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.

- 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 1. Butanol fermentation.](image)

- 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.

![Figure 2. A scale of common metabolites.](image)
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

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Traits</th>
<th>Hypothesis</th>
</tr>
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<tbody>
<tr>
<td>Brewers Yeast</td>
<td>Common model organism that is suggested to be electroactive</td>
<td>Not electroactive</td>
</tr>
<tr>
<td><em>Clostridium Pasteurianum</em></td>
<td>Natural producer of butanol and other <em>Clostridia</em> show evidence of electroactivity</td>
<td>Electroactive</td>
</tr>
<tr>
<td><em>Shewanella oneidensis</em></td>
<td>Known electroactive species</td>
<td>Electroactive</td>
</tr>
</tbody>
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- One focus was trouble shooting the H cell.
- pH and temperature control systems were needed.

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.

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

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

**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

- 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.

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

Growing and testing the *S. oneidensis* positive control to reevaluate 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 nonelectroactive species.

*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


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Thank you