Electrofermentation with Recombinant *S. Cerevisiae* for Increasing Fermentation Yields

Experiences and experimental results from a bioscience summer internship at IFF's Genencor Technology Center in Palo Alto, CA

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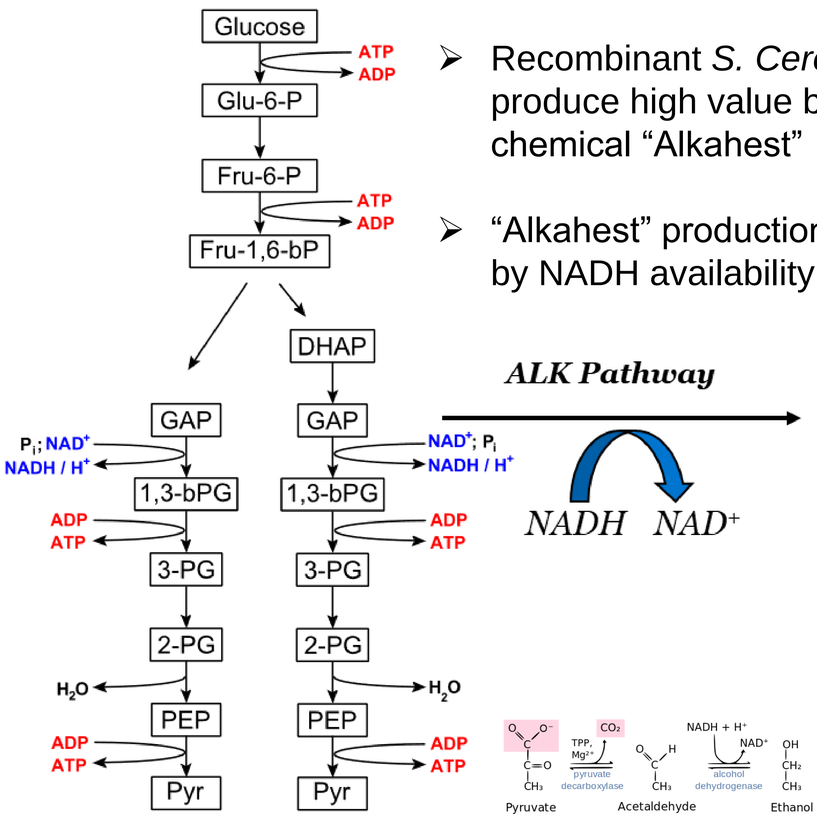
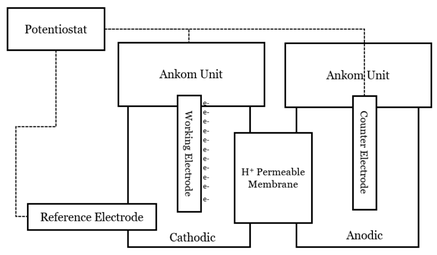
Abstract

Advances in metabolic engineering and strain engineering have allowed for many specialty and bulk chemicals to be produced from a feedstock of biomass. This is accomplished by adding biosynthetic pathways to the engineered organism that exist alongside the native metabolic pathways. There are many barriers that restrict the yield of these processes; one of which is the deficit of reducing power in the form of NADH. This shortage has two causes: 1.) the production step where NAD+ is reduced to NADH must be bypassed to produce the target fermentation product and 2.) both the native and engineered pathways utilize this coenzyme in its reduced form. To bypass this bottleneck, electrofermentation seeks to add reducing power by directly providing electrons to the process in a form that can be uptaken by the organism. This project was completed over the course of a summer in Palo Alto, California at the Genencor technology center of IFF. A novel electrofermentation unit was designed, constructed, and tested with various recombinant strains of S. Cerevisiae. Using this novel fermentation setup was shown to increase both the yield and production rate of the desired fermentation product.

IFF's Genencor Technology Center, 925 Page Mill Road, Palo Alto CA. Photo Courtesy of M. Alberto.

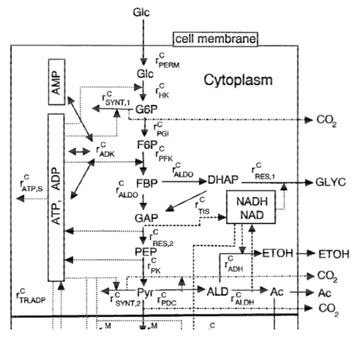
Electrofermentation: Powering Bacteria with Electricity

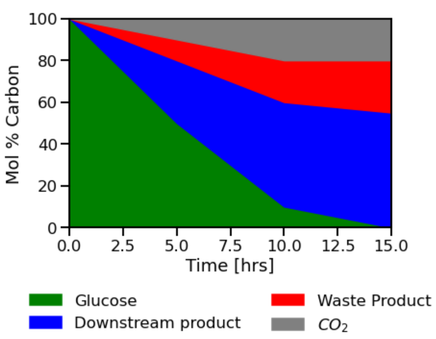




- Recombinant *S. Cerevisiae* produce high value bulk chemical "Alkahest"
- "Alkahest" production limited by NADH availability
- Conduct fermentation in a fuel cell analogue, with biotic cathode and abiotic anode
- Provide reducing power with free electrons from an external current
- Electrons enter bacteria through a chemical redox mediator
- Excess electrons increase reducing power and flux through ALK pathway

Optimizing Carbon Flux





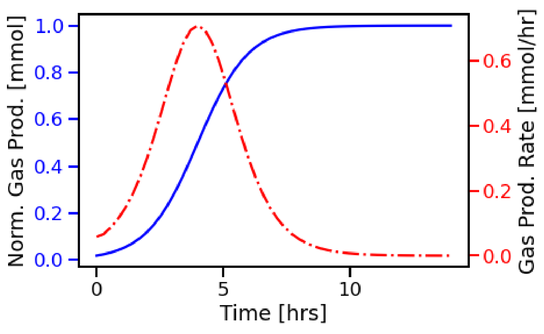
- Real time fermentation monitoring, HPLC, and existing mathematical models used to optimize carbon flux

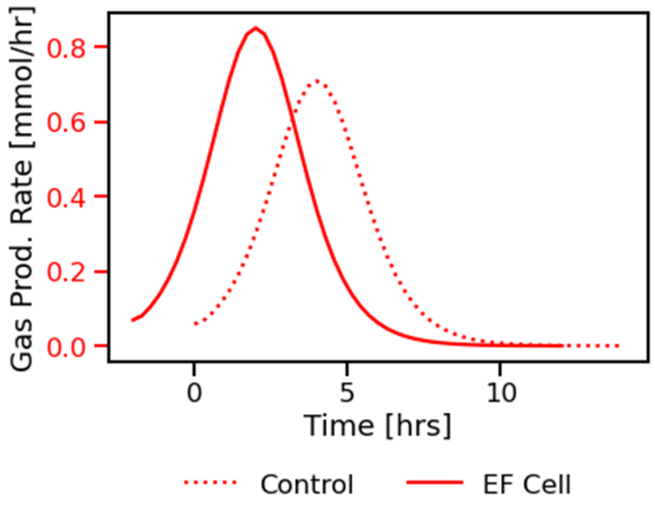
Novel electrofermentation setup designed, built and tested during the internship

Results*: Increase in Metabolic Rate and Alkahest Yield

**data shown not real experimental data to comply with company policy*

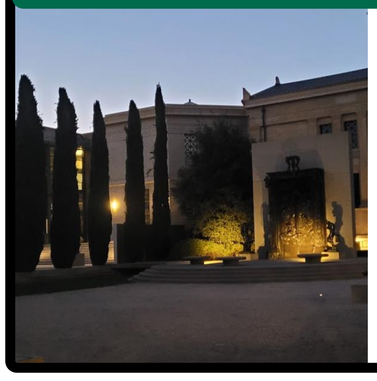
- EF increased the metabolic rate of recombinant strains
- Metabolic rate increased with both applied voltage in EF and expression level of ALK pathway in *S. Cerevisiae*





$$n = \frac{P(t)V}{RT}$$

Acknowledgements and References



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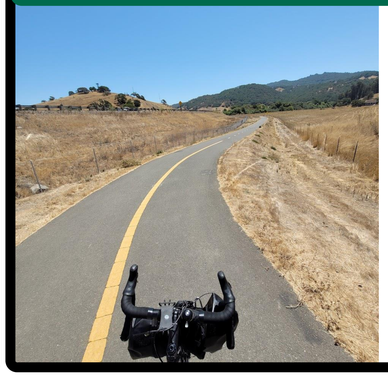
Conclusions



- Internship provided proof of concept to pursue EF for "Alkahest" as a full project
- Company plans to file for IP rights on system
- Represented CSU in a cohort of students from universities across the country (Hopkins, UCLA, WashU)
- Plan to continue education with a doctorate in bioengineering

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