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Stable isotope composition of biogas allows early warning of complete process failure as a result of ammonia inhibition in anaerobic digesters

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HIGHLIGHTS

- Anaerobic co-digestion of chicken manure and maize silage was investigated.
- A stable process was achieved at very high total ammonia nitrogen concentrations.
- Isotope composition of produced biogas was used as process monitoring parameter.
- The novel isotope fingerprinting approach pre-warned the complete process failure.

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ABSTRACT

Four 15-L lab-scale continuous stirred tank reactors were operated under mesophilic conditions to investigate the effect of ammonia inhibition. Stable isotope fingerprinting of biogas was applied as a process monitoring tool. Ammonia inhibition was initiated by amendment of chicken manure to maize silage fed reactors. During the accumulation of ammonia, the concentration of volatile fatty acids increased while the biogas production and pH decreased. However, in one reactor, an inhibited steady state with stable gas production even at high ammonia levels was achieved, while the other reactor proceeded to complete process failure. A depletion of the $\delta^{13}CH_4$ and $\delta^{13}CO_2$ values preceded the process inhibition. Moreover, the stable isotope composition of biogas also forecasted the complete process failure earlier than other standard parameters. The stable isotope analyses of biogas have a potential for mechanistic insights in anaerobic processes, and may be used to pre-warn process failure under stress conditions.

1. Introduction

Poultry production is increasing along with the world population and, consequently, has a global impact on the environment (Sakar et al., 2009). Of the many options for handling poultry waste, the most popular practice is land application as fertilizer (Bejan et al., 2013). However, raw chicken manure is rich in nitrogen and easily degradable carbon and direct application can burn plant leaves and cause tall, bushy plants without fruit production. In addition, careless land application may pollute surface water bodies and groundwater with oxygen demanding substances, such as ammonia and organic nutrients, as well as with pathogens and odorous compounds (Kelleher et al., 2002). Eutrophication as a result of nutrient pollution may disrupt normal functioning of aquatic ecosystems. Since poultry waste may provide organic matter for microorganisms in the biogas process, anaerobic digestion (AD) has become a promising alternative method, which minimizes pollution and yields renewable energy. Anaerobic digestion proved also to be an effective way of controlling pathogens including the most frequent *Eimeria tenella* causing coccidiosis among livestock (Lee and Shih, 1988). However, the AD of nitrogen-rich compounds results in the formation of ammonia, which above critical concentrations can inhibit the process, especially the methanogenesis and therefore decrease overall biogas production. Co-digestion with nitrogen-depleted and carbon-rich sources can be one strategy to control ammonia inhibition.

The general term ammonia used in this paper includes ionized ammonium ions (NH_4^+) and un-ionized free ammonia (FA, NH_3). This parameter is expressed in many articles based on the mass

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of nitrogen as total ammonia nitrogen (TAN) composed of free ammonia nitrogen (FAN) and ammonium nitrogen (NH_4^+–N). Free ammonia has been considered as the main factor for inhibition during digestion of nitrogen rich substrates (Chen et al., 2008). It is known to act by changing microbial intracellular pH and inhibiting essential enzymes (Wittmann et al., 1995). Therefore physico-chemical parameters, especially pH and temperature, which influence the balance of ammonium to free ammonia, were in the focus of several previous investigations (Garcia and Angenent, 2009).

Since the aceticlastic methanogens are more sensitive to process disturbances in AD, hydrogenotrophic methanogenesis is considered to be the main methanogenic pathway under ammonia inhibition (Angelidaki and Ahring, 1993). However, a recent conflicting finding indicated that aceticlastic methanogenesis, by the activity of *Methanosarcinaceae* spp., was dominant at high ammonia and acetate concentrations under mesophilic and thermophilic conditions established by single drastic increase in batch tests (Fotidis et al., 2013). Although aceticlastic methanogens are considered to play a significant role for the stability of AD, syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis was found to be the predominant pathway in many stable systems (Hao et al., 2012; Lebuhn et al., 2008).

Presently, VFA concentration is the most common process parameter used to assess AD since it reflects the balance between acid producers and consumers. VFA accumulation to a toxic level is usually accompanied by a drop in pH and followed by instability of AD. However, pH decrease does not always follow VFA accumulation due to the buffering capacity of biogas reactors (Ward et al., 2008) to which high ammonia concentrations substantially contribute. Low proportions of methane in the biogas usually indicate instable process performance, but pH changes can affect the carbon dioxide content without influencing methane production. Moreover, even with elevated VFA concentrations, a stable biogas process is possible (Wang et al., 2009).

As an alternative to standard process parameters (specific gas production, gas composition, VFA concentration, pH) stable isotope fingerprinting of the produced biogas has been suggested as a potential monitoring technology, especially for changes of methanogenic processes in biogas reactors (Nikolausz et al., 2013). Measurement of the isotopic composition based on optical spectrometry allows online monitoring of the carbon isotope composition of the produced methane even in large scale biogas reactors (Keppler et al., 2010). The determination of stable isotope characteristics of the produced biogas allows a rough identification of the predominant methanogenic pathway in anaerobic digesters. This is because methanogenesis is associated with isotope fractionation due to the enzymatic preference of molecules containing lighter isotopes (Whiticar, 1999). As methane formation from H_2 and CO_2 results in larger isotope fractionation than aceticlastic methanogenesis, the isotope signature of methane can be used to distinguish methanogenic pathways (Conrad, 2005).

Changes in the stable isotope composition of methane expressed as delta ^{13}C–CH_4 has even be used to follow short-term activity changes of methanogens (Lv et al., 2014). The apparent fractionation factor ($\alpha C = (\delta^{13}CO_2 + 10^3)/(\delta^{13}CH_4 + 10^3)$) is another possible indicator to roughly identify the dominant methanogenic pathway. According to literature suggestions $\alpha C > 1.065$ indicates that hydrogenotrophic methanogenesis is the predominant pathway, while $\alpha C < 1.025$ indicates aceticlastic methanogenesis (Conrad, 2005; Galand et al., 2010).

The effect of ammonia on the biogas process was reviewed in detail in recent articles (Chen et al., 2008; Yenigün and Demirel, 2013). It is clear from these reviews that most of the studies used batch cultures (Fotidis et al., 2013) or sparsely controlled pilot-scale plants (Karthikeyan and Visvanathan, 2012) and in many cases ammonia was introduced as ammonium salts (mainly NH_4Cl) (Rajagopal et al., 2013). Moreover, a detailed analysis of a continuous co-digestion process to assess the long-term effect of increasing TAN concentration has not yet been performed with stable isotope tools.

The aim of this study was to follow ammonia inhibition by co-digesting dry chicken waste with maize silage in laboratory-scale semi-continuous anaerobic biogas reactors until complete process failure. The applicability of stable isotope fingerprinting as an early warning tool was also assessed.

Table 1
Time schedule and amount of daily maize silage and chicken waste addition.

Feeding regimes	Reactor No.	Maize silage					Chicken waste					Total OLR[c] (g_{VS}/L Day)
		TS[a] (%)	VS[b] (%$_{TS}$)	Input (g/Day)	OLR (g_{VS}/L Day)	m NH_4^+–N (g/L Day)	TS[a] (%)	VS[b] (%$_{TS}$)	Input (g/Day)	OLR (g_{VS}/L Day)	m NH_4^+–N (g/L Day)	
1–26 days	R4.22	35.30	96.10	117.91	4.00	0.26	–	–	–	–	–	4.00
	R4.23	35.30	96.10	117.91	4.00	0.26	–	–	–	–	–	4.00
	R4.24	35.30	96.10	117.91	4.00	0.26	–	–	–	–	–	4.00
	R4.25	35.30	96.10	117.91	4.00	0.26	–	–	–	–	–	4.00
27–54 days	R4.22	35.30	96.10	98.23	3.33	0.22	42.60	61.30	15.00	0.39	0.28	3.72
	R4.23	35.30	96.10	98.23	3.33	0.22	42.60	61.30	15.00	0.39	0.28	3.72
	R4.24	35.30	96.10	117.91	4.00	0.26	–	–	–	–	–	4.00
	R4.25	35.30	96.10	117.91	4.00	0.26	–	–	–	–	–	4.00
55–83 days	R4.22	35.83	95.65	85.87	2.94	0.19	42.60	61.30	30.00	0.78	0.56	3.72
	R4.23	35.83	95.65	85.87	2.94	0.19	42.60	61.30	30.00	0.78	0.56	3.72
	R4.24	35.83	95.65	116.72	4.00	0.26	–	–	–	–	–	4.00
	R4.25	35.83	95.65	116.72	4.00	0.26	–	–	–	–	–	4.00
84–105 days	R4.22	30.24	95.42	89.14	2.57	0.20	42.60	61.30	45.00	1.18	0.84	3.75
	R4.23	30.24	95.42	89.14	2.57	0.20	42.60	61.30	45.00	1.18	0.84	3.75
	R4.24	30.24	95.42	138.62	4.00	0.31	–	–	–	–	–	4.00
	R4.25	30.24	95.42	138.62	4.00	0.31	–	–	–	–	–	4.00
106–119 days	R4.22	35.20	97.35	67.29	2.31	0.15	42.60	61.30	60.00	1.57	1.12	3.88
	R4.23	35.20	97.35	67.29	2.31	0.15	42.60	61.30	60.00	1.57	1.12	3.88
	R4.24	35.20	97.35	116.73	4.00	0.26	–	–	–	–	–	4.00
	R4.25	35.20	97.35	116.73	4.00	0.26	–	–	–	–	–	4.00

[a] TS: Total Solids.
[b] VS: Volatile Solids.
[c] Total OLR: the sum of OLR of maize silage and chicken manure.