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Research paper

The impact of inoculum source, inoculum to substrate ratio and sample preservation on methane potential from different substrates

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ABSTRACT

Batch experiments were conducted to evaluate the impact of inoculum sources, inoculum to substrate (IS) ratio and storage conditions on the potential and production rate of methane (CH_4) from different substrates: wheat straw, whole crop maize, cattle manure, grass and cellulose.

The results of the test with four inocula and four substrates indicated that inoculum source could have a significant impact on both CH_4 potential (BMP) and the kinetics parameters of different substrates. The two inocula showing the highest BMP and production rates in each period were those coming from a feeding with more than 70% of animal manure under thermophilic conditions. The impact of the IS ratio in the range 0.25–2.5, in terms of g volatile solids (VS) substrate/g VS inoculum, depended on substrate type. Maize silage was more affected to changes in the IS ratio than wheat straw. The optimal IS ratio range for maize was 1.0–1.5, however, a wider IS range can be used in wheat straw (0.5–2.5). The impact of freezing and drying depended on biomass type. Freezing, drying and ensiling of grass increased the CH_4 yield compared to fresh grass. Drying of maize had no impact while freezing reduced the CH_4 potential. Drying and freezing had no impact on straw.

1. Introduction

The total global energy consumption is expected to grow by one third during the next 25 years which will increase the pressure on fossil energy and the requirement for a higher contribution from renewable energy. There is an increasing interest in renewable energy, such as anaerobic digestion, due to its economic and environmental benefits [1,2]. Ultimate methane (CH_4) potential (B_0), which is the maximum CH_4 yield that can be obtained from a substrate, is a widely used parameter for correct design and budgeting of full-scale biogas plants as well as for assessment of the influence of biogas plants on greenhouse gas (GHG) emissions. In addition, B_0 is a key parameter when calculating CH_4 emission from animal slurry according with the IPCC [3] procedure. However important differences are shown in literature among B_0 when compared within the same substrate, probably because differences in methodologies.

Owen et al. [4], Angelidaki et al. [5], and ISO-11734 1995(E) [6] recommended different procedures for B_0 determination but no procedure has been defined as a standard method [7,8]. Therefore, a precise determination of the B_0, and how this value can be affected according to different methodologies used during the determination in the laboratory is essential. In order to choose a procedure, it is important to know the effect of each parameter on B_0 determination. On this regard several works have been publish evaluating the effect of specific parameters on B_0 determination [7,8]. From these works it is concluded that one of the most important parameters affecting B_0 is the inoculum, not only the inoculum source, but also the amount of the inoculum added, what is called inoculum to substrate ratio (IS ratio). It is clearly shown that IS ratio can affect not only the biodegradability but also the CH_4 production rate or hydrolysis rate, calculated from first-order kinetics models [7]. However, it is not clear how this factor affects the results, and if this depends on substrate composition.

Sample preservation before the B_0 test is also another factor affecting biodegradability and thus B_0 results [9,10]. However, scarce information about this issue can be found in literature.

In this work we aimed to study the effect of three of the major points of controversy among B_0 methodologies: inoculum source, IS ratio and sample preservation, by means three anaerobic batch experiments. In each batch experiment, one of the three factors was evaluated by using different substrates in a factorial design, in order

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to determine whether substrate composition could influence the results. Inocula from four biogas plants in Denmark working with different temperatures and substrates were used to investigate the impact of inoculum source. The impact of the IS ratio on volatile solids (VS) basis was evaluated in the range 0.25–2.5 VS of substrate/g VS inoculum. The storage methods used to test the effect of sample preservation were drying, freezing and ensiling. The substrates used were wheat straw, whole crop maize, cattle manure, grass and cellulose.

2. Materials and methods

2.1. Substrates and inocula

Table 1 shows the origin, sample preservation conditions and characteristics of the substrates used.

Whole crop maize (Artist) was harvested and chopped at Research Centre Foulum (Aarhus University, Denmark) in October 2011. It was ensiled for one year in a plan silo in Foulum (Denmark). Fresh whole crop maize (Adept) was harvested and chopped at Research Centre Foulum (Aarhus University, Denmark) in October 2012. This maize was stored in a refrigerator at 5 °C for a couple of days without additives before use.

Wheat straw was harvested in eastern Jutland, Denmark, in the summer of 2011. The straw was homogenized by hammer-milling (Cormall HDH 770, Sønderborg, Denmark). The size of the straw particles was in the range 0.5–3 cm. The baled wheat straw was stored in a bale at room temperature.

The grass sample contained 17% clover and 83% ryegrass and was harvested in October 2012 at Research Centre Foulum (Aarhus University, Denmark). The grass was chopped to particle size 0.5–10 cm with a machine from Landtechnik Weihenstephan (Versuchshacksler no. 008, Austria).

Cattle manure (Holstein) was collected from the livestock building at Research Centre Foulum (Aarhus University, Denmark). The cows were fed 9.76% wheat, 65.85% late grass silage and 24.39% rapeseed meal. Cattle manure was stored at −20 °C and before use it was thawed at room temperature. The total VFA was 2.5 ± 0.3 g/L.

The cellulose was powdered cotton linters with a particle size of 50 μm (Sigma Aldrich, USA).

The inocula were collected from four different biogas plants. All inocula were collected on the same day, and pre-incubated for 15 days at their corresponding initial temperatures in order to deplete the residual biodegradable organic material (degasification), according to the recommendation of Angelidaki et al. [5]. After degasification, they were filtered with a 1-mm screen. Table 2 shows the origin, feed composition storage conditions and chemical composition of the inocula used.

2.2. Experiments

2.2.1. Impact of inoculum source (experiment 1)

This experiment was conducted in a factorial design in which four inocula (Horsens, Bånlev, Foulum and Thorsø) and four substrates (wheat straw, maize silage, cattle manure and cellulose) were used.

The dry matter (DM) dose of substrate to each bottle was fixed (4 ± 0.1 g DM per bottle), resulting in an IS ratio in the range 0.79 (Bånlev) −1.21 (Foulum).

2.2.2. Impact of IS ratio (experiment 2)

This experiment was conducted in a factorial design in which six IS ratios (0.25, 0.5, 1, 1.5, 2 and 2.5) were tested with two different substrates (wheat straw and fresh whole crop maize) using inoculum from Foulum whose characteristics are shown in Table 2.

The IS range was chosen according to literature [7]. The two substrates tested in this experiment were selected based on the fact that wheat straw and maize are products commonly added to anaerobic digestion plants in Denmark and also on the results from experiment 1 by using inoculum from Foulum.

2.2.3. Experiment 3: impact of sample preservation

This experiment was conducted in a factorial design in which three different storing methods were used: Drying, freezing and vacuum ensiling. These methods were selected based on normal procedure in real plants and laboratories. Storing biomass samples in refrigerator and freezer and drying are commonly used methods in laboratories, before a B_0 analysis; while ensiling is widely used for storing of crops for biogas production. Three substrates were used: fresh whole crop maize, wheat straw and grass. These substrates were selected because they are commonly used as co-substrates in anaerobic digestion plants.

Drying was carried at 50 ± 1 °C for about 69 h. Freezing consisted of a storage period of 6 day at −18 ± 1 °C in plastic bags. Vacuum ensiling was only used on grass, using the Webomatic CT100 model I22 vacuum machine (Genpack A/S, Denmark). The fresh chopped grass was ensiled in a plastic bag under vacuum for 33 days at 5 ± 0.5 °C.

The batch experiment was carried at thermophilic conditions (53 ± 1 °C) for 91 days using an inoculum from Foulum. In this experiment the IS ratio in terms of gVS/gVS was 1 ± 0.02.

Table 1
Origin, sample preservation conditions and characteristics in terms of dry matter (DM), volatile solids (VS) and pH and experimental distribution of the substrates used.

	Origen	Sample preservation	DM %†	VS %	pH	Experiment
Substrate	Maize	Fresh	30.7	28.8		2 3
		Frozen	30.5	29.5		3
		Dry	96.1	93.0		3
		Ensiled	35.8	32.4		1
	Wheat straw	Baled	90.1	85.8		1 2 3
		Frozen	92.2	88.0		3
		Dry	96.9	92.4		3
	Grass	Fresh	19.8	17.1		3
		Frozen	20.3	17.5		3
		Dry	95.4	82.5		3
		Ensiled	20.3	17.4		3
	Cattle manure	Frozen	11.33	9.96	6.84	1
	Cellulose	Dry				1

%† percentage on wet weight basis.