

## Understanding how communities of uncultured microbes catalyze biotransformations at the meta omic era and beyond

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### Résumé:

In environmental biotechnological processes such as wastewater treatment plants or anaerobic digesters, the natural abilities of environmental microbes are intensively exploited in order to convert, stabilize or recycle the different liquid and solid organic waste streams produced by our societies. These complex communities of microbes are catalyzing the bioconversion of organic substances and nutrients into desired outputs and therefore constitute the "microbial engine" of the process. The study of these communities has long been hindered by the difficulty to isolate and grow in the laboratory the microorganisms involved in the various bioconversion processes. The advent of molecular techniques during the 90s, based on the amplification and analysis of 16S rDNA molecules, progressively uncovered the diversity and dynamics of microbes inhabiting these engineered environments. During the 2000s, combination of isotopic tracer and molecular microbiology techniques further enabled to document into more details the key microbial populations involved in various degradation processes in situ. Today, a new turning point has been reached with the application of high throughput meta omic techniques. These approaches give an unprecedented access to detailed microbial functions sustaining process performances and shed light on what the process engineers used to call the microbial "black box". Some examples, taken from the study of anaerobic digestion processes, are given below to illustrate how these approaches could help us to progressively build a more holistic view of these microbiomes. Associated emerging challenges related to meta-data treatment should however also be outlined. Beyond correlation analysis and data reduction strategies, I also particularly want to advocate the need for more conceptual approaches for knowledge integration into a comprehensive framework that could be used to manage ecosystems services and foster innovation.

Metaproteomics is aiming to characterize the proteins expressed by the whole community. For process engineering, proteins constitute particularly relevant biomarkers as they are directly supporting the catalytic activities responsible for biotransformation processes. However, protein identification is constrained by the current lack of relevant metagenomic databases that could be used to efficiently match the mass spectra generated by high throughput protein analyses of AD samples. Nevertheless, in order to more precisely evaluate the insights that could be gained relying solely on publically available protein databases, we have carried out a metaproteomic survey of thermophilic lab-scale batch digesters degrading cellulose (Lu et al., 2014). By multiplying LC MS analytical replicates on various protein fractions, we were able to demonstrate the expression of more than 500 distinct non-redundant microbial functions involved in hydrolytic, fermentative and methanogenic processes and contributing to the methanisation of cellulose, the most abundant renewable biopolymer on earth. A high proteolytic activity catalyzed by members of the Genus *Coprothermobacter*, possibly acting as

scavengers of the abundant exocellular enzymatic pool required for cellulose hydrolysis, was also revealed. This finding illustrates how unexpected competitive interactions within community members and direct informative functional insights can be evidenced by metaproteomics. The attractiveness of this approach should increase in the future as sequence databases are growing exponentially.

In order to document community function relationships, metatranscriptomics is another particularly promising approach as it proposes to sequence only the genes expressed, therefore focusing on the genomic information relevant in a particular context. We have recently undertaken the metatranscriptomic survey of an industrial scale (5m<sup>3</sup>) Expanded Granular Sludge Bed (EGSB) pilot reactor, generating more than 18 million reads from 7 sampling events spanning over a 6 month period (Grégory Marandat et al., 2014). Sequence analysis allowed the identification of 500-1600 unique expressed microbial functions per sample covering all the major steps of C/N/S microbial conversion pathways. Influence of reactor operational parameters such as sulfate load or onset of micro aeration on the gene expression profiles associated to methane or sulfur metabolisms could especially be evidenced, demonstrating that this approach has the power to resolve functionally relevant information on industrial scale facilities.

Although these results illustrate how meta omic approaches can give invaluable insights on the functioning of AD microbiome, several hurdles have still to be overcome. In particular, the main research bottlenecks are gradually shifting from the development of analytical methodologies to the elaboration of in silico approaches aiming at extracting the relevant information from these large datasets and linking the various meta omic analyses into an inclusive picture. Today, this work largely relies on multivariate statistics and data reduction. Beside and beyond this ongoing work, another even greater challenge we have to face is the integration of all these new information into an ecologically consistent modelling framework. To this extent, it is noteworthy that our ability to model the AD microbiome has not taken advantage of this new knowledge and relies on a rather rudimentary ground. Expert models such as the Anaerobic Digestion Model 1 (Batstone et al., 2002) describe microbial dynamics using the phenomenological Monod equation (Monod, 1942), which does not allow taking into account one of the main characteristics of AD microbial communities: their plasticity and adaptability to a variation of thermodynamic conditions. It is indeed striking to notice that despite the diversity and complexity of ecological interactions therein, the functional response of microbial communities to given thermodynamic conditions tends to always converge towards a state of maximum Gibbs free energy dissipation, suggesting that a thermodynamic principle might be governing the ecological dynamics of those systems, possibly in relation to the "Maximum Power Principle" formerly proposed by Lotka (Lotka, 1922a; Lotka, 1922b). This observation has led us to consider a new thermodynamic modelling framework and to propose a microbial growth equation that relates microbial growth rate to available energy (Desmond-Le Quemener & Bouchez, 2014). In the future, following this track, we believe that the integration of the ecological rules sustaining the functioning of the AD microbiome into this thermodynamic framework could allow us to elaborate more predictive models and to progressively set up the principles of a sound ecological engineering of AD microbiome, that could take full advantage of the microbiote self-organizing capacities in order to maximize services and foster innovation.

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