



## Research article

## Anaerobic digestion of sulfate-acidified cattle slurry: One-stage vs. two-stage



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## ABSTRACT

Two strategies to include acidified cattle manure (AcCM) in co-digestion with normal cattle manure (CM) are presented in this work. The strategies are a single thermophilic (50 °C) continuous stirred tank reactor (CSTR) anaerobic digestion and a two-step (65 °C + 50 °C) CSTR process. In both strategies, two different inclusion levels of H<sub>2</sub>SO<sub>4</sub>-acidified CM (10% and 20%) in co-digestion with normal CM were tested and compared with a control CSTR fed only CM.

Important enhancement of methane (CH<sub>4</sub>) yield and solid reductions were observed in the thermophilic one-step CSTR working with 10% AcCM. However, a higher inclusion level of AcCM (20%) caused volatile fatty acid accumulation in the reactor and a more than 30% reduction in CH<sub>4</sub> production. In terms of CH<sub>4</sub> production, when 10% of AcCM was co-digested with 90% of CM, the two-step anaerobic co-digestion yielded less than the single step. During the first step of the two-step CSTR process, acidogenesis and a partial sulfate reduction were achieved. However, sulfide stripping between the first and the second step must be promoted in order to advance this technology.

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## 1. Introduction

Slurry acidification with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is used in Denmark at farm level to decrease ammonia (NH<sub>3</sub>) emission (Kai et al., 2008; Dai and Blanes-Vidal, 2013). Important reductions in carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and hydrogen sulfide (H<sub>2</sub>S) emissions have also been observed during storage of acidified slurries with H<sub>2</sub>SO<sub>4</sub> (Petersen et al., 2012; Dai and Blanes-Vidal,

2013). Acidification with H<sub>2</sub>SO<sub>4</sub> is considered an appropriate technology to conserve initial slurry composition and to avoid microbial activity during storage (Ottosen et al., 2009); the reasons, however, are less evident.

There are different possible explanations for the observed cessation of microbial activity in H<sub>2</sub>SO<sub>4</sub>-acidified slurries, but it is primarily due to the sudden decrease in slurry pH and the toxicity of the subsequent organic acids and sulfide (H<sub>2</sub>S) generated (Chen et al., 2008). However, in an anaerobic digestion of animal slurry with a low H<sub>2</sub>SO<sub>4</sub> concentration and no apparent change in the pH of the effluent, a significant reduction was nevertheless observed in microbial activity (Moset et al., 2012a, b). The explanation for the absence of microbial activity in those cases could be because of the creation of an imbalance by the H<sub>2</sub>S produced from H<sub>2</sub>SO<sub>4</sub> to the symbiotic relationships between different microbial groups (fermentative microorganisms, acidogens, acetogens, sulfate-reducing bacteria (SRB) and methanogens) that are formed to completely degrade the organic matter (Hulshoff Pol et al., 1998).

Surprisingly, these previous works carried out by Moset et al. (2012a, b) also showed that low inclusion levels of H<sub>2</sub>SO<sub>4</sub>-acidified slurries can increase CH<sub>4</sub> production in anaerobic reactors. Therefore, more research is needed to understand the microbial

**Abbreviations:** AcCM, acidified cattle manure; CH<sub>4</sub>, methane; CM, cattle manure; CO<sub>2</sub>, carbon dioxide; CSTR, continuous stirred tank reactor; H<sub>2</sub>S, hydrogen sulfide; H<sub>2</sub>SO<sub>4</sub>, sulfuric acid; NH<sub>3</sub>, ammonia; PR, pilot reactor 100 L total capacity; PR1, control pilot reactor 100 L total capacity fed cattle manure and working at 50 °C; PR2, one stage pilot reactor 100 L total capacity fed with mixture of acidified and non-acidified cattle manure and working at 50 °C; PR3, pilot reactor (100 L total capacity) used as second step of a two stages anaerobic digestion; R65, laboratory reactor with 20 L working capacity working at 65 °C used as first step of a two-stage anaerobic digestion; SRB, sulfate-reducing bacteria; TAN, total ammonia nitrogen; TS, total solids; VFA, volatile fatty acids; VS, volatile solids; VSc, corrected volatile solids.

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**Table 1**  
Average  $\pm$  standard deviation of the influents used to feed the reactors in each period: cattle manure and acidified cattle manure (N = 4 in Period 1 and 3 and N = 3 in period 2).

	Period 1		Period 2		Period 3	
	Cattle manure		Cattle manure	Acidified cattle manure	Cattle manure	Acidified cattle manure
TS (g L <sup>-1</sup> slurry)	76.6 $\pm$ 6.01		67.6 $\pm$ 10.84	76.5 $\pm$ 0.65	70.3 $\pm$ 0.50	92.2 $\pm$ 0.80
VS (g L <sup>-1</sup> slurry)	62.9 $\pm$ 3.59		56.2 $\pm$ 8.99	58.2 $\pm$ 4.08	57.7 $\pm$ 0.41	73.4 $\pm$ 8.97
VSc (g L <sup>-1</sup> slurry)	71.0 $\pm$ 2.77		60.9 $\pm$ 9.76	66.7 $\pm$ 3.95	63.7 $\pm$ 1.98	80.7 $\pm$ 8.92
pH	6.9 $\pm$ 0.12		7.1 $\pm$ 0.07	5.5 $\pm$ 0.33	7.1 $\pm$ 0.05	5.2 $\pm$ 0.07
TAN (g L <sup>-1</sup> slurry)	1.5 $\pm$ 0.05		1.3 $\pm$ 0.14	1.5 $\pm$ 0.36	1.7 $\pm$ 0.26	1.9 $\pm$ 0.35
Sulfate (g L <sup>-1</sup> slurry)	n.a. <sup>a</sup>		2.1 $\pm$ 0.11	10.3 $\pm$ 2.32	2.4 $\pm$ 0.28	11.1 $\pm$ 1.24
VFA (g L <sup>-1</sup> slurry)	8.5 $\pm$ 0.23		5.9 $\pm$ 0.99	8.4 $\pm$ 0.11	6.3 $\pm$ 0.60	7.4 $\pm$ 0.33
Acetic/VFA (%)	51.76		57.62	51.67	66.67	68.92
VFA/VSc (%)	11.97		9.69	12.59	9.89	9.17

<sup>a</sup> Not analyzed.

**Table 2**  
Operational parameters of the reactors used in this work.

	Control	One-stage anaerobic digestion	Two-stages anaerobic digestion	
Reactors	Pilot reactor 1 (PR 1)	Pilot reactor 2 (PR 2)	Laboratory reactor (R65)	Pilot reactor 3 (PR3)
Feed period 2	100% <sup>b</sup> CM	90% <sup>b</sup> CM + 10% <sup>c</sup> AcCM	90% <sup>b</sup> CM + 10% <sup>c</sup> AcCM	
Feed period 3	100% <sup>b</sup> CM	80% <sup>b</sup> CM + 20% <sup>c</sup> AcCM	80% <sup>b</sup> CM + 20% <sup>c</sup> AcCM	
<sup>a</sup> HRT (days)	20	20	4	16
Temperature (°C)	50	50	65	50
Reactors working volume (L)	100	100	20	80
Feed volume (L day <sup>-1</sup> )	5	5	5	5 from R65

<sup>a</sup> Hydraulic retention time.

<sup>b</sup> Raw cattle manure.

<sup>c</sup> Acidified cattle manure.

dynamics in anaerobic digestion of H<sub>2</sub>SO<sub>4</sub>-acidified slurries and to suggest technology that can improve the synergism between acidification and anaerobic digestion of animal manure.

In the present work we hypothesize that a two-stage thermophilic anaerobic digestion process could be an appropriate technology for acidified cattle manure; not only for the separation of SRB from methanogens, since as Vallero et al. (2004) showed, SRB can outcompete methanogens when the temperature increases from 55 to 65 °C in a methanol-fed sulfidogenic UASB operated at 55 °C, but also because a first thermophilic step has been shown to be an appropriate technology for lignocellulosic substrates in a two-step anaerobic digestion process (Gavala et al., 2003, Orozco et al., 2013).

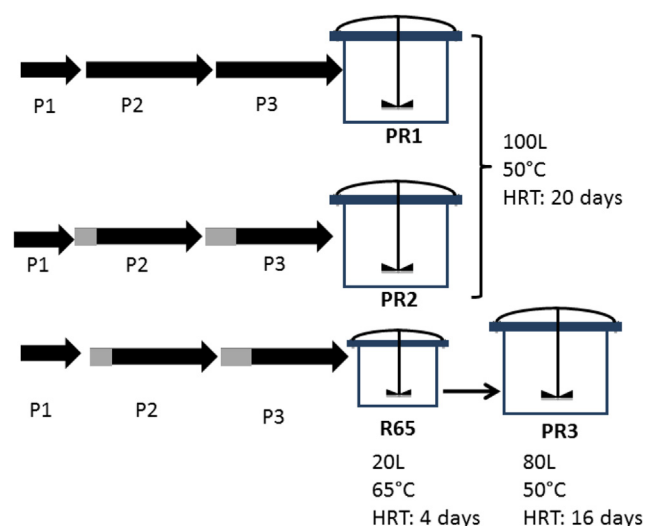
The desired two-step anaerobic digestion mechanism designed in this work consists of a first step at 65 °C (low thermal hydrolysis), followed by a second step at the normal thermophilic conditions of 50 °C. Biological sulfur removal (sulfate reduction and H<sub>2</sub>S volatilization) and hydrolysis will potentially be achieved in the first step. A reduction in the methanogenesis inhibition by H<sub>2</sub>S or even a methanogenesis enhancement could then be expected in the second step through increased availability of substrates produced in step 1 (hydrolysis). A comparable technology has been successfully tested in sulfur-rich tannery wastewater (Genschow et al., 1996) and acrylic fiber manufacturing wastewater (Li et al., 2012) where simple organic substrates are used. To our knowledge, the application of the concept in complex and slowly biodegradable substrates like cattle manure has not been investigated.

Therefore, the aims of the present work were, firstly, to determine the inhibitory thresholds for anaerobic digestion of acidified cattle manure in a long-term operation and, secondly, to evaluate the efficiency of a two-stage anaerobic digestion process in terms of biological sulfur removal and CH<sub>4</sub> yield with different inclusion levels of acidified slurry. To this end, two different levels of acidified cattle manure (10% and 20%) were tested in CSTRs in a long-term study.

## 2. Materials and methods

### 2.1. Substrates and anaerobic digesters

The two cattle manures (CM) used in this experiment were obtained at the experimental farms at Research Centre Foulum (Aarhus University, Denmark). Table 1 shows the composition of the influents used to feed the reactors in each period. In this experiment, acidified cattle manure (AcCM) was produced



**Fig. 1.** Schematic diagram of the experiment. P1: period 1 (28 days), P2: period 2 (49 days), P3: period 3 (43 days), PR1: reactor working with raw manure. PR2: reactor working with a mixture acidified + non acidified cattle manure. R65 and PR3: the first and second step, respectively, of the two-step anaerobic reactor. Period 2: PR2 and R65 were fed 10% acidified cattle manure +90% cattle manure, Period 3: PR2 and R65 were fed 20% acidified cattle manure +80% cattle manure.

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