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Short Communication

Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment

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HIGHLIGHTS

- Granules from four brewery waste UASB digesters were electrically conductive.
- *Geobacter*, *Syntrophobacter* and *Desulfovibrio* spp. were the dominant members.
- Granules conductivity was moderately correlated with only *Geobacter* spp. abundance.
- This study suggest that *Geobacter* spp. are a major contributor to conductivity.

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ABSTRACT

Prior investigation of an upflow anaerobic sludge blanket (UASB) reactor treating brewery wastes suggested that direct interspecies electron transfer (DIET) significantly contributed to interspecies electron transfer to methanogens. To investigate DIET in granules further, the electrical conductivity and bacterial community composition of granules in fourteen samples from four different UASB reactors treating brewery wastes were investigated. All of the UASB granules were electrically conductive whereas control granules from ANAMMOX (ANaerobic AMMonium OXidation) reactors and microbial granules from an aerobic bioreactor designed for phosphate removal were not. There was a moderate correlation ($r = 0.67$) between the abundance of *Geobacter* species in the UASB granules and granule conductivity, suggesting that *Geobacter* contributed to granule conductivity. These results, coupled with previous studies, which have demonstrated that *Geobacter* species can donate electrons to methanogens that are typically predominant in anaerobic digesters, suggest that DIET may be a widespread phenomenon in UASB reactors treating brewery wastes.

1. Introduction

The discovery that *Methanosaeta* (Rotaru et al., 2014a) and *Methanosarcina* (Rotaru et al., 2014b) can accept electrons via direct interspecies electron transfer (DIET) has challenged the long-held assumption that H_2 and formate are the primary interspecies electron carriers in the conversion of organic matter to methane. *Methanosaeta* and/or *Methanosarcina* species are often the most abundant methanogens in many anaerobic digesters, which has been attributed to their effectiveness in converting acetate to methane (De Vrieze et al., 2012; van Haandel et al., 2013). In fact, until recently (Rotaru et al., 2014a) conversion of acetate to methane was considered to be the sole strategy for *Methanosaeta* to conserve energy to support growth (van Haandel et al., 2013).

However, in defined co-cultures both *Methanosaeta harundinacea* (Rotaru et al., 2014a) and *Methanosarcina barkeri* (Rotaru et al., 2014b) directly accepted electrons from *Geobacter metallireducens* for the reduction of carbon dioxide to methane. Although,

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many of the mechanistic details are yet to be elucidated, DIET required that *G. metallireducens* express the electrically conductive pili (Malvankar and Lovley, 2014) that have previously been shown to be necessary for DIET in other co-cultures (Summers et al., 2010). Metatranscriptomic analysis of a laboratory upflow anaerobic sludge blanket (UASB) reactor treating simulated brewery waste suggested that *Geobacter* species were highly expressing genes for pili and that *Methanosaeta* species were actively reducing carbon dioxide to methane with electrons received via DIET (Rotaru et al., 2014a). Further evidence for the importance of DIET were the findings that: (1) the granules were electrically conductive (Morita et al., 2011), with a metallic-like conductivity similar to that of *Geobacter* pili (Malvankar and Lovley, 2014); (2) *Geobacter* and *Methanosaeta* were the predominant and most metabolically active microbes in the granules (Morita et al., 2011; Rotaru et al., 2014a); (3) methanogens capable of metabolizing H_2 or formate accounted for less than 10% of the methanogens in the granules (Morita et al., 2011; Rotaru et al., 2014a); and (4) the ability of the granules to metabolize H_2 or formate was poor (Morita et al., 2011), further suggesting that interspecies H_2 or formate were not important avenues for interspecies electron exchange.

Design, analysis, and operation of UASB reactors, other upflow anaerobic bioreactors, and other forms of anaerobic digesters have been based on the assumption that syntrophic microbes oxidize alcohols and fatty acids with the release of reducing equivalents as H_2 and/or formate, which are then the electron donors for the reduction of carbon dioxide to methane (Sieber et al., 2012). However, if DIET is prevalent, then alternative strategies for optimizing anaerobic digestion may be required. As an initial survey of the possibility of DIET in UASB reactors, we examined a fundamental property associated with DIET, which is electrical conductivity of granules, in brewery UASB reactor samples that were studied previously (Werner et al., 2011).

2. Methods

2.1. Conductivity measurements

Fourteen deep frozen (−80 °C) UASB reactor granules from a previous study (Werner et al., 2011) at four different brewery locations (U1–U4) were examined. Preliminary studies with previously described granules (Morita et al., 2011) demonstrated that freezing and thawing granules did not impact on their conductivity. As previously described (Morita et al., 2011; Summers et al., 2010), granule conductivity was measured with two gold electrodes separated by 50 μm non-conductive gap. The granules were placed on the gold electrodes, spanning the non-conductive gap. Voltage was applied across the gap with a Keithley 2400 source meter. Voltage was scanned from −0.3 V to +0.3 V in steps of 0.025 V, then from +0.3 V to −0.3 V. For each measurement, current was measured 10 s after setting the voltage to allow the exponential decay of the transient ionic current in the gap and to measure steady state electronic current.

The conductivity of a similar quantity of microbial granules from an aerobic bioreactor (polyphosphate/glycogen- accumulating organisms; PAO/GAO) and an anaerobic ANAMMOX reactor was also evaluated. In each instance liquid medium without the granules served as a conductivity measurement control.

2.2. Taxonomic analysis 454-sequence reads

Bacterial 16S rRNA gene sequences (Accession No. SRA029112) obtained from the UASB reactors using 454-sequencing were downloaded and were processed for genus level taxonomic assignment as described earlier (Werner et al., 2011). The "Pearson correlation coefficient (r)" between conductivity and different bacterial genera was calculated using "CORREL" function in an Excel worksheet.

2.3. Chemical analysis

The chemical composition for two samples from different time periods for U1 were measured. The samples were taken after the equalization tank in which sugars are converted into volatile fatty acids (VFAs) and before the UASB reactors. Chemical analysis, such as chemical oxygen demand (COD) was measured by titrimetric method (Clesceri et al., 1998) and pH was measured using a pH meter. Individual VFAs were analyzed on a gas chromatograph (GC) (HP 5890 Series II, Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) with a ramp temperature program (initial temperature 70 °C for 2 min; temperature ramp 12 °C per min to 200 °C; final temperature 200 °C for 2 min), and a capillary column (NUKOL, Fused Silica Capillary Column, 15 m × 0.53 mm × 0.50 μm film thickness; Supelco Inc., Bellefonte, PA). The injection port was set to 200 °C and the detector, 275 °C. The ethanol was also measured with a HP 5890 Series II GC. For the quantification of alcohols, a custom-made packed bed glass column was used, 1.8 m × 2 mm i.d. (Supelco). The support matrix of this column was Chromosorb W/AW80 over 100 mesh; phases were preconditioned: phase A was 10% Carbowax-20M; phase B was 0.1% phosphoric acid. Glass Purecol inlet liners, 2 mm i.d. were installed (Supelco). The inlet and detector temperatures were 220 °C and 240 °C, respectively. The column temperature program was 100 °C for 2 min, a temperature ramp of 40 °C/min to 180 °C where the temperature was kept for 5 min. Sugars were measured with a high-pressure liquid chromatograph (600 HPLC, Waters, Milford, MA) using an Aminex HPX-87H column (Bio-Rad, Hercules, CA) at a temperature of 60 °C, and a 5 mM sulfuric acid eluent at a flow rate of 0.6 mL/min. Metabolites were detected via a refractive index (RI) detector (410 Differential Refractometer, Waters).

3. Results and discussion

3.1. Conductivity measurements

Granules were black with a diameter between 0.5 and 2 mm with a morphology similar that was previously described (Morita et al., 2011). The conductivity of the granules ranged from 0.8 to 36.7 μS/cm (Fig. 1), which was consistently significantly higher than the conductivity of granule-free bioreactor samples. This range of conductivities is broader but comparable to previously reported conductivities (6–7 μS/cm) for the granules from an industrial-size bioreactor and a laboratory-scale bioreactor initiated with granules from the industrial system (Morita et al., 2011). In contrast, granules from two ANAMMOX reactors and the granules from an aerobic bioreactor with polyphosphate/glycogen-accumulating organisms (PAO/GAO), did not have detectable conductivity above controls (data not shown).

With the exception of digester U4, the conductivity varied substantially in samples collected at different times (Fig. 1). There was no correlation between granule conductivity with the specific rate of methanogenesis ($r = 0.03$) and methanogenic activity ($r = -0.084$) in the digesters (Fig. 2d).

3.2. Taxonomic assignment of 454-sequence reads

To evaluate potential relationships between the composition of the microbial community and granule conductivity, taxons accounting for >1% of the relative abundance of the total bacterial