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## Original Research Article

## Influence of sheep manure addition on biogas potential and methanogenic communities during cow dung digestion under mesophilic conditions

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## ABSTRACT

The efficient treatment of animal slurries can support the bioenergy management and environmental protection; however, the low biogas yield and quality are the major constraints. The object of this paper is to investigate how the co-digestion of sheep manure and cow dung by not using inoculum influences the performance of the process and determine the methanogenic communities at the end of the experiment. Biochemical Methane Potential essays were conducted in mesophilic conditions in order to determine the biogas-methane potential. Enhanced biogas production was achieved from the mono-digestion of cow dung with 104.3 NmL biogas g<sup>-1</sup> VS and the co-digestion of cow dung and sterilized sheep manure with a lower biogas yield of 89.0 NmL biogas g<sup>-1</sup> VS.

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## 1. Introduction

Dairy industry is now a significant global industry and it contributes to nearly 7% of total agricultural production value [1]. It is also one of the most important industries in the Netherlands. With the expansion of the big farms, a lot of herds now lead to large amount of livestock manure which causes serious environmental problems such as greenhouse gas (GHG) emissions, surface water contamination, and animal related pathogens [2,3]. Among livestock manure, the majority is produced by cattle. As ruminants (mainly dairy and beef cattle) contribute the largest proportion (61%) to livestock-related GHG emissions [4,5], there is an increased pressure to reduce their carbon footprint.

Anaerobic digestion (AD) produces biogas for heat and power as well as solid residue - so-called digestate-which can also be used as organic fertilizer in agricultural activities [6]. The basic steps of the organic mass conversion to biogas are illustrated in Fig. 1. The mono-digestion of cattle manure is proved to be reasonable because it contains bacteria needed in the fermentation phase as well as degradable materials such as carbohydrate and lipids. But on

the other hand, the fermentation of cattle manure alone often results in low biogas production and sometimes reaches only quarter of the theoretical biogas yield. Moreover, compared with other farm animals, the biogas yield of cattle is lower because of its lignin complexes from fodder that are very resistant to AD [7]. It is critical to find another proper substrate to co-digestion with cattle manure in order to balance the nutrition and dilute the limitations in the AD process.

This paper chooses sheep manure (SM) for co-digestion with cow dung (CD). Cestonaro et al. [8] previously used the co-digestion of sheep bedding with cow manure without inoculum at room temperature and found that when adding 50% or more cow manure, it would increase the biogas production and improves the digestate quality. Alvarez and Liden [9] used the co-digestion of llama, cow manure and SM for improving methane production and found that co-digestion was better than the mono-digestion among those kinds of animal manure. However, previous studies of cow manure co-digestion with SM do not take into account the AD without inoculum. So there is a need to see the performance of co-digestion of sheep and cow manure without inoculum in order to investigate the interactions of the microorganisms present in the animal slurries. The object of this paper is to investigate how the co-digestion of SM and CD by not using inoculum influences the performance of the process and determine the methanogenic communities at the end of the experiment.

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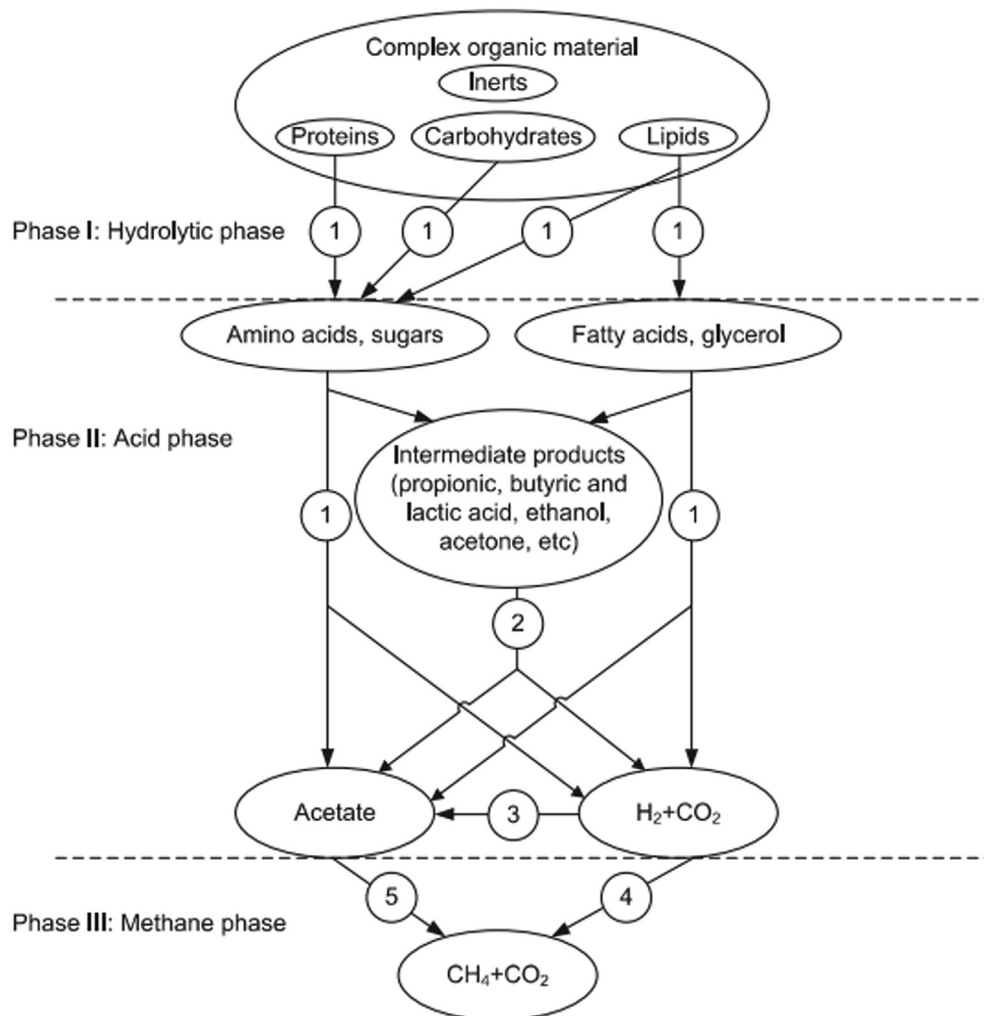


Fig. 1. Steps related with anaerobic digestion of organic materials.

## 2. Materials and methods

### 2.1. Origin of inoculum and substrates

Animal slurries were used as substrates in the experiments. Fresh CD and SM were collected from a farm in Groningen (Netherlands). Two different materials were selected for experimental essays as they are considering important source for agricultural bioenergy production. Two additional samples of cow manure and SM were undergone autoclaving (10 min) in order to eliminate the microorganisms. Pecorini et al. [10] report that short-term autoclaving does not influence the hydrolysis of cellulosic fraction of non-biodegradable substances. Their characteristics in terms of volatile solids (VS), total solids (TS) and chemical oxygen demand (COD) are given in Table 1. All the substrates were

**Table 1**  
Characteristics of the substrates.

Feedstocks*	TS (g kg <sup>-1</sup> )	VS (g kg <sup>-1</sup> )	COD (g kg <sup>-1</sup> )	TS/VS	COD/VS
Cow dung (CD)	121.3	107.2	134.7	1.1	1.3
Sheep manure (SM)	252.8	213.8	349.2	1.1	1.5
Sterilized cow dung (SCD)	135.4	121.7	186.6	1.2	1.6
Sterilized sheep manure (SSM)	237.4	199.0	250.2	1.2	1.3

undergone agitating for 5 min before final feed in order to increase the active surface of the particles. The substrates were stored at 4 °C prior to use.

### 2.2. Experimental essays

The experiment carried out in batch mode using the water displacement method for measuring the biogas produced. The biogas potential was based on the total volume of biogas produced during the degradation period and is defined as NmL biogas g<sup>-1</sup> VS added. Fig. 2 represents the set-up employed for the experimental procedure. In our set-up, 500 mL serum bottles were used for the essays (Fig. 2), flushed with N<sub>2</sub> for 2 min in order to maintain anaerobic conditions, placed in an incubator at a constant mesophilic temperature (36 ± 1 °C) and shaken at 150 rpm during the experimental period of the assay. Two tests studied with CD and SM digestion, the other three tests studied mixtures of CD:SM, CD:SSM (sterilized sheep manure) and SCD:SM with ratio 1:1 based on VS concentrations. Stocks samples (substrates solutions) were prepared and the serum bottles were filled starting with the substrates, followed by the addition of distilled water in order to achieve a working volume of 350 mL. No inoculum or additional external nutrients/trace elements was added to the serum bottles. The experimental conditions and the content of the reactors are given in Table 2.

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