyrate. Figure 1 illustrates the dense packing of microorganisms and micro-colonies in the granule structure. As pointed out by

Table 3 | Detection and quantification of syntrophic bacteria in anaerobic reactors

Reactor type	Reactor feed and operational conditions	Syntrophic bacteria abundance	Detection/Quantification method	Reference
Mesophilic laboratory-scale UASB reactor	Industrial wastewater from a brewery $OLR = 4\ g_{COD}\ L^{-1}\ day^{-1}$; $T = 30\ ^{\circ}C$	*Syntrophobacter* spp. 5–10%	FISH (probe SYN835)	Férnandez *et al.* (2008)
Thermophilic full-scale anaerobic reactor	Municipal sewage sludge $COD_{in} = 59\ g\ L^{-1}$; $T = 53\ ^{\circ}C$	*Pelotomaculum* spp. 0.1%	Real-time PCR (primers DEM116f-Ih820)	Imachi *et al.* (2006)
Thermophilic UASB laboratory-scale reactor	Synthetic wastewater containing sucrose, acetate, propionate, and peptone or yeast extract $COD_{in} = 4\ g\ L^{-1}$; $T = 55\ ^{\circ}C$	*Pelotomaculum* spp. 1.1%	Real-time PCR (primers DEM116f-Ih820)	Imachi *et al.* (2006)
		0.5 ± 0.2%	FISH (probe Ih820)	
Thermophilic laboratory-scale anaerobic reactor	Wastewater from clear liquor manufacture $COD_{in} = 12\ g\ L^{-1}$; $T = 55\ ^{\circ}C$	*Pelotomaculum* spp. 3.9%	Real-time PCR (primers DEM116f-Ih820)	Imachi *et al.* (2006)
		4.1 ± 1.1%	FISH (probe Ih820)	
Mesophilic UASB laboratory-scale reactor	Synthetic wastewater $COD_{in} = 2\ g\ L^{-1}$; $T = 35\ ^{\circ}C$	*Pelotomaculum* spp. 0.1%	Real-time PCR (primers DEM116f-Ih820)	Imachi *et al.* (2006)
		<0.1%	FISH (probe Ih820)	
Mesophilic full-scale anaerobic reactor	Municipal sewage sludge $COD_{in} = 58\ g\ L^{-1}$; $T = 35\ ^{\circ}C$	*Pelotomaculum* spp. 0.3%	Real-time PCR (primers DEM116f-Ih820)	Imachi *et al.* (2006)
Mesophilic laboratory-scale reactor (inoculated with sludge from an anaerobic digester from municipal wastewater treatment plant)	Synthetic wastewater made from powdered whole milk $OLR = 1.5\ g_{COD}\ L^{-1}\ day^{-1}$; $T = 37\ ^{\circ}C$	*Syntrophobacter*, *Smithella* spp. 2.7–7.3%	MAR-FISH (probes SmiSR354, SmiLR150, Synbac824)	Ariesyady *et al.* (2007b)
		Syntrophomonadaceae detected but not quantified	MAR-FISH (probe Synm700)	
Mesophilic full-scale anaerobic digester (two reactors)		*Syntrophobacter fumaroxidans* 0.27 ± 0.14% /0.37 ± 0.12%	Membrane hybridization (probe S.fum464)	McMahon *et al.* (2004)
		Syntrophobacter pfennigii 0.33 ± 0.08% /0.48 ± 0.08%	Membrane hybridization (probe S.pfn460)	
		Syntrophobacter wolinii 0.44 ± 0.12% /0.43 ± 0.11%	Membrane hybridization (probe S.wol223)	
		Smithella propionica <0.2% /0.28 ± 0.29%	Membrane hybridization (probe S.pro450)	
		Syntrophomonadaceae 0.42 ± 0.16% /1.76 ± 0.25%	Membrane hybridization (probe Synm700)	

(*continued*)

Table 3 | continued

Reactor type	Reactor feed and operational conditions	Syntrophic bacteria abundance	Detection/Quantification method	Reference
Mesophilic laboratory-scale anaerobic digester	Synthetic mixture of organic fraction of municipal solid waste, primary sludge and waste activated sludge OLR= 9.4 kg_{VS} m^{-3} day^{-1}; T= 37 °C	*Syntrophobacter fumaroxidans* 0.25 ± 0.05% *Syntrophobacter pfennigii* 0.67 ± 0.07% *Syntrophobacter wolinii* 0.47 ± 0.06% *Smithella propionica* 0.23 ± 0.04% *Syntrophomonadaceae* 0.65 ± 0.03%	Membrane hybridization (probe S.fum464) Membrane hybridization (probe S.pfn460) Membrane hybridization (probe S.wol223) Membrane hybridization (probe S.pro450) Membrane hybridization (probe Synm700)	McMahon *et al.* (2001)
Mesophilic full-scale anaerobic digester plant	Excess sludge of domestic wastewater treatment facility OLR= 2.5 kg m^{-3} day^{-1}; T= 40 °C	*Syntrophus, Smithella propionica* 2.0 ± 0.7% *Syntrophobacter* 0.5 ± 0.4% *Syntrophomonas* 1.5 ± 0.5%	FISH (probe SmiSR354) FISH (probe Synbac824) FISH (probe Synm700)	Ariesyady *et al.* (2007a)
Mesophilic full-scale anaerobic reactor	Mixture of swine and cattle manure and a variety of industrial organic waste streams	*Syntrophomonadaceae* 0.2–1% *Syntrophomonas* 0.2–1%	Membrane hybridization (probe Synm700) Membrane hybridization (probe Syn126)	Hansen *et al.* (1999)
Mesophilic full-scale anaerobic reactors (four reactors)	Edible tallow refinery wastewater Poultry slaughterhouse wastewater Brewery wastewater Potato processing wastewater	*Syntrophomonadaceae* 3% <0.01% <0.01% <0.01%	FISH (probe Butox)	Menes & Travers (2006)
Anaerobic lagoon	Woolscouring wastewater	*Syntrophomonadaceae* <0.01%	FISH (probe Butox)	Menes & Travers (2006)
Mesophilic full-scale anaerobic digester plant	Excess sludge of domestic wastewater treatment facility OLR= 2.5 kg m^{-3} day^{-1}; T= 40 °C	*Syntrophus, Smithella propionica* 2.0 ± 0.7% *Syntrophobacter* 0.5 ± 0.4% *Syntrophomonas* 1.5 ± 0.5%	FISH (probe SmiSR354) FISH (probe Synbac824) FISH (probe Synm700)	Ariesyady *et al.* (2007a)

OLR: Organic loading rate; COD: Chemical oxygen demand; FISH: Fluorescence in situ hybridization; MAR-FISH: Microautoradiography combined with FISH; Real-time PCR: Real-time polymerase chain reaction.

Schink & Thauer (1988) by bringing hydrogen-producing bacteria into close proximity with hydrogen-consuming methanogens the distance that hydrogen has to diffuse from the producing to the consuming microorganisms becomes shorter, and this results in higher conversion rates. Thus, the compact microbial structure in methanogenic granular sludge is functional. It has been observed that when the structure is disrupted the specific methanogenic activity with propionate decreases considerably, while the specific methanogenic activity with acetate is not affected (Grotenhuis *et al.* 1991; Schmidt & Ahring 1995).

Another factor that affects the specific activity is the size of the granules. When granules are too large, the centre of the granules will become substrate limited. Moreover, biogas may accumulate in the granules that are weakened in structure inside. This may cause a disintegration of the mature granules into smaller particles that grow again. In this way, the average size of the granules is determined by the wastewater composition.

In the granules competition for space takes place as well. To efficiently degrade fatty acids, the microorganisms in the granule should mainly consist of acetogenic fatty acid-degrading bacteria and acetoclastic and hydrogenotrophic methanogens. Fluorescence *in situ* hybridization (FISH) has been used to study the structural organization of anaerobic granular sludge (Harmsen *et al.* 1996; Sekiguchi *et al.* 1999). In these studies, a close association between *Syntrophobacter* species and hydrogenotrophic methanogens belonging to the *Methanomicrobiales* group has been observed. Nevertheless, microbial community analysis with molecular techniques has shown that granular sludge of full-scale reactors rarely harbours high numbers of acetogenic bacteria (Table 3). This suggests that fatty acid-degrading communities establish high specific conversion rates, and that even higher conversion rates can be achieved by minimizing the numbers of primary fermenters in the granules.

Still, due to the limitations in quantification of individual groups of bacteria in mixed anaerobic biomass, fluxes can hardly be attributed to concentrations of specific microorganisms. Consequently, conversion rates are expressed per amount of lumped biomass in terms of volatile suspended solids (VSS). Herewith, it remains unclear if energy is shared equally between the partner microorganisms and what the mutual effects of different acetogenic substrates are on their degradation. Detailed quantitative insight is required about the electron fluxes between the partner organisms in anaerobic sludges and the way they share the chemical energy available in the conversion of substrates. Relatively novel molecular biology-based tools allow for quantification of different trophic groups in anaerobic environments and combined with flux measurements will allow for a detailed understanding of anaerobic conversion processes. Based on the improved understanding of electron transfer in anaerobic environments it can readily be envisioned that the anaerobic digestion process can still be improved significantly.

CONCLUSIONS

High-rate methanogenesis is required to treat industrial wastewaters efficiently and conserve the chemical energy in the organic pollutants as biogas. In the complete conversion of organic pollutants to methane and carbon dioxide, syntrophic methanogenic communities of bacteria and archaea play an indispensable role. Both Gram-negative (deltaproteobacteria) and Gram-positive bacteria with the ability to grow with propionate and butyrate in syntrophy with methanogens have been described. Some of the described bacteria have been obtained in pure culture, while others are only maintained in syntrophic co-culture with a defined methanogen. In high-rate methanogenic bioreactors propionate-degrading and butyrate-degrading acetogenic bacteria form microcolonies with methanogens. The short distances between the bacteria and methanogens are favourable for transfer of metabolites from bacteria to archaea and result in high conversion rates. To further improve the rate of methane formation with mixtures of fatty acids as substrates the underlying mechanisms of interspecies electron transfer in syntrophic communities need to be better understood. In addition, insight is needed into how by process engineering the compact structure of syntrophic communities in granules can be obtained and maintained.

ACKNOWLEDGEMENT

Our research is funded by grants from the division of Chemical Sciences (CW) and Earth and Life Sciences (ALW) of The Netherlands Organisation for Scientific Research (NWO) and by the Technology Foundation (STW), the applied science division of NWO.

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First received 16 September 2011; accepted in revised form 5 March 2012