

Cranfield University

Cranfield Water Science Institute

School of Applied Sciences

PhD

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Yadira Bajón Fernández

**Carbon dioxide utilisation in anaerobic digesters as
an on-site carbon revalorisation strategy**

Supervisors:

Prof. Elise Cartmell

Dr. Ana Soares

Peter Vale

November 2014

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ABSTRACT

The increasing carbon footprint of the water and organic waste sectors has led to water utilities to voluntarily include carbon mitigation approaches within their strategic plans and to an increase in research aimed at mitigating carbon dioxide (CO₂) emissions. Injection of CO₂ in anaerobic digesters (ADs) for its bioconversion into methane (CH₄) has been identified as a potential solution. However, previous literature provided limited knowledge of the carbon benefits obtainable and presented conflicting information regarding the mechanisms of CO₂ utilisation.

This thesis investigated the potential of injecting exogenous CO₂ into ADs for its bioconversion into CH₄ to reduce CO₂ emissions from water and organic waste facilities. Batch laboratory scale and continuous pilot-scale ADs enriched with CO₂ were operated. A substrate dependant response to exogenous CO₂ was reported for the first time and potential CO₂ savings of up to 34% and 11% were estimated for sewage sludge and food waste batch ADs, respectively, injected with CO₂ before the digestion process. Higher benefits in CH₄ production were observed in sewage sludge ADs than in food waste units. An up to 2.4 fold increase in CH₄ production during the 24 hours following saturation with CO₂ was obtained in sewage sludge units, while benefit was limited to 1.16 fold in food waste ADs. Microbial community analyses were performed to elucidate CO₂ fate mechanisms. An increase of up to 80% in the activity of *Methanosaetaceae* (obligate acetoclastic methanogen) was observed in sewage sludge ADs periodically enriched with CO₂. *Methanosaetaceae* was scarce (4.3±1.7%) in food waste units, which was attributed to an inhibitory concentration of ammonia (4 g·L⁻¹ NH₄-N). Based on *Archaea* analyses and on monitoring hydrogen (H₂) and volatile fatty acids (VFA) speciation dynamics in a pilot-scale AD, it was proposed that exogenous CO₂ is reduced by homoacetogenesis (Wood-Ljungdahl mechanism) and the acetate generated by this route is converted to CH₄ by acetoclastic methanogenesis.

Gas to liquid mass transfer was identified as limiting of the amount of dissolved CO₂ loaded to an AD and the complex rheology of anaerobically digested media as detrimental for transfer performance. An increase of apparent viscosity (μ_a) from 130 to 340 cPo (typical variability of sewage sludge) reduced gas transfer efficiency (GTE) by 6 percentage points. The use of bubble columns was identified as suitable for further scaled-up units. Injection of CO₂ could be performed in the digestate recirculation loop of single phase ADs or in the first phase of two phase ADs (TPADs), with CO₂ sourced from off-gas of biogas upgrading technologies. It has been demonstrated that bioconversion of CO₂ in ADs can reduce carbon footprint and increase CH₄ production, with the possibility of becoming an on-site carbon revalorisation strategy.

RESUMEN (TRANSLATION OF THE SECTION ABSTRACT)

La creciente huella de carbono de los sectores del agua y residuos orgánicos ha llevado a las empresas del sector a incluir voluntariamente estrategias de mitigación de carbono en sus planes estratégicos y a un aumento de la investigación enfocada a reducir la emisión de dióxido de carbono (CO₂). La inyección de CO₂ en digestores anaeróbicos (DAs) para su bioconversión a metano (CH₄) ha sido identificada como una posible solución. Sin embargo, investigaciones anteriores ofrecen escasa información de los beneficios obtenibles y presentan información contradictoria en relación a los mecanismos de utilización de CO₂.

Esta tesis ha investigado el potencial de inyectar CO₂ exógeno en DAs para su bioconversión a CH₄ para reducir la huella de carbono del tratamiento de aguas residuales y residuos orgánicos. Se han operado DAs enriquecidos con CO₂ a escala de laboratorio y de planta piloto. Se ha obtenido una respuesta dependiente del sustrato tratado y se ha estimado una reducción de CO₂ de hasta un 34% y 11% en DAs discontinuos saturados con CO₂ que tratan lodos de depuradora y residuos alimentarios, respectivamente. Se ha observado un mayor beneficio en la producción de CH₄ en DAs de lodos de depuradora que con residuos alimentarios. Se ha obtenido un aumento de hasta 2.4 veces en la producción de CH₄ durante las 24 horas posteriores a saturar con CO₂ DAs de lodos de depuradora, mientras que el beneficio máximo en residuos alimentarios ha sido de 1.16 veces. Se han desarrollado análisis microbianos para elucidar los mecanismos de utilización de CO₂, obteniéndose un incremento de hasta un 80% en la actividad de *Methanosaetaceae* (arquea metanogénica acetoclástica) en DAs tratando lodos de depuradora enriquecidos periódicamente con CO₂. *Methanosaetaceae* se ha encontrado en escasa proporción en residuos alimentarios (4.3±1.7%) debido a su inhibición por amoníaco (4 g·L⁻¹ NH₄-N). En base al análisis de arqueas y a la concentración de hidrógeno (H₂) y ácidos volátiles grasos en un DA a escala piloto, se ha propuesto que el CO₂ exógeno es reducido por homoacetogénesis (ruta Wood-Ljungdahl) y el acetato generado es convertido a CH₄ por metanogénesis acetoclástica. La transferencia de masa gas-líquido es un factor limitante de la cantidad de CO₂ disuelto introducida en un DA y la reología de sustratos digeridos anaeróbicamente dificulta dicha transferencia. Un incremento de viscosidad aparente (μ_a) de 130 a 340 cPo (típico en lodos de depuradora) puede reducir en 6 puntos de porcentaje la eficiencia de transferencia de gas. Se ha considerado adecuado el uso de columnas de burbujas para disolver CO₂ en unidades a mayor escala. Ha sido concluido que la inyección de CO₂ puede realizarse en la recirculación de material digerido en un DA de fase única o en la primera fase de un DA de dos fases. El CO₂ inyectado podría capturarse de los gases de salida de varias tecnologías de enriquecimiento de biogás. Se ha demostrado que la bioconversión de CO₂ en DAs puede reducir la huella de carbono y aumentar la producción de CH₄, teniendo así potencial para ser una estrategia de revalorización de carbono in-situ.

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ABBREVIATIONS

AD	Anaerobic digestion / Anaerobic digester
ANOVA	Analysis of variance
C2ES	Center for climate and energy solutions
CCS	Carbon capture and storage
CHP	Combined heat and power
CIWM	Chartered Institution of Wastes Management
CMC	Carboxymethyl cellulose sodium salt
DC	Digester control
DECC	Department of Energy and Climate Change
DEFRA	Department for Environment, Food and Rural Affairs
DGC	Downflow gas contactor
DI	Deionized (water)
DIAD	Driving innovation in anaerobic digestion
DMEA	Di-methyl ethanol amine
DO	Dissolved oxygen
Dperiodic	Anaerobic digester enriched periodically with CO ₂
Dsingle	Anaerobic digester enriched once with CO ₂
EEA	European Environment Agency
EPSRC	Engineering and Physical Sciences Research Council
ESEM	Environmental scanning electron microscope
FAN	Free ammonia nitrogen
FISH	Fluorescence in situ hybridisation
FiT	Feed in tariff
GDP	Gross domestic product
GHG	Greenhouse gas
GTE	Gas transfer efficiency
GTR	Gas transfer rate
HPLC	High performance liquid chromatography
IA	Intermediate alkalinity
IEA	International Energy Agency
MEA	Mono ethanol amine
MFC	Mass flow controller
NAD	Nicotine adenine dinucleotide
NADH	Reduced form of nicotine adenine dinucleotide
NERC	Natural Environment Research Council

NOAA	National Oceanic and Atmospheric Administration
OLR	Organic loading rate
ORP	Oxidation reduction potential
PA	Partial alkalinity
PBS	Phosphate buffer solution
PFA	Paraformaldehyde solution
RR	Ripley ratio
SAO	Syntrophic acetate oxidation
SCCS	Scottish Carbon Capture & Storage
sCOD	Soluble chemical oxygen demand
TA	Total alkalinity
TAN	Total ammonia nitrogen
TPAD	Two-phase anaerobic digestion / Two-phase anaerobic digester
TS	Total solids
TUM	Technical University of Munich
TVFA	Total volatile fatty acids
UASB	Upflow anaerobic sludge blanket
USEPA	United States Environmental Protection Agency
VFA	Volatile fatty acids
VS	Volatile solids
WAS	Waste activated sludge
WRI	World Resource Institute
WWTP	Wastewater treatment plant
XRD	X-ray diffraction
Abbreviations for the sections in Spanish	
CAC	Captura y almacenamiento de carbono
DA	Digestión anaerobia/ digestor anaerobio
GEI	Gases de efecto invernadero

All abbreviations are introduced within the text and re-introduced for each individual chapter.

NOTATIONS

Ca^{2+}	Calcium
CaCO_3	Calcium carbonate
CH_4	Methane
C	Concentration in the liquid phase
C^*	Solubility
C_{model}	Concentration estimated with equation 2 of Chapter 6
C_0	Concentration at time zero
C_{sensor}	Concentration measured by the probe
C_t	Concentration at time t
CO_2	Carbon dioxide
CO_2e	Carbon dioxide equivalents
CO_3^{2-}	Carbonate
$(\text{CO}_2)_{\text{digestate}}$	CO_2 dissolved in the digestate at the end of the digestion period
$(\text{CO}_2)_{\text{biogas}}$	CO_2 released with the biogas
$(\text{CO}_2)_{\text{in}}$	CO_2 dissolved in the material to digest after the CO_2 injection
D_L	Diffusion coefficient
\dot{F}_{CO_2}	Incoming CO_2 mass flow rate
H_2	Hydrogen
H^+	Proton
HCO_3^{2-}	Bicarbonate
H_2CO_3	Carbonic acid
K_H	Henry's constant
$k_{L,a}$	Volumetric liquid phase mass transfer coefficients
$(k_{L,a})_T$	Volumetric mass transfer coefficient at temperature T
$(k_{L,a})_{U_G}$	Volumetric mass transfer coefficient obtained with U_G
$(k_{L,a})_{20}$	Volumetric mass transfer coefficient at 20°C
$(k_{L,a})_\mu$	Volumetric mass transfer coefficient obtained with a liquid phase with μ
n	Coefficient depending on the theory for interfacial mass transfer considered between the gas and the liquid phases
N_2	Nitrogen
NH_3	Ammonia
NH_4HCO_3	Ammonium bicarbonate
$(\text{NH}_4)_2\text{CO}_3$	ammonium carbonate
$\text{NH}_2\text{COONH}_4$	ammonium carbamate
O_2	Oxygen

p_{CO_2}	Carbon dioxide partial pressure
p_{H_2}	Hydrogen partial pressure
P_T	Total pressure
y_{CO_2}	Carbon dioxide molar fraction
S°	Entropy at standard conditions
sCOD	Soluble chemical oxygen demand
t_f	Characteristic time of the mass transfer
t_{95}	Time to reach 95% of the equilibrium solubility
U_G	Superficial gas velocities
U_{trans}	Transition superficial gas velocity between bubbly and churn-turbulent flow regimes
V	Volume of liquid inside of the bubble column
α	parameter to assess the impact of μ_a in mass transfer when normalised against DI water
$\dot{\gamma}$	Shear rate
$\dot{\gamma}_{av}$	Average shear rates
ΔG°	Gibbs free energy at standard conditions
$\Delta G_{38^\circ C}$	Gibbs free energy at a temperature of 38°C
ΔH°	Reaction enthalpy at standard conditions
ζ	Response time of the probe
O	Parameter to assess the impact of U_G , when normalised against $0.61 \text{ cm}\cdot\text{s}^{-1}$
μ	Dynamic viscosity
μ_a	Apparent dynamic viscosity
$(\mu_a)_{av}$	Average dynamic apparent viscosity

All notations are introduced within the text and re-introduced for each individual chapter.

CHAPTER 1
INTRODUCTION

1. INTRODUCTION

1.1. BACKGROUND

The concentration of carbon dioxide (CO₂) in the atmosphere has increased from 280 ppm in the preindustrial era to *ca.* 400 ppm in 2014 (NOAA, 2014). The most well-known impact of increased CO₂ atmospheric concentration is climate change, with a rise of 3.6°C in temperature over preindustrial levels expected in the long term even when announced greenhouse gas (GHG) mitigation commitments are implemented (IEA, 2013). Anthropogenic emissions are the main contributor to this problem (IEA, 2013), with the energy sector contributing to 72% of global emissions (2011 data (WRI, 2014)). Decarbonisation of the energy supply and increased efficiency of energy use are global priorities required to meet national (e.g. Climate Change Act) and international (Kyoto Protocol) GHG mitigations targets. However, the carbon emissions associated with the supply of energy have remained constant at *ca.* 57.1 tonnes CO₂·TJ⁻¹ over the last 25 years (IEA, 2013), indicating that sustainable energy generation has not yet been achieved at global level. Therefore, GHG mitigation measures are required from individual industrial sectors in order to achieve global impact. However, the water sector in the European Union for example has an increasing carbon footprint, which is partly attributed to the tightened quality standards resulting from the implementation of the Water Framework Directive (European Commission, 2009; Georges et al., 2009).

Emissions of GHG from the water sector at world level have been estimated to account for 3-10% of total global emissions (McGuckin et al., 2013), with the electricity demand of this sector accounting for 4% of total national consumption in the USA and for *ca.* 3% in UK (Rothausen and Conway, 2011). Therefore, there is potential for this sector to contribute towards GHG mitigation (McGuckin et al., 2013; USEPA, 2012), with water utilities voluntary including carbon mitigation strategies within their strategic plans. Increase in renewable energy production through the anaerobic digestion (AD) of sewage sludge is considered one of the most promising solutions to be implemented (DEFRA, 2007) to support carbon reduction measures. However its rapid growth has led to an increase in the generation of biogenic CO₂ with biogas, which is emitted to atmosphere with process exhaust streams. Biogas derived CO₂ has in turn been identified as the CO₂ stream most readily available to be captured for storage or valorisation within the wastewater treatment process (Byrns et al., 2013). Avoidance of emission of biogas CO₂ would be accounted as negative emissions to be deducted from overall carbon accounting inventories, which makes the development of management solutions for this stream of evident value. Emissions of biogas CO₂ from sewage sludge treatment have been estimated at 270,000 tonnes CO₂ per annum for the UK water

industry alone (Byrns et al., 2013). This is likely to increase with the predicted raise in AD implementation for the treatment of alternative substrates (e.g. manure, community waste). Biogas CO₂ from European ADs treating municipal solid waste have been estimated at *ca.* 980,000 tonnes CO₂ per annum (Bajón Fernández et al., 2015). Therefore, management of biogas CO₂ could not only counteract the increasing carbon footprint of the water sector but potentially help support organic waste treatment becoming a negative CO₂ emission process.

Carbon capture and storage (CCS) in geological or oceanic reservoirs is considered one of the most cost effective GHG mitigation strategies in sectors like energy, where clusters of centralised emitters are encountered (DECC, 2012). However, the scattered location of AD sites prevents a common pipeline infrastructure for CO₂ transport to be installed, raising the need to develop new on-site carbon revalorisation strategies applicable to the water and organic waste sectors. Alternatives based on biogenic carbon sequestration are being investigated, with CO₂ bioconversion to methane (CH₄) in anaerobic processes identified as the solution with a higher anticipated simplicity for implementation (Byrns et al., 2013). However, its potential for CO₂ uptake and full-scale applicability remain unclear, partly conditioned by the lack of understanding of exogenous CO₂ fate and of studies investigating the needs and means for scaled-up implementation.

1.2. AIMS AND OBJECTIVES

The thesis reports research funded by the Engineering and Physical Sciences Research Council (EPSRC), Severn Trent Water, WRAP and the Lorch Foundation. The aim is to understand the potential benefits of injecting exogenous CO₂ into ADs treating different substrates, with a view of utilising CO₂ bioconversion into CH₄ as an on-site carbon revalorisation strategy to reduce the carbon footprint of the water and organic waste sectors. Consequently, the following objectives were identified:

1. To review the need to reduce GHG emissions from the water and organic waste sectors and identify how CO₂ emissions could be reduced.
2. To assess at laboratory and pilot scale the feasibility of CO₂ injection in ADs treating food waste and sewage sludge, both with respect to carbon uptake and renewable energy production.
3. To elucidate the mechanisms of utilisation of exogenous CO₂ in ADs.
4. To investigate the influence of liquid viscosity and rheology on CO₂ gas to liquid mass transfer, in the context of CO₂ dissolution into anaerobically digested substrates.
5. To review the CO₂ streams of the water and organic waste sectors that could be bioconverted to CH₄ in anaerobic processes.

6. To examine the feasibility, potential benefits and practicalities (systems and points of injection) of implementing CO₂ bioconversion in scaled-up ADs.

1.3. THESIS PLAN

Each research section has been structured into chapters based on published, submitted or in preparation papers, which together constitute this thesis. All chapters have been written by the first author, Yadira Bajón Fernández, and edited by Professor Elise Cartmell, and Dr. Ana Soares with exception of Chapter 6 which was edited by Professor Bruce Jefferson. All laboratory work was undertaken by Yadira Bajón Fernández, with exception of the last stages of the fluorescence in situ hybridisation (FISH) laboratory protocol (Chapter 4), for which samples were sent to an external contractor, and for part of the mass transfer tests of Chapter 6 where there was laboratory support from the sixth author. Part of this PhD thesis was completed as a result of a three month stay of Yadira Bajón Fernández in the Technical University of Munich (TUM), Germany, as required for obtaining the international doctorate mention.

The connection between the thesis chapters is schematised in Figure 1.1. Chapter 2 constitutes a review of the need and means to reduce the increasing emissions of CO₂ from the water and organic waste sectors. Enrichment of ADs with additional CO₂ for this to be bioconverted to CH₄ was identified as a suitable option to minimize carbon footprint. Hence, the state of the art, mechanisms of CO₂ utilisation and areas where further knowledge was required in relation to CO₂ enrichment of ADs were identified and discussed. Chapter 2 was submitted to *Bioresource Technology - Bajón Fernández, Y., Soares, A., Koch, K., Vale, P. and Cartmell, E., A review of re-use and valorisation of by-product CO₂ gas streams from anaerobic digestion sites* - and it is under review.

Chapter 3 is a feasibility study completed to determine the potential benefits of injecting exogenous CO₂ into ADs treating sewage sludge or food waste. Chapter 3 was published in *Bioresource Technology - Bajón Fernández, Y., Soares, A., Villa, R., Vale, P. and Cartmell, E., (2014). Carbon capture and biogas enhancement by carbon dioxide enrichment of anaerobic digesters treating sewage sludge or food waste. Bioresource Technology, 159, 1-7.* The most remarkable outcome of this chapter was the finding of a substrate dependant response of ADs to an injection of exogenous CO₂, with higher benefits in carbon uptake and CH₄ production recorded for sewage sludge than for food waste ADs. The ammonia concentration in food waste units was within the levels reported as toxic for acetoclastic methanogens. Therefore, it was hypothesised that the higher benefit in sewage sludge ADs was due to an increased activity of acetoclastic methanogens in response to CO₂ injections.

Chapter 4 studies the mechanisms by which additional CO₂ can be utilised in anaerobic processes, with consideration of both chemical and biological assimilation pathways. Chapter 4 was submitted to Water Research - *Bajón Fernández, Y., Soares, A., Vale, P. and Cartmell, E. Enhancing the anaerobic digestion process through carbon dioxide enrichment: mechanisms of utilisation* - and it is under review. In this chapter it was confirmed that different methanogenic communities were predominant in ADs treating different substrates, with obligate acetoclastic methanogens being the main contributor to the total *Archaea* population in sewage sludge ADs and scarce in food waste units. Furthermore, the initial hypothesis (CO₂ injection enhances the acetoclastic route of CH₄ formation) was confirmed, as activity of *Methanosaetaceae* (obligate acetoclastic methanogens) was up to 80% higher in sewage sludge ADs periodically enriched with CO₂ than in control units. The question of whether the enhanced *Methanosaetaceae* activity was 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) could not be fully elucidated at this stage. However, it was hypothesised that exogenous CO₂ was reduced by the Wood-Ljungdahl pathway, leading to formation of acetate that acted as substrate for acetoclastic methanogenesis.

Chapter 5 investigates the possibility of implementing CO₂ bioconversion in up-scaled units, with special emphasis on the suitability of using an external bubble column to perform the required CO₂ gas to liquid mass transfer. Further insight in fate of exogenous CO₂ is also provided. Chapter 5 is in preparation to be submitted to the special issue “Carbon Neutral” of Water Research - *Bajón Fernández, Y., Green, K., Schuler, K., Soares, A., Vale, P. and Cartmell, E. Biological carbon dioxide utilisation in food waste anaerobic digesters*. In this research section it was considered that monitoring volatile fatty acids (VFAs) dynamics and headspace hydrogen (H₂) levels in continuously operated ADs could contribute to elucidate whether injection of CO₂ alters the initial stages of the digestion process, as hypothesised in Chapter 4. An increasing trend in the headspace H₂ concentration of the pilot-scale AD periodically enriched with CO₂ was observed, which stabilised at a value 2.5 fold higher than the control unit. This increase was attributed to the dissolution of CO₂ and to an alteration of acetogenesis. The H₂ level did not increase further in spite of sequential CO₂ injections, which was believed due to its assimilation by the Wood-Ljungdahl pathway and supportive of the hypothesis of Chapter 4. The use of bubble columns for dissolving exogenous CO₂ into anaerobic digesting media was identified as suitable. A significant carbon uptake was recorded for the test AD injected with exogenous CO₂. However, monitoring of dissolved CO₂ levels indicated that only *ca.* 25% of solubility values were achieved in the exit of the bubble column used for CO₂ injection. This indicated that gas to liquid mass transfer

was a limiting factor of the CO₂ to be loaded to an AD and the need to further understand mass transfer in fluids of complex rheology like anaerobically digested substrates was identified.

Chapter 6 addresses the gap in knowledge regarding the impact of viscosity and rheology in gas to liquid mass transfer retardation. Synthetic fluids were utilised to confidently decouple the impact of viscosity and rheology from other process variables. Rheological and operational conditions that imitate gas to liquid mass transfer in anaerobically digesting media were included within those tested. Chapter 6 was submitted to *Chemical Engineering Journal* - *Bajón Fernández, Y., Cartmell, E., Soares, A., McAdam, E., Vale, P., Darche-Dugaret, C. and Jefferson, B. Gas to liquid mass transfer in viscous fluids* - and it is under review. This research confirmed that viscosity variations can significantly hinder gas to liquid mass transfer efficiency and evidenced that the extent of this impact is different for Newtonian and non-Newtonian (e.g. sewage sludge) fluids. Mass transfer retardation was predominantly associated with alterations in the hydrodynamics of the system and a new flow regime (slug-annular flow) was described. This is considered of great interest for designing full-scale systems relying on gas to liquid mass transfer in fluids of complex rheology, because it evidences the need to account for viscosity variations in order to avoid a reduction of active volume.

Chapter 7 assesses the potential for implementing bioconversion of CO₂ in ADs at full scale sites. Systems where benefits are likely to be observed, means of implementation and potential benefits are identified in view of increasing readiness for implementation. Chapter 6 is in preparation to be submitted to *Environmental Technology* - *Bajón Fernández, Y., Soares, A., Koch, K., Vale, P. and Cartmell, E. Carbon dioxide enrichment of anaerobic digesters as an on-site carbon revalorisation strategy: considerations and requirements for implementation*.

Chapter 8 is the overall discussion of the thesis. Appendix 1 includes pictures of the experimental rigs operated during this research.

Within the course of this PhD two research proposals were written for Phase 1 and Phase 2 of the *Driving innovation in anaerobic digestion* (DIAD) programme run by WRAP. Both proposals were awarded, with a financial contribution of £25k and £75k, respectively. The proposals and reports summarising the research findings were written by Yadira Bajón Fernández and edited by Professor Elise Cartmell and Dr. Ana Soares. The experimental work corresponding to both proposals was undertaken by Yadira Bajón Fernández. The report of Phase 1 is accessible in WRAP's website (<http://www.wrap.org.uk/node/16676>) and that corresponding to Phase 2 is under review and will be available shortly.

In addition, during the course of this research Yadira Bajón Fernández has designed, purchased and operated a macerator-pump system to be retrofitted into the 1.5 m³ AD demonstrator available at Cranfield University. Specifications for designing a CO₂ injection system that could be retrofitted to this AD demonstrator were also provided to WRK Design and Services Ltd. (Birmingham, UK). Images and information of both systems can be found in Appendix 1 and Appendix 2, respectively.

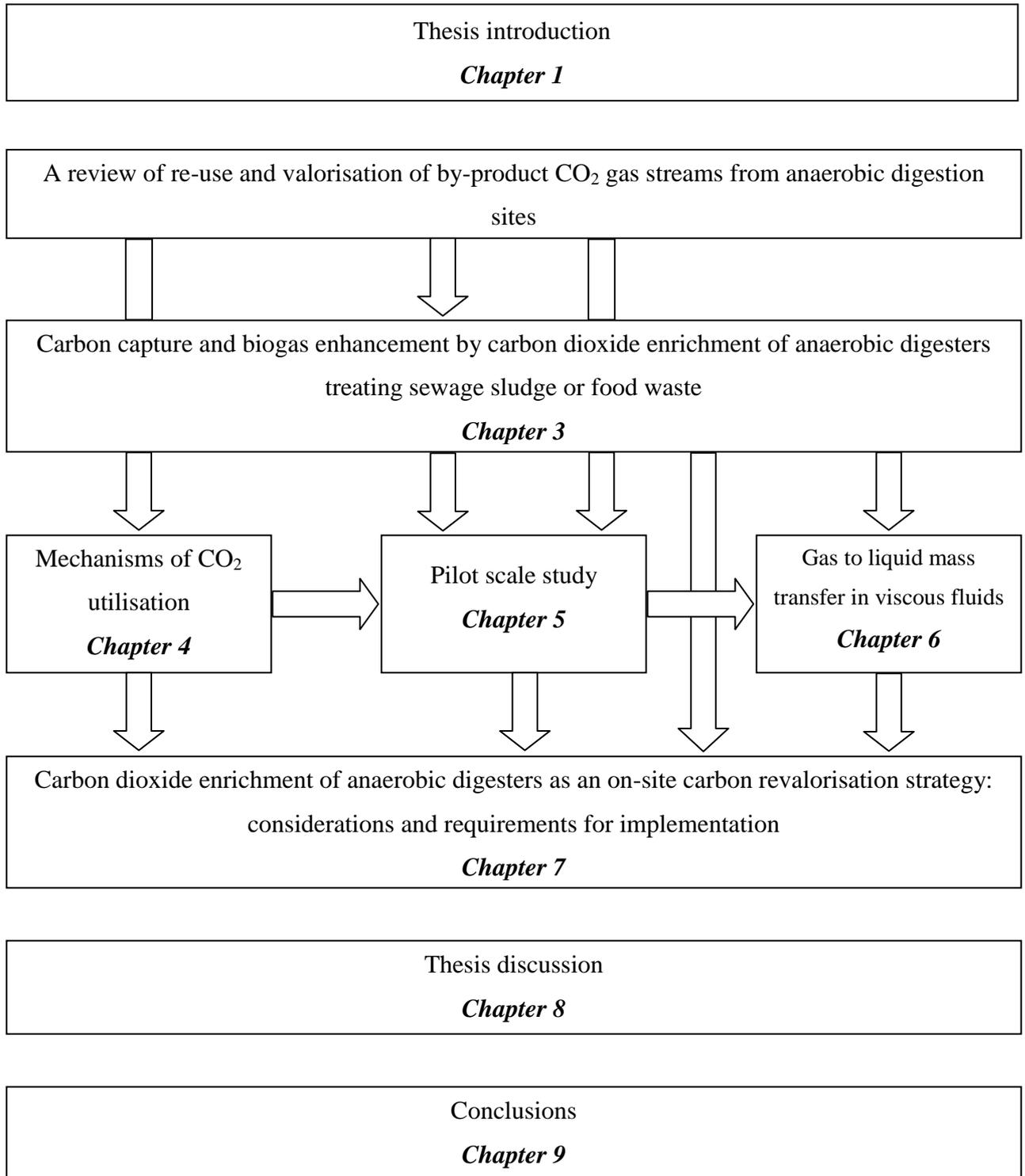


Figure 1.1. Thesis structure as a flow chart.

1.4. REFERENCES

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1.5. INTRODUCCIÓN (TRANSLATION OF THE SECTION INTRODUCTION)

1.5.1. Contexto

La concentración de dióxido de carbono (CO₂) en la atmósfera ha aumentado desde 280 ppm en la era preindustrial a *ca.* 400 ppm en 2014 (NOAA, 2014). El impacto más conocido de un aumento en la concentración de CO₂ atmosférico es el cambio climático. Un incremento de 3.6°C en la temperatura sobre el nivel preindustrial ha sido estimado a largo plazo incluso si las medidas propuestas de mitigación de gases de efecto invernadero (GEI) son implementadas (IEA, 2013). El mayor contribuyente a este problema son las emisiones de origen antropogénico (IEA, 2013), siendo el sector energético responsable del 72% de las emisiones globales (dato de 2011, (WRI, 2014)). La descarbonización del suministro energético y el aumento de la eficiencia del uso de energía son prioridades globales necesarias para cumplir con los objetivos nacionales (p. ej. Climate Change Act) e internacionales (Protocolo de Kyoto) de mitigación de GEI. Sin embargo, la emisión de carbono asociada con el suministro de energía se ha mantenido constante en *ca.* 57.1 t CO₂·TJ⁻¹ durante los últimos 25 años (IEA, 2013), lo que indica que una generación sostenible de energía aún no ha sido obtenida a nivel global. Es por ello por lo que medidas de mitigación de GEI son necesarias en sectores individuales para obtener un impacto global. Sin embargo, el sector del agua en la Unión Europea, por ejemplo, presenta una creciente huella de carbono, en parte debido a los restrictivos estándares de calidad establecidos en la Directiva marco del agua (European Commission, 2009; Georges et al., 2009).

La emisión de GEI en el sector del agua a nivel mundial ha sido estimada responsable de un 3-10% de las emisiones totales globales (McGuckin et al., 2013), siendo la demanda eléctrica de este sector un 4% del consumo nacional en los Estados Unidos y *ca.* 3% en Reino Unido (Rothausen and Conway, 2011). Es por ello evidente el potencial que presenta este sector para contribuir a la mitigación de GEI (McGuckin et al., 2013; USEPA, 2012), lo que ha llevado a empresas de agua a incluir voluntariamente estrategias de reducción de carbono en sus planes estratégicos. El aumento de la producción de energía renovable mediante digestión anaerobia (DA) de lodos de depuradora es considerada una de las soluciones más prometedoras para contribuir a la reducción de emisión de carbono (DEFRA, 2007). Sin embargo, su rápido crecimiento ha conllevado un aumento en la generación de CO₂ contenido en biogás, el cual es emitido a la atmósfera. El CO₂ contenido en biogás ha sido identificado como la corriente de CO₂ más fácilmente capturable para su almacenamiento o valorización entre las generadas durante el tratamiento de aguas residuales (Byrns et al., 2013). La prevención de emisión de CO₂ derivado de biogás es contabilizada como emisión negativa a

descontar de inventarios de carbono, lo que evidencia el valor de desarrollar soluciones para esta corriente. La emisión de CO₂ derivado de biogás producido en el tratamiento de lodos de depuradora ha sido estimada en 270,000 toneladas de CO₂ anuales en la industria del agua en Reino Unido (Byrns et al., 2013). Este valor aumentará con la rápida implementación de DA para el tratamiento de otros substratos (p. ej. estiércol, residuos sólidos urbanos). La emisión de CO₂ de biogás generado en DAs europeas que tratan residuos sólidos urbanos se ha estimado en *ca.* 980,000 toneladas de CO₂ anuales (Bajón Fernández et al., 2015). La prevención de emisión de este CO₂ podría contrarrestar la creciente huella de carbono del sector del agua y contribuir a que el tratamiento de residuos orgánicos llegue a ser un proceso de emisión negativa de CO₂.

La captura y almacenamiento de carbono (CAC) en reservorios geológicos u oceánicos es considerada una de las estrategias de mitigación de GEI más rentables en el sector energético, donde existen aglomeraciones de emisores (DECC, 2012). Sin embargo, la ubicación dispersa de la plantas de DA impide la instalación de una estructura de tuberías común para el transporte de CO₂, lo que requiere el desarrollo de nuevas estrategias de revalorización de carbono in situ aplicables a los sectores del agua y los residuos orgánicos. Están siendo investigadas alternativas basadas en el secuestro biogénico de carbono, siendo la bioconversión de CO₂ a metano (CH₄) en procesos anaerobios la solución con una mayor simplicidad de implementación (Byrns et al., 2013). Sin embargo, su potencial para capturar CO₂ y su aplicabilidad a gran escala no han sido dilucidados, en parte debido a la falta tanto de conocimiento sobre los mecanismos de utilización de CO₂ exógeno como de estudios que investiguen los requisitos y medios para una implementación a gran escala.

1.5.2. Metas y objetivos

Esta tesis incluye investigación financiada por Engineering and Physical Sciences Research Council (EPSRC), Severn Trent Water, WRAP y Lorch Foundation. La finalidad es entender los potenciales beneficios de inyectar CO₂ exógeno en DAs que tratan diferentes substratos, con la intención de utilizar la bioconversión de CO₂ a CH₄ como una estrategia de valorización de carbono in situ para reducir la huella de carbono de los sectores del agua y residuos orgánicos. En consecuencia, se han identificado los siguientes objetivos:

1. Examinar la necesidad de reducir GEI en los sectores del agua y de los residuos orgánicos e identificar como se podría reducir la emisión de CO₂.
2. Evaluar a escala de laboratorio y de planta piloto la factibilidad de inyectar CO₂ en DAs que tratan residuos alimentarios y lodos de depuradora, tanto en relación a la captura de carbono como a la producción de energía renovable.
3. Dilucidar los mecanismos de utilización de CO₂ exógeno en DAs.

4. Investigar la influencia de viscosidad y reología en la transferencia de masa gas líquido de CO₂, en el contexto de disolver CO₂ en substratos digeridos anaeróbicamente.
5. Examinar las corrientes de CO₂ disponibles en los sectores del agua y residuos orgánicos que podrían ser bioconvertidas a CH₄ en procesos anaeróbicos.
6. Examinar la factibilidad, beneficios potenciales y aspectos prácticos (sistemas y puntos de inyección) de implementar bioconversión de CO₂ en DAs de gran escala.

1.5.3. Estructura de la tesis

Cada sección de esta investigación ha sido estructurada en un capítulo basado en artículos publicados, enviados para publicación o en preparación, los cuales constituyen esta tesis. Todos los capítulos han sido escritos por la primera autora, Yadira Bajón Fernández, y editados por la catedrática Elise Cartmell y Dr. Ana Soares; a excepción del Capítulo 6 que ha sido editado por el catedrático Bruce Jefferson. Todo el trabajo experimental ha sido llevado a cabo por Yadira Bajón Fernández, a excepción de las últimas etapas del protocolo experimental de hibridación fluorescente in situ (fluorescence in situ hybridisation – FISH), para las cuales las muestras fueron enviadas a un laboratorio externo, y de parte de los test de transferencia de masa del Capítulo 6, donde el sexto autor contribuyó en el laboratorio. Parte de esta tesis de doctorado ha sido completada durante una estancia de tres meses de Yadira Bajón Fernández en la Universidad Técnica de Múnich (TUM), Alemania, en cumplimiento de los requerimientos para la obtención del título de doctor internacional.

La conexión entre los capítulos de la tesis está esquematizada en la Figura 1.1 (Figure 1.1). El Capítulo 2 constituye una revisión bibliográfica de la necesidad y los medios para reducir la creciente emisión de CO₂ en los sectores del agua y residuos orgánicos. El enriquecimiento de DAs con CO₂ para su bioconversión a CH₄ ha sido identificada como una opción adecuada para minimizar la huella de carbono. Por lo tanto, el estado de la técnica, los mecanismos de utilización de CO₂ y las áreas donde se requería conocimiento adicional en relación con el enriquecimiento de DAs con CO₂ han sido identificadas y discutidas. El Capítulo 2 ha sido enviado a Bioresource Technology – *Bajón Fernández, Y., Soares, A., Koch, K., Vale, P. and Cartmell, E., A review of re-use and valorisation of by-product CO₂ gas streams from anaerobic digestion sites* –y está en revisión.

El Capítulo 3 es un estudio de viabilidad realizado para determinar los beneficios potenciales de inyectar CO₂ exógeno en DAs que tratan lodos de depuradora y residuos alimentarios. El capítulo 3 ha sido publicado en Bioresource Technology - *Bajón Fernández, Y., Soares, A., Villa, R., Vale, P. and Cartmell, E., (2014). Carbon capture and biogas*

enhancement by carbon dioxide enrichment of anaerobic digesters treating sewage sludge or food waste. Bioresource Technology, 159, 1-7. El resultado más notable de este capítulo ha sido el hallazgo de una respuesta a la inyección de CO₂ dependiente del sustrato tratado en DAs, con mayores beneficios en la captura de carbono y la producción de CH₄ encontrados para lodos de depuradora que para residuos alimentarios. La concentración de amoníaco en los DAs que tratan residuos alimentarios se encontraba dentro de los niveles tóxicos para arqueas metanogénicas acetoclásticas. Por lo tanto, se planteó la hipótesis de que el mayor beneficio obtenido en DAs tratando lodos de depuradora fuera debido a un aumento de actividad de las arqueas metanogénicas acetoclásticas derivada de la inyección de CO₂.

El Capítulo 4 estudia los mecanismos por los que CO₂ adicional puede ser utilizado en procesos anaerobios, considerando rutas de asimilación químicas y biológicas. El Capítulo 4 ha sido enviado a Water Research - *Bajón Fernández, Y., Soares, A., Vale, P. and Cartmell, E. Enhancing the anaerobic digestion process through carbon dioxide enrichment: mechanisms of utilisation* – y está en revisión. En este capítulo se ha confirmado la diferencia en comunidades metanogénicas presentes en diferentes sustratos, siendo las arqueas metanogénicas acetoclásticas las más abundantes en DAs tratando lodos de depuradora y escasas en residuos alimentarios. Además, ha sido confirmada la hipótesis inicial (inyección de CO₂ favorece la ruta acetoclástica de formación de CH₄), dado que la actividad de *Methanosaetaceae* (arquea metanogénica acetoclástica) fue hasta un 80% mayor en DAs tratando lodos de depuradora sometidos a inyecciones periódicas de CO₂ que en los DAs control. En esta etapa no fue posible clarificar si el aumento de la actividad de *Methanosaetaceae* era debido a un impacto directo del CO₂ en estas arqueas o a una alteración de las etapas previas del proceso de digestión (p. ej. acidogénesis y acetogénesis). Se planteó la hipótesis de que CO₂ exógeno fuera reducido en la ruta Wood-Ljungdahl, formando acetato utilizable como sustrato por las arqueas metanogénicas acetoclásticas.

El Capítulo 5 investiga la posibilidad de implementar bioconversión de CO₂ en DAs a mayor escala, con especial énfasis en la idoneidad de utilizar una columna de burbujas externa para llevar a cabo la transferencia de CO₂ gas líquida requerida. Se incluye también información adicional en relación a los mecanismos de utilización de CO₂ exógeno. El Capítulo 5 está siendo preparado para su envío a la publicación especial “Carbon Neutral” de Water Research - *Bajón Fernández, Y., Green, K., Schuler, K., Soares, A., Vale, P. and Cartmell, E. Biological carbon dioxide utilisation in food waste anaerobic digesters.* En esta etapa se consideró que monitorear la evolución de ácidos grasos volátiles (volatile fatty acids – VFAs) y la concentración de hidrógeno (H₂) en la fase gaseosa de DAs operados en modo continuo, podría contribuir a dilucidar si la inyección de CO₂ altera las etapas iniciales del proceso de digestión, tal y como se planteó en el Capítulo 4. Se ha observado una tendencia

creciente en la concentración de H₂ de un DA a escala piloto enriquecido con CO₂ de forma periódica y su estabilización en un valor 2.5 veces mayor que en el DA control. Este incremento ha sido atribuido a la disolución de CO₂ y a una alteración de la etapa de acetogénesis. El nivel de H₂ no continuó aumentando a pesar de inyectar CO₂ periódicamente, lo que se ha atribuido a su asimilación por la ruta Wood-Ljungdahl y ha respaldado la hipótesis del Capítulo 4. Se ha concluido que el uso de columnas de burbujas para la disolución de CO₂ exógeno en substratos digeridos anaeróbicamente es adecuado. Se ha obtenido una captura significativa de carbono en el DA test inyectado con CO₂ exógeno. Sin embargo, el monitoreo del nivel de CO₂ disuelto ha indicado que tan sólo un *ca.* 25% del valor de solubilidad fue obtenido en la salida de la columna de burbujas usada para inyectar CO₂. Esto ha evidenciado que la transferencia de masa gas líquido es un factor limitante del CO₂ disuelto en DAs y la necesidad de entender la transferencia de masa en fluidos de reología complicada como es el caso de substratos digeridos anaeróbicamente.

El Capítulo 6 está enfocado a cubrir la falta de conocimiento en relación al impacto de viscosidad y reología en la transferencia de masa. Se ha utilizado fluidos sintéticos para desacoplar el impacto de la viscosidad y reología de otras variables. Se han incluido condiciones de operación y reológicas que imitan la transferencia de masa en substratos digeridos anaeróbicamente. El Capítulo 6 ha sido enviado a *Chemical Engineering Journal* - *Bajón Fernández, Y., Cartmell, E., Soares, A., McAdam, E., Vale, P., Darche-Dugaret, C. and Jefferson, B. Gas to liquid mass transfer in viscous fluids* – y está en revisión. Esta investigación ha confirmado que variaciones de viscosidad pueden dificultar la transferencia de masa gas líquido y ha evidenciado que el grado de esta alteración es diferente en fluidos Newtonianos y no-Newtonianos (p. ej. lodos de depuradora). La obstrucción de transferencia de masa ha sido primordialmente asociada con alteraciones en las condiciones hidrodinámicas del sistema y se ha identificado un nuevo régimen de flujo (slug-annular flow). Este hallazgo es de alto valor para el diseño de sistemas de transferencia de masa de gran escala que traten con fluidos de reología complicada, porque evidencia la necesidad de considerar variaciones de viscosidad para evitar una reducción del volumen activo.

El Capítulo 7 evalúa el potencial de implementar bioconversión de CO₂ en DAs en plantas a escala real. Se han identificado los sistemas donde podría implementarse, los medios de implementación y los potenciales beneficios. El Capítulo 6 está siendo preparado para su envío a *Environmental Technology* - *Bajón Fernández, Y., Soares, A., Koch, K., Vale, P. and Cartmell, E. Carbon dioxide enrichment of anaerobic digesters as a non-site carbon revalorisation strategy: considerations and requirements for implementation.*

El Capitulo 8 es la discusión general de la tesis. En el Apéndice 1(Appendix 1) se han incluido imágenes de los reactores construidos y operados en esta investigación.

Durante el curso de este doctorado se han escrito dos propuestas de investigación para la fase 1 y fase 2 del programa *Driving innovation in anaerobic digestion* (DIAD) llevado a cabo por WRAP. Las dos propuestas fueron dotadas con una contribución económica de aproximadamente 25.000 y 75.000 libras, respectivamente. Las propuestas y los informes describiendo los hallazgos de las investigaciones han sido escritos por Yadira Bajón Fernández y editados por la catedrática Elise Cartmell y Dr. Ana Soares. El trabajo experimental correspondiente a ambas propuestas ha sido llevado a cabo por Yadira Bajón Fernández. Se puede acceder al informe correspondiente a la fase 1 a través de la página web de WRAP (<http://www.wrap.org.uk/node/16676>) y el correspondiente a la fase 2 está en revisión y estará disponible a corto plazo.

Además, durante el transcurso de esta investigación Yadira Bajón Fernández ha diseñado, comprado y operado un sistema macerador-bomba para que sea incorporado en el DA de 1.5 m³ disponible en Cranfield University. También se han proporcionado a WRK Design and Services Ltd. (Birmingham, Reino Unido) las especificaciones para el diseño de un sistema de inyección de CO₂ a incorporar al mencionado DA de 1.5 m³. Imágenes e información de los sistemas se encuentran recogidas en los Appendix 1 y Appendix 2, respectivamente.

CHAPTER 2

LITERATURE REVIEW – A REVIEW OF RE-USE AND VALORISATION OF BY-PRODUCT CO₂ GAS STREAMS FROM ANAEROBIC DIGESTION SITES

2. A REVIEW OF RE-USE AND VALORISATION OF BY-PRODUCT CO₂ GAS STREAMS FROM ANAEROBIC DIGESTION SITES

HIGHLIGHTS

- Atmospheric CO₂ has risen from 280 ppm in the preindustrial era to *ca.* 400 ppm today.
- Emissions of GHG from water sector account for 3-10% of total world emissions.
- CO₂ from European municipal solid waste ADs estimated at *ca.* 980,000 t·annum⁻¹.
- Bioconversion of CO₂ is one of the feasible solutions for on-site CO₂ revalorisation.
- Research needed to increase readiness for implementation of CO₂ bioconversion in ADs.

ABSTRACT

Concentration of carbon dioxide (CO₂) in the atmosphere has increased from 280 ppm in the preindustrial era to *ca.* 400 ppm in 2014, necessitating development of carbon management strategies if greenhouse gas (GHG) targets are to be met. The energy sector is the main contributor to GHG emissions, with the decarbonisation of energy supplies an international priority. Anaerobic digestion (AD) supports the production of renewable energy. However, its increased implementation raises emissions of biogenic CO₂ with biogas, which is estimated at *ca.* 980,000 t·annum⁻¹ from European municipal solid waste ADs. Bioconversion of CO₂ into methane in ADs is a potential on-site CO₂ revalorisation strategy. This review examines the carbon benefits of this strategy, the mechanisms of CO₂ utilisation and the need for further research to help support future implementation.

KEYWORDS

Greenhouse gas, carbon dioxide, revalorisation, anaerobic digestion, bioconversion.

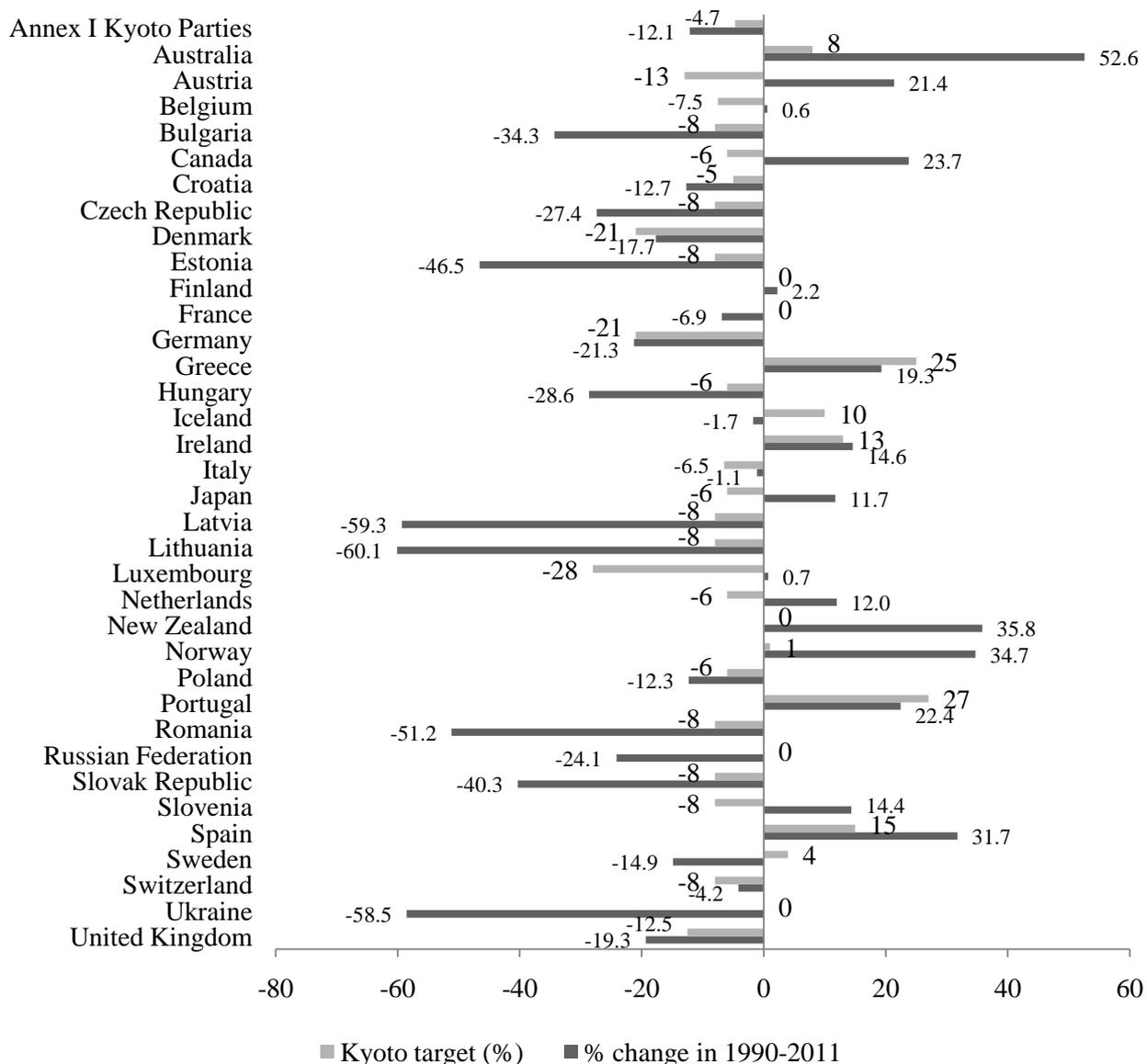
2.1. MOTIVATION AND TARGETS FOR GHG REDUCTION

Carbon dioxide (CO₂) concentration in the atmosphere has increased from 280 ppm in the preindustrial era to *ca.* 400 ppm in 2014 (NOAA, 2014), with an average rise of 2 ppm per year during the last decade (IEA, 2013a). This rise has been attributed to anthropogenic emissions (IEA, 2013a; United Nations, 1998), leading to legislation targeting greenhouse gas (GHG) reduction both at national and international levels. The Kyoto Protocol, signed in 1997

and effective since 2005, was the first treaty stating legally binding GHG reduction targets. It was divided into two commitment periods, 2008-2012 and 2013-2020, stating a GHG reduction target of 4.7% for industrialised countries as a group for the first phase. A combined GHG reduction target of 8% was stated for European countries (EU-15), which was further distributed in a burn-sharing agreement (Figure 2.1). The Kyoto Protocol has in turn led to the development of national legislation in each specific country, aimed at compiling or tightening the stated targets. For example, a GHG reduction target of 12.5% when compared against the 1990 baseline was agreed for the UK as part of the Kyoto Protocol, while an additional legally binding GHG target was set by the Climate Change Act 2008, which stated a 80% reduction below base levels by 2050 (34% by 2020).

Fuel combustion related CO₂ emissions for countries with emission targets in the first commitment period (Annex I Kyoto Parties) reduced from 8,778 to 7,714 megatons CO₂ (MtCO₂) between 1990 and 2011 (Figure 2.2 (a)) (IEA, 2013a), which implied a 12.1% reduction and hence compliance with the 4.7% target. The UK CO₂ emissions were reduced by 19.3% compared to the baseline by 2011 (IEA, 2013a), with a drop from 549.3 MtCO₂ in 1990 to 443.0 MtCO₂. Despite these positive trends, there is a need for further GHG mitigation efforts, since part of the goals for the Kyoto Protocol first commitment period were achieved by utilising emissions trading schemes (EEA, 2012) or as a consequence of the global economic recession (IEA, 2013a), rather than due to operation of industrial processes in a more sustainable manner. Furthermore, equivalent world CO₂ emissions experienced an increase of 49.3% for the same period, increasing from 20,989 to 31,342 MtCO₂ (Figure 2.2 (b)) (IEA, 2013a), which was attributed to a combined growth in population (32%) and in per capita gross domestic product (GDP) (48%) (IEA, 2013a). The energy sector is the main contributor to GHG emissions, being responsible for 72% of the world GHG emissions in 2011 (WRI, 2014). The World Energy Outlook estimated, in its New Policies Scenario, that global electricity demand will increase by 70% over 2010 levels by 2035 (IEA, 2012), with CO₂ emissions from combustion of fossil fuel reaching 37,200 MtCO₂ even when the announced GHG mitigation commitments are implemented (IEA, 2013b). This would in turn lead to a temperature increase of 3.6°C, requiring stringent measurements to be implemented if the target of maintaining global temperature increases below 2°C over preindustrial levels is to be met (IEA, 2013a). As a response, decarbonisation of energy supply is identified as one of the most pressing needs to improve carbon footprint and mitigate climate change (DECC, 2012a). Carbon intensity of energy supply was reduced by 8.6% for Annex I Kyoto Parties between 1990 and 2011 (Figure 2.2 (a)) (IEA, 2013a). However, this parameter remained constant at a world level for the same period (*ca.*57.1 tCO₂·TJ⁻¹ (IEA, 2013a)) (Figure 2.2 (b)), evidencing that sustainable energy generation has not yet been achieved at a global level. The

share of energy with fossil fuel origin has indeed remained relatively constant over the last 40 years, constituting over 80% of the world energy supply (IEA, 2013a).



Note: Annex I Kyoto Parties includes Australia, Austria, Belgium, Bulgaria, Canada, Croatia, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Japan, Latvia, Liechtenstein, Lithuania, Luxembourg, Monaco, the Netherlands, New Zealand, Norway, Poland, Portugal, Romania, Russian Federation, the Slovak (IEA, 2013a).

Figure 2.1. Kyoto GHG reduction targets for the 2008-2012 commitment period and percentage change in CO₂ emissions from fuel combustion between 1990 and 2011.

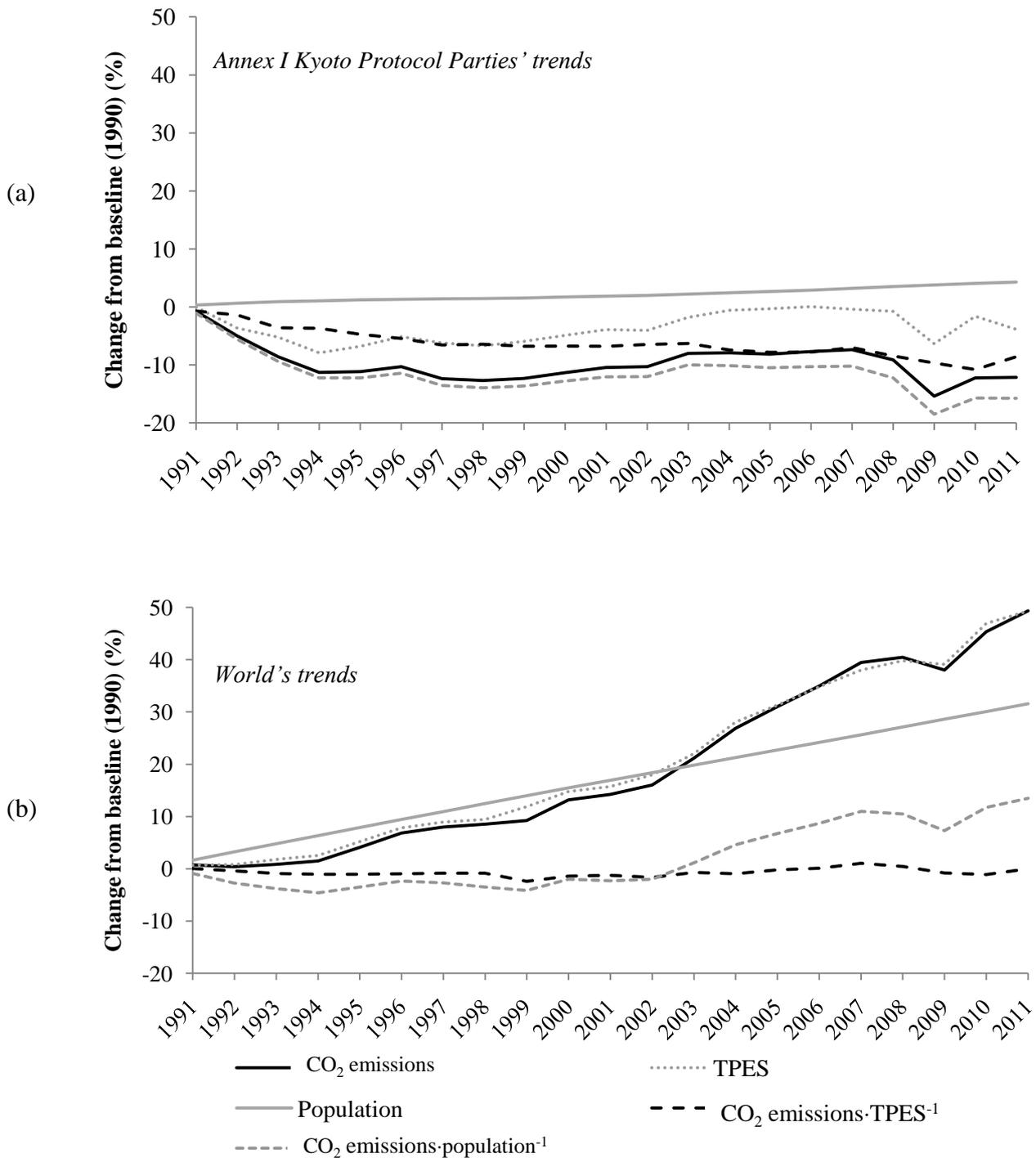


Figure 2.2. (a) Annex I Kyoto Protocol Parties' and (b) world's trends in CO₂ emissions from fuel combustion, energy demand and population growth for the period 1990 to 2011. TPES: total primary energy supply. Source: (IEA, 2013a).

Energy consumption of the water sector presents an increasing energy demand, contrary to GHG legislation aims. Electricity demand of this sector accounts for 4% of total national consumption in the USA and for *ca.* 3% in UK (Rothausen and Conway, 2011). Energy usage for operation of water and wastewater treatment sites at a UK level increased from 8,290 GWh in 2007/2008 (Water UK, 2008a) to 9,016 GWh in 2010/2011 (Water UK,

2012), in part as a consequence of the tightened quality standards resulting from the endorsement of the Water Framework Directive in 2000 (European Commission, 2009; Georges et al., 2009; Severn Trent Water, 2010; Water UK, 2009). Emissions of CO₂ are expected to increase by over 110,000 tonnes per year (Georges et al., 2009) from energy demand and emissions from additional processes needed to upgrade more wastewater treatment plants to meet the most recent quality standards. As a result, the potential of the water sector to contribute towards GHG emissions mitigation has been highlighted at national (e.g. UK (DEFRA, 2008), USA (USEPA, 2012)) and global level (McGuckin et al., 2013) and five key strategies that the water industry could implement to mitigate its carbon footprint have been outlined by the Environment Agency (Georges et al., 2009). Solutions based both on pollution prevention (*source control strategies* and *renewable energy production*) and on pollution reduction (*least-carbon end-of-pipe technologies*, *higher operational efficiencies* or *lower energy treatment processes*) have been proposed (Georges et al., 2009). This has in turn led to a surge in research aimed at capturing emitted CO₂ and at developing treatment technologies with a low energy demand and increased renewable energy production. An important strategy towards mitigating GHG emissions is the implementation of anaerobic digestion (AD), which is regarded favourably due to the production of renewable energy and the stabilisation of waste into a digestate that can be used as a fertilizer, which offsets GHG emissions from usage of energy with fossil fuel origin (DEFRA, 2007). This review addresses options for mitigating the increasing carbon footprint of the water sector, with special focus on on-site carbon revalorisation strategies applicable to CO₂ emitted with biogas, which is rising as a result of the increased use of AD.

2.2. IDENTIFICATION AND QUANTIFICATION OF CO₂ EMISSIONS FROM THE WATER AND ORGANIC WASTE SECTORS

Emissions of GHG from the water sector have been estimated to account for 3-10% of total world emissions (McGuckin et al., 2013), which evidences the potential for this sector to contribute towards GHG mitigation (McGuckin et al., 2013; USEPA, 2012). Biogenic CO₂ emissions are not accounted for in carbon inventories. However, its reduction is considered as a negative release to be deducted from overall carbon emissions, which makes carbon management strategies for biogenic CO₂ suitable to mitigate carbon footprint (Byrns et al., 2013). A detailed quantification of biogenic CO₂ emissions per source in the water sector has not yet been reported at world or European level. However, more detailed data are available at national scale. To illustrate, emissions of GHG from the UK water sector were estimated to be over 5 MtCO₂ equivalents (MtCO₂e) during 2010-2011 (Water UK, 2012), which accounts for ca. 1% of the total national GHG emissions (Water UK, 2009). Approximately 56% of the emissions were attributable to wastewater treatment (2005-2006 data (DEFRA, 2008)).

Biogenic CO₂ emissions resulting from UK wastewater treatment have in turn been estimated at 2 MtCO₂ per annum (Byrns et al., 2013), which if reduced would contribute to reduce the sector's carbon footprint. Specific sources of biogenic CO₂ emissions were previously quantified by Byrns et al., (2013) at a UK level (Table 2.1). Aerobic wastewater treatment was identified as the main source of CO₂, with a contribution of 1-1.1 MtCO₂ per annum from activated sludge or biological filters. Emissions of CO₂ with the biogas generated in ADs were estimated at 0.27 MtCO₂ per annum (Byrns et al., 2013), when considering a total sludge production of 1,762,000 tonnes as dry solids (Water UK, 2008b) of which 66% is anaerobically digested. Combustion of methane (CH₄) from biogas in combined heat and power (CHP) engines or flares was quantified as 0.5 MtCO₂ per annum. Energy recovery by combustion of sludge was estimated responsible for the annual emission of 0.26 MtCO₂. Considering these figures, the contribution of aerobic wastewater treatment towards on-site biogenic CO₂ emissions is three to four times higher than that of sludge incineration or of CO₂ confined in biogas produced by ADs. However, biogas CO₂ was identified as the direct emission most easily available to be recovered within the wastewater flowsheet (Byrns et al., 2013), due to a concentration up to 40 times higher than the CO₂ generated in aerobic processes (Table 2.1). Furthermore, the point source nature of this stream would reduce the costs for capture, when compared with emissions from open systems such as activated sludge. The development of carbon management strategies for biogas derived CO₂ (CO₂ inherent in biogas and from combustion of CH₄) would imply a *ca.* 38% reduction in the release of biogenic CO₂ from the UK water sector (0.77 MtCO₂ per annum) (Table 2.1). This potential for avoidance of CO₂ emissions in the water sector relates to current AD infrastructure and will further increase as biogenic CO₂ emissions rise in response to the higher implementation of this technology. A total of 8,960 ADs were operational in Europe in 2010, of which 6,800 were located in Germany (C2ES, 2011).

Table 2.1. Estimated biogenic CO₂ emissions in UK from wastewater treatment. Source: Byrns et al., (2013).

	CO ₂ production (MtCO ₂ ·annum ⁻¹)	CO ₂ concentration (% v/v)	confined stream
Aerobic treatment	1-1.1	0.8	NO
Biogas of anaerobic digestion	0.27	35	YES
Combustion of biogas (CHP or flares)	0.5	8-15	YES
Incineration of sludge	0.26	^(a)	YES

^(a) Depending on the amount of air used for incineration.

The benefits of developing carbon management strategies for AD derived CO₂ are even higher when considering the increasing implementation of AD for treating alternative substrates (e.g. manure, community waste). To illustrate, the number of ADs treating

municipal solid waste in Europe increased from 53 in 1999 (De Baere, 2006) to 171 in 2010 (De Baere and Mattheeuws, 2008), which implied an increase in treating capacity from 1,037,000 to 5,204,000 t·annum⁻¹ (sites with capacity over 3,000 t·annum⁻¹ considered (De Baere, 2006)). Attending to this capacity, emissions of biogas CO₂ from European ADs treating municipal solid waste can be estimated at *ca.* 0.98 MtCO₂ per annum if a biogas yield of 300 m³ biogas·tonne⁻¹ (Georges et al., 2009) with 60% CH₄ concentration is considered. Within the UK, the number of AD sites outside of the water sector increased from two in 2005 to 140 in middle 2014 (NNFCC, 2014; WRAP, 2012). Biogenic CO₂ emissions from ADs, both within and outside of the water sector, are expected to further increase because of the government support towards the use of AD (DEFRA, 2007) and the UK legal requirement to reduce by 2020 the biodegradable waste derived to landfill to 35% of 1995 levels (CIWM, 2010). Table 2.2 compiles information of the current AD infrastructure in the UK outside of the water sector, where size is considered as per electricity generation capacity and CO₂ emissions have been estimated based on an electrical yield of 2.1 KWhe·m⁻³ biogas (energy yield of 6.1 KWhe·m⁻³ biogas with 35% CHP electrical efficiency) and a biogas composition of 65% CH₄ and 35% CO₂ (0.63 kg CO₂·m⁻³ biogas at standard conditions). Emissions of CO₂ with biogas from ADs outside of the water sector are hence estimated at 0.31 MtCO₂ per annum (industrial sites not accounted), with ADs treating community waste accounting for 66% (Table 2.2). Sequestration or valorisation of biogas CO₂ would hence further increase the carbon benefits of the AD process, contributing towards energy supply decarbonisation. This in turn would help mitigating the negative trend in GHG emissions of the water sector and improving the carbon footprint of the organic waste sector.

Table 2.2. Number of AD sites outside of the water sector currently in UK classified per electrical production capacity and associated CO₂ emissions contained in the biogas produced. Industrial ADs have not been considered. Data extracted from NNFCC, (2014).

Electrical capacity range (kWe)	Agricultural waste			Community waste		
	Number of sites ^(a)	Combined capacity (kWe)	CO ₂ produced with biogas (tonnes CO ₂ ·year ⁻¹)	Number of sites ^(b)	Combined capacity (kWe)	CO ₂ produced with biogas (tonnes CO ₂ ·year ⁻¹)
kWe ≤ 250	20	1,563	4,035	4	324	837
250 < kWe ≤ 500	18	8,248	21,298	11	5,059	13,063
500 < kWe ≤ 1000	7	6,500	16,784	7	5,848	15,101
1000 < kWe ≤ 2000	11	16,051	41,447	19	28,960	74,781
2000 < kWe ≤ 3000	3	7,700	19,883	7	16,600	42,865
kWe ≥ 3000	0	0	0	5	21,300	55,001

^(a) One AD site registered as operational but without reported electricity production capacity has not been accounted for.

^(b) Two AD sites registered as operational but without reported electricity production capacity have not been accounted for.

2.3. OPTIONS FOR IMPLEMENTATION OF CARBON CAPTURE AND STORAGE OR REVALORISATION STRATEGIES IN THE WATER AND ORGANIC WASTE SECTORS

Carbon capture and storage (CCS) in geological or oceanic reservoirs is proposed as one of the most cost effective technologies for CO₂ management both at UK and worldwide level. Its economic feasibility is further enhanced when coupled with enhanced gas or oil recovery (DECC, 2012a). The International Energy Agency has estimated that CCS has the potential to provide a sixth of the carbon emission reduction targets required by 2050 (IEA, 2014). The feasibility of a commercial-scale implementation of CCS and the availability of storage capacity in the UK seabed have been evidenced; however, widespread implementation has not yet been achieved (DECC, 2012a). The need to develop a pipeline infrastructure to transport CO₂ to the final reservoirs is the most pressing challenge to be addressed before an extensive application can be considered (DECC, 2012a, 2012b). The viability of implementing this required pipe layout for transporting to reservoirs the CO₂ emitted in the energy sector has been investigated and certified as economically feasible. The existence of clusters of centralised emission sources (power and industrial plants) close to a potential storage site (DECC, 2012a) has raised the possibility of installing a shared CO₂ transportation infrastructure, which would benefit from the pipeline's economy of scale and hence reduce overall transport costs. Figure 2.3 (a) illustrates the location of the largest CO₂ sources in the UK (power and industrial plants) and the potential storage sites as identified by the Energy Technologies Institute and reported by DECC (2012a). Clear aggregations of CO₂ emitters are located in Scotland, Yorkshire, Teesside and the Irish Sea. The existence of clusters of large stationary sources of GHG is also evident in other developed countries (e.g. USA (USEPA, 2014)). However, the performance of a similar preliminary assessment comparing the location of AD sites and potential CO₂ reservoirs in the UK (Figure 2.3 (b)), evidences the particular transportation challenge to be addressed in sectors like water or organic waste, where individual sites are scattered. The development of a common pipeline infrastructure for handling CO₂ emissions of the water and organic waste sectors appears unrealistic and the varied size of the sites hinders that these can benefit from the pipelines economy of scale. Table 2.2 compiles information of the current UK AD infrastructure outside of the water sector, providing evidence of the different production capacity of AD plants. To illustrate, the 53 AD sites registered in UK treating community waste in 2014, are distributed with 8%, 21%, 13%, 36%, 13% and 9% having an electrical production capacity below 250 kWe, 250 <kWe ≤ 500, 500 <kWe ≤ 1000, 1000 <kWe ≤ 2000, 2000 <kWe ≤ 3000 and kWe ≥ 3000, respectively. Relative costs for CO₂ transportation are higher when dealing with emissions from small sources and it is estimated that constructing pipelines for

power stations with a capacity below 300 MW would increase cost for transporting CO₂ by a factor of 5 (DECC, 2012b). The varied size and scattered location of AD sites would likely require a tanker based transport approach for the estimated 575,000 tonnes of CO₂ emitted per annum from ADs treating agricultural, community waste and sewage sludge, which will not prove feasible in the long term. It is not within the scope of this review to further address the current status of CCS in geological or oceanic reservoirs, however, further information can be found in online references (Global CCS Institute, 2014; SCCS, 2014), which evidence that the maturity of CCS in full-scale projects and the growth in the installation of AD sites are completely decoupled.

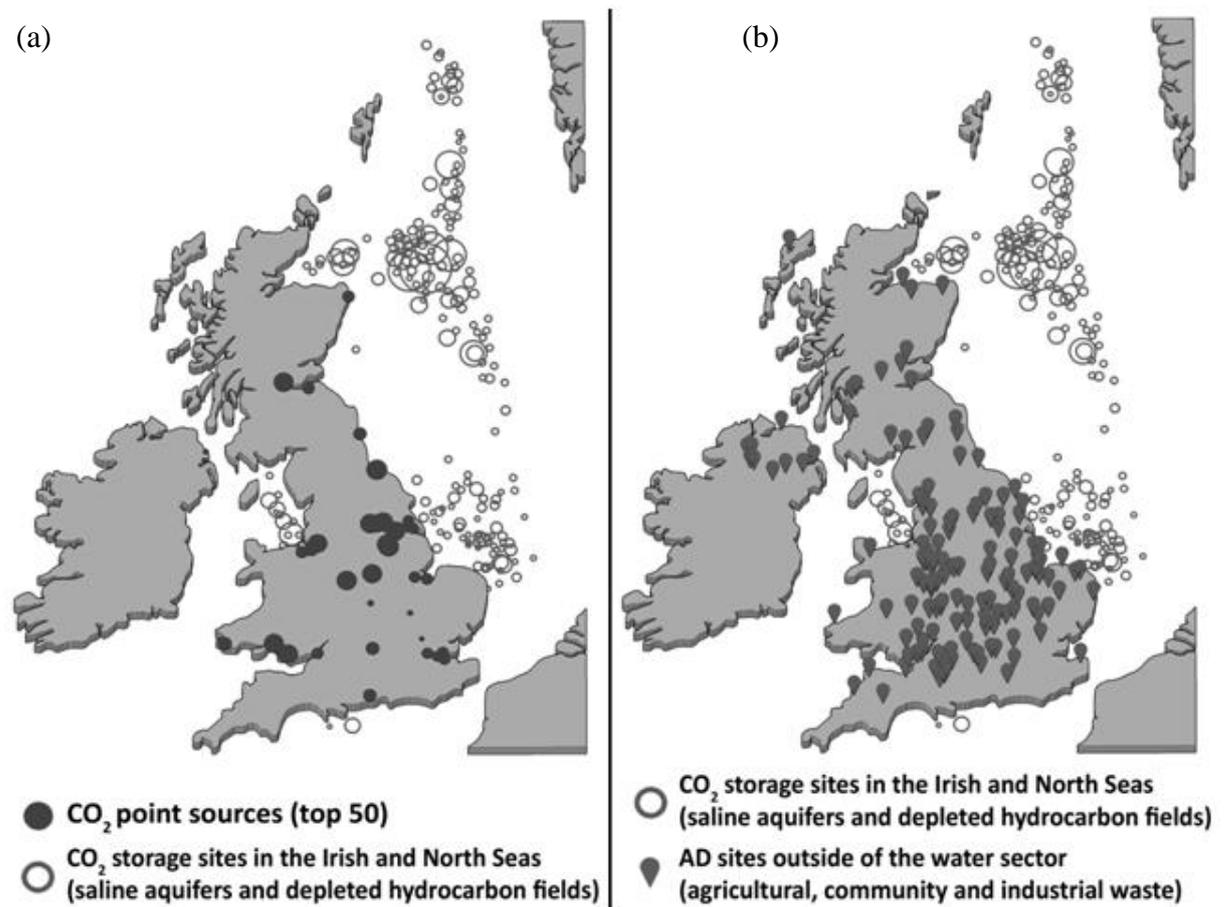


Figure 2.3. (a) Main CO₂ emitters (power and industrial plants) in the UK and location of potential CO₂ storage sites, adapted from DECC (2012a). (b) AD sites in UK outside of the water sector and location of potential CO₂ storage sites. Adapted from NNFCC (2014a) and DECC (2012a).

Alternative strategies for management of biogas CO₂ emissions are hence required. Particularly appealing are those that promote on-site CO₂ revalorisation as opposed to off-site storage, since they avoid transport and compression of CO₂. Potential opportunities for biogenic carbon sequestration have been discussed by leading practitioners of the sector, such

as in the technology strategy board in 2011 (NERC, 2011), where the carbon sequestration potential of biochar and algae, amongst others, were discussed. The need for further basic research (proof of concept) and for increasing technology readiness before a widespread implementation can be considered were stated. Byrns et al. (2013) further studied on-site carbon management alternatives and identified utilisation of CO₂ for growth of algae and addition of CO₂ to anaerobic processes for its bioconversion to CH₄ by methanogenic *Archaea* as the most economically feasible options. Both alternatives would have a double benefit in GHG emissions, since they imply a direct uptake of CO₂ and an increase in renewable energy production associated with the digestion of the grown algae or the bioconversion of CO₂ to CH₄. This increase in renewable energy production places these carbon management strategies in a leading position when compared with conventional CCS alternatives, since they aim for CO₂ revalorisation as opposed to storage or sequestration. Besides, an increase in renewable energy production would contribute towards the decoupling of energy demand from combustion of fossil fuels, main source of GHG anthropogenic emissions (IEA, 2013a). Furthermore, it is in line with the government commitments to expand energy recovery from waste through AD (DEFRA, 2007) and to provide 15% of the UK energy and 10% of the energy used in the transport sector from renewable sources by 2020 (European Union and Council, 2009). When CO₂ utilisation for algae growth and bioconversion to CH₄ are compared, the later strategy appears particularly attractive because of its anticipated simplicity for implementation. Minimal capital costs are envisaged if injection of CO₂ in ADs for its bioconversion to CH₄ could be performed through already installed gas mixing systems, which would constitute a significant advantage for potential full-scale implementation (Bajón Fernández et al., 2012). Furthermore, carbon management through bioconversion to CH₄ has the added benefit of intermediate products (e.g. algae) not being generated. Previous studies investigating bioconversion of CO₂ to CH₄ in anaerobic processes, possible mechanisms of CO₂ utilisation and potential impacts in operation of ADs are reviewed in the following section.

2.4. STATE OF THE ART OF BIOCONVERSION OF CO₂ IN ADs AS AN ON-SITE CARBON MANAGEMENT STRATEGY

2.4.1. Previous evidence

Bioconversion of CO₂ to CH₄ has been studied for different systems and applications, including, within others, electrochemical bioreactors (Jeon et al., 2009), fixed bioreactors enriched with hydrogen (H₂) (Lee et al., 2012), bioconversion in deep subsurface aquifers (Leu et al., 2011) or biogas upgrading units (Martin et al., 2013). Investigations considering onsite CO₂ bioconversion to CH₄ as a GHG management strategy in real anaerobic matrices

and without addition of exogenous H₂ are, however, scarce (Table 2.3). Studies addressing bioconversion of CO₂ into CH₄ in a sewage sludge matrix were first reported in the 1990's. Sato and Ochi (1994) measured associated increases in CH₄ production when CO₂ concentration in the headspace of ADs was controlled both in laboratory and pilot scale units. Increases of up to 30% in specific CH₄ yield were reported when maintaining CO₂ concentrations of 60% v/v in semicontinuous operating ADs treating waste activated sludge. The extent of CO₂ uptake or the mechanisms by which this could be biotransformed into CH₄ were not thoroughly investigated. Alimahmoodi and Mulligan, (2008) explored the impact of enriching with CO₂ the influent to a laboratory scale upflow anaerobic sludge blanket (UASB) reactor, estimating that 69-86% of the CO₂ dissolved could be utilised in the process. Salomoni et al., (2011) measured a 25% increased specific CH₄ yield when continuously injecting CO₂ into the first stage of a two-phase anaerobic digestion (TPAD) process. A CO₂ uptake of up to 46% of that injected was estimated. Bioconversion of CO₂ to CH₄ in ADs has not yet been given significant attention according to the scarcity of published literature employing real matrices. However, the references available have evidenced that a significant benefit in CH₄ production and CO₂ uptake can be achieved, being CO₂ bioconversion identified as one of the most promising strategies for reducing the water sector carbon footprint (Byrns et al., 2013).

Table 2.3. References addressing CO₂ bioconversion in anaerobic systems.

Substrate treated	Anaerobic system used	Scale of the reactor	Operational conditions	CO ₂ injection	y _{CO₂} ^(c)	Increase in CH ₄ yield or production rate	Increase in CH ₄ content of the biogas	CO ₂ uptake	Mechanism of CO ₂ utilisation suggested	Ref.
Waste activated sludge		6 L	T = 35°C; HRT ^(b) = 10.8 d; semicontinuous operation of the ADs	Daily CO ₂ enrichment with gas mixing line		30% increased specific CH ₄ yield (m ³ CH ₄ · kg VS ⁻¹) with CO ₂ concentrations of 60% v/v				(Sato and Ochi, 1994)
Synthetic solutions	UASB reactor	1 L working volume	T = 35°C	Dissolved in the influent to unit, which was treated with KOH to maximise the dissolution	1	CH ₄ rate increased from ca. 3.5 g COD·d ⁻¹ to ca. 7 g COD·d ⁻¹ for a system with acetic acid as solely VFA		69-86%	Hydrogenotrophic methanogenesis	(Alimahmoodi and Mulligan, 2008)
Stabilised sludge	TPAD	Ten units of 1.8 L working volume per phase	T = 25°C in first phase and T = 42°C in second phase; HRT for each phase = 6 days	Continuous injection of 1.5 L CO ₂ ·d ⁻¹				Up to 40% of input	Transformation of CO ₂ into short-chain VFAs by Wood-Ljungdahl pathway	(Francioso et al., 2010)
Synthetic solution, simulating stream from EOR ^(a) process	Two-phase reactor	2 L	T = 35°C, pH of 2.5-4.5	CO ₂ was present in the initial waste stream, without additional CO ₂ being injected				Up to 98% CO ₂ removal	Hydrogenotrophic methanogenesis	(Alimahmoodi and Mulligan, 2011)

Substrate treated	Anaerobic system used	Scale of the reactor	Operational conditions	CO ₂ injection	y _{CO₂} ^(c)	Increase in CH ₄ yield or production rate	Increase in CH ₄ content of the biogas	CO ₂ uptake	Mechanism of CO ₂ utilisation suggested	Ref.
Mixed primary and secondary sludge	TPAD	580 and 630 L working volume for first and second phase, respectively	T = 25°C in first phase and T = 42°C in second phase; HRT first stage = 8.3 d and HRT second stage = 9.0 d	Continuous CO ₂ injection in bottom of the first phase via internal tubing; CO ₂ load = 0.49 m ³ ·d ⁻¹ (0.035 m ³ CO ₂ ·h ⁻¹ ·m ⁻³ working volume)	1	25% increased specific CH ₄ yield (0.279 to 0.35 m ³ CH ₄ ·kg VSS ⁻¹)	64% average CH ₄ content vs. 60% of the control	46% of the input (229 L·d ⁻¹)	Transformation of CO ₂ into short-chain VFAs by Wood-Ljungdahl pathway	(Salomoni et al., 2011)
Food waste or sewage sludge	Single phase AD	0.7 L working volume	T = 38°C, batch mode	Saturation at start of batch process via Pyrex diffusers	0.3, 0.6, 0.9	<ul style="list-style-type: none"> • Food waste ADs: Up to 13% increased CH₄ yield • Sewage sludge: 2.0-2.4 fold increase in CH₄ production during 24 hours following CO₂ injection 	Concentration not altered	<ul style="list-style-type: none"> • Food waste: 3-11% • Sewage sludge: 8-34% 	Transformation of CO ₂ into short-chain VFAs by Wood-Ljungdahl pathway followed by acetoclastic methanogenesis	(Bajón Fernández et al., 2014)

^(a) Enhanced oil recovery.

^(b) Hydraulic retention time.

^(c) CO₂ molar fraction.

2.4.2. Possible mechanisms of CO₂ utilisation in the digestion process

The complexity and multiplicity of reactions involved in ADs means that it is difficult to identify the mechanisms by which additional CO₂ could be utilised and bioconverted to CH₄ (Alimahmoodi and Mulligan, 2008; Bajón Fernández et al., 2014). Potential benefits in carbon footprint associated with bioconversion of CO₂ in anaerobic processes have been reported as an overall term in which all the reactions where CO₂ could be utilised or produced are included, without specifically considering the individual reactions. This section aims to review the mechanisms by which CO₂ could be utilised and to identify impacts other than CH₄ production that CO₂ injection could have in the AD process.

2.4.2.1. Biological bioconversion to CH₄

There is conflicting information in the literature regarding the means by which CO₂ can be bioconverted to CH₄ in an anaerobic process. There are references supporting an increase in hydrogenotrophic or acetoclastic methanogenesis. Alimahmoodi and Mulligan, (2008) studied the impact of bioconversion of CO₂ in the CH₄ production and CO₂ uptake capacity of a laboratory scale UASB reactor. External CO₂ gas was injected in three types of synthetic influents containing a different proportion of short-chain volatile fatty acids (VFAs): (1) acetic acid, (2) acetic, propionic and butyric acids and (3) propionic and butyric acids. In every case a higher CH₄ production rate was observed when the influent was enriched with CO₂. The improvement was more noticeable for the system containing solely acetic acid. This was attributed to additional CO₂ being reduced to CH₄ by hydrogenotrophic methanogens (Figure 2.4), which were considered able to utilise VFAs as an alternative supply of H₂. Francioso et al., (2010) and Salomoni et al., (2011) studied the influence of injecting CO₂ into the first stage of a TPAD process at laboratory and pilot scale, respectively. The 40-46% CO₂ uptake observed in both cases was attributed to an increased carbon assimilation by the Wood-Ljungdahl pathway, which leads to formation of acetate that can in turn be utilised in acetoclastic methanogenesis (Figure 2.4).

The mechanism based on hydrogenotrophic methanogenesis relies on the reduction of CO₂ with H₂ by hydrogenotrophic *Archaea* (Table 2.4). Several studies have successfully exploited hydrogenotrophic methanogenesis in order to produce CH₄ (Lee et al., 2012) or as a means to upgrade biogas within the AD itself (Luo et al., 2012) or in external reactors (Luo and Angelidaki, 2012). Although these studies have an equivalent principle of enhancing methanogenic reactions as when injecting CO₂ into ADs, the results are not directly comparable since in the former additional H₂ was also added. This resulted in a selective enrichment of the methanogenic community with hydrogenotrophic methanogens (Lee et al., 2012; Luo and Angelidaki, 2012). When CO₂ has been stated to encourage acetoclastic methanogenesis, this has generally been attributed to a higher substrate availability (VFAs)

for this reaction to take place as a consequence of homoacetogenesis being encouraged via de Wood-Ljungdahl pathway (Salomoni et al., 2011). Utilisation of CO₂ by the Wood-Ljungdahl pathway consists on its reduction in the methyl branch and carbonyl branch (Figure 2.5) that constitute this mechanism of CO₂ fixation. In the methyl branch one molecule of CO₂ is reduced to formate, which is the precursor for the formation of a methyl group in the shape of methyl-H₄folate after reduction with four more electrons. In the carbonyl branch, one molecule of CO₂ is reduced by two electrons to carbon monoxide. The methyl and carbonyl groups are then condensed with coenzyme A to form acetyl-CoA, which can be assimilated as cellular carbon or converted to acetylphosphate, which in turn leads to acetate formation. Acetoclastic methanogenesis would then be encouraged because of a higher substrate availability. The steps in the Wood-Ljungdahl pathway are summarised in Figure 2.5 and can be further consulted in the review by Ragsdale and Pierce (2008a).

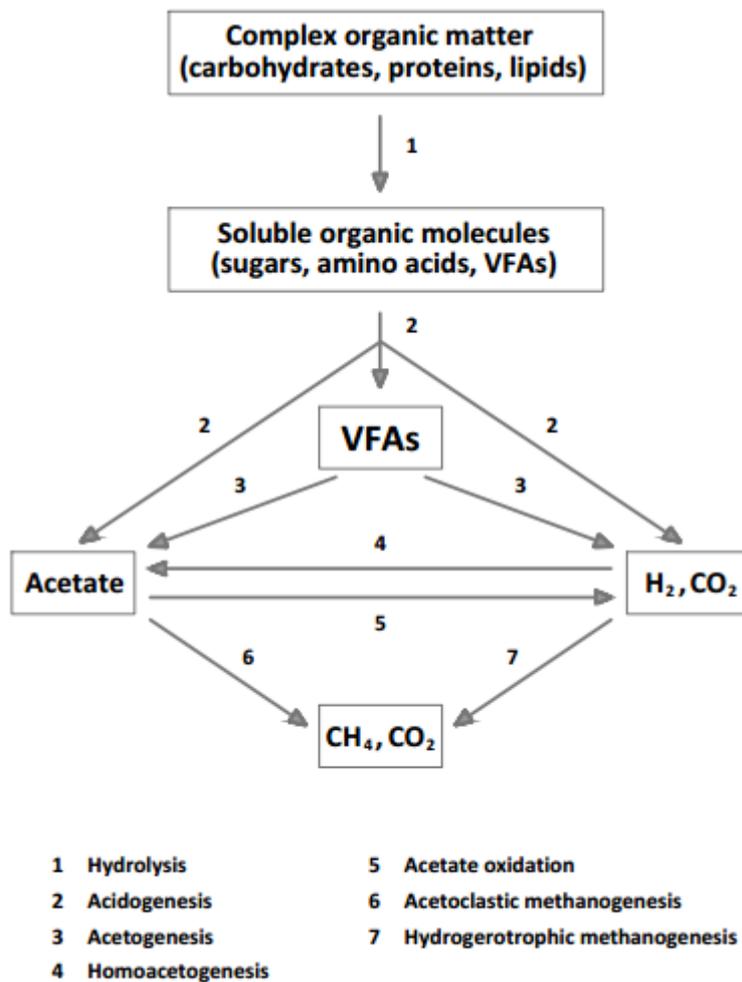


Figure 2.4. Schematic of stages of the anaerobic digestion process. Adapted from Demirel (2014).

When the energy yield of both hydrogenotrophic methanogenesis and homoacetogenic acetogenesis is considered (Table 2.4), the first mechanism of CO₂ utilisation appears more likely since the conversion of CO₂ and H₂ to CH₄ implies a higher energy efficiency for the microorganisms than when converted to acetate (Ragsdale and Pierce, 2008). However, it must be remarked that the study of Salomoni et al., (2011) was performed in a TPAD system where a good phase separation (based on pH, VFA levels and produced gas composition) was in operation. In this scenario no methanogenic activity was expected in the first reactor (acid phase), where the CO₂ was injected, hence it is unlikely that hydrogenotrophic methanogenesis was the reason for the utilisation of additional CO₂. Bajón Fernández et al., (2014) reported a substrate dependant response of ADs to an injection of CO₂, with higher benefits in CH₄ production observed in sewage sludge digesters compared with food waste units. Since the ammonia (NH₃) concentration observed in food waste ADs reached reported toxicity levels for obligate acetoclastic methanogens, the higher benefit in sewage sludge ADs was attributed to an increase in the activity of these methanogens as a response to CO₂ injection. However, in no case was experimental evidence supporting the proposed hypothesis provided (e.g. microbial community analyses). This requires further investigation before the mechanisms of utilisation of exogenous CO₂ in ADs can be elucidated.

Table 2.4. Reactions, reaction enthalpy at standard conditions (ΔH°), entropy at standard conditions (S°), Gibbs free energy at standard conditions (ΔG°) and Gibbs free energy at a temperature of 38°C ($\Delta G_{38^\circ\text{C}}$) for hydrogenotrophic methanogenesis, homoacetogenesis and acetoclastic methanogenesis.

Process	Overall reaction	ΔH° (kJ·mol ⁻¹)	S° (kJ·mol ⁻¹ ·K ⁻¹)	ΔG° (kJ·mol ⁻¹)	$\Delta G_{38^\circ\text{C}}$ (kJ·mol ⁻¹) ^(a)
Hydrogenotrophic methanogenesis	$\text{CO}_2(\text{g}) + 4 \text{H}_2(\text{g}) \rightleftharpoons \text{CH}_4(\text{g}) + 2\text{H}_2\text{O}(\text{l})$	-253.0	-0.410	-130.7	-125.4
Homoacetogenesis	$2 \text{CO}_2(\text{g}) + 4 \text{H}_2(\text{g}) \rightleftharpoons \text{CH}_3\text{COO}^-(\text{aq}) + \text{H}^+(\text{aq}) + 2\text{H}_2\text{O}(\text{l})$	-270.7	-0.724	-54.9	-45.4
Acetoclastic methanogenesis	$\text{CH}_3\text{COO}^-(\text{aq}) + \text{H}^+(\text{aq}) \rightleftharpoons \text{CH}_4(\text{g}) + \text{CO}_2(\text{g})$	+17.7	+0.313	-75.8	-79.7

(a) Gibbs free energy corrected for mesophilic conditions by using Gibbs-Helmholtz equation and considering that enthalpy of formation is constant with temperature.

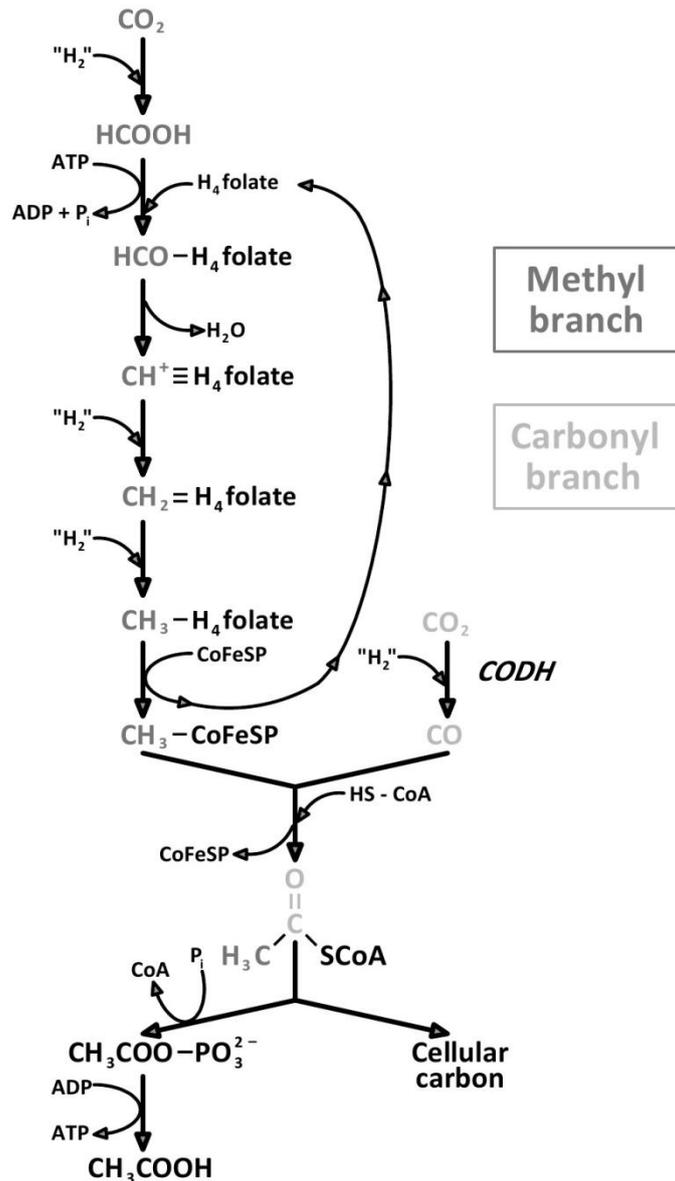


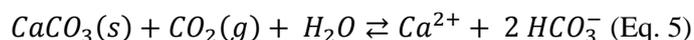
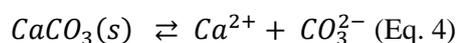
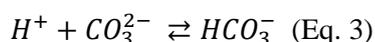
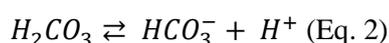
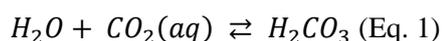
Figure 2.5. Wood-Ljungdahl pathway for homoacetogenic acetogenesis. “H₂” is used to represent requirement of two electrons and two protons. Adapted from Ragsdale and Pierce, (2008b).

The need to understand the mechanisms by which CO_2 can be transformed into CH_4 in ADs is required to understand the types of systems in which CO_2 bioconversion could be applied with positive benefits. Recent studies indicate that acetoclastic methanogenesis can be severely inhibited in ADs where substrate degradation leads to high NH_3 concentrations (e.g. putrescible waste, manure, food waste) (Banks et al., 2012; Demirel, 2014; Koch et al., 2010; Vavilin et al., 2008) resulting in lower contribution to total CH_4 production than the commonly accepted 70% (Conrad, 1999). If coupling of homoacetogenesis by the Wood-Ljungdahl pathway and acetoclastic methanogenesis proves to be the mechanism of CO_2 bioconversion, the inhibition of this pathway in protein rich substrates could be a limit for

implementing CO₂ bioconversion in ADs in sectors like organic waste, unless NH₃ concentration could be reduced. Further information therefore needs to be gathered regarding the CO₂ fate, with emphasis on the possibility of a substrate dependant response based on the predominance of different methanogenic communities. This in turn would contribute to determining the substrates and industrial sectors in which it could be successfully applied and the potential GHG savings achievable if a widespread implementation could be achieved.

2.4.2.2. Chemical utilisation pathways

Part of the CO₂ injected into ADs has the potential to react chemically to form or dissolve carbonated compounds. Calcium carbonate (CaCO₃) can form in anaerobic processes, potentially leading to scaling problems and reduced specific methanogenic activity (Chen et al., 2008). Addition of CO₂ to a media containing calcium (Ca²⁺) has the potential to precipitate CaCO₃ (solubility 15 mg·L⁻¹ at 25°C (Patnaik, 2003)). However, at the pH conditions present in ADs (6.5 - 7.5), an increased solubility of this compound is expected. Dissolution of CO₂ in an aqueous media leads to formation of carbonic acid (Eq. 1), which in turns dissociates to protons (H⁺) and bicarbonates (HCO₃²⁻) (Eq. 2). The majority of the H⁺ formed is then neutralized by reacting with carbonates (CO₃²⁻) (Eq. 3), further increasing the concentration of HCO₃⁻. Part of the HCO₃⁻ can in turn be converted to CO₃²⁻, however, under the pH conditions present in ADs (6.5 - 7.5 pH units), the HCO₃⁻ form is expected to dominate. The overall impact of injecting CO₂ in ADs would hence be a reduction in the CO₃²⁻ concentration, which would displace the CaCO₃ equilibrium to the ionized form (Eq. 4) and contribute to reduce any potential scaling problems associated with this precipitate. The overall reaction can be described as (Eq. 5).



Chemical reaction of CO₂ with aqueous NH₃ leads to formation of ammonium carbonated species, which is industrially exploited in processes aimed at CO₂ capture for carbon mitigation (Bai and Yeh, 1997; Zhuang et al., 2012). Within the possible reaction products, ammonium bicarbonate (NH₄HCO₃) is the most likely to precipitate in a system maintained at pH of *ca.* 8 and mesophilic conditions when a high CO₂/NH₃ ratio is maintained (Bai and Yeh, 1997; Darde et al., 2010; Zhuang et al., 2012). The high solubility of this compound in aqueous solutions (366 g·L⁻¹ at 40°C (Patnaik, 2003)), requires further

investigation to determine if precipitation would occur in ADs with a high NH_3 concentration (e.g. treating food waste or manure).

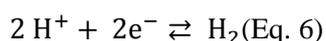
The study of the potential formation of ammonium precipitates is of particular interest when considering the fertiliser properties of NH_4HCO_3 (Zhuang et al., 2012), which could enhance the soil conditioning or fertilizer potential of AD digestates. Furthermore, the high concentrations of NH_3 resulting from hydrolysis of protein rich substrates, leads to toxicity for acetoclastic methanogenesis in ADs (Banks et al., 2011; Chen et al., 2008; Rajagopal et al., 2013). Therefore, processes with potential to reduce free NH_3 levels would be beneficial.

2.4.3. Other potential impacts of CO_2 injection in ADs

Aside from the reported increase in CH_4 production and uptake of CO_2 , which will benefit renewable energy production and carbon footprint, CO_2 could influence the AD process in other manners, as discussed below.

2.4.3.1. Increase in H_2 levels

Injection of CO_2 in ADs has the potential to alter H_2 concentration in several ways. On one hand, utilisation of additional CO_2 by hydrogenotrophic methanogens would imply a consumption of H_2 (Table 2.4). On the other hand, dissolution of CO_2 in aqueous media alters the carbonate equilibrium, increasing the concentration of HCO_3^- and releasing H^+ as per Eq. 2. Due to the low oxidation reduction potential (ORP) characteristic of ADs, typically below -200 mV (Gupta et al., 1994), the H^+ released can react with available electrons to form H_2 (Eq. 6). The overall impact in the AD H_2 concentration would be determined by the extent to which both individual processes take place.



The role of H_2 as an intermediate and electron carrier in several reactions of the AD process makes its concentration influence the relative abundance of other intermediate (e.g. VFAs) or end products (e.g. CH_4) (Cord-Ruwisch et al., 1997). When moderate, an increase in H_2 will be buffered by H_2 consuming metabolisms, i.e. hydrogenotrophic methanogenesis, homoacetogenic acetogenesis or sulphate reducing reactions. However, in cases where the H_2 assimilatory capacity of the system is saturated, several unfavourable effects for the digester operation have been reported, namely inhibition of fermentation reactions by obligate syntrophic bacteria and a shift in the regeneration pathway of the cofactor nicotine adenine dinucleotide (NAD) (Collins and Paskins, 1987; Harper and Pohland, 1986). In the first case, propionic and butyric acid degradation reactions are thermodynamically favoured at H_2 partial pressures below 10^{-4} atm and 10^{-3} atm, respectively (Cord-Ruwisch et al., 1997; Harper and Pohland, 1986; Kidby and Nedwell, 1991). Higher H_2 concentrations will hence hinder degradation of higher VFAs into acetic acid, leading to an accumulation of the former and to

a reduction of pH that can inhibit methanogenesis (Harper and Pohland, 1986). In the second case, a shift in the regeneration pathway of the reduced form of nicotine adenine dinucleotide (NADH) will also contribute to an acidification of the digesting media. As part of the catabolic reactions taking place in AD (Figure 2.6), NAD is reduced to NADH during the oxidative decarboxylation of pyruvic acid to acetyl CoA (Harper and Pohland, 1986). Re-oxidation of NADH for the process to continue is accomplished by the reduction of H^+ and release of H_2 gas (Collins and Paskins, 1987). An increase in the H_2 concentration of an AD hinders this regeneration pathway and necessitates different electron disposal mechanisms. Both the fermentation of acetyl-CoA to butyric acid (Harper and Pohland, 1986) and fermentation of pyruvic acid to propionate, lactate or butyrate as opposed to acetate (Collins and Paskins, 1987) have been reported as alternative NADH regeneration pathways at elevated H_2 concentrations. The slower assimilation of these VFAs can lead to their accumulation and to a pH drop in the system.

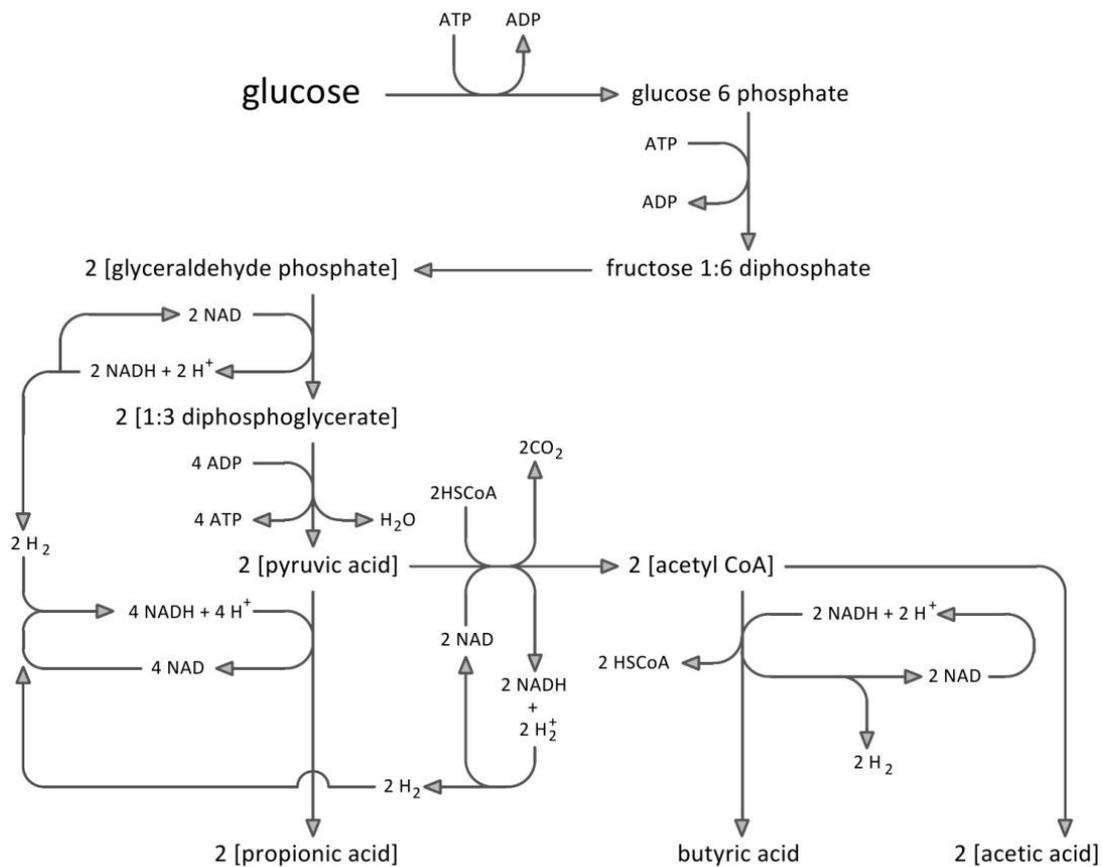


Figure 2.6. Hydrogen-regulated catabolic pathways in anaerobic processes, exemplified for glucose. Adapted from (Harper and Pohland, 1986).

Previous references studying bioconversion of CO₂ in ADs (Table 2.3) have not reported H_2 concentrations or alluded to a possible process inhibition due to H_2 . This suggests

that any potential formation of H₂ associated with the acidic properties of CO₂ and the low ORP characteristic of ADs was buffered by the system H₂ assimilatory capacity.

2.4.3.2. Ammonia stripping

Several studies have investigated the possibility of controlling NH₃ inhibition in ADs treating substrates with a high protein content (e.g. food waste) by stripping it with biogas (Abouelenien et al., 2010; Guštin and Marinšek-Logar, 2011; Serna-Maza et al., 2014; Walker et al., 2011). Significant reductions in free NH₃ levels in the digesting material have been reported, particularly when digestate temperature is maintained over 70°C and lime or sodium hydroxide are dosed in order to maintain pH close to 10 (Guštin and Marinšek-Logar, 2011; Serna-Maza et al., 2014). The possibility of coupling NH₃ toxicity control with CO₂ bioconversion to CH₄ in ADs was introduced by Budzianowski, (2012). Although findings reported for NH₃ stripping with biogas are likely to be transferable when utilising CO₂ concentrated streams (e.g. impact of temperature, pH and gas flowrate in removal performance), potential particular implications of utilising a different stripping gas still need to be investigated. In particular, any pH drop associated with a dissolution of CO₂ would modify the equilibrium between total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) (Koch et al., 2010), influencing the availability of the second form for it to be degassed.

2.4.3.3. Alteration in alkalinity levels

Dissolution of CO₂ is not expected to alter total alkalinity since it releases the same amount of positive (H⁺) and negative equivalents (CO₃²⁻ and HCO₃⁻), as demonstrated mathematically by Pankow (1991). However, in presence of a high amount of inorganic cations or NH₃, carbonated precipitates may be formed, leading to a modification of the alkalinity available in the liquid phase. This would be reflected in total alkalinity analysis when done in supernatant after centrifugation (majority of solids removed), but would not increase or reduce the alkalinity available within an AD. In agreement with this, Francioso et al., (2010) reported that total alkalinity was not affected when injecting CO₂ into the first stage of a TPAD.

2.4.3.4. Increase in dissolved CO₂ levels

Injection of CO₂ in ADs may potentially increase CO₂ dissolved levels in the final digestate if not fully utilised in the AD process. If this is the case, additional CO₂ could be released in later stages of the sludge treatment process, contributing towards uncontrolled GHG emissions. From previous studies assessing the potential benefits of bioconversion of CO₂ in anaerobic processes (Table 2.3) only Alimahmoodi and Mulligan, (2008) reported dissolved CO₂ concentrations in the system effluent for various influent CO₂ levels. Dissolved

CO₂ discharged with the effluent of the operated UASB reactor appeared fairly constant, with discharge rates of 0.3-0.7 g CO₂·d⁻¹ for influent dissolved CO₂ rates of 8.6-25.1 g CO₂·d⁻¹ for a system containing acetic acid as only VFA (Alimahmoodi and Mulligan, 2008). Effluent CO₂ was estimated by applying carbonate equilibrium reactions for a measured pH and alkalinity. It appears that no study has actually reported direct dissolved CO₂ recordings in effluents of an anaerobic process enriched with CO₂, which would be recommended.

2.4.4. Potential implications of a full-scale application

Implementation of CO₂ bioconversion in ADs at full-scale would lead to benefits on the renewable energy generation capacity of the process and on its carbon footprint, being the later enhanced both due to the direct CO₂ uptake and to the offset of energy with fossil fuel origin. A preliminary assessment of the additional benefits achievable can be completed based on literature available data. Uptakes of CO₂ between 3 and 98% have been reported (Table 2.3) with typically 40-46% when considering ADs operating continuously. If the solubility of CO₂ in the anaerobically digesting material is considered as 1071 mg·L⁻¹, based on values for aqueous solutions at mesophilic conditions, an uptake of 40% of CO₂ in an influent saturated with a partial pressure of CO₂ of 1 atm would imply a normalised CO₂ assimilation of 0.43 kg CO₂ per tonne substrate treated. This figure for CO₂ utilised is considered a conservative estimate, since an uptake of 40% of CO₂ can have different GHG implications depending on the point in the AD flowsheet where CO₂ is injected. If the influent AD material is saturated by injecting CO₂, a 0.43 kg CO₂ uptake will be obtained per tonne of substrate derived into the AD process, when considering performances reported in literature. However, if CO₂ is dissolved in the bulk of the AD unit (e.g. by a potential use of gas mixing systems), the whole content of the AD will be enriched with CO₂. If CO₂ injection is applied in a continuous or periodic basis, the digesting material would be enriched with CO₂ several times in a hydraulic retention time, which would proportionally increase the total amount of CO₂ dissolved and hence the benefits attainable in carbon footprint. However, the rate at which ADs can assimilate exogenous CO₂ has not been thoroughly investigated yet, which would determine the frequency of CO₂ injection in order to ensure that dissolved CO₂ levels in the effluent are not raised.

Further environmental benefits are envisaged when the increase in CH₄ production resulting from CO₂ bioconversion is considered. Attending to previous literature, an increase in CH₄ yield of 30% appears sensible when considering continuously operated ADs (Table 2.3), which could raise CH₄ yield by 54 m³ per tonne of substrate treated when considering a base value of 180 m³·tonne⁻¹ (300 m³ biogas·tonne⁻¹ (Georges et al., 2009) with 60% CH₄ concentration). An offset of CO₂ emissions from prevention of energy usage with fossil fuel

origin of $536 \text{ g CO}_2\cdot\text{kWh}^{-1}$ (IEA, 2013a) can be considered. This would imply that an increase of $54 \text{ m}^3 \text{ CH}_4\cdot\text{tonne}^{-1}$ offsets the emission of 101 kg CO_2 per tonne of substrate treated, when considering an energy yield from CH_4 of $10 \text{ kWh}\cdot\text{m}^{-3}$ and a CHP electrical efficiency of 35%.

Savings of CO_2 from diverting from landfill one tonne of food waste to be treated by AD have been estimated at 430 kg CO_2 (DEFRA, 2007). Revalorisation of the CO_2 contained in biogas by bioconversion in ADs would lead to additional benefits when the CO_2 utilised is considered as a negative emission and the offset of GHG emissions from usage of energy with fossil fuel origin is considered. Bioconversion of CO_2 in ADs has therefore the potential to increase CO_2 savings from diverting one tonne of waste from landfill by *ca.* 24% (430 kg CO_2 estimated by DEFRA (2007) to 531 kg CO_2), when considering literature data. Additional incentives to study the potential of CO_2 bioconversion in ADs are present when considering the support of the UK government towards an increase of renewable energy production and the feed-in tariff schemes, which currently support renewable energy from ADs with a total capacity greater than 500 kW with $0.122\text{€}\cdot\text{kWh}^{-1}$ (Ofgem, 2014).

2.5. REQUIREMENT FOR FURTHER WORK

The increasing emissions of biogenic CO_2 from ADs needs to be addressed to support GHG mitigation objectives and to provide an appropriate closed loop strategy to utilise “waste” CO_2 . While the increasing implementation of AD has positive impacts in renewable energy production, there is not a route of utilisation for the CO_2 emitted with the biogas. This is normally vented to atmosphere with exhaust gases and has been estimated to account for over 0.58 MtCO_2 per annum for the UK water and organic waste sectors. The scattered location and varied size of AD sites, however, makes CO_2 transportation a limiting factor for implementing solutions based on storage in oceanic or geological reservoirs. On-site carbon management strategies are hence being investigated, with a current focus on biogenic solutions. Utilisation of CO_2 for growth of algae and bioconversion to CH_4 by methanogenic *Archaea* have been identified as the most feasible options for on-site carbon management (Byrns et al., 2013), with the later predicted to be more easily implemented.

The potential of CO_2 bioconversion in ADs to act as an on-site carbon management strategy while enhancing renewable energy production has been experimentally evidenced (Alimahmoodi and Mulligan, 2008; Bajón Fernández et al., 2014; Salomoni et al., 2011; Sato and Ochi, 1994). However, several aspects need to be addressed in order to increase readiness for implementation. Examination of the literature presents conflicting information regarding the mechanisms of utilisation of additional CO_2 , being proposed both an enhancement of the acetoclastic (Bajón Fernández et al., 2014; Francioso et al., 2010) and of the hydrogenotrophic (Alimahmoodi and Mulligan, 2008) routes of CH_4 formation. However, in

no case have microbial community analysis been reported in order to support the proposed hypothesis. Understanding the fate of exogenous CO₂ in ADs is essential to determine overall benefits achievable in carbon footprint reduction and to determine the systems in which CO₂ bioconversion could be implemented. Enrichment of anaerobic processes with CO₂ has consistently been reported to enhance CH₄ production, hence evidencing biological conversion of exogenous CO₂. However, part of the CO₂ has the potential to remain in unstable forms that can be released in the later treatment of digestates. Investigation of the potential of additional CO₂ to form carbonated precipitates and to increase CO₂ dissolved levels in the final digestates is essential to confidently determine benefits in carbon footprint reduction. Injection of CO₂ is expected to increase CaCO₃ solubility (as a consequence of CO₃²⁻ consumption) and has the potential to form NH₄HCO₃, which could contribute to avoid inhibition of methanogenic activity as a result of scaling (Chen et al., 2008) and to increase the fertiliser potential of the final digestate, respectively. However, the thermal instability of NH₄HCO₃, which decomposes at 60°C (Patnaik, 2003), could lead to subsequent carbon emissions. Understanding the mechanism of CO₂ utilisation is also essential to determine the systems in which CO₂ bioconversion could be implemented. Inhibition of acetoclastic methanogenesis in ADs with a high NH₃ concentration would limit implementation of CO₂ bioconversion in substrates with a high protein content if coupling of homoacetogenesis and acetoclastic methanogenesis proves to be the mechanism of CO₂ bioconversion.

Byrns et al., (2013) identified the need for the benefits of CO₂ bioconversion in ADs to be demonstrated in full-scale trials. While the proof of concept has been developed at laboratory or pilot scale, there is no study addressing the practicalities of an up-scaled implementation. In particular, the need for contacting CO₂ with digestate or substrate material while achieving a significant CO₂ gas to liquid mass transfer needs to be addressed. The possibility of utilising already existing gas mixing systems of ADs to inject additional CO₂ would ease full scale implementation and enable retrofitting CO₂ enrichment of ADs without incurring in additional pumping costs. However, the risk of diluting the AD's headspace with non dissolved CO₂ needs to be considered, since variations in biogas quality would lead to a detriment in the performance of CHP engines. Furthermore, it is postulated that CO₂ would be preferably utilised in dissolved form, which would require a better understanding of CO₂ gas to liquid mass transfer in fluids of complex rheology like anaerobically digested substrates.

2.6. CONCLUDING REMARKS

Bioconversion of CO₂ in ADs has the potential to reduce the carbon footprint of the water and organic waste sectors, with a concomitant increase in renewable energy generation and hence a contribution towards energy production decarbonisation. However, the scarce

literature available addressing CO₂ bioconversion in ADs, necessitates a better understanding before a widespread implementation can be considered. The mechanisms of CO₂ utilisation and the technologies for contacting CO₂(g) and digesting fluids with an efficient mass transfer, are some of the aspects that need to be further investigated to confidently quantify benefits in carbon footprint and to increase readiness for implementation.

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CHAPTER 3

CARBON CAPTURE AND BIOGAS ENHANCEMENT BY CARBON DIOXIDE ENRICHMENT OF ANAEROBIC DIGESTERS TREATING SEWAGE SLUDGE OR FOOD WASTE

3. CARBON CAPTURE AND BIOGAS ENHANCEMENT BY CARBON DIOXIDE ENRICHMENT OF ANAEROBIC DIGESTERS TREATING SEWAGE SLUDGE OR FOOD WASTE

HIGHLIGHTS

- The benefits of CO₂ enrichment on anaerobic digestion were evidenced.
- Sewage sludge and food waste anaerobic digesters were examined.
- First 24 hour CH₄ production increased 11-16% for food waste and 96-138% for sludge.
- A mechanism of CO₂ utilisation has been hypothesised.
- Estimated potential CO₂ reductions of 8-34% for sludge and of 3-11% for food waste.

ABSTRACT

The increasing concentration of carbon dioxide (CO₂) in the atmosphere and the stringent greenhouse gases (GHG) reduction targets, require the development of CO₂ sequestration technologies applicable for the waste and wastewater sector. This study addressed the reduction of CO₂ emissions and enhancement of biogas production associated with CO₂ enrichment of anaerobic digesters (ADs). The benefits of CO₂ enrichment were examined by injecting CO₂ at 0, 0.3, 0.6 and 0.9 molar fractions into batch ADs treating food waste or sewage sludge. Daily specific methane (CH₄) production increased 11 to 16% for food waste and 96 to 138% for sewage sludge over the first 24 hours. Potential CO₂ reductions of 8 to 34% for sewage sludge and 3 to 11% for food waste were estimated. The capacity of ADs to utilise additional CO₂ was demonstrated, which could provide a potential solution for onsite sequestration of CO₂ streams while enhancing renewable energy production.

KEYWORDS

Anaerobic digestion, food waste, sewage sludge, carbon dioxide sequestration, methane enhancement.

3.1. INTRODUCTION

Carbon dioxide (CO₂) emissions to the atmosphere need to be reduced if targets for CO₂ reduction are to be met (e.g. UK Climate Change Act 2008). Conventional carbon capture and storage (CCS) is based on the long term storage of this compound in geological or

ocean reservoirs (Xu et al., 2010). This still has high associated costs and significant limitations linked to the potential risk of leaking from storage sites (Holloway, 2007). Moreover, the need to transport the CO₂ makes the proximity of source and reservoir a limiting factor. Therefore, the implementation of CCS is more feasible in large centralised sources which benefit from the pipeline's economy of scale (Middleton and Eccles, 2013).

The UK water industry emitted over 5 million tonnes of greenhouse gases (GHG) as CO₂ equivalents (CO₂e) during 2010-2011 (Water UK, 2012), of which 56% can be attributed to wastewater treatment (DEFRA, 2008). However, the varied size and scattered location of organic waste and wastewater treatment plants (WWTPs), make the implementation of CCS particularly challenging in the water or waste sectors. This necessitates the development of alternative solutions for CO₂ capture and long term storage. Additionally, the increased implementation of upgrading technologies for the biogas produced in anaerobic digesters (ADs) (Weiland, 2010), results in the production of CO₂ concentrated streams. This further raises the need to develop new carbon storage or utilisation technologies applicable to the wastewater and waste sectors.

Biogenic carbon sequestration methods (e.g., microalgae, biochar) are being studied as alternatives to geological or oceanic reservoirs. However, in general, their capacity for CO₂ sequestration or their large-scale applicability needs to be further investigated (NERC, 2011). A few studies have considered the potential of CO₂ biological conversion in anaerobic processes, reporting benefits both in terms of carbon uptake and renewable energy production. Alimahmoodi and Mulligan (2008) stated a 69-86% CO₂ uptake when dissolving this gas in the influent to an upflow anaerobic sludge blanket (UASB) reactor. Salomoni et al. (2011) further confirmed the potential of CO₂ biological conversion in two phase anaerobic digestion (TPAD), and observed a 25% methane (CH₄) yield enhancement when bubbling CO₂ into the first stage. Sato and Ochi (1994) stated associated benefits of up to 30% increased specific CH₄ yields when enriching ADs treating sewage sludge with CO₂.

Therefore, the capacity of ADs to transform CO₂ into CH₄ could result in the onsite treatment of CO₂ concentrated streams and potential increases in CH₄ production. Although the benefits of CO₂ enrichment of ADs have been evidenced, the scarcity of the literature available requires further research before its full potential can be estimated. Furthermore, the increasing practice to treat food waste or mixed substrates, also needs to be considered in relation to the benefits of CO₂ enrichment.

This paper assessed the impact of CO₂ injection in batch ADs treating food waste or sewage sludge. Renewable energy production, CO₂ utilisation and digestate quality were studied. Firstly, absorption tests were completed to estimate the gas-liquid contact time

required to reach CO₂ equilibrium conditions between the liquid phase and the injected gas. Secondly, the impact of CO₂ enrichment in batch ADs treating food waste and sewage sludge was assessed for CO₂ molar fractions (y_{CO_2}) of 0.3, 0.6 and 0.9 (0.4, 0.8 and 1.6 bar CO₂ partial pressures [p_{CO_2}]). Lastly, the time required to recover from any initial acidification due to CO₂ injection was determined for sewage sludge by monitoring the pH of sacrificial ADs.

3.2. MATERIALS AND METHODS

3.2.1. Description of the anaerobic digester equipment

Each batch AD unit consisted of a 1 L glass bottle with a four port cap (Fisher Scientific, Loughborough, UK). Two ports were used for gas injection by means of Pyrex diffusers with a porosity of 3 and 15 mm diameter (Fisher Scientific, Loughborough, UK). When running absorption tests, one port was acting as pressure release and the fourth port was blocked (Figure 3.1 a). When conducting CO₂ enrichment tests in ADs, one port was blocked with a 17mm septa (Thames Restek UK Ltd., Buckinghamshire, UK), allowing gas sample extraction for composition analysis, and the last port was connected to a MilliGascounter (Litre Meter Ltd., Buckinghamshire, UK) for biogas volume recording (Figure 3.1 b). When running sacrificial ADs for pH monitoring, one port was used for daily sample extraction of the liquid phase. The ADs were continuously stirred and placed in a temperature controlled water bath (38±0.5°C).

3.2.2. Absorption tests methodology

The contact time required to ensure CO₂ equilibrium conditions between the gas injected and the sewage sludge or food waste, was estimated by conducting oxygen (O₂) absorption tests with air, and converting the results to CO₂ using diffusion coefficients, as previously suggested by Garcia-Ochoa and Gomez (2009). In order to account for the viscosity variability of food waste and sewage sludge, tests with different liquid viscosities were performed. Glycerol was used as a viscosity enhancer, because of the extensive information available of its impact on aqueous solutions (Jordan et al., 1956). Tests in deionized (DI) water with air flow rates of 0.5, 1.0 and 1.7 L·min⁻¹ and tests with a fixed air flow rate (1.0 L·min⁻¹) and mixtures of glycerol in DI water of 10, 30, 50 and 70% weight (glycerol ≥98%; Fisher Scientific, Loughborough, UK) were performed. The air flow rate was controlled by a mass flow controller (MFC) (Premier Control Technologies, Norfolk, UK). The dissolved oxygen (DO) was monitored using a DO probe (HACH LDO101; Camlab, Cambridge, UK) connected to a meter device (HACH HQ30d; Camlab, Cambridge, UK).

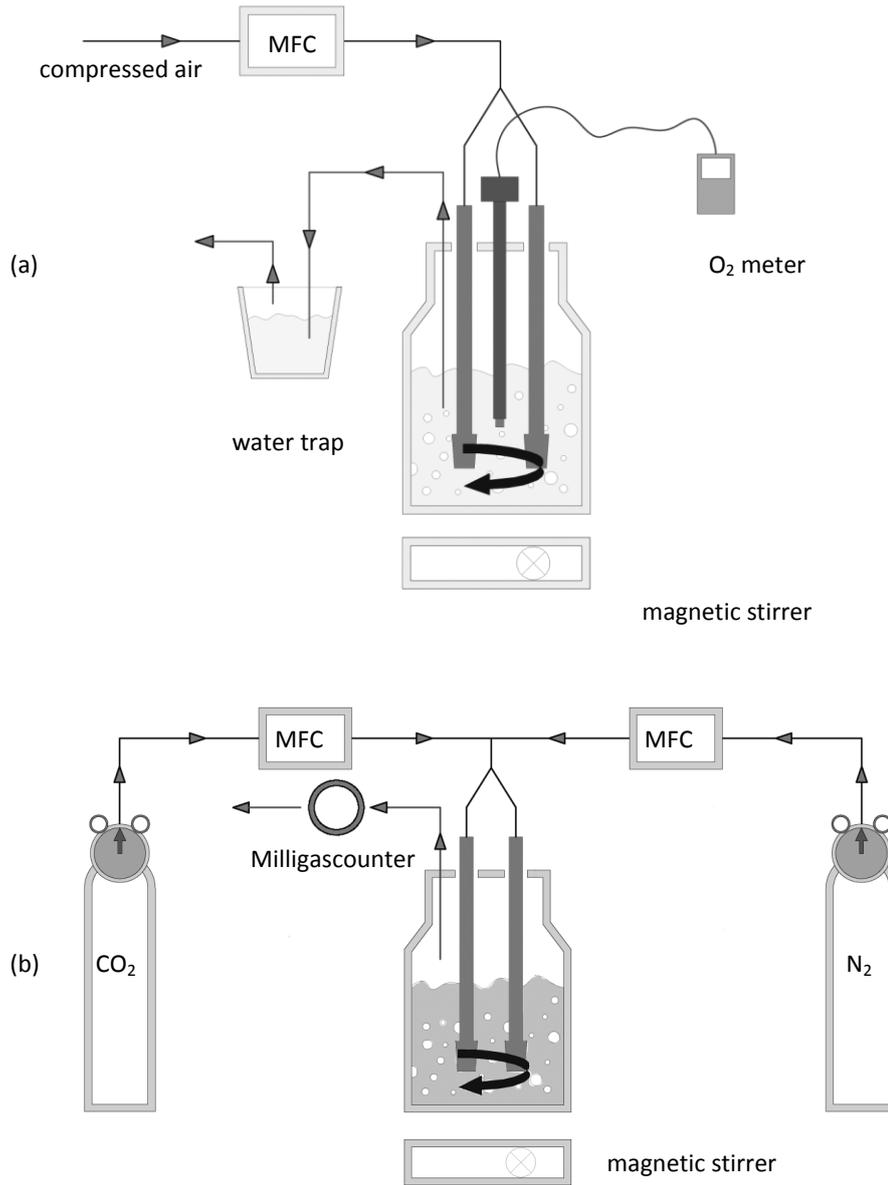


Figure 3.1. Schematic representation of the experimental rig used (a) for the absorption tests and (b) for the operation of ADs enriched with CO₂.

The gas to liquid mass transfer was described using Eq. 1 and corrected for the time to reach 95% of the equilibrium solubility by Eq. 2. Considering similar equations for CO₂ and O₂, and relating the volumetric mass transfer coefficients ($k_L a$) of both gases with the ratio of their diffusion coefficients (Eq. 3), a relationship between the times to reach equilibrium solubility with CO₂ and with O₂ was obtained (Eq. 4). The film theory for interfacial mass transfer was considered, which states $n=1$. The diffusion coefficients for CO₂ in water-glycerol mixtures used in Eq. 4 were $2.6 \cdot 10^{-5}$, $1.7 \cdot 10^{-5}$, $7.2 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$ for glycerol concentrations of 0, 25 and 50% weight, respectively. The values used for O₂ were $3.0 \cdot 10^{-5}$, $3.4 \cdot 10^{-5}$, $1.6 \cdot 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ for glycerol concentrations of 0, 25 and 50% weight, respectively.

These diffusion coefficients were obtained from those reported by Brignole and Echarte (1981) and Jordan et al. (1956), for CO₂ and O₂ respectively, after correction for a temperature of 38°C as per Díaz et al. (1987).

$$\ln\left(\frac{C^*-C_t}{C^*-C_0}\right) = -k_L a \cdot t \text{ (Eq. 1)}$$

$$\ln(0.05) = -k_L a \cdot t_{95} \text{ (Eq. 2)}$$

$$(k_L a)_{CO_2} = (k_L a)_{O_2} \cdot \left[\frac{(D_L)_{CO_2}}{(D_L)_{O_2}}\right]^n \text{ (Eq. 3)}$$

$$(t_{95})_{CO_2} = (t_{95})_{O_2} \cdot \frac{(D_L)_{O_2}}{(D_L)_{CO_2}} \text{ (Eq. 4)}$$

Where $k_L a$: volumetric liquid phase mass transfer coefficient (s⁻¹), D_L : diffusion coefficient (m²·s⁻¹), n : coefficient depending on the theory for interfacial mass transfer considered between the gas and the liquid phases, t_{95} :time to reach 95% of the equilibrium solubility (s), C^* :solubility (mg·L⁻¹), C_0 :concentration at time zero (mg·L⁻¹), C_t : concentration at time t (mg·L⁻¹).

3.2.3. Methodology for enriching the digesters with CO₂

Batch ADs treating food waste or sewage sludge were operated with an inoculum to substrate volatile solids (VS) ratio of 2:1 and a working volume of 700 ml. Macerated and digested food waste were collected from a full scale UK AD site treating 30,000 tonnes of organic waste per year. Thickened waste activated sludge (WAS) and digested sewage sludge were collected from a full scale UK WWTP serving a 2.5 million population equivalent.

The material in each AD was enriched with a different p_{CO₂} before starting the digestion process, (Table 3.1). Mixtures of CO₂ and nitrogen (N₂) were used to regulate the p_{CO₂}, and only N₂ was used in the control units. The N₂ and CO₂ were supplied from gas cylinders (BOC, Manchester, UK) and were controlled by MFCs (Premier Control Technologies, Norfolk, UK).

The duration of the CO₂ injection was determined from the results of the absorption tests. The control ADs for food waste and sewage sludge were bubbled with N₂ from 5 up to 20 minutes to ensure that any increase in performance was not due to an initial improved mixing of substrate and inoculum.

Table 3.1. Gas injection conditions used for enrichment with CO₂ of the material to digest in batch ADs.

		DC ^e	D0.3 ^f	D0.6 ^g	D0.9 ^h
	y _{CO₂} ^a	0.00	0.30	0.60	0.89
Food waste	p _{CO₂} ^b (bar)	0.0±0.0	0.4±0.0	0.8±0.1	1.6±0.3
	n ^c	2	2	2	2
Sewage sludge	p _{CO₂} (bar)	0.0±0.0	0.5±0.1	n/a	1.7±0.0
	n	3	3	n/a	2
Sacrificial AD	p _{CO₂} (bar)	0.0±0.0	0.4±0.1	n/a	1.3±0.2
	n	6 ^d	3	n/a	3

^aCO₂ molar fraction

^bCO₂ partial pressure

^cNumber of replicates

^dThree ADs bubbled with nitrogen for 0.5 min and three for 20 minutes

^eControl digester

^fDigesters enriched with y_{CO₂}=0.3

^gDigesters enriched with y_{CO₂}=0.6

^hDigesters enriched with y_{CO₂}=0.9

Lastly, sacrificial ADs treating sewage sludge were operated under the same conditions (Table 3.1) and their pH evolution was monitored daily. The effect of the N₂ injection time on the initial conditions of the control ADs was studied by operating two types of sacrificial controls: bubbled with N₂ for 0.5 min and 20 min. A gas flow rate of 1.0 L·min⁻¹ was used in all the reactors.

3.2.4. Analytical methods

The materials were analysed on commencement and at the end of the AD operation for soluble chemical oxygen demand (sCOD), total solids (TS) and VS (APHA, 2005). To obtain the solid free fraction, samples were centrifuged in a Falcon 6/300 refrigerated centrifuge (MSE UK Ltd., London, UK) at 4700 g and 19°C for 20 minutes, and the supernatant was centrifuged again for 40 minutes under the same conditions. The final supernatant was vacuum filtered through 1.2 µm pore size glass microfiber filters GF/C (WhatmanTM, Kent, UK) and then through 0.45 µm pore size syringe-drive filter units (MilliporeTM, Billerica, United States).

The volume of gas produced and its composition were recorded daily by means of MilliGascounters (Litre Meter Ltd., Buckinghamshire, UK) and a CSi 200 Series Gas Chromatograph (Cambridge Scientific Instruments Ltd., Witchford, UK), respectively.

The CO₂ generated during the entire batch digestion process was calculated as per the following mass balance, which was compared for control and test ADs to estimate the reduction of CO₂ emissions:

$$(CO_2)_{generated} = (CO_2)_{digestate} + (CO_2)_{biogas} - (CO_2)_{in} \quad (\text{Eq. 5})$$

Where:

$(CO_2)_{digestate}$: CO₂ dissolved in the digestate at the end of the digestion period (mg). Obtained with the headspace concentration of digestate samples allowed to reach equilibrium conditions with the gas phase and Henry's law.

$(CO_2)_{biogas}$: CO₂ released with the biogas (mg), at 20°C and 1atm.

$(CO_2)_{in}$: CO₂ dissolved in the material to digest after the CO₂ injection (mg). Calculated based on Henry's law, considering the partial pressure of each injection (Table 3.1) and assuming CO₂ solubility of 1071 mg·L⁻¹.

Statistically significant differences between ADs were identified through an analysis of variance (ANOVA), where the AD performances (e.g., CH₄ yield, daily CH₄ production) were the dependent variables and y_{CO_2} or p_{CO_2} were the factors. Statistica software version 11 (StatSoft Ltd., Bedford, UK) was used.

3.3. RESULTS AND DISCUSSION

3.3.1. Estimation of gas-liquid contact time to achieve CO₂ equilibrium during enrichment

The results from the absorption tests demonstrated that equilibrium of the liquid phase with O₂ in air was achieved in 2-4 minutes for all the air flow rates and viscosities tested. The diffusion coefficients for CO₂-water-glycerol and O₂-water-glycerol reported by Brignole and Echarte (1981) and Jordan et al.(1956) were used, after correction for mesophilic temperatures as per Díaz et al. (1987). A ratio of diffusion coefficients of O₂ to CO₂ of 1.2, 2.0 and 2.3 was obtained for glycerol concentrations of 0, 25 and 50% weight, respectively, demonstrating that the gas-liquid contact time required with CO₂ was 1.2-2.3 times higher than with O₂. Considering Eq. 4 and an O₂ to CO₂ diffusion coefficients ratio of 2.3, a gas-liquid contact time over 9 minutes was required to reach equilibrium conditions with the CO₂ enriched gas, for the system in place. Due to the scarcity of published diffusion coefficients for high glycerol concentrations, food waste and sewage sludge, and due to the added complexity of the bicarbonate equilibrium in ADs, a safety factor was applied. A CO₂ injection time of 20 minutes was used when enriching with CO₂ the materials to digest in the test ADs.

This methodology and equilibrium time were validated by injecting CO₂ enriched streams into sewage sludge and food waste, and monitoring the pH change. In both cases a gas injection of 20 minutes ensured that equilibrium conditions were achieved.

From the sacrificial ADs operation (Figure 3.2), the duration of N₂ injection on the control ADs significantly affected the initial pH, with longer injection times (20 minutes) increasing the pH by 0.9 units. Therefore, it was concluded that the starting pH of the ADs bubbled with N₂ for only 0.5 minutes, was more comparable to that measured in the test ADs before CO₂ injection.

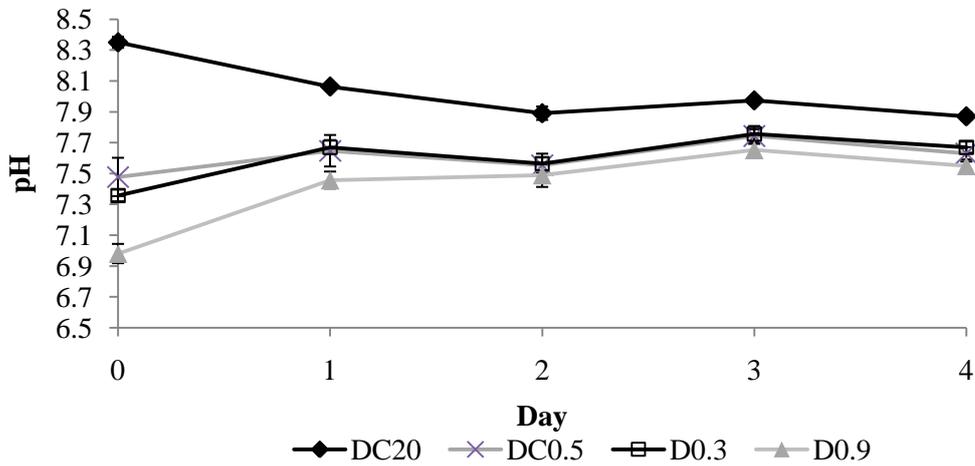


Figure 3.2. Evolution of pH in sacrificial ADs treating sewage sludge. The data at day zero represent the pH after the gas injection. DC20: digesters control bubbled with N₂(g) for 20 minutes, DC0.5: digesters control bubbled with N₂(g) for 0.5 min, D0.3: digesters enriched with $y_{CO_2}=0.3$, D0.6: digesters enriched with $y_{CO_2}=0.6$, D0.9: digesters enriched with $y_{CO_2}=0.9$. The error bars represent the standard deviation between replicates.

3.3.2. Assessment of digestion performance: renewable energy enhancement and digestate quality

Biogas and CH₄ production data are summarised in Table 3.2 and Figure 3.3. All the ADs enriched with CO₂ and treating food waste obtained higher CH₄ yields than the controls. More specifically, the ADs enriched with $y_{CO_2}=0.9$ achieved a 13% improvement (p-value of 0.04) on CH₄ yield, whilst an 8% and 5% increase (p-value of 0.15 and 0.29, respectively) was observed for ADs bubbled with $y_{CO_2}=0.3$ and $y_{CO_2}=0.6$, respectively. During the first 24 hours after CO₂ injection, the increase in daily CH₄ production was 14, 11 and 16% for $y_{CO_2}=0.3$, 0.6 and 0.9, respectively (p-value of 0.03, 0.06, 0.02, respectively) (Table 3.2).

Despite the increase in the final yield, there were no significant differences in the distribution of the CH₄ production over time for food waste ADs (Figure 3.3 a). During the first 48 hours of digestion, the control units achieved 47±3% of their final CH₄ yield, which was similar to that of the test reactors: 49±0%, 48±0% and 46±2%, for $y_{CO_2}=0.3$, 0.6, 0.9, respectively.

Conversely, the test ADs treating sewage sludge experienced an increase of 96% and 138% (p-value of 0.007 and 0.001, respectively) in the CH₄ production 24 hours after the CO₂

injection, when enriched with $y_{\text{CO}_2}=0.3$ and $y_{\text{CO}_2}=0.9$, respectively. However, this initial boost was not maintained throughout the batch digestion period, leading to no benefit in the final CH_4 yield when compared with the control ADs (Table 3.2). Therefore, there was a significantly different distribution of the CH_4 production of control and test sewage sludge ADs over time (Figure 3.3 b). The test ADs achieved over 60% of the CH_4 yield during the first 48 hours of the digestion process, whilst the control ADs attained less than 40% (Table 3.2).

Since the material to digest was enriched with CO_2 only at the start of the digestion process, lower CH_4 yield improvements than the 30% achieved by Sato and Ochi (1994) or the 25% reported by Salomoni et al. (2011) when injecting CO_2 periodically into ADs, were observed. However, if the enhancement in the sewage sludge ADs over the first 48 hours following CO_2 enrichment was considered, significantly higher benefits were achieved in this study. Nevertheless, the comparison with previous studies was limited because of the difference of substrates treated and reactor type used (i.e., continuous or batch, single or two phased, UASB or AD).

Table 3.2. pH at start and end of the digestion process, batch ADs performance and removal efficiencies. Format as *average ± standard deviation*.

	Food waste					Sewage sludge			
	Mixture to AD	DC ^a	D0.3 ^b	D0.6 ^c	D0.9 ^d	Mixture to AD	DC ^a	D0.3 ^b	D0.9 ^d
Liquid phase									
pH	7.6±0.0	8.4±0.0	8.4±0.0	8.4±0.0	8.4±0.0	7.2±0.0	7.7±0.0	7.7±0.1	7.7±0.0
Removal efficiencies									
TS (%)	-	16.6±0.7	17.2±0.2	16.0±0.5	17.3±1.1	-	19.2±0.1	25.2±1.2	18.6±0.5
VS (%)	-	26.1±0.1	25.6±0.1	25.4±1.8	26.0±0.8	-	27.2±0.0	32.6±0.4	26.5±0.4
sCOD (%)	-	22.7±6.4	33.2±12.9	16.1±3.3	21.7±2.3	-	29.9±4.6	43.3±6.7	34.9±8.3
Biogas and methane yields									
CH ₄ yield (ml CH ₄ ·(g VS) ⁻¹)	-	172±12	186±8	182±1	195±6	-	101±2	94±2	103±10
% of the CH ₄ yield achieved during the first 48hours of digestion	-	47±3	49±0	48±0	46±2	-	39±10	63±4	61±7
Biogas yield (ml biogas·(g VS) ⁻¹)	-	267±13	281±8	279±3	303±9	-	183±8	171±4	189±5
% of the biogas yield achieved during the first 48 hours of digestion	-	52±3	53±0	53±0	52±1	-	37±7	61±3	62±4
Average CH ₄ content in the biogas (%)	-	68±1	70±1	69±1	69±0	-	57±1	56±1	55±2
Enhancement of ADs enriched with CO₂									
Increase in normalised CH ₄ yield (%)	-	-	8.0	5.5	13.3	-	-	-6.6	2.2
Increase in CH ₄ production during the first 24 hours (%)	-	-	14.4	11.1	16.3	-	-	95.9	137.9
Increase in biogas yield (%)	-	-	5.1	4.5	13.2	-	-	-6.3	3.5

^aControl digester

^bDigesters enriched with $y_{CO_2}=0.3$

^cDigesters enriched with $y_{CO_2}=0.6$

^dDigesters enriched with $y_{CO_2}=0.9$

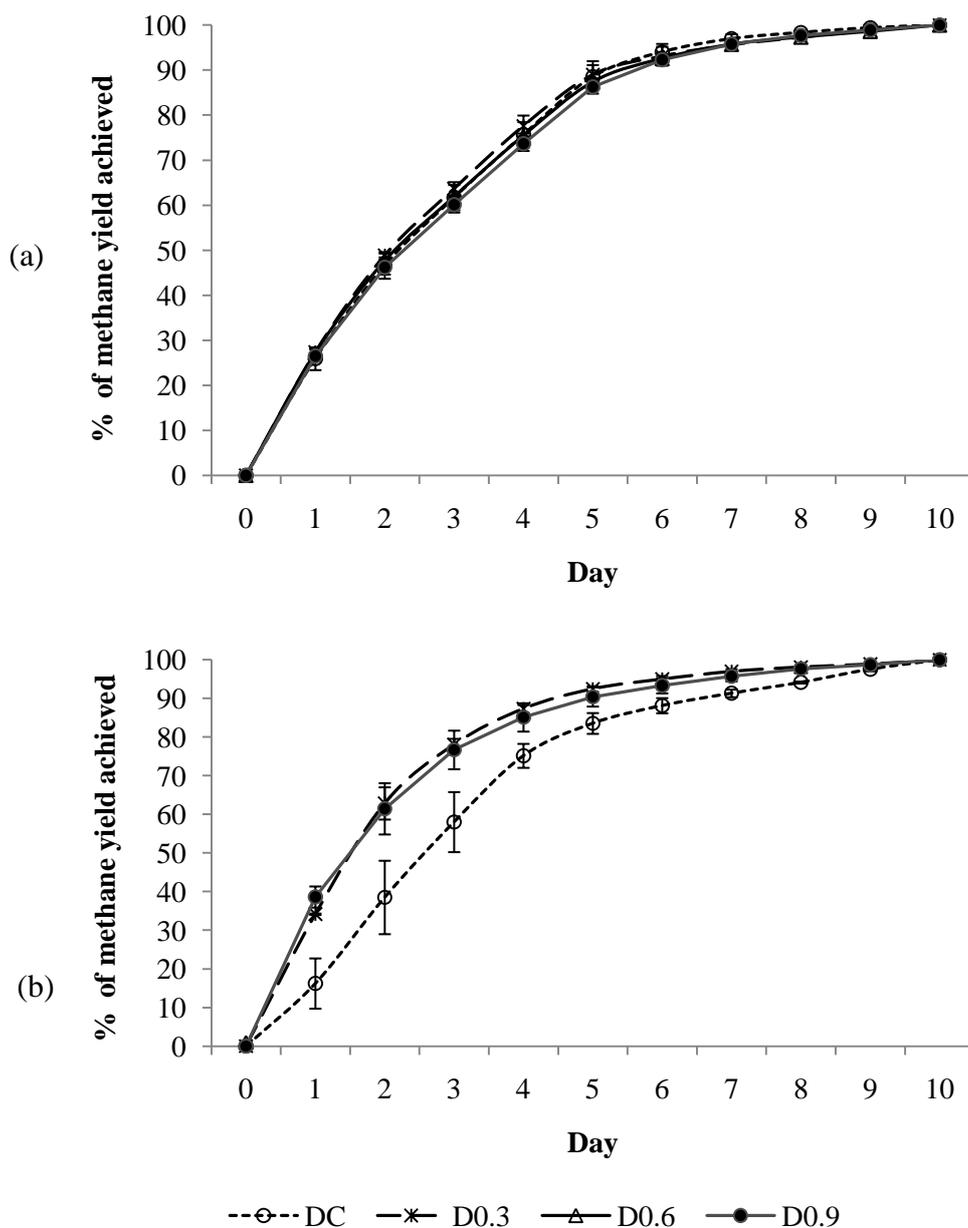


Figure 3.3. Distribution of the CH₄ production over time, as percentage of the CH₄ yield achieved by each day of the digestion process, when treating food waste (a) and sewage sludge (b). DC: digesters control, D0.3: digesters enriched with $y_{CO_2}=0.3$, D0.6: digesters enriched with $y_{CO_2}=0.6$, D0.9: digesters enriched with $y_{CO_2}=0.9$. The error bars represent the standard deviations.

The ADs enriched with higher CO₂ concentrations achieved greater enhancements, with the exception of $y_{CO_2}=0.6$ when treating food waste, which led to similar benefits than $y_{CO_2}=0.3$. For the two substrates treated, the best performance was obtained when using $y_{CO_2}=0.9$ (p_{CO_2} of 1.6-1.7 bar). This finding differs from the study of Sato and Ochi (1994), who reported an optimum performance at $y_{CO_2}=0.6$ and related the reduction in yield at higher concentrations with a possible drop in pH. Again the comparison of the results is limited,

since only data of the CO₂ concentrations were reported in that study whilst the amount of CO₂ dissolved is determined by the p_{CO₂}. Moreover, different alkalinities (buffering capacities) in the material to digest would lead to a different impact of the CO₂ injection in the pH.

In this study, no difference in the pH of the digestate of tests and controls for any of the substrates treated was observed (Table 3.2), which confirmed that the initial acidification associated with CO₂ injection was overcome during the digestion process. Moreover, the lowest pH achieved in the sacrificial ADs after bubbling sewage sludge with CO₂ during 20 minutes, was 7.0±0.1 (Figure 3.2), which is above the pH of 6 stated as inhibitory by Gerardi (2003).

Similar solids removals for the units enriched with CO₂ and the controls were observed, with the exception of sewage sludge ADs enriched with y_{CO₂}=0.3, where the VS removal increased by 20% (Table 3.2). Sato and Ochi (1994) reported no benefit in the VS removal when enriching periodically with CO₂ in laboratory scale ADs (6 L) treating WAS. However, the same study observed an increase of solids reduction from 39.7% to 45.4% when digesting mixed sludge (primary and WAS) in pilot-scale units with periodic CO₂ injection.

In all the ADs treating sewage sludge and enriched with CO₂, the sCOD removal during the entire batch digestion period was enhanced. The removal of sCOD reached 43.3±6.7% and 34.9±8.3% when enriching with y_{CO₂}=0.3 and y_{CO₂}=0.9, respectively, whilst 29.9±4.6% was recorded for the control units. Only on ADs bubbled with y_{CO₂}=0.3 was observed an increased sCOD removal when digesting food waste (Table 3.2).

3.3.3. CO₂ utilisation in the batch digesters

The benefits in carbon footprint were initially quantified with a CO₂ mass balance (Eq. 5) of the batch ADs, which considered the CO₂ dissolved in the material to digest after the enrichment, the CO₂ dissolved in the final digestates and that released with the biogas. The first two terms were estimated for equilibrium conditions with the p_{CO₂} of each injection (Table 3.1) or the headspace concentration, respectively, and assuming CO₂ solubility of 1071 mg·L⁻¹. The CO₂ in the biogas was obtained from the daily monitoring data of biogas production and composition.

The mass balance allowed a preliminary comparison between the carbon footprint of the batch ADs enriched with CO₂ and the control units. The contribution of each of the reactions in which CO₂ was produced or consumed was gathered in the overall CO₂ emission term, as similarly reported by Alimahmoodi and Mulligan (2008).

The comparison between control and test ADs suggested CO₂ overall reductions of 8 and 34% for sewage sludge ADs enriched with y_{CO_2} of 0.3 and 0.9, respectively. Similarly, benefits of 3, 10 and 11% were estimated for food waste ADs enriched with y_{CO_2} of 0.3, 0.6 and 0.9, respectively. If scaled-up, these carbon benefits associated with CO₂ enrichment of ADs, could significantly contribute towards the target to reduce GHG emissions at least an 80% by 2050 compared to 1990 (Climate Change Act, 2008). However, the benefits of CO₂ enrichment in the GHG emissions of ADs needs to be further investigated and quantified.

The complexity of the reactions taking place in ADs makes high the uncertainty regarding the mechanism of action by which the CO₂ could be utilised and bioconverted to CH₄. Besides, part of the CO₂ could have been transformed into other species (e.g., ammonia bicarbonates) rather than converted to CH₄, which further hinders stating a single mechanism of CO₂ utilisation. Alimahmoodi and Mulligan (2008) attributed the benefits to the encouragement of the hydrogenotrophic route for CH₄ production. On the contrary, Francioso et al. (2010) sustained that CO₂ boosts the volatile fatty acids (VFA) formation by combining with reducing compounds in the early stages of the digestion process according to the Wood–Ljungdahl pathway. In this study, CO₂ enrichment resulted in different CH₄ production patterns over time for food waste and sewage sludge ADs, which can help to hypothesize a CO₂ mechanism of action.

The initial increase in CH₄ production was significantly more pronounced when treating sewage sludge than when treating food waste, as stated before in terms of the production over the first 24 hours (Table 3.2). Several studies have reported an inhibition of the acetoclastic methanogens at high ammonia concentrations (Banks et al., 2011, 2012; Borja et al., 1996; Rajagopal et al., 2013; Schnürer and Nordberg, 2008; Walker et al., 2011), making the hydrogenotrophic methanogenesis the dominant route for CH₄ formation. This has been demonstrated for food waste ADs, where the hydrolysis of proteins leads to inhibitory levels of ammonia (Banks et al., 2008; Chen et al., 2008; Mata-Alvarez, 2003; Schnürer and Nordberg, 2008; Siles et al., 2010; Walker et al., 2011). In this study, total ammonia concentration in digestates was around 4 g·L⁻¹ NH₄-N in food waste ADs, which was higher than the 3 g·L⁻¹ NH₄-N reported as completely inhibitory (Rajagopal et al., 2013) for the acetoclastic route of CH₄ formation. Thus, it is considered that the main mechanism of CH₄ production in the food waste ADs was hydrogenotrophic methanogenesis preceded by syntrophic acetate oxidation (SAO).

If the acetoclastic route is considered to be inhibited, the more moderate improvement of the CH₄ yield in food waste ADs could be due to the CO₂ being reduced by hydrogenotrophic methanogens. If only partly inhibited, the acetoclastic pathway could have

been enhanced, leading to moderate benefits since it would have a much lower contribution to the CH₄ formation than the commonly accepted 70% (Conrad, 1999).

The ammonia content in the digestates of sewage sludge ADs (1.1 g·L⁻¹ NH₄-N) did not reach inhibitory levels, hence it is likely that both mechanisms of CH₄ formation were active when digesting this substrate. Consequently, the increased CH₄ formation in the sewage sludge ADs may be due to the enhancement of the acetoclastic pathway of CH₄ formation, likely due to an encouragement of the Wood-Ljungdahl mechanism in which CO₂ is reduced and acetate is formed (Muller, 2003; Ragsdale and Pierce, 2008). Salomoni et al. (2011) reported a 25% increased specific CH₄ yield when injecting CO₂ into the first stage of a TPAD treating sewage sludge. Since an efficient phase separation was stated, an injection into the first stage was also attributed to an encouragement of the acetogenic metabolism.

For both substrates the benefits were more emphasized during the first 48 hours of digestion, which may be due to the CO₂ having been utilised to the levels prior to the enrichment or to other substrate limitation. The recovery from any initial acidification during the first 24-48 hours of digestion (Figure 3.2) may indicate that CO₂ was utilised up to the levels prior to the enrichment. This could support the possibility of CO₂ enrichment by periodic injections, which could potentially maintain the benefits observed over the 24 hour period following CO₂ enrichment throughout the digestion process. However, the pH evolution is due to a combination of reactions (e.g., VFA formation/consumption) and not only due to CO₂ utilisation, therefore further testing would be required.

3.4. CONCLUSIONS

The effect of CO₂ enrichment of ADs was investigated for food waste and sewage sludge. An enhancement of CH₄ production was observed, demonstrating the potential of ADs to utilise additional CO₂. When treating food waste, CO₂ enrichment increased the CH₄ yield by up to 13%. For sewage sludge, CH₄ production increases of 96-138% were obtained during the first 24 hours of digestion. Associated CO₂ reductions of 3 to 11% for food waste and 8 to 34% for sewage sludge were estimated. The different substrate response to CO₂ observed could indicate that CO₂ enrichment enhanced the acetoclastic pathway of CH₄ formation.

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CHAPTER 4

ENHANCING THE ANAEROBIC DIGESTION PROCESS THROUGH CARBON DIOXIDE ENRICHMENT: MECHANISMS OF UTILISATION

4. ENHANCING THE ANAEROBIC DIGESTION PROCESS THROUGH CARBON DIOXIDE ENRICHMENT: MECHANISMS OF UTILISATION

HIGHLIGHTS

- The mechanisms of CO₂ utilisation in ADs were investigated.
- Biological CO₂ utilisation through acetoclastic methanogenesis was found dominant.
- *Methanosaetaceae* activity was 80% higher in sludge ADs with periodic CO₂ injection.
- *Methanosaetaceae* scarcity in food waste ADs limited benefits of CO₂ enrichment.

ABSTRACT

Carbon dioxide (CO₂) enrichment of anaerobic digesters (ADs) is a potential solution to manage CO₂ concentrated streams generated in the water and organic waste processing industries, with concomitant benefits in methane (CH₄) production. In this study the possible mechanisms of additional CO₂ utilisation in ADs were investigated, considering both chemical and biological pathways. Periodic CO₂ injections were determined to affect the activity of methanogenic communities in ADs. Up to 80% increased activity of *Methanosaetaceae* was observed in sewage sludge ADs enriched periodically with CO₂, indicating acetoclastic methanogenesis as the CO₂ to CH₄ conversion mechanism, likely preceded by an enhanced acetate production through the Wood-Ljungdahl pathway. The contribution of *Methanosaetaceae* to the total *Archaea* population in food waste ADs was scarce (4.3±1.7%), which is in accordance with the limited benefit obtained when applying CO₂ enrichment in ADs treating this substrate. Inorganic CO₂ consumption was observed not to occur to a significant extent. Implementation of CO₂ enrichment could be an effective carbon revalorisation strategy in systems where *Methanosaeta* species are present.

KEYWORDS

Acetoclastic, carbon uptake, CO₂ revalorisation, food waste, greenhouse gases, sewage sludge.

4.1. INTRODUCTION

Anaerobic digestion (AD) is a biological process that aims at stabilizing organic wastes while generating biogas with a 50-75% methane (CH₄) and a 50-25% carbon dioxide (CO₂) content (AEBIOM, 2009). Biogas has traditionally been utilised by combusting it in combined heat and power (CHP) engines or upgrading it to biomethane for this to be

incorporated into the gas grid, which is an increasing practice. In both cases, these processes aim for the CH₄ calorific value to be utilised, without offering a route for utilisation of produced CO₂. Due to the widespread use of AD to treat sewage sludge, its growing implementation for stabilising other organic substrates (e.g. food waste, slurries, crops and mixed substrates) (Bajón Fernández et al., 2012) and the requirement to meet more stringent CO₂ reduction targets (Climate Change Act, 2008), there is a need to investigate and implement technologies for storing or utilising the CO₂ contained in biogas.

Carbon capture and storage in geological or oceanic reservoirs is currently considered as one of the most promising solutions for dealing with CO₂ emissions (DECC, 2012). However, this technique relies on transporting CO₂ to a final reservoir, which may limit its application for storing CO₂ produced in ADs, since organic waste and wastewater treatment plants are sparsely located and of varied size. Biological conversion of CO₂ to CH₄ in anaerobic processes (e.g. upflow anaerobic sludge blanket (UASB) reactors, ADs) has been stated as a potential alternative for onsite treatment of CO₂ concentrated streams, with the added benefit of enhancing renewable energy production. Alimahmoodi and Mulligan (2008) reported a 69-86% CO₂ uptake when enriching the influent of a UASB reactor with CO₂. Salomoni et al. (2011) evidenced the potential of CO₂ biological conversion in a two phase anaerobic digestion process and stated a 25% CH₄ yield increase when enriching with CO₂ the first stage. Bajón Fernández, et al. (2014) studied the benefits of CO₂ enrichment of ADs treating different substrates and observed a substrate dependant response to additional CO₂, with an estimated reduction of CO₂ emissions in batch units of 8-34% for sewage sludge and of 3-11% for food waste.

Although the capacity of anaerobic processes to utilise additional CO₂ has been demonstrated, previous studies have focused on quantifying the associated increase in CH₄ production, while a deep understanding of the mechanism by which the additional CO₂ is utilised is still lacking. Uptake of CO₂ has been reported as an overall term involving the contribution of all the reactions in which it may be consumed or produced (Alimahmoodi and Mulligan, 2008; Bajón Fernández et al., 2014), which enables a comparison between units with and without CO₂ enrichment, but fails to elucidate the mechanism behind CO₂ utilisation. The scarce literature available regarding CO₂ utilisation routes presents conflicting information in relation to its use in the hydrogenotrophic (Alimahmoodi and Mulligan, 2008) or acetoclastic (Francioso et al., 2010; Bajón Fernández et al., 2014) pathways for CH₄ formation and is based on hypotheses rather than on experimental evidence. Hence, a better understanding of the fate of additional CO₂ injected in ADs is required to help improve the process and verify that CO₂ is being utilised rather than captured as instable forms with the risk of re-release.

This paper investigates the utilisation of CO₂ injected into ADs by considering its impact on analytical parameters of the final digestate, its potential to form or dissolve carbonated precipitates and its biotransformation to CH₄. Firstly, digestates of food waste and sewage sludge ADs with and without CO₂ enrichment were compared in terms of pH, alkalinity and ammonia concentration, within other parameters. Secondly, environmental scanning electron microscope (ESEM) and X-ray diffraction (XRD) were utilised to detect the potential of CO₂ enrichment to form or dissolve carbonated precipitates in food waste ADs. Lastly, fluorescence in situ hybridisation (FISH) was used to detect differences in methanogenic microbial populations (*Methanosaetaceae*, *Methanobacteriaceae* and *Methanosarcinaceae*) between different digestates.

4.2. MATERIALS AND METHODS

4.2.1. Description and operation of anaerobic digesters

Nine batch AD units were operated for each of the two substrates treated: food waste and sewage sludge. Three operating conditions were tested in triplicates: control units, units enriched with CO₂ at the start of the digestion process and units enriched with CO₂ periodically. Each AD consisted of a 1 L bottle with a four port cap (Fisher Scientific, Loughborough, UK). Two ports were used for gas injection (CO₂ or nitrogen (N₂)) through Pyrex diffusers with a porosity of 3 and 15 mm diameter (Fisher Scientific, Loughborough, UK). One port was coupled to a “Y” shaped tubing connector from which one exit was connected to a MilliGascounter (Litre Meter Ltd., Buckinghamshire, UK) for biogas volume recording and the other was fitted with a septa for gas sampling. The last port was used for digestate sampling. The ADs were continuously stirred and immersed in a temperature controlled water bath maintained under mesophilic conditions (temperature of 38±0.5°C).

A working volume of 700 mL and an inoculum to substrate volatile solids (VS) ratio of 2:1 were used in the batch ADs. The materials to operate food waste units (macerated and digested food waste) were collected from a UK AD site treating 30,000 tonnes of organic waste per year. The materials for sewage sludge ADs (thickened waste activated sludge and digested sewage sludge) were from a UK wastewater treatment plant serving a 2.5 million population equivalent.

Gas with a CO₂ molar fraction (y_{CO_2}) of 0.9 (partial pressure (p_{CO_2}) of 1.3±0.2 bar) was bubbled through the test ADs (both enriched once or periodically with CO₂) for 20 minutes at the start of the digestion process. The control ADs were bubbled with N₂ for 5 minutes. These gas injection times were considered suitable based on the results reported by Bajón Fernández et al., (2014). The ADs operated with a periodic CO₂ injection regime were enriched once every 48 hours for 5 minutes with gas of $y_{CO_2}=0.9$. Both gases (N₂ and CO₂)

were supplied from gas cylinders (BOC, Manchester, UK) and their flowrate ($1.0 \text{ L}\cdot\text{min}^{-1}$ combined flowrate) was controlled by mass flow controllers (Premier Control Technologies, Norfolk, UK).

4.2.2. Ammonium or calcium carbonated precipitates detection

The potential formation of carbonated precipitates in ADs enriched with CO_2 was examined in digestate samples collected from a pilot-scale AD of 100 L working volume treating food waste. Analysis were performed in digestates of ADs treating food waste, as opposed to sewage sludge, because of the higher ammonia content associated with this substrate. Total ammonia concentration of digestate samples was $1798\pm 124 \text{ mg}\cdot\text{L}^{-1} \text{ NH}_4\text{-N}$.

Samples of unaltered digestate and of digestate bubbled with CO_2 during several hours were frozen at -80°C and freeze dried in an Alpha 1-2 LD freeze dryer (Martin Christ, Osterode am Harz, Germany) in order to condition the samples for XRD tests. Freeze drying was preferred over a heating process because of the thermal instability of ammonium carbonated species (Patnaik, 2003). In order to reduce the noise of the XRD spectra, part of the digestate was centrifuged at 4000 g for 20 minutes, the supernatant centrifuged again for 40 minutes and the new supernatant separated for analysis. Both unaltered supernatant and supernatant bubbled with CO_2 during several hours were freeze dried for XRD tests as previously described.

The diffraction spectra of the samples was obtained by XRD using a D5005 unit (Bruker, Coventry, UK) with a 2-theta range of $10\text{-}90^\circ$, a step size of 0.04° and step time of one second. The spectrum of samples with and without CO_2 enrichment were compared for potential differences in calcium carbonate (CaCO_3), ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$), ammonium bicarbonate (NH_4HCO_3) and ammonium carbamate ($\text{NH}_2\text{COONH}_4$). Three sites per dried sample were imaged with a XL30 ESEM (FEI, Oregon, USA) in order to obtain the weight percentage of present elements.

4.2.3. Fluorescence in situ hybridisation (FISH)

Food waste and sewage sludge samples were fixed for FISH analysis immediately after collection from the batch ADs. Sample aliquots (2 mL) were centrifuged at 4000 g for 10 minutes and the pellet re-suspended in 750 μL of phosphate buffer solution (PBS), treated with 250 μL of 4% paraformaldehyde solution (PFA) and incubated overnight at 4°C . The incubated sample was centrifuged under the same conditions and re-suspended in PBS twice to remove any residual PFA. Absolute ethanol was added to the final sample, which was then kept at -20°C until further analysis. On defrosting, 5 μL of fixed sample were placed on gelatin coated well slides, incubated at 46°C during 10 minutes and dehydrated for 3 minutes in each 50, 80 and 100% ethanol solutions. Each sample was loaded in 5 different wells.

Hybridisation was carried out at 46°C for 2 hours with 5µL of previously preheated hybridisation buffer and 1µL of each oligonucleotide probe. Hybridized samples were then rinsed and incubated in preheated (48°C) washing buffer for 20 minutes. Slides were finally rinsed with distilled water and air-dried in darkness. Details of the 16S rRNA-targeted oligonucleotide probes (Sigma-Aldrich, Madrid, Spain) used are summarised in Table 4.1. The following microorganisms were targeted: *Bacteria* with Eub338-Mix probe (Eub338, Eub338-II and Eub338-III in equimolar proportion), *Archaea* with Arch915 probe, *Methanosaetaceae* with Mx825 probe and *Methanobacteriaceae* with Mbac1174 probe. In food waste samples *Methanosarcinaceae* was targeted with MS1414 probe. The helper oligonucleotides hMS1395 and hMS1480 were used to improve the accessibility of the *Methanosarcinaceae* targeting probe to its binding site, as previously suggested by Crocetti et al., (2006). These helpers were added during hybridisation in equimolar proportion to the MS1414 probe. Non Eub338 probe was used as negative control. A poor cell wall permeability of the oligonucleotide probes was observed in food waste samples, which was addressed by adding freeze-thaw cycles consisting of 5 minute steps at -80°C and 60°C to the experimental protocol (Narihiro and Sekiguchi, 2011; Sekiguchi et al., 1999).

Images for FISH were acquired with a confocal laser scanning microscope (Nikon CS-1). Six images per well were captured at random positions, leading to 30 images per sample. In food waste samples there was a great spatial distribution of the targeted cells in the z-axis. In order to obtain representative images, z-stack images were generated by compiling fields collected every 0.5µm of the z-axis. A magnification of X600 was used in every case. The fluorescence of the images was enhanced with EZ-C1 software (Nikon, Melville, New York) and signal quantification was completed with daime software version 2.0 (Vienna, Austria). The biovolume fraction was calculated by setting *Methanosaetaceae* (Mx825), *Methanobacteriaceae* (Mbac1174) or *Methanosarcinaceae* (MS1414) as specific probes in relation to the general *Archaea* probe (Arch915).

Table 4.1. Oligonucleotide probes used for FISH analysis, with details of sequence and target microbial group.

Probe	Probe sequence (5' – 3')	Target microbial group	Fluorochrome	Colour	Reference
Eub338 ^a	GCTGCCTCCCGTAGGAGT	<i>Bacteria</i>	FITC	Green	(Amann et al., 1990)
Eub338-II ^a	GCAGCCACCCGTAGGTGT	<i>Bacteria</i>	FITC	Green	(Daims et al., 1999)
Eub338-III ^a	GCTGCCACCCGTAGGTGT	<i>Bacteria</i>	FITC	Green	(Daims et al., 1999)
Arch915	GTGCTCCCCCGCCAATTCCT	<i>Archaea</i>	Cy3	Red	(Stahl and Amann, 1991)
Mx825	TCGCACCGTGGCCGACACCTAGC	<i>Methanosaetaceae</i>	Cy5	Blue ^c	(Raskin et al., 1994)
Mbac1174	TACCGTCGTCCACTCCTTCCTC	<i>Methanobacteriaceae</i>	Cy5	Blue ^c	(Raskin et al., 1994)
MS1414 ^b	CTCACCCATACCTCACTCGGG	<i>Methanosarcinaceae</i>	Cy5	Blue ^c	(Raskin et al., 1994)
hMS1395 ^b	GGTTTGACGGGCGGTGTG	<i>Methanosarcinaceae helper</i>	-	-	(Crocetti et al., 2006)
hMS1480 ^b	CGACTTAACCCCTTGC	<i>Methanosarcinaceae helper</i>	-	-	(Crocetti et al., 2006)
Non Eub338	ACTCCTACGGGAGGCAGC	Non bacteria	FITC	-	(Wallner et al., 1993)

^a Eub338, Eub338-II and Eub338-III used in equimolar proportion as Eub338-Mix.

^b Probe only used in food waste samples.

^c Purple fluorescence when co-hybridized with Arch915.

4.2.4. Analytical methods

Ammonia, soluble chemical oxygen demand (sCOD), volatile fatty acids (VFA), alkalinity, total solids (TS) and VS (APHA, 2005) were analysed at the beginning and end of the digestion process. Ammonia, sCOD and VFA tests were completed in the solid free fraction of the samples. This was obtained by filtering through 0.45 μm pore size syringe-drive filters (MilliporeTM, Billerica, United States) with the methodology reported by Bajón Fernández et al. (2014). Ammonia and sCOD were quantified with Spectroquant test kits (VWR, Lutterworth, UK). Quantification of VFA was completed by high performance liquid chromatography (HPLC) performed in a Shimadzu VP Series unit (Milton Keynes, UK). The concentration of acetic acid, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid and iso-valeric acid were quantified and their sum reported as total VFA (TVFA) concentration. The methodology for VFA determination was similar to that of Soares et al. (2010) adapted for a HPLC run time of 60 minutes. Alkalinity was measured in the supernatant obtained after a double centrifugation process: samples centrifuged at 4700 g for 20 minutes and the supernatant centrifuged again for 40 minutes under the same conditions. It was expressed as $\text{mg CaCO}_3 \cdot \text{L}^{-1}$ and evaluated as partial alkalinity (PA) by titration to pH 5.75 (Jenkins et al., 1983) and as total alkalinity (TA) by titration to pH 4.3 (APHA, 2005). The volume of biogas produced in the ADs and its composition were monitored daily with MilliGascounters (Litre Meter Ltd., Buckinghamshire, UK) and a CSi 200 Series Gas Chromatograph (Cambridge Scientific Instruments Ltd., Witchford, UK), respectively.

4.3. RESULTS AND DISCUSSION

4.3.1. Biogas production following CO₂ enrichment and digestate characterisation

All the sewage sludge test ADs achieved a 2.1-2.4 fold increased CH₄ production in the 24 hours following the first CO₂ enrichment, which replicates the 2.4 fold increase obtained by Bajón Fernández et al., (2014) when using a similar p_{CO_2} for enrichment (ca. 1.3 bars). In ADs enriched periodically with CO₂, a second injection boosted the daily CH₄ production by 1.3-1.5 fold. However, the batch nature of the ADs led to the bulk of the CH₄ yield to be produced during the first days of operation (Astals et al., 2013), which resulted in lower increases for subsequent CO₂ injections. Overall, the test sewage sludge ADs (both enriched once and periodically with CO₂) achieved ca. 30% of their CH₄ yield during the first 48 hours of the digestion process, while the control ADs attained ca. 13%. Higher benefits were associated with CO₂ enrichment in sewage sludge ADs than in food waste units, where there was a more limited impact on CH₄ yield as previously evidenced by Bajón Fernández et

al., (2014), which reported a moderate increase in CH₄ yield (5-13%) in ADs treating food waste.

Digestate characterisation data and removal efficiencies obtained for the different ADs are outlined in Table 4.2. The alkalinity, ammonia, VFA, TS and VS content of the digestates was not significantly different between ADs with and without CO₂ enrichment. Only sCOD removal in ADs treating sewage sludge was increased from 23.4±2.3% for control ADs to 27.9±2.1% and 35.9±2.5% for ADs enriched with CO₂ once and periodically, respectively. A single CO₂ injection did not alter the pH of food waste units, whereas a slight decrease in the pH of the digestate of ADs enriched periodically with CO₂ was observed, of 0.4-0.5 pH units for food waste ADs and 0.7 pH units for sewage sludge ADs (Table 4.2). This pH drop could potentially be avoided by injecting CO₂ less frequently, since any acidification associated with CO₂ enrichment of sewage sludge ADs could be overcome in around 48 hours (Bajón Fernández et al., 2014). However, in every case the final pH was above the value of 6 which is stated as inhibitory for methanogenesis by Gerardi (2003) and no inhibitory effects in the digestion process were observed.

Table 4.2. Digestate characterisation and removal efficiencies for food waste and sewage sludge ADs. Format as *average ± standard deviation*.

	Food waste				Sewage sludge			
	Mixture to AD	DC ^a	Dsingle ^b	Dperiodic ^c	Mixture to AD	DC ^a	Dsingle ^b	Dperiodic ^c
Liquid phase								
pH	7.5±0	8.3±0.1	8.4±0.0	7.9±0.1	7.5±0	8.1±0.1	8.1±0.1	7.4±0.1
Ammonia (NH ₄ -N mg·L ⁻¹)	3350±0	3963±103	3998±297	4138±138	798±0	1194±49	1149±34	1185±27
TA (mg CaCO ₃ ·L ⁻¹)	-	15854±349	16083±581	16444±668	3367±0	4637±32	4663±5	4674±93
PA (mg CaCO ₃ ·L ⁻¹)	-	13569±969	14394±418	14287±694	2665±0	3796±140	3923±42	3641±64
TVFA (mg·L ⁻¹) ^d	2424±0	797±54	825±55	861±124	423±0	201±13	310±43	288±28
Acetic acid (as % of TVFA)	52±0	0±0	0±0	0±0	39±0	0±0	0±0	0±0
Removal efficiencies								
TS (%)	n/a	27.4±1.8	25.5±0.3	29.4±2.6	n/a	20.8±0.8	18.9±0.9	20.9±1.6
VS (%)	n/a	36.3±1.7	36.8±1.0	39.6±1.3	n/a	29.5±0.6	27.9±1.2	28.0±2.5
sCOD (%)	n/a	51.8±4.0	52.0±2.1	48.1±9.6	n/a	23.4±2.3	27.9±2.1	35.9±2.5

^a DC: Digester control.

^b Dsingle: Digesters enriched with $y_{CO_2}=0.9$ once at the start of the batch process.

^c Dperiodic: Digesters enriched periodically with $y_{CO_2}=0.9$.

^d Calculated as sum of acetic acid, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid and iso-valeric acid.

4.3.2. Potential formation of ammonium and calcium carbonated precipitates when enriching ADs with CO₂

Several tests were performed in the liquid and solid fractions of digestate samples in order to assess the potential of CO₂ enrichment to form or dissolve ammonium or calcium carbonated precipitates and to alter the ammonia concentration and TA levels. A similar ammonia concentration in the solid free fraction of test and control digestates was observed (ca. 4 g·L⁻¹ NH₄-N for food waste and ca. 1.2 g·L⁻¹ NH₄-N for sewage sludge) (Table 4.2), implying that neither ammonia desorption nor precipitation of ammonium carbonated species were substantial when enriching ADs with CO₂. Within the ammonia carbonated precipitates which could be formed (e.g., NH₂COONH₄, (NH₄)₂CO₃, NH₄HCO₃), NH₄HCO₃ is the most likely to be present in a wet environment under mesophilic conditions, a pH of ca. 8 and excess CO₂ (Bai and Yeh, 1997; Darde et al., 2010). However, this compound is highly soluble in water (366 g·L⁻¹ at 40°C (Patnaik, 2003)) which explains that it was not precipitated in spite of the significant ammonia concentration in the food waste digestate samples studied (ca. 1.8 g·L⁻¹ NH₄-N) and the excess of CO₂ available.

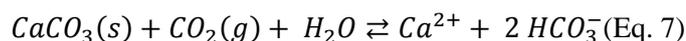
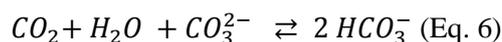
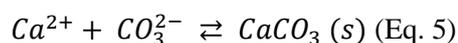
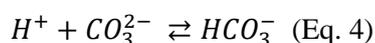
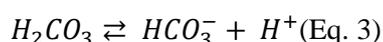
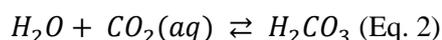
Dissolution of CO₂ was not expected to directly affect TA since it forms the same amount of molar equivalents from protons (H⁺) and carbonated species (carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻)); as mathematically demonstrated by Pankow, (1991). However, precipitation or dissolution of CaCO₃ would alter TA because of the two molar equivalents of the CO₃²⁻ group. Hence, analysis of TA has previously been identified as suitable to determine calcification rates (Chisholm and Gattuso, 1991). In this study, measurement of TA was performed in the supernatant after centrifugation (bulk of solids removed), which implies that formation or dissolution of carbonated precipitates would have altered the CO₃²⁻ or HCO₃⁻ to be accounted for in the titration. Removal of solids before titration avoided the re-dissolution of any precipitate present in the sample when adding acid.

Although CaCO₃ can naturally precipitate during digestion processes (Van Langerak et al., 1999), addition of CO₂ is expected to increase its solubility. Dissolution of CO₂ in an aqueous solution leads to the formation of carbonic acid (H₂CO₃) (Eq. 1 and Eq. 2), which dissociates forming H⁺ and HCO₃⁻ (Eq. 3). The hydrogen ions formed are in turn neutralized by reacting with CO₃²⁻, forming a higher amount of HCO₃⁻ (Eq. 4). Dissociation of HCO₃⁻ into CO₃²⁻ and H⁺ is possible (Eq. 4), although HCO₃⁻ is the predominant species at the pH of ca. 8 measured in the samples. The reduction in the concentration of CO₃²⁻ would alter the CaCO₃ equilibrium, increasing the solubility of this compound (Eq. 5). The overall effect of adding CO₂ to an aqueous solution would hence be an increase in the concentration of total dissolved inorganic carbon, HCO₃⁻ and dissolved CO₂ and a reduction of CO₃²⁻ concentration and pH,

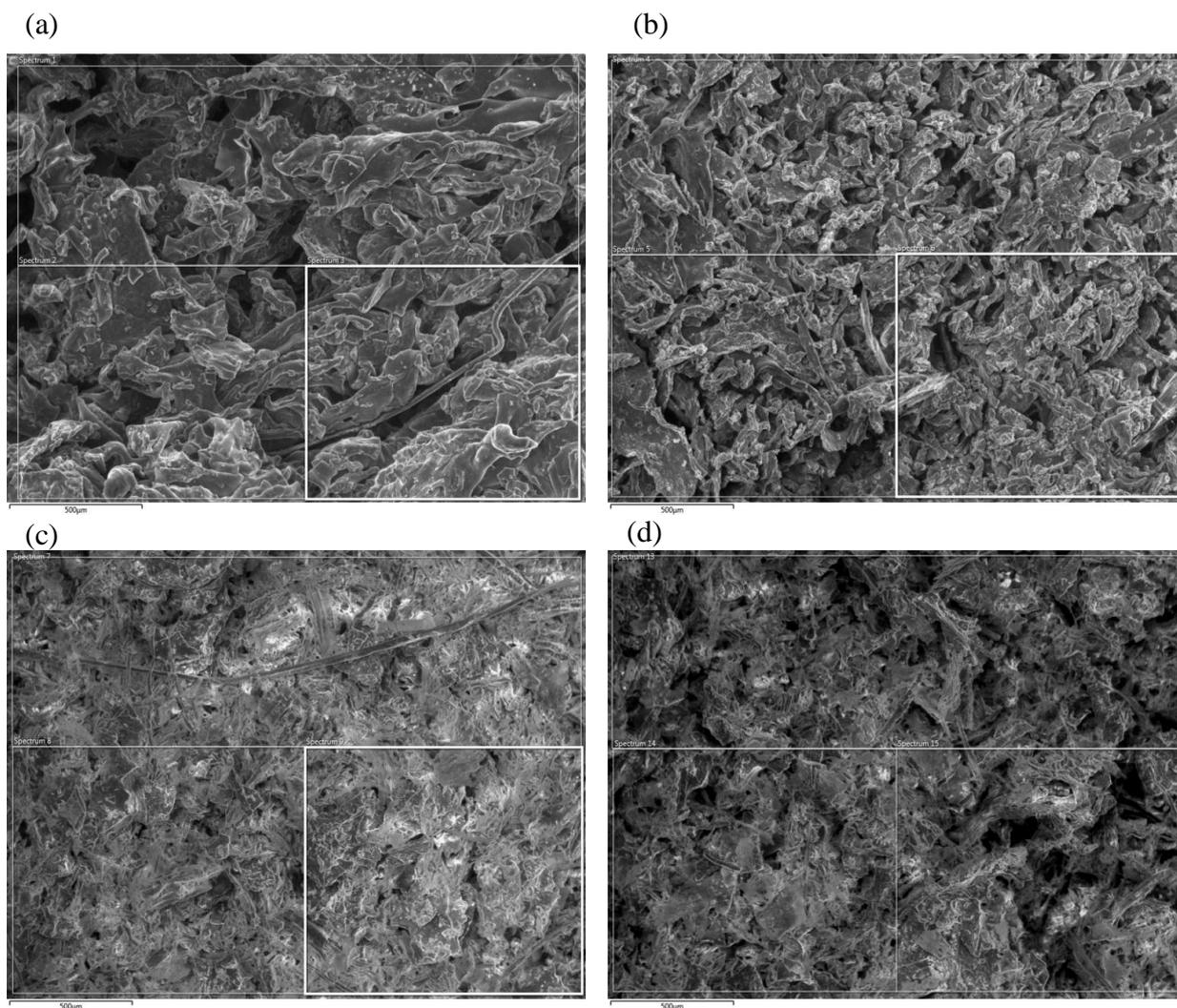
with the overall reaction of Eq. 6. In the presence of calcium, the consumption of CO_3^{2-} would in turn displace the CaCO_3 equilibrium towards the ionized form (Eq. 5). The overall reaction can be described with Eq. 7. The consistency in the TA values measured in control and test ADs both for sewage sludge and food waste (Table 4.2), suggested that precipitation or dissolution of CaCO_3 did not happen to a significant extent in the tests performed.

$$[\text{CO}_2(aq)] = \frac{p_{\text{CO}_2}}{k_H} \text{ (Eq. 1)}$$

Where k_H : Henry's constant for CO_2 , which relates its solubility in the liquid phase and its partial pressure in the gas phase



The elemental composition obtained by ESEM in freeze dried samples of food waste digestates (Figure 4.1) further supported that there was no enrichment or depletion of carbon and oxygen of the solid fraction of digestates bubbled with CO_2 . Carbon and oxygen accounted for $49.1 \pm 0.3\%$ and $32.1 \pm 0.8\%$, respectively, of the total weight elemental composition of the digestates' solid phase (Figure 4.1). Similar values of $47.6 \pm 0.2\%$ and $32.0 \pm 0.9\%$ were obtained when the digestate was bubbled with CO_2 for several hours prior to freeze drying. Consistent results were obtained when the tests were completed in the digestates' supernatant, where carbon and oxygen were found to account for $45.7 \pm 0.9\%$ and $32.9 \pm 1.3\%$, respectively, when CO_2 was not injected and for $43.2 \pm 0.4\%$ and $33.2 \pm 0.3\%$ when enriching the supernatant with CO_2 . Furthermore, no significant differences between the XRD spectrum of samples with and without CO_2 enrichment were observed to indicate formation or dissolution of carbonated precipitates.



	Ca (%)	O (%)	Na (%)	Mg (%)	Al (%)	Si (%)	P (%)	S (%)	Cl (%)	K (%)	Ca (%)	Fe (%)
Supernatant of digestate without CO ₂ enrichment ^a	45.7±0.9	32.9±1.3	8.6±0.1	0.4±0.0	-	0.1±0.0	0.4±0.0	0.1±0.0	6.4±0.6	3.2±0.1	2.1±0.1	-
Supernatant of digestate enriched with CO ₂ ^a	43.2±0.4	33.2±0.3	8.7±0.1	0.4±0.0	-	0.2±0.1	0.5±0.0	0.2±0.0	7.5±0.2	4.2±0.1	2.0±0.1	-
Digestate without CO ₂ enrichment	49.1±0.3	32.1±0.8	4.5±0.2	0.2±0.0	0.3±0.1	0.4±0.2	0.9±0.0	0.7±0.0	4.9±0.3	3.3±0.2	2.8±0.3	0.8±0.2
Digestate enriched with CO ₂	47.6±0.2	32.0±0.9	4.9±0.5	0.3±0.0	0.2±0.0	0.4±0.0	1.0±0.1	0.7±0.0	5.6±0.3	3.7±0.2	3.0±0.3	0.8±0.0

^a Supernatant obtained after a double centrifugation process as described in the *materials and methods* section.

Figure 4.1. ESEM images and elemental analysis of food waste AD digestates with and without CO₂ enrichment. Images correspond to: (a) supernatant of digestate without CO₂ enrichment, (b) supernatant of digestate enriched with CO₂, (c) digestate without CO₂ enrichment, (d) digestate enriched with CO₂. Format of elemental composition as average ± standard deviation with results in weight percentage.

4.3.3. Microbial populations diversity by FISH analysis

4.3.3.1. Microbial populations in batch ADs treating sewage sludge

A good fluorescence signal from all the rRNA-targeted probes was obtained in samples from ADs treating sewage sludge (Figure 4.2). *Methanosaetaceae* was the predominant *Archaea* in all the samples, accounting for $86.4 \pm 12.1\%$ of the *Archaea* population at the start of the batch digestion process (Table 4.3). Since *Methanosaetaceae* are obligate acetoclastic methanogens (formation of CH_4 by conversion of acetate), its major dominancy among the *Archaea* population evidenced a higher contribution of acetoclastic methanogenesis to the total CH_4 production than the commonly accepted 70% (Hansen et al., 1998). The relative abundance of *Methanobacteriaceae* at the start of the digestion trials was low ($11.0 \pm 4.1\%$ of the *Archaea* population) (Table 4.3), which confirmed the minor contribution of the hydrogenotrophic pathway towards total CH_4 formation. This is in agreement with previous studies reporting *Methanosaeta* sp. as the predominant methanogen in sewage sludge ADs (Vavilin et al., 2008).

Cell aggregates hybridised by the *Archaea* probe (Arch915) and not co-hybridised with the *Methanosaetaceae* (Mx825) or *Methanobacteriaceae* (Mbac1174) probes were clearly observed with Cy3 fluorochrome at the start of the digestion process (Figure 4.3 (a) and (b)). This clustered cell structure has been consistently associated with agglomerations of *Methanosarcina* sp. (Demirel and Scherer, 2008; Lübken et al., 2007; Vavilin et al., 2008). An oligonucleotide probe targeting *Methanosarcinaceae* in sewage sludge samples was not used. Nevertheless, visual observation showed a minor contribution of *Methanosarcinaceae* to the total *Archaea* population.

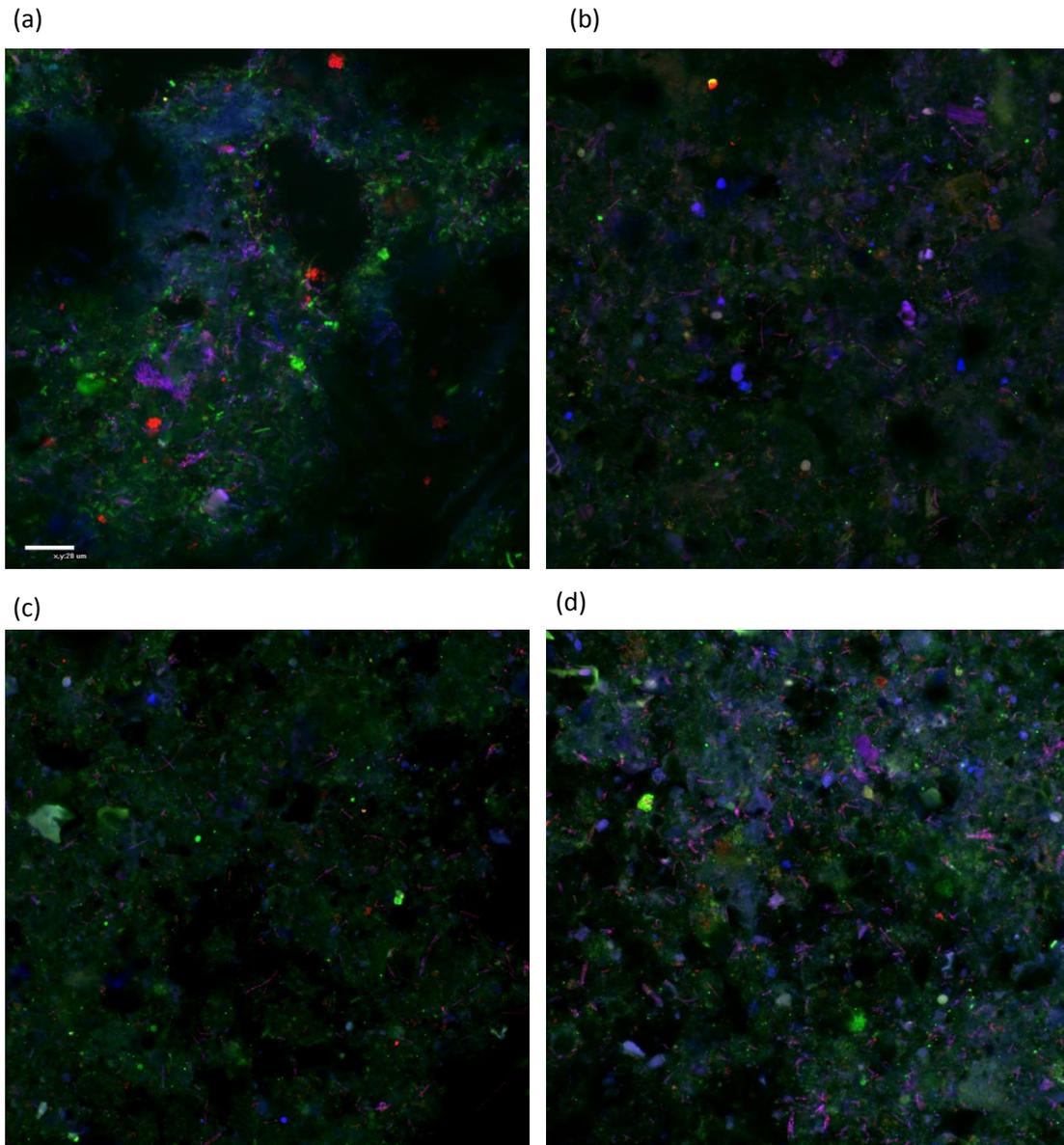


Figure 4.2. FISH images obtained for sewage sludge ADs at the start of the batch digestion process (a) and at the end for control ADs (b), ADs enriched once with CO₂ (c) and ADs enriched periodically with CO₂ (d). Cells in purple are *Methanosaetaceae* co-hybridised by the *Archaea* (Arch915) and the *Methanosaetaceae* (Mx825) probes.

This image is included in the CD in the back cover of this thesis.

Table 4.3 Percentage of volume of *Methanosaetaceae*, *Methanobacteriaceae* and *Methanosarcinaceae* when compared with the total *Archaea* population in sewage sludge and food waste ADs. Reported as *average ± standard deviation* of 30 images obtained per sample.

	Sewage sludge ADs ^a		Food waste ADs		
	<i>Methanosaetaceae</i> (%)	<i>Methanobacteriaceae</i> (%)	<i>Methanosaetaceae</i> (%)	<i>Methanobacteriaceae</i> (%)	<i>Methanosarcinaceae</i> (%)
Start of ADs	86.4±12.1	11.0±4.1	4.3±1.7	1.8±0.7	19.4±9.8
Digestate of control ADs	98.8±21.6	7.2±1.8	5.4±1.7	3.1±1.8	21.9±9.0
Digestate of ADs with single CO ₂ injection	83.8±12.4	8.0±2.6	5.8±2.1	2.1±0.8	32.4±15.1
Digestate of ADs with periodic CO ₂ injections	94.9±17.5	6.0±1.8	3.5±1.0	1.1±0.6	14.3±6.4

^a An oligonucleotide probe targeting *Methanosarcinaceae* was not used in sewage sludge samples.

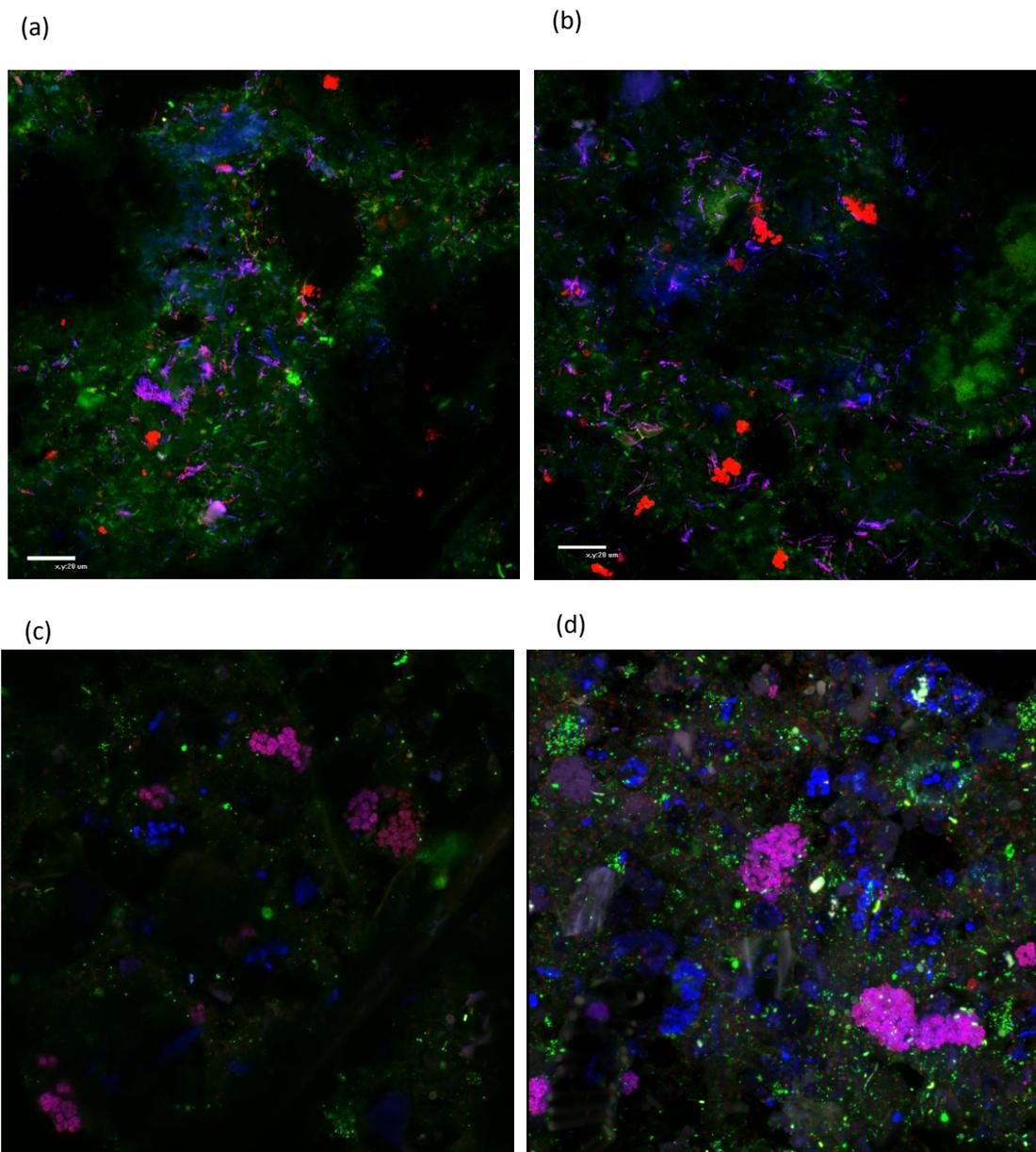


Figure 4.3. FISH images obtained for sewage sludge ((a) and (b)) and for food waste ((c) and (d)) at the start of the digestion process. In sewage sludge samples *Methanosarcinaceae* cells were those stained in red, only hybridized by the *Archaea* probe (Arch 915) and with a characteristic cluster structure. In food waste samples *Methanosarcinaceae* cells were stained in purple since they were co-hybridised by the *Archaea* (Arch 915) and the *Methanosarcinaceae* (MS1414) probes. Images (a), (b) and (c) correspond to a single field and image (d) to a z-stack image.

This image is included in the CD in the back cover of this thesis.

At the end of the batch digestion, the activity of both *Bacteria* and *Archaea* species were clearly reduced when compared to the start of the process. The rRNA attributable to *Methanosaetaceae* and *Methanobacteriaceae* cells was reduced by 65% and 53%, respectively, in control ADs; which was attributed to the batch nature of the tests leading to a decrease in the bacteria cells' activity with time and a reduction of the cellular rRNA content.

Starving cells have previously been reported to derive faint fluorescent signals during FISH analysis (Pernthaler et al., 2002). However, the reduction of *Bacteria* and *Archaea* activities during the batch digestion was less pronounced in ADs enriched with CO₂ (Figure 4.2 and Figure 4.4). In digestate samples of ADs enriched once with CO₂, the activity of *Methanosaetaceae* and *Methanobacteriaceae* at the end of the batch process was 46% and 24% higher (*p*-value of 0.002 and 0.256, respectively) than for the control units, respectively. The benefits were more emphasized in ADs enriched periodically with CO₂, with the fluorescence count attributable to *Methanosaetaceae* and *Methanobacteriaceae* being 80% and 32% higher (*p*-value of 0.001 and 0.125, respectively) than for the control ADs (Figure 4.2 and Figure 4.4). *Methanosarcina* spp. cell clusters were not noticeable in any of the digestate samples at the end of the digestion process (control, enriched with CO₂ once and periodically).

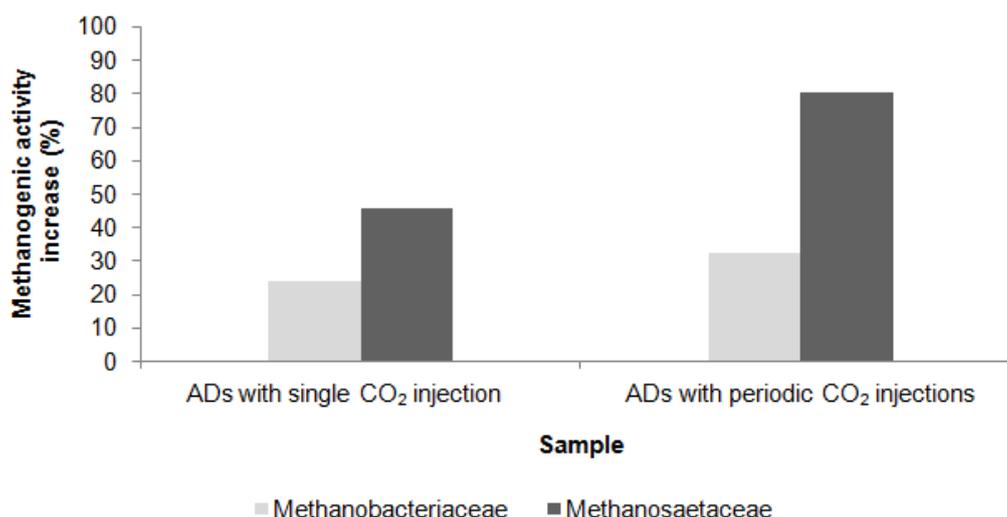


Figure 4.4. *Methanosaetaceae* and *Methanobacteriaceae* activity in digestate of test ADs (single and periodic CO₂ injections) compared to digestate of control ADs (no CO₂ injection) for sewage sludge units.

4.3.3.2. Microbial populations in batch ADs treating food waste

A weak signal of the *Bacteria* probe (Eub338-Mix) and a blue unspecific fluorescence with Cy5 fluorochrome were obtained when performing FISH tests in food waste samples with the same protocol as for sewage sludge. *Bacteria* (Eub338-Mix), *Archaea* (Arch915) and *Methanosaetaceae* (Mx825) probes were tested separately in order to detect any incompatibilities between them that could explain the blue fluorescence. However, the same blue staining was obtained in every case with Cy5 fluorochrome, suggesting that it was related to fluorescence from debris. The weak signal from the Eub338-Mix probe was attributed to a poor penetration into the bacteria cells. In order to improve cell wall permeability, freeze-thaw cycles consisting of 5 minute steps at -80°C and 60°C were

completed in the samples previously fixed with 4% PFA. A significantly enhanced hybridisation was achieved when five cycles were completed, as previously reported by Sekiguchi et al., (1999) and Narihiro and Sekiguchi, (2011). This additional step did not hinder hybridisation by any methanogen targeting probe and hence the method for FISH analysis was amended for all the food waste samples.

A great spatial distribution of the targeted cells was observed when collecting images at random positions on the gelatin coated well slides, both in the xy-plane and the z-axis. *Methanosarcinaceae* clusters were found mainly in the centre of the xy-plane and higher positions in the z-axis. On the contrary, *Bacteria* and other *Archaea* cells (*Methanosaetaceae* and *Methanobacteriaceae*) were predominant in the edge of the wells and lower positions of the z-axis. In order to account for this variability, images were collected at random positions of the xy-plane every 0.5µm of the z-axis and these fields were merged in a single z-stack image. A total of 30 z-stack images were used to complete the quantification analysis. The spatial distribution observed in the z-axis is evident in a video included as *supplementary data*.

Methanosarcinaceae was found to have the greatest contribution to the total *Archaea* population within the methanogens studied, accounting for 19.4±9.8% at the start of the digestion process (Table 4.3). The relative abundance of *Methanosaetaceae* and *Methanobacteriaceae* was estimated as minimal, with values of 4.3±1.7% and 1.8±0.7%, respectively (Table 4.3). The significant presence of *Methanosarcinaceae* is in good agreement with the results of Vavilin et al., (2008), who concluded that this *Archaea* is the prevailing methanogen in putrescible waste ADs. The scarcity of *Methanosaetaceae* was associated with the high total ammonia concentration of food waste ADs (ca. 3.4 g·L⁻¹ NH₄-N at the start of the digestion process) (Table 4.2), since levels >3 g·L⁻¹ NH₄-N have been reported as completely inhibitory for acetoclastic methanogens (Rajagopal et al., 2013). However, *Methanosarcina* related species present a higher tolerance to ammonia toxicity, due to the formation of big *Archaea* clusters (Calli et al., 2005; Vavilin et al., 2008). These clusters reduce ammonia diffusion due to a high volume to surface area ratio (Wiegant and Zeeman, 1986) and protect the cells located inside the clusters from inhibitors present in the fluid matrix (Macario et al., 1999). Furthermore, when comparing the size of *Methanosarcinaceae* agglomerates observed in food waste and sewage sludge samples (Figure 4.3), the morphology of these *Archaea* appeared to be affected by the ammonia concentration as larger clusters formed with higher ammonia contents. *Methanosarcinaceae* were dispersed in smaller aggregates in sewage sludge, where the total ammonia concentrations (0.8 g·L⁻¹ NH₄-N) did not reach inhibitory levels.

Furthermore, *Methanosarcina* spp. was shown to have a higher tolerance to acetate than *Methanosaeta* spp. (Griffin et al., 1998). Indeed, *Methanosarcina* spp. is often found in environments with acetate concentrations $>60 \text{ mg}\cdot\text{L}^{-1}$ as per Schmidt et al. (2000) and $>200 \text{ mg}\cdot\text{L}^{-1}$ as per Zheng and Raskin (2000). In food waste ADs the acetic acid concentration was determined to be 7.7 times that of sewage sludge units (Table 4.2), which may have led to a further inhibition of *Methanosaetaceae* populations.

In contrast with sewage sludge units, enrichment with CO_2 in food waste ADs did not lead to a clear increase in the activity of the methanogenic *Archaea* studied. The contribution of *Methanosarcinaceae* to the total *Archaea* population was estimated at $21.9\pm 9.0\%$, $32.4\pm 15.1\%$ and $14.3\pm 6.4\%$ for digestate samples of control ADs, ADs enriched once and periodically with CO_2 , respectively. The low activity of *Methanosaetaceae* and *Methanobacteriaceae* observed at the start of the digestion process was maintained for all the digestates. *Methanosaetaceae* accounted for $5.4\pm 1.7\%$, $5.8\pm 2.1\%$ and $3.5\pm 1.0\%$ of the *Archaea* population in digestate samples of control ADs and test ADs with single and periodic CO_2 injections, respectively. The contribution of *Methanobacteriaceae* was quantified at $3.1\pm 1.8\%$, $2.1\pm 0.8\%$ and $1.1\pm 0.6\%$ for control ADs and units with single and periodic CO_2 injections.

4.3.3.3. Mechanisms of CO_2 utilisation following AD enrichment based on FISH analysis

The complexity of the reactions involved in AD makes it difficult to elucidate the possible mechanisms of utilisation of additional CO_2 , which has led to conflicting information in the literature. Several studies have associated the benefits of CO_2 enrichment to a boost of the acetoclastic route of CH_4 formation (Francioso et al., 2010; Bajón Fernández et al., 2014), while others refer to an increase in hydrogenotrophic methanogenesis (Alimahmoodi and Mulligan, 2008). However, in no case have microbial community analyses been reported to confirm the proposed mechanism of utilization.

The benefit of CO_2 enrichment of ADs was found to be dependent upon the substrate treated, with increases in CH_4 production after a CO_2 injection being higher for ADs treating sewage sludge than for food waste. This finding was attributed to possible differences in the methanogenic populations and used to initially hypothesize a CO_2 mechanism of utilisation in a previous study by the same authors (Bajón Fernández et al., 2014). The high ammonia concentration in food waste digestates ($4 \text{ g}\cdot\text{L}^{-1} \text{ NH}_4\text{-N}$) led to speculate that obligate acetoclastic methanogens were inhibited for this substrate. On the contrary, ammonia concentrations in sewage sludge ADs ($1.2 \text{ g}\cdot\text{L}^{-1} \text{ NH}_4\text{-N}$) did not reach the inhibitory levels reported by Rajagopal et al. (2013). Therefore, both acetoclastic and hydrogenotrophic

metabolic pathways were understood to be active. It was hence hypothesized that the higher benefits associated with CO₂ enrichment in sewage sludge treating ADs could be attributable to an increase in acetoclastic methanogenesis. The microbial population analysis developed in the present study, provides experimental evidence to support this hypothesis. *Methanosaetaceae* was confirmed to be the dominant methanogen in sewage sludge ADs (86.4±12.1% at the start of the batch process) but was found to be limited in food waste units (4.3±1.7%), where *Methanosarcinaceae* was more abundant (19.4±9.8%). Since *Methanosaetaceae* is an obligate acetoclastic methanogen, its major dominancy among the *Archaea* population in sewage sludge implied that acetoclastic methanogenesis was the major contributing pathway for CH₄ formation. *Methanosarcinaceae* was more abundant in food waste. This is a versatile *Archaea* that can undertake methanogenesis by both acetoclastic and hydrogenotrophic routes (Galagan et al., 2002). Hence, its predominance among the *Archaea* species studied in food waste ADs, prevents a clear conclusion regarding which was the prevailing metabolic pathway of CH₄ formation.

Periodic CO₂ injections in sewage sludge ADs influenced methanogenic communities and led to an 80% and 32% increased fluorescence signal from *Methanosaetaceae* and *Methanobacteriaceae*, respectively, when compared with control units. *Methanosarcinaceae* clusters were not observed at the end of the batch tests completed with sewage sludge, irrespective of whether CO₂ was injected or not. These findings indicate that benefits in CH₄ production associated with CO₂ enrichment were mainly associated with a boost of *Methanosaetaceae* communities. The limited benefits of CO₂ enrichment observed in food waste ADs, where *Methanosarcinaceae* was found to be the predominant within the methanogens studied, further confirmed that this *Archaea* was not responsible for the benefits associated with CO₂ enrichment and that the possibility of applying CO₂ enrichment of ADs as a CO₂ revalorisation strategy should be applied when *Methanosaeta* species are present.

In the acetoclastic pathway of CH₄ formation (Eq. 8) CO₂ is a product rather than a substrate, which implies that the observed activity enhancement of *Methanosaetaceae* following CO₂ injection must be associated with an indirect impact. It appears possible that additional CO₂ injected into ADs was reduced by the Wood-Ljungdahl pathway, leading to formation of acetate that acted as substrate for acetoclastic methanogenesis.



4.4. CONCLUSIONS

The mechanisms for CO₂ utilisation in ADs were studied by investigating its impact on analytical parameters, on formation or dissolution of carbonated precipitates and on microbial communities. The comparison of ammonia and TA levels in digestates of control

and tests ADs revealed that chemical utilisation of additional CO₂ did not occur to a significant extent. This was further confirmed by completing XRD and ESEM tests in digestate samples, since an enrichment or depletion in carbon and oxygen in the solid fraction was not observed when injecting CO₂. However, an alteration in the activity of the methanogenic communities present in the ADs was obtained, with the activity of *Methanosaetaceae* being 80% higher in ADs where CO₂ was injected periodically. The added CO₂ was therefore shown to be biologically utilised by acetoclastic methanogenesis to produce CH₄, which was likely preceded by an enhanced acetate formation through the Wood-Ljungdahl pathway.

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CHAPTER 5

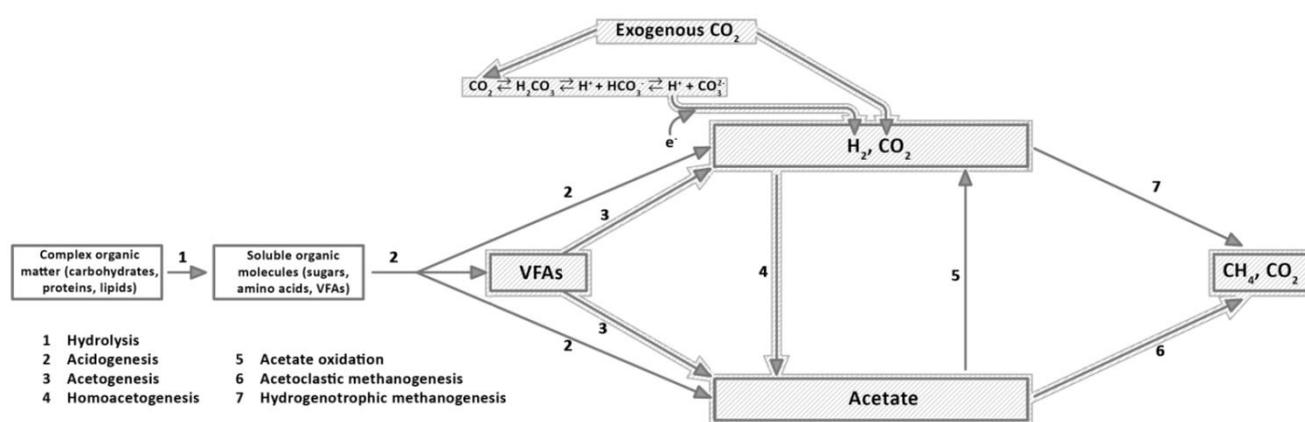
BIOLOGICAL CARBON DIOXIDE UTILISATION IN FOOD WASTE ANAEROBIC DIGESTERS

5. BIOLOGICAL CARBON DIOXIDE UTILISATION IN FOOD WASTE ANAEROBIC DIGESTERS

HIGHLIGHTS

- A pilot-scale AD with CO₂ injection treating food waste operated for the first time.
- Injection of CO₂ with a bubble column achieved without diluting the AD's headspace.
- Concentration of H₂ increased by 2.5 fold after four CO₂ injections.
- A mechanism of CO₂ utilisation has been proposed.

GRAPHICAL ABSTRACT



ABSTRACT

Carbon dioxide (CO₂) enrichment in anaerobic digestion (AD) was previously identified as a potential on-site carbon revalorisation strategy to reduce the carbon footprint of the water and organic waste sectors and improve methane (CH₄) production. However, the lack of studies utilising non-laboratory-scale units limits implementation. In this study a pilot-scale AD treating food waste was injected with CO₂ using a bubble column, which resulted in negligible CH₄ losses or headspace dilution. A 2.5 fold increase in hydrogen (H₂) concentration after four CO₂ injections was observed, which was attributed to CO₂ dissolution and to an alteration of acidogenesis and acetogenesis. Further increases in H₂ concentration did not occur, most probably due its assimilation by the Wood-Ljungdahl pathway, which is stimulated by availability of exogenous CO₂. Acetate generated by this pathway was most likely utilised by the increased activity observed for obligate acetoclastic *Archaea*.

KEYWORDS

Anaerobic digestion, bubble column, carbon dioxide utilisation, food waste, hydrogen.

5.1. 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 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., 2015a). Implementation of revalorisation strategies for biogas CO₂ has been suggested as 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 (Bajón Fernández et al., 2015a).

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 (Bajón Fernández et al., 2015a). 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 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 CO₂ enrichment could be retrofitted 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 bioconverted 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., 2015b). In this case, an increase in acetoclastic methanogenic activity (*Methanosaetaceae*) 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 and 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.

5.2. MATERIALS AND METHODS

5.2.1. 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 cone base with a total volume of 193 L, of which 106 L were liquid working volume (Figure 5.1). 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).

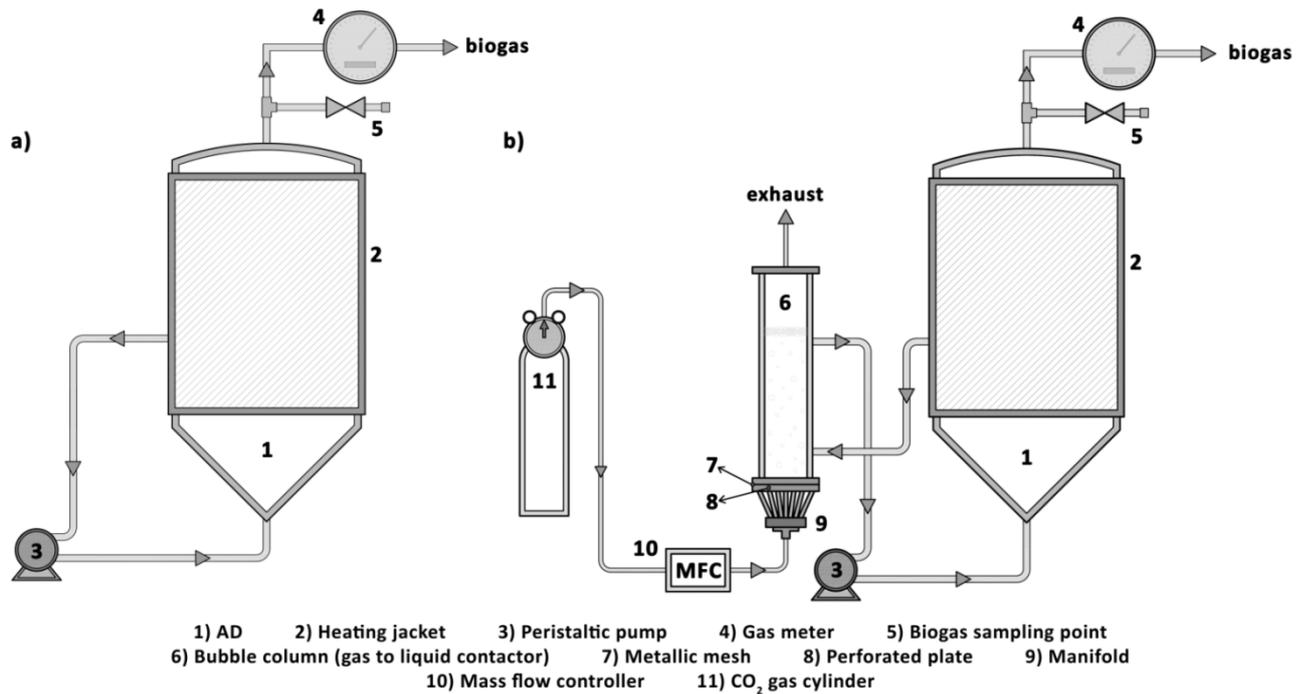


Figure 5.1. Schematic representation of the pilot-scale experimental rig. (a) Conventionally operated AD and (b) AD retrofitted with an external bubble column for CO₂ injection.

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. 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 $2.8 \pm 0.3 \text{ kg VS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ 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 (Figure 5.1). The column was operated with a 7 L working volume and CO₂ and digestate contact was in a co-current mode (Figure 5.1). 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 means of a mass flow controller (MFC) (Premier Control Technologies, Norfolk, UK), fixed at $1.5 \text{ L} \cdot \text{min}^{-1}$ 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 and 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 (Figure 5.1) were monitored online with BCP sensors (Bluesens, Herten, Germany), which were connected to a logging computer using BacVis software (Bluesens, Herten, Germany) for data recording.

5.2.2. Analytical methods

The composition and volume of biogas produced by the ADs were measured daily by means of a LMSXi multifunction gas analyser (Gas Data, Coventry, England) and TG05/5 gas meters equipped with a totalizer (Ritter, Bochum, Germany). 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 was completed according to APHA (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 $1.2 \text{ }\mu\text{m}$ pore size microfiber filters GF/C (WhatmanTM, Kent, UK). The solid free fraction was then obtained with a last filtration stage through $0.45 \text{ }\mu\text{m}$ pore size syringe-drive filters (MilliporeTM,

Billerica, United States). Ammonia was quantified with Spectroquant test kits. High performance liquid chromatography (HPLC) (Shimadzu VP Series unit, Milton Keynes, UK) was utilised to quantify 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. 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. 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 4.3, respectively. Ripley ratio ($RR = IA/PA$) was used as an indicator of digestion stability, with OLR being temporary reduced or the feed's pH further increased when $RR > 0.3$. 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).

5.3. RESULTS

5.3.1. Substrate characterisation

The feed substrate was characterised by an ammonia concentration of $361 \pm 20 \text{ mg} \cdot \text{L}^{-1}$ $NH_4\text{-N}$, a TVFA content of $21114 \pm 1723 \text{ mg} \cdot \text{L}^{-1}$, of which acetic acid was $81 \pm 5\%$; and a pH of 3.6 ± 0.5 (Table 5.1). The pH was raised to 5.7 ± 0.6 units by addition of sodium hydroxide or calcium carbonate (Fisher Scientific, Loughborough, UK) and, when required, water was added to adjust the material's solids concentration. The final pre-conditioned substrate contained $9.7 \pm 1.4\%$ TS, of which VS = $83.1 \pm 9.8\%$.

Table 5.1. Characterisation of digester feed, digestate and headspace of the control and tests ADs.

	Feed	Control AD	Test AD	
			Before CO ₂ enrichment	With CO ₂ enrichment
<i>Digestate monitoring</i>				
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·L ⁻¹ NH ₄ -N)	361±20	1855±205	1798±124	1807±166
VFA concentration (mg·L ⁻¹)	21114±1723	10707±313 ^(a)	9470±739 ^(a)	3662±44 ^(b)
<i>Headspace monitoring</i>				
CH ₄ production rate (m ³ CH ₄ ·(kg VS _{fed} ·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 Figure 5.3.

^(b) Value on day 153. For VFA dynamics refer to Figure 5.3.

5.3.2. Assessment of digestion performance: digestate quality and renewable energy enhancement

A stable digestion was observed in both 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 $1.8\text{-}1.9\text{ g}\cdot\text{L}^{-1}$ $\text{NH}_4\text{-N}$ in both cases. Prior to CO_2 injection a TVFA concentration of $10707\pm 313\text{ mg}\cdot\text{L}^{-1}$ with $67\pm 3\%$ acetic acid and of $9470\pm 739\text{ mg}\cdot\text{L}^{-1}$ with $57\pm 6\%$ acetic acid were recorded in the control and test ADs, respectively (day 134 of operation). The high VFA levels did not hinder process performance. Digestion stability was monitored as per RR, 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 and normal feeding rates resumed after few days. The average VS reduction of the substrate was of 74% and 76% for the control and test ADs, respectively, from the time of commencement of CO_2 enrichment until completion of the trials.

Control and test ADs were operated in a similar manner until day 148, when CO_2 injection into the test unit commenced. During the period without CO_2 enrichment a specific CH_4 production rate of $0.53\pm 0.16\text{ m}^3\text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$ and a CH_4 concentration of $68.3\pm 5.7\%$ for the control AD and of $0.45\pm 0.05\text{ m}^3\text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$ and a CH_4 content of $68.8\pm 3.4\%$ 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). After CO_2 enrichment of the test AD commenced, CH_4 production was $0.56\pm 0.13\text{ m}^3\text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$ with a CH_4 content of $68.5\pm 3.4\%$. This was recorded from 24 hours after the first CO_2 injection until completion of the trials. When compared with the CH_4 production rate before injection ($0.45\pm 0.05\text{ m}^3\text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$), it implied a *ca.* 20% improvement (p -value of 0.058), which is in agreement with previous literature in the topic (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 ($0.56\pm 0.13\text{ m}^3\text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$) with that of the control unit ($0.53\pm 0.16\text{ m}^3\text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$). This suggests that any improvement was not appreciable due to the natural variability of the performance of the biological process (high standard deviation).

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 of 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 (Figure 5.2), with an average value of the daily recordings of 320 ± 153 ppm between days 149 and 225 of operation. 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). A stable operation of the test AD was observed in spite of the H_2 concentration reaching values of over 600 ppm on several occasions (Figure 5.2).

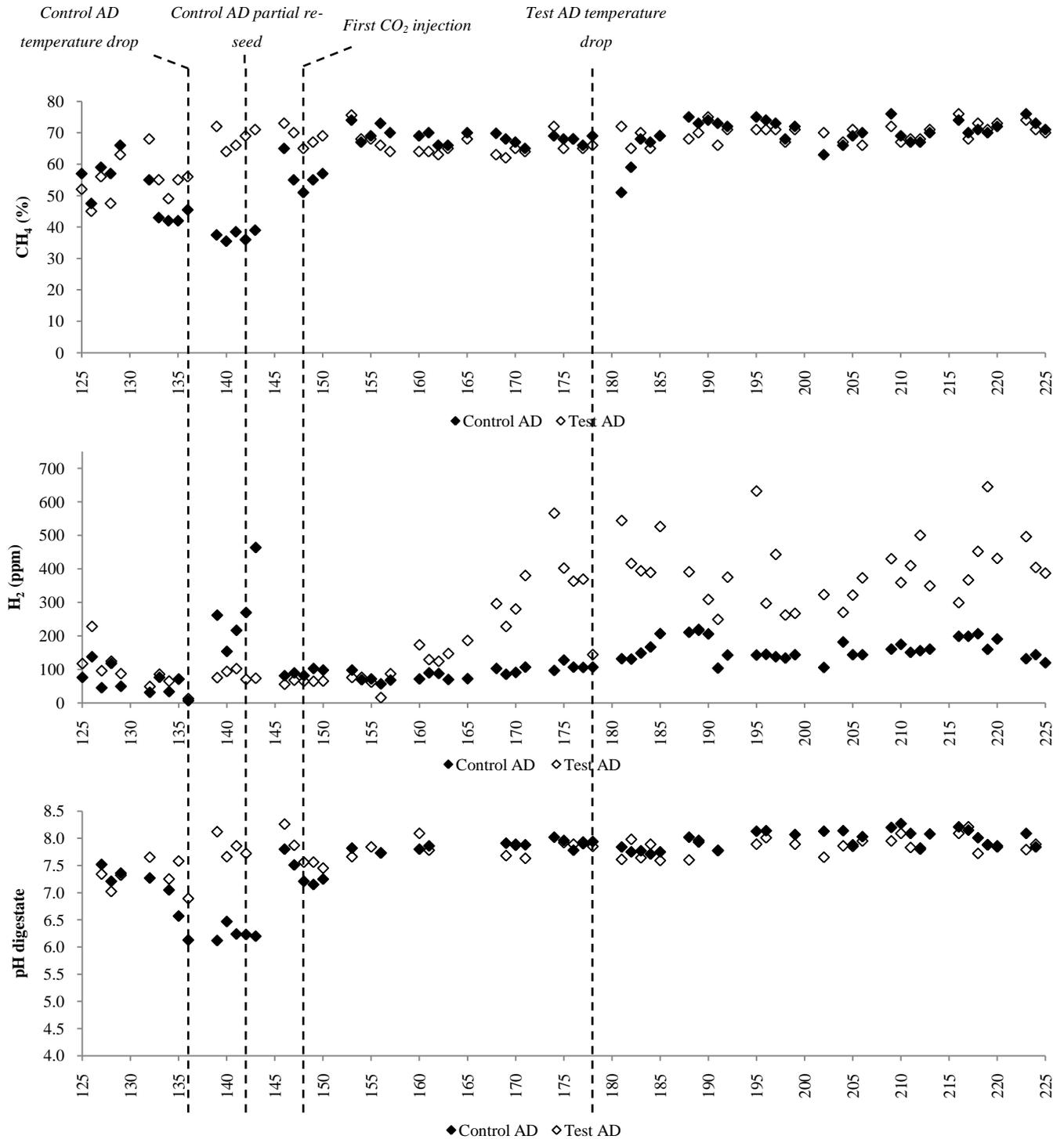


Figure 5.2. Evolution of CH_4 and H_2 biogas concentration and digester pH in the control and test ADs during the pilot scale digestion trials.

During each CO₂ 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 CO₂ injection was not applied. Bajón Fernández et al., (2015b) previously reported a decrease of 0.4-0.5 pH units in digestates of food waste laboratory scale ADs periodically enriched with CO₂. The time for recovering pH levels prior to CO₂ enrichment are lower in the present study (≤24 hours) than in previous investigations. This was attributable to the lower efficiency of the gas to liquid mass transfer system used in pilot scale (i.e. bubble column) than in laboratory scale trials (i.e. sintered diffusers), which led to lower levels of dissolved CO₂.

Interestingly, two failures of the heating system occurred during the experimental period. 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 CH₄ production rate dropping by over an order of magnitude and oscillating between 2.8·10⁻² and 6.1·10⁻² m³ CH₄·(kg VS_{fed}·d)⁻¹ over the six days following the disturbance. A drop in H₂ concentration to 7 ppm was recorded the day when the temperature disturbance occurred. 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 17235±147 mg·L⁻¹ as opposed to the 10707±313 mg·L⁻¹ recorded on day 134 and the 9435±686 mg·L⁻¹ obtained in the test AD (Figure 5.3). The increase in TVFA concentration resulted from a rise in all the individual VFA tested except n-valeric acid. When comparing day 134 and day 139 of operation an increase in acetic and propionic acids concentration of 44% and 40%, respectively, was experienced (Figure 5.3). Of note was the 3.8 and 4.3 fold increase in iso-butyric and n-butyric acids level, respectively. Calcium carbonate was dosed 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. No evidence of recovery was observed in the control AD after further dosing alkalinity and stopping substrate feeding for four days. 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. This was performed with digestate collected from the same full-scale site from which the initial inoculum was

collected, in order to reduce the acclimation period required to reach stable 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 VS_{fed}·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 hours 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, a drop in H₂ content of the headspace was recorded the day of the disturbance, together with an immediate reduction in CH₄ production rate and a drop in the pH of the digesting media. Nevertheless, alterations were significantly lower than those previously recorded for the control AD, with a reduction in H₂ content from 369 ppm of the previous day (day 177 of operation) to 144 ppm, a CH₄ production rate decrease from 0.67 to 0.46 m³ CH₄·(kg VS_{fed}·d)⁻¹ and a pH drop of 0.24 units. An increase of TVFA concentration from 2473±153mg·L⁻¹ to 4764±145mg·L⁻¹ between days 175 and 178 was recorded. The extent of this change in TVFA level was within the normal variability observed during the entire digestion period (Figure 5.3) and 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 for a complete recovery of the initial digestion performance to be achieved, without any re-seeding required. A CH₄ production rate of 0.71 and 0.75 m³ CH₄·(kg VS_{fed}·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, which 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 (Figure 5.2). This further indicates that the higher H₂ content in the test AD was associated with the periodic addition of exogenous CO₂.

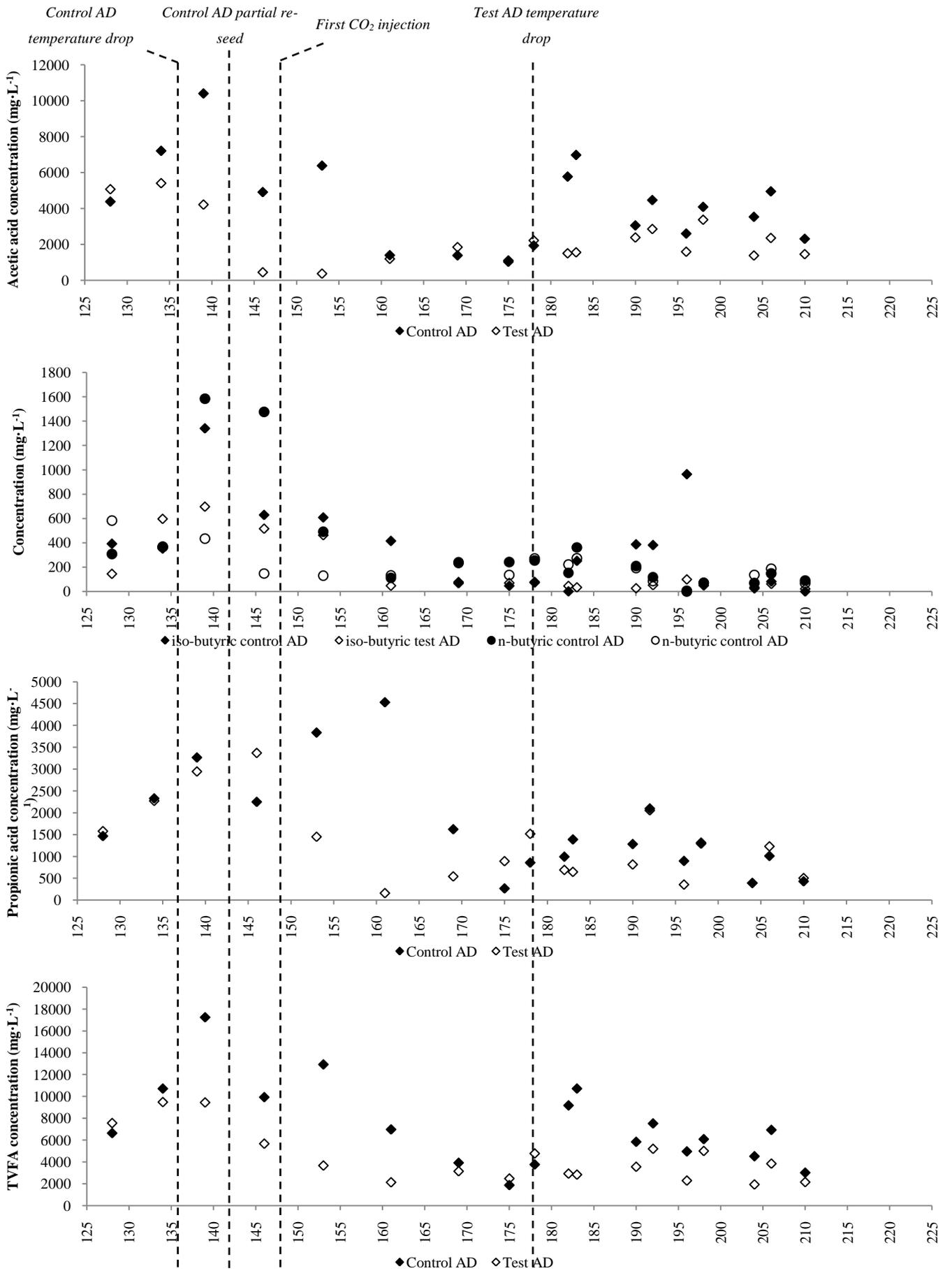


Figure 5.3. Dynamics of total and individual VFA digestate concentrations for control and test ADs during the pilot scale digestion trials.

5.3.3. 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 Figure 5.4. An average dissolved CO₂ concentration of $2.0\text{E-}3 \pm 5.3\text{E-}4 \text{ kmol}\cdot\text{m}^{-3}$ was recorded in the liquid phase of the control AD during the digestion trial. A similar value of $2.0\text{E-}3 \pm 5.9\text{E-}4 \text{ kmol}\cdot\text{m}^{-3}$ 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 $6.1\text{E-}3 \pm 1.4\text{E-}3 \text{ kmol}\cdot\text{m}^{-3}$ in the material exiting the bubble column (Figure 5.4). The mass transfer system was operated for an hour per CO₂ enrichment, with a reduced speed of the peristaltic pump in the recirculation loop of the AD in order for the whole digesting material to be contacted with incoming CO₂ within this time. Hence, all the content of the test AD was enriched with an additional $4.0\text{E-}3 \text{ kmol CO}_2\cdot\text{m}^{-3}$, implying an input of *ca.* $4.0\text{E-}4 \text{ kmol}$ of exogenous CO₂ (18455 mg CO₂) per enrichment when considering the working volume of the unit (106 L). Monitoring the dissolved CO₂ concentration confirmed the rapid utilisation of additional CO₂, since levels dropped from $6.1\text{E-}3 \pm 1.4\text{E-}3 \text{ kmol}\cdot\text{m}^{-3}$ obtained after enrichment to $2.0\text{E-}3 \pm 5.9\text{E-}4 \text{ kmol}\cdot\text{m}^{-3}$ within 24 hours. This utilisation rate of CO₂ matched the overcome of the slight acidification due to CO₂ enrichment in the 24 hours following a CO₂ injection. The additional CO₂ dissolved in the test AD was considered to be bioconverted to CH₄, as previously demonstrated in a previous study by the same authors (Bajón Fernández et al., 2015b). In that study periodic CO₂ injections into sewage sludge batch ADs led to an activity of *Methanosaetaceae* up to 80% higher than in control units, which was attributed to exogenous CO₂ indirectly benefiting acetoclastic methanogenesis. It was proposed that exogenous CO₂ was reduced to acetate by the Wood-Ljungdahl pathway, which could have enhanced activity of obligate acetoclastic *Archaea* (e.g. *Methanosaetaceae*) because of a higher substrate (acetate) availability.

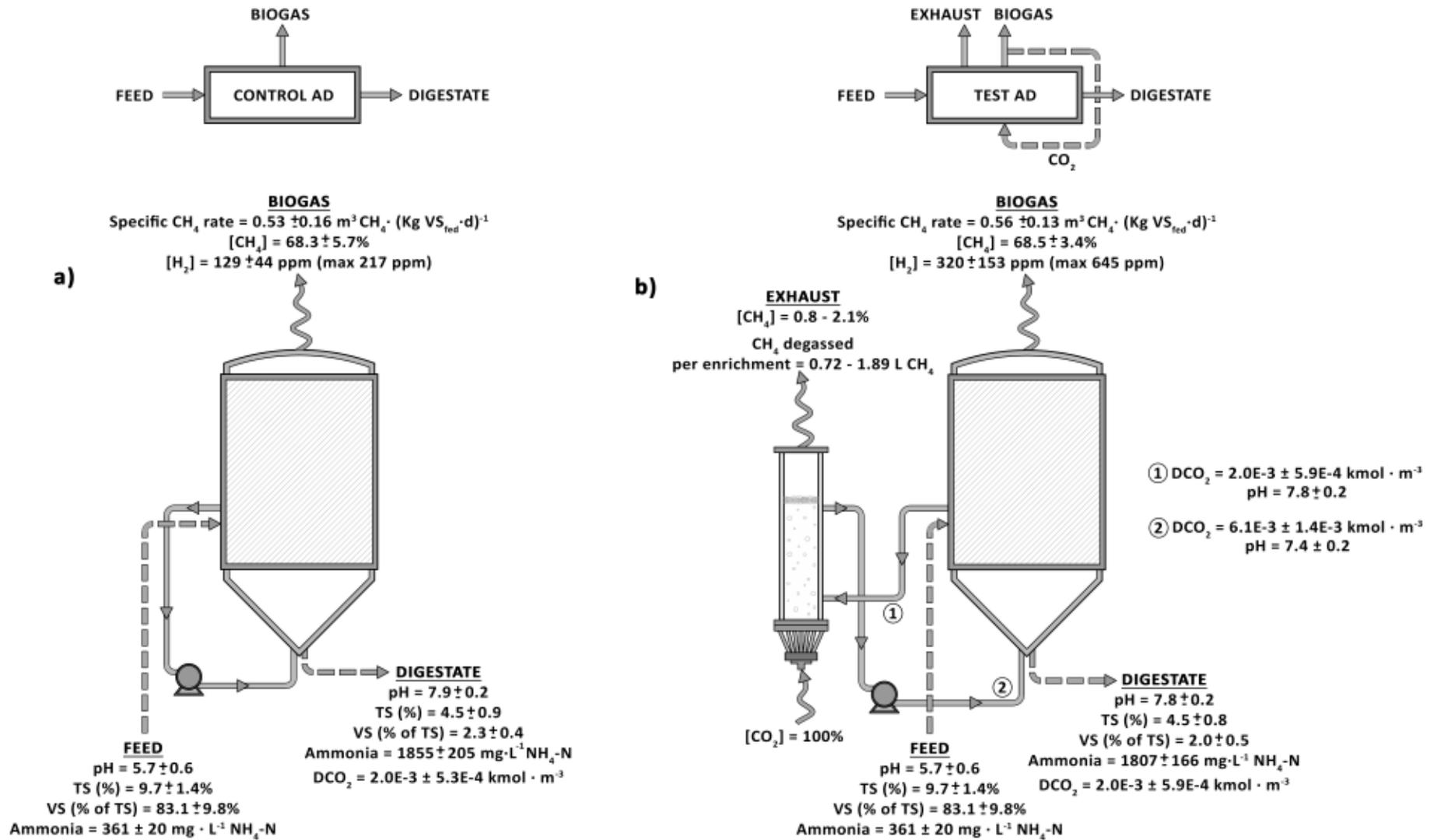


Figure 5.4. Schematic summary of performance of (a) control and (b) test ADs in terms of digestate quality and biogas production. Recorded dissolved CO_2 concentrations are also included.

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 (Figure 5.4). 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 hours) 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 5.1). 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 would not have a significant positive benefit in controlling ammonia inhibition, which contradicts previous literature investigating free ammonia removal in ADs by stripping it with biogas (Abouelenien et al., 2010; Serna-Maza et al., 2014; Walker et al., 2011). The disagreement with previous studies was attributed to the influence of operating conditions in the performance of a mass transfer system for ammonia removal, since a pH close to 10 and temperatures over 70°C are required to effectively control ammonia toxicity in ADs by biogas stripping (Serna-Maza et al., 2014). Besides, the extent of ammonia desorption would be influenced by gas flowrate and bubble size, which highly affect mass transfer efficiency.

5.4. DISCUSSION

5.4.1. 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 CO₂ levels

by a 3 fold (from $2.0\text{E-}3\pm 5.9\text{E-}4 \text{ kmol}\cdot\text{m}^{-3}$ to $6.1\text{E-}3\pm 1.4\text{E-}3 \text{ kmol}\cdot\text{m}^{-3}$). However, the solubility of CO_2 at 38.5°C in aqueous solutions with a CO_2 partial pressure (p_{CO_2}) of 1atm is $2.4\text{E-}2 \text{ kmol}\cdot\text{m}^{-3}$ ($1071 \text{ mg}\cdot\text{L}^{-1}$) (Green and Perry, 2008). Therefore, the operated bubble column achieved only *ca.* 25% of the CO_2 that could have been dissolved at p_{CO_2} 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. This in turn determines the contribution towards reduction of carbon footprint that can be achieved 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, which would increase dissolved CO_2 levels and hence potential for carbon assimilation.

5.4.2. 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 $0.45\pm 0.05 \text{ m}^3 \text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$ before any CO_2 was applied. When this value is considered as a baseline, the CH_4 production rate observed during the time when CO_2 enrichment was applied ($0.56\pm 0.13 \text{ m}^3 \text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$) 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, an average specific CH_4 production of $0.53\pm 0.16 \text{ m}^3 \text{ CH}_4\cdot(\text{kg VS}_{\text{fed}}\cdot\text{d})^{-1}$ was recorded in the control AD over the studied period. When this value is considered as the baseline, no significant benefit of the CH_4 production (p -value of 0.261) associated with CO_2 enrichment was observed. This suggests that any improvement was not appreciable due to the natural variability of the performance of the biological process (high standard deviation).

To note was the impact observed in relation to the H_2 content of the biogas produced. This reached a baseline 2.5 fold higher in the test AD compared to the control unit (p -value < 0.001) during the period when CO_2 was periodically injected. This impact of CO_2 injection was not intuitively anticipated since CO_2 would be expected to consume available H_2 by hydrogenotrophic methanogenesis. This effect 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 H₂ concentration have been reported in response to process disturbances, 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). This in turn leads to an active hydrolysis, acidogenesis and acetogenesis inside the AD 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 of ADs (Rodríguez et al., 2006). Fluctuations of 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 (Figure 5.2) over a ≤ 23 hour 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 led 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 (Figure 5.2). 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 dynamics of VFA speciation within the AD. The increase in H₂ concentration of the control AD was simultaneous to a sudden increase in TVFA concentration (Figure 5.3), which reached 17235±147 mg·L⁻¹ on day 139. The major rise in individual VFA concentration was that of n-butyric acid (4.3 fold), although increases in acetic, propionic, iso-butyric and iso-valeric acids were also recorded (Figure 5.3). 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 H₂ would have resulted in unfavourable conditions for acetogenesis itself, leading to accumulation of VFAs of higher number of carbons (Figure 5.3). Eventually process failure occurred (sour AD) and a partial re-seed for stability recovery was required. The increase in H₂ concentration implied a H₂ partial pressure (p_{H2}) of 4.6·10⁻⁴ atm to be reached in the control unit (atmospheric pressure considered inside the AD), which is within the reported ranges reported to be

thermodynamically unfavourable for acetogenesis (10^{-4} atm and 10^{-3} atm for propionic and butyric acid, respectively (Cord-Ruwisch et al., 1997; Harper and Pohland, 1986; Kidby and Nedwell, 1991)).

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 (Figure 5.2 and Figure 5.3). In fact, TVFA and acetic acid were quantified at $3662 \pm 44 \text{ mg} \cdot \text{L}^{-1}$ and $369 \pm 18 \text{ mg} \cdot \text{L}^{-1}$, respectively, on day 153, which was lower than average values maintained during the entire digestion trials (Figure 5.3). In this case methanogenesis was not inhibited. 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 (Bajón Fernández et al., 2015a) 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 ($< -200 \text{ mV}$ (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 (Figure 5.5). 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., 2015b), 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.

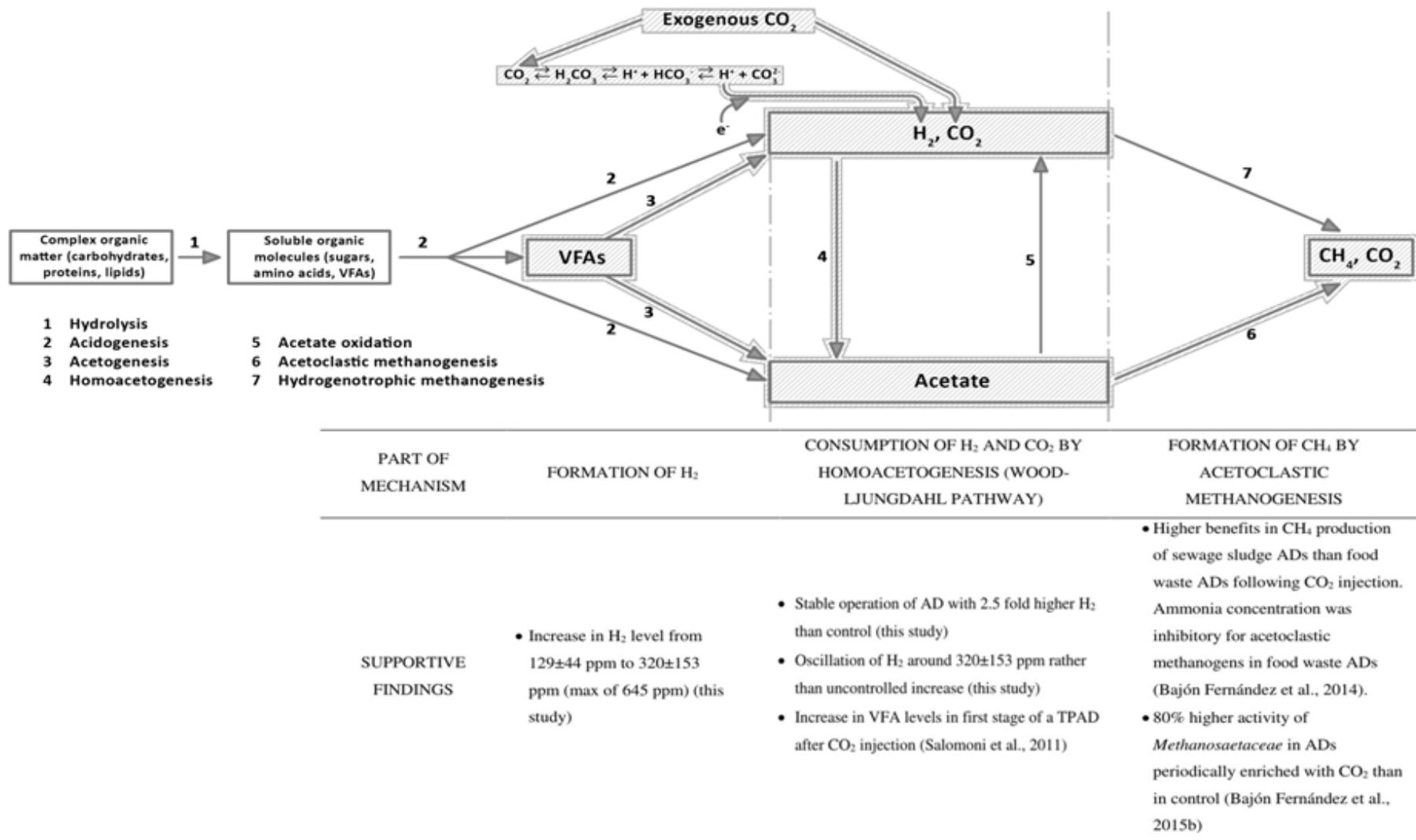


Figure 5.5. Proposed mechanism of exogenous CO₂ utilisation in ADs, with findings supporting each of the proposed stages.

It is of note that the H₂ concentration oscillated around 320±153 ppm, with peaks over 600 ppm but without a continuously increasing trend in spite of CO₂ being injected periodically. The fact that H₂ concentration did not increase further, suggests that additional H₂ produced was consumed in the AD. Assimilation of H₂ was likely by the Wood-Ljungdahl pathway of CO₂ fixation. This metabolic pathway can be stimulated by the availability of exogenous CO₂ (Misoph and Drake, 1996) and requires eight electrons and eight protons for each two molecules of CO₂ assimilated, which can be supplied by consumption of H₂ (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. Assimilation of H₂ by hydrogenotrophic methanogens was considered not to happen to a significant extent, since these *Archaea* have been reported to have a minimal contribution to the total amount of CH₄ formed at ammonia levels of 1.2 g·L⁻¹ NH₄-N (Bajón Fernández et al., 2015b), which is close to the 1.8-1.9 g·L⁻¹ NH₄-N maintained in the pilot plant unit.

It is then proposed that CO₂ leads to a boost of H₂ production, derived from the protons formed when dissolving CO₂ in the aqueous media, from a boost of obligate acetogenesis or from a combination of both (Figure 5.5). 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 acetoclastic 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 Figure 5.5, including previous findings that support the suggested hypothesis. Further work will be required to support or reject the proposed mechanism. In particular, bacteria analyses to understand the potential impact of CO₂ injection in acetogenesis are of great interest.

5.5. CONCLUSIONS

The practicalities of implementing CO₂ enrichment in up-scaled ADs 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%). An exogenous CO₂ uptake of 4.0E-3 kmol CO₂·m⁻³ per enrichment was recorded, which could be augmented if the bubble column mass transfer efficiency was increased. 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 likely uptaken by Wood-

Ljungdahl pathway and the acetate generated by this in turn assimilated by an increased activity of obligate acetoclastic *Archaea*.

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CHAPTER 6

GAS TO LIQUID MASS TRANSFER IN VISCOUS FLUIDS

6. GAS TO LIQUID MASS TRANSFER IN VISCOUS FLUIDS

HIGHLIGHTS

- The impact of μ_a on mass transfer was studied for different liquid rheologies.
- Reduction of $k_L a$ with μ_a was non-linear for Newtonian and non-Newtonian fluids.
- Impact of μ_a and U_G in $k_L a$ was predominantly influenced by changes in hydrodynamics.
- Slug-annular flow was formed in shear thinning fluids, with a reduced active volume.
- Rheology and μ_a need to be considered in design of sludge mass transfer systems.

ABSTRACT

The increase of studies relating on gas to liquid mass transfer in digested sludge (shear thinning fluid) necessitates a better understanding of the impact of apparent viscosity (μ_a) and rheology in process performance. Mass transfer retardation due to μ_a variations was investigated in a pilot scale absorption bubble column for Newtonian and shear thinning fluids with varied superficial gas velocities (U_G). A non-linear reduction of mass transfer efficiency with increasing μ_a was observed, being the impact higher at low μ_a ranges and high U_G . An increase of 114 cPo in μ from 1.01 to 115 cPo in glycerol solutions saturated with $U_G = 1.73 \text{ cm}\cdot\text{s}^{-1}$ led to a reduction of 96% in $k_L a$ ($\alpha = 0.04$), while a comparable raise from 115 to 229 cPo implied a reduction of 52% ($\alpha = 0.02$).

Slug-annular flow regime was identified for shear thinning fluids of high μ_a (1.0 and 1.5% carboxymethyl cellulose sodium salt solutions), where bubble buoyancy was conditioned by the μ of the fluid at rest and the active volume for mass transfer was reduced because of the presence of stagnant areas. Conditions imitating the rheological variability of anaerobically digested sewage sludge were included within those tested, being a reduction in gas transfer efficiency of 6 percentage points (from $7.6 \pm 0.3\%$ to $1.6 \pm 0.1\%$) recorded when increasing μ_a from 130 to 340 cPo. It is thus recommended that rheology and μ_a variability are accounted for within the design of gas to liquid mass transfer systems involving digested sewage sludge, in order to avoid reductions in process performance and active volume.

KEYWORDS

Mass transfer, viscous fluids, anaerobic digestion.

6.1. INTRODUCTION

Unit processes that utilise mass transfer in gas-liquid systems are widely used in both the environmental and industrial sectors in processes such as polyester production (Xiong et al., 2003), fermentation broths cultivation (Badino et al., 2001), xanthan gum production

(García-Ochoa et al., 2000) and wastewater treatment. The fluids being considered are often of non simple rheology and exhibit localised variation in viscosities (μ) making understanding of such systems difficult. A recent new area of consideration relates to the anaerobic digestion of organic waste and municipal wastewater in processes such as ammonia stripping from digestate to reduce toxicity (Walker et al., 2011) and carbon dioxide (CO_2) enrichment of sewage sludge or food waste for enhanced biogas production and carbon uptake (Bajón Fernández et al., 2014). For instance, sewage sludge is considered a fluid of complex matrix exhibiting pseudoplastic rheological behaviour (Baudez et al., 2011; Eshtiaghi et al., 2012), with its apparent viscosity (μ_a) being highly affected by temperature (Hammadi et al., 2012), solid content (Brar et al., 2005; Goel et al., 2004; USEPA, 1979) or shear history (Honey and Pretorius, 2000). Typical apparent viscosities of such sludges are commonly thought to range between 150-400 cPo (based on a 2-4% total solid content), although ranges as wide as 50-1000 cPo have been reported (Table 6.1). This μ_a variability and complex rheology challenges mass transfer process design based on empirical correlations obtained for Newtonian fluids and necessitates an understanding of the impact that viscous and rheological variations can have in process performance.

Increased μ_a has consistently been reported to lead to mass transfer retardation (Ozbek and Gayik, 2001) and its impact reported in terms of volumetric mass transfer coefficient (k_La) reduction. However, gas to liquid mass transfer in viscous fluids is still poorly understood, particularly when considering fluids with non-Newtonian rheological behaviour (Martín et al., 2008). Although different fluids have been investigated, the comparison between studies in order to gain a broad understanding of the importance of μ_a and rheology on k_La is limited, due to the dependence of k_La on experimental set-up and considerations used for its calculation. Sparger type (Heijnen and Van't Riet, 1984), probe position (Gourich et al., 2008), probe dynamics (Gourich et al., 2008), assumptions for calculation (Gourich et al., 2008) and data truncation (Merchuk et al., 1990) can substantially affect k_La . This necessitates the development of studies that address the impact on mass transfer of μ_a , rheological behaviour and operational conditions with a single experimental set-up and reported assumptions for calculations.

Table 6.1. Rheological properties and viscosities reported for sludge. Where TS: total solids, SRT: solids retention time, SS: suspended solids.

Reference	Material	Material characterisation	Fluid behaviour	μ_a (cPo)	Conditions
(Goel et al., 2004)	Digested sludge	TS = 2.3% SRT = 58 d	pseudoplastic	200-400 cPo	
(Brar et al., 2005)	Fermented sludge	TS = 4%	pseudo-plastic thixotropic	≤ 150 cPo	T = 25°C $\dot{\gamma} = 36.71 \text{ s}^{-1}$
(Wu, 2010)	Liquid manure	$2.5\% \leq \text{TS} \leq 12.1\%$	pseudoplastic (if TS $\geq 2.5\%$)	<ul style="list-style-type: none"> • 6- 8 cPo if TS = 2.5% • 10- 30 cPo if TS = 5.4% • 250-2930 cPo if TS = 12.1% 	T = 35°C
(Frost, 1983)	Anaerobically digested sludge	SS = 24 kg·m ⁻³		37-406 cPo	
(Eshtiaghi et al., 2012)	Thickened digested sludge	TS = 3.23%		50-1000 cPo	T = 25°C
(USEPA, 1979)	Anaerobically digested sludge	$2\% \leq \text{TS} \leq 8\%$	pseudoplastic thixotropic	<ul style="list-style-type: none"> • ≤ 310 cPo if TS $\leq 4\%$ • 310-625cPo if TS = 4-5% 	μ_a at 30 rpm

The gap in knowledge is further evidenced when the reasons behind the impact of μ_a on mass transfer are considered. Several mechanisms have been proposed in the literature, such as poorer gas distribution (Ozbek and Gayik, 2001), formation of larger bubbles (Wicaksana et al., 2006), reduced bubble breakup due to a higher stability (Wilkinson et al., 1993), higher resistance for gas to liquid transfer (Badino et al., 2001), reduced coalescence efficiency due to a lower drainage velocity of the liquid film between bubbles (Martín et al., 2008), reduced bubble oscillation (Martín et al., 2008) or increased residence time (García-Abuín et al., 2013). Several of these mechanisms are dependent on the hydrodynamics inside of the bubble column, which have been reported as key for understanding mass transfer in multiphase reactors (Kantarci et al., 2005; Segur and Oberstar, 1951). The transition superficial gas velocity (U_{trans}) between bubbly and churn-turbulent flow regimes has been reported of particular interest to understand mass transfer (Hyndman et al., 1997). However, the effect of liquid μ_a and rheology in U_{trans} has not been extensively investigated so far (Kantarci et al., 2005). A better understanding is hence required to achieve a better design and process operation in industrial sectors where the performance of gas to liquid mass transfer systems can be hindered by μ_a variations.

This paper assessed the impact of μ_a on CO₂ gas to liquid mass transfer and hydrodynamics for fluids of different rheologies (Newtonian and non-Newtonian), with a

single experimental set-up and reported assumptions for $k_L a$ calculations. Absorption tests with varied μ_a , rheologies and gas flowrates were performed in a high aspect ratio bubble column, where $k_L a$ and flow regime patterns were investigated while considering dissolved gas measuring probe dynamics. Results are discussed in the basis of differences between fluids of varied rheologies and particularised for those conditions imitating the behaviour of anaerobically digested sewage sludge.

6.2. MATERIALS AND METHODS

6.2.1. Fluids selection and solutions preparation for the absorption tests

Absorption tests with liquid phases of different μ_a and rheological behaviours were performed, including those operational conditions mimicking the operation of ADs. The fluids to be used were selected based on having different rheologies, published μ_a data availability and stable μ_a to pH variations. Glycerol ($\geq 98\%$; Fisher Scientific, Loughborough, UK) and carboxymethyl cellulose sodium salt (CMC) ($M_w = 700,000$; Sigma-Aldrich, Dorset, UK) were chosen as Newtonian and non-Newtonian fluids, respectively. CMC is a shear thinning fluid, which follows the Cross model for power-law fluids (Eshtiaghi et al., 2012). It suitably mimics the rheological properties of digested sludge in steady state at high shear rates ($\dot{\gamma} > 20 \text{ s}^{-1}$) (Eshtiaghi et al., 2012). The μ_a of the solutions tested was obtained from the studies of Segur and Oberstar (1951) and Eshtiaghi et al. (2012) for glycerol and CMC, respectively.

The solutions to be used in the absorption tests were prepared by dissolving the correct amount of chemical in deionized (DI) water at room temperature. The CMC solutions were prepared under continuous stirring at 300-400 rpm using a RW20 digital paddle stirrer (IKA, Staufen, Germany). Any CMC aggregate was dissolved by stirring the solution at 200-250 rpm overnight.

6.2.2. Absorption experimental rig description

A high aspect ratio bubble column (2m tall and 10.1 cm diameter) was used to develop CO_2 absorption tests (Figure 6.1). The column was filled up to 1.7 meters with the liquid phase. A manifold divided the incoming gas stream into seven inlets, which were connected to a perforated plate placed at the bottom of the column. A metallic mesh with 0.5 mm hole size was mounted on top of the plate, acting as a finer diffuser. $\text{CO}_2(\text{g})$ was used for the absorption tests and $\text{N}_2(\text{g})$ was bubbled between replicates to achieve full desorption of the CO_2 . A sufficient resting time was allowed for any bubbles entrapped in the liquid phase to be removed. $\text{N}_2(\text{g})$ and $\text{CO}_2(\text{g})$ were supplied from gas cylinders (BOC, Manchester, United Kingdom) and their flowrate regulated by mass flow controllers (MFC) (Premier Control Technologies, Norfolk, United Kingdom). The free CO_2 concentration in the liquid phase was monitored using an InPro5000(i) dissolved CO_2 sensor (Mettler Toledo Ltd.,

Leicester, UK) installed in the centre of the cross sectional area at 1.5 meters from the diffusion mesh and connected to a Multi-parameter Transmitter M400 (Mettler Toledo Ltd., Leicester, UK). Each absorption test was developed at least in duplicates and in triplicates in the majority of the cases.

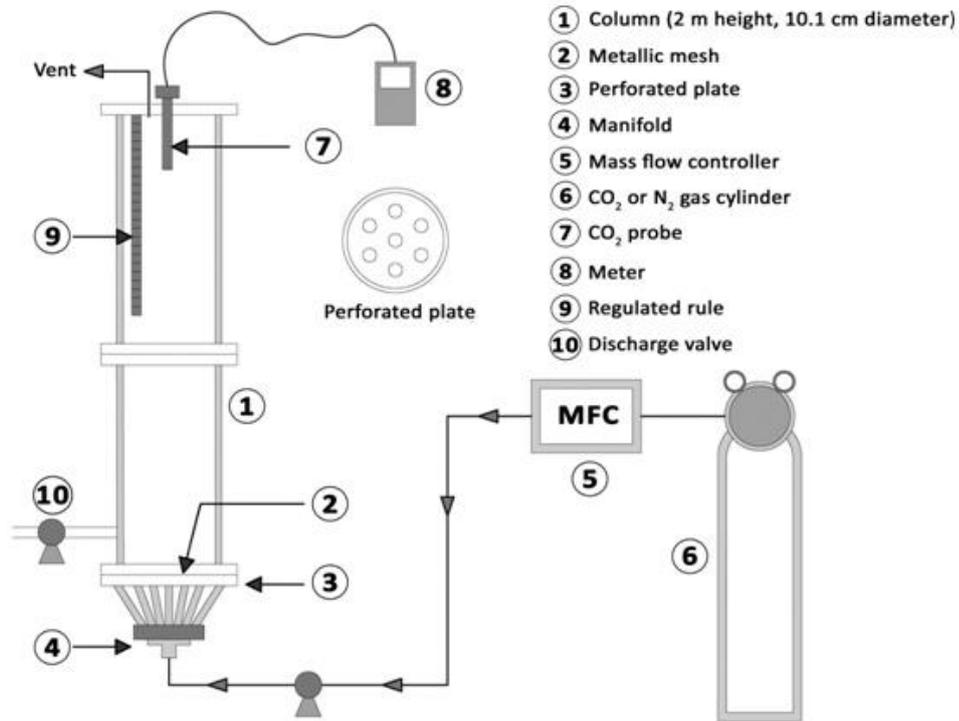


Figure 6.1. Set-up of the pilot scale bubble column used for absorption tests.

The characteristics of the liquid phases and operational conditions (superficial gas velocities (U_G) and associated average shear rates ($\dot{\gamma}_{av}$) obtained with Eq. 1 (Nakanoh and Yoshida, 1980)) used in the absorption tests are compiled in Table 6.2.

$$\dot{\gamma}_{av} = 50 \cdot U_G \text{ (Eq. 1)}$$

Where U_G is the superficial gas velocity in $\text{cm} \cdot \text{s}^{-1}$.

A flow regime map for DI water:glycerol solutions was obtained by observation of the hydrodynamics in the column for each μ_a tested, for U_G between 0.16 and $3.7 \text{ cm} \cdot \text{s}^{-1}$ increased with a step of $0.2 \text{ cm} \cdot \text{s}^{-1}$. Flow regimes were categorised as imperfect bubbly, churn-turbulent or slug flow as described by Kantarci et al. (2005). The gradual transition between regimes for increasing U_G (Hyndman et al., 1997) was categorised as a transition range. The U_{trans} , considered for the first U_G leading to churn-turbulent flow regime, was also determined for all the liquid phases tested.

Table 6.2. Operational conditions used in the absorption tests.

		U _G (cm·s ⁻¹)					
		0.61	1.73	2.85			
		Ȳ _{av} (s ⁻¹)					
		30.5	86.5	142.5			
		concentration (% weight)	μ _a (cPo) at T of test	concentration (% weight)	μ _a (cPo) at T of test	concentration (% weight)	μ _a (cPo) at T of test
glycerol		0	0.9	0	1.0	0	0.9
		10	1.4	10	1.3	10	1.4
		30	2.4	30	2.4	30	2.4
		50	5.8	50	5.7	50	6.2
		70	22.1	70	23.3	70	24.4
		87	124	87	115	87	120
		90	215	90	229	90	219
CMC		0.5	200	0.5	140	0.5	130
		1.0	710	1.0	420	1.0	340
		1.5	1900	1.5	900	1.5	650

Note: Differences in μ of solutions of same glycerol concentration are due to correction as per the temperature of each test.

6.2.3. Considerations for the calculation of volumetric mass transfer coefficients

The calculation of $k_L a$ from CO₂ absorption tests was based on the iterative resolution of Eq. 2. This equation was obtained from the combination of the Whitman's two-film model equation (Eq. 3) and that modelling the response time of the probe (ζ) as a first order process (Eq. 4). Eq. 2 has been widely used in this or equivalent forms in the literature (Gourich et al., 2008; Van't Riet, 1979), when the probe's dynamics cannot be neglected. Consideration of the probe dynamics was necessary since the characteristic time of the mass transfer $t_f = (k_L a)^{-1}$ did not meet the criterion $t_f > 10\zeta$ (Gourich et al., 2008). An average ζ of 91.5 s was identified as suitable for all the conditions tested. This was measured as the time required to reach 63% of the final solubility when submitted to a step change in CO₂ concentration (Royce and Thronhill, 1991).

$$C_{sensor} = C^* + \frac{C^* - C_0}{1 - \zeta k_L a} \cdot \left[\zeta k_L a \cdot \exp\left(\frac{-t}{\zeta}\right) - \exp(-k_L a \cdot t) \right] \text{ (Eq. 2)}$$

$$\frac{dC}{dt} = k_L a (C^* - C) \text{ (Eq. 3)}$$

$$\frac{dC_{sensor}}{dt} = \frac{(C - C_{sensor})}{\zeta} \text{ (Eq. 4)}$$

Where C : concentration in the liquid phase; C^* : solubility as equilibrium CO_2 concentration at infinite time; C_0 : concentration at time zero; C_{sensor} : concentration measured by the probe; $k_{L,a}$: volumetric mass transfer coefficient; ζ : probe's response time.

The impact on mass transfer of other species of the bicarbonate equilibrium apart from dissolved CO_2 was negligible, since the pH was below 5.9 at the end of all the absorption tests performed (Royce and Thronhill, 1991). The impact that CO_2 depletion on the gas phase has on $k_{L,a}$ was considered to be offset with the axial pressure variation in the column (Gourich et al., 2008). Experimental data between 20-98% of the final solubility were used for $k_{L,a}$ determination (Gourich et al., 2008; Merchuk et al., 1990). This truncation discarded the data with a higher ratio of signal to noise (Merchuk et al., 1990) and is in accordance with the literature recommendation of a 30% maximum truncation of the total saturation value (Bouaifi et al., 2001; Merchuk et al., 1990). It was evident that the adjustment between the second order mass transfer model (Eq. 2) and the real experimental data was higher if the first part of the data set was discarded (Figure 6.2), being the error defined as per Eq. 5. The $k_{L,a}$ values were corrected to 20°C with Eq. 6, considering a value of c of 1.0192 (Bewtra et al., 1970).

$$\text{Error} = \frac{\text{ABS}(C_{\text{model}} - C_{\text{sensor}})}{C_{\text{sensor}}} \times 100 \text{ (Eq. 5)}$$

$$(k_{L,a})_{20} = \frac{(k_{L,a})_T}{c^{(T-20)}} \text{ (Eq. 6)}$$

Where C_{model} : concentration estimated with Eq. 2; C_{sensor} : concentration measured by the probe; $(k_{L,a})_T$: volumetric mass transfer coefficient at temperature T ; $(k_{L,a})_{20}$: volumetric mass transfer coefficient at 20°C .

The results are reported as per alpha (α) and theta factors (O). Alpha factor (Eq. 7) was defined as a parameter to assess the impact of μ_a in mass transfer, when normalised against DI water. Theta factor (Eq. 6) was stated as a parameter to assess the impact of U_G , when normalised against the lowest one tested in the saturation tests: $0.61 \text{ cm}\cdot\text{s}^{-1}$.

$$\alpha = \frac{(k_{L,a})_{\mu}^{20^\circ\text{C}}}{(k_{L,a})_{\text{water}}^{20^\circ\text{C}}} \text{ (Eq. 7)}$$

$$\text{O} = \frac{((k_{L,a})_{U_G})_{20^\circ\text{C}}}{((k_{L,a})_{0.61})_{20^\circ\text{C}}} \text{ (Eq. 8)}$$

Where $(k_{L,a})_{U_G}$: volumetric mass transfer coefficient obtained with U_G ; $(k_{L,a})_{\mu}$: volumetric mass transfer coefficient obtained with a liquid phase with μ .

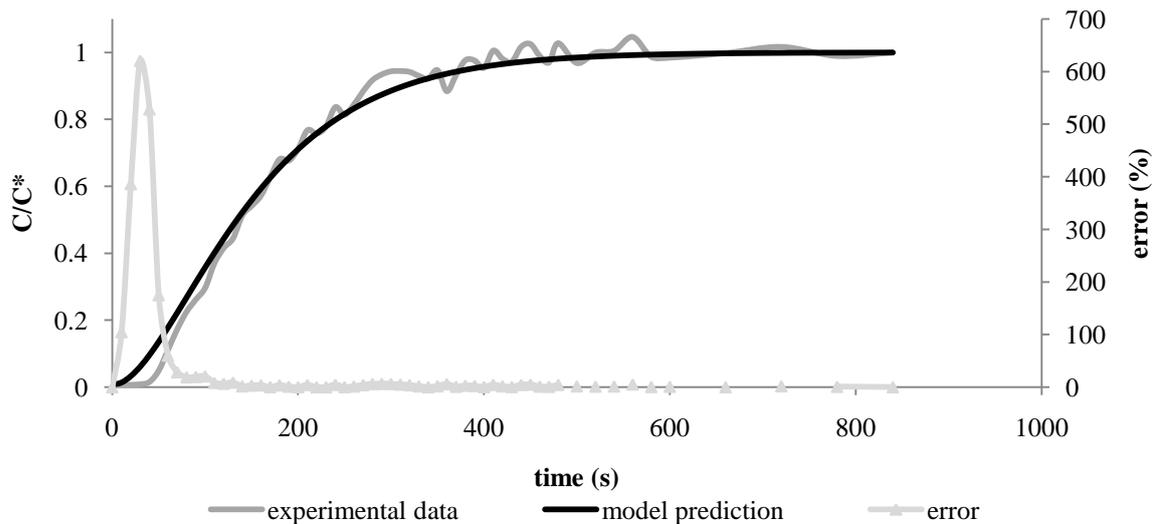


Figure 6.2. Example of absorption experimental data and adjustment of model obtained with Eq. 2. Error is defined as per Eq. 5. Data corresponding to a 30% weight glycerol solution saturated with CO₂ (g) at medium $U_G=1.73 \text{ cm}\cdot\text{s}^{-1}$.

6.3. RESULTS AND DISCUSSION

6.3.1. Impact of viscosity and superficial gas velocity on mass transfer

A significant reduction of $k_L a$ with μ_a was observed for the two fluids and all the operational conditions tested. The relationship between μ_a and $k_L a$ was non linear, with the normalised decrease of $k_L a$ per μ_a increase ($\Delta k_L a / \Delta \mu_a$) being lower at higher μ_a of the liquid phase (Figure 6.3). To illustrate, for absorption tests in glycerol solutions with medium U_G ($1.73 \text{ cm}\cdot\text{s}^{-1}$), an increase of 114 cPo in μ from 1.01 to 115 cPo led to a reduction of 96% in $k_L a$ ($\alpha=0.04$), while a comparable raise from 115 to 229 cPo generated a reduction of 52% ($\alpha=0.02$) (Figure 6.3 a). Similarly, $k_L a$ was reduced by 94% ($\alpha=0.06$) with an increase of μ_a from 0.92 to 130 cPo in tests with CMC solutions at high U_G ($2.85 \text{ cm}\cdot\text{s}^{-1}$), while an increase from 130 to 340 cPo reduced $k_L a$ by 43% ($\alpha=0.04$).

The impact of μ_a in $k_L a$ was greater with higher U_G , both for CMC and glycerol, as evidenced by the lower alpha factor obtained with increasing U_G for a given liquid μ_a (Figure 6.3). For glycerol solutions with a μ of ca. 120 cPo (87% glycerol), alpha factors of 0.05, 0.04 and 0.02 were obtained for U_G of 0.61, 1.73 and $2.85 \text{ cm}\cdot\text{s}^{-1}$, respectively. For equivalent μ_a ranges in CMC solutions (i.e. 0.5% CMC) alpha factors of 0.18, 0.13 and 0.06 were obtained for increasing U_G of 0.61, 1.73 and $2.85 \text{ cm}\cdot\text{s}^{-1}$. The higher impact of μ_a experienced when operating with higher U_G was attributed to variations in the flow regime inside of the system, as explained below (Figure 6.4).

When comparing different CMC solutions of close μ_a , a lower alpha factor was obtained for increasing CMC concentrations. Alpha was 0.03 for absorption tests performed in 1.5% CMC solutions with U_G of $2.85 \text{ cm}\cdot\text{s}^{-1}$ (650 cPo) and 0.12 for 1.0% CMC solutions bubbled with U_G of $0.61 \text{ cm}\cdot\text{s}^{-1}$ (710 cPo) (Figure 6.3). This indicates a higher negative impact of μ_a for increased CMC concentrations and was attributable to mass transfer being affected by the μ of the fluid at rest (as opposed to by μ_a determined with $\dot{\gamma}_{av}$), which offered a greater resistance to CO_2 gas to liquid transfer (Badino et al., 2001) and affected fluid hydrodynamics as explained below.

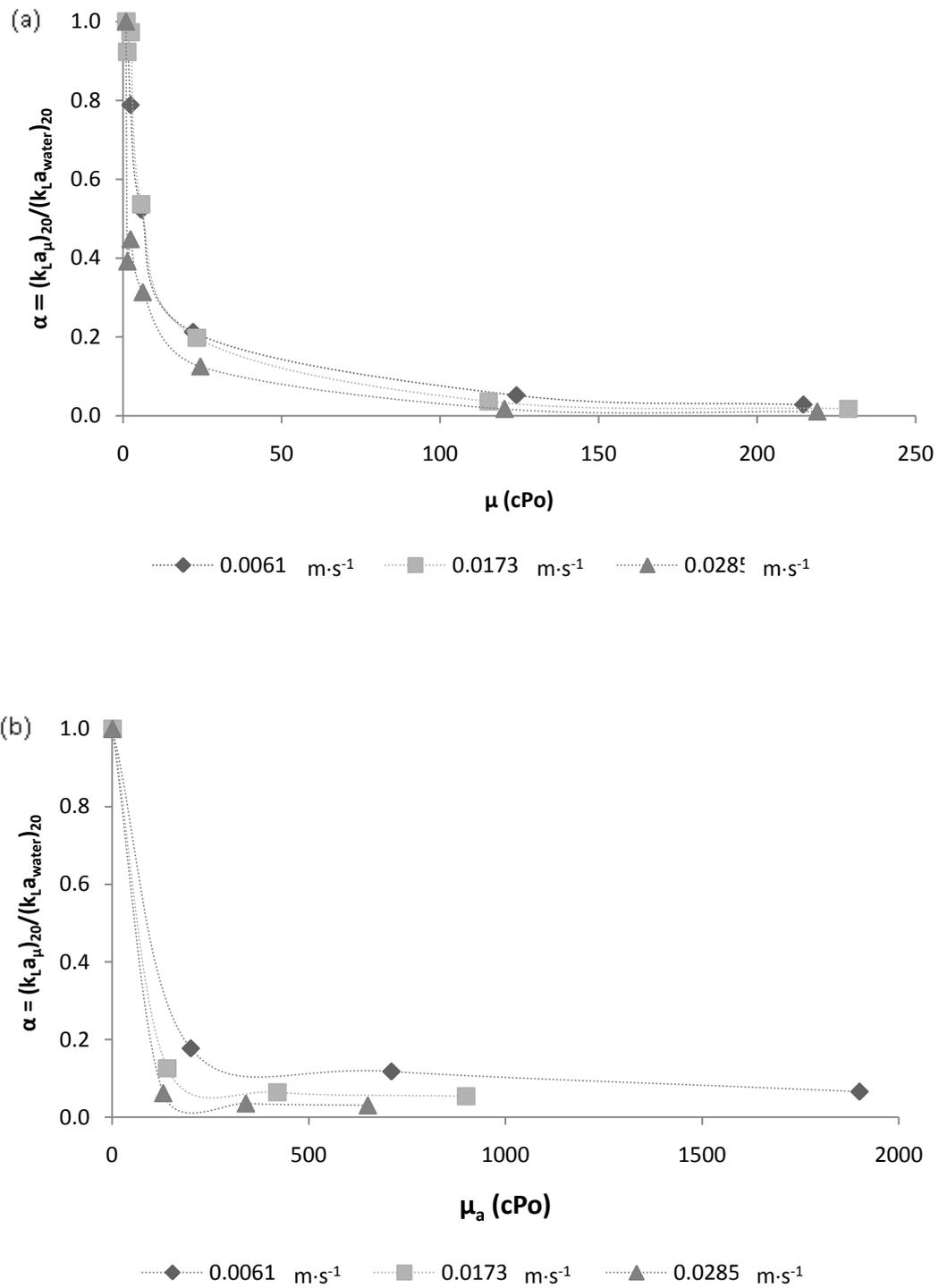


Figure 6.3. Impact of μ_a on $k_L a$ for (a) DI water:glycerol solutions and (b) DI water:CMC solutions.

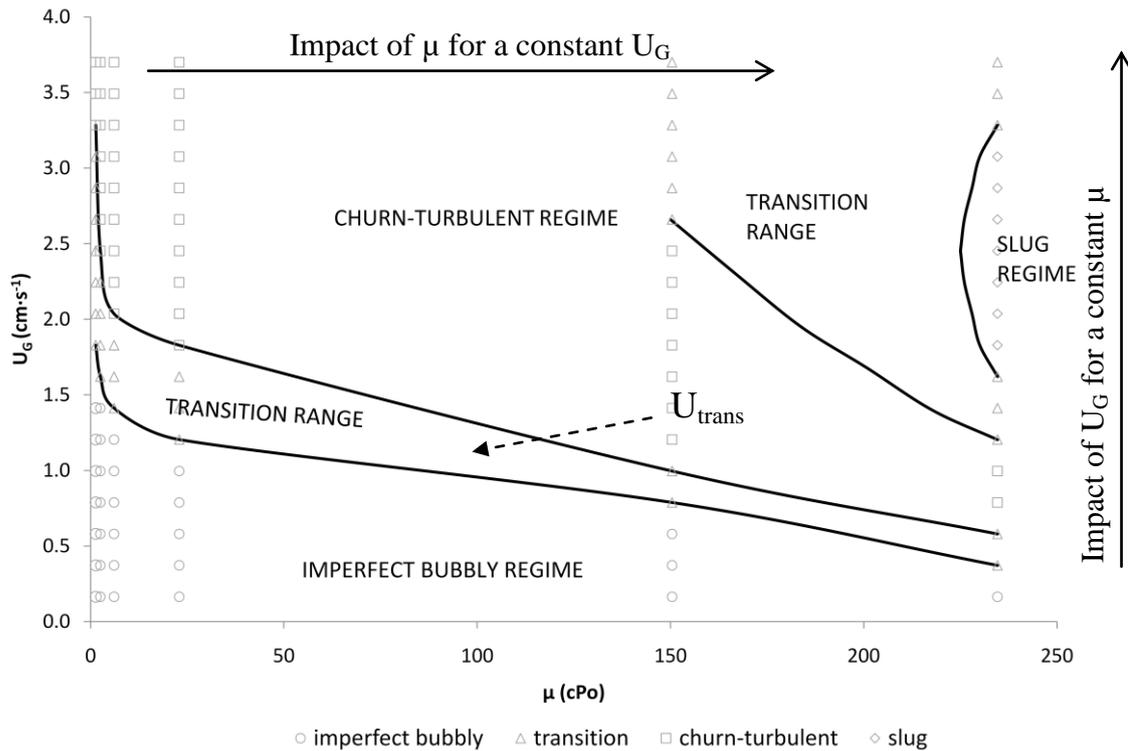


Figure 6.4. Flow regime map for DI water:glycerol solutions of different viscosities. Obtained in a bubble column of 10.1 cm diameter and 2 m height.

As far as gas flowrate is concerned, an increase in U_G enhanced mass transfer for all the experimental conditions tested, being the benefits (reported as O) higher for lower μ_a of the liquid phase (Figure 6.5). For DI water tests (μ of 0.9-1 cPo) theta factor was 3.1 and 7.1 for medium ($1.73 \text{ cm}\cdot\text{s}^{-1}$) and high U_G ($2.85 \text{ cm}\cdot\text{s}^{-1}$), respectively. For glycerol solutions with a μ of ca. 115 cPo (i.e. 87% glycerol), theta factor was 2.1 and 2.3 for medium and high U_G , respectively. An increase in U_G led to formation of bigger and more numerous bubbles and to a higher turbulence inside of the column (Figure 6.4). The enhanced mass transfer obtained for increasing U_G (Figure 6.5), suggests that the impact of the effects positively affecting $k_{L,a}$ (number of bubbles and higher turbulence) was greater than that hindering process efficiency (reduced specific surface area per bubble). The reduced benefit obtained when increasing U_G through liquid phases of higher μ_a (Figure 6.5) was attributed to a greater part of the bubbles' oscillation energy being dispersed as viscous dissipation (Martín et al., 2008), which lead to a reduced turbulence and flow regimes less efficient for mass transfer as described below.

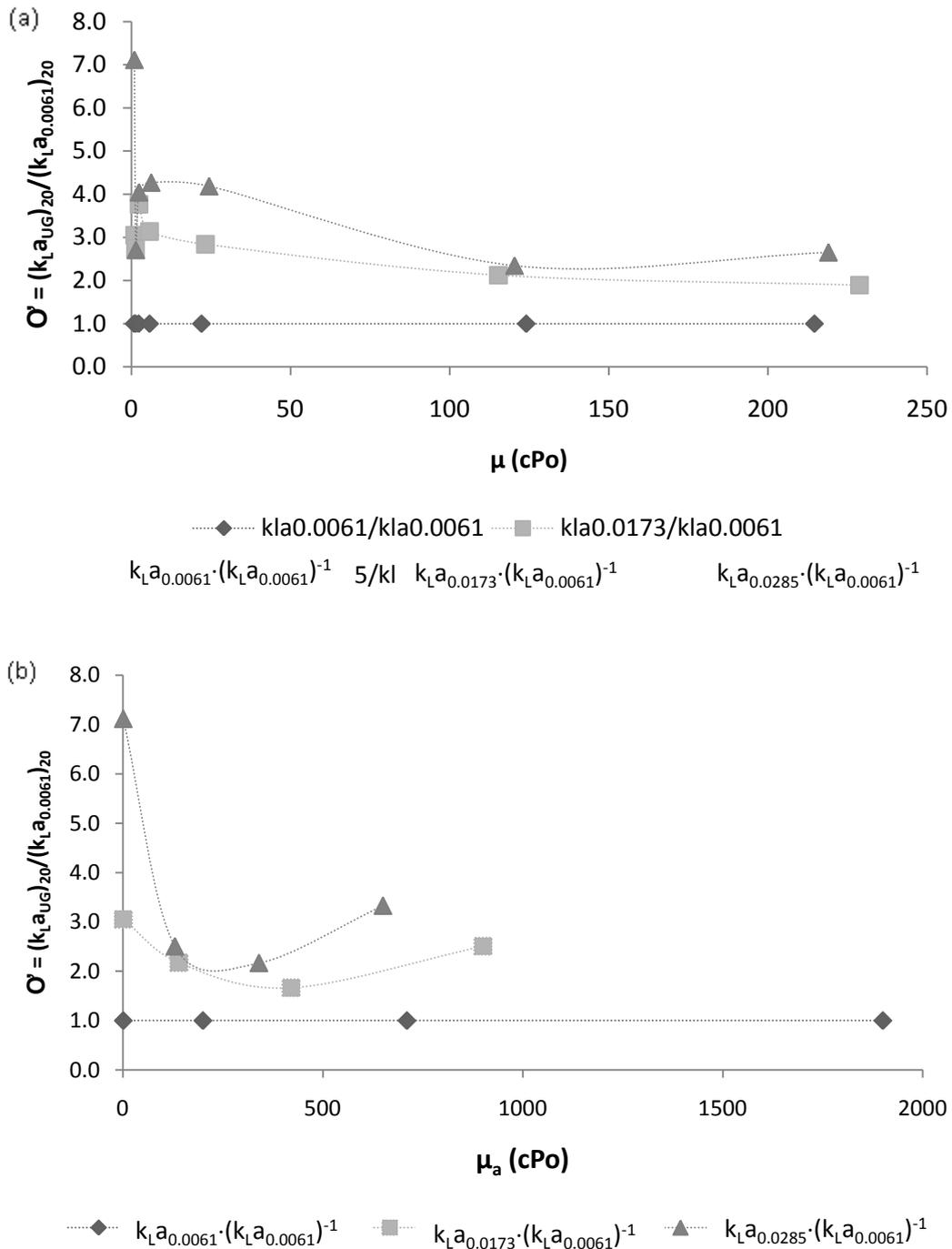


Figure 6.5. Impact of U_G on $k_L a$ for (a) DI water:glycerol solutions and (b) DI water:CMC solutions. Values normalised with the lowest U_G used in the absorption tests ($U_G=0.61 \text{ cm}\cdot\text{s}^{-1}$).

Similar trends of enhanced mass transfer for higher U_G and reduced benefits at higher μ_a of the liquid phase were obtained for CMC solutions (Figure 6.5 b). Theta was 2.2 and 2.5 for 0.5% CMC solutions (130-140 cPo) as opposed to the 3.1 and 7.1 values recorded for DI water (1.0 cPo), with medium and high U_G (1.73 and 2.85 $\text{cm}\cdot\text{s}^{-1}$), respectively. However, an irregularity in the trend was observed for the highest CMC concentration tested (650-

1900 cPo). Theta factors of 1.7 and 2.2 were obtained for 1.0% CMC solutions (340-710 cPo) for medium ($1.73 \text{ cm}\cdot\text{s}^{-1}$) and high ($1.73 \text{ cm}\cdot\text{s}^{-1}$) U_G , respectively (Figure 6.5 b), while significantly higher values (2.5 and 3.3, respectively) were recorded for 1.5% CMC solutions (650-1900 cPo).

6.3.2. Impact of viscosity and superficial gas velocity on hydrodynamics

Hydrodynamics in bubble columns strongly influence mass transfer (Hyndman et al., 1997; Kantarci et al., 2005) and hence need to be further understood in relation to μ_a and rheological variations. For comparable μ_a between the Newtonian and non-Newtonian fluids tested, like CMC 0.5% with low U_G and glycerol 90%, the flow regime inside of the column was similar (transition to churn-turbulent). However, bubble size was bigger for CMC solutions (greater z-component), which indicated that bubble size was affected by the non-Newtonicity of the CMC solutions with the μ of the fluid at rest influencing the minimum bubble buoyancy required for detachment.

A significant variation in the hydrodynamics inside of the bubble column was observed when μ_a or U_G were changed. Imperfect bubbly, churn-turbulent and slug flow regimes were identified as defined by Bouaifi et al. (2001) and the boundaries between them in glycerol solutions compiled in a flow regime map (Figure 6.4). At low U_G ($<0.37 \text{ cm}\cdot\text{s}^{-1}$) imperfect bubbly regime was maintained irrespective of μ , although a reduction on the number of bubbles and bigger bubble size was observed for increasing μ when maintaining a constant U_G . An increase in U_G deviated the pattern towards transition regime, where the initial homogeneous flow was disturbed and a more intense turbulence was observed. The U_G leading to transition regime was reduced with increasing μ of the liquid phase, varying between 1.89 and $0.37 \text{ cm}\cdot\text{s}^{-1}$ for glycerol solutions with μ of 1.4 and 235 cPo , respectively. A further increase in U_G led to fully developed churn-turbulent regime, with U_{trans} being dependent on the μ of the liquid phase. Fully developed churn-turbulent regime was observed with $U_G > U_{\text{trans}} = 3.1 \text{ cm}\cdot\text{s}^{-1}$ for glycerol solutions with μ of 1.3 cPo (10% glycerol) and with values as low as $U_G > U_{\text{trans}} = 0.79 \text{ cm}\cdot\text{s}^{-1}$ for glycerol solutions with μ of 234 cPo (90% glycerol).

For $\mu < 124 \text{ cPo}$ (87% glycerol) a further increase in U_G led to an increased level of turbulence without deviating the flow regime from churn-turbulent. At higher liquid μ ($\geq 124 \text{ cPo}$) a new transition range was observed, which was characterised by slug bubbles occupying all the column cross sectional area but presenting a high instability (Figure 6.6 a). For the highest μ tested for glycerol (ca. 230 cPo) fully developed slug flow regime was obtained in the top part of the column with U_G from 1.6 to $3.3 \text{ cm}\cdot\text{s}^{-1}$ (Figure 6.6 b). Further

increases of gas flowrate lead to breakage of the slug bubbles and a transition flow with mixed characteristics of slug flow and churn-turbulent regimes.

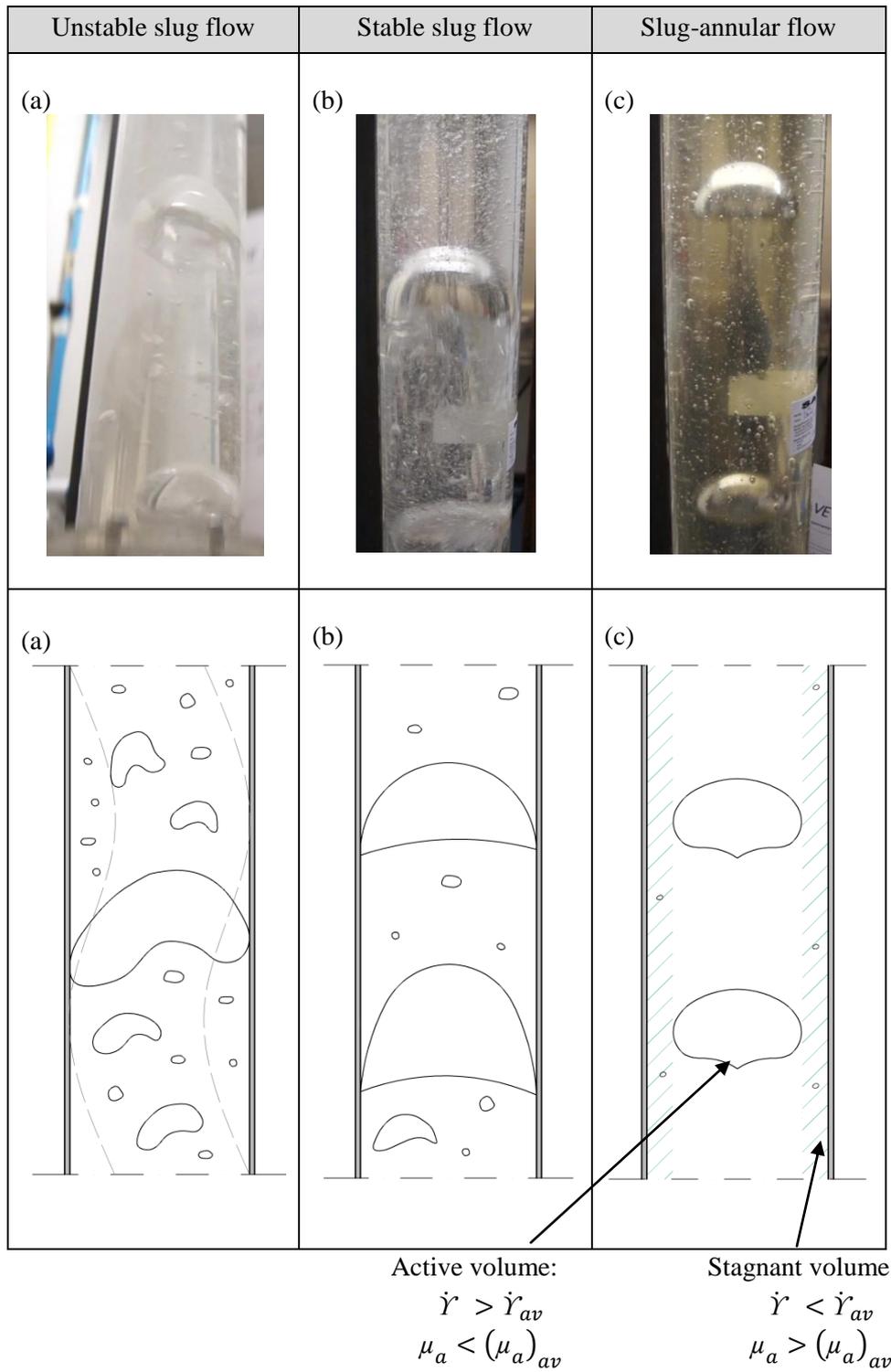


Figure 6.6. Types of slug flow regimes observed: (a) unstable slug flow or transition between churn-turbulent and slug flow regimes observed for 87% glycerol solutions with $U_G = 2.45 \text{ cm}\cdot\text{s}^{-1}$ ($\mu = 124 \text{ cPo}$), (b) stable slug flow observed for 90% glycerol solutions with $U_G = 1.83 \text{ cm}\cdot\text{s}^{-1}$ ($\mu_a = 220 \text{ cPo}$) and (c) slug-annular flow observed for 1.0% CMC solutions with $U_G = 1.62 \text{ cm}\cdot\text{s}^{-1}$.

Similarly, for 0.5% CMC solutions imperfect bubbly regime was observed inside of the bubble column for low U_G ($\leq 0.58 \text{ cm}\cdot\text{s}^{-1}$), while transition and churn-turbulent flows were observed when increasing U_G . Fully developed churn-turbulent regime was observed for $U_G > U_{\text{trans}} = 0.996 \text{ cm}\cdot\text{s}^{-1}$, which is close to the values obtained for glycerol with similar μ_a (87% glycerol, Figure 6.4). For 1.0% CMC solutions with $U_G \leq 2.24 \text{ cm}\cdot\text{s}^{-1}$ and 1.5% CMC solutions with $U_G \leq 3.28 \text{ cm}\cdot\text{s}^{-1}$ the bubbles generated at the bottom of the column merged into bigger bubbles and led to a single raising chain in the centre of the column. A lack of oscillation for single bubbles rising in stagnant CMC solutions has been previously reported (Wenyuan et al., 2010) and associated with the solutions' high μ_a (Li et al., 2012), which leads to a great level of viscous dissipation. The volume active for mass transfer appeared to be limited to the central plume of raising bubbles, with non-mixed areas of higher μ_a in the outer annulus. Conventional slug flow (slugs covering all the cross sectional area of the column) was reached for $U_G \geq 2.45 \text{ cm}\cdot\text{s}^{-1}$ in the case of 1.0% CMC solutions and for $U_G \geq 3.49 \text{ cm}\cdot\text{s}^{-1}$ in tests performed in 1.5% CMC solutions.

The regime observed for 1.0% CMC solutions with $U_G \leq 2.24 \text{ cm}\cdot\text{s}^{-1}$ and 1.5% CMC solutions with $U_G \leq 3.28 \text{ cm}\cdot\text{s}^{-1}$ can be named as slug-annular flow (Figure 6.6 c), where oblate ellipsoidal caps, skirted or inverted tear drop shaped bubbles (Figure 6.7) were effectively stabilised by the non-mixed fluid in the outer annulus, which appeared to apply a dragging force imitating a wall effect. The slug-annular flow found in this study partly resembles the pseudo-slug flow described in horizontal pipes, where a continuous liquid film is formed on the pipe wall and slugs touching the top of the pipe are only formed occasionally, being the gas stream normally confined within the pipe's core (Hewitt and Roberts, 1969; Jagota et al., 1973). However, the analogy is limited since gravity leads to a non-symmetric flow pattern in horizontal pipes while axisymmetric flow pattern can be considered in vertical systems (Jagota et al., 1973). Besides, the slugs formed in pseudo-slug flow in horizontal pipes tend to have a temporal nature (Lin and Hanratty, 1987), while slugs observed in this study travelled the majority of the column height without significant disturbances. Annular regime has previously been described for vertical tubes (Hewitt and Roberts, 1969). However, the gas phase was previously envisaged as continuous and the liquid as partly dispersed (Jagota et al., 1973). The new slug-annular flow described in this study is characterised by liquid occupying the entire column but being divided into two separate regions: outer stagnant annulus and liquid contained in the centre of the column, being the latest disturbed only by ascendant slugs of gas (Figure 6.6 c). Small stagnant gas bubbles were found entrapped in the external annulus close to the column wall, which further confirmed that non-mixed areas with scarce contribution to mass transfer were present. The inner part of the bubble column was characterised by $\dot{\gamma} > \dot{\gamma}_{av}$ and μ_a lower than that

predicted with $\dot{\gamma}_{av}$ (Figure 6.6 (c)), while the stagnant liquid areas found in the outer annulus where subjected to a $\dot{\gamma} < \dot{\gamma}_{av}$ that lead to μ_a higher than in the bulk of the fluid. A non-uniform $\dot{\gamma}$ and μ_a distribution in the cross sectional area of bubble columns with CMC solutions was previously reported by Nishikawa et. al. (1977) for $U_G < 4 \text{ cm}\cdot\text{s}^{-1}$ when studying heat transfer.

In slug-annular regime bubble size and bubble shape were conditioned by both U_G and μ_a . In 1.0% CMC solutions oblate ellipsoidal caps with a short tail were observed (Figure 6.7 a), which progressively transformed into skirted bubbles when U_G was increased (Figure 6.7 e). Quasi spherical shaped bubbles with a short tail (inverted tear drop) were characteristic of 1.5% CMC solutions, with an increase in U_G leading to bigger bubbles (reduced outer annulus) with similar shape (Figure 6.7). When different CMC solutions of similar μ_a are compared, a bigger bubble size was observed for the highest CMC concentration. Figure 6.7 (a and f) evidences the presence of bigger bubbles for tests with 1.5% CMC solutions at high U_G ($\mu_a = 650 \text{ cPo}$) than for 1.0% CMC at low U_G ($\mu_a = 710 \text{ cPo}$), being the bubbles flatter for reduced CMC concentrations. Part of this difference in bubble size could be attributed to the higher gas flowrate required with 1.5% CMC solutions to achieve a μ_a comparable to 1.0% CMC (shear thinning fluid). However, bubble volume was considerably bigger for higher CMC concentrations even when an equivalent U_G was applied (Figure 6.7 (a), (c), (e) VS Figure 6.7 (b), (d) (f)). Bubbles formed in solutions of higher CMC concentration consistently presented a higher vertical component ($c > a$ in Figure 6.7), which evidenced the influence of the μ of the fluid at rest in buoyancy for detachment and bubble shape. In unmixed shear thinning fluids (e.g. CMC), the at rest μ appears to effectively act as a pseudo-yield stress that needs to be overcome by bubble buoyancy. A higher bubble vertical component and reduced cross sectional area lead to a more favourable balance between drag force (function of bubble cross sectional area) and buoyancy (function of bubble volume), which allows the bubble to overcome the opposing forces and rise in a stable plume with a reduced active volume for mass transfer. This concept is in accordance with previous references reporting bubble shape to be influenced by yield stress (Sikorski et al., 2009) and with the mathematical simulations of Tsamopoulos et al. (2008), which predicted an elongation of the vertical component of bubbles when formed in fluids of increasing yield stress.

The stable plume of rising bubbles of the slug-annular flow allowed a visual observation of the coalescence process for 1.0% and 1.5% CMC solutions. At lower μ_a the rising bubbles collided more frequently with others in a higher position, resulting in coalescence. On the contrary, bubbles formed in 1.5% CMC solutions tended to maintain their relative distance in the vertical chain of bubbles throughout the height of the column and

coalescence was not observed apart from that taking place close to the diffusion device. Retardation in the drainage of the liquid film between bubbles for higher μ_a was hence evidenced, which appeared responsible for a reduced bubble coalescence and break-up.

6.3.3. Mechanistic understanding

Variations of $k_L a$ associated with μ_a and rheological changes were related with the influence of these on bubble buoyancy, bubble shape, viscous dissipation of bubble oscillation energy (turbulence level), retardation in drainage of liquid film between bubbles (coalesce and break-up) and at rest μ in shear thinning fluids; all of which in turn determined the hydrodynamics in the system. The reduction in mass transfer efficiency associated with a μ_a raise was hence attributed to a combination of effects. The increase in the size of the bubbles formed at higher μ_a implied a reduction of specific surface area with negative implications in $k_L a$ (García-Abuín et al., 2013). Besides, bubbles tended to coalescence close to the diffusion mesh, leading to bigger bubbles in the bulk of the column and resulting in slug flow or annular-slug flow for some of the higher μ_a tested (Figure 6.4 and Figure 6.7). Slug flow regime is associated with poor mass transfer (Jones, 2007), leading to lower $k_L a$ values. Furthermore, higher μ_a led to a reduced turbulence and radial oscillation, due to a partial absorption of oscillation energy as viscous dissipation (Martín et al., 2008) and limiting the positive impact of turbulence on mass transfer. A flow without radial dispersion was hence observed for the highest μ_a tested, where bubbles appeared stable in a vertical chain and the probability of collision leading to bubble break-up was clearly reduced (Martín et al., 2008; Wilkinson et al., 1993). The higher impact of μ_a in $k_L a$ at higher U_G (lower alpha factor for increasing U_G for a given liquid μ_a (Figure 6.3)) was again related with the hydrodynamics inside of the bubble column. This can be illustrated with glycerol solutions, where a change of μ for the lowest U_G tested ($0.61 \text{ cm}\cdot\text{s}^{-1}$) did not deviate the flow pattern from imperfect bubbly. On the contrary, at higher U_G , an increase in μ changed the hydrodynamics from imperfect bubbly ($<1.4 \text{ cPo}$) to transition regime, churn-turbulent regime and even slug flow (ca. 230 cPo). Since slug flow is the least favourable regime for mass transfer (Jones, 2007), the variation of hydrodynamics towards regimes of lower turbulence explains the varied impact of μ for different gas flowrates.

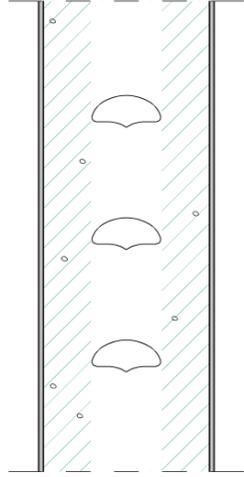
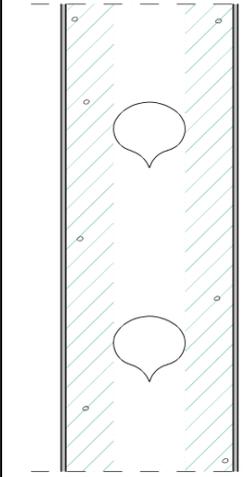
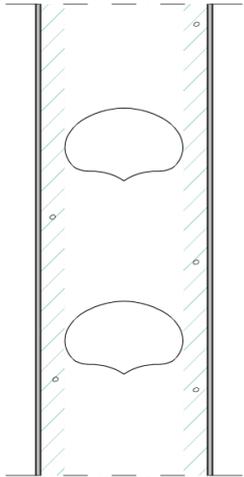
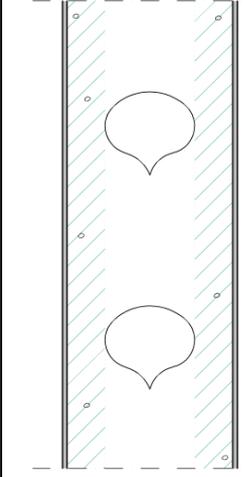
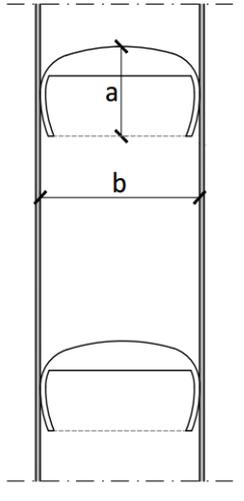
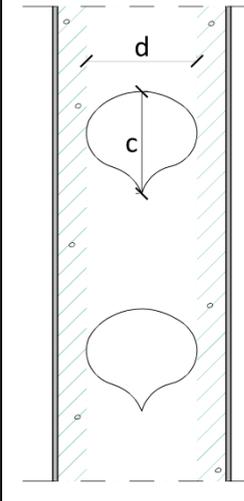
1.0% CMC image	1.0% CMC schema	1.5% CMC image	1.5% CMC schema	U_G (cm·s ⁻¹)
(a) 		(b) 		0.58
(c) 		(d) 		
(e) 		(f) 		2.87 $a < c$ $b > d$

Figure 6.7. Bubble shape, bubble size and evolution of liquid working volume actively used for gas to liquid mass transfer in the slug-annular flow regime observed in shear thinning fluids (1.0% CMC and 1.5% CMC solutions).

Mass transfer was enhanced with higher U_G , with the benefit being reduced at higher μ_a of the liquid phase both for Newtonian (glycerol) and non-Newtonian (CMC) solutions (Figure 6.5 b). However, an irregularity in the trend was observed for the highest CMC concentration tested (1.5%), where higher theta factors than for lower concentrations of CMC were obtained (2.5 and 3.3 for U_G of $1.73 \text{ cm}\cdot\text{s}^{-1}$ and $1.73 \text{ cm}\cdot\text{s}^{-1}$, respectively, for 1.5% CMC solutions and 1.7 and 2.2 for 1.0% CMC). This irregularity was due to the particular implications that the shear thinning nature of CMC solutions has in the system hydrodynamics and the influence that the μ of the fluid at rest has in bubble buoyancy. Slug flow was achieved in 1.0% CMC solutions at a lower U_G than for 1.5% CMC solutions, 2.45 and $3.49 \text{ cm}\cdot\text{s}^{-1}$, respectively. Therefore, for the same $U_G = 2.85 \text{ cm}\cdot\text{s}^{-1}$ the bubbles formed in 1.0% CMC solutions were characterised by a higher cross sectional area and a lower vertical component than for 1.5% CMC solutions, where bubbles had a higher z-component and stagnant areas characteristic of slug-annular flow were present in the outer annulus of the column (Figure 6.6). Consequently, a lower volume of liquid was actively involved in mass transfer for 1.5% CMC solutions, which effectively implied a higher gas rate (amount of gas per liquid volume treated) and hence a greater positive impact of increased U_G in $k_{L,a}$ (higher \dot{O}). The two liquid regions described in slug-annular flow are visualised as decoupled in terms of mass transfer. While increased $k_{L,a}$ are expected in the inner core (higher gas rate), mass transfer in the outer stagnant annulus appeared virtually limited to diffusion mechanisms. It must be considered that the dissolved CO_2 probe was fixed in the centre of the cross sectional area of the bubble column, being the $k_{L,a}$ values reported obtained for the area active for mass transfer in the case of slug-annular regime.

6.3.4. Practical implications for gas to liquid mass transfer in sludge

The conditions that would imitate the common operation of sewage sludge ADs (μ and $\dot{\gamma}$) were included within those tested. A range of μ_a of 150-400 cPo was considered to cover the variability observed in digested sludge with a 2-4% total solid content (Table 6.1) (Eshtiaghi et al., 2012; Frost, 1983; Goel et al., 2004; USEPA, 1979). A range of $50\text{-}80 \text{ s}^{-1}$ covers the $\dot{\gamma}$ most frequently used in design of full scale ADs (USEPA, 1979) and was represented by the absorption tests performed at low and medium U_G , $\dot{\gamma}_{av} = 30.5 \text{ s}^{-1}$ and $\dot{\gamma}_{av} = 86.5 \text{ s}^{-1}$, respectively. Since CMC has been reported to suitably mimic the rheological properties of digested sludge in steady state at high $\dot{\gamma}$ (above 20 s^{-1}) (Eshtiaghi et al., 2012), the results of this study give indication of the trends expected when performing mass transfer in digested sludge, for applications such as CO_2 uptake by biotransformation to methane (Bajón Fernández et al., 2014) or control of ammonia toxicity in ADs (Walker et al., 2011).

It was concluded that fluid μ_a and rheology need to be thoroughly characterised and included in the design of any mass transfer system involving digested sludge, since a poor characterisation of these variables can have a double negative implication in the performance of a gas to liquid mass transfer system. On the one hand, a variation of μ_a that has not been accounted for in the design stage, could significantly reduce the performance of the process. A reduction of 43% in $k_L a$ was obtained with a μ_a increase from 130 to 340 cPo (0.5 to 1.0% CMC), which is within the reported range of μ_a variability in digested sludge (Table 6.1). Gas flowrate could be increased as a means to compensate for the reduced performance. Theta factors of 1.7-2.5 were obtained when increasing U_G from 0.61 to 1.73 $\text{cm} \cdot \text{s}^{-1}$ and of 2.2-3.3 when applying 2.85 $\text{cm} \cdot \text{s}^{-1}$ for CMC solutions. However, this required 3 to 5 times higher gas flowrates, with the associated increased operational costs that may offset the benefits of an enhanced $k_L a$. When assessing μ_a impact in terms of the diffusion system performance, an increase of μ_a from 130 to 340 cPo in CMC solutions implied a reduction in gas transfer rate (GTR) (Eq. 8) from $31.4 \pm 1.4 \text{ mg} \cdot \text{s}^{-1}$ to $19.0 \pm 0.3 \text{ mg} \cdot \text{s}^{-1}$ and in gas transfer efficiency (GTE) (Eq. 9) from $7.6 \pm 0.3\%$ to $1.6 \pm 0.1\%$. Since sewage sludge μ_a is highly influenced by solid content (Brar et al., 2005) and temperature (Hammadi et al., 2012), these need to be particularly characterised and accounted for since the design stage of a gas to liquid mass transfer system.

$$GTR (\text{mg} \cdot \text{s}^{-1}) = (k_L a)_{20^\circ\text{C}} \cdot (C^* - C) \cdot V \quad (\text{Eq. 8})$$

$$GTE (\%) = SGTR \cdot (\dot{F}_{\text{CO}_2})^{-1} \cdot 100 \quad (\text{Eq. 9})$$

Where C: concentration in the liquid phase; C*: solubility as equilibrium CO_2 concentration at infinite time; \dot{F}_{CO_2} : incoming CO_2 mass flow rate; $(k_L a)_{20}$: volumetric mass transfer coefficient at 20°C ; V: volume of liquid inside of the bubble column.

On the other hand, this study evidenced the existence of a slug-annular flow in shear thinning fluids with rheological behaviour comparable to that of anaerobically digested sewage sludge (e.g. CMC solutions). In this hydrodynamic regime, slug-like bubbles appeared stabilised by stagnant liquid of high μ_a rather than by the column wall itself, challenging the common statement of slug flow being limited to the operation of laboratory scale bubble columns of small diameter (Hyndman et al., 1997). A stable plume of rising bubbles without radial oscillation and a reduced volume of liquid actively involved in mass transfer were hence observed for 1.0% and 1.5% CMC solutions. The reduced viscous forces in the central part of the column ($\mu_a < (\mu_a)_{av}$) led to higher alpha factors (lower impact of μ_a) for CMC than for glycerol solutions of similar μ_a (Figure 6.3), when placing the dissolved CO_2 probe in the centre of the cross sectional area. However, the stagnant areas of reduced

mass transfer efficiency present in the outer annulus of the column should be avoided in a full scale operation. In practical terms, the appearance of slug-annular flow in a volume of shear thinning fluid with CO₂ injection could lead to a reduced active volume for mass transfer due to the system operating as several individual bubble columns separated by stagnant areas. Whereas k_La would be higher than predicted in each column core, mass transfer in the outer annulus would be mainly limited to that associated with diffusion mechanisms. In order for this to be avoided, special consideration should be given towards the design of efficient gas distribution systems in mass transfer processes involving digested sludge, with an increased number of diffusion devices per surface area than when dealing with Newtonian fluids of similar μ_a . Alternatively, additional mixing devices capable of disturbing the fluid stagnant areas could be considered.

6.4. CONCLUSIONS

Mass transfer retardation due to increased μ_a was investigated for Newtonian and shear thinning fluids. A non-linear reduction of mass transfer efficiency with increasing μ_a was observed, being the impact higher at low μ_a ranges and high U_G .

The impact of both μ_a and U_G in k_La was predominantly influenced by changes in the system hydrodynamics through an alteration of bubble buoyancy, bubble shape, turbulence level and drainage of the liquid film between bubbles. Slug-annular flow was observed for shear thinning fluids of high μ_a , including rheological conditions imitating digested sewage sludge, where k_La was conditioned by the μ of the fluid at rest and the active liquid volume for mass transfer was reduced because of the presence of a stagnant outer annulus. Particular emphasis should be placed in selecting the number of diffusion systems per unit area to be used in a mass transfer system involving sewage sludge, in order to avoid a reduction in process performance and active volume as a consequence of viscous or rheological variations.

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CHAPTER 7

CARBON DIOXIDE BIOCONVERSION IN ANAEROBIC DIGESTERS AS AN ON-SITE CARBON REVALORISATION STRATEGY: CONSIDERATIONS AND REQUIREMENTS FOR IMPLEMENTATION

7. CARBON DIOXIDE BIOCONVERSION IN ANAEROBIC DIGESTERS AS AN ON-SITE CARBON REVALORISATION STRATEGY: CONSIDERATIONS AND REQUIREMENTS FOR IMPLEMENTATION

HIGHLIGHTS

- Implementation of CO₂ bioconversion in full-scale ADs investigated.
- Injection of CO₂ is feasible in single phase ADs or first stage of TPADs.
- Benefits in carbon footprint expected in ADs with ammonia < *ca.* 1500 mg·L⁻¹ NH₄-N.
- CO₂ could be sourced from off-gas streams of biogas upgrading units.
- Injection of exogenous CO₂ recommended in digestate recirculation loop.

ABSTRACT

Bioconversion of carbon dioxide (CO₂) into methane (CH₄) in anaerobic digesters (ADs) has been stated a more feasible alternative than carbon capture and storage (CCS) in reservoirs for reducing the carbon footprint of the water and organic waste sectors. This study addressed the potential of implementing this carbon revalorisation strategy at full-scale. Bioconversion of CO₂ was identified as feasible in ADs with ammonia concentrations below *ca.* 1500 mg·L⁻¹ NH₄-N, with injection of exogenous CO₂ performed in the digestate recirculation loop of single phase ADs or in the first phase of two phase ADs (TPADs). It was concluded that CO₂ could be sourced from off-gas streams of biogas upgrading technologies. When theoretically scaled up to a single phase AD of 5200 m³, potential CO₂ savings of 1805-18050 tonnes·annum⁻¹ and increases of 4.7-47% in CH₄ production could potentially be achieved, with benefits parameterized for a CO₂ gas to liquid transfer efficiency of 8-80%.

KEYWORDS

Bioconversion, carbon mitigation, CO₂ revalorisation, implementation.

7.1. DRIVERS FOR GREENHOUSE GAS MITIGATION AND POTENTIAL BENEFITS OF CARBON DIOXIDE BIOCONVERSION IN ANAEROBIC DIGESTERS

Carbon dioxide (CO₂) emissions in the water sector are increasing contrary to greenhouse gas (GHG) mitigation aims. Combustion of fossil fuels for energy generation has been recognized as the main source of GHG anthropogenic emissions (IEA, 2013) and decarbonisation of energy supply and increased operational energy efficiencies identified as key strategies towards GHG targets compliance (Georges et al., 2009). However, energy

demand of the water sector presents an increasing trend, in part due to the treatment requirements associated with more stringent water quality standards (European Commission, 2009). This has led the water sector to contribute in 3-10% to total world GHG emissions (McGuckin et al., 2013). To illustrate, energy demand of the UK water sector increased by 8.7% (726 GWh) between 2007 and 2011 (Water UK, 2012, 2008) and carbon emissions in this sector are envisaged to rise by over 110,000 tonnes per annum (Georges et al., 2009). In response, carbon management strategies that can offset the increasing carbon footprint of the water sector are being investigated. Increased generation of renewable energy through anaerobic digestion (AD) (DEFRA, 2007; Georges et al., 2009) and on-site revalorisation of biogenic CO₂ streams are credited as one of the most promising solutions (Byrns et al., 2013). Bioconversion of CO₂ to methane (CH₄) in anaerobic processes has been identified as one of the most viable on-site revalorisation alternatives for CO₂ streams generated during wastewater treatment (Bajón Fernández et al., 2015c; Byrns et al., 2013).

The benefits of anaerobic CO₂ bioconversion in ADs have been particularly highlighted (Bajón Fernández et al., 2015c, 2014) in view of the widespread implementation of AD technology and the government encouragement towards its further growth (DEFRA, 2007). The majority of sludge produced in the UK water industry is already treated with digestion processes, with an estimated emission of 270,000 tonnes of biogas CO₂ per annum (Byrns et al., 2013). In addition, implementation of ADs treating alternative substrates (e.g. food waste, energy crops, manure) is growing, with an increase in the number of ADs treating municipal solid waste in Europe from 53 in 1999 (De Baere, 2006) to 171 in 2010 (De Baere and Mattheeuws, 2008). Biogas CO₂ emitted by ADs treating municipal solid waste in Europe has been estimated at *ca.* 980,000 tonnes CO₂ per annum (Bajón Fernández et al., 2015c). Valorisation of these emissions would hence contribute to counteract the increasing GHG emissions of the water sector and has the potential to turn organic waste treatment into a carbon negative process.

The benefits of bioconversion of CO₂ to CH₄ have been demonstrated at laboratory (Alimahmoodi and Mulligan, 2008; Bajón Fernández et al., 2014; Sato and Ochi, 1994) and pilot scale (Salomoni et al., 2011). It was estimated that the CO₂ savings from diverting one tonne of waste from landfill to be treated by AD could be increased by *ca.* 24% when operating ADs enriched with CO₂ (Bajón Fernández et al., 2015c). Associated increments in CH₄ yields of 25-30% in continuous ADs enriched with CO₂ (Salomoni et al., 2011; Sato and Ochi, 1994) and of up to two fold raises in CH₄ production following saturation with CO₂ in batch laboratory scale ADs (Bajón Fernández et al., 2014) have been reported. In view of these potential benefits in carbon uptake and renewable energy generation, it is considered suitable to identify how CO₂ bioconversion in ADs could be implemented in full-scale

facilities. This paper analyzes the potential on-site CO₂ sources to be utilised, the AD systems and configurations in which CO₂ bioconversion could be applied and areas requiring further research in order to increase readiness for implementation.

7.2. IMPLEMENTATION FEASIBILITY

7.2.1. Systems where CO₂ bioconversion could be implemented

The impact of injecting exogenous CO₂ into ADs is dependent on the feed substrate (Bajón Fernández et al., 2015d, 2014), which indicates that benefits in carbon footprint and renewable energy generation resulting from bioconversion of CO₂ into CH₄ may be limited to certain systems. Review of previous references studying the mechanisms of exogenous CO₂ utilisation in ADs can provide insight regarding the AD configurations and substrates in which bioconversion of CO₂ can be applied as a carbon management strategy. Periodic injections of CO₂ in ADs have been reported to increase the activity of *Methanosaetaceae* (Bajón Fernández et al., 2015d), suggesting a boost in the acetoclastic pathway for CH₄ formation as these are obligate acetoclastic methanogens. This increased activity has been hypothesised as due to CO₂ altering early stages of the digestion process (i.e. acidogenesis and acetogenesis), which indirectly benefits acetoclastic methanogens. The benefits in CH₄ production obtained by Salomoni et al., (2011) when injecting CO₂ into the first phase of a two phase anaerobic digestion (TPAD) process, support an indirect impact in methanogenesis, as CH₄ producing *Archaea* were not present in the acid phase where CO₂ was injected. Furthermore, Bajón Fernández et al. (2015b) reported a 2.5 fold increase in the headspace hydrogen (H₂) concentration of an AD periodically enriched with CO₂, which was attributed to the dissolution of CO₂ and to an alteration of acetogenesis. Since H₂ level did not increase further in spite of further CO₂ injections, this was believed assimilated by homoacetogenesis (via the Wood-Ljungdahl mechanism), which is stimulated by exogenous CO₂ and produces acetate that could stimulate acetoclastic methanogenesis because of a higher substrate availability. Under this scenario, the application of CO₂ bioconversion as a carbon mitigation strategy appears suited to anaerobic systems where both homoacetogenic bacteria and acetoclastic methanogens are present.

In relation to the configuration of AD systems, the reduction of CO₂ by homoacetogenic bacteria requires CO₂ injection in single phase ADs or in the first phase of a TPAD process, where hydrolysis, acidogenesis and acetogenesis take place. Injection of exogenous CO₂ into the second phase of a TPAD is less suitable, since an efficient phase separation would prevent growth of homoacetogenic bacteria in this reactor. With regard to the substrates treated, ammonia concentration in ADs treating substrates with a high protein content can reach levels toxic for acetoclastic methanogenesis (Banks et al., 2011; Schnürer

and Nordberg, 2008). Concentrations of 1500-3000 mg·L⁻¹ NH₄-N have been reported as partly inhibitory for these *Archaea* and over 3000 mg·L⁻¹ NH₄-N as completely toxic (Rajagopal et al., 2013). Therefore, implementation of CO₂ bioconversion appears best suited in units with ammonia concentrations below *ca.* 1500 mg·L⁻¹ NH₄-N, such as ADs treating sewage sludge or co-digesting this with other substrates. Operation of ADs treating solely animal waste or food waste rich in proteins could lead to ammonia inhibitory levels (Banks et al., 2008; Siles et al., 2010; Walker et al., 2011). Consequently, implementation of CO₂ bioconversion with these substrates is less feasible unless ammonia control strategies are implemented (e.g. ammonia stripping (Walker et al., 2011), dilution of substrate with water (Banks et al., 2008) or chemical precipitation (Chen et al., 2008)).

7.2.2. Possible gas streams to be used for CO₂ bioconversion in ADs

7.2.2.1. Required characteristics of CO₂ stream

In order to apply CO₂ bioconversion in ADs the suitability of on-site available gas streams to be injected into an anaerobic process needs to be considered. The CO₂ partial pressure (p_{CO_2}) of the stream injected needs to be higher than that of the AD under normal operation, in order to enable an efficient mass transfer of additional CO₂ for bioconversion. When examining previous studies, the p_{CO_2} of the injected stream can alter the potential of carbon uptake and associated CH₄ production (Bajón Fernández et al., 2014; Sato and Ochi, 1994). Higher benefits for increased p_{CO_2} have been reported, with the best performance obtained when using a p_{CO_2} =1.6-1.7 bar (CO₂ molar fraction (y_{CO_2}) of 0.9), because of a higher dissolved CO₂ available for bioconversion than for lower p_{CO_2} tested (Bajón Fernández et al., 2014). The p_{CO_2} of a gas stream can be increased by either raising its y_{CO_2} or the total pressure (P_T) at which it is injected (Eq. 1). Since ADs are normally operated at pressure close to atmospheric in the headspace (up to 0.02 bar overpressure (Chen et al., 2014)), dissolving CO₂ at much higher pressure is not considered suitable as it would be partly released inside the AD until an equilibrium level with the operational pressure is achieved. Therefore, it is recommended that any adjustment in p_{CO_2} required to implement CO₂ bioconversion in ADs is achieved by altering the y_{CO_2} of the available gas streams, while maintaining a relative low pressure for injection.

$$p_{CO_2} = y_{CO_2} \cdot P_T \text{ (Eq. 1)}$$

Additionally, the presence of contaminants such as oxygen (O₂) and the accessibility of the gas streams to be recovered need to be considered when selecting on-site sources of CO₂ with the potential to be injected into ADs for carbon footprint mitigation.

7.2.2.2. Identification of suitable streams

The potential of CO₂ streams produced on-site to be revalorised through CO₂ bioconversion in ADs was assessed based on their y_{CO_2} and on the presence of contaminants (mainly O₂) that could have a negative impact in the AD process. When considering the flowsheet of a wastewater treatment plant (WWTP) the main sources of CO₂ on-site were considered as follows:

- a) Gas produced during aerobic wastewater treatment: Biogenic CO₂ emissions produced during aerobic wastewater treatment have been identified as the major source of CO₂ emissions within a WWTP (Byrns et al., 2013). However, the low CO₂ concentration of these streams (0.8% v/v (Byrns et al., 2013)), its O₂ content and the difficulties and associated costs for capturing a stream produced in open systems, limit its revalorisation by bioconversion in ADs.
- b) Gas produced in the first phase of TPADs: The conversion of complex organic matter into soluble substrates in the acid phase of a TPAD produces a gas stream with y_{CO_2} of ca. 0.75 and a total contribution to the overall gas flowrate between 6% (*communication with site operators*) and 60% (Rubio-Loza and Noyola, 2010).
- c) Biogas: The biogas produced during the AD process has a y_{CO_2} of 0.30-0.45. Recirculation of this stream into the AD is not considered suitable for it to be revalorised by CO₂ bioconversion since the p_{CO_2} of the gas injected needs to be higher than that in the AD; in order to enable a step change in the dissolved CO₂ level of the liquid phase. Injection at a higher P_T would increase the driving force for dissolution of exogenous CO₂. However, as discussed before, an increase in y_{CO_2} is a preferred option.
- d) Combined heat and power (CHP) engine exhaust: Combustion of biogas in CHPs for production of electricity and heat generates a stream containing the CO₂ initially present in the biogas and that derived from the combustion of CH₄ to CO₂. This stream, however, does not have the potential to be revalorised by CO₂ bioconversion in ADs because its CO₂ content is diluted with the N₂ and excess O₂ of the air used for combustion, which leads to y_{CO_2} of 0.05-0.15 (Byrns et al., 2013; GE Power & Water, 2014).
- e) Off-gas of biogas upgrading processes: The biogas produced in ADs normally has a concentration of CH₄ of 50-75% (AEBIOM, 2009) with 25-50% CO₂. Biogas upgrading technologies aim to obtain a CH₄ enriched gas stream that can be injected into the gas grid, while the CO₂ initially present in the biogas is emitted with process waste streams. Different upgrading technologies will result in different y_{CO_2} in the off-gas released during biogas upgrading and are hence individually considered below.

a. Absorption

Absorption upgrading technologies are based in the dissolution of CO₂ in a liquid by contacting this with biogas in a column. The higher solubility of CO₂ than CH₄ leads to a wet gas with a high CH₄ concentration (which can be dried to produce a purified CH₄ stream) and a liquid stream with a high content of dissolved CO₂. Two different absorption technologies can be implemented based on the interaction between the liquid used and the CO₂ to be removed: physical and chemical absorption. The former relies on binding CO₂ with the contacting liquid by physical forces. Both water (water scrubber) or organic solvents (e.g. polyethylene glycol) can be used, with CO₂ having a higher solubility in the later. Chemical absorption in turn is based in the chemical reaction between biogas CO₂ and the liquid solvent (e.g. mono ethanol amine (MEA) or dimethyl ethanol amine (DMEA)) (Bailón Allegue and Hinge, 2012; Petersson and Wellinger, 2009). In both types of absorption the bulk of CO₂ initially present in the biogas is finally encountered dissolved in the absorbing liquid. The means by which this liquid is regenerated determine the final concentration of CO₂ in the process off gas stream.

In physical absorption units regeneration is generally accomplished by desorption of CO₂ in flash tanks, where a lower pressure than in the absorption column is maintained for CO₂ to be released. The gas leaving the flash tanks contains mainly CO₂, with the rest being CH₄ (Vienna University of Technology, 2012) and has hence the potential to be used for bioconversion in ADs. In systems where the water or organic solvent is recirculated to the absorption stage, the liquid stream is further transferred to a desorption column where it is contacted with a counter flow of air that desorbs CO₂. This process dilutes the final CO₂ concentration in the off gas and derives into a high O₂ content, limiting suitability for CO₂ bioconversion in ADs. When chemical absorption is used, the stronger bond of CO₂ to the liquid media generally requires alternative regeneration strategies, which frequently imply a heating or vacuum step for CO₂ to be released (Bailón Allegue and Hinge, 2012). While this incurs in a higher cost for regeneration, it leads to an almost pure CO₂ off-gas stream (Persson, 2003; Vienna University of Technology, 2012).

There is a lack of publically available data and a different performance is expected for the same technology when considering different suppliers (Bailón Allegue and Hinge, 2012). This prevents a unique CO₂ concentration in the off-gas to be reported. However, both the gas streams resulting from regeneration of solutions used in physical and chemical absorption have been stated to have a high CO₂ purity and could hence potentially be used for CO₂ bioconversion in ADs. In the first case the stream generated

in the flash tank is considered as opposed to that from the desorption column, which is expected to have a low concentration of CO₂ ($\leq 30\%$) and high O₂ content ($\leq 20\%$) resulting from the air injection (*communication with site operators*).

b. Pressure swing adsorption

This upgrading technology is based on the capacity of an adsorption media to selectively retain certain components of a gas stream. For removal of CO₂ from biogas generally activated carbon or zeolites are used, which are placed in a column where biogas is injected from the bottom and biomethane is obtained from the upper section. When the absorption material is close to be saturated, this is regenerated by a sequential reduction of the column's pressure. The gas desorbed during the initial pressure drops contains a small percentage of CH₄ and is hence normally recirculated for further upgrading (Vienna University of Technology, 2012). However, the gas desorbed when further decreasing the column's pressure is mainly constituted of CO₂ and could potentially be used for its bioconversion to CH₄ in ADs. A pressure swing adsorption plant consists of several columns that are simultaneously operated in different working stages: adsorption, depressurisation, regeneration and pressure increase (Bailón Allegue and Hinge, 2012).

c. Membrane separation

The operation of membrane upgrading systems is based on the varied permeation rate of different molecules through permeable membranes. The separation is usually performed at pressures of 25-40 bars (Persson, 2003) although operation at pressures of around 8 bars can be achieved (Pettersson and Wellinger, 2009). High permeation area is normally achieved by operating in parallel modules of hollow-fibre-shaped membranes (Vienna University of Technology, 2012). The process exhausts are a CH₄ rich retentate (biomethane) and a CO₂ rich permeate. Concentration of CH₄ varies depending on membrane type and system's configuration, but CH₄ concentrations in the retentate of 92-99% are feasible to be achieved (Bailón Allegue and Hinge, 2012). The CO₂ concentrated stream leaving the membrane (permeate) could potentially be used for CO₂ bioconversion in ADs.

d. Cryogenic upgrading

This upgrading technology is based on the different liquefaction points of individual components present in a mixture of gases. In the case of biogas upgrading, CO₂ condensates at a lower pressure and higher temperature than CH₄, which enables

separation of both gases and recovery of CO₂ in liquid or solid form. The sublimation point of pure CO₂ at a pressure of 1 atm is at 194.65K, although higher pressures or lower temperatures are generally required when present in a mixture like biogas (Petersson and Wellinger, 2009). This technique is still under development, although pilot-scale installations upgrading biogas to a purity of 96% and recovering almost pure CO₂ are already being operated (Bailón Allegue and Hinge, 2012).

Table 7.1 summarises the CO₂ concentration expected in the gas exhaust from different biogenic CO₂ sources on a WWTP and the aspects that may limit its utilisation for bioconversion in ADs. There is a lack of reported data regarding exhaust CO₂ concentrations in streams from biogas upgrading units, which needs to be addressed by a more open reporting by suppliers, researchers and sites operators. This would in turn help to determine the best suitable streams to be used for CO₂ bioconversion, although it is already anticipated that streams from several upgrading systems would be suitable (Table 7.1).

Table 7.1. Sources of CO₂ available within a WWTP and its potential for bioconversion in ADs.

Stream	y _{CO₂}	Limiting factor	Potential use for bioconversion in ADs
CHP exhaust	0.05-0.15	Dilution of exhaust with N ₂ and O ₂ of the air used for combustion	No
Gas from first phase of TPAD	0.75	<ul style="list-style-type: none"> • Increase in pressure required to obtain driving force for mass transfer • Flowrate can be low in certain systems 	Yes
Biogas CO ₂	0.30-0.45	High increase in pressure required to obtain driving force for mass transfer	No
Aerobic wastewater treatment	0.008	Low CO ₂ concentration and unconfined stream	No
Off-gas from absorption biogas upgrading (flash tanks or regeneration of chemical absorbent)	^a		Yes
Off-gas from absorption biogas upgrading (exhaust from desorption column)	0.3	High O ₂ and low CO ₂ contents	No
Off-gas from pressure swing adsorption biogas upgrading	^a		Yes
Off-gas from membrane biogas upgrading (retentate)	^a		Yes
Off-gas from cryogenic biogas upgrading	0.98 ^a		Yes

^a Dependent on operation, configuration and manufacture specifications. However, concentrated CO₂ streams are attainable.

7.2.3. Possible points of CO₂ injection in an AD

Several points within the AD flowsheet can be considered for contacting CO₂ with the digesting material: the substrate input to the digester; via gas mixing systems; or using the digestate recirculation loop used for AD mixing. In this section the suitability of using these points to inject exogenous CO₂ for it to be bioconverted into CH₄ was investigated. As concluded in the previous section, CO₂ could be sourced from the off-gas of different biogas upgrading systems or from the gas produced in the first phase of a TPAD (Table 7.1). Combination of the potential CO₂ sources and points for injection lead to several alternatives for implementation (Table 7.2). The potential benefit obtainable with each injection point was assessed for alternative (a) of Table 7.2, where a two stage membrane module installed in a single phase AD has been considered to exemplify a base scenario. However, this should be seen as an exemplification for the purpose of comparing the rest of alternatives, knowing that

different systems like a TPAD process with CO₂ sourced from off-gas of biogas upgrading and/or gas produced in the first phase could also be possible (Table 7.2 alternative (b) and (c)).

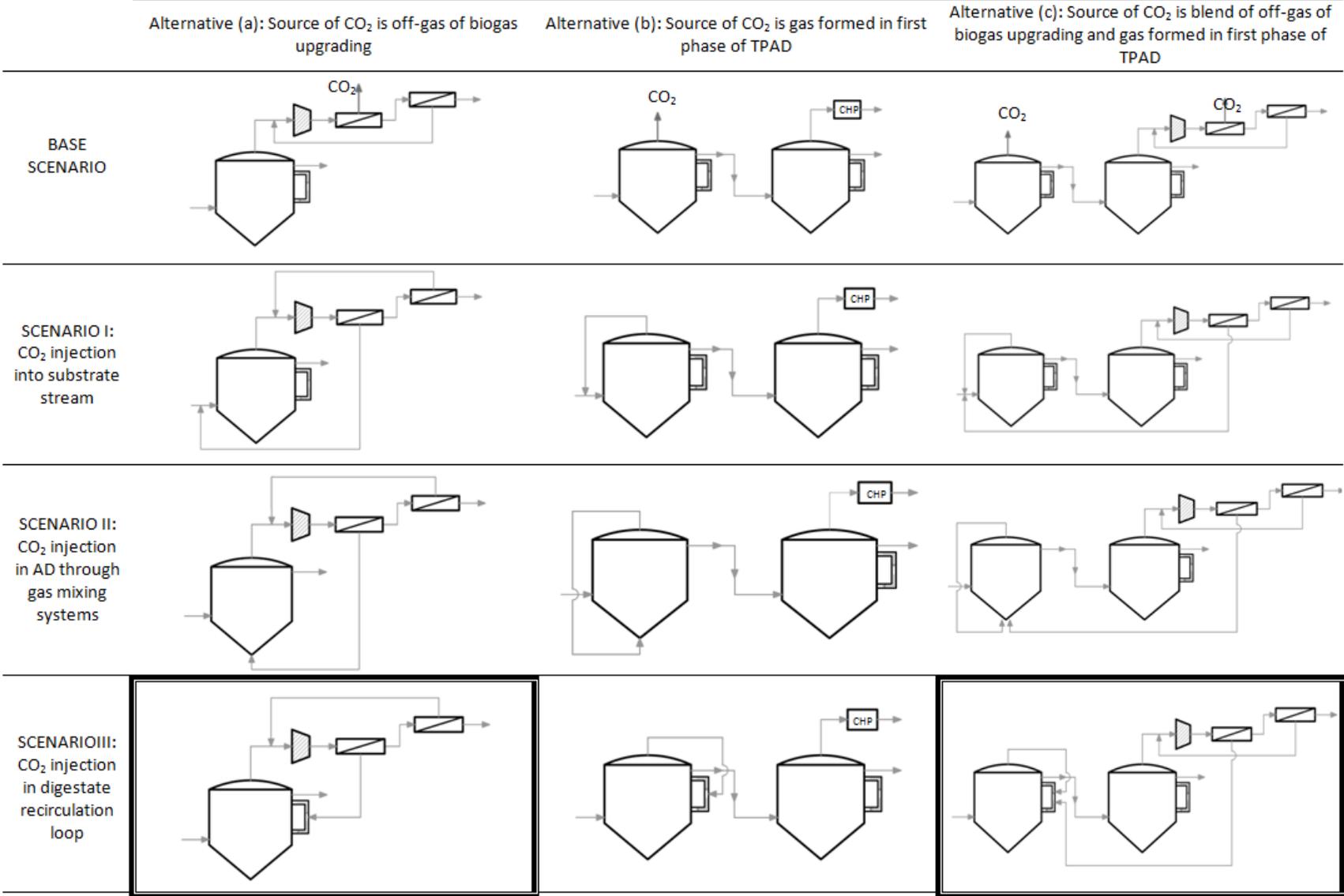
In scenarios I, II and III the possibility of injecting CO₂ into the substrate stream, gas mixing systems and digestate recirculation loop have been contemplated, respectively (Table 7.2). The suitability was assessed based on CO₂ gas to liquid mass transfer efficiency, flexibility of operation (CO₂ loading) and potential impact to the normal operation of the AD. Those injection points initially considered feasible have been further evaluated by assessing the potential uptake of CO₂ and improvement in CH₄ production that could be achieved when compared with a base scenario. Calculation of the maximum CO₂ that can be dissolved has been based on considering solubility of aqueous solutions at mesophilic temperatures and P_T =1 atm (supersaturation conditions not considered). Associated benefits in CH₄ production were calculated based on the stoichiometry derived from considering the reduction of two molecules of CO₂ by the Wood-Ljungdahl metabolic pathway to form one molecule of acetate (Eq. 2), which is then converted to a molecule of CH₄ and one molecule of CO₂ by acetoclastic methanogenesis (Eq. 3). Hence, the overall formation of one molecule of CH₄ per each molecule of CO₂ additionally reduced was considered. For the reasons described in *section 2.1*, only scenarios where both homoacetogenic bacteria and acetoclastic methanogens are expected to be present were considered. In every case the ammonia concentration was considered to be below toxicity levels for acetoclastic methanogenesis.

Homoacetogenesis: $2 \text{CO}_2(\text{g}) + 4 \text{H}_2(\text{g}) \rightleftharpoons \text{CH}_3\text{COO}^-(\text{aq}) + \text{H}^+(\text{aq}) + 2\text{H}_2\text{O}(\text{l})$ (Eq. 2)

Acetoclastic methanogenesis: $\text{CH}_3\text{COO}^-(\text{aq}) + \text{H}^+(\text{aq}) \rightleftharpoons \text{CH}_4(\text{g}) + \text{CO}_2(\text{g})$ (Eq. 3)

A scenario with a single phase AD and energy production with CHP engines has not been considered, as it is envisaged that no CO₂ stream suitable to be revalorised through CO₂ bioconversion into CH₄ would be available (Table 7.1).

Table 7.2. Potential scenarios for implementation of exogenous CO₂ bioconversion into ADs considered, with recommended implementation alternatives highlighted.



7.2.3.1. Base scenario

This scenario considers the operation of a 5200 m³ single phase AD operated with a hydraulic retention time (HRT) of 16 days and mixed by external pumping of digesting material with a turnover of 30 minutes. The CH₄ yield has been calculated based on a biogas yield of 0.3 m³·kg⁻¹ of waste treated (Georges et al., 2009) and CH₄ and CO₂ concentrations of 60% and 40%, respectively. Biogas upgrading has been exemplified with a two-stage dense membrane separation unit with internal recirculation. The permeate of the second stage (CH₄ concentration of 95%) is the biomethane to be incorporated into the national grid and the retentate of the first stage constitutes the CO₂ source available for its bioconversion into CH₄, with a y_{CO_2} of 0.77. Under these conditions it was estimated that the operation of this single phase AD would lead to the production of 4063 m³·h⁻¹ of biogas, of which 1439 m³·h⁻¹ of CO₂ would be recovered with the membrane upgrading system in a stream with a y_{CO_2} of 0.77 (Figure 7.1).

7.2.3.2. Scenario I: CO₂ injection into substrate stream

Scenario I is based on dissolving the CO₂ available identified in the base scenario into the substrate influent to the AD. The resulting dissolved CO₂ in the digesting material (inside the AD) could not be directly controlled, because CO₂ loading would be limited by the maximum CO₂ that can be dissolved in the incoming substrate with the operational HRT. When considering a p_{CO_2} of 0.77 atm in the CO₂ enriched stream resulting from the membrane upgrading system and a CO₂ solubility of 1071 mg·L⁻¹ (supersaturation conditions not considered), the amount of CO₂ that can be dissolved in the substrate stream is 825 g CO₂·tonne⁻¹. This implies an uptake of CO₂ of 11 kg·h⁻¹ when considering the size (5200 m³) and HRT (16 d) of the AD described in the base scenario.

A stoichiometric conversion of the additional CO₂ into CH₄ would lead to an increase in CH₄ production of 6.2 m³·h⁻¹. While this scenario implies the direct uptake of 98 tonnes of CO₂ per annum and an additional *ca.* 54500 m³ of CH₄ per annum when considering the full-scale AD described, the relative increase in CH₄ production is limited to *ca.* 0.25%. The additional CO₂ to be loaded into the unit is restricted to the amount that can be dissolved in the incoming substrate without altering the system's HRT. Furthermore, the high solids content and lack of homogeneity of the substrate is expected to significantly hinder gas transfer efficiency (GTE), which would significantly reduce the estimated benefits if dissolved CO₂ solubility levels are not achieved.

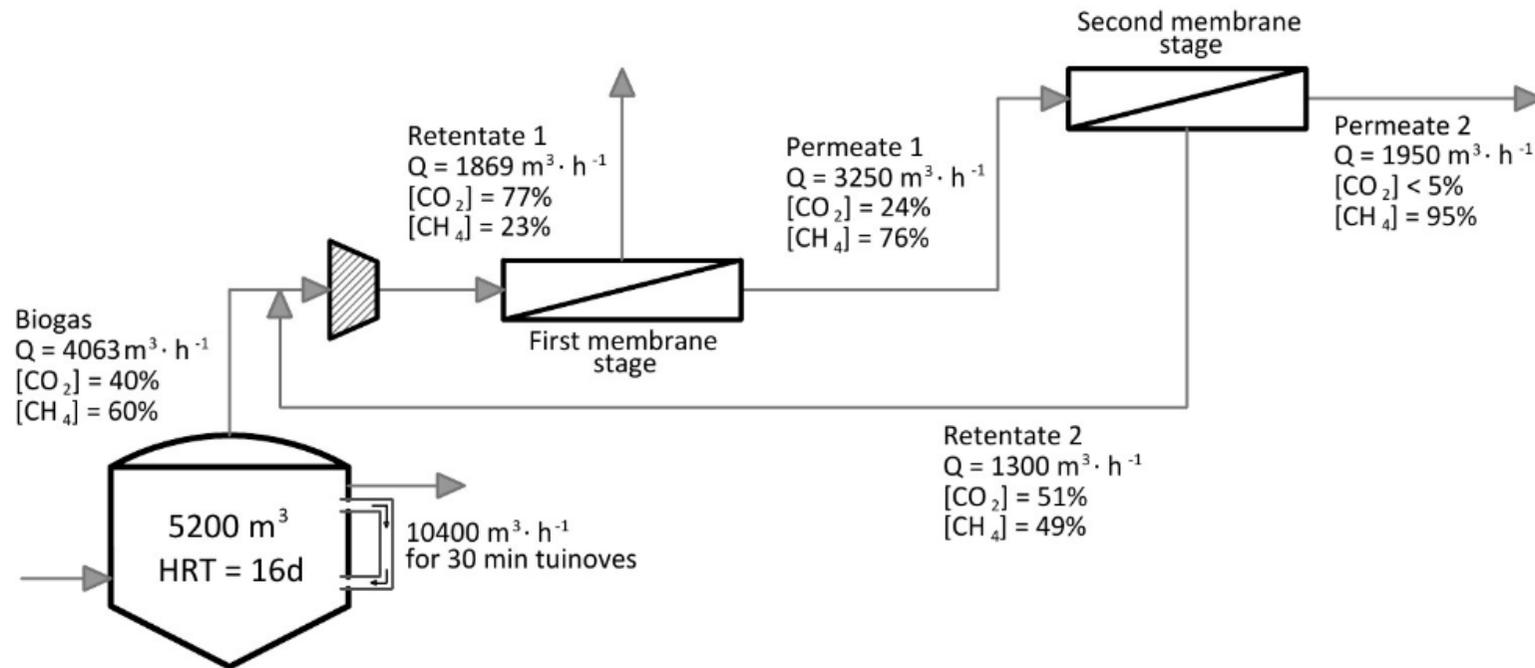


Figure 7.1. Summary of base scenario considered for benchmarking potential benefits of CO₂ bioconversion in ADs. A single phase AD with biogas upgrading by a two stage membrane module has been considered.

7.2.3.3. Scenario II: CO₂ injection through gas mixing systems

Injection of CO₂ through gas mixing systems would minimise the capital investment required for implementation of CO₂ bioconversion. However, in systems where energy recovery is undertaken by means of CHP engines, the injection of CO₂ enriched streams through gas mixing systems is necessarily limited by the risk of dilution of the AD's headspace. The release of any non dissolved CO₂ to the AD headspace would significantly offset the performance of the CHP engines, which require a relatively constant grade of CH₄ for operating efficiently. This issue will not be encountered when injecting CO₂ into the first phase of a TPAD (if only the headspace of the second phase is connected to CHP engines) or when biogas upgrading as opposed to CHP engines is used. However, a further limitation of injection of exogenous CO₂ through gas mixing systems is the low GTE expected, because of the systems been designed for mixing rather than specifically for gas to liquid mass transfer (reduced bubble specific surface area).

7.2.3.4. Scenario III: CO₂ injection into the digestate recirculation stream

Scenario III is based on dissolving the additional CO₂ into the digestate recirculation stream used for mixing or heating the AD content. Since a turnover of *ca.* 30 minutes is generally applied, the amount of CO₂ that can be dissolved would be significantly higher than in scenario I. Furthermore, GTE would benefit from a reduced solid content and increased homogeneity when compared to injection into the incoming substrate stream (scenario I) and systems specifically designed for mass transfer could be used (as opposed to scenario II). Implementation of CO₂ injection in this point of the AD flowsheet would be limited to the existence of an external recirculation loop where the gas to liquid mass transfer system could be installed.

A typical turnover rate of 30 min implies a digestate flowrate of 10400 m³·h⁻¹ when considering an AD of 5200 m³. This stream would have an initial dissolved CO₂ concentration of 428 mg·L⁻¹ when considering equilibrium conditions with the AD headspace (40% CO₂). This value can be considered a conservative estimate since lower dissolved CO₂ levels can be present in the digesting media of an AD (Bajón Fernández et al., 2015b; Lindberg and Rasmuson, 2006). This initial concentration would enable an additional dissolution of 396 mg CO₂·L⁻¹ until solubility levels associated with a p_{CO₂}=0.77 are achieved. When considering solubility levels in all the AD content are achieved, this would enable *ca.* 1147 m³ CO₂·h⁻¹ (2061 kg CO₂·h⁻¹) to be transferred into the liquid phase, which requires a GTE of *ca.* 80% to be achieved when considering the 1439 m³·h⁻¹ CO₂ estimated as available from the membrane upgrading system (Figure 7.1). Previous studies have, however, highlighted the complex rheology of digested sewage sludge (Baudez et al., 2011; Eshtiaghi

et al., 2012) and the impact that viscosity variations can have in GTE (Bajón Fernández et al., 2015a; Ozbek and Gayik, 2001). Values of GTE as low as 1.6-7.6% have been reported when performing absorption tests in solutions with viscosity ranges imitating those typically found in digested sludge (Bajón Fernández et al., 2015a). When applied to the current scenario a GTE of 80% would imply 18051 tonnes of CO₂ per annum to be dissolved in the AD and an increase in CH₄ production of 47% (1147 m³ CH₄·h⁻¹) when considering full time availability, continuous CO₂ injection and a 1:1 stoichiometric conversion of CO₂ to CH₄. Benefits would be reduced by a tenfold if GTE of 8% is considered, which would decrease carbon benefits to 1805 tonnes CO₂ uptaken·annum⁻¹ and CH₄ increase to 4.7% respect to base scenario. The importance of understanding gas to liquid mass transfer in fluids of complex rheology and of accounting for possible viscosity variations during the design of a gas to liquid mass transfer system is hence evident (Bajón Fernández et al., 2015a).

When considering the advantages, disadvantages (Table 7.3) and potential benefits (Table 7.4) of each of the gas injection points considered, injection of exogenous CO₂ in the digestate recirculation loop of an AD appears the recommended option (Table 7.2). However, it needs to be considered that injection point selection is to be based on existing AD configuration and that a hindered GTE associated with some operational conditions (e.g. solid content of substrate streams or bubble size of the gas injection system) would imply a reduced gas to liquid mass transfer rate but not a reduced bioconversion of CO₂ to CH₄ once the desired CO₂ levels are achieved.

Table 7.3. Possible points for exogenous CO₂ injection into ADs considered, advantages and disadvantages identified.

Stream	Advantages identified	Disadvantages identified	Systems to which implementation is limited
Substrate stream	Potential easier implementation	<ul style="list-style-type: none"> • Reduced mass transfer efficiency • Loading of CO₂ to AD limited by HRT 	
AD through gas mixing systems	Reduced solids content and increased homogeneity	<ul style="list-style-type: none"> • Reduced mass transfer efficiency • Injection in AD will dilute headspace's CH₄ content 	It could be implemented in first phase of TPAD if headspace is not connected to CHP
Digestate recirculation loop	Reduced solids content and increased homogeneity		External digestate recirculation loop needs to be available

Table 7.4. Potential benefits of CO₂ bioconversion in ADs for each scenario considered.

Scenario	Brief description	Source of CO ₂ considered	CO ₂ available estimation (m ³ ·h ⁻¹)	Initial CO ₂ dissolved concentration considered in liquid stream to enrich with CO ₂ (mg·L ⁻¹)	Maximum CO ₂ that can be uptaken (tonnes·annum ⁻¹)	Relative increase in CH ₄ production (%) ^b	Main limitation
Base scenario	Single phase AD	biogas upgrading	1439	n/a	n/a	n/a	n/a
Scenario I	Single phase AD with CO ₂ injected in incoming substrate	biogas upgrading	1439	0	98	0.25	CO ₂ loading function of HRT and mass transfer efficiency
Scenario III	Single phase AD with CO ₂ injected in digestate recirculation	biogas upgrading	1439	428	1805-18051 ^a	4.7-47 ^a	mass transfer efficiency

^a Range due to consideration of GTE of 8% and 80%.

^b Considered 1:1 CO₂ to CH₄ conversion stoichiometry.

7.2.4. Means of injection

The potential of implementing CO₂ bioconversion in scaled-up ADs requires investigating possible technologies that could be utilised to perform the required gas to liquid mass transfer. Previous related studies are mainly based in laboratory scale experimental set-ups (Alimahmoodi and Mulligan, 2008; Bajón Fernández et al., 2014; Francioso et al., 2010; Sato and Ochi, 1994) with only few of them employing pilot-scale units (Bajón Fernández et al., 2015b; Salomoni et al., 2011). When these last are considered, injection of CO₂ has been performed through internal tubing or by an external bubble column, with the later reported as suitable because of its flexibility of operation and low CH₄ loss achievable during CO₂ injection (Bajón Fernández et al., 2015b). Usage of bubble columns for contacting gas with digesting media has been reported as suitable in other applications like ammonia stripping for reducing toxicity in ADs (De la Rubia et al., 2010; Walker et al., 2011) or in-situ CH₄ enrichment (Lindberg and Rasmuson, 2006). Guštin and Marinšek-Logar, (2011) utilised a packed column for contacting digestate with air for ammonia removal. However, in this case the material was previously centrifuged for suspended solids removal. When this precondition step is not included, it is anticipated that solids accumulation in the system will difficult process operation and hence columns without packing material are considered more suitable for a potential full-scale implementation of CO₂ bioconversion in ADs.

Conventional bubble columns or commercial versions of this technology operating with a similar principle but with enhanced gas to liquid mass transfer could potentially be utilised. A CO₂ injection system that could be retrofitted to an operational 1.5 m³ AD was designed during this project, as a means to retrofit the AD demonstrator available at Cranfield University with bioconversion of exogenous CO₂. Performance of an analysis based on Kepner Tregoe methodology indicated that the Downflow Gas Contactor (DGC) supplied by WRK Design and Services Ltd (Birmingham, UK) was a suitable technology to be used for this particular application. The technologies considered included jets, conventional bubble columns, DGC of WRK and fluidic oscillator of Sheffield University. The parameters accounted for when selecting the CO₂ injection technology were, within others, efficient mass transfer, operational flexibility, ease of operation and low time of commissioning. However, these parameters and the relative importance attributed to each of them would vary depending on the system where it is to be retrofitted. The Kepner Tregoe analysis performed is included in Appendix 2. The final design of the selected system is also included in Appendix 2.

7.3. CONCLUSIONS: RECOMMENDED ALTERNATIVE AND POTENTIAL BENEFITS

Bioconversion of carbon dioxide in ADs has the potential of becoming an on-site carbon management solution with concomitant benefits in renewable energy production. Its application is recommended in systems where both homoacetogenic bacteria and acetoclastic methanogens are present, which in turn limits implementation to systems where ammonia concentration is maintained below *ca.* 1500 mg·L⁻¹ NH₄ (threshold for inhibition of acetoclastic methanogens). Carbon footprint benefits are predicted when CO₂ is injected in single phase ADs or in the first phase of a TPAD process. As far as the gas injection point is concerned, injection of CO₂ in digestate recirculation streams is recommended where possible, because of the reduced solid content and increased homogeneity which favours gas to liquid mass transfer. The gas stream produced in the first phase of a TPAD and the off-gas of several upgrading technologies have been identified as potential streams to be revalorised through CO₂ bioconversion in ADs (Table 7.1). Injection of exogenous CO₂ through an external bubble column is considered suitable.

When considering injection of CO₂ into the digestate recirculation loop of a 5200 m³ AD with a HRT of 16 days, a maximum uptake of 18051 tonnes of CO₂ per annum has been estimated as possible if a GTE of 80% can be achieved. The high viscosity of anaerobically digested substrates (e.g. sewage sludge) can hinder the benefits obtainable in carbon footprint because of a reduced GTE, with a maximum uptake of 1805 tonnes CO₂ uptaken·annum⁻¹ if GTE is limited to 8%. Assimilation of this CO₂ by homoacetogenesis (Wood-Ljungdahl mechanism) followed by acetoclastic methanogenesis with a 1:1 stoichiometric conversion of CO₂ to CH₄ would imply an increase of 4.7-47% (GTE of 8% and 80%, respectively) in CH₄ production when compared to the base scenario. If an energy yield from CH₄ of 10 kWh·m⁻³ CH₄ and a CHP electrical efficiency of 35% are considered, the higher CH₄ production would imply an increase in electrical output of 3.5-35 GWh per annum. With a feed in tariff (FiT) of 9.49 p·kWh⁻¹ (Ofgem, 2014) a revenue of £334k - £3340k per annum could potentially be generated.

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CHAPTER 8
THESIS DISCUSSION

8. THESIS DISCUSSION

Contrary to greenhouse gas (GHG) mitigation aims, the water sector is experiencing an increasing carbon footprint, partly due to the higher energy demand required to meet the tightened quality standards resulting from the implementation of the Water Framework Directive (European Commission, 2009). To illustrate, the energy usage for operation of water and wastewater treatment sites in the UK increased by 8.7% between 2007 and 2011 (Water UK, 2012, 2008). Emissions of GHG from the water sector at world level have been estimated to account for 3-10% of total global emissions (McGuckin et al., 2013), making evident the potential of this sector to contribute towards GHG mitigation (McGuckin et al., 2013; USEPA, 2012). This has led to a surge in research aimed at capturing emitted carbon dioxide (CO₂), at developing treatment technologies with a low energy demand and at increasing renewable energy production. Biogas CO₂ produced in anaerobic digesters (ADs) is the stream most readily available to be captured for storage or valorisation within the wastewater treatment flowsheet (Byrns et al., 2013). However, the scattered location of AD sites is a limiting factor to implement carbon capture and storage (CCS) techniques relying on transporting this CO₂ to a final reservoir. Injection of CO₂ in ADs for its bioconversion into methane (CH₄) has been identified as a solution which could be implemented (Byrns et al., 2013). However, previous studies have been insufficient to determine the potential of this technique to become an on-site carbon management strategy.

This research involved assessing the potential benefits obtainable when injecting CO₂ in ADs treating different substrates (sewage sludge and food waste). Operation of laboratory scale batch ADs enabled an initial quantification of the benefits in carbon footprint and concomitant increases in CH₄ formation obtainable when saturating the digesting material with CO₂ streams of different concentrations. The performance of the tests with real sewage sludge and food waste samples enabled a substrate dependant response to injections of exogenous CO₂ to be identified. This was attributed to differences in the methanogenic communities present in ADs treating different substrates and allowed a hypothesis for CO₂ utilisation to be proposed. It was suggested that exogenous CO₂ was reduced in early stages of the digestion process, likely by the Wood-Ljungdahl mechanism, leading to formation of acetate that in turn stimulated acetoclastic methanogenesis. Previous literature presents conflicting information regarding the mechanisms by which exogenous CO₂ can be utilised in anaerobic processes, with studies supporting a boost of hydrogenotrophic methanogenesis and of acetoclastic methanogenesis available. To elucidate CO₂ fate in ADs, microbial community analyses were performed for the first time in sewage sludge and food waste ADs enriched with CO₂ by using fluorescence in situ hybridisation (FISH). These confirmed the initially proposed hypothesis (encouragement of acetoclastic methanogenesis) and enabled the

suitability of implementing CO₂ bioconversion for different substrates to be assessed. It was considered that monitoring of headspace hydrogen (H₂) concentration and volatile fatty acids (VFA) dynamics in continuously operated ADs could provide further insight regarding CO₂ fate, with regard of its reduction by the Wood-Ljungdahl mechanism. This was done under controlled conditions using real food waste in a pilot-scale system where both a control and a test AD were operated. These tests also allowed the suitability of using bubble columns to inject exogenous CO₂ to be evaluated. Furthermore, operation of a pilot-scale AD retrofitted with CO₂ injection permitted the identification of CO₂ gas to liquid mass transfer as a critical parameter limiting dissolved CO₂ levels achievable in the AD. It was hypothesised that the high viscosity and complex rheology of anaerobically digested material could hinder mass transfer efficiency, which was confirmed with absorption tests performed in a pilot-scale bubble column operated with liquid phases of Newtonian and non-Newtonian rheology. All the information was then used to investigate the possibility of implementing CO₂ bioconversion as an on-site carbon revalorisation strategy in full-scale units, by considering potential CO₂ sources, means and points of injection and estimating potential benefits.

8.1. POTENTIAL OF BIOCONVERSION OF CO₂ IN ADs TO CONTRIBUTE TO GHG MITIGATION

Previously reported studies addressing the capacity of anaerobic processes to uptake exogenous CO₂ were insufficient to determine the potential contribution of these towards carbon footprint reduction. In the majority of cases, conditions of CO₂ injection were not described and in no case was reported the CO₂ partial pressure (p_{CO_2}) utilised for injection into ADs, which is determining of the dissolved CO₂ concentration achievable in the liquid phase. It was hence hypothesised that injection of CO₂ with different p_{CO_2} would lead to different benefits in carbon uptake. The different benefits achievable for ADs treating different substrates were investigated for the first time. Batch laboratory scale ADs were operated (Chapter 3) to determine the potential of ADs to uptake CO₂, for which it was ensured that CO₂ saturation levels were achieved in the digesting media for initially quantifying carbon savings with respect to control units. Overall CO₂ reductions of 8% and 34% were estimated for sewage sludge ADs saturated with p_{CO_2} of 0.5 and 1.7 bar, respectively (CO₂ molar fractions (y_{CO_2}) of 0.3 and 0.9, respectively). Benefits of 3%, 10% and 11% in CO₂ emissions were estimated for food waste ADs saturated with p_{CO_2} of 0.4, 0.8 and 1.6 bar, respectively (y_{CO_2} of 0.3, 0.6 and 0.9, respectively). Comparison with the scarce previous studies injecting CO₂ into ADs is limited because CO₂ uptaken was reported as a percentage of CO₂ injected ((40% (Francioso et al., 2010), 46% (Salomoni et al., 2011)), which is not a comparable parameter between studies, rather than in relation to CO₂ dissolved. Furthermore, p_{CO_2} of injection was not reported.

The laboratory trials also provided information of the potential benefits achievable in CH₄ production. Increases of 14%, 11% and 16% in CH₄ production during the first 24 hours after saturating with p_{CO₂} of 0.4, 0.8 and 1.6 bar, respectively, were obtained in food waste units (y_{CO₂} of 0.3, 0.6 and 0.9, respectively). Increases of 96% and 138% in the same parameter were obtained for sewage sludge ADs enriched with p_{CO₂} of 0.5 and 1.7 bar, respectively (y_{CO₂} of 0.3 and 0.9, respectively). This is the first study investigating CO₂ bioconversion in food waste units and to report a substrate dependant response to injection of exogenous CO₂. When compared with the improvements of 30% in CH₄ yield reported by Sato and Ochi, (1994) for sewage sludge ADs enriched with a gas stream of 60%, the benefits reported in this research are considerably higher. This was attributed to a higher dissolution of CO₂ in the liquid phase because of the use of sintered diffusers and because it was ensured that equilibrium with the gas injected was reached. Salomoni et al., (2011) stated an increase in specific CH₄ yield of 25% when continuously injecting CO₂ in the first phase of a two-phase anaerobic digester (TPAD). However, performance was compared with a single phase AD and, therefore, it did not enable quantification of the enhancement related to the bioconversion of CO₂ into CH₄ and to the use of different AD configurations.

The laboratory tests also enabled to determine that 24-48 hours were enough for any acidification associated with CO₂ saturation to be recovered, raising the question of whether periodic CO₂ injections could maintain the benefits obtained over the 24 hours period following a single CO₂ saturation. The operation of batch laboratory scale ADs with periodic CO₂ injections ratified the reproducibility of the benefits previously recorded for sewage sludge units. However, the moderate benefit previously observed in food waste ADs (Chapter 3) was not replicated (Chapter 4), which was attributed to the variability of food waste depending on source. It was confirmed that periodic CO₂ injections could further increase CH₄ production during the digestion process. However, the batch nature of the tests reduced the benefits of sequent injections (Chapter 4) because the bulk of the CH₄ yield was produced during the first days of operation (Astals et al., 2013). Increases of 1.3-1.5 fold in CH₄ production were obtained after the second injection of CO₂. It was hence evident the value of assessing the potential of CO₂ to be utilised in continuously operated units. The substrate dependant response to exogenous CO₂ was attributed to differences in methanogenic communities and helped elucidating its mechanisms of utilisation.

8.2. MECHANISMS OF UTILISATION OF EXOGENOUS CO₂ IN ADs

Previous studies have reported the potential of anaerobic processes to uptake exogenous CO₂ as an overall term (Alimahmoodi and Mulligan, 2008; Chapter 3). This was suitable to identify the contribution of CO₂ enrichment of ADs to GHG mitigation. However,

it did not provide insight in CO₂ fate mechanisms, required for process understanding and optimisation. Indeed, previous literature presents conflicting information with regard to the means by which CO₂ could be bioconverted to CH₄. References supporting both an encouragement of hydrogenotrophic (Alimahmoodi and Mulligan, 2008) and of acetoclastic methanogenesis (Francioso et al., 2010) were available, although none of them provided experimental evidence to support the research hypothesis. The substrate dependant response to exogenous CO₂ observed in sewage sludge and food waste ADs (Chapter 3 and Chapter 4) enabled an initial hypothesis to be proposed, as follows. Production of CH₄ after saturation of the digesting material with CO₂ was significantly more enhanced in ADs treating sewage sludge than food waste. This was hypothesised due to differences in the CH₄ producing *Archaea* between both substrates. The ammonia concentration in food waste ADs was quantified at 4 g·L⁻¹ NH₄-N, which is above the 3 g·L⁻¹ NH₄-N stated as completely inhibitory for acetoclastic methanogens (Rajagopal et al., 2013). On the other hand, the ammonia concentration in sewage sludge ADs was 1.2 g·L⁻¹ NH₄-N, which suggested that both acetoclastic and hydrogenotrophic methanogenesis were actively contributing towards total CH₄ formation. It was hence hypothesized that the higher benefits observed in sewage sludge treating ADs was due to an encouragement of acetoclastic methanogenesis. Therefore, microbial community analyses in ADs enriched with CO₂ were performed for the first time in order to confirm the proposed hypothesis (encouragement of acetoclastic methanogenesis). *Methanosaetaceae* (Mx825), *Methanobacteriaceae* (Mbac1174) and *Methanosarcinaceae* (MS1414) *Archaea* were targeted with oligonucleotide probes and its biovolume fraction calculated in relation to signal from a general *Archaea* probe (Arch915). A clear difference in *Archaea* populations between substrates was detected. *Methanosaetaceae* (obligate acetoclastic methanogen) was confirmed the dominant methanogen in sewage sludge ADs, contributing in 86.4±12.1% to the total *Archaea* population. However, *Methanosaetaceae* was found scarce in food waste units, representing 4.3±1.7% of the total *Archaea* community. *Methanosarcinaceae* was found to have the greatest contribution to the total *Archaea* population within the methanogens studied in food waste ADs (Table 4.3, Chapter 4), which is in agreement with previous studies stating this as predominant in putrescible waste ADs (Vavilin et al., 2008). A higher activity of *Methanosaetaceae* (up to 80%) was observed in sewage sludge ADs where CO₂ was injected periodically, which confirmed an encouragement of acetoclastic methanogenesis in response to exogenous CO₂. Furthermore, evidence was provided of ammonia toxicity being the reason for the lack of *Methanosaetaceae* communities in food waste ADs, because of the morphology encountered for *Methanosarcinaceae* cells. This *Archaea* was found to form large clusters in food waste matrices, while they appeared dispersed in smaller aggregates in sewage sludge samples

(Figure 4.3, Chapter 4). This different morphology was attributed to a cluster structure reducing ammonia permeation inside the individual cells (Macario et al., 1999), because of the higher volume to surface area ratio (Wiegant and Zeeman, 1986).

It is of note, however, that method development for FISH analysis was more restrictive in food waste samples due to a more complicated matrix for probe permeation and fluorescence detection. Inclusion of five freeze-thaw cycles, consisting of 5 minute steps at -80°C and 60°C of the samples previously fixed with 4% PFA, were required to enhance probe hybridisation (Narihiro and Sekiguchi, 2011; Sekiguchi et al., 1999). This greatly increased the image quality and enabled *Archaea* populations between food waste samples to be compared (Chapter 4). However, sum of the fluorescence corresponding to the three specific *Archaea* tested (*Methanosaetaceae*, *Methanobacteriaceae* and *Methanosarcinaceae*) constituted only 19 to 40% of the total *Archaea* signal emitted by Arch915 probe. This could be attributed to *Archaea* other than those tested being present in food waste samples or to an incomplete probe hybridisation. Previous studies have described the complexity of completing FISH analysis in food waste matrices and have reported sums of individual methanogens lower than 100% (Zhang et al., 2013). Nevertheless, the images acquired were suitable to compare *Archaea* evolution trends and images more representative (z-stack) and of higher quality than some previous studies were obtained. The high matrix complexity of food waste samples was further evidenced by the non-uniform spatial distribution of *Archaea* cells within the samples, which was not encountered in sewage sludge. *Methanosarcinaceae* clusters were mainly found in the centre of the xy-plane and higher positions in the z-axis, while *Methanosaetaceae* and *Methanobacteriaceae* were predominant in the edge of the wells and lower positions of the z-axis. Therefore, it was required to use z-stack images for quantification, as opposed to single fields, in order to account for this variability. A video evidencing this spatial distribution is included in a CD in the back cover of this thesis.

Microbial analyses confirmed the initial proposed hypothesis (exogenous CO₂ enhances acetoclastic methanogenesis) but could not elucidate whether the increased obligate acetoclastic activity was due to a direct impact of CO₂ on this *Archaea* or to an indirect alteration through previous stages of the AD process. It was hypothesised that CO₂ was reduced by the Wood-Ljungdahl pathway of CO₂ fixation (Ragsdale and Pierce, 2008) and that monitoring of VFA and H₂ levels in continuously operated ADs could help to confirm this alternation in the initial stages of the digestion process. Operation of pilot-scale ADs treating food waste and retrofitted with CO₂ injection provided further insight with regard to CO₂ fate in ADs. An increase of 2.5 fold in the headspace H₂ concentration of the unit enriched with CO₂ was obtained when compared to the control reactor. Peaks of H₂ over 600 ppm were obtained, which are within the reported ranges for acetogenesis to become

thermodynamically unfavoured (Cord-Ruwisch et al., 1997; Harper and Pohland, 1986; Kidby and Nedwell, 1991)). Indeed, H₂ concentrations of 464 ppm were recorded in the control AD when digester failure occurred as a consequence of a temperature drop (Chapter 5). However, no reduction in CH₄ production was observed in the test AD when operating at a baseline of H₂ concentration of 320±153 ppm with peaks > 600 ppm. Indeed, the test AD operated stably with a CH₄ production rate of 0.56±0.13 m³ CH₄·(kg VS_{fed}·d)⁻¹, a CH₄ content of 68.5±3.4% and a pH of 7.8±0.2 units. This unit also proved to have a higher stability towards process disturbances, with a temperature drop of 12.5°C not affecting process performance in the long term and without corrective actions (e.g. partial re-seed) being required to recover process stability. The increase in H₂ production following periodic CO₂ injections was believed to be attributed to the release of protons as a result of dissolution of an acid gas in an aqueous media and to an alteration of obligate acetogenesis. This supports an indirect impact of CO₂ in acetoclastic communities, because of a higher substrate (acetate) availability due to the reduction of CO₂ via the Wood-Ljungdahl mechanism (Ragsdale and Pierce, 2008). This has been reported to be stimulated by exogenous CO₂ (Misoph and Drake, 1996). Further microbial analysis targeting homoacetogenic bacteria should be considered to confirm this proposed mechanism. These findings have helped to elucidate the mechanisms of CO₂ utilisation, in particular in relation to different AD substrates and configurations which can help support the implementation of CO₂ enrichment.

8.3. EFFECT OF AD SUBSTRATES IN CO₂ BIOCONVERSION

Previous references addressing the bioconversion of CO₂ in anaerobic processes have not provided a wider perspective as to its suitability for a range of substrates (Alimahmoodi and Mulligan, 2011, 2008; Francioso et al., 2010; Salomoni et al., 2011; Sato and Ochi, 1994). The findings of this research regarding the fate of exogenous CO₂ in ADs provides further insight in this area. The proposed mechanism of CO₂ utilisation (reduction of CO₂ by homoacetogenesis through the Wood-Ljungdahl pathway and utilisation of the generated acetic acid by acetoclastic methanogens), potentially limits implementation to systems where both homoacetogenic bacteria and acetoclastic *Archaea* are present. Ammonia concentrations in ADs treating substrates with a high protein content inhibit acetoclastic methanogenesis (Banks et al., 2008), which was confirmed in this research by the low contribution of *Methanosaetaceae* to the total population of *Archaea* found in food waste samples with ammonia concentration of 4 g·L⁻¹ NH₄-N (Chapter 4). The maximum total ammonia concentration for implementation of CO₂ bioconversion with additional formation of CH₄ is believed to be within the range of 1.5-3.0g·L⁻¹ NH₄-N (Chapter 7), which covers reported levels of partial to total inhibition of acetoclastic methanogenesis (Rajagopal et al., 2013). Units treating sewage sludge or co-digesting this with other substrates are expected to

maintain total ammonia concentration below this range. However, operation of ADs treating solely animal waste or food waste rich in proteins are likely to maintain ammonia inhibitory or toxic levels (Banks et al., 2008; Siles et al., 2010; Walker et al., 2011). Implementation of CO₂ bioconversion with these substrates may not be feasible unless ammonia control strategies are implemented (e.g. ammonia stripping (Walker et al., 2011), dilution of substrate with water (Banks et al., 2008) or chemical precipitation (Chen et al., 2008)). Injection of CO₂ was not found to contribute to ammonia stripping in a significant manner (Chapter 4 and Chapter 5). This does not contradict references using gas injection as an ammonia stripping strategy when considering that those normally require altering the digestate temperature and pH conditions to achieve substantial ammonia removals (Guštin and Marinšek-Logar, 2011; Serna-Maza et al., 2014).

If ammonia concentration is considered critical for obtaining an enhanced CH₄ production in response to CO₂ injection, benefits associated with CO₂ injection in the pilot-scale trials (1.8 g·L⁻¹ NH₄-N) should have been similar to those obtained in laboratory scale units treating sewage sludge (1.2 g·L⁻¹ NH₄-N). However, an obvious increase in CH₄ production was not recorded in the pilot-scale food waste AD operated with CO₂ injection (Chapter 5). This could be attributed to a lack of *Methanosaetaceae* communities in the inoculum used for AD start-up or most likely to CO₂ gas to liquid mass transfer efficiency limiting the benefits achievable in CH₄ production. Dissolved CO₂ levels in the exit of the bubble column used for CO₂ injection reached only *ca.* 25% of solubility values (Chapter 5). Although an uptake of 4.0E-3 kmol CO₂·m⁻³ per injection (once every 48 hours) was obtained, the amount of dissolved CO₂ limited the obtainable benefit in CH₄ production, which could not be confidently quantified when compared with the normal variability of the biological process (high standard deviation). Previous laboratory tests were operated ensuring that CO₂ saturation conditions were achieved in the digesting media (Chapter 3), hence the higher CO₂ uptakes and CH₄ production increases obtained for ADs operated under a similar ammonia concentration. Identification of dissolved CO₂ as a critical parameter raised the importance of understanding CO₂ gas to liquid mass transfer in digesting media and of its efficiency to be increased in scaled-up units for benefits similar to those obtained at laboratory scale to be achievable.

8.4. IMPORTANCE OF UNDERSTANDING MASS TRANSFER FOR IMPLEMENTING CO₂ BIOCONVERSION IN ADs

The efficiency of CO₂ gas to liquid mass transfer influences the amount of dissolved CO₂ loaded into an AD for this to be bioconverted to CH₄. Gas to liquid mass transfer systems are widely used in both environmental and industrial sectors. However, gas to liquid mass

transfer in viscous fluids is still poorly understood, particularly when considering fluids with non-Newtonian rheological behaviour (Martín et al., 2008). Anaerobically digesting materials like sewage sludge are accounted as non-Newtonian fluids of complex rheology (Baudez et al., 2011; Eshtiaghi et al., 2012) and present a variable apparent viscosity (μ_a) as a function of temperature (Hammadi et al., 2012), solid content (Brar et al., 2005; Goel et al., 2004; USEPA, 1979) or shear history (Honey and Pretorius, 2000). This prevents mass transfer systems involving anaerobically digested media to be designed based on empirical correlations obtained for Newtonian fluids; and necessitates an understanding of the impact that viscous and rheological variations can have in process performance (Chapter 6). Typical μ_a of sewage sludge is reported between 150-400 cPo, but values as varied as 50 to 1000 cPo have been reported (Table 6.1, Chapter 6) (Brar et al., 2005; Eshtiaghi et al., 2012; Frost, 1983; Goel et al., 2004; USEPA, 1979).

Absorption tests performed in a pilot-scale bubble column (10.1 cm diameter and 2 meter tall) allowed investigation of mass transfer retardation due to μ_a variations for fluids of different rheologies (Newtonian and shear thinning). Clear synthetic fluids (i.e. glycerol and carboxymethyl cellulose sodium salt (CMC)) were employed to decouple the impact of μ in mass transfer efficiency from other process variables like solids content. It was confirmed a reduction on mass transfer efficiency in response to μ_a increases, which influences the minimum buoyancy required for bubble detachment and strongly influences system hydrodynamics (Chapter 6). For the first time, it was stated that an equivalent increase in μ_a has different implications in fluids of Newtonian and non-Newtonian rheology. Mass transfer was found to be conditioned by the μ of the fluid at rest in shear thinning fluids (like sewage sludge), as opposed to by μ_a determined with average shear rate ($\dot{\gamma}_{av}$). This at rest μ influenced fluid hydrodynamics and offered a greater resistance for CO₂ gas to liquid transfer (Badino et al., 2001). A new hydrodynamic regime (slug-annular flow) was encountered for shear thinning fluids, which was characterised by a rising chain of bubbles in the centre of the column stabilised by non-mixed fluid present in the outer annulus, as opposed to by the column wall. The conditions that would imitate the common operation of sewage sludge ADs (μ and $\dot{\gamma}$) were included within those tested: μ_a of 150-400 cPo (Table 6.1 and Table 6.2, Chapter 6) (Eshtiaghi et al., 2012; Frost, 1983; Goel et al., 2004; USEPA, 1979) and $\dot{\gamma}$ of 50-80 s⁻¹ (USEPA, 1979). Therefore the findings are of great value for the design of full-scale mass transfer systems involving anaerobically digested media. It was concluded that to fully characterise the liquid phase involved in mass transfer in a site by site basis is required. Failure to do so can have a negative implication in the performance of a gas to liquid mass transfer system. On the one hand an increase of μ_a from 130 to 340 cPo (which is within typical variability of sewage sludge) can reduce volumetric mass transfer coefficients (k_{La}) by

43% and gas transfer efficiency (GTE) by 6 percentage points (Chapter 6). Gas flowrate could be increased as a means to compensate for the reduced performance, but the increased operational costs associated with the extra pumping may offset the benefits of enhancing $k_L a$. On the other hand, development of slug-annular flow would imply a reduced active volume for mass transfer. In order to avoid this, special care should be given to the design of gas distribution systems in units dealing with non-Newtonian fluids. A higher number of diffusers per surface area may be required to avoid a reduced active volume for mass transfer.

8.5. PRACTICALITIES FOR IMPLEMENTATION AND IMPLICATIONS OF THE WORK

Previous research in the field of CO₂ bioconversion in anaerobic processes has highlighted the lack of studies addressing the practicalities of implementation in up-scaled systems (Byrns et al., 2013). This research has provided data in relation to full-scale implementation by determining potential CO₂ streams to be utilised, means and conditions for injection into ADs and potential benefits obtainable at full-scale. The operation of ADs saturated with different p_{CO_2} indicated that ADs enriched with higher P_{CO_2} achieved greater enhancements in CO₂ uptake and CH₄ production (Chapter 3). For both types of substrates treated (food waste and sewage sludge) the best performance in terms of CO₂ uptake and CH₄ production was obtained when using a $p_{CO_2}=1.6-1.7$ bar ($y_{CO_2}=0.9$). This differs from the findings of Sato and Ochi, (1994) who indicated that reduction of yields were observed for y_{CO_2} over 0.6, which was determined as an optimum. However, in that study p_{CO_2} were not reported, which determines dissolved CO₂ levels as opposed to y_{CO_2} . Furthermore, in that study the reduced performance at higher concentrations was attributed to a drop in pH, while in this research any pH drop was below 0.6 units in all the laboratory and pilot scale ADs operated with exogenous CO₂ injection. Furthermore, the pH drop was always overcome within 24-48 hours and values below pH 6, which was stated as inhibitory by Gerardi, (2003), were never obtained. Hence it was concluded that CO₂ streams with a higher CO₂ content would lead to a greater carbon mitigation. Off-gas of several biogas upgrading technologies (e.g. pressure swing adsorption, membranes (Table 7.1, Chapter 7)) are considered the most feasible sources of on-site CO₂ within the wastewater treatment flowsheet (Chapter 7). For the first time, possible points of injections were also investigated, based on knowledge gained in relation to CO₂ fate and mass transfer. Injection into the digestate recirculation loop of an AD was determined as the most suitable point to retrofit a gas to liquid mass transfer system for CO₂ bioconversion. This is a result of the reduced solids content, less μ variations than in the substrate stream and lower limitation of the CO₂ loaded as per AD hydraulic retention time (HRT).

Bioconversion of CO₂ to CH₄ in ADs has been postulated to occur through the reduction of CO₂ by homoacetogenesis (via the Wood-Ljungdahl pathway) to form acetate, which is in turn utilised by acetoclastic methanogens (Chapter 4 and Chapter 5). Based on this, injection of CO₂ into ADs should be performed in single phased ADs or in the first stage of TPAD, in order for both homoacetogenic bacteria and acetoclastic methanogens to be present. As far as the means for injection are concerned, use of external bubble columns is considered suitable, because conditions that enhance mass transfer (bubble size, flow rates, contact time) are easily modifiable and blockages are less likely to occur than in alternative systems like packed columns. Other studies have reported the suitability of contacting gas with anaerobically digesting media in bubble columns for other applications like ammonia stripping in ADs (De la Rubia et al., 2010; Walker et al., 2011) or in-situ CH₄ enrichment (Lindberg and Rasmuson, 2006). Commercial versions of this technology could be used. To illustrate, the Downflow Gas Contactor (DGC) supplied by WRK Design and Services Ltd (Birmingham, UK) was identified as suitable for implementing CO₂ bioconversion in the 1.5 m³ AD demonstrator available at Cranfield University. Design and images of the commissioned DGC system can be consulted in Appendix 1 and Appendix 2.

The potential benefits in carbon footprint and renewable energy production derived from injection of exogenous CO₂ into ADs were determined to be dependent on several factors, e.g. methanogenic communities present, mass transfer efficiency, CO₂ concentration of the gas stream. Potential benefits were illustrated by considering a single phase AD with biogas upgrading in a two stage membrane unit with internal recirculation between modules. The retentate stream of the first module was considered a source of CO₂ with 77% concentration. A 5200 m³ AD operated with a HRT of 16 days and a 30 minutes turnover was considered. Associated benefits in CH₄ production were calculated based on a stoichiometric conversion (1:1) of additionally dissolved CO₂ into CH₄, as derived from combination of the Wood-Ljungdahl pathway with acetoclastic methanogenesis (Chapter 7). Under these conditions and the assumptions compiled in Chapter 7, a maximum uptake of 1805-18051 tonnes CO₂·annum⁻¹ and a relative increase in CH₄ production of 4.7-47% are achievable when compared to an AD without injection of additional CO₂.

8.6. REFERENCES

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CHAPTER 9
CONCLUSIONS

9. CONCLUSIONS

The conclusions are provided in relation to the objectives stated in the section 1.2 AIMS AND OBJECTIVES.

Objective 1: To review the need to reduce greenhouse gas (GHG) emissions from the water and organic waste sectors and identify how carbon dioxide (CO₂) emissions could be reduced.

- Energy demand of the water sector is increasing, which contributes to raise its carbon footprint. Energy demand of the UK water sector increased by 8.7% (726 GWh) between 2007 and 2011 (Water UK, 2012, 2008). Emissions of CO₂ are expected to increase by over 110,000 tonnes per year (Georges et al., 2009). Biogenic emissions of CO₂ from treatment of organic waste are also growing as the number of anaerobic digesters (ADs) treating alternative substrates increases. The number of ADs treating municipal solid waste in Europe increased from 53 in 1999 (De Baere, 2006) to 171 in 2010 (De Baere and Mattheeuws, 2008).
- Biogas CO₂ is the direct emission of CO₂ most readily available to be recovered within a treatment flowsheet (Byrns et al., 2013). Emissions of CO₂ from ADs treating agricultural, community waste and sewage sludge in the UK were estimated at 575,000 tonnes of CO₂ emitted per annum (Chapter 2).
- Implementation of carbon capture and storage (CCS) for management of biogas CO₂ is not feasible because the scattered location of AD sites limits CO₂ transportation to the final reservoir. Exogenous CO₂ injected into ADs can be bioconverted to methane (CH₄), acting as an on-site carbon revalorisation strategy. However, previous references in this area were insufficient to determine the potential CO₂ savings achievable and did not consider requirements for an un-scaled implementation.

Objective 2: To assess at laboratory and pilot scale the feasibility of CO₂ injection in ADs treating food waste and sewage sludge, both with respect to carbon uptake and renewable energy production.

- A potential CO₂ reduction of 8 to 34% was estimated for sewage sludge ADs saturated with molar fractions (y_{CO_2}) of 0.3 and 0.9, respectively, at the start of the digestion process. Benefits of 3, 10 and 11% were estimated for food waste ADs enriched with y_{CO_2} of 0.3, 0.6 and 0.9, respectively (Chapter 3).

- The capacity of ADs to bioconvert exogenous CO₂ to CH₄ is dependent on the substrate treated. Higher benefits in CH₄ production were observed in sewage sludge ADs (up to 2.4 fold) compared to food waste units (up to 1.16 fold) during the 24 hours following saturation with CO₂ (Chapter 3).
- Benefits in sewage sludge units were reproducible, while a higher variability in food waste units was observed (Chapter 3 and Chapter 4).
- Injection of CO₂ at a partial pressure (p_{CO₂}) of 1.6-1.7 achieved the highest carbon uptake and CH₄ enhancement within the p_{CO₂} tested (Chapter 3).
- An exogenous CO₂ uptake of 4.0E-3 kmol CO₂·m⁻³ per CO₂ injection (every 48 hours) was recorded in pilot-scale trials, which could be augmented if the bubble column mass transfer efficiency was increased (Chapter 5)

Objective 3: To elucidate the mechanisms of utilisation of exogenous CO₂ in ADs.

- Utilisation of CO₂ in ADs was confirmed to proceed via biological pathways, with inorganic CO₂ consumption not observed to occur to a significant extent.
- *Methanosaetaceae* accounted for 86.4±12.1% of the *Archaea* population in sewage sludge ADs (1.2 g·L⁻¹ NH₄-N) and was poorly represented in food waste units (4.3±1.7% and 4 g·L⁻¹ NH₄-N), where *Methanosarcinaceae* was the main contributor to total *Archaea* within those tested (19-40%). Differences were attributed to ammonia inhibition in food waste units. Since *Methanosaetaceae* is an obligate acetoclastic methanogen this was the main pathway for CH₄ formation in sewage sludge ADs. However, *Methanosarcinaceae* is a versatile *Archaea* and hence radiolabelling tests are required to determine CH₄ formation pathways where it is present (Chapter 4).
- *Methanosaetaceae* activity increased by 80% in ADs with ammonia concentration of 1.2 g·L⁻¹ NH₄-N (sewage sludge) submitted to periodic (once every 48 hours) CO₂ injections (Chapter 4).
- A 2.5 fold increase in hydrogen (H₂) concentration was observed after four CO₂ injections in a pilot-scale food waste AD. This was attributed to CO₂ dissolution and to an alternation of acetogenesis. Additional H₂ was likely utilised via the Wood-Ljungdahl pathway (Chapter 5).

- It was postulated that exogenous CO₂ is reduced by homoacetogenesis (Wood-Ljungdahl mechanism) and the acetate generated by this route is converted to CH₄ by acetoclastic methanogenesis.

Objective 4: To investigate the influence of liquid viscosity (μ) and rheology on CO₂ gas to liquid mass transfer, in the context of CO₂ dissolution into anaerobically digested substrates.

- Efficiency of gas to liquid mass transfer limits the amount of dissolved CO₂ loaded to an AD. Rheology and μ need to be characterised on a site by site basis and considered in the design of CO₂ gas to liquid mass transfer systems in order to avoid reductions in process performance and active volume for mass transfer. An increase of apparent viscosity (μ_a) from 130 to 340 cPo (which is within typical variability of sewage sludge) reduced volumetric mass transfer coefficient (k_La) by 43% and gas transfer efficiency (GTE) by 6 percentage points in a pilot-scale bubble column (Chapter 6).
- A new hydrodynamic regime (slug-annular flow) was identified for shear-thinning fluids of high μ . This is characterised by a chain of bubbles rising in the centre of the column and stagnant areas of reduced mass transfer efficiency in the outer annulus. Therefore, a higher number of gas injectors per surface area are required when dealing with non-Newtonian than with Newtonian fluids, in order to avoid a reduced active volume for mass transfer (Chapter 6).

Objective 5: To review the CO₂ streams of the water and organic waste sectors that could be bioconverted to CH₄ in anaerobic processes.

- It was identified that CO₂ could be sourced from off-gas of several biogas upgrading technologies (absorption, pressure swing adsorption, membranes, cryogenic upgrading) or from a two-phase AD (TPAD) process (Chapter 7).

Objective 6: To examine the feasibility, potential benefits and practicalities (systems and points of injection) of implementing CO₂ bioconversion in scaled-up ADs.

- Injection of CO₂ in ADs is expected to lead to benefits in carbon footprint in systems where both homoacetogenic bacteria and acetoclastic *Archaea* are present .
- Bioconversion of CO₂ was identified as feasible in ADs with ammonia concentrations below *ca.* 1500 mg·L⁻¹ NH₄-N (threshold for inhibition of

acetoclastic methanogens), with injection of exogenous CO₂ performed in the digestate recirculation loop of single phase ADs or in the first phase of TPADs.

- The use of bubble columns for dissolving exogenous CO₂ into anaerobic digesting media is considered suitable, since injection can be performed without dilution of the AD's headspace and a low loss of CH₄ (≤ 0.4 %) (Chapter 5).
- It has been demonstrated that bioconversion of CO₂ in ADs can reduce carbon footprint and increase CH₄ production, with the possibility of becoming an on-site carbon revalorisation strategy.

9.1. CONCLUSIONES (TRANSLATION OF THE SECTION *CONCLUSIONS*)

Las conclusiones corresponden a los objetivos establecidos en la sección 1.5.2 Metas y objetivos.

Objetivo 1: Examinar la necesidad de reducir los gases de efecto invernadero (GEI) en los sectores del agua y de los residuos orgánicos e identificar como se podría reducir la emisión de dióxido de carbono (CO₂).

- La demanda de energía del sector del agua está aumentado, lo que contribuye a un incremento de su huella de carbono. La demanda energética del sector del agua en el Reino Unido aumentó un 8.7% (726 GWh) entre 2007 y 2011 (Water UK, 2012, 2008). Se ha estimado que la emisión de CO₂ seguirá aumentando en 110,000 toneladas anuales (Georges et al., 2009). La emisión de CO₂ biogénico durante el tratamiento de residuos orgánicos también está aumentando, debido a la implementación de digestores anaeróbicos (DAs) que tratan estos substratos. El número de DAs que tratan residuos sólidos urbanos en Europa ha aumentado de 53 en 1999 (De Baere, 2006) a 171 en 2010 (De Baere and Mattheeuws, 2008).
- El CO₂ contenido en biogás es la corriente de CO₂ más fácilmente capturable entre las generadas durante el tratamiento de aguas residuales (Byrns et al., 2013). Se ha estimado que la emisión de CO₂ en DAs que tratan residuos de agricultura, residuos sólidos urbanos o lodos de depuradora en Reino Unido es de 575,000 toneladas de CO₂ anuales (Capítulo 2).
- La implementación de captura y almacenamiento de carbono (CAC) para CO₂ derivado de biogás no es factible debido a la ubicación dispersa de las plantas de DA, lo que limita el transporte de CO₂ a reservorios. Inyección de CO₂ en DAs

para su bioconversión a metano (CH_4), puede ser una estrategia in-situ de valorización de carbono. Sin embargo, referencias anteriores en esta área eran insuficientes para determinar el potencial de reducción de emisiones de CO_2 alcanzable y no consideraban los requisitos para una implementación a gran escala.

Objetivo 2: Evaluar a escala de laboratorio y de planta piloto la factibilidad de inyectar CO_2 en DAs que tratan residuos alimentarios y lodos de depuradora, tanto en relación a la captura de carbono como a la producción de energía renovable.

- Se ha estimado una reducción potencial de CO_2 de entre 8 a 34% en DAs que traten lodos de depuradora saturados con una fracción molar de CO_2 (y_{CO_2}) de 0.3 y 0.9, respectivamente, al comienzo del proceso de digestión. Se han estimado beneficios de 3, 10 y 11% en DAs que traten residuos alimentarios enriquecidos con y_{CO_2} de 0.3, 0.6 y 0.9, respectivamente (Capítulo 3).
- La capacidad de DAs de bioconvertir CO_2 exógeno a CH_4 depende del substrato tratado. Se han obtenido mayores beneficios en la producción de CH_4 en DAs que tratan lodos de depuradora (un aumento de hasta de 2.4 veces) que en unidades de residuos alimentarios (un aumento de 1.16 veces) durante las 24 horas posteriores a saturar con CO_2 (Capítulo 3).
- Los beneficios observados con lodos de depuradoras son más reproducibles que con residuos alimentarios (Capítulo 3 y Capítulo 4).
- La inyección de CO_2 con una presión parcial (p_{CO_2}) de 1.6-1.7 resultó en la mayor captura de carbono e incremento de CH_4 entre las p_{CO_2} estudiadas (Capítulo 3).
- Se ha obtenido una captura de CO_2 exógeno de $4.0\text{E-}3 \text{ kmol CO}_2 \cdot \text{m}^{-3}$ por cada inyección de CO_2 (cada 48 horas) en unidades a escala piloto. Este valor podría ser incrementado con un aumento de la eficiencia de transferencia de masa en la columna de burbujas (Capítulo 5).

Objetivo 3: Dilucidar los mecanismos de utilización de CO_2 exógeno en DAs.

- La utilización de CO_2 en DAs tiene lugar por medios biológicos, siendo un consumo inorgánico de CO_2 no observado.
- Se ha determinado que *Methanosaetaceae* constituye un $86.4 \pm 12.1\%$ de la totalidad de arquea en DAs que tratan lodos de depuradora ($1.2 \text{ g} \cdot \text{L}^{-1} \text{ NH}_4\text{-N}$) y su presencia es escasa en unidades que tratan residuos alimentarios ($4.3 \pm 1.7\%$ y

4 g·L⁻¹ NH₄-N), donde *Methanosarcinaceae* es la arquea más abundante entre las estudiadas (19-40%). Las diferencias se han atribuido a la inhibición por amoníaco en DAs tratando residuos alimentarios. Dado que *Methanosaetaceae* es una arquea metanogénica acetoclástica, esta es la ruta de producción de CH₄ más abundante en DAs tratando lodos de depuradora. Sin embargo, *Methanosarcinaceae* es una arquea versátil por lo que se requieren análisis con compuestos radiomarcados para determinar las rutas de formación de CH₄ (Capítulo 4).

- La actividad de *Methanosaetaceae* aumentó un 80% en DAs con concentración de amoníaco de 1.2 g·L⁻¹ NH₄-N (lodos de depuradora) sometidos a inyecciones periódicas de CO₂ (Capítulo 4).
- La concentración de hidrógeno (H₂) aumentó 2.5 veces tras cuatro inyecciones de CO₂ en un DA de residuos alimentarios a escala piloto. Esto se ha atribuido a la disolución de CO₂ y a una alteración de acetogénesis. El H₂ adicional fue probablemente asimilado en la ruta Wood-Ljungdahl (Capítulo 5).
- Se ha postulado que el CO₂ exógeno es reducido por homoacetogénesis (ruta Wood-Ljungdahl) y el acetato generado por esta ruta es convertido a CH₄ vía metanogénesis acetoclástica.

Objetivo 4: Investigar la influencia de viscosidad (μ) y reología en la transferencia de masa gas líquido de CO₂, en el contexto de disolver CO₂ en substratos digeridos anaeróbicamente.

- La eficiencia de transferencia de masa gas-líquido limita la cantidad de CO₂ disuelto alimentado a un DA. Reología y μ han de ser caracterizadas en cada planta y consideradas durante el diseño de sistemas de transferencia de masa de CO₂ para evitar reducciones en el rendimiento del proceso y en el volumen activo para la transferencia. Un aumento de viscosidad aparente (μ_a) de 130 a 340 cPo (típica variabilidad en lodos de depuradora) redujo el coeficiente volumétrico de transferencia de masa (k_La) en un 43% y la eficiencia de transferencia de gas en 6 puntos de porcentaje en una columna de burbujas a escala piloto (Capítulo 6).
- Ha sido identificado un nuevo régimen de flujo (slug-annular flow) para fluidos pseudoplásticos de alta μ . Este está caracterizado por una cadena de burbujas ascendentes en el centro de la columna y áreas estancas de reducida eficiencia para la transferencia de masa en el anillo exterior. Por lo tanto, para evitar una

reducción del volumen activo, se requiere un mayor número de inyectores de gas por unidad de superficie cuando se utilizan fluidos no-Newtonianos que con fluidos Newtonianos (Capítulo 6).

Objetivo 5: Examinar las corrientes de CO₂ disponibles en los sectores del agua y residuos orgánicos que podrían ser bioconvertidas a CH₄ en procesos anaeróbicos.

- El CO₂ inyectado en DAs podría capturarse de los gases de salida de varias tecnologías de enriquecimiento de biogás o de un proceso de DA de dos fases (Capítulo 7).

Objetivo 6: Examinar la factibilidad, beneficios potenciales y aspectos prácticos (sistemas y puntos de inyección) de implementar bioconversión de CO₂ en DAs a gran escala.

- La inyección de CO₂ en DAs puede obtener beneficios en la huella de carbono en sistemas donde estén presentes tanto bacterias homoacetogénicas como arqueas acetoclásticas.
- Se ha identificado que la bioconversión de CO₂ es factible en DAs con una concentración de amoníaco inferior a *ca.* 1500 mg·L⁻¹ NH₄-N (límite para la inhibición de arqueas metanogénicas acetoclásticas), con inyección de CO₂ exógeno en la recirculación de material digerido en un DA de fase única o en la primera fase de un proceso de dos fases.
- Se ha considerado adecuado el uso de columnas de burbujas para disolver CO₂ exógeno en material digerido anaeróbicamente, dado que la inyección puede llevarse a cabo sin diluir la fase gaseosas del DA y con una baja pérdida de CH₄ (≤0.4 %) (Capítulo 5).
- Se ha demostrado que la bioconversión de CO₂ en DAs puede reducir la huella de carbono y aumentar la producción de CH₄, teniendo así potencial para ser una estrategia de revalorización de carbono in-situ.

9.2. REFERENCES

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CHAPTER 10
APPENDICES

10. APPENDICES

10.1. APPENDIX 1: IMAGES OF EXPERIMENTAL RIGS

10.1.1. Laboratory scale anaerobic digestion rig

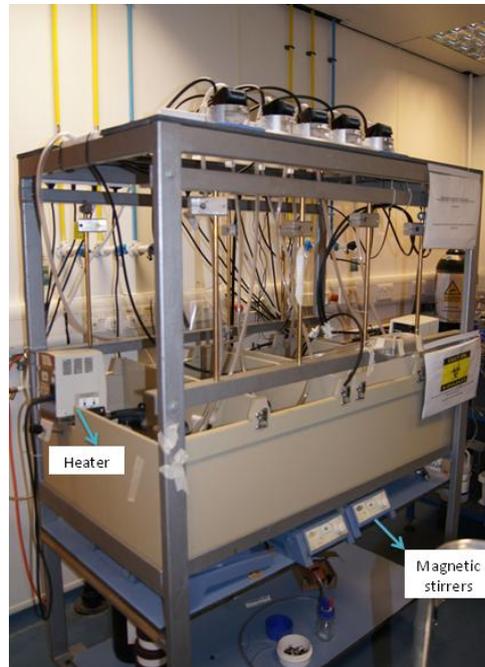


Figure A1.1. Rig used for the saturation tests (Chapter 3) and operation of CO₂ enriched ADs at laboratory scale (Chapter 3 and Chapter 4).



Figure A1.2. MilliGascounters used for recording biogas production of laboratory scale ADs (Chapter 3 and Chapter 4).

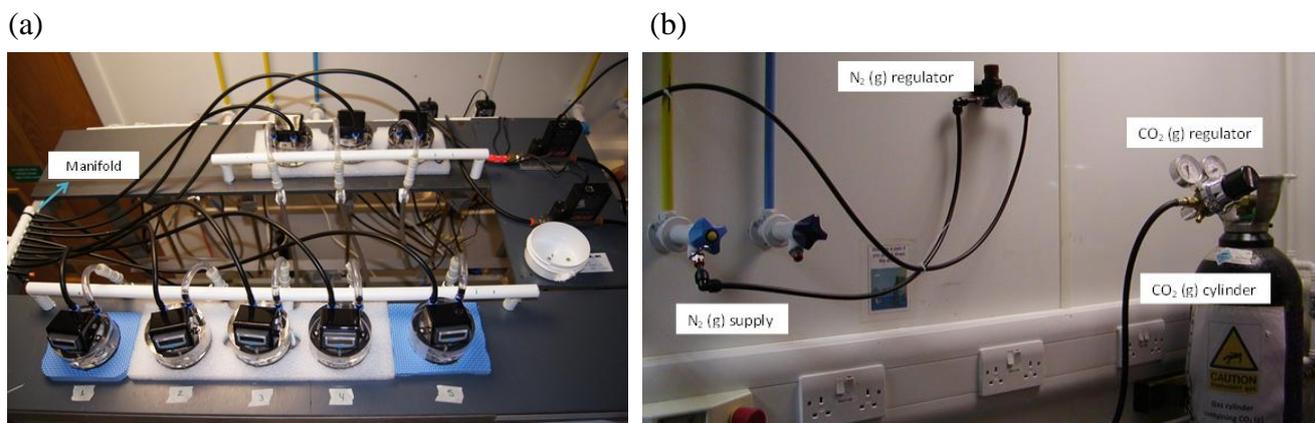


Figure A1.3. (a) Top view of the laboratory scale experimental rig in which the position of the MilliGascounters, mass flow controllers (MFC) and manifold is appreciated. (b) N₂ and CO₂ supplies and regulators.

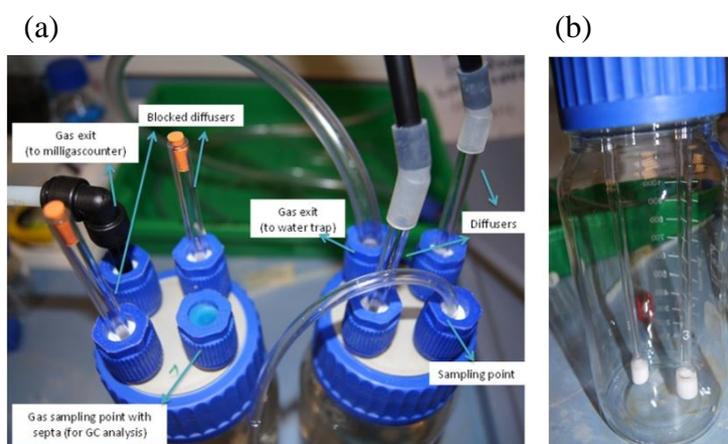


Figure A1.4. (a) Left hand side: Four port lid as used for operation of ADs enriched with CO₂ (Chapter 3). Right hand side: Four port lid as used for saturation tests (Chapter 3). (b) Diffusers used for CO₂ injection.

10.1.2. Pilot scale anaerobic digestion rig and macerator



Figure A1.5. Pilot scale ADs with peristaltic pumps for recirculation of digestate.



Figure A1.6. Macerator-pump system used for macerating food waste and control panel.

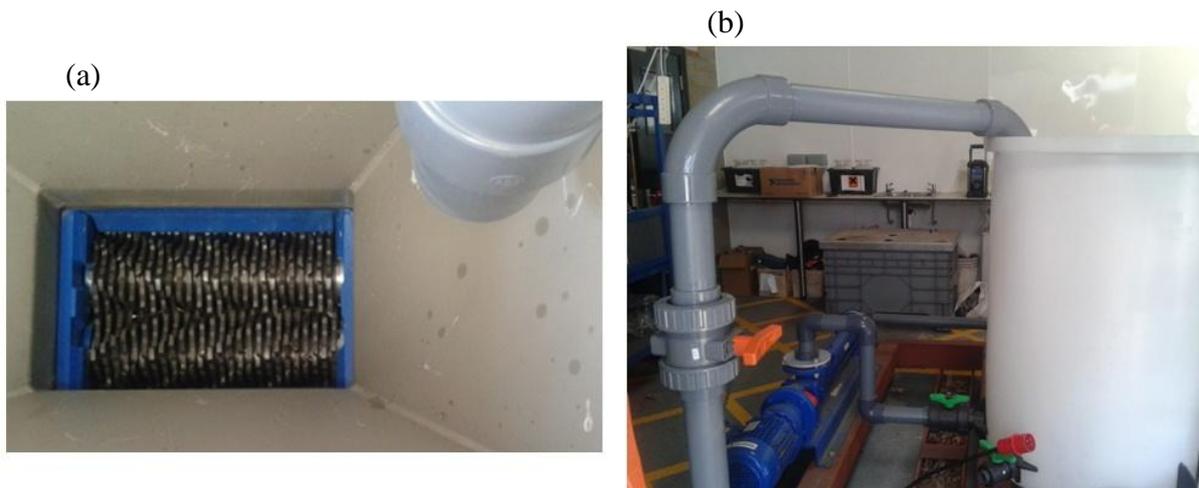


Figure A1.7. (a) Detail of the blades inside of the macerator hopper. (b) Detail of the connexion between the macerator and the wetting tank of the 1.5 m³ AD demonstrator available at Cranfield University.

10.1.3. Bubble column used for gas to liquid mass transfer tests

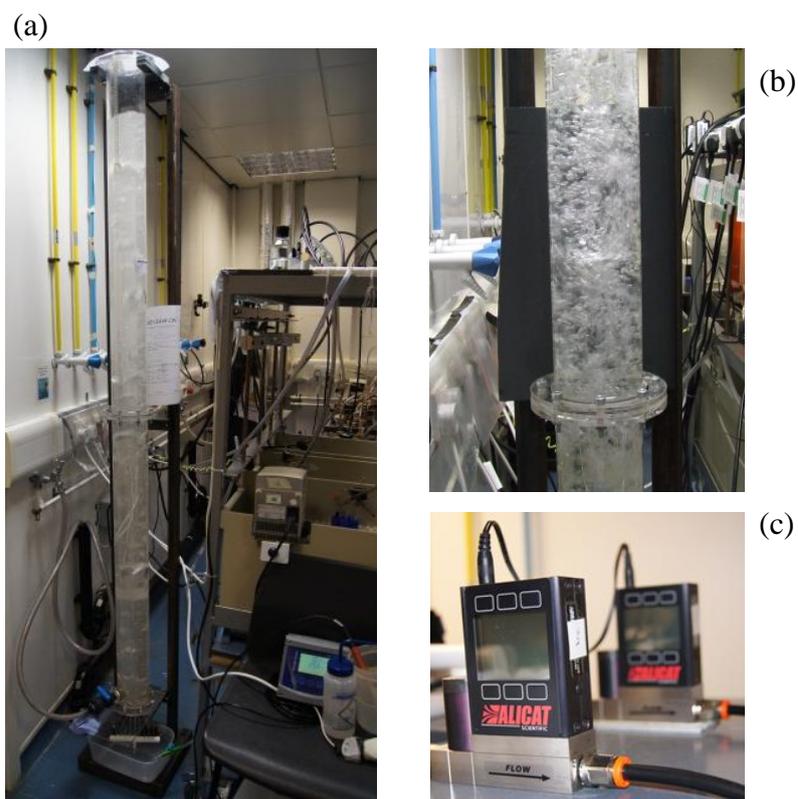


Figure A1.8. (a) and (b) Bubble column used for gas to liquid mass transfer absorption tests. (b) Mass flow controllers (MFCs) used of injection of CO₂ and N₂.

10.1.4. Downflow gas contactor (DGC)

(a)



(b)



Figure A1.9. Downflow gas contactor (DGC) for retrofitting CO₂ injection into the 1.5 m³ AD demonstrator available at Cranfield University.

(a)



(b)



Figure A1.10. (a) Detail of the gas analysers in the gas exhaust of the DGC and of liquid exits and sampling points. (b) Level of gas dispersion that DGC can achieve in water.

(a)



(b)



Figure A1.11. Images of the 1.5 m³ AD demonstrator available at Cranfield University, for which the macerator-pump system and the DGC were designed.

10.2. APPENDIX 2: SELECTION OF THE CO₂ INJECTION POINT AND INJECTION TECHNOLOGY BY KEPNER-TREGOE DECISION MAKING TECHNIQUE

This document aims to establish the most suitable injection point for contacting food waste and/or sludge with CO₂ gas at a convenient partial pressure in the anaerobic digestion (AD) demonstrator of 1.5 m³ available at Cranfield University. A suitable technology for contacting food waste with CO₂ has also been selected. The assessment is based on the Kepner Tregoe decision making methodology. For each of the decisions made the following steps have been performed (Kepner and Tregoe, 2006).

1. State the decision
2. Develop objectives
3. Classify the objectives in MUSTs and WANTS
4. Weight the WANTS. The most important objective is allocated a 10 and the rest are weighted in comparison with it.
5. Generate alternatives and provide a short description
6. Screen the alternatives through the MUSTs
7. Compare the alternatives against the WANTS. A 10 is given to the alternative that is closest to meet the objective and score the rest by comparison. We are searching for the best alternatives within the available, not an ideal one.
8. Identify adverse consequences
9. Make the best balances choice

10.2.1. Injection point selection

This section aims to determine the point of the pilot plant flowsheet in which the contact between the food waste and/or sludge and the CO₂ should take place. The injection points considered were: macerator outlet, water or liquors used for adjustment of the feed's solids content, wetting tank, recirculation loop used for mixing the wetting tank, inlet to the AD (outlet of wetting tank), AD digester and recirculation loop used for mixing the anaerobic digester (Figure A2.1).

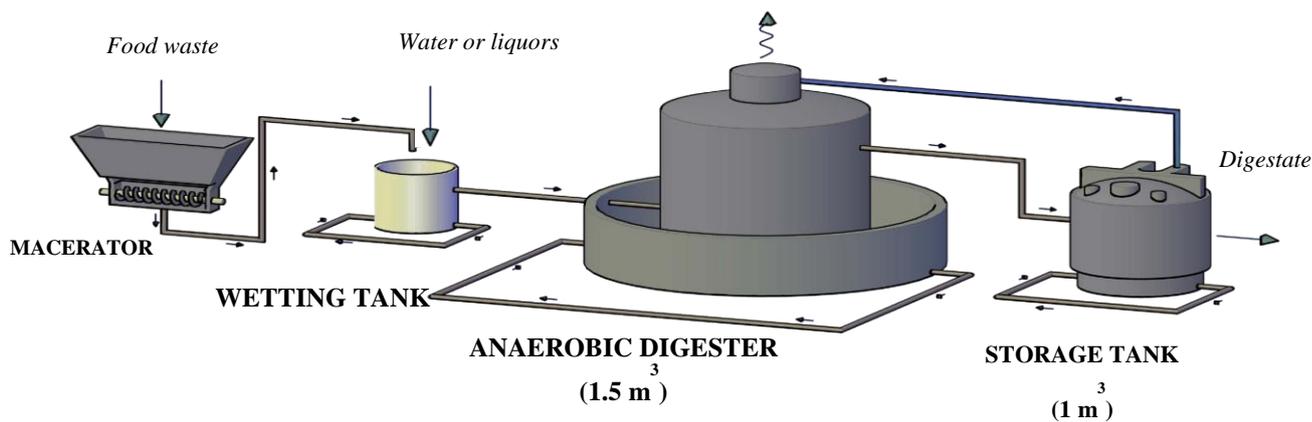


Figure A2.1. Diagram of the 1.5 m³ AD demonstrator available at Cranfield University.

Table A2.1. Criteria considered for selection of the CO₂ injection point.

Objective	Type	Explanation
No need to stop AD in case of maintenance	must	If the injection technology requires any maintenance, there would be not need to stop and re-start the AD. Several start-ups could limit the deliverability of project on time and cause failures.
No contact with the atmosphere after CO ₂ injection	must	In order to avoid an unknown change of the conditions reached with the injection.
Significant liquid fraction available	must	A significant amount of liquid fraction needs to be available for gas-liquid mass transfer to take place.
Minimal changes after the CO ₂ injection	want	Any significant change of temperature or pressure should be avoided not to alter the conditions after the CO ₂ injections. At least any change should be known so that it can be compensated.
Easy operation and low risk of blockage	want	Points of the flowsheet where the material is thicker or less homogeneous are considered more likely to cause operational problems such as blockages
Easy accessibility for maintenance	want	The research nature of the project increases the likelihood of maintenance to be required, being an easy accessibility essential. However, since Must 1 rejects any alternative that needs to stop the AD for maintenance, a really high weight is not given.
Operational flexibility	want	Those points of the flowsheet in which the sludge or waste flowrate is controllable would facilitate a variation of the CO ₂ load. The dissolved CO ₂ could be adjusted by changing the pressure of injection, although it may not be a preferred option.
Efficient mass transfer	want	Points of the flowsheet where the material is thicker or less homogeneous are considered more challenging for a controlled and reproducible mass transfer of the CO ₂ to a dissolved form
Easy installation (low disruption to control AD operation)	want	Any technology that can be installed without disrupting the AD working as a control would be preferred. However, since the installation would take place once, the weight given is lower than for other objectives.
Possible installation of any technology	want	Some points of the flowsheet limit the technologies that can be applied, for instance for footprint reasons. It would be preferred not to limit the technology to be used at this stage.

Table A2.2. Analysis for selection of the CO₂ injection point.

<i>Alternative</i>		Macerator outlet		Water or liquors for solids adjustment		Wetting tank		Wetting tank recirculation loop		Inlet to the AD		AD		AD recirculation loop	
<i>Musts</i>	No need to stop AD in case of maintenance	GO		GO		GO		GO		GO		NO GO		GO	
	No contact with the atmosphere after CO ₂ injection	GO ^(a)		GO ^(a)		GO ^(a)		GO ^(a)		GO		GO		GO	
	Significant liquid fraction available	NO GO		GO		GO		GO		GO		GO		GO	
<i>Want objectives</i>	<i>Weight</i>	<i>S</i>	<i>WS</i>	<i>S</i>	<i>WS</i>	<i>S</i>	<i>WS</i>	<i>S</i>	<i>WS</i>	<i>S</i>	<i>WS</i>	<i>S</i>	<i>WS</i>	<i>S</i>	<i>WS</i>
Minimal changes after the CO ₂ injection	10	-	-	2	20	2	20	2	20	10	100	-	-	10	100
Easy operation and low risk of blockage	10	-	-	10	100	3	30	2	20	3	30	-	-	6	60
Operational flexibility	8	-	-	3	24	1	8	4	32	4	32	-	-	10	80
Efficient mass transfer	7	-	-	10	70	4	28	4	28	4	28	-	-	6	42
Easy accessibility for maintenance	6	-	-	10	60	3	18	10	60	10	60	-	-	10	60
Easy installation (low disruption to control AD operation)	5	-	-	10	50	10	50	10	50	10	50	-	-	9	45
Possible installation of any technology	2	-	-	10	20	3	6	9	18	9	18	-	-	9	18
		Total =	-	Total =	344	Total =	160	Total =	228	Total =	318	Total =	-	Total =	405

^(a) Wetting tank could be closed

As per the analysis above, injection of CO₂ in the AD recirculation loop was selected.

10.2.2. Injection technology selection

The objectives considered for this selection refer to the performance, the ease of installation and use; and the possibility of a further scale-up of the CO₂ injection technologies. A brief description of each objective is given in Table A2.3, while the weights allocated are included in Table A2.4.

Table A2. 3. Criteria considered for selection of the CO₂ injection technology.

Objective	Type	Explanation
Could be implemented as per H&S at Cranfield	must	The installation and operation of the injection technology must not imply an unacceptable risk to the operators.
Delivery time < 12 weeks	must	
Fit for purpose	must	Technology envisaged as suitable for dissolving CO ₂ into food waste.
Efficient mass transfer	want	Technologies with previously reported (or envisaged) efficient gas to liquid mass transfer are preferred so that a high percentage of the CO ₂ injected is utilised.
Operational flexibility and control	want	Preferred technologies that permit variable gas and liquid flow rates, gas bubbles size and CO ₂ injection pressure and concentration, so that the gas to liquid mass transfer can be modified.
Measurable carbon footprint	want	Systems that permit to quantify the CO ₂ in and out of the gas-liquid contactor are preferred, because of the importance of understanding the CO ₂ mass balance.
Ease of installation	want	
No need for auxiliary equipment	want	Technologies that do not require additional units apart from those included by the supplier are preferred.
Supplier's support on installation and start-up	want	
Low time of commissioning	want	Preferred reduced time since the technology is delivered until is ready for operation - low need of assembly and preparation once on site.
Ease of start-up	want	
Easy operation	want	The CO ₂ injection technology should be as automated as possible and ideally operated by one single person.
Low risk of blockage	want	Alternatives that can handle high solids content and solids of big size are preferred - valued the no presence of critical parts that can be blocked or the possibility of any critical part to be conservatively sized in the design stage.
Easy maintenance and suppliers' support	want	Plant reliability, simplicity of maintenance and easy access to critical parts with minimal disruption to the AD operation. Supplier's support in case of failure also considered.
Injection point flexibility	want	Technologies that can be relatively easy transferred from different points of the AD flowsheet are preferred.
Possible scale-up	want	Possibility of using the same technology in a potential full-scale application or at least of representatively up-scaling the obtained results.

The CO₂ injection alternatives considered are:

10.2.2.1. Jets

Installation of jets through which the CO₂ enriched gas stream would be bubbled into a tank containing food waste. The tank would be retrofitted in a bypass of the AD recirculation loop and would be acting as a continuously stirred tank reactor (CSTR) in which the mixing is achieved by gas injection. The tank would be fed by the progressing cavity pump already in place and the material would leave by overflowing. The gas injection would require compressors and suitable connections between the gas source and the jets.

10.2.2.2. Conventional bubble column

Design of a column acting as a gas-liquid contactor in which the liquid is the continuous phase and the gas is dispersed through it as bubbles with high specific surface area (for mass transfer enhancement). The column would be retrofitted in a bypass of the AD recirculation loop after the progressing cavity pump already available, which would continuously feed the column. Special care would have to be taken while selecting the liquid inlet connections for avoiding blockages and the gas disperser, which would highly affect the bubbles surface area and therefore the mass transfer rate of CO₂ from the gas to the liquid phase.

10.2.2.3. Downflow gas contactor (DGC)

Installation of the downflow gas contactor (DGC) supplied by WRK Design and Services Ltd. It consists of a flooded column in which the gas is injected co-currently through a specially designed orifice. The intense shear generates a gas-liquid dispersion in which the mass transfer is highly enhanced. The unit would be retrofitted in a bypass of the AD recirculation loop and designed by WRK as per the specifications given by Cranfield University, which include the characteristics of the fluids to treat and the level of control and monitoring required. WRK offers support in the installation and commissioning of the system.

10.2.2.4. Fluidic oscillator (Sheffield University)

This alternative would use the microbubble generator by fluidic oscillation designed at Sheffield University. The oscillator would be placed in a tank or column flooded with the material of the AD recirculation loop. The progressing cavity pump already in place would feed the waste continuously to the system, which is likely to leave the contactor by overflowing design. Only the gas disperser would be supplied by Sheffield University, being the gas injection system, column or tank and rest of devices designed and assembled by Cranfield University.

Table A2.4. Analysis for selection of the CO₂ injection technology.

<i>Alternative</i>		Jets		Conventional bubble column		DGC of WRK		Fluidic oscillator of Sheffield University	
Musts	Could be implemented as per H&S at Cranfield	GO		GO		GO		GO	
	Delivery time < 12 weeks	GO		GO		GO		GO	
	Fit for purpose	GO		GO		GO		GO	
Want objectives	Weight	S	WS	S	WS	S	WS	S	WS
Efficient mass transfer	10	3	30	7	70	10	100	2	20
Operational flexibility and control	10	4	40	9	90	10	100	4	40
Measurable carbon footprint	7	5	35	10	70	10	70	5	35
Ease of installation	4	5	20	1	4	10	40	5	20
No need for auxiliary equipment	6	4	24	1	6	10	60	4	24
Supplier's support on installation and start-up	5	1	5	1	5	10	50	2	10
Low time of commissioning	4	7	28	3	12	10	40	7	28
Ease of start-up	5	10	50	5	25	7	35	9	45
Easy operation	7	10	70	6	42	8	56	10	70
Low risk of blockage	7	10	70	6	42	7	49	5	35
Easy maintenance and suppliers' support	6	10	60	5	30	7	42	8	48
Injection point flexibility	8	9	72	9	72	10	80	9	72
Possible scale-up	7	10	70	7	49	7	49	2	14
		Total A =	574	Total B =	517	Total C =	771	Total D =	461

As per the analysis above, the DGC technology supplied by WRK Design and Services Ltd. was selected. One of the last stages of the Kepner Tregoe methodology is the *identification of adverse consequences*, which aims to detect potential risks that were not considered before. In the case of installation of the DGC technology the following risks have been identified:

Table A2.5. Potential risks and preventive measures of the selected CO₂ injection technology.

Potential risk	Preventive measures	Likelihood	Impact
Blockage of the liquid pipes or entry zone due to the heterogeneous properties of the food waste	<ul style="list-style-type: none"> • Special consideration given to the high solids content and particle size of the food waste during the technology design • Cranfield University would provide solids size information to WRK and real samples of the material once the AD is operating as a control • Easy access for maintenance of any potentially blocked parts stated as a requirement since the design stage 	High	Low (easy access considered)
Incapacity of the system to treat the high flowrate of the AD recirculation loop	<ul style="list-style-type: none"> • Flowrates considered during design • Unit to be installed in a bypass of the AD recirculation loop, for possible adjustments to treat part of the flowrate rather than all 	Medium	Medium
Not efficient mass transfer achieved (system not used with food waste before)	<ul style="list-style-type: none"> • Installation in the recirculation loop of the AD, where material is more homogeneous • Modifiable flowrates and possibility of including a recirculation stream, to modify gas-liquid contact time 	Low	Medium

Different configurations for the installation of the DGC have been considered and narrowed to the two of Figure A2.2, which would enable a high degree of flexibility in terms of CO₂ loading to the AD and the possibility of operating with or without CO₂ injection.

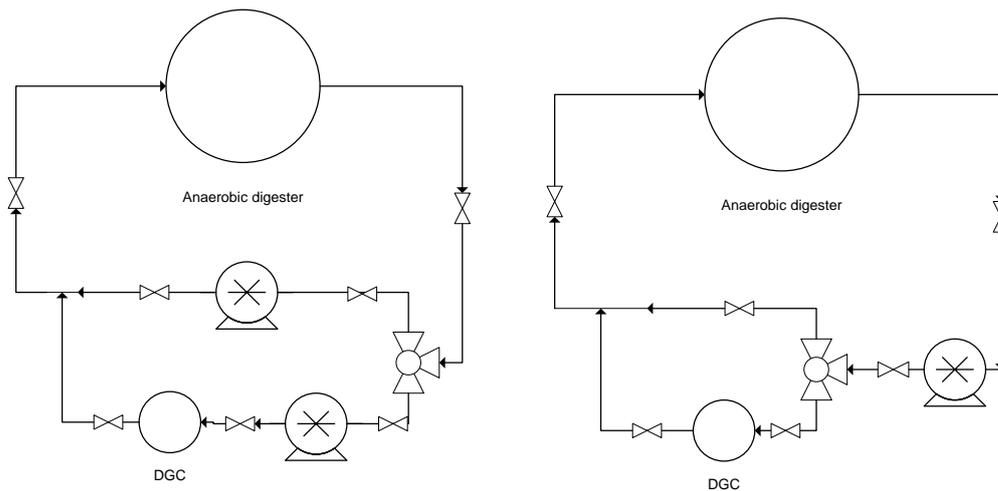


Figure A2.2. Possible configurations for installation of the selected CO₂ injection technology in the digestate recirculation loop of the 1.5 m³ AD demonstrator available at Cranfield University.

10.2.3. Selected CO₂ injection technology outline and specifications

The DGC supplied by WRK Design and Services Ltd was the technology selected as a possible means to apply CO₂ enrichment in the 1.5 m³ AD demonstrator available at Cranfield University. Its design consisted of a flooded column in which the gas is injected concurrently in the top through a specially designed orifice. The intense shear generates a gas-liquid dispersion in which the mass transfer is highly enhanced. The unit could potentially be retrofitted in a bypass of an AD recirculation loop. The main specifications of the system provided by the supplier were as follows and the flowsheet is included in Figure A2.3.

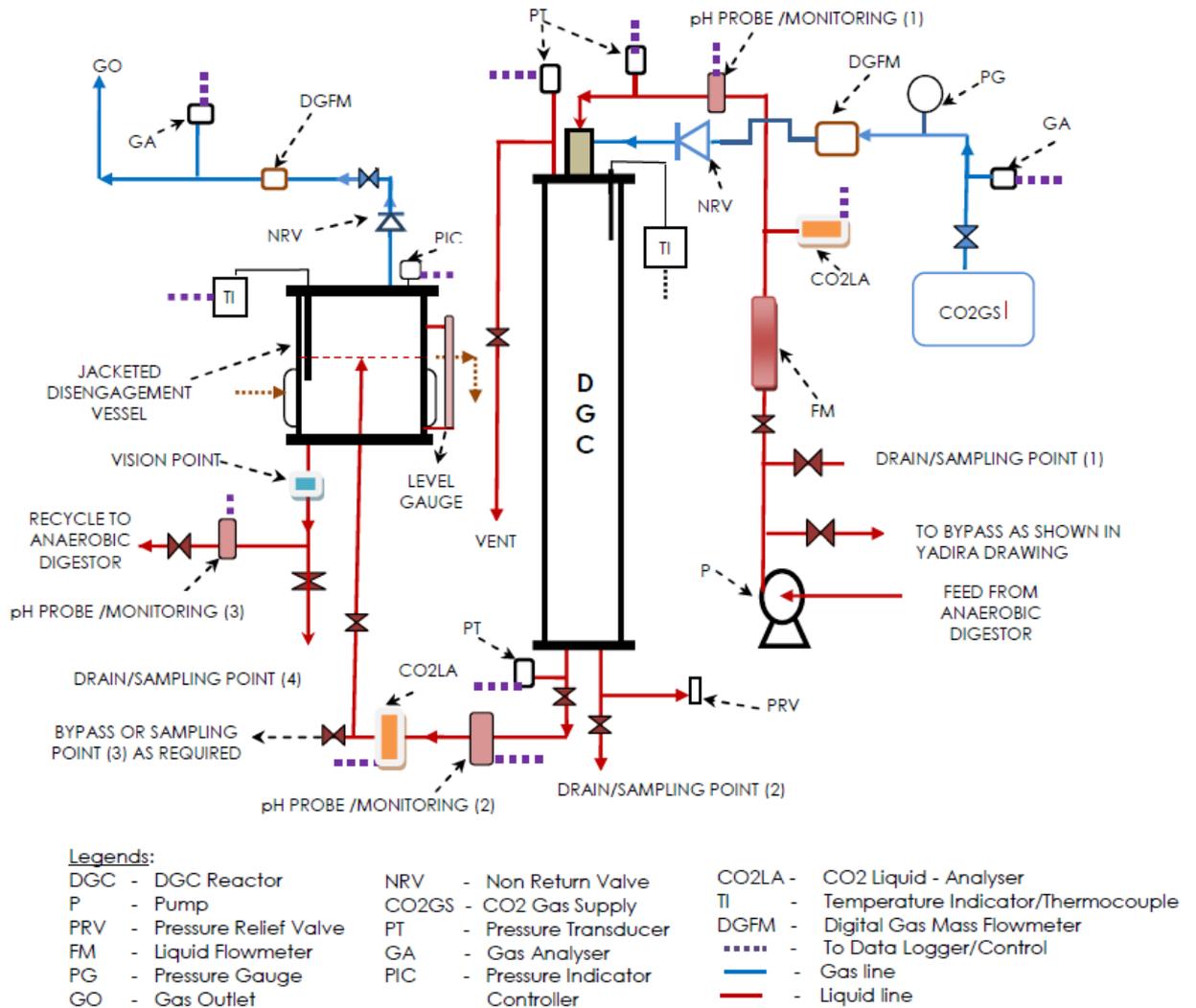


Figure A2.3: Specifications of DGC CO₂ injection system designed for the 1.5 m³ AD demonstrator.

- 1) The liquid feed from the recycle loop could be directed through a liquid flowmeter into the DGC reactor
- 2) The operating pressure of the DGC reactor could be varied and controlled by a regulating valve at the outlet of the reactor column.
- 3) The inlet CO₂ gas is fed into the DGC reactor through the specially designed inlet connected to the top of the DGC reactor. There is a pressure drop created which allows the CO₂ to be fed into the DGC. The inlet pressure of the CO₂ would depend on the liquid inlet flowrate and pressure and operating pressure of the DGC required, which could be varied.
- 4) The liquid with the absorbed CO₂ flows through the bottom outlet of the DGC into the disengagement vessel.

- 5) Unabsorbed gas (if any) from the DGC, disengages in the disengagement vessel and is vented out through a non-return valve.
- 6) The rate of absorption would vary depending on liquid flowrate, temperature and operating pressure in the DGC. The CO₂ gas absorption levels would depend on the equilibrium solubility level of CO₂ in the digestate liquid at the temperature and pressure of operation.
- 7) The following parameters could be varied:
 - Liquid flowrate
 - Inlet gas (CO₂ or gas mixture) flowrate
 - Operating pressure of the DGC
 - Operating pressure of gas (dependent on operating pressure of DGC)

The main dimensions and specifications were as follows:

- a) DGC reactor: column height: 1.75 m; diameter: 10 – 15 cm
- b) Disengagement vessel: Height: 0.65 m; Diameter: 0.3 m

10.2.4. References

Kepner, C.H., Tregoe, B.B., 2006. The new rational manager: an updated edition for a new world. New Jersey.