Impact of operational parameters on reactor performance and microbial community development during pilot-scale low temperature anaerobic digestion wastewater treatment

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Abstract
The anaerobic digestion (AD) of wastewaters is usually performed under mesophilic or thermophilic temperature conditions. Yet, many industrial wastewaters are discharged at lower temperatures. Low temperature AD (Lt-AD) was already proven to be possible at laboratory scale with associated biogas production. In this project, the development of the microbial community was analysed over time and the impact of operational parameters was evaluated. It was observed that the microbial community development was shaped by HRT changes and was significantly altered by pH shocks. Furthermore, the feasibility and application of Lt-AD to treat dairy-processing wastewater was demonstrated at pilot scale. Stable COD removal efficiencies of greater than 80%, with stable, high-quality, biogas (methane > 80%) was observed. Reactor performance was not affected by HRT changes, but lower COD removals where observed when shock loadings increased the reactor pH above 8.5.

Keywords: low-temperature anaerobic digestion, biomethane, microbial community, operational parameters

Session: Microbiology of anaerobic digestion/ (meta)genomic research

Introduction
High-rate anaerobic digestion (AD) is an efficient process to treat waste and wastewaters and is commonly employed under mesophilic (25-37°C) or thermophilic (45-60°C) temperature conditions. This infers energy consumption in the form of heat, as many industrial wastewaters are discharged at much lower temperatures. Low temperature AD offers an economically attractive alternative that was considered unfeasible until recently. Although many reports have been published on long-term replicated trials at laboratory scale, very few pilot and full-scale studies have been published to date (McKeown et al., 2012).
The AD process relies on a complex network of microorganisms with different physiologies, nutritional requirements and different responses to environment alterations, such as pH or temperature variations (Venkiteshwaran et al., 2015). Low temperature AD relies on the adaptation of microorganisms to the low temperatures. Several studies investigated such adaptation at laboratory-scale (Gunnigle et al., 2015; Keating et al., 2018). Yet, our knowledge about the development of microbial communities at pilot and full-scale, or how they respond under environmental stresses, such as variations in the operational parameters, is very scarce.

The aim of this project was to study the feasibility of high-rate, low temperature AD at pilot scale and to investigate the impact of operational parameters, such as pH or hydraulic retention time (HRT) on reactor performance and on the development of microbial communities.

Materials and Methods
The pilot scale unit had a total volume of 2 m$^3$ and was a scale up of the reactor described by Keating et al. (2018). The trial was performed on an industrial dairy processing site using a wastewater stream, following dissolved air flotation separation of fats. The pH was automatically controlled in-line and kept at 7.5±0.2 for the inlet flow by adding NaOH or HCl to a homogenisation tank. However, due to technical problems with the acid’s dosage, the reactor was exposed to three pH shocks were the pH inside the reactor was higher than 8.5 for a few days. The first shock was at days 70 – 75, the second one at days 246 – 251 and the third at days 277 - 283. The reactor was seeded with anaerobic granular sludge from a previous AD digestion reactor for the treatment of slaughterhouse wastewater. The trial was carried out over a period of 291 days and the HRT over this trial was reduced, in a stepwise manner, from an initial 36 hours down to 6 hours. Total and Soluble COD were measured using a kit of Reagecon (Shannon, Ireland) testing medium range COD (0 - 1500 mg/L). The methane content in the biogas was determined by gas chromatography according to standard methods (APHA and AWWA, 2005). Sludge samples were taken from the reactor at regular intervals during the trial and were instantly frozen in liquid nitrogen and stored at -80°C prior to DNA/RNA extraction, according to the method of Griffiths et al. (2000). For each time point sample, DNA/RNA was extracted in triplicate. Library preparation and Hiseq data analysis was performed as described elsewhere (Ramiro-Garcia et al., 2016).

Results and Conclusions
The reactor performance was not strongly affected by the imposed HRT reductions. Total COD removal varied between 49 and 71%, while the average soluble COD removal oscillated
between 71 and 84.3%. The methane content in the biogas during the trial ranged between 73.4 ± 29.5% and 89.6 ±3.2 % (Table 1). COD removal efficiency and biogas methane content lowered when pH increased above 8.5 (Figure 1). Especially during days 246-251, where the pH remained around 9.7 - 9.8 for four days due to a technical issue, no COD removal was observed, and methane content dropped to ~15%. Once the pH returned to 7.5, the COD removal and methane content quickly returned to match previous values.

Table 1 – Summary of the reactor trial.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
<th>Phase 5</th>
<th>Phase 6</th>
<th>Phase 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (h)</td>
<td>36</td>
<td>30</td>
<td>24</td>
<td>18</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Total COD Removal (%)</td>
<td>70.7 ± 14.2</td>
<td>52.9 ± 24.4</td>
<td>70 ± 14.2</td>
<td>67.8 ± 12.3</td>
<td>55.1 ± 14.4</td>
<td>49.3 ± 23.5</td>
<td>52.5 ± 14.3</td>
</tr>
<tr>
<td>Soluble COD Removal (%)</td>
<td>82.2 ± 10.1</td>
<td>74.3 ± 23.1</td>
<td>83.6 ± 15.7</td>
<td>84.3 ± 7.4</td>
<td>82.4 ± 14.8</td>
<td>70.9 ± 29.6</td>
<td>83.2 ± 13.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 0.3</td>
<td>8.0 ± 0.7</td>
<td>7.6 ± 0.3</td>
<td>7.6 ± 0.2</td>
<td>7.8 ± 0.6</td>
<td>8.2 ± 0.7</td>
<td>8.1 ±.5</td>
</tr>
<tr>
<td>Average methane content (%)</td>
<td>83.2 ± 3.3</td>
<td>85.5 ± 6.1</td>
<td>85.4 ± 8.9</td>
<td>83.1 ± 5.6</td>
<td>80.1 ± 5.8</td>
<td>73.3 ± 29.5</td>
<td>89.6 ± 3.2</td>
</tr>
</tbody>
</table>

The microbial community was analysed by 16S rRNA (both DNA- and cDNA-based) sequence analysis the trial. Results indicated that *Methanobacteriaceae* species (up to 10%), *Methanosetaeaceae* species (up to 23%), unclassified members of VadinHA17 (up to 29%), and unclassified members of *Synergistaceae* (up to 21%) were, relatively, the most abundant groups. On the other hand, cDNA results showed that members of *Methanosetaeaceae* species and unclassified members of VadinHA17, with relative abundances up to 47 and 30% respectively, were the main active members. Moreover, a clear shift of the microbial communities, both at DNA and cDNA level, related with HRT changes was revealed by principal component analysis of the data (Figure 2). It was also observed that the microbial community was affected when pH values where higher than 8.5 and clear shifts could be observed after days 75, 214 and 242 (Figure 2). After the longer exposure to high pH (days 246-250), a reduction of overall archaea, both at DNA (from 25-35% to 10-11%) and cDNA
(from 35-50% to 11%) level was observed and corresponded to a decreased in the methane content in the biogas. Once the pH returned to 7.5, the system was able to recover, and archaea populations started to increase again.

The pilot trial performed in this work revealed how operational parameters, such as pH and HRT, can impact system performance and microbial community composition. Regardless of the changes to its composition, the microbial community performed in similar fashion as before the perturbations, indicating possible functional redundancy, but also the resilience of the main active core that remained constant for almost all trial. Furthermore, the trial validates the feasibility and robustness of low temperature AD as a process to treat real industrial wastewaters at ambient temperature.

References


