Investigation of Microbial Electroactivity for Electro Fermentation

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Background

Fermentation is the anaerobic biological process where glucose is broken down into organic acids and alcohols using electron powered redox reactions.

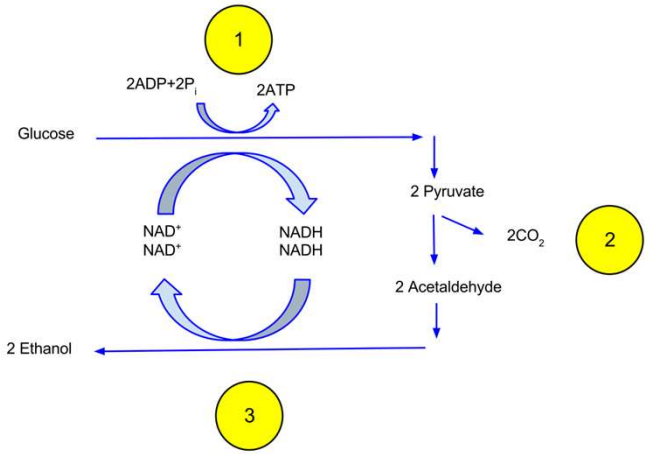


Figure 1. Butanol fermentation.

- A metabolite is a substance formed by a cell for metabolic purposes.
- We focused on highly reduced metabolites.
- A cell requires a significant quantity of free electrons to produce these molecules

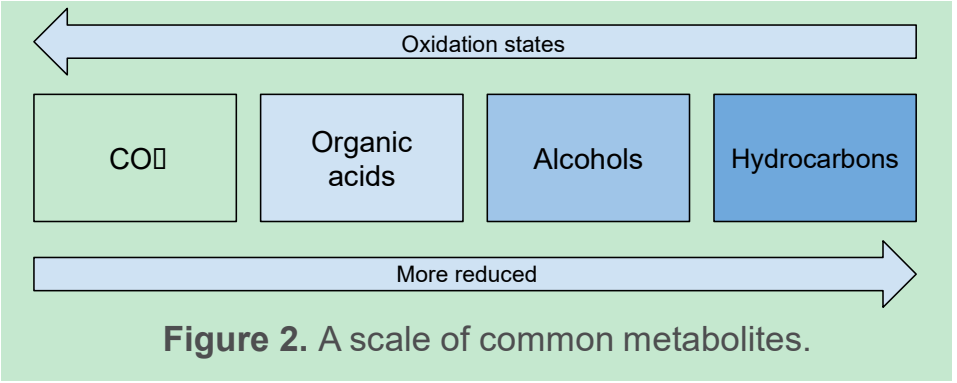


Figure 2. A scale of common metabolites.

- Useful biofuels, industrial solvents, and many other applications.
- Often produced petrol-chemically.
- Microbial production of metabolites is limited by high costs and low production.
- Decreases the process's sustainability and practicality.

Introduction

The goal of this research project is to improve the yield of metabolites from microbial production by increasing the flux of metabolic electrons into microbes through electro stimulated fermentation (e-ferm).

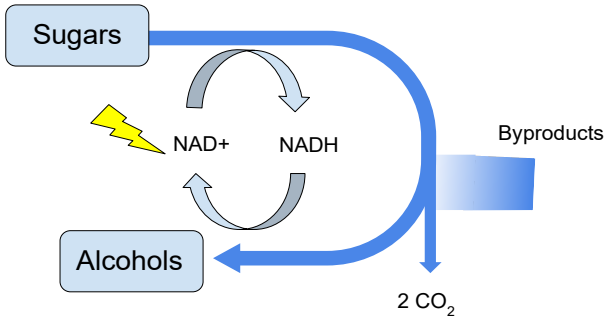
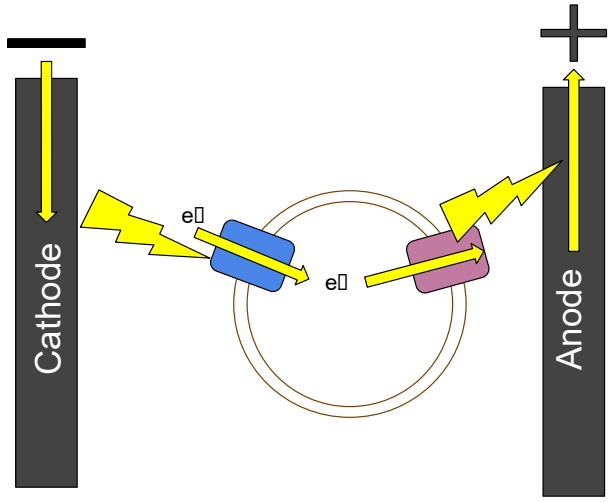


Figure 3. The general electro fermentation process.

Figure 4. Cathodic and anodic extracellular electron transfer.



- E-ferm changes the number of electrons available in a cell.
- When more electrons are available a cell will produce more highly reduced molecules.

Experiment – H Cell Reactor Design

Organism	Traits	Hypothesis
Brewers Yeast	Common model organism that is suggested to be electroactive	Not electroactive
Clostridium Pasteurianum	Natural producer of butanol and other *Clostridia* show evidence of electroactivity	Electroactive
Shewanella oneidensis	Known electroactive species	Electroactive

- One focus was trouble shooting the H cell.
- pH and temperature control systems were needed.

Methods:

- Noticed that the e-ferm pH and temperature was not stable
- Tested how the applied potential was altering the pH
- Designed a control system that worked in the anaerobic chamber to test and control the pH and purchased a temperature control apparatus
- Used both control systems to optimizing the e-ferm experiments and found that the current H cell apparatus is not capable of controlling pH and must be reconfigured

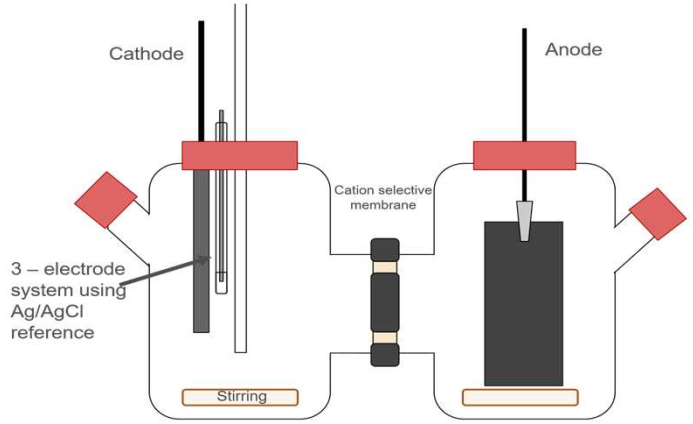


Figure 5. The H cell or 3 electrode e-ferm system[1]

Experiment – Cyclic Voltammetry

- Cyclic voltammetry (CV) measures the current an electrochemical species creates
- Cycles a working electrode's potential and measures the resulting current[2].
- Characterizes the behavior of electroactive organisms.

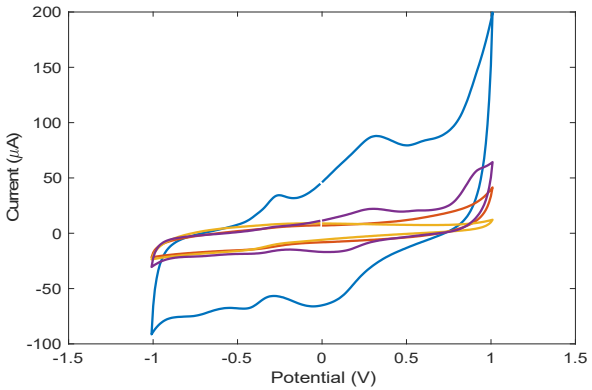


Figure 7. Sample CV scan showing a Faradaic and Capacitive current.

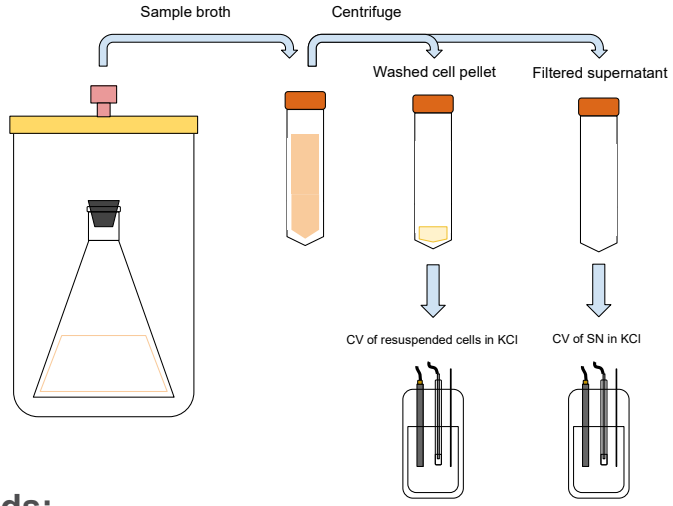
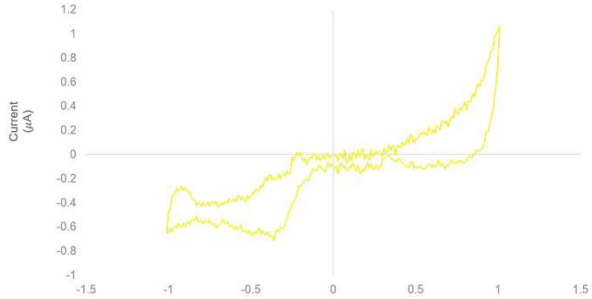


Figure 6. Steps required to prepare a cell culture for CV.

Methods:

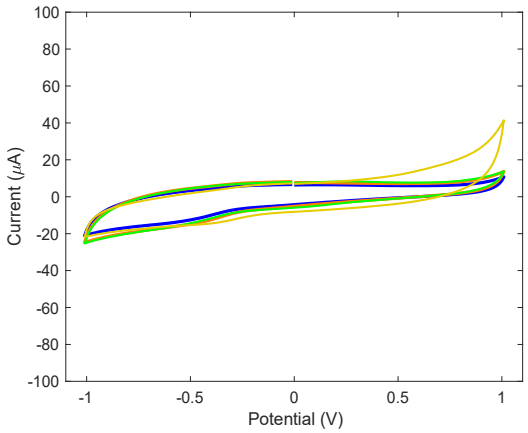
- Inoculated mediums with the organism and let them grow for ~48 hours in e-ferm conditions
- Prepared the cultures for CV (Figure 6)
- Took numerous CV scans with different scan rates or electrodes
- Compared the CV scans

Figure 8. Ambiguous CV results. Caused us to reevaluate our CV techniques and apparatus using a positive control (*S. oneidensis*).



Results

Figure 10. CV of *S. cerevisiae* which includes yeast grown without an applied potential. It appears that yeast is not electroactive.



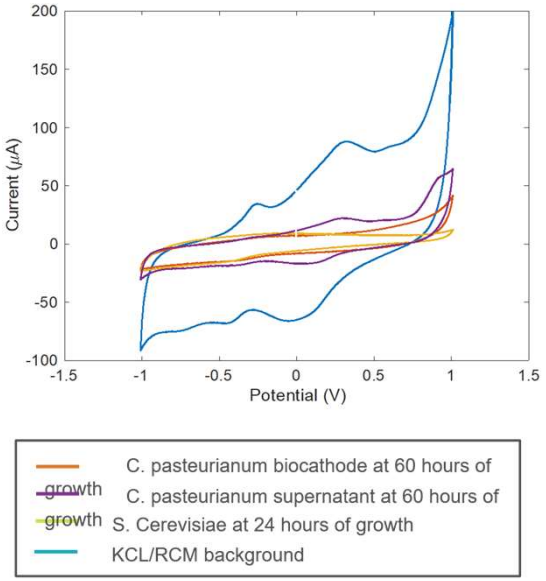


Figure 9. CV scans of the electroactivity of *C. pasteurianum*. Which indicate potential redox humps but are not conclusive evidence yet.

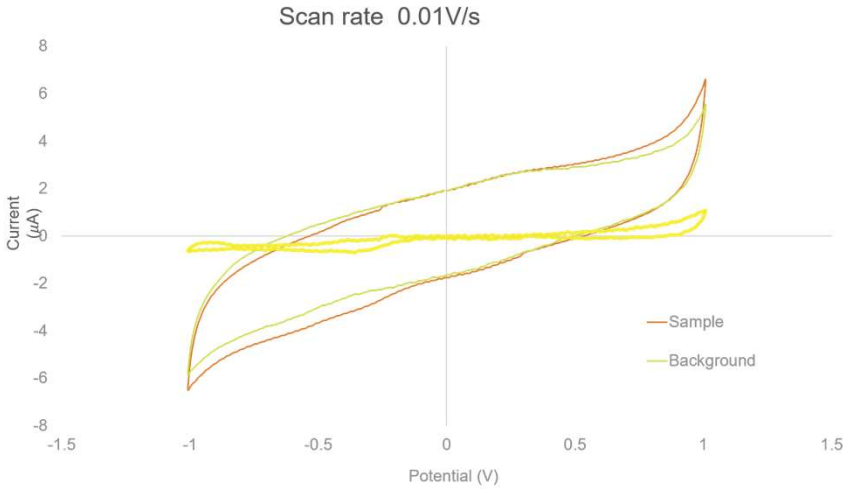


Figure 11. Ambiguous CV scan.

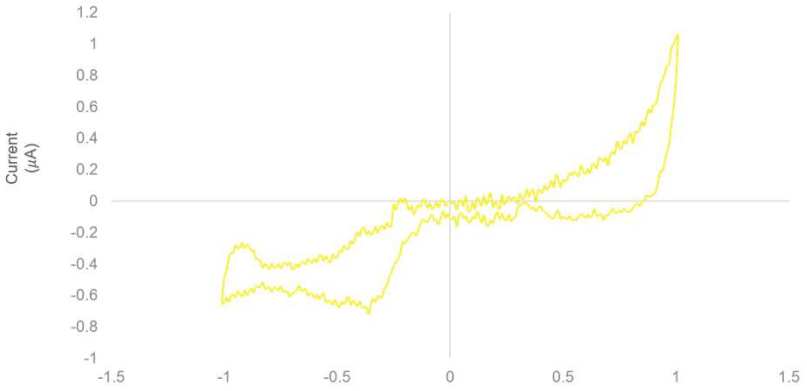


Figure 12. Close-up of the ambiguous CV scan.

Results

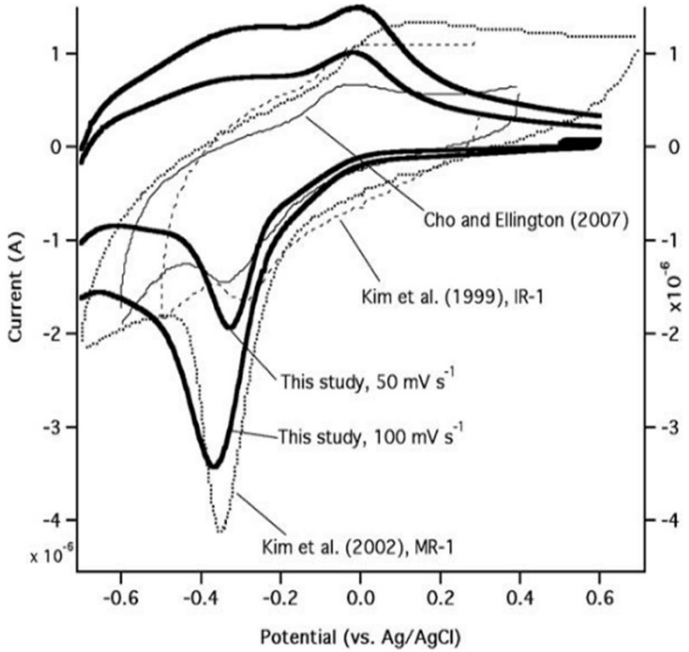


Figure 13. The expected results for *S. oneidensis* positive control. From Meitl et al.

- Most of our results led to more questions.
- No data indicates that yeast is electroactive, but more testing must be done with a positive control before conclusions can be drawn.
- Our ambiguous CV results led us to a positive control (*S. oneidensis*) which will likely bring more clarity and confidence to our current data.
- Controlling pH is not possible in the current H cell. Other options are currently being explored.
- The temperature control system was successful.

Next Steps

Growing and testing the *S. oneidensis* positive control to reevaluated the current techniques.

Exploration for an alternative H cell designs that allows for pH control.

Testing *S. oneidensis.*

Testing *C. pasteurianum* to conclude if it is an electroactive species.

Conclusions

As of current data, yeast appear to be a nonelectoactive specifies.

S. oneidensis will be the positive control.

Alternative H cell designs is needed to control pH.

The temperature control apparatus was successful.

SURE Experience Benefits

The opportunity to be mentored by someone with knowledge about the next phase of life.

The opportunity to watch, understand, and partake in the research process.

The opportunity to have experience with basic and more complex lab practices.

References & Acknowledgements

[1] Shin, H., Zeikus, J. & Jain, M. Electrically enhanced ethanol fermentation by Clostridium thermocellum and Saccharomyces cerevisiae. *Appl. Microbiol. Biotechnol.* 58, 476–481 (2002).

[2] Ross, D. E., Flynn, J. M., Baron, D. B., Gralnick, J. A. & Bond, D. R. Towards Electrosynthesis in Shewanella: Energetics of Reversing the Mtr Pathway for Reductive Metabolism. *PLoS One* **6**, e16649 (2011).

[3] Leisa A. Meitl, Carrick M. Eggleston, Patricia J.S. Colberg, Nidhi Khare, Catherine L. Reardon, Liang Shi, *Electrochemical interaction of Shewanella oneidensis MR-1 and its outer membrane cytochromes OmcA and MtrC with hematite electrodes,* Geochimica et Cosmochimica Acta, Volume 73, Issue 18, 2009, Pages 5292-5307.

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Thank you

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