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Online monitoring of stable carbon isotopes of methane in anaerobic digestion as a new tool for early warning of process instability

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HIGHLIGHTS

- $\delta^{13}C_{CH4}$-values act as early warning for process disturbances in AD.
- Monitoring of $\delta^{13}C_{CH4}$-values identifies microbial activities within AD.
- First implementation of $\delta^{13}C$ laser spectrograph to biogas digestion.

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ABSTRACT

Effective control of anaerobic digestion in biogas plants requires the monitoring of process sensitive and rapid response parameters in order to ensure efficient biogas production and to prevent potential process failure. In this study, stable carbon isotopes of methane ($\delta^{13}C_{CH4}$) produced in a full-scale continuous stirred-tank reactor were investigated as a potential new monitoring tool for this purpose. Over a six-month period with variable organic loading rates, $\delta^{13}C_{CH4}$-values were measured online by a portable high-precision laser absorption spectrometer. During a stress period of consecutive high organic loading, $\delta^{13}C_{CH4}$-values early indicated process changes in contrast to traditionally monitored parameters where a change was observed some five to ten days later. Comparison of the stable isotope values with data from microbial analyses showed a distinct relationship between the quantity of potentially acetoclastic methanogens and $\delta^{13}C_{CH4}$-values. This finding indicates an association between dominant methanogenic pathways and carbon isotope values.

1. Introduction

Energy production by anaerobic digestion makes an important contribution to the renewable energies of Germany. With approximately 8000 large-scale anaerobic digesters, Germany leads the European nations for energy production using this technology. However, currently the construction of new plants has declined and, instead, the focus has turned to increasing the efficiency of existing biogas plants by a ‘power on demand’-concept. This concept requires a high flexibility in digester loading and thus it is essential to establish effective monitoring in order to reach optimal process control of anaerobic digestion (AD). An ideal key process indicator should not only show the actual state of AD but also indicate process changes at an early stage. In this respect, the process parameter should respond rapidly to process disturbances, for example to those induced by overloading conditions or process inhibitors.

In the field of AD a number of different online/offline parameters are currently employed as indicators of process state. Important parameters include gas composition and gas production, pH, volatile fatty acids (VFA), ratio of volatile organic acids to the total inorganic carbonate (VOA/TIC), and microbial composition. All these parameters have been widely researched and discussed for their suitability to act as process performance indicators. For example various opinions exist on the relevance of the quantity and ratio of VFAs as process indicators (Nielsen et al., 2007; Boe et al., 2010). Generally, most of the current parameters used for process control have deficiencies such as the availability of adequate sensors or online instruments, complex interpretations, time-consuming measurements, or a delayed response to process change or instability. Moreover, as many of the above mentioned parameters are measured in the liquid phase they are not independent of the actual mixing conditions. Thus, a high-resolution, fast-responding and, easy-to-interpret monitoring parameter for which

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realtime measurements can be performed inside the fermenter headspace is much sought for rigorous control of the operation of AD.

Analysis of stable carbon isotope values, usually expressed as $\delta^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{VPDB\text{-}standard}}$, is a well-established tool in environmental sciences for the reconstruction of distinct biochemical reactions (Whiticar, 1999; Conrad, 2005). In this context, the amount of fractionation mainly depends on the specific reaction pathway. During the last step of AD, methanogenesis, CH_4 is produced by different metabolic pathways depending on the methanogenic communities present. The quantity and activity of the methanogens is dependent on the environmental and operational conditions of the fermentation process (Karakashev et al., 2004; Nettmann et al., 2010; Munk et al., 2010). Major known pathways of CH_4 formation are acetoclastic methanogenesis during which CH_4 is produced by acetate cleavage (Eq. (1)) and hydrogenotrophic methanogenesis where CO_2 is reduced to CH_4 with H_2 as electron donor (Eq. (2)).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (1)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2)$$

Some studies investigating the methanogenic population of AD detected a strict hydrogenotrophic cluster containing the orders Methanomicrobiales, Methanobacteriales as well as Methanococcales and an acetoclastic branch of the order Methanosarcinales (Nettmann et al., 2010; Stantscheff et al., 2014). The latter may be subdivided into exclusively acetoclastic representatives (family Methanosaetaceae) and the metabolic versatile family Methanosarcinaceae (hydrogenotrophic, acetoclastic and methylotrophic). Unlike hydrogenotrophic methanogens, acetoclastic methanogens discriminate the isotopically heavier carbon to a lesser degree resulting in a more enriched isotopic signature of CH_4 (Conrad, 2005). Thus, CH_4 produced by acetoclastic methanogenesis has a carbon isotopic signature between −15‰ and −30‰ whereas CH_4 produced by hydrogenotrophic methanogenesis is slightly more depleted with values between −40‰ and −60‰ (Whiticar, 1999). Specific microbial species such as *Methanosaeta* sp., an obligate acetoclastic methanogen, are known to be less robust to stress conditions (De Vrieze et al., 2012). Therefore the microbial species present and their relative loads are of considerable importance in AD. Since change in predominant methanogenic pathways will be reflected by a change in the $\delta^{13}C$-values, this parameter might act as an early warning of process disturbances.

Recently, stable isotope techniques for the reconstruction of methanogenic pathways in AD were applied to biogas basic research (Qu et al., 2009; Vavilin, 2010; Lv et al., 2014). However, isotope fingerprinting techniques using $\delta^{13}C$-values of methane ($\delta^{13}C_{CH4}$) and carbon dioxide ($\delta^{13}C_{CO2}$) were mostly limited to small scale batch experiments (Laukenmann et al., 2010; Nikolausz et al., 2013; Polag et al., 2013a). First measurements at a two-stage experimental biogas plant highlighted the potential of $\delta^{13}C$-analysis as an indicator for distinct anaerobic degradation processes (Gehring et al., 2015). However, sampling was only undertaken once a week and isotope measurements were carried out by using regular GC–IRMS-techniques.

New developments of optical methods based on laser absorption spectroscopy have enabled the continuous measurement of stable carbon isotopes with significant precision. Recently, good agreement, within given analytical uncertainties, was reported in a study comparing an optical technique with mass spectrometry for measurements on CH_4 from an anaerobic digester (Keppler et al., 2010). In that study the optical technique was suggested as a suitable online tool for biogas monitoring studies. A first application of this technique at a semi-scale biogas reactor (2500 L) at the Bavarian State Research Center for Agriculture showed promising results (Polag et al., 2013b).

Here, for the first time, $\delta^{13}C_{CH4}$ measured by laser absorption spectroscopy has been employed for continuous high-resolution long-term monitoring of a full-scale anaerobic digester to study the effects from highly variable organic loading. Additionally, $\delta^{13}C_{CH4}$-values were compared to conventional monitoring parameters such as gas composition, pH, and VFA. Moreover, in order to determine the relationship between isotopic shifts of the CH_4 and the microbial processes within the digestate, bacterial and archaeal community structure were analyzed using quantitative real-time PCR (qPCR) approaches.

2. Methods

2.1. Performance of anaerobic digester

A full-scale continuous stirred-tank reactor (CSTR) located at the Deutsches Biomasseforschungszentrum (DBFZ) in Leipzig, Germany was used for this study. The reactor with a total volume of 190 m^3 and an effective fill level of approximately 155 m^3 was operated under mesophilic conditions (40 °C ± 0.5). For a one month period prior to the starting date of the monitoring period the OLR of the reactor was low with values between 2 kg VS $(m^3\,d)^{-1}$ and 3 kg VS $(m^3\,d)^{-1}$. During the experimental period between 01.07.2014 and 20.12.2014 (180 days) the reactor was fed with a mixture of maize silage (average daily load: 2500 kg, average dry matter content: 30%) and cattle manure (average daily load: 1.6 m^3, average dry matter content: 8.4%). The reactor was operated under highly variable loading conditions in order to obtain a high range of biochemical conditions including under- and overfeeding conditions. Thus, the amount of volatile solids fed to the digester was chosen in order to get organic loading rates (OLRs) between 0.4 kg VS $(m^3\,d)^{-1}$ and 13.5 kg VS $(m^3\,d)^{-1}$. Depending on the substrate input, hydraulic retention time varied between 161 and 15 days, respectively. Biogas production ranged between 11 m^3/h and 45 m^3/h. Gas composition (CH_4, CO_2, and H_2) was measured continuously by infrared and electrochemical sensors (Awite GmbH, Langenbach, Germany). Gas yields are presented in norm liters per kg of volatile solids (VS). Norm conditions are 273.15 K and 101.325 kPa.

2.2. Laboratory analysis

Digestate samples from the CSTR were removed daily for laboratory analysis. Measurements included pH, organic dry matter content (ODM), ammonium-nitrogen (NH_4-N), VFA, and total inorganic carbon (TIC). Table 1 shows the average, minimum

Table 1
Reactor digestate average, minimum and maximum values for pH, organic dry matter content (ODM), ammonium-nitrogen (NH_4-N), and total inorganic carbon (TIC).

	pH	ODM [% DM]	NH_4 (g/L)	VFA (g/L)	VOA/TIC	Acetic acid (mg/L)	Propionic acid (mg/L)
Average value	7.34	80.4	1.68	4	0.47	1781	296
Min value	6.51	75.8	1.06	1.8	0.18	53	3
Max value	7.82	84.9	3.46	9.8	1.85	6297	2555