Summary for Public Release

Name of the applicant: The Ohio State University

Project director/principal investigator: Prof. Shang-Tian Yang

Project title: Metabolic and process engineering of solventogenic clostridia for stable, continuous *n*-butanol production from lignocellulosic biomass hydrolysates

Project objectives: The goal of this project is to develop robust solventogenic clostridia with enhanced stability and longevity for continuous production of *n*-butanol from lignocellulosic biomass in an integrated bioprocess at an economically competitive cost of less than $2.50/gal or $3.0/GGE (gal gasoline equivalent) suitable for biofuel application.

Project Description:
The Ohio State University, University of Cincinnati, Lawrence Berkeley National Laboratory, Southern Illinois University Edwardsville, and Tuskegee University will collaborate on the research to understand and mitigate bottlenecks limiting biobutanol production from lignocellulosic sugars in clostridial fermentation. Current production of biobutanol from sugar in clostridial fermentation is limited by its low product titer, productivity, and yield and poor process stability and short production duration. This project will initially focus on the understanding of mechanisms and factors (at genetic, metabolic, and process levels) causing large process performance variability and short or limited culture longevity in clostridial fermentation. First, selected clostridial strains will be evaluated under different fermentation (pH and C6/C5 sugars) and stress (oxygen, glucose limitation, butanol and hydrolysate inhibitors) conditions to study their effects on growth/fermentation kinetics and culture stability/longevity at different scales with population and multi-omics analyses. The results will be used to identify genes/enzymes contributing to the culture heterogeneity, production variability, and short production duration or longevity. Then, genome-engineered strains with mediated/aborted sporulation and reduced sensitivity to inhibitors or environmental stress will be created through the design-build-test-learn (DBTL) cycle until achieving >50% improvements in longevity or production duration (>1 month) and TRY (titer >18 g/L; rate >2 g/L·h; yield >0.3 g/g). A robust and genetically stable clostridial strain that can efficiently use all hydrolysate sugars (C5 and C6) in bioreactor cultures without significant carbon catabolite repression (CCR) while maintaining high TRY under stresses of oxygen, metabolites (butanol and butyrate), and hydrolysate inhibitors will be developed. The robust strain will allow continuous operation for an extended period (>1 month) without culture degeneration and can thus be used in a continuous fermentation with *in-situ* butanol recovery by gas stripping/pervaporation, which can also alleviate butanol cytotoxicity. Rational metabolic engineering with multi-omics analyses will help us better understand how and why fermentation performance varies and deteriorates in bioreactors at scale and how clostridia can be engineered for reduced variability with improved performance and prolonged production. The bioprocess including feedstock pretreatment, enzymatic hydrolysis, fermentation with *in-situ* butanol separation, and product purification will be evaluated and optimized at a bench scale and then demonstrated at a pre-pilot scale with techno-economic analysis to verify that *n*-butanol can be produced from lignocellulosic biomass at $2.5/gal or $3.0/GGE (gallon gasoline equivalent) with >70% reduction in GHG emissions. The successful development of biobutanol production technology will thus enhance the economic viability of the rural area by turning abundant agricultural residues and processing wastes into a green biofuel product.