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

Denis M.1, Druilhe C.2, Le Maréchal C.1, Repérant E.1, Boscher E.1, Nagard B.1, Rouxel S.1, Rezezevara T.1, Martin L.1, Pourcher A.-M.2
1Anses - Laboratoire de Ploufragan, Ploufragan, France,
2IRSTEA, Nîmes, France

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.), 95.8% (Salmonella and L. 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% (Salmonella and Listeria monocytogenes) and in all three BGP, except C. botulinum which was not detected in manures of BGP1 and 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

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Manure</th>
<th>Digestate</th>
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<tbody>
<tr>
<td>Campylobacter</td>
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<td>Salmonella</td>
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<td>L. monocytogenes</td>
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<td>C. difficile</td>
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<td>C. botulinum</td>
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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 45.2 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 aerobic digestion, the average level of pathogens decreased between manure and digestate by 2 Log (Salmonella spp.), 0.3 Log (L. monocytogenes), 2.1 Log, (Campylobacter spp.), 0.4 Log (C. difficile) and 0.1 Log (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 (Keaney 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.

Acknowledgments

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References


Materials and Methods

In 2018, a total of 250 fattening pigs from 25 farms (10 pigs per farm) were sampled in an abattoir in North-West Germany. One sample of ham muscle, one sample of liver tissue and one sample of the muscle of the diaphragm pillar were collected from each pig during the slaughter process. Each animal was tagged individually and samples were taken at different stages of the slaughter line. Livers were collected and stored in boxes during the slaughter process as usual until sampling, all samples were chilled and transported to the institute’s laboratory. Muscle samples from the diaphragm pillar were stored at -30 °C and liver and ham muscle samples were stored at -80 °C until laboratory examination.

To determine the seroprevalence, meat juice from the diaphragm pillar samples was serologically tested for HEV antibodies using the Priocheck® HEV Antibody porcine ELISA Kit (Thermo Fisher Scientific®, USA) according to the manufacturer’s manual. The liver and muscle samples were analysed for the presence of HEV RNA by real-time RT-PCR according to Jothikumar et al. (2006) after RNA extraction with the N3easy® Mini Qiacube Kit (Qiagen®, Germany).

For each pig the antibody status will be gathered and herd status will be analysed, too. Afterwards, the presence of HEV antibodies for each animal will be compared with the presence of viral RNA in the liver and the muscle.

Results

In total, 62% (155/250) of the meat juice samples were positive for antibodies against HEV at a single animal basis. At herd level, 72% (18/25) of the herds were positive. Herds were considered to be positive, if at least one of the ten samples was positive.

For the herd seroprevalence four groups, according to the serological detection rate, were defined. The herds investigated were allocated to one of these groups using their antibody prevalence (Table 1).

<table>
<thead>
<tr>
<th>serological detection rate</th>
<th>Proportion of herds (n/N)</th>
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<tbody>
<tr>
<td>0% (HEV seronegative)</td>
<td>28% (7/25)</td>
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<tr>
<td>10%-30% (low prevalence)</td>
<td>8% (2/25)</td>
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<tr>
<td>60%-90% (high to very high prevalence)</td>
<td>16% (4/25)</td>
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<tr>
<td>100% (all samples are HEV seropositive)</td>
<td>48% (12/25)</td>
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</table>

Table 1: Allocation of herds according to the antibody status