Overcoming the Gas–Liquid Mass Transfer of Oxygen by Coupling Photosynthetic Water Oxidation with Biocatalytic Oxyfunctionalization

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Abstract: Gas–liquid mass transfer of gaseous reactants is a major limitation for high space–time yields, especially for O₂-dependent (bio)catalytic reactions in aqueous solutions. Herein, oxygenic photosynthesis was used for homogeneous O₂ supply via in situ generation in the liquid phase to overcome this limitation. The phototrophic cyanobacterium Synechocystis sp. PCC6803 was engineered to synthesize the alkane monooxygenase AlkBGT from Pseudomonas putida GPo1.

Gas–liquid mass transfer defines the performance and efficiency of reactions in liquids with gaseous reactants. This is especially true for (bio)catalysts operating in aqueous solutions, where O₂ is one of the most prominent gaseous reactants. As an oxidant for oxidative catalysis, O₂ is of great importance for the production of value-added chemicals and pharmaceuticals. For the efficient use of O₂ as a reactant, harsh reaction conditions with high temperatures and/or pressures are typically necessary. Such conditions may lead to severe safety and selectivity issues, often resulting in low reaction yields. They typically also necessitate highly regulated, elaborate, and thus expensive process control regimes. Mild reaction conditions, high selectivities, and high yields are generally desirable for oxidative production processes and achieved most efficiently by enzyme catalysis. However, low gas–liquid mass transfer rates unfortunately constitute major limitations under such mild conditions. Furthermore, the application of enzymes in whole cells, which is advantageous for oxygenases, suffers from a competition for O₂ between the target reaction and respiration. A technical solution for increasing the O₂ gas–liquid mass transfer rate under ambient conditions is the utilization of O₂-enriched air. Yet, O₂ mass transfer is basically limiting the space–time yields of processes with high oxidation rates, especially in the production of bulk chemicals. To improve O₂ mass transfer, various reactor concepts with different modes of gaseous reactant supply have been proposed. Examples include the utilization of bubble columns, gas-permeable membranes, segmented flow microreactors, or falling film microreactors.

Herein, we report a novel concept based on oxygenic photosynthesis for the homogeneous supply of O₂ to an oxidation reaction. To date, several studies have investigated the coupling of light-driven electron activation to (enzymatic) reactions, both chemically and biotechnologically. However, light-driven water oxidation has not been considered for the homogeneous supply of O₂. Photosynthesis generates O₂ in situ within an aqueous liquid phase from water. This has the potential to basically overcome gas–liquid mass transfer limitations. Light-driven photosynthetic water oxidation is the core of our concept, delivering O₂ homogeneously within cells to the catalytically active oxygenase enzyme, thus driving the oxyfunctionalization reaction (Figure 1).

The well-studied phototrophic cyanobacterium Synechocystis sp. PCC 6803 was chosen as the source for delivering O₂. It was engineered for the synthesis of alkane monooxygenase AlkBGT originating from Pseudomonas putida GPo1 (hereinafter referred to as Syn6803 pAH042; see the Supporting Information for experimental procedures). The highly regioselective terminal oxyfunctionalization of nonanoic acid methyl ester served as the model oxidation reaction. It constitutes an industrially relevant example for the production of polymer building blocks from renewables (Figure 1).

Syn6803 pAH042 produced ca. 65 μmol mol⁻¹ g⁻¹ CDW (Table 1) and demonstrates the functionality of the biocatalyst. However, a specific oxidation rate of 1.3 ± 0.1 μmol min⁻¹ g⁻¹ CDW was still measured in the dark, showing that reduction equivalents were supplied at almost the same rate with and without light (Table 1). Obviously, the catabolism of storage compounds enabled substantial NAD(P)H regeneration in the dark.

Upon successful construction of the functional phototrophic whole-cell biocatalyst, we evaluated the oxidation reaction for exclusive utilization of photosynthetically gen-
concentration does not exceed 3.7 in water:

Specific rates for the hydroxylation of nonanoic acid methyl ester (NAME) to ω-hydroxynonanoic acid methyl ester (H-NAME).

Table 1: Specific rates for the hydroxylation of nonanoic acid methyl ester to ω-hydroxynonanoic acid methyl ester and O₂ evolution of Syn6803 pAH042.

| Conditions                  | Specific production rate [μmol min⁻¹ gCDW⁻¹] |
|-----------------------------|---------------------------------------------|
| Aerobic, irradiated         | 1.5 ± 0.2                                   |
| Aerobic, in the dark        | 1.3 ± 0.1                                   |
| Anaerobic, irradiated      | 0.9 ± 0.1                                   |
| Anaerobic, in the dark      | 0.0                                         |
| Anaerobic, irradiated, OER  | 3.7 ± 0.5                                   |

Specific product formation rates are given with respect to the product formed after [a] 20 or [b] 30 min. [c] The specific O₂ evolution rate (OER) was determined within the aqueous phase in a sealed, gas-free glass chamber in the absence of substrate. Average values and standard deviations of at least two independent biological replicates are given.

Figure 1. Homogenous O₂ evolution coupled to an oxygenase-catalyzed oxyfunctionalization reaction. Water is oxidized by the photosynthetic cyanobacterium Synechocystis sp. PCC6803, yielding O₂ and activated reduction equivalents. The heterologously introduced alkane monoxygenase system AlkBGT captures both O₂ and the reduction equivalents, and catalyzes the regiospecific oxyfunctionalization of nonanoic acid methyl ester (NAME) to ω-hydroxynonanoic acid methyl ester (H-NAME).

Figure 2. In situ supply of photosynthetically generated O₂ to the oxidizing enzyme AlkBGT in Syn6803 pAH042. The biotransformation experiment was performed under anaerobic conditions under irradiation (-----) or in the dark (-----). Average values and standard deviations of two independent biological replicates are given. CDW = cell dry weight.

Evolution rate of 3.7 ± 0.5 μmol min⁻¹ gCDW⁻¹, corresponding to 100% of O₂ available in the system (assuming no photorespiration), nearly 25% of the photosynthetically generated O₂ was captured for terminal hydroxylation of NAME.

Diffusion of photosynthetically generated O₂ may affect the reaction efficiency of the terminal hydroxylation and theoretically results in gas–liquid mass transfer processes within the assay system. The specific O₂ accumulation rate in the aqueous phase was calculated to be 0.01 μmol min⁻¹ gCDW⁻¹ assuming immediate O₂ diffusion from the aqueous to the gaseous phase (aqueous/gaseous ratio 1:10, Henry volatility for O₂ in water: \( H_2O = c_\text{aq}/c_\text{gas} = 0.0297 \) at 25°C).\(^{12}\) Thus the effective O₂ concentration does not exceed 0.6 μM within 30 min of reaction time (applied biomass concentration: 2 gCDW L⁻¹). In contrast, Michaelis constants (\( K_m \)) of oxygenases with respect to O₂ are typically in the range of 10–60 μM.\(^{13} \) This, together with the high fraction of O₂ captured by the monoxygenase (25%), suggests that the photosynthetically generated O₂ is concentrated within the microbial cell and captured in situ by the monoxygenase before diffusing out of the cell. Although O₂ can in principle diffuse across cellular membranes, the lipid bilayer system seems to pose a physical barrier that is beneficial for the intracellular oxidation process. These results are proof of concept for the in situ coupling of photosynthetic O₂ evolution to O₂-dependent oxidation reactions. The photosynthetic light reaction was used for the intracellular supply of both activated reduction equivalents and O₂.

These results might be the starting point for the development of various efficient photosynthesis-driven oxyfunctionalization reactions. In the present case, future optimizations include an increase in the AlkBGT level in the cyanobacterial
whole-cell biocatalyst.\[13\] This is obvious from comparing the transformation rates of NAME into H-NAME catalyzed by E. coli W3110 carrying the very plasmid pAH042 (10.0 ± 0.1 μmol min\(^{-1}\) g\(_{\text{CDW}}\)^{-1}; see S4 in the Supporting Information) with those of E. coli that strongly express alkBGT (104–128 μmol min\(^{-1}\) g\(_{\text{CDW}}\)^{-1}).\[14\] Other targets are electron channeling and improved cultivation and biocatalyst concepts. The cyanobacterial photosynthetic metabolism supports the supply of activated reduction equivalents at high rates (123 μmol min\(^{-1}\) g\(_{\text{CDW}}\)^{-1}).\[36\] Yet, the O\(_2\) evolution rate determined in this study implies a photosynthetic activity of only 3.7 μmol min\(^{-1}\) g\(_{\text{CDW}}\)^{-1}. This corresponds to a specific NAD(P)H regeneration rate of 7.4 μmol min\(^{-1}\) g\(_{\text{CDW}}\)^{-1}. The theoretical maximum of this rate was estimated to be 850 μmol min\(^{-1}\) g\(_{\text{CDW}}\)^{-1} (assumptions for PSII: \(k_{\text{ps}} = 1000 \text{ s}^{-1}\), 10 mg g\(_{\text{CDW}}\)^{-1}, \(M_{W} = 350 \text{ kDa}\)).\[37,38\] With high biomass concentrations (40 g g\(_{\text{CDW}}\)^{-1}), a theoretical maximum of 2040 mmol L\(^{-1}\) h\(^{-1}\) would be possible for the oxygen supply rate. This translates into a volumetric mass transfer coefficient \(k_{L}A\) of 4533 h\(^{-1}\) for a bioreactor operated at 2.5 atm, 30°C, and a residual O\(_2\) concentration of 100 μM (typical conditions for large-scale biocatalyst operation).\[34\] In contrast, the \(k_{L}A\) values of large-scale biocatalysts are on the order of 200 h\(^{-1}\).\[35a\] In addition, the use of photoautotrophic instead of chemoheterotrophic organisms largely relieves the competition for O\(_2\) between oxygenation and respiration.

The development of photobiocatalysts enabling the generation of high biomass concentrations with high oxygen evolution activity is key for the future applicability of the presented concept.\[46\] Biofilm cultivation in capillary microreactors constitutes one possible solution to increase the cyanobacterial biomass concentration.\[47\] Stable cyanobacterial biofilm cultivation has recently been achieved over several weeks with retention of the photosynthetic activity throughout the biofilm. Reaction optimization addressing the key issue of photobiocatalyst development has the potential to facilitate currently oxygen-transfer-limited selective hydroxylation processes for the biocatalytic functionalization of hydrocarbons.\[45\] In summary, the in situ coupling of oxygenic photosynthesis to oxidizing enzymes provides a novel and safe access to O\(_2\) as a reactant for designing new reactions for oxidation catalysis.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biocatalysis · oxidoreductases · oxyfunctionalization · oxygen mass transfer · photosynthesis

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