Neglecting the inoculum of anaerobic digesters: a missed opportunity?

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Abstract

Every year the knowledge about the microbial ecology of anaerobic digestion increases but despite that, the inoculation step of anaerobic digesters still remains empirical. Anaerobic digestion remains a microbial process without any microbial engineering. In this review we try to understand why putting in practice this knowledge remains difficult by identifying bottlenecks of this unsuccessful story.

Introduction

Twenty years ago the microbiology of anaerobic digestion was a black box and microbial inoculation was empirical. Today with the application of concepts of microbial ecology, the appropriation of metadata through high-throughput sequencing and modelling, the microbiology of anaerobic digestion is much better known but the inoculation of digesters remains empirical.

In the meantime, food- and pharma-industries have exploited the potential of specific sets of microorganisms (e.g. in brewing, wine production, bread and yoghurt making and antibiotic production). It results that optimized ferments and inocula are readily available for a variety of desired processes. Bioengineers in these industries compose their biological resources to their needs, reproducibly, over and over. In contrast, for anaerobic digestion and also for other pollution removal applications like wastewater treatment, any biological management of the microbial resource based on optimized microbial communities has not become current practice.

In anaerobic digestion and the wastewater treatment context, there is no defined seed community to which management strategies can be applied. These ecosystems are open to continuous contamination by the organic matter to be treated. The current thinking is that the appropriate microbial resource for treating the waste in these engineered ecosystems is supplied by the microbial community residing in the waste itself. Management is only possible by physico-chemical means (e.g., pre-treatment of the waste, temperature adjustment, control of hydraulic retention times).

Starting from this statement, we propose to review past experiments dealing with inoculation, to draw some conclusions about experimental failures, and finally to propose some future research perspectives. This contribution will provide an alternative thinking by developing concepts of microbial resource management for optimizing the biogas production in a continuous reactor environment. We think there is room for improvement of anaerobic digestion by the analysis of failed experiments and by including the inoculation step in the scientific analysis.

Difference between anaerobic digestion and actual success stories where targeted inoculation is used

Batch versus continuous processes

The wishful thinking that the targeted inoculation of a microbial process may be successful originates from other industrial processes such as the dairy industry. However, processes like yoghurt making are not comparable with anaerobic digestion because of how bioreactors are operated in two situations: influent streams in anaerobic digestion are treated continuously while the food- and pharma-industries operate batch reactors. The microbial community in a batch process can be manipulated at the point of departure. The small set of optimized microorganisms may interact during the development of the reaction (Mounier et al 2008) and this initial choice of ferments strongly governs the output of the process, regardless of whether the optimized ferments are outcompeted by indigenous microorganisms.

Pure culture alone versus pure culture within a complex ecosystem

The huge metabolic versatility of microbes to produce various enzymes and compounds is widely exploited for example in the food- or pharma-industries. This great potential primarily relies on a pure culture approach. Attempts to introduce 'brilliant' strains within a complex microbial ecosystem often failed (Bouchez et al 2000). This assertion is true not only for anaerobic digestion but also for all microbial ecosystems. The explanations of these failures are not really known but may partly be explained by predation (protozoa and virus) and by the lack of fitness of 'brilliant' strains from well-controlled lab environments compared to indigenous microorganisms that are already used to struggle for life and able to cope with environmental fluctuations.

Perennial versus engineered bioreactors

Another critical aspect may be the time dedicated for selection and adaptation as for example in animal guts. In the animal context, the vertical transmission of the microbiote from one animal generation to the next allows precise adaptation and a co-evolution between animal (process) and microbial community (Godon et al., 2013). Thus, each gut microbiote is 500 000 000 years old. In contrast, in anaerobic digesters selection and adaptation of the microbial community over comparable time spans did not occur. The maximum numbers of vertical transmission steps are once from mother reactor to offspring reactors such as for UASB granules. The microbial communities of anaerobic digestiors are at most a few years old, duration far below the evolutionary time necessary for a real adaptation to the environment.

Can we manage the microbial resource in anaerobic digesters at all? If yes, at what point, how and who?

Despite many trials found in the literature or the availability of some commercial products, the question of whether it is possible or not to improve the microbial community of an anaerobic digester has not been answered yet. This question is obviously tackled and answered on the side of process engineering by engineers but not on the microbial engineering side by the microbiologists.

At what point during reactor operation can we improve by the microbial resource?

Two possibilities for management of the microbial community may be imagined: an inoculation effect when starting a reactor, or a continuous addition of microbes during the operation. Following the animal example, the inoculation effect is essential, after initial colonization, the microbiote establishes a barrier (barrier effect) (Young et al., 2014) which prevents the integration of new members of the microbial community. To overcome this barrier such as for probiotic in human nutrition, a continuous feed of optimized ferments (e.g., *Lactobacillus* in yoghurt) is required.

One might expect that the development of a working anaerobic digestion community may occur from virtually any ecosystem if we provide anaerobic conditions and the required nutrients for microbial growth over a sufficient length of time. The question then is what part of the seed community will be sorted to provide an efficient ecosystem for anaerobic digestion. If the seed community comes from an aerobic environment, a tiny fraction of the community will be able to adapt to anaerobic digestion. During the enrichment process, there is room for microbial improvement of the community.

Role of the diversity

Microbial diversity is often measured from 16S rDNA inventories. The richness (i.e., the number of species) is recorded most of the time (Sundberg et al., 2013) albeit it is prone to biased results as compared to Shannon and Simpson diversity indices (Haegeman et al., 2013). The microbial diversity may be used as a proxy for process performance (Quéméneur et al., 2011), may correlate with functional parameter or may enquire early warning of process failure. However, we do not yet have an answer if changes in diversity are cause or consequence of the digester malfunctioning. Thus, diversity would not be a possible driver and would not be handled.

Inoculation strategy

Before concluding on any inoculation strategies from published data, one major bias comes from the small number of inoculum or digesters tested and from the variability observed between replicates. Thus, the sludge used as seed inoculum evolves over time in terms of community structure and methane production, whether inoculated or not. Deciphering the respective role of community changes and inoculation may be difficult. Another problem which impairs experimental results is the relatively short length of experiments, most of the time on the order of less than 3 months. This corresponds merely to the startup of the digester, but could not be associated with a steady state (Konopka et al., 2007). Moreover, the review of the literature is highly biases because negative results are rarely published.

Super-bacteria strategy

Based on the idea of missing organisms, a large number of microbes were isolated and used for bioaugmentation of anaerobic digesters. Most of published papers show conclusive evidence of increase of the methane yield (Bagi et al., 2007; Zhang et al., 2015). However, this promising approach was impaired by: (i) the huge microbial diversity and the functional redundancy associated in all anaerobic digesters which are in disagreement with the missing microbes concept (Riviere et al., 2009), (ii) the evidence of non-capability for exogenous microbes to survive in complex ecosystems (Bouchez et al., 2000), (iii) the absence of application of successful bioaugmentation at the industrial stage.

Super-inoculum strategy

In contrast to pure culture bioaugmentation, another strategy more closely to the reality of ecology concept were to use inocula already know to be efficient. Animal digestive systems are good candidates with a significantly higher rate of biodegradation (Weimer et al., 2009). Among them, rumen microbiote was often tested alone or in co-inoculation (Gijzen et al., 1987)(Blasig et al., 1992) and despite variable results on laboratory experiments, molecular tools revealed that no ruminal microbial species are able to settle in the bioreactors (Chapleur et al., 2014).

Inocula from diverse anaerobic digesters were also compared. Most of the results have shown a strong dynamic of microbial communities over time, strong structural difference between microbial communities and variable methane production between the inocula (De Vrieze et al., 2015). However, in the context of natural fluctuations of microbial populations (Fernandez et al., 1999)(Zumstein et al., 2000) the stability of such variations of methane production remains to be confirmed over time.

Overall description strategy

For 20 years, boosted by molecular tools and massive sequencing of DNA descriptive knowledge on the microbiology of anaerobic digestion has significantly increased. Gradually, we were able to describe the species present, the genes, the expressed genes and finally the proteins. Promises to justify the acquisition of this vast amount of knowledge were ultimately a proactive management of anaerobic digestion. Thus, anaerobic digestion is no longer a black box. The wealth of information has replaced the black box but the proactive management remains an unfulfilled promise.

Neglected questions

Propagation and storage of microbial consortia

Before you can use high-performance complex inocula for industrial digesters, we must solve some related issues that are rarely discussed. First, how to propagate such complex inoculum keeping both functions and microbial community structures? Second, how can we store performing complex inoculum for further inoculations? It remains a real challenge that has to be evaluated in future studies.

Definition and classification of the relevant ecosystemic services

The goal of microbial improvement should be to select the more robust and efficient microbial ecosystems. But, the quality or the performance of an inoculum depends on the expected function. One inoculum performing exceptionally well with one substrate may be outperformed by other inoculums when changing the nature of the substrate feed. Which selection criteria to be chosen to rank inoculums is the right question to ask (i.e., resilience after a toxic addition, resistance to overloads, versatility towards the nature of the organic matter used as feed, biodegradation of persistent organic pollutants, etc.). However, the unique measurable service is the yield of produced biogas. The others relevant services such as resistance, resilience, barrier effect against pathogens etc. cannot be really measured and by this way selected. The links between these different ecological services are unknown, thus for industrial application a biogas gain in exchange for a loss of stability is acceptable?

Conclusion

Research on the inoculation of anaerobic digesters is not over yet. The selection of a suitable inoculum strategy to start anaerobic digesters is Animal guts, who already achieved the use of the right microbiota for anaerobic digestion, remain a relevant source of inspiration for engineers. At last, human microbiote replacement is the example of a promising methods (using feces from healthy donor to treat recurrent *Clostridium difficile* infection of a patient (Cammarota et al., 2014) which demonstrate the feasibility and the potential of suitable inoculation.

Acknowledgements

This work was supported by the French Institute for Agricultural and Food Research (INRA).

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