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Establishing an in vivo cascade in cyanobacteria for light-driven redox biocatalysis on gram scale

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PURPOSE OF THE ABSTRACT

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Cyanobacteria are ideal host organisms for truly sustainable whole-cell biocatalysis, as both reduction equivalents and O2 are provided in vivo via water oxidation with light as sole energy source. 1 This attracted the coupling of redox reactions, especially oxygenases, to the photosynthetic light reaction via NADPH and O2 in recombinant cyanobacteria. Introducing heterologous reactions into microbial hosts often suffers from reactant toxicity. Based on a recombinant Synechocystis sp. PCC 6803 strain harboring a Baeyer-Villiger monooxygenase (BVMO)2, we could implement the first artificial light-driven redox cascade for the conversion of cyclohexanone to the polymer building block 6-hydroxyhexanoic acid. BVMO and lactonase co-expression, both from Acidovorax sp. CHX100, enabled this two-step conversion with an activity of up to 63.1 ±1.0 U gCDW-1 without accumulating inhibitory ε-caprolactone. Thereby, one of the key limitations of biocatalytic reactions, i.e., reactant inhibition or toxicity, was overcome. In 2 L stirred-tank-photobioreactors, the process could be stabilized for 48 h, forming 23.50 ± 0.84 mM (3.11 ± 0.12 g L-1) 6-HA. The high specificity enabling a product yield (YP/S) of 0.96 ± 0.01 mol mol-1 and the remarkable biocatalyst-related yield of 3.71 ± 0.21 g6-HA gCDW-1 illustrate the potential of producing this non-toxic product in a synthetic cascade. The fine-tuning of the energy burden on the catalyst was found to be crucial, which indicates a limitation by the metabolic capacity of the cells possibly being compromised by biocatalysis-related reductant withdrawal. Product balancing revealed that up to 16% of intracellular reductant can sustainably be branched off without inflicting biocatalyst growth. This study shows the feasibility of light-driven biocatalytic cascade operation in cyanobacteria and highlights respective metabolic limitations and engineering targets.

FIGURES

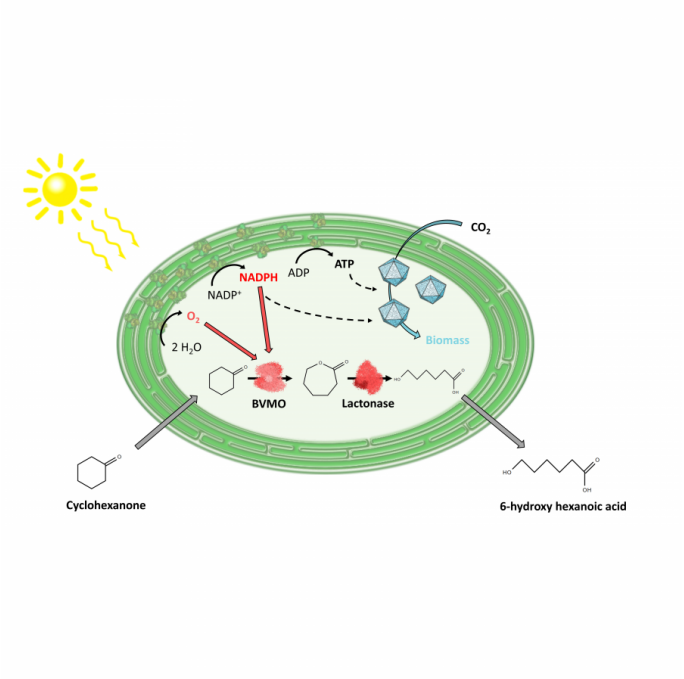

FIGURE 1

Light-driven redox biocatalysis in cyanobacterial whole-cells via an in vivo cascade

With light as only enery source, reduction equivalents and O2 derived from water splitting are utilized by heterologously expressed Bayer-Villiger-moonooxygenase and lactonase to convert cyclohexanone via caprolacton to 6-hydroxyhexanoic acid.

FIGURE 2

KEYWORDS

Photo-biotechnology | Biocatalytic cascade | Redox biocatalysis | Light-driven biocatalysis

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