



Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Quantifying the percentage of methane formation via acetoclastic and syntrophic acetate oxidation pathways in anaerobic digesters

Ying Jiang^{a,*}, Charles Banks^b, Yue Zhang^b, Sonia Heaven^b, Philip Longhurst^a

^a Centre for Bioenergy & Resource Management, School of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, UK

^b Faculty of Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, UK

ARTICLE INFO

Article history:

Received 21 October 2016

Revised 28 March 2017

Accepted 2 April 2017

Available online xxxxx

Keywords:

Food waste

Anaerobic digestion

Methanogenic pathway

Radioactive isotope labelling

Ammonia inhibition

ABSTRACT

Ammonia concentration is one of the key factors influencing the methanogenic community composition and dominant methanogenic pathway in anaerobic digesters. This study adopted a radiolabelling technique using [2-¹⁴C] acetate to investigate the relationship between total ammonia nitrogen (TAN) and the methanogenic pathway. The radiolabelling experiments determined the ratio of ¹⁴CO₂ and ¹⁴CH₄ in the biogas which was used to quantitatively determine the percentage of CH₄ derived from acetoclastic and syntrophic acetate oxidation routes, respectively.

This technique was performed on a selection of mesophilic digesters representing samples of low to high TAN concentrations (0.2–11.1 g kg⁻¹ wet weight). In high TAN digesters, the ratio between ¹⁴CO₂ and ¹⁴CH₄ was in the range 2.1–3.0; indicating 68–75% of methane was produced via the hydrogenotrophic route; whereas in low ammonia samples the ratio was 0.1–0.3, indicating 9–23% of methane was produced by the hydrogenotrophic route. These findings have been confirmed further by phylogenetic studies.

© 2017 Published by Elsevier Ltd.

1. Introduction

Anaerobic digestion (AD) has gained popularity over the last decade as a waste management strategy with the added benefit of producing next generation renewable energy in the form of methane (Mata-Alvarez, 2003). Numerous studies have been carried out to optimise the process, increase efficiency and evaluate potential substrates. Food waste in particular has received considerable attention due to its widespread availability and high energy content on dry weight basis. A wide range of prior research shows, however, that substrates including food waste and other farm waste streams which have high nitrogen concentration are prone to unstable digestion. This is due to the inhibitory effect of free ammonia nitrogen (FAN) released during protein hydrolysis and the breakdown of urea in animal manure (Breure et al., 1986; Kayhanian, 1999; McCarty, 1964; Yenigün and Demirel, 2013). It has been reported on a number of occasions and at different scales of operation that a characteristic build-up of volatile fatty acids (VFA) can occur when digesting food waste which can lead to process failure (Banks et al., 2008; Banks and Zhang, 2010; Neiva Correia et al., 2008).

It is now acknowledged that the anaerobes involved in the AD process exhibit different threshold tolerances towards ammonia. Amongst these microorganisms, the methanogens are generally more sensitive than the anaerobic bacterial species (Gallert et al., 1998; McCarty, 1964); within methanogens the acetoclastic methanogens are recognised as being more sensitive to ammonia than hydrogenotrophic methanogens, with the latter reported to have a similar, or in some cases, more tolerance to high ammonia levels compared to syntrophic acetate-oxidizing bacteria (Wang et al., 2015). As a result, ammonia concentration can be one of the most important factors that influence methanogen community structure and could alter the dominant methanogenic pathway in the digester as reported in a number of previous studies (Fotidis et al., 2014; Karakashev et al., 2006; Schnürer et al., 1997; Schnürer and Nordberg, 2008; Westerholm et al., 2012).

The microbial community structure in digesters can be examined using a variety of techniques including targeted DNA amplification and sequencing, and fluorescence *in situ* hybridisation (FISH). These methods are now well established and commonly used to assess the microbial population structure within samples from a wide range of environments and processes. Studies looking at anaerobic digesters (Karakashev et al., 2006, 2005; Nettmann et al., 2010, 2008) have found that the microbial population structure is very dependent on environmental conditions and is

* Corresponding author.

E-mail address: Y.Jiang@cranfield.ac.uk (Y. Jiang).

influenced by factors such as the concentration of VFA and ammonia as well as temperature. A comprehensive study surveyed methanogen diversity in 15 full-scale biogas plants using the FISH technique (Karakashev et al., 2005). The study concluded that in digesters operating with high ammonia and VFA concentrations the methanogen population was dominated by members of the *Methanosarcinaceae*; whereas digesters operating at low levels of ammonia and VFA were dominated by members of the *Methanosaetaceae*. Another study used FISH and quantitative polymerase chain reaction (PCR) methods to investigate methanogen community composition in six full-scale commercial digesters treating nitrogen-rich poultry faeces and/or maize silage, and observed that in five of the digesters the hydrogenotrophic *Methanomicrobiales* spp. were dominant (Nettmann et al., 2010). The sixth digester, which had only been operational for a short period, had a significantly lower (1.7 g kg^{-1} wet weight (WW)) concentration of total ammonia nitrogen (TAN) and the acetoclastic *Methanosaetaceae* spp. was found to be dominant. Determining metabolic functionality by analysis of the community structure, however, depends on a clearly known association between a specific metabolic pathway and a particular species or genera. Where such associations exist molecular biology techniques can be an effective tool to monitor change and associate this to particular characteristics, as demonstrated by targeting specific methanogenic spp. It is, however, known (Collins et al., 2003; de Bok et al., 2006; Nettmann et al., 2010, 2008) that a large proportion of methanogens commonly observed in anaerobic digesters belong to the genus *Methanosarcina* which can utilise acetate, methyl compounds, and CO_2/H_2 to produce methane (Galagan et al., 2002). The metabolic pathway to methane production when *Methanosarcina* is the dominant methanogenic species thus remains unclear (Collins et al., 2003). To resolve this uncertainty of methanogenic pathway when there is a large representation of *Methanosarcina*, it is therefore desirable to have a direct method to determine the degree of each methanogenic pathway in anaerobic digesters.

A radiolabelling method was established using ^{14}C labelled sodium acetate ($^{14}\text{CH}_3\text{COONa}$) and has been applied successfully in a number of studies carried out by Schnürer et al. (1999), Schnürer and Nordberg (2008), Karakashev et al. (2006, 2005) and Fotidis et al. (2013). The method determines the ratio of radioactive ^{14}C in CO_2 and CH_4 in the biogas, following the addition of $^{14}\text{CH}_3\text{COONa}$ into a digester sample. This $^{14}\text{CO}_2/^{14}\text{CH}_4$ ratio provides a rapid indication of the degree of syntrophic methanogenesis occurring in the anaerobic microbial consortium. It also avoids the high analysis cost and lengthy sample preparation commonly associated with molecular biology methods. To the best of the authors' knowledge, however, in the previous literature $^{14}\text{CO}_2/^{14}\text{CH}_4$ ratio was used as a qualitative indicator and an $^{14}\text{CO}_2/^{14}\text{CH}_4$ ratio of 1 was accepted as the threshold to distinguish the dominant methanogen pathway (Fotidis et al., 2013). Based on this previous radiolabelling work, the current study uses stoichiometric calculation to explore the correlation between $^{14}\text{CO}_2/^{14}\text{CH}_4$ ratio and the percentage of acetoclastic and hydrogenotrophic methanogenic pathways that contribute to the final methane formation from acetate as substrate in a mixed culture AD system.

To study the influence of ammonia concentration to $^{14}\text{CO}_2/^{14}\text{CH}_4$ ratio, ^{14}C studies were carried out on digester samples from food waste digesters operating at various ammonia nitrogen concentrations and from a digester treating municipal wastewater biosolids (MWB) with a low ammonia nitrogen concentration. This MWB digester had initially been used to inoculate all of the food waste digesters. To complement the ^{14}C study a methanogenic community analysis was also performed on one high nitrogen food waste digester samples and on the MWB digester sample. This used a pyrosequencing technique using a

fragment of the methyl Co-A reductase gene (*mcrA*) which is common to all known methanogens.

2. Materials & methods

2.1. Digester operation and sampling

Digester samples were collected from eight digesters (F1–8) which had been operating for over two years on source segregated domestic food waste. The full operational history of these digesters and analytical methods for key operational parameters including pH, VFA, TAN and daily biogas production has been described in detail elsewhere (Banks et al., 2012). Samples were taken between days 760–780, when total ammonia nitrogen (TAN) concentrations were in the range $3.5\text{--}5.3 \text{ g N kg}^{-1}$ WW. Digester samples were also collected from three pairs of digesters (R1–6) which had been operating for over 1 year. R1 and 2 were fed on food waste similar to that used for digester F1–8; however the total Kjeldahl nitrogen (TKN) level was artificially increased from $\sim 7 \text{ g N kg}^{-1}$ WW to 12 g kg^{-1} WW with urea (Reagent grade, Sigma Aldrich, UK). R3–R6 were fed with low nitrogen food waste (TKN = 2.08 g N kg^{-1} WW) which was specially prepared following a recipe described in Yirong (2014). All the digesters described above were operated at mesophilic temperature ($36 \text{ }^\circ\text{C}$).

Additionally, a low nitrogen digester sample was collected from a mesophilic digester treating municipal wastewater biosolids with digester TAN at 1.58 g N kg^{-1} WW (Millbrook Wastewater Treatment plant, Southampton, UK).

A total of nine digester samples were prepared for dosing with isotope labelled acetate as the feed. A summary of the key operational parameters at the time of sampling associated with the digesters from which the samples were taken is given in Table 1: these include TAN and VFA concentration and pH.

2.2. ^{14}C radiolabelling experiment

2.2.1. Sample preparation

The experimental method for radiolabelling tests was adopted and adapted from Schnürer et al. (1999) and Fotidis et al. (2013). 50 g of each digester sample was transferred into a 250 mL conical flask containing 100 mL of the non-selective culture medium, and thoroughly mixed. The medium recipe was as described in (Zinder and Koch, 1984), and consisted of the following (g L^{-1}): NH_4Cl , 1; NaCl , 0.1; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.4, and sodium acetate, 0.25. Five trace element solutions of the following concentrations (g L^{-1}): $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 4.03; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.184; $\text{NiCl}_2 \cdot 2\text{H}_2\text{O}$, 4.11; $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 0.18 and Na_2SeO_3 , 0.219; were prepared individually and 0.1 mL of each solution was added into 1 L of the medium. The medium solution was boiled under N_2 and autoclaved (15 min, $120 \text{ }^\circ\text{C}$) before use.

45 mL aliquots of the sample/culture medium mix were dispensed into crimp top serum bottles with a capacity of 119 mL. The specified quantity (see Table 2) of the $^{14}\text{CH}_3\text{COONa}$ (MP biomedical, Solon, OH, United States) solution was added into each mix. The headspace of the serum bottle was flushed with N_2/CO_2 (80:20) (BOC, UK) before sealing with a crimp cap with PTFE coated silicon septum. The serum bottles were then placed in an incubator (Hybaid Maxi 14, Thermo Scientific, UK) at $37 \text{ }^\circ\text{C}$ with orbital shaking at $\sim 50 \text{ RPM}$ for $\sim 12 \text{ h}$ to allow biogas to be produced. The test was carried out in duplicate for each of the digester samples.

2.2.2. Scintillation counting

After incubation, a 10% mix of oxygen in nitrogen was sparged into the liquid and headspace of the serum bottle at a flow rate of 30 mL min^{-1} for 45 min. The sparge gas from the serum bottle

Download English Version:

<https://daneshyari.com/en/article/8870381>

Download Persian Version:

<https://daneshyari.com/article/8870381>

[Daneshyari.com](https://daneshyari.com)