SUPPLEMENTARY INFORMATION

Comparative Life Cycle Assessment of NAD(P)H Regeneration Technologies

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Supplementary information consists of 7 Figures and 6 Tables.

**Biocatalytic NADH Regeneration (Bio)**

This system involved the use of a carbon nanotube-lined quartz column to immobilise hydrogenase, NAD$^+$ reductase and L-alanine dehydrogenase. A H$_2$-saturated solution of NAD$^+$, pyruvate and ammonium chloride was flowed over the immobilised enzymes, resulting in the production of alanine with concurrent NADH regeneration. Hydrogenase was used to convert H$_2$ to protons and electrons, whereby the protons and electrons formed are then used by NAD$^+$ reductase to reduce NAD$^+$ to NADH. L-alanine dehydrogenase was used as the production enzyme to convert pyruvate to L-alanine using NADH. When data for some materials (e.g., ferrocene) was not available, the data for the constituent components (i.e., iron and cyclopentadiene) were included instead.

![Carbon Nanotube Schematic](image)

Figure S1: Schematic representation of Bio.
Table S1: Material inputs for Bio.

| Material                        | Mass (kg) |
|--------------------------------|-----------|
| Ammonium chloride              | 6.00E-07  |
| Bis Tris buffer                | 1.06E-05  |
| Cyclopentadiene                | 2.59E-04  |
| Hydrogenase                    | 1.20E-07  |
| Hydrogen                       | 3.24E-06  |
| Iron                           | 1.12E-04  |
| Lactic acid                    | 8.40E-08  |
| L-Alanine dehydrogenase        | 3.00E-07  |
| NAD$^+$                        | 4.98E-08  |
| NAD$^+$ reductase               | 4.80E-08  |
| Sodium formate                 | 9.98E-08  |
| Toluene                        | 7.41E-03  |
| Water                          | 1.50E-03  |

**Homogeneous Catalytic NADH Regeneration (Homo)**

An organometallic Rh complex was used to regenerate NADH from NAD$^+$, utilising sodium formate as the hydrogen source. Horse liver alcohol dehydrogenase (HLADH) was then used to convert 4-phenylbutan-2-one to the corresponding alcohol. Again, when the data of the niche ligands was unavailable, the data for the constituent components were used instead.

![Schematic representation of Homo](image)

Figure S2: Schematic representation of Homo.
| Material                                           | Mass (kg)    |
|---------------------------------------------------|--------------|
| Benzylacetone                                     | 2.06E-06     |
| Bromine                                           | 3.60E-08     |
| Butene                                            | 1.26E-08     |
| Cyclopentadiene                                   | 6.75E-08     |
| Dichloromethane                                   | 6.78E-05     |
| Ethyl acetate                                     | 1.99E-08     |
| Horse liver alcohol dehydrogenase                 | 2.60E-06     |
| NAD⁺                                              | 6.64E-07     |
| Phenanthroline                                    | 9.20E-08     |
| Phosphoric acid                                   | 9.80E-06     |
| Potassium carbonate                               | 1.38E-05     |
| Rhodium                                           | 5.15E-08     |
| Sodium formate                                     | 8.40E-06     |
| Water                                             | 1.00E-03     |

**Electrocatalytic NADH Regeneration (Electro)**

A cholesterol-modified gold amalgam electrode was used as the regeneration system, and this was coupled to the enzymatic reduction of pyruvate to D-lactate via D-lactate dehydrogenase (LDH).

![Figure S3: Schematic representation of Electro.](image-url)
**Table S3: Material inputs for Electro.**

| Material               | Mass (kg) |
|------------------------|-----------|
| Cholesteryl oleate     | 4.52E-06  |
| D-Lactate dehydrogenase| 3.00E-06  |
| Gold                   | 6.95E-03  |
| Hexane                 | 1.50E-05  |
| Lactic acid            | 1.14E-04  |
| Mercury                | 1.36E-02  |
| NAD$^+$                | 4.14E-07  |
| Phosphoric acid        | 2.45E-03  |
| Potassium carbonate    | 3.46E-03  |
| Sodium formate         | 9.97E-05  |
| Water                  | 1.00E-01  |

**Photocatalytic NADH Regeneration (Photo)**

A two-dimensional isotype heterojunction photocatalyst, named quantum dots@flake graphitic carbon nitride, was used to regenerate NADH under visible light irradiation. Using triethanolamine as a sacrificial electron donor, the regeneration system was coupled to the enzymatic reduction of formaldehyde to methanol via yeast alcohol dehydrogenase (YADH).

![Figure S4: Schematic representation of Photo.](image)

**Table S4: Material inputs for Photo.**

| Material                      | Mass (kg) |
|-------------------------------|-----------|
| NAD$^+$                       | 2.66E-04  |
| Methanal                      | 7.20E-06  |
| Melamine                      | 1.32E-05  |
| Phosphoric acid               | 1.96E-04  |
| Potassium carbonate           | 2.76E-04  |
| Triethanolamine               | 2.26E-03  |
| Urea                          | 3.30E-05  |
| Water                         | 2.00E-02  |
| Yeast alcohol dehydrogenase   | 2.00E-06  |
Heterogeneous Catalytic NADH Regeneration (Hetero)

A platinum supported on aluminium oxide catalyst was used in the presence of hydrogen to reduce NAD$^+$ to NADH. The regeneration system was then coupled to the enzymatic reduction of propanal to propanol via alcohol dehydrogenase.

![Schematic representation of Hetero.](image)

Figure S5: Schematic representation of Hetero.

| Material                  | Mass (kg)   |
|---------------------------|-------------|
| Alcohol dehydrogenase     | 1.00E-06    |
| Aluminium oxide           | 2.48E-05    |
| Hydrochloric acid         | 2.70E-07    |
| Hydrogen                  | 5.85E-03    |
| NAD$^+$                   | 2.50E-04    |
| Nitric acid               | 3.20E-07    |
| Nitrogen                  | 1.13E-03    |
| Phosphoric acid           | 4.12E-04    |
| Platinum                  | 2.50E-07    |
| Potassium carbonate       | 2.90E-04    |
| Propanal                  | 2.90E-05    |
| Water                     | 7.50E-02    |
Figure S6: Characterisation results of the impacts to the ReCiPe endpoint categories for the entire reaction for (a) Bio (b) Homo (c) Electro (d) Photo and (e) Hetero.

Figure S7: Characterisation results of the impacts to the ReCiPe endpoint categories for the synthesis of the catalysts in (a) Bio (b) Homo (c) Electro (d) Photo and (e) Hetero.
Table S6: Life cycle inventory data of chemicals, grouped into metals, enzymes, chemicals required for catalyst synthesis and chemicals required in the reaction, respectively.

| Chemical                  | ALOP (m2a) | GWP100 (kg CO$_2$-eq) | FDP (kg oil-eq) | FETPinf (kg 1,4-DCB-eq) | HETPinf (kg-P-eq) | IRP_HE (kg-U-eq) | METPinf (kg 1,4-DCB-eq) | MDP (kg-Fe-eq) | TAP100 (kg-SO$_2$-eq) | ULOP (m2a) |
|---------------------------|------------|------------------------|-----------------|--------------------------|-------------------|-------------------|-------------------------|---------------|------------------------|-------------|
| Platinum                  | 515.81     | 26835                  | 8666.8          | 3289.6                   | 130360            | 1431.8            | 3013.5                  | 134350        | 2138                   | 559.17      |
| Gold                      | 543.79     | 15486                  | 4851            | 16827                    | 851000            | 1678              | 15493                   | 75839         | 161.4                  | 2860        |
| Rhodium                   | 3988.3     | 83934                  | 29005           | 24858                    | 312800            | 4984              | 21513                   | 141130        | 3263                   | 900880      |
| Iron                      | 0.0972     | 0.8332                 | 0.2315          | 0.10873                  | 0.70707           | 0.09458           | 0.09664                  | 0.26424       | 0.004647               | 0.35253     |
| Mercury                   | 1.2795     | 12.268                 | 2.0612          | 0.5067                   | 66839             | 0.1639            | 76.54                    | 0.06488       | 0.07686                | 0.10054     |
| ADH                       | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| D-Lactate dehydrogenase   | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| HLADH                     | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| Hydrogenase               | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| L-Alanine dehydrogenase   | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| NAD reductase             | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| YADH                      | 5.6793     | 7.0334                 | 1.9397          | 0.12071                  | 3.8434            | 1.6366            | 0.11079                  | 0.47822       | 0.053722               | 0.086694    |
| Aluminium oxide           | 0.021902   | 1.6379                 | 0.40847         | 0.25392                  | 1.1125            | 0.04864           | 0.22462                  | 0.066963      | 0.01229                | 0.017829    |
| Bromine                   | 0.09697    | 5.217                  | 1.7034          | 0.0331                   | 1.21              | 0.2661            | 0.03149                  | 0.10796       | 0.01674                | 0.02289     |
| Butene                    | 0.00015    | 1.534                  | 1.532           | 0.00091                  | 0.0134            | 0.000266         | 0.000718                 | 0.000761      | 0.00355                | 0.00017     |
| Cholesteryl oleate        | 0.029055   | 1.8225                 | 1.428           | 0.013956                 | 0.46307           | 0.078935         | 0.012196                 | 0.057448      | 0.0066758              | 0.010604    |
| Cyclopentadiene           | 0.029055   | 1.8225                 | 1.428           | 0.013956                 | 0.46307           | 0.078935         | 0.012196                 | 0.057448      | 0.0066758              | 0.010604    |
| Benzylicacetone           | 0.029055   | 1.8225                 | 1.428           | 0.013956                 | 0.46307           | 0.078935         | 0.012196                 | 0.057448      | 0.0066758              | 0.010604    |
| Dichloromethane           | 0.029055   | 1.8225                 | 1.428           | 0.013956                 | 0.46307           | 0.078935         | 0.012196                 | 0.057448      | 0.0066758              | 0.010604    |
| Ethyl acetate             | 0.1326     | 2.586                  | 1.643           | 0.0281                   | 1.0289           | 0.2006           | 0.0253                   | 0.1492        | 0.01123                | 0.02298     |
| Compound       | Value 1  | Value 2  | Value 3  | Value 4  | Value 5  | Value 6  | Value 7  | Value 8  |
|----------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Hexane         | 0.03225 | 0.611   | 1.029   | 0.0072  | 0.2708  | 0.1891  | 0.0065  | 0.0499  |
| Hydrochloric acid | 0.07147 | 0.60894 | 0.22036 | 0.015431| 0.78867 | 0.2121  | 0.018028| 0.078801|
| Hydrogen       | 0.041481| 2.0365  | 1.7434  | 0.010772| 0.40072 | 0.17319 | 0.010225| 0.035178|
| Melamine       | 0.117   | 5.11    | 2.166   | 0.0484  | 1.8945  | 0.3048  | 0.04706 | 0.33614 |
| Nitric acid    | 0.024872| 3.1583  | 0.3019  | 0.010073| 0.45323 | 0.049194| 0.010692| 0.20324 |
| Nitrogen       | 0.025162| 0.24517 | 0.069994| 0.0056092|0.17735 | 0.14007 | 0.0052849|0.0043299|
| Phenanthroline | 0.029055| 1.8225  | 1.428   | 0.013956| 0.46307 | 0.078935| 0.012196| 0.057448|
| Toluene        | 0.001243| 1.5461  | 1.5826  | 0.00143 | 0.02227 | 0.00395 | 0.001512| 0.003005|
| Urea           | 0.06656 | 3.2979  | 1.4398  | 0.02816 | 1.1186  | 0.17526 | 0.02745 | 0.0202  |

(Table S6 continued)