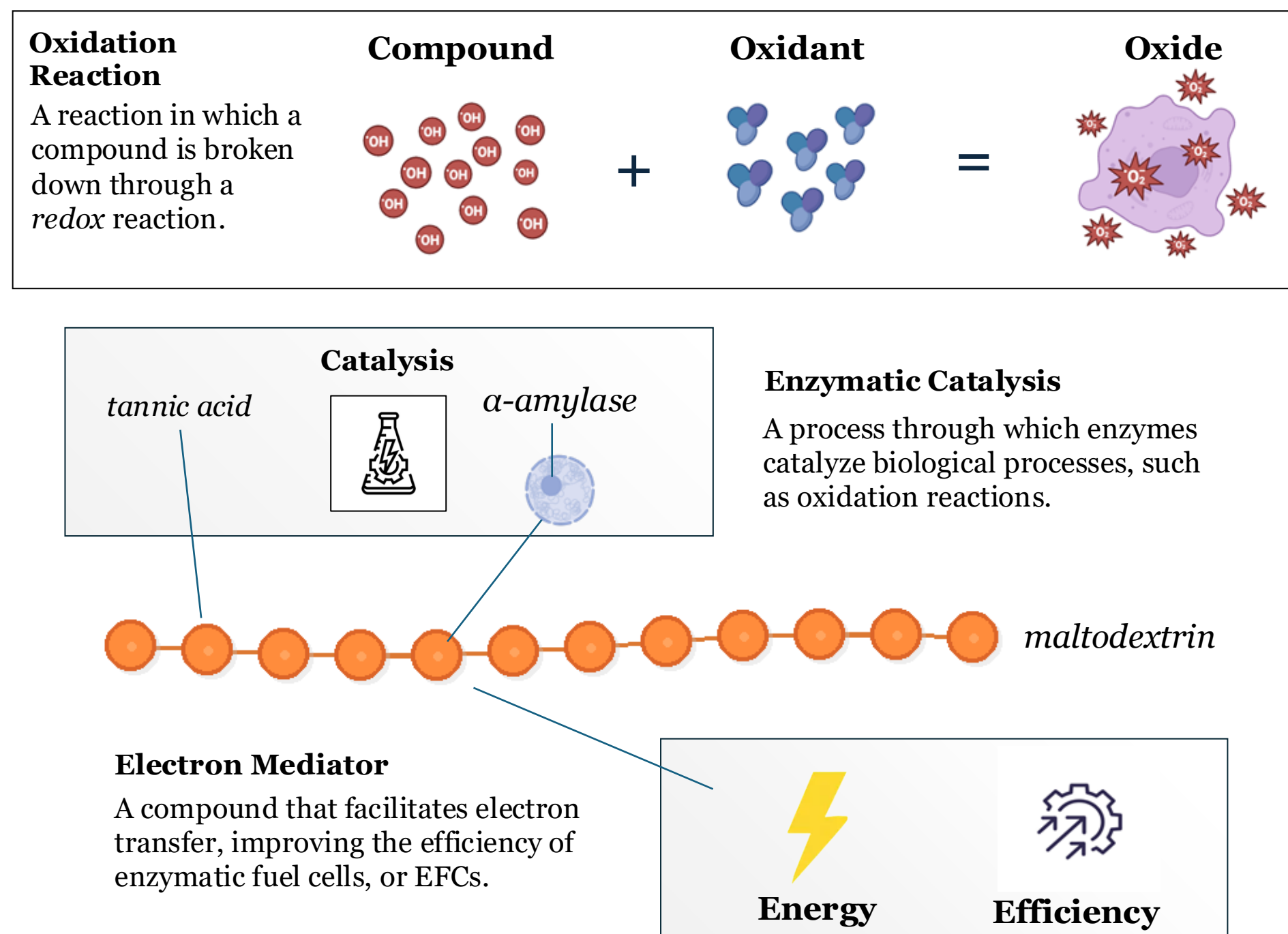
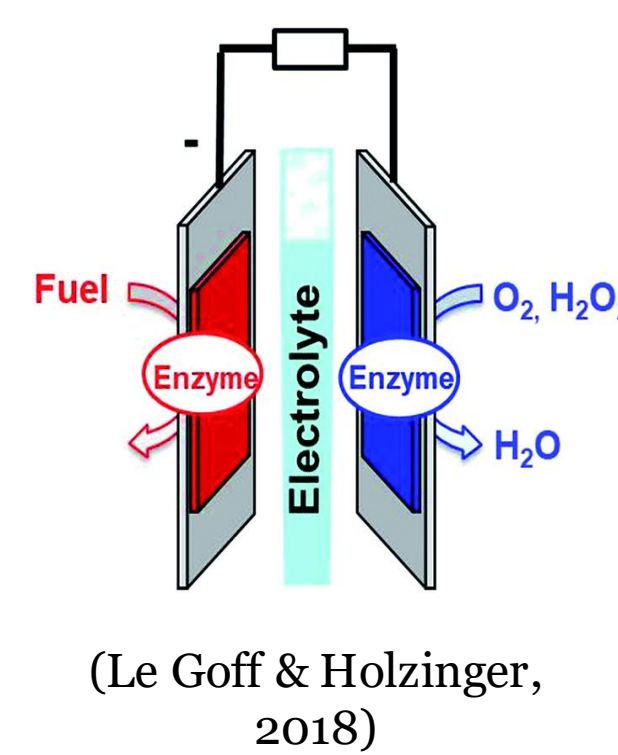


GRAPHICAL ABSTRACT



INTRODUCTION

- Lithium-ion batteries (LIBs) are the primary form of energy storage globally
- One kg of LIB produced causes 17.63 kg-CO₂-eq. emissions (Clemente et al., 2025)
- Fuel cells are a promising LIB alternative
 - However, they are under-researched
- Enzymatic fuel cells have higher theoretical energy densities than LIBs (Oyarce et al., 2013)
- Developing efficient EFCs provides a sustainable, long-term LIB alternative



RESEARCH OBJECTIVES

ENGINEERING NEED: Lithium-ion batteries are the main form of global energy storage, but cause water pollution and accelerating climate change during mining.

OBJECTIVE: The engineering goal of this project is to engineer a low-cost enzymatic fuel cell (EFC) that can produce sustainable electricity.

GOAL 1

This project aims to study how fuel sources affect EFC performance.

GOAL 2

This project aims to study how an electron mediator affects EFC performance.

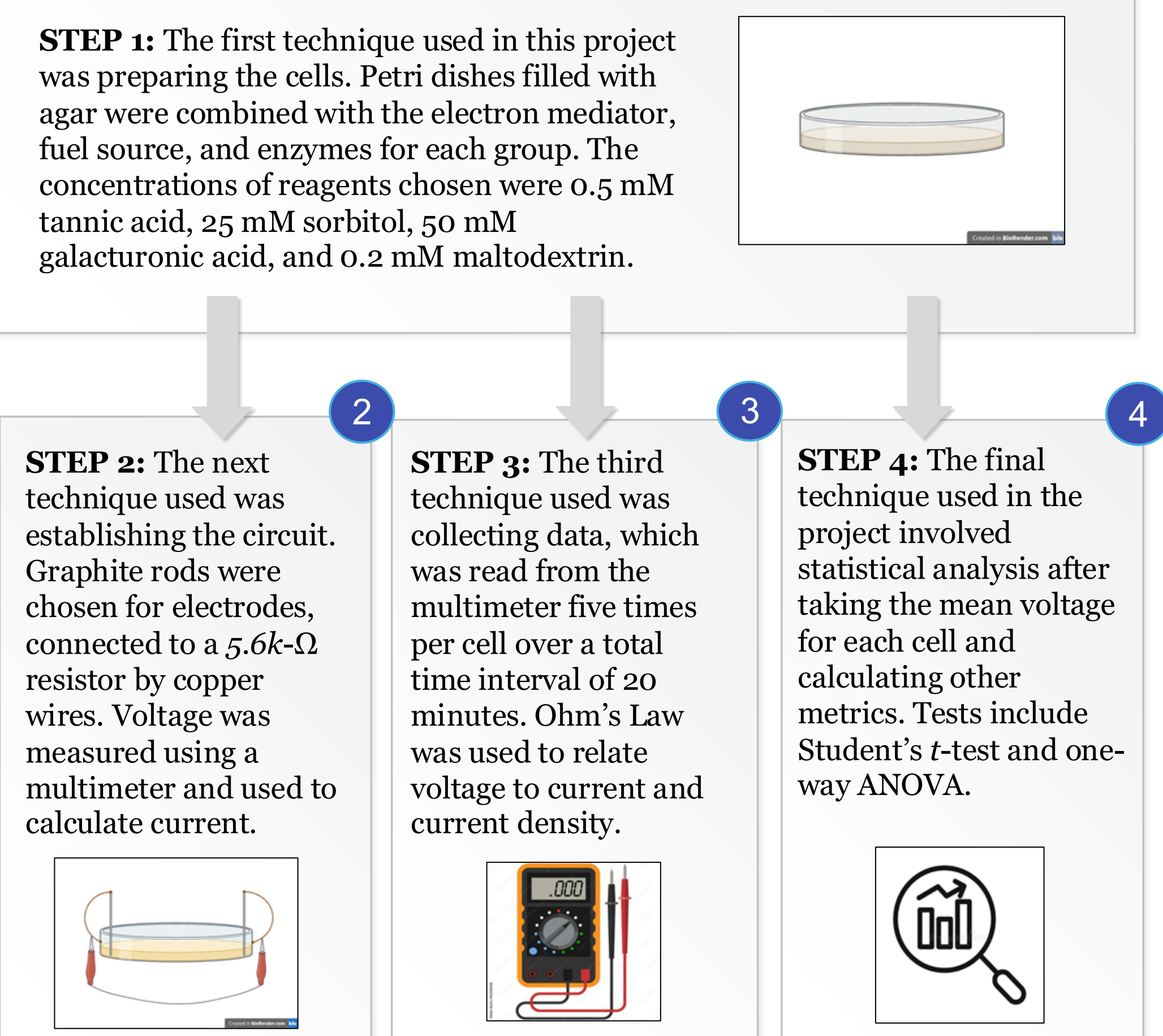
GOAL 3

This project aims to test novel fuel sources and system design.

ENGINEERING CRITERIA

- The engineering criteria for this project were evaluated in four parts: performance, cost, safety, and usability
- Many existing EFCs focus heavily on some, but not all, of these parts
- This project tests a model focused on maximizing performance across all criteria types
- Future EFC research can draw inspiration from the framework proposed in this project

METHODOLOGY



PROJECT SIGNIFICANCE

- Current EFCs are **highly limited** in the fuels that they use and are **rigid** in their designs due to **bulky membranes** and **electrode materials**
- This project proposes a **membraneless** design for **enhanced cellular flexibility**, along with **lower production costs**
- This project also explores the use of **novel fuel sources**, namely **sorbitol** and **galacturonic acid**, that have **higher theoretical energy densities** than those used in current designs, like maltodextrin and glucose
- Electron mediators** are used to **control** electrode interactions and **electron transfer**, but are **underutilized** in EFC research
- This project uses **tannic acid** as a **novel electron mediator** to provide a **higher peak energy production capability**, despite less advanced surrounding infrastructure compared to current models
- This project **successfully provides a framework** for developing **low-cost EFCs** with comparable performance to those on the market
- Galacturonic acid** was deemed to be **more effective** as a fuel source in mediated EFCs due to **higher energy density** and improved enzyme interactions **compared to maltodextrin**
- This project determines the **optimal approach** for engineering EFCs in a manner that has **not yet been adopted** in formal research

STATISTICAL ANALYSIS

- A two-sample *t*-test was conducted for each fuel source's enzyme-non-enzyme-group pair. The resulting *p*-values were 0.028, 0.020, and 0.0085, respectively. This shows strong evidence of enzymes significantly improving mean cellular voltage.
- A one-way ANOVA test was performed to determine if there was a difference in current density across the six groups. The resulting *p*-value was 0.0035, showing strong evidence that one group had a significantly different current density.
- To determine the cause of these differences, a Tukey's HSD test was performed. The test confirmed that the differences were caused by the presence of enzymes, as the *p*-values were around 0.001. This confirms the effect of enzymatic catalysis on cellular performance.
- Finally, three two-sample *t*-tests were used to compare current density across enzyme-non-enzyme pairs for each fuel source. The results mirrored significance for voltage comparisons, showing the correlation between voltage and current density.

RESULTS

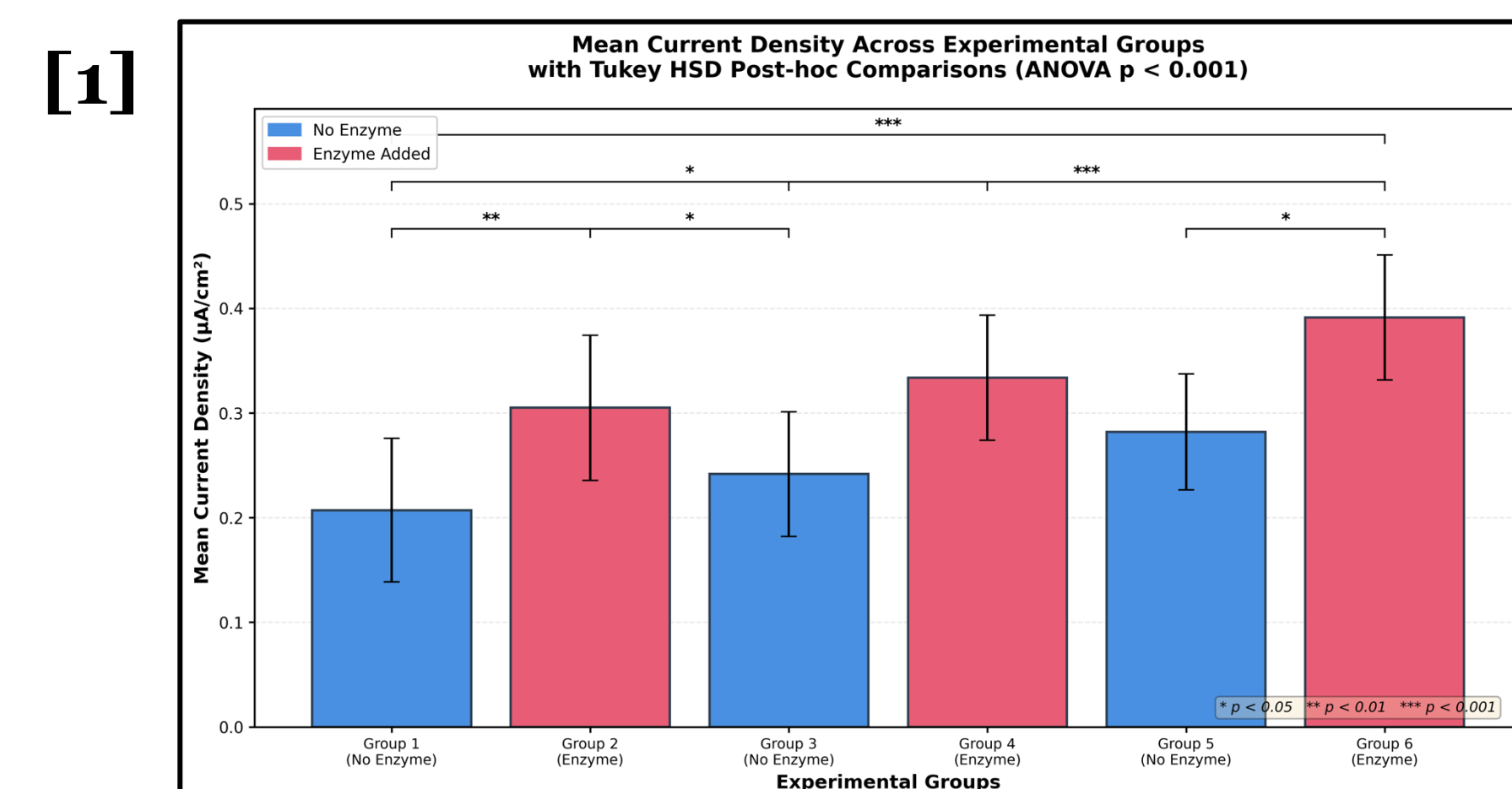


Figure 1 shows the results of Tukey's HSD analysis of differences in mean current density as shown from a One-Way ANOVA.

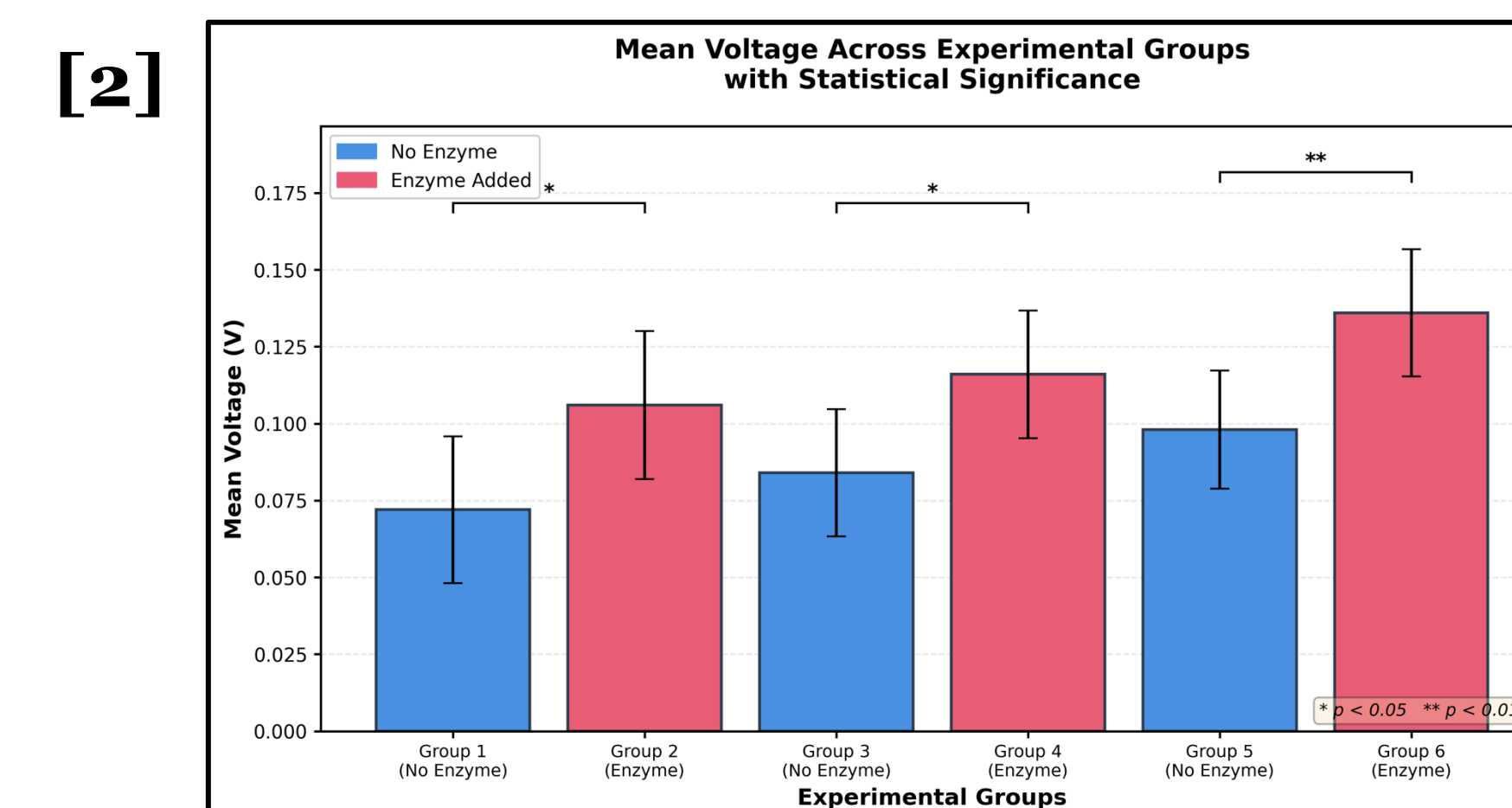


Figure 2 shows the results of Student's *t*-test for analysis of mean voltage across testing groups.

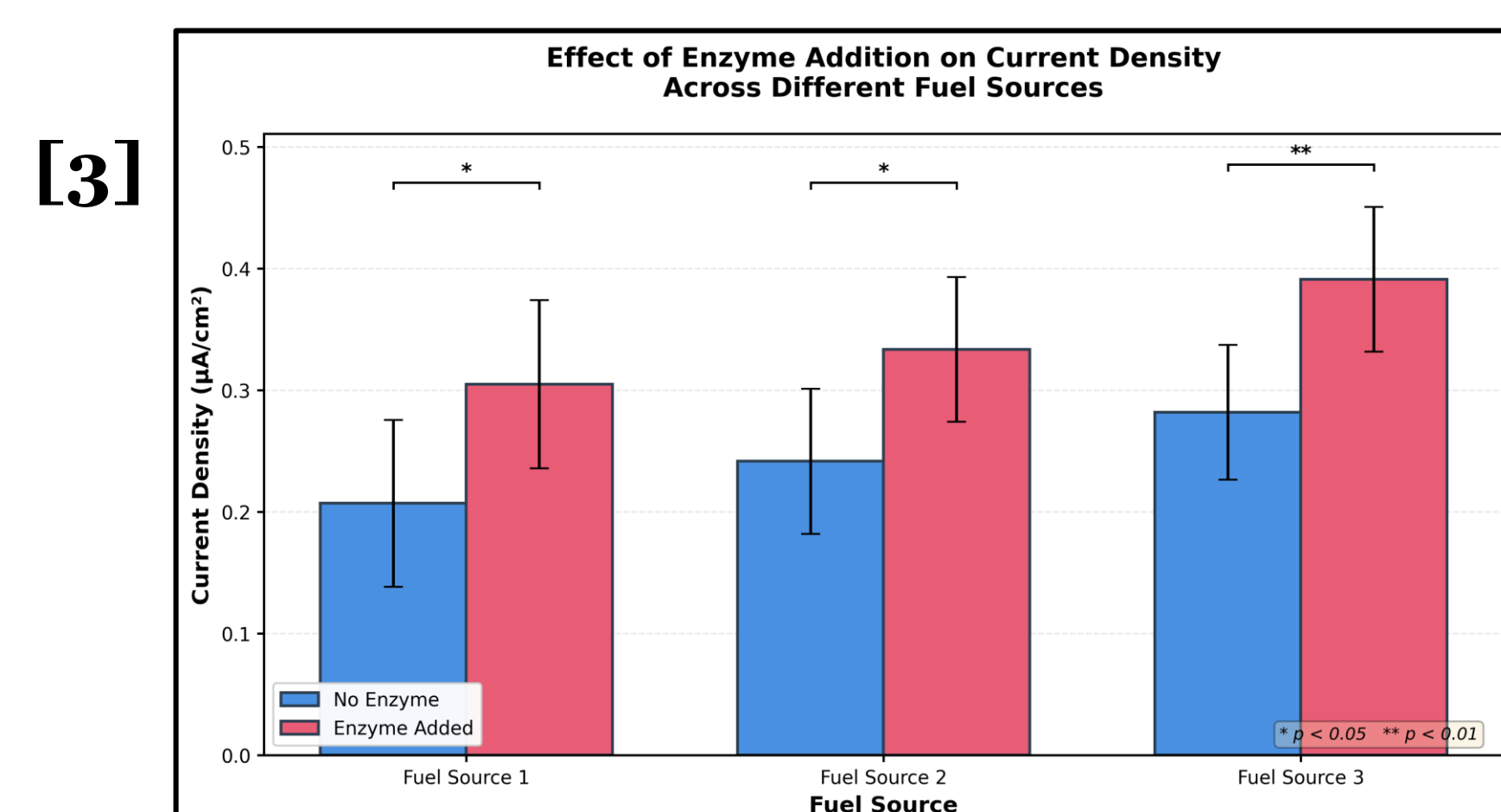


Figure 3 shows the results of Student's *t*-test for analysis of the effect on enzymes on mean current density grouped by fuel source.

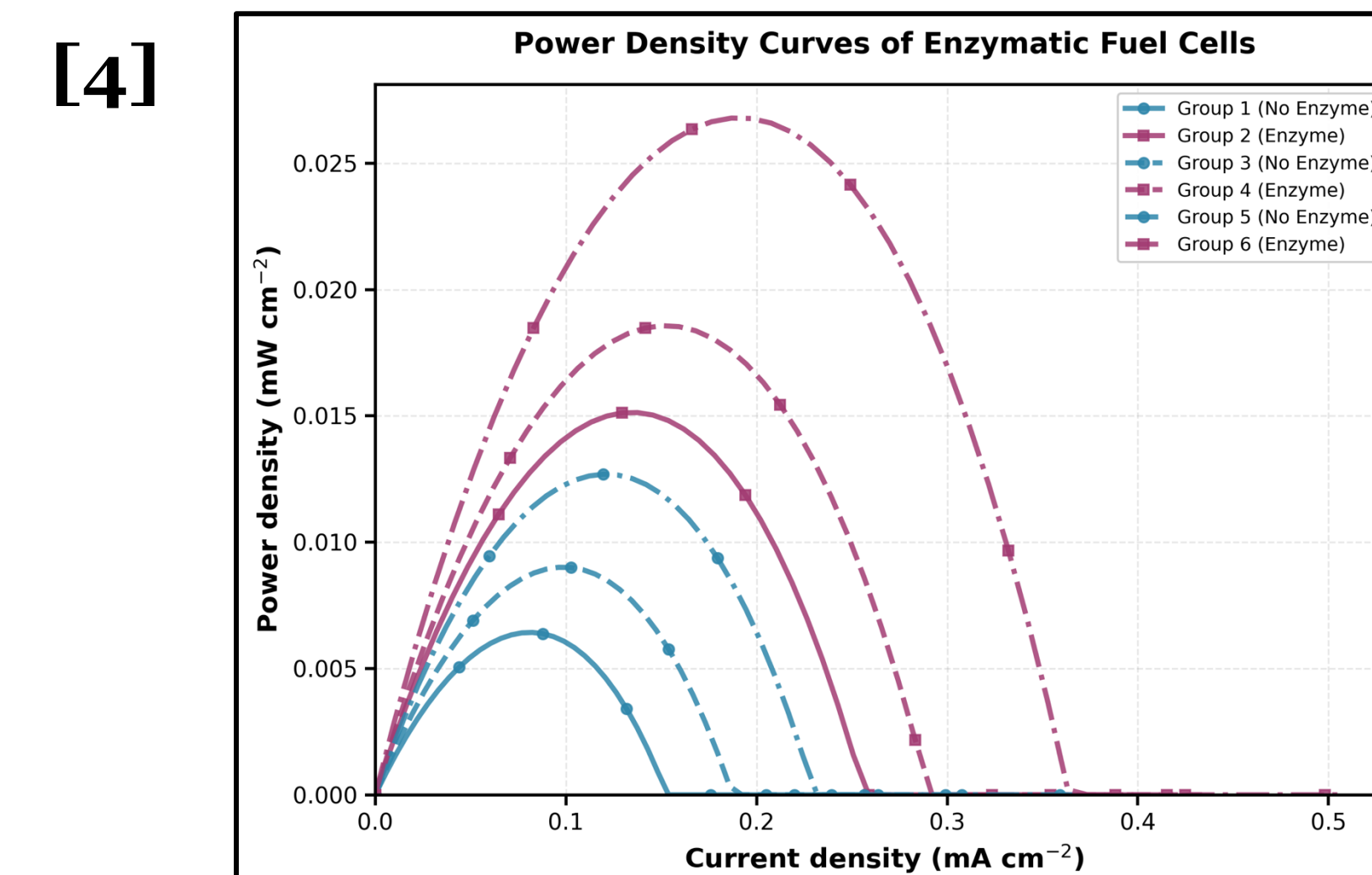


Figure 4 shows the results of polynomial regression methods used to create power density curves for each group.

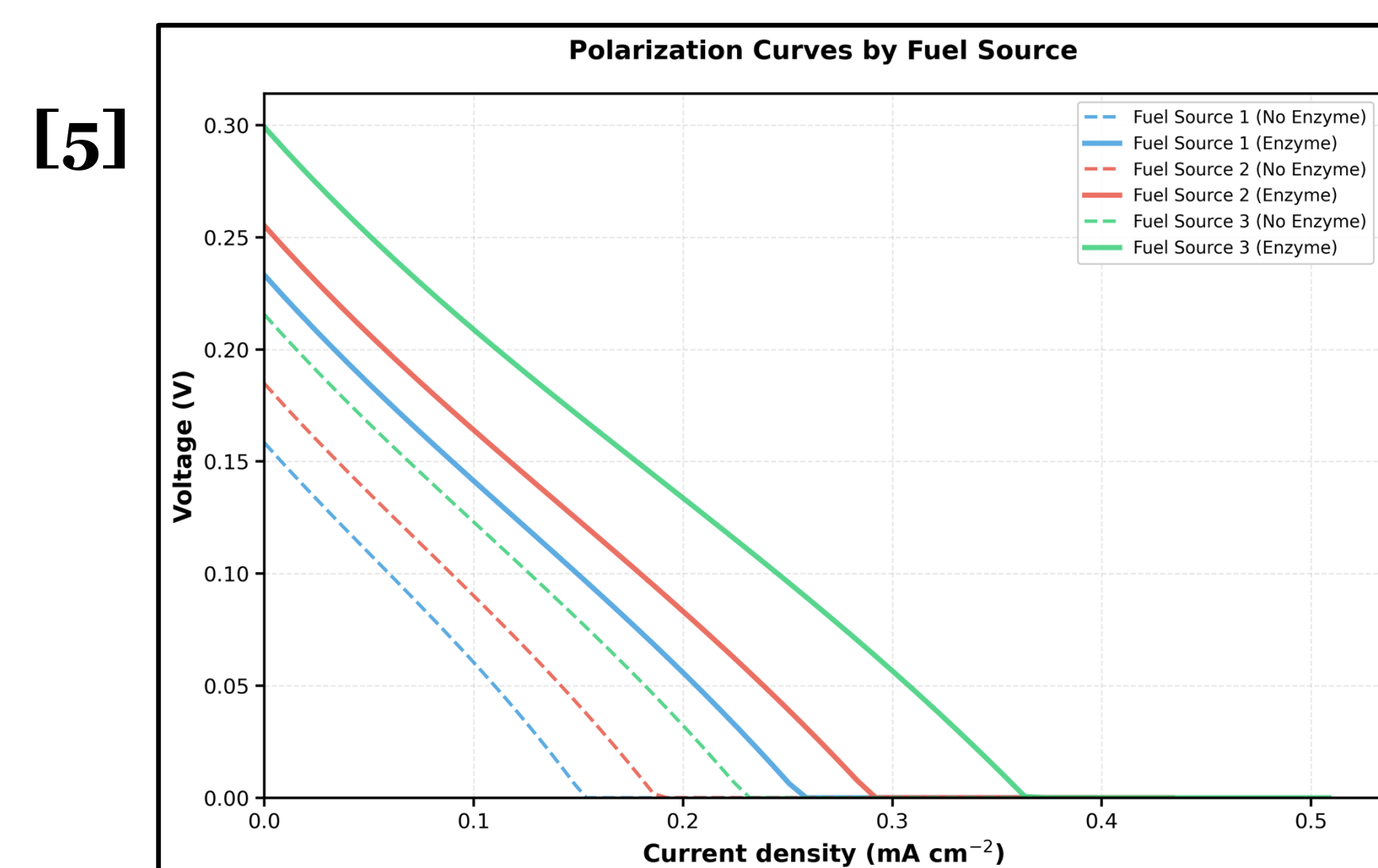


Figure 5 shows the linear relationship between voltage and current.

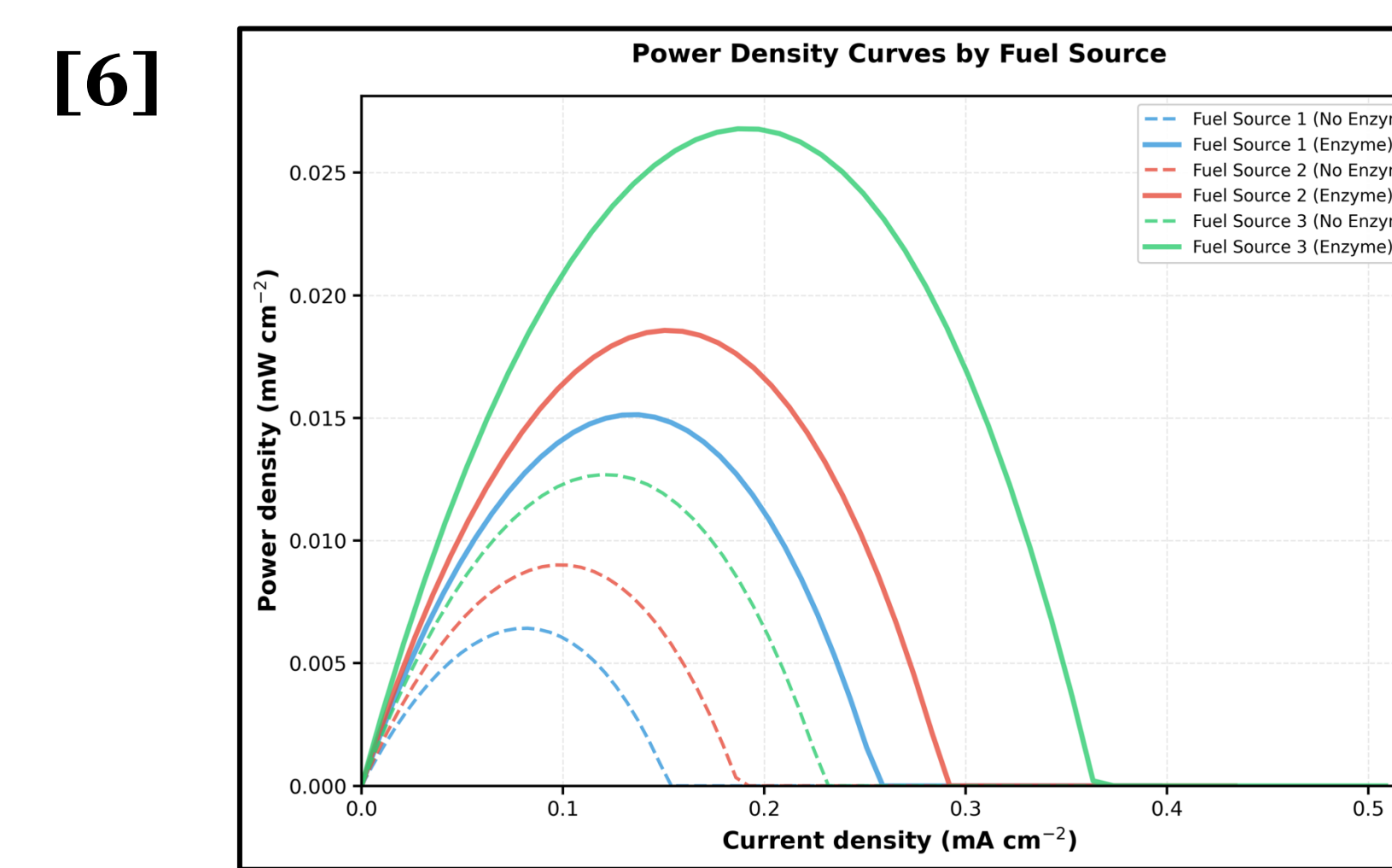


Figure 6 shows the parabolic relationship between power and current density.

ANALYSIS

- The **addition** of fuel source-specific **enzymes** proved to be extremely important in improving **cellular performance** and **efficiency**
- Statistical testing showed **highly significant differences** between **enzyme** and **non-enzyme** groups for the **same fuel sources**
- Galacturonic acid** and **sorbitol** provided **higher mean voltages** and **current densities** compared to the control
- This project provides a **framework** for testing **novel fuel sources**, thereby **accelerating** progress in EFC research
- Overall, this project **successfully determined** the **optimal system design** for non-energy-intensive energy production in an EFC

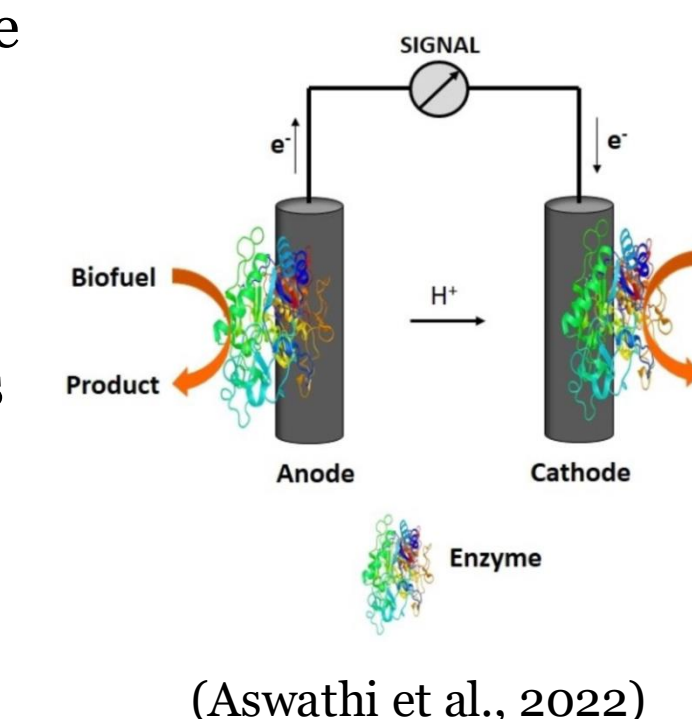
CONCLUSION

Developing EFCs with Novel Fuel Sources

- Expanding EFC research beyond current fuel sources can accelerate innovation and improve cellular efficiency long-term

Accelerating Research into LIB Alternatives

- As lithium becomes scarce in the future, this project outlines methods to develop EFC alternatives
- Future EFC research can further explore galacturonic acid and sorbitol as effective fuel sources for long-term energy generation



KEY REFERENCES

Korkut, S. & Kiliç, M.S. (2015). Design of a mediated enzymatic fuel cell to generate power from renewable fuel sources. *Environmental Technology*, 37(2). <https://doi.org/10.1080/09593330.2015.1065007>

Oyarce, A., Gonzalez, C., Lima, R. B., Lindström, R.W., Lagergren, C., & Lindbergh, G. (2013). Direct sorbitol proton exchange membrane fuel cell using moderate catalyst loadings. *Electrochimica Acta*, 116, 379–387. <https://doi.org/10.1016/j.electacta.2013.11.070>

Rengaraj, S., Mani, V., Kavanagh, P., Rusling, J., & Leech, D. (2011). A membrane-less enzymatic fuel cell with layer-by-layer assembly of redox polymer and enzyme over graphite electrodes. *Chemical Communications* (43). <https://doi.org/10.1039/C1CC15002B>

Zhu, Z., Tam, T., Sun, F., You, C., & Zhang, Y. (2014). A high-energy-density sugar biobattery based on a synthetic enzymatic pathway. *Nature Communications*, 5(1). <https://doi.org/10.1038/ncomms4026>

Le Goff, A. & Holzinger, M. (2018). Molecular engineering of the bio/nano-interface for enzymatic electrocatalysis in fuel cells. *Sustainable Energy Fuels*, 2, 2555–2566. <https://doi.org/10.1039/C8SE00374B>

Aswathi, M., Ganesh, V., & Berchmans, S. (2022). Metal-Organic Framework-Based Electrode Platforms in the Assembly of Biofuel Cells and Self-Powered Sensors. *ChemElectroChem*. <https://doi.org/10.1002/celec.202200276>

