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Research Article

Effectiveness of Biochar in the Short-Term Abatement of GHGs and NH_3 Emissions from Digestate and Slurry

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Abstract

Management of livestock wastes significantly contribute to gaseous losses in the form of N_2O, CH_4 and NH_3, causing threats to soil health and climate. Several strategies reducing the environmental impact of wastes storage are available today. Among them, we tested the effectiveness of adding biochar alone and in combination with bio acidification on gaseous emissions from livestock slurry and digestate in a short-term laboratory simulation. We simultaneously analyzed CO_2, CH_4, N_2O and NH_3 emissions and the main microbial groups involved in their production, and the impact of different storage treatments. Digestate showed twice NH_3 emissions and half CH_4 emissions than slurry, according to the NH_4^+ and C availability of the substrates and abundance of microbial communities. Biochar favoured oxidative conditions that reduced CH_4 emissions slightly from slurry and largely from digestate, but increased CO_2 losses from slurry. The combination of biochar with lactic acid was effective in reducing NH_3 emissions from both slurry and digestate. However, it triggered C losses (CO_2 and CH_4 emissions) due to a fast microbial response to increased labile C availability.

Introduction

Manure management is responsible for 5.7 % and 4.3 % of global livestock methane (CH_4) and nitrous oxide (N_2O) emissions, respectively [1]. Additional losses take place in the form of ammonia (NH_3) emissions that have a severe impact on eutrophication and acidification processes, and in particulate matter formation [2]. On the other side, livestock wastes are valuable sources of

nutrients and organic matter that maintain soil fertility and crop production [3]. The potential use of such wastes as alternatives to mineral fertilizers open new opportunities in a circular economy perspective [4]. Several techniques that prevent emissions and preserve N in livestock wastes are today available, as reviewed by Ambrose et al. [5] for CH_4 and by Kupper et al. [6] for NH_3. Among them, anaerobic digestion is designed to optimize conversion of available carbon (C) into biogas and obtain an ammonium (NH_4^+) enriched product, producing low N_2O [7], but high NH_3 emissions during storage [6,8,9]. Gaseous emissions are the result of microbial processes directly involved in CH_4, N_2O and NH_3 [10] production and consumption. Few research focused comprehensively on environmental feedback of livestock wastes [11] and a better understanding of biological processes and microbial functioning is fundamental to better setup ad hoc solutions.

Biochar was successfully used to reduce CH_4, N_2O and NH_3 emissions from manure [12]. However, clear evidence on the net balance of gaseous emissions are still scarce, and contrasting effects on each gas were reported [11]. Schmidt [13] suggested the combined use of biochar and lactic acid bacteria to treat liquid slurry to obtain several environmental benefits. Laboratory-scale testing was successful to compare different treatments in short-term simulated storage [11,14-16]. This work evaluated at laboratory-scale the effectiveness of adding biochar alone and in combination with bio-acidification on gaseous exchanges from livestock slurry and digestate. Despite the short-term duration of the experiment, the focus was directed to comprehensively analyze gaseous losses and microbial communities and processes which are at the base of gas production.

Materials and Methods

Lab-scale set up

The experiment was conducted in a controlled environment (20 °C Temperature) at Fondazione Minoprio (Italy) in February - March 2022. Eighteen tanks of 20 L capacity and hermetical closure were prepared and positioned within the greenhouse in a randomized design. Six combination treatments in tri-replicates were set up: slurry, digestate, slurry + biochar (SluB), digestate + biochar (DigB), slurry + biochar + lactic acid (SluLacB) and digestate + biochar + lactic acid (DigLacB). 10 L of digestate or slurry were used in each tank, 500 g of biochar (DigB and SluB) and a mix with 0.03 g *Lactobacillus plantarum* 14D/CSL (Lactosil 3.0, CLS srl), lactic acid (20 mL), 25 g glucose and 25 g saccarose (DigLacB and SluLacB). Characteristics of digestate, slurry and biochar are reported in Lagomarsino et al. [17]. Production of CO_2, CH_4, N_2O and NH_3 was monitored for two weeks, at 0, 0.8, 1, 2, 3, 4, 7, 9, 11 and 14 days from the substrate's addition. Temperature within the tanks was measured at every single measurement time with portable instruments (Hanna thermometer, Part Code HI7661).

GHGs emissions measurements

Gas measurements were conducted with a DX4040 FTIR Gas Analyzer (Gasmet Technologies Oyd), detecting gaseous compounds by absorbance of infrared radiation at 10 s intervals (Powell and Vadas, 2016). The tanks were left open between each measurement to avoid saturation and were closed during measurements by a lid equipped with two valves for instrument connection via two Teflon tubes. The tank headspace air was pulled into the FTIR and then returned back into the tank through the outlet with an on-board sample pump (maximum pressure is 1.0 bar and maximum flow is 2.0 l min-1), creating a dynamic chamber with a closed loop air circulation required for measuring cumulative gas concentrations [15], which allowed for reliable NH_3 measurements [9]. Consecutive measurements of the individual gas concentrations over time were performed until NH_3 saturation in the chamber's headspace was observed, at least for 10 minutes, reading gas concentrations every minute. Gas fluxes were calculated from linear increase of gas concentration ($R^2 > 0.75$) versus time plot, headspace volume, and emitting volume. Significant differences in GHGs productions were assessed by analysis of variance (ANOVA) followed by Fisher LSD post-hoc test ($p<0.05$) using Statistica 7 (StatSoft).

Microbial analyses

Total DNA was extracted from 0.5 mg of slurry or digestate samples collected at the end of experimentation using the Fast DNA Spin Kit for soil (Biomedicals). The microbial communities were analyzed by denaturing gradient gel electrophoresis (DGGE) using primers for the bacterial and methanogenic archaeal 16S rRNA gene [18,19]. DGGEs were carried out and analyzed as previously described by Pastorelli et al. [20]. Real-time PCR was used for microbial groups quantification [21]. Bacteria and methanogenic archaea were quantified using primers for 16S rRNA gene [22,23]. Denitrifiers were quantified using primers for *nirK* and *nosZ* marker genes [24]. Nitrifiers were quantified using primer for *amoA* marker gene, distinguishing archaea and bacteria [25]. Significant differences were assessed by ANOVA.

Results

Gas production

CO_2 emissions increased sharply in the first 24 hours of incubation (Figure 1, top). The highest peaks were observed from SluLacB and DigLacB. The initial peaks declined rapidly in the first 24 hours. The effects of treatments remained consistent along measurements, with the maximum emissions from DigLacB and SluLacB > SluB > Slurry > DigB and Digestate (Table 1). CH_4 emissions raised after the third day in all treatments, except for DigB that peaked at the beginning but then remained very low throughout the storage period, with a reducing effect of biochar (Figure 1, middle). The maximum rates were observed from SluLacB (from 24 hours onwards) and DigLacB (from 72 hours onwards). CH_4 emissions from slurry and digestate were reduced by sole biochar addition but strongly increased with the combination of biochar and lactic acid (Table 1). NH_3 emission rates were on average higher in the first 24 hours of storage, with a progressive reduction after the fourth day of storage (Figure 1, bottom). Independent of treatments, emissions from digestate were about double those from slurry throughout the monitoring period. The effect of treatments (Table 1) was clear from the beginning with a constant reduction of emissions from digestate and slurry with biochar plus lactic acid combination. N_2O emissions remained very low, around 0 values throughout the 2 weeks of monitoring, without significant effects of treatments and were not reported.

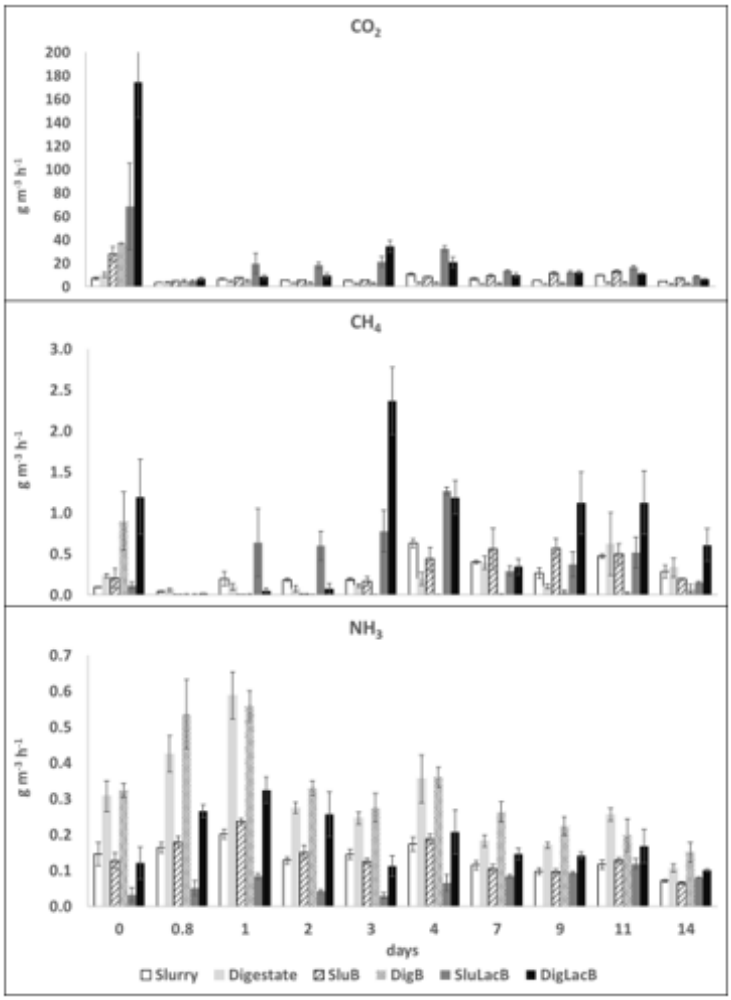

Figure 1: CO_2 (top), CH_4 (middle) and NH3 (bottom) emissions rates in the six storage combinations.

Table 1: Percentage effects of biochar and biochar + lactic acid treatments on untreated slurry or digestate on of CO_2, CH_4 and NH_3 fluxes at each measurement time. *p<0.05, **p<0.01, ***p<0.001.

Days		0	0.8	1	2	3	4	7	9	11	14
CO_2 production											
Slurry	Biochar	+290$^{n.s.}$	+34$^{n.s.}$	+19$^{n.s.}$	+2$^{n.s.}$	+6$^{n.s.}$	-21$^{n.s.}$	+42*	+115***	+32*	+56**
	Biochar + Lactic acid	+858***	+32$^{n.s.}$	+200**	+233***	+309**	+201***	+100***	+132***	+65***	+93***
Digestate	Biochar	+260$^{n.s.}$	+28$^{n.s.}$	+14$^{n.s.}$	+6$^{n.s.}$	+11$^{n.s.}$	0$^{n.s.}$	+19$^{n.s.}$	+32$^{n.s.}$	+13$^{n.s.}$	+26$^{n.s.}$
	Biochar + Lactic acid	+1588***	+66**	+77$^{n.s.}$	+177*	+1169***	+486***	+307***	+432***	+228***	+201***
CH_4 production											
Slurry	Biochar	+111$^{n.s.}$	-88***	-96$^{n.s.}$	-93$^{n.s.}$	-14$^{n.s.}$	-30$^{n.s.}$	+39$^{n.s.}$	+115$^{n.s.}$	+4$^{n.s.}$	-31$^{n.s.}$
	Biochar + Lactic acid	+21$^{n.s.}$	-76**	+222*	+222***	+304*	+102**	-27$^{n.s.}$	+41$^{n.s.}$	+9$^{n.s.}$	-46$^{n.s.}$
Digestate	Biochar	+283*	-87**	-91$^{n.s.}$	-93$^{n.s.}$	-95$^{n.s.}$	-98$^{n.s.}$	-98*	-63$^{n.s.}$	-96*	-77*
	Biochar + Lactic acid	+410**	-73***	-47$^{n.s.}$	+3$^{n.s.}$	+2007***	+506***	-12$^{n.s.}$	+971***	+81$^{n.s.}$	+83*
NH_3 production											
Slurry	Biochar	-13$^{n.s.}$	+10$^{n.s.}$	+17$^{n.s.}$	+18$^{n.s.}$	-13$^{n.s.}$	+8$^{n.s.}$	-8$^{n.s.}$	-2$^{n.s.}$	+10$^{n.s.}$	-7$^{n.s.}$
	Biochar + Lactic acid	-78**	-69*	-58*	-67*	-79**	-62*	-26$^{n.s.}$	-5$^{n.s.}$	+2$^{n.s.}$	+14$^{n.s.}$
Digestate	Biochar	+5$^{n.s.}$	+26$^{n.s.}$	-5$^{n.s.}$	+20$^{n.s.}$	+12$^{n.s.}$	+1$^{n.s.}$	+44***	+32*	-22$^{n.s.}$	+41*
	Biochar + Lactic acid	-61***	-38**	-45***	-7$^{n.s.}$	-54***	-42**	-20$^{n.s.}$	-17$^{n.s.}$	-34*	-6$^{n.s.}$

Microbial community

The analysis of similarity (ANOSIM) conducted on DGGE profiles showed that the type of substrate (slurry or digestate) has a significant role in shaping the bacterial and methanogenic archaeal communities (R=1, p<0.001 and R=0.9, p<0.001, respectively) regardless the addition of biochar or lactic acid (R=0.13, p>0.05 and R=-0.04, p<0.001, respectively). The canonical correspondence analysis (CCA) showed the microbial communities of slurry or digestate, clearly and significantly separated with respect to axis 1. The microbial communities of digestate were mainly correlated to NH_3 emissions, while CH_4 emissions were the least relevant variable in separating the bacterial and archaeal communities (Figure 2). Untreated slurry showed the highest bacterial and methanogenic archaeal 16S rRNA gene copy values while the lowest values were recorded in SluLacB. Treated and untreated digestate samples showed similar 16S rRNA gene copy values for both bacteria and methanogenic archaea (Table 2). The *nirK* and *nosZ* genes showed a similar trend with the highest values found in the slurry and the lowest in the digestate (Table 2). However, in no case significant differences were recorded.

The *amoA* gene showed values below the detection threshold.

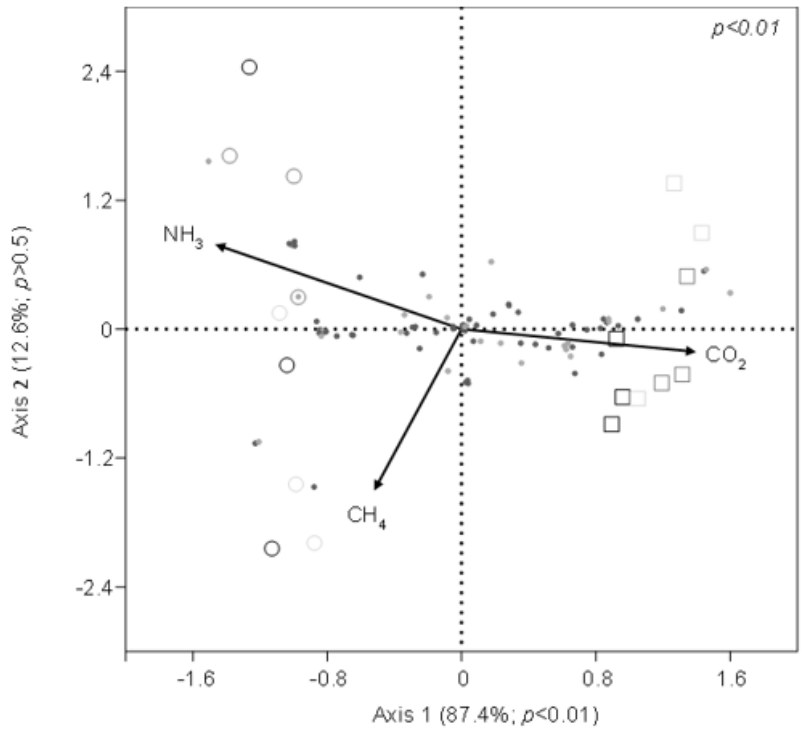

Figure 2: CCA plot of microbial communities from digestate (circle) and slurry (square) generated by bacterial (grey dots) and methanogenic archaeal (light grey dots) DGGE profiles and GHG emissions (vectors). Treatment: no treatment, black; biochar addition, dark grey; biochar and lactic acid, light grey.

Table 2: Real time PCR results (gene copy gr^{-1}). Values are means with standard error in parenthesis.

	Bacteria (16S rRNA)		Methanogenic archaea (16S rRNA)		Denitrifiers (nirK)		Denitrifiers (nosZ)	
Slurry	$1.62\ 10^{10}$	($5.1\ 10^{9}$)	$1.55\ 10^{7}$	($4.0\ 10^{6}$)	$5.79\ 10^{6}$	($1.8\ 10^{6}$)	$4.24\ 10^{6}$	($3.1\ 10^{6}$)
SluB	$7.69\ 10^{9}$	($1.5\ 10^{9}$)	$9.90\ 10^{6}$	($2.8\ 10^{6}$)	$3.53\ 10^{6}$	($1.1\ 10^{6}$)	$8.32\ 10^{5}$	($3.3\ 10^{5}$)
SluBLac	$6.42\ 10^{9}$	($1.8\ 10^{9}$)	$6.46\ 10^{6}$	($1.7\ 10^{6}$)	$2.98\ 10^{6}$	($1.1\ 10^{6}$)	$1.36\ 10^{6}$	($6.1\ 10^{5}$)
Digestate	$1.21\ 10^{10}$	($1.8\ 10^{9}$)	$1.10\ 10^{7}$	($3.1\ 10^{6}$)	$2.47\ 10^{6}$	($9.0\ 10^{5}$)	$5.45\ 10^{5}$	($2.0\ 10^{5}$)
DigB	$1.11\ 10^{10}$	($3.3\ 10^{9}$)	$1.05\ 10^{7}$	($3.3\ 10^{6}$)	$4.36\ 10^{6}$	($1.7\ 10^{6}$)	$1.47\ 10^{6}$	($8.9\ 10^{5}$)
DigBLac	$1.23\ 10^{10}$	($4.9\ 10^{9}$)	$1.33\ 10^{7}$	($4.2\ 10^{6}$)	$5.31\ 10^{6}$	($4.0\ 10^{6}$)	$2.20\ 10^{6}$	($2.0\ 10^{6}$)

Discussion

Even if anaerobic digestion is considered one promising technique to reduce NH_3 emissions from untreated slurry [26], increasing rates of NH_3 emission from digestate than slurry were detected, driven by the higher NH_4+ content, as observed by Zilio et al. [27] and reviewed by Kupper et al. [6]. Conversely, digestion of livestock wastes was an effective strategy to reduce CH_4 emissions [5], as confirmed by a contextual lower abundance of methanogenic communities in digestate than in slurry. Similar results were obtained by Aguirre-Villegas et a. [7] that observed higher NH_3 and lower CH_4 emissions from digestate than untreated manure. N_2O fluxes were negligible during all storage period, independent of treatments and waste type, demonstrating a minor contribution of this gas during storage (lower than 3%), which was however prevalent after application to soil [17]. Accordingly, the *amoA* gene at the end of the incubation period was below the detection limit for both nitrifying bacteria and archaea, which may limit the generation of N_2O [28], while contributing to NH_3 emission [29]. Several studies report negligible values of N_2O in the absence of a dry encrusted surface [30-32], and Park et al. [33] suggested to ignore N_2O emissions from non-aerated manure storage in GHG inventories.

The use of biochar during the storage of livestock wastes to reduce their environmental impact is rapidly increasing, thanks to the biochar capacity to absorb gases and liquids [12,13]. The effect of biochar varied depending on

a. The type of livestock waste (slurry or digestate),

b. The type of gas and

c. The storage time, highlighting the complexity of a system where feedbacks are interacting each other.

Overall, an increase of CO_2 emissions from slurry and a decrease of CH_4 emissions from either slurry or digestate was evident. Biochar is reported to improve oxidative conditions [34] and reduced methanogenic archaea (Table 2), which drove the CH_4 reduction. Ammonia emissions were little affected by biochar, unless an increasing trend from digestate after the first week, probably supported by the release of NH_4^+ initially sorbed [35]. Indeed, biochar was found to reduce NH_3 emissions from manure, but this effect was mostly related to composting [12] or surface application [11]. The manipulation of the balance between NH_3 and NH_4^+ by adding acids and lowering the pH value of the manure successfully reduced NH_3 emissions [32,36] and CH_4 [37]. Moreover, promising results on NH_3 emissions reduction have been recently obtained through biochar activation with additives such as acids or oxidants that can improve sorption capacities [38-40]. The decrease of NH_3 observed in our simulation supported those findings, confirming the combination of biochar and lactic acid as a promising strategy for both slurry and digestate. Conversely, lactic acid increased both CO_2 and CH_4 emissions, this last with huge peaks, suggesting the occurrence of a fast microbial response to availability of labile C substrates added, which triggered microbial activity and C losses. In fact, this increase was not related to a larger bacteria or methanogenic communities, which remained stable among treatments.

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