Do Effective Micro-Organisms Affect Greenhouse Gas Emissions from Slurry Crusts?

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Abstract-Slurry crusts form on the slurry surface and act as a primary barrier to gaseous emissions and could also be a zone where CH₄ is consumed by methane-oxidising bacteria present. However, slurry crusts have also been reported as sources of nitrous oxide emissions. This study evaluated methane oxidation rate and nitrous oxide emissions from a 8 months developed slurry crust followed by 8 weeks application of a mixed microbial consortia (effective microorganism; EM®). There was no clear evidence of CH₄ oxidation following EM[®] application. Whilst there was no significant reduction of N₂O fluxes from EM[®]-treated crusts, there was a tendency for lower N₂O emissions from EM[®]-sprayed crusts. N₂O emissions were greater than CH₄ consumption, resulting in net greenhouse gas (GHG) emissions of between 13.8-46.7 mg CO₂ eq. g⁻¹ DM hr⁻¹. We conclude that it is important to consider net GHG emissions (CO₂ eq.) when reporting CH₄ oxidation from slurry crusts.

Index Term—slurry crust, methane oxidation, nitrous oxide, effective microorganisms, GHG mitigation

I. INTRODUCTION

Methane (CH₄) oxidation is an important process, which removes CH₄ from the atmosphere [1], [2]. Although most (90%) CH₄ oxidation occurs in the troposphere [3], CH₄ oxidation by methanotrophic bacteria or methane oxidizing bacteria (MOB) by soil [4] and slurry crusts [2], [5], [6] should also be considered important, as we have the ability to manage these CH₄ sinks. Methane oxidation within livestock systems can occur by CH₄ oxidation in the slurry crust, which develops after the prolonged storage of undisturbed slurry. This may help to achieve IPCC [7] targets to reduce greenhouse gas (GHG) emissions by more than 25-40% by the year 2020, compared to year 1990.

Methane oxidation in the crust occurs when methane oxidising bacteria (MOB), specialized methylotroph prokaryotes, establish within the crust and utilize CH_4 as a sole carbon and energy source [4]. These microbes are either type I or II MOB characterized by defining their

ability to produce methane monooxygenases (MMO), an enzyme that catalyses CH_4 oxidation to methanol (CH_3OH). The methanol produced is further oxidized to formaldehyde (HCHO) by methanol dehydrogenase through either the Rump or the Serine pathway [4]. Methane oxidation by slurry crusts offers a potentially important sink for the CH_4 generated by the bulk of the liquid slurry beneath it, but may increase the rate of nitrous oxide (N_2O) emission due to nitrification and subsequent denitrification of NH_4^+ in the crust microenvironment [8].

The positive effect of applying effective microorganisms (EM) to soil and wastewater are widely known but their impact on slurry crust processes remains unknown. It is known that EM contain a complex mixture of different microbial species, which when applied to slurry crusts may result in competition with resident crust microorganisms. This paper investigates the potential of EM to reduce net GHG emissions from slurry stores by either enhancing CH_4 oxidation or reducing N_2O emissions.

II. METHODOLOGY

Fresh mixed beef and cattle slurry was collected from the reception pit of a commercial farm and stored in 6 separate 90 litres 48 cm diameter barrels to allow the crust to develop. The studies were setup during the winter (December 2013) and placed under a shed at the Henfaes Research Centre, Bangor University, UK. Effective micro-organism (EM[®]) provided by Effective Microorganism Limited, Exeter UK were diluted 10-fold and sprayed onto the surface of the newly formed crust at 28 ml m⁻² starting in April 2014, twice weekly for the next 8 weeks. The control untreated crust received the same application rate of H₂O. There were three barrels of each treatment.

The crust was carefully removed from the barrels, crushed, homogenized, sieved to pass 10 mm, and characterised in the laboratory. Methane oxidation and N_2O emission rates from the crusts were measured by sampling the headspace gas in a closed vessel system. Briefly, 5 g of homogenized crust from each treatment

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were incubated in 0.7 litre sealable vessels at 4, 20 and 22 $^{\circ}$ and supplied with 5.1 ml of 4% CH₄, to achieve a final headspace CH₄ concentration of 300 ppm. Headspace gas sampling (20 ml) was carried out at time 0, 6, 24, 48 (t-0, t-6, t-24, t-48) hours and the samples stored for 1 week before analysis using a Perkin Elmer Clarus 580 Gas Chromatograph (GC) linked to a Perkin Elmer TurboMatrix 110 auto sampler. The GC was equipped with megabore capillary Q PLOT columns run at 50 $^{\circ}$ C and fitted with a flame ionization detector (FID) with methanizer (350 $^{\circ}$ C) for detection of CH₄. N₂O was detected via an electron capture detector (375 $^{\circ}$ C). O₂ free N₂ was used as the carrier gas.

III. DATA ANALYSIS

The oxidation rate of CH₄ in the slurry crusts at different incubation temperatures were analysed by General Linear Model, ANOVA using Minitab 16 software (Minitab Ltd., Coventry, UK). N₂O emission rates were determined as the difference between the t-0 and t-6 hour sampling points and differences in flux rates analysed by t-test. The limit for statistical significance was set at P < 0.05.

IV. RESULTS

Overall, the moisture content of the crusts varied slightly due to differences in contact with the main body of the slurry. The surface of the crusts were grey to black in colour, while underneath they were grey-brown (Fig. 1). No grass or fibrous material was seen on the upper surface, but some hay, fibrous or grains could be seen underneath, with maggots also present in certain crusts. Analysis of the crusts indicated that they were slightly alkaline, possessed a high electrolyte content (Table I). NO₃⁻ concentrations in the crust were also significantly lower in the EM[®] treatment.

Although CH₄ oxidation activity was observed in the slurry crust, there was no consistent effect of EM[®] on CH₄ consumption rates (Fig. 2). CH₄ oxidation rate fluctuated during the incubation period as summarized in Table II. In most cases, the oxidation rate was higher during the first 6 hrs and decreased thereafter, occasionally resulting in low rates of CH₄ emission. This was clearly seen for the 22 °C incubation temperature. There was no significant difference in oxidation rate in the two treatments measured at incubation times of either 6, 24 or 48 hrs (P = 0.051, P=0.097 and P=0.457). The maximal rate of methane oxidation was 2.52 µg CH₄ g⁻¹ DM hr⁻¹ from the control crust incubated at 30 °C.



N₂O emissions from the slurry crusts were of equal significance to CH₄ oxidation in this study. Emission rates from the EM[®] treated crusts were generally lower than from the control treatment, although they were not significantly different (*P*>0.05). N₂O emission rates increased with temperature from 46.5 to 157.1 μ g N₂O g⁻¹ DM hr⁻¹ at 4 °C and 30 °C respectively. These high N₂O flux rates resulted in a positive net GHG emissions ranging from 13.8 to 46.7 mg g⁻¹ CO₂ eq. DM hr⁻¹ (Table II).

V. DISCUSSION

Methane oxidation by slurry crusts is an important process, as it can represent a significant sink for CH_4 immediately after being emitted from the underlying liquid slurry. Methane oxidation has also been shown to be positively related to the depth of the slurry crust [9]. According to [10], slurry crusts have the potential to oxidise 80% of the CH_4 emitted during storage. During our study, we assumed that atmospheric O₂ levels did not become depleted during the 48 hr incubation and did not influence CH_4 oxidation rate [5].



Figure 1. Images of crusts formed on a 90 litre slurry storage barrel after 6 months and after treatment with EM[®] additives. Panel A shows the crust and underlying slurry while Panel B shows an intact slurry crust.

TABLE I.	PHYSIOCHEMICAL PROPERTIES OF THE SLURRY CRUSTS
TREATED	WITH EITHER EFFECTIVE MICROORGANISMS (EM [®]) OR
	WATER

Doromotor	Crust type			
Farameter	EM treated	H ₂ O treated		
pH	9.1 ±0.3	9.0 ±0.5		
EC (mS cm ⁻¹)	6.7 ±0.5	7.8 ±1.9		
DM (% FM)	57.1 ±1.8	51.2 ±0.1		
VS (% DM)	45.6 ±6.4	30.7 ±7.6		
Total C (g kg ⁻¹ FM)	14.9 ±0.93	20.2 ±2.04		
Total N (g kg ⁻¹ FM)	1.61 ±0.13	2.22 ±0.14		
NH4 ⁺ (g kg ⁻¹ FM)	0.67 ± 0.15	0.72 ± 0.25		
NO_3^- (mg kg ⁻¹ FM)	18.2 ±0.8	63.5 ±45.9		

DM, Dry matter; EC, electrical conductivity; VS, volatile solid; Values represent means \pm SEM, n = 5.





Figure 2. Methane concentration in the headspace above the slurry crust. A depletion of CH₄ indicates microbial oxidation. Panel A. 4 $^{\circ}$ incubation; Panel B. 10 $^{\circ}$ incubation; Panel C. 30 $^{\circ}$ incubation. Values represent mean ± SEM (*n* = 3). EM[®]- Effective microorganisms, Ctrl- control (treated with water).

TABLE II. METHANE OXIDATION FLUX, N₂O EMISSION RATES AND NET GREENHOUSE GAS (GHG) EMISSIONS RECORDED FROM SLURRY CRUSTS TREATED EITHER WITH EFFECTIVE MICROORGANISMS (EM^{\odot}) OR WITH WATER (CTRL).

Incubation temperature (°C)	Treatment	Average CH_4 oxidation rate during 48 hrs observation (ng g ⁻¹ DM hr ⁻¹)			N_2O emission rate (µg g ⁻¹ DM hr ⁻¹)	Net GHG emitted (CO ₂ eq.) (mg g ⁻¹ DM hr ⁻¹)
		6	24	48		
4	EM	309.9 ±330.5	288.6 ±140.5	-45.0 ±78.0	46.6 ±23.8	13.8
	Ctrl	680.0 ±77.7	256.1 ±66.0	0.9 ±38.3	69.7 ±4.3	20.7
22	EM	654.0 ±633.4	-213.0 ±58.8	-108.2 ± 101.0	115.9 ±43.3	34.5
	Ctrl	-1115.7 ±730.8	-548.5 ±445.1	-437.5 ±416.2	121.5 ±29.9	36.2
30	EM	-694.1 ±204.4	150.4 ±103.3	77.2 ±105.3	146.3 ±30.8	43.6
	Ctrl	1201.2 ±674.6	190.4 ±192.7	97.0 ±188.7	157.1 ±41.8	46.7
<i>P</i> value (n=3)		0.051	0.097	0.475		0.176

Negative (-) CH₄ oxidation rate indicating more CH₄ was released than removed from the headspace. Net GHG was calculated based on N₂O emission substrate maximum CH₄ oxidation rate recorded based on CO₂ equivalent by calculating to global warming potential (GWP) as guided by IPCC (2013) as CH₄ = 34, N₂O = 298 GWP, [13]. Total net emission was not counting the CO₂ emission during the respiration. EM[®]- Effective microorganisms, Ctrl- control untreated. Values represent mean \pm SEM (n = 3).

No previous studies have investigated the influence of EM^{\circledast} on greenhouse gas emissions from slurry crusts. However, ref [11] proposed that EM works by (i) competing with harmful microorganisms, and (ii) by production of beneficial substances that promote the health of the microbial environment (e.g. enzymes, organic acids, amino acids, hormones and antioxidants). This effect is thought to be achieved by 5 microbial groups present in the EM consortia: photosynthetic bacteria, lactic acid producing bacteria, yeast, fungi and *Actinomycetes*. Our study has indicated that N₂O emissions were numerically lower from the EM[®] treatment, coinciding with a low crust NO₃⁻ content. This suggests that some slurry processes may be affected by the addition of EM[®].

A. Methane Oxidation

In our study, the maximum CH₄ oxidation rate was 2.52 µg CH₄ g⁻¹ DM hr⁻¹, which is within the range reported by [5], [6]. This oxidation rate was for a starting headspace concentration of between 250-300 ppm, which is similar to CH₄ concentrations observed underneath crusts. Previous work [5] reported that the CH₄ oxidation rate was 0.08-0.4 µg CH₄ g⁻¹ DM hr⁻¹ at normal moisture content, and 0.16-1.11 µg CH₄ g⁻¹ DM hr⁻¹ when the crust was partially dried (assuming an OM content of 79%; data not shown). Similarly, if we assume 1 g is equal to a surface area of 1 cm², our oxidation rate represents 0.61 g m⁻² day⁻¹, which is much lower when compared to 4.5 g

 $m^{-2} day^{-1}$ [12]. Oxidation rates are also known to fluctuate during the year (with season) as reported by [12]. Therefore, oxidation rates could be 20-times greater in the summer compared to a winter climate. We observed a similar influence of temperature on CH₄ oxidation rate.

There was no significant difference in the rate of CH₄ oxidation from crusts treated with and without EM[®], thus the impact of EM[®] on CH₄ oxidation appears minimal. Despite this, slightly lower oxidation rates were observed from crusts treated with EM® at all incubation temperatures. There was an unusual oxidation rate (negative oxidation, i.e. emission) from control crust incubated at room temperature and we could not explain this phenomenon. It could be due to micro-anaerobic condition of the crust as a result of wetting after the crust had fallen back into the liquid slurry prior to use in the incubation study. In addition, MOB growth and activity is affected by pH and temperature, and most MOBs that have been isolated are mesophiles [14]. The crust pH in our study was alkaline (pH 9.1) and might have impaired the activity of MOB community as the optimum pH range reported is 3.5-8.0 [4].

B. Nitrous Oxide Emissions

The oxidation potential of slurry crusts is considered an important factor in controlling rates of CH_4 emissions, however, the loss of N through N₂O emissions from crusts is not well understood and this could offset the benefits of enhanced CH_4 oxidation in terms of CO_2 eq. During slurry storage, N_2O losses have been considered very low or near zero with an emission factor of 0.0007% of the initial N in the slurry store, and thus can be considered negligible [15]-[18]. Meanwhile the N_2O emission factor from slurry with the presence of crust is reported at 0.005% [16]. Significant N_2O emissions from stored slurry store has been reported by [18]-[20] when crust have developed, however, the factors regulating N_2O loss were not explored.

Our results show that N₂O emissions outweigh rates of CH_4 oxidation, when expressed on a CO_2 eq. basis. The pH of the slurry crust (range of 8.2 and 9.1) recorded here is within the optimum range (pH 7-9) for nitrifying bacteria [21]. Hence, following O₂ diffusion into the crust, N₂O emissions may have been the result of nitrification by either ammonia oxidizing bacteria (AOB), nitriteoxidizing bacteria (NOB), eukarya and archea [20], [21], and also from denitrification in anaerobic microenvironments of the NO_3^- that was produced [22]. Reference [6] explored N₂O distribution in slurry crusts using a micro-sensor, and showed that the source of N₂O emission was in the upper part of the crust layer, which is likely to lie 3-10 mm underneath the surface depending on the crust thickness. This is possibly promoted by O₂ rapid O_2 diffusion into this layer from air above the store [6]. The sub-oxic or anoxic zones in the middle of the crust could promote nitrification and or denitrification activities, thus accumulating N₂O at that particular depth. In addition, the N₂O flux is affected by moisture content; reduced water condition increases N2O emission and vice versa [23]. Temperature also influences the N₂O emission rate, with higher emissions in warmer conditions [24]; we found a similar trend with greater N₂O emissions from crusts incubated at the higher temperatures (Table II). Nitrous oxide emission from slurry crust was recorded by [25] at 0.44 μ g N₂O cm⁻³ day⁻¹ during winter and 0.48 μ g N_2O cm⁻³ day⁻¹ in summer. Assuming that 1 cm³ is equal to 0.45 g, their emission rate is lower compared to the measurements made here. This is possibly due to differences in crust specific surface area and moisture content resulting in different oxic/anoxic conditions in the crust. A recent study by [24] from crusted pig slurry showed cumulative N_2O emission 40 g m⁻² with the highest recorded rate being 8 μ g cm⁻² hr⁻¹ during a 58 day monitoring period. Nitrous oxide emission in our study were numerically lower from crusts treated with EM[®], although this was not significantly different to the control due to the limited replication (n) and large variance (±SEM). Similar observations were reported by [19] during slurry storage treated with EM[®] at 0.1% ($^{v}/_{v}$) concentration.

C. Impact of Slurry Crusts from a GHG Mitigation Perspective

CH₄ oxidation by slurry crusts could be considered as a cost-effective strategy to reduce CH₄ emissions [5]. Firstly, crusts act as a natural barrier for CH₄ emission as explored by [5], [12] and [6], [7], however, our data indicate the possibility of CH₄ being released from the crust when conditions are anoxic as well as low rates of CH₄ oxidation.

When N₂O emissions are taken into account, the net GHG emissions calculated as GHG CO₂ equivalents were lowest from slurry held under cold climatic conditions (4 °C; 13.8 mg CO₂ eq. g^{-1} DM hr⁻¹). This value does not include CO₂ emitted during aerobic and anaerobic respiration from the crust medium. In order to gain negative net GHG emission (CO_2 eq.), there is a need to maximize the rate of CH_1 oxidation within the crust combined with a reduction in N₂O emissions that counteract the effect of oxidation. Methane oxidation in the crust can be promoted by adding floating substances that help to promote crust development, e.g. woodchip, leca, peat or straw [8], [26], [27]. Appropriate inoculum or cultured methanotrophs can also be applied with optimum moisture (60-70%) combined with an incubation temperature which promotes oxidation activity [28].

In addition, slurry crusts can act as a barrier against agronomically important losses of N, via NH_3 volatilisation [29]. Approaches to enhance slurry crust formation are being practiced in livestock farms in Denmark (>80%) as a way to reduce NH_3 volatilization [5]. This is being promoted due to regulations imposed by the Danish government however, it may have a secondary benefit by enhancing GHG reductions through CH_4 oxidation, assuming that N_2O emissions are not increased.

VI. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, we were unable to demonstrate that EM^{\circledast} application to slurry crusts could consistently improve CH_4 oxidation or, reduce N_2O emission. Keeping a crust as a natural barrier to reduce CH_4 emissions from slurry stores is not recommended if higher net GHG emissions (CO_2 eq.) result from greater N_2O emissions in comparison to rates of CH_4 oxidation (although NH_3 emissions would be reduced). Fixed slurry covers on lagoons that create anaerobic conditions will inhibit N_2O production (as nitrification of slurry NH_4^+ is minimised), and the CH_4 produced can be trapped and utilized as natural gas or flared off as CO_2 .

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