Advances in Electrochemical Cofactor Regeneration: Enzymatic and Non-Enzymatic Approaches

Yoo Seok Lee[*1], Rokas Gerulskis[1], Shelley D. Minteer**

Department of Chemistry, University of Utah 315 S 1400 E, Salt Lake City, UT 84112, USA;

[1]Co-first authors

ORCID

Shelley D. Minteer: 0000-0002-5788-2249

Yoo Seok Lee: 0000-0002-8840-0206

Rokas Gerulskis: 0000-0003-0370-679X

***Corresponding Author**

Shelley D. Minteer (minteer@chem.utah.edu)

Yoo Seok Lee (lys302601@gmail.com)

Abstract

Nicotinamide adenine dinucleotide(NAD(P)H) is a metabolically interconnected redox cofactor serving as a hydride source for the majority of oxidoreductases, and consequently constituting a significant cost factor for bioprocessing. Much research has been devoted to the development of efficient, affordable, and sustainable methods for the regeneration of these cofactors through chemical, electrochemical, and photochemical approaches. However, the enzymatic approach using formate dehydrogenase is still the most abundantly employed in industrial applications, even though it suffers from system complexity and product purity issues. In this review, we summarize non-enzymatic and enzymatic electrochemical approaches for cofactor regeneration, then discuss recent developments to solve major issues. Issues discussed include Rh-catalyst mediated enzyme mutual inactivation, electron-transfer rates, catalyst sustainability, product selectivity and simplifying product purification. Recently reported remedies are discussed, such as heterogeneous metal catalysts generating H^+ as the sole byproduct or high activity and stability redox-polymer immobilized enzymatic systems for sustainable organic synthesis.

INTRODUCTION

Enzymatic catalysis systems have widespread use in environmental applications, organic synthesis, and pharmaceutical manufacturing due to the exceptional selectivity and turnover frequencies of enzyme-catalyzed reactions. [1-3] The majority of oxidoreductases, enzymes that catalyze redox reactions, [4] employ nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated form ($NADP^+$) as a cofactor to receive and transfer hydrides between independently catalyzed reactions (Figure 1). Commercial enzymatic redox transformations require stoichiometric amounts of NAD(P)H, and the high cost of NADH (>$20/g) [5,6] and NADPH ($500/g) [7] has motivated research aimed at their regeneration in both the reduction and oxidation directions. This review provides a compilation of important developments in both enzymatic and non-enzymatic cofactor

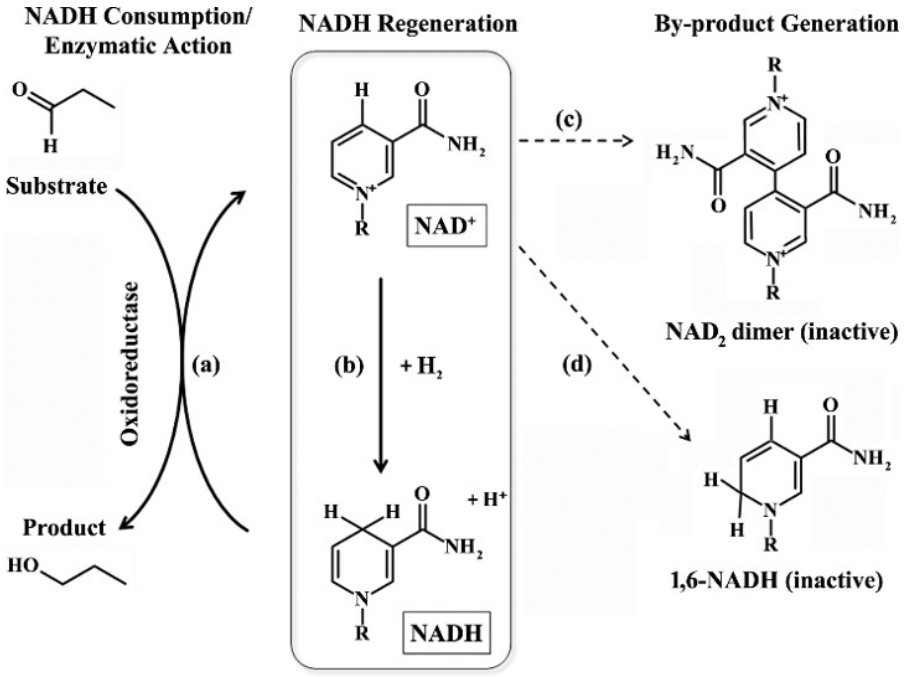

Figure 1. (a) NADH is utilized as a cofactor (reducing agents) to donate hydride for oxidoreductase biotransformation; (b) NAD^+ reduction to NADH; (c) formation of enzymatically inactive NAD_2 dimer and (d) 1,6-NADH. Reprinted with permission from ref. [8]. Copyright 2016, American Chemical Society.

regeneration systems. Enzymatic systems presented include both electrochemical and non-electrochemical approaches. The three types of non-enzymatic systems presented employ (1) inorganic reductants (e.g. $Na_2S_2O_4$ and $NaBH_4$), [9] (2) photocatalysts (e.g., copolymers and carbon nitride), [10-12] and/or (3) electrocatalysis (including both direct electrochemical regeneration [13,14] and indirect regeneration using homogeneous or heterogeneous inorganic complexes [15-20]).

Non-enzymatic Electrochemical Cofactor Regeneration

In one of the first methods presented for the non-enzymatic regeneration of NADH, Wienkamp et al. [16] employed the two-electron transfer agent $[Rh(bpy)_2]^+$ generated from $[Rh(bpy)_3]^{3+}$ at a graphite cathode. The use of a $[Rh(bpy)_3]^{3+}$ mediator decreased the overpotential required for NAD^+ reduction by 250 mV when compared to the direct reduction on the electrode surface (-0.65 V compared to over -0.9 V vs. SHE). This decrease minimizes the production of enzymatically inactive NAD_2 dimers, while avoiding the use of enzymes minimizes productivity losses arising from enzyme inactivation. More recently, Zhang et al. [21] demonstrated in a photoelectrochemical system that employing Si nanowires (SiNW) rather than planar Si at the cathode with dissolved Cp*Rh(bpy) shifts this overpotential yet further, from -0.35 V (onset vs. RHE, peaking at -0.65V) in Si sheets, to +0.25 V (peaking at -0.05V) in the SiNW electrode. This corresponded to a catalytic rate of 1.5 mM cm^{-2} h^{-1} at -0.5V (vs. SHE) and nearly 100% Faradaic efficiency using the SiNW electrode. To advance this technology, many kinds of metal based catalysts including iron [22], platinium-gold [23], rhenium [24,25], copper-mercury [26], and iridium [27] have been actively investigated in the past 20 years, while others have focused on the role of Rh-ligand identity [28], all with the aim of increasing cofactor conversion kinetics and

product yield. Gopalan et al. [29] were the first to utilize core–shell metal nanoparticles as a catalyst for the conversion of NADH to NAD^+, aiming to make use of the excellent catalytic and optical [30] properties of this catalyst. Since then, many various core–shell architectures have received attention, especially combinations employing a more abundant and cheaper metal core with a noble metal shell. The use of noble metal shells protects the core metal from degradation, but can also improve the catalytic properties due to strain and ligand effects of the core metal on the supported noble metal [31,32].

Ali et al. [33] developed a Pt and Ni nanoparticle-patterned electrode with the aim of improving radical protonation kinetics. The undesired production of inactive NAD_2 dimers in many direct regeneration systems results from the proximal reduction of multiple NAD^+ molecules at the electrode surface, which dimerize with one another before they can be protonated. The Pt and Ni nano-particles provide 'active' adsorbed hydrogen at the electrode surface to improve radical protonation kinetics and minimize this radical dimerization. The Pt nanoparticle patterned electrode demonstrated a 100% increase in the amount of NADH regenerated with a yield of 65 ± 1.9% compared to the 32 ± 0.02% in the control conversion using a pure electrode. 98% recovery of enzymatically active 1,4-NADH was demonstrated at -1.6V vs MSE, an impressive selectivity given that direct regeneration methods typically demonstrate significant loss to NAD^+ reduction side reactions [34,35].

Photoinduced cofactor conversion has attracted attention as a cost-effective and sustainable approach by eliminating the need for an external power supply by using available solar energy. Nam et al [36] utilized proflavine, a photosensitizer with high stability and turnover numbers under photolysis conditions. Triethanolamine was used as a sacrificial electron donor, and $[Cp^*Rh(bpy)(H_2O)]^{2+}$ was applied as an organometallic electron mediator to minimize the rate of

the dimer and isomer-yielding side-reactions of NAD^+ reduction. This system showed 63.4% maximum yield and an initial turnover frequency (TOF) of 127.8 h^{-1} for NADH regeneration under Xenon arc lamp and lower TOFs under colored LEDs. Liu et al. [11] proposed an NADH reduction system using a graphitic carbon nitride electrode employing $[Cp^*Rh(bpy)H_2O]^{2+}$and a frustule structure constructed using sustainable diatomaceous earth. Again, with triethanolamine as a sacrificial electron donor, the NADH yield reached nearly 100%, with 1,4-NADH as the sole product. This was ascribed to the enhanced light trapping and scattering of the diatom layer promoting high photocatalytic efficiency.

Enzymatic Electrochemical Cofactor Regeneration

Compared to homogeneous organometallic complexes for catalyzing NADH regeneration, enzyme systems typically demonstrate superior regioselectivity, low overpotentials, greener catalytic conditions (temperature, pressure, pH), and higher turnover frequencies. [9,37] For these reasons, enzymatic NADH regeneration is the main regeneration method used by industry. Enzymatic NADH regeneration systems typically employ a dehydrogenase, which reduces NAD^+ by extracting a hydride from a more affordable substrate, oxidizing it concomitant to NAD^+ reduction. For example, formate dehydrogenase (FDH), which oxidizes formate to CO_2, is the most common NADH regenerating dehydrogenase employed at the industrial scale. FDH has been employed to regenerate NADH for the production of several tons per year of L-tert-leucine using leucine dehydrogenase [38]. It was employed to support phenylalanine dehydrogenase mediated manufacture of the antihypertensive drug omapatrilat, (Bristol-Myers Squibb), [39] and it is also employed to support the enzymatic conversion of asymmetric ketones to polyols for the production of optically-active homogeneous catalysts, although this process is still being developed for use at

industrial scales. [40] The TOF of FDH from *Candida boidinii* is reported as 3900 h^{-1}, more than three times higher than the TOF of the fastest reported organometallic catalyst (a variant of Cp*Rh(bpy)). [28,41] Other dehydrogenases with high potential for widespread application include [42,43] glucose dehydrogenase (oxidizing glucose to gluconolactone) and phosphite dehydrogenase (oxidizing phosphite to phosphate). The former shows more versatility in employing NAD^+ or $NADP^+$, and higher activity and stability compared to FDH, while the latter reaction does not cause a shift in pH, avoiding the cost of acid/base addition. [44] One of the most significant disadvantages of dehydrogenase-mediated NADH regeneration is the requirement for continual addition of co-substrate (e.g., formate) to the bioreactor, whose often incomplete consumption requires downstream purification steps. [9,45] This issue can be resolved electrochemically by employing diaphorase, an enzyme that reduces NAD^+ to NADH. Direct or mediated bioelectrocatalysis can then be used for regeneration without consuming any other co-substrates. In spite of this advantage, the potential of diaphorase to replace existing commercial systems has rarely been investigated.

There are a few enzymes that can directly transfer electrons between their cofactors and the electrode surface (direct electron transfer, DET). [46] However, for electron tunneling rates to be sufficient for physiologically relevant TOFs, direct electrode-enzyme contact (<14 Å) is required, [47,48] placing an upper limit on catalytic efficiency as a function of electrode surface area. The use of a dissolved redox mediator as an electron transferring agent increases tunneling rates, but this leads to extra product purification steps. [49] Dinh et al. demonstrated a diaphorase with surface-conjugation of the mediator ethyl carboxyethyl viologen (ECV) [50], demonstrating 126% improved NAD^+ conversion versus diaphorase in 0.1 mM ECV, after 3h electrolysis at -0.85 V vs Ag/AgCl. This performance increase is a side-benefit to a regeneration system requiring minimal

NADH purification resulting from electrode-conjugation of both catalyst and mediator. Unfortunately, redox mediators often suffer from degradation issues mandating frequent replacement or are not commercially available and require complex syntheses and purifications for use. [34,51,52] Svenja et al. [53] compared different mediators as electron transferring agents in a scalable electrochemical reactor driving $NAD(P)^+$ regeneration, discussing relative electron transfer efficiencies and required overpotentials.

Recent Pioneering Developments for Cofactor Regeneration

All large-scale systems for cofactor regeneration must consider the environmental sustainability of component manufacture, the cost of downstream purification, and the role in system productivity played by the interaction of components with byproducts and other components. [18,54-56] Although the environmental sustainability of proposed regeneration methods is rarely quantified in original literature, a recent work by Saba et al. [57] meticulously compared the e-factor (regeneration process cleanliness as quantified by kg_{waste}/kg_{NADH}) of dozens of NAD(P)H regeneration systems. Several recent works demonstrate NAD^+ reduction catalysts producing little byproduct on top of construction without the use of environmentally costly rare metals. Wang et al. [8] proposed, for the first time, an *in situ* NAD^+ conversion to NADH using a heterogeneous Pt/Al_2O_3 catalyst that uses H_2 as its hydride source and generates H^+ as the sole byproduct, as shown in Figure 2. Subsequently, Ali et al. [7] improved upon their previous work investigating Ni and Pt NP protonation kinetics by introducing a system employing Ni nanoparticle-dotted multi-walled carbon nanotubes, demonstrating 98% selectivity for functional 1,4-NADH with H^+ as the sole byproduct. These systems exhibit improvements in stability, selectivity, purification, waste minimization, and sustainability (minimizing the use of rare or toxic metal complexes).

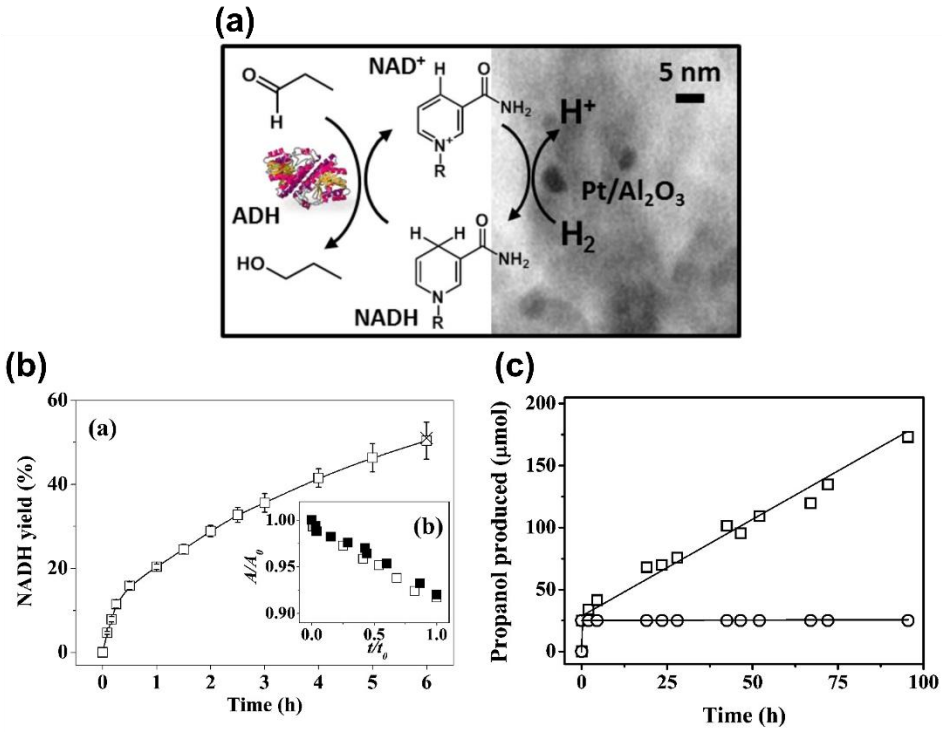

Figure 2. (a) Scheme presenting NADH regeneration using a heterogeneous catalyst (Pt/Al_2O_3) and H_2 coupled with an enzymatic reduction. (b) Variation of NADH yield as a function of time (□) as determined by ^1H NMR (×). (b) NADH yield validation using enzymatic assay: NADH produced experimentally (□) and from a prepared mixture using commercial NADH and NAD^+ (■). (c) Continuous enzymatic reduction of propanal to propanol coupled with in situ NADH regeneration by Pt/Al_2O_3 in a fed-batch system. Reprinted with permission from ref. [6]. Copyright 2016, American Chemical Society.

A loss of NAD^+ to side-products is characteristic of direct regeneration systems, which minimize environmental impact through sustainably manufacturable catalysts at the cost of poor selectivity for 1,4-NADH over reduction side-products. One approach to overcome this drawback is the employment of renalase, [58] an enzyme which catalyzes the oxidation of enzymatically inactive 1,4- and 1,6-NADH (but not NAD_2) back to NAD^+. Recent work [59] has made strides in increasing the soluble expression levels and catalytic activity of recombinant renalase, so the future of sustainable direct regeneration systems looks more promising than ever. While NADH-

regenerating Rh complexes demonstrate superior stereoselectivity compared to direct regeneration approaches, and often longer lifetimes compared to enzymatic systems, these catalysts not only have an environmental cost resulting from Rh use, but suffer from a well-established side reaction with -SH and -NH_2 groups on enzymes which leads to mutual inactivation of both catalysts in under 24 hours. [18,54-56]. The last half-decade produced copious studies focused on minimizing this interaction through physical barriers. An immobilization procedure proposed by Zhang et al. [60] demonstrated porous carbon electrodes with covalently attached $[Cp^*Rh(bpy)Cl]^+$ as shown in Figure 3. When employed with D-sorbitol dehydrogenase and galactitol dehydrogenase, the system showed stable NADH production for over 90 hours. Himiyama et al. instead employed a periodic mesoporous organosilica (PMO) to pack Cp*Rh(bpy), allowing the diffusion of substrates while excluding interactions with large proteins [61]. While the addition of bovine serum albumin decreased the 6 hour conversion % of cyclohexen-1-one by 83% when using free [Cp*Rh(bpy)Cl]Cl as catalyst, this loss of activity was only 9% when employing the PMO-modified catalyst. Morra and Pordea modified an ADH which naturally coordinates Zn to instead encapsulate a Cp*Rh-based catalyst [57]. This method proved to decrease enzyme activity loss after 24 hours from 80% in systems employing traditional Cp*Rh catalysts to less than 20% in those employing the protein conjugate. Zhang et al. investigated a photo-enzymatic system in which Cp*Rh(bpy) receives electrons for NAD^+ reduction from light-activated graphitic carbon nitride (GCN) [62], which is also able to inactivate enzymes but through an electron hole-transferring mechanism. These authors employed a Cp*Rh(bpy)-embedded, size-excluding TiO_2 coating on GCN. 1h of illumination decreased ADH activity by 90% in the presence of GCN and 100% in the presence of Cp*Rh(bpy) (interestingly, only 13% without illumination), but only decreased activity by 33% when ADH was protected using the TiO_2 layer.

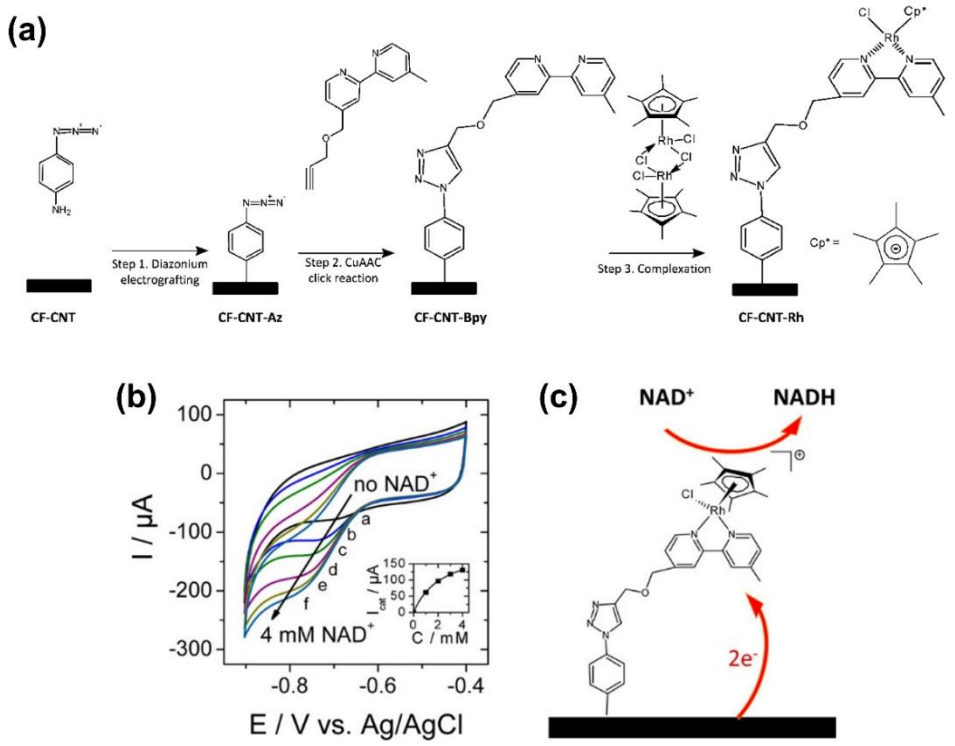

Figure 3. (a) Covalent bonding of (2,2′-bipyridyl) (pentamethylcyclopentadienyl)-rhodium complex at the carbon electrode surface (b) Cyclic voltammetric response for NAD^+ reduction under gradual addition of NAD^+. (c) Schematic representation of NADH regeneration mediated by a CF-CNT-Rh electrode. Reprinted with permission from ref. [60]. Copyright 2017, American Chemical Society.

One approach for cofactor regeneration with electron transfer efficiency and purification cost in mind is the use of redox polymers for enzyme immobilization. A redox polymer consists of an insulating polymeric backbone with covalently bound redox mediators as side chains and serves the function of facilitating rapid electron transfer to the catalyst through self-exchange based conduction. The advantage of redox-polymer mediated enzyme-electrode immobilization lies in the ability to retain enzyme and redox mediating species on the electrode when the new reactant solution is replenished, which allows for long-term reuse of electrodes and minimizes purification issues. [63-66] A recent work demonstrated a NAD regeneration system in which a

naphthoquinone redox polymer oxidized NADH non-enzymatically. [67] The only redox polymers offering NAD^+ reduction capability are viologen-based, but these have not seen widespread application owing to stability and selectivity issues [50]. Quah et al. [68] proposed a benzylpropyl-

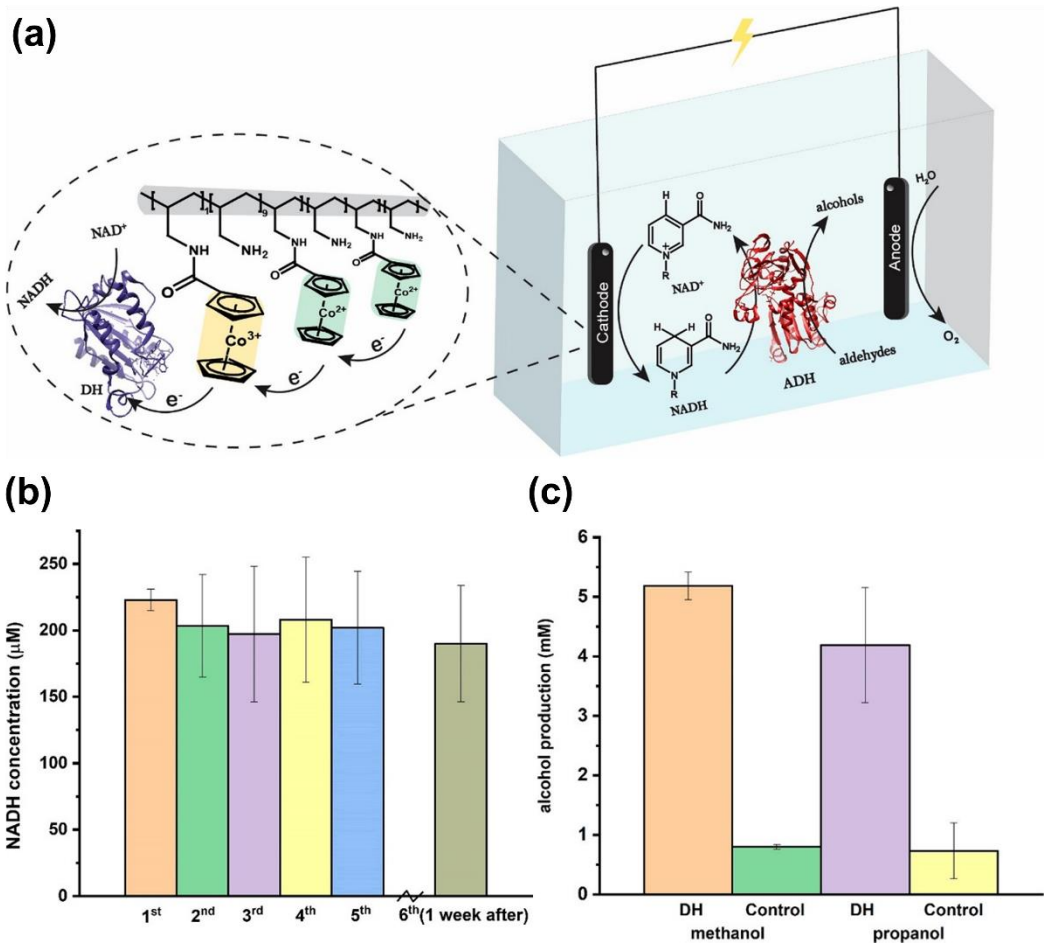

Figure 4. (a) Cc-PAA polymer-immobilized DH system capable of NADH production from NAD^+ with alcohol dehydrogenase for biofuel production. (b) Regeneration yield of NADH from NAD^+ over one week for stability test of DH/Cc-PAA (c) Production of methanol and propanol in the presence of DH and with DH replaced with BSA (control). Reprinted with permission from ref. [69]. Copyright 2019, American Chemical Society.

viologen (BPV) redox polymer to support NAD^+/NADH regeneration with diaphorase. The relatively positive potential of BPV (−0.27 V vs. SHE) compared to the cofactor (−0.32 V vs SHE) makes this polymer more suitable for cofactor oxidation than cofactor reduction. Yuan et al. [69] subsequently proposed a cobaltocene modified poly(allylamine) polymer (Cc-PAA) immobilized diaphorase system showing superior operational stability, Faradaic efficiency (near-100%), durability, turnover frequency, selectivity, and a mild overpotential for bioactive NADH production over one week, as shown in Figure 4.

Conclusion

$NAD(P)^+$ and NAD(P)H are highly metabolically interconnected redox cofactors serving the majority of oxidoreductase-catalyzed reactions. This interconnectivity means that the regeneration of these cofactors is critical to the economically feasible enzymatic synthesis of high value-added products. Enzymatic cofactor conversion has historically demonstrated exceptional turnover frequencies and product selectivity, but has been held back by product impurity arising from substrate requirements and catalyst mobility. Although performance has not yet crossed traditional systems, diaphorase-based systems, especially with the maturation of redox polymer research, are a promising avenue to address these issues. Molecular catalysts, though demonstrating increasing turnover frequencies and product selectivity, still suffer from undesired interactions with system components, and are still largely fabricated using unsustainable rare metals. Direct electrochemical regeneration systems, though exceptionally simple and homogenous, have historically also shown the lowest turnover frequencies and product selectivity. These drawbacks of direct electrochemical regeneration methods are in decline with the advent of novel nanostructured electrodes with performance unprecedented in the category. The unveiling of novel catalyst and system

architectures shows rapid performance improvements in all three categories, and the rapid evolution of regeneration methods promises exciting developments in the efficiency of regeneration methods and the scope of their parallel biosynthetic pathways.

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Conflict of interest statement

Nothing declared.

Reference and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Bornscheuer U, Huisman G, Kazlauskas R, Lutz S, Moore J, Robins K: **Engineering the third wave of biocatalysis**. *Nature* 2012, **485**:185-194.

2. Lee YS, Lim K, Minteer SD: **Cascaded Biocatalysis and Bioelectrocatalysis: Overview and Recent Advances**. *Annual Review of Physical Chemistry* 2021, **72**:467-488.

*** Paper of special interest – an important recent review that describes in details the multi-enzyme catalysis and electrocatalysis with cofactor regeneration system.**

3. Chen H, Simoska O, Lim K, Grattieri M, Yuan M, Dong F, Lee YS, Beaver K, Weliwatte S, Gaffney EM: **Fundamentals, Applications, and Future Directions of Bioelectrocatalysis**. *Chemical Reviews* 2020, **120**:12903-12993.

*** Paper of special interest – a very comprehensive and current review on microbial and enzymatic electrochemical system with cofactor regeneration system.**

4. Gamenara D, Seoane G, Méndez PS, de María PD: *Redox biocatalysis: fundamentals and applications*. John Wiley & Sons; 2012.

5. Faber K: **Biocatalytic applications**. In *Biotransformations in organic chemistry*. Springer; 2011:31-313.

6. Satoh Y, Tajima K, Tannai H, Munekata M: **Enzyme-catalyzed poly (3-hydroxybutyrate) synthesis from acetate with CoA recycling and NADPH regeneration in vitro**. *Journal of bioscience and bioengineering* 2003, **95**:335-341.

7. Ali I, Ullah N, McArthur MA, Coulombe S, Omanovic S: **Direct electrochemical regeneration of enzymatic cofactor 1, 4-NADH on a cathode composed of multi-walled carbon nanotubes decorated with nickel nanoparticles**. *The Canadian Journal of Chemical Engineering* 2018, **96**:68-73.

**** Paper of outstanding interest – exceptional 1,4-NADH selectivity among direct regeneration methods.**

8. Wang X, Yiu HH: **Heterogeneous catalysis mediated cofactor NADH regeneration for enzymatic reduction**. *ACS Catalysis* 2016, **6**:1880-1886.

9. Wu H, Tian C, Song X, Liu C, Yang D, Jiang Z: **Methods for the regeneration of nicotinamide coenzymes**. *Green Chemistry* 2013, **15**:1773-1789.

10. Oppelt KT, Gasiorowski J, Egbe DAM, Kollender JP, Himmelsbach M, Hassel AW, Sariciftci NS, Knör Gn: **Rhodium-coordinated poly (arylene-ethynylene)-alt-poly (arylene-vinylene) copolymer acting as photocatalyst for visible-light-powered NAD+/NADH reduction**. *Journal of the American Chemical Society* 2014, **136**:12721-12729.

11. Liu J, Antonietti M: **Bio-inspired NADH regeneration by carbon nitride photocatalysis using diatom templates**. *Energy & Environmental Science* 2013, **6**:1486-1493.

12. Huang J, Antonietti M, Liu J: **Bio-inspired carbon nitride mesoporous spheres for artificial photosynthesis: photocatalytic cofactor regeneration for sustainable enzymatic synthesis**. *Journal of Materials Chemistry A* 2014, **2**:7686-7693.

13. Ali I, Omanovic S: **Kinetics of electrochemical reduction of NAD+ on a glassy carbon electrode**. *International Journal of Electrochemical Science* 2013, **8**:4283-4304.

14. Ali I, Khan T, Omanovic S: **Direct electrochemical regeneration of the cofactor NADH on bare Ti, Ni, Co and Cd electrodes: The influence of electrode potential and electrode material**. *Journal of Molecular Catalysis A: Chemical* 2014, **387**:86-91.

15. Steckhan E, Herrmann S, Ruppert R, Dietz E, Frede M, Spika E: **Analytical Study of a Series of Substituted (2, 2'-bipyridyl)(pentamethylcyclopentadienyl) rhodium and-iridium Complexes with Regard to their Effectiveness as Redox Catalysts for the Indirect Electrochemical and Chemical Reduction of NAD (P)+**. *Organometallics* 1991, **10**:1568-1577.

16. Wienkamp R, Steckhan E: **Indirect electrochemical regeneration of NADH by a bipyridinerhodium (I) complex as electron-transfer agent**. *Angewandte Chemie International Edition in English* 1982, **21**:782-783.

*** Paper of special interest – First non-enzymatic regeneration of NADH approach, employing $[Rh(bpy)_3]^{3+}$ mediator.**

17. Hollmann F, Witholt B, Schmid A: **[Cp∗ Rh (bpy)(H2O)] 2+: a versatile tool for efficient and non-enzymatic regeneration of nicotinamide and flavin coenzymes**. *Journal of Molecular Catalysis B: Enzymatic* 2002, **19**:167-176.

18. Hildebrand F, Lütz S: **Stable electroenzymatic processes by catalyst separation**. *Chemistry–A European Journal* 2009, **15**:4998-5001.

19. Lu LQ, Li Y, Junge K, Beller M: **Iron-Catalyzed Hydrogenation for the In Situ Regeneration of an NAD (P) H Model: Biomimetic Reduction of α-Keto-/α-Iminoesters**. *Angewandte Chemie* 2013, **125**:8540-8544.

20. Yan YK, Melchart M, Habtemariam A, Sadler PJ: **Organometallic chemistry, biology and medicine: ruthenium arene anticancer complexes**. *Chemical communications* 2005:4764-4776.

21. Zhang B, Xu S, He D, Chen R, He Y, Fa W, Li G, Wang D: **Photoelectrochemical NADH regeneration is highly sensitive to the nature of electrode surface**. *The Journal of Chemical Physics* 2020, **153**:064703.

22. Fukuzumi S, Kotani H, Lee Y-M, Nam W: **Sequential electron-transfer and proton-transfer pathways in hydride-transfer reactions from dihydronicotinamide adenine dinucleotide analogues to non-heme oxoiron (IV) complexes and p-chloranil. Detection of radical cations of NADH analogues in acid-promoted hydride-transfer reactions**. *Journal of the American Chemical Society* 2008, **130**:15134-15142.

23. Damian A, Maloo K, Omanovic S: **Direct electrochemical regeneration of NADH on Au, Cu and Pt-Au electrodes**. *Chemical and biochemical engineering quarterly* 2007, **21**:21-32.

24. Kobayashi A, Takatori R, Kikuchi I, Konno H, Sakamoto K, Ishitani O: **Formation of novel 1: 1 adducts accompanied by regioselective hydride transfer from transition-metal hydrido complexes to NAD (P) models**. *Organometallics* 2001, **20**:3361-3363.

25. Kobayashi A, Konno H, Sakamoto K, Sekine A, Ohashi Y, Iida M, Ishitani O: **Transition Metal Complexes Coordinated by an NAD (P) H Model Compound and their Enhanced Hydride-Donating Abilities in the Presence of a Base**. *Chemistry–A European Journal* 2005, **11**:4219-4226.

26. Munteanu G, Dempsey E, McCormac T, Munteanu C: **Fast cyclic voltammetry of redox system NAD+/NADH on the copper nanodoped mercury monolayer carbon fiber electrode**. *Journal of Electroanalytical Chemistry* 2012, **665**:12-19.

27. Maenaka Y, Suenobu T, Fukuzumi S: **Efficient catalytic interconversion between NADH and NAD^+ accompanied by generation and consumption of hydrogen with a water-soluble iridium complex at ambient pressure and temperature**. *Journal of the American Chemical Society* 2012, **134**:367-374.

28. Ganesan V, Sivanesan D, Yoon S: **Correlation between the structure and catalytic activity of [Cp* Rh (substituted Bipyridine)] complexes for NADH regeneration**. *Inorganic chemistry* 2017, **56**:1366-1374.

*** Paper of special interest – Discusses structural correlations of Rh ligand identity and catalytic activity, relevant to further catalyst optimization.**

29. Gopalan A, Ragupathy D, Kim H-T, Manesh K, Lee K-P: **Pd (core)–Au (shell) nanoparticles catalyzed conversion of NADH to NAD+ by UV–vis spectroscopy—a kinetic analysis**. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2009, **74**:678-684.

30. Caruso F: **Nanoengineering of particle surfaces**. *Advanced materials* 2001, **13**:11-22.

31. Bognitzki M, Hou H, Ishaque M, Frese T, Hellwig M, Schwarte C, Schaper A, Wendorff JH, Greiner A: **Polymer, metal, and hybrid nano-and mesotubes by coating degradable polymer template fibers (TUFT process)**. *Advanced Materials* 2000, **12**:637-640.

32. Peng X, Schlamp MC, Kadavanich AV, Alivisatos AP: **Epitaxial growth of highly luminescent CdSe/CdS core/shell nanocrystals with photostability and electronic accessibility**. *Journal of the American Chemical Society* 1997, **119**:7019-7029.

33. Ali I, Gill A, Omanovic S: **Direct electrochemical regeneration of the enzymatic cofactor 1, 4-NADH employing nano-patterned glassy carbon/Pt and glassy carbon/Ni electrodes**. *Chemical engineering journal* 2012, **188**:173-180.

34. Barin R, Rashid-Nadimi S, Biria D, Asadollahi MA: **Direct electrochemical regeneration of 1, 4-NADH at the copper foam and bimetallic copper foam**. *Electrochimica Acta* 2017, **247**:1095-1102.

35. Kadowaki J, Jones T, Sengupta A, Gopalan V, Subramaniam V: **Copper oxide-based cathode for direct NADPH regeneration**. *Scientific reports* 2021, **11**:1-12.

*** Paper of special interest – work presenting a photoelectrochemical regeneration of NADPH employing a nanostructured Ni–Cu_2O–Cu heterolayer material, showing the low overpotential (− 0.75 V versus Ag/AgCl) without dimer production.**

36. Nam DH, Park CB: **Visible Light-Driven NADH Regeneration Sensitized by Proflavine for Biocatalysis**. *ChemBioChem* 2012, **13**:1278-1282.

37. Lauterbach L, Lenz O, Vincent KA: **H2-driven cofactor regeneration with NAD (P)+-reducing hydrogenases**. *The FEBS journal* 2013, **280**:3058-3068.

38. Schmid A, Hollmann F, Park JB, Bühler B: **The use of enzymes in the chemical industry in Europe**. *Current Opinion in Biotechnology* 2002, **13**:359-366.

39. Schroer K, Luef KP, Hartner FS, Glieder A, Pscheidt B: **Engineering the Pichia pastoris methanol oxidation pathway for improved NADH regeneration during whole-cell biotransformation**. *Metabolic engineering* 2010, **12**:8-17.

*** Paper of special interest – work presenting a new engineering strategy for improved NADH regeneration based on the *Pichia pastoris* methanol oxidation.**

40. Wandrey C, Liese A, Kihumbu D: **Industrial biocatalysis: past, present, and future**. *Organic Process Research & Development* 2000, **4**:286-290.

41. Lee S, Choe H, Cho DH, Yoon SH, Won K, Kim YH: **Communication—Highly Efficient Electroenzymatic NADH Regeneration by an Electron-Relay Flavoenzyme**. *Journal of The Electrochemical Society* 2016, **163**:G50.

42. Shaked Ze, Whitesides GM: **Enzyme-catalyzed organic synthesis: NADH regeneration by using formate dehydrogenase**. *Journal of the American Chemical Society* 1980, **102**:7104-7105.

43. Gröger H, Borchert S, Kraußer M, Hummel W: **Enzyme-Catalyzed Asymmetric Reduction of Ketones**. *Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology* 2009:1-16.

44. Moore JC, Pollard DJ, Kosjek B, Devine PN: **Advances in the enzymatic reduction of ketones**. *Accounts of chemical research* 2007, **40**:1412-1419.

45. Morthensen ST, Sigurdardóttir SB, Meyer AS, Jørgensen H, Pinelo M: **Separation of xylose and glucose using an integrated membrane system for enzymatic cofactor regeneration and downstream purification**. *Journal of Membrane Science* 2017, **523**:327-335.

46. Ghindilis AL, Atanasov P, Wilkins E: **Enzyme-catalyzed direct electron transfer: Fundamentals and analytical applications**. *Electroanalysis* 1997, **9**:661-674.

47. Page CC, Moser CC, Chen X, Dutton PL: **Natural engineering principles of electron tunnelling in biological oxidation–reduction**. *Nature* 1999, **402**:47-52.

48. Lee YS, Baek S, Lee H, Reginald SS, Kim Y, Kang H, Choi I-G, Chang IS: **Construction of uniform monolayer-and orientation-tunable enzyme electrode by a synthetic glucose dehydrogenase without electron-transfer subunit via optimized site-specific gold-binding peptide capable of direct electron transfer**. *ACS applied materials & interfaces* 2018, **10**:28615-28626.

49. Kim S, Yun S-E, Kang C: **Electrochemical evaluation of the reaction rate between methyl viologen mediator and diaphorase enzyme for the electrocatalytic reduction of NAD+ and digital simulation for its voltammetric responses**. *Journal of Electroanalytical Chemistry* 1999, **465**:153-159.

50. Dinh TH, Lee SC, Hou CY, Won K: **Diaphorase-viologen conjugates as bioelectrocatalysts for NADH regeneration**. *Journal of The Electrochemical Society* 2016, **163**:H440.

*** Paper of special interest – work presenting the concept of mediator conjugated enzymatic NADH regeneration considering the environmental sustainability of component manufacture and the cost of downstream purification.**

51. Ren L, Hardy CG, Tang C: **Synthesis and solution self-assembly of side-chain cobaltocenium-containing block copolymers**. *Journal of the American Chemical Society* 2010, **132**:8874-8875.

52. Ruff A, Szczesny J, Zacarias Sn, Pereira IsA, Plumeré N, Schuhmann W: **Protection and reactivation of the [NiFeSe] hydrogenase from Desulfovibrio vulgaris Hildenborough under oxidative conditions**. *ACS energy letters* 2017, **2**:964-968.

53. Kochius S, Magnusson AO, Hollmann F, Schrader J, Holtmann D: **Immobilized redox mediators for electrochemical NAD (P)+ regeneration**. *Applied microbiology and biotechnology* 2012, **93**:2251-2264.

54. Poizat M, Arends IW, Hollmann F: **On the nature of mutual inactivation between [Cp* Rh (bpy)(H2O)] 2+ and enzymes–analysis and potential remedies**. *Journal of Molecular Catalysis B: Enzymatic* 2010, **63**:149-156.

***Paper of special interest – quantitative introduction to Rh-catalyst mediated mutual inactivation in enzyme systems.**

55. Lutz J, Hollmann F, Ho TV, Schnyder A, Fish RH, Schmid A: **Bioorganometallic chemistry: biocatalytic oxidation reactions with biomimetic NAD+/NADH co-factors and [Cp* Rh (bpy) H]+ for selective organic synthesis**. *Journal of organometallic chemistry* 2004, **689**:4783-4790.

56. Walcarius A, Nasraoui R, Wang Z, Qu F, Urbanova V, Etienne M, Göllü M, Demir AS, Gajdzik J, Hempelmann R: **Factors affecting the electrochemical regeneration of NADH by (2, 2′-bipyridyl)(pentamethylcyclopentadienyl)-rhodium complexes: Impact on their immobilization onto electrode surfaces**. *Bioelectrochemistry* 2011, **82**:46-54.

57. Saba T, Burnett JW, Li J, Wang X, Anderson JA, Kechagiopoulos PN, Wang X: **Assessing the environmental performance of NADH regeneration methods: A cleaner process using recyclable Pt/Fe3O4 and hydrogen**. *Catalysis Today* 2020, **339**:281-288.

**** Paper of outstanding interest – quantification of regeneration process wastefulness. Has potential to shift perspectives of regeneration method quality if wastefulness quantification becomes more widespread.**

58. Beaupre BA, Hoag MR, Roman J, Försterling FH, Moran GR: **Metabolic function for human renalase: oxidation of isomeric forms of β-NAD (P) H that are inhibitory to primary metabolism**. *Biochemistry* 2015, **54**:795-806.

59. Morrison CS, Paskaleva EE, Rios MA, Beusse TR, Blair EM, Lin LQ, Hu JR, Gorby AH, Dodds DR, Armiger WB: **Improved soluble expression and use of recombinant human renalase**. *Plos one* 2020, **15**:e0242109.

**** Paper of outstanding interest – discusses methods for improved expression of renalase, whose employment in direct regeneration systems is critical to improving product selectivity to an industrially practical level.**

60. Zhang L, Vila N, Kohring G-W, Walcarius A, Etienne M: **Covalent immobilization of (2, 2′ -bipyridyl)(pentamethylcyclopentadienyl)-rhodium complex on a porous carbon electrode for efficient electrocatalytic NADH regeneration**. *ACS Catalysis* 2017, **7**:4386-4394.

*** Paper of special interest – may be one of the more straightforward methods of Rh catalyst immobilization which may be employed to minimize mutual inactivation.**

61. Himiyama T, Waki M, Maegawa Y, Inagaki S: **Cooperative Catalysis of an Alcohol Dehydrogenase and Rhodium-Modified Periodic Mesoporous Organosilica**. *Angewandte Chemie International Edition* 2019, **58**:9150-9154.

62. Zhang S, Zhang Y, Chen Y, Yang D, Li S, Wu Y, Sun Y, Cheng Y, Shi J, Jiang Z: **Metal Hydride-Embedded Titania Coating to Coordinate Electron Transfer and Enzyme Protection in Photo-enzymatic Catalysis**. *ACS Catalysis* 2020, **11**:476-483.

*** Paper of special interest – work presenting the photo-enzymatic alcohol production system employing metal hydride-embedded titania coating on graphitic carbon nitride, showing a protection of alcohol dehydrogenase from deactivation by the graphitic carbon nitride core.**

63. Yuan M, Sahin S, Cai R, Abdellaoui S, Hickey DP, Minteer SD, Milton RD: **Creating a Low-Potential Redox Polymer for Efficient Electroenzymatic CO_2 Reduction**. *Angewandte Chemie* 2018, **130**:6692-6696.

64. Lee YS, Yuan M, Cai R, Lim K, Minteer SD: **Nitrogenase Bioelectrocatalysis: ATP-Independent Ammonia Production Using a Redox Polymer/MoFe Protein System**. *ACS Catalysis* 2020, **10**:6854-6861.

65. Lee YS, Ruff A, Cai R, Lim K, Schuhmann W, Minteer SD: **Electroenzymatic nitrogen fixation using a MoFe protein system immobilized in an organic redox polymer**. *Angewandte Chemie International Edition* 2020, **59**:16511-16516.

66. Szczesny J, Ruff A, Oliveira AR, Pita M, Pereira IsA, De Lacey AL, Schuhmann W: **Electroenzymatic CO_2 fixation using redox polymer/enzyme-modified gas diffusion electrodes**. *ACS Energy Letters* 2020, **5**:321-327.

67. Abdellaoui S, Milton RD, Quah T, Minteer SD: **NAD-dependent dehydrogenase bioelectrocatalysis: the ability of a naphthoquinone redox polymer to regenerate NAD**. *Chemical Communications* 2016, **52**:1147-1150.

68. Quah T, Milton RD, Abdellaoui S, Minteer SD: **Bioelectrocatalytic NAD+/NADH inter-conversion: transformation of an enzymatic fuel cell into an enzymatic redox flow battery**. *Chemical Communications* 2017, **53**:8411-8414.

*** Paper of special interest – work presenting the diaphorase/benzylpropylviologen redox polymer based NAD^+/NADH regeneration system.**

69. Yuan M, Kummer MJ, Milton RD, Quah T, Minteer SD: **Efficient NADH regeneration by a redox polymer-immobilized enzymatic system**. *ACS Catalysis* 2019, **9**:5486-5495.

** Paper of outstanding interest – work presenting the cobaltocene modified poly(allylamine) polymer immobilized diaphorase system showing superior operational stability