Method for determination of methane potentials of solid organic waste

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Abstract

A laboratory procedure is described for measuring methane potentials of organic solid waste. Triplicate reactors with 10 grams of volatile solids were incubated at 55 °C with 400 ml of inoculum from a thermophilic biogas plant and the methane production was followed over a 50-day period by regular measurements of methane on a gas chromatograph. The procedure involves blanks as well as cellulose controls. Methane potentials have been measured for source-separated organic household waste and for individual waste materials. The procedure has been evaluated regarding practicality, workload, detection limit, repeatability and reproducibility as well as quality control procedures. For the source-separated organic household waste a methane potential of 495 ml CH$_4$/g VS was found. For fat and oil a lag-phase of several days was seen. The protein sample was clearly inhibited and the maximal methane potential was therefore not achieved. For paper bags, starch and glucose 63, 84 and 94% of the theoretical methane potential was achieved respectively. A detection limit of 72.5 ml CH$_4$/g VS was calculated from the results. This is acceptable, since the methane potential of the tested waste materials was in the range of 200–500 ml CH$_4$/g VS. The determination of methane potentials is a biological method subject to relatively large variation due to the use of non-standardized inoculum and waste heterogeneity. Therefore, procedures for addressing repeatability and reproducibility are suggested.

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1. Introduction

Several batch methods exist for measuring methane potentials of waste. The basic approach is to incubate a small amount of the waste with an anaerobic inoculum and measure the methane generation, usually by simultaneous measurements of gas volume and gas composition. However, the technical approaches in terms of pretreatment of the sample, inoculum, gas measurement technique and incubation vary significantly among the published methods (Adani et al., 2001; Eleazer et al., 1997; Harries et al., 2001; Heerenklage and Stegmann, 2001; Owen et al., 1979; Owens et al., 1993). Some of these differences originate from the purpose of measuring the methane potential and from the type of waste samples measured. Most of the methods are geared to either very homogenous samples, such as sewage sludge, or to samples with low methane potentials, such as waste from old landfills or biologically pretreated waste.

We have adapted and modified existing procedures, in particular inspired by Angelidaki and Ahring (1997), to an easy-to-operate method of determining methane potentials of solid waste samples generating high amounts of methane. Here, the procedure and the experiences obtained from measuring methane potentials of more than 100 waste samples during a 2-year period are described.

The method was used to characterize organic waste, separated from household waste, regarding methane potential as relevant in the context of treatment by anaerobic digestion. The main goal was to determine the methane potential in terms of STP (STP: standard
temperature and pressure) ml CH₄ per gram of organic waste expressed as volatile solids (VS). The determination should be reliably with a reasonable incubation period and with a minimum workload. The methane generation as a function of time may also be of interest for identification of inhibition or adaptation. These priorities have fostered a procedure including extensive homogenization of the solid waste sample, a large inoculum, incubation at 55 °C for 50 days, and direct measurement by a gas chromatograph (GC) of CH₄ mass produced.

2. Materials and methods

2.1. Equipment and supplies

The following equipment and supplies were used:

- Two-liter glass bottles with a thick rubber septum (chlorobuthyle rubber, Apoden Nordic, Copenhagen, Denmark) were used as reactors. The exact volume of each bottle was determined by weighing the water contained in the bottle.
- An incubator at 55 °C for the incubation.
- Inoculum from a thermophilic biogas plant.
- A 1 ml glass syringe with pressure lock (VICI, Precision Sampling Inc., Baton Rouge, LA, USA) to allow sampling of a fixed volume at actual pressure from the reactors.
- Gas chromatograph (Shimadzu GC 14A) equipped with a thermal FID detector and methane standards (0.5–100%±5% rel.) in nitrogen (Mikrolab, Aarhus, Denmark). A linear calibration curve based on 5 different methane concentrations (0.5–100%±5% rel. in nitrogen) was used for calibration. For every analytical series, one methane standard (30% in nitrogen) was measured. The actual content of methane in the samples was calculated based on this standard.
- A 1:1 mix of Avicel (Fluka, Sigma-Aldrich, Vallensbæk Strand, Denmark) and cellulose powder (Bie & Berntsen, Rødovre, Denmark) was used as standard substrate in the controls.
- Gas mixture of 80% N₂ and 20% CO₂ (alternatively, N₂-gas can be used)

2.2. Preparation of the waste

Significant heterogeneity and a wide range of particle sizes characterize solid organic waste separated from household waste. However, only 10 grams (DM) of waste was used in the measurement in order to manage the gas collection in a practical way. Thus, homogenization of the waste is very important to ensure representative sampling.

The field sampling procedure is described by Jansen et al. (2004), while the procedure applied in the laboratory on a sample of 20–30 kg of shredded organic waste, which is the typical outcome of the field sampling procedure, is described here.

In the laboratory, the waste sample was first blended in a large industrial blender (KW A/S, Humlebæk, Denmark) to reduce particle size and mix the sample. This was done without addition of water. A large sub-sample (1–2 kg) was taken to determine the dry matter content of the original sample. Thereafter, water was added to the blender (the dry matter content is typically about 25–30%) and the sample was further blended. After homogenization, a sub-sample of about 1 kg was transferred to a high-speed blender (Ultra Turrax, T45/N, Jake & Kunkel KG, IKA Werk, Staufen in Breisgau, Germany), diluted to a dry matter content of 10% and blended for about 5 minutes. The sample then had an appearance as thick gravy and a small sub-sample could easily be drawn for determination of dry matter, volatile solids and for the methane-potential measurement. The sample may be frozen and stored for later determination of the methane potential.

2.3. Inoculum

An active inoculum from a thermophilic biogas plant was needed. Inoculum from Vegger Biogasanlæg, Aalborg, was used. Vegger Biogasanlæg is a biogas plant in Denmark treating primarily manure together with some industrial organic waste and occasionally organic household waste. The plant operates at 55 °C.

The inoculum was transported in 25-liter containers by a delivery service guarantying delivery within 48 h. The temperature drops to ambient temperature during delivery, but is always kept above the freezing point. In order to readapt the inoculum to 55 °C, ensure degradation of easy degradable organic matter still present in the inoculum and remove dissolved methane, the inoculum was stored with an anaerobic headspace for three days in the 55 °C incubator.

2.4. Set-up of measurement

Fig. 1 shows the set-up of the experiment.

The test was carried out as triplicate batch experiments. Triplicates were used because the method is a biological test method using inoculum from full-scale biogas plants (varying quality) and the test material (waste) is relatively heterogeneous.

During stirring of the inoculum, 400 ml of inoculum was transferred to all reactors (the reactors were placed on a scale). For each waste sample, three reactors were picked randomly and during stirring each reactor was supplied with 100 ml of sample (10% DM, 80–90% VS). Each reactor then contained about 2 g VS/100 ml
solution, which is suitable to avoid acidification of the process. After set-up the reactors were flushed for 2 minutes with an anaerobic gas containing 80% N₂ and 20% CO₂ to ensure anaerobic conditions in the headspace of the batches. The mixed gas was used to prevent pH-change in the water-phase due to removal of CO₂ from the headspace of the reactors. However, it is possible to use pure N₂ gas for flushing.

The headspace of each batch was calculated by subtracting the added amount of inoculum and substrate (assuming the density of substrate and inoculum was 1 g/mL) from the volume of the bottle.

Thereafter, the reactors were placed in the incubator at 55 °C (±1 °C). During the experiment the batches were occasionally shaken and moved around in the incubator to compensate for any minor variation in temperature in different parts of the incubator. The incubation time was 50 days to ensure full degradation of the degradable organic matter.

For each run, three blanks with only water and inoculum were included to measure the methane production originating from the inoculum. In addition, three cellulose samples were performed to test the quality of the inoculum.

The reactors were monitored 15–20 times during the experiment. After the 50-day incubation period, pH was measured in the reactors and samples for volatile fatty acids (VFA) and nitrogen content were taken. These samples were typically frozen and only analyzed if the methane potential was low and inhibition by ammonia or VFA accumulation was suspected.

2.5. Monitoring

The methane content in the headspace of the reactors was measured regularly (15–20 times) throughout the 50 days of incubation. During the first week, daily measurements were necessary. Later it was sufficient to measure once a week.

Gas samples (0.2 ml) were taken from the headspace of the reactors through the septum with a syringe with pressure lock (see Fig. 1). The pressure lock was closed after the needle of the syringe had penetrated the septum and was inside the reactor headspace, making it possible to sample a fixed volume of gas at the actual pressure in the reactor. The syringe was redrawn and the sample was injected directly into the gas chromatograph where the mass of methane was measured. From the fixed volume sampled and the measured mass of methane in the sample, the methane content in the reactor headspace can be calculated without measuring the actual pressure in the bottle.

The amount of gas removed due to the measurements was relatively small (the volume of all gas samples was less than 0.7% of the headspace) and the results were thus not significantly affected by the induced change in actual headspace pressure.

The amount of substrate in the reactors produced approximately 6 l of biogas during the 50 days of incubation. It was therefore necessary to release gas during the experiment to avoid build-up of too high pressure in the reactor leading to leakage of gas. The pressure was always kept below 2 bars, and any significant pressure build-up was easily identified from the shape of the rubber septum. By inserting a hospital needle in the rubber stopper, the pressure was released. This was done under a hood and the amount released was calculated from measurement of the methane content in the headspace of the reactor before and after the release. During the first week the gas was released 3–4 times due to a very high gas production. Later the gas was released only occasionally. Typically 80–90% of the methane potential was produced during the first 8–10 days. However, some organic waste may be slowly degradable and the measurements should therefore be continued for a total of 50 days to ensure achievement of the maximal methane potential. If the theoretical methane potential is reached within a short time, it can be considered to shorten the length of the experiment.

2.6. Data treatment and presentation

Based on the volume of the headspace of each reactor and CH₄-content per 0.2 ml of headspace measured directly on the GC, the produced amount of methane was determined. The measurements, including the gas releases, were transferred into accumulated CH₄ as a function of incubation time.
The methane production from the inoculum (blanks) was subtracted from the methane production of the waste samples. The result thus represents only the methane production from the waste and not from the inoculum. At very low gas productions the uncertainty on the results increases, because the difference between the waste samples and the control samples might not be significant. If the methane potential is low the ratio of waste sample to inoculum should be increased.

The actual temperature \( T_m \) and atmospheric pressure \( P_m \) were recorded at every measurement of methane \( X_m \). These values were used to determine the gas content at standard temperature and pressure \( X_{STP} \) according to:

\[
X_{STP} = \frac{X_m \cdot T_{\text{Standard}} \cdot P_m}{T_m \cdot P_{\text{Standard}}}
\]

Thus, the results are given as produced methane per gram VS at standard conditions (STP: \( 0^\circ \text{C}, 1 \text{ atmosphere} \)) versus time.

3. Results and discussion

The DTU-methane-potential-measurement has been used routinely on source-separated organic household waste and occasionally on specific organic waste fractions, for example paper, fat and protein. All the results below are given at STP conditions.

3.1. Examples of results

Fig. 2 shows examples of triplicate curves measured for organic waste separated from household waste (including inoculum), for blanks containing only inoculum and water and for a control containing cellulose as a reference substrate and inoculum. The triplicates are fair regarding the final level of produced methane, although the development over time may vary between triplicates. The large amount of inoculum used gives a fast methane production, but the fact that the inoculum itself produces significant amounts of methane limits the detection limit of the procedure (see later). Fig. 3 shows, for the same data as presented in Fig. 2, the accumulated methane production for the waste after correction for the methane produced by the inoculum. This is the actual result of the measurement. The methane potential is defined as the maximum of produced methane during the 50 days of the experiment (the methane production rate can decline due to inhibition). In Fig. 3 the methane potential was on average 495 ml CH\(_4\)/g VS for the waste sample and 379 ml CH\(_4\)/g VS for the cellulose sample.

Fig. 4 shows examples of triplicate curves for food oil (rape seed oil), pork fat, protein (gelatine), paper bags for collecting organic household waste and chemically produced starch and glucose. The theoretical methane potential calculated by assuming full degradation is also shown for each substance. The degradation of the fat was apparently inhibited, possibly by accumulation of intermediates (Angelidaki and Ahring, 1992). The inhibition resulted in highly variable methane production curves for the triplicates, although the final methane potential was determined with reasonable precision. For both fat and oil a significant lag phase (two weeks) was seen. The curves for protein peaked and decreased slightly as the degradation proceeded, probably reflecting inhibition by ammonia (Angelidaki, 2002), and the methane potential determined as the maximum produced methane varied significantly among the triplicates. For paper bags, starch and glucose the deviation between the triplicates was small. The methane potentials achieved for glucose and starch were 94 and 84% of the theoretical potentials. However, only 63% of the
3.2. Workload

The introduced method seems practical without excessive work. A good working procedure is obtained by running series with 10–15 waste samples, which results in a total of 36–51 reactors, including blanks, cellulose controls and samples (all in triplicates). The time estimates are based on our current experiences:

- Preparation of bottles and inoculum: 1–2 days per series
- Preparation of samples: 1–2 hours per sample
- Set up of reactors in triplicate: 0.3–0.5 hours per sample
- Monitoring involves determination of CH$_4$ 15–20 times during the 50 days of incubation. Furthermore, the gas must be released 5–6 times for each sample during the experiment. The time used for the measurements depends on the gas chromatograph used. The average time used per sample was 1–1.5 day (15–20 measurements, 5–6 gas releases).
- Data evaluation and presentation using an existing MS Excel-sheet: 1 h per sample.

3.3. Inoculum

The inoculum contains many particles and is not a homogeneous suspension. Thus, a careful set-up procedure is essential to prevent variations between the samples that originate from variations in the inoculum. The inoculum is thus continuously stirred and distributed to all the bottles in the beginning of the set-up and three bottles are randomly chosen for each sample (triplicates).

It was necessary to discard all data from one experimental series because the cellulose controls or the blanks showed too large variation among triplicates, indicating that the distribution of inoculum to the reactors was not sufficiently homogenous.

3.4. Substrate concentration

The substrate concentration of 2 g VS/100 ml in the reactors is a compromise of, on one hand, the need to use a large sample to have good representativity and to get a high easy-to-measure gas production, and, on the other hand, to avoid too large and impractical volumes of reactors and gas production and keep the solution dilute to avoid inhibition from accumulation of VFA and ammonia. If signs of inhibition are observed,
as for example for protein in Fig. 4, new measurements with a lower substrate concentration and dilute inoculum should be made.

3.5. Statistical performance

Although the experience with the methods is still limited regarding the number of samples that have been determined, the first statistical evaluation of the performance of the DTU-method for determination of methane potentials of organic solid waste is provided. The relative limited number of samples involved in the statistical analysis leads to fairly coarse estimates of uncertainties and large confidence intervals. As more samples are determined these estimates most likely will improve.

3.5.1. Detection limit

The uncertainty of the blanks, i.e. the triplicates with water and inoculum, determines the detection limit of the methane potential measurement, since the average of the blanks is subtracted from the average of the three samples. The mean and standard deviation of triplicate blanks from 6 series of measurements was 5.79 and 0.408 ml CH₄/ml solution giving a relative standard deviation of 7.04%. The detection limit (DL) given as the limit above which one can quantify a difference between a sample and a blank sample with a probability of 95%, can be calculated from:

\[
DL = t(v) \cdot s \cdot \sqrt{\frac{1}{n_1} + \frac{1}{n_2}},
\]

where \( t(v) \) is the 95% quantile in the Student \( t \)-distribution with \( v \) degrees of freedom; \( s^2 \) is the estimated variance of the blanks; \( n_1 \) is the number of replicates used to measure the sample; \( n_2 \) is the number of replicates used to measure the blanks.

In the present case the degrees of freedom are 12 and the numbers \( n_1 \) and \( n_2 \) are 3 derived from the use of triplicates for any measurements. Using \( s = 0.408 \) ml CH₄/ml solution from above the following is obtained:

\[
DL = 2.1788 \cdot 0.408 \cdot 2 \cdot \sqrt{\frac{1}{3} + \frac{1}{3}} = 72.5 \text{ ml CH}_4/\text{g VS} (1.45 \text{ ml CH}_4/\text{ml solution } \times 500 \text{ ml solution}/10 \text{ g VS}).
\]

This detection limit is acceptable since most samples are in the range of 200–500 ml CH₄/g VS. If samples with lower methane potentials are measured the detection limit can be lowered by increasing the ratio of sample to inoculum in the set-up of the measurements.

3.5.2. Repeatability and reproducibility

Repeatability and reproducibility are determined on the basis of seven series of triplicate measurements of the methane potential of cellulose. The average and the standard deviation of the average were 377.2 and 9.3 ml CH₄/gVS respectively, and the average standard deviation within triplicates was 38.7 ml CH₄/gVS.

The repeatability \( (r) \) is defined as “the uncertainty of repeated measurements of the same sample within the same analytical series” and can be found from the recommendation in the ISO 5725, 1994 standard yielding:

\[
r = 1.96 \cdot \sqrt{\frac{2}{3}} \cdot s_r = 2.77s_r
\]

where \( s_r = \) is the standard deviation within series given above as 38.7 ml CH₄/gVS.

The repeatability is then 107 ml CH₄/gVS, which is the 95% envelope for absolute difference between two single measurements of the same sample. The corresponding repeatability for the an average of three samples, \( r_3 \) is:

\[
r_3 = 1.96 \cdot \sqrt{\frac{2}{3}} \cdot s_r = 1.60s_r = 62 \text{ ml CH}_4/\text{g VS}
\]

The reproducibility of the measurement can be found from the same seven experiments of the methane potential of cellulose as presented above. The reproducibility \( (R) \) reflects the full uncertainty, since each average is determined in separate series, i.e. different inoculum, different days of set-up and different monitoring. Using ISO 5725 the same formulas appear as above:

\[
R = 1.96 \cdot \sqrt{2} \cdot s_R = 2.77s_R
\]

where \( s_R \) is the standard deviation including variation within and between the series, found to be 41.8 ml CH₄/gVS:

\[
R_3 = 1.96 \cdot \sqrt{\frac{2}{3}} \cdot s_R = 1.60s_R
\]

The reproducibility \( R \) is then \( 116 \) ml CH₄/gVS and \( R_3 = 67 \) ml CH₄/gVS. The reproducibility states that two measurements of the same sample performed according to the described method but on different days in 95% of all cases will be 67 ml CH₄/gVS or less apart from each other.
3.5.3. Quality control
The performance of the controls (the cellulose samples) is used as a quality control in each series of measurements (same inoculum, set-up the same day, and monitored the same days). The quality control addresses both the variation among the three individual cellulose samples and the variation of the observed average from previously observed cellulose methane potentials.

If the range (largest minus smallest value) of the three individual control samples is high, it may reflect lack of a homogeneous inoculum and the measurements should be disregarded. Based on classic control chart theory as described in standard text books on statistical control (e.g. Juran, 1976), the upper control limit for the range of triplicate control samples is the average range times 2.5739. The average range of the triplicate controls in the seven series was 60.6 ml CH₄/g VS, which gives an average of the three cellulose samples is outside these control limits, the series should be disregarded.

If the average of the three control samples deviates too much from a previously determined average, it may suggest that the inoculum, although evenly distributed in the reactors, was inhibited or of other reasons produced too little methane. Assuming standard Gaussian assumptions the upper and lower limit for the average of the three control samples can be derived from standard textbooks on statistical process control (e.g. Juran, 1976). Based on an average triplicate range of 60.6 ml CH₄/g VS the limits can be calculated as the overall average ± 1.025-60.6 ml CH₄/g VS equalling 315 and 439 ml CH₄/g VS, respectively. This means that if the average of the three cellulose samples is outside these control limits, the series should be disregarded.

3.5.4. Deviating results
In addition to signs of inhibition some samples also have shown negative methane potentials. Due to an inhibitory effect from the substrate the samples produced less methane than the blanks (inoculum and water). This effect has mainly been seen in experiments with single waste fractions such as newspaper and water). This effect has mainly been seen in experiments along the lines described to ensure the reproducibility and repeatability.

The determination procedure suggests that the test is run for 50 days to obtain the maximal methane potential for slowly degrading waste. Where time is a key factor and past experience shows that the maximal methane potential is obtained fast, the procedure may be shortened. However, as the current experience is limited it is suggested that the test is always run for 50 days.

The test reactors must be monitored regularly to obtain a good representation of the methane generation as a function of time. This makes the procedure laborious, but the use of pressure tight syringes and direct measurements of methane on a GC reduce the workload. The fact that volumes at atmospheric pressures do not need to be determined is a time-saving factor.

The suggested procedure is simple and has the potential of being commonly used, both for measurement of methane potentials and for studies on enhancement and inhibition of methane potentials. It is suggested that such experiences are reported to build a broad database regarding this important waste characteristic.

4. Conclusions
A laboratory procedure is described for measuring methane potentials of organic solid waste showing high methane potentials, for example in the context of treating the waste by anaerobic digestion. The lower detection limit of the presented method based on experiences from about 100 sample is of the order of 70 ml CH₄/g VS, which suggests that modifications (primarily ratio of inoculum to sample) must be made if waste samples with low methane potentials are to be measured. The biological approach to determining methane potentials leads to substantial uncertainty in the determination, and triplicate samples must be used as a minimum. The variable nature of the inoculum and the heterogeneity of the waste make it important to use control procedures along the lines described to ensure the reproducibility and repeatability.

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