

# Measurements of $^{13}\text{C}/^{12}\text{C}$ Methane from Anaerobic Digesters: Comparison of Optical Spectrometry with Continuous-Flow Isotope Ratio Mass Spectrometry

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Received February 10, 2010. Revised manuscript received May 12, 2010. Accepted May 26, 2010.

Methane production by anaerobic digestion of biomass has recently become more attractive because of its potential for renewable energy production. Analytical tools are needed to study and optimize the ongoing processes in biogas reactors. It is considered that optical methods providing continuous measurements at high temporal resolution of carbon isotope ratios of methane ( $\delta^{13}\text{C}_{\text{CH}_4}$ ) might be of great help for this purpose. In this study we have tested near-infrared laser optical spectrometry and compared it with conventional continuous-flow isotope ratio mass spectrometry (CF-IRMS) using several methane carbon isotope standards and a large number of biogas samples from batch anaerobic reactors. Results from measurements on these samples were used to determine and compare the precision of the two techniques and to quantify for systematic offsets. With pure standards analytical precision of measurements for  $\delta^{13}\text{C}_{\text{CH}_4}$  was found to be in the range of 0.33 and 0.48‰, and 0.09 and 0.27‰ for the optical method and CF-IRMS, respectively. Biogas samples showed an average mean deviation of  $\delta^{13}\text{C}_{\text{CH}_4}$  of 0.38‰ and 0.08‰ for the optical method and CF-IRMS, respectively. Although the tested laser optical spectrometer showed a dependence of  $\delta^{13}\text{C}_{\text{CH}_4}$  on  $\text{CH}_4$  mixing ratio in the range of 500 to 8000 ppm this could be easily corrected. After correction, the  $\delta^{13}\text{C}_{\text{CH}_4}$  values usually varied within 0.7‰ from those measured by conventional CF-IRMS and thus results from both methods agreed within the given analytical uncertainties. Although the precision of the conventional CF-IRMS is higher than the tested optical system, both instruments were well within the acceptable  $\delta^{13}\text{C}_{\text{CH}_4}$  precision required for biogas methane measurements. The advantages of the optical system are its

simplicity of operation, speed of analysis, good precision, reduced costs in comparison to IRMS, and the potential for field applications.

## Introduction

The analysis of stable carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) of methane ( $\delta^{13}\text{C}_{\text{CH}_4}$ ) is a useful tool in atmospheric research (1, 2). Traditional measurement is made by continuous flow-isotope ratio mass spectrometry (CF-IRMS) for which the analytical precision ( $1\sigma$  external) is reported to be better than 0.1‰ (3–5). As an alternative, Bergamaschi et al. (6) showed that a laser-based method (long-path diode laser absorption spectrometer) could be a valid approach to precisely measure  $^{13}\text{C}/^{12}\text{C}$  ratios of atmospheric methane ( $\text{CH}_4$ ) with a reported mean deviation of  $\delta^{13}\text{C}_{\text{CH}_4}$  of 0.44‰. Subsequently, several laboratory demonstrations of carbon and hydrogen isotope ratio measurements have been reported for  $\text{CH}_4$  using different laser and spectroscopic techniques (7–11). Nevertheless, CF-IRMS and, until recently, optical spectrometry techniques were limited to laboratory-based applications. Robust portable optical instruments potentially suitable for field applications are now commercially available.

Methane production by anaerobic fermentation of biomass in a controlled reactor is becoming more and more important due to its potential for renewable energy production (12, 13). However, analytical tools are required to investigate and optimize the processes occurring in biogas reactors. Measuring the carbon isotope ratios of  $\text{CH}_4$  in biogas plants could be of great assistance to aid in better understanding of the processes involved in anaerobic biomass digestion and biogas production. Optical methods might provide a useful tool to measure online  $\delta^{13}\text{C}_{\text{CH}_4}$  values in anaerobic digesters with high time resolution.

The purpose of this work was to verify if a commercially available optical system could be used to determine the carbon isotopic signatures of  $\text{CH}_4$  within the precision range of conventional CF-IRMS, and to assess the offset between the two analytical tools.

## Materials and Methods

**Methane Reference Gases.** Four calibrated  $\text{CH}_4$  standards gases (H-iso1, T-iso1, B-iso1, and L-iso) were used for these experiments. Three provided by Isometric Instruments (Victoria, Canada) had  $\text{CH}_4$  concentrations of ca. 2500 ppmv (diluted in hydrocarbon free air) with  $\delta^{13}\text{C}_{\text{CH}_4}$  values of  $-23.9 \pm 0.2\text{‰}$  (H-iso1),  $-38.3 \pm 0.2\text{‰}$  (T-iso1), and  $-54.5 \pm 0.2\text{‰}$  (B-iso1). Furthermore, T-iso1 was also available at a concentration of ca. 25000 ppmv. High purity  $\text{CH}_4$  gas (methane 3.5, Linde, Pullach, Germany), which was calibrated against the  $\text{CO}_2$ -working reference gas, was used as the fourth  $\text{CH}_4$  isotope standard (L-iso,  $\delta^{13}\text{C}_{\text{CH}_4} = -41.29 \pm 0.07\text{‰}$ ).

**Biogas Samples Collected from Anaerobic Batch Reactors.** Gas samples from anaerobic digestion of biomass were collected from a series of 2-L batch reactors that were fed with two different organic substrates. The substrates were maize, dried whole plant material ground to pass a 10 mm sieve, and cellulose as a micro-crystalline pure chemical. The cellulose represents a material that degrades slowly (almost 100% acid detergent fiber, ADF), while the maize, which consisted mainly of 25% ADF and 44% starch, was expected to degrade faster (for further details see ref 14). Each treatment (control, maize, or cellulose) comprised four replicates with three parallels each. The batch reactors were filled with inoculum, a fresh sludge taken from a semiscale biogas plant, and  $\text{H}_2\text{O}$  plus 20 g of substrate in the case of the maize and

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cellulose treatments. The control (sequence I1–16) contained 1.2 L of inoculum and 0.3 L of H<sub>2</sub>O, while the other treatments contained substrate plus 0.6 L each of inoculum and H<sub>2</sub>O (maize: sequence M1–16; cellulose: sequence C1–16). All batch reactors were incubated in temperature-controlled chambers at 38 ± 0.5 °C. Gas samples were collected on a daily basis from each batch reactor over a period of 30 days. The volume of sample collected varied depending on the actual rate of biogas production and the CH<sub>4</sub> content. The CH<sub>4</sub> content of the biogas in the batch reactor was continuously monitored by an infrared sensor (measurement range 0–100% vol/vol, measurement error ±2%) attached to a gas analyzer (Awite Bioenergie GmbH, Langenbach, Germany). During the first 10 days 24 mL of biogas was collected with low to moderate CH<sub>4</sub> concentrations depending on the treatment. Later 5 mL of biogas was adequate because CH<sub>4</sub> concentration was mostly above 70% (v/v). The gas samples were collected with a gastight syringe and directly transferred to a 12-mL vacutainer (Exetainers, Labco Limited, GB). The volume in each vacutainer was made up to 24 mL with ambient air. Samples were stored at room temperature (20–25 °C) and measured by both methods within two months following collection.

**Sample Analysis by Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS).** Analyses of methane <sup>13</sup>C/<sup>12</sup>C by CF-IRMS were performed with an HP 6890N gas chromatograph (Agilent, Santa Clara, CA) equipped with an auto sampler A200S (CTC Analytics, Zwingen, Switzerland), coupled to a Delta<sup>PLUS</sup>XL isotope ratio mass spectrometer (ThermoQuest Finnigan, Bremen, Germany) via an oxidation reactor [ceramic tube (Al<sub>2</sub>O<sub>3</sub>), length 320 mm, 1.0 mm i.d., with Cu/Ni/Pt wires inside (activated by oxygen), reactor temperature 960 °C] and a GC Combustion III Interface (ThermoQuest Finnigan, Bremen, Germany). The gas chromatograph (GC) was fitted with a CP-PoraPLOT Q capillary column (Varian, Palo Alto, CA) (27.5 m × 0.25 mm i.d., *d*<sub>f</sub> 8 μm). The following GC conditions were employed: split injection (10:1), injector temperature 200 °C; oven temperature 30 °C isothermal. Methane-free helium was used as carrier gas and set to 1.8 mL/min constant flow. The retention times for CH<sub>4</sub> and CO<sub>2</sub> were 141 and 180 s, respectively.

All <sup>13</sup>C/<sup>12</sup>C values are expressed in the conventional δ notation in per mil versus VPDB using the following equation:

$$\delta^{13}\text{C} = \left( \frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}} \right) - 1 \quad (1)$$

A tank of high-purity carbon dioxide (carbon dioxide 4.5, Messer Griesheim, Frankfurt, Germany) with a calibrated δ<sup>13</sup>C value of −23.63‰ (VPDB) was used as a working reference gas (calibrated at MPI for Biogeochemistry in Jena, Germany).

To determine the suitability of the vacutainers employed for sample storage, CF-IRMS measurements were conducted on a set of biogas samples prior to and following their storage for an eight month period at room temperature (20–25 °C). Good reproducibility was observed between samples before and after storage and was mostly in the range of ±0.5‰. Samples were analyzed five times (*n* = 5) and the average standard deviations of the CF-IRMS measurements were in the range 0.01 to 0.27‰ for all CH<sub>4</sub> measurements.

**Sample Analysis by GYRO δCH<sub>4</sub> Optical Spectrometer.** Optical measurements of <sup>13</sup>CH<sub>4</sub>/<sup>12</sup>CH<sub>4</sub> were made using the portable, 19-in. rack-mount GYRO δCH<sub>4</sub> optical spectrometer, termed “GYRO” here (Isometric Instruments, Victoria, Canada). The GYRO utilizes specific, near-infrared absorption lines to measure the abundances of the individual <sup>12</sup>CH<sub>4</sub> and <sup>13</sup>CH<sub>4</sub> isotopologues in the gas to calculate the δ<sup>13</sup>C<sub>CH<sub>4</sub></sub>. The theory that describes the operation of the instrument has been described earlier (15). In brief, the instrument is based on Off-Axis ICOS, a cavity enhanced laser absorption

technique that employs an optical cavity as a measurement cell which contains the sample gas. The optical cavity is composed of two high-reflectivity mirrors (100 ppm optical loss, typical). In the present work, a single distributed feedback diode laser (telecommunications grade, 10 mW output power, 2 MHz line width), operating near 1.65 μm, is used as the tunable light source that is injected into the optical cavity. The laser light transmitted through the cell is collected using an aspheric lens and focused onto an amplified photodetector. The laser is wavelength tuned over a spectral region (less than 1 cm<sup>−1</sup>) that spans selected rovibrational absorption features of both <sup>12</sup>CH<sub>4</sub> and <sup>13</sup>CH<sub>4</sub> in the 2ν<sub>3</sub> overtone band of CH<sub>4</sub>. The voltage from the photodetector is digitized and subsequently analyzed by an on-board computer. The high-resolution absorption spectra, recorded in real time, are analyzed using nonlinear least-squares (chemometric) algorithms, to determine the respective absorbances due to <sup>12</sup>CH<sub>4</sub> and <sup>13</sup>CH<sub>4</sub>. The measured absorption spectra of the two isotopomers, together with measured pressure and temperature in the measurement cell, are used to quantitatively determine the mixing ratios of <sup>12</sup>CH<sub>4</sub> and <sup>13</sup>CH<sub>4</sub> and thus δ<sup>13</sup>C<sub>CH<sub>4</sub></sub>.

In contrast to IRMS, the GYRO can measure δ<sup>13</sup>C<sub>CH<sub>4</sub></sub> directly as CH<sub>4</sub>, i.e., GC partitioning, combustion of CH<sub>4</sub> to CO<sub>2</sub>, or water-removal preparatory steps are not required. The <sup>13</sup>C/<sup>12</sup>C measurements in methane are not affected by water vapor or CO<sub>2</sub>. The GYRO can operate in continuous-flow or static/injection (sample hold) modes. The nondestructive nature of the analysis permits repeated measurements of the same injected sample. With the GYRO's acquisition rate of ca. 200 Hz hundreds of discrete measurements generate an average reading value on a typical 1 Hz reporting rate. The GYRO is a self-contained unit that is capable of analyzing samples within minutes of start up and does not require a high vacuum system, special power, or supply gases. Typically the GYRO should be calibrated just prior to its use, but if a small drift in accuracy of measurement is tolerable, as will be shown here, then calibration can be less frequent.

The GYRO was housed in an air-conditioned room at a temperature of 22 ± 0.5 °C. The recommended CH<sub>4</sub> concentration measurement range of the GYRO, without the optional dilution module, is 1000 to 10000 ppm. Since biogas samples from anaerobic fermenters can contain up to 70% (v/v) CH<sub>4</sub> they must be diluted prior to measurement. Dilutions in this study were performed using Tedlar gas sampling bags, where the sample gas was placed and diluted with ambient air in a ratio of 1:200. Diluted samples usually had CH<sub>4</sub> mixing ratios between 2000 and 4000 ppm.

Gas samples were measured with the GYRO employed in the manual injection mode. Prior to measurement, the optic cell was first automatically flushed with sample gas from the sample chamber to eliminate any possible memory effects from the previous sample. The GYRO was then set to automatically evacuate the sample chamber and optic cell before 25 mL of the diluted sample gas was manually injected by syringe through the injection port into the GYRO sample chamber. The optic cell was then re-evacuated and the sample gas was allowed to enter into the optic cell. Pumping was continued and when the operating pressure of the optic cell was 20 Torr measurement commenced. The reported δ<sup>13</sup>C<sub>CH<sub>4</sub></sub> measurements are based on the integration of ca. 20 000 discrete measurements over the 120-s measurement period. The average δ<sup>13</sup>C<sub>CH<sub>4</sub></sub> standard deviation of the GYRO was in the range of 0.2–0.4‰. Each sample injection and measurement was repeated 3–6 times. Following the analysis of every fifth sample, a reference gas of known δ<sup>13</sup>C<sub>CH<sub>4</sub></sub> value was measured to monitor the stability of the GYRO.

**TABLE 1. Methane Reference Gases (ca. 2500 ppm in air) Analyzed for  $\delta^{13}\text{C}_{\text{CH}_4}$  Values on the GYRO on Three Different Days over a 6-Day Period**

sample	08/05/2009 (day 1)		08/06/2009 (day 2)		08/10/2009 (day 6)	
	$\delta^{13}\text{C}$ (‰)	mean SD	$\delta^{13}\text{C}$ (‰)	mean SD	$\delta^{13}\text{C}$ (‰)	mean SD
B-iso1	-54.2		-54.6		-55.8	
B-iso1	-54.6		-55.4		-56.3	
B-iso1	-54.7		-55.4		-56.4	
B-iso1	-54.9		-54.6		-56.0	
B-iso1	-54.3	-54.55	-55.4	-55.1	-55.3	-56.02
B-iso1	-54.6	0.26	-55.2	0.39	-56.3	0.42
T-iso1	-36.4		-38.5		-38.7	
T-iso1	-36.6		-38.2		-39.4	
T-iso1	-36.9		-38.0		-38.8	
T-iso1	-37.4		-38.2		-39.2	
T-iso1	-37.4	-37.03	-37.9	-38.2	-39.0	-38.97
T-iso1	-37.5	0.47	-38.4	0.23	-38.7	0.29
H-iso1	-23.1		-23.5		-24.9	
H-iso1	-21.7		-23.8		-24.1	
H-iso1	-22.1		-23.7		-24.1	
H-iso1	-23.6		-24.1		-24.3	
H-iso1	-22.9	-22.62	-23.5	-23.65	-23.7	-24.18
H-iso1	-22.3	0.71	-23.3	0.28	-24.0	0.40

**TABLE 2. Mean  $\delta^{13}\text{C}_{\text{CH}_4}$  Values of Methane Reference Gases Measured by CF-IRMS**

sample	$\delta^{13}\text{C}$ (‰)	SD ( $n = 10$ )
B-iso1	-54.39	0.24
T-iso1	-38.57	0.09
H-iso1	-23.93	0.27

## Results and Discussion

A broad range of  $\delta^{13}\text{C}_{\text{CH}_4}$  measurements, including reference gases and samples collected from anaerobic digesters were performed to verify the deviation between CF-IRMS and the optical method. In a first experiment, both analytical systems were compared using three  $\text{CH}_4$  isotope reference gases (H-iso1, T-iso1, and B-iso1) with  $\delta^{13}\text{C}_{\text{CH}_4}$  values of  $-23.9 \pm 0.2\text{‰}$ ,  $-38.3 \pm 0.2\text{‰}$ , and  $-54.5 \pm 0.2\text{‰}$  (same mixing ratio of  $\sim 2500$  ppm  $\text{CH}_4$ ). Measurements were made with the GYRO spanning a day of manual injections on three different occasions; day 1, day 2, and day 6. The GYRO was not recalibrated at any time during this experiment. Measurements using CF-IRMS were conducted at day 1. Tables 1 and 2 display the results for the three different  $\text{CH}_4$  standards where data presented are as the raw values, i.e., not corrected for any effects. Precisions are given as the standard deviations ( $1\sigma$ ) of the mean values.

The mean  $\delta^{13}\text{C}_{\text{CH}_4}$  precision of the GYRO measured for the three individual isotope standards over the 6-day period ranged from 0.33‰ to 0.46‰ with a maximum SD of 0.71‰ (Table 1). The precision obtained by CF-IRMS was better than that of the GYRO, with a mean  $\delta^{13}\text{C}_{\text{CH}_4}$  precision ranging from 0.09 to 0.27‰ (Table 2). The SDs observed here by the optical measurements are similar to those reported by Bergamaschi et al. (6) who when employing a long-path diode laser absorption spectrometer measured  $\delta^{13}\text{C}_{\text{CH}_4}$  values with a mean deviation of 0.44‰.

There was a drift in the actual  $\delta^{13}\text{C}_{\text{CH}_4}$  value (mean of 0.84‰) measured with the optical method over the 6-day experimental time period. It should be noted that this drift was larger than the drift usually observed for CF-IRMS measurements. However, this can be easily resolved by the daily running of a standard  $\text{CH}_4$  calibrant gas on the GYRO. This greatly reduces the calibration/reference gas requirements in comparison with the IRMS which requires reference gas measurements with each run.

The mixing ratio of  $\text{CH}_4$  in the biogas from anaerobic digesters usually ranges from 20 to 80% (v/v). Therefore, it is necessary that the analyzed stable isotope ratios are independent of the  $\text{CH}_4$  mixing ratios in the gas sample or that the dependence is quantitatively known and reproducible. To get accurate  $\delta^{13}\text{C}_{\text{CH}_4}$  values the dependence on concentration was investigated in a series of experiments using the aforementioned isotope reference gases.

The four isotope reference  $\text{CH}_4$  gases at different mixing ratios corresponding to  $\text{CH}_4$  concentrations ranging between 500 and 2500 ppm were analyzed with the GYRO. With CF-IRMS measurements, no significant change in  $\delta^{13}\text{C}_{\text{CH}_4}$  is generally observed over a very wide range of mixing ratios with peak areas ranging from 1 to 20 Vs which corresponds to  $\text{CH}_4$  concentrations of roughly 1000–30000 ppm. However, with the GYRO a dependence of the  $\delta^{13}\text{C}_{\text{CH}_4}$  value on  $\text{CH}_4$  mixing ratio was clearly found (Figure 1). A definite trend toward lower  $\delta^{13}\text{C}_{\text{CH}_4}$  values was observed with decreasing mixing ratios. This corresponded to a  $\delta^{13}\text{C}_{\text{CH}_4}$  mean shift of 3.1‰ per 1000 ppm change in the  $\text{CH}_4$  mixing ratio. Figure 1 demonstrates that this linear concentration dependence remained constant over the range of  $\delta^{13}\text{C}_{\text{CH}_4}$  values from  $-54.5$  to  $-23\text{‰}$ , i.e., the slopes for each of the four reference gases were the same. The linear regressions from each of the four reference gases (spanning a  $\delta^{13}\text{C}_{\text{CH}_4}$  range of 32‰) gave a mean slope of  $0.0031 \pm 0.00027\text{‰}/\text{ppm}$ .

Using the linear equations presented in Figure 1, the value of the  $\text{CH}_4$  mixing ratio corresponding to each reference gas  $\delta^{13}\text{C}_{\text{CH}_4}$  value was calculated. The average value of the calculated  $\text{CH}_4$  mixing ratio was  $2848 \pm 250$  ppm. Based on this value and the slope of the average linear regression, i.e.,  $0.0031 \pm 0.00027\text{‰}/\text{ppm}$   $\text{CH}_4$ , eq 2 can be used to correct  $\delta^{13}\text{C}_{\text{CH}_4}$  values of samples with  $\text{CH}_4$  mixing ratios  $< 2848$  ppm:

$$\Delta\delta^{13}\text{C}_{\text{CH}_4 \text{ correction1}}(\text{‰}) = (2848 - \text{mixing ratio}_{\text{measured}}) \times 0.0031 \quad (2)$$

The manufacturer of the standard GYRO instrument used for this study claims that it is capable of measuring  $\delta^{13}\text{C}_{\text{CH}_4}$  in gas samples with  $\text{CH}_4$  mixing ratios ranging from 1000 to 10 000 ppm (1000 to 100 000 ppm with dilution module). To verify this assertion, another series of experiments was performed using two of the  $\text{CH}_4$  reference gases ( $\delta^{13}\text{C}_{\text{CH}_4} = -38.3 \pm 0.2\text{‰}$  and  $-41.3 \pm 0.07\text{‰}$ ) where  $\delta^{13}\text{C}_{\text{CH}_4}$  values were measured across the mixing ratio range 500–8000 ppm. Results of these measurements are presented graphically in Figure 2.

Again, as expected, the same dependence of  $\delta^{13}\text{C}_{\text{CH}_4}$  with mixing ratio of  $\text{CH}_4$  between 500 and 2500 ppm was observed. However, a clear change in the slope of the trend line was noted when  $\text{CH}_4$  mixing ratios were between 2500 and 3000 ppm. For  $\text{CH}_4$  mixing ratios between 2800 and 8000 ppm a linear trend of the regression lines of both standard gases was again apparent, but now with much smaller slopes (0.00064 and 0.00071) than those observed when  $\text{CH}_4$  mixing ratios were between 500 and 2500 ppm. This change in the trendline slopes means that at the higher mixing ratios there was an average  $\delta^{13}\text{C}_{\text{CH}_4}$  shift of 0.675‰ for a  $\text{CH}_4$  mixing ratio change of 1000 ppm. As also seen in Figure 1, the regression lines of the two standard gases in Figure 2 have essentially the same slope and thus are not dependent on the  $\delta^{13}\text{C}_{\text{CH}_4}$  of the gas over the range measured. The procedure for correcting the GYRO<sub>raw</sub> data measured at higher mixing ratios is analogous to that described for mixing ratios  $< 2500$  ppm  $\text{CH}_4$  (eq 2). Using the linear equations in Figure 3, a  $\text{CH}_4$  mixing ratio of 2920 ppm (mean from both regression lines) was calculated for the  $\delta^{13}\text{C}_{\text{CH}_4}$  of the two reference gases. Based on this value and the mean slope of the two regression lines ( $0.000675\text{‰}/\text{ppm}$   $\text{CH}_4$ ) eq 3 was derived and used to

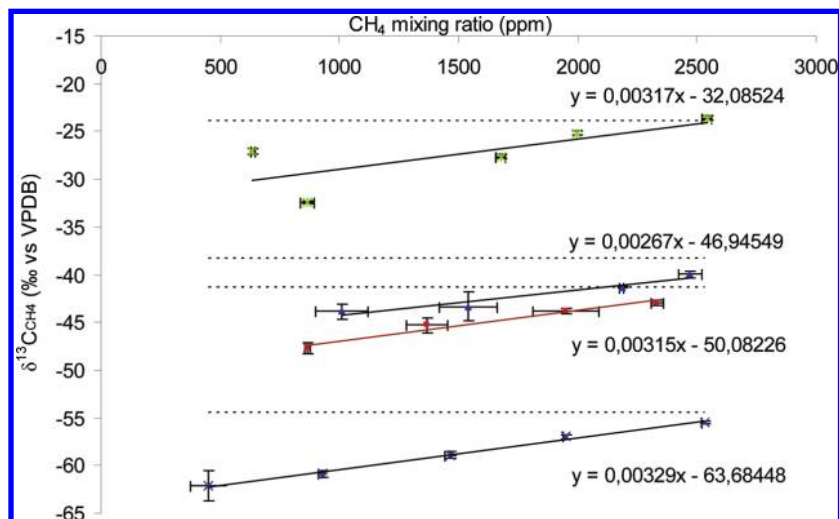


FIGURE 1.  $\delta^{13}\text{C}_{\text{CH}_4}$  values as a function of  $\text{CH}_4$  mixing ratio (500–2500 ppm) in the gas sample through the GYRO. For reference, dashed lines show the calibrated  $\delta^{13}\text{C}_{\text{CH}_4}$  values of the gas samples (–23.9, –38.3, –41.3, and –54.5‰). Data show the linear regression parameters. Error bars show  $1\sigma$  standard deviation.

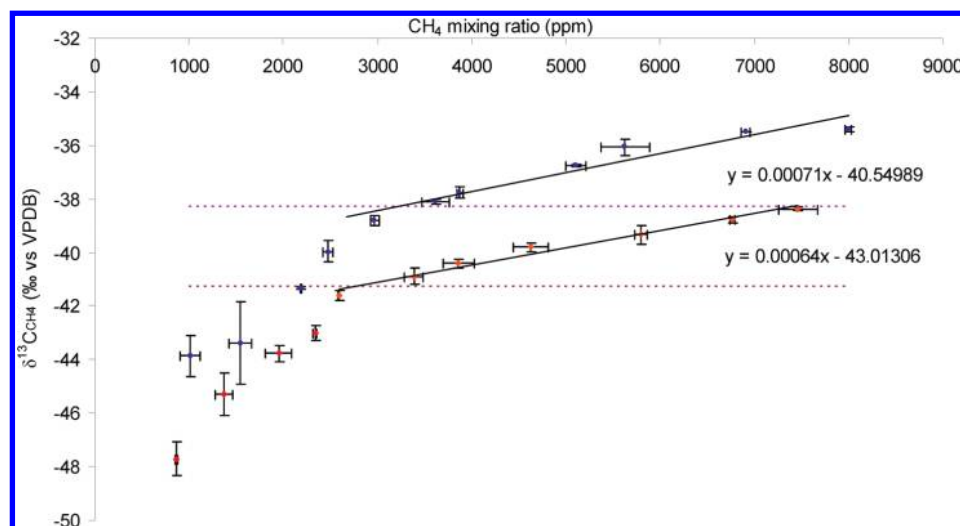


FIGURE 2. Linearity of measured  $\delta^{13}\text{C}_{\text{CH}_4}$  as a function of  $\text{CH}_4$  mixing ratio (500–8000 ppm) in the gas sample manually injected into the GYRO. For reference, dashed lines show the calibrated  $\delta^{13}\text{C}_{\text{CH}_4}$  values of the gas samples (–38.3 and –41.3‰). Data show the linear regression parameters. Error bars show  $1\sigma$  standard deviation.

correct the  $\delta^{13}\text{C}_{\text{CH}_4}$  of samples with  $\text{CH}_4$  mixing ratios ranging from 2920 to 8000 ppm:

$$\Delta\delta^{13}\text{C}_{\text{CH}_4 \text{ correction}_2}(\text{‰}) = (\text{mixing ratio}_{\text{measured}} - 2920) \times 0.000675 \quad (3)$$

Although the reasons that account for the change of slope of the trend line are as yet unknown, it is important to point out that the concentration dependence was always repeatable and thus a factor could be justifiably and easily applied to correct the raw data. When eqs 2 and 3 are applied for data correction, accuracy of  $\delta^{13}\text{C}_{\text{CH}_4}$  measurements should then be adequate so as to enable employment of the GYRO as an optical method to measure  $\delta^{13}\text{C}_{\text{CH}_4}$  in biogas samples. Methane in biogas typically ranges from –40 to –70‰ dependent on substrate and type and degree of methanogenesis. However, verification of the approach is necessary by measurement of biogas samples from anaerobic digesters.

Toward this end a further series of experiments was conducted where  $\delta^{13}\text{C}_{\text{CH}_4}$  values of samples collected from an anaerobic batch reactor (see Materials and Methods section) were measured using both CF-IRMS and GYRO (Figure 3). Before measurement all samples were diluted so

that the  $\text{CH}_4$  mixing ratios ranged from 1000 to 2200 ppm. The results for all biogas samples measured during this study showed an average mean deviation of  $\delta^{13}\text{C}_{\text{CH}_4}$  of 0.38‰ and 0.08‰ for the optical method and CF-IRMS, respectively. These standard deviations are similar to those recently reported for environmental samples (3, 5–7). However, as can be seen from Figure 3 there is a large  $\delta^{13}\text{C}_{\text{CH}_4}$  offset between the CF-IRMS data and the  $\text{GYRO}_{\text{raw}}$  data. The  $\delta^{13}\text{C}_{\text{CH}_4}$  deviations ( $\Delta_{\text{IRMS-GYRO}_{\text{raw}}}$ ) for all samples lie within the range 0.4–7.2‰, depending on the  $\text{CH}_4$  mixing ratio of the sample. Thus use of only the uncorrected  $\text{GYRO}_{\text{raw}}$  data could lead to substantial  $\delta^{13}\text{C}_{\text{CH}_4}$  errors, especially for the samples with low  $\text{CH}_4$  mixing ratios. However, when the  $\text{GYRO}_{\text{raw}}$  data are corrected using eq 2 the  $\delta^{13}\text{C}_{\text{CH}_4}$  deviations ( $\Delta_{\text{IRMS-GYRO}_{\text{corrected}}}$ ) are reduced to  $\leq 1.6\%$ . Moreover, after correction 87% of the data points for the CF-IRMS and GYRO now overlap within the given analytical uncertainties (CF-IRMS 0.3‰, GYRO 0.5‰) (Figure 3).

A further two series of gas samples were collected from batch experiments of anaerobic batch reactors and analyzed by both the optical and IRMS techniques. One sequence, M1–16, was collected from an anaerobic digester that was fed maize (C4 plant), and a second sequence, C1–16, was

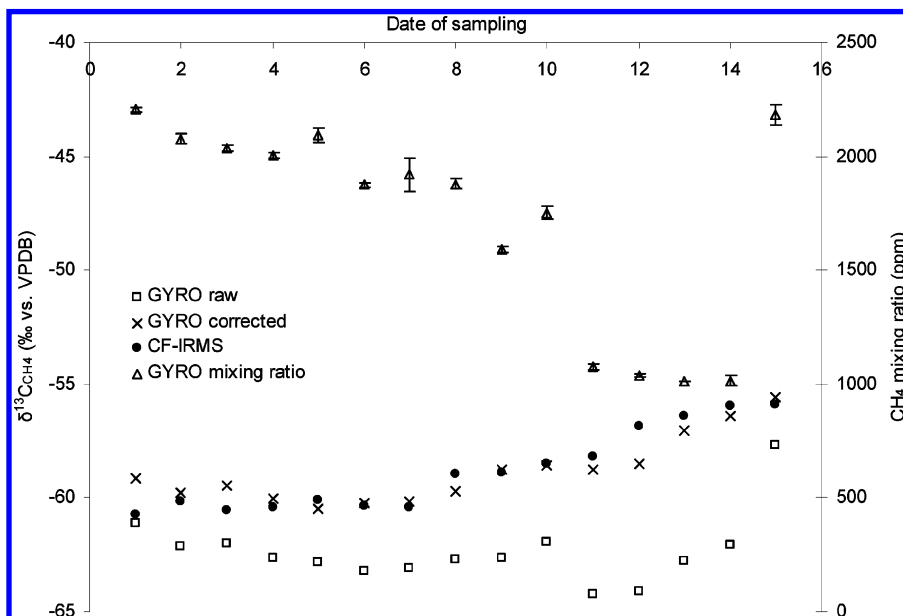


FIGURE 3.  $\delta^{13}\text{C}_{\text{CH}_4}$  values from anaerobic batch reactors (sequence I1–16) measured by CF-IRMS and the GYRO. Mixing ratios of  $\text{CH}_4$  were measured by the GYRO. Error bars show  $1\sigma$  standard deviation.

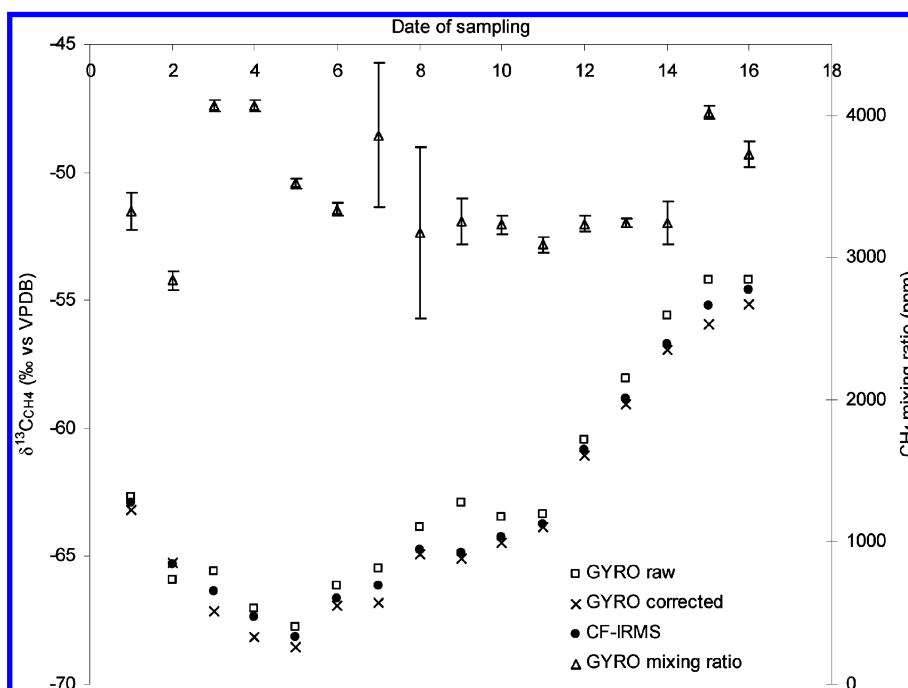


FIGURE 4.  $\delta^{13}\text{C}_{\text{CH}_4}$  values and mixing ratios of  $\text{CH}_4$  from anaerobic batch reactors fed with cellulose (sequence C1–16) measured by CF-IRMS and the GYRO. Mixing ratios of  $\text{CH}_4$  were measured by the GYRO. Error bars show  $1\sigma$  standard deviation.

collected from a digester fed with cellulose derived from C3 plants. As these samples contained between 40 and 70% (v/v)  $\text{CH}_4$ , they were first diluted using the Tedlar bag method so that the  $\text{CH}_4$  mixing ratios were in the range of 3000–5000 ppm prior to the measurement. The  $\Delta_{\text{IRMS-GYROraw}}$  from sequence M1–16 and C1–16 was calculated to range from  $-2$  to  $0.6\text{‰}$  and  $-1.9$  to  $2.2\text{‰}$ , respectively. As expected for uncorrected GYRO<sub>raw</sub> values, the  $\Delta_{\text{IRMS-GYROraw}}$  increased with higher  $\text{CH}_4$  mixing ratios in the sample. Furthermore, larger values for  $\Delta_{\text{IRMS-GYROraw}}$  were also observed when the error of the respective concentration measurement was high (e.g., data points day 7–9 and day 8–13 in Figures 4 and 5, respectively). Unfortunately, we cannot provide an explanation for the large error of concentration measurements of some of the biogas samples measured by the GYRO. However, after applying eq 3 to the data, the  $\Delta_{\text{IRMS-GYROcorrected}}$  values

ranged from  $-0.58$  to  $0.45\text{‰}$  for M1–16 and  $-0.77$  to  $0.1\text{‰}$  for C1–16 (Figures 4 and 5, respectively). Thus with correction for concentration dependence, all  $\delta^{13}\text{C}_{\text{CH}_4}$  measurements from both the M1–16 and C1–16 sequences measured by GYRO agree well, within the given analytical uncertainties (CF-IRMS  $0.3\text{‰}$ , GYRO  $0.5\text{‰}$ ), with that by CF-IRMS.

We tested the recently developed GYRO  $\delta\text{CH}_4$  optical spectrometer from Isometric Instruments, and corroborate that it is capable of making reliable routine  $\delta^{13}\text{C}_{\text{CH}_4}$  measurements in biogas samples. Although this model of the GYRO  $\delta^{13}\text{C}_{\text{CH}_4}$  was found to have a dependence on  $\text{CH}_4$  mixing ratio, this could be simply mathematically corrected. Once corrected, the  $\delta^{13}\text{C}_{\text{CH}_4}$  values were similar to those measured by conventional CF-IRMS, within stated instrument precisions. For correction it is essential to measure the  $\text{CH}_4$  mixing ratio of the sample, which can also be performed by the GYRO. While the precision

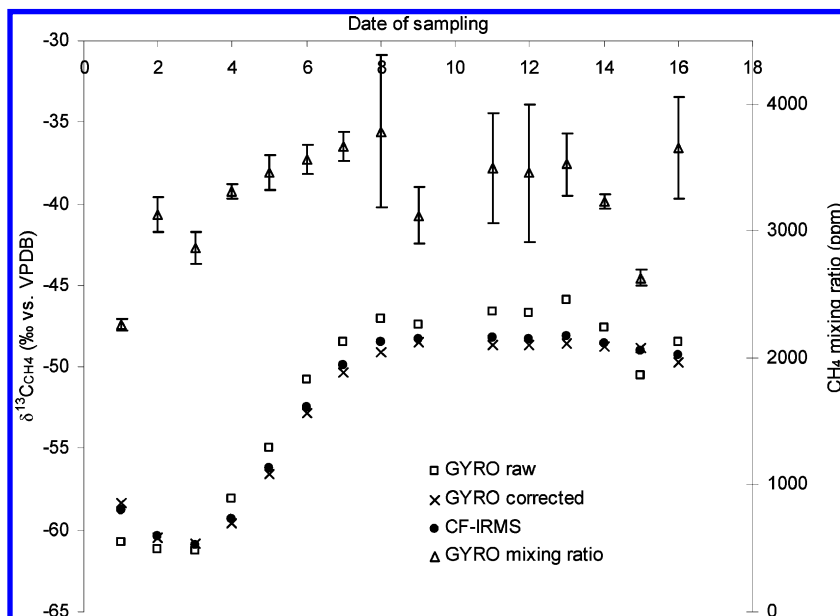


FIGURE 5.  $\delta^{13}\text{C}_{\text{CH}_4}$  values and mixing ratios of  $\text{CH}_4$  from anaerobic batch reactors fed with maize (sequence M1–16) measured by CF-IRMS and the GYRO. Mixing ratios of  $\text{CH}_4$  are shown for samples analyzed by the GYRO. Error bars show  $1\sigma$  standard deviation.

of the conventional CF-IRMS is higher than the optical system under test, results from both instruments were well within the acceptable  $\delta^{13}\text{C}_{\text{CH}_4}$  precision required for biogas  $\text{CH}_4$  measurements. The advantages of the optical system are in its simplicity of operation, speed of analysis, good precision, and reduced costs in comparison to IRMS. Thus the optical system has a large potential for field applications as it is also portable and produces data at a high time resolution. The combination of the GYRO with occasional sample analysis by CF-IRMS offers a high degree of confidence in the measurements. Monitoring of the isotopic evolution of  $\text{CH}_4$  might be a powerful tool to rapidly identify changes occurring in the processes of anaerobic digestion. Our experiments in which the temporal evolution of stable carbon isotopes were determined for  $\text{CH}_4$  in anaerobic batch digesters demonstrate how this method could be utilized for process analysis in biogas reactors. Results of the isotopic  $\text{CH}_4$  evolution shows large isotope dynamics for maize and cellulose (Figures 3–5) indicating a temporal change in degradation pathways and/or a change in the relative contribution from different carbon fractions within the substrate. Applications might range from the identification of different reaction pathways to degradation kinetics by the analysis of isotopically labeled substances. Generally, biomass degradation into energetically usable  $\text{CH}_4$  offers great potential as a renewable energy supply. However, continuous stability of the processes is not guaranteed, because effects like acidification can lead to a decrease in gas production. The lack of significant control parameters in online measurements is problematic (12). It is envisaged that monitoring of  $\delta^{13}\text{C}_{\text{CH}_4}$  might provide valuable information of the anaerobic digester to counteract any obstructive or limiting processes at an early stage and thus improve outputs. In this context, one future application could be the use of optical methods as a permanent monitoring tool in continuously operating biogas reactors. The planned next application will be the online  $\delta^{13}\text{C}_{\text{CH}_4}$  measurement by the GYRO in operational anaerobic digester systems.

### Acknowledgments

We thank three anonymous reviewers for their comments which greatly improved the quality of this manuscript. We thank the Institute for Agricultural Engineering and Animal Husbandry (LfL) in Freising/Germany for conducting the batch experiments and providing the biogas samples. We are grateful to Willi Brand and Michael Rothe from the Max Planck Institute for Bio-

geochemistry for calibration of the  $\text{CO}_2$  working reference gas for the IRMS. We acknowledge Douglas Baer for providing technical details of the optical method and Jack Hamilton for carefully reading the manuscript.

### Supporting Information Available

More technical details about the optical method. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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ES100460D