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Reduction of sporulating and non-sporulating pathogens during anaerobic digestion of livestock manure in biogas plants

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Introduction

In the current context of developing renewable energies and recovering organic waste, on-farm anaerobic digestion (AD) represents a major challenge for the agricultural sector (energy and organic recovery of livestock manure and agricultural substrates). In France, most of biogas plants fed with manure operate at mesophilic conditions converting organic matter to biogas and by-product degradation, i.e. digestate. This digestate is usually spread as fertilizer on land after transformation or storage. Farm animals like pig, bovine and poultry are known to be reservoirs of various pathogenic microorganisms responsible of animal or human infections (Denis et al., 2011; Boscher et al., 2012, Souillard et al., 2014 and 2015, Moono et al., 2016; Gosling et al., 2018; Thépault et al., 2018). Because these pathogens can survive in manure, their fate during mesophilic AD appears to be a matter of public health concern.

In this study, we investigated the effect of mesophilic AD on the level of sporulating pathogens (*Clostridioides difficile* and *Clostridium botulinum*) and non-sporulating pathogens (*Salmonella* spp, *Listeria monocytogenes* and *Campylobacter* spp.).

Material and Methods

Our study was carried out on three on-farm biogas plants (BGP1, BGP2 and BGP3), two filled with pig manure (BGP1 and BGP3) and one with bovine manure (BGP2). Over one-year, they were visited eight times each. At each visit, three replicates of both inputs (manure) and digestates were collected for detection and enumeration (MPN/g) of *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp., *Clostridioides difficile* and *Clostridium botulinum*. A total of 144 samples (72 inputs, 72 digestates) were analyzed.

Results

All the pathogens were detected in manure at a frequency of 33.3% (*C. botulinum*), 88% (*C. difficile*), 92% (*Campylobacter* spp.), and 95.8% (*Salmonella* and *Listeria monocytogenes*) and in all three BGP, except *C. botulinum* which was not detected in manures of BGP1 and BGP2.

The pathogens were also detected in digestate at a frequency of 37.5% (*Campylobacter* spp.), 79.2% (*C. botulinum*), 83.3% (*L. monocytogenes*), 87.5% (*Salmonella* spp.) and 100% (*C. difficile*). However, no *Campylobacter* spp. could be isolated from digestates of BGP2.

In manure, the level in MPN/g varied in mean from 249 to 368 for *Campylobacter*, from 1.1 to 359.1 for *Salmonella*, from 3.1 to 145.9 for *L. monocytogenes*, from 0.5 to 234.5 for *C. difficile* and from 0 to 3.5 for *C. botulinum* (Fig. 1).

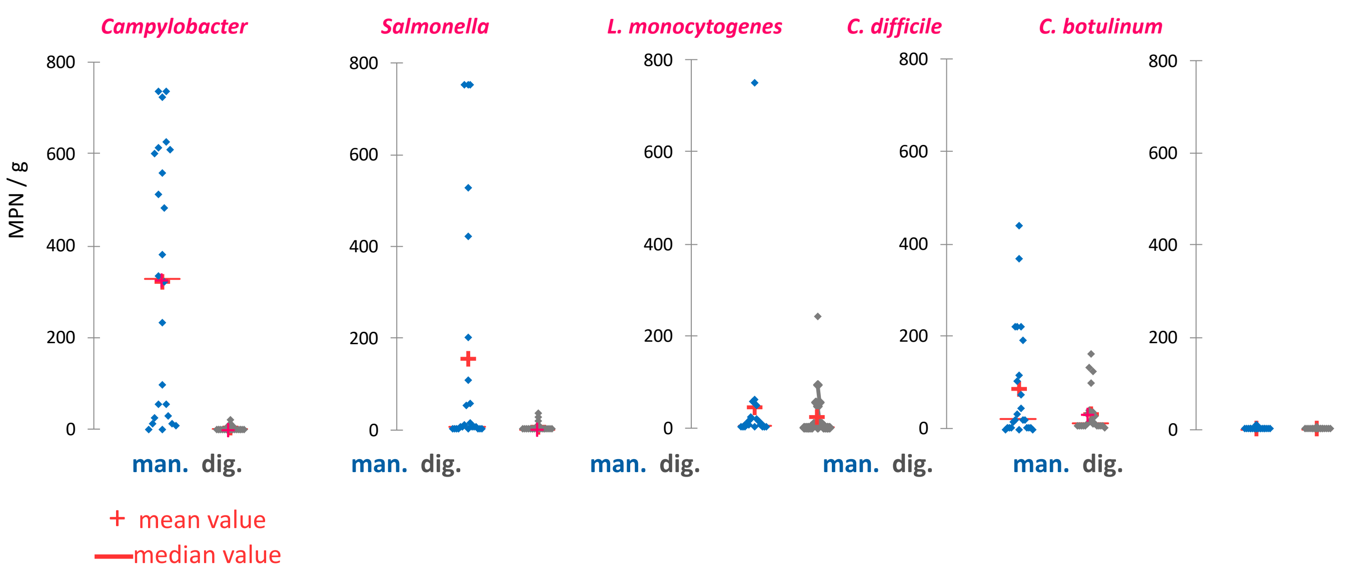

Figure 1: Concentrations of the pathogens in manures and digestates

In raw digestate, the level in MPN/g varied in mean from 0 to 6.3 for *Campylobacter*, from 1.1 to 6.9 for *Salmonella*, from 3 to 45.7 for *L. monocytogenes*, from 8.2 to 80.1 for *C. difficile* and from 0.3 to 2.4 for *C. botulinum* (Fig. 1). Concentration of *C. botulinum* was therefore very low in both samples, manure and raw digestate, with a maximum of 13 MPN/g.
During AD, the average level of pathogens decreased between manure and digestate by 2 Log_{10} (*Salmonella* spp.), 0.3 Log_{10} (*L. monocytogenes*), 2.1 Log_{10} (*Campylobacter* spp.), 0.4 Log_{10} (*C. difficile*) and 0.1 Log_{10} (*C. botulinum*).

Discussion and Conclusion

Our study showed that non-sporulating pathogens like *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp. can be detected in digestate after anaerobic digestion like in previous studies (Kearney et al., 1993; Bonetta et al., 2011; Orzi et al., 2015) suggesting that these pathogens can survive this process, even if their concentrations are reduced during the process. *C. botulinum* concentration was very low, whether in manures or in digestates, which confirms study of Froschle et al, (2015). In this study, *C. difficile* was also frequently detected in digestate with similar levels of *C. difficile* concentration.
With this one-year survey, we demonstrated that mesophilic AD does not lead to bacterial growth and even reduced concentration of sporulating and non-sporulating pathogens. Thus, such treatment of livestock manure can be effective in reducing the presence of these pathogens, and reduce consequent spreading in the environment after post-treatment (eg. storage or post-digestion) of digestates.

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