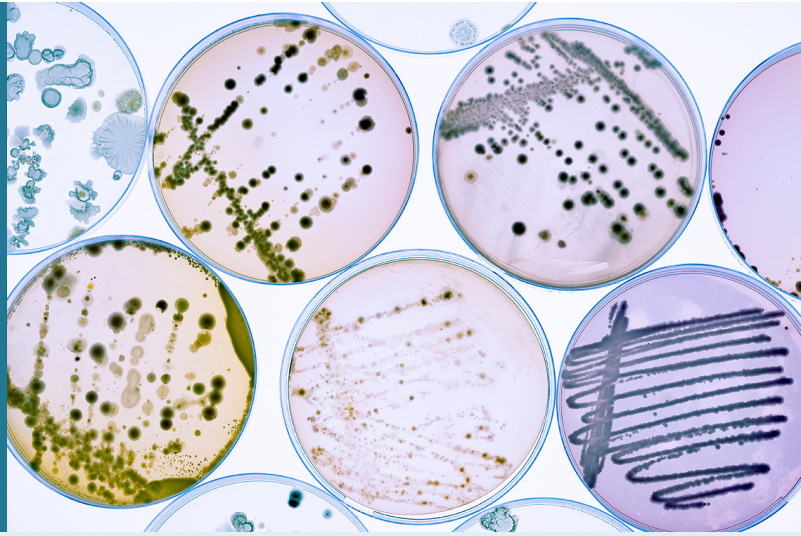


## Microbiome Engineering



The Microbiome Engineering group works at the interface of engineering, microbiology, and computational biology to develop bio-based processes to produce bioenergy and biomolecules from biomass. The group is especially interested in the process of anaerobic digestion and biomethanation, studying both natural and engineered systems. In addition, the group has been applying its internally optimized molecular biology tools to study, in a high-throughput manner, microbiomes from lab-scale reactors and full-scale energy units, as well as communities in soil, animal guts, cave deposits, etc.

### Main expertise fields

- Anaerobic digestion (AD) is the most advanced technology to convert carbon-rich biomass, including lignocellulosic waste, into energy (methane-rich biogas) and value-added products, such as biofertilizers. Methane-rich biogas can be further upgraded to natural gas quality, via a process called biomethanation.
- Lignocellulose degradation. Lignocellulose is the major structural component of plant biomass, but a considerable fraction of this natural material is wasted because of its recalcitrance. The group characterizes microbial communities from biomass-rich habitats, with the objective of further valorizing their lignocellulolytic capacities for industrial applications.
- Soil Microbiome. The soil microbiome regroups mainly bacteria, archaea, viruses, fungi, protists, and other small eukaryotes such as diatoms. Soil microorganisms play key roles in carbon and nutrient cycling by decomposing soil organic matter and transforming important nutrients for soil fertility, thus determining the productivity of agroecosystems. In addition, despite their importance in food security and climate change, most of the soil microbes remain largely uncharacterized.
- Wastewater treatment and pollutant removal, focusing on a biological process (e.g. aerobic granular sludge reactors) or the removal of recalcitrant compounds via adsorption or membrane operations.

### Research challenges

In LIST, along with microbiome characterization, the group develops innovative reactor designs and process monitoring tools to boost the process of biomass (organic waste, including lignocellulose) and gaseous waste valorization, contributing to the development of the biorefinery sector, as explained more specifically in the following points:

- To characterise the microbiome, the group relies on high-throughput sequencing, employing metagenomics and metatranscriptomics, as well as metaproteomics and metabolomics. By doing this, the group has recently shown that propionate oxidizing bacteria of the candidate phylum Cloacimonetes, can largely improve the resilience of the anaerobic digestion process, greatly boosting methane production from carbon-rich waste, at high organic loading rates. Currently, the group exploits their potential as a healthy implant of the anaerobic digestion reactors.
- The group has proposed exploiting the potential of multivariate statistical process monitoring on biogas composition to detect free ammonia intoxication at an early stage in anaerobic digestion reactors;
- Recently, the group has developed a dense membrane gas capture reactor to promote efficient biomethanation under mesophilic conditions. Currently a pilot-scale prototype has been implemented on a farm and tested under real-scale conditions. Preliminary lab-scale experiments have proven high rates of CO<sub>2</sub> conversion to methane, with the methane content in the biogas > 95% (i.e. natural gas quality biogas).

The Microbiome Engineering has investigated the highly specialized higher termite gut system, anaerobic digestion microbiome and soil. The most interesting outcomes include the following:

- The characterization of the microbial lignocellulolytic potential allowed the group to create extensive databases and identify potentially industrially relevant enzymes, including new endoglucanases, xylanases, mannanases, and other enzymes with auxiliary functions in lignocellulose degradation.
- For the first time, the group has identified and characterized multifunctional cellulases from the termite gut microbes, which are capable of simultaneously cleaving linkages between cellulose, xylans and mannans moieties.
- The application of an integrated omics approach to the anaerobic digestion microbiome allowed the group to identify members of the phylum Planctomycetes as previously unexplored robust biomass degrading bacteria, of high industrial relevance.

One of the group's missions is to promote anaerobic digestion residue as an environmentally benign biofertilizer which could contribute to the mitigation of nitrate leaching in agricultural soils and promote carbon sequestration in grasslands. The most interesting outcomes are detailed below:

- The diversity of bacterial communities is pH dependent, however, arable soils have more diverse microbiomes than grasslands and forests.
- Granulated biogas residue is a biofertilizer with the potential to supply nutrients to soil biota over time, and promote carbon sequestration in grassland soils, and thereby advance agricultural sustainability while contributing to climate change mitigation.
- The (partial) substitution of chemical fertilizers by raw digestate reduces the concentration of nitrate in the soil without having a negative impact on the yield and N content of the biomass.

### Application areas

- Biochemical methane potential (BMP) measurements and lab-scale and larger scale fermentations and biological methanation assessments.
- Characterizations of microbial communities (microbiomes and viromes - whole viral communities) and assessment of their metabolic potential with omics and bioinformatic data analysis.
- Isolation and characterization of aerobic and anaerobic bacteria of industrial relevance.
- Discovery, production and characterization of carbohydrate active enzymes for tailored solutions.

### Main assets

- [cLOMICS](#)
- [Microbiome Designer](#)
- [Persephone](#)
- [OPTILYS](#)
- [Metasoil](#)
- [M2ex](#) "Exploiting metal-microbe applications to expand the circular economy"

### Equipment

- Lab-scale and pilot-scale anaerobic digestion reactors, including 200 continuously stirred-tank reactors (CSTRs) with 2L capacity; four CSTRs with 100L capacity; eight anaerobic baffled reactors (ABRs) with 2L capacity and four compartmented ABRs with 100 L capacity.
- Five lab-scale and one pilot scale dense membrane gas capture reactor (DMGC).
- Short- (Illumina) and long-read sequences and other molecular biology tools.
- Two in-house servers and easy access to the LIST HPC facilities and other HPC resources available in Luxembourg.
- Anaerobic and microaerophilic cultivation chambers for the isolation and growing of microorganisms. The complete cultivation infrastructure includes several hundreds of sealed cultivation bottles, and instrumentation for anaerobic media generation, incubators, shakers, etc.

### Selected publications

- Westerholm, M., Calusinska, M and Dolfing, J. [Syntrophic propionate oxidizing bacteria in methanogenic systems](#) (2022) *FEMS Microbiology Reviews*, Volume 46, Issue 2, fuab057
- Calusinska, M., Marynowska, M., Bertucci, M. et al. [Integrative omics analysis of the termite gut system adaptation to Miscanthus diet identifies lignocellulose degradation enzymes](#) (2020) *Commun Biol* 3, 275
- Calusinska M, Goux X, Fossepre M, Muller EEL Wilmes P and Delfosse P. [A year of monitoring 20 mesophilic full-scale bioreactors reveals the existence of stable but different core microbiomes in bio-waste and wastewater anaerobic digestion systems](#) (2018) *Biotechnol Biofuels* 11:196
- Plattes, M., Goux, X., O'Nagy, O., Untereiner, B., Fernandez Lahore, H.M. [Zero-dimensional modelling, and bacterial characterization of an aerobic granular sludge reactor](#) (2023) *Journal of Chemical Technology and Biotechnology*, 98 (2), pp. 369-380.

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