CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN978-88-95608- 56-3; **ISSN** 2283-9216
DOI: 10.3303/CET1864066

Syntrophic Microorganisms Interactions in Anaerobic Digestion (Ad): a Critical Review in the Light of Increase Energy Production

Carlos E. Gomez Camacho, Bernardo Ruggeri*

Politecnico di Torino, Dep. of Applied Science and Technology (DISAT), C/so Duca degli Abruzzi 24, 10129 Torino, Italy; bernardo.ruggeri@polito.it

Despite the worldwide growth of Anaerobic Digestion (AD) plants able to produce biogas, further investigations on the cooperation of syntrophic organisms within mixed cultures are necessary. Microbiomes in AD bioreactors depend on process conditions, heterogeneities and on the intrinsic characteristics of microorganisms. However, *chemotropic* organisms obtain energy by extracting an electron flow from different organic compounds that are successively passed through different Red-Ox cycles, supported by enzymes under different red-ox potentials (E°). Oxidation-reduction cycles are present not only at intracellular level, but also as interspecies (exocellular) electron exchange, mediated through chemical compounds (Shuttled Interspecies Electron Transfer, SIET) or based on electrical interactions between bacteria (Direct Interspecies Electron Transfer, DIET). Metal particles, namely Fe, via anaerobic corrosion (AC) generates Fe^{2+} and *e-* that interact with the biological system suggesting that a higher energy recovery is achievable, due to modification of syntrophic relationships and to positive bio-stimulatory effects. This review addresses the state-of-the-art of exocellular syntrophy among microorganisms SIET and DIET, introduces the AC of Fe° under the operative conditions, summarizes the different laboratory-scale studies that have been performed with metals particles and hypothesizes energy improvement mechanisms in the light of enhancement of AD performances.

1. Introduction

Energy conservation within the AD process is a complex phenomenon strongly dependent on *mutualism* among mixed consortia of microorganisms. The widespread use of this technique in different areas and the versatility that it offers for energy-recovering under bio-H_2 and bio-CH_4 using different substrates is very ample. From the energy sustainability point of view, AD is an advantageous process because it provides a relative inexpensive technique to valorize the energy contained in organic wastes (OW) and transform it into biogas, a gas mixture containing mainly CH_4 and CO_2. Desired biogas composition is around 70 % v/v in methane, but real yields are in the range of 40-60 % *v/v* with an important unavoidable fraction of carbon dioxide (> 30%). Biogas, in fact, is curious because it is constituted essentially of two gases where C atoms exhibits the maximum difference in their oxidation state: -4 in CH_4 totally reduced, whereas +4 in the CO_2 the most oxidized state. Microorganisms can be widely classified according to their metabolic requirements. *Heterotrophs* use always organic carbon coupled either to organic (*chemoorganotrophs*) or inorganic compounds (*chemolithotrophs*), or when sunlight is used as energy source, they are classified as *photoheterotroph.* Differently, *autotrophs* can grow on CO_2 as carbon source, using the energy contained in sunlight (*photoautotroph*) or different compounds (*chemoautotroph).* Within the microbial consortia of AD, microorganisms of interest belong to *Archaea* and *Bacteria* domains, hence prokaryotes, *chemotropic* either *chemoorganotrophs* or *chemoautotroph.* For chemotrophs, the degradation of substrates inside a bacterial cell produces energy and electrons that are released mainly from the broken of C-C and C-H bonds of substrates. The others vital functions of the cell, that require energy, are known as anabolism. For the good functioning of the cell, catabolic reactions are coupled to anabolic processes and the energy flux from the first is fed into growth and maintenance mechanisms. The extracted chemical energy, in the form of an electron flow is mediated at intracellular level by different intracellular redox mediators (i.e. ferredoxins, flavodoxins,

Please cite this article as: Gomez Camacho C.E., Ruggeri B., 2018, Syntrophic microorganisms interations in anaerobic digestion (ad): a critical review in the light of increase the energy production, Chemical Engineering Transactions, 64, 391-396 DOI: 10.3303/CET1864066

rubredoxins, NAD, FAD) that are continuously reduced and oxidized, at different redox potential, depending on the metabolism conditions of each specie (Thauer *et al.*, 2008), by different enzymatic groups.
Biogas production through AD is a task carried out by different groups of microorganisms, whose actions are concatenated: first comes the *hydrolysis* of large molecules (i.e. polysaccharides, lipids, proteins and nucleic acids) into monomers and the successive primary fermentation (*acidogenesis*) that converts these substances to short-chain organic acids or volatile fatty acids (VFA) as butyric, propionic, n-butyric, lactic, acetic and formic acids and gases as H_2 and CO_2 to provide the necessary metabolites for the last two steps of the AD process: *acetogenesis* and *methanogenesis*. The acetogenic phase is responsible for the further oxidation of VFA into acetate, releasing an electron flow, which is ultimately used for the methane production. Mixed cultures used in AD exhibit a great level of complexity and organization, where each group of microorganisms (i.e. *hydrolytic, acidogenic, acetogenic and methanogenic*) has a defined function during the fermentation, which cannot be replaced by other group, hence it is important to maintain significant levels of each. In fact, the process conditions of a bioreactor are carefully chosen taking into consideration the microorganisms growth requirements so that they can exercise their role within the complex biological system. Among all the phases present, the *methanogenesis* requires slightly different optimal conditions compared to the other phases. Such conditions are, for example, a narrower pH range, strictly anaerobic conditions with redox < -330 mV, salinity, the need of micronutrients (Ni, Co, Mo, Se) and the presence of the intermediate compounds necessary for the metabolic pathways of *hydrogenothropic* and *acetoclastic methanogenesis*. Each methanogenic pathway, presented in Table 1 (i.e. reactions 7 and 8) requires different substrates, which are mainly produced during *acetogenesis*; hydrogenothropic pathway fixes CO_2 and H_2 into CH_4, while the acetoclastic one cleaves acetate molecules into CH_4 and CO_2 (Gottardo *et al.*, 2015).

2. Overall Energy Balance in AD processes

The transfer of chemical energy within biological systems is carried out through redox reactions, where (mainly) carbon molecules do not remain in the same oxidation state. Electrons are extracted from the oxidized compound and are accepted by a reductive agent. Typically, oxidation and reduction reactions in these types of systems exchanged not only e^- but whole atoms of H_2.

Table 1: Key fermentation and methanogenic reactions in AD

	Name	Reaction	[a] $\Delta G^{0'}$ (kJ/mol)	Pairs of Electrons
1.	Methane Combustion	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	-827	4
2.	Ethanol oxidation	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	+10	2
3.	Propionate oxidation	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$	+72	3
4.	Butyrate oxidation	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	+48	2
5.	Acetate oxidation	$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 4H_2$	+95	4
6.	Homoacetogenesis	$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	-95	4
7.	Hydrogenotrophic pathway	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-131	4
8.	Acetoclastic pathway	$CH_3COOH \rightarrow CH_4 + CO_2$	-36	2
9.	Fe° oxidation and CO_2 reduction	$4Fe^\circ + 8H^+ + CO_2 \rightarrow CH_4 + 4Fe^{2+} + 2H_2O$		4

[a] *Data calculated from* (Thauer, Jungermann and Decker, 1977)

A general approach used in biochemistry to study the direction and feasibility of metabolic pathways is the evaluation of the standard change of Gibbs free energy ($\Delta G°$) of a given reaction at standard physiological conditions ($\Delta G°'$). Values of $\Delta G°'$, hence, represent the driving force of the process and can be expressed by means of the Nerst Equation ($\Delta G°' = -nF\Delta E°'$) in terms of electrochemical potential ($\Delta E°'$). This difference in energy content between reactants and products, expressed as $\Delta E°'$ or $\Delta G°'$, provides valuable information about the studied system: *i)* indicates in which direction the reaction will proceed (i.e. *exergonic* or *endergonic*) and *ii)* allows knowing the energy level of the final product. Besides, considering the thermodynamic relation $\Delta G = \Delta H - T\Delta S$, the efficiency and performance of the AD systems is commonly assessed by evaluating the $\Delta_f H$ or Low Heating Value (LHV_{biogas}) of the resulting gas, compared to the initial energy content of the substrate $LHV_{substrate}$ (Malavè *et al.*, 2017).

3. Syntrophic relations

Within AD systems, between the *acetogenic* and the *methanogenic* phase, there have been identified syntrophic relationships where both groups of microorganisms strongly depend on each other. Bryant *et al.*, (1967) first evinced these type of relation, proposing the "*Interspecies Electron Transfer*" theory. The observation of a *Methanobacillus omelianskii* culture, an anaerobic methanogenic culture that was thought to

be pure, lead to the conclusion that there were two strains involved for CH_4 production that established a syntrophic relation. That is, the first strain "*S organism*" was able to oxidize ethanol, producing H_2 used by the second hydrogen-consuming bacterium, strain *Methanobacterium bryantii* M.o.H, to produce CH_4. During the separated culture of the two species, H_2 production was lower and CH_4 generation non-existent. These results first suggested that the syntrophic relation among these microorganisms was based on electron transfer: fermentative bacteria used as final electron carrier H^+, producing H_2 that was captured by methanogens to form CH_4 (reactions 2 and 7 from Table 1). The syntrophic relation, in that system, was not only limited to the electron transfer but also to the maintenance of a low hydrogen partial pressure (P_{H2}) that is necessity to favor thermodynamically the further production of hydrogen from fermentative bacteria.

It should be noted from Table 1, that the acetogenic phase (i.e. the oxidation of VFA and alcohols into acetate) takes place under endergonic conditions, at the expense of the next step, methanogenesis. This means that the syntrophic relation between acetogenic bacteria and methanogenic archaea is based on the overall free energy balance and in the trade of electrons and metabolites that these two groups exchange. The term of syntrophy, in AD context, is used then to describe the thermodynamically interdependent lifestyle where the degradation of a compound is couple to a successive reaction that takes place in other microorganism, and where specific conditions, such as metabolites concentrations (i.e. mainly H_2, formate and acetate) are maintained at specific values (Mcinerney, Sieber and Gunsalus, 2010). The syntrophic oxidation of different compounds has been widely revised in literature using substrates such as butyrate, propionate (Schmidt and Ahring, 1995), branched-chain fatty acids (Stams *et al.*, 2012), among others.

4. Interspecies Electron Transfer (IET)

IET can be seen as the most important phenomenon contributing to syntrophic relations in AD. Different studies (Stams *et al.*, 2006) summarize the different types of interspecies electron transfer in methanogenic environments. The exocellular interspecies electron transfer, in general, can be classified into two types:

a) Shuttled Interspecies Electron Transfer (SIET): an exchange mechanism mediated through chemical shuttle molecules (*electron carriers*), mainly referred to H_2 and formate,
b) Direct Interspecies electron transfer (DIET): a mechanism that does not involve chemical shuttles (shuttle-free), but rather relies on cytochromes, electrical conductive pili or aggregates formation to take place;

Evidence suggest that SIET is not only limited to H_2 and formate, but also a wide variety of compounds can serve as electron shuttles (e.g. sulphur compounds, humic acid complexes, flavins, cysteine, among others) for different types of microorganisms. Morris *et al.* (2013) further classified shuttle compounds as soluble or insoluble depending of their nature. Each microbial consortium presents different conditions for SIET, but general observations of AD sludges suggest that the main shuttles compounds are H_2 and formate. As noted before, fermentative bacteria release an electron flow derived from the degradation of substrates that reduce H^+ to H_2 or CO_2 to formate ($HCOO^-$). One important consideration must be done concerning the diffusion of these carries in the system. The formation of granules or aggregates within the anaerobic system has a strong effect on IET, for both types of transfer (SIET and DIET). Evidently, the diffusion of the carries compounds and the distance between syntrophic partners considerably influence which type of transport might occur. Schmidt and Ahring (1995) suggested that average distance between cells in intact granules is 2 to 3 μm and it was also noted that after disintegration the distance can reach up to 10 μm. An early study conducted by Boone, Johnson and Liu (1989) examined the diffusion of both carriers, H_2 and formate, for a *Syntrophomonas wolfei* and *Methanobacterium formicicum* in anoxic conditions and interspecies diffusion of H_2 and formate were estimated, demonstrating that IET not kinetically limited by enzymatic formation or consumption of these species but rather thermodynamically influenced by the involved species. Felchner-Zwirello, Winter and Gallert (2013) used a propionic acid enriched culture to study the interactions between aggregation and IET H_2 transfer. For this system, formate contribution to CH_4 generation was low and H_2-SIET accounted for most of the production. It was determined that over time, granules reached a maximum size (54 μm), where distance between electron acceptors and donors could be as short as 0.29 μm and neither diffusion constituted a limiting step. On the other hand, DIET relies on the physical interaction of the involved species. The general idea of direct electron transfer follows the same principle: one microorganism serves as an electron donor and/or other as electron acceptor, mediated by different structures such as electrochemical active enzymes, redox mediators, cytochromes, pili-like filaments and/or the formation of mixed aggregates of the involved species. Most of the research related to DIET has been focused on the bacterial *Geobacter* and *Shewanella* spp. According to Thauer *et al.* (2008), the presence of cytochromes in methanogens is almost limited to the *Methanosarcinales* (i.e. *Methanosarcina*, *Methanosaeta* and *Methanolobus*) and the reduction of CO_2 with H_2 (i.e.*hydrogenotrophic* pathway), proceeds differently for each case. Different evidence suggest that *Geobacter Sulfurreducens'* ability for extracellular electron transfer requires not only pili-like structures,

but also different types of cytochromes, that are required for electrical interactions with other species and for Fe^{3+} reduction (Shrestha and Rotaru, 2014). Moreover, expression of different types of cytochromes has been observed depending on whether *Geobacter Sulfurreducens* serves as cathode or anode during the electron exchange. Rotaru *et al.* (2012) studied a co-culture of *Pelobacter carbinolicus* and *Geobacter sulfurreducens* using three different modified strains for the experiments; their results showed that the primary carrier is H_2, and the observations suggested that SIET requires less coordination with syntrophic partners than DIET, probably because of the faster metabolizing via SIET and the less energetic investment for producing proteins necessary to generate the electrical connections/structures required for DIET.

5. Fe° supplementation to AD

IET interactions, in its broadest sense, can serve to understand the interaction of different metal particles or metal oxides with the biological system, particularly serving as electron sink for heterotrophic metabolisms or as electron supply for autotrophic pathways. Nowadays, different studies suggest that the supplementation of different metals might provide positive results enhancing the AD process, particularly due to the *Exogenous Electron Transfer* between the biological phase and inorganic compounds. Several metals and oxide have been tested (Co, Ni, Fe_3O_4) and among them, the most tested material so far is Fe°, nano-scale or micro-sized iron. The corrosion of Fe° has been studied independently, in particular to understand the behavior under different conditions (e.g. aerobic, anaerobic, sediments, etc.) and verify the average lifetime (Reardon, 1995). A first approximation of Fe° anaerobic corrosion (AC) is the study of the system Fe-H_2O under the AD conditions, evaluating the thermodynamic stable form of Fe, represented commonly in E_H *vs.* pH plots (Pourbaix Diagrams). Nevertheless, fermentation broths also comprise the biological phase as well as other organic and inorganic species, which can influence the speciation of the corrosion products. Considering the semi-reaction of zero valent iron (ZVI) corrosion at standard conditions (25 °C, pH = 7, a_{Fe2+}=1), which is $Fe° \rightarrow Fe^{2+} + e^-$, it presents a standard potential of E°'(V)= -0,440, which is similar to key biological reactions taking place in AD.Currently, obtained results exhibit not unified trend, as it can be seen on Table 2, sometimes different outputs appear contradictory and no study provides a holistic explanation capable of correlating experimental results with theoretical predictions. Different studies have tested metals particles (Table 2) ranging from a few nanometers up to the range of millimeters, and consequently exposed to diverse specific surfaces. However, higher reactivity based on the structure and size has not been directly related to cytotoxicity for all micro and nanoparticles (Karlsson *et al.*, 2009). To the best of the author's knowledge, cytotoxicity of nano- and micro-sized particles for the methane-producing microbial communities has not been fully assessed yet.

6. Conclusions

AD is seen today as successful technology for bioenergy production through OW energy valorization therefore the techniques directed towards the improvement of its performance must be kept economically and environmentally sustainable; probably due to this fact, some of the revised studies used waste metal oxides, iron powders, magnetite, waste scrap iron and even rusty iron scraps. However, the reviewed studies suggest that electrochemical activity of AD consortia is a global microbial phenomenon widely expressed in different AD systems, probably also in nature overall. The present review presents the results and the state-of-the-art concerning the empirical observations, but the direct explanation of enhancement mechanism for methane production, increased performance and the CH4 enrichment in the resulting biogas has not been fully clarified. Furthermore, the performance improvement for the studied systems might have multi-causal motivations, which responds to the action of the metal particles in the different stages of AD; the corollary of metal supplementation can be divided into four large blocks: i) enzymatic enhancement promoted by metals availability in the culture media, ii) effect on H2 threshold and population dynamics, iii) boost of electroactivity due to higher SIET, DIET and EET and iv) improvement of operating parameters. All these factors seem to the contribute of natural AD redox cycles interactions with redox cycles of metals particles with an overall energy production that is evidenced through the increase in biogas production and the higher percentage of methane in it. Therefore, syntrophy inside AD is a key phenomenon to understand by deeper analysis of electrochemical interactions among microorganisms and metals particles. More research is needed to assess effects of metals supplementations on biological systems, mechanisms for microbiological iron use and uptake, the speciation products of corrosion, the stability of micro- or nano- particles, optimal dosages and the scale-up feasibility of this technique to different levels from bioreactor to natural environments. Natural carbon cycles include the photosynthetic reduction of CO_2 into organic forms, using solar energy, to be subsequently re-oxidized by chemotrophs (or industrial activities), extracting the energy embedded in carbon molecules.

Reference	Vs_1 (g/L)	Substrate	Size	Added Compound	Doses (g/L)	Relevant results
(Yang, Guo and Hu 2013)	-	Glucose (Prepared medium)	55 nm	Fe°	0 0.06 0.56 1.68	- Inhibition from 1 mM NZVI (0.056 g/L)
			212 µm	Fe°	1.68	- ZVI increase methane production (around 10 %)
(Feng *et al.*, 2014)	6.54	Waste activated sludge	0 2 mm	Fe°	0 1 4 20	- Production of VFAs was enhanced by 37.3% (at 4 g/L) - V_{CH4} increased 43.5% (at 20 g/L) - % CH_4 went from 58,5 % to 68,9 % (at 20 g/L)
(Cruz Viggi *et al.*, 2014)	0.2	Prepared Medium	100-150 nm	Fe_3O_4 (Magnetite)	0 0 35	- CH_4 production rate increased by 30 % - Electron Transfer calculations suggest DIET
(Zhang *et al.*, 2014)	56.7	Dewatered sewage sludge	0 2 mm	Fe° powder	0 1 6 8 10 14 20	- Fe rusty scrap had better performance - Hypothesized Fe^{3+} microbial reduction - CH_4 production increased 13 % (Fe° powder at 10 g/L), 23 % for the clean scrap and almost 30 % for the rusty scrap
			~ 8mm	Clean scrap Fe	0 1 6 8 10 14 20	
			~ 8 mm	Rusty scrap Fe	10	
(Zhen *et al.*, 2015)	21.9	Waste activated sludge	2 mm	Fe°	0 1.1 2.2 5.5 1´ 22	- CH_4 production increased 38.3 % (at 22 g/L) - Fe° effects are higher at mesophilic conditions (35 °C) - % CH_4 went from 60,9 % to 68,9 %
(Hu *et al.*, 2015)	18.4	Waste activated sludge	50 nm	Fe°	0 1 7 3.3 10 16.7	- CH_4 yield enhanced by 60.5 % - Fe° addition favoured PO_4^{3-} removal - % CH_4 increased 37.4% (at 3.3 g/L)
			10 mm	Waste Fe scraps	0 33.3 66.7 100	
(Suanon *et al.*, 2016)	47.6	Dewatered sewage sludge	50 nm	Fe°	0 5 10	- CH_4 content increased up to 15 % (at 10 g/L magnetite) - Decreased gas yield at 10 g/L for Fe° and Fe_3O_4
			20 nm	Fe_3O_4 (Magnetite)	5 10	
(Abdelsalam *et al.*, 2016)	58.5	Cattle dung slurry	28 nm	Co	0.00´	- Ni was the most effective nanoparticle - For each case, CH_4 production was increased more than 60 % - Bio-stimulatory effects of nanoparticles (Co, Ni, Fe, Fe_3O_4)
			17 nm	Ni	0.002	
			9 nm	Fe	0.02	
			7 nm	Fe_3O_4	0.02	

References

Abdelsalam, E. et al. (2016) 'Comparison of nanoparticles effects on biogas and methane production from anaerobic digestion of cattle dung slurry', Renewable Energy. Elsevier Ltd, 87, pp. 592–598. doi: 10.1016/j.renene.2015.10.053.

Boone, D. R., Johnson, R. L. and Liu, Y. (1989) 'Diffusion of the Interspecies Electron Carriers H2 and Formate in Methanogenic Ecosystems and Its Implications in the Measurement of Km for H2 or Formate Uptake', 55(7), pp. 1735–1741.

Bryant, M. P. et al. (1967) 'Methanobacillus omelianskii, a symbiotic association of two species of bacteria', Archiv für Mikrobiologie, 59(1–3), pp. 20–31. doi: 10.1007/BF00406313.

Cruz Viggi, C. et al. (2014) 'Magnetite particles triggering a faster and more robust syntrophic pathway of methanogenic propionate degradation', Environmental Science and Technology, 48(13), pp. 7536–7543. doi: 10.1021/es5016789.

Felchner-Zwirello, M., Winter, J. and Gallert, C. (2013) 'Interspecies distances between propionic acid degraders and methanogens in syntrophic consortia for optimal hydrogen transfer', Applied Microbiology and Biotechnology, 97(20), pp. 9193–9205. doi: 10.1007/s00253-012-4616-9.

Feng, Y. et al. (2014) 'Enhanced anaerobic digestion of waste activated sludge digestion by the addition of zero valent iron', Water Research, 52, pp. 242–250. doi: 10.1016/j.watres.2013.10.072.

Gottardo, M. et al. (2015) 'Hydrogen and methane production from biowaste and sewage sludge by two phases anaerobic codigestion', Chemical Engineering Transactions, 43, pp. 379–384. doi: 10.3303/CET1543064.

Hu, Y. et al. (2015) 'Enhancing the CH4 yield of anaerobic digestion via endogenous CO2 fixation by exogenous H2.', Chemosphere. Elsevier Ltd, 140, pp. 34–9. doi: 10.1016/j.chemosphere.2014.10.022.

Karlsson, H. L. et al. (2009) 'Size-dependent toxicity of metal oxide particles-A comparison between nano- and micrometer size', Toxicology Letters, 188(2), pp. 112–118. doi: 10.1016/j.toxlet.2009.03.014.

Malavè, A. C. L. et al. (2017) 'Experimental tests on commercial Sweet Product Residue (SPR) as a suitable feed for anaerobic bioenergy (H2+CH4) production', Waste Management, 71, pp. 626–635. doi: 10.1016/j.wasman.2017.06.011.

Mcinerney, M. J., Sieber, J. R. and Gunsalus, R. P. (2010) 'Syntrophy in Anaerobic Global Carbon Cycles', Current Opinion in Biotechnology, 20(6), pp. 623–632. doi: 10.1016/j.copbio.2009.10.001.Syntrophy.

Morris, B. E. L. et al. (2013) 'Microbial syntrophy: Interaction for the common good', FEMS Microbiology Reviews, 37(3), pp. 384–406. doi: 10.1111/1574-6976.12019.

Reardon, E. J. (1995) 'Anaerobic corrosion of granular iron: measurement and interpretation of hydrogen evolution rates.', Environmental science & technology, 29(12), pp. 2936–45. doi: 10.1021/es00012a008.

Rotaru, A. E. et al. (2012) 'Interspecies electron transfer via hydrogen and formate rather than direct electrical connections in cocultures of Pelobacter carbinolicus and Geobacter sulfurreducens', Applied and Environmental Microbiology, 78(21), pp. 7645–7651. doi: 10.1128/AEM.01946-12.

Schmidt, J. E. and Ahring, B. K. (1995) 'Interspecies Electron Transfer during Propionate and Butyrate Degradation in Mesophilic , Granular Sludge', 61(7), pp. 2765–2767.

Shrestha, P. M. and Rotaru, A.-E. (2014) 'Plugging in or going wireless: strategies for interspecies electron transfer.', Frontiers in microbiology. Frontiers Media SA, 5, p. 237. doi: 10.3389/fmicb.2014.00237.

Stams, A. J. M. et al. (2006) 'Exocellular electron transfer in anaerobic microbial communities', Environmental Microbiology, 8(3), pp. 371–382. doi: 10.1111/j.1462-2920.2006.00989.x.

Stams, A. J. M. et al. (2012) 'Syntrophic Degradation of Fatty Acids by Methanogenic Communities', in Microbial Technologies in Advanced Biofuels Production. Boston, MA: Springer US, pp. 127–142. doi: 10.1007/978-1-4614-1208-3_8.

Suanon, F. et al. (2016) 'Effect of nanoscale zero-valent iron and magnetite (Fe3O4) on the fate of metals during anaerobic digestion of sludge', Water Research, 88, pp. 897–903. doi: 10.1016/j.watres.2015.11.014.

Thauer, R. K. et al. (2008) 'Methanogenic archaea: ecologically relevant differences in energy conservation', Nature Reviews Microbiology, 6(8), pp. 579–591. doi: 10.1038/nrmicro1931.

Thauer, R. K., Jungermann, K. and Decker, K. (1977) 'Energy conservation in chemotrophic anaerobic bacteria.', Bacteriological reviews, 41(1), pp. 100–180. doi: 10.1073/pnas.0803850105.

Yang, Y., Guo, J. and Hu, Z. (2013) 'Impact of nano zero valent iron (NZVI) on methanogenic activity and population dynamics in anaerobic digestion', Water Research, 47(17), pp. 6790–6800. doi: 10.1016/j.watres.2013.09.012.

Zhang, Y. et al. (2014) 'Enhanced high-solids anaerobic digestion of waste activated sludge by the addition of scrap iron', Bioresource Technology. Elsevier Ltd, 159, pp. 297–304. doi: 10.1016/j.biortech.2014.02.114.