The effect of the dairy feed additive monensin on the stability of manure-based anaerobic digesters

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Abstract
Dairy farmers can supplement the feed additive monensin to dairy cow diet rations to improve milk production efficiency. Few long-term studies have been conducted on the effect of monensin on AD performance for manure treatment. Here, we performed a year-long, laboratory-scale study in which four, 4.5-L continuously stirred anaerobic digesters (CSADs) were subjected to different monensin dosing strategies. We used manure from dairy cows that were dosed with increasing amounts of monensin (feed-dosed manure) or manure from monensin-free cows with monensin directly added afterwards (direct-dosed manure). We found that, despite slow increases in the monensin concentration in the feed-dosed manure fed to two CSADs, there was a small, but significant negative impact on the biogas production rate (~10-15%). This occurred at the highest feed-dosed manure levels from which the biogas production did not recover fully over the operating period, even though the CSADs maintained a stable performance. Analysis of the manure substrate, which was fed to the digesters, indicated that differences in substrate composition, such as gross energy, could have contributed to the reduced biogas production rate. Another CSAD fed the direct-dosed manure was able to acclimate to high monensin doses (5 $mg \cdot L^{-1}$) when the monensin dose was gradually increased. Such high concentrations of monensin are not anticipated at the dairy farm. Therefore, farm-based ADs should maintain stable performances with a possible reduced biogas production yield.

Keywords
Monensin, anaerobic digestion, antimicrobial, antibiotic, ionophore, methane, biogas, manure

INTRODUCTION

Monensin is an ionophore that is given to dairy cows to increase their milk production efficiency. Previous studies have shown a decrease in biogas production upon first introduction of monensin to anaerobic digesters (ADs) (Varel & Hashimoto 1981; Thaveesri *et al.* 1994). These studies indicated that monensin indirectly inhibits methanogenesis by depleting the precursor acetic acid. However, a follow-up, six-month study by Varel and Hashimoto (1982) indicated the capability of ADs fed monensin-laden manure to adapt to this ionophore when the hydraulic retention time (HRT) was slowly shortened.

Our study aimed to test a strategy for farmers to introduce monensin into their ADs in a manner that would minimize any upset to the methane production of the system and overall stability. This study also aimed to assess whether monensin degrades faster in monensin acclimated digesters *vs.* digesters that had not been acclimated to monensin. A previous study (Angenent *et al.* 2008) on the effect of tylosin on anaerobic digestion of swine waste found that the prophylactic antimicrobial tylosin was degraded rapidly by an acclimated digester microbiome.

MATERIALS AND METHODS

Seven dairy cows were fed the same diet except that three cows received a ration with monensin (Rumensin® 90 Premix, Elanco Animal Health) while four cows (control cows) received no monensin. Every two weeks, the monensin dose to the three cows was increased 100 $mg \cdot day^{-1}$ from ~200 to 500 $mg \cdot day^{-1}$ in a stepwise manner (M200-M500). The manure from the two groups of dairy cows was collected at the appropriate time points and stored frozen (-23°C) for later use.

Four, 4.5-L continuously stirred anaerobic digesters (CSADs) were fed semi-continuously (every two days) with manure at a target organic loading rate (OLR) of 2 g $VS \cdot L_{CSAD}^{-1} \cdot d^{-1}$. The systems were all operated at a 25-day HRT (except during startup (Table 1**Error! Reference source not found.**)) and a temperature of 37±1°C for the operating period of the study. The four CSADs (R1 to R4) were subjected to different monensin dosing strategies. Each of the different manure solutions were diluted based on volatile solids (VS) concentrations to achieve the same OLR for all CSADs. We used either manure from control cows (M0), monensin-fed cows (feed-dosed manure substrate; M200-M500), or from control cows with the direct addition of monensin (direct-dosed manure substrate) as monensin reference standard (Elanco Animal Health). R2 and R3 were fed feed-dosed manure according to the different stepwise increases (Table 1**Error! Reference source not found.**). R4 was fed direct-dosed manure with increasing monensin concentrations, initially by modelling to the monensin concentrations for the R2 feed (Period 2) and later to more rapid monensin concentration increases (Periods 3 and 4). In Period 5, R1 was no longer operated as the control CSAD and was fed direct-dosed manure with stepwise increases in monensin.

Table 1. Manure substrates fed to digesters in periods 1-5.

Period	Startup	1	2	3	4	5
Days	1-114	115-202	203-264	265-280	281-306	307-383
R1	M0					1 $mg \cdot L^{-1}$ to 5 $mg \cdot L^{-1}$*
R2	M0		M200 to M500**	M500		
R3	M0		M200 to M400**	M500		***
R4	M0		Added to R2 levels*	1 $mg \cdot L^{-1}$*	5 $mg \cdot L^{-1}$*	***

*These digesters were fed direct-dosed manure with the concentration of monensin added as given in the table (the dose was increased by 1 $mg \cdot L^{-1}$ every two weeks in Period 5 for R1). **These digesters were fed feed-dosed manure for which every two weeks a higher monensin concentration manure was used (except in Period 2 for R3 for which the digester was maintained at M400 for 36 days and only increased to M500 at the start of period 3). ***R4 was discontinued on day 306 and R3 on day 355.

We measured pH and biogas production (daily) and methane content in biogas and the following analysis for the effluent (weekly): 1. total and volatile solids (TS and VS); 2. total and individual volatile fatty acids (VFAs); 3. soluble chemical oxygen demand (COD); 4. alkalinity; and 5. total ammonium levels, according to Standard Methods (Eaton *et al.* 2005). We also characterized M0-M500. Statistical analyses were performed using the Tukey HSD model for comparing multiple means by pairwise comparisons in RStudio (v0.96.316). Monensin half-life studies were performed using serum bottles containing effluent from R1-R4. Monensin was added to each bottle and samples were collected throughout the operating period of the bottle. Monensin levels in the animal feed, manure substrate, half-life test samples, and digester effluent were assayed using liquid chromatography by an independent laboratory.

RESULTS AND DISCUSSION

Monensin excretion from dairy cows

In our study, a linear correlation was found between the measured monensin concentration in the consumed feed and in the manure, which indicated an excretion rate of 13% (R^2=0.81) of the parent monensin. The calculated average concentrations of monensin in the prepared feed-dosed manure substrates were 0.093±0.003, 0.125±0.003, 0.145±0.005, and 0.195±0.004 $mg \cdot L^{-1}$ for M200, M300, M400, and M500, respectively.

Decreased biogas production at higher feed dosed manure levels

A significant effect ($p<0.05$) of the feed-dosed manure on the biogas production was observed when feeding the higher feed-dosed manure levels (M400 and M500) to R2 and R3. Specific biogas production rates were reduced by 14% for R2 and R3 compared to the control (R1) in Period 2 when R2 and R3 were both fed M400 (days 231 to 244) (Figure 1). These percentages were calculated by using the mean value of the last five data points of this period. Similarly, during Period 4 (days 281 to 305) when R1 remained the control CSAD and R2 and R3 were fed M500, the specific biogas production rate was 15% and 9% lower for R2 and R3 compared to R1, respectively. For all CSADs, the methane content of the biogas was approximately equal (average: 54.70±1.98% from Period 1 to the end of study), except for declines in methane content for R4 during Period 4 and for R1 during days 325-341. In the last 25 days of Period 1, when the digesters were all fed the same M0, ANOVA analysis showed a significant difference between the biogas production rates for the CSADs. Closer analysis (Tukey HSD model) revealed that only R4 was significantly different from R2 and R3 (note: R1 compared to R3 obtained a p-value of 0.04). During the first 100 days of our study, we believe that R4 was plagued by small leaks in the gas lines and a larger leak, which were all repaired.

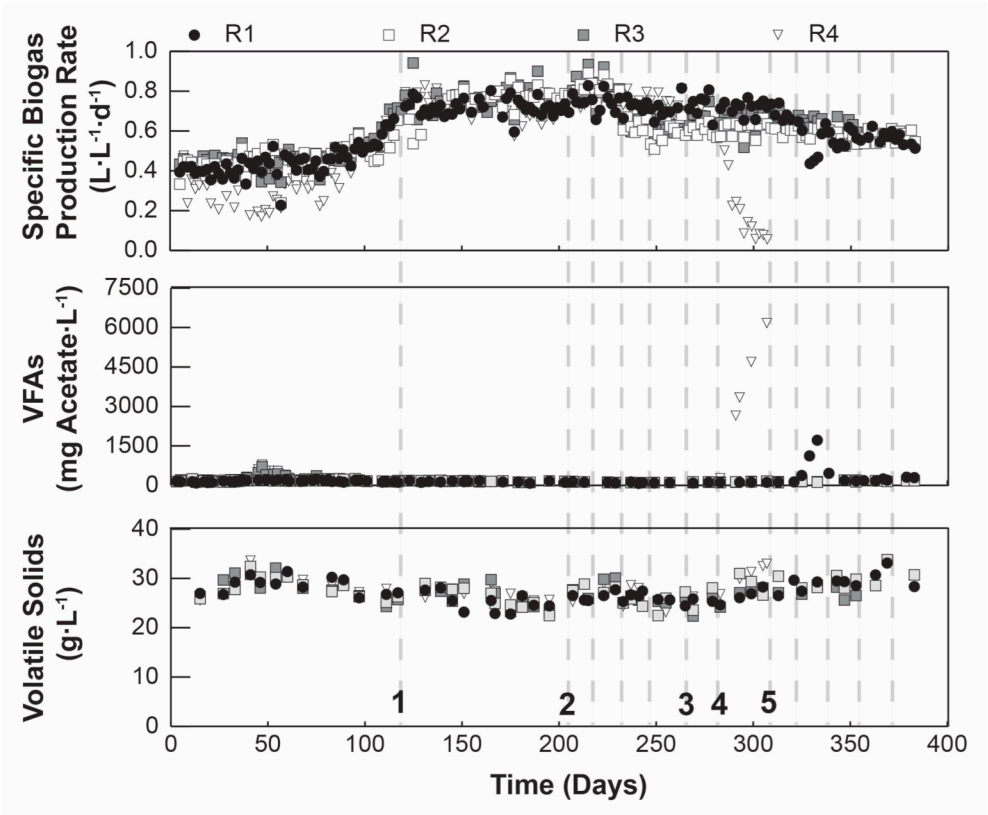

Figure 1. Specific biogas production rates, VFAs concentrations, and VS levels for R1-R4. Numbers indicate the separate periods, while dashed lines indicate changes in CSAD conditions within periods (*i.e.,* increase in feed-dosed manure level every two weeks for R2 in Period 2).

No significant differences were noted between the effluent VFAs or VS levels of all the CSADs during Periods 1-3, indicating stable performances. Despite the use of direct-dosed manure for R4 that modelled R2 substrate monensin concentrations, no significant decrease in R4 biogas production was observed during Period 2. A different biogas production with the same concentrations of monensin could indicate that the lower biogas production rates for R2 and R3 *vs*. R1 (control) were due to difference in the quality of M400 and M500 compared to M0. We, therefore, hypothesized that the decreased biogas production rates for R2 and R3 was, at least in part, due to differences in the manure substrate composition, rather than only a direct effect of the

monensin to digester performance. Indeed, a preliminary analysis (Tukey HSD model) revealed significant differences between some of the feed-dosed manure substrates and M0 in terms of pH, alkalinity, total ammonium, acetic to propionic acid ratios, gross energy, and total VFAs.

However, at this point we cannot rule out a direct effect of monensin on biogas production in R2 and R3 during Period 2, especially since we found significant differences between the biogas production rates for R2 and R3 *vs*. the control (R1). In addition, another study had already found a negative effect on biogas production in laboratory-scale upflow anaerobic sludge blanket (UASB) reactors treating synthetic wastewater at monensin concentrations of only 0.1 $mg \cdot L^{-1}$ (Thaveesri *et al*. (1994). Therefore, the low concentrations of monensin in M400 of 0.145 $mg \cdot L^{-1}$ could explain the first onset of biogas production rate drops in R2 and R3. Regardless, at these concentrations we observed stable performances by maintaining very low concentrations of total VFAs. The anticipated low concentrations of monensin in the manure at dairy farms should, according to our study, not cause unstable digester performance.

Acclimation of system to monensin

We never observed a full recovery of biogas production to pre-monensin levels in R2 and R3 for these low concentrations of monensin, which had been observed in a study by Varel and Hashimoto (1982). However, we did observe acclimation at higher monensin concentrations. When the monensin dose fed to R4 was rapidly increased from 1 to 5 $mg \cdot L^{-1}$, a considerable increase in total VFAs and corresponding decrease in specific biogas production was observed (Figure 1), resulting in unstable performances. Subsequently, when monensin was dosed to R1 (from then on not a control CSAD) more gradually (Period 5 in Figure 1), the digester acclimated to the monensin, with the first performance drop starting on day 325 and then recovering to R2 and R3 performance levels. Measurements of the influent and effluent monensin concentrations for all digesters indicate that monensin was not rapidly degraded in the digester system. These findings suggest that the microbiomes of the digesters acclimate to monensin rather than rapidly degrading it.

Future Work

To gain further insight into the effect of monensin on the CSADs, we will characterize the microbial community dynamics. We are performing 16S rRNA gene sequencing using barcoded primers (V4 region) with Illumina MiSeq for the weekly biomass samples that we have collected.

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