Simultaneous biomethanisation of endogenous and imported CO_2 in organically loaded anaerobic digesters

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Abstract

In-situ biomethanisation reduces the CO_2 in biogas to CH_4 via direct H_2 injection into an anaerobic digester, but volumetric methane production (VMP) is limited by organic loading. Ex-situ biomethanisation, where gaseous substrates are fed to pure or mixed cultures of hydrogenotrophic methanogens, offers higher VMP but requires an additional reactor and supply of essential nutrients. This work combined the two approaches in a novel hybrid application achieving simultaneous in-situ and ex-situ biomethanisation within an organically-loaded anaerobic digester receiving supplementary biogas. Conventional stirred-tank digesters were first acclimated to H_2 addition, increasing biogas methane content from 50% to 95% and VMP from 0.86 to 1.51 L L^{-1} day^{-1} at a moderate loading rate of 3 g organic chemical oxygen demand per L per day (g COD_{org} L^{-1} day^{-1}). Externally-produced biogas was then added to demonstrate simultaneous biomethanisation of endogenous and imported CO_2. This further increased VMP to 2.76 L L^{-1} day^{-1} without affecting organic substrate degradation. In-situ CO_2 reduction can alter digester pH by reducing bicarbonate buffering: the combined process operated stably at around pH 8.0 with 3-5% CO_2 in the headspace. Microbial community analysis indicated the process was mediated by bacterial syntrophic acetate oxidation and highly enriched hydrogenotrophic methanogenic archaea (up to 97% of the archaeal

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population). This approach presents the opportunity to retrofit a single digester for H_2 injection to convert and upgrade biogas from several others, minimising capital and operating costs by utilising both existing infrastructure and waste-derived feedstock nutrients for simultaneous biogas upgrading and power-to-methane.

Keywords: CO_2 biomethanisation; Anaerobic digestion; Biogas upgrading; Power-to-gas; Hydrogenotrophic methanogens.

1 Introduction

Biomethanisation of CO_2 is a microbially-catalysed process that directly converts CO_2 and H_2 into CH_4 via hydrogenotrophic methanogenesis. It has attracted significant scientific attention in the past decade, as it offers one of the few viable alternatives for storing surplus electricity from renewable sources (e.g. photovoltaic and wind energy) when consumer demand is low. If this electricity is fed into the grid it can cause imbalances, leading to damage and power outages [1]. In many cases it is therefore not utilised, representing a loss of resource: but it can be used to produce H_2 by electrolysis of water in the so-called power-to-gas process. Subsequent conversion of this H_2 to CH_4 involves an energy loss, but provides a fuel gas with a higher volumetric energy density that is easier to handle and more compatible with current energy infrastructure.

Much of the research to date on the power-to-methane route has focused on reduction of imported CO_2 using pure or mixed cultures of hydrogenotrophic methanogens in bioreactors designed for this purpose [2-10]. Since the CO_2 is from an external source, this type of process is known as ex-situ biomethanisation. The maximum reported volumetric methane production (VMP) for such a system at laboratory scale is 40 L CH_4 per L working volume of digester per day (L L^{-1} day^{-1}) [2]. To achieve these high volumetric conversion rates and promote the mass

transfer of H_2, which has a low solubility (~1.35 $\times 10^{-5}$ v/v) in aqueous media [11], different reactor configurations have been investigated. These include conventional continuous stirred-tank reactors (CSTR) [5, 6, 12]; and also packed column reactors [7, 8] where extended gas-liquid contact times can be achieved, and biofilm/trickle bed reactors [3, 10] where the gases are directly in contact with the culture as a wetted biofilm.

Although high methane productivities can be achieved in these ex-situ processes, two main obstacles need to be overcome for successful commercialisation. Firstly, the reactor configurations have to be scaled up without loss in performance; and secondly, low-cost synthetic nutrient media for the growth of hydrogenotrophic methanogens have to be formulated, while the effluent generated may have to be treated in a downstream process. A recent review[13] also concluded that in high rate reactors there is likely to be a trade-off between the conversion rate and the methane percentage of the product gas, as H_2 mass transfer becomes limiting: for example, one laboratory-scale study found that at a VMP of 40 L L^{-1} day^{-1} the methane percentage was 50%, compared to 95% at a VMP of 15 L L^{-1} day^{-1} [2]. If CO_2 reductive biomethanisation is to have a role in producing high calorific value fuel gas, the minimum methane content should typically be above 90%. In Europe a minimum of 95% CH_4 is required for direct gas grid injection [14]. More research is thus required to optimise these ex-situ processes, but they offer a high potential for carbon utilisation if conversion rates can be further increased and process scale-up issues resolved.

Anaerobic digestion (AD) of biomass for energy production represents a significant CO_2 source, with production in Europe of around 18 billion m^3 natural gas equivalent in 2015 [15]. As the AD industry moves away from combined heat and power applications to biomethane as a source of heat and vehicle fuel, there is an increasing requirement for biogas upgrading. Conventional upgrading separates and discards the CO_2, with the loss of this portion of the feedstock carbon. An alternative is the direct injection of H_2 into a digester processing organic

substrates. In this case the CO_2 present in the produced biogas is reduced in-situ to methane by an indigenous hydrogenotrophic methanogenic population. Conceptually this process is attractive, as no additional reactors are needed, and the nutrients required by the methanogens are already present in the organic feed. The additional CH_4 has an energy value of around 78% of the original H_2 [16], and there is a further saving of the energy required for conventional gas upgrading which is equivalent to approximately 10% of the original methane production [17]. The resulting increase in methane output, with relatively little change in parasitic energy requirements for mixing and pumping etc, makes the process energetically attractive. The VMP, however, is limited by the digestion process itself, i.e. by the rate at which input volatile solids (VS) can be converted into biogas. Most commercial processes operate at organic loading rates (OLR) of 4-5 kg VS m^{-3} day^{-1} and the volume of CO_2 produced that can be reacted with externally introduced H_2 is thus constrained by the applied carbon loading. The highest VMP achieved for in-situ biomethanisation to date is 1.53 L L^{-1} day^{-1} [18], although it has been reported that H_2 injection can upgrade the biogas quality to 96-98% CH_4 [19, 20].

Although the applied organic loading rate is limited, it may be possible to increase the overall methane production capacity by increasing the input of gaseous carbon from external sources. This could be in the form of biogas from other digesters, in which case the biogas upgrading function for multiple digesters could be performed by a single unit. This depends upon the capacity to increase the gaseous carbon conversion through enriching the hydrogenotrophic population without detrimental effects on organic carbon conversion or digestion stability. Potential problems are that reduction of the headspace CO_2 content can cause pH swings in in-situ digesters due to changes in the bicarbonate equilibrium [21]; while high H_2 partial pressures can suppress the degradation of propionic acid, as has been observed in previous studies [22, 23].

If these issues can be avoided the concept has wide areas of application, in particular for enhancing methane yields from a given organic carbon input. Examples include the cultivation

of energy crops, where the amount of photosynthetically-fixed carbon is determined by plant type and environmental conditions, and biomethanisation could thus increase methane yield by as much as 70% per hectare. Likewise, for waste and wastewater treatment plants receiving a fixed carbon load based on population equivalent, the methane output could be increased by 60% or more. The process thus represents a first move towards utilisation of CO_2 as opposed to capture for storage. Although there are many studies on both in-situ and ex-situ biomethanisation, to date no work on simultaneous hybridisation of these processes has been reported and the limitations on introduction of external CO_2 to an in-situ process in the form of imported biogas are not known.

In the current work, to demonstrate the novel simultaneous in-situ and ex-situ biomethanisation process, digesters were first run to provide baseline data on methane productivity and performance, then acclimated to H_2 addition to allow enrichment of the hydrogenotrophic population and establishment of in-situ biomethanisation. Once this had been achieved, an additional load of externally-produced CO_2 was added to realise ex-situ and in-situ biomethanisation simultaneously. In parallel with performance monitoring, characterisation of the microbial population was carried out to show any changes qualitatively, and where possible to quantify the increase in numbers of hydrogenotrophic methanogens. A lower limit for biogas CO_2 content to prevent the onset of instability as a result of pH increases due to deficiencies in bicarbonate buffering was also established.

2 Materials and Methods

2.1 Digester operation

Eight identical 1-L digesters (designated D1-8) were inoculated with 500 mL of digestate from Millbrook Wastewater Treatment Plant, Southampton, UK. The inoculum characteristics are summarised in Table S1. Each digester was mixed using a 3-blade impeller 40 mm in diameter

which was shaft-driven by a geared DC motor at 200 rpm. The digesters were maintained at 37 ^{o}C in a water bath. An organically-rich synthetic substrate was used as the feed, the composition of which is given in Table S2. The substrate was fed to the digesters at different concentrations but with the same volume addition to provide a constant hydraulic retention time (HRT) of 15 days. The four pairs of digesters received organic loading rates (OLR) of 2 (D1&2), 3 (D3&4), 4 (D5&6) and 5 (D7&8) g COD_{org} L^{-1} day^{-1}. The feedstock was added manually once per day, resulting in a semi-continuous operating mode with a feed cycle of 24 hours.

The study consisted of three phases defined in terms of gaseous input. In phase I there was no H_2 injection, and the digesters were configured as conventional continuous stirred-tank reactors (CSTR) with daily organic feed input (Figure 1a). The digesters were operated for 4-6 HRT in this phase to ensure stable conditions and allow collection of baseline data on CO_2 production, pH and methanogenic community structure before progressing to phase II.

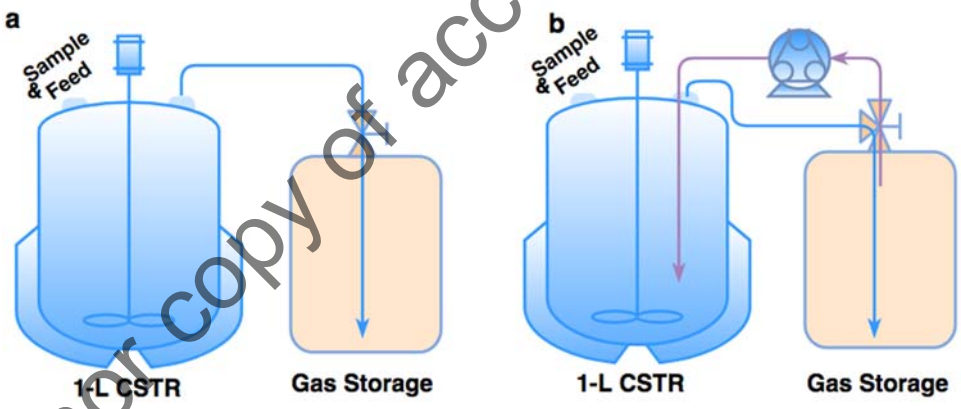

Figure 1 Schematic of digester configurations in phase I (a) and phase II and III (b)

In phase II, H_2 was introduced into the digesters incrementally to enrich the population of hydrogenotrophic methanogens, and to allow stoichiometric CO_2 reduction in proportion to the capacity of this population, without H_2 or VFA accumulation. The volume of H_2 injected into the digesters was calculated according to the CO_2 production at each applied OLR in phase I. The theoretical maximum volume of H_2 required ($V_{H2,theo}$) was taken as four times the baseline

volume of CO_2 produced. At the beginning of phase II, 10% of $V_{H2,theo}$ was injected, followed by 20%, 30%, 50%, 75% and 100%. In practice gas addition was achieved by dispensing the quantity of H_2 required for the daily feed cycle into gas-impermeable aluminium/mylar foil bags using an EL-Flow® Prestige mass flow controller (Bronkhorst, UK). Each day after the introduction of the organic substrate the gas bag containing H_2 was connected to the digester. Gas was pumped in at a rate of 8 mL min^{-1} through a sparge tube located beneath the impeller, by means of a peristaltic pump. The digester headspace was connected directly into the gas bag, providing a continuous gas injection and recycling loop (Figure 1b) in which the residual H_2 was continuously diluted by enriched biomethane. At the end of each 24-hour feed cycle the gas bag was removed and the gas volume and composition were measured. Phase II was operated for 13-19 HRT in total.

In phase III, an external source of CO_2 (mixture of CH_4/CO_2 at 50/50 v/v, BOC Group, UK) was injected to simulate the addition of biogas from other digesters as a supplementary CO_2 source, with additional H_2 to balance this. The same operating procedure was used as in phase II except that at the beginning of each daily feed cycle, the gas bag also held the supplementary CH_4/CO_2 mix and the H_2 required to convert 100% of this additional imported CO_2 load as well as 95% of the endogenous CO_2. The volume of imported CO_2 added at the beginning of phase III was 25% of the phase I baseline endogenous CO_2. This was incrementally increased to 50%, 100%, 150%, 175% and 200%, corresponding to a total CO_2 availability (endogenous plus imported) rising from 125 to 300% of the baseline value. Phase III ran for 6-7 HRT in total.

2.2 Analytical methods

Total solids (TS) and volatile solids (VS) were determined by Standard Method 2540 G and total Chemical Oxygen Demand (tCOD) by Standard Method 5220 C [24]. Total Kjeldahl Nitrogen (TKN) was converted to total ammonium nitrogen (TAN) by acid digestion in a

BÜCHI K-435 Digestion Unit, with H_2SO_4 and K_2SO_4 as the reactants and $CuSO_4$ as the catalyst. TAN was measured using a BÜCHI Distillation Unit K-350 with NaOH addition, followed by collection of the distillate in boric acid indicator and titration with 0.25 N H_2SO_4. Elemental composition (C, H, N, S) was determined using a FlashEA 1112 Elemental Analyser (Thermo Finnigan, Italy), following the manufacturer's recommended procedures and using a Birch Leaf standard. Digestate samples for dissolved inorganic carbon (DIC) analysis were centrifuged and then filtered through syringe membrane filters (0.2µm pore size), and the filtrate measured using a total organic carbon analyser (Shimadzu TOC-V_{CPH}) from filtered digestate samples, following the manufacturer's recommended procedures with sodium carbonate and sodium bicarbonate as standards. VFA concentrations in the digestate were measured by gas chromatography (Shimadzu GC-2010) using a flame ionisation detector and a capillary column (SGE BP-21) with nitrogen as the carrier gas. Samples were acidified to 10% formic acid and measured against mixed standards of 50, 250 and 500 mg L^{-1} of acetic, propionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids. Biogas composition was determined using a MG#5 Gas Chromatograph (SRI Instruments, USA) with a thermal conductivity detector (TCD). The instrument had two linked analytical lines with CH_4 and CO_2 separated by a Porapak Q column (80/100 mesh, 6ft), while H_2 was separated by a molecular sieve 5A column (6ft). The GC was calibrated with standard gases from BOC Ltd, UK: CH_4 (>99.95%), 65% CH_4/35% CO_2, 50% CH_4/50% CO_2 and CO_2 (>99.95%) (v/v). Biogas volumes were measured using a weight-based water displacement gasometer [25]. Gas volumes are reported at a standard temperature and pressure of 0 ^oC and 101.325 kPa.

The response of the microbial community to the gradual increase in H_2 injection in digesters at 2 and 3 g COD_{org} L^{-1} day^{-1} was monitored by 16S rRNA amplicon sequencing. At the end of each increment in H_2 input, digestate samples were taken which were then subjected to DNA extraction, polymerase chain reaction (PCR) amplification and sequencing to determine the

relative abundance of different microbial groups. Results reported are the average for duplicate digesters. Detailed methods and data analysis for microbial community profiling can be found in the supplementary information.

3 Results and Discussion

3.1 Phase I - conventional AD for determination of baseline conditions

The phase I digester operation established baseline values for performance and stability parameters at the applied OLR. After an initial period of acclimatisation to the synthetic feedstock, all digesters stabilised at their respective loadings (Figure S1). Table 1 shows average values for key parameters in each pair of digesters for the last 10 days of phase I when the digesters were regarded as being in steady state. At OLR from 2-4 g COD_{org} L^{-1} day^{-1} the daily biogas production (V_{Biogas}) was proportional to OLR and there were no significant differences in biogas composition between the digesters, indicating uninhibited digestion of the synthetic substrate. The measured biogas composition was very close to the theoretical value of 49% CH_4 / 51% CO_2 calculated from the feedstock elemental composition using the Buswell equation [26]. At the highest loading of 5 g COD_{org} L^{-1} day^{-1} digesters D7&8 showed a lower lower biogas methane content than D1-D6, reflected in a marginally lower specific methane production (SMP, L g^{-1} COD_{org}), suggesting that the digesters may have been approaching their loading limit under these operating conditions.

Table 1. Summary of operational and performance parameters for digesters at steady state for the last 10 days of phase I (average of pair ± standard deviation)

Digester	pH	V_{biogas}	CH_4	CO_2	SMP	V_{CO2}	$V_{H2,theo}$
		mL day^{-1}	% v/v	% v/v	L g^{-1} COD_{org}	mL day^{-1}	mL day^{-1}
D1&2 (2) *	7.12 ± 0.02	589 ± 22	50.0 ± 0.7	47.2 ± 0.6	0.294 ± 0.012	278 ± 11	1112 ± 43
D3&4 (3)	7.32 ± 0.03	867 ± 18	50.0 ± 0.2	47.3 ± 0.3	0.289 ± 0.006	410 ± 8	1639 ± 35
D5&6 (4)	7.38 ± 0.02	1159 ± 30	49.7 ± 0.2	47.9 ± 0.7	0.288 ± 0.007	555 ± 16	2220 ± 64
D7&8 (5)	7.38 ± 0.02	1434 ± 36	48.5 ± 0.6	49.1 ± 0.9	0.278 ± 0.008	704 ± 24	2816 ± 97

*: Values in brackets denote digester OLR in g COD_{org} L^{-1} day^{-1}

3.2 Phase II – in-situ biomethanisation of endogenously-produced CO_2

H_2 input started at 10% of $V_{H2,theo}$ and was increased to the next level when complete conversion of H_2 had been achieved and maintained for at least a week. Monitoring of the VFA profile of the digesters was used as an indicator of the success of this gradual acclimatisation strategy; the results showed that throughout the transition period there was no propionate accumulation, in agreement with previous acclimatisation studies [19, 27]. The performance of reductive CO_2 biomethanisation was assessed based on the methane percentage in the product biogas, and also on the SMP. These are presented in Figure 2 for digesters D1-D4 operated at 2 and 3 g COD_{org} L^{-1} day^{-1}, and in Figure 3 for D5-D8 operated at 4 and 5 g COD_{org} L^{-1} day^{-1}. The results show the biogas methane content for all eight digesters increased from ~50-52%, to ~93-95% by the end of phase II. The maximum methane content of 99% was obtained in digester D3 on day 231 (Figure 3) at 100% of $V_{H2,theo}$. As expected, however, this performance could not be sustained due to a fall in bicarbonate buffering caused by the reduction of CO_2 partial pressure and an associated increase in pH, which rose to 8.45 and led to an accumulation of VFA in the digesters (Figure 4c). The H_2 input was therefore decreased to 95% of $V_{H2,theo}$, which resulted in 3-5% CO_2 remaining in the biogas, and sufficient bicarbonate buffering to prevent pH increasing beyond the optimum range for digestion.

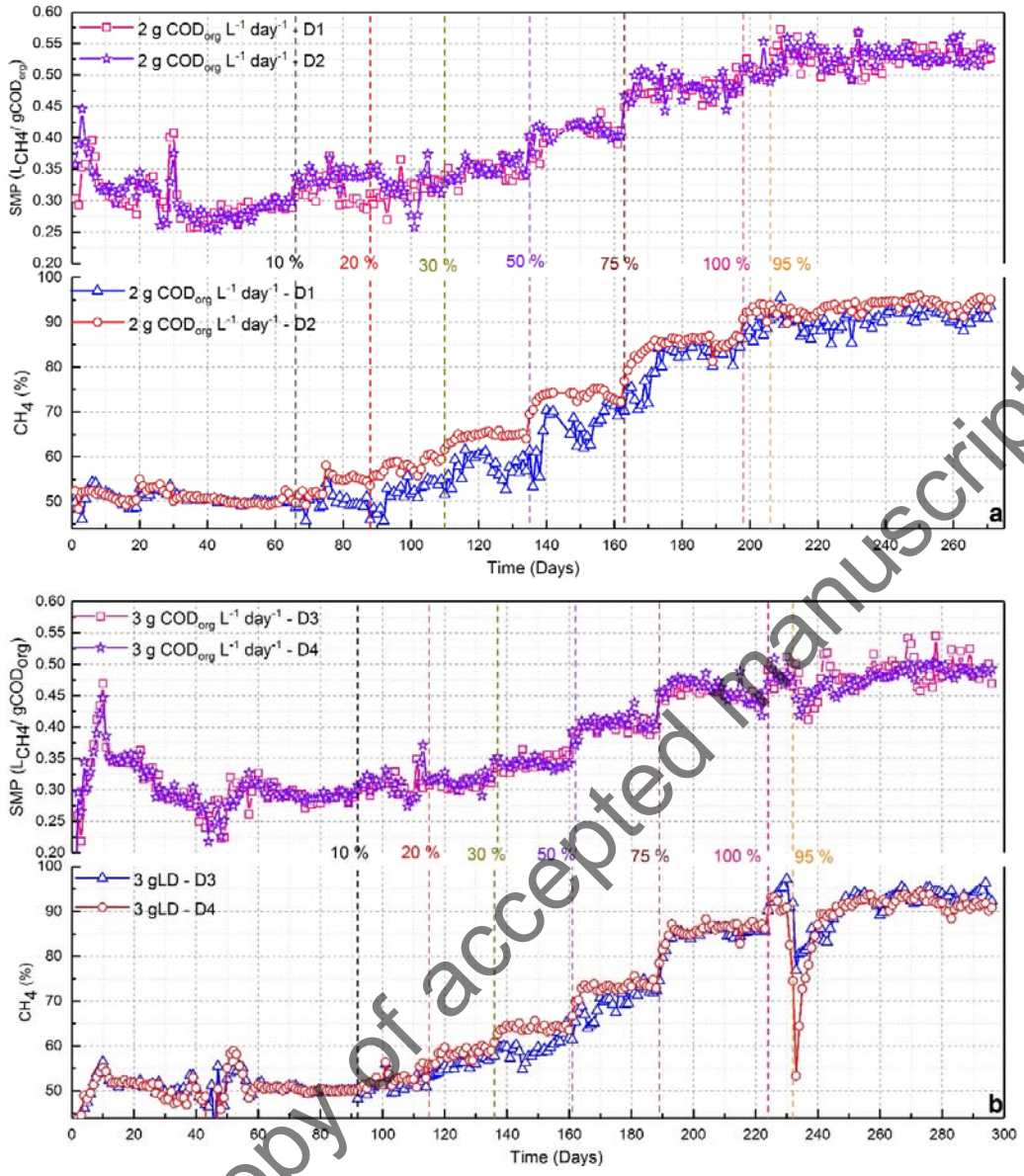

Figure 2 Biogas methane content and SMP in phases I and II as a function of time and H_2 input (vertical dashed lines) for digesters at (a) 2 and (b) 3 g COD_{org} L^{-1} day^{-1}

During this final period no H_2 was detected and the biogas typically contained 95% CH_4 and 3-4% CO_2, with 1-2% unaccounted-for volume which could be attributed to water vapour and the common impurities of biogas such as H_2S, and NH_3 [28]. The methane content of 95% is comparable to the reported maximum range of 92-98% from other CO_2 biomethanisation processes [10, 19, 20, 29, 30].

There were some notable differences between digesters operating at different loading rates, as shown from the total VFA concentration and its compositional profiles in Figure S2-3. For D7&8 at the highest loading rate of 5 g COD_{org} L^{-1} day^{-1} a longer H_2 acclimatisation period was needed, and some VFA accumulation was observed at the highest H_2 input. Total VFA concentrations were around 2 g L^{-1} and mainly consisted of acetic acid (Figure S3b). These were persistent, and sufficient to cause a pH drop and some signs of instability in the digesters (Figure 3b, day 249 onwards), preventing the establishment of long-term stable operation at a methane concentration of ~95%. VFA accumulation was also seen in D5&6 at 4 g COD_{org} L^{-1} day^{-1} (Figure S3a), but this was eventually reduced, and stable operation was achieved (Figure 3a, day 327 onwards) with a biogas methane content of 95%. There is no definitive reason why acetic acid should be persistent in D7&8, although one possibility may be an enrichment of homoacetogenic bacteria converting H_2 and CO_2 into acetic acid coupled with a decreasing population of acetoclastic methanogens [30]. Even during phase I some signs of instability were observed in D7&8: it is likely that these digesters were challenged at the higher loading with a very readily degradable substrate, and simply did not have the capacity to convert this applied load effectively to gaseous end products at the applied HRT.

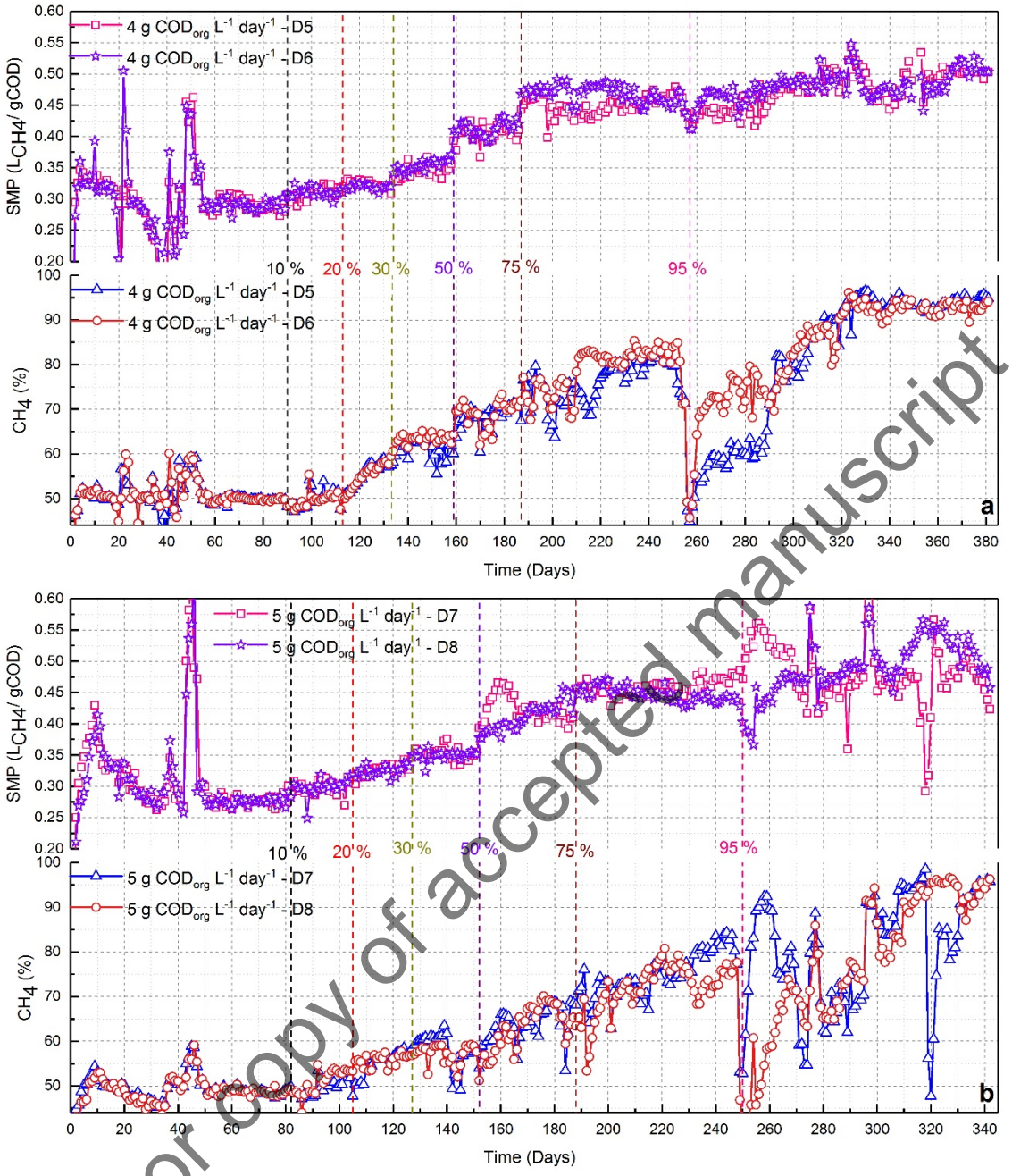

Figure 3 Biogas methane content and SMP in phases I & II as a function of time and H_2 input (vertical dashed lines) for digesters at (a) 4 and (b) 5 g COD_{org} L^{-1} day^{-1}.

The SMP in phase I (without H_2 injection, as shown in Table 1) increased as a result of H_2 injection to 0.525, 0.502, 0.505 and 0.475 L g^{-1} COD_{org} for digesters operated at 2, 3, 4 and 5 g COD_{org} L^{-1} day^{-1}, respectively. This was lower than might be expected based simply on the phase I value plus additional SMP from organically-derived CO_2 present in the biogas. For a $V_{H2,theo}$ of 95% the expected SMP values would be 0.552, 0.544, 0.548 and 0.544 L g^{-1} COD_{org}-

[1]. This difference could be accounted for in part by a proportion of the organic carbon being used for the growth of extra biomass needed to realise the in-situ CO_2 biomethanisation [13]. This is supported by a carbon mass balance calculation. The daily carbon input (C_{fed}) from the sucrose, yeast extract and urea was equal to 0.404 [0.606] g C for digesters operated at 2 g COD_{org} L^{-1} day^{-1} [with figures in brackets denoting values at 3 g COD_{org} L^{-1} day^{-1}]. The daily carbon output consisted of the carbon in the biogas (C_{gas}), carbon output as total organic carbon in the digestate (mostly as microbial biomass, $C_{biomass}$), and carbon output as dissolved inorganic carbon (DIC) in the digestate ($C_{inorganic}$). In steady state conditions at the end of phase II the average output of C1 gases was 0.550 [0.799] L day^{-1}, corresponding to a daily C_{gas} output of 0.295 [0.428] g C for digesters operated at 2 [3] g COD_{org} L^{-1} day^{-1}. The average VS concentration was 7.2 [9.9] g L^{-1}, giving a daily wastage of 0.24 [0.33] g VS. The digestate composition was not analysed; but if it is assumed that this VS is entirely composed of microbial biomass with a typical carbon content of 47% [31], the daily $C_{biomass}$ output was 0.115 [0.158] g C. The average DIC concentration of the digesters was 0.295 [0.452] g L^{-1}, corresponding to a daily $C_{inorganic}$ output of 0.010 [0.015] g C. Hence, the total daily output of carbon was around 0.420 [0.602] g C, equivalent to 104% [99%] of the input values. From Figure 4 it can be seen that the VS content of the digestate rose slightly during the course of phase II, while the ratio of gaseous carbon output to organic carbon input fell slightly, reflecting this growth in microbial population at the expense of carbon in the gaseous feed.

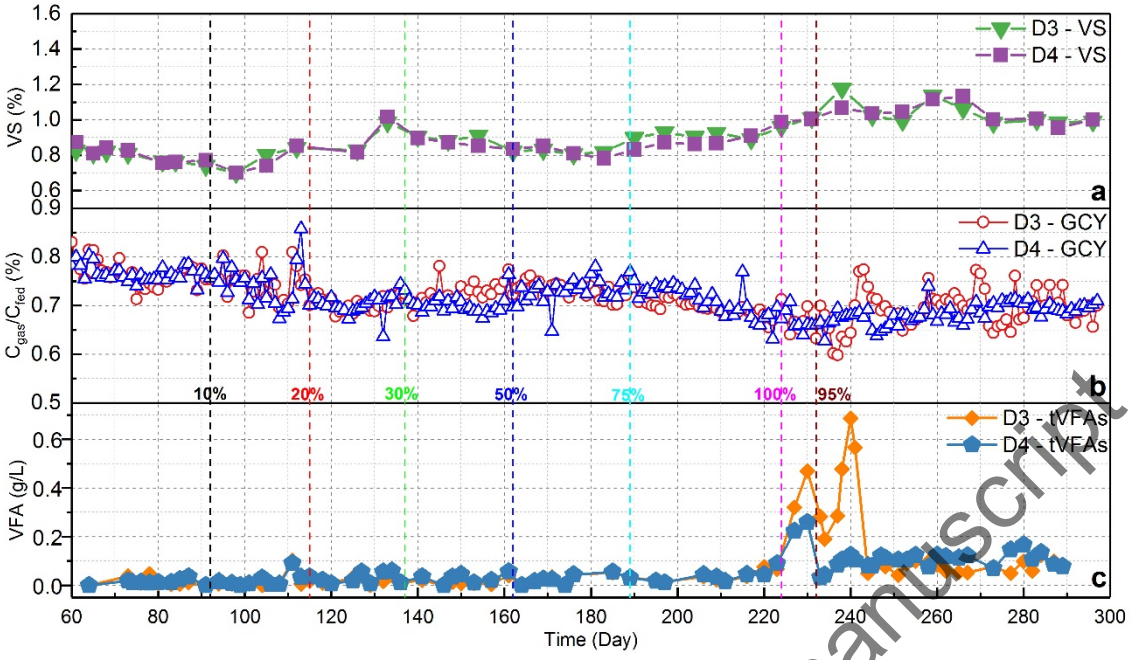

Figure 4 Monitoring parameters for digesters at 3 g COD_{org} L^{-1} day^{-1} during phase II: (a) volatile solids, (b) ratio C_{gas}/C_{fed} and (c) tVFA with H_2 input shown as vertical dashed lines

The mode of operation of the digesters with gas recirculation was successful in achieving a high conversion of endogenously-produced CO_2, indicating that there were no H_2 mass transfer limitations in this phase. This supports the findings of others who have also used gas recirculation to extend gas-liquid contact time as the main measure for facilitating mass transfer in both ex-situ and in-situ biogas upgrading systems [10, 18, 30]. The mass transfer of H_2 into the digestate might also be enhanced by the favourable concentration gradient created as a result of the activity of the growing population of hydrogenotrophic methanogens (as shown in section 3.4) which continually reduce the H_2 concentration in solution.

The digesters operated at 4 and 5 g COD_{org} L^{-1} day^{-1} were not progressed to Phase III due to the instabilities encountered at the end of phase II, which may have been associated with the relatively high organic loading rate for the specific feedstock type and operating conditions.

3.3 Phase III - simultaneous biomethanisation of endogenous and imported CO_2

The main aim of the research was realised by increasing the applied carbon load to the digester through adding externally-produced CO_2 in the form of biogas. The process thus simulated taking biogas from several digesters and using a single digester to convert the endogenously-produced and imported biogas CO_2 into an upgraded biomethane. A mixture of CH_4/CO_2 together with additional H_2 was injected into the digesters operated at 2 and 3 g COD_{org} L^{-1} day^{-1}. The results in Figure 5 show that the SMP (excluding exogenous CH_4 in the injected biogas) increased in line with the imported CO_2 load applied, from 0.525 to 0.975 L g^{-1} COD_{org} in digesters D1&2 at the end of phase III, and from 0.502 to 0.920 L g^{-1} COD_{org} in digesters D3&4. Biogas methane concentrations remained stable at 93-96%. Comparing the SMPs at the end of phase I and III, methane productivities rose from 0.294 to 0.975 L g^{-1} COD_{org} in D1&2 and from 0.289 to 0.920 L g^{-1} COD_{org} in D3&4, an overall increase of more than 3-fold.

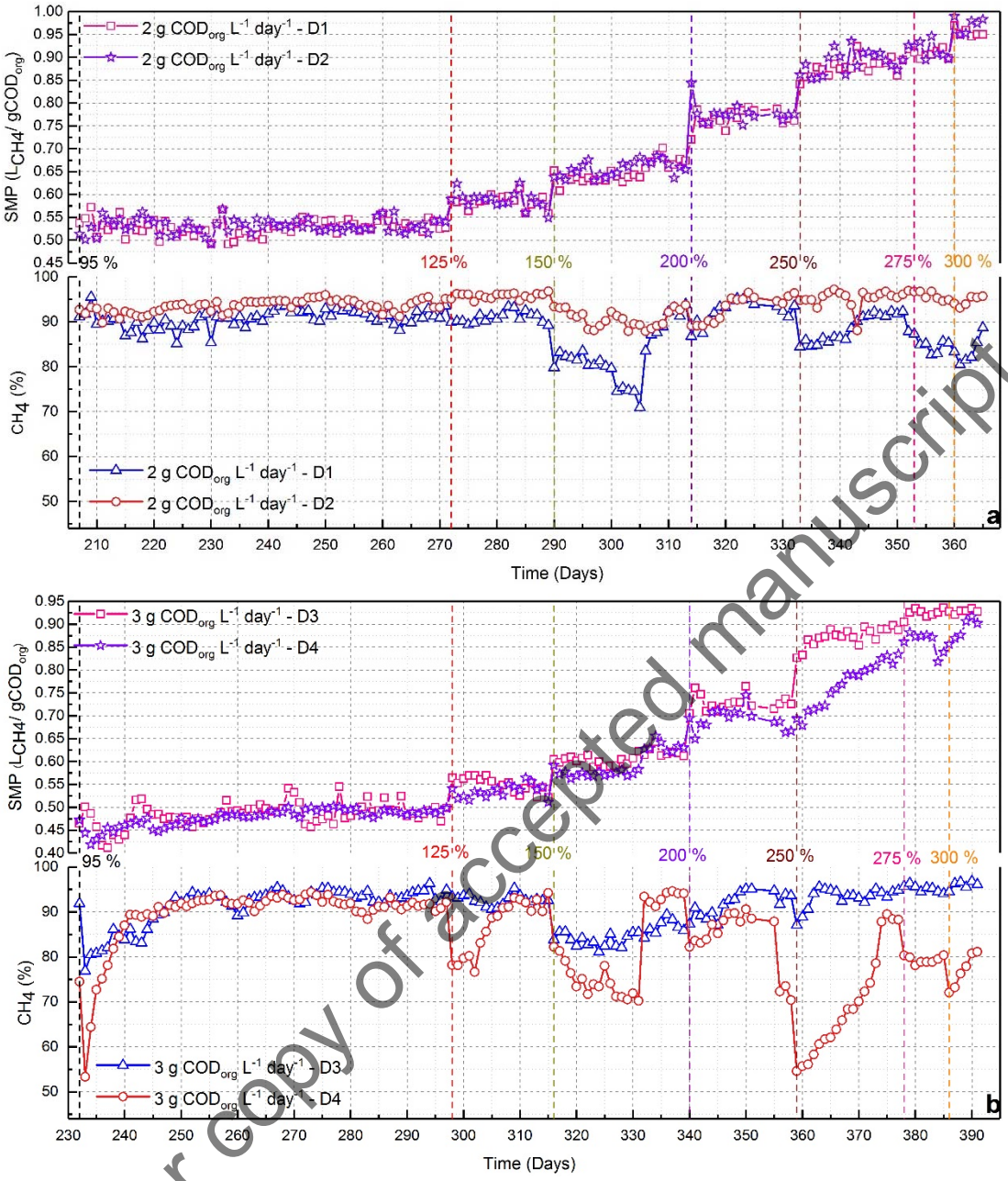

Figure 5 Biogas methane content and SMP (excluding exogenous CH_4) in phase III for digesters operated at 2 (a) and 3 g COD_{org} L^{-1} day^{-1} (b). Vertical dashed lines show total CO_2 availability (endogenous plus imported) as a % of endogenous CO_2 in phase I

3.4 Microbiological analysis of Phase I, II, and III digesters

Analysis of the microbial community using 16S rRNA sequencing for samples from the digesters at 2 and 3 g COD_{org} L^{-1} day^{-1} showed that the average relative abundance of archaea in the total microbial community increased from 1.12 ± 0.12% prior to H_2 injection to 4.41 ± 0.10% at the end of phase II in D1&2, and from 2.15 ± 0.01% to 4.76 ± 0.58% in D3&4 (Figure

6). Samples taken from the same digesters at the end of phase III with both endogenous and imported CO_2 showed a further enrichment of archaea to 6.26 ± 0.49% for D1&2 and 9.38 ± 0.53% for D3&4. A similar value was reported for a mixed culture ex-situ biomethanisation system receiving only gaseous feedstocks, where the archaea accounted for 7.3% of the total microbial community [32].

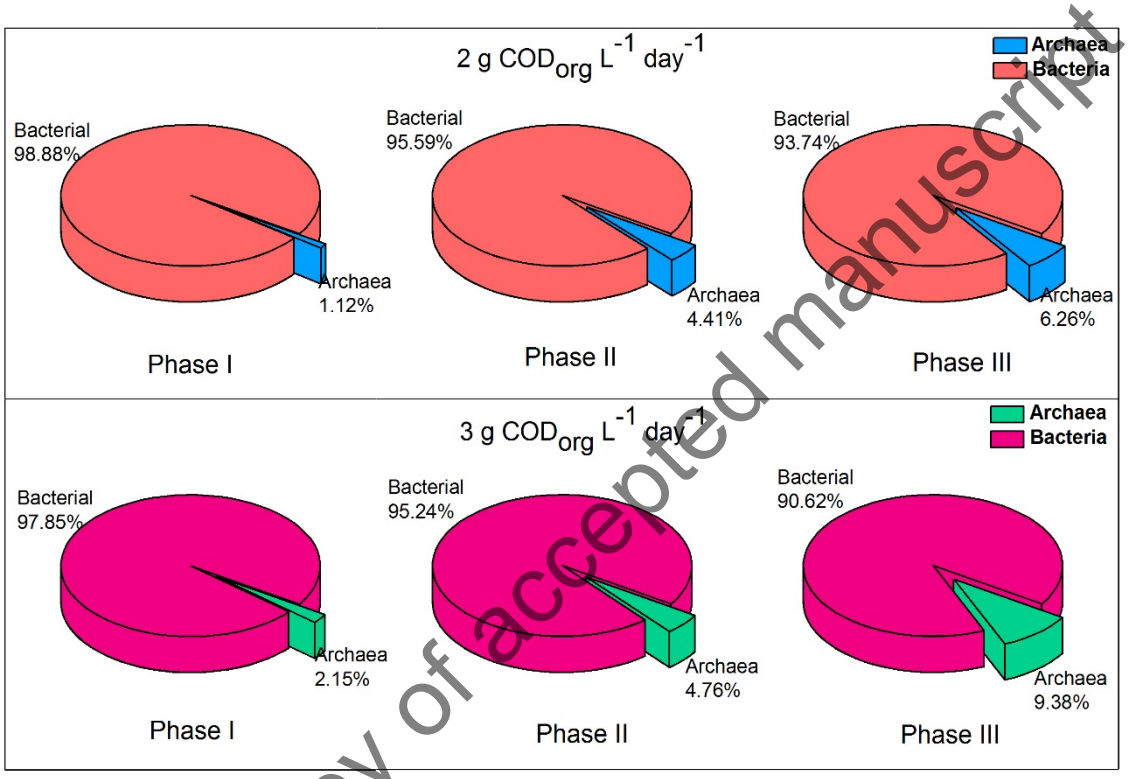

Figure 6 Relative abundance of archaea in the total microbial community at different phases for digesters operated at 2 and 3 COD_{org} L^{-1} day^{-1} (data represent mean for two digesters)

The archaeal community amplicon sequences were assigned to four of the seven orders of methanogens [33], as shown in Figure 7 for the digesters at 2 and 3 g COD_{org} L^{-1} day^{-1}. Prior to H_2 injection, the archaeal community in the digesters operated at 2 g COD_{org} L^{-1} day^{-1} was dominated by the hydrogenotrophic *Methanomicrobiales* (51.5 ± 4.7%) with acetoclastic *Methanosaetaceae* (the sole family detected from the order *Methanosarcinales*) (26.5 ± 1.67%), methylotrophic *Thermoplasmatales* (all from the order *Methanomassiliicoccales* [34-36], 18.6 ± 2.65%) and hydrogenotrophic *Methanobacteriales* (3.4 ± 0.46%) accounting for the balance of

archaea. A similar distribution was seen for the digesters at 3 COD_{org} L^{-1} day^{-1}. When external H_2 was injected the relative abundance of hydrogenotrophic methanogens increased, indicating gradual enrichment of this fraction of the community; by the end of phase II hydrogenotrophic methanogens collectively made up over 93% of the archaeal community and by the end of phase III this had increased to 97% at both 2 and 3 COD_{org} L^{-1} day^{-1}. There was a significant reduction in the proportion of methanogens reliant on the cleavage of acetate (*Methanosaetaceae)* or known not to be hydrogenotrophic (*Thermoplasmatales*), the latter found in phase I in unusually high abundance for anaerobic digestion. The digesters at 4 and 5 g COD_{org} L^{-1} day^{-1} showed similar changes during phase II to those seen at 2 and 3 g COD_{org} L^{-1} day^{-1}, as shown in Figure S4. Profiling of the archaeal community at genus level showed that at the end of phase II and III, the dominant methanogen was hydrogenotrophic *Methanoculleus* sp. (Figure S5), also reported by others as a highly abundant member of methanogens in CO_2 reductive biomethanisation systems [37].

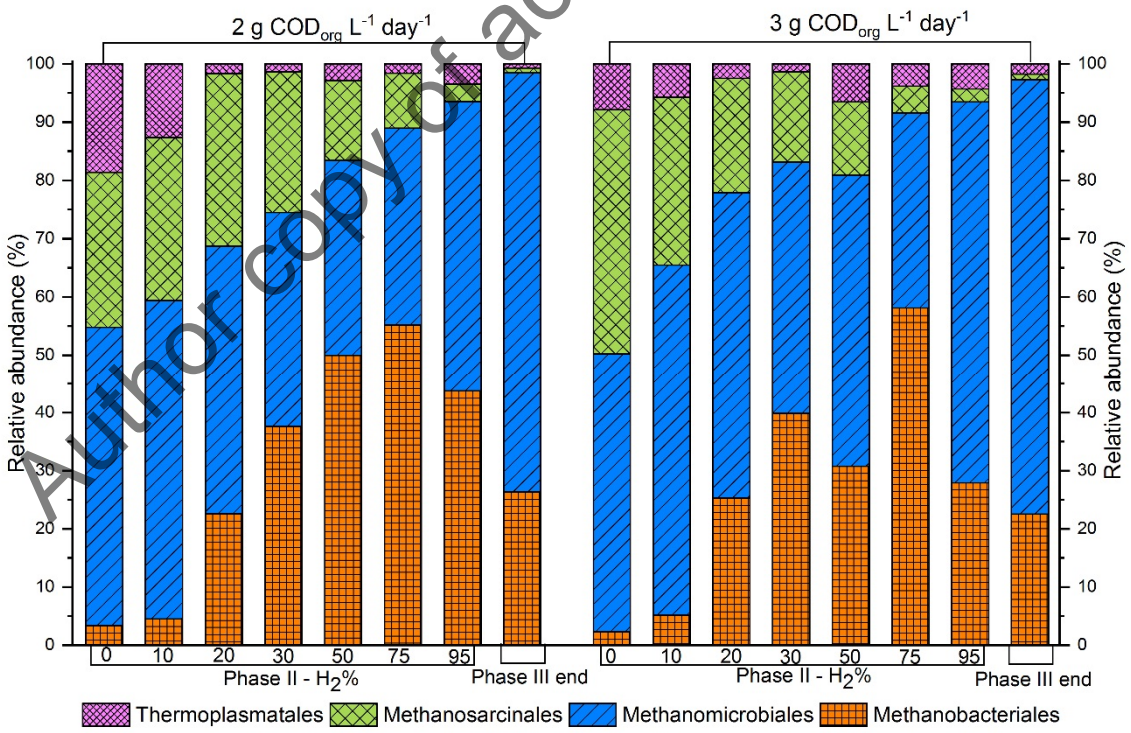

Figure 7. Average distribution of archaeal community in phase II and III for duplicate digesters operated at 2 and 3 g COD_{org} L^{-1} day^{-1}

The limitations of 16S rRNA sequencing mean that the abundances of methanogens can only be expressed relative to the total archaea, making it challenging to pinpoint specific pathways within the system. If, however, it is assumed that the digestate VS content consists entirely of microbial biomass, and abundance correlates to % VS, the relative mass of acetoclastic and hydrogenotrophic methanogens can be estimated as shown in Table 2. Since the VS content at OLR 2 [3] g COD_{org} L^{-1} day^{-1} rose from 6.5 to 7.2 [7.6 to 9.9] g VS L^{-1} between the end of phases I and II, while the estimated archaeal biomass only rose from 0.07 to 0.32 [0.16 to 0.47] g VS L^{-1}, it is clear that only a small proportion of this increase in VS can be accounted for by the growth in the methanogenic population. At the same time, the proportion of acetoclastic methanogens showed a decline.

Table 2 Estimated biomass concentration of methanogens in digesters operated at 2 and 3 g COD_{org} L^{-1} day^{-1} based on average digestate VS content in the final 10 days of phases I, II and III

	2 g COD_{org} L^{-1} day^{-1}			**3 g COD_{org} L^{-1} day^{-1}**		
Phase	**I**	**II**	**III**	**I**	**II**	**III**
Digestate VS (% wt)	0.65	0.72	0.74	0.76	0.99	0.99
Total microbial concentration (g kg^{-1})	6.50	7.20	7.40	7.60	9.90	9.90
Archaea fraction in total microbes (%)	1.12	4.41	6.26	2.15	4.76	9.38
Archaea concentration (g kg^{-1})	0.07	0.32	0.46	0.16	0.47	0.93
Acetoclastic fraction in archaea (%)	26.50	2.97	0.82	41.89	2.23	0.94
Hydrogenotrophic fraction in archaea (%)	54.82	93.53	98.45	50.24	93.45	97.29
Acetoclastic concentration (mg kg^{-1})	18.55	9.50	3.77	67.02	10.48	8.74
Hydrogenotrophic concentration (mg kg^{-1})	38.37	299.29	452.87	80.38	439.22	904.79

It is possible that the decline in the acetoclastic population and pathway is compensated for by an increase in syntrophic acetate oxidation (SAO) in which acetate is oxidised into CO_2 and H_2, which are subsequently converted into CH_4 by the growing population of hydrogenotrophic

methanogens [38, 39]. Some evidence that could support this is the population increase of the family *Thermoanaerobacteraceae* which includes two known SAO bacteria, namely *Thermacetogenium phaeum* and *Tepidanaerobacter acetatoxydans* [39]. An increase of approximately 8% in relative abundance of *Thermoanaerobacteraceae* was observed at the end of phase III in digesters operated at 2 and 3 g COD_{org} L^{-1} day^{-1} (Figure 8). An increase in hydrogenotrophic methanogens in the *Methanoculleus* genus with a concurrent rise in *Thermoanaerobacteraceae* was also observed in another biomethanisation study [37].

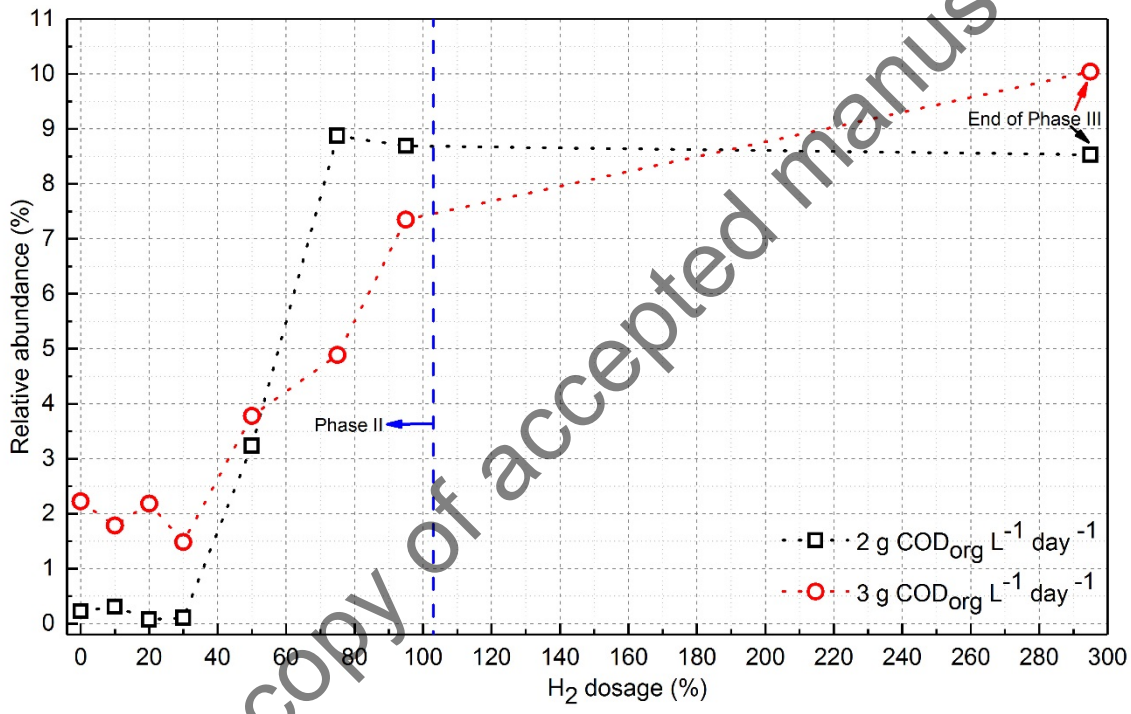

Figure 8 Relative abundance of bacteria family *Thermoanaerobacteraceae* to total microbial community during phase II and III in digesters operated at 2 and 3 COD_{org} L^{-1} day^{-1}

3.5 Influence of H_2 injection on digester pH and bicarbonate buffering

The greatest risk to stable in-situ biomethanisation in CSTR-type digesters with suspended cultures is a rise in pH as a result of a fall in the bicarbonate buffering strength due to depletion of headspace CO_2 [21]. The problem has been consistently encountered by others [10, 18, 19, 21], and likewise in this study significant pH increases were observed in all eight digesters during phase II. The upper limit of pH at which anaerobic digestion is not inhibited is generally

acknowledged to be around pH 8.5 [40-42], and a pH near to this was recorded for digester D3 in phase II on day 231, when $V_{H2,theo}$ reached 100% and almost complete CO_2 removal occurred. The CH_4 content of the biogas on that day exceeded 99%, but as mentioned above this was not sustainable, and VFA accumulation was evident as shown in Figure 4c and Figure S2b.

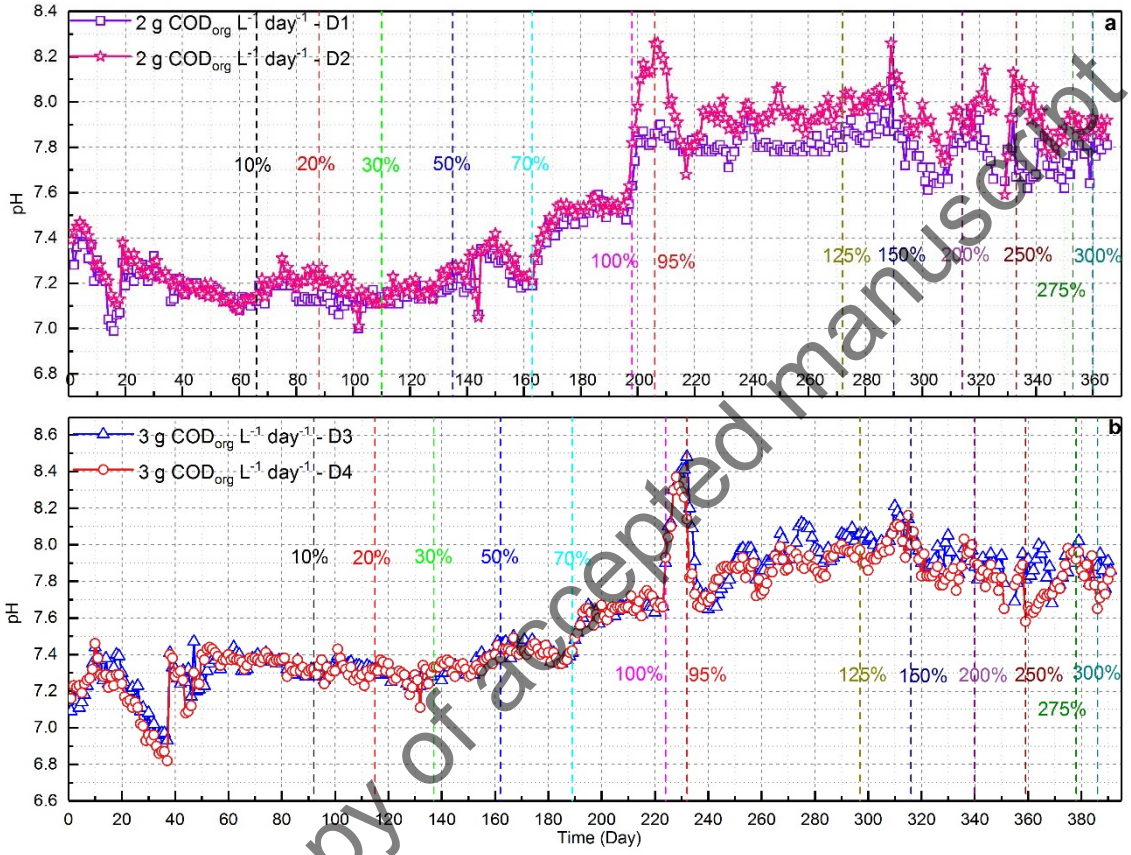

Figure 9 pH of digesters at (a) 2 and (b) 3 COD_{org} L^{-1} day^{-1} during phase II and III; H_2 inputs are shown by vertical dashed lines

Figure 9 shows the pH evolution against time in the digesters operated at 2 and 3 g COD_{org} L^{-1} day^{-1} and Figure S6 shows those of digesters operated at 4 and 5 g COD_{org} L^{-1} day^{-1}. The pH of the digesters during phase III remained relatively stable with increasing external CO_2 load, although values were generally higher than those seen when using only endogenously-produced CO_2 in phase II. Digester pH is closely related to the partial pressure of CO_2 in the final biogas (P_{CO2}) as it is determined by the equilibria between carbonic acid, bicarbonate and carbonate alkalinity as well as ammonium/ammonia. Henry's constant (K_h) regulates

dissolution of CO_2 into the liquid phase and the hydration constant (k_H) determines the concentration of carbonic acid (H_2CO_3), which then dissociates into H^+, HCO_3^- and CO_3^{2-} in two steps (Figure 10).

Figure 10. Schematic illustration of bicarbonate and ammonia buffering within AD

It is possible to estimate pH based on P_{CO2} for a closed system with pure water as the liquid phase, as all the constants used in the equations are defined and it can be assumed that the rule of charge neutrality applies. That system does not, however, fully represent the complex conditions in a digester, where all cations, anions, ammonia and VFA would need to be considered and any resulting expression would also be very complex. An empirical modelling approach was therefore adopted to relate pH to the change in P_{CO2} using data from digesters D1-D4.

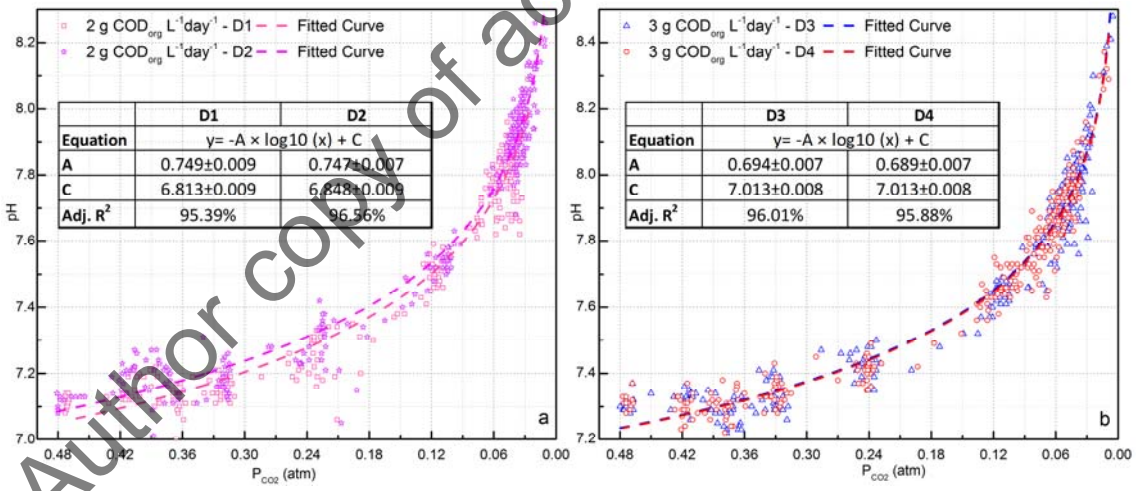

Figure 11. pH as a function of CO_2 partial pressures for digesters D1&2 (dots, a) and D3&4 (dots, b) during the three phases of experiment, with curve fitted to equation 4

Figure 11 shows the relationship of digester pH against P_{CO2} during the three phases. It is clear that at lower $V_{H2,theo}$ additions while headspace CO_2 was still relatively high (up to 30%), pH remained fairly stable. At higher % $V_{H2,theo}$ additions, however, pH rose sharply. The data were fitted to a logarithmic model, as in the definition of pH, with the following equation:

$$pH_t = -A \cdot \log_{10} P_{CO2} + C \quad (4)$$

Where pH_t denotes the pH at $P_{CO2,t}$; C is a constant related to the digestate characteristics and A is an adjustment factor that may be related to the total alkalinity strength, especially total ammonia nitrogen (TAN) concentration. The good fit between the simulated curve and the experimental data confirms that the pH rise is essentially caused by the fall in P_{CO2} in the biogas. It also explains why pH levels were quite stable during the whole of phase III when methane content remained stable between 93-96%. This was achieved by controlling the P_{CO2} around 3%, which successfully maintained pH values below 8.0 in the digesters at 2 and 3 g COD_{org} L^{-1} day^{-1}. In the digesters at 4 and 5 COD_{org} L^{-1} day^{-1} with TAN concentrations of 0.9 and 1.6 g N L^{-1} respectively, the methane content was also successfully increased to 95% without triggering pH values above 8.1. This may not be the case for digesters with higher TAN concentrations, for example those running on food waste [43], chicken manure [44] or slaughterhouse waste [45] feedstocks, which tend to operate at pH values above 8. In this situation the pH may exceed 8.5 before the biogas methane content reaches 95%, and measures such as ammonia removal may be required if a high methane content is targeted [44, 46].

3.6 Volumetric methane production

The VMP values for digesters operated at 3 g COD_{org} L^{-1} day^{-1} were 0.86, 1.51 and 2.76 L L^{-1} day^{-1} at the end of phase I, II and III, respectively. The phase III VMP of 2.76 L L^{-1} day^{-1} exceeded that reported in other in-situ biomethanisation studies [18, 19, 27, 47] and in many ex-situ systems [3, 4, 6-8, 10, 12, 29, 30, 37]. It was, however, lower than the maximum previously reported VMP of 4.62 L L^{-1} day^{-1} [6] using a thermophilic CSTR system with gas diffusers. Kim et al [36] also achieved a VMP of 4.5-5.0 L L^{-1} day^{-1} with mass transfer promoted via gas recirculation and a gas dissolution device. There is no indication in the results reported here that the hydrogenotrophic metabolic capacity had been reached: this VMP simply reflects the highest loading applied in phase III, and it is possible that higher values could be obtained.

With respect to other reactor types, the maximum reported VMP in up-flow digesters was 2.37 L L^{-1} day^{-1} [29, 30], for packed bed digesters 1.79-8.14 L L^{-1} day^{-1} [8, 48], for membrane reactors 3.80-8.84 L L^{-1} day^{-1} [9, 49], and for trickle bed reactors 1.17-2.52 L L^{-1} day^{-1} [3, 4, 10]. All of these fall far short of the value reported by Savvas et al. [7] who achieved 40 L L^{-1} day^{-1} in a plug-flow biofilm reactor. While high methane productivities have been achieved in laboratory-scale reactors, there is as yet no large-scale commercialisation of any of the innovative designs proposed [12].

3.7 Potential applications

The results confirm that the abundance of hydrogenotrophic methanogens in conventional anaerobic digesters can be enriched using the endogenously-produced CO_2 without affecting the functionality of the microbial community in degrading organic substrates. The VMP of test digesters operated at 3 COD_{org} L^{-1} day^{-1} was increased from 0.86 to 1.51 and then 2.76 L L^{-1} day^{-1} from phase I to III, with CO_2 content reducing from 47% to 5%. This indicates that industrial digesters working at conventional OLR and HRT on liquid or semi-solid feeds have the potential to triple their current VMP by reacting both endogenous and imported CO_2 with H_2, opening up new opportunities with potential commercial significance for the AD industry.

This approach could provide a cost-effective scenario for power-to-methane as it mitigates the need to construct dedicated bioreactors and may reduce the required capital investment. There is a further bonus in that in-situ systems both with and without external CO_2 additions do not require a supply of synthetic nutrient medium to sustain biomass growth, thus minimising operating expenditure. The most likely process limitation is the availability of carbon that can be converted, as this is dependent on the feedstock to the digestion plant or on that which can be imported from other sources. If the existing AD capacity in Europe were coupled to grid-based or local renewables, the biomethane production from existing organic feedstocks could

potentially be increased by around 70%, corresponding to utilisation of around 26 M tonnes of CO_2 per year and decarbonising the equivalent amount of electricity production.

It should be noted that the presence of methane in the biogas reduces the partial pressure of convertible gases and thus the efficiency of gas-liquid mass transfer; hence, retrofitting would involve providing efficient mass transfer methods and devices (e.g. gas recirculation and diffusers) [10, 13]. In the current trial, however, simple recirculation was an effective strategy. Scale-up may give improved mass transfer due to greater hydrostatic pressures, while higher viscosity digestates in full-scale digesters may also increase gas-liquid contact times and thus aid H_2 mass transfer.

4 Conclusions

The study confirmed that hydrogen addition to a CSTR digester using simple recirculation of gases from the headspace could increase the methane content from 50% to over 95% if the system was acclimated gradually, allowing the hydrogenotrophic population to increase in relation to the increasing gaseous H_2 load applied. It was necessary to maintain some buffering from CO_2 partial pressure to prevent an inhibitory rise in pH, and stable operation with pH around 8.0 was achieved by maintaining 3-5% of P_{CO2}. Addition of imported CO_2 to digesters operated at 2 and 3g COD_{org} L^{-1} day^{-1} achieved simultaneous biomethanisation of CO_2 from endogenous and external sources. This allowed the volumetric methane production to increase from 0.86 L L^{-1} day^{-1} without H_2 to 2.76 L L^{-1} day^{-1} with an external CO_2 load equal to twice the endogenous CO_2. Analysis of the microbial community structure showed that methanogenic abundance increased by over 4-fold in response to the CO_2 and H_2 injection. Over 97% of the methanogenic community was shown to be hydrogenotrophic. The results showed that the capacity of a digester for CO_2 reduction can be increased beyond the constraints of the organic substrate, and that this did not interfere with substrate degradation. The

implication is that a single modified digester could upgrade the biogas from multiple digesters, thus minimising capital and operating costs and providing a cost-effective solution for power-to-methane applications using existing anaerobic digestion infrastructure.

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgements

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