Automated equipment for anaerobic sludge parameters determination

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Abstract Methanogenic activity, anaerobic biodegradability and toxicity are key parameters in the design and operation of anaerobic bioreactors. A large variety of methods exist for the determination of these parameters but a normalized method has not been established so far. This paper presents the development of an automated manometric system for the determination of these anaerobic sludge parameters. The system is based on monitoring the production of methane by using a pressure transducer that measures the pressure in a gas-collecting chamber of known adjustable volume, which is independent of the space where biogas production takes place. The evolution of pressure generated by the accumulation of methane relates to the conversion of COD. In this way, the methanogenic activity of the sludge can be determined, as well as the biodegradability of solids and liquid, as well as the methanogenic toxicity of compounds. The equipment permits gas sampling, as well as extraction and introduction of liquid, without losing the anaerobic conditions. Various assays have been conducted to test the reliability and reproducibility of the obtained results, showing a high level of both. The methanogenic activities obtained in the assays ranged between 0.1 and 1.8 g COD g^{-1} VSS d^{-1}, and the biodegradability of the organic compounds tested ranged between 20 and 90%.

Keywords Anaerobic assays; biodegradability; manometric method; specific methanogenic activity; toxicity/inhibition

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Introduction

The anaerobic processes of organic matter degradation have a large number of applications, like domestic and industrial wastewater treatment. During the last years, anaerobic digestion of solid residues, in particular of the organic fraction of solid domestic waste has experienced a notable increase.

To evaluate the viability of anaerobic treatment, as well as to follow the operation of biological treatment plants, it is required to gain information on the basic parameters of the water to be treated and the sludge to be used (Field *et al.*, 1988). Those parameters can be assessed in biological sludge assays to quantify: the activity of the sludge, the anaerobic biodegradability of the wastewater and the toxicity of compounds present in the wastewater.

The assays will deliver data on the viability of the process and the optimal loading rate to realize a faster start-up can be determined. Furthermore, by taking periodical measures it will be able to detect periods of inhibition.

The methods used for the determination of these parameters are based: (i) on the rate of substrate consumption; or (ii) on the rate of product formation. The majority of the existing methods measure biogas, either by liquid displacement or by pressure measurements, or by gas chromatography.

By gas chromatography, the biogas methane and carbon dioxide content can be measured (Dolfing and Bloemen, 1985; Soto *et al.*, 1993; Angelidaki, 2002). The analysis is very precise and can be used for detecting low concentrations but it is also complex and time-consuming. Methods based on measuring the quantity of produced methane are simpler. They measure either the produced volume of biogas at a constant pressure

(liquid displacement methods) or they measure the increased pressure at a constant volume (manometric methods). The liquid displacement method was the first method to be developed and one can encounter a large number of these methods described in the literature, either manual ones (Owen *et al.*, 1979; Valcke and Verstraete, 1983; Cohen, 1992) or automatically controlled ones (Van der Berg *et al.*, 1974; Rozzi *et al.*, 1983). Manometric methods were based on a variety of the Warburg respirometer. Later, methods have been developed using pressure transducers, thereby facilitating its automation (Miller and Wolin, 1974; James *et al.*, 1990; Chernicharo and Campos, 1991; Ince *et al.*, 1995; Angelidaki *et al.*, 1998).

Despite the large variety of existing methods, normalized activity and toxicity assays have not yet been established. Various biodegradability assays have been proposed (ISO 11734, 1995; ECETOC, 1998). In the present study, we present a automated manometric method with a high level reliability and reproducibility.

Materials and methods

Experimental set-up

The proposed method derives from a method to test aerobic biodegradability that was modified to be adapted to anaerobic processes (James *et al.*, 1990). It can be classified as an automated manometric anaerobic batch assay. The system is based on following the production of methane by using a transducer that measures the pressure in a gas-collecting chamber of known volume, which is independent of the space in which the anaerobic conversions take place. From the pressure data one can calculate the produced volume of methane.

The experimental set-up (Figure 1) consists of a digester ($V = 530$ mL) connected to a gas-collecting chamber ($V = 1500$ mL) that is partially filled with an alkaline solution to absorb the biogas' carbon dioxide once the biogas crosses the barrier that separates the small digester gas headspace and the gas-collecting chamber. The pressure transducers, located in the gas-collecting chamber, measure the pressure, which is registered by an on-line data-acquisition system connected to a computer. The system is equipped with gaseous and liquid sample points, from which samples can be taken and without losing anaerobic conditions. In this way, it is possible to follow the COD, TOC, volatile fatty acids and pH in parallel with the pressure.

The advantages of the automated method are various: the easiness to perform and replicate the assay and the comfort for the researcher when taking into account that the computer registers all data. Moreover, continuous monitoring offers rapid detection of anomalies.

The principal novelty of the method, in contrast to existing manometric methods, is that the pressure is being measured in the gas-collecting chamber instead of the gas headspace in the digester. This minimizes the digester's gas headspace volume, by means of

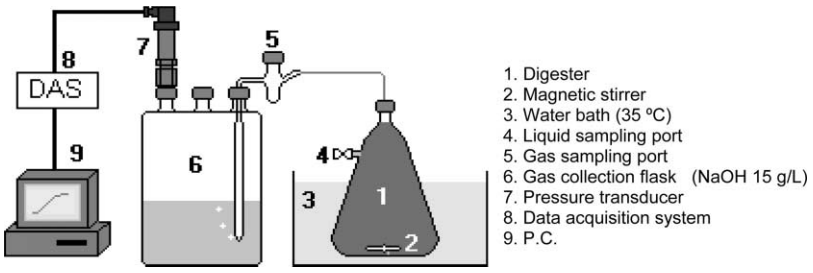

Figure 1 Schematic representation of the experimental set-up

which it is no longer necessary to flush the system with an oxygen-free gas before performing the assay, thereby avoiding large periods of adaptation. When the expected gas production is low, it is easily possible to adjust the pressure build-up in time to the range of the pressure transducer by reducing the free volume in the gas-collecting chamber.

As the produced biogas flows through an alkaline solution in the gas-collecting chamber, the carbon dioxide is absorbed. In this way, the pressure build-up in the gas-collection chamber is directly related to the production of methane.

Procedure

Leakage is one of the major problems associated with manometric methods. The system's pressure tightness was regularly checked by filling it with gas at the maximum operational pressure (1.6 bar) and monitoring its pressure for several days. The equipment was considered to be in good condition if the pressure drop was less than 1%.

The procedure of the anaerobic batch assays was as follows: (1) addition of solutions of nutrients, sulfide (to reduce the redox potential), and buffer; (2) addition of sludge; (3) addition of substrate (neutralized volatile fatty acids (VFA) solution or, for biodegradation tests, a sample of the wastewater or compound to degrade), as well as, in case of toxicity tests, addition of the inhibitor to be tested; (4) addition of water up to a volume of a little bit more than the marker at 530 mL and introduction of alkaline solutions (15 g $NaOH\,L^{-1}$) to the gas-collecting chambers up to a chosen volume (normally 600 mL); (5) adjustment of the digester pH to 7.0–7.2; (6) closing of the digesters and placing them, together with the gas-collecting chambers, in the thermal baths (at 35 °C); (7) first sample after 30 minutes of incubation, leaving the liquid volume of the digesters at 530 mL; (8) connecting the digesters to the gas-collectors, starting the data-logging program and (9) set initial pressure to zero and start magnetic stirring.

The assay can be considered finished when an almost constant pressure is obtained or, in case of activity assays, when the slope of methane production versus time starts to decrease. Due to the heterogeneity of the sludge, repetition of the assay is recommended, and also a control without substrate will have to be incorporated, to correct for endogenous methanogenic activity.

Calculation of the methane production from the pressure data

The pressure in the gas-collection chamber is continuously measured and registered at fixed time intervals. To calculate the produced volume of methane from the pressure data, Eq. 1 is used.

$$V = K * (P_f - P_i) \tag{1}$$

where:

V = produced volume of methane under standard conditions (0 °C and 1 atm) (mL)

P_f = final pressure (mbar); P_i = initial pressure (mbar)

K = calibration factor (ml $mbar^{-1}$)

Since the gas headspace volume in the digester is negligible in comparison with the gas headspace volume in the gas-collecting chamber (V_g) and the solubility of methane is rather low, the calibration factor can be calculated from Eq. 2:

$$K = \frac{V_g \cdot (273/T)}{P_0} \tag{2}$$

where:

V_g = gas headspace volume in the gas-collection chamber (mL)

T = incubation temperature (K),

P_0 = normal pressure (mbar)

The value of K has been calculated both experimentally and theoretically, with differences less than 1%. The use of the alkaline solution greatly simplifies the calculation as it rules out the necessity to correct for dissolution of carbon dioxide and to monitor the gas composition in time. Yet, if desired, it is still possible to use the equipment for performing conventional manometric anaerobic assays, simply by leaving out the alkaline solution.

Chemicals and reagents

The composition of the solutions added to the digester were taken from Field *et al.* (1988), including: neutralized VFA solution (acetate:propionate:butyrate 4:1:1 on COD basis), macronutrient solution, trace element solution, sodium sulfide solution, bicarbonate solution, yeast extract

Sludge

The sludge samples used for the assays came from several industrial anaerobic bioreactors treating sugar, brewery and paper mill wastewater.

Analytical methods

Standard methods (APHA, 1995) were used to analyze: solids and volatile suspended solids, (methods 2540D, 2540E and 2540G), VFA by GC, (method 5560D), total and soluble COD, (method 5220C), biogas composition by GC, (method 2720C), pH and redox potential.

Results and discussion

The described assay has been used since February 2003 to determine the methanogenic activity and the biodegradability of industrial wastewater samples. For this purpose, Eq. 2 was used in combination with measured VSS and COD or TOC data.

Evaluation of the results

To evaluate the reproducibility, a series of assays was performed in triplicate. The plot directly obtained from the computer is shown in Figure 2, the activities calculated from

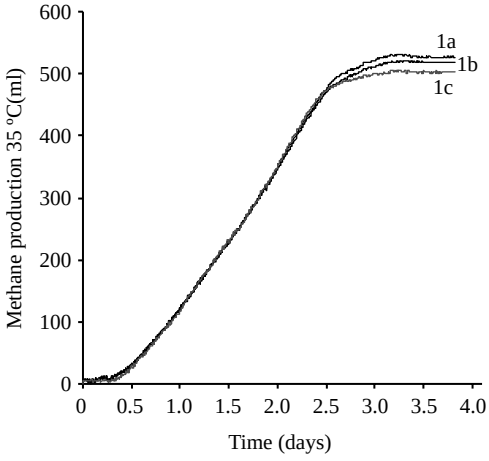

Figure 2 Methane production vs. time plots of a triplicate methanogenic activity assay

this results fit to 0.46 ± 0.01 g COD g^{-1} VSS d^{-1}, thereby showing the high reproducibility of the method, with a standard deviation lower than 2.5%. To assess the method VFA samples were analyzed at different time intervals. The experimental VFA concentration converted into COD is shown in Figure 3, thereby demonstrating that the activity calculated from COD removal is identical to the one calculated from methane production.

To verify if the gas production calculated from the pressure increase is related with the COD removal, at the end of the assays the residual COD was analyzed and the theoretical methane production calculated. Figure 4 shows the excellent agreement between the volume of methane calculated from pressure increase and from COD removal.

The obtained values for the specific methanogenic activity under various conditions tested ranged between 0.18 and 1.8 g COD g^{-1} VSS d^{-1}. In all cases, linear regression of the straight part of the methane production vs. time plots had R^2 values higher than 0.995.

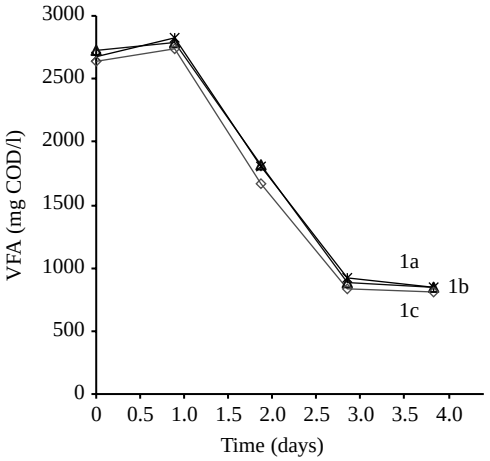

Figure 3 Degradation of VFA in the triplicate methanogenic activity assay of Figure 2

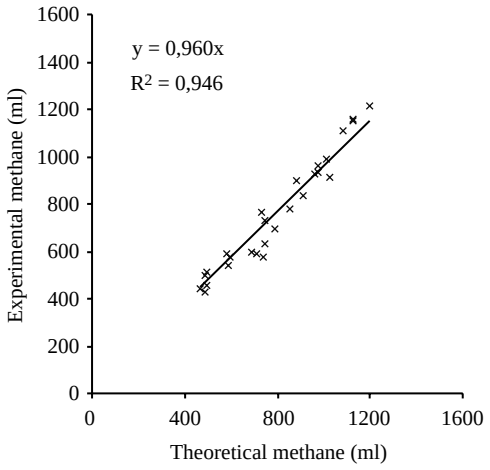

Figure 4 Comparison between the theoretical methane production (on basis of COD removal) and the experimentally measured methane production. All data are normalized for standard conditions (T = 0 °C, P = 1 atm.)

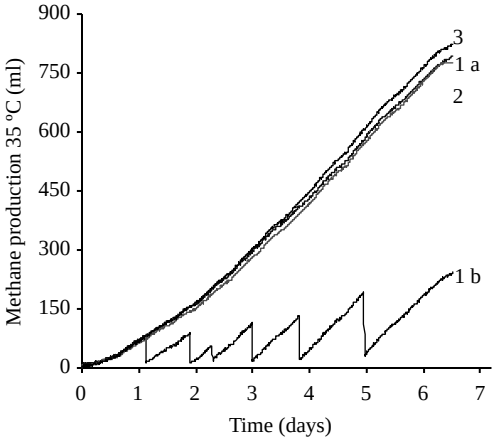

Figure 5 Pressure influence on methane production. 1b. Assay with intermittent pressure release. 2 and 3. Assays without pressure release. 1a. Accumulative methane production in assay 1b

Influence of pressure

To test the effect of pressure, the cumulative methane production in a series of assays performed in the usual way (without pressure release) was compared to that in a series of assays in which the pressure was regularly released. The results of this test (Figure 5) show that the slope of the intermittent methane production vs. time curve is similar each time after pressure release.

Moreover, the cumulative plot of the intermittent curve showed no difference with the methane vs. time curve of the assay without pressure release. Therefore, it is obvious that the pressure does not affect the results of the assay within the range of pressure encountered in the system.

Different biodegradability assays were performed to check industrial and domestic solid and liquid wastes. The average biodegradation values obtained ranged between 20 and 90%.

Conclusions

An automated manometric method has been developed for performing anaerobic assays. The method was successfully applied for determining the specific methanogenic activities of several sludge types and for assessing the anaerobic biodegradability of several industrial wastewater samples. The obtained results demonstrate the high reliability and reproducibility of the method.

Automation permits a procedure that is independent of manual readings and the direct mathematical treatment of data.

The separation between the gas-collecting chamber and the digester facilitates sampling and provides flexibility with choosing the volume of the gas-collector.

Acknowledgements

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