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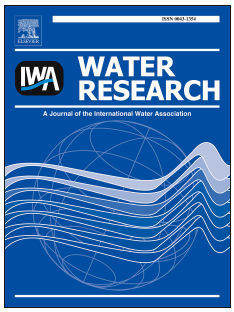

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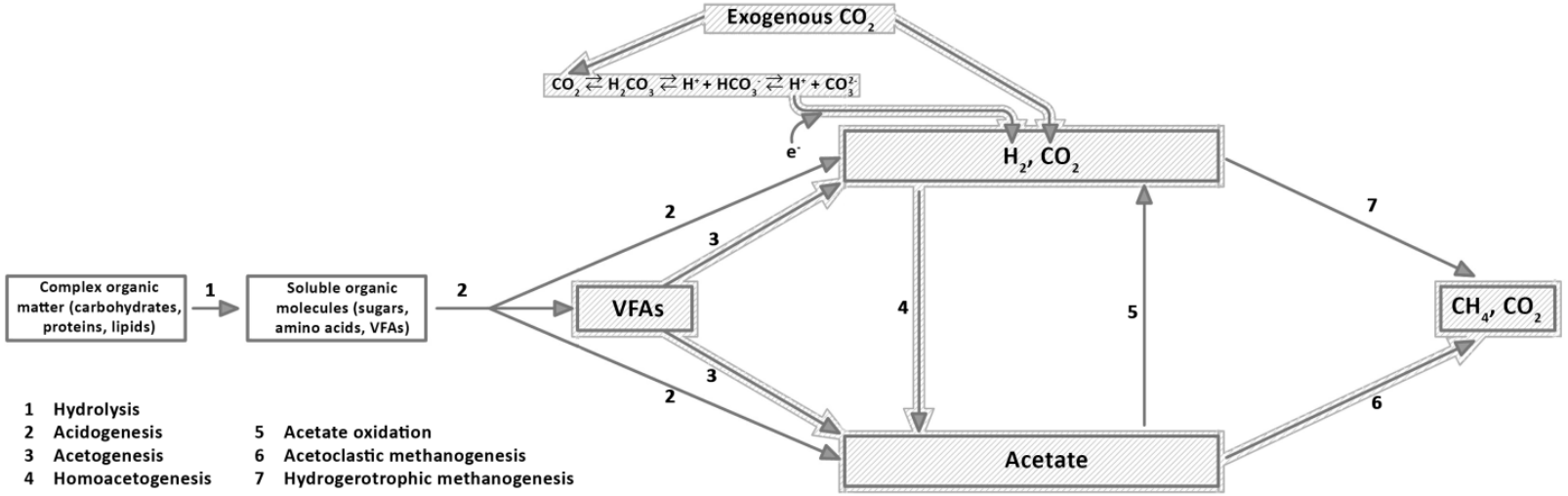

** Marked path is a hypothesis for conversion of exogenous CO_2*

BIOLOGICAL CARBON DIOXIDE UTILISATION IN FOOD WASTE ANAEROBIC DIGESTERS

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ABSTRACT

Carbon dioxide (CO_2) 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 CO_2 utilisation. Two pilot-scale ADs treating food waste were monitored for 225 days, with the test unit being periodically injected with CO_2 using a bubble column. The test AD maintained a CH_4 production rate of 0.56±0.13 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ and a CH_4 concentration in biogas of 68% even when dissolved CO_2 levels were increased by a 3 fold over the control unit. An additional uptake of 0.55 kg of exogenous CO_2 was achieved in the test AD during the trial period. A 2.5 fold increase in hydrogen (H_2) concentration was observed and attributed to CO_2 dissolution and to an alteration of the acidogenesis and acetogenesis pathways. A hypothesis for conversion of exogenous CO_2 has been proposed, which requires validation by microbial community analysis.

KEY WORDS

Carbon dioxide utilisation, anaerobic digestion, food waste, hydrogen, bubble column

1.1. INTRODUCTION

Anaerobic digestion (AD) stabilises organic wastes while producing biogas with a 50-75% methane (CH_4) and 50-25% carbon dioxide (CO_2) 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 CH_4 for its use as biomethane. The remaining CO_2 present in the biogas, however, is emitted to the atmosphere with exhaust gas streams. Biogenic emissions of CO_2 from ADs just in the UK have been estimated at 0.27 $MtCO_2$ per annum for the water sector (Byrns et al., 2013) and at 0.31 $MtCO_2$ per annum for ADs treating agricultural and community waste (industrial sites not accounted) (Bajón Fernández et al., *n.a.*). Emissions of CO_2 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 CO_2 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 CO_2 is limited by the requirement to transport CO_2 from AD sites in scattered location. New biogenic carbon sequestration methods such as the enrichment of anaerobic processes with CO_2 (for its bioconversion into CH_4) are therefore being investigated as a method to utilise on-site CO_2 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 CO_2. However, these previous studies have focussed on proving the concept of carbon uptake or assessing associated benefits in CH_4 formation at laboratory scale. In the case of ADs treating food waste, CO_2 enrichment has so far only been studied in batch one litre units (Bajón Fernández et al., 2014). Therefore, there is limited information available for scaled-up units. As a consequence, the means by which CO_2 enrichment could be retrofitted into scaled-up units have not yet been addressed. There is a need to investigate suitable technologies for completing CO_2 enrichment of ADs without incurring any dilution of the headspace CH_4 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 CO_2 can be bioconverted to CH_4 have not been fully elucidated. Several studies have hypothesised mechanisms of CO_2 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 CO_2 where conditions specifically favouring development of hydrogenotrophic methanogens were not applied (Bajón Fernández et al., *n.a*). In this case, an increase in acetoclastic methanogenic activity (*Methanosaetaceae*) as a result of periodic CO_2 injections was reported. Nevertheless, the question of whether this increase is due to a direct impact of CO_2 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 CO_2 enrichment into ADs and the fate of exogenous CO_2. For the first time, a pilot-scale AD rig (106 L) treating food waste and adapted for CO_2 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 CO_2 uptake when comparing ADs with and without CO_2 enrichment. A comprehensive discussion on the mechanisms of CO_2 utilisation is included based on monitoring hydrogen (H_2) levels and volatile fatty acid (VFA) speciation dynamics.

1.2. MATERIALS AND METHODS

1.2.1. Description and operation of the AD rig retrofitted with CO_2 enrichment

Two pilot-scale semi-continuous ADs treating food waste were operated for 225 days. Each unit consisted of a cylindrical section and a cone 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 minutes. 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 seven 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 homogenizing it with a T 25 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 $\cdot m^{-3} \cdot d^{-1}$ 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 CO_2 commenced on the test AD. Enrichment with CO_2 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 CO_2 and digestate contact was in a co-current mode (Fig. 1). Injection of CO_2 (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 CO_2 dissolution into the digesting material. The CO_2 flowrate into the column was controlled by means of a mass flow controller (MFC) (Premier Control Technologies, Norfolk, UK), fixed

at 1.5 $L \cdot min^{-1}$ and supplied from gas cylinders (BOC, Manchester, UK). The external bubble column was retrofitted as a side process and connected for each CO_2 injection, whereas the test AD operated similarly to the control AD during the rest of the time. Injection of CO_2 was done three times per week, because up to 48 h may be required for the AD to recover from any acidification associated with CO_2 injection (Bajón 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 CO_2 within an hour. Concentrations of CO_2 and CH_4 in the column exhaust (Fig. 1) were monitored with online sensors (BCP sensors, Bluesens, Herten, Germany), which were connected to a logging computer using BacVis software (Bluesens, Herten, Germany) for data recording.

1.2.2. Analytical methods

The volume of biogas produced by the ADs was measured continuously with a TG05/5 gas meter equipped with a totalizer (Ritter, Bochum, Germany) connected to each of the units (Fig. 1). Data of biogas produced were logged daily. The biogas composition was analysed on a daily basis by means of a LMSXi multifunction gas analyser (Gas Data, Coventry, England), which had individual measurement cells for CH_4, CO_2 and H_2 concentration. The H_2 measurement cell was suitable for ranges of 0 to 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 minutes at 4700 *g* and 19°C. The supernatant was centrifuged again under the same conditions for 40 minutes and vacuum filtered through 1.2 µm pore size microfiber filters GF/C (Whatman™, Kent, UK). The solid free fraction was then obtained with a last filtration stage through 0.45 µm pore size syringe-drive filters (Millipore™, Billerica, United States). High performance liquid chromatography (HPLC) (Shimadzu 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 = ∑ 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 minutes.

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 minutes and the supernatant centrifuged again for 40 minutes 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 CO_2 were monitored in the control AD, the test AD and the exit of the CO_2 injection column by utilising an InPro5000(i) dissolved CO_2 sensor (Mettler Toledo Ltd., Leicester, UK) connected to a multi-parameter transmitter M400 (Mettler Toledo Ltd., Leicester, UK).

1.3. RESULTS

1.3.1. Substrate characterisation

The pre-conditioned feed substrate (after maceration, homogenization and solids adjustment) was characterised by an ammonia concentration of 361±20 mg·L^{-1} NH_4-N, a TVFA content of 21114±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%.

1.3.2. 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 1.8-1.9 $g \cdot L^{-1}$ NH_4-N in both cases. Prior to CO_2 injection a TVFA concentration of 10707±313 $mg \cdot L^{-1}$ with 67±3% acetic acid and of 9470±739 $mg \cdot L^{-1}$ 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 CH_4 output of this study. A specific CH_4 production rate of 0.53±0.16 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ and a CH_4 concentration of 68.3±5.7% for the control AD and of 0.45±0.05 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ and a CH_4 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 CO_2 injection into the test unit commenced. After CO_2 enrichment of the test AD commenced, CH_4 production was 0.56±0.13 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ with a CH_4 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 commencement of CO_2 enrichment until completion of the trials. Concentration of H_2 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 CO_2 (12 days of operation after commencement of CO_2 enrichment), an increase in the H_2 content on the test AD was observed, with a value of 173 ppm recorded on day 160 of operation. An increasing trend in H_2 content of biogas was observed with subsequent CO_2 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 H_2 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 CH_4 production measurements, was observed in spite of the H_2 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 hours led to a temperature drop of 11°C. An immediate alteration in biogas production was observed, with the CH_4 production rate dropping by over an order of magnitude and oscillating between $2.8 \cdot 10^{-2}$ and $6.1 \cdot 10^{-2}$ m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ over the six days following the disturbance.

When mesophilic conditions resumed a sudden increase of H_2 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 17235±147 $mg \cdot L^{-1}$ as opposed to the 10707±313 $mg \cdot L^{-1}$ recorded on day 134 and the 9435±686 $mg \cdot L^{-1}$ obtained in the test AD (Fig. 3). Individual VFA increases when comparing day 139 over day 134 of operation of 1.4, 1.4, 3.8 and 4.3 fold were recorded for acetic, propionic, iso-butyric and n-butyric acids, respectively (Fig. 3). Calcium carbonate was dosed in order to increase the pH, which had dropped to *ca.* 6 units. However, the high CO_2 content of the headspace (up to 65%), the inhibited CH_4 production rate, the RR considerably over the desired value of 0.3 and the rise in H_2 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 H_2 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 CH_4 production rate of 0.46 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ 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 $mg \cdot L^{-1}$ on day 161.

The heating system of the test AD failed for ≤ 23 hours on day 178 (31 days after CO_2 enrichment was started), leading to a temperature drop of 12.5°C. Similar to the experience in the control AD, an immediate reduction in CH_4 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 CH_4 production rate decrease from 0.67 to 0.46 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ and a pH drop of 0.24 units. An increase of TVFA concentration from 2473±153$mg \cdot L^{-1}$ to 4764±145$mg \cdot L^{-1}$ 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 re-start 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 CH_4 production rate of 0.71 and 0.75 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ 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 CO_2 injection. This potential benefit of CO_2 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 H_2 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 H_2 during the week following recovery (Fig. 2).

1.3.3. Impact of CO_2 enrichment on dissolved CO_2 and digestate's ammonia concentration

The dissolved CO_2 and ammonia levels recorded in the digestate of both control and test ADs are presented in Fig. 4. An average dissolved CO_2 concentration of 2.0E-3±5.3E-4 $kmol \cdot m^{-3}$ was recorded in the liquid phase of the control AD during the digestion trial. A similar value of 2.0E-3±5.9E-4 $kmol \cdot m^{-3}$ was obtained for the test AD when measuring dissolved CO_2 inside of the unit or in the inlet to the bubble column used for mass transfer. Each enrichment with CO_2 led to dissolved CO_2 levels of 6.1E-3±1.4E-3 $kmol \cdot m^{-3}$ 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 $CO_2 \cdot m^{-3}$, implying an input of *ca.* 4.0E-4 kmol of exogenous CO_2 (18455 mg CO_2) 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 CO_2 were assimilated by the test AD during the trial period. Monitoring the dissolved CO_2 concentration confirmed the rapid utilisation of additional CO_2, since levels dropped from 6.1E-3±1.4E-3 $kmol \cdot m^{-3}$ obtained after enrichment to 2.0E-3±5.9E-4 $kmol \cdot m^{-3}$ within 24 hours. This utilisation rate of CO_2 matched the overcome of the slight acidification due to CO_2 enrichment in the 24 hours following a CO_2 injection. During each CO_2 injection a pH drop of 0.4 to 0.6 units was experienced between the inlet and outlet of the bubble column. This pH reduction was consistently overcome within 24 hours, 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 CO_2 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 CO_2 in the AD's headspace and the amount of CH_4 stripped from the digesting material during the mass transfer process. The CO_2 content in the produced biogas, which was recorded daily, did not increase; with non-dissolved CO_2 being released with the exhaust of the bubble column only. The extent to which CH_4 was degassed during the mass transfer process was quantified by measuring on-line the CH_4 content of the gas exhaust of the bubble column (Fig. 4). Concentrations between 0.8 and 2.1% of CH_4 were measured, which implied a release of 0.72-1.89 L CH_4 per CO_2 enrichment (every 48 hours) when considering the incoming CO_2 flowrate of 1.5 $L \cdot min^{-1}$. When compared to the average of 235±49 L CH_4 produced per day by the test AD, the loss of CH_4 in the mass transfer system accounted for ≤0.4 % and was hence considered to be negligible.

Periodic injections of CO_2 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 $mg \cdot L^{-1}$ NH_4-N before CO_2 enrichment and 1807±166 $mg \cdot L^{-1}$ NH_4-N during the rest of the digestion trials. This seems to indicate that injection of CO_2 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).

1.4. DISCUSSION

1.4.1. Suitability of injecting CO_2 into ADs with an external bubble column

The majority of previous studies investigating CO_2 injection into ADs have been completed at laboratory scale only, without the suitability of injecting CO_2 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 CO_2 in ADs through examining biogas quality, amount of CH_4 lost and mass transfer efficiency. Non-dilution of AD headspace and the low amount of CH_4 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 CO_2 dissolved determines the contribution towards reduction of carbon footprint that can be achieved (negative carbon release if dissolving CO_2 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 (Ozbek 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 CO_2 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 CO_2 levels and hence potential for carbon assimilation.

1.4.2. Impact of CO_2 injection in AD performance and mechanisms of utilisation based on VFA and H_2 dynamics

The test AD achieved an average CH_4 production rate of 0.45±0.05 m^3 CH_4·(kg VS_{fed}·d)$^{-1}$ before any CO_2 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 CH_4 production rate observed during the time when CO_2 enrichment was applied (0.56±0.13 m^3 CH_4·(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 CO_2 enrichment (0.56±0.13 m^3 CH_4·(kg VS_{fed}·d)$^{-1}$) with that of the control unit (0.53±0.16 m^3 CH_4·(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 H_2 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 CO_2 was periodically injected. The observed increased H_2 production can be used to further understand the mechanisms of CO_2 utilisation because of the role of H_2 as an electron carrier and intermediate product in several reactions of the digestion process (Cord-Ruwisch et al., 1997). Sudden increases in H_2 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 hydrolysis, acidogenesis and acetogenesis with an associated release of H_2 (Guwy et al., 1997). The fast response to system destabilizations and the recovery of initial H_2 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 H_2 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 H_2 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 ≤ 23 hour period, with H_2 rapidly returning to initial levels once the disturbance was overcome. On the contrary, an increase in H_2 concentration was observed in the test AD following four CO_2 injections, which lead to a new H_2 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 H_2 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 H_2 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 CO_2 injection affecting the microbial process in a more permanent manner. The different nature of both H_2 alternations was further evident when attending to the dynamics of VFA speciation within the AD. The increase in H_2 concentration of the control AD was simultaneous to a sudden increase in TVFA concentration (Fig. 3), which reached

17235±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_{H2}) 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_{H2} in the control unit reached these unfavourable conditions, with a value of $4.6 \cdot 10^{-4}$ atm (atmospheric pressure considered inside the AD). This is 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 (Fig. 2 and Fig. 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 *Methanosaetaceae* (obligate acetoclastic methanogen) has been reported to increase after periodic CO_2 injections in ADs (Bajón Fernández et al., *n.a.*), 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 previously observed.

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 aqueous media, from a boost of obligate acetogenesis or from a combination of both (Fig. 5). Part of the additional H_2 formed is then assimilated in the AD, leading to a steady operation as opposed to a continuously increasing H_2 level. Assimilation of additional H_2 is likely to occur through the Wood-Ljungdahl pathway, which has a preference for exogenous CO_2. The additional acetic acid formed by this pathway would then be assimilated by acetoclastic methanogenesis, which has been reported to have an increased activity when subjected to periodic CO_2 injections. The proposed mechanism of CO_2 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 CO_2 injection in acetogenesis are of great interest.

1.5. CONCLUSION

The capacity of ADs treating food waste to utilise exogenous CO_2 was tested and the practicalities of an up-scaled implementation and mechanisms of CO_2 utilisation were investigated. Injection of CO_2 through an external bubble column was suitable, as the headspace was not diluted and CH_4 loss during

384 injection was negligible (≤0.4%). A CH_4 production rate of 0.56±0.13 m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d)^{-1}$ was recorded
385 for an AD periodically enriched with CO_2. An additional uptake of 0.55 kg of exogenous CO_2 in the test
386 AD during the trial period was calculated, which could be augmented if the bubble column mass transfer
387 efficiency was increased, hence augmenting the potential benefits in CO_2 mitigation. A 2.5 fold increase in
388 H_2 concentration was observed after four CO_2 injections, likely due to CO_2 dissolution or an alternation of
389 acidogenesis/acetogenesis. Additional H_2 was believed uptaken by Wood-Ljungdahl pathway and the
390 acetate generated by this in turn assimilated by an increased activity of obligate acetoclastic *Archaea*. This
391 proposed hypothesis of exogenous CO2 conversion requires verification with microbial community
392 analysis.

393

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472

Table 1. Characterisation of digester feed, digestate and headspace of the control and tests ADs.

			Test AD	
	Feed	Control AD	Before CO_2 enrichment	With CO_2 enrichment
Parameter monitoring				
pH	5.7±0.6	7.9±0.2	7.8±0.2	7.8±0.2
TS (%)	9.7±1.4	4.5±0.9	3.1±0.4	4.5±0.8
VS (% of TS)	83.1±9.8	51.0±8.9	65.8±7.3	44.6±5.3
VS (% of wet matter)	8.0±1.2	2.3±0.4	2.0±0.1	2.0±0.5
Ammonia ($mg \cdot L^{-1}$ NH_4-N)	361±20	1855±205	1798±124	1807±166
VFA concentration ($mg \cdot L^{-1}$)	21114±1723	10707±313 [(a)]	9470±739 [(a)]	3662±44 [(b)]
Headspace monitoring				
CH_4 production rate (m^3 $CH_4 \cdot (kg\ VS_{fed} \cdot d^{-1})$)	-	0.53±0.16	0.45±0.05	0.56±0.13
CH_4 concentration (%)	-	68.3±5.7	68.8±3.4	68.5±3.4
H_2 concentration (ppm)	-	129±44	75±15	320±153

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

[(a)] Value on day 134. For VFA dynamics refer to Fig. 3.
[(b)] Value on day 153. For VFA dynamics refer to Fig. 3.

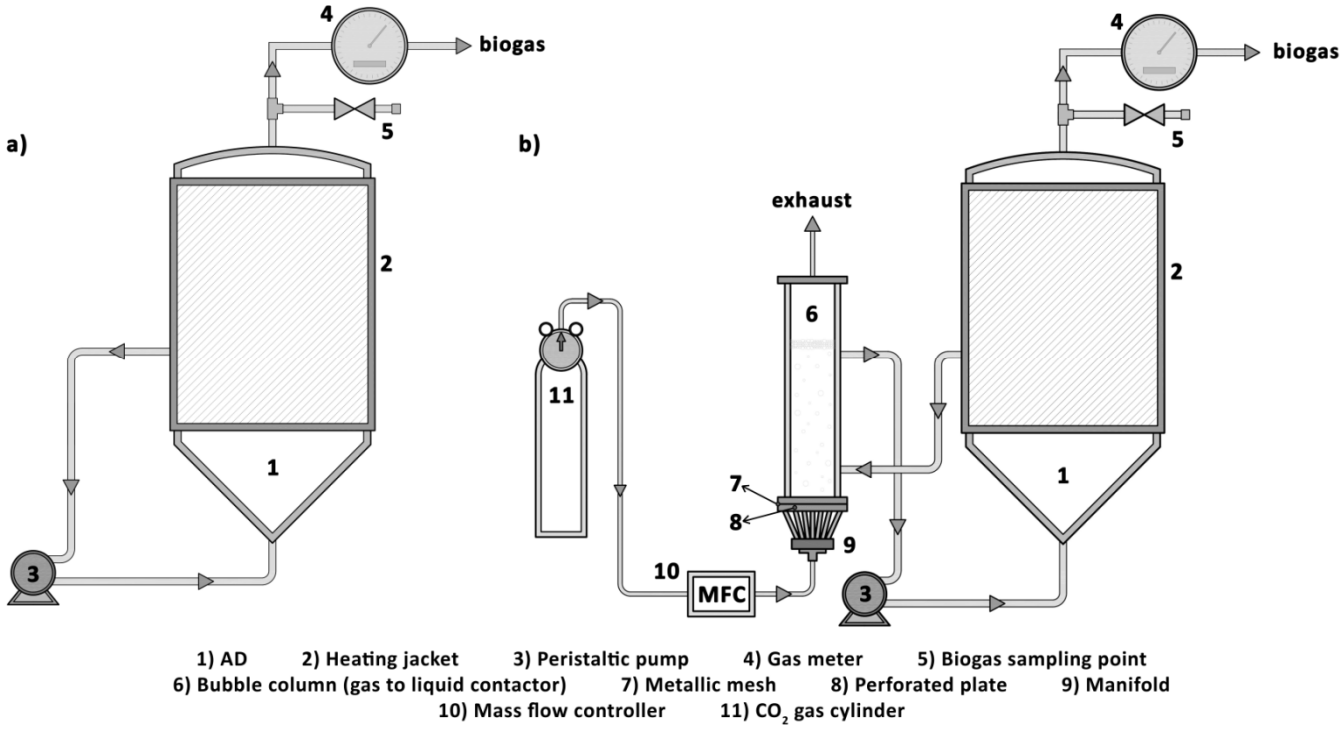


Fig. 1. Schematic representation of the pilot-scale experimental rig. (a) Conventionally operated AD and (b) AD retrofitted with an external bubble column for CO_2 injection. MFC: mass flow controller.

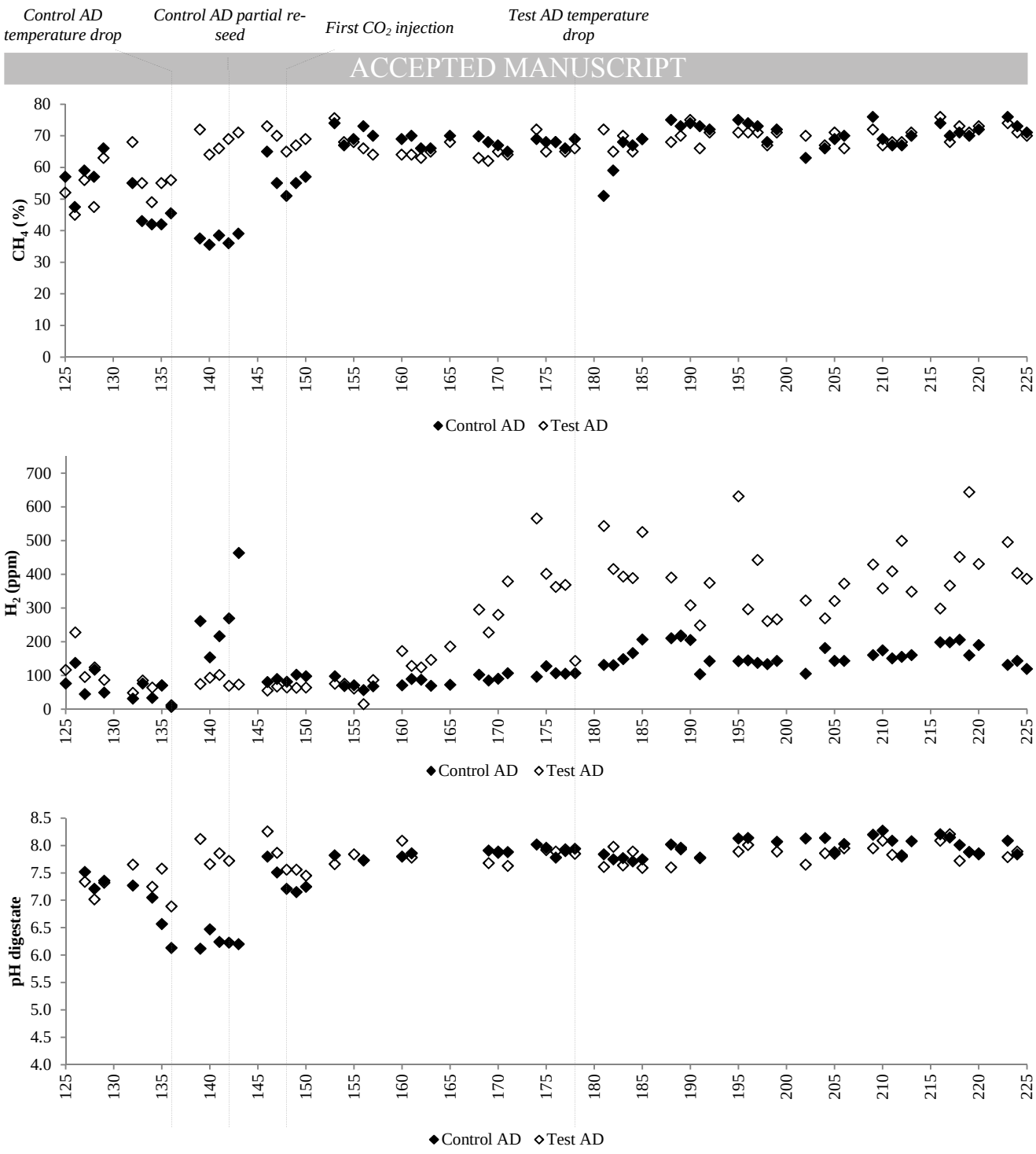


Fig. 2. Evolution of CH_4 and H_2 biogas concentration and digester pH in the control and test ADs during the pilot scale digestion trials.

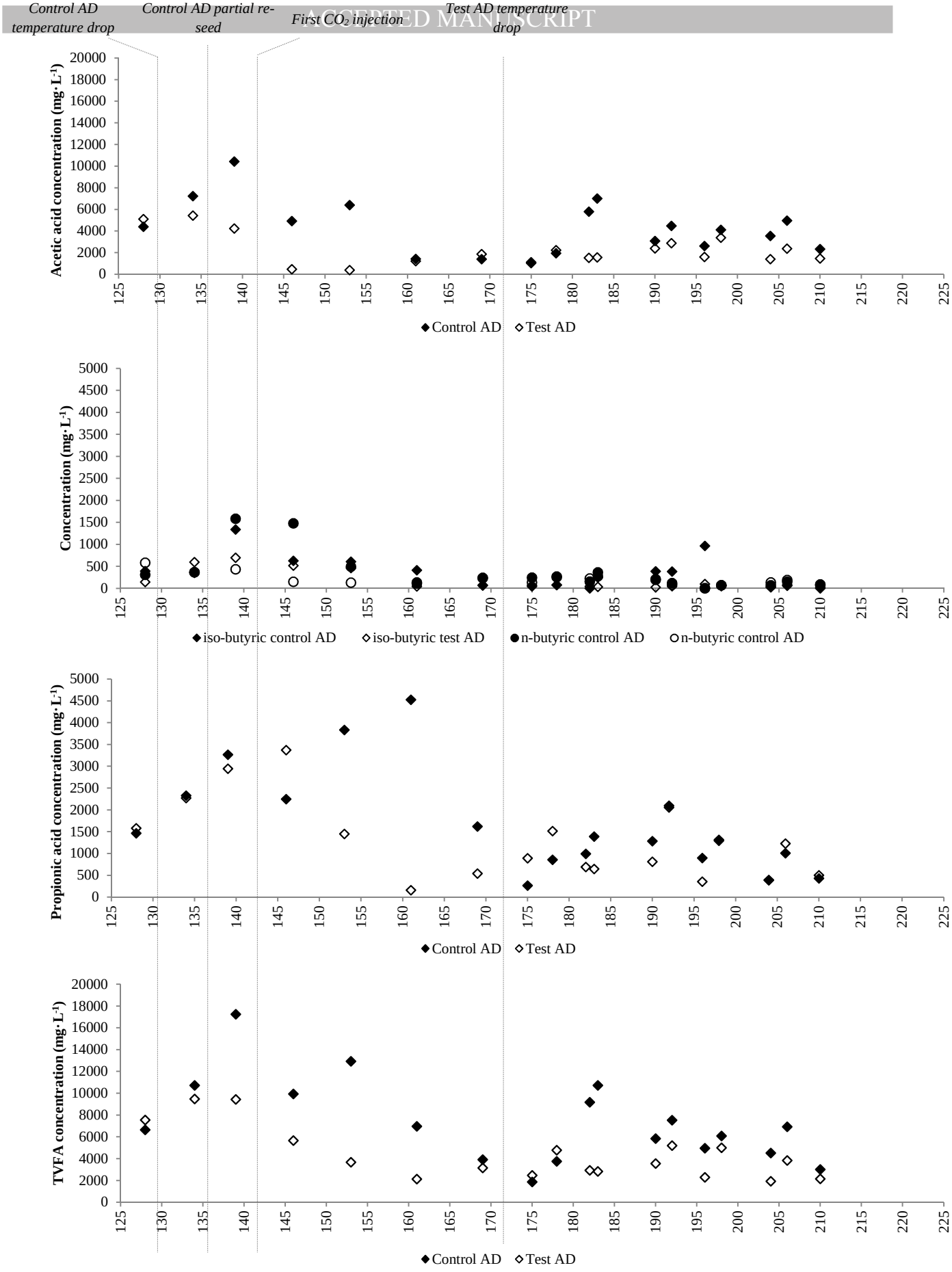


Fig. 3. Dynamics of total and individual VFA digestate concentrations for control and test ADs during the pilot scale digestion trials.

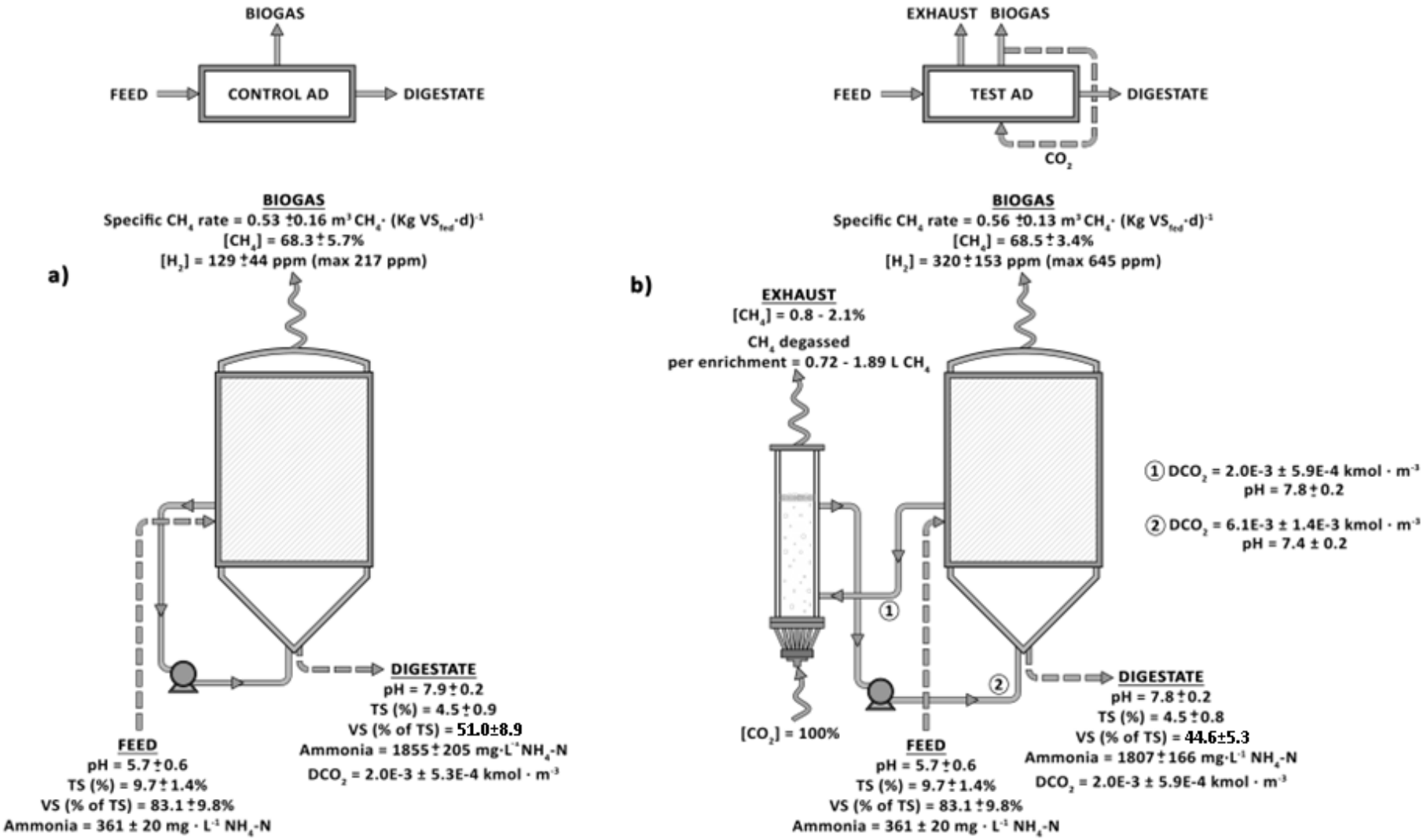
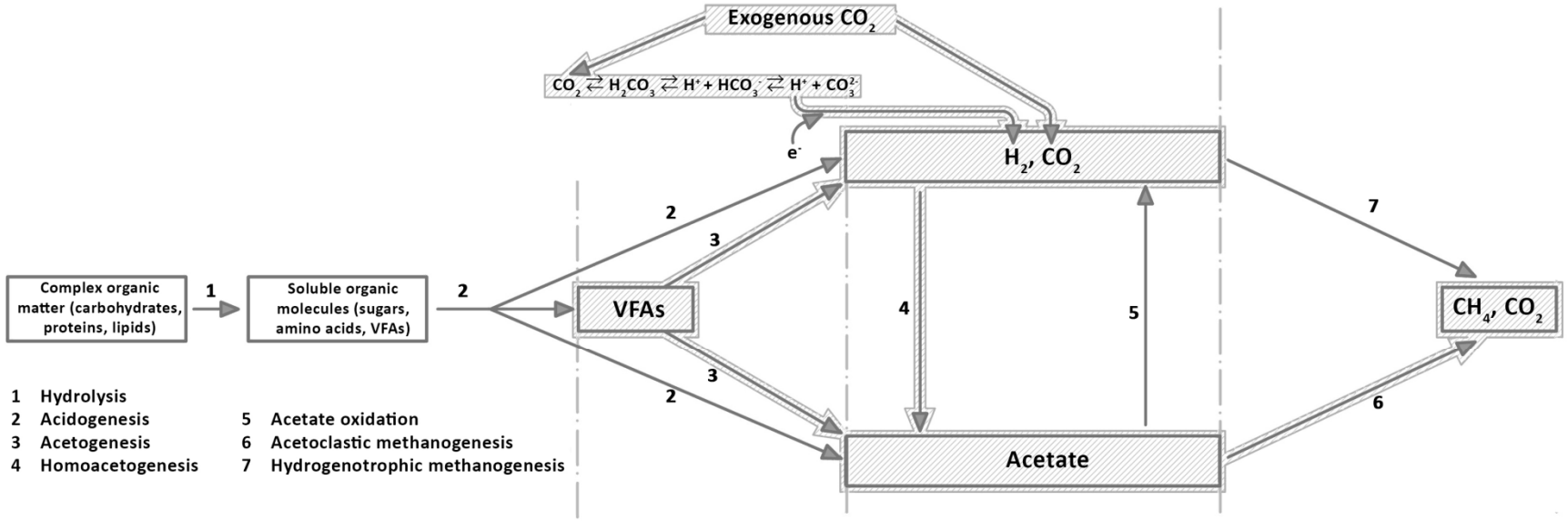


Fig. 4. Schematic summary of performance of (a) control and (b) test ADs in terms of digestate quality and biogas production. Recorded dissolved CO_2 concentrations are also included.



PART OF MECHANISM	FORMATION OF H_2	CONSUMPTION OF H_2 AND CO_2 BY HOMOACETOGENESIS (WOOD-LJUNGDAHL PATHWAY)	FORMATION OF CH_4 BY ACETOCLASTIC METHANOGENESIS
SUPPORTIVE FINDINGS	• Increase in H_2 level from 129±44 ppm to 320±153 ppm (max of 645 ppm) (this study)	• Stable operation of AD with 2.5 fold higher H_2 than control (this study) • Oscillation of H_2 around 320±153 ppm rather than uncontrolled increase (this study) • Increase in VFA levels in first stage of a TPAD after CO_2 injection (Salomoni et al., 2011)	• Higher benefits in CH_4 production of sewage sludge ADs than food waste ADs following CO_2 injection. Ammonia concentration was inhibitory for acetoclastic methanogens in food waste ADs (Bajón Fernández et al., 2014). • 80% higher activity of *Methanosaetaceae* in ADs periodically enriched with CO_2 than in control (Bajón Fernández et al., *submitted*)

Fig. 5. Hypothesised mechanism of exogenous CO_2 utilisation in ADs, with findings supporting each of the proposed stages.

HIGHLIGHTS

- Utilisation of exogenous CO_2 in ADs was investigated
- A pilot-scale AD with CO_2 injection treating food waste operated for the first time
- Injection of CO_2 with a bubble column achieved without diluting the AD's headspace
- Concentration of H_2 increased by 2.5 fold after four CO_2 injections
- A mechanism of CO_2 utilisation has been proposed