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Methane production improvement by modulation of solid phase immersion in dry batch anaerobic digestion process: Dynamic of methanogen populations

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HIGHLIGHTS

- The high immersion of cattle manure improved the biogas production.
- Methanosarcinaceae was a relevant bio-indicator of the methane production.
- Methanosarcinaceae and Methanobacteriales dominated in dry AD process on manure.
- DNA extraction and qPCR were adapted to quantify methanogens in dry batch AD.
- The development of methanogens was significantly enhanced in the solid phase.

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ABSTRACT

Several 60 L dry batch anaerobic digestion (AD) reactors were implemented with or without liquid reserve on cattle manure. The immersed part modulation of cattle manure increased the methane flow of about 13%. The quantitative real time PCR and the optimized DNA extraction were implemented and validated to characterize and quantify the methanogen dynamic in dry batch AD process. Final quantities of methanogens converged toward the same level in several inocula at the end of AD. Methanogen dynamic was shown by dominance of Methanosarcinaceae for acetotrophic methanogens and Methanobacteriales for the hydrogenotrophic methanogens. Overall, methanogens populations were stabilized in liquid phase, except Methanosaetaceae. Solid phase was colonized by Methanomicrobiales and Methanosarcinaceae populations giving a support to biofilm development. The methane increase could be explained by a raise of Methanosarcinaceae population in presence of a total contact between solid and liquid phases. Methanosarcinaceae was a bio-indicator of the methane production.

1. Introduction

Anaerobic digestion (AD) process is achieved by a consortium of microorganisms degrading organic matter of waste to produce a biogas composed of 50–70% of methane (valorized in electricity and heating) and a digestate with high agronomic values (used as a fertilizer). Several technologies of dry AD process are implemented according to the process temperature, the step number, the reactor configuration and the presence of mixing system. Dry anaerobic digestion generally occurs on waste with a total solid content superior to 15% (agricultural waste, food waste and industrial waste) (Karthikeyan and Visvanathan, 2013). Liquid phase is usually minimized in dry AD process, implying several constraints in process as humidity, temperature and microorganism repartitions. The liquid quantity improves biogas production (El-Mashad et al., 2006) by transporting and spreading microorganisms and metabolites in media (El-Mashad et al., 2006; Kusch et al., 2009; Shahriari et al., 2012). Increasing the recirculation frequency of inoculum improves the methane production until the second peak of methane flow in dry AD process (André et al., 2015).

A linear relation between water content and methanogenic activity has been established by Le Hyaric et al. (2011). Moreover, a high total solid content impacts on biogas production (Abbassi-Guendouz et al., 2012).

These phenomena understanding are possible due to the molecular biology methods developed since the 90's. Furthermore, anaerobic microorganisms cultivation is difficult. Biomolecular

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tools can be used to monitor the process, to optimize anaerobic digestion and detect a potential drift of the process (Karakashev et al., 2005; Yu et al., 2005; Demirel and Scherer, 2008; Lee et al., 2009; Ike et al., 2010; Traversi et al., 2011, 2012; Merlino et al., 2013; Williams et al., 2013; Guo et al., 2014; Town et al., 2014).

Methanogenic groups include the acetotrophic methanogens which transform acetate into methane and carbon dioxide (Ferry, 1992), and with the hydrogenotrophic methanogens which transform hydrogen and carbon dioxide into methane. The acetotrophic methanogens are always present in AD process. Operating and environmental parameters influence the activity and the diversity of methanogens. Karakashev et al. (2005) have used fluorescence in situ hybridization to observe the methanogenic diversity of 15 Danish biogas plants treating 50–500 tons of feedstock per day. These biogas plants have been operated at different conditions (temperature, hydraulic retention time and biogas production) implying the dominance of different methanogens orders. Diversity of methanogens depends on inoculum source and waste. Hydrogenotrophic methanogens ensure the stability and the efficiency of the AD process (Demirel and Scherer, 2008). A comparison between a single stage thermophilic and a two stage thermophilic AD process treating cattle manure has shown a similar range of hydrogenotrophic methanogens demonstrating that the reactor configuration in this case does not have any impact on the process (Nielsen et al., 2004).

Most of the studies analyze methanogenic dynamic in liquid processes; few data are available on dry processes. Degueurce et al. (2016) have quantified the bacterial and the archaeal communities on cattle manure in dry AD process. Dry batch AD process could be monitored, optimized and managed by using molecular biology.

The aim of this study was to optimize a dry batch AD process by modulation of the immersed part of cattle manure by using biomolecular tools, firstly to observe the consequence on the methane production and, secondly to understand methanogenic dynamic according to the reactor configuration. 60 L dry batch anaerobic reactors were implemented with two different configurations, with or without liquid reserve modulating the solid phase immersion. As a first step, DNA extractions of inoculum and cattle manure were optimized changing several parameters (thermal shock, mechanic shock). The quantitative real time PCR (qPCR) of methanogenic populations targeting 16S rRNA was implemented to quantify total Archaea, four orders of methanogens whether Methanococcales, Methanomicrobiales, Methanobacteriales, Methanosarcinales, and two families such as Methanosaetaceae and Methanosarcinaceae. An external validation of DNA extraction and qPCR method were made by an external laboratory. Methanogenic compositions of inoculum used in these experiments have then been compared to fresh manure and inoculum at the end of anaerobic digestion. The dynamic of methanogen populations was studied in order to explain the methane production difference between two reactor configurations according to the immersion of solid phase.

2. Methods

2.1. Implementation of dry anaerobic digestion

2.1.1. Chemical and microbial characterizations

Total solid (TS) and volatile solid (VS) contents were analyzed on cattle manure (22.0 ± 0.5% TS; 89.4 ± 0.6% VS) and inoculum (2.10 ± 0.5% TS; 54.2 ± 0.3% VS) at 105 °C during 12 h and 550 °C during 2 h, respectively relative to the standard methods (APHA, 1998; AFNOR NF U44-160, 1985). Analyses were made in duplicate.

Before anaerobic digestion, cattle manure (around 1 kg) and inocula (around 1 L) were sampling according to a sampling plan (Gy, 1988), in order to analyze the methanogen dynamics by the qPCR method (see Section 2.3). Analyses were made in triplicate.

2.1.2. Dry batch anaerobic digestion at 60 L pilot scale

The reactors were made of polyethylene barrels (internal diameter: around 36 cm; height: 60 cm; working volume: around 60 L). Anaerobic digestion was carried out on 21 kg of cattle manure from the Institut Polytechnique LaSalle Beauvais farm. 22 kg of inoculum corresponding to a liquid phase of the previous batch has been added to enhance the biogas production. The word "inoculum" used in these experiments refers to: (i) the liquid phase added in the reactor at the beginning (inoculum t_0), (ii) the liquid phase that has been recirculated on solid phase during AD process, (iii) the liquid phase sampled at the end of AD (inoculum t_{30}).

Anaerobic digestion was carried out at 37 ± 1 °C in mesophilic condition. In both configurations (see Section 2.1.3), inoculum was recirculated at the top of the reactor at a constant rate (3.33 L/2 h) using a peristaltic pump (Masterflex, USA). The biogas production was measured with the AMPTS I system (Bioprocess control, Sweden). A gas analyzer (Multitec 540, Sewerin, Germany) was used daily to determine the biogas composition (methane, carbon dioxide, hydrogen sulfide, oxygen) (Fig. 1(A) and (B)). Batch processes have been monitored for 30 days except sacrifices (described in Section 2.1.3).

2.1.3. Reactor configuration and sampling

Two reactor configurations were implemented under the following descriptions:

In the first configuration (Fig. 1(A)) only one reactor was implemented, cattle manure and inoculum were partially in contact (12 L of inoculum immerging about 33% of solid phase). In fact, a plastic support at the bottom created a liquid reserve of around 10 L. In this experiment, the inoculum was sampled (around 200 mL) in the recirculation flow at different moment of the biogas production described below (Fig. 1(C)). The inoculum sampled was replaced by a previous inoculum to keep an inoculum level constant in the reactor. The digestate sampling at the top of the reactor (a layer around 15 cm, around 1 kg) and inoculum were analyzed by qPCR method, by use a sampling protocol to obtain a representative mass (Gy, 1988).

In the second configuration (Fig. 1(B)), cattle manure and inoculum were placed in the reactor without liquid reserve. The totality of inoculum was always in contact with the substrate, representing an immersion of the solid phase around 50%. In this part, five reactors were started in parallel and sacrificed at specific times of methane flow production such as: t_0: before anaerobic digestion; 1P: first peak of methane flow; S: slack flow; 2P: second peak of methane flow; t_{30}: end of methane flow production (Fig. 1(C)) as previously described by André et al. (2015). Inoculum was sampled in flow recirculation (around 200 mL) and digestate was sampled at the top of reactor (a layer around 15 cm, around 1 kg) after opening reactors to qPCR analysis, by use a sampling protocol to obtain a representative mass (Gy, 1988).

2.2. DNA extraction

2.2.1. Substrates preparation before DNA extraction

Inoculum (2 mL) was put in a microtube and was centrifuged (16,100g during 5 min). Cells were resuspended in 1 mL of deionised water and centrifuged again (16,100g during 5 min). Around 0.15 g of solid was then again resuspended in 100 μL of water (Lee et al., 2009). Samples were added in a Powerbead tube (Mo bio laboratories, Inc., USA) and the extraction protocol was applied.