

Microbiome Structure and Dynamics in Novel Fixed-Bed Biofilm Systems for Medium Chain Fatty Acids Production from Organic Waste

Alessio Massimi^{ab}, Barbara Tonanzi^{ac}, Maurizio Petruccioli^b, Simona Rossetti^a, Simona Crognale^{ac*}.

^a *Water Research Institute, National Research Council of Italy, CNR-IRSA, Area Della Ricerca RM1, Via Salaria Km 29.300, Monterotondo, 00015 Rome, Italy*

^b *Department for Innovation in Agroforestry and Biological Systems (DIBAF), University of Tuscia, Viterbo, Italy*

^c *National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133 Palermo, Italy*

* simona.crognale@irsa.cnr.it

The microbial valorization of organic waste into medium-chain fatty acids (MCFAs) represents a promising resource recovery strategy. However, its efficiency is often limited by competition between primary fermenters and chain-elongating bacteria, with the former favored in the commonly-used fluidized-bed systems due to their higher growth rates and substrate uptake. To overcome this limitation, this study developed a novel single-stage fixed-bed biofilm reactor for the co-fermentation of food waste (FW) and waste activated sludge (WAS). Several tests were conducted under mesophilic conditions (35 °C) with a 4-day hydraulic retention time and varying organic loading rates (OLRs of 6.2, 12.4, and 18.75 gVSL⁻¹d⁻¹), without external pH control or the addition of external electron donors. Initially, two support-to-inoculum ratios (1:3 and 1:1 w:V) were tested using a mixture of FW and WAS at a ratio of 60:40 (on VS basis). After identifying 1:1 w:V as the best-performing ratio, additional tests were carried out using different substrates (i.e., FW:WAS at a ratio of 80:20, and FW liquid extract combined with WAS at a 60:40 ratio) combined with different feeding frequencies (i.e., 2, 3, and 5 days per week). Control reactors without filling material were operated in parallel for each tested conditions. A comprehensive chemical and microbiological characterization of biofilm and suspended biomass was performed using complementary advanced techniques (e.g., Illumina and Nanopore sequencing, microscopy-based analysis, and real-time quantification). By promoting microbial selection and biomass retention, fixed-bed biofilm systems enhanced system stability and MCFAs production and consistently outperformed controls, representing an effective strategy for valorizing complex organic waste into high-value chemicals.