Summary for public release

Project Title: Understanding and Controlling Cell-to-Cell Variability for Robust Bioconversion
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Team: Washington University in St. Louis, Texas A&M University, Prairie View A&M University (HBCU), and Danimer Scientific.
Industrial Partners: Danimer Scientific.

Summary: To support BETO's mission on microbial conversion of lignocellulosic biomass into affordable low-carbon biofuels and bioproducts, it is critical to improve both microbial conversion efficiency (i.e. productivity, yield) and robustness. The **objective** of this project is to advance those items by exploiting non-genetic factors contributing to cell-to-cell variability in bioproduction and batch-to-batch reproducibility. This project will identify the major factors that cause cell-to-cell variability in industrial-relevant microbial strains—*Pseudomonas putida* and *Rhodococcus opacus*, and further reduce the variability to empower robust and efficient conversion of hydrolysate and lignin derived from lignocellulosic biorefinery. **This project will employ** multiple specific metabolite biosensors and use microfluidics-assisted time-lapse microscopy to track hundreds to thousands of living single cells, simultaneously measuring the variability in bioproduction, enzyme expression, ATP concentrations, metabolic precursor levels, and cell growth rates to understand the source of bioproduction heterogeneity. We will then develop gene circuits to reduce bioproduction variability and to improve the efficiency and robustness for the conversion of biomass hydrolysate into biodiesel and biodegradable plastic precursors. The **impact** of this project is significant because it will illuminate the factors causing non-genetic cell-to-cell variability in bioproduction, providing a systematic and quantitative understanding on the propagation of cellular noise to bioproduction in industrial-relevant microbial strains. Moreover, this project will offer multiple transformative strategies to reduce bioproduction variability, thus reducing failed fermentation batches and enhancing overall bioconversion yield and efficiency. These strategies are evolutionary as they can be applied to a broad set of industrial-relevant microbial production systems, reducing batch variability while improving robustness, cost competitiveness, and productivity of bioconversion. The project will also achieve the FOA's requirements by reducing the variations by 60% and increasing bioconversion efficiency, titer, and rate by 100%. The innovative process will be scaled up by our commercial partner, Danimer Scientific, as informed by our technoeconomic and life cycle analyses. Ultimately, the project has the potential to inform a broad range of bioprocess design and transform modern biorefinery with better economics, robustness and sustainability.