Reactor performance and microbial community dynamics during anaerobic biological treatment of wastewaters at 16–37 °C

Sharon McHugh *, Micheal Carton, Gavin Collins, Vincent O’Flaherty

Microbial Ecology Laboratory, Department of Microbiology, National University of Ireland, Galway, Ireland

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Abstract

The anaerobic biological treatment of volatile fatty acid (VFA) – and sucrose – based wastewaters was investigated in two anaerobic bioreactors, R1 and R2, over a 300-day trial period. During the trial, the operating temperature of both reactors was lowered, in a stepwise fashion, from 37 to 16 °C. The VFA-fed reactor maintained an excellent level of performance, regardless of operating temperature, reaching COD removal efficiencies of 95% at 18 °C, and a biogas methane content in excess of 70% at 16 °C, at an imposed OLR of 20 kg COD m⁻³ d⁻¹. However, an increase in the applied liquid upflow velocity to the bottom chamber of the reactor from 5 to 7.5 m h⁻¹ on day 236 resulted in a considerable decline in reactor performance. COD removal efficiencies in excess of 80% were achieved by the sucrose-fed reactor at 18 °C, at an imposed OLR of 20 kg COD m⁻³ d⁻¹. An increase in the liquid upflow velocity applied to the sucrose-fed reactor resulted in enhanced reactor performance and stability, with respect to decreasing temperature. The different responses of both reactors to increased upflow velocity was associated with variations in the microbial population structure of the sludges, as determined by culture-independent molecular approaches, specifically the presence of high levels of \( \delta \)-Proteobacteria and hydrogenotrophic methanogens in the VFA-fed biomass. High levels of \( \textit{Methanomicrobiales} \) sp., in particular \( \textit{Methanococcus parvum} \) sp., were observed in both R1 and R2 during the trial. There was a distinct shift from acetoclastic methanogenic dominance to hydrogenotrophic dominance in both reactors in response to a decrease in the operating temperature.

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Keywords: Psychrophilic anaerobic digestion; 16S rRNA genes; Microbial community dynamics; T-RFLP; Hydrogenotrophic methanogens

1. Introduction

Psychrophilic anaerobic digestion is an attractive option for the treatment of wastewaters discharged at low or ambient temperatures, offering technical and economical benefits over more conventional treatment processes [1,2]. The potential drawbacks previously associated with low temperature reactor operation, such as low biogas production rates and poor stability, have been largely overcome by the introduction of novel reactor designs, such as the expanded granular sludge bed (EGSB) reactor [3], and improved modes of operation. However, much scope still exists for the application of further bioengineering innovations, which will allow for the implementation of psychrophilic anaerobic digestion on a more global basis for the treatment of a wide variety of wastewaters.

In particular, advanced research into the structure, function and biological properties of the microbial communities involved in psychrophilic anaerobic digestion is essential, in order to fully understand the microbial ecology of the process and to allow future improvement and optimisation of the technology. The success, or otherwise of an anaerobic treatment system is entirely dependant on the actions and interactions of complex microbial communities. However, many of the current bioreactor designs and operating routines have very little, if any, support from a microbiological point of view [4]. Furthermore, at present very little is known about low temperature methanogenesis or psychrophilic...
methanogenic communities. Anaerobic microbial degradation of organic matter has been reported at temperatures as low as 2 °C [5], however conflicting reports have been obtained regarding the pathways of degradation under low temperature conditions and the microbial trophic groups involved [6,7]. The application of nucleic acid-based molecular techniques to the study of the microbial populations involved in psychrophilic anaerobic digestion should provide a greater insight into the process, aiding the successful implementation of this treatment technology.

In the present study, the feasibility of psychrophilic anaerobic digestion was investigated by monitoring the effect of temperature decrease on reactor performance and sludge biomass activity in two anaerobic bioreactors over a 300-day period. In addition, the microbial population structure and dynamics of both reactors during the trial were investigated using molecular microbial ecology approaches.

2. Materials and methods

2.1. Reactor design and experimental setup

Two 3.8 l laboratory-scale upflow reactors, R1 and R2, were used in this study. An effluent recirculation facility was applied to the lower chamber of each reactor to give a liquid upflow velocity of 3.5 m h⁻¹ in this section. This was increased for R1 and R2 to 5.0 and 7.5 m h⁻¹ on days 118 and 236 of the study, respectively. The top chamber of each reactor was designed as a fixed-film section, comprising randomly packed polyethylene ring-shaped matrix pieces. The initial temperature of both reactors was 37 °C and this was decreased during the trial to 30, 25, 20, 18 and 16 °C on days 45, 98, 167, 184 and 198, respectively. R1 was fed a sucrose-based wastewater, prepared by dissolving table sugar (>99.9% sucrose; Irish Sugar Ltd., Co. Carlow, Ireland) in tap water to a final concentration of 10 g chemical oxygen demand (COD) l⁻¹. R2 was fed a synthetic wastewater, consisting of acetate, ethanolate, butyrate and propionate, at a COD ratio of 1:1:1:1 to a total of 10 g COD l⁻¹. The wastewaters were buffered with NaHCO₃ (10 g l⁻¹) and supplemented with macro- (10 ml l⁻¹) and micro- (1 ml l⁻¹) nutrients, as recommended by Shelton and Tiedje [8]. Throughout the trial, both R1 and R2 were operated at a hydraulic retention time (HRT) of 12 h, corresponding to an organic loading rate (OLR) of 20 kg COD m⁻³ d⁻¹. Samples of reactor effluent and biogas were routinely taken for volatile fatty acids (VFAs), ethanol, COD and CH₄ determination, as described previously [9].

Both reactors were initially seeded with a 10-year-old unfed sludge, consisting of mesophilic granular sludges obtained from full-scale industrial wastewater digesters in the Netherlands (Paques B.V.), to a final sludge concentration of 13.9 g volatile suspended solids (VSS) l⁻¹. Prior to the commencement of this study, both R1 and R2 were operated at 37 °C for 200 days. The operational data for this period are described in detail in a separate paper by the same authors (unpublished data; manuscript submitted). Throughout this initial 200-day period, R1 was fed the VFA-based wastewater for 177 days and the sucrose-based wastewater for the final 23 days, with the opposite feeding regime employed for R2.

2.2. Determination of specific methanogenic activity

Maximum specific methanogenic activity (SMA) profiles of sludge samples taken from R1 and R2 on days 165 and 300, and also from the biomass removed from the fixed-biofilm section of each reactor on day 300, were determined using the pressure transducer technique [1,10]. Briefly, the test procedure involved the measurement of the biogas pressure increase developing in sealed vials fed with the non-gaseous substrates, ethanol (30 mM), propionate (30 mM), butyrate (15 mM) and acetate (30 mM), or of the pressure decrease in vials pressurized with H₂/CO₂ (80:20) to 1 atm. Tests were carried out, in triplicate, at 37, 22 and 15 °C.

2.3. DNA extraction

Sludge samples were initially crushed with a pestle and DNA extracted from the crushed sludge, in triplicate, using the MoBio Soil DNA extraction kit (Cambio, Cambridge, UK), according to the manufacturer’s instructions. Cell lysis efficiency was determined using a modified version of the method of Bitton et al. [11], as described previously [1].

2.4. Generation of 16S rDNA clone libraries, amplified rDNA restriction analysis (ARDRA) and phylogenetic classification

Archaeal and bacterial clone libraries were generated by PCR amplification of 16S rRNA genes using the archaeal primers 21F (5’-TTCCGTTATCGGCGG-3’) [12] and 958R (5’-YCCGCGTGCAG-3’) [13] and the bacterial primers 27F (5’-GAGTTTGATCCTGGCTCAG-3’) [13] and 1392R (5’-ACGAGCCGAGGTGTGCGCC-3’) [14]. Reaction mixtures (50 µl) contained NH₄ buffer (16 mM (NH₄)₂SO₄, 67 mM Tris·HCl (pH 8.8 at 25 °C), 0.01% Tween-20), 200 µM dNTP (dATP, dCTP, dGTP, dTTP), 100 ng of each primer, 100 ng template DNA, 0.5 U Taq DNA polymerase and 3.0 mM MgCl₂ (archaeal PCR) or 1.5 mM MgCl₂ (bacterial PCR). The PCR conditions were initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturing (95 °C, 1 min), annealing (55 °C – archael; 52 °C – bacterial, 1 min) and primer extension.
PCR products were electrophoresed on 0.8% agarose and bands of the correct size were cut out and eluted using Snap minicolumns (Invitrogen, Groningen, The Netherlands).

The purified DNA amplicons were ligated into pCR® 2.1-TOPO (Invitrogen) plasmids and the ligation products transformed into E. coli TOP 10 competent cells using kanamycin selection. Plasmid inserts were amplified by PCR using the M13 PCR set [1]. Insert-containing clones were restricted using HaeIII, grouped into their restriction profiles and representatives from selected OTUs were chosen for gene sequencing. Template DNA was prepared from overnight cultures of selected clones using an alkaline miniprep kit (Qiagen, Heidelberg, Germany). Sequencing was performed on a Licor gel sequencer using vector specific primers (MWG Biotech, Milton Keynes, UK). The resultant sequence data were compared to nucleotide databases using basic local alignment search tool (BLASTn) as described previously [15]. The presence of chimeric amplification products was screened for using the Ribosomal Database Project (RDP) Chimera_Check software package [16]. None were present in the data generated from this study. Sequence data for the retrieved sequences was manually aligned to sequences obtained from the RDP. The phylogenetic inference package Paup* 4.0b8 was used for all phylogenetic analyses [17]. The partial 16S rRNA gene sequences determined in this study were deposited in the Genbank database under accession numbersAY231301 – AY231364.

2.5. Terminal restriction fragment length polymorphism (T-RFLP)

PCR was carried out, as above, but with fluorescently labelled forward and reverse primers. The forward primers, 21F and 27F, were 5′-hexachloroflourescein (HEX) labelled and the reverse primers, 958R and 1392R, were 5′ – 6′ – carboxyflourescein (FAM) labelled. All oligonucleotide primers were obtained from Oswe!, Southampton, UK. The PCR reactions were carried out in triplicate for each sample and resulting products grouped together, ethanol precipitated and resuspended in 20 μl of sterile water prior to restriction. Restrictions were carried out separately with the tetrameric endonucleases HhaI and Alul for 6 h at 37 °C using 10 μl of DNA and 10 units of enzyme to produce a mixture of variable length end-labelled 16S rRNA gene fragments. The lengths of the terminal restriction fragments (>50 bp) were determined by comparison with internal standards using an automated ABI PRISM™ Genetic Analyzer (Oswel Genetic Analysis, UK) and GeneScan® 3.1 analysis software (Applied Biosystems, Foster City, CA), as described previously [1]. Predicted TRF lengths were determined for each sequence obtained through clone library analysis by locating the primer and enzyme restriction site within the retrieved sequences and counting the number of base pairs in the terminal fragment. In addition, predicted TRF lengths of known archaeal and bacterial species were obtained using the TAP-TRFLP tool of the RDP database [18].

3. Results

3.1. Operational performance of R1 and R2

During the initial 45 days of this study, a steady increase in COD removal efficiencies, from 60% to 90%, was observed for R1, the sucrose-fed reactor (Fig. 1(a)). During this period, the methane content of the biogas remained relatively stable at approximately 50% and effluent VFA concentrations remained low (<500 mg l⁻¹). Following the initial decrease in operating temperature, from 37 to 30 °C on day 45, a drop in both COD removal efficiencies (to 50–60%) and in the methane content of the biogas was observed. This was associated with an immediate rise in effluent propionate levels, which remained high until day 70, after which time a decrease in propionate concentration and a parallel increase in acetate and butyrate levels was noted. An increase in COD removal efficiency was also observed from day 70 for R1, with the system reaching COD removal efficiencies in excess of 75% by day 75. A further temperature decrease (to 25 °C) on day 98 also resulted in a reduction in R1 reactor performance, with a decrease in COD removal efficiency to 40% (Fig. 1(a)) and a reduction in the methane content of the biogas to 20%, the lowest values obtained during the trial. The decrease in reactor performance was associated with elevated effluent concentrations of VFA, with acetate, propionate and butyrate concentrations of 1433, 1065 and 286 mg l⁻¹, respectively, recorded on day 100. An applied increase in the liquid upflow velocity of the system to 5 m h⁻¹ on day 118 resulted in a considerable improvement in reactor performance with an increase in R1 COD removal efficiency to approximately 75% (Fig. 1(a)). Lowering the operational temperature of R1 to 20 °C, and then to 18 °C, on days 167 and 184, respectively, did not appear to have a detrimental effect on reactor performance with COD removal efficiencies in excess of 75% consistently achieved under these temperature conditions (Fig. 1(a)). Moreover, the biogas methane content and effluent VFA concentrations remained stable in response to these temperature decreases. After 28 HRTs at 18 °C, the operating temperature of R1 was lowered by a further 2–16 °C. This resulted in a considerable decline in R1 COD removal efficiency to 50–60% and an increase in effluent VFA concentrations, particularly acetate. However,
following this perturbation, stable reactor performance was achieved with a slow, steady increase recorded in COD removal efficiency, to levels of approximately 70% after a 30-day period (Fig. 1(a)). In an attempt to further improve the treatment capacity of the reactor, the applied liquid upflow velocity in the bottom chamber of R1 was increased to 7.5 m h\(^{-1}\) on day 236, resulting in an increase in both COD removal efficiency (Fig. 1(a)) and in the biogas methane content, with stable and efficient performance levels achieved for the final 20 days of the study.

The COD removal efficiency of R2, the VFA-fed reactor, remained very stable (<80%) for the initial 240 days of the study, regardless of operating temperature, with the highest removal efficiencies achieved at 18 °C (average – 94.5%; Fig. 1(b)). R2 effluent VFA concentrations remained low for the first 240 days of R2 operation, with the most prominent VFA, acetate, generally detected at levels <800 mg l\(^{-1}\), with propionate (<400 mg l\(^{-1}\)) and butyrate (<100 mg l\(^{-1}\)) also detected. Furthermore, the methane content of the biogas produced by R2 increased over the initial 60 days of the trial to >70%, and it remained stable at this level for the remainder of the study, with the highest values obtained during operation at 16 °C (average – 74%). The increase in applied liquid upflow velocity on day 236 had a significant effect on R2 reactor performance, resulting in considerable increases in effluent propionate, butyrate and in particular, acetate levels, and a subsequent decline in R2 COD removal efficiency to 40% (Fig. 1(b)). By day 480, however, the performance of R2 had recovered with effluent VFA concentrations >1000 mg l\(^{-1}\) and stable COD removal efficiency of 80% (Fig. 1(b)).

### 3.2. Specific methanogenic activity profiles of R1 and R2 biomass samples

The seed sludge originally used to inoculate R1 and R2 displayed low SMA values towards all substrates tested, with no propionate activity detected at 22 °C (Table 1), although considerable methanogenic activity developed within the biomass in both reactors over the 200-day trial period prior to commencement of this study. In the present study, R1 sludge bed biomass ex-
hibited a higher methanogenic activity than R2 biomass with H2/CO2 at all temperatures tested. R2 biomass, conversely, exhibited higher activities for all soluble substrates, with the exception of ethanol at 22 and 15 °C on day 300 (Table 1), demonstrating the influence of feed composition on the methanogenic activity of anaerobic sludge. The biofilm sample removed from the upper chamber of R1 on day 300 exhibited a higher activity than R2 on all substrates tested. The values for the SMA profiles of the fixed biofilm samples recorded at 37 °C were generally lower than those obtained from the sludge bed biomass (Table 1). A temperature optimum of 37 °C, with respect to SMA, was recorded for all R1 and R2 sludge samples tested, with lower activities recorded as the test temperature was decreased, implying that a true psychrophilic biomass had not developed during the trial (Table 1). However, the development of a psychrotolerant biomass was observed in both reactors, with activity at 15 °C detected in R1 and R2 biomass on day 300 of the trial. In addition, attempts to set up an anaerobic bioreactor at 18–20 °C using the original seed sludge from this study as inoculum were unsuccessful, with no significant COD removal or biogas production achieved over a six month trial period (data not shown). The absence of propionate activity from the sucrose-fed R1 biomass at 22 °C on day 165 correlates with the results obtained during the previous 200-day trial period (unpublished data). However, by day 300 of the trial, a comparable activity to that obtained at 37 °C was obtained at 22 °C, indicating the adaptation of the propionate-degrading bacteria within the sludge to the lower temperatures during the course of the study.

3.3. Archaeal population dynamics within R1 and R2 biomass

Analysis of the T-RFLP profiles of biomass samples removed from R1 and R2 on days 45 (operating temperature 37 °C), 142 (operating temperature 25 °C), 197 (operating temperature 18 °C) and 300 (operating temperature 16 °C) revealed a comparable shift in the archaeal community structure within both bioreactors. In R1, the initial predominant archaeal species were *Methanosarcina* sp. (555; 137 – Table 2), although these were subsequently replaced as the major archaeal group by *Methanosaeta* sp. (187; 582 – Table 2) by day 142. However, by day 197, and for the remainder of the study, members of the hydrogenotrophic *Methanomicrobiales* group (516; 122 – Table 2) were the predominant methanogens detected in R1 biomass samples. Similarly for R2, the initially dominant *Methanosarcina* sp. were replaced by *Methanomicrobiales* sp. during the trial, with the population shift occurring by day 142. However, by day 197, and for the remainder of the study, members of the hydrogenotrophic *Methanomicrobiales* group (516; 122 – Table 2) were the predominant methanogens detected in R2 biomass samples. The predominance of *Methanomicrobiales* sp. in the final biomass samples from R1 and R2 was also demonstrated using clone library analysis, with clones showing a

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### Table 1

<table>
<thead>
<tr>
<th>Substrate/test temperature (°C)</th>
<th>Seed sludge (a)</th>
<th>Day 165</th>
<th>Day 300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>Acetate</td>
<td>7.9</td>
<td>0.9</td>
<td>104.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>10.9</td>
<td>1.1</td>
<td>197.1</td>
</tr>
<tr>
<td>Butyrate</td>
<td>10.7</td>
<td>2.1</td>
<td>71.9</td>
</tr>
<tr>
<td>Propionate</td>
<td>6.4</td>
<td>ND*</td>
<td>13.2</td>
</tr>
<tr>
<td>H2/CO2</td>
<td>27.8</td>
<td>1.5</td>
<td>212.5</td>
</tr>
</tbody>
</table>

*ND*: no activity detectable.

\(a\) Original seed sludge used to inoculate R1 and R2.

### Table 2

<table>
<thead>
<tr>
<th>Days</th>
<th>R1</th>
<th>R2</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>45</td>
<td>555</td>
<td>211</td>
</tr>
<tr>
<td>142</td>
<td>187</td>
<td>211</td>
</tr>
<tr>
<td>197</td>
<td>516</td>
<td>211</td>
</tr>
<tr>
<td>300</td>
<td>516</td>
<td>211</td>
</tr>
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</table>
strong similarity to *Methanocorpusculum parvum* accounting for 84.2% and 90.6% of total clones analysed for R1 and R2 biomass, respectively. In total, 107 clones were analysed and 10 OTUs were observed within each library. In addition to *M. parvum* – like clones, sequences closely related to *Methanoseta* sp. (R1 – 12.7% of total clones analysed; R2 – 4.4% of total clones analysed) were also detected in both sludges. Clones correlating to *Methanosarcina* sp. (3.2%) were detected in R1 sludge biomass only, while clones affiliated to *Methanospirillum hungatei* (4.5%) were only detected in R2 biomass. Phylogenetic classification of the sequences obtained with known sequences from the RDP is illustrated in Fig. 2.

3.4. Bacterial population dynamics within R1 and R2 biomass

The bacterial populations present within R1 and R2, as determined by T-RFLP analysis, appeared to be considerably more diverse and dynamic than the archaeal populations, even during periods of stable reactor performance, as reported in several previous studies [1,19]. Variations in bacterial population structure between reactors and sampling times were observed during the trial, although no clear pattern could be discerned. Many recurring peaks were present, including 292 and 301 (*Hha* reverse), 339 and 349 (*Alu* reverse) and 240 (*Alu* forward) (Table 3). However, many of the peaks

Fig. 2. Phylogenetic classification of archaeal sequences obtained from R1 and R2 biomass, sampled on day 300. The tree was constructed with evolutionary distances calculated based on the Kimura-2 model and the neighbour joining method of Saitou and Nei [26]. Three bacterial sequences were defined as outgroups during phylogenetic reconstruction. Numbers at nodes represent bootstrap values (100 replicates).
within the bacterial TRF profiles had poor phylogenetic resolution, with several distinct unrelated bacterial groups generating TRFs of equal size and, therefore, the sequences obtained from clone library analysis were used to obtain more phylogenetically informative results. Of the clones analysed from the R1 library, 10 of the OTUs (51% of total clones analysed) were closely affiliated to the Gram positive bacteria, in particular the *Bacillus–Lactobacillus–Streptococcus* group (Fig. 3). The remaining clones showed strong similarity to *Bacteroides* sp. (30%), *Spirochaeta* sp. (6%), TMF phylum (8%) and 5% were closely related only to uncultured clones for which phylogenetic information is unknown, although phylogenetic classification suggested that these clones (R1_4; R1_20; R1_21; R1_24; Fig. 3) were affiliated with the Gram positive bacteria. Similarly, R2 biomass samples contained sequences affiliated to *Bacteroides* sp. (33.4%), Gram positive bacteria (7.2%) and *Spirochaeta* sp. (1.2%), as well as a large number of unaligned clones (29.6%). However, additional clones

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### Table 3
Length (bp) of the predominant peaks in the bacterial T-RFLP profiles generated from R1 and R2 biomass

<table>
<thead>
<tr>
<th>Days</th>
<th>R1</th>
<th>R2</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>45</td>
<td>142</td>
<td>272</td>
</tr>
<tr>
<td>142</td>
<td>200</td>
<td>349</td>
</tr>
<tr>
<td>197</td>
<td>252</td>
<td>339</td>
</tr>
<tr>
<td>300</td>
<td>240</td>
<td>339</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Phylogenetic classification of bacterial sequences obtained from R1 biomass sampled on day 300. The tree was constructed with evolutionary distances calculated based on the Kimura-2 model and the neighbour joining method of Saitou and Nei [26]. Three archaeal sequences were defined as outgroups during phylogenetic reconstruction. Numbers at nodes represent bootstrap values (100 replicates).
related to δ-Proteobacteria (25.9%), green sulphur bacteria (1.2%) and green non-sulfur bacteria (1.2%) were also present (Fig. 4).

4. Discussion

Current economic, social and environmental trends have necessitated the development of high-rate, low-cost waste treatment systems. In this study, psychrophilic anaerobic digestion was demonstrated to provide the potential for such a system. Treatment efficiencies in excess of 95% and 80% were achieved at 18 °C and imposed organic loading rates of 20 kg COD m⁻³ d⁻¹ for VFA- and sucrose-based wastewaters respectively, results which are comparable and, in some cases, superior to those obtained at mesophilic temperatures [20,21]. No specific shift towards psychrophilic microorganisms was detected throughout the trial, with R1 and R2 biomass on day 300 displaying mesophilic temperature optima against all substrates tested by SMA analysis. However, due to the poor phylogenetic resolution of many bacterial TRFs [22], subtle population changes within bacterial groups and/or the presence of low numbers of psychrophilic microorganisms may have been overlooked.

A distinct shift in archael community structure was observed in both R1 and R2 during the trial with a proliferation of Methanomicrobiales sp. and a parallel decrease in Methanosarcina sp./Methanosaeta sp. observed. The reason for the shift in population structure from acetate-utilising methanogens to hydrogen-utilising methanogens is unclear. However, it has been suggested that in stressed systems, a syntrophic relationship between an acetate-utilising organism and a hydrogen-utilising methanogen serves as the major route of methane production from acetate [23]. The combination of high loading rate and low operating temperature may have resulted in a stressed state within the reactors, encouraging the propagation of hydrogenotrophic methanogens. In a similar study by the same authors [24], a similar proliferation of M. parvum-like organisms, coupled with a decrease in the relative abundance of acetoclastic methanogens, was observed in an EGSB bioreactor treating whey-based wastewater at 12–20 °C, following an increase in OLR. This further suggests the contribution of low temperature and high loading rate to the shift in archael population structure. The positive effect of low temperature on hydrogenotrophic methanogens, due to the decrease in H₂ threshold concentrations [6], may also have contributed to the emergence of Methanomicrobiales sp. during the trial, as may have changes within the microbial composition of the higher trophic groups. Regardless of the reason to why the proliferation occurred, the results do show the importance of hydrogenotrophic methanogens in the low temperature anaerobic degradation of organic matter.

The increase in upflow velocity to R1 resulted in improved performance, presumably due to enhanced substrate-biomass contact within the reactor, and also appeared to confer on the system a better ability to deal with decreased temperature. However, the increase in applied liquid upflow velocity (from 5.0 to 7.5 m h⁻¹) to R2 had an adverse effect on reactor performance, with a significant decrease in COD removal efficiencies and an increase in effluent VFA concentrations observed. The reason for the differing responses to this parameter change by R1 and R2 is likely due to variations within the microbial community structure of both reactors. A considerable number of δ-Proteobacteria sequences were detected in the R2 bacterial clone library only, with peaks corresponding to the reverse TRF lengths of this group (338 – Alu, 301 – Hha) predominant in the TRF profiles generated from R2 samples taken subsequent to the upflow velocity increase. Members of this group, such as Syntrophobacter wolinnii, are known to be important in propionate degradation and concomitant acetate production in anaerobic digesters. The
increased upflow velocity in R2 may have allowed for the proliferation of these acetogenic bacteria, due to increased substrate–biomass contact within the reactor. This would account for the low levels of propionate (<200 mg l⁻¹) and butyrate (below the detection threshold) detected in R2 effluent following the applied upflow velocity increase, and the subsequent rise in effluent acetate concentrations. The low density of acetoclastic methanogens present within R2 biomass was presumably inadequate for sufficient degradation of the high levels of generated acetate, resulting in the accumulation of this VFA in R2 effluent and the subsequent decline in reactor performance. Previous studies have suggested that high densities of both acetoclastic and hydrogenotrophic methanogens are necessary for balanced and successful anaerobic biological treatment, particularly during perturbations to the system [24,25]. The results of this trial suggest that although methanogenic populations comprised predominantly of hydrogenotrophs are capable of achieving high COD removal efficiencies within anaerobic bioreactors (<95% COD removal in R2), the absence of acetoclastic methanogens within an anaerobic reactor infers an increased susceptibility to process disturbance on the system.

Previously, anaerobic bioreactor trials were monitored exclusively by material balances, such as pH, COD removal efficiency and biogas methane content. Although all of these parameters are essential elements in the successful operation of anaerobic biological wastewater treatment systems, this study illustrates that analysis of the microbial composition of anaerobic bioreactors during reactor operation can provide a greater understanding of the process, potentially explaining reactor performance and system disturbance. In the present study, it was demonstrated that bacterial communities display high levels of complexity and dynamism, even during periods of functional stability. As a consequence, it would appear that biomonitoring of the archaean community dynamics within anaerobic bioreactors is more effective in assessment of the response of the reactor biomass to environmental change. However, with respect to bacterial populations, more specific primer–enzyme combinations should be used to monitor specific bacterial groups or species within sludge biomass, allowing for the generation of more informative results than those obtained in the present study using universal primers. Molecular techniques are now an invaluable tool for the characterisation of complex microbial communities and, when used in conjunction with process engineering and physiological measurements, they will allow a more extensive knowledge and understanding of the physiology and biochemistry of the microbial populations involved in anaerobic digestion. This will allow for the identification of potential bottlenecks and problems, the development of novel high-rate treatment systems and ultimately, the increased efficiency and exploitation of anaerobic technologies.

References


