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Ability of anaerobic granules for metal-mediated direct interspecies electron transfer

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The long-established efficacy of granular sludge for high-rate anaerobic wastewater treatment is namely due to the granule compactness and cells proximity that facilitate metabolites exchange and interspecies electron transfer (IET). It has long been known that granulation is aided by metal cations and precipitates (Guiot et al. 1988), namely as cations help binding negatively charged cells together to form microbial nuclei and cation precipitates serve as surface for adhesion of bacteria (Yu et al. 2000, Show 2006). It has also been shown more recently that granulation may benefit from the addition of conductive materials (granular activated carbon (GAC), charcoal, metallic minerals), through mineral-mediated direct interspecies electron transfer (mDIET) (Shrestha and Rotaru 2014, Zhao et al. 2015, Dubé and Guiot 2015). DIET excluding hydrogen and formate, could happen between obligate H_2-producing acetogenic (OHPA) bacteria and methanogenic archaea in some environments (Stams et al. 2006), including brewery wastewater anaerobic granules where *Geobacter* sp. play a key role (up to 30% of all bacteria) (Morita et al. 2011).

To further investigate the question whether DIET could occur in *Geobacter*-deprived granules, specific activities of disintegrated granules, where *Geobacter* represented only 0.2% of total bacteria, with and without electrically conductive and non-conductive microparticles were compared to each other, and to those of the whole granules. Three materials were tested: GAC (conductive), stainless steel (ProPak) (highly conductive) and porcelain (non-conductive). GAC is known to help methanogenesis during digester start-up or recovery (Liu et al. 2012, Lü et al. 2016). GAC provides a large specific surface area for biofilm development, just like porcelain, while ProPak offers the greatest conductivity but a lower specific surface area for colonisation.

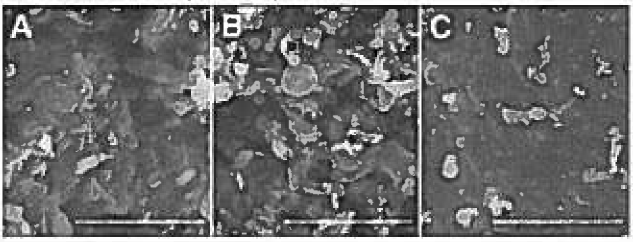
The layered architecture of granules, which promotes the physical proximity between syntrophic cells (Guiot et al. 1992), could also promote DIET, as previously seen in granules formed with *Geobacter* species (Summers et al. 2010). Consequently, any disruption of such a structure should result in reduced methane-producing specific activities (MPA), and in most cases, reduced substrate-consuming activities (SCA) also. As shown in Table 1, the disintegration of the granules had a negative effect, resulting in a decrease of the ethanol SCA and MPA at 41% and 38%, respectively, of those of the integral granules. When incubated with ProPak and GAC, the cells from disintegrated granules had a higher MPA, reaching 190±10% and 175±13% of the MPA obtained with disintegrated granules without microparticles, respectively. When porcelain (non-conductive) microparticles were added, the MPA was reduced at 65±4% of the control without microparticles. Thus, the conductivity of the microparticles added to cells from disintegrated granules seems instrumental and suggests the reality of mDIET.

Scanning electron microscopy (SEM) clearly showed that GAC and porcelain microparticles were colonized to a greater extent than the stainless steel microparticles by the end of assay. By contrast, SEM showed that ProPak was ineffective for cell colonisation, where most cells observed were still separated (Figure 1). This was expected since the former ones presented a higher porosity and ruggedness than the stainless steel. The porosity and ruggedness of non-conductive microparticles (e.g. porcelain) facilitated cells from disintegrated anaerobic granules to reform a biofilm without the methanogenic activity to be recovered as compared to that of whole original granules. On the contrary, cells in the presence of non-porous but highly conductive microparticles (e.g. ProPak) did recover a significant level of the whole granule activity without reformation of biofilm. This suggests that anaerobic granule architecture facilitates IET not only because of the reduced distance of diffusion for the electron-transfer molecules, but likely also because they provide an enabling matrix for DIET.

Table 1. Specific activities of both ethanol consumption (SCA) and methane production (MPA) obtained with disintegrated granules without and with conductive and non-conductive microparticles

Microbial materials	Ethanol SCA		MPA	
	mmol/gVSS·d	%	mmol/gVSS·d	%
Whole granules	5.4 ± 0.3	245 ± 18	5.2 ± 0.3	260 ± 15
Disintegrated granules	2.2 ± 0.1	100	2.0 ± 0.1	100
Disintegr. gran. + GAC	5.4 ± 0.4	245 ± 9	3.5 ± 0.4	175 ± 13
Disintegr. gran. + stainless steel	2.1 ± 0.3	95 ± 15	3.8 ± 0.3	190 ± 10
Disintegr. gran. + porcelain	2.1 ± 0.4	95 ± 20	1.3 ± 0.0	65 ± 4

Figure 1. Scanning electron microscope images of colonized GAC (A), porcelain (B), and ProPak (C) microparticles at the end of the assays. Bar = 5 µm.



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