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

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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.

Keywords:
Direct interspecies electron transfer
Syntrophy
Granules
UASB reactor
Conductive pili

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 H2 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 harundinea (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,
many of the mechanistic details are yet to be elucidated, DIET required that Geobacter species 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 was 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 H₂ 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 H₂ or formate was poor (Morita et al., 2011), further suggesting that interspecies H₂ 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 H₂ 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 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, taxa accounting for >1% of the relative abundance of the total bacterial community
Communities were considered. These abundant taxons could be assigned to genus level (Geobacter, Syntrophobacter spp., Desulfovibrio, and Kosmotoga spp.), class level (Clostridia, Epsilonproteobacteria, Anaerolineae, Streptococcus, and Betaproteobacteria), and phylum level (Bacteroidetes, Synergistetes, Spirochaetes, Acidobacteria, Actinobacteria, Caldisericra, Chlorobi, Planctomycetes, and OP9). Geobacter species were one of the most dominant bacterial genera in all 14 granular samples from the four UASB reactors, accounting for 2.3–29% of the microbial community. There was a moderate correlation ($r = 0.67$) between the percentage of Geobacter species and the conductivity of the granules despite the fact that several of the anaerobic bioreactors with high Geobacter abundance contained granules with low conductivities (Fig. 2a). Studies on the conductivity of Geobacter biofilms have demonstrated that conductivity can vary over 50-fold based on the growth conditions and strain variation and is correlated with the abundance of PilA, the structural pilin protein (Malvankar et al., 2011). The degree of pili expression in Geobacter species can differ greatly in response to environmental conditions (Reguera et al., 2005). Thus, proteomic studies that quantify the abundance of pili protein actually in granules, though technically difficult, may be a better approach than Geobacter cell abundance to elucidate the role of Geobacter pili in contributing to granule conductivity.

Fig. 1. Conductivity measurements of granules from UASB reactors (U1–U4) at different months a year 2008, the conductivity of respective granule-free media as a control was also measured, which was very low compared to granules conductivity and is not shown in the figure. Error bar represents an average conductivity of three biological replicates ± SE.

Fig. 2. Correlation between conductivity and abundance of genus Geobacter, Syntrophobacter, Desulfovibrio and methanogenic activity. Each dot represents the percentage of bacteria present in an individual sample from each of four UASB reactors from different brewery locations.
Sulfate reducing bacteria, such as Desulfovibrio and Syntrophobacter spp., were also abundant in many of the UASB reactor samples. Both of these genera are capable of switching between sulfidogenic and syntrophic lifestyles (Meyer et al., 2013). Syntrophobacter species were abundant in twelve granular samples out of fourteen and accounted for 0.64–15.8% of the total community. The possible role of the Syntrophobacter species is to metabolize propionate to acetate with transfer of reducing equivalents to methanogens (Sieber et al., 2012). Gene expression studies (Worm et al., 2011) and genome analysis (Sieber et al., 2012) have provided strong evidence that the transfer of reducing equivalents between Syntrophobacter species and methanogens proceeds via H₂ and formate. Therefore, Syntrophobacter species are not expected to participate in DIET. Indeed, there was little correlation (r = 0.22) between the abundance of Syntrophobacter species and granule conductivity (Fig. 2b).

Desulfovibrio spp. comprised >1% of the total community in only two of the fourteen granules samples from the four UASB reactors and their possible role could be the transfer of reducing equivalents to methanogens via H₂. Desulfovibrio vulgaris strain H mediates electron transfer via H₂ (Walker et al., 2009) in co-culture with methanogens, such as Methanococcus maripaludis S2 (Walker et al., 2009), M. barkeri (Scholten et al., 2007) or Methanobacterium formicicum (Bryant et al., 1977) with lactate as electron donor. The correlation between the abundance of Desulfovibrio spp. and granule conductivity was very low (r = 0.19; Fig. 2c).

There was no correlation between the abundance of remaining bacterial groups and granule conductivity. These included Bacteroides (8.5–29.4% of total community), which are known proteolytic bacteria, responsible for the degradation of protein and subsequent fermentation of amino acids into propionate, acetate, carbon dioxide, and hydrogen as major end products (Smith et al., 2006), and Clostridia species (3.95–10.90% of total community), which are generally considered to be involved in processes such as hydrolysis, fermentation, and syntrophy (Klocke et al., 2007). Other abundant phyla included Synergistetes (0.78–9.3%), Spirochaetes (3.92–44.94%), and Epsilonproteobacteria (0.2–6.9%). These are all reported to be abundant groups in UASB reactors in earlier studies (Morita et al., 2011), but are not expected to be involved in DIET.

3.3. Ethanol concentration in brewery UASB influent

To verify whether a considerable ethanol concentration is a characteristic of brewery wastewater, we obtained two time point samples from U1. Ethanol concentrations were as high as acetate and greater than propionate and butyrate concentration in the samples collected after the pre-equalization tank and before the UASB reactor (Table 1), suggesting ethanol as an important component of an anaerobic brewery wastewater. Studies with defined co-cultures and anaerobic digesters have demonstrated that G. metallireducens is highly effective in oxidizing ethanol to acetate with electron disposal via DIET to GGeobacter sulfurreducens (Summers et al., 2010), M. haurundaeceae (Rotaru et al., 2014a), and M. barkeri (Rotaru et al., 2014b), however, its ability to metabolize other organic compounds, such as fatty acids and aromatic compounds that also must first be converted to acetate in methanogenic environments has yet to be fully investigated. A diversity of microbes that metabolize fatty acids and aromatic compounds to acetate with the production of H₂ have been described (Sieber et al., 2012), but it is not yet known if any of these organisms are capable of DIET. The role of DIET is yet to be determined in an anaerobic digestion of wastewaters from sources other than breweries, where ethanol can account for a much lesser proportion of the complex organic content in the influent to the digester and coming out of the equalization tanks.

4. Conclusions

These results, coupled with previous findings (Morita et al., 2011; Rotaru et al., 2014a), indicate that Geobacter species play an important role in DIET in anaerobic bioreactors treating brewery wastes, suggesting that brewery waste treatment with anaerobic upflow bioreactors should be designed with consideration of what factors might best promote DIET. For example incorporation of conductive materials such as granulated activated charcoal, biochar or iron minerals (Shrestha and Rotaru, 2014) during anaerobic bioreactor operation might accelerate and/or improve the stability of anaerobic digestion for brewery wastes.

Acknowledgements

This research was supported by the Office of Naval Research Grant No. N00014-13-1-0550 and the Cornell University Agricultural Experiment Station federal formula funds, project number NYC-123444, received from the USDA National Institutes for Food and Agriculture. The authors thank Robbert Kleerebezem, PhD (Delft University) for sending ANAMMOX and PAO/GAO granules, Robert Cleeton (Anheuser-Busch Inbev) for sending samples, and for Lauren Harroff (Cornell University) for chemical analysis of samples.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.10.004.

References


Table 1

Sample characteristics for U1 when sampled after the pre-equalization tank (with pH control) and before the UASB reactor.

<table>
<thead>
<tr>
<th>Sampling dates</th>
<th>pH</th>
<th>Total COD (g O₂/L)</th>
<th>Soluble COD (g O₂/L)</th>
<th>Ethanol (g/L)</th>
<th>Acetate (g/L)</th>
<th>Propionate (g/L)</th>
<th>n-Butyrate (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec., 2013</td>
<td>6.6</td>
<td>2.3</td>
<td>1.5</td>
<td>0.36</td>
<td>0.35 (±0.01)*</td>
<td>0.25 (±0.01)*</td>
<td>0.26 (±0.01)*</td>
</tr>
<tr>
<td>Jan., 2014</td>
<td>6.5</td>
<td>2.8</td>
<td>2.3</td>
<td>0.42</td>
<td>0.40 (±0.04)*</td>
<td>0.33 (±0.01)*</td>
<td>0.11 (±10⁻³)*</td>
</tr>
</tbody>
</table>

* Standard error for two GC measurements of same sample.