Phototrophs, electricity, and the circular economy

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Abstract
Climate change poses numerous global challenges. Tackling them will require responses at the level of policy, culture, and technology[1]. One of the most valuable tools available has the advantage of almost 4 billion years of modification and fine-tuning via evolution: microbes. By taking advantage of microbiological processes, we can build technologies that will revolutionize our economies. One such technology is microbial electrosynthesis (MES). MES takes advantage of bacteria that consume electrons via a process called extracellular electron uptake (EEU). By feeding microbes electricity, we can produce a range of bio-commodities such as bioplastic and biofuel using substrates like organic carbon-rich wastes or even carbon dioxide (CO_2). However, before such a technological feat is scalable, we must understand the fundamental mechanisms at play and develop proof-of-concept prototypes. Only then can we design systems capable of economically feasible, industrial scale bioproduction. Here, we discuss the underlying biology, highlight our lab's work pertaining to EEU and MES, and outline future directions.

Extracellular Electron Transfer in Nature
The Nobel Prize-winning biochemist Albert Szent-Györgyi observed that "life is nothing but an electron looking for a place to rest." He recognized that the movement of electrons is vital to the biochemistry at the heart of biology; from the smallest bacteria to the largest mammals, all life on earth relies on electron transfer. Decades later, researchers learned that some microbes exchange electrons with solid phase conductive substances such as minerals and electrically poised electrodes. These "electroactive" microbes broadly comprise two groups: exoelectrogens, which transfer electrons from cell to surface via reductive extracellular electron transfer (EET) and electrotrophs, which take up electrons via extracellular electron uptake (EEU). Both exoelectrogens and electrotrophs play vital roles in Earth's biogeochemical cycles. For example, photosynthetic iron oxidizing bacteria (photoferrotrophs) use EEU to oxidize iron, resulting in insoluble iron oxide depositions that constitute banded iron formations[2]. On the other hand, iron reducing microbes oxidize organic matter while respiring iron (III) via EET[3]. Efforts to understand electroactive microbes rely on the availability of tractable model organisms, which, in the case of EEU, remained elusive for some time.

Uncovering a model for EEU
In 2005, the photoferrotroph *Rhodopseudomonas palustris* TIE-1 was isolated from an iron-rich mat in Woods Hole, Massachusetts, USA[4]. Almost a decade later, TIE-1 was shown to conduct EEU by quantifying electron uptake in bioelectrochemical cells (BECs) with poised cathodes as electron donors[5]. Here, TIE-1 generated a cathodic current of 1.5 $\mu A\ cm^{-2}$ under illumination. Furthermore, the phototrophic iron oxidation operon *pioABC* played a significant role in mediating EEU. Importantly, mass balance calculations suggested an electron sink during EEU other than biomass alone. Gene expression data showed an increase in Rubisco form I expression during illuminated EEU. Together, these data showed that TIE-1 conducts EEU in a *pioABC*-dependent manner, and that photosynthetic CO_2 fixation mediated by Rubisco may be an important electron sink.

Building on this work, we sought to understand how TIE-1 acquired electrons via EEU through the PioABC machinery. In addition to the data above, another study showed that Δ*pioABC* mutant cannot perform photoferrotrophy, suggesting a relationship between PioABC-mediated EEU and photosynthetic iron oxidation[6]. We demonstrated that the decaheme cytochrome *c* protein PioA and the outer membrane porin PioB form a complex allowing EEU across the outer membrane from soluble iron and poised electrodes[7]. Additionally, PioAB homologs were found in other phototrophs, such as *Rhodomicrobium vannielii* and *Rhodomicrobium udaipurense*, both of which conduct photoferrotrophy and EEU from poised electrodes. This suggested that EEU and photoferrotrophy may be distributed across broad groups of freshwater phototrophs.

Further work in TIE-1 revealed that electrons acquired via EEU enter the photosynthetic electron transport chain and that EEU results in a highly reducing intracellular environment.[8] In this study, we fabricated micro-BECs with indium tin oxide working electrodes that allowed for bioelectrochemical analyses and confocal microscopy of TIE-1 biofilms. By focusing exclusively on surface-attached cells, we reduced confounding factors such as planktonic cells, extracellular enzymes, and abiotic reactions that occur in bulk BECs. Consequently, we observed that Rubisco form I expression was induced in wild-type biofilms, suggesting that *de novo* growth via photosynthetic CO_2 fixation may occur on the electrode surface. Secondary ion mass spectrometry allowed us to trace carbon assimilation and revealed that labeled carbon is enriched in cells attached to poised electrodes, suggesting that electrons from EEU are used for CO_2 fixation. Finally, by knocking out both *Rubisco* genes, we abrogated current uptake in TIE-1. Altogether, this strongly suggests that photosynthetic CO_2 fixation is the primary electron sink during EEU.

Sustainable Bioproduction with TIE-1

EEU can be leveraged for bio-commodity production via microbial electrosynthesis (MES)[9]. During MES, CO_2 is converted to organic carbon compounds using electrotrophs as biocatalysts. In the case of photoautotrophic EEU, MES uses renewable inputs: light, CO_2, and electricity **(Figure 1)**. Consequently, photosynthetic electrotrophs like TIE-1 are promising biocatalysts for sustainable MES. Moreover, TIE-1's metabolic plasticity and its genetic tractability make it a promising model for exploring industrial applications. For example, our lab used TIE-1 to make biofuel (*n*-butanol) via MES[10]. We achieved this by introducing the *n*-butanol biosynthesis pathway into TIE-1, and improved production by deleting the electron-consuming nitrogen fixation pathway. Coupled with a bioelectrochemical platform that used solar panel-generated electricity, we achieved efficient biofuel production. This provides a foundation for carbon-neutral *n*-butanol synthesis using sustainable resources.

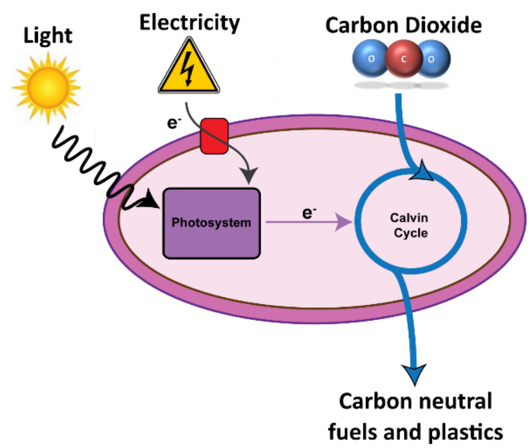

Figure 1. Simplified overview of bio-commodity production via microbial electrosynthesis with photoautotrophic microbes (e.g., *R. palustris* TIE-1).

We also investigated TIE-1's ability to produce bioplastic (polyhydroxybutyrate, or PHB) which acts as an intracellular carbon and energy reserve for bacteria. PHBs are a promising alternative to petroleum-based plastics; they are thermoresistant, moldable, biocompatible, and biodegradable polyesters that have been used in fields including agriculture, aerospace, biomedicine, infrastructure, and electrical engineering. Our study showed that TIE-1 produced PHB under various growth conditions, with the highest electron yields during MES and highest specific PHB productivity during photoferrotrophy[11]. This was the first systematic exploration of PHB production under these conditions and could lead to sustainable bioproduction using abundant, renewable resources.

Improving MES

Research on improving MES has focused on cathode modifications, the biology of EEU, and isolating electrotrophs from the environment. Our laboratory demonstrated that modifying electrodes with iron-based composites can increase EEU. Using an immobilized iron-based redox mediator called Prussian Blue, we achieved a 3.8-fold increase in cathodic current uptake[12]. We also synthesized a composite of magnetite nanoparticles and reduced graphene oxide, which we electrodeposited onto a carbon felt cathode[13]. This resulted in 5-fold higher EEU and 4.2-fold higher PHB production relative to unmodified carbon felt – 20 times higher than unmodified graphite. From a biological perspective, genetically modified strains will be a powerful tool. Engineered strains lacking resource-consuming pathways, overexpressing bio-commodity synthesis pathways, or expressing different EEU proteins from other organisms may further increase yields and efficiency. In this vein, we recently enhanced PHB production 5-fold in TIE-1 by overexpressing the Rubisco enzyme[14]. Finally, bioprospecting for novel strains capable of EEU will further expand our biological toolbox. We recently demonstrated that the photosynthetic purple non-sulfur bacteria *Rhodomicrobium vannielii* and *Rhodomicrobium udaipurense* produce PHB with far greater per-cell PHB production (productivity), electron conversion efficiency, and carbon conversion efficiency than TIE-1[15]. This suggests that biological chassis with desirable characteristics await discovery and characterization.

EEU in the Marine Wetlands

EEU is not only relevant for biotechnology, but also ecology. Though EEU is common across diverse ecosystems, the potential for pEEU in marine organisms remains largely unexplored[16]. We addressed this knowledge gap by isolating 15 novel strains of marine anoxygenic phototrophs belonging to the genus *Rhodovulum* from microbial mats in the Trunk River estuary in Woods Hole, Massachusetts, USA[17,18]. These isolates perform photoferrotrophy, and one strain that we examined performs pEEU from poised electrodes. Further investigation revealed that this strain uses a previously unknown diheme cytochrome *c* protein, EeuP, for pEEU. Homologs of EeuP were identified in a range of marine microbes. Together, this suggests that photoferrotrophy and pEEU could play an important role in biogeochemical cycling of iron and carbon in marine ecosystems.

Conclusions and Perspectives

The latest report from the Intergovernmental Panel on Climate Change is frank: Climate change is "widespread, rapid, and intensifying" and mitigating global warming requires "limiting cumulative CO_2 emissions, reaching at least net zero CO_2 emissions," and reductions in other greenhouse gases[19]. Plastic waste poses a similar challenge, with global plastic volume reaching ~6.3 billion megatons in 2015 and expected to reach 12 billion megatons by 2050[20]. Innovations that push us closer to a circular economy can address these challenges. The goal of a circular economy is to minimize negative externalities and waste using a systems-level approach to economic organization that accounts for the flow of renewable and non-renewable materials[21]. This framework forces us to consider the broader impacts of MES technologies (e.g., does implementing an MES platform at industrial scales simply shift emissions from plastic production to feedstock or water use?). While MES should play a role in decarbonization, implementing it without careful consideration of externalities will not yield truly sustainable solutions.

For MES, the primary question is one of scalability: How do we transfer this from laboratory to industry while striking a balance between costs and output? One bottleneck is EEU efficiency; low electron uptake means low product formation, and current densities above 50 – 100 mA cm^{-2} may be required for most MES applications[22]. Biofilms also play an important role in achieving higher current densities; advancements in 3D-printed biofilms can maximize EEU efficiency by considering parameters like biofilm thickness, density, and spatial organization. Mathematical modelling of MES may clarify electrochemical and biological dynamics, leading to improved reactor designs. Additionally, bioprospecting, genetic engineering and synthetic biology will yield novel strains with enhanced EEU capabilities and resilience to diverse conditions[23]. Finally, it is important to remember the ecological significance of EEU. EEU in the environment is severely understudied, and our early endeavors suggest that it is quite widespread in ecologically meaningful environments. Wetlands play important roles in global carbon cycles, and understanding EEU within this context will help future conservation efforts and potentially inform nature-based solutions to climate change.

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