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Improving Gas Fixation in Acetogenic Bacteria - Thesis Technical Summary

Waste gases are an increasing concern for their impact on the environment. This includes CO_2 produced from industry and transportation, and methane from distributed oil wells and shale gas formations. Generally, these gases are released to the environment, or CH_4 is burned to CO_2, as CH_4 is a more harmful greenhouse gas than CO_2. Researchers are increasingly interested in methods to fix these two gases into valuable fuels and chemicals so as to achieve a sustainable economy. However, any such technology must be economically viable to ensure widespread adoption in the regions that these gases are released.

Yet biological gas fixation has many limitations that currently prevent practical implementation. This includes biological limitations such as slow growth rates, and low biomass and product titers. Process limitations are also a concern, as full conversion is desirable and gas mass transfer can be a substantial rate limiting step. Reactors must also be simple and cheap due to the expected cost differential between the reducing equivalents (electricity, H_2, etc.) and the products (fuels and commodity chemicals). This thesis approaches the limitations of gas fixation from different perspectives to overcome biological limitations and design low cost bioreactors.

Syngas fermentation via the Wood-Ljungdahl pathway (WLP) is receiving growing attention as a possible platform for the fixation of CO_2 and renewable production of fuels and chemicals. Unlike other carbon fixation pathways, such as the Calvin-Benson cycle, the WLP fixes CO_2 into acetate with the net production of ATP. However, the WLP operates near the thermodynamic limit of life, resulting in minimal ATP production and long doubling times. This calls into question the feasibility of producing high-energy compounds at industrially relevant levels. In this thesis, we investigated the possibility of co-utilizing nitrate as an inexpensive additional electron acceptor to enhance ATP production during autotrophic growth of *Clostridium ljungdahlii*. In contrast to other acetogens tested, growth rate (0.084 ± 0.002 vs 0.048 ± 0.003 hr^{-1}) and final biomass titer (0.323 ± 0.001 vs 0.148 ± 0.011 OD_{660nm}) were improved for *C. ljungdahlii* growing on a mixture of H_2 and CO_2 when supplemented with nitrate. Volumetric CO_2 fixation rates also increased (0.47 ± 0.01 vs 0.38 ± 0.02 mmol C hr^{-1} L^{-1}). Transcriptomic analysis, $^{13}CO_2$ labeling, and an electron balance were employed to understand how electron flux was partitioned between CO_2 and nitrate. The pool size of the key carbon building block, acetyl-CoA was also increased by 2.8 ± 0.9-fold in the presence of nitrate. Furthermore, the ATP/ADP ratio was increased by 5.3 ± 0.9-fold.

Finally, we proposed a pathway for enhanced ATP production from nitrate, and use this as a basis to calculate theoretical yields for a variety of products. This work demonstrated a viable strategy for the decoupling of ATP production from carbon dioxide fixation, which will serve to significantly improve the CO_2 fixation rate and the production metrics of other chemicals from CO_2 and H_2 in this host. Future metabolic engineering could greatly increase the yield to those predicted in the theoretical analysis.

Methane, like CO_2, also contributes greatly to climate change; as a gas, methane is more difficult to transport than liquid or solid fuels, despite burning more cleanly. Due to the distributed nature

of natural gas, there is interest in developing small scale gas-to-liquid technologies to convert methane into hydrocarbons. Yet they still require relatively high flow rates (1,000 Mscf/day). While this would be sufficient for high flow rate wells, there are also substantial reserves of methane whose location or flow rates and pressures are too low for economic production (70%). Researchers believe biology can fill this niche (low flow rate wells at or below 66 Mscf/day). Appropriate reactor design could provide sufficient mass transfer to biologic or inorganic catalysts.

In the second half of the thesis, we propose using the natural gas well as the bioreactor, negating capital costs, and leveraging the gas pressure for mass transfer. We evaluated the 'Deep Well Reactor's feasibility by developing mathematical models that simulated mass transfer and explored how operating parameters impacted ethanol production. The results showed sufficient mass transfer for 100% conversion, despite minimal complexity. In the theoretical reactor, reactions rates of 9.6×10^{-3} mol m^{-3} s^{-1} would be sufficient for full conversion. Current aerobic methanotrophs and inorganic catalysts could provide sufficient reaction rates. However, they would require addition of O_2 or other oxidizing agents such as H_2O_2. Conversely, anaerobic methanotrophs rates must be improved by 1200-fold. With an appropriate catalyst, this technology allows the recovery of methane at flow rates an order of magnitude lower than current technologies.