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Short Communication

Early assessment of a rapid alternative method for the estimation of the biomethane potential of sewage sludge

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HIGHLIGHTS

- Fast fluorescence-based method to investigate biodegradability of sewage sludge.
- Proportionality between fluorescence and metabolic activity of anaerobic sludge.
- Assessment of nineteen municipal sewage sludge by this method and by AMPTS.
- Similar results between AMPTS and rapid alternative method for BMP measurement.

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ABSTRACT

This short communication briefly presents a rapid method using a fluorescent redox indicator, similar to resazurin, in order to estimate the biodegradability of sewage sludge during anaerobic digestion (AD). The biodegradability and by extension the Biochemical Methane Potential (BMP) of nineteen municipal sludge samples (primary, biological and tertiary) were investigated and estimated in only 48 h. Results showed the relevance to follow the metabolic activity of anaerobic sludge by the kinetic of probe reduction. The extended lag phase of inoculum indicated an impact of pre-treatments on enzyme activity. The comparison with Automatic Methane Potential Test System II (AMPTS) confirmed the estimated values of BMP according to an uncertainty limit of 25%. These first results highlight the interest of this rapid assay as a preliminary tool of the biodegradability of sewage sludge in anaerobic digestion.

1. Introduction

The global population growth increases the demand for water resources. Consequently, many research programs on tomorrow's wastewater treatment plants are conducted on each process step in order to answer water and energy challenges (Ruffino et al., 2015). For that purpose, the MOCOPEE (MOdeling, Control and Optimization of wastewater treatment ProcEssEs) project focuses on applied metrology, modeling and on remote monitoring of wastewater treatment process (www.mocopee.com).

Anaerobic digestion (AD) is a natural biological process where micro-organisms break down organic matter in the absence of oxygen. Since few decades, AD is recognized as a renewable energy having a huge potential. The anaerobic digestion process for wastewater treatment plants (WWTPs) is an economical process and is considered as being a major and essential part of WWTPs (Appels et al., 2008). Indeed, AD plays an important role thanks to its abilities to produce a net energy gain in the form of methane gas, to reduce the volume, the pathogens content and the odour of sewage sludge (Silvestre et al., 2015).

The BMP assay is an important tool and the most commonly used for the assessment of the anaerobic biodegradability of sewage sludge produced during wastewater treatment (Aquino et al., 2008; Mottet et al., 2010). The BMP assay requires 20–60 days to measure the BMP of samples. Nevertheless, the required time is a major inconvenience in answering operational requests in WWTPs. To address this lack of a rapid and pertinent tool, many developments of alternatives approaches were made: modeling with the IWA ADM1 model (Batstone et al., 2002), or methods based only

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on organic matter characterization using spectroscopy (Jimenez et al., 2014; Lesteur et al., 2010). Recently, new tools, based on fluorescence measurement, have been developed in order to answer operational requests such as toxicity (Chen et al., 2015) or for high-throughput characterization of more than 32 samples simultaneously in 48 h, for digester operation, the Envital® kit. More precisely, these technologies use a probe where the kinetics of redox indicator reduction is directly correlated with the activity of anaerobic sludge (Chen et al., 2015). Furthermore, the few BMP data of sewage sludge based on the existing literature are variable (Carrère et al., 2010; Jensen et al., 2014). According to Wang et al. (2015a), it is very difficult to compare these varying results because there are many differences in instrumentation, protocols, and different experimental conditions. The MOCOPEE research program has worked on the determination of the BMP of sewage sludge with the Automatic Methane Potential Test System II, since 2013. The aim is to provide a sewage sludge data base and to developed mathematic models to predict methane production.

The objective of this short communication is to succinctly present a rapid assay based on fluorescence, the Envital® kit, in order to estimate anaerobic biodegradability of sewage sludge and to compare it to AMPTS, in the context of MOCOPEE research program.

2. Methods

2.1. Substrates and inoculum: characteristics and preparation

2.1.1. Characteristics

The inoculum, an anaerobic sludge, was systematically collected from the same secondary digester on a WWTP next to Paris (interdepartmental association for sewage disposal of Paris conurbation, SIAAP) but not necessarily the same day for both technologies. Indeed, the Envital® experiments were carried out in parallel of the AMPTS analysis but also at different times. Thus, different inocula were used between the two technologies. In the same way, samples were collected from the WWTPs and at different locations in the process: primary settler (primary sludge), biological tank or biofilter (biological sludge), tertiary settler with chemicals (tertiary sludge). Combinations of primary and biological sludge were sampled (mixed sludge). Physical and chemical properties of the samples and inoculum, Total Solid (TS), Volatile Solid (VS), and Chemical Oxygen Demand (COD), have been measured according to standardized methods (APHA, 2005) (Table1).

2.1.2. Preparation

For the AMPTS analysis, fresh samples and fresh inoculum have been added in glass bottles of 500 mL each, following the [I]/[S] ratio of 3 based on VS content. This ratio is widely quoted in the literature (Jackowiak et al., 2011) and advised by the manufacturer. 40 mL of each sample was frozen in order to create a sample collection stored at −20 °C for the comparison with Envital® assay in microplate. The freezing allowed to stabilize and conserve the organic matter on several weeks. Just before the Envital® analysis, 40 mL of samples were gently thawed at room temperature and, then suspended and diluted in distilled water. For the Envital® analysis, the anaerobic sludge, used as inoculum, was pre-incubated at 35 °C after collection from the mesophilic digester for 24 h. The pre-incubation of the anaerobic sludge allowed to reduce its organic content before using it in the BMP tests. After incubation, the inoculum was prepared by filtration with a specific filter supplied in the kit in order to remove organic matter in suspension.

Table 1
Principals characteristics of sewage sludge and inoculum.

Sewage sludge	TS (g/L) (mean ± sd*)	VS (%TS) (mean ± sd*)	COD (g/gVS) (mean ± sd*)
Primary sludge	30.7 ± 11.6	79.3 ± 3.1	1.8 ± 0.1
Biological sludge (tank)	34.2 ± 2.2	81.1 ± 4.3	1.5 ± 0.4
Biological sludge (biofilter)	42.7 ± 16.9	72.3 ± 10.6	1.5 ± 0.4
Mixed sludge	37.0 ± 0.4	77.4 ± 4.7	1.7 ± 0.2
Tertiary sludge	29.7 ± 12.1	79.3 ± 3.1	1.8 ± 0.3
Inoculum (anaerobic sludge)	22.7 ± 2.8	62.2 ± 6.6	1.7 ± 0.1

* sd: standard deviation.

2.2. AMPTS

In a first step, the study of characterization of sludge samples was carried out using AMPTS II since 2013 (Bioprocess Control, Lund, Sweden). This technology allows automatic and real-time measurements of the biogas production during 20–40 days at 35 °C ± 0, 2. Each reactor is mixed by a stirring rod in a circular manner by a motor located on the top of the reactor (rotation speed = 80 rpm – agitation frequency: 60 s ON/60 s OFF). The biogas produced was further led to a scrubbing reactor by flexible tubing. The acid gases (CO_2, H_2S) were trapped by an alkali solution (NaOH, 4 M) and the remaining biogas (methane) passed into the measuring cell, containing a gas counter based on the principle of liquid displacement (Bassard et al., 2014). Gas bubbles generate impulsions. These impulsions were recorded and translated in Nml CH_4/gVS by AMPTS® v5 software. The temperature and pressure sensors are incorporated for a correction online of results. Blanks containing only inoculum and without substrate were used to correct for background methane potential.

2.3. Envital® kit

The Envital® kit, developed by Envolure, was used on all samples following the recommended procedure with a given specific ratio for: fluorescent probe, buffer solution, samples or standards and inoculum in separate wells of 96-well microplates. In the same way, blanks were used to correct for background methane potential. To ensure anaerobic conditions, the wells were sealed immediately with a specific reagent and a microplate lid supplied in the kit. Excepted for inoculum, all the reagents, standards and materials are provided in the kit. Analysis was carried out at 35 °C during 48 h. Fluorescence was recorded every 30 min by a fluorescence microplate reader (FLx800, BioTek®, USA) at λEx. = 540 nm and λEm. = 600 nm with a gain of 35. There was a shaking before each reading. The raw fluorescence data were translated in Nml CH_4/g VS thanks to the range of standard solutions (developed by AMS Envolure, France) according to a linear regression model (Fig. 1).

3. Results and discussion

3.1. Fluorescence and anaerobic biodegradability with Envital® technology

WWTPs generate different sludge as a by-product of different processes (primary, biological and tertiary treatment) used in the sewage treatment. Four sludge produced at different steps in the process were chosen in order to assess the ability of fluorescence technology to distinguish the different sewage sludge. Fig. 2(a) shows the cumulative fluorescence intensity resulting from the metabolic activity of anaerobic sludge. The control (blanks) represented the endogenous metabolic activity without addition of organic matter. The cumulative fluorescence intensity curves corresponding to sludge samples were much higher than the control.