

**Project Title:** Towards Economical Cell-free Isobutanol Production

**Applicant:** Invizyne Technologies, Inc.

**Principle Investigator:** Tyler Korman, PhD

**Major Participants:** Invizyne Technologies, University of California, Los Angeles

Replacing high volume petroleum fuels with renewables could have a significant environmental impact even as energy consumption is projected to increase 28% by 2040. Replacing petroleum fuels, however, is economically challenging as cost is critical in a high volume, low margin market—a challenge that has so far stymied most “New to Market” renewable fuels. Achieving stringent cost targets requires extremely efficient and robust conversion of biomass feed-stocks into fuel. So far, microbial conversion approaches comparable to yeast-based ethanol production have not yet been able to achieve the stringent production parameters required to match the low petroleum fuels costs. Recently, cell-free methods have gained considerable interest as an alternative way to meet the challenge. The cell free approach makes use of the cell’s sophisticated machinery (e.g. enzymes) to convert sugar into chemicals without having to worry about maintaining cell viability.

Invizyne Technologies is seeking to use cell free systems to break through biological barriers to achieve efficient, economical bio-based chemical production. Invizyne has developed proprietary methods to resolve energy imbalances that allow cell-free reactions proceed for long periods of time without intervention. Additionally, by removing cellular constraints such as toxicity and competing metabolism, our cell-free approach can be used to produce not only biofuels, but provide an alternative, viable route to cost-effective production of many other low value/high volume chemicals that compete with petroleum products.

In this project, we propose to show the potential of the cell-free approach by developing a cell free process with disruptive production metrics, a full order of magnitude beyond current cell-based methods. Specifically, our goal will be to expand upon our previous proof-of-concept experiments to produce the second generation biofuel, isobutanol, from low cost inputs at meaningful scale and increased productivity. In collaboration with the Bowie Lab at UCLA we will improve cell-free capabilities using a combination of enzyme and process engineering. By the completion of this project, we expect to demonstrate a working prototype system that, with further development, has the potential to provide a commercially competitive biofuel.