Composting Recycled Manure Solid: Impact on Bioaerosols in Dairy Farms

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INTRODUCTION

Bedding handling in dairy barns results in high levels of exposure to organic particles suspended in the air (bioaerosols) for dairy producers.[1] Since traditional bedding materials are expensive, Eastern Canadian dairy producers are looking for alternative materials, such as recycled manure solids (RMS).[2] To produce RMS, fresh cow manure is separated into a solid and a liquid fraction. The undigested fibres in the solid fraction is then used as bedding.[3] Since produced on the farm, the RMS are economically suitable and large amounts are available for producers.[4] However, RMS can represent a potential risk for human and animal health. If bedding bacterial burden increases, pathogens might be in higher concentrations in dairy barns and spreading of infections can be facilitated. Yet, dairy producers sanitize RMS using various composting and/or digestive methods,[4] but the impact of those methods on occupational exposure to bioaerosols is still unknown.

Long-term and frequent exposure to bioaerosols and organic dust are responsible for the prevalence of respiratory problems in dairy producers, such as extrinsic allergic alveolitis (farmer's lung), chronic bronchitis and asthma.[5] Extrinsic allergic alveolitis can be caused by the hay-decaying actinomycete *Saccharopolyspora rectivirgula*, while aspergillosis from occupational exposure in farms can develop in immunosuppressed individuals when exposed to the mould *Aspergillus fumigatus*.[5] *Klebsiella pneumonia* and *Legionella pneumophila* can cause respiratory infections[6-7], but the presence of these bacteria are still to be quantified in the air of dairy barns. RMS and its associated microbial burden could then lead to higher bioaerosol and respiratory pathogen exposure.

Fournel et al. 2019 is the first study to our knowledge to document bioaerosols in dairy barns associated with RMS use.[8] Since different methods are used to sanitize RMS, four different RMS composting methods were tested at an experimental scale, with the objective to identify the method leading to the less generation of bioaerosols, for a subsequent transfer of the technology to commercial-scale buildings.

METHODOLOGY

The experimental setup and design were detailed in Fournel et al. 2019.[8] Briefly, four different 10-day composting methods of piled RMS were tested in ten confined and environmentally-controlled experimental chambers in July 2017 (IRDA, QC, Canada). For the Static Windrow (SW) method, RMS were left undisturbed in piles for 10 day while the Turned Windrow (TW) method consisted in piled RMS turned daily in chambers. For the 24-hour drum-composting (DC24) method, RMS were drum composted for 24 h prior to be loading in piles for 10 d of maturation. Finally, the 72 h drum-composting for (DC72) method involved the 10 d aging of 72 h drum composted RMS. Three experimental chambers were used to evaluate the SW composting method, three for TW, two for DC24, and two contained a RMS pile resulting from a 72 h drum-composting (DC72).

Air was sampled at days 0 (when RMS were piled), 5 and 10. Coriolisμ Biological Air Sampler (200 L/min, 10 min, Bertin Corp.) was used to analyze by culture airborne microorganisms as described in Fournel et al. 2019[8] while SASS 3100® Dry Air Sampler (300 L/min, 10 min, Research International) was used for DNA extraction and molecular biology methods[9] Total bacteria, *Penicillium* and *Aspergillus* moulds, as well as human pathogens were quantified by real-time quantitative polymerase chain reaction (qPCR) using Integrated DNA Technologies primers and probes (Table 1).

Table 1. Airborne microorganisms quantified by PCR.

Microorganisms	**Targeted gene**	**Annealing temperature (°C)**	**References**
Total bacteria	16S rDNA	62	Bach et al., 2002 [10]
Penicillium/ Aspergillus	ITS1	60	Haugland et al., 2004 [11]
Aspergillus fumigatus	ITS1	60	Haugland et al., 2004 [11]
Klebsiella pneumoniae	phoE	60	Shannon et al., 2007 [12]
Legionella pneumophila	mip	57	Joly et al., 2006 [13]
Saccharopolyspora rectivirgula	16S rDNA	59.6	Schafer et al., 2011 [14]

Results for total bacteria are expressed in *E. coli* equivalent genomes/m^3 of air (*E. coli* eq/m^3), while *Penicillium/Aspergillus* concentrations in *Aspergillus fumigatus* equivalent genome/m^3 of air (*A. fumigatus* eq/m^3) as the genomic DNA of these microorganisms were used to construct standard curves.

All results are shown in mean ± sem. Several statistical models were used to get the best-fitted model for covariance structure and likelihood ratio tests were carried out among models. Comparisons of Akaike's information criterion were also obtained. Brown and Forsythe's

variation of Levene's was used as well as Shapiro-Wilk test on the error distribution from the statistical model was used after a Cholesky factorization. All data were log-transformed and p-values were calculated using these values. For values below the quantification limits, a non-parametric mixed statistical model on longitudinal data was performed, as in Brunner et al 2002.

RESULTS AND DISCUSSION

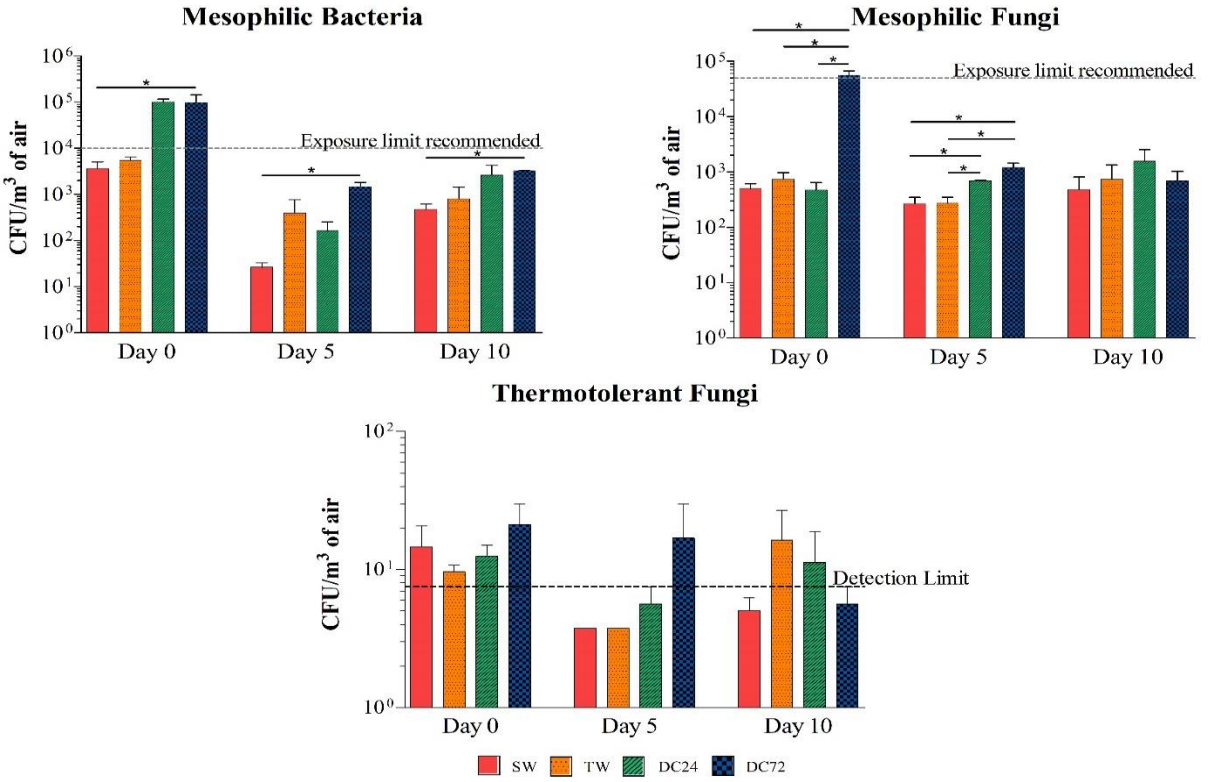
At day 0, airborne culturable mesophilic bacteria concentrations for SW and TW were lower than the recommended exposure limit for an 8 h shift recommended by the National Labor Inspection of Denmark of 10^4 CFU/m^3 of air[15]. For culturable mesophilic fungi, SW, TW and DC24 led to lower values than the recommenced exposure limit of 5×10^4 CFU/m^3 by Dutkiewicz in Poland.[16] SW was also characterized by lower total bacteria and *Penicillium/Aspergilus* concentrations than DC24 and DC72 for at day 0. Five days after piling RMS, the composting method SW resulted in lower amounts of culturable mesophilic bacteria than DC72. Airborne culturable mesophilc fungi concentrations for SW and TW were lower than for DC24 and DC72. SW, TW and DC24 led to concentrations below the detection limit for day 5. For total bacteria and *Penicillium/ Aspergillus*, SW and TW concentrations were also lower than the detection limit. For day 10, airborne culturable mesophilic bacteria concentrations for SW were lower than DC72. For culturable thermotolerant fungi concentration, SW and DC72 were below the detection limit. At day 10, SW and TW showed lower levels of total bacteria than DC24 and DC71, while SW levels were below the detection limit for *Penicillium/Aspergillus* (Table 2, Figure 1).

Table 2. Airborne total bacteria and *Penicillium/Aspergillus* in chambers containing RMS composted by static windrow (SW), by daily turned windrow (TW), in a rotating drum for 24 h (DC24) or in a rotating drum for 72 h (DC72)

Microorganisms/ Composting method	**Total bacteria** (*E. coli* eq/m^3)	***Penicillium/ Aspergillus*** (*A. fumigatus* eq/m^3)
Day 0		
SW	**$9.5\times10^{6\ a}$**	$5.7\times10^{2\ a}$
TW	**$1.1\times10^{7\ a}$**	$9.6\times10^{2\ ab}$
DC24	$3.4\times10^{7\ b}$	**$3.9\times10^{2\ bc}$**
DC72	$4.0\times10^{7\ b}$	$3.8\times10^{3\ c}$
Day 5		
SW	**$< 4.2\times10^{3\ a}$**	**$< 8.3\times10^{1\ a}$**
TW	**$< 4.2\times10^{3\ a}$**	**$< 8.3\times10^{1\ ab}$**
DC24	$2.6\times10^{5\ b}$	$3.1\times10^{2\ bc}$
DC72	$1.4\times10^{6\ b}$	$9.2\times10^{1\ c}$
Day 10		
SW	$3.6\times10^{5\ a}$	**$< 8.3\times10^{1\ a}$**
TW	$1.2\times10^{6\ a}$	**$1.1\times10^{2\ a}$**
DC24	$2.6\times10^{6\ b}$	$3.5\times10^{2\ bc}$
DC72	**$3.3\times10^{5\ c}$**	$2.1\times10^{2\ c}$

[a-e] Within the same day (0, 5 or 10) and microorganism (bacteria or *Penicillium/Aspergillus*), values with the same superscript do not differ ($P<0.05$). The lower concentrations are in green.

Figure 1. Culturable airborne microorganisms in chambers containing RMS composted by static windrow (SW), by daily turned windrow (TW), in a rotating drum for 24 h (DC24) or in a rotating drum for 72 h (DC72) (* : P<0.05)



SUMMARY

The static windrow (SW) composting method seems to be preferable since generating low concentrations of bioaerosols. However, while environmentally-controlled chambers allowed to experimentally determining the best RMS composting method, the associated technologies need to be scale up for commercial dairy barns and the consequent impact on bioaerosols evaluated.

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