

METHODS FOR REGENERATION OF NAD(P)H COFACTORS

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Oxidoreductase enzymes require cofactors for their operation in stoichiometric amount. Approximately half of them need NAD(P)H cofactors, which should be regenerated for the cost-efficient implementation of the enzymatic processes. In this minireview regeneration methods are presented including chemical, electrochemical, biological (enzymatic), photocatalytic techniques.

Keywords: oxidoreductase enzymes, (electro)chemical regeneration

1. Introduction

Oxidoreductase enzymes belong to the group of EC 1. They catalyze the transfer of electrons from one molecule (reductant or electron donor) to another (oxidant or electron acceptor) [1]–[5]. NAD(P)-dependent oxidoreductases perform key functions in several fundamental biological processes, such as cellular respiration, photosynthesis, and bioenergetics. Their high chemical specificity and selectivity have enabled the widespread application in industrial biocatalysis, pharmaceutical manufacturing, biosensor development, and environmental technologies [6],[7]. The oxidoreductase enzymes include, for instance dehydrogenases, oxidases, peroxidases, and reductases.

This group of enzymes usually requires cofactors for the operation in stoichiometric amount. Approximately half of these enzymes utilize nicotinamide-type cofactors [6], such as NADH or NADPH. These cofactors are composed of two separate structural units (*Figures 1 and 2*): the nicotinamide moiety is responsible for the redox activity, (i.e. electron donor or acceptor functions) while the adenosine-dinucleotide unit (adenosine diphosphate ribose moiety) serves to distinguish the anabolic and catabolic metabolisms.

From another aspect NADH is called a dinucleotide because it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine nucleobase and the other contains nicotinamide.

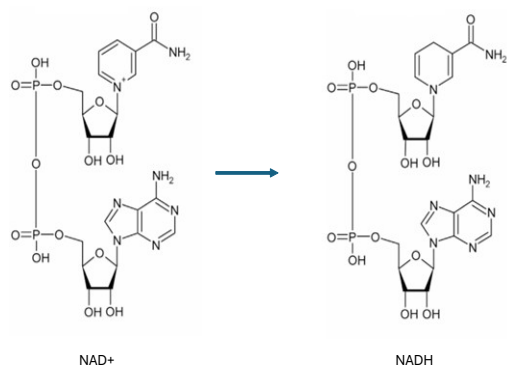
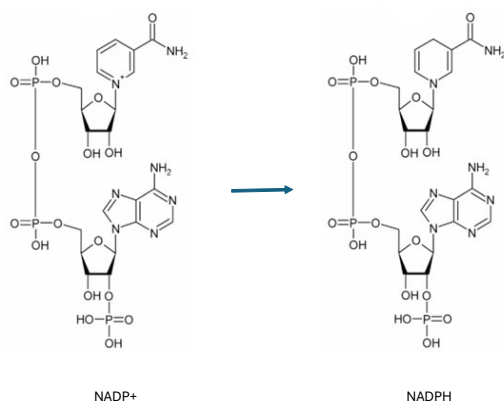
During the enzymatic reactions these cofactors continuously oxidized or reduced, therefore their effective regeneration is essential [5],[6]. Since the production of cofactors is extremely expensive, it is economically unfeasible to provide (i.e. add) cofactors in stoichiometric amounts in scaled-up industrial systems. Thus, intensive research work has been conducted to develop different effective techniques for in-situ cofactor regeneration.

2. Characterization of the regeneration process

In an enzymatic reduction cycle NAD(P)H is a reductant, that is oxidized to NAD(P)⁺. Thus, cofactor regeneration means [8]–[10] that a proton and two electrons should be transferred to NAD(P)⁺. Hence the oxidized form, NAD(P)⁺ can be transformed into the reduced form (*Figures 1 and 2*). The proton and the two electrons are usually provided by a so-called sacrificial substrate.

The efficiency and industrial relevance of cofactor regeneration systems are commonly assessed using five key criteria [8]–[11]:

- selectivity,
- activity,
- effectiveness,
- sustainability,
- further practical aspects.

Figure 1: Conversion of NAD⁺ to NADHFigure 2: Conversion of NADP⁺ to NADPH

Regarding selectivity it should be noted that other compounds (byproducts) may be formed during regeneration that are inactive: e.g. NAD₂ dimer [10]. This phenomenon might cause cofactor loss and should therefore be avoided.

The activity of cofactor regeneration is generally calculated using the *turnover frequency*: the number of moles of cofactor formed per mole of active site per unit of time.

The efficiency of the process can be evaluated by the *turnover number*. It defines the number of moles of product formed per mole of cofactor per unit of time. For characterization of the complete reaction course, the *total turnover number* (TTN) can be applied, which shows the number of moles of product formed per mole of cofactor. In general, TTNs greater than 1,000 are considered necessary for the process economically viable.

3. Regeneration strategies

Cofactor regeneration techniques can be divided into four main groups:

- biological methods,
- chemical methods,
- electrochemical methods,
- photocatalytic methods.

3.1. Biological methods

Cofactor regeneration by biological methods includes microbial and enzymatic techniques. For example, Stevenson et al. [12] applied yeast cells for regeneration. Recently *microbial cell factories* [13] have been studied where intracellular cofactor regeneration can be realized.

Enzymatic techniques are more widely used because of their advantages.

The first report on enzymatic regeneration of cofactors was presented almost 70 years ago (!). In 1957 Rafter and Colowick, researchers from the McCollum-Pratt Institute (Johns Hopkins University, Baltimore, Maryland, USA) investigated ethanol as a substrate and alcohol dehydrogenase (ADH) as the enzyme to regenerate cofactors [14].

Since 1957 tremendous number of papers have been published in this area, because enzymatic regeneration is a favorable approach [11],[15] offering very good selectivity and compatibility, operation under mild conditions, with low energy consumption. The techniques applied can be classified into two main groups:

- (i) coupled enzyme system, which utilize a second (i.e. regeneration) enzyme, and an associated sacrificial hydride donor (e.g., glucose),
- (ii) coupled substrate systems, where one enzyme serves both substrate reduction and cofactor regeneration.

The most widely used enzymes for cofactor regeneration include glucose dehydrogenases (GDHs), formate dehydrogenases (FDHs), alcohol dehydrogenases (ADHs), and phosphite dehydrogenases (PTDHs).

The manufacture of trimethyl L-leucine (Degussa) is presented in Figure 3 as an example of an industrial biocatalytic processes [6],[8], in which leucine dehydrogenase catalyzes the reductive amination of trimethyl pyruvic acid using ammonium ions. During the

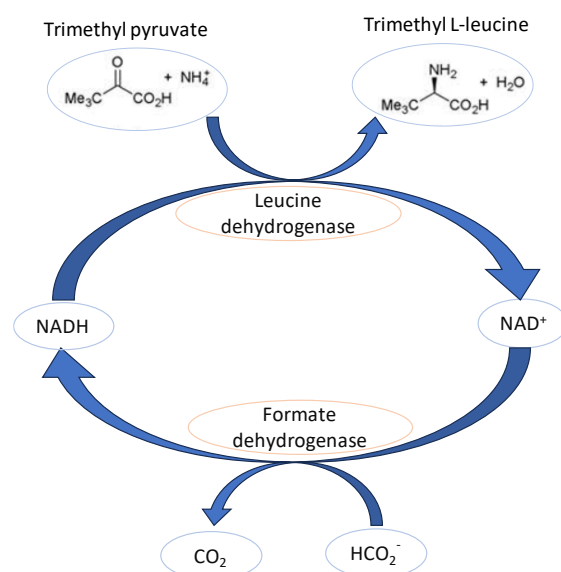


Figure 3: Example for industrial application

reaction, the NADH cofactor is oxidized to NAD^+ and must be continuously regenerated to maintain process efficiency. In this process, cofactor recycling is achieved by a second enzyme, formate dehydrogenase.

This example illustrates the general principle of enzyme-driven cofactor regeneration, where an auxiliary regeneration enzyme operates in conjunction with a sacrificial electron donor to continuously recycle the oxidized cofactor. FDH is among the most widely applied regeneration enzymes owing to high selectivity, operational robustness, and favorable reaction thermodynamics [8],[10]. In the FDH-catalyzed reaction, formate serves as the electron donor and is oxidized to carbon dioxide (CO_2), while NAD^+ is simultaneously reduced to NADH. The irreversible release of CO_2 from the reaction medium shifts the equilibrium toward product formation and minimizes accumulation making this approach attractive for industrial biocatalytic processes [15].

A representative example of substrate-coupled cofactor regeneration was reported by Lajtai-Szabo et al. [16], where horse-liver alcohol dehydrogenase enzyme was used (Figure 4) for the biotransformation of racemic 2-phenylpropionaldehyde to (S)-2-phenyl-1-propanol, as well as for regeneration of the cofactor NAD^+ using ethanol as a sacrificial substrate.

3.2. Chemical methods

Chemical cofactor regeneration uses chemical compounds that enable electron transfer. The selected chemical agent initially binds reversibly to e.g. the oxidized form of the cofactor, transporting electrons from it to an oxidative reactant, thereby regenerating the cofactor to its reduced form [8]-[11]. In the first studies, simple reducing compounds, such as $\text{Na}_2\text{S}_2\text{O}_4$ were used, however the TTN values were quite low, and these compounds had other drawbacks [9],[10]. Therefore, other methods, namely catalytic approaches, were introduced. Both homogeneous and heterogeneous catalytic regeneration methods have been investigated [10],[11].

3.3. Electrochemical methods

Electrochemical cofactor regeneration utilizes electrical energy to supply electrons for the reduction of NAD(P) to NAD(P)H . Depending on the system configuration, regeneration can proceed either directly at the electrode surface or indirectly through redox mediators and coupled enzymatic reactions [11,17,18].

Compared with chemical regeneration methods, electrochemical systems offer several advantages, including the use of inexpensive electricity, precise control of the applied potential, and straightforward, real-time process monitoring. The reduction of NAD(P) generally proceeds via a sequential single-electron transfer mechanism involving a radical intermediate. Following the first electron transfer, a $\text{NAD(P)}^\cdot$ radical is formed. This highly reactive intermediate can either undergo a second electron transfer and protonation to

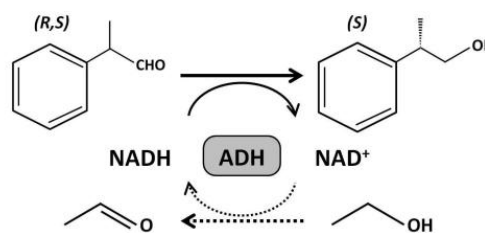


Figure 4: Coupled substrate system by using alcohol dehydrogenase enzyme

generate the enzymatically active 1,4- NAD(P)H or participate in side reactions leading to enzymatically inactive cofactor dimers (NAD_2 / NADP_2) and non-functional NAD(P)H isomers [17],[19].

Since dimerization is often kinetically favored, direct regeneration on unmodified electrodes typically exhibits poor selectivity. Consequently, considerable efforts have been devoted to developing modified electrode materials, electrocatalysts, and mediator-assisted systems to suppress dimer formation and improve selectivity toward the active 1,4- NAD(P)H form [17,18,19].

3.4. Photocatalytic methods

Photocatalytic regeneration utilizes light energy to drive the reduction of NAD(P)^+ and represents an attractive approach for sustainable cofactor recycling. In these systems, semiconductor photocatalysts absorb photons to generate electron-hole (e^-/h^+) pairs. The photoexcited electrons are transferred either directly or through electron-transfer mediators to NAD(P)^+ , leading to NAD(P)H formation [11]. Concurrently, the co-generated valence band holes (h^+), representing electron vacancies left behind after photoexcitation, must be rapidly quenched by a sacrificial electron donor (hole scavenger) to prevent cofactor overoxidation and photocatalyst deactivation.

Common photocatalysts include TiO_2 , CdS , and more recently ZnIn_2S_4 -based materials. Recent studies have demonstrated that polarized ZnIn_2S_4 photocatalysts can efficiently utilize selective alcohol oxidation to quench photogenerated holes while simultaneously driving NADH regeneration [20]. Furthermore, biomimetic leaf-inspired microreactors have been developed to enhance light utilization and electron-transfer efficiency across these multiphase systems [21]. Although photocatalytic systems offer the possibility of utilizing solar energy as the primary driving force for cofactor regeneration, challenges related to photocatalyst stability, low quantum efficiency caused by electron-hole recombination, and the selective formation of active 1,4- NAD(P)H continue to limit their industrial application.

Although considerable progress has been achieved in recent years, photocatalytic cofactor regeneration has not yet reached industrial implementation. The development of more stable photocatalysts, improved charge-separation strategies, and highly selective regeneration systems remains necessary to establish

photocatalysis as a competitive alternative to enzymatic cofactor recycling.

4. Economic aspects and industrial feasibility of cofactor regeneration

The economics of NADH-driven biocatalytic processes rely heavily on effective cofactor regeneration. The reduction of acetophenone to (R)-1-phenylethanol was selected to demonstrate this point. Production of 1 kg of product (MW = 122.17 g mol⁻¹) corresponds to 8.19 mol of product and therefore requires 8.19 mol of NADH under stoichiometric conditions. Considering the conservative price of NADH to be 25 €/g and its molecular weight to be 663.4 g/mol, the economic cost of cofactor usage through stoichiometry would be approximately 136,000 €/kg product, which illustrates that the process is economically not viable without cofactor recycling [22],[23].

To assess the economic impact of cofactor regeneration, an effective turnover number (TTN_{eff}) was defined as the amount of product formed per total amount of cofactor supplied to the process, including cofactor replenishment due to irreversible losses:

$$TTN_{eff} = \frac{n_{product}}{n_{cofactor,initial} + n_{cofactor,replenished}} \quad (1).$$

TTN values in the range of 10^4 – 10^5 are generally considered desirable for industrial implementation [23],[24]. Assuming $TTN_{eff} = 10,000$, the net NADH cost drops drastically to 13.6 €/kg⁻¹ product.

For comparison, the theoretical electrical energy required for the *electrochemical regeneration* of 8.19 mol NADH was estimated using Faraday's law and the two-electron reduction of NAD⁺ to NADH. Assuming a cell voltage of 1.2 V, the required electrical energy is approximately 0.53 kWh. Using a representative European industrial electricity price of 0.15 €/kWh⁻¹ based on Eurostat data for non-household consumers [24], the corresponding electricity cost is approximately 0.09 €/kg⁻¹ product [25]. This value is more than two orders of magnitude lower than the estimated cofactor cost at $TTN_{eff} = 10,000$ (13.6 €/kg⁻¹ product), indicating that electricity consumption itself is not the primary economic limitation of electrochemical cofactor regeneration. However, direct electrochemical reduction is often associated with low effective TTN values due to limited selectivity, particularly the formation of inactive dimers (NAD₂) and non-functional NADH isomers, which increase irreversible cofactor losses and necessitate cofactor replenishment [26],[27].

Using the previously introduced FDH-based *enzymatic* regeneration system as an example, formate is oxidized to CO₂ while NAD⁺ is reduced to NADH. Regeneration of 8.19 mol NADH therefore requires 8.19 mol formate, corresponding to approximately 0.56 kg sodium formate. Assuming a bulk sodium formate price of 1–2 €/kg⁻¹, the electron donor cost remains below 1 €/kg⁻¹ product. Another benefit of using FDH for regeneration is that its by-product, CO₂, as

mentioned earlier. In contrast, alternative regeneration systems, such as glucose dehydrogenase, generate soluble by-products that may complicate downstream processing [28],[29].

Nevertheless, for industrial-scale production, there are additional parameters that affect the economics of the process, such as volumetric productivity, product concentration, enzyme stability and downstream processing, which may constitute an appreciable portion of the total production cost [23],[25]. With a value of $TTN_{eff} = 10,000$, the cost of cofactors and sodium formate is approximately 14–15 €/kg⁻¹ of product. Consequently, the economic attractiveness of FDH-based regeneration arises not from exceptionally low substrate costs, but from its high selectivity, efficient cofactor utilization, and compatibility with industrial-scale biocatalytic processes.

Interestingly, the simplified economic assessment presented here suggests that, assuming identical TTN_{eff} values, the direct costs associated with cofactor regeneration are comparable for electrochemical and FDH-based enzymatic systems (approximately 14 €/kg⁻¹ product). Consequently, the economic competitiveness of the two technologies is determined primarily by the achievable effective turnover number, regeneration selectivity, and long-term cofactor stability rather than by the cost of electricity or sacrificial substrates.

Currently, *enzymatic* regeneration represents the most widely applied industrial approach owing to its excellent selectivity, high cofactor utilization efficiency, and proven scalability [22,29,30]. Consequently, enzymatic systems can achieve high TTN_{eff} values while minimizing cofactor degradation. Nevertheless, electrochemical regeneration remains an attractive alternative because it fundamentally differs from conventional enzymatic approaches. Instead of relying on sacrificial substrates and auxiliary regeneration enzymes, reducing equivalents can be supplied directly as electrons from an external electrical source, potentially generated from renewable energy. This feature may simplify process design, reduce reagent consumption, and improve the overall sustainability of future biocatalytic manufacturing processes.

Therefore, the primary objective of future research is not merely to reduce electricity consumption, which already contributes negligibly to the total process cost, but rather to improve regeneration selectivity and cofactor stability. Achieving effective TTN values comparable to those obtained in enzymatic systems would enable electrochemical regeneration to combine the operational simplicity of electrically driven processes with the economic performance currently associated with enzymatic cofactor recycling. Under such conditions, electrochemical regeneration could become a competitive and sustainable alternative for industrial biocatalytic applications.

5. Future directions

Cofactor-dependent enzymatic reactions (redox processes) are generally more challenging to implement at an industrial scale than cofactor-independent biotransformations because they require continuous cofactor recycling in addition to the target catalytic reaction. Nevertheless, several successful large-scale processes have been developed, particularly for producing high-value chiral compounds, pharmaceuticals, and fine chemicals, where the exceptional selectivity of oxidoreductases justifies the additional process complexity. To date, enzymatic regeneration remains the only cofactor recycling strategy that has achieved widespread industrial implementation, primarily due to its high selectivity, excellent cofactor utilization efficiency, and successful implementation at industrial scale [10]. In contrast, electrochemical and photocatalytic regeneration technologies remain largely at the laboratory or pilot scale, mainly because of challenges associated with regeneration selectivity, cofactor degradation, and the efficient formation of biologically active 1,4-NAD(P)H.

Current research efforts focus on overcoming these limitations through the development of novel regeneration enzymes, improved enzyme engineering strategies, enhanced thermostability, and increased catalytic efficiency [31]. In parallel, emerging concepts such as self-sufficient artificial metabolic cells, enzyme immobilization technologies, artificial cofactors, and integrated multi-enzyme systems are being explored to further improve regeneration efficiency and process sustainability [32]. More recently, cofactor engineering has emerged as a key research area aimed at optimizing intracellular cofactor availability and regeneration. By addressing the mismatch between cofactor supply and metabolic demand, cofactor engineering is increasingly recognized as a central enabling technology for next-generation green biomanufacturing and sustainable biocatalytic production processes [30]. In this context, cofactor engineering is considered a key strategy for “revolutionizing green biomanufacturing by overcoming the fundamental bottleneck between limited supply/regeneration of cellular energy cofactors and the high demand of efficient bioproduction” [22].

Ultimately, the future success of cofactor-dependent biocatalysis will depend not only on catalyst performance but on the development of cofactor regeneration technologies capable of combining high regeneration selectivity, long-term cofactor stability, and economically viable TTN_{eff} values.

REFERENCES

- [1] Price, N. C., & Stevens, L. (1999). *Fundamentals of enzymology: The cell and molecular biology of catalytic proteins* (3rd ed.). Oxford University Press.
- [2] Awasthi, S. (2022). *Introduction to enzymology*. Arcler Press
- [3] Singh, R. S., Singh, T., & Pandey, A. (2019). Microbial enzymes—An overview. In R. S. Singh, R. R. Singhania, A. Pandey, & C. Larroche (Eds.), *Biomass, biofuels, biochemicals: Advances in enzyme technology* (pp. 1–40). Elsevier. <https://doi.org/10.1016/B978-0-444-64114-4.00001-7>
- [4] Kermasha, S., & Eskin, M. N. A. (Eds.). (2021). *Enzymes: Novel biotechnological approaches for the food industry*. Academic Press. <https://doi.org/10.1016/C2013-0-13507-6>
- [5] Pandey, A., Webb, C., Soccol, C. R., & Larroche, C. (Eds.). (2006). *Enzyme technology*. Springer.
- [6] Vidal, L. S., Kelly, C. L., Mordaka, P. M., & Heap, J. T. (2018). Review of NAD(P)H-dependent oxidoreductases: Properties, engineering and application. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1866(2), 327–347. <https://doi.org/10.1016/j.bbapap.2017.11.005>
- [7] Yuan, B., Yang, D., Qu, G., Turner, N. J., & Sun, Z. (2024). Biocatalytic reductive aminations with NAD(P)H-dependent enzymes: enzyme discovery, engineering and synthetic applications. *Chemical Society Reviews*, 53(1), 227–262. <https://doi.org/10.1039/D3CS00391D>
- [8] Uppada, V., Bhaduri, S., & Noronha, S. B. (2014). Cofactor regeneration – an important aspect of biocatalysis. *Current Science*, 106(7), 946–957. <https://www.currentscience.ac.in/Volumes/106/07/0946.pdf>
- [9] Woodyer, R. D., Johannes, T. W., & Zhao, H. (2006). Regeneration of cofactors for enzyme biocatalysis. In: Pandey, A., Webb, C., Soccol, C. R., & Larroche, C. (Eds.), *Enzyme Technology* (pp. 85–103). Springer. https://doi.org/10.1007/978-0-387-35141-4_5
- [10] Weckbecker, A., Gröger, H., & Hummel, W. (2010). Regeneration of nicotinamide coenzymes: Principles and applications for the synthesis of chiral compounds. In C. Wittmann, & R. Krull (Eds.), *Biosystems engineering I: Creating superior biocatalysts* (pp. 195–242). Springer. https://doi.org/10.1007/10_2009_55
- [11] Wang, X., Saba, T., Yiu, H. H. P., Howe, R. H., Anderson, J. A., & Shi, J. (2017). Cofactor NAD(P)H regeneration inspired by heterogeneous pathways. *Chem*, 2(5), 621–654. <https://doi.org/10.1016/j.chempr.2017.04.009>
- [12] Stevenson, E., Ibbotson, P. G., & Spedding, P. L. (1993). Regeneration of NADH in a bioreactor using yeast cells immobilized in alginate fiber: I. Method and effect of reactor variables. *Biotechnology and Bioengineering*, 42(1), 43–49. <https://doi.org/10.1002/bit.260420107>
- [13] Fang, Z., Jayaraman, N., Lv, Y., & Luo, Z. (2023). Editorial: Cofactor regeneration technologies for microbial cell factory. *Frontiers in Microbiology*, 14, Article 1196002. <https://doi.org/10.3389/fmicb.2023.1196002>

- [14] Rafter, G. W., & Colowick, S. P. (1957). Enzymatic preparation of DPNH and TPNH. In S. P. Colowick, & N. O. Kaplan (Eds.), *Methods in enzymology* (Vol. 3, pp. 887–890). Academic Press. [https://doi.org/10.1016/S0076-6879\(57\)03471-0](https://doi.org/10.1016/S0076-6879(57)03471-0)
- [15] Bachosz, K., Zdarta, J., Bilal, M., Meyer, A. S., & Jesionowski, T. (2023). Enzymatic cofactor regeneration systems: A new perspective on efficiency assessment. *Science of the Total Environment*, 868, Article 161630. <https://doi.org/10.1016/j.scitotenv.2023.161630>
- [16] Lajtai-Szabó, P., Bagó, T.B., & Nemestóthy, N. (2022). Production of chiral (S)-2-phenyl-1-propanol by enantioselective biocatalysts. *Hungarian Journal of Industry and Chemistry*, 50(1), 23–28. <https://doi.org/10.33927/hjic-2022-05>
- [17] Li, Y., Liu, G., Kong, W., Zhang, S., Bao, Y., Zhao, H., Wang, L., Zhou, L., & Jiang, Y. (2024). Electrocatalytic NAD(P)H regeneration for biosynthesis. *Green Chemical Engineering*, 5(1), 1–15. <https://doi.org/10.1016/j.gce.2023.02.001>
- [18] Chen, H., Simoska, O., Lim, K., Grattieri, M., Yuan, M., Dong, F., Lee, Y. S., Beaver, K., Weliwatte, S., Gaffney, E. M., & Minter, S. D. (2020). Fundamentals, applications, and future directions of bioelectrocatalysis. *Chemical Reviews*, 120(23), 12903–12993. <https://doi.org/10.1021/acs.chemrev.0c00472>
- [19] Aamer, E., Thöming, J., Baune, M., Reimer, N., Dringen, R., Romero, M., & Bösing, I. (2022). Influence of electrode potential, pH and NAD⁺ concentration on electrochemical NADH regeneration. *Scientific Reports*, 12, Article 16380. <https://doi.org/10.1038/s41598-022-20508-w>
- [20] Xing, F., Xue, X., Li, J., Liu, J., Wang, W., Dong, W., Yuan, H., & Liu, J. Sustainable photocatalytic biological cofactor regeneration fueled by selective alcohol oxidation over polarized ZnIn₂S₄. *ACS Catalysis*, 14(15), 11366–11377. <https://doi.org/10.1021/acscatal.4c01703>
- [21] Wang, L., Bao, H., Lin, H., Yang, C., Song, J., & Huang, X. (2022). An easy fabricated biomimetic leaf microreactor for photocatalytic nicotinamide adenine dinucleotide (NADH) regeneration. *Applied Catalysis A: General*, 641, Article 118685. <https://doi.org/10.1016/j.apcata.2022.118685>
- [22] Chenault, H. K., & Whitesides, G. M. (1987). Regeneration of nicotinamide cofactors for use in organic synthesis. *Applied Biochemistry and Biotechnology*, 14(2), 147–197. <https://doi.org/10.1007/BF02798431>
- [23] Sharma, V. K., Hutchison, J. M., & Allgeier, A. M. (2022). Redox biocatalysis: quantitative comparisons of nicotinamide cofactor regeneration methods. *ChemSusChem*, 15(22), Article e202200888. <https://doi.org/10.1002/cssc.202200888>
- [24] Eurostat (n.d.). *Electricity price statistics for non-household consumers*, European Union. Retrieved June 10, 2026 from https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_price_statistics
- [25] Lee, Y. S., Gerulskis, R., Minter, S. D. (2022). Advances in electrochemical cofactor regeneration: enzymatic and non-enzymatic approaches. *Current Opinion in Biotechnology*, 73, 14–21. <https://doi.org/10.1016/j.copbio.2021.06.013>
- [26] Wolfe, K. D., Alahuhta, M., Himmel, M. E., Bomble, Y. J., Jennings, G. K., & Cliffel, D. E. (2024). Long-term stability of nicotinamide cofactors in common aqueous buffers: implications for cell-free biocatalysis. *Molecules*, 29(22), Article 5453. <https://doi.org/10.3390/molecules29225453>
- [27] Al-Shaibani, M. A. S., Sakoleva, T., Živković, L. A., Austin, H. P., Dörr, M., Hilfert, L., Haak, E., Bornscheuer, U. T., & Vidaković-Koch, T. (2024). Product distribution of steady-state and pulsed electrochemical regeneration of 1,4-NADH and integration with enzymatic reaction. *ChemistryOpen*, 13(8), Article e202400064. <https://doi.org/10.1002/open.202400064>
- [28] Tishkov, V. I., & Popov, V. O. (2026). Protein engineering of formate dehydrogenase. *Biomolecular Engineering*, 23(2–3), 89–110. <https://doi.org/10.1016/j.bioeng.2006.02.003>
- [29] Maier, A., Mguni, L. M., Ngo, A. C. R., & Tischler, D. (2024). Formate dehydrogenase: recent developments for NADH and NADPH recycling in biocatalysis. *ChemCatChem*, 16(21), Article e202401021. <https://doi.org/10.1002/cctc.202401021>
- [30] Lu, Z., Zhang, Z., Chen, P., Xu, Q., & Xue, F. (2026). Cofactor engineering powers next-generation biomanufacturing. *Trends in Biotechnology*. <https://doi.org/10.1016/j.tibtech.2026.03.010>
- [31] Jia, Q., Zheng, Y.-C., Li, H.-P., Qian, X.-L., Zhang, Z.-J., & Xu, J.-H. (2022). Engineering isopropanol dehydrogenase for efficient regeneration of nicotinamide cofactors. *Applied and Environmental Microbiology*, 88(9), Article e00341-22. <https://doi.org/10.1128/aem.00341-22>
- [32] Lopez-Gallego, F., Jackson, E., & Betancor, L. (2017). Heterogeneous systems biocatalysis: The path to the fabrication of self-sufficient artificial metabolic cells. *Chemistry: A European Journal*, 23(71), 17841–17849. <https://doi.org/10.1002/chem.201703593>