Harnessing the Power of Methylotrophs for Bioelectrocatalysis: Lanthanum-dependent methanol dehydrogenase in Methylobacterium Extorquens

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Introduction

Bioelectrocatalysis is a rapidly growing field that focuses on using biological systems to power electrochemical reactions. Just like humans, the catabolic profiles of most organisms are built upon oxidation and reduction of chemical species. In humans, the majority of these redox reactions occur in the inner mitochondrial matrix, where the oxygen finally gets reduced to power the production of ATP for cellular use. This leads to the production of CO_2 and water. In plants, a similar process takes place in the thylakoid membranes of chlorophyll after prior consumption of CO_2 to produce oxygen, which ends up being used up by humans.
Methylotrophs are exotic microorganisms that are known for their consumption and breakdown of organic compounds such as methane, methanol, as well as other two and three carbon species to produce energy. Since methylotrophs are very adaptable and resilient to harsh conditions, scientists are actively exploring the option of employing them as a means to generate energy for several purposes including biofuel cells, biosensors, and many other biotechnological applications.
In this review article, the goal is to provide a current state of knowledge on the biolectrocatalysis of methylotrophs as well as recent advances elucidating the mechanisms of bioelcectrocatalytic oxidation in other exoelectrogens and how that can be harnessed to power devices. This becomes more important as the need to
mitigate environmental climate problems. Methane is one of the most abundant gases in the atmosphere that has significant effects on global warming. Presenting methane to methylotrophs for metabolism . The first being slowing down ozone depletion and the second being powering systems in a more conservative manner.

Enzymatic biofuel-cells

Enzymatic biofuels refer to a type of biofuel that is manufactured using enzymes. Their ability to generate energy depends on catalytic activities of the enzymes involved. Compared with traditional biofuel cells, enzymatic biofuel cells appear to be more advantageous given their diversity and ability to operate based on a variety of catalysts. Even though the optimization of biofuel cells has witnessed limitations, building enzymatic electrodes has been extensively studied. Currently, the most significant setbacks include the development of immobilizing materials, methods of immobilizing enzymes, optimizing enzyme electrode structures and shapes, and improving enzyme biocatalytic activity.
Wu et. al. has also explored the use of Laccase and NAD+ dependent dehydrogenase cascades as

biocatalysts on carbon nanodot electrodes to build methanol/oxygen enzymatic biofuel cell. They were able to efficiently assemble multienzymes-biocatalyzing electrodes for deep oxidation of methanol as well as DET-type electrode for four-electron reduction of oxygen.

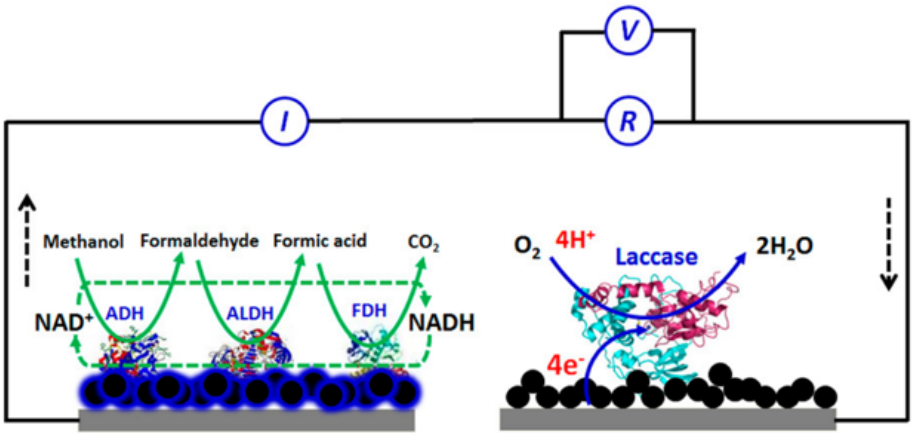

Figure 1. Schematic illustration of membraneless methanol/O_2 biofuel cell at carbon nanodots electrodes.[1]

Electrochemistry of Methanol dehydrogenase

Methylotrophs, particularly Methylobacterium Extorquens, have been extensively examined given their expressions of xoxf, the gene that codes for mxaI and mxaF, the small and large subunits of methanol dehydrogenase (MDH). The genome of the AM1 strain genome contained MxaF, XoxF1, and XoxF2. Both homologs of XoxF are crucial for the expression of MDH. The XoxF is a pyrroloquinoline quinone (PQQ)- dependent periplasmic alcohol dehydrogenase. The MxaF subunit of MDH has active sites residues and the afore mentioned PQQ prosthetic group which coordinates with Ca^{2+}. Currently, our lab is exploring the use of La^{3+} in lieu of Ca^{2+} as a cofactor for MDH. Prior studies such as Nakagawa et. al. in 2012, they were able to show that XoxF1 functions as a La^{3+}-dependent MDH.

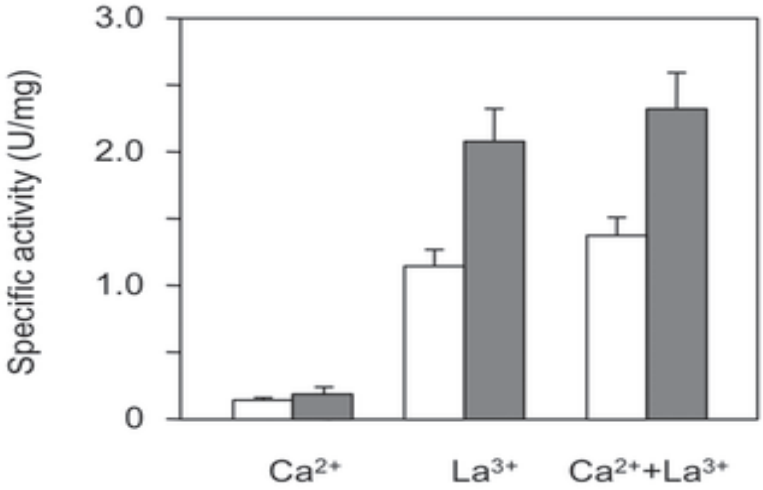

Figure 2. MDH activity in strain AM1 grown on methanol and succinate media with Ca^{2+} and/or La^{3+}.

The region downstream of XoxF encodes the cytochrome c, analogous to that found in human mitochondria, are responsible for electron transfer for energy production. Several mechanisms through which methylotrophs harness energy by oxidizing methanol has been proposed. Most of which include the serine and citric acid cycles as depicted in (Figure?)

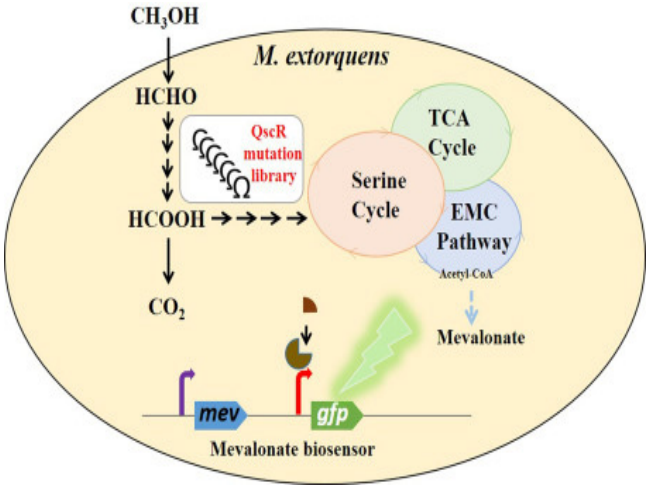

Figure 3. Biosensor-assisted transcriptional regulator engineering (SATRE) approach in *M. extorquens* AM1. QscR regulates the serine cycle to shift the carbon flux toward acetyl-CoA (Ac-CoA) accumulation. A heterologous mevalonate synthesis pathway responsible for converting acetyl-CoA into mevalonate was introduced into *M. extorquens* along with a mevalonate biosensor. The high mevalonate production mutants were screened out by fluorescence-activated cell sorting (FACS).
(This figure was modified from Liang, W., Cui, L., Cui, J., Yu, K., Yang, S., Wang, T., Guan, C., Zhang, C., Xing, X., 2017. Biosensor-assisted transcriptional regulator engineering for *Methylobacterium extorquens* AM1 to improve mevalonate synthesis by increasing the acetyl-CoA supply. Metab. Eng. 39, 159–168.)

Just like the human tricarboxylic acid cycle metabolized several compounds the create ATP by electron transfer, electrons obtained from methylotrophic catabolism of methanol is a great avenue for harnessing electrical power.

Developmental problems associated with Enzymatic biofuel cells.

As indicated earlier, enzymatic biofuels utilize enzyme catalyzed redox reactions to generate power. This means that there needs to be a succinct mechanism through which electrons generated from these enzymes are able to bind electrodes for further transfer. Of the many setbacks that could be expected in building enzymatic biofuel cells, this is most difficult to overcome as it predicts not just the longevity of the cells, but their efficiency and durability as well.

A myriad of research is ongoing regarding the optimum enzyme immobilization techniques on electrode. Our group over the past few months has been working on using tetrabutylammonium bromide (TBAB) modified Nafion® polymer. Essentially, having the enzymes bound to the electrodes leads to a better signal than having enzymes in solution. This is because electrons from electrode-bound enzymes are less likely to get repurposed as compared to their in-solution counterparts. This was one of the key things Dutta et.al. paid close attention to while designing

their single-enzyme biofuel cells (Figure). Specifically, they ensured direct electron transfer through electron tunneling between the T1 site on the anode (laccase Cu) and the T2/T3 site on cathode (laccase Cu) of a single enzyme. Per their results, this minimized electron transfer intermediates as the spatial distance between the T1 and T2/T3 redox centers were minimized. Subsequently, ABTS mediated electron transfer proved to be a great method of enhancing electron transfer between the active sites of enzymes and electrode.

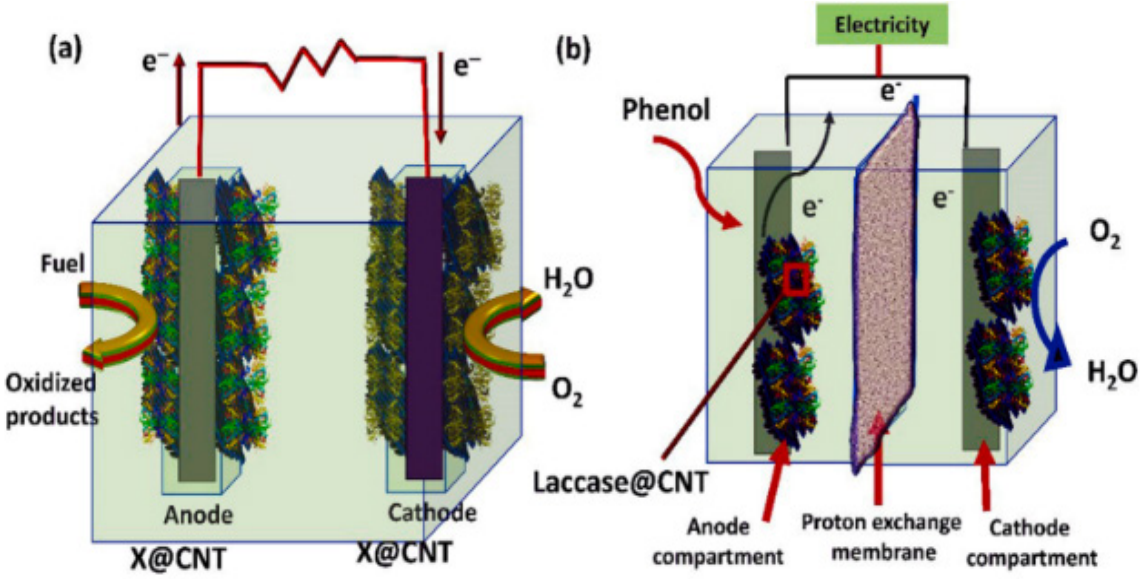

Figure 4. A comparative depiction of fundamental framework of traditional EBFC (a) and single-enzyme EBFC (b).

Possible solutions

Previously mentioned are several problems that pose hurdles in the quest to assemble biofuel cells. A few hypothetical solutions have been brought forth by various electrochemists for tackling hurdles ranging from low electron transfer efficiency between microbial cells and electrodes to issues related to longevity of the biofuel cells.

One very promising solution to these issues has to do with the incorporation of electron conduits in non-native exoelectrogens.[xxx] Exoelectrogens are simply microorganisms capable of generating electrical energy through electron transfer. *M. Extorquens* is an example. This solution would require an intersection of synthetic biology and some engineering as the reprogramming or even genetic adjustments of biological species will be required. In recent years, . This approach is not a one size fits all solution. Instead, every microbial species is approached differently depending on the cells' extracellular electron transfer mechanism. Of the most commonly used non-native exoelectrogens for industrial and pharmaceutical applications are E. Coli and P. Putida.

This solution is becoming more feasible given the immense growth in synthetic biology. It becomes easier for model organisms' genetic background to Another advantage this approach provides is the possible use of the created exoelextrogens as chassis strains to create new cell factories through metabolic modifications.

Electron flux enhancement may also be another approach toward fine-tuning biofuel cells. There are about five different but successive steps through which electrons are generated from substrate oxidation. Through synthetic biology, each step could be modified to enhance the extracellular electron transfer pathway, increasing the flux of electrons through the systems.

Conclusion

Enzyme based biofuel cells are still under development and look more promising in their prospective use as biosensors for testing the presence of metabolites *in vivo*. In any case optimization of electron transfer within microorganisms as well as between microorganisms and electrodes is essential for effectively constructing a cell. As noted above, various methods of enhancing bioelectrocatalysis are being investigated. Some of the most promising efforts include making biofilms such as TBAB modified nafion to immobilize enzymes or microbes onto the surface of electrodes to enhance direct electron transfer. Other very promising avenues also include biologically modified microorganisms such that their extracellular electron transfer efficiency is optimized as much as possible. This could include genetically modifying the microorganisms to metabolize a large array of molecules efficiently or altering cofactors involved in enzymatic activity as the substitution of lanthanum for calcium showed higher enzyme activity.

References

Guozhi Wu, Yue Dan Zhao, Pinghua Ling, and Feng Gao
ACS Applied Materials & Interfaces **2017** 9 (46), 40978-40986
DOI: 10.1021/acsami.7b12295

Chistoserdova, L.; Kalyuzhnaya, M. G.; Lidstrom, M. E. The Expanding World of Methylotrophic Metabolism. *Annual Review of Microbiology* **2009**, 63 (1), 477–499. https://doi.org/10.1146/annurev.micro.091208.073600.

Nakagawa, T.; Mitsui, R.; Tani, A.; Sasa, K.; Tashiro, S.; Iwama, T.; Hayakawa, T.; Kawai, K. A Catalytic Role of XoxF1 as La3+-Dependent Methanol Dehydrogenase in Methylobacterium Extorquens Strain AM1. *PLoS ONE* **2012**, 7 (11), e50480. https://doi.org/10.1371/journal.pone.0050480.

Skovran, E.; Palmer, A. D.; Rountree, A. M.; Good, N. M.; Lidstrom, M. E. XoxF Is Required for Expression of Methanol Dehydrogenase in Methylobacterium Extorquens AM1. *Journal of Bacteriology* **2011**, 193 (21), 6032–6038. https://doi.org/10.1128/jb.05367-11.

Chistoserdova, L.; Lidstrom, M. E. Molecular and Mutational Analysis of a DNA Region Separating Two Methylotrophy Gene Clusters in Methylobacterium Extorquens AM1. *Microbiology* **1997**, 143 (5), 1729–1736. https://doi.org/10.1099/00221287-143-5-1729.

Dutta, S.; Patil, R.; Dey, T. Electron Transfer-Driven Single and Multi-Enzyme Biofuel Cells for Self-Powering and Energy Bioscience. *Nano Energy* **2022**, 96, 107074. https://doi.org/10.1016/j.nanoen.2022.107074.

Chen, H.; Simoska, O.; Lim, K.; Grattieri, M.; Yuan, M.; Dong, F.; Lee, Y. S.; Beaver, K.; Weliwatte, S.; Gaffney, E. M.; Minteer, S. D. Fundamentals, Applications, and Future Directions of Bioelectrocatalysis. *Chemical Reviews* **2020**, 120 (23), 12903–12993. https://doi.org/10.1021/acs.chemrev.0c00472.

Davidson, V. L. Electron Transfer in Quinoproteins. Archives of Biochemistry and *Biophysics* **2004**, 428 (1), 32–40. https://doi.org/10.1016/j.abb.2004.03.022.

Dutta, S.; Patil, R.; Dey, T. Electron Transfer-Driven Single and Multi-Enzyme Biofuel Cells for Self-Powering and Energy Bioscience. *Nano Energy* **2022**, 96, 107074. https://doi.org/10.1016/j.nanoen.2022.107074.

Kalimuthu, P.; Daumann, L. J.; Pol, A.; Op den Camp, H. J. M.; Bernhardt, P. V. Electrocatalysis of a Europium Dependent Bacterial Methanol Dehydrogenase with Its Physiological Electron Acceptor Cytochrome c_{GJ}. Chemistry – *A European Journal* **2019**. https://doi.org/10.1002/chem.201901461.

Chen, H.; Dong, F.; Minteer, S. D. The Progress and Outlook of Bioelectrocatalysis for the Production of Chemicals, Fuels and Materials. *Nature Catalysis* **2020**, 3 (3), 225–244. https://doi.rg/10.1038/s41929-019-0408-2.

Hall, C. L.; Lambeth, J. D. Studies on Electron Transfer from General Acyl-CoA Dehydrogenase to Electron Transfer Flavoprotein. J*ournal of Biological Chemistry* **1980**, 255 (8), 3591–3595. https://doi.org/10.1016/s0021-9258(19)85743-5.

Umasankar, Y.; Ramasamy, R. P. Enhanced Electron Transfer in Enzymatic Bioelectrodes by a Poly(Vinyl Alcohol) N-Methyl-4(4′-Formylstyryl) Pyridinium Methosulfate Acetal Cationic Polymer. *ChemElectroChem* **2014**, 1 (11), 1834–1839. https://doi.org/10.1002/celc.201402186.

Bourourou, M.; Elouarzaki, K.; Lalaoui, N.; Agnès, C.; Le Goff, A.; Holzinger, M.; Maaref, A.; Cosnier, S. Supramolecular Immobilization of Laccase on Carbon Nanotube Electrodes Functionalized with (Methylpyrenylaminomethyl) Anthraquinone for Direct Electron Reduction of Oxygen. Chemistry - *A European Journal* **2013**, 19 (28), 9371–9375. https://doi.org/10.1002chem.201301043.