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Biological carbon dioxide utilisation in food waste anaerobic digesters

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A B S T R A C T
Carbon dioxide (CO2) enrichment of anaerobic digesters (AD) was previously identified as a potential on-site carbon revalorisation strategy. This study addresses the lack of studies investigating this concept in up-scaled units and the need to understand the mechanisms of exogenous CO2 utilisation. Two pilot-scale ADs treating food waste were monitored for 225 days, with the test unit being periodically injected with CO2 using a bubble column. The test AD maintained a CH4 production rate of 0.56 ± 0.13 m³ CH4/(kg VS fed d)−1 and a CH4 concentration in biogas of 68% even when dissolved CO2 levels were increased by a 3 fold over the control unit. An additional uptake of 0.55 kg of exogenous CO2 was achieved in the test AD during the trial period. A 2.5 fold increase in hydrogen (H2) concentration was observed and attributed to CO2 dissolution and to an alteration of the acidogenesis and acetogenesis pathways. A hypothesis for conversion of exogenous CO2 has been proposed, which requires validation by microbial community analysis.
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1. Introduction

Anaerobic digestion (AD) stabilises organic wastes while producing biogas with a 50–75% methane (CH4) and 50–25% carbon dioxide (CO2) concentration. The calorific value of the biogas can then be used by combustion in combined heat and power (CHP) engines or by selectively separating the CH4 for its use as biomethane. The remaining CO2 present in the biogas, however, is emitted to the atmosphere with exhaust gas streams. Biogenic emissions of CO2 from ADs just in the UK have been estimated at 0.27 MtCO2 per annum for the water sector (Byrns et al., 2013) and at 0.31 MtCO2 per annum for ADs treating agricultural and community waste (industrial sites not accounted) (Bajón Fernández et al., Submitted for publication a). Emissions of CO2 with biogenic origin are not considered within carbon accounting inventories, however, its reduction is considered as a negative carbon release. Therefore, implementation of revalorisation strategies for biogas CO2 could be an option to counteract the increasing greenhouse gas (GHG) emissions of the water sector (Byrns et al., 2013) and to further reduce the carbon footprint of AD in the organic waste sector.

Implementation of carbon capture and storage (CCS) is feasible in the energy sector (DECC, 2012). However, its implementation for handling biogas CO2 is limited by the requirement to transport CO2 from AD sites in scattered location. New biogenic carbon sequestration methods such as the enrichment of anaerobic processes with CO2 (for its bioconversion into CH4) are therefore being investigated as a method to utilise on-site CO2 concentrated gas streams. The capacity of upflow anaerobic sludge blanket (UASB) reactors (Alimahmoodi and Mulligan, 2008), single stage anaerobic digesters (ADs) (Bajón Fernández et al., 2014; Sato and Ochi, 1994) and two phase ADs (Salomoni et al., 2011) has previously been examined to utilise additional CO2. However, these previous studies have focussed on proving the concept of carbon uptake or assessing associated benefits in CH4 formation at laboratory scale. In the case of ADs treating food waste, CO2 enrichment has so far only been studied in batch 1 L units (Bajón Fernández et al., 2014). Therefore, there is limited information available for scaled-up units. As a consequence, the means by which CO2 enrichment could be retrofit into scaled-up units have not yet been addressed. There is a need to investigate suitable technologies for completing CO2 enrichment of ADs without incurring any dilution of the headspace CH4 content. The anticipated simplicity for implementation (Byrns
et al., 2013) and the possibility of transferring gas to liquid technologies already used in other industrial sectors (e.g. bubble columns) have been suggested but not investigated. Furthermore, the mechanisms by which CO₂ can be biocarboxenised to CH₄ have not been fully elucidated. Several studies have hypothesised mechanisms of CO₂ utilisation (Alimahmoodi and Mulligan, 2008; Bajón Fernández et al., 2014; Francioso et al., 2010). However, only one has reported microbial community data in ADs enriched with CO₂ where conditions specifically favouring development of hydrogenotrophic methanogens were not applied (Bajón Fernández et al., Submitted for publication b). In this case, an increase in acetoclastic methanogenic activity (Methanosacetaceae) as a result of periodic CO₂ injections was reported. Nevertheless, the question of whether this increase is due to a direct impact of CO₂ in Archaea communities or to an indirect benefit through an alteration of previous stages of the digestion process (i.e. acidogenesis and acetogenesis) remains unclear.

This study investigated both the practicalities of an up-scaled implementation of CO₂ enrichment into ADs and the fate of exogenous CO₂. For the first time, a pilot-scale AD rig (106 L) treating food waste was adapted for CO₂ enrichment through an external bubble column was operated and compared to a standard unit. Results are discussed in terms of digestate quality, biogas production and CO₂ uptake when comparing ADs with and without CO₂ enrichment. A comprehensive discussion on the mechanisms of CO₂ utilisation is included based on monitoring hydrogen (H₂) levels and volatile fatty acid (VFA) speciation dynamics.

2. Materials and methods

2.1. Description and operation of the AD rig retrofitted with CO₂ enrichment

Two pilot-scale semi-continuous ADs treating food waste were operated for 225 days. Each unit consisted of a cylindrical section and a conical base with a total volume of 193 L, of which 106 L were liquid working volume (Fig. 1). Each AD was continuously stirred with an external peristaltic pump (series 600, Watson Marlow, Cornwall, UK) operated to achieve a recirculation rate of 30 min. The ADs were maintained at mesophilic conditions (38.5 °C) with a heating jacket over the cylindrical section (LMK Thermosafe, Haverhill, UK).

Both ADs were inoculated with digestate collected from a full-scale UK AD site treating 48,000 tonnes of organic waste per year. The units were fed on a daily basis (Monday to Friday) with a mixture of organic waste collected from local supermarkets and catering facilities. This waste was manually segregated to remove any inorganic content and macerated on-site with a series ‘A’ Muncher macerator (Mono Pumps Ltd., Manchester, UK) connected to a progressing cavity pump (W range, Mono Pumps Ltd., Manchester, UK). A loop in the system allowed the material to be recirculated into the macerator until a homogenous mixture with a maximum particle size of 6 mm was achieved. The substrate solids content was varied by adding water. On day 122 of operation a change of substrate source was required. Feed material was then collected weekly from the full-scale UK AD site where the inoculum was previously sourced from. Substrate was stored for a maximum of 7 days at 4 °C until the day of its use, when it was warmed to 22–30 °C before feeding to the ADs. Consistent quality of the substrate was then ensured by sieving it through a 6.3 mm aperture size sieve (Endecotts Ltd., London, UK) and homogenising it with a T25 DS 2 digital ultra-turrax disperser (IKA, Staufen, Germany). The material’s pH was raised to ca. 5.7 by addition of sodium hydroxide or calcium carbonate (Fisher Scientific, Loughborough, UK). Micronutrients were added daily into both ADs at a dosing rate of 50 ml of TEA 310 solution per tonne of volatile solids (VS) fed (Omex Environmental Ltd., King’s Lynn, UK). An organic loading rate (OLR) of 2.8 ± 0.3 kg VS m⁻³ d⁻¹ was applied with a hydraulic retention time (HRT) of ca. 29 days.

Both ADs were operated in an identical manner until day 148, when enrichment with CO₂ commenced on the test AD. Enrichment with CO₂ was performed by installing a 1 m tall and 10 cm diameter bubble column in the recirculation loop of the test AD (Fig. 1). The column was operated with a 7 L working volume and CO₂ and digestate contact was in a co-current mode (Fig. 1). Injection of CO₂ (g) was performed at the bottom of the column through a perforated plate connected to a manifold that divided the incoming gas stream into seven inlets. A metallic mesh with 0.5 mm hole size was placed on top of the perforated plate, which allowed generation of smaller gas bubbles in order to enhance gas to liquid mass transfer and hence CO₂ dissolution into the digesting material. The CO₂ flowrate into the column was controlled by

Fig. 1. Schematic representation of the pilot-scale experimental rig. (a) Conventionally operated AD and (b) AD retrofitted with an external bubble column for CO₂ injection. MFC: mass flow controller.
measured using a mass flow controller (MFC) (Premier Control Technologies, Norfolk, UK), fixed at 1.5 L min⁻¹ and supplied from gas cylinders (BOC, Manchester, UK). The external bubble column was retrofitted as a side process and connected for each CO₂ injection, whereas the test AD operated similarly to the control AD during the rest of the time. Injection of CO₂ was done three times per week, because up to 48 h may be required for the AD to recover from any acidification associated with CO₂ injection (Bajan Fernández et al., 2014). During each injection the speed of the pump in the AD’s recirculation loop was reduced in order to increase the gas to liquid contact time in the column. The whole AD content was contacted with the incoming CO₂ within an hour. Concentrations of CO₂ and CH₄ in the column exhaust (Fig. 1) were monitored with online pore size micro gas analyser (Gas Data, Coventry, England), which had individual measurement cells for CH₄, CO₂ and H₂ concentration. The H₂ measurement cell was suitable for ranges of 0–1000 ppm. The digestate of each unit was analysed daily for pH and up to twice per week for total solids (TS), VS, ammonia, VFAs and alkalinity. Statistically significant differences were identified by performing F-test and t-test in order to confirm the rejection of the null hypothesis. Analysis of TS and VS were completed according to the American Public Health Association (2005).

Ammonia and VFAs were analysed in the solid free fraction of the samples. To obtain this fraction, samples were centrifuged in a Falcon 6/300 centrifuge (MSE UK Ltd., London, UK) for 20 min at 4700 g and 19°C. The supernatant was centrifuged again under the same conditions for 40 min and vacuum filtered through 1.2 μm pore size microfilter filters GF/C (Whatman™, Kent, UK). The solid free fraction was then obtained with a final filtration stage through 0.45 μm pore size syringe-drive filters (Millipore™, Billerica, United States). High performance liquid chromatography (HPLC) (Shimazu VP Series unit, Milton Keynes, UK) was utilised to quantify the concentration of acetic acid, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid and iso-valeric acid, whose sum was reported as total VFA (TVFA) concentration (TVFA = Σ individual acids). An equivalent methodology to that reported by Soares et al. (2010) was used with the exception of the HPLC run time, which was set at 60 min.

Ammonia was quantified with Spectroquant test kits. Alkalinity was measured in the supernatant resulting from a double centrifugation process in which digestate samples were centrifuged at 4700 g for 20 min and the supernatant centrifuged again for 40 min under similar conditions. Partial alkalinity (PA) and intermediate alkalinity (IA) were monitored by titrating to a pH of 5.75 and of 4.30, respectively. Ripley ratio (RR = IA/PA) was used as an indicator of digestion stability, with a value lower than 0.3 considered indicative of stable operation (Ripley et al., 1986). When higher values were measured, the OLR was temporary reduced or the feed’s pH further increased and normal feeding resumed after few days. The feed substrate was also characterised for pH, TS, VS, ammonia and VFA on a regular basis. Dissolved levels of CO₂ were monitored in the control AD, the test AD and the exit of the CO₂ injection column by utilising an InPro5000(i) dissolved CO₂ sensor (Mettler Toledo Ltd., Leicester, UK) connected to a multi-parameter transmitter M400 (Mettler Toledo Ltd., Leicester, UK).

### 3. Results

#### 3.1. Substrate characterisation

The pre-conditioned feed substrate (after maceration, homogenisation and solids adjustment) was characterised by an ammonia concentration of 361 ± 20 mg L⁻¹ NH₄-N, a TVFA content of 21,114 ± 1723 mg L⁻¹, of which acetic acid was 81 ± 5%; and a pH of 3.6 ± 0.5 (Table 1). The pH was raised to 5.7 ± 0.6 units by addition of sodium hydroxide or calcium carbonate and, when required, water was added to adjust the material’s solids concentration. The final substrate contained 9.7 ± 1.4% TS, of which the VS were 83.1 ± 9.8%.

#### 3.2. Assessment of digestion performance (digestate quality and renewable energy enhancement) and process resilience

A stable digestion process was observed in both control and test ADs, with digestate characterised by a pH of 7.9 ± 0.2 and 7.8 ± 0.2 for control and test ADs, respectively, and a total ammonia concentration of 1.8–19 g L⁻¹ NH₄-N in both cases. Prior to CO₂ injection a TVFA concentration of 10,707 ± 313 mg L⁻¹ with 67 ± 3% acetic acid and of 9470 ± 739 mg L⁻¹ with 57 ± 6% acetic acid were recorded in the control and test ADs, respectively (day 134 of operation). The high VFA levels did not hinder process performance, as previously reported by other authors (Banks et al., 2011) and verified by the CH₄ output of this study. A specific CH₄ production rate of 0.53 ± 0.16 m³ CH₄ (kg VSfed⁻¹ d⁻¹) and a CH₄ concentration of 68.3 ± 5.7% for the control AD and of 0.45 ± 0.05 m³ CH₄ (kg VSfed⁻¹ d⁻¹) and a CH₄ content of 68.8 ± 3.4% for the test unit were recorded. The variation between units was attributed to the biological nature of the process but was not statistically significant (p-value of 0.280).

Control and test ADs were operated in a similar manner until day 148, when CO₂ injection into the test unit commenced. After CO₂ enrichment of the test AD commenced, CH₄ production was 0.56 ± 0.13 m³ CH₄ (kg VSfed⁻¹ d⁻¹) with a CH₄ content of 68.5 ± 3.4% (Table 1). The average VS reduction of the substrate in the control AD was of 74% and of 76% in the test AD from the time of CO₂ enrichment until completion of the trials. Concentration of H₂ in the headspace was quantified at 84 ± 5 ppm and 75 ± 15 ppm for control and test ADs, respectively, as average up to day 148. However, after four enrichments with CO₂ (12 days of operation after commencement of CO₂ enrichment), an increase in the H₂ content on the test AD was observed, with a value of 173 ppm recorded on day 160 of operation. An increasing trend in H₂ content of biogas was observed with subsequent CO₂ injections (Fig. 2), with an average value of the daily recordings of 320 ± 153 ppm between days 149 and 225 of operation (Table 1). This implied a statistically significant 2.5 fold increase (p-value < 0.001) when compared to the average H₂ content of the control AD over the entire trial period (129 ± 44 ppm) (Table 1). A stable operation of the test AD, assessed with RR and CH₄ production measurements, was observed in spite of the H₂ concentration reaching values of over 600 ppm on several occasions (Fig. 2).

Interestingly, two failures of the heating system occurred during the experimental period, which enabled investigation of process resilience to disturbances. A failure in the heating jacket of the control AD on day 136 of operation for <23 h led to a temperature drop of 11°C. An immediate alteration in biogas production was observed, with the CH₄ production rate dropping by over an order of magnitude and oscillating between 2.8 10⁻² and 6.1 10⁻² m³
Table 1
Characterisation of digester feed, digestate and headspace of the control and tests ADs.

<table>
<thead>
<tr>
<th>Parameter monitoring</th>
<th>Feed</th>
<th>Control AD</th>
<th>Test AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.7 ± 0.6</td>
<td>7.9 ± 0.2</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>pH</td>
<td>9.7 ± 1.4</td>
<td>4.5 ± 0.9</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>TS (%)</td>
<td>83.1 ± 9.8</td>
<td>51.0 ± 8.9</td>
<td>65.8 ± 7.3</td>
</tr>
<tr>
<td>VS (% of TS)</td>
<td>8.0 ± 1.2</td>
<td>2.3 ± 0.4</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Ammonia (mg L⁻¹ NH₄-N)</td>
<td>361 ± 20</td>
<td>1855 ± 205</td>
<td>1798 ± 124</td>
</tr>
<tr>
<td>VFA concentration (mg L⁻¹)</td>
<td>21,114 ± 1723</td>
<td>10,707 ± 313</td>
<td>9470 ± 739</td>
</tr>
<tr>
<td>Headspace monitoring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₄ production rate (m³ CH₄ (kg VS fed d⁻¹))</td>
<td>--</td>
<td>0.53 ± 0.16</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td>CH₄ concentration (%)</td>
<td>--</td>
<td>68.3 ± 5.7</td>
<td>68.8 ± 3.4</td>
</tr>
<tr>
<td>H₂ concentration (ppm)</td>
<td>--</td>
<td>129 ± 44</td>
<td>73 ± 15</td>
</tr>
</tbody>
</table>

Data corresponding to days of temperature drop have not been considered for average values.

- Value on day 134. For VFA dynamics refer to Fig. 3.
- Value on day 153. For VFA dynamics refer to Fig. 3.

Fig. 2. Evolution of CH₄ and H₂ biogas concentration and digester pH in the control and test ADs during the pilot scale digestion trials.
CH₄ (kg VSfed·d⁻¹) over the six days following the disturbance. When mesophilic conditions resumed a sudden increase of H₂ was experienced, which ranged from 262 ppm on the day of temperature correction to 464 ppm 7 days afterwards. A RR of 11.5 was measured on day 139 of operation, in spite of substrate addition being suspended immediately after the temperature drop. Analysis of VFA on day 139 evidenced an accumulation of acids, with TVFA reaching values of 17,235 ± 313 mg L⁻¹ as opposed to the 10,707 ± 313 mg L⁻¹ recorded on day 134 and the 9435 ± 686 mg L⁻¹ obtained in the test AD (Fig. 3). Individual VFA increases when comparing day 139 over day 134 of operation 1.4, 1.4, 3.8 and 4.3 fold were recorded for acetic, propionic, isobutyric and n-butyric acids, respectively (Fig. 3). Calcium carbonate was dissolved in order to increase the pH, which had dropped to ca. 6 units. However, the high CO₂ content of the headspace (up to 65%), the inhibited CH₄ production rate, the RR considerably over the desired value of 0.3 and the rise in H₂ content indicated that methanogenesis activity was severely inhibited. Acetogenesis and methanogenesis were hence considered to be completely decoupled and a partial re-seed (80%) of the control AD was required on day 142 of operation. Signs of recovery were observed from day 146, when a H₂ content in biogas of 81 ppm and a pH in digestate of 7.8 were recorded. A performance comparable to that previous to the temperature disturbance was achieved within 11 days from the partial re-seed (day 154 of operation), when a CH₄ production rate of 0.46 m³ CH₄ (kg VSfed·d⁻¹) was obtained. Alkalinity tests confirmed the recovery of digester stability since a RR of 0.82 on day 153 and of 0.30 on day 161 were recorded. Concentration of TVFA was 6969 ± 591 mg L⁻¹ on day 161.

The heating system of the test AD failed for <23 h on day 178 (31 days after CO₂ enrichment was started), leading to a temperature drop of 12.5 °C. Similar to the experience in the control AD, an immediate reduction in CH₄ production rate and a drop in the pH of the digesting media were recorded. Nevertheless, alterations were significantly lower than those previously recorded for the control AD, with a CH₄ production rate decrease from 0.67 to 0.46 m³ CH₄ (kg VSfed·d⁻¹) and a pH drop of 0.24 units. An increase of TVFA concentration from 2473 ± 153 mg L⁻¹ to 4764 ± 145 mg L⁻¹ between days 175 and 178 was recorded, which was within the normal variability observed during the entire digestion period (Fig. 3). No specific sign of VFA accumulation associated with methanogenic activity inhibition was obtained. The immediate restart of the heating jacket and the suspension of substrate addition during two days sufficed to recover the initial digestion performance, without any re-seeding required. A CH₄ production rate of 0.71 and 0.75 m³ CH₄ (kg VSfed·d⁻¹) was recorded on days 182 and 183 of operation, respectively. The different behaviour of the control and test ADs to a situation of stress for methanogenic communities could be associated with a higher resistance of these Archaea in ADs retrofitted with CO₂ injection. This potential benefit of CO₂ injection in process resilience could prove beneficial in food waste ADs, which are commonly associated with operational problems (Banks et al., 2011), and should be further investigated. Concentration of H₂ in the test AD on recovery from the temperature drop stabilised again to a baseline higher than that of the control unit, with values oscillating between 380 and 550 ppm of H₂ during the week following recovery (Fig. 2).

3.3. Impact of CO₂ enrichment on dissolved CO₂ and digestate’s ammonia concentration

The dissolved CO₂ and ammonia levels recorded in the digestate of both control and test ADs are presented in Fig. 4. An average dissolved CO₂ concentration of 2.0E-3 ± 5.3E-4 kmol m⁻³ was recorded in the liquid phase of the control AD during the digestion trial. A similar value of 2.0E-3 ± 5.9E-4 kmol m⁻³ was obtained for the test AD when measuring dissolved CO₂ inside of the unit or in the inlet to the bubble column used for mass transfer. Each enrichment with CO₂ led to dissolved CO₂ levels of 6.1E-3 ± 1.4E-3 kmol m⁻³ in the material exiting the bubble column (Fig. 4). Hence, all the content of the test AD was enriched with an additional 4.0E-3 kmol CO₂ m⁻³, implying an input of ca. 4.0E-4 kmol of exogenous CO₂ (18.455 mg CO₂) per enrichment when considering the working volume of the unit (106 L). When a frequency of injection of three times per week is considered, it is calculated that 0.55 kg of exogenous CO₂ were assimilated by the test AD during the trial period. Monitoring the dissolved CO₂ concentration confirmed the rapid utilisation of additional CO₂, since levels dropped from 6.1E-3 ± 1.4E-3 kmol m⁻³ obtained after enrichment to 2.0E-3 ± 5.9E-4 kmol m⁻³ within 24 h. This utilisation rate of CO₂ matched the overcompensation of the slight acidification due to CO₂ enrichment in the 24 h following a CO₂ injection. During each CO₂ injection a pH drop of 0.4–0.6 units was experienced between the inlet and outlet of the bubble column. This pH reduction was consistently overcome within 24 h, with an average pH of 7.9 ± 0.2 and 7.8 ± 0.2 maintained in control and test ADs, respectively. This implied no alteration of the pH with respect to the period when CO₂ injection was not applied.

The suitability of utilising a co-currently operated bubble column for gas to liquid mass transfer was assessed by examining the concentration of CO₂ in the AD’s headspace and the amount of CH₄ stripped from the digesting material during the mass transfer process. The CO₂ content in the produced biogas, which was recorded daily, did not increase; with non-dissolved CO₂ being released with the exhaust of the bubble column only. The extent to which CH₄ was degassed during the mass transfer process was quantified by measuring on-line the CH₄ content of the gas exhaust of the bubble column (Fig. 4). Concentrations between 0.8 and 2.1% of CH₄ were measured, which implied a release of 0.72–1.89 L CH₄ per CO₂ enrichment (every 48 h) when considering the incoming CO₂ flowrate of 1.5 L min⁻¹. When compared to the average of 235 ± 49 L CH₄ produced per day by the test AD, the loss of CH₄ in the mass transfer system accounted for < 0.4% and was hence considered to be negligible.

Periodic injections of CO₂ in the test AD did not vary the concentration of ammonia in the digesting material to a significant extent (Table 1). Average total ammonia concentration was 1798 ± 124 mg L⁻¹ NH₄-N before CO₂ enrichment and 1807 ± 166 mg L⁻¹ NH₄-N during the rest of the digestion trials. This seems to indicate that injection of CO₂ did not have a significant positive benefit in controlling ammonia inhibition, which agrees with previous literature stating that increased pH and temperatures are required for an efficient free ammonia removal in ADs by stripping it with biogas (Serna-Maza et al., 2014; Walker et al., 2011).

4. Discussion

4.1. Suitability of injecting CO₂ into ADs with an external bubble column

The majority of previous studies investigating CO₂ injection into ADs have been completed at laboratory scale only, without the suitability of injecting CO₂ through existing gas mixing systems or by means of external mass transfer units having been investigated for scaled-up systems. This study provides an insight into the effectiveness of using an external bubble column to inject CO₂ in ADs through examining biogas quality, amount of CH₄ lost and mass transfer efficiency. Non-dilution of AD headspace and the low amount of CH₄ degassed during the enrichment (< 0.4%), indicated
the suitability of employing an external bubble column for performing the required gas to liquid mass transfer. As far as efficiency of the system is concerned, operation of the bubble column increased the dissolved CO$_2$ levels by a 3 fold (from 2.0E-3 ± 5.9E-4 kmol m$^{-3}$ to 6.1E-3 ± 1.4E-3 kmol m$^{-3}$). However, the solubility of CO$_2$ at 38.5 °C in aqueous solutions with a CO$_2$ partial pressure (p$_{CO2}$) of 1atm is 2.4E-2 kmol m$^{-3}$ (1071 mg L$^{-1}$) (Green and Perry, 2008). Therefore, the operated bubble column achieved only ca. 25% of the CO$_2$ that could have been dissolved at p$_{CO2}$ of 1atm. This indicates the important role that CO$_2$ gas to liquid mass transfer plays in the amount of CO$_2$ which can be dissolved in an anaerobic process when implementing CO$_2$ enrichment. In turn the amount of

Fig. 3. Dynamics of total and individual VFA digestate concentrations for control and test ADs during the pilot scale digestion trials.
CO₂ dissolved determines the contribution towards reduction of carbon footprint that can be achieved (negative carbon release if dissolving CO₂ with biogenic origin) and the potential increase in renewable energy production. The complex rheology of anaerobically digested material (Baudez et al., 2011; Eshtiaghi et al., 2012) and the strong impact of viscosity on mass transfer retardation (Ozpek and Gayik, 2001) requires a better understanding in order for mass transfer systems involving these fluids to be designed and operated in an efficient manner. The use of bubble columns for dissolving exogenous CO₂ into anaerobic digesting media is considered suitable because of a lower risk of clogging than other technologies. Besides, efficiency of mass transfer could be increased by a greater gas to liquid contact time, a reduced bubble size or a higher incoming gas flowrate (Kantarci et al., 2005), which would increase dissolved CO₂ levels and hence potential for carbon assimilation.

4.2. Impact of CO₂ injection in AD performance and mechanisms of utilisation based on VFA and H₂ dynamics

The test AD achieved an average CH₄ production rate of 0.45 ± 0.05 m³ CH₄/(kg VS fed d⁻¹) before any CO₂ was applied, which is within the order of magnitude reported in the literature for domestic food waste (Banks et al., 2011). When this value is considered as a baseline, the CH₄ production rate observed during the time when CO₂ enrichment was applied (0.56 ± 0.13 m³ CH₄/(kg VS fed d⁻¹)) implied a ca. 20% improvement (p-value of 0.058), which is in agreement with performances previously reported in the literature (Salomoni et al., 2011; Sato and Ochi, 1994). However, no significant benefit (p-value of 0.261) was recorded when comparing the performance of the test AD with CO₂ enrichment (0.56 ± 0.13 m³ CH₄/(kg VS fed d⁻¹)) with that of the control unit (0.53 ± 0.16 m³ CH₄/(kg VS fed d⁻¹)). This suggests that any improvement was not appreciable due to the natural variability of the performance of the biological process (i.e. high standard deviation).

Of note was the impact observed in relation to the H₂ content of the biogas produced, which reached a baseline 2.5 fold higher in the test AD than in the control unit (p-value < 0.001) during the period when CO₂ was periodically injected. The observed increased H₂ production can be used to further understand the mechanisms of CO₂ utilisation because of the role of H₂ as an electron carrier and intermediate product in several reactions of the digestion process (Cord-Ruwisch et al., 1997). Suddenly increases in H₂ concentration have been reported in response to process disturbances, such as changes in the feed quality or loading rate (Kidby and Nedwell, 1991; Mosey and Fernandes, 1989) and when feeding a digestion process with unfermented material of a labile nature (Kidby and Nedwell, 1991). The sudden increase in readily available substrate in turn leads to an active hydrolisis, acidogenesis and acetogenesis with an associated release of H₂ (Guwy et al., 1997). The fast response to system destabilizations and the recovery of initial H₂ levels shortly after the disturbance is overcome, has led several authors to study the possibility of using it as a control parameter in ADs (Rodríguez et al., 2006). Fluctuations in H₂ with return to initial concentrations are hence considered indicative of specific events or transition phenomena, rather than of long-term alterations (Mosey and Fernandes, 1989).

During the pilot plant trials of this study two types of disturbances in biogas H₂ levels were observed. An increase from 84 ± 5 ppm to 464 ppm was recorded in the control AD when the temperature dropped by 11 °C (Fig. 2) over a ≤ 24 h period, with H₂ rapidly returning to initial levels once the disturbance was overcome. On the contrary, an increase in H₂ concentration was observed in the test AD following four CO₂ injections, which lead to a new H₂ baseline (320 ± 153 ppm) to be maintained during the rest of the trial period and to sporadic peaks of up to 645 ppm (Fig. 2). The rapid variation in H₂ level in the control AD was an indicator of process disturbance. This was overcome when normal operation conditions were re-established and agrees with the previously mentioned literature findings. The increased H₂ production of the test AD, however, was maintained over 65 days of operation (until the experimental trials were concluded), and was assumed to be associated with CO₂ injection affecting the microbial process in a more permanent manner. The different nature of both H₂ alternations was further evident when attending to the
The increase in H$_2$ concentration of the control AD was simultaneous to a sudden increase in TVFA concentration (Fig. 3), which reached 17,235 ± 147 mg L$^{-1}$ on day 139. Accumulation of VFA indicated that hydrolysis, acidogenesis and acetogenesis were taking place in spite of the temperature drop, while the acid assimilatory capacity of methanogenic communities was inhibited. Progression of fermentation without an efficient assimilation of acetic acid and H$_2$ would have resulted in unfavourable conditions for acetogenesis itself, leading to accumulation of VFAs of higher number of carbons (Fig. 3). Propionic and butyric acid degradation reactions have been reported to be thermodynamically unfavoured at H$_2$ partial pressure ($p_{H_2}$) over 10$^{-4}$ atm and 10$^{-3}$ atm, respectively (Cord-Ruwisch et al., 1997; Harper and Pohland, 1986; Kidby and Nedwell, 1991; Labatut et al., 2014). The $p_{H_2}$ in the control unit reached these unfavourable conditions, with a value of 4.6·10$^{-4}$ atm (atmospheric pressure considered inside the AD). This in turn lead to a hindered degradation of propionic and butyric acids, which accumulated on the system reducing the digester’s pH (Fig. 2). Eventually process failure occurred (sour AD) and a partial re-seed for stability recovery was required.

On the contrary, the increase in H$_2$ concentration in the test AD was not related to a rising trend in TVFA or individual VFA concentrations (Figs. 2 and 3). In fact, TVFA and acetic acid were quantified at 3662 ± 44 mg L$^{-1}$ and 369 ± 18 mg L$^{-1}$, respectively, on day 153, which was lower than average values maintained during the entire digestion trials (Fig. 3). The increase in H$_2$ was considered resulting from injection of CO$_2$ (only variable modified) and was attributed to a boost of H$_2$ producing mechanisms rather than to a reduced H$_2$ assimilatory capacity. Two mechanisms could have led to the increased H$_2$ production observed. On the one hand, dissolution of CO$_2$ in the aqueous media could have contributed to an increased H$_2$ concentration as a result of CO$_2$ forming carbonic acid that releases protons when dissociated into carbonate and bicarbonate species. At the low oxidation reduction potential found in ADs (<-200 mV (Gupta et al., 1994)) the protons could react to form H$_2$. On the other hand, the H$_2$ increase could have resulted from its production by acetogenesis (Fig. 5). In this case, an increase in acetic acid would have been expected, similar to that recorded in the control unit, unless the acetic acid assimilatory capacity of the system was enhanced. The activity of Methanosaetaeae (obligate acetoclastic methanogen) has been reported to increase after periodic CO$_2$ injections in ADs (Bajon Fernandez et al., Submitted for publication b), hence being likely to have had the capacity to assimilate additional acetate. Further investigation needs to be undertaken to determine the contribution of both pathways to the formation of additional H$_2$. By either mechanism the additional H$_2$ would have been formed in the liquid phase. The limited mass transfer of H$_2$ between the liquid and gas phases (Guwy et al., 1997) explained that four injections of CO$_2$ were required before an impact in the headspace’s H$_2$ content was evident and that pH was recovered between injections while H$_2$ levels did not drop to the baseline of the control AD.

It is of note that the H$_2$ concentration oscillated around 320 ± 153 ppm, with peaks over 600 ppm but without a continuously increasing trend in spite of CO$_2$ being injected periodically. The fact that H$_2$ concentration did not increase further, suggests that additional H$_2$ produced was consumed in the AD. Assimilation of H$_2$ could occur by the Wood-Ljungdahl pathway of CO$_2$ fixation. This metabolic pathway can be stimulated by the availability of exogenous CO$_2$ (Misoph and Drake, 1996) and requires eight electrons and eight protons for each two molecules of CO$_2$ assimilated, which can be supplied by consumption of H$_2$ (Ragsdale and Pierce, 2008). This pathway leads to the generation of acetate, which in turn would have been assimilated by the enhanced acetoclastic methanogenesis.

It is then proposed that CO$_2$ leads to a boost of H$_2$ production, derived from the protons formed when dissolving CO$_2$ in the

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**Fig. 5.** Hypothesised mechanism of exogenous CO$_2$ utilisation in ADs, with findings supporting each of the proposed stages.
aqueous media, from a boost of obligate acetogenesis or from a combination of both (Fig. 5). Part of the additional H2 formed is then assimilated in the AD, leading to a steady operation as opposed to a continuously increasing H2 level. Assimilation of additional H2 is likely to occur through the Wood-Ljungdahl pathway, which has a preference for exogenous CO2. The additional acetic acid formed by this pathway would then be assimilated by aceticlastic methanogenesis, which has been reported to have an increased activity when subjected to periodic CO2 injections. The proposed mechanism of CO2 assimilation is summarised in Fig. 5, including previous findings that support the suggested hypothesis. Further work will be required to support or reject the proposed mechanism. In particular, microbial community analyses to understand the potential impact of CO2 injection in acetogenesis are of great interest.

5. Conclusion

The capacity of ADs treating food waste to utilise exogenous CO2 was tested and the practicalities of an up-scaled implementation and mechanisms of CO2 utilisation were investigated. Injection of CO2 through an external bubble column was suitable, as the headspace was not diluted and CH4 loss during injection was negligible (<0.4%). A CH4 production rate of 0.56 ± 0.13 m3 CH4 (kg VSref d−1)−1 was recorded for an AD periodically enriched with CO2. An additional uptake of 0.55 kg of exogenous CO2 in the test AD during the trial period was calculated, which could be augmented if the bubble column mass transfer efficiency was increased, hence augmenting the potential benefits in CO2 mitigation. A 2.5 fold increase in H2 concentration was observed after four CO2 injections, likely due to CO2 dissolution or an alternation of acidogenesis/acetogenesis. Additional H2 was believed uptaken by Wood-Ljungdahl pathway and the acetate generated by this in turn emulated by acetoclastic methanogenesis, which has been reported to be augmented the potential benefits in CO2 mitigation. A 2.5 fold increase in H2 concentration was observed after four CO2 injections, likely due to CO2 dissolution or an alternation of acidogenesis/acetogenesis. Additional H2 was believed uptaken by Wood-Ljungdahl pathway and the acetate generated by this in turn emulated by acetoclastic methanogenesis, which has been reported to be augmented the potential benefits in CO2 mitigation. A 2.5 fold increase in H2 concentration was observed after four CO2 injections, likely due to CO2 dissolution or an alternation of acidogenesis/acetogenesis. Additional H2 was believed uptaken by Wood-Ljungdahl pathway and the acetate generated by this in turn emulated by acetoclastic methanogenesis, which has been reported to be augmented the potential benefits in CO2 mitigation. 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