

Reducing Carbon Emission in Liquid Biofuel Production

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One of the main contributors to global warming is the emission of greenhouse gases from burning fossil fuels. In addition, petroleum-based fuels are a significant source of air pollutants, such as polycyclic aromatic hydrocarbons and NO_x. Because of these shortcomings, renewable biofuel production has attracted considerable scientific and commercial interest. Currently, most biofuels are produced from microbial fermentation. This process also produces CO₂ as a by-product, which limits the overall reduction in CO₂ emission.

To solve this issue, we identified non-oxidative glycolysis (NOG) as a potential alternative metabolic pathway to reduce carbon emission because it converts glucose to acetyl-coenzyme A without carbon loss. After selecting suitable enzymes, constructing the pathway, and demonstrating its activity using a variety of assays, we also optimized its efficiency by debottlenecking the rate-limiting steps, knocking out competitive pathways, and evolving the engineered strain to overcome its growth defect. To evaluate the reduction in CO₂ emission, we designed, constructed, and validated a continuous CO₂ monitoring pipeline. Using the pipeline, we showed that the final engineered strain showed a 70% reduction in CO₂ emission compared to the wild-type control strain.

We then optimized the biofuel production in the engineered strain. Using a secondary substrate showed a synergistic effect and increased lipid titer by about 2-fold. Using computational simulations, we showed that such effects can be attributed to the balance of carbon source and energy supply, which enables rational designs of substrate co-feeding schemes.

Overall, this thesis is a concrete step towards zero-carbon biofuel production. It demonstrated the reduction of CO₂ during fermentation, developed a CO₂ monitoring system, and provided directions for the design of substrate co-feeding schemes.

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