Journal of Microbiological Methods 91 (2012) 89–92

Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Methanogenic activity tests by Infrared Tunable Diode Laser Absorption Spectroscopy

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ARTICLE INFO

Article history:
Received 9 June 2012
Received in revised form 18 July 2012
Accepted 18 July 2012
Available online 3 August 2012

Keywords:
Anaerobic degradation
Measurement
Methane concentration
Methanogenesis

ABSTRACT

Methanogenic activity (MA) tests are commonly carried out to estimate the capability of anaerobic biomass to treat effluents, to evaluate anaerobic activity in bioreactors or natural ecosystems, or to quantify inhibitory effects on methanogenic activity. These activity tests are usually based on the measurement of the volume of biogas produced by volumetric, pressure increase or gas chromatography (GC) methods. In this study, we present an alternative method for non-invasive measurement of methane produced during activity tests in closed vials, based on Infrared Tunable Diode Laser Absorption Spectroscopy (MA-TDLAS). This new method was tested during model acetoclastic and hydrogenotrophic methanogenic activity tests and was compared to a more traditional method based on gas chromatography. From the results obtained, the CH_4 detection limit of the method was estimated to 60 ppm and the minimum measurable methane production rate was estimated to $1.09{\cdot}10^{-3}$ mg l^{-1} h^{-1}, which is below CH_4 production rate usually reported in both anaerobic reactors and natural ecosystems. Additionally to sensitivity, the method has several potential interests compared to more traditional methods among which short measurements time allowing the measurement of a large number of MA test vials, non-invasive measurements avoiding leakage or external interferences and similar cost to GC based methods. It is concluded that MA-TDLAS is a promising method that could be of interest not only in the field of anaerobic digestion but also, in the field of environmental ecology where CH_4 production rates are usually very low.

1. Introduction

Anaerobic degradation of organic matter proceeds via a number of microbial processes, including hydrolysis, acidogenesis and acetogenesis; which produce hydrogen, CO_2, formate, acetate and ammonium (Muyzer and Stams, 2008). As the last step of anaerobic digestion, methanogens produce CH_4 from H_2 and CO_2 (hydrogenotrophic pathway), acetate (acetoclastic pathway) or methylated compounds (methylotrophic pathway) (Liu and Whitman, 2008). In sewage sludge digesters, about 70% of CH_4 is produced via acetoclastic pathway and the other 30% is produced by the hydrogenotrophic pathway (Kruger et al., 2005; Pavlostathis and Giraldogomez, 1991). With soluble substrates, methanogenesis is generally considered as the rate limiting step and methanogens are also regarded as the microbial community most sensitive to the environmental or operational conditions. Thus, CH_4 production rate is an important parameter which informs about the all anaerobic process and is often used (i) to quantify or characterize anaerobic digestion processes such as wastewater treatment or soil remediation or (ii) to evaluate anaerobic processes in natural environments such as soils, peatlands or aquatic ecosystems.

CH_4 production rate is usually measured during methanogenic activity (MA) tests, using several procedures that have been exhaustively listed by Souto et al. (2010) and earlier by Soto et al. (1993). Despite a large diversity, MA tests are usually based on one of the three following methods; (i) the recovery and measurement of the volume of biogas produced, combined with the determination of CH_4 content of the biogas, (ii) the measurement of CH_4 produced in closed vials by gas chromatography (GC) or (iii) the measurement of pressure increase in closed vials, combined with the determination of CH_4 content of the biogas.

These methods are largely used on a daily basis in countless applications and give confident results but they also have some drawbacks. Methods based on recovery and measurement of the volume of biogas produced require a measurement device for each MA test and are limited to the measurement of relatively high methanogenic activities; i.e. producing a measurable volume of biogas. On the contrary, methods based on gas chromatography are very sensitive but are time demanding which limit the number of test vials that can be processed together. Methods based on pressure can be applied to very large number of samples but require the quantification of CH_4 content of the biogas produced and are limited to relatively high methanogenic activities, although high sensitivity pressure sensors have been developed. Pressure based methods are also of limited interest for hydrogenotrophic MA tests.

A relatively new technology with the potential for improving determinations of dissolved gas measurement is Infrared (IR) Tunable Diode Laser Absorption Spectroscopy (TDLAS). TDLA spectrometers are based on the emission and reflection, back to a detector, of a laser beam. Along

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http://dx.doi.org/10.1016/j.mimet.2012.07.022

the laser beam path, the presence of the target gas modifies the laser spectrum through the absorption of light in a specific wavelength range, which is detected by the instrument upon return of the laser beam. This modification of the laser spectrum can be used to quantify the concentration of the target gas with a sensitivity as low as 1 ppm meter (ppmm), defined as the minimum concentration detectable multiplied by the path length. A modification to the commercially available open path technique allows the measurement of the gas concentration within the headspace of a closed cell, providing a convenient technique for non-invasive determinations of methane concentration. A method, based on the same principle has been previously described for the determination of dissolved methane concentration in aquatic ecosystems (Sepulveda-Jauregui et al., in press).

In this paper, we developed and tested a method based on TDLAS, called MA-TDLAS, for the determination of methane production rate during MA tests. We put emphasis on quantifying the precision and accuracy of the method by comparing the results obtained by MA-TDLAS to classical gas chromatography method.

2. Methods

2.1. MA-TDLAS prototype

We used a commercial infrared TDLAS (GasFinder 2.0, Boreal Laser Inc., Edmonton, Canada) to detect and quantify gaseous CH_4 produced during MA tests. The GasFinder 2.0 is a portable instrument with 1 ppmm sensitivity and a measurement frequency of 1 s^{-1}. We modified the GasFinder 2.0 to support a frame for a closed glass vial, used as MA test vial and a laser reflector, perfectly aligned with the laser beam source (Fig. 1). This design allowed the laser beam to cross the superior section (headspace) of the MA test vial before being reflected back to the detector, crossing again through the MA test vial on the return path. Standard MA test vials of 100 ± 1.0 mL volume were custom made from Schott Duran 3.3 borosilicate glass having a 38 mm external diameter, 34 mm internal diameter, 104 mm length and 1.473 refraction index. Additionally, each MA test vial was fabricated with a serum vial type bottle neck at the bottom to allow sealing with a 20 mm inner diameter rubber stopper and aluminum crimp cap. GasFinder 2.0 was not initially designed for measurement of gas concentration within a glass enclosure or with such a short path length; therefore all measurements were made with reference to a calibration curve.

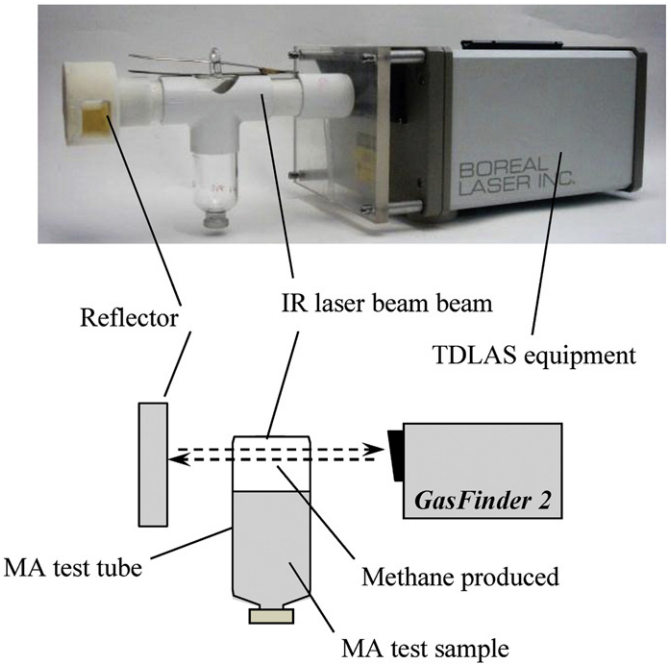

Fig. 1. Schematic of MA-TDLAS measurement method and prototype.

2.2. Methanogenic activity tests

MA-TDLAS method was assessed during MA tests done with anaerobic sludge obtained from a full scale upflow anaerobic sludge blanket (UASB) plant treating urban wastewater (Metropolitan Autonomous University, Mexico). Experiments were conducted in MA test vials containing 60 ml of sludge and 40 ml headspace. In order to test MA-TDLAS method over a wide range of methanogenic activity, three sludge concentrations (X) were used; namely 1.021, 0.102 and 0.010 $g_{VSS}\ l^{-1}$ obtained by diluting the original sludge sample with mineral medium (Park et al., 2010). It should be noted that due to granular nature of the original sludge, dilutions as well as concentrations were approximate. Medium preparation and inoculation were done under strict anaerobic conditions by continuous flushing with He (99.998%, Infra, Mexico) upon vials closure.

Two distinct MA tests were performed; (1) Hydrogenotrophic MA tests, where headspace of test vials was replaced by H_2/CO_2 (80/20, Infra, México) as carbon and energy source (Sorensen and Ahring, 1993) and (2) Acetoclastic MA tests by addition of 3.0 g l^{-1} of acetate as carbon and energy source (Park et al., 2010) and with He (99.998%, Infra, México) as headspace. Each experimental condition (3 sludge concentrations, each with 2 different substrates) was tested in triplicate, each MA test vial being treated independently from the others (total of 18 MA test vials). All MA test vials were incubated at 37 °C without shaking. CH_4 produced by methanogenesis was measured each hour by MA-TDLAS, according to the following procedure; (1) control MA test vials containing standard CH_4 concentrations were read for calibration, (2) each MA test vial containing sample was vigorously shaken for 10 s to allow for phase equilibrium and then immediately placed in the laser beam path, (3) a stable MA-TDLAS reading was typically observed within 5 seconds, (4) five readings for each MA test vial were done and (5) a new calibration was performed after measurement of all test vials, to ensure reading stability.

In order to compare results obtained by MA-TDLAS with a more conventional method, CH_4 concentration in MA test vials were also measured in triplicate by gas chromatography (GC) using a Clarus-500 (Perkin Elmer, Mexico) chromatograph equipped with a FID detector and an Elite-QPlot column (Perkin Elmer, Mexico). Significant difference between results was determined using the Tukey–Kramer's multiple comparison (TK) tests performed after analyses of variance ($\alpha < 0.05$) using the NCSS 2000 Statistical Analysis System software (Number Cruncher Statistical Systems, USA).

3. Results and discussion

We first tested the MA-TDLAS method against GC, by measuring seven MA test vials containing 60 ml of distilled water and different CH_4 concentrations, ranging from 2500 to 25000 ppm. CH_4 concentration in the headspace of each test vial was measured, first in triplicate by GC and then, in quintuplicate by TDLAS. Fig. 2 shows the results observed. A linear correlation was observed between both techniques ($R^2 > 0.99$), with a slope of 1.00. These results confirm that CH_4 concentration in the headspace of MA test vials can be measured by TDLAS.

MA-TDLAS method was then tested during actual MA tests, under both acetoclastic and hydrogenotrophic conditions, with three sludge concentrations. Fig. 3 shows the average CH_4 concentration increased observed for each sludge concentration, during acetoclastic (Fig. 3A) and hydrogenotrophic (Fig. 3B) tests. As observed, TDLAS methods allowed the detection of a clear CH_4 production during 10 hours of experimental time.

Table 1 shows methanogenic activities, measured by TDLAS and by GC during acetoclastic and hydrogenotrophic MA tests. Except for the higher sludge concentration measured, no significant difference was