Title: Waste-Derived Carbon Utilization in *Yarrowia lipolytica*: Quantitative Insights for Sustainable Biotechnology

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The transition toward a circular and sustainable bioeconomy increasingly depends on biotechnological processes capable of converting waste streams into valuable products. A major challenge in this context is understanding how microorganisms process unconventional, waste-derived substrates at the cellular level. *Yarrowia lipolytica*, a non-conventional yeast already used in several industrial applications, is particularly attractive because it can grow on a wide variety of low-value carbon sources that are inaccessible to many traditional production organisms.

Among such substrates are short-chain fatty acids generated during anaerobic digestion of organic waste, as well as small molecules derived from plastic degradation, such as ethylene glycol. These compounds represent promising, sustainable alternatives to sugar-based feedstocks, but their intracellular fate and contribution to cellular growth remain poorly understood, limiting their rational use in industrial bioprocesses.

In this work, metabolic flux analysis combined with stable carbon isotope tracing (^{13}C-MFA) was applied to quantitatively follow how carbon from waste-derived substrates is redistributed inside *Y. lipolytica*. By integrating measurements of substrate uptake, by-product secretion, and isotopic labeling patterns in biomass components, this approach reconstructs carbon flow through central metabolic processes involved in energy generation and biomass formation. The analysis reveals how different waste-derived substrates interact metabolically and how carbon is dynamically partitioned between intracellular pools.

Overall, this study highlights the importance of quantitative metabolic approaches to understand how *Y. lipolytica* transforms waste-carbon into cellular material. Such knowledge is essential for developing more efficient and predictable biotechnological processes that rely on waste substrates, reducing dependence on food-grade resources and supporting sustainable industrial biotechnology.