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Determination of the fractions of syntrophically oxidized acetate in a mesophilic methanogenic reactor through an ^{12}C and ^{13}C isotope-based kinetic model

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ABSTRACT

In order to accurately describe the carbon flow in anaerobic digestion processes, this work investigates the acetate degradation pathways through the use of stable carbon isotope analysis and a mathematical model. Batch assays using labeled ^{13}C acetate were employed to distinguish the acetate consumption through methanogenic Archaea and acetate-oxidizing Bacteria. Suspended and sessile biomass, with over 400 days of retention time, from a mesophilic (36.5 °C) upflow anaerobic filter was used as inocula in these assays. A three-process model for acetoclastic methanogenesis and syntrophic acetate oxidation (SAO) was developed to allow for a precise quantification of the SAO contribution. The model distinguishes carbon atoms in light and heavy isotopes, ^{12}C and ^{13}C, respectively, which permitted the simulation of the isotope ratios variation in addition to gas production, gas composition and acetate concentrations. The model indicated oxidized fractions of acetate between 7 and 18%. Due to the low free ammonia inhibition potential for the acetoclastic methanogens in these assays these findings point to the biomass retention times as a driven factor for the SAO pathway. The isotope-based kinetic model developed here also describes the $\delta^{13}C$ variations in unlabeled assays accurately and has the potential to determine biological ^{13}C fractionation factors.

1. Introduction

The further development of anaerobic technologies requires a comprehensive understanding of the complex biological processes involved in the conversion of organic matter to biogas. Acetate is the main intermediate of this complex chain of processes and identified as the precursor of at least two thirds of the methane production (Ferry, 2010). The acetate conversion to methane occurs through two known pathways: either through the acetoclastic methanogenesis or through a syntrophic process combining the anaerobic acetate oxidation with hydrogenotrophic methanogenesis. Due to the high Gibbs free energy associated to the acetate oxidation step the contribution of the syntrophic pathway is commonly disregarded in literature for mesophilic conditions. Under thermophilic conditions the energetic constraints are more favorable for an anaerobic oxidation of acetate (Schink, 1997). Indeed, the importance of the SAO pathway has been recently confirmed predominantly for thermophilic conditions (Hao et al., 2012, 2011; Ho et al., 2014; Liu and Conrad, 2010; Lü et al., 2013; Rui et al., 2011). Furthermore, the mechanisms of syntrophic acetate oxidation (SAO) are still not fully understood (Angelidaki et al., 2011) and neither are all the trophic groups involved yet known (Hattori, 2008).

In comparison to acidogenic and methanogenic processes, information about kinetics of acetate oxidation is scarce. Despite the discussions regarding its inclusion into the Anaerobic Digestion Model No. 1 (ADM1; see Batstone et al., 2002), the acetate oxidation is neglected in most of the anaerobic digestion models with only a few exceptions (see Shimada et al., 2011; Vavilin, 2012a, 2012b; Wett et al., 2014). Yet, it is clear that the overall methane production rates from acetate through the syntrophic oxidation pathway are significantly lower than from acetoclastic methanogenesis

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(Karakashev et al., 2006; Schnürer and Nordberg, 2008). Thus, reduced hydraulic retention times (HRT) diminishes the syntrophic acetate oxidation rates (Shigematsu et al., 2004; Westerholm et al., 2012), while high solid retention times (SRT) are likely to favor the slow growing acetate oxidizers (Westerholm et al., 2010).

In spite of their slow growth rates, acetate oxidizers tolerate much higher free ammonia concentrations (NH_3) than acetoclastic methanogens (Schnürer and Nordberg, 2008; Westerholm et al., 2012). This higher NH_3 tolerance potentially explains the dominance of hydrogenotrophic methanogens in agricultural and industrial biogas plants, and consequently the presence of acetate oxidizers, over acetoclastic methanogens (Karakashev et al., 2005; Nettmann et al., 2010). Indeed, Polag et al. (2013) found that syntrophic acetate oxidation can even completely dominate the acetate degradation in the mesophilic fermentation of maize silage. These evidences indicate that syntrophic acetate oxidation is largely underestimated with respect to the design and operation of biomethanisation technologies. Consequently, analytical methods to determinate the methanogenic pathways and the amounts of SAO are an essential element towards a further understanding of the CH_4 formation in anaerobic plants. Methods based on labeled isotopes or the natural abundance of carbon isotopes analysis are able to quantify the different methane precursors, mainly acetate and carbon dioxide (Conrad, 2005), or identify specific acetate degraders through stable isotope probing (Liu and Conrad, 2010; Rui et al., 2011). Otherwise, the acetate oxidation occurrence can only be indirectly determined by microbiological analysis (e.g.: absence of acetoclastic methanogens; Nettmann et al., 2010) or by the specific inhibition of acetoclastic methanogens (e.g: Penning and Conrad, 2006).

The use of methyl or carboxyl labeled acetate isotopes is based on the fact that in the acetoclastic methanogenesis (*ma*) methane originates only from the methyl carbon in acetate (Zehnder et al., 1980). Differently, after the acetate oxidation (*ca*) both carbon atoms, methyl and carboxyl, are available for the hydrogenotrophic methanogenesis (*mc*) in the form of carbon dioxide:

$$2\ \underline{C}H_3COOH \xrightarrow{ma} 2\ \underline{C}H_4 + 2\ CO_2 \quad (1)$$

$$2\ \underline{C}H_3COOH + 4\ H_2O \xrightarrow{ca} 2\ \underline{C}O_2 + 2\ CO_2 + 8\ H_2 \xrightarrow{mc} \underline{C}H_4 + CH_4 + \underline{C}O_2 + CO_2 + 4\ H_2O \quad (2)$$

Equations (1) and (2) have the same net reaction, with two moles of labeled [2-$^{13}\underline{C}$]acetate or [2-$^{14}\underline{C}$]acetate (identified by the underlined carbon atom) being converted into two moles of CH_4 and two moles of CO_2. Only labeled methane is present in acetoclastic methanogenesis (Equation (1)), while the SAO (Equation (2)) yields a uniform distribution of the labeled carbons in methane and carbon dioxide. The addition of the radioactive carbon isotope, ^{14}C, allows for a straightforward data interpretation, since there is no presence of ^{14}C other than the added tracer, i. e. no mixing with other sources occurs. By contrast, by adding labeled substrates with heavy stable carbon, ^{13}C, the stoichiometry of Equation (2) becomes more complex. The CO_2 originating from the labeled acetate is immediately mixed into the inorganic carbon pool from the reactor (about 1.1% of the naturally occurring carbon is ^{13}C; Farquhar et al., 1989) , making its direct distinction from this background carbon source virtually impossible (Hori et al., 2011). Thus, a more elaborate experimental design and/or a more detailed description of the phenomena involved is required in order to provide a precise pathway distinction in experiments with labeled [^{13}C]acetate.

In this paper, we investigate the occurrence of acetate oxidation in an anaerobic mesophilic reactor (first described in Gehring et al., 2015) with moderate free ammonia concentrations. This results in a low inhibition for the acetoclastic methanogens. Hence, this investigation focuses on the contribution of the biomass' long retention times to the SAO pathway. We designed an upflow anaerobic filter (UAF), which allowed us to obtain representative samples of sessile and suspended biomass separately. Through batch assays with labeled [^{13}C]acetate the fraction of acetate oxidation was determined for each biomass fraction. Additionally, different acetate concentrations were used in the batch tests to determine a possible pathway shift under stress conditions caused by acidification of the system as observed by thermophilic conditions (Hao et al., 2012, 2011; Lü et al., 2013). To determine the amounts of oxidized acetate, we developed a model including the acetoclastic methanogenesis and the syntrophic acetate oxidation processes. The model distinguishes between light and heavy carbon isotopes (^{12}C, ^{13}C). This permitted the simulation of the isotope ratios variation in addition to gas production, gas composition and acetate concentrations. Kinetic and equilibrium isotope effects (KIE and EIE) involving the carbon atoms were also included.

2. Materials and methods

2.1. Upflow anaerobic filter – UAF

The UAF reactor consisted of a double jacket cylindrical glass reactor with a diameter of 200 mm and a volume of 11.0 L with a headspace of 1.5 L. 50 polyvinyl chloride (PVC) stripes (395 × 18 × 3 mm) were used as biofilm carriers (BC), comprising 0.83 m^2 of total area for biofilm growth in the reactor. Each biofilm carrier was fixed by a single screw and could be separately removed from the reactor without disturbing the remaining biomass in the system. A sample point at the bottom of the UAF was foreseen for collection of the suspended biomass. A constant recirculation rate of 70 L d^{-1} was maintained between the UAF and a storage tank - a glass reactor without temperature control and average fill volume of 7.0 L. The UAF system was operated in a closed cycle with three thermophilic leach bed reactors (LBRs) fed exclusively with maize silage. The resulting organic loading rates (OLR) to the UAF varied from 1.2 to 17.0 g_{COD} L^{-1} d^{-1}. Ammonia nitrogen concentrations (NH_4) were relatively constant with values below 0.6 g_N L^{-1} and the pH values were also mostly constant on average 7.3 ± 0.2 standard deviation (SD). In Fig. 1a and b the UAF system and the BCs disposition are depicted. Detailed descriptions of the two-stage system as well as operational results are provided in Gehring et al. (2015).

2.2. Batch assays

The batch assays were conducted in glass bottles with 300 ± 1 mL total volume (nominal volume of 250 mL; Schott Duran® Pressure Plus, Germany) with online manometric determination of the gas production. A pressure transmitter (ATM.ECO; STS, Switzerland), was fixed in a stainless steel connector, which had a manual valve for gas sampling. Start acetate concentrations ranged from 1.3 g L^{-1} to 3.2 g L^{-1}. Acetate concentrations around 1 g L^{-1} are recommended for acetoclastic activity tests (Batstone and Jensen, 2011), and the higher dosage should reproduce the overload conditions in the parent reactor. The liquid fill volumes from the assays, which varied from 100 to 160 mL, were adjusted to obtain the maximal gas production for sampling without exceeding 1.5 bar of overpressure during the assay conduction. This limit avoids high CO_2 partial pressures within the system, which affect the pH values. No significant effects on the isotopic equilibrium for the CO_2 and CH_4 gases exists for this pressure range (Harting, 1978; Szaran, 1997). A 10 g L^{-1} glacial acetate solution (99–100% pure,