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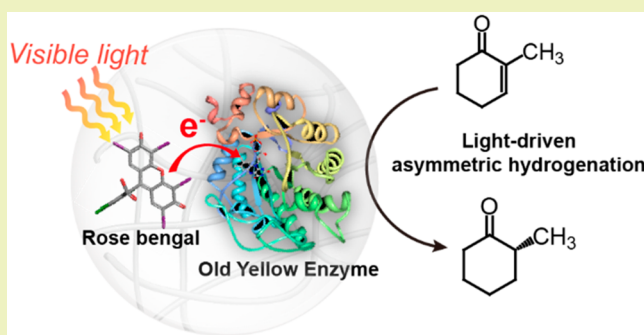
Light-Harvesting Dye–Alginate Hydrogel for Solar-Driven, Sustainable Biocatalysis of Asymmetric Hydrogenation

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Supporting Information

ABSTRACT: We report visible light-driven, asymmetric hydrogenation of C=C bonds using an ene-reductase from *Thermus scotoductus* SA-01 (TsOYE) and a light-harvesting dye (rose bengal, RB) co-immobilized in an alginate hydrogel. Highly efficient encapsulation of RB in alginate hydrogel was achieved using the intrinsic affinity between TsOYE and RB, which allowed for the construction of robust RB-TsOYE-loaded alginate capsules. In the absence of NADH, the photobiocatalytic system facilitated asymmetric reduction of 2-methylcyclohexenone to an enantiopure (*R*)-2-methylcyclohexanone (*ee* > 99%; max. conversion, 70.4%; turnover frequency, 1.54 min^{−1}; turnover number, 300.2) under illumination. A series of stability tests revealed a significant enhancement of TsOYE's robustness in alginate hydrogel against heat and chemical denaturants. This study provides insight into a greener and sustainable approach of cofactor-free OYE catalysis for producing value-added chemicals using light energy.

KEYWORDS: Photobiocatalysis, Ene-reductase, Asymmetric hydrogenation, NADH-free bioprocess, Alginate hydrogel



INTRODUCTION

Biocatalytic transformation is an attractive route for ecofriendly synthesis of chemicals. The outstanding catalytic activities and specificities (e.g., chemo-, regio-, stereo-, and enantio-selectivities) of enzymes make them a green alternative to conventional catalysts for producing value-added chemicals under mild reaction conditions without generating unwanted byproducts.^{1–3} Despite the fascinating features of biocatalysis, many challenges—such as limited stability of enzymes against environmental stresses, complex purification steps, and difficulties in reuse—remain in cost-effective and practical applications. The immobilization of biocatalysts is often considered as a solution to address the issues by enhancing enzyme stability and sustainability, allowing for easy recovery and reuse of key reaction components.^{4–7}

Recent efforts of combining biocatalysis with photocatalysis take a step forward to greener and more sustainable synthesis of chemicals using solar energy.^{8–12} In this study, we report an all-in-one photobiocatalytic platform for asymmetric hydrogenation driven by an ene-reductase from *Thermus scotoductus* SA-01 (TsOYE) encapsulated in light-harvesting alginate hydrogel. The ene-reductases from the family of Old Yellow Enzymes (OYEs) catalyze chiral reduction of activated C=C double bonds.^{13–15} During the biotransformation, the reduced form of OYE-bound flavin mononucleotide (FMNH₂) trans-

fers two electrons and two protons to the activated C=C double bond via a Michael-type reaction, which is usually mediated by a reduced form of expensive nicotinamide adenine dinucleotide cofactor (NADH).^{16,17} Different approaches for light-driven OYE-catalysis have been reported for the reduction of alkenes. For example, Burai et al. reported OYE-catalyzed reduction of ketosiphorone at a rate of approximately 20 μM h^{−1} using quantum dots.¹⁸ Recently, carbon nanodots were applied to OYE photobiocatalysis to enhance the reaction rate as high as 4 mM h^{−1}.¹⁹ On the other hand, OYE-containing recombinant cyanobacteria were employed for light-driven alkene reduction with approximately 2 mM h^{−1} of the production rate.²⁰

Our previous work demonstrated that direct photoactivation of OYEs using xanthene derivatives, such as rose bengal (RB), as a photosensitizer could drive OYE-catalyzed reactions in a cofactor-free manner.²¹ The simultaneous reduction of RB and TsOYE facilitated the asymmetric reduction of C=C bonds in the absence of NADH, allowing for the synthesis of enantiopure (*ee* > 99%) products at the rate of 2.3 mM h^{−1}. Spectroscopic and electrochemical assays verified spontaneous binding of RB molecules—in a higher affinity than NADH—to

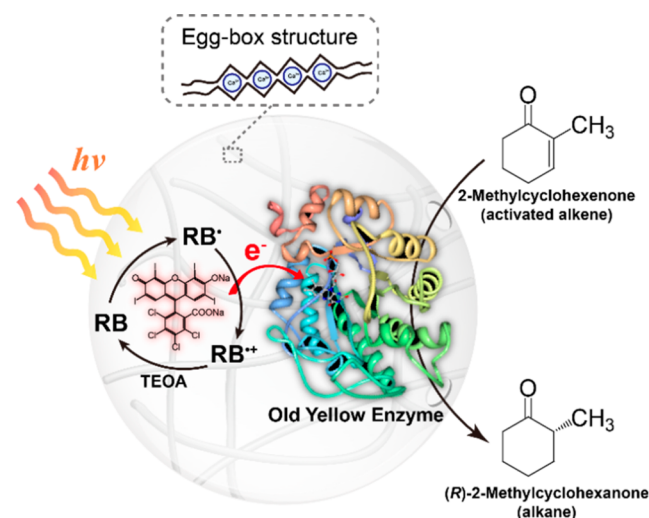
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the flavin prosthetic group of TsOYE. In addition, the isoelectric point (pI) of TsOYE (7.83) and pK_a values of RB (1.89 and 3.93) further indicate electrostatic affinity between TsOYE and RB at pH 7.5.^{22,23}

Alginate is a sea-weed-derived polysaccharide consisting of 1,4-linked β -D-mannuronic (M) and α -L-guluronic (G) acid blocks, which has been widely used for the encapsulation of biologicals because of low cost, biocompatibility, and facile gelation process. The ionic cross-linking, a most well-known method for forming alginate hydrogel, occurs through electrostatic interactions of the carboxyl groups of G blocks with divalent cations (e.g., Ca^{2+} , Ba^{2+} , Sr^{2+}), inducing an egg-box gel structure.²⁴ As depicted in Scheme 1, we encapsulated

Scheme 1. Schematic Illustration of a RB and TsOYE Encapsulated Alginate Capsule for OYE Photobiocatalysis^a



^aThe TsOYE catalyzes asymmetric reduction of activated 2-methylcyclohexenone into (R)-2-methylcyclohexanone through photosensitization of RB in the alginate hydrogel.

TsOYEs in the ionically cross-linked alginate beads, followed by a spontaneous post-hybridization of RB to the TsOYEs in the hydrogel. The direct transfer of photoinduced electrons from RB to the enzyme-bound flavin prosthetic group using triethanolamine (TEOA) as an electron donor drives asymmetric reduction reactions, which occur within the alginate beads under illumination of a white light-emitting diode (LED).

RESULTS AND DISCUSSION

We simply treated the cell-free extract by a one-step heat treatment to obtain TsOYEs for this study. TsOYE-encapsulated alginate (TsOYE/alginate) beads were prepared by mixing TsOYEs with an aqueous alginate solution (2 w/v %), which was applied dropwise to a 100 mM $CaCl_2$ gelling medium (Figures S1, S2). The TsOYE-containing beads exhibited a pale yellow color, distinguishable from pure alginate beads. According to our spectroscopic analyses (Figure S3), over 90% of encapsulated TsOYE remained in alginate beads after 24 h of immersion in deionized water. The results show that alginate hydrogel encapsulates TsOYEs stably within the beads without any significant leakage problem. We compared the loading efficiencies of RB in bare alginate and TsOYE-containing alginate beads. For the experiments, we

incubated the alginate beads in RB solutions for 1 h under mild stirring, collected supernatants, and measured the loading efficiency of RB using a spectrophotometer.

As shown in Figure 1A, the loaded amount of RB was different by a factor of 2 by the presence of TsOYEs in the

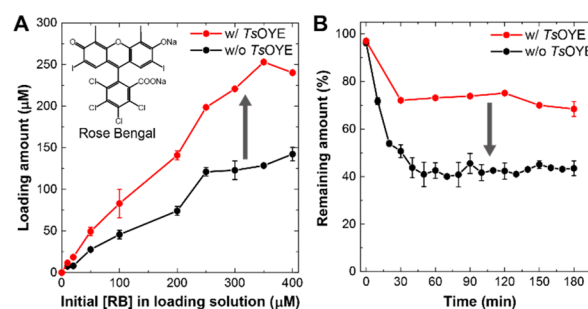


Figure 1. (A) RB-loading capacity of pure alginate beads and TsOYE-containing alginate beads. (B) Remaining amount of RB in RB/alginate beads and RB-TsOYE/alginate beads after 3 h of leaching test.

alginate hydrogel. We attribute the result to spontaneous association of RB molecules with TsOYE in addition to physical entrapment of RB within the hydrogel. We also examined the effect of TsOYE on the leaching of encapsulated RB by immersing RB/alginate and RB-TsOYE/alginate beads in deionized water. More than a half of the RB molecules were lost after 3 h when loaded in bare alginate beads; in contrast, over 70% of loaded RB were retained in the TsOYE/alginate hydrogel (Figure 1B). Previous studies reported the problem of encapsulating small molecules within the ionically cross-linked alginate hydrogels that have a relatively large pore size (~ 5 nm).^{25,26} Thus, additional coatings (or hybridization) with other supporting materials have been pursued to enhance the efficacy of small molecule entrapment.^{27–29} Our results indicate that facile immobilization of small RB molecules in alginate hydrogel can be achieved without any additional process using the intrinsic binding affinity between RB and TsOYE. The stably encapsulated TsOYE attracted RB molecules, resulting in a remarkably enhanced loading efficiency with a minimal leaching.

We investigated electrochemical responses of TsOYE and RB encapsulated in alginate hydrogel using protein film voltammetry. We prepared a TsOYE/alginate film on a glassy carbon electrode by a drop-casting method. As displayed in Figure 2A, a clear peak for the reduction of the prosthetic FMN group in TsOYE was observed at -0.45 V (vs Ag/AgCl) in agreement with the literature.^{30,31} Approximately 50 mV of a negative shift occurred in the presence of RB within alginate hydrogel, which indicates the interaction between RB and the immobilized TsOYE. Additional analyses verified that alginate itself did not affect the electrochemical properties of RB and TsOYE (Figure S4). The encapsulated TsOYE and RB showed a distinguishable light response from that of either TsOYE or RB (Figure 2B). At an applied potential of -0.6 V (vs Ag/AgCl), an anodic photocurrent of $0.6 \mu A$ was generated in the presence of both TsOYE and RB in the alginate film. We attribute the result to the transfer of photoexcited electrons from RB to the enzyme-bound FMN, resulting in an increased anodic photocurrent. Note that the enzyme or RB alone did not show significant anodic photocurrents ($<0.1 \mu A$). The series of electrochemical and photochemical analyses verified

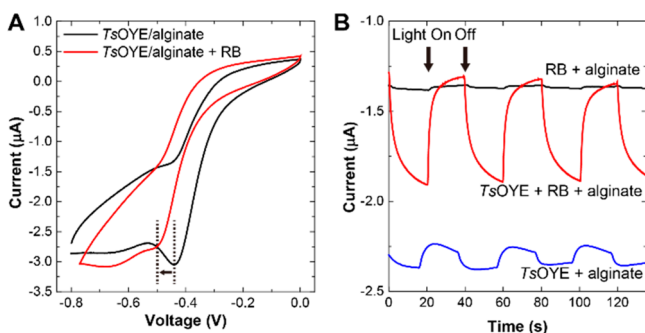


Figure 2. (A) Cyclic voltammetric diagrams of *TsOYE* in alginate film in the presence and the absence of RB. (B) Photocurrent response of *TsOYE* in alginate film at an applied potential of -0.6 V (vs Ag/AgCl, 3 M NaCl). A MOPS buffer (50 mM, pH 7.5) was used with Ag/AgCl and Pt as a reference electrode and a counter electrode, respectively.

the photosensitized, coreduction property of *TsOYE* and RB in alginate hydrogel, which occurred through direct electron transfer between spontaneously hybridized *TsOYE* and RB.

We studied catalytic activities of RB/*TsOYE* hydrogels under illumination of a white LED (power: 5.22 mW/cm^2) using 2-methylcyclohexen-1-one (MCH) as a model substrate (10 mM). The RB-*TsOYE*/alginate beads converted MCH into enantiopure ($ee > 99\%$) (*R*)-2-methylcyclohexanone with 51% of conversion $\{[\text{product}]/[\text{substrate}]_{\text{initial}} \times 100 (\%) \}$ after 3 h of illumination (Figure 3A). We attribute the

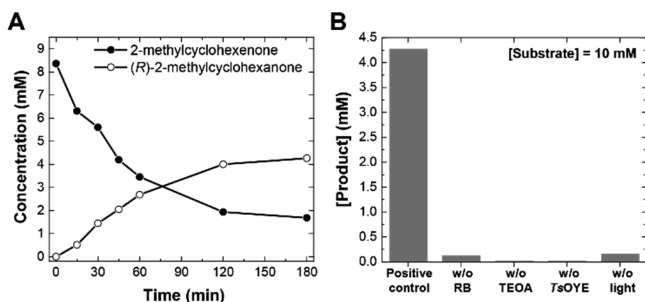


Figure 3. (A) Conversion of 2-methylcyclohexenone to (*R*)-2-methylcyclohexanone by RB-*TsOYE*/alginate beads. Reaction conditions: $[2\text{-methylcyclohexenone}] = 10 \text{ mM}$, $[TsOYE]_{\text{in alginate}} = 21 \mu\text{M}$, $[RB]_{\text{in alginate}} = 140 \mu\text{M}$, 200 mM TEOA buffer (pH 7.5), reaction temperature = 30°C , white LED illumination. (B) Control experiments about the effect of each reaction component. A 50 mM MOPS buffer (pH 7.5) was used for the without-TEOA case.

mismatch of mass balance between the substrate consumption and product formation to strong volatility of the chemicals at the reaction conditions. We also conducted a series of control experiments to test the role of key photobiocatalytic components [i.e., enzyme (*TsOYE*), photosensitizer (RB), electron donor (TEOA), and light source].

As displayed in Figure 3B, a positive control (i.e., with all the components) generated 4.26 mM of enantiopure (*R*)-2-methylcyclohexanone, whereas none or a negligible amount ($<0.15 \text{ mM}$) of the product was detected in the absence of any single component. The results show that RB-*TsOYE*/alginate hydrogel functions as an efficient light-driven biocatalytic platform for asymmetric hydrogenation of $\text{C}=\text{C}$ bonds. We also examined the dependency of product formation on TEOA concentration. As shown in Figure S5, the overall reaction rate

was proportional to the concentration of TEOA up to 100 mM, which indicates that an excess amount of TEOA is necessary for the system. According to the literature,^{19,32–34} a high surplus of electron donors (e.g., TEOA) is often needed to drive multiple electron-transfer steps in photocatalytic systems due to back electron transfer and charge recombination problems.

We further examined the effect of the loaded RB's concentration on the activities of *TsOYE*. With the increasing amount of the loaded RB at a fixed *TsOYE* concentration (21 μM) in alginate, the reaction conversion, turnover frequency (TOF), and turnover number (TON) increased gradually (Figure 4), which saturated to maximum values (66.9%

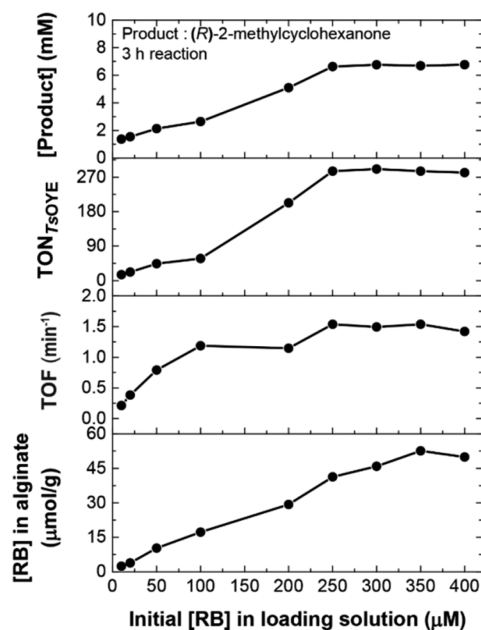


Figure 4. Photobiocatalytic activities of RB-*TsOYE*/alginate beads with different initial RB concentrations in the loading solution. TOF and TON_{TsOYE} values for the reduction of 2-methylcyclohexenone were determined after 30 min and 3 h of reactions, respectively.

conversion, 1.54 min^{-1} TOF, and 291.2 TON) due to the limitation of the RB-loading capacity ($\sim 51 \mu\text{mol/g}$) within alginate beads. Free *TsOYE* and RB dissolved in a buffer solution showed 78.8% conversion at the saturation point (RB/*TsOYE* = 5) (Figure S6), similarly to our previous report (i.e., 76% conversion at RB/*TsOYE* = 5).²¹ In contrast, a lower conversion (66.9%) and a higher RB/*TsOYE* molar ratio (RB/*TsOYE* = 9.5) were observed at the saturation point of the initial reaction rate for the *TsOYE* encapsulated in the alginate hydrogel. According to the literature,⁴ encapsulation within a hydrogel matrix limits the enzyme's mobility, blocks the active site of the enzyme, and hinders mass transfer of substrates and products. We attribute the almost 2-fold increase in the saturation RB/*TsOYE* ratio to the suppressed *TsOYE* mobility and substrate accessibility that should cause an increase in the required RB amount in the hydrogel to reach the saturation point.

The *TsOYE*s encapsulated in alginate hydrogel exhibited significantly enhanced stability against external stresses (e.g., heat, chemical denaturants). When we conducted stability tests in terms of relative conversion ($\text{RC} = \text{conversion}/\text{conversion}_{\text{max}} \times 100$), the immobilized *TsOYE* showed strong

robustness against heat (RC \sim 80% at 60 °C) (Figure 5A), whereas free TsOYE lost over a half of its initial conversion

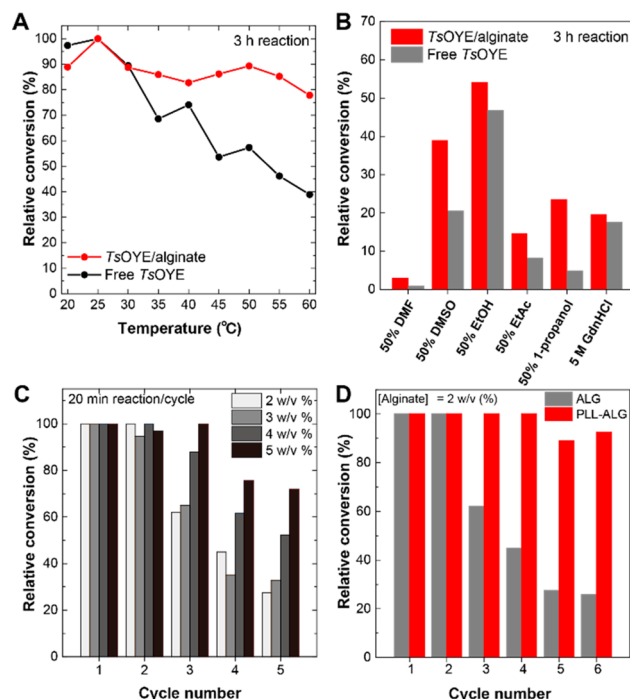


Figure 5. Series of stability tests for RB-TsOYE/alginate beads in terms of relative conversion upon (A) temperature change and (B) solvent addition. Series of recycle tests for RB-TsOYE/alginate beads by (C) varying alginate concentration and (D) coating a poly-L-lysine layer. [Substrate] = 10 mM, [TEOA] = 200 mM, reaction temperature = 30 °C.

(RC \sim 40% at 60 °C). Note that the loss of free TsOYE's catalytic activity during prolonged incubation at high temperature has been reported previously. According to the literature,³⁵ residual activity of TsOYE decreased gradually with the increasing temperature due to an unknown reason. The volatility issue of the substrate and product further contributes to the low conversion of TsOYE-driven catalysis at high temperature. Furthermore, the immobilization also enhanced TsOYE's resistance to chemical denaturants such as dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtAc), 1-propanol, and guanidine hydrochloride (GdnHCl) (Figure 5B). In particular, we observed significant enhancement of TsOYE stability against DMSO and 1-propanol with doubled and quadrupled RC values, respectively. Our results show that TsOYE becomes robust against heat and chemical denaturants when encapsulated in alginate beads. We further investigated the reusability of RB-TsOYE/alginate beads through a series of recycling experiments. The RB-TsOYE/alginate beads (alginate concentration, 2 w/v%) showed a good retention of RC in the first few cycles but lost most of their initial conversion over nine reaction cycles (with only about 10% of remaining RC) (Figure S7). We ascribe the result to the degradation of RB through irreversible photobleaching under illumination.^{36–38} Figure S8 supports RB's photobleaching problem accelerated in the presence of TEOA, the electron donor.

Furthermore, a partial leakage of RB shown in Figure 1B became more significant with the increasing number of cycles. We attempted to enhance the reusability by (1) increasing

alginate concentration, (2) supplementing fresh RBs, and (3) coating alginate beads with a poly-L-lysine layer.

As shown in Figure 5C, higher alginate concentration reduced RB leakage significantly by forming a denser gel network and improved the recyclability of RB-TsOYE/alginate beads with a retention of RC over 50% after five cycles. We also found a dramatic enhancement of reusability—nearly 100% of its initial conversion until the ninth cycle—by supplementing fresh RBs (Figure S7). In addition, the electrostatic interaction between poly-L-lysine and RB at pH 7.5 could reduce the loss of RB, resulting in approximately 90% RC after six cycles (Figure 5D). To sum up, the long-term reusability of RB-TsOYE alginate hydrogel was hampered by the intrinsic limitation of RB. Future studies will focus on addressing the photoresistance issue and the scalability of the light-driven biocatalytic process to a preparative level. For example, carbon nanotubes³⁹ and carbon-based quantum dots^{34,40} are promising alternatives due to their resistance to photobleaching. Also, phosphine oxide-containing fluorescein, which is derived from xanthene dyes similar to RB, exhibited a superior photobleaching resistance according to the literature.⁴¹ Finally, chemically modified RB derivatives with hopefully increased photobleaching stability may also represent an attractive solution.

We further tested a cell-free extract instead of heat-purified TsOYE to demonstrate the sustainability of the system. We found that the cell-free extract shows a similar negative shift in the presence of RB, which indicates an electrochemical interaction between the cell-free extract and RB within an alginate hydrogel (Figure S10A, B). We also observed visible light-driven, cell-free-extract-catalyzed reduction of 2-methylcyclohexenone into an enantiopure ($ee > 99\%$) product, though the overall catalytic activity was lowered (17.4% conversion and 53.5 TON_{TsOYE}) than that of heat-purified TsOYE (Figure S10C, D). Overall, our results show that the RB-TsOYE/alginate system is applicable to a cell-free extract and support the sustainability of the photobiocatalytic platform.

CONCLUSION

NADH-free, light-driven TsOYE biocatalysis for asymmetric hydrogenation is demonstrated using cost-effective and biocompatible alginate hydrogel. The spontaneous RB-TsOYE association within alginate hydrogel matrix enabled efficient and stable immobilization of both RB and TsOYE, resulting in the formation of light-harvesting all-in-one beads with excellent catalytic performances of TsOYE. The reduction of 2-methylcyclohexenone was achieved through direct electron transfer and coreduction of TsOYE and RB in alginate hydrogel. The RB-TsOYE/alginate beads exhibited a catalytic performance of maximum 70.4% conversion of (R)-2-methylcyclohexanone ($ee > 99\%$) and 300.2 TON_{TsOYE}, while the limited mobility of TsOYE and substrate accessibility induced a 2-fold increase in the saturation ratio of RB/TsOYE compared to the free enzyme. We revealed enhancements of stability and reusability of immobilized RB and TsOYE, which hints at greener applications of OYEs for solar-driven synthesis of value-added chemicals.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.9b01075.

Experimental details for TsOYE preparation and encapsulation in alginate hydrogel, optical images of alginate beads, spectroscopic analyses for TsOYE leakage test, additional cyclic voltammograms of alginate film, time course data and control experiments for OYE photobiocatalysis, and examination of RB's photo-bleaching. (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Aldridge, S. Industry backs biocatalysis for greener manufacturing. *Nat. Biotechnol.* **2013**, *31*, 95–96.
- (2) Sheldon, R. A.; Woodley, J. M. Role of biocatalysis in sustainable chemistry. *Chem. Rev.* **2018**, *118*, 801–838.
- (3) Rudroff, F.; Mihovilovic, M. D.; Gröger, H.; Snajdrova, R.; Iding, H.; Bornscheuer, U. T. Opportunities and challenges for combining chemo- and biocatalysis. *Nat. Catal.* **2018**, *1*, 12–22.
- (4) Jochems, P.; Satyawali, Y.; Diels, L.; Dejonghe, W. Enzyme immobilization on/in polymeric membranes: status, challenges and perspectives in biocatalytic membrane reactors (BMRs). *Green Chem.* **2011**, *13*, 1609–1623.
- (5) van Pelt, S.; Sheldon, R. A. Enzyme immobilisation in biocatalysis: why, what and how. *Chem. Soc. Rev.* **2013**, *42*, 6223–6235.
- (6) DiCosimo, R.; McAuliffe, J.; Poulou, A. J.; Bohlmann, G. Industrial use of immobilized enzymes. *Chem. Soc. Rev.* **2013**, *42*, 6437–6474.
- (7) Barbosa, O.; Torres, R.; Ortiz, C.; Berenguer-Murcia, Á.; Rodrigues, R. C.; Fernandez-Lafuente, R. Heterofunctional supports in enzyme immobilization: From traditional immobilization protocols to opportunities in tuning enzyme properties. *Biomacromolecules* **2013**, *14*, 2433–2462.
- (8) Mifsud, M.; Gargiulo, S.; Iborra, S.; Arends, I. W. C. E.; Hollmann, F.; Corma, A. Photobiocatalytic chemistry of oxidoreductases using water as the electron donor. *Nat. Commun.* **2014**, *5*, 3145.
- (9) Brown, K. A.; Harris, D. F.; Wilker, M. B.; Rasmussen, A.; Khadka, N.; Hamby, H.; Keable, S.; Dukovic, G.; Peters, J. W.; Seefeldt, L. C.; King, P. W. Light-driven dinitrogen reduction catalyzed by a CdS:nitrogenase MoFe protein biohybrid. *Science* **2016**, *352*, 448–450.
- (10) Mersch, D.; Lee, C. Y.; Zhang, J. Z.; Brinkert, K.; Fontecilla-Camps, J. C.; Rutherford, A. W.; Reisner, E. Wiring of photosystem II to hydrogenase for photoelectrochemical water splitting. *J. Am. Chem. Soc.* **2015**, *137*, 8541–8549.
- (11) Rowe, S. F.; Le Gall, G.; Ainsworth, E. V.; Davies, J. A.; Lockwood, C. W. J.; Shi, L.; Elliston, A.; Roberts, I. N.; Waldron, K. W.; Richardson, D. J.; Clarke, T. A.; Jeuken, L. J. C.; Reisner, E.; Butt, J. N. Light-driven H₂ evolution and C = C or C = O bond hydrogenation by *Shewanella oneidensis*: A versatile strategy for photocatalysis by nonphotosynthetic microorganisms. *ACS Catal.* **2017**, *7*, 7558–7566.
- (12) Lee, S. H.; Choi, D. S.; Kuk, S. K.; Park, C. B. Photobiocatalysis: Activating redox enzymes by direct or indirect transfer of photoinduced electrons. *Angew. Chem., Int. Ed.* **2018**, *57*, 7958–7985.
- (13) Toogood, H. S.; Scrutton, N. S. Discovery, characterization, engineering, and applications of ene-reductases for industrial biocatalysis. *ACS Catal.* **2018**, *8*, 3532–3549.
- (14) Stuermer, R.; Hauer, B.; Hall, M.; Faber, K. Asymmetric bioreduction of activated C = C bonds using enoate reductases from the Old Yellow Enzyme family. *Curr. Opin. Chem. Biol.* **2007**, *11*, 203–213.
- (15) Hollmann, F.; Arends, I. W. C. E.; Holtmann, D. Enzymatic reductions for the chemist. *Green Chem.* **2011**, *13*, 2285–2313.
- (16) Opperman, D. J.; Sewell, B. T.; Litthauer, D.; Isupov, M. N.; Littlechild, J. A.; van Heerden, E. Crystal structure of a thermostable Old Yellow Enzyme from *Thermus scotoductus* SA-01. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 426–431.
- (17) Toogood, H. S.; Gardiner, J. M.; Scrutton, N. S. Biocatalytic reductions and chemical versatility of the Old Yellow Enzyme family of flavoprotein oxidoreductases. *ChemCatChem* **2010**, *2*, 892–914.
- (18) Burai, T. N.; Panay, A. J.; Zhu, H.; Lian, T.; Lutz, S. Light-driven, quantum dot-mediated regeneration of FMN to drive reduction of ketosiphorone by Old Yellow Enzyme. *ACS Catal.* **2012**, *2*, 667–670.
- (19) Kim, J.; Lee, S. H.; Tieves, F.; Choi, D. S.; Hollmann, F.; Paul, C. E.; Park, C. B. Biocatalytic C = C bond reduction through carbon nanodot-sensitized regeneration of NADH analogues. *Angew. Chem., Int. Ed.* **2018**, *57*, 13825–13828.
- (20) Königer, K.; Baraibar, A. G.; Mügge, C.; Paul, C. E.; Hollmann, F.; Nowaczyk, M. M.; Kourist, R. Recombinant cyanobacteria for the asymmetric reduction of C = C bonds fueled by the biocatalytic oxidation of water. *Angew. Chem., Int. Ed.* **2016**, *55*, 5582–5585.
- (21) Lee, S. H.; Choi, D. S.; Pesic, M.; Lee, Y. W.; Paul, C. E.; Hollmann, F.; Park, C. B. Cofactor-free, direct photoactivation of enoate reductases for the asymmetric reduction of C = C bonds. *Angew. Chem., Int. Ed.* **2017**, *56*, 8681–8685.
- (22) Opperman, D. J.; Piater, L. A.; van Heerden, E. A novel chromate reductase from *Thermus scotoductus* SA-01 related to Old Yellow Enzyme. *J. Bacteriol.* **2008**, *190*, 3076–3082.
- (23) Batistela, V. R.; Pellosi, D. S.; de Souza, F. D.; da Costa, W. F.; de Oliveira Santin, S. M.; de Souza, V. R.; Caetano, W.; de Oliveira, H. P. M.; Scarmínio, I. S.; Hioka, N. pK_a determinations of xanthene derivatives in aqueous solutions by multivariate analysis applied to UV-Vis spectrophotometric data. *Spectrochim. Acta, Part A* **2011**, *79*, 889–897.
- (24) Pawar, S. N.; Edgar, K. J. Alginate derivatization: A review of chemistry, properties and applications. *Biomaterials* **2012**, *33*, 3279–3305.
- (25) Boonthekul, T.; Kong, H. J.; Mooney, D. J. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. *Biomaterials* **2005**, *26*, 2455–2465.
- (26) Zhao, J.; Zhao, X.; Guo, B.; Ma, P. X. Multifunctional interpenetrating polymer network hydrogels based on methacrylated alginate for the delivery of small molecule drugs and sustained release of protein. *Biomacromolecules* **2014**, *15*, 3246–3252.
- (27) Bouhadir, K. H.; Alsberg, E.; Mooney, D. J. Hydrogels for combination delivery of antineoplastic agents. *Biomaterials* **2001**, *22*, 2625–2633.

- (28) Zhang, X.; Hui, Z.; Wan, D.; Huang, H.; Huang, J.; Yuan, H.; Yu, J. Alginate microsphere filled with carbon nanotube as drug carrier. *Int. J. Biol. Macromol.* **2010**, *47*, 389–395.
- (29) Zhang, J.; Wang, Q.; Wang, A. In situ generation of sodium alginate/hydroxyapatite nanocomposite beads as drug-controlled release matrices. *Acta Biomater.* **2010**, *6*, 445–454.
- (30) Fleming, B. D.; Tian, Y.; Bell, S. G.; Wong, L.; Urlacher, V.; Hill, H. A. O. Redox properties of cytochrome P450_{BM3} measured by direct methods. *Eur. J. Biochem.* **2003**, *270*, 4082–4088.
- (31) Okamoto, A.; Hashimoto, K.; Nealson, K. H.; Nakamura, R. Rate enhancement of bacterial extracellular electron transport involves bound flavin semiquinones. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 7856–7861.
- (32) Zhang, X.; Jin, Z.; Li, Y.; Li, S.; Lu, G. Efficient photocatalytic hydrogen evolution from water without an electron mediator over Pt–rose bengal catalysts. *J. Phys. Chem. C* **2009**, *113*, 2630–2635.
- (33) Park, J. H.; Lee, S. H.; Cha, G. S.; Choi, D. S.; Nam, D. H.; Lee, J. H.; Lee, J. K.; Yun, C. H.; Jeong, K. J.; Park, C. B. Cofactor-free light-driven whole-cell cytochrome P450 Catalysis. *Angew. Chem., Int. Ed.* **2015**, *54*, 969–973.
- (34) Hutton, G. A. M.; Reuillard, B.; Martindale, B. C. M.; Caputo, C. A.; Lockwood, C. W. J.; Butt, J. N.; Reisner, E. Carbon dots as versatile photosensitizers for solar-driven catalysis with redox enzymes. *J. Am. Chem. Soc.* **2016**, *138*, 16722–16730.
- (35) Bernard, J.; van Heerden, E.; Arends, I. W. C. E.; Opperman, D. J.; Hollmann, F. Chemoenzymatic reduction of conjugated C = C double bonds. *ChemCatChem* **2012**, *4*, 196–199.
- (36) Lee, P. C. C.; Rodgers, M. A. J. Laser flash photokinetic studies of rose bengal sensitized photodynamic interactions of nucleotides and DNA. *Photochem. Photobiol.* **1987**, *45*, 79–86.
- (37) Neckers, D. C.; Linden, S. M. Bleaching Studies of rose bengal onium salts. *J. Am. Chem. Soc.* **1988**, *110*, 1257–1260.
- (38) Rauf, M. A.; Marzouki, N.; Korbahti, B. K. Photolytic decolorization of rose bengal by UV/H₂O₂ and data optimization using response surface method. *J. Hazard. Mater.* **2008**, *159*, 602–609.
- (39) Heller, D. A.; Baik, S. H.; Eurell, T. E.; Strano, M. S. Single-walled carbon nanotube spectroscopy in live cells: Towards long-term labels and optical sensors. *Adv. Mater.* **2005**, *17*, 2793–2799.
- (40) Li, D.; Han, D.; Qu, S.; Liu, L.; Jing, P.; Zhou, D.; Ji, W.; Wang, X.; Zhang, T.; Shen, D. Supra-(carbon nanodots) with a strong visible to near-infrared absorption band and efficient photothermal conversion. *Light: Sci. Appl.* **2016**, *5*, e16120.
- (41) Fukazawa, A.; Suda, S.; Taki, M.; Yamaguchi, E.; Grzybowski, M.; Sato, Y.; Higashiyama, T.; Yamaguchi, S. Phospha-fluorescein: a red-emissive fluorescein analogue with high photobleaching resistance. *Chem. Commun.* **2016**, *52*, 1120–1123.