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Factors controlling headspace pressure in a manual manometric BMP method can be used to produce a methane output comparable to AMPTS

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Abstract

The manual manometric biochemical methane potential (mBMP) test uses the increase in pressure to calculate the gas produced. This gas production may be affected by the headspace volume in the incubation bottle and by the overhead pressure measurement and release (OHPMR) frequency. The biogas and methane yields of cellulose, barley, silage and slurry were compared with three incubation bottle headspace volumes (50, 90 and 180 ml; constant 70 ml total medium) and four OHPMR frequencies (daily, each third day, weekly and solely at the end of experiment). The methane yields of barley, silage and slurry were compared with those from an automated volumetric method (AMPTS). Headspace volume and OHPMR frequency effects on biogas yield were mediated mainly through headspace pressure, with the latter having a negative effect on the biogas yield measured and relatively little effect on methane yield. Two mBMP treatments produced methane yields equivalent to AMPTS.

Keywords: Anaerobic digestion, Biogas, Headspace volume, Methane, Pressure release frequency

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Abbreviations

ABAI	Anaerobic Biodegradation, Activity and Inhibition
AD	Anaerobic digestion
AFBI	Agri-Food and Biosciences Institute
AMPTS	Automatic methane potential test system
ANOVA	Analysis of variance
BMP	Biochemical methane potential
F	Frequency with which overhead pressure was measured and released
IWA	International Water Association
k	First order decay constant
mBMP	Manual manometric biochemical methane potential
NA	Not available
OHPMR	Overhead pressure measurement and release
P	Level of statistical significance
P day	Time at which the maximum pressure was recorded during the mBMP test
P_{Max}	Maximum pressure measured during the mBMP test
SEM	Standard error of the mean
T_{50}	Time taken to produce 50% of the total gas (Half-life)

TS	Total solids
U	Maximum methane or biogas production rate
V	Headspace volume
VS	Volatile solids
λ	Lag phase

1. Introduction

The biochemical methane potential (BMP) test is an anaerobic batch digestion process which is commonly used to determine the biogas and methane yields from organic substrates. The two most commonly used BMP test methods are the manometric and volumetric methods. In the manometric method the volume is kept constant and an increase in the overhead pressure is measured and used to calculate the amount of gas produced. In the volumetric method the pressure is kept constant and the volume of produced gas is measured by a displacement volume device (Valero et al., 2016)). There is no single universally accepted standard method to conduct the BMP test although several guidelines are published such as VDI 4630 guideline (2006), the method by members of the ABAI of the IWA (Angelidaki et al., 2009), and the updated guidelines from ABAI group (Holliger et al., 2016). These guidelines recommend both manometric and volumetric methods for the BMP test.

Although the manometric method is widely used, its parameters (incubation bottle size, maximum pressure limit and overhead pressure measurement and release (OHPMR) frequency) vary with different guidelines. For example, the VDI 4630 guideline (2006) recommends an incubation bottle size of 500 - 2000 ml for homogeneous substrates and 10 l - 20 l for heterogeneous substrates whereas Holliger et al. (2016) recommend an incubation bottle size of 100 ml for homogeneous substrates and 500 - 2000 ml for heterogeneous substrates. Both these guidelines have no direct recommendation for the OHPMR frequency but identify a maximum overhead pressure 100 hPa (VDI 4630 guideline, 2006) and 3000 hPa (Holliger et al., 2016) that should not be exceeded during the BMP test.

The manual manometric method (mBMP) can have a lower capital cost but a higher labour input than either the automated manometric or the volumetric methods. In the mBMP method it may be difficult to pinpoint the maximum overhead pressure achieved if readings are only taken once daily. Researchers using the mBMP have used different incubation bottle sizes and OHPMR frequencies (Ferrer et al., 2008; Hosseini Koupaie et al., 2014; McEniry et al., 2014; Nolan et al., 2016) but important descriptive details of these parameters are not always provided.

The methane yield of a particular substrate can be impacted by various factors including, but not limited to, inoculum, inoculum to substrate ratio, buffering system, substrate to buffer ratio, operating temperature, duration of the assay and the specific BMP technique employed. A wide range of methane yields have been reported, even for a relatively homogeneous and industrially synthesized feedstock such as cellulose (Raposo et al., 2011). However, in the inter-laboratory study (19 participating laboratories) reported by Raposo et al. (2011), laboratories using manometric BMP methods reported lower methane yields from cellulose than those using volumetric BMP methods. Furthermore, when compared within controlled experiments, McEniry et al. (2014), Nolan et al. (2016) and Wang et al. (2014) reported a lower methane yield from cellulose using the mBMP method compared to an automated volumetric method i.e. AMPTS (http://www.bioprocesscontrol.com/products/ampts-ii/). Also, Logan et al. (2002) reported a lower biogas yield with a manometric method compared to a respirometer (a variation of the volumetric method).

Biogas and methane yields with the mBMP method may be affected by the overhead pressure. The latter can be altered by differences in headspace volume in the incubation bottle and/or by the frequency of pressure release associated with the OHPMR frequency regime adopted. There is limited literature that thoroughly assesses the influence of these factors on biogas and methane yield. However, Yilmaz (2015) reported enhanced biogas yield for glucose with a lowering of the headspace pressure. Furthermore, Valero et al. (2016) suggested that the influence of overhead pressure on methane yield varied with the substrate used. The innovation in this study is that other papers have not compared a manual manometric method (with varied headspace volume and OHPMR frequency) with an automated volumetric method for assessing the biomethane potential values of energy crops and slurry. By undertaking these comparisons with substrates of contrasting anaerobic digestion characteristics this study provides the opportunity to identify manual manometric methods that best replicate the methane outputs obtained with an automated volumetric method.

The objectives of the present study were to compare the effects of different headspace volumes and the frequency of pressure release associated with different OHPMR frequency regimes on biogas and methane yields using a mBMP test, and to compare the outputs for these mBMP treatment combinations with the output for an industry standard automated volumetric method i.e. AMPTS. In order to broaden the circumstances under which these comparisons were made, contrasting substrates (cellulose, barley, silage and slurry) with different digestion profiles were used.

2. Materials and methods

2.1 Substrates

Silage was prepared from the first cut of perennial ryegrass (*Lolium perenne* L.) while whole barley (*Hordeum vulgare* L.) grains were purchased from a livestock feed merchant. Both silage and barley samples were dried at 40°C for 48 h in an oven with forced air circulation and then milled (Wiley mill; 1 mm pore screen). These dried and milled samples were used for the BMP assay. Cellulose powder was obtained from Sigma-Aldrich (product id. 22184). The cattle slurry was collected from a tank under a roofed slatted-floor cattle building at Teagasc, the Irish Agricultural and Food Development Authority Research Centre in Grange, County Meath, Ireland. It was produced by cattle consuming grass silage *ad libitum* and consisted of faeces and urine. The collected cattle slurry was thoroughly mixed and stored at -20°C until required. The inoculum was obtained from an on-farm anaerobic digestion (AD) reactor digesting cattle slurry and grass silage at the Agri-Food and Biosciences Institute in Hillsborough (AFBI), Co. Down, Northern Ireland. This was de-gassed in an incubator for 5 d at 37°C. The inoculum was then mixed with a wooden spatula and, under a continuous flow of N_2, filtered through a 2 mm pore sieve. The total solids (TS) and volatile solids (VS) of the four substrate samples were measured according to Standard Methods 2540 G (APHA, 2005). The TS of cellulose, barley, silage, slurry and inoculum was 966, 846, 901, 136 and 48 g kg^{-1}, respectively. While, the VS of cellulose, barley, silage, slurry and inoculum was 1000, 924, 978, 794 and 715 g kg^{-1}VS, respectively.

2.2 mBMP

The biogas and methane yields were determined in triplicate incubation bottles for each of the four substrates in each of three different volume serum bottles (i.e. 120, 160 and 250 ml) and were subjected to each of four gas sampling and gas pressure release frequencies throughout incubation, using the method described in McEniry and

O'Kiely (2013) with a few minor adjustments. The relative design and shape of all the bottles were similar but they differed in the diameter of their base and in height. The outer base diameter × height of the 120, 160 and 250 ml bottles were 52 mm × 95 mm, 54 mm × 108 mm and 64 mm × 117 mm, respectively. The inoculum and substrate were added at a 2:1 VS inoculum-to-substrate gravimetric ratio to provide a total organic loading of 10 g VS kg^{-1} total medium. Micro- and macro-mineral solutions were also added to prevent mineral nutrient deficiency (McEniry & O'Kiely, 2013). The final total medium volume of each bottle was adjusted to 70 ml using distilled water. The headspace volume in 120, 160 and 250 ml bottles was 50, 90 and 180 ml, respectively. Three blank replicates (inoculum only) were also prepared for each different bottle volume set at each sampling frequency. All bottles were flushed with N_2 gas for about 1 min and sealed with butyl rubber stoppers and aluminium crimp caps. Bottles were incubated at 37°C for 35 d and mixed daily by manual swirling. The overhead pressure in the incubation bottles was measured, and gas was released to equilibrate to atmospheric pressure, at four different frequencies i.e. daily, each third day, weekly and only after 35 d incubation, using a detachable pressure transducer (Tracker 220, Gems Sensors and Controls, Basingstoke, UK) and Vaseline® lubricated needle.

Thus 180 mBMP incubation bottles were used as follows:
[(three headspace volumes × four OHPMR) × triplicate replication] for each of four substrates and one blank, where headspace volumes were 50, 90 and 180 ml, OHPMR were daily, each third day, weekly and only after 35 d incubation, the substrates were cellulose, barley, silage and slurry and the blank was inoculum only.

For 50 ml headspace bottles designated to be sampled only after 35 d incubation, overhead pressure was not measured at day 35 because the high pressure had ruptured the butyl rubber stopper on the incubation bottles. Thus 15 incubation bottles did not survive the study, leaving the data from 165 incubation bottles for statistical analyses. The biogas produced was estimated using the equation:

$$\text{Gas produce (ml)} = \frac{\text{vh}}{\text{Pa}} \times \text{Pt}$$

where, vh is the headspace volume (ml), Pa is the atmospheric pressure (hPa) and Pt is the gas headspace pressure (hPa).

The methane concentration of biogas was determined using a Shimadzu GC-2014 gas chromatograph with a flame-ionisation detector equipped with a glass column (2.1 m × 5.0 mm × 3.2 mm packed with molecular sieve 5A 60/80 mesh). The temperatures in the column, injector and detector were 120, 150 and 170 °C, respectively, with helium as the carrier gas. Evaluation of biogas and methane yield included a correction for inert gas, a correction for inoculum-induced gas production and a normalisation of gas output (normalised litres) to standard temperature and pressure (273 K, 1013 hPa) conditions.

2.3 AMPTS

The methane yield of three substrates (silage, barley and slurry) was also determined using a volumetric gas production method i.e. the Automated Methane Potential Test System II (AMPTS; Bioprocess Control AB, Lund, Sweden). To avoid possible confounding due to factors such as differences in substrate or inoculum, sub-samples of the same substrate and inoculum used in the mBMP system were simultaneously used in the AMPTS. The AMPTS employed similar characteristics to the mBMP system where feasible i.e. both systems started at the same date and continued for 35 d, using triplicate samples of each substrate, and using the same inoculum-to-substrate ratio, buffer, blanks, flushing with N_2 and incubation at 37°C. However, each AMPTS bottle (500 ml total volume; 400 ml working volume and 100 ml headspace) was equipped with an individual mechanical mixer (60 revolutions per min; for 10 min after a 10 min pause; repeat) and the biogas produced in each bottle passed through a second bottle (one per incubation bottle, containing 3 M NaOH which retains CO_2 and H_2S while allowing methane to pass through). The upgraded gas was sent to a flow measurement device (one for each incubation bottle) which measures gas through water displacement. A specific volume (approximately 10 ml) of methane caused the tipping device to tip. This movement was recorded via a digital pulse and output was recorded in a software package as volume of methane produced. For each tipping the pressure and temperature were recorded to allow normalization of the methane produced (normalised litres) to standard temperature and pressure (273K, 1013 hPa) conditions. AMPTS is further described in McEniry et al. (2014) and Bioprocess Control Sweden AB (2014).

Thus there were 12 AMPTS bottles: [three substrates and one blank] × triplicate replication, where the substrates were barley, silage and slurry and blank was inoculum only.

2.4 Kinetics

First and second order kinetics were run in Matlab® R2009a software, as described by Wall et al. (2013). The average decay constant or k value for both biogas and methane were determined using first-order kinetics:

$$y(t) = y_m x(1 - e^{(-kt)})$$

where, y(t) is the cumulative methane (or biogas) yield at time t (L kg^{-1} VS), y(m) is the methane (or biogas) yield at the end of the 35 d batch test (L kg^{-1} VS), t is the time (d) and k is the first order decay constant (d^{-1}).

Lag phase (λ), half-life (T_{50}) and maximum production rate (U) for both biogas and methane were calculated using second-order kinetics:

$$y = y_{max}.\exp\left\{-\exp\left[U.\frac{e}{y_{max}}.(\lambda - t) + 1\right]\right\}$$

where, y is the cumulative methane (or biogas) yield (L kg^{-1} VS), y_{max} is the predicted methane (or biogas) yield at the end of the 35 d batch test (L kg^{-1} VS), U is the maximum methane (or biogas) production rate (L kg^{-1} VS d^{-1}), λ is the lag phase (d) and t is the time (d).

2.5 Statistical analysis

The data were analysed using the MIXED procedure in SAS 9.3. Methane yield, biogas yield and kinetics data for the mBMP system were analysed as a split plot design with incubation bottle headspace volume as the main plot and OHPMR frequency as the sub plot. The methane yield and methane kinetics from mBMP and AMPTS were compared using a one-way classification where Dunnett's adjustment was used to correct for multiple comparisons effects when comparing all means to the AMPTS control.

Within each substrate, linear regression and R^2 values were derived for the relationships between the P_{Max} and each of biogas yield, methane yield and methane proportion of treatment means using the 'format trendline' for XY scatter graphs within Microsoft Excel.

3. Results

3.1 Cellulose

Mean and standard error of the mean (SEM) values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 1, and the corresponding levels of significance are presented in Table 5. Biogas yield and the associated λ, k and U values increased with an increase in headspace volume, although the scale of this response was greater as the OHPMR frequency declined. In contrast, T_{50} and P_{Max} declined with an increase in headspace volume, and the scale of this response was greater as the OHPMR frequency declined. Biogas yield decreased with a reduction in OHPMR frequency. Declining OHPMR frequency reduced λ and k when the headspace volume was 50 ml. The U value decreased but T_{50} and P_{Max} increased with a reduction in OHPMR frequency.

Increasing headspace volume did not significantly alter methane yield for daily OHPMR frequency but it resulted in an increase during weekly and solely after 35 d OHPMR frequencies. The k value decreased with an increase in headspace volume for each third day OHPMR frequency but it increased for the solely 35 d OHPMR frequency. The U value decreased with an increase in headspace volume when the OHPMR was done daily or each third day but it increased for the weekly OHPMR frequency. Reducing OHPMR frequency reduced methane yield when headspace volume was 50 ml. λ decreased with decline in OHPMR frequency when the headspace volume was 90 ml. The U value declined as OHPMR frequency declined when the headspace volume was 50 or 90 ml.

3.2 Barley

Mean and SEM values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 2, and the corresponding levels of significance are presented in Table 5. Biogas yield and the associated λ, k and U increased with an increase in headspace volume, although the scale of this response was generally greater as the OHPMR frequency declined. In contrast, T_{50} and P_{Max} declined with an increase in headspace volume and the scale of this response was greater as the OHPMR frequency declined. Biogas yield decreased with a reduction in OHPMR frequency when the headspace volume was 50 or 90 ml. The k value decreased while P_{Max} increased with a reduction in OHPMR frequency. Methane% declined with an increase in headspace volume during daily and each third day OHMP frequency.

Increasing headspace volume did not significantly alter methane yield for daily OHPMR frequency but it resulted in an increase during each third day, weekly and solely after 35 d OHPMR frequencies. The associated kinetic parameters k and U generally increased while λ and T_{50} generally decreased with an increase in headspace volume. No clear effect of OHPMR frequency on methane yield emerged.

The comparisons of methane production for mBMP and AMPTSs are shown in Table 3. Five of the 11 mBMP treatments had methane yields that differed ($P<0.05$) from AMPTS, but the differences between the two systems for associated kinetic parameters followed contrasting patterns.

3.3 Silage

Mean and SEM values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 3, and the corresponding levels of significance are presented in Table 5. Biogas yield and the associated λ, k and U increased with an increase in headspace volume, although the scale of this response was generally greater as the OHPMR frequency declined. In contrast, T_{50} and P_{Max} declined with an increase in headspace volume, and the scale of this response was greater as the OHPMR frequency declined. Biogas yield decreased with a decline in OHPMR frequency when the headspace volume was 50 or 90 ml. The k and U values generally decreased while P_{Max} increased with a reduction in OHPMR frequency.

The methane yield and the associated U value increased with an increase in headspace volume except for daily OHPMR frequency. The k value increased while T_{50} decreased with an increase in headspace volume. Methane yield showed a variable response to declining OHPMR frequency across the three headspace volumes. The λ value generally decreased with a decline in OHPMR frequency. The k value increased while T_{50} decreased with a decline in OHPMR frequency when the headspace volume was 90 or 180 ml.

The comparisons of methane production for mBMP and AMPTSs are shown in Table 4. Eight of the 11 mBMP treatments had methane yields that differed ($P<0.05$) from AMPTS, but the differences between the two systems for associated kinetic parameters followed contrasting patterns.

3.4 Slurry

Mean and SEM values for biogas yield, methane yield and their associated kinetic parameters are presented in Table 4, and the corresponding levels of significance are presented in Table 5. Biogas yield increased with an increase in headspace volume except for daily OHPMR frequency, and the scale of this response was highest for weekly OHPMR frequency. The associated kinetic parameters k and U generally increased while λ, T_{50}, methane% and P_{Max} generally decreased with an increase in headspace volume. Biogas yield generally decreased with a decline in OHPMR frequency when the headspace volume was 50 or 90 ml. The U value decreased while T_{50}, methane% and P_{Max} generally increased with a decline in OHPMR frequency.

There was no main effect of headspace volume or OHPMR frequency on methane yield, although individual treatment differences did occur. The significant effects on the associated kinetic parameters generally did not follow a linear progression in response to either headspace volume or OHPMR frequency.

The comparisons of methane production for mBMP and AMPTSs are shown in Table 3. Two of the 11 mBMP treatments had methane yields that differed ($P<0.05$) from AMPTS, but the differences between the two systems for associated kinetic parameters followed contrasting patterns.

Table 1. Biogas yield, methane yield and kinetic parameters when cellulose was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

Table 2. Biogas yield, methane yield and kinetic parameters when barley was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

Table 3. Biogas yield, methane yield and kinetic parameters when silage was was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

Table 4. Biogas yield, methane yield and kinetic parameters when slurry was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

Table 5. The level of significance (P) for biogas yield, methane yield and kinetic parameters for cellulose, barley, silage and slurry.

4. Discussion

The four substrates provided contrasting chemical compositions of their VS thereby broadening the conditions under which the objectives were assessed. The progressive decline in biogas yields in the mBMP test from similarly high values with cellulose and barley, intermediate values with silage and lowest values with slurry suggest a matching decline in AD of VS. This progression at least partially reflects the negative effects of corresponding increases in lignifications. These differences in extent of AD were accompanied by contrasting kinetics of digestion, with barley showing a particularly short lag phase and a rapid early rate of AD whereas slurry had a relatively long lag phase and slow early rate of AD.

The substrates also differed in the methanogenic nature of their digested VS (i.e. methane proportion in biogas) in the order slurry > silage > barley > cellulose (daily OHPMR frequency for 180 ml headspace bottles). Published methane proportions for slurry, silage, barley and cellulose are 56-62%, 54-56% (Triolo et al., 2011), 53% (Biteco, 2017) and 55-56% (Holliger et al., 2016; Wang et al., 2014), respectively.

4.1 mBMP

The VDI 4630 guideline (2006) recommends that when cellulose is digested in a BMP test it should produce a biogas yield of at least 80% of its theoretical maximum yield (i.e. 592 to 600 L kg^{-1} VS (VDI 4630 guideline, 2006)). In the present mBMP test this was achieved with eight of twelve treatments imposed, and these were mainly treatments that exhibited lower P_{max} values. However, all of the P_{max} values for the treatments imposed on cellulose and on the other three substrates exceeded the recommended maximum pressure of 100 hPa in VDI 4630 (2006) but most were below the maximum pressure of 3000 hPa recommended by the ABAI guideline group (Holliger et al., 2016)

In the present study, the effects of altering headspace volume, OHPMR frequency or both factors on biogas yield were most likely mediated through their individual or combined effects on headspace pressure. Using P_{max} as an estimate of the maximum headspace pressure that occurred, it is clear that a progressive increase in maximum headspace pressure correspondingly reduced biogas yield (Figure 1). Although this relationship was evident with all four substrates the apparent rate of decline in biogas yield was greatest for the substrate that also had the greatest yield at low headspace pressure (i.e. cellulose) and lowest for the substrate with the lowest biogas yield at low headspace pressure (i.e. slurry).

The negative impact of headspace pressure on biogas yield could be due to increased solubilisation of carbon dioxide in the medium as headspace pressure increased. According to Henry's Law, when the partial pressure of carbon dioxide increases in the headspace an increasing amount of this gas will dissolve in the medium and thus less of it will be released at the time of OHPMR. This agrees with the findings of a recent meta-analysis of methodological factors affecting *in vitro* rumen fermentation systems (Maccarana et al., 2016) where increasing headspace pressure also resulted in reduced gas production. Whereas an increased concentration of carbon dioxide might be expected to reduce the pH of the medium, potentially perturbing some microbial activity, the robust buffering provided to the medium in this study appeared to prevent such a change in pH.

A negative effect of presumably very high P_{max} values was evident with the treatment that combined the smallest headspace volume (50 ml) with the lowest

OHPMR frequency (solely after 35 d). In this case, the butyl rubber stopper on all the incubation bottles ruptured resulting in loss of data for this treatment.

The methane yield for the 11 successfully completed treatments with cellulose ranged from 70-112% and 65-99% of the minimum yields recommended by VDI 4630 (2006) and Holliger et al. (2016), respectively. The similar methane yields recorded for cellulose, barley and silage but the lower yields for slurry (during daily OHPMR frequency for 180 ml headspace volume) relate to corresponding published values of 259 to 366 L kg^{-1} VS for cellulose (McEniry & O'Kiely, 2013; Wang et al., 2014), 304-380 L kg^{-1} VS (Biteco, 2017; Braun, 2007; Heiermann et al., 2002; Rudolf et al., 2009), 229-400 L kg^{-1} VS (McEniry & O'Kiely, 2013; Wall et al., 2013) and 125-239 (Triolo et al., 2011; Wall et al., 2013).

The weak relationship between headspace pressure and methane yield contrasts with the clear negative relationship between headspace pressure and biogas yield (Figure 1). Since biogas is composed mainly of carbon dioxide and methane their different responses to increasing headspace pressure likely reflect the combined effects of the much greater solubility of carbon dioxide than methane (88 ml CO_2 per 100 ml H_2O vs. 3.5 ml CH_4 per 100 ml H_2O; O'Neil (2013)) and their different Henry's Law solubility constants $3.3x10^{-2}$ mol m^{-3} hPa^{-1} for CO_2 and $1.4x10^{-3}$ mol m^{-3} hPa^{-1} for CH_4; Sander (2015)). The latter indicate that a markedly greater increase in solubility of carbon dioxide occurs in response to an increase in its partial pressure than occurs for methane. This, in turn, should result in an increase in the concentration of methane in biogas as headspace pressure increases, and Figure 2 shows that this occurred. These findings agree with Maccarana et al. (2016) who also reported that increasing headspace pressure had little effect on methane yield but increased the concentration of methane in the headspace gases in *in vitro* rumen digestion systems.

Headspace pressure effects alone appear not to provide a full explanation for methane yield outcomes. For example, for the three substrates that produced higher methane yields than slurry increasing headspace volume generally increased methane yield when OHPMR frequency was less than daily, but for slurry that produced a lower methane yield the headspace volume did not have a clear effect. In contrast, OHPMR frequency had little direct effect on methane yield. Thus the two factors (headspace volume and OHPMR frequency) seem to differ in the mechanisms by which they affect methane yield.

A direct comparison of the results of the present study and those of Yilmaz (2015) is difficult since different substrates, headspace volumes and OHPMR frequencies were used. However, when glucose (Yilmaz (2015)) and cellulose (present study) were used as substrates, there was a general trend for methane yield to increase in response to increasing headspace volume for each third day OHPMR frequency. Also, reducing OHPMR frequency reduced methane yield only for incubation bottles with the smallest headspace volume. The results of the present study also agree with Valero et al. (2016) that headspace pressure can differentially influence the methane yield with contrasting substrates.

4.2 mBMP vs. AMPTS

Although the methane yields produced for many OHPMR frequency and headspace volume combinations when cellulose was digested by mBMP test were below VDI 4630 guideline (2006) and (Holliger et al. (2016)) targets, the values obtained for barley, silage and slurry were 59-115%, 67-111% and 84-118% of the corresponding values recorded using AMPTS. Furthermore, the similar methane yields for barley and silage but the much lower yield for slurry when using AMPTS was repeated with eight of the 11 successfully completed mBMP treatments.

Taking the methane yields obtained using AMPTS as reference target values, two of the mBMP treatments produced comparable yields to AMPTS across the three contrasting substrates (Table 6). First, when the mBMP test had an each third day OHPMR frequency and a headspace volume of 180 ml it produced 100, 96 and 98% of the methane yields recorded using AMPTS for barley, silage and slurry, respectively. Furthermore, the methane yield relativities for barley, silage and slurry reflected those obtained by AMPTS (barley:silage:slurry of 1.53:1.50:1.00 and 1.50:1.54:1.00 for this mBMP treatment and AMPTS, respectively). Second, when the mBMP test had an OHPMR solely after 35 d and a headspace volume of 180 ml it produced 100, 97 and 103% of the methane yields of AMPTS for barley, silage and slurry, respectively and had a barley:silage:slurry methane yield relativity of 1.45:1.45:1.00. For these two mBMP treatments, the option of each third day OHPMR frequency plus 180 ml headspace volume requires a greater and more frequent labour input but provides the opportunity to produce digestion kinetics results. It also poses a lower risk of septum failure due to high headspace pressure accumulation.

Table 6. Methane yields using mBMP for 180 ml headspace volume bottles and OHPMR frequencies of each third day and solely after 35 d and the corresponding yields with AMPTS.

Figure 1. Relationships between maximum pressure measured for cellulose, barley, silage and slurry and biogas and methane yields during 35 day anaerobic digestion.

Figure 2. Relationships between maximum pressure measured for cellulose, barley, silage and slurry and methane proportion in biogas during 35 day anaerobic digestion.

5. Future perspective

Judicious consideration is required when selecting a BMP technique as the decision can impact on the methane yields recorded and on the relative values attributed to different substrates.

This study highlights the importance of using substrates with contrasting digestion characteristics when assessing the effects of factors of interest on biogas and methane output. Furthermore, it is important that resultant publications should report the headspace volume, OHPMR frequency and other relevant factors used in their BMP tests. Finally, where an accurate estimate of biogas yield is required, it is recommended that the duration of mBMP tests be extended sufficiently to allow dissolved CO_2 be retrieved.

6. Conclusion

Headspace volume and OHPMR frequency affected headspace pressure and the latter had a negative effect on biogas yield in a mBMP test. Headspace pressure had relatively little effect on methane yield but had a clear positive effect on methane concentration.

Accepting the methane yields obtained using the AMPTS system as reference target values, two mBMP treatments replicated these targets – OHPMR frequencies of each third day or solely after 35 d, in each case with a headspace volume of 180 ml (70 ml total medium).

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8. References

1. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci Technol*, **59**(5), 927-34.
2. APHA. 2005. Standard methods for the examination of water and wastewater. *American Public Health Association, American Water Works Association, Water Environment Federation, Washington, USA.*
3. Bioprocess Control Sweden AB. 2014. AMPTS II (Automatic Methane Potential Test System): Operation and maintenance manual. Sweden.
4. Biteco. 2017. Out of biogas from different types of substrates http://www.biteco-energy.com/biogas-yield/ (accessed on 14-01-2017).
5. Braun, R. 2007. Anaerobic digestion: a multi-faceted process for energy, environmental management and rural development. in: *Improvement of crop plants for industrial end uses*, Springer, pp. 335-416.

6. Ferrer, I., Ponsá, S., Vázquez, F., Font, X. 2008. Increasing biogas production by thermal (70°C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal*, **42**(2), 186-192.
7. Heiermann, M., Plöchl, M., Linke, B., Schelle, H. 2002. Preliminary evaluation of some cereals as energy crops for biogas production. *Renewable Energy: Renewables World's Best Energy Option. Proceedings of the World Renewable Energy Congress VII*, **29**,107-116.
8. Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffiere, P., Carballa, M., de Wilde, V., Ebertseder, F., Fernandez, B., Ficara, E., Fotidis, I., Frigon, J.C., de Laclos, H.F., Ghasimi, D.S., Hack, G., Hartel, M., Heerenklage, J., Horvath, I.S., Jenicek, P., Koch, K., Krautwald, J., Lizasoain, J., Liu, J., Mosberger, L., Nistor, M., Oechsner, H., Oliveira, J.V., Paterson, M., Pauss, A., Pommier, S., Porqueddu, I., Raposo, F., Ribeiro, T., Rusch Pfund, F., Stromberg, S., Torrijos, M., van Eekert, M., van Lier, J., Wedwitschka, H., Wierinck, I. 2016. Towards a standardization of biomethane potential tests. *Water Sci Technol*, **74**(11), 2515-2522.
9. Hosseini Koupaie, E., Barrantes Leiva, M., Eskicioglu, C., Dutil, C. 2014. Mesophilic batch anaerobic co-digestion of fruit-juice industrial waste and municipal waste sludge: process and cost-benefit analysis. *Bioresour Technol*, **152**, 66-73.
10. Logan, B.E., Oh, S.E., Kim, I.S., Van Ginkel, S. 2002. Biological hydrogen production measured in batch anaerobic respirometers. *Environ Sci Technol*, **36**(11), 2530-5.

11. Maccarana, L., Cattani, M., Tagliapietra, F., Schiavon, S., Bailoni, L., Mantovani, R. 2016. Methodological factors affecting gas and methane production during in vitro rumen fermentation evaluated by meta-analysis approach. *J Anim Sci Biotechnol*, **7**(1), 35.
12. McEniry, J., Allen, E., Murphy, J.D., O'Kiely, P. 2014. Grass for biogas production: The impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems. *Renew Energy*, **63**, 524-530.
13. McEniry, J., O'Kiely, P. 2013. Anaerobic methane production from five common grassland species at sequential stages of maturity. *Bioresour Technol*, **127**(0), 143-50.
14. Nolan, P., Luostarinen, S., Doyle, E.M., O'Kiely, P. 2016. Anaerobic digestion of perennial ryegrass prepared by cryogenic freezing versus thermal drying methods, using contrasting in vitro batch digestion systems. *Renew Energy*, **87**, 273-278.
15. O'Neil, M.J. 2013*. The Merck index: an encyclopedia of chemicals, drugs, and biologicals.* RSC Publishing.
16. Raposo, F., Fernández-Cegrí, V., De la Rubia, M., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J. 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J. Chem. Technol. Biotechnol.*, **86**(8), 1088-1098.
17. Rudolf, B., Peter, W., Arthur, W. 2009. Biogas from Energy Crop Digestion. in: *IEA Bioenergy*, Vol. Task 37 - Energy from Biogas and Landfill Gas.

18. Sander, R. 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmos. Chem. Phys.*, **15**(8).

19. Triolo, J.M., Sommer, S.G., Moller, H.B., Weisbjerg, M.R., Jiang, X.Y. 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. *Bioresour Technol*, **102**(20), 9395-402.

20. Valero, D., Montes, J.A., Rico, J.L., Rico, C. 2016. Influence of headspace pressure on methane production in Biochemical Methane Potential (BMP) tests. *Waste Manag*, **48**, 193-8.

21. VDI 4630 guideline. 2006. Fermentation of organic materials. Characterisation of the substrates. Sampling, collection of material data, fermentation test. VDI-Handbuch Energietechnik.

22. Wall, D.M., O'Kiely, P., Murphy, J.D. 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. *Bioresour Technol*, **149**, 425-31.

23. Wang, B., Nges, I.A., Nistor, M., Liu, J. 2014. Determination of methane yield of cellulose using different experimental setups. *Water Sci Technol*, **70**(4), 599-604.

24. Yilmaz, V. 2015. A straightforward method: Biochemical methane potential assay. *Renewable Energy Research and Applications (ICRERA), 2015 International Conference on*. IEEE. 148-150.

Table 1. Biogas yield, methane yield and kinetic parameters when cellulose was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each third day			Weekly			After 35 days		SEM[1]
V (ml)	50	90	180	50	90	180	50	90	180	90	180	
Biogas												
L kg^{-1} VS	611.6^{d}	626^{d}	713.3^{e}	543.9^{c}	616.9^{d}	701.9^{e}	434.9^{b}	604.9^{d}	679.5^{e}	126.2^{a}	590.5^{cd}	15.96
λ[2]	1.4^{c}	1.5^{c}	1.8^{cd}	0.4^{b}	1.6^{cd}	2.1^{d}	-1.2^{a}	0.4^{b}	1.5^{c}			0.19
k[3]	0.088^{de}	0.088^{de}	0.097^{f}	0.069^{b}	0.083^{cd}	0.091^{ef}	0.057^{a}	0.078^{c}	0.094^{ef}			0.0026
U[4]	39.3^{cd}	40^{cd}	54.4^{e}	23.3^{b}	36.5^{c}	50.5^{e}	12.8^{a}	29.4^{b}	43.9^{d}			2.13
T_{50}[5]	9.3^{ab}	9.4^{ab}	8.3^{a}	12.7^{d}	10.1^{bc}	9.1^{ab}	24.6^{e}	10.9^{c}	9.4^{ab}			0.49
CH_4%[6]	49.0^{bcd}	46.6^{ab}	42.1^{a}	49.0^{bcd}	49.0^{bcd}	42.2^{a}	52.9^{cde}	48.4^{bc}	51.5^{bcd}	57.8^{e}	54.0^{de}	1.80
P_{Max}[7]	676.6^{d}	358.3^{b}	210.1^{a}	1340.1^{f}	892.6^{e}	486.8^{c}	1889.9^{h}	1629.2^{g}	946.2^{e}	2618.4^{j}	2217.1^{i}	31.45
P day[8]	9	9	9	9	9	9	14	14	14	35	35	0
Methane												
L kg^{-1} VS	299.7^{de}	291.4^{cd}	300.3^{de}	266.7^{c}	302.5^{de}	296.4^{de}	230.5^{b}	292.5^{cde}	349.7^{f}	74.5^{a}	319.0^{e}	9.21
λ[2]	4.2^{c}	9.2^{f}	3.8^{c}	4.3^{cd}	6.0^{e}	6.5^{e}	5.1^{d}	1.5^{a}	2.9^{b}			0.30
k[3]	0.072^{d}	0.056^{b}	0.069^{d}	0.080^{e}	0.066^{cd}	0.060^{bc}	0.048^{a}	0.068^{d}	0.080^{e}			0.0025
U[4]	34.9^{f}	29.2^{e}	20.8^{d}	21.9^{d}	18.0^{c}	17.1^{c}	8.0^{a}	13.3^{b}	21.4^{d}			0.66
T_{50}[5]	11.7^{ab}	15.2^{d}	12.6^{abc}	11.2^{a}	12.5^{abc}	13.3^{c}	38.8^{e}	13.1^{bc}	11.3^{a}			0.47

[1] SEM is Standard error of mean, [2] λ is the lag phase (d), [3] k is the first order decay constant (d^{-1}), [4] U is the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}), [5] T_{50} is half-life i.e. time taken (d) to produce 50% of the gas production, [6] CH_4% is the methane proportion in biogas (vol. vol.$^{-1}$), [7] P_{Max} (hPa) is the maximum pressure measured during the mBMP test, and [8] P day is the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically (P>0.05) not different from each other.

Table 2. Biogas yield, methane yield and kinetic parameters when barley was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each 3 day			Weekly			After 35 days		AMPTS	SEM[1]	
V (ml)	50	90	180	50	90	180	50	90	180	90	180		ANOVA	Dunnett's
Biogas														
L kg^{-1} VS	612.4^{de}	642.2^{e}	701.1^{f}	551.5^{bc}	620.0^{de}	736.9^{f}	386.7^{a}	604.0^{de}	700.9^{f}	523.1^{b}	583.0^{cd}	-	14.43	-
λ^2	-2.8^{a}	-2.8^{a}	-1.9^{bc}	-1.2^{cd}	-1.0^{d}	-0.6^{d}	-2.2^{ab}	0.5^{e}	0.6^{e}	-	-	-	0.29	-
k^3	0.094^{cd}	0.109^{e}	0.136^{f}	0.074^{b}	0.094^{cd}	0.113^{e}	0.056^{a}	0.085^{bc}	0.104^{de}	-	-	-	0.004	-
U^4	28.3^{c}	33.4^{d}	47.4^{e}	22.1^{b}	32.1^{cd}	49.3^{e}	10.6^{a}	33.7^{d}	47.9^{e}	-	-	-	1.66	-
$T_{50}{}^5$	8.1^{ab}	6.9^{ab}	5.5^{a}	12.2^{c}	8.8^{b}	7.0^{ab}	26.4^{d}	9.4^{bc}	7.8^{ab}	-	-	-	0.96	-
$CH_4\%^6$	51.5^{c}	48.8^{b}	44.0^{a}	54.4^{d}	53.2^{d}	47.5^{b}	53.7^{d}	50.9^{c}	57.2^{e}	60.2^{f}	60.1^{f}	-	0.52	-
$P_{Max}{}^7$	673.2^{d}	550.0^{c}	348.0^{a}	1248.9^{g}	821.6^{e}	462.4^{b}	1901.3^{i}	1694.3^{h}	902.1^{f}	3871.4^{k}	2206.3^{j}	-	12.13	-
P day^8	1	1	1	12	12	12	14	14	14	35	35	-	0	-
Methane														
L kg^{-1} VS	315.2^{bc}	313.2^{bc}	$\mathbf{308.4}^{bc}$	$\mathbf{300.1}^{b}$	329.8^{cd}	350.0^{d}	$\mathbf{207.5}^{a}$	$\mathbf{307.6}^{bc}$	$\mathbf{401.3}^{e}$	314.8^{bc}	350.6^{d}	349.7	8.75	8.76
λ^2	6.4^{cd}	$\mathbf{3.0}^{ab}$	$\mathbf{4.5}^{bc}$	7.1^{d}	$\mathbf{2.3}^{ab}$	$\mathbf{2.0}^{ab}$	8.1^{d}	$\mathbf{1.5}^{a}$	$\mathbf{1.1}^{a}$	-	-	9.20	0.90	0.87
k^3	$\mathbf{0.044}^{a}$	$\mathbf{0.070}^{bc}$	$\mathbf{0.066}^{b}$	$\mathbf{0.066}^{b}$	$\mathbf{0.074}^{cd}$	$\mathbf{0.077}^{d}$	$\mathbf{0.045}^{a}$	$\mathbf{0.074}^{cd}$	$\mathbf{0.089}^{e}$	-	-	0.056	0.0023	0.0023
U^4	$\mathbf{9.7}^{a}$	$\mathbf{16.7}^{b}$	$\mathbf{16.9}^{b}$	$\mathbf{17.2}^{bc}$	$\mathbf{17.2}^{bc}$	$\mathbf{19.1}^{c}$	$\mathbf{7.9}^{a}$	$\mathbf{16.2}^{b}$	$\mathbf{24.2}^{d}$	-	-	29.2	0.71	0.72
$T_{50}{}^5$	20.9^{b}	12.6^{a}	13.5^{a}	12.8^{a}	12.3^{a}	11.5^{a}	$\mathbf{49.5}^{c}$	11.0^{a}	9.5^{a}	-	-	15.2	1.73	1.64

[1] SEM is Standard error of mean, [2] λ is the lag phase (d), [3] k is the first order decay constant (d^{-1}), [4] U is the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}), [5] T_{50} is half-life i.e. time taken (d) to produce 50% of the gas production, [6] $CH_4\%$ is the methane proportion in biogas (vol. vol.$^{-1}$), [7] P_{Max} (hPa) is the maximum pressure measured during the mBMP test, and [8] P day is the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically

(P>0.05) not different from each other. The values in bold, within a row, are statistically (P<0.05, using Dunnett’s adjustment) different from AMPTS values.

Table 3. Biogas yield, methane yield and kinetic parameters when silage was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each 3 day			Weekly			After 35 days		AMPTS	SEM[1]	
V (ml)	50	90	180	50	90	180	50	90	180	90	180		ANOVA	Dunnett's
Biogas														
L kg^{-1} VS	550.4^{c}	602.2^{e}	619.8^{e}	523.5^{c}	596.3^{de}	691.3^{f}	407.0^{b}	560.1^{cd}	614.5^{e}	332.3^{a}	550.7^{c}	-	13.95	-
λ[2]	-0.5^{b}	-0.4^{b}	0.3^{c}	-0.7^{b}	-0.4^{b}	0.1^{c}	-2.7^{a}	0.4^{c}	0.3^{c}	-	-	-	0.13	-
k[3]	0.101^{cd}	0.108^{de}	0.144^{f}	0.083^{b}	0.097^{c}	0.116^{e}	0.065^{a}	0.092^{bc}	0.116^{e}	-	-	-	0.0034	-
U[4]	32.6^{c}	38.8^{d}	56.7^{g}	24.5^{b}	33.5^{c}	49.5^{f}	12.7^{a}	32.1^{c}	44.6^{e}	-	-	-	1.38	-
T_{50}[5]	8.1^{cd}	7.4^{bc}	5.8^{a}	10.4^{f}	8.7^{de}	7.2^{b}	16.6^{g}	9.2^{e}	7.3^{b}	-	-	-	0.24	-
CH_4%[6]	55.0^{cd}	52.6^{c}	46.9^{a}	56.4^{d}	55.3^{d}	49.7^{b}	59.1^{e}	54.5^{cd}	64.6^{f}	67.8^{g}	63.5^{f}	-	0.85	-
P_{Max}[7]	543.8^{d}	322.4^{b}	186.6^{a}	1196.2^{f}	765.1^{e}	453.7^{c}	1896.5^{h}	1493.9^{g}	792.9^{e}	3267.9^{j}	2155.3^{i}	-	22.38	-
P day[8]	9	9	9	9	9	9	14	14	14	35	35	-	-	-
Methane														
L kg^{-1} VS	**302.7**^{bc}	**316.7**^{cd}	**290.5**^{b}	**295.1**^{bc}	330.0^{de}	343.4^{e}	**240.7**^{a}	**305.2**^{bc}	**397.2**^{f}	**224.9**^{a}	349.6^{e}	358.7	8.43	8.13
λ[2]	**5.4**^{g}	**2.3**^{e}	3.2^{f}	**1.7**^{d}	**2.5**^{e}	**2.7**^{ef}	**-0.5**^{a}	**1.1**^{c}	**0.5**^{b}	-	-	3.80	0.20	0.20
k[3]	0.064^{b}	0.068^{bc}	0.072^{cd}	0.071^{c}	0.077^{de}	**0.081**^{e}	**0.058**^{a}	**0.088**^{f}	**0.100**^{g}	-	-	0.069	0.0021	0.002
U[4]	18.8^{d}	**16.3**^{c}	18.6^{d}	**14.2**^{b}	18.4^{d}	21.1^{e}	**7.6**^{a}	**18.3**^{d}	**24.9**^{f}	-	-	20.8	0.61	0.59
T_{50}[5]	13.7^{d}	12.2^{bc}	11.8^{bc}	12.9^{cd}	11.9^{bc}	11.1^{b}	21.1^{e}	**9.6**^{a}	**8.7**^{a}	-	-	12.6	0.47	0.45

[1] SEM is Standard error of mean, [2] λ is the lag phase (d), [3] k is the first order decay constant (d^{-1}), [4] U is the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}), [5] T_{50} is half-life i.e. time taken (d) to produce 50% of the gas production, [6] CH_4% is the methane proportion in biogas (vol. $vol.^{-1}$), [7] P_{Max} (hPa) is the maximum pressure measured during the mBMP test, and [8] P day is the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically (P>0.05) not different from each other. The values in bold, within a row, are statistically (P<0.05, using Dunnett's adjustment) different from AMPTS values.

Table 4. Biogas yield, methane yield and kinetic parameters when slurry was *in vitro* batch digested in incubation bottles differing in the frequency with which overhead pressure was measured and released (F) and differing in headspace volume (V).

F	Daily			Each 3 day			Weekly			After 35 days		AMPTS	SEM[1]	
V (ml)	50	90	180	50	90	180	50	90	180	90	180		ANOVA	Dunnett's
Biogas														
L kg^{-1} VS	371.6^{bc}	413.4^{de}	387.7^{cd}	372.6^{bc}	387.1^{cd}	445.1^{ef}	296.3^{a}	362.4^{bc}	462.0^{f}	309.5^{a}	348.1^{b}	-	12.66	-
λ[2]	4.2^{bc}	3.6^{a}	3.6^{a}	4.5^{c}	4.2^{bc}	3.6^{a}	4.5^{c}	4.2^{bc}	3.8^{ab}	-	-	-	0.16	-
k[3]	0.071^{bc}	0.071^{bc}	0.092^{d}	0.067^{b}	0.070^{bc}	0.074^{c}	0.051^{a}	0.070^{bc}	0.070^{bc}	-	-	-	0.0014	-
U[4]	24.7^{cde}	25.9^{de}	33.5^{g}	22.2^{bc}	23.8^{bcd}	29.2^{f}	11.4^{a}	21.8^{b}	27.5^{ef}	-	-	-	0.97	-
T_{50}[5]	11.6^{bcd}	11.4^{bc}	9.4^{a}	13.2^{e}	12.5^{cde}	11.1^{b}	21.6^{f}	12.7^{de}	12.4^{cde}	-	-	-	0.37	-
CH_4%[6]	63.9^{c}	58.1^{b}	50.8^{a}	65.0^{c}	60.3^{b}	51.4^{a}	70.5^{d}	58.4^{b}	59.5^{b}	71.0^{d}	69.4^{d}	-	0.99	-
P_{Max}[7]	547.7^{d}	328.4^{b}	184.8^{a}	1223.1^{g}	705.6^{e}	423.6^{c}	1866.9^{i}	1464.2^{h}	852.9^{f}	3195.5^{j}	1835.6^{i}	-	12.08	-
P day[8]	11	11	11	12	12	12	14	14	14	35	35	-	-	-
Methane														
L kg^{-1} VS	237.5^{de}	239.7^{de}	**196.5**^{a}	242.3^{e}	233.4^{de}	228.9^{cde}	208.7^{ab}	211.6^{abc}	**275.0**^{f}	219.6^{bcd}	241.2^{e}	233.4	7.17	6.9
λ[2]	**3.6**^{bc}	**2.8**^{a}	**3.5**^{ab}	5.6^{d}	6.3^{de}	6.4^{e}	**4.1**^{bc}	**4.2**^{bc}	**4.3**^{c}	-	-	6.40	0.24	0.24
k[3]	**0.073**^{de}	**0.079**^{f}	**0.077**^{ef}	**0.065**^{bc}	**0.063**^{b}	**0.061**^{b}	**0.053**^{a}	**0.072**^{de}	**0.069**^{cd}	-	-	0.044	0.0018	0.0018
U[4]	**19.1**^{d}	**18.9**^{d}	**20.7**^{e}	**15.7**^{c}	**15.8**^{c}	**15.7**^{c}	8.3^{a}	**13.3**^{b}	**16.5**^{c}	-	-	9.7	0.55	0.52
T_{50}[5]	**11.8**^{ab}	**11.1**^{a}	**11.4**^{a}	**13.5**^{cd}	**13.8**^{d}	**13.6**^{d}	19.7^{e}	**12.2**^{abc}	**12.8**^{bcd}	-	-	20.9	0.42	0.45

[1] SEM is Standard error of mean, [2] λ is the lag phase (d), [3] k is the first order decay constant (d^{-1}), [4] U is the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}), [5] T_{50} is half-life i.e. time taken (d) to produce 50% of the gas production, [6] CH_4% is the methane proportion in biogas (vol. vol.$^{-1}$), [7] P_{Max} (hPa) is the maximum pressure measured during the mBMP test, and [8] P day is the time (d) at which the maximum pressure was recorded during the mBMP test. Values with the same superscript, within a row, are statistically

(P>0.05) not different from each other. The values in bold, within a row, are statistically (P<0.05, using Dunnett's adjustment) different from AMPTS values.

Table 5. The level of significance (P) for biogas yield, methane yield and kinetic parameters for cellulose, barley, silage and slurry.

F^1	Cellulose			Barley			Silage			Slurry		
V^2 (ml)	F	V	FxV	F	V	FxV	F	V	FxV	F	V	FxV
Biogas												
L kg^{-1} VS	***	***	***	***	***	***	***	***	***	***	***	***
λ^3	***	***	***	***	***	***	***	***	***	*	***	NS
k^4	***	***	***	***	***	NS	***	***	*	***	***	***
U^5	***	***	**	***	**	***	***	***	**	***	***	***
$T_{50}{}^6$	***	***	***	***	***	***	***	***	***	***	***	***
$CH_4\%^7$	**	***	NS	***	***	***	***	***	***	***	***	***
$P_{Max}{}^8$ (hPa)	***	***	***	***	***	***	***	***	***	***	***	***
Methane												
L kg^{-1} VS	***	***	***	***	*	***	***	***	***	NS	NS	***
λ^3	***	***	***	**NS**	*******	**NS**	***	NS	***	***	NS	NS
k^4	***	*	***	***	***	***	***	***	***	***	***	***
U^5	**	***	***	***	***	***	NS	***	***	***	***	***
$T_{50}{}^6$	***	***	***	***	***	***	*	***	***	***	***	***

[1] F is overhead pressure measurement and release frequency, [2] V is headspace volume, [3] λ is the lag phase (d), [4] k is the first order decay constant (d^{-1}), [5] U is the maximum methane or biogas production rate (L CH_4 or biogas kg^{-1} VS d^{-1}), [6] T_{50} is half-life i.e. time taken (d) to

produce 50% of the gas production, [7] CH_4% is the methane proportion in biogas (vol. vol.$^{-1}$) and [8] P_{Max} (hPa) is the maximum pressure measured during the mBMP test.

Table 6. Methane yields using mBMP for 180 ml headspace volume bottles and OHPMR frequencies of each third day and solely after 35 d, and the corresponding yields with AMPTS.

	Methane yield (L kg^{-1} VS)		
	mBMP		AMPTS
	Each 3 day OHPMR	After 35 d OHPMR	
Cellulose	296.4	319.0	NA
Barley	350.0	350.6	349.7
Silage	343.4	349.6	358.7
Slurry	228.9	241.2	233.4

OHPMR is overhead pressure measurement and release and NA is not available.

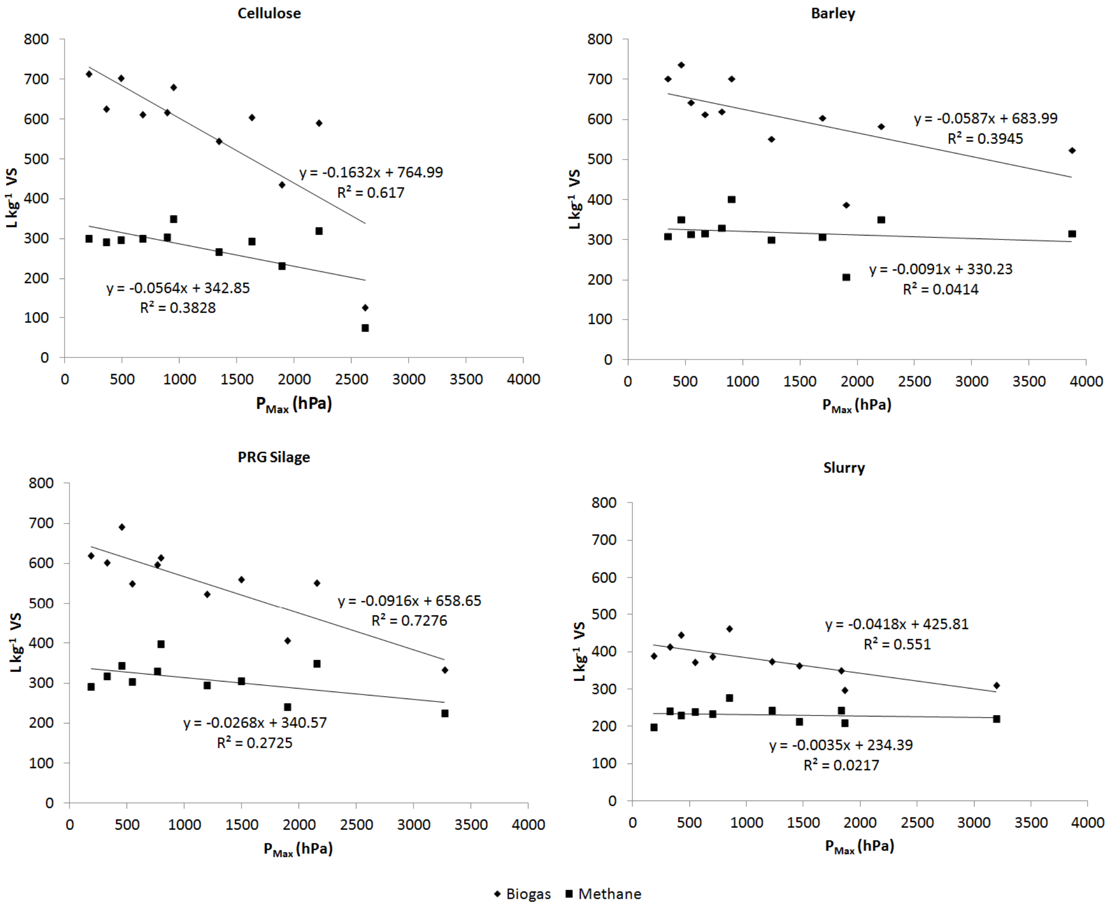


Figure 1. Relationships between maximum pressure measured for cellulose, barley, silage and slurry and biogas and methane yields during 35 day anaerobic digestion.

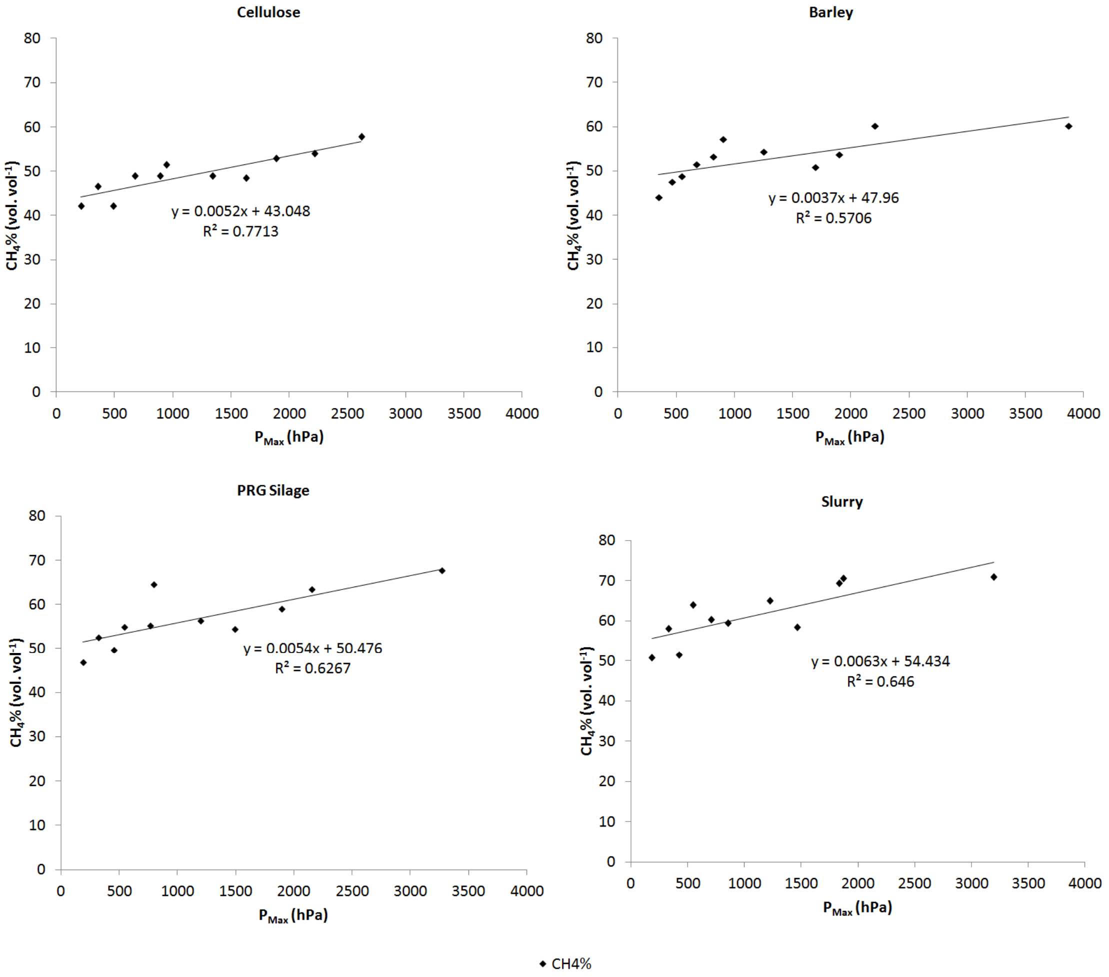


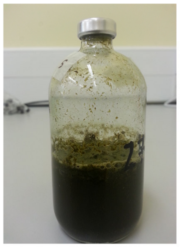
Figure 2. Relationships between maximum pressure measured for cellulose, barley, silage and slurry and methane proportion in biogas during 35 day anaerobic digestion.

Highlights

- Manometric biomethane potential (mBMP) and volumetric (AMPTS) tests were compared.
- In the mBMP, headspace volume and pressure release frequency were assessed.
- In the mBMP, greater maximum pressure reduced biogas yield but not methane yield.
- Two mBMP treatments had similar methane yields to AMPTS.

Manual manometric BMP

Headspace volume
X
Pressure release
frequency



VS

Automatic volumetric BMP (AMPTS)

Barley **Silage** **Slurry** **Cellulose**