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IMPLICATIONS OF SYNTROPHIC INTERACTIONS IN THE STRUCTURING OF BIOGAS-PRODUCING MICROBIAL COMMUNITIES

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Anaerobic digestion (AD) is an attractive process in the context of sustainable development since it combines waste treatment and renewable energy production. It is carried out by interdependent microbial guilds, which sequentially degrade organic substrates into simple products such as CO₂, H₂, and CH₄. Environmental selection pressure is recognized to shape the microbial populations associated with the AD process, but the significance of microbial interactions in AD microbial community assembly is less well understood. Our group studied the microbial communities in two full-scale anaerobic digesters (CDSR and UASB), both treating the same brewery wastewater. Their metagenomic analysis revealed similarities in bacterial composition but unexpected differences in dominant archaeal members (Spatola Rossi 2024). Bacteria utilize brewery effluent as a substrate, whereas methanogenic archaea rely on bacterial metabolic byproducts. Methanogenic archaea benefit from interactions with syntrophic bacteria, taking advantage of the hydrogen produced by fermentation and favoring bacterial growth near the thermodynamic limits. This led to the hypothesis that syntrophic interactions are determinants of the abundance of archaea species. From a top-down approach (Spatola Rossi 2024) we selected five bacterial species, distinctive for each anaerobic reactor and capable of syntrophic interactions, and the three most abundant archaeal species. We hypothesize that if syntrophic interactions define the community structure, synthetic communities built with syntrophic bacteria distinctive for each digester will lead to the dominance of the corresponding archaeal species. To detect and characterize microbial interactions, we compared microbial growth rates of the selected species, both axenically and in co-culture, finding a positive result (increase in growth rate when in co-culture) for *Levilinea saccharolytica* with *Methanobacterium formicicum*, and negative ones for *Aminiphilus circumscriptus* with *M. formicicum* and *A. circumscriptus* with *Aminomonas paucivorans*. With the selected strains, we set up synthetic communities (SC) resembling the composition of the two full-scale digesters starting from equal abundances and following its course over time by fluorescent *in situ* hybridization (FISH) and confocal microscopy. At the endpoint, we found significant differences in both SC, with *Methanobrevibacter arboriphilus* as the dominant Achaea in CDSR-like SC and *Methanobacterium oryzae* being dominant in

UASB-like SC ($p < 0.05$) confirming our prediction. Our results support the hypothesis that microbial interactions play an important role in the structuration of the archaeal community of anaerobic digestion.

Palabras clave: biogas - interactions - syntrophy