Building a computational and experimental toolkit to predict microbial performance in large-scale bioreactors

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The biological conversion of waste and biogenic feedstocks into fuels and chemicals plays a pivotal role in fostering a sustainable bioeconomy, and a wide range of such bioprocesses have been successfully demonstrated at the laboratory scale. However, the transition from bench- to industrial-scale operations poses significant challenges, as performance of these processes often decreases significantly. Thus, addressing scale-up issues emerges as a primary concern for advancing the bioeconomy. Bench-scale bioreactors, due to efficient mixing and high surface area to volume ratios, facilitate homogeneous conditions for crucial process controls such as heat transfer, pH control, aeration, and nutrient distribution. Yet, the translation of these ideal conditions to large-scale processes encounters numerous engineering hurdles that hinder uniformity. We hypothesize that the gradients observed in industrial-scale reactors create microenvironments leading to increased cell heterogeneity and decreased microbial performance.

To gain a deeper understanding of this "valley of death" between laboratory and industrial-scale bioprocesses, we are employing a scale-down methodology to two industrially relevant bioprocesses: muconate production in *Pseudomonas putida* and butyrate production in *Clostridium tyrobutyricum*. This approach enables us to examine how anticipated microenvironments impact bioprocess efficiency on a smaller scale, while also exploring any phenotypic and genotypic variations that might emerge under stress conditions. By integrating computational fluid dynamics and genome-scale models, we will predict microenvironmental conditions, thus guiding our experimental configurations in bench scale bioreactors. Subsequently, the data collected will inform machine learning models, aiding in the prediction and validation of process performance at the pilot scale (1,500 L). The use of both aerobic and anaerobic processes for the development of this pipeline will help to establish a host-agnostic framework that can be implemented across a range of bioprocesses. Ultimately, this project is establishing an experimental and computational methodology to mitigate the risks associated with bioprocess scale-up.