

Genome-centric metagenomics and community-scale flux balance analysis reveal microbial dynamics in bio-electrochemical systems

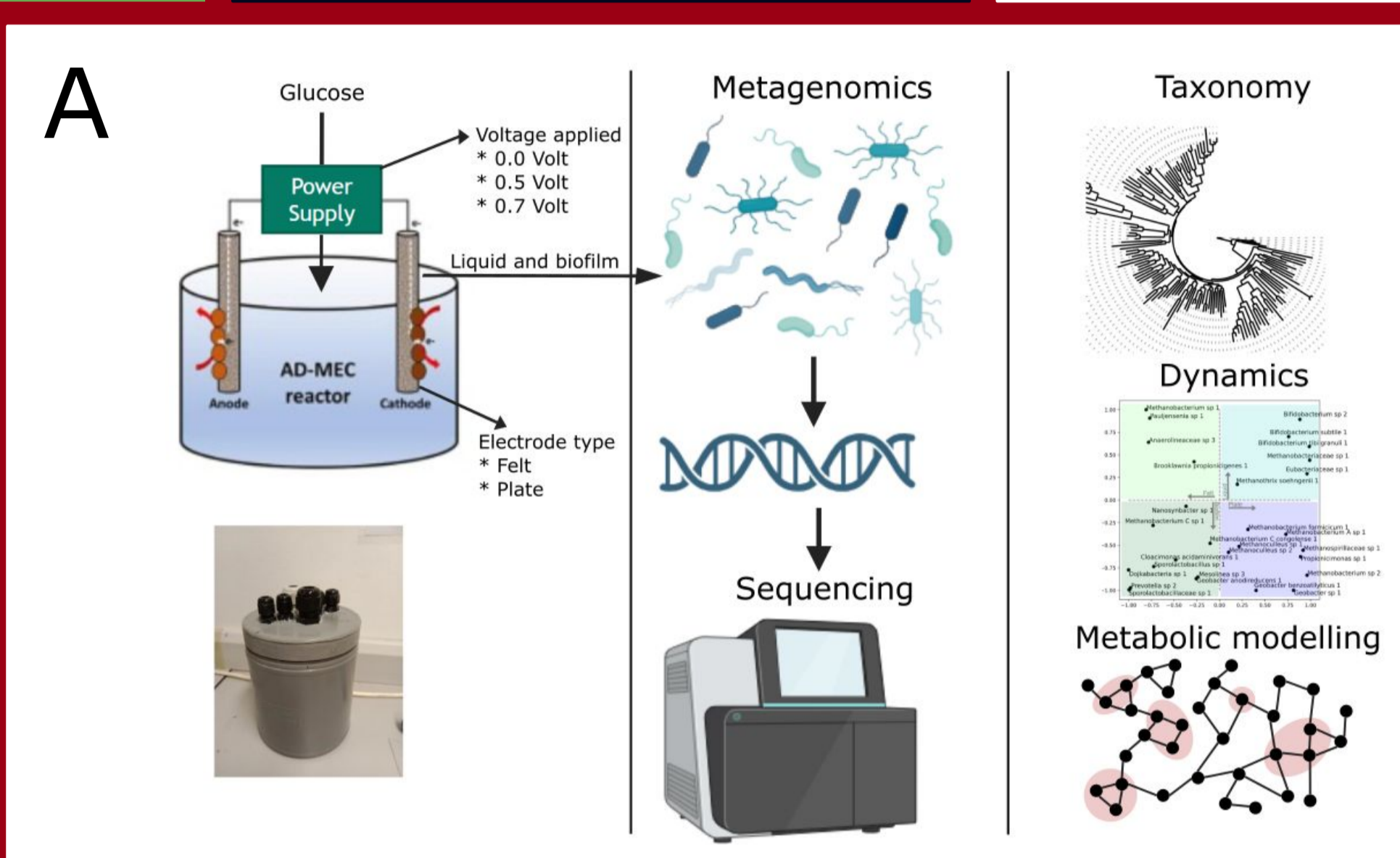
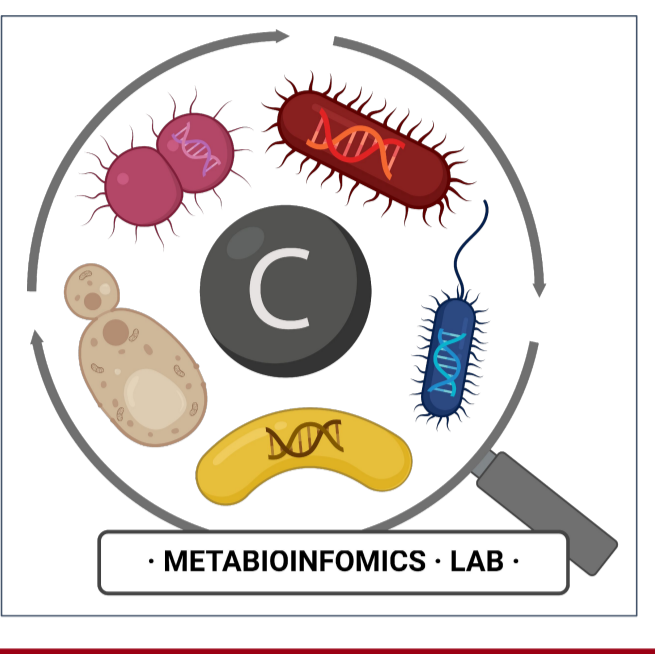
Genomics and Bioinformatics Research Unit

Davide Sanguineti¹, Maria Chiara Valerin¹, Luca Francescato¹, Dolores Hidalgo², Miguel Angel Sanchez², Guido Zampieri¹, Stefano Campanaro¹, Rudolphus Antonius Timmers², Laura Treu¹

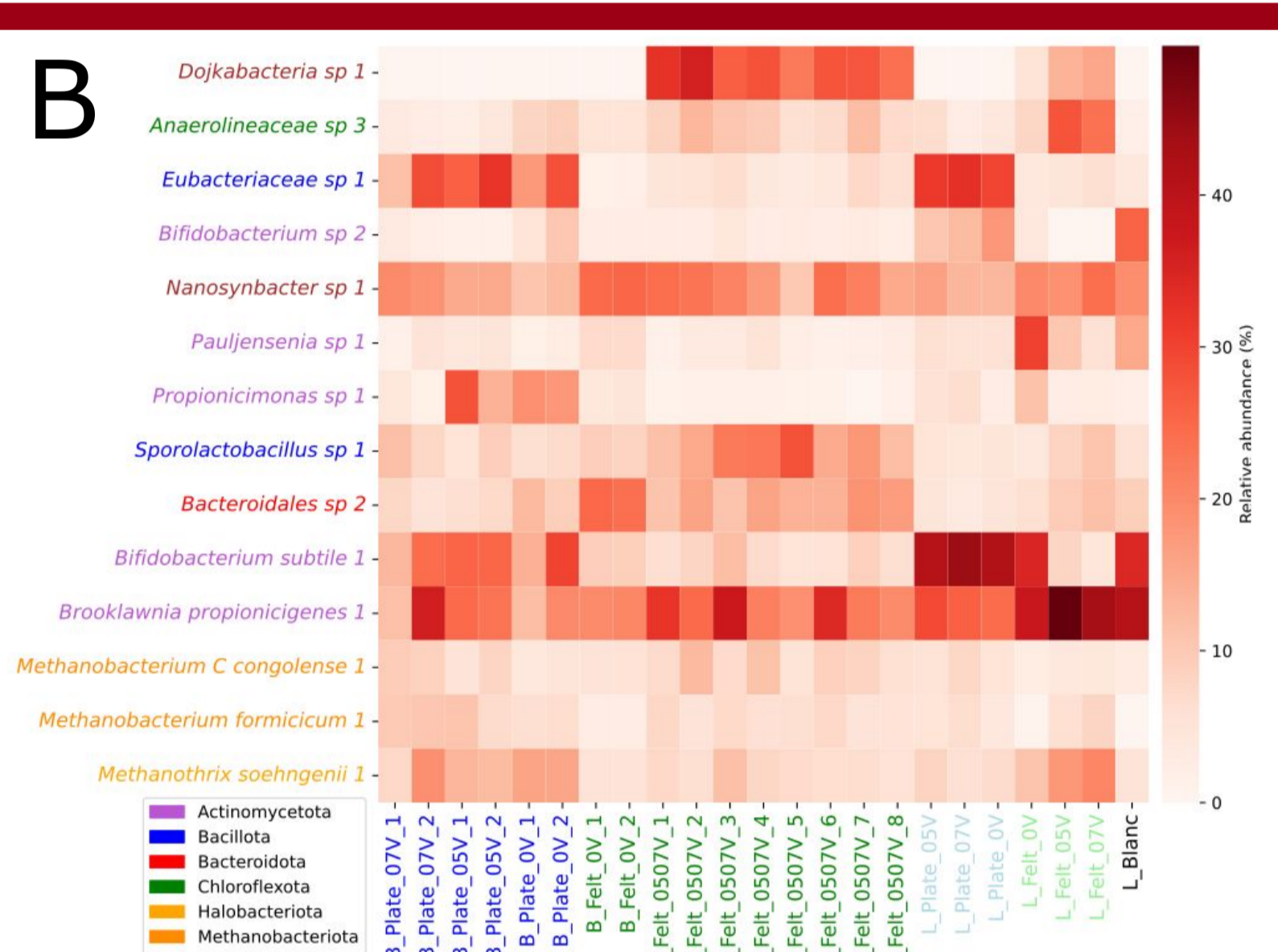
(1) Department of Biology, University of Padova, Via U. Bassi 58/b, 35121, Padua, Italy

(2) Circular Economy Area, CARTIF Technology Centre, 47151 Valladolid, Spain

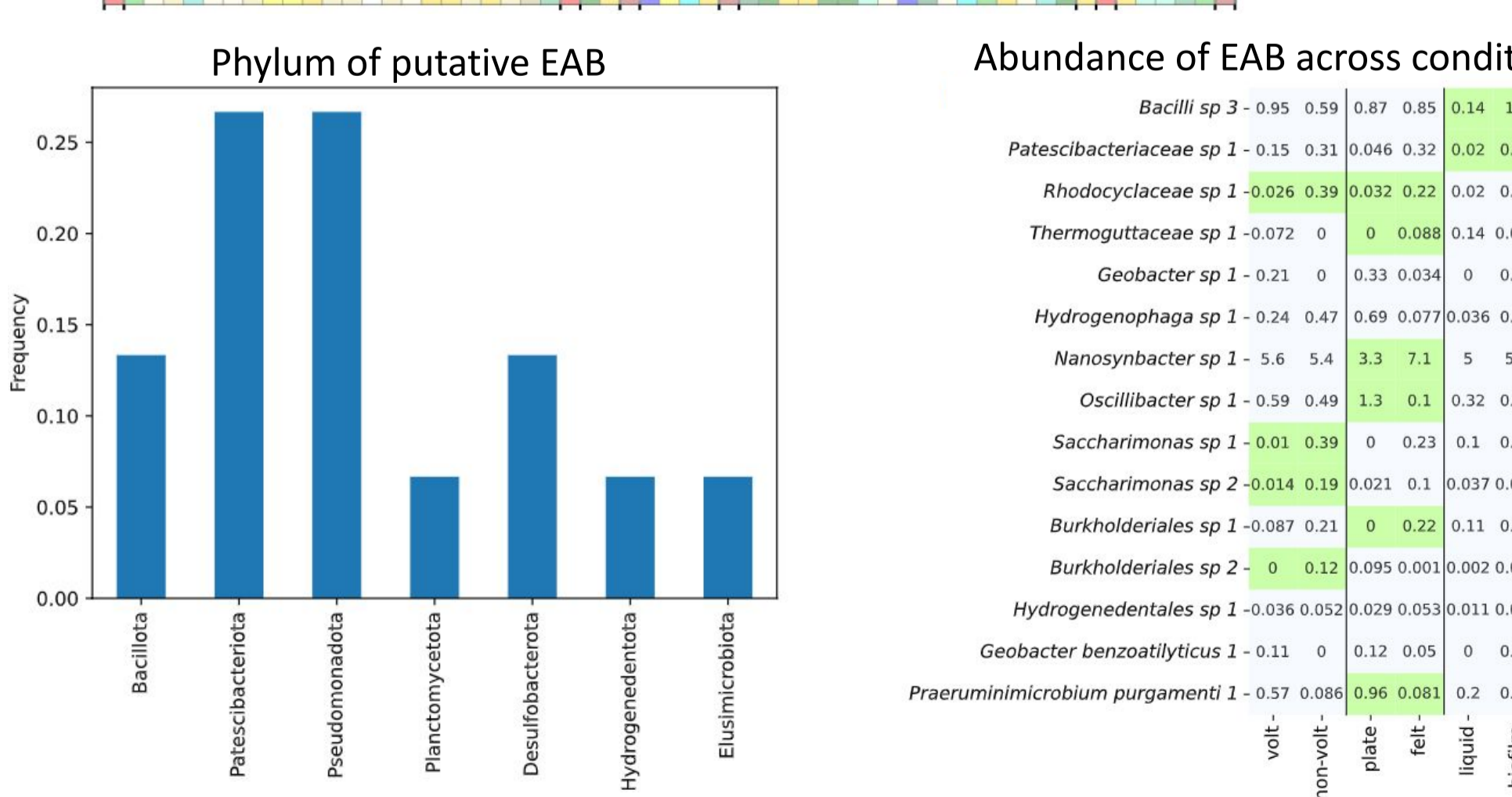
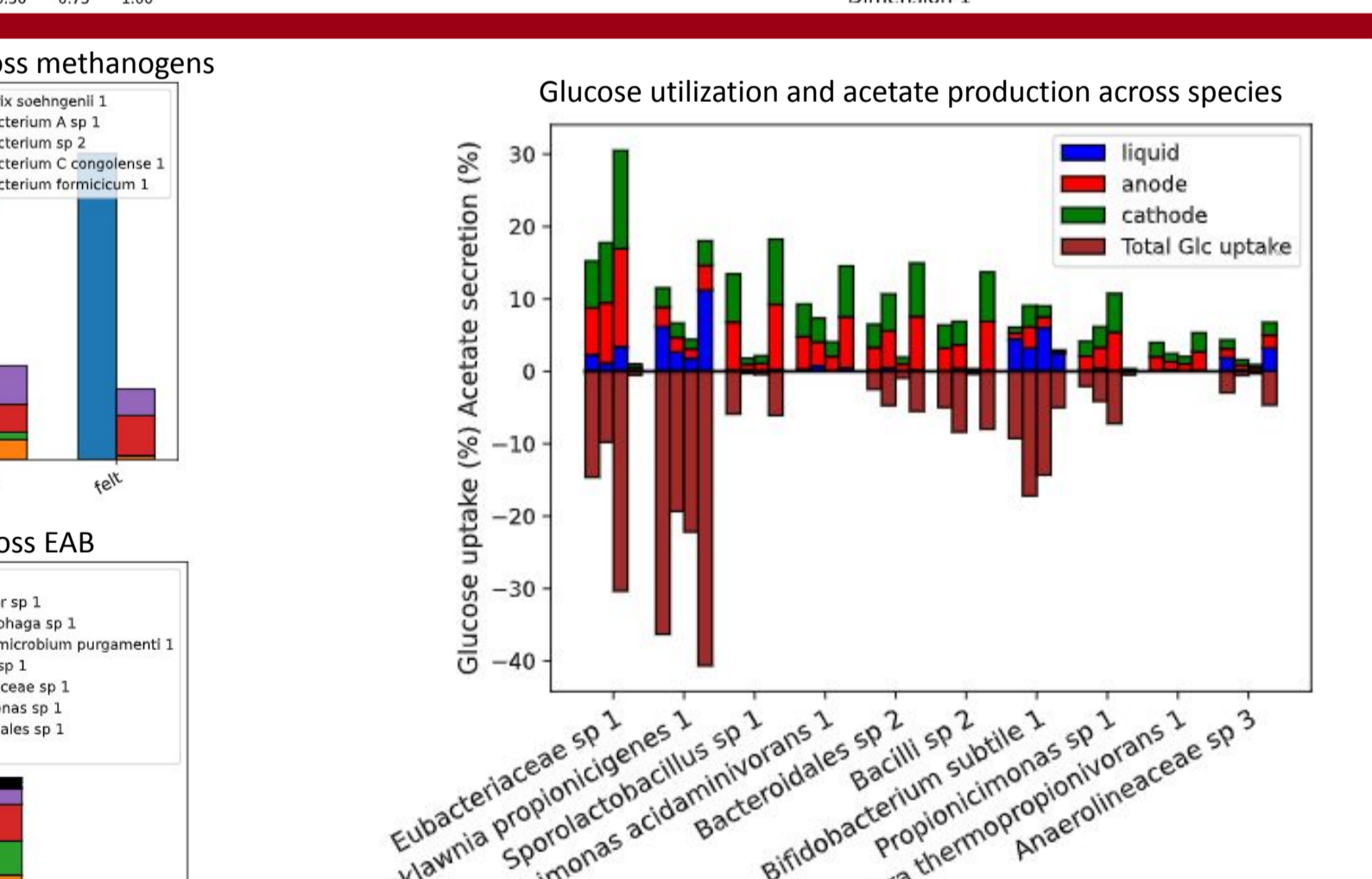
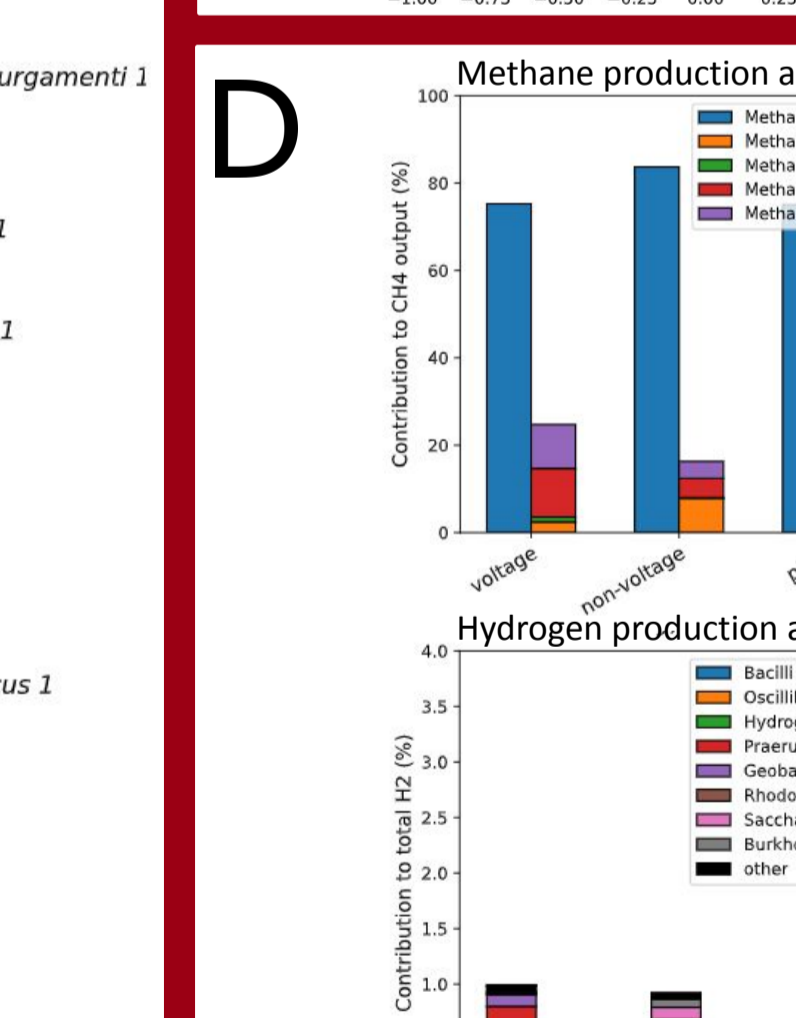
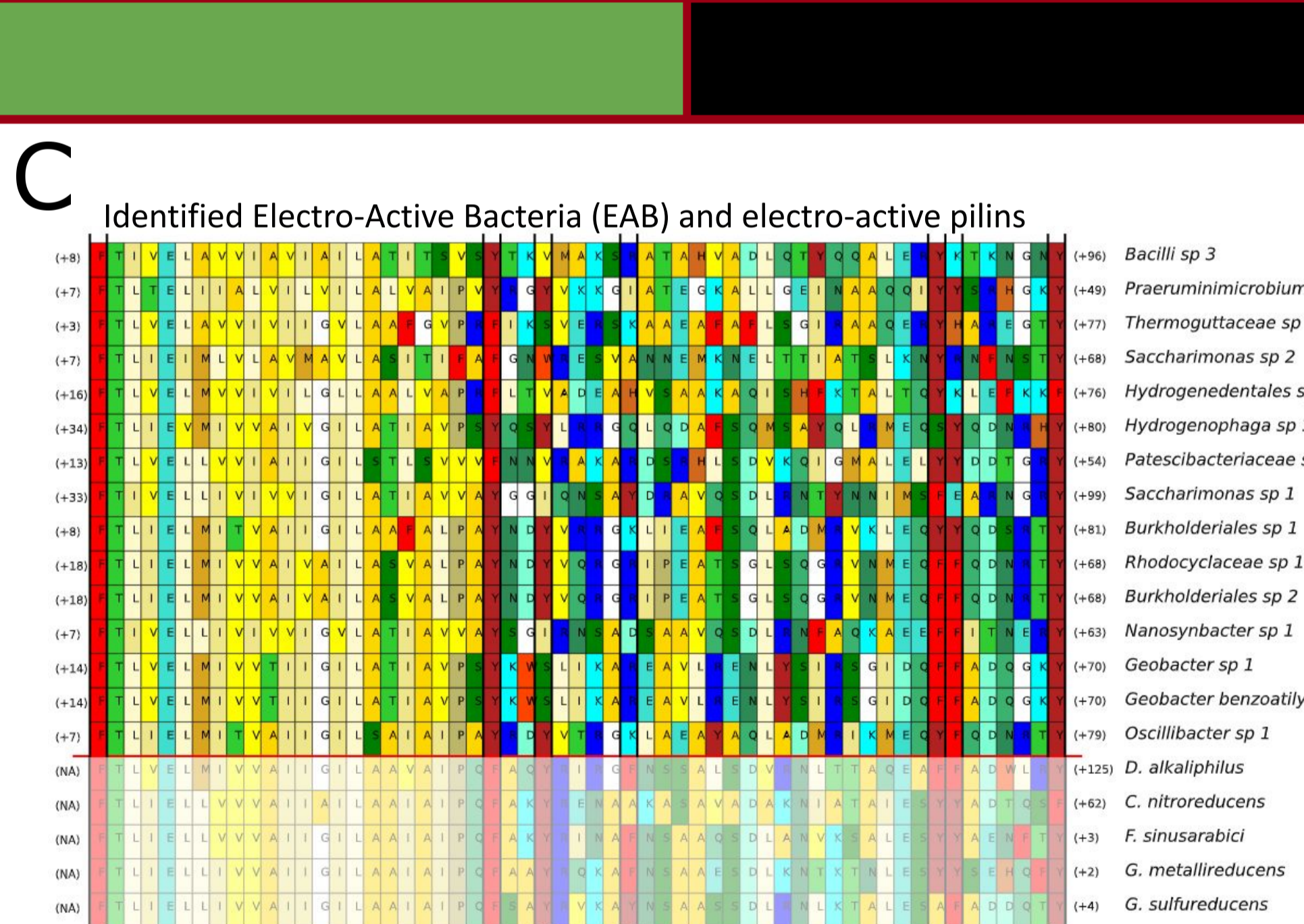
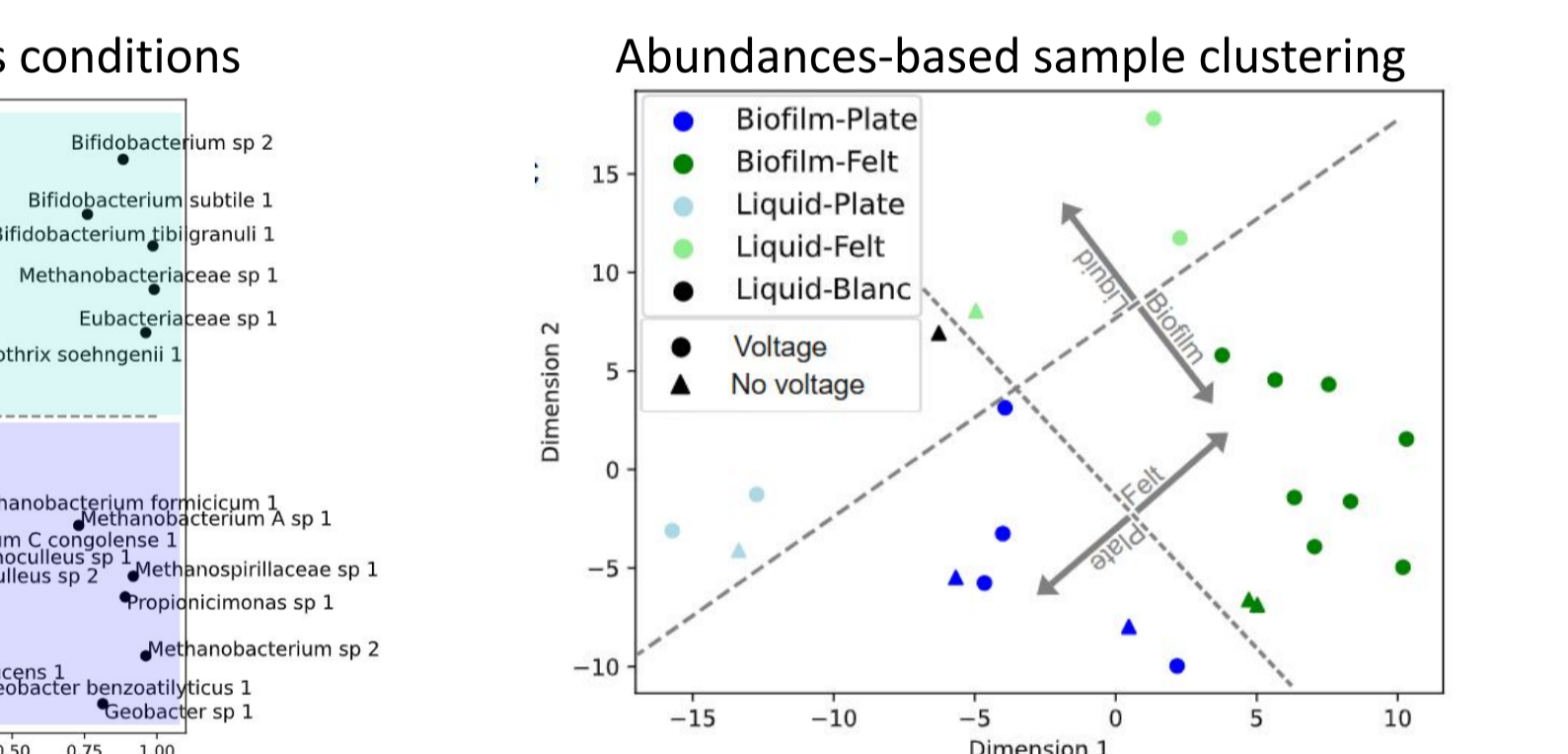
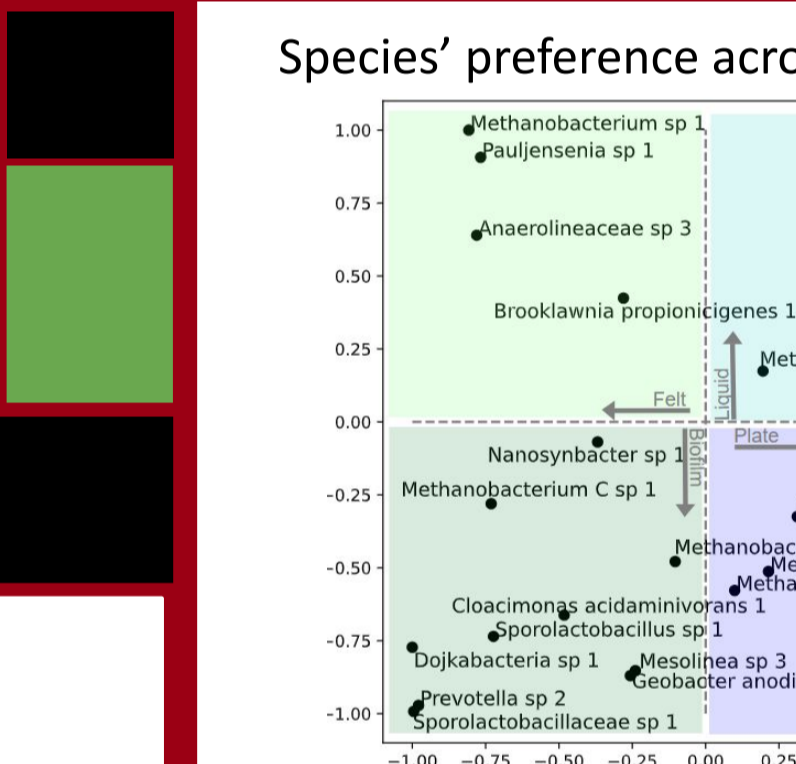
Anaerobic Digestion (AD) is a process of syntrophic organic matter degradation for biomethane production. Coupling AD with a **Microbial Electrolysis Cell (AD-MEC)** was shown to enhance **biomethane generation efficiency**. An AD-MEC system consists of an anode and a cathode placed in an AD reactor, with microbes thriving both in the liquid phase and as biofilms on electrode surfaces. At the anode, microbes anaerobically **oxidize** organic material, generating **electrons** that flow through the circuit. This flow drives positive ions toward the cathode, where protons are reduced to **generate H₂**. Hydrogenotrophic methanogens utilize H₂ to reduce **CO₂ to CH₄**. Unveiling the microbial “dark-matter” associated with this process could open to intelligent optimization of biomethane production. In this work, **six lab-scale AD-MEC reactors** were operated at different conditions, and biological samples were taken at the end of the experiment. We applied **metagenomics** and **metabolic modelling** to analyze microbial composition and activity and associating it to operational conditions.



Panel B. AD-MEC microbiome.
Top) Relative abundance (RA) of dominant species across conditions. B (biofilm) and L (liquid) indicate sample origin. Fermentative *B. subtilis* and *B. propionigenes* dominate the planktonic community across samples, with differential preference for Plate or Felt electrodes.
Bottom-left) Preference of relevant bacteria for a given condition, calculated comparing mean RAs: electrogenic species such as *Geobacter* spp. are enriched in the biofilm, similarly to methanogenic archaea.
Bottom-right) Multidimensional scaling using species' RAs in each sample. Sample origin (Liquid/Biofilm) and electrode type (Plate/Felt) clearly determine community composition, with applied voltage being less relevant.



Panel A. Overview. Six lab-scale Anaerobic Digestion coupled Microbial Electrolysis Cell (AD-MEC) were operated for 100 days at different levels of applied voltage and electrode type (Plate/Felt), and fed with glucose. Microbial samples were collected from both the liquid phase and the biofilm attached to the electrode surfaces. Metagenomics was used to reconstruct the community composition to associate taxonomy, functional pathways and conditions. System-level metabolic analyses were performed using community-level Flux Balance Analysis, starting from the genome-scale models of identified species.



Panel C. Electro-Active Bacteria. Top) 15 Putative EAB were identified by searching electrogenic pilins in their genomes using amino acids composition criteria. Transparent-shaded sequences correspond to known EAB, while relevant positions are marked by black bars. Bottom-left) Phylum-representation among identified putative EAB. Y-axis represents their fraction associated to the corresponding phylum. The epibiont phylum *Patescibacteriota* has the highest representation, together with *Pseudomonadota*. Bottom-right) Average RA of putative EAB across binary conditions. Green-coloured squares indicate significant difference in the condition pair. Application of voltage seems not to clearly favour EAB fitness.

Panel D. Community-level metabolic modelling. Top-left) Fraction of CH₄ generated by a given methanogen, according to metabolic modelling results. Acetoclastic methanogenesis (*M. soehngenii*) is consistently the dominant pathway. Bottom-left) Fraction of hydrogen generated from the activity of a given EAB. Across conditions, electrogenic mechanisms contribute to just a small fraction of the total hydrogen production in the system. Right) Fraction of acetate generated by a given species across conditions, and fraction of utilized glucose (negative percentage). Glucose-utilizers are responsible for the largest amount of generated acetate, and therefore of the total CH₄ generated.