Pathogen decay or growth in anaerobic digesters: a question with no possible answer? Elements of understanding for non-microbiologists

Jean-Jacques Godon, Nathalie Wéry, Nicolas Bernet

Godon, J.-J., Wéry, N., Bernet, N. (2018). Pathogen decay or growth in anaerobic digesters: a question with no possible answer? Elements of understanding for non-microbiologists. Presented at 15. IWA World Conference on Anaerobic Digestion (AD-15), Beijing, CHN (2017-10-17 - 2017-10-20).

Pathogen decay or growth in anaerobic digesters: a question with no possible answer? Elements of understanding for non-microbiologists.

J. J. Godon, N. Wery and N. Bernet.
LBE, INRA, 102 avenue des Etangs, 11100, Narbonne, France

(E-mail: *jean-jacques.godon@inra.fr*)

Abstract
The goal of this contribution is to analyze the relevance of measuring anaerobic digestion effectiveness to the elimination of pathogens. Elements such as the variation in abiotic and biotic conditions, indicators and measurement tools are analyzed in light of experimental data. We conclude that measuring the effectiveness of anaerobic digesters in eliminating pathogens seems an unrealistic challenge. We recommend measuring the pathogen risk only though analyzing the presence of pathogens in the digestate, and not via the decontamination efficiency of the process.

Keywords
Pathogen, survival, anaerobic digestion, digestate,

CONTEXT

Anaerobic digesters transform biomass into biogas and digestate. A large part of the biomass used is animal and human fecal wastes, and digestates are often spread in the environment. The behavior of pathogenic entities in this cycle is a relevant question.

QUESTION

Anaerobic digestion requires very specific biotic (living organism interactions) and abiotic conditions (process parameters). Can the 'undesirable' microbial entities (pathogenic bacteria, antibiotic resistance genes, viruses, prions, etc.) survive under these conditions, which often last for long periods of time, and how? And how can we evaluate this survival?

RESPONSE ELEMENTS

Anaerobic digestion

The anaerobic digestion process depends on a wide variety of conditions. The feeding, sizes and retention times are very variable. To this is added various pre- and post-treatments. Anaerobic digestion also takes place in a very wide variety of animal digestive systems. Two identical anaerobic digesters do not exist. However, biotic and abiotic parameters that influence the decay of pathogens present some common features.

Abiotic parameters in anaerobic digestion

The main original properties of anaerobic digestion are the low redox potential and the lack of oxygen. The pH is generally moderate and the temperature is mesophilic (around 35°C) or thermophilic (around 55°C). Except for thermophilic temperatures, these parameters are rather good for microbial survival (Pandey et al., 2015).

Biotic parameters in anaerobic digestion

The role of biotic parameters is mainly unknown in the microbial world and a fortiori in anaerobic ecosystems. Predation by protozoa and by viruses (bacteriophages) plays an important role in for example marine microbial ecosystems (Suttle, 2007), but is not quantified yet in anaerobic digestion ecosystems. Anaerobic conditions can decrease the presence of protozoa but not that of viruses. The presence of bactericidal agents can also be involved (Mateu et al., 1992). Moreover, due to the variety of inocula and the diversity of the process, the microbiota of each digester is different; microbiologically speaking there are never two identical anaerobic digesters.

Most of the pathogenic entities in anaerobic digesters come from human- and animal feces. Thus they have already undergone an anaerobic period in the human and animal digestive tracts. Animal digestive tracts have biotic and abiotic parameters similar to those of anaerobic digesters. The antagonistic role of the animal ecosystem towards pathogens has been widely studied. Parameters that act on pathogens, notably the microbiota-induced immune system (Brown and Clarke, 2017), are absent in anaerobic digesters.

Diversity of pathogenic entities

Pathogenic entities include living microbes (bacteria or eucarya), genes (e.g. antibiotic resistance genes) and proteins (e.g. prions). Currently, it is impossible to follows all of these. Several indicators or representatives must be chosen to mimic the behavior of all entities. Out of convenience, the same fecal indicators as for water were recommended, but their survival in anaerobic conditions is dramatically different from that in aerobic conditions (wastewater treatment plant vs drinking water treatment…). Therefore, indicators for anaerobic digestion cannot be a copy and paste of those used for aerobic systems.

Measuring tools

Life or death is not obvious from a microbial- or viral point of view. Thus, the use of different measuring tools leads to different views of the status of life and death, which remains an unsolved issue and is the topic of active debate within the scientific community. To summarize, methods based on culture (petri dishes) detect living organisms able to grow, but not those in a reversible state called VNBC (Viable But Non Culturable) (Pinto et al., 2015). Methods based on DNA detection of specific targets in environmental samples, such as quantitative PCR, detect intact DNA from living cells but also from dead cells and even free DNA (Hassard et al., 2016).

Experimental approaches

The great variability of digester parameters, the matrix heterogeneity and sampling makes it very difficult to do *in situ* measurements. Most published data were obtained on perfectly controlled laboratory devices and inoculation with laboratory cultures (Avery et al., 2014). Concerning phages, no inactivation was found after one month in mesophilic conditions (Mateu et al., 1992)). For coliforms in mesophilic conditions, ^{2}log reduction was not observed to be related with retention time (Mateu et al., 1992) but elsewhere, a similar reduction was found to be linked to retention time (Pandey et al., 2016; Pandey and Soupir, 2011). Antibiotic resistant bacteria from animal feces were shown to be reduced but persistent in mesophilic conditions (Resende et al., 2014). Many factors are invoked to explain such reduction including pH, bactericidal agents (Mateu et al., 1992), ammonia, feedstock characteristics, volatile fatty acids, protein, fats, and carbohydrates. The impacts of these factors on inactivation kinetics are unclear. The only clear active factor is the temperature (thermophilic condition) (Pandey and Soupir, 2011). It is important to notice that almost all microbial analyses were done using culturing approaches, and inactivation correspond to a lack of bacterial growth (cf. Measuring tools above).

Few works use culture independent methods such as qPCR to follow the decay within anaerobic digesters. Maynaud et al (2016) have shown drastic differences in persistence between *C. coli*, *Salmonella* and *L. monocytogenes* in digestates. They show also a strong discrepancy between results of qPCR assays and the culture method (Maynaud et al., 2016).

CONCLUSION AND FUTURE DIRECTIONS

The measurement of pathogen dynamics, decay or growth, in anaerobic digesters seems neither relevant nor possible. Each digester is different for its microbiology, for its substrate and its processes; the imperfect laboratory data on batch conditions cannot be extrapolated. Finally, the indicators used are not relevant and the measuring tools do not allow concluding on inactivation or not. However, the data gained on the digestive systems of animals will allow a better understanding of the processes involved (e.g. predation). Finally, only measurements in the digestate can guarantee its microbiological 'quality'. Regulations should take those facts more into consideration.

REFERENCES

Avery, L.M., Anchang, K.Y., Tumwesige, V., Strachan, N., Goude, P.J., 2014. Potential for Pathogen reduction in anaerobic digestion and biogas generation in Sub-Saharan Africa. Biomass Bioenergy 70, 112–124. doi:10.1016/j.biombioe.2014.01.053

Hassard, F., Gwyther, C.L., Farkas, K., Andrews, A., Jones, V., Cox, B., Brett, H., Jones, D.L., McDonald, J.E., Malham, S.K., 2016. Abundance and Distribution of Enteric Bacteria and Viruses in Coastal and Estuarine Sediments-a Review. Front. Microbiol. 7. doi:10.3389/fmicb.2016.01692

Mateu, A., MataAlvarez, J., Pares, R., 1992. Enterobacterial and viral decay experimental-models for anaerobic-digestion of piggery waste. Appl. Microbiol. Biotechnol. 38, 291–296.

Maynaud, G., Pourcher, A.-M., Ziebal, C., Cuny, A., Druilhe, C., Steyer, J.-P., Wéry, N., 2016. Persistence and Potential Viable but Non-culturable State of Pathogenic Bacteria during Storage of Digestates from Agricultural Biogas Plants. Front. Microbiol. 7, 1469. doi:10.3389/fmicb.2016.01469

Pandey, P.K., Biswas, S., Vaddella, V.K., Soupir, M.L., 2015. Escherichia coli persistence kinetics in dairy manure at moderate, mesophilic, and thermophilic temperatures under aerobic and anaerobic environments. Bioprocess Biosyst. Eng. 38, 457–467. doi:10.1007/s00449-014-1285-3

Pandey, P.K., Cao, W., Wang, Y., Vaddella, V., Castillo, A.R., Souza, A., del Rio, N.S., 2016. Simulating the effects of mesophilic anaerobic and aerobic digestions, lagoon system, and composting on pathogen inactivation. Ecol. Eng. 97, 633–641. doi:http://dx.doi.org/10.1016/j.ecoleng.2016.10.047

Pandey, P.K., Soupir, M.L., 2011. Escherichia coli inactivation kinetics in anaerobic digestion of dairy manure under moderate, mesophilic and thermophilic temperatures. AMB Express 1, 18. doi:10.1186/2191-0855-1-18

Pinto, D., Santos, M.A., Chambel, L., 2015. Thirty years of viable but nonculturable state research: Unsolved molecular mechanisms. Crit. Rev. Microbiol. 41, 61–76. doi:10.3109/1040841X.2013.794127

Resende, J.A., Silva, V.L., de Oliveira, T.L.R., de Oliveira Fortunato, S., da Costa Carneiro, J., Otenio, M.H., Diniz, C.G., 2014. Prevalence and persistence of potentially pathogenic and antibiotic resistant bacteria during anaerobic digestion treatment of cattle manure. Bioresour. Technol. 153, 284–291. doi:http://dx.doi.org/10.1016/j.biortech.2013.12.007

Suttle, C.A., 2007. Marine viruses - major players in the global ecosystem. Nat. Rev. Microbiol. 5, 801–812. doi:10.1038/nrmicro1750