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Persistence of *Salmonella* Derby and *Listeria monocytogenes* in digestates derived from pig and dairy farms

Anne-Marie POURCHER<sup>1,2</sup>, Christine ZIEBAL<sup>1,2</sup>, Géraldine MAYNAUD<sup>3</sup>, Céline DRUILHE<sup>1,2</sup>,  
Eric LATRILLE<sup>3</sup>, Dominique PATUREAU<sup>3</sup>, Julie JIMENEZ<sup>3</sup>, Nathalie WERY<sup>3</sup>

1 Irstea, UR GERE, 17 avenue de Cucillé, CS 64427, F-35044 Rennes, France

2 Université européenne de Bretagne, F-35000 Rennes, France

3 INRA, LBE UR050, Avenue des Etangs, F-11100 Narbonne, France

\*Correspondance: [anne-marie.pourcher@irstea.fr](mailto:anne-marie.pourcher@irstea.fr)

## Summary

Digestates produced from the anaerobic digestion (AD) of manure can be reused as fertilizer. However the potential presence of pathogens in these organic waste products (OWP) and their ability to regrow during long-term storage may constitute a health hazard. This study aims to investigate the factors which may influence the survival of pathogenic bacteria during storage of by-product of AD. Eight OWP were sampled from agricultural AD plants. They were inoculated with *Salmonella* Derby and *L. monocytogenes* and incubated at 24°C. The results show a decline in the number of the two strains during storage (4.5-6 Log reduction in 7-20 days). Pathogen survival has been analyzed with regards to the degree of stabilization of organic matter and the residual microbial activity. The results showed that both biotic and abiotic factors have an effect on the survival of the pathogens and that the survival of *Salmonella* Derby is not influenced by the same factors as *L. monocytogenes*.

## Introduction

Livestock manures are co-digested with various organic feedstocks to enhance the biogas production. The resulting digestates are interesting feedstocks for nutrient recovery. However, questions remain on technical and environmental impacts of biogas plants. Indeed, digestates used as organic fertilizers may contain pathogens which may be spread together with the digestate onto agricultural soils. Thermophilic AD is more effective for pathogen inactivation than mesophilic AD (Smith *et al.*, 2005). Nevertheless, even after thermophilic AD, there is a risk of recontamination and regrowth of pathogens during the storage of digestates (Bagge *et al.*, 2005). Although the most important factors affecting the survival of pathogens during AD have been reported, there is little information on the behaviour of pathogens during storage of OWP. This study aims to investigate abiotic and biotic factors that may influence the survival of pathogenic bacteria during storage of organic waste products (OWP) obtained from on-farm biogas plants.

## Material and method

Eight types of OWP were sampled from mesophilic on-farm AD processes: three raw digestates (R3, R4, R9), two liquid fractions of digestates (L2, L7) and three composted solid fraction of digestates (C1, C5, C7). OWP (125 mL) were inoculated with rifampicin-resistant strains of *L. monocytogenes* and *Salmonella* Derby at an initial level of 10<sup>6</sup>-10<sup>7</sup> CFU/g. They were stored at 24°C for 41 days. *L. monocytogenes* and *Salmonella* were enumerated using Palcam and XLD agar supplemented with 100 mg/L of rifampicin and 50 mg/L of cycloheximide. Media were incubated at 37°C for 72h and 24h, respectively.

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The degree of stabilization of organic matter (OM) was determined by a chemical sequential extraction based on a modified Van Soest method. Two compartments were extracted: (i) the easily accessible fraction of OM assimilated to particulate extracellular OM and (ii) the slowly accessible fraction assimilated to humic substance-like, cellulose and hemicellulose. The non extractible fraction was assimilated to lignin-like compounds. Microbial activities were estimated by (i) the residual microbial activity measured by methane production during 40 days under anaerobic conditions, and (ii) the non-specific enzymatic activity performed by fluorescein diacetate (FDA) hydrolysis.

## Results and discussion

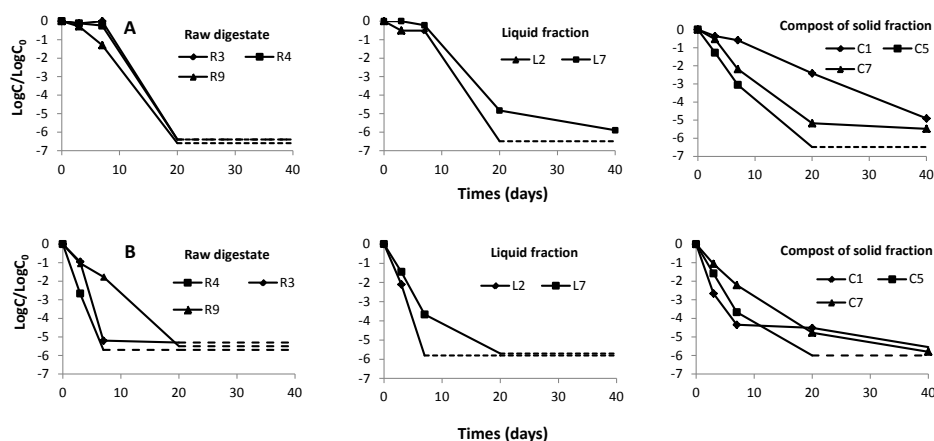
Digestate characteristics of the investigated plants are presented in Table 1.

Table 1. Characteristics of the OWP

sample	pH	Moisture content %	Fraction easily accessible %	Fraction slowly accessible %	Fraction No extractible %	CH <sub>4</sub> prod. (L/Kg VM)	FDA activity (µg/g RM)
R3	8.1	96	44.3	12.3	43.4	35.9	73.5
R9	7.7	90	9.5	30.5	60.0	53.7	168
R4	8	91	33.1	13.8	53.2	64.4	300
L2	8.3	94	45.0	13.2	41.8	53.1	83
L7	7	89	62.9	22.2	14.9	40.9	117
C1	7.3	25	4.0	53.4	42.6	19.1	182
C5	7.8	56	6.9	77.8	15.3	17.5	270
C7	8	59	5.4	39.7	54.9	0	233

The liquid fraction and the raw digestates contain a high level of easily accessible fraction of organic matter which was not degraded during the anaerobic digestion, independently of the hydraulic retention time (ranged between 20 and 90 days). Their residual methane productions is in the same order of magnitude than those reported by Rico *et al.* (2011) for digestates of liquid fraction of dairy manure. As expected, composted digestates contain the lowest easily accessible fraction and the lowest residual methane production. The lowest enzymatic activity, measured by FDA hydrolysis, is observed in the liquid fractions.

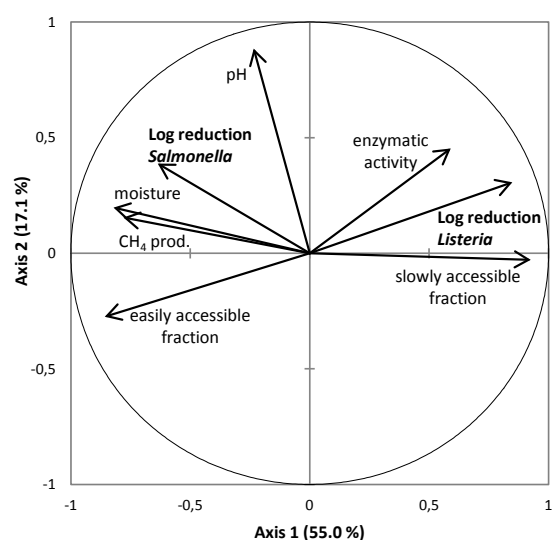
The log reduction of both bacteria ranged between 4.5 and 6 (Fig.1) within 20 days.



**Figure 1.** Kinetics of survival of *L. monocytogenes* (A) and *Salmonella* Derby (B) in 8 OWP. Dotted lines indicate the limit of detection.

Overall, *L. monocytogenes* survived longer than *Salmonella*. This is in accordance with the shorter persistence of *Salmonella*, compared to that of *L. monocytogenes*, observed in soil by Brennan *et al.* (2014). Interestingly, the level of *L. monocytogenes* remained stable during the first week of incubation in raw digestates and in liquid fractions whereas it decreased in composted digestates which had less easily accessible fraction (Table 1). However, *L. monocytogenes* was still detected in two of the composted digestates after 41 days. No lag phase was observed for *Salmonella* which survived longer in composted digestates.

A principal component analysis was performed on variables describing the behaviour of the strains and the characteristics of the OWP (Fig. 2).



**Figure 2.** PCA of data for chemical, biochemical and microbial characteristics of the 8 OWP.

The persistence of the strains was estimated by their log reduction after 7 days. The decay of *Salmonella* is positively correlated to the moisture content and to the anaerobic activity (residual CH<sub>4</sub> production) whereas the decay of *L. monocytogenes* was correlated to the slowly accessible fraction and negatively correlated to the easily accessible fraction of the OM (humic substance-like, cellulose and hemicellulose).

## Conclusion

The decay of *L. monocytogenes* and *Salmonella* is influenced by a combination of biotic and abiotic factors including substrate accessibility to microbes and microbial activity. Nevertheless, other factors such as microbial diversity may also interact and will be further analysed.

## Acknowledgements

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