Photobiocatalytic alcohol oxidation using LED light sources†

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The photocatalytic oxidation of NADH using a flavin photocatalyst and a simple blue LED light source is reported. This in situ NAD+ regeneration system can be used to promote biocatalytic, enantioselective oxidation reactions. Compared to the traditional use of white light bulbs this method enables very significant reductions in energy consumption and CO2 emission.

Oxidation reactions employing alcohol dehydrogenases are experiencing an increased interest in the field of biocatalysis.1–4 While the toolbox of selective enzymes is growing constantly, the choice of efficient in situ regeneration systems of the oxidized nicotinamide cofactors to sustain the catalytic cycle is still comparably limited.5 This is particularly true for regeneration systems employing molecular oxygen as a terminal oxidant, which is attractive from the high thermodynamic driving force and the innocuous nature of the by-products.6–12 As early as 1973, Jones and coworkers proposed using simple flavins to catalyse the aerobic oxidation of reduced nicotinamide cofactors.13 The slow reaction kinetics of the hydride transfer from NAD(P)H to the oxidised isoalloxazine moiety rendered the system impractical as significant molar surpluses of the flavin ‘catalyst’ were necessary to attain full conversion within a reasonable time frame. More recently, we have reported that this limitation (low reaction rates) can be overcome simply by applying visible light to the reaction system.14 The overall rate-limiting hydride transfer step from NAD(P)H to the oxidised flavin could be accelerated by photoexcitation of the flavin catalyst. The oxidised flavin catalyst is regenerated spontaneously through aerobic reoxidation yielding hydrogen peroxide (Scheme 1).14,15

During these proof-of-concept experiments we have utilised a simple, commercially available light-white bulb. Despite its simplicity, this setup severely suffered from heat generated by the bulb. As a consequence not only a significant amount of electrical power was deviated into heat generations but also additional cooling of the reaction mixture was necessary to maintain optimal reaction conditions for the enzyme reaction.

With the advent of cheap light emitting diode (LED) light sources we became interested in using those to promote photobiocatalytic oxidation reactions. Compared to the use of traditional light sources LEDs bear the promise of double energy efficiency: first, LEDs enable a more efficient conversion of electrical power into light energy (only a narrow range of wavelengths is emitted instead of an entire spectrum containing ‘useless’ wavelengths) and, second, due to the absence of thermal effects, also less thermostating is needed.

As commercial LEDs come with three individual colours (blue, green and red exhibiting wavelength maxima at 465, 519, and 631 nm respectively, Fig. 1A) we investigated each wavelength to promote the photochemical oxidation of NADH.

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It should be mentioned here that in accordance with the mechanism proposed in Scheme 1, no conversion of NADH was observed in the absence of either, the photocatalyst, a light source or under anaerobic reaction conditions. As shown in Fig. 1B only blue light (λ_{max} of 465 nm) significantly accelerated the overall reaction while green light (λ_{max} of 519 nm) yielded significantly lower rates and red light (λ_{max} of 631 nm) had almost no accelerating influence as compared to ‘dark’ conditions. In terms of turnover frequencies the flavin catalysts performed <5, 30 and 680 catalytic cycles per hour under red, green and blue light irradiation, respectively. This trend is in agreement with the relative overlap of the LED emission spectra and the FMN absorption spectrum (Fig. 1A).

In terms of light intensity, the LED-based photocatalytic system was well-behaved (Fig. 2) and the overall rate of the system correlated directly with the intensity of the LED light source.

Another advantage of the LED system is the missing thermal effect on the reaction system as compared to the white light bulb. While with the first no significant temperature change was observed (in the absence of external cooling), the latter lead to a temperature increase of more than 30 °C within one hour under the same conditions (Fig. S9†).

Encouraged by these results we decided to apply the LED-based photocatalytic NADH oxidation system to the horse liver alcohol dehydrogenase (HLADH) catalysed oxidative lactonisation of ω,ω-diols such as meso-3-methyl-1,5-pentanediol (Scheme 2). In this system, HLADH catalyses the enantioselective two-step oxidation of the diol starting material yielding enantiopure (ee > 98%) (S)-4-methyltetrahydro-2H-pyran-2-one using the oxidised nicotinamide cofactor (NAD^+) as...
primary hydride acceptor. *In situ* regeneration of NAD$^+$ from NADH is achieved using the photocatalytic system outlined in Scheme 1.

Pleasingly, smooth conversion of the starting material into the enantiopure lactone product was observed (Fig. 3).

Within the first 2 hours of the reaction the mass balance of the reaction was closed (sum of all reagents 10 ± 0.2 mM) and decreased to 8 mM after 4 hours. This observation is most likely explained by the slow, spontaneous hydrolysis of the lactone product to the corresponding hydroxy acid. 17 This finding is also supported by the significant acidification of the reaction mixture especially at higher substrate loadings ([Fig. S10](#)). Further optimisation of the reaction conditions to minimise or prevent this undesired side reaction are currently underway. For example, a tighter pH control and *in situ* extraction of the lactone product are promising approaches to circumvent the undesired hydrolysis.

The catalytic activity of the photocatalyst was 122 h$^{-1}$ and thereby significantly lower than determined before (Fig. 2). Partially this can be attributed to the higher pH value of the reaction medium. 11

<table>
<thead>
<tr>
<th>Contribution</th>
<th>White light bulb</th>
<th>LED</th>
</tr>
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<tbody>
<tr>
<td>Water</td>
<td>877</td>
<td>3920</td>
</tr>
<tr>
<td>Buffer (glycine)</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>NAD</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>FMN</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>HLADH</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate$^c$</td>
<td>1973</td>
<td></td>
</tr>
<tr>
<td>CO$_2$ from cooling$^b$</td>
<td>40.4</td>
<td>0</td>
</tr>
<tr>
<td>CO$_2$ from light source$^b$</td>
<td>40 780</td>
<td>3920</td>
</tr>
</tbody>
</table>

$^a$ Used as extraction solvent for GC analysis. $^b$ To estimate the CO$_2$ emissions due to electricity consumption, we used the average European CO$_2$ emission intensity (see ESI for further details).
terms of energy efficiency as futile wavelengths and heat generation are omitted. Taking together the lower power consumption with the increased catalytic activity of the LED system a more than 90% decrease of energy consumption (and corresponding CO₂ emissions) can be achieved. Furthermore, the significant thermal effect of the white light bulb also necessitates additional cooling of the reaction mixture, which can be reduced or even omitted in case of the LEDs.

**Conclusions**

Today, photocatalysis is in focus of catalysis research. Comparably few studies deal with photocatalysis to promote biocatalytic reactions, a ‘combination of two intrinsically green technologies’. However, neither biocatalysis nor photocatalysis are environmentally sustainable per se. A (self-)critical evaluation of the possible environmental impact is necessary to substantiate ‘green claims’ and to identify bottlenecks as a basis for improved reaction setups en route to truly sustainable procedures.

In this study, we have demonstrated that simple LEDs are well-suited to substitute conventional light sources in flavin-based NAD⁺ regeneration systems to promote dehydrogenase-catalysed oxidation reactions. Particularly, the significantly deceased energy demand of LED systems make it attractive envisioning environmentally acceptable syntheses.

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**Notes and references**