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Mitigating Greenhouse Gas and Ammonia Emissions from Stored Slurry through the Addition of Brewing Sugar and a Biological Additive

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSBB designed the study, analysed and interpreted the data, managed literature searches and prepared first draft of the manuscript. Finally, all authors read and approved the final manuscript.

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ABSTRACT

Livestock slurry stores are a key source of ammonia (NH_3) and greenhouse gas (GHG) emissions. This study evaluated the potential to reduce NH_3, CO_2, CH_4 and N_2O emissions by adding effective microorganisms (EM) and brewing sugar to beef cattle slurry in a replicated small-scale (1 litre slurry volume) experiment. The effect of EM and brewing sugar was explored at two concentrations (5 $^v/_w$ and 10% $^w/_w$ respectively) and in two environments (cold and warm) over a period of 30 days slurry storage. Greenhouse gas emissions were measured by taking headspace samples from the closed vessels over a 1 hour period, whilst relative NH_3 loss was quantified at the same time by placing an acid trap within the closed headspace. Brewing sugar addition induced 'self-acidification' of the slurry, via lactic acid production and accumulation, resulting in a decrease in slurry pH from pH 7.8 to <4.5. This was effective in lowering average NH_3 loss in the cold and warm environments by 40% and 70%, respectively. Methane emissions were also reduced following the addition of brewing sugar, by up to 75%, resulting in a reduction in the cumulative total GHG (N_2O + CH_4 + CO_2) emission (expressed as CO_2 equivalent; CO_2e) of 34% and 85%, respectively. The total greenhouse gas emission (CO_2e) during slurry storage was dominated by CH_4, representing at least 59% of total CO_2e emitted. Effective microorganisms had little impact on NH_3 and GHG emission, and are not deemed a useful mitigation strategy for these gases.

Keywords: Slurry storage; greenhouse gas; ammonia; mitigation; additives.

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1. INTRODUCTION

Livestock production contributes between 7% and 18% of global anthropogenic greenhouse gas (GHG) emissions [1]. Both methane (CH_4) and nitrous oxide (N_2O) are potent GHG, and are estimated to contribute possess between 34 and 298 times (respectively) the global warming potential (GWP, over 100 years) relative to carbon dioxide (CO_2) [2]. In addition, current manure management practice is also responsible for 13% and 23% of total UK NH_3 loss during storage and following application of slurry to land [3]. The need to reduce GHG emissions therefore represents a major challenge for the livestock industry whilst simultaneously meeting the increasing demand for livestock products due to the burgeoning and more affluent population.

Slurry based livestock systems are commonplace, offering easy handling and storage of animal-derived waste. Methane emission from slurry is the main GHG produced during storage, being the end product of anaerobic decomposition [4]. Efforts to reduce CH_4 emission include the holding of slurry at cooler temperatures which slow the methanogenesis process [5]. For example, a possible 74% reduction in CH_4 emission was observed by storing pig slurry at 10°C relative to 20°C [6]. This is further supported by the observation that CH_4 release from stored slurry was lower under winter and spring climates conditions [7]. Slurry removal from the tank during the summer may further reduce CH_4 emission by 9% to 10% [8]. Similarly, frequent and complete slurry removal from the storage tank will lower CH_4 emission due to the lower methanogen inoculum and the prolonged methanogenic lag phase [9]. Generally, the natural or promoted formation of a surface crust on the slurry should be avoided to reduce nitrification and thus prevent the formation of NO_3^-, the precursor for N_2O emission [5]. However, a crust will also act as passive barrier and can reduce NH_3 and CH_4 emission [10–11] and may also act as CH_4 sink by supporting methanotrophs and CH_4 oxidation [12-14]. In some of the best systems, airtight, artificial covers are used to trap CH_4 as biogas, while simultaneously minimizing NH_3 losses [5,15].

The solid and liquid components of slurry can be mechanically separated to remove the organic fraction, which is responsible for promoting both N_2O and CH_4 formation (5). Reports have shown that the combined CH_4 and N_2O emission (expressed as CO_2e) from both the separated solid and liquid slurry is not always lower, suggesting that this approach may not be efficient unless anaerobic digestion of the solid fraction is taken into consideration [5]. At the same time, net NH_3 loss from the separated liquid fraction also increases by 44% [16].

A reduction in CH_4 and NH_3 production during slurry storage can also be achieved by the forced acidification of the matrix by the addition of strong acid such as sulphuric acid (H_2SO_4), hydrochloric acid (HCl), lactic acid ($C_3H_6O_3$) or nitric acid (HNO_3) [17,18]. Storing slurry below pH 5.5 inhibits CH_4 emission by 67 to 87%, and a higher inhibition rate is further expected if continuous in-house acidification is performed on the slurry [17–19]. Lowering the pH can also inhibit NH_3 emissions by >95% [20,21], while others have reported reductions of 40 to 70% [17,21,22]. To ensure the most effective and economic process is undertaken by farmers, it is important to use the most appropriate acid to prevent undesirable environmental effects such as an increase in N_2O generation [23,24]. There is also a need to replace the use of concentrated acids to overcome health and safety implications and to explore new GHG and NH_3 mitigation strategies. In addition, reducing NH_3 volatilization during slurry storage will benefit farmers by retaining more available nitrogen (N) for crop uptake when slurry is applied to soil [18,22,25]. The addition of an organic carbon source has been shown to reduce slurry pH, however, its impact on GHG emissions remains poorly understood [26,27].

In comparison to acid addition, there has been relatively little research on other potential additives which can be added to slurry to reduce GHG emissions. [28,29] showed that the addition of effective microorganisms (EM) to stored cattle slurry significantly lowered NH_3 and N_2O emissions by 20 and 17%, respectively, while no change was seen for CH_4. However, [28] did not observe any effect of EM addition on NH_3 and N_2O emissions from swine slurries, possibly due to an insufficient inoculum of introduced microorganisms needed to overcome the dominance of the intrinsic slurry microbial population [30,31].

Despite the mitigation methods described above, the practice is limited to large cattle farm holdings and enterprises with large herd size.

Therefore, the practice is deemed as impractical and uneconomical for small medium entrepreneurs with small herd size, not to mention the possible association with the legal, health and safety issues. Meanwhile, mitigation practice for small-medium cattle farms remains underexplored and requires further attention. This study may help the current move to reduce the global GHG emission as advocated by the Kyoto Protocol (1997) under the United Nations Framework Convention on Climate Change (UNFCCC). This aim of this study was to determine the effect of two additives with different modes of action on GHG and NH_3 emissions from stored slurry. Cattle slurry was amended with either a carbohydrate source to induce 'self-acidification' of the slurry, or an EM bio-inoculum (at greater concentration than used by Amon et al. [28,29] or a combination of the two treatments. The emissions of GHGs and NH_3 were measured over 30 days under two different environments to simulate storage conditions in different climates; a winter climate and a controlled 30°C temperature (i.e. cold and warm).

2. METHODOLOGY

2.1 Slurry Preparation and Experimental Design

Fresh cattle slurry was collected from the reception pit of a commercial beef cattle farm near Henfaes Research Centre, Bangor University, UK. Slurry was stored for 3 days in 200 litre plastic tanks, and coarse material (e.g. uneaten grass silage/hay) was removed manually prior to the start of the experiment. The experimental design for the experiment comprised three additive treatments, 10% $^{w}/_{w}$ brewing sugar (Better Brew, Hambleton Bard, UK), 5% $^{v}/_{w}$ activated EM (Actiferm EM®, Effective micro-organisms UK, Exeter, UK), a combined 10% brewing sugar + 5% EM treatment, and a control (no additive) slurry. The four treatments are termed Sugar, EM, Sugar+EM and Control throughout this paper. One litre of each treatment was placed in a 2 litre plastic storage vessels. There were two temperature regimes; a 'cold' environment where the kilner jars were kept outdoors under cover during December 2013 at the Bangor University farm, and a 'warm' environment where the kilner jars were housed in a 30°C incubator (Clarkson F10400160 Incubator, Chula Vista CA, USA) at the same location.

2.2 Slurry Physiochemical Observation

2.2.1 Slurry dry matter and volatile solids content

Slurry dry matter (DM) was determined by drying *ca.* 10.0 g slurry at 80°C (24-48 hr), before further drying at 105°C to constant weight (24-36 hr). The volatile solids (VS) content was measured on dried samples as loss-on-ignition at 450°C for 16 hr in a muffle furnace (Carbolite CWF 1200, Carbolite Ltd, UK).

2.2.2 Volatile fatty acid and lactic acid determination

Slurry samples for volatile fatty acid (VFA) determination were prepared following the methods of [32] and [33]. Briefly, 15 mL of slurry was centrifuged at 4000 rpm (Eppendorf 5810R, UK) at 15°C for 30 minutes, 2 mL of the supernatant was then mixed with 400 uL metaphosphoric acid (HPO_3) and incubated for 30 minutes at 4°C. Samples were then centrifuged again at 10000 rpm at 15°C for 20 minutes. The supernatant was transferred into a GC vial and an internal standard of 2-ethyl butyric acid (300 mM) internal standard was added. The sample was then injected into a Varian 3380 gas chromatograph (GC) fitted with a Free Fatty Acid Phase (FFAP) column (25 m x 0.32 mm x 0.5 µm) (Agilent J&W GC column) and a flame ionization detector with a split ratio 1:10. The GC was supplied with nitrogen (N_2) carrier gas with a column flow rate of 1.4 ml min^{-1}, head temperature 250°C, column oven 80°C (0.2 s) ramped at 20°C min^{-1} to 170°C (3.2 min), and then ramped at 65°C min^{-1} to 240°C (4.5 min). The lactic acid content of the supernatant was determined using a D-/L-Lactic Acid (D-/L-Lactate) rapid assay kit (Megazyme, Co. Wicklow, Ireland).

2.2.3 Total carbon (C) and nitrogen (N)

The total C and N content were measured on fresh slurry samples by a TruSpec® CN analyzer (Leco Corp, St Joseph, MI).

2.2.4 Slurry sampling through the experiment

Periodic sampling was carried out to determine the slurry pH, oxidation redox potential (ORP), moisture loss and slurry temperature. The air temperature was recorded eight times a day continuously using an Ibutton® (Thermochron, USA), whilst slurry temperature, pH and ORP

were measured using a portable pH/ORP/ temperature meter with electrodes/probes (model HI 991003, Hanna Instruments, USA). Slurry moisture loss was recorded by measuring slurry weight loss.

2.2.5 Ammonia volatilisation

Relative NH_3 volatilisation was determined by placing an acid trap, 0.02 M orthophosphoric acid (H_3PO_4), into the sealed (non-ventilated) headspace above the slurry when the vessel was sealed for GHG sampling [34]. A disposable pasteur pipette was modified to hold 3 mL H_3PO_4 during the one-hour vessel closure period. The NH_4^+ content of the H_3PO_4 represented the relative amount of NH_3 volatilized during the incubation period. The NH_4^+ concentration in the H_3PO_4 acid was analysed as described by [35]. Briefly, prior to incubation at 30°C, 6% Na_2EDTA, Na-salicylate-nitroprusside and hypochlorite solution was added (15, 60 and 30 µL). Na-salicylate-nitroprusside solution consists of 7.8% ($^w/_v$) Na-salicylate and 0.125% ($^w/_v$) Na-nitroprusside while hypochlorite solution (pH 13) contains 2.96% ($^w/_v$) NaOH, 9.96% K_2HPO_4 ($^w/_v$) and 10% ($^v/_v$) Na-hypochlorite. Absorbance readings were measured using a microplate reader (Biotek Power Wave XS, Winooski, USA) at a wavelength of 667nm and analysed by Gen 5 software Biotech (Instruments, Inc, USA).

2.2.6 Greenhouse gas sampling

The slurry vessels were sealed with airtight lids fitted with butyl rubber septa at the time of each sampling. The headspace volume above the slurry surface was *ca.* 1000 mL. The lid was left in place for one hour, during which three subsequent samples were withdrawn, at times 0, 30 and 60 minutes. 20 mL gas sample were withdrawn using a syringe (Therumo, UK Ltd) with a 25G 16 mm needle and transferred into 20 mL pre-evacuated gas chromatograph (GC) glass vials. Gas samples were kept at room temperature until analysed using a Perkin Elmer Clarus 580 Gas Chromatograph equipped with a flame ionization detector, and methanizer to allow detection of both CH_4 and CO_2, and an electron capture detector for N_2O analysis. The GC was linked to a Perkin Elmer Turbo Matrix 110 auto sampler. Cumulative emissions for 30 d periods were calculated by interpolating between measurements using the trapezoidal rule, based on fluxes obtained during five occasional sampling.

3. RESULTS AND DISCUSSION

3.1 Slurry Characteristics

At the start of the experiment, the slurry DM was 10.7% (±0.02), while the VS content was 81.4% (±0.30). The DM content was higher than a typical cattle slurry (6%; [36]), but the slurry was taken from the reception pit at a time of low rainfall. After the 30 d storage period, the brewing sugar treatments had significantly greater DM content than the control (Table 1 and Table 2). This was also the case for the VS content, total C and N in the Sugar and Sugar+EM treatments in both temperature regimes (Table 1 and Table 2). The high DM values were associated with the brewing sugar addition. The practical disadvantage of a thicker slurry is that it may require a more powerful pump during slurry transfer, especially in field applications [37]. However, thicker slurry promotes faster crust formation [12,38], which can be useful as a natural, cheap passive barrier for NH_3 emission (10). In contrast, thinner slurry (lower % DM) can infiltrate soils easier; thus reducing potential NH_3 volatilisation after spreading slurry [39,40].

Although there was some moisture loss during the 30 d experiment, this was not significantly different between the treatments possibly due to the short observation period, with 22-28% loss recorded from the cold and 34-39% loss from the warm storage environments. At both temperatures, a crust or a layer of floating solid material was observed on the slurry surface, and started to develop after 7 d [38,41]. A crust is often seen on any undisturbed slurry with DM content >2% [10–11,41] and it was seen to be softer for the brewing sugar treated slurries (Sugar, Sugar+EM) than the other treatments.

3.2 Changes in Slurry Characteristics during the Storage Period

The average ambient temperature during the cold (winter) storage was 10°C, while the warm storage air temperature was constant, at 30°C. As can be seen, slurry temperatures largely paralleled ambient environments in both storage environments during the 30 days storage period (Figs. 1a and b), which is similar to observations of [42] at >1000 m^3 storage capacity.

Table 1. Influence of sugar and effective microorganism addition on the characteristics of cattle slurry stored for 30 d at 10°C

Perimeters	Control	Sugar	EM	Sugar+EM
pH	7.4±0.11^{b}	4.7±0.03^{a}	7.3±0.19^{b}	4.6±0.02^{a}
Redox potential (mV)	-225.2±11.9^{b}	-61.4±16.7^{a}	-274.2±9.4^{b}	-65.8±17.3^{a}
Dry matter (% FWt)	10.6±0.38^{b}	16.3±0.41^{a}	10.2±0.27^{b}	17.8±1.96^{a}
Volatile solid (% DM^{-1})	79.1±2.85^{b}	88.3±0.39^{b}	78.7±1.33^{b}	89.8±1.96^{b}
Ammonium-N (g N kg^{-1} FWt)	0.8±0.01	1.0±0.16	0.9±0.01	0.8±0.01
Nitrate-N (g N kg^{-1} FWt)	< .01	< .01	< .01	< .01
Total C (g C kg^{-1} FWt)	42.6±2.67^{b}	84.1±1.68^{a}	42.2±3.34^{b}	82.9±2.34^{a}
Total N (g N kg^{-1} FWt)	4.5±0.17^{b}	5.3±0.27^{a}	4.9±0.33^{b}	5.6±0.32^{a}

Means within the same row with no common superscript differ significantly (P ≤ .05). Data represent Mean ± SEM, n=5

Table 2. Influence of sugar and effective microorganism addition on the characteristics of cattle slurry stored for 30 d at 30°C

Perimeters	Control	Sugar	EM	Sugar+EM
pH	7.8±0.10^{b}	4.1±0.12^{a}	7.4±0.20^{b}	3.8±0.10^{a}
Redox potential (mV)	-342.4±10.0^{b}	-68.3±12.9^{a}	-304.4±10.0^{b}	-52.6±10.9^{a}
Dry matter (% FWt)	11.2±0.27^{b}	16.7±0.80^{a}	10.3±0.23^{b}	15.5±0.20^{a}
Volatile solid (% DM^{-1})	77.9±0.29^{b}	86.8±0.04^{a}	78.1±0.50^{b}	86.7±0.15^{a}
Ammonium-N (g N kg^{-1} FWt)	1.0±0.16	0.6±0.01	0.8±0.01	0.7±0.17
Nitrate-N (g N kg^{-1} FWt)	< .01	< .01	< .01	< .01
Total C (g C kg^{-1} FWt)	50.0±2.03^{b}	84.3±1.98^{a}	43.7±1.15^{b}	82.4±3.18^{a}
Total N (g N kg^{-1} FWt)	4.5±0.17^{b}	5.3±0.27^{a}	4.9±0.33^{b}	5.6±0.32^{a}

Means within the same row with no common superscript differ significantly (P ≤ .05). Data represent Mean ± SEM, n=5

The brewing sugar addition resulted in significant decreases in slurry pH either in the presence or absence of the EM, compared with the EM and Control treatments. The rate of 'self-acidification' was significantly greater at 30°C (than at 10°C), with a rapid decrease in slurry pH to 5-6 within 2 days, and a further decrease in pH to pH 4.3 for the brewing sugar treatments by day seven. This compares with a more gradual pH decrease to pH 4.4 at day 21 in cold storage conditions (Figs. 1c and d). This is clearly differentiated by the quadratic regression equation fitted for the rate of pH decrease: where the acidification rates were -0.30 pH units day^{-1} ($R^2=0.844$) and -0.221 pH units day^{-1} ($R^2=0.997$) at the warm and cold storage conditions, respectively.

The lower pH in the brewing sugar treatments suggests induced acidification as a result of anaerobic decomposition of the carbohydrate source and an accumulation of organic acids [43,44]. This is supported by the trend of the slurry ORP values for the brewing sugar treatments (Figs. 1e and f). The ORP level increased from -231.6 mV on day 0 to -37.8 mV on day 21 in the cold storage conditions, and increased at a faster rate to -52 mV (mean) on day 7 in the 30°C environment. However, the ORP level of the EM and Control treatments remained unchanged (P > .05) at below -230 mV for the duration of the experiment. The acidified slurry showed a negative correlation between ORP and pH at -0.89 and -0.80 (P < .05) at cold and warm storage, respectively.

3.3 Lactic Acid and Volatile Fatty Acid Content

Lactic acid production by homo/hetero lactic acid fermentation [45] and accumulation in the Sugar and Sugar+EM treatments possibly explains the pH decreases during storage following addition of brewing sugar. This is similar to the hydrolysis of carbohydrate such as kitchen waste in anaerobic digesters, where fermentation results in lactic acid production [46]. The amount of lactic acid found in the these treatments at the end of the experiment were between 4.2 to 4.8 g L^{-1} (Table 3), while in the EM and Control treatments the concentrations were <0.1 g L^{-1}. Meanwhile, there was no indication of the different treatments on slurry VFA content (Appendix 1), with values within the normal range (1.21 and 17.0 g L^{-1}) as stated by previous authors [33,47–48].

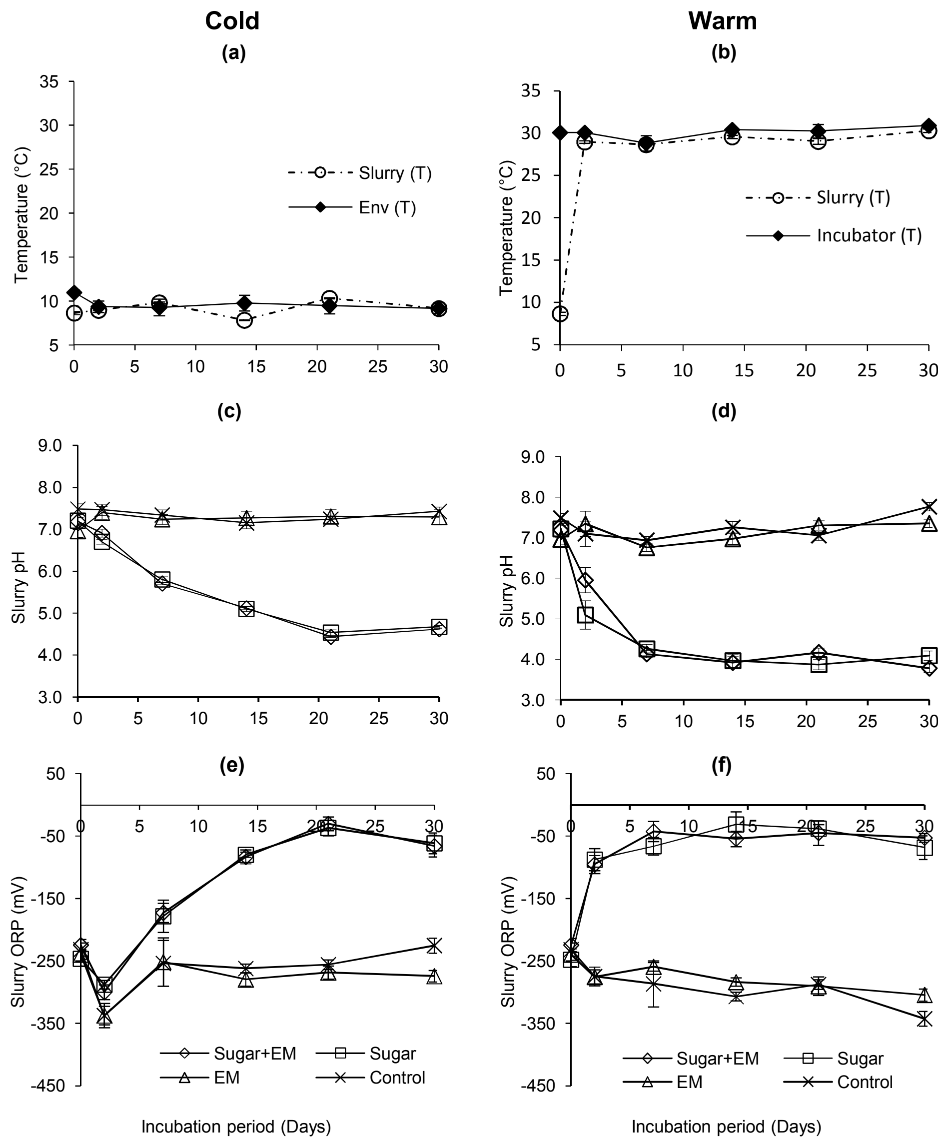

Fig. 1. Influence of sugar and effective microorganism addition on changes in slurry temperature (a, b), pH (c, d) and ORP (e, f) during the 30 d storage period under cold (10°C) and warm (30°C) environments. Data represent Mean ± SEM, *n*=5

3.4 Ammonia Volatilisation

The cumulative indicative NH_3 losses during this short storage period (30 days) are shown in Fig. 2 and Table 4. The average cumulative N loss through volatilized NH_3 recorded from 30°C incubation were nearly 2.6 times greater than from the cold storage, as NH_3 partitioning to the vapour phase increases with temperature [49]. The highest NH_3 volatilisation in warm storage recorded from Control, followed by EM treated slurry as both slurries were recorded at mean pH 7.6 compared to Sugar and Sugar+EM treated slurries at pH <4.3, which recorded 51% and 45% lower emission respectively, (compared to Control). Similarly, there were high cumulative indicative NH_3 emissions from the cold storage conditions from the untreated (Control) and EM treated slurries. The lowest cumulative emission recorded from brewing sugar addition (Table 4). These lower emissions was relatively greater than warm storage equivalent to 64% and 70% in both Sugar and Sugar+EM respectively. The large reduction in NH_3 volatilisation from the brewing sugar added treatments in both storage condition (cold and warm) was likely due to

reduction of slurry pH, to <5.5 [5,17,21,38]. Overall, the more acidic the slurry, the greater the reduction in NH_3 losses. This is supported by [50], who showed that NH_3 losses were reduced by 77% at pH 5.0, compared with a reduction of 50% at pH 6.0. Our results corroborate similar findings from [26] where sucrose application to slurry reduced the pH to <5.0 and greater acidification (pH <4.0) was seen following the addition of bio-waste at 50 g L^{-1}.

As can be seen in Fig. 2, there were lower NH_3 volatilisation rates from all slurry treatments after day 15 until the end of the storage period. This lower volatilisation was associated with the development of a surface crust that acted as a natural barrier to airflow and emissions [10–11,38,41,51]. A surface crust forms easily on slurry with a DM content >2% in the absence of surface disturbance (wind, pumping etc.) [11].

Studies using sulphuric acid (H_2SO_4) acidification of slurry to pH 5.5, have resulted in larger NH_3 reductions, e.g. by 95% [21]. The large NH_3 volatilisation inhibition effect of mineral acid addition is most probably the result of the instant/immediate acidification that occurs when concentrated mineral acids are used, and particularly when the slurry is stored over longer periods [17,21]. Yet acidification by H_2SO_4 could increase hydrogen sulphide gas (H_2S) production significantly during entire storage [17], again with important implications for odour emission and farm worker health and safety.

Table 3. Influence of sugar and effective microorganism addition on the production of lactic acid in cattle slurry after a 30 d incubation period

Treatment	Lactic acid ($C_3H_6O_3$) concentration (g L^{-1} FWt)	
	Cold, 10°C	Warm, 30°C
Control	< 0.1[b]	< 0.1[b]
Sugar	4.24±0.05[a]	4.74±0.03[a]
EM	< 0.1[b]	< 0.1[b]
Sugar+EM	4.18±0.10[a]	4.80±0.04[a]

Means within the same column with no common superscript differ significantly (P ≤ .05). Data represent Mean ± SEM, n=5

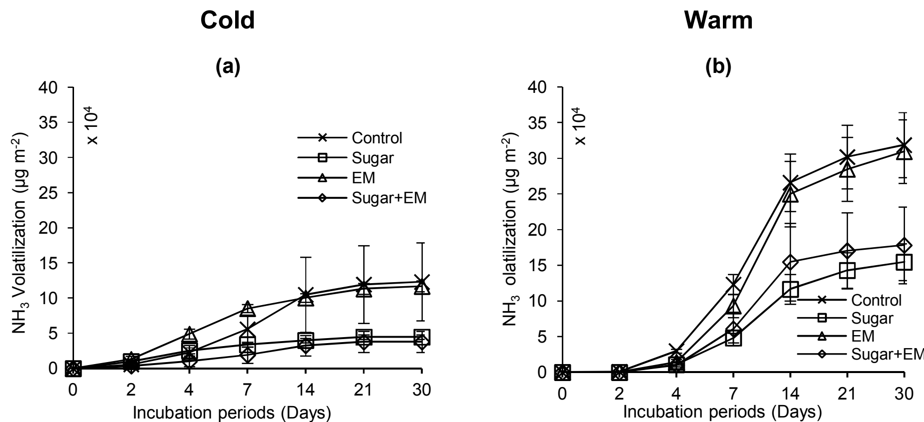

Fig. 2. Influence of sugar and effective microorganism addition in cumulative ammonia volatilisation during the 30 d storage period under cold (10°C) and warm (30°C) environments. Data represent Mean ± SEM, *n*=5

Table 4. Influence of sugar and effective microorganism addition in inhibition of ammonia emission from cattle slurry after a 30 d incubation period under cold (10°C) and warm (30°C) environments

Treatment	Cold, 10°C		Warm, 30°C	
	Ammonia emission (mg m^{-2})	Percentage inhibition compared to Ctrl (%)	Ammonia emission (mg m^{-2})	Percentage inhibition compared to Ctrl (%)
Control	123.2±55.4	0.0	318.8±17.5[b]	0.00
Sugar	44.7±3.2	63.7	154.6±24.2[a]	51.5
EM	116.9±7.8	5.1	309.6±18.3[b]	2.9
Sugar+EM	37.8±14.9	69.3	178.1±32.1[a]	44.1

Means within the same column with no common superscript differ significantly (P ≤ .05). Data represent Mean ± SEM, n=5

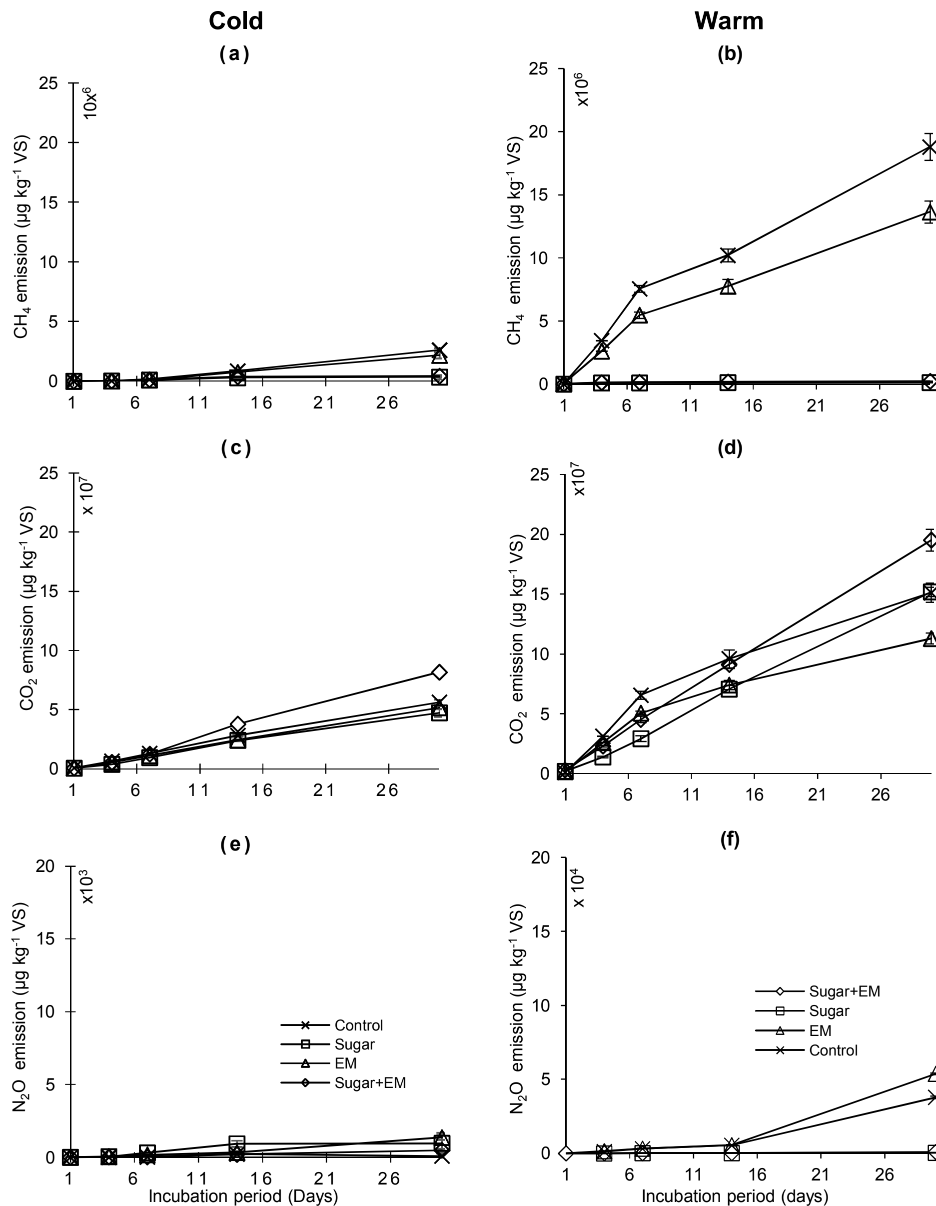

Fig. 3. Influence of sugar and effective microorganism addition in cumulative greenhouse gas emissions during the 30 day storage period under cold (10°C) and warm (30°C) environments. Data represent Mean ± SEM, *n*=5

3.5 Greenhouse Gas Emission

The slurry stored at the higher temperature resulted in higher CH_4 and other gaseous (CO_2 and N_2O) emissions than from the slurry stored in the colder conditions (Fig. 3 and Table 5) as a result of higher microbial activity, hydrolysis and biodegradation activity by mesophilic microbes than psychrophilic microbes [52]. [53] also noted the influence of temperature on CH_4 emissions from slurry stores, while lower emissions from cool climates suggest production was predominantly from psychrophilic methanogens [54].

In this study, the key finding from both temperature environments was that the cumulative CH_4 emitted was significantly reduced ($P < .05$) from the 'self-acidified' slurry treatments following brewing sugar addition. This effect was

Table 5. Relative comparison of slurry greenhouse gas emission (CH_4, CO_2, N_2O and CO_2e) influenced by the addition of sugar and effective microorganism after a 30 d incubation period under cold (10°C) and warm (30°C) environments

Storage	Greenhouse gas emission ($g\ kg^{-1}$ VS CO_2e)				Proportion CH_4/GHG (%)	CH_4 inhibition (%)	GHG inhibition (%)
	CH_4	CO_2	N_2O	GHG CO_2e			
Cold, 10°C							
Control	88.65	56.08	0.03	144.8	61.2	0.0	0.0
Sugar	11.49	47.31	0.29	59.1	19.4	87.0	59.2
EM	73.53	51.68	0.41	125.6	58.5	17.0	13.2
Sugar+EM	14.55	81.59	0.14	96.3	15.1	83.6	33.5
Warm, 30°C							
Control	639.32	151.18	11.29	801.8	79.7	0.0	0.0
Sugar	4.79	151.88	0.23	156.9	3.1	99.3	80.4
EM	464.16	112.96	16.04	593.2	78.3	27.4	26.0
Sugar+EM	7.98	195.15	0.27	203.4	3.9	98.8	74.6

greatest in slurry stored at warmer temperatures (ca. 99% reduction) compared to those stored in cold temperatures (ca. 85% reduction) (Fig. 3a and 3b, and Table 5). Anaerobic fermentation of brewing sugar resulted in accumulation of lactic acid, which subsequently decreased the slurry pH. The lower pH then inhibited CH_4 production by the acetoclastic methanogens [55]. As can be seen in Table 5, CH_4 represented between 59 and 80% of the total GHG CO_2e emitted from the non-acidified slurry, so it is important to evaluate mitigation strategies during slurry storage.

Overall, CH_4 fluxes were lower in the cooler environment compared to emissions from slurry incubated at warmer temperatures. Previous finding [56] showed that CH_4 emissions from stored slurry were higher, by 130%, at 25°C compared to 5°C. Therefore, another CH_4 emission mitigation strategy would be to site slurry stores in cooler places (e.g. in the shade of buildings and trees), or to actively cool slurry stores, which is consistent with the findings of Husted (1994) as reviewed by [57].

A CH_4 reduction of 30% to 46% is achievable when slurry is cooled considerably compared to non-cooled slurry [58]. Under warmer condition, mitigation of CH_4 emission from slurry stores can be reduced by emptying the storage tank frequently to reduce the methanogen inoculum and the mass of available carbon used by the methanogens [5,8,59–60]. Methanogens are facultative anaerobes, so increased oxygen (O_2) concentration inhibit CH_4 production. Hence, frequent emptying of slurry stores and spreading slurry on land will reduce total CH_4 emissions, as would high rate aeration of the slurry [61].

In contrast to the effects of brewing sugar on CH_4 and NH_3 emissions, the addition of Actiferm EM® resulted in only a small reduction in CH_4 emission between 17% and 27% in both storage conditions, hence the GHG CO_2e was reduced by 13 and 26%, respectively (Fig. 3a, 3b and Table 5). [62] suggested that EM addition benefits the slurry environment by supplying beneficial organisms, enhancing the microbial diversity of the slurry. The EMs are thought to compete with harmful microorganisms by releasing beneficial substances such as enzymes, organic acids, amino acids, hormones and antioxidants that promote the health of the slurry environment [31]. However, [29] reported that EM addition led to a small increase in CH_4 emission. Difference in the microbial composition of commercial EM products, and different EM concentrations applied in different studies could explain the differences between our results and those of others, e.g. [29].

The effect of additives (brewing sugar and EM) on CO_2 and N_2O emission difference in both storage condition was insignificant, however their emission was proportionally higher from warmer storage. Slurry N_2O emission probably were from the slurry crust where microbial nitrification denitrification can take place and would be greater under mesophilic conditions [14,63].

It was noticed that the effect of net GHG CO_2 emission was lower with the use of additive, demonstrating between 13% and 80% GHG decrement under both temperature regimes (Table 5). The GHG inhibition was found greater during warm storage is contributed by the high CH_4 inhibition and relatively similar to mineral acid acidification such as H_2SO_4 or HCl, however

it requires a deeper exploration as this present data based on small scale and short period observations.

4. CONCLUSION

Manure management is a key source of GHG and NH_3 emissions, hence research is exploring strategies to reduce these emissions from different parts of the manure management chain, including slurry storage. We have shown that the 'self-acidification' of slurry as a result of adding brewing sugar can successfully reduce NH_3, CH_4 and total GHG emissions during slurry storage. Acidification can be observed as early as the second day after addition of the carbon source in a warm environment (30°C), and after day 5 in the cold environment as a result of lactic acid accumulation. The success in reducing CH_4 emission in 'self-acidification' stored slurry by 84% and 99% during the cold and warm storage environments subsequently reduced the total GHG CO_2e emission by 34% to 80%, considering CH_4 is the major contributor in slurry GHG emission. Overall, slurry 'self-acidification' reduced NH_3 emissions significantly; retaining 40 to 70% more plant available N (NH_4^+) for recycling to soil than the non-acidified slurry (Control, EM). However, addition of EMs to slurry had little impact on NH_3 and CH_4 emissions. Replacing brewing sugar with easily fermented carbohydrate sources, e.g. waste products from food processing, could represent a cost effective treatment additive to help reduce CH_4 and NH_3 emissions from slurry stores, and is worthy of further research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Appendix 1. Influence of sugar and effective microorganism addition on the slurry volatile fatty acid content slurry after a 30 d incubation period

i. Volatile fatty acid content of slurries after 30 d cold storage at 10°C

Volatile fatty acid type (gL^{-1})	Treatments			
	Control	**Sugar**	**EM**	**Sugar+EM**
Acetic acid	5.11±0.4	4.59±0.4	3.34±0.3	4.90±0.5
Propionic acid	2.65±0.2	2.40±0.2	1.51±0.1	1.93±0.2
Iso-butyric acid	0.10±0.02	0.10±0.02	0.15±0.01	0.23±0.02
Butyric acid	0.83±0.1	0.75±0.07	0.64±0.1	0.95±0.1
Iso-valeric acid	0.20±0.02	0.17±0.02	0.23±0.02	0.36±0.03
Valeric acid	0.03±0.00	0.03±0.00	0.11±0.01	0.16±0.02
Total VFA	8.93±0.7	8.04±0.8	5.98±0.6	8.53±0.8

ii. Volatile fatty acid content of slurries after 30 d warm storage at 30°C

Volatile fatty acid type (gL^{-1})	Treatments			
	Control	**Sugar**	**EM**	**Sugar+EM**
Acetic acid	3.57±0.959	3.25±0.124	4.03±0.103	3.26±0.910
Propionic acid	2.05±0.444	1.24±0.336	2.07±0.273	1.22±0.266
Iso-butyric acid	0.44±0.097	0.10±0.143	0.40±0.115	0.09±0.014
Butyric acid	0.80±0.212	1.42±0.080	0.89±0.183	1.00±0.251
Iso-valeric acid	0.60±0.134	0.14±0.197	0.55±0.157	0.12±0.021
Valeric acid	0.31±0.072	0.22±0.036	0.38±0.030	0.22±0.055
Total VFA	7.76±1.919	6.36±0.917	8.33±0.861	5.91±1.516

No significant differences ($P > .05$) in VFA concentrations were observed at either storage temperature. Data represent Mean ± SEM, n=5

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