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Solar Activation of Fungus Wearing Photothermal Clothes

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1. Supplementary Methods

Chemicals and Materials.

The fungal cells of *Aspergillus oryzae AS3.800* were received as lyophilized powder from Shanghai Jiachu Biological Engineering Co., Ltd. Pyrrole and Iodide were purchased from Shanghai Zane Energy Chemical Co., Ltd. Ferric chloride ($FeCl_3$) was purchased from Tianjin Damao Chemical Reagent Factory. The potato dextrose agar (PDA medium) was purchased from Beijing Land Bridge Technology Co., Ltd. and the potato dextrose broth (PDB) was purchased from Shanghai Bio-way Technology Co., Ltd. The water-soluble starch and the potassium iodide regent were purchased from Sinopharm Chemical Reagent Co., Ltd. The Coomassie Brilliant Blue regent (PA102) was purchased from Tiangen Biotech (Beijing) Co., Ltd.

Characterization methods.

Optical images were recorded by a Digital Camera (Canon EOS 70D). Cell viability was characterized on a Fluorescence Microscope (Zeiss Scope A1). Surface morphologies of fungal cells were characterized by Scanning Electron Microscope (SEM, JEOL-6700). UV-Vis-NIR absorption spectra were recorded on a spectrophotometer (Shimadzu UV-3600PLUS) and the solid material absorption was obtained from an Integrating Sphere Spectrophotometer (Shimadzu UH4150). The solar simulator with tunable power density was supplied from Crowmtech, INC (Solarbeam-02-3A). The temporal temperature was detected by a Thermocouple (Thermometer UT325). The infrared absorption was characterized by a Fourier Transform Infrared Spectrometer (Bruker

Tensor 27) and the Zeta Potential was recorded on a dynamic light scattering detector (Malvern NanoZS90).

Inoculum culture of *Aspergillus Oryzae*.

The lyophilized powder was activated to make enormous living *Aspergillus Oryzae AS3.800* (*Asp*) fungal cells by adopting the slant culture activation[1]. Typically, the lyophilized powder was primarily dissolved in 0.1 mL sterile water and then the solution was added into a 5 mL solid potato dextrose agar (PDA) slant. Sealed with a diaphragm, the slant was incubated at 28 ^{o}C for 3 days, and afterwards, white velvet shaped hyphae could be seen on the surface. An inoculating loop was subjected to transfer hyphae from the culture plant to a 50 mL potato dextrose broth (PDB) for another one-day incubation. The broth became turbid as the consequence of generation of living fungal cells in the broth. Then, the *Asp* liquor was subjected to centrifugation (5000 rpm, 2 min) and the sediment was washed by phosphate buffer saline (PBS) twice to remove the culture medium. By diluting the as-prepared solution with OD_{600} as 1.0, the turbidity unified mother liquor of the *Asp* was ready for further uses.

Fabrication of the polypyrrole-coated *Asp* hybrid solution.

The polypyrrole-coated *Asp* hybrid was fabricated via a two-step method (as illustrated in Scheme 1). Firstly, ferric ions were primarily adsorbed on the *Asp* cell surface. To be specific, 2 mL of the *Asp* mother liquor was centrifugated (5000 rpm, 2 min) and the sediment was soaked in 2 mL of the $FeCl_3$ aqueous solution (10 mM),

which was further placed in a constant temperature oscillator (150 rpm) at 25°C for 2h adsorption. Then, the solution was centrifuged for 2 min and washed by 2 mL PBS solution twice for fully removing the excessive unbounded Fe^{3+}. After the absorption of ferric ions, 100 μL of the pyrrole was added subsequently to the cell solution. Upon adding 5 μL HCl (0.1 M) to keep pH at 5.0, the mixture was then incubated sealed in the orbital shake (250 rpm) at 28 °C for 24h. Polypyrrole was in-situ formed on *Asp* cell surface to serve as a photothermal cloth, which accounts for the darkened solution color. Excessive pyrrole and uncombined polypyrrole were removed by repeated washing with PBS solution.

Concentration unification of the *Asp* and polypyrrole-coated *Asp* solutions.

For the sake of unifying fungal cell concentration in a more accurate way, an electronic scale was used to prepare standard concentration of *Asp* and polypyrrole-coated *Asp* solution by evaluating the weight difference among the Asp solutions and pure water. Specifically, 100 mL of the measured Asp solution was weighed after 100 mL of the pure water. The concentration of the *Asp* mother liquor (OD_{600} =1) and fresh polypyrrole-coated *Asp* solution were estimated as 1.7 mg/mL and 1.9 mg/mL separately. By centrifuging and concentrating, 10 mg/mL of the *Asp* and polypyrrole-coated *Asp* solution were fabricated. In the following experiments, all of the *Asp* and polypyrrole-coated *Asp* cell solutions were fresh for further uses.

The morphology characterization of fungal cells.

SEM observation was applied to characterize the surface morphology of fungal cells and illustrate the polypyrrole cloth closely dressed on the cells. Because fungal cells could be collapsed in the vacuum environment, the structure of fungal cells would be not easy to be identified unless special freezing of surface morphology. Specifically, 2 mL of the *Asp* mother liquor was centrifugated at 5000 rpm primarily followed with protein solidification upon adding 2 mL of 4% methanol aqueous solution into the centrifugal tube. After solidification at 28^{o}C for 12h, the tube was subjected to centrifugation and the sediment was washed by 25%, 50%, 75% and 95% ethanol aqueous ordinally to remove the residual solvent. Then 10 μL of the as-prepared liquid was added onto silicon wafer. The sample was prepared for SEM characterization after the total evaporation of solvents.

Evaluation of fungal cell viability.

The Fungal cell viability was evaluated according to the fluorescence staining method. Fungal cells were stained by a mixture of fluorescein Diacetate (FDA) and propidium Iodide (PI)[2]. PI could go across the dead cytomembrane to stain the DNA and emit red fluorescence, while FDA could release fluorescein with the help of nonspecific esterase and only the living cells possessing living cytomembrane are able to acquire a long-time fluorescence. In a typical dyeing process, 5 μL of the PDA (300 μg/mL) and PI (300 μg/mL) solution were added together into 1 mL of the polypyrrole-coated *Asp* hybrid solution for 30 min staining and then centrifugated and washed with deionized water twice. By exciting blue light (488nm) and green light (532nm), the sample gave

green and red fluorescence emission which represent living and dead cells accordingly. The relative cell viability was estimated by counting the green/red ratio of cell spots.

Budding reproduction of polypyrrole-coated *Asp* cells.

Living *Asp* cells are able to form new generations by budding reproduction. To further prove the maintaince of high *Asp* viability, the budding reproduction ability of polypyrrole-coated *Asp* cells was investigated. Typically, 100 μL of the polypyrrole-coated *Asp* standard liquor (10 mg/mL) was subjected into 50 mL of the potato dextrose broth (PDB) for a 24h cultivation at 28^{o}C. It was confirmed that new generation of the *Asp* cells were successfully grown up in the broth as the solution turned extremely turbid after cultivation. Then, the cultivating broth was centrifugated and washed by PBS (0.1 mol/L) solution twice to remove culture medium thoroughly. Following the characterization method as stated above, the surface micromorphology of the bud and the budding process were confirmed by SEM observation. Both mother cells and daughter cells are easy to be distinguished as the daughter cells are naked without polypyrrole clothes.

Photothermal conversion test.

The photothermal test was accomplished in a refrigerator cabinet to realize a constant temperature of 15^{o}C to mimic a cold ambient environment. 2 mL of the *Asp* standard liquor and the polypyrrole-coated *Asp* standard solution (10 mg/mL) were fixed in two cuvettes separately under the solar light simulator. The light power with variable power densities was recorded by an optical power meter. Illumination time was set as

30 min. The temporal temperature change in the mother liquor and the hybrid solution were monitored by a thermocouple dipped in the liquid.

The quantification of α-amylase production from *Asp* cells.

The α-amylase production was quantified by the well-known Coomassie Brilliant Blue method[3]. In a traditional process, the BSA protein was selected as a model protein to build a standard working curve. Specifically, 10-50 μL of the BSA standard solution was injected into six cuvettes and PBS solution was added to fulfill the solution to 150 μL and then 2.85 mL of the Coomassie standard solution was added. The BSA content has great effect on the solution color. *Asp* liquor was developed at different temperature based on the method as noted above. Then, α-amylase was extracted from the *Asp* mother liquor by following the $(NH_4)_2SO_4$/ PEG double water phase method[4]. Typically, 5 mL of the 50% PEG solution and 4 mL of the mother liquor cultivated at different temperature was mixed with 3 mL of the $(NH_4)_2SO_4$ saturated solution. The mixture was put on a vortex for rigorous agitation and then subjected to centrifugation for 5 min. The α-amylase was concentrated within liquor supernatant. By dyeing with the Coomassie Brilliant Blue agents, the relative protein content was quantified according to the absorbance of the supernatant liquid.

The characterization of α-amylase activity by starch decomposition.

The α-amylase activity is positively correlated with the rate of starch decomposition. Based on the chromogenic reaction between the iodine and the starch, more concentrated starch solution corresponds to deeper color. As the consequence of the

continuous decomposition of starch under the catalysis by α-amylase, the starch-iodine solution gradually faded into transparent. The standard calibration curve was obtained to ascertain the relationship of UV-vis/NIR absorption spectra of the iodine-starch solution with the amount of residual starch. The maximum absorption wavelength of the starch-iodine solution was chosen at 598 nm, which was then selected as the characteristic testing wavelength. The linear equation was fitted as A_{598}= 0.290 m + 0.0063, R^2=0.997. The starch-iodine test was used to monitor the degree of starch hydrolysis by the α-amylase under solar illumination and the concentration of the KI/I_2 solution was 200 mg/L (200 μL). The concentration of starch and polypyrrole-coated *Asp* hybrid solution were 10 g/L (2 mL) and 10 mg/mL (20 μL), respectively. After light illumination for 300s, the absorbance of the starch-iodine solution at the wavelength of 598 nm was measured by using the UV-Vis/NIR spectrometer.

Comprehensive demonstration of photothermal-tailored biocatalysis.

Photothermal conversion is envisioned able to accelerate the metabolism of *Asp* cells and the comprehensive catalytic ability was evaluated by the starch decomposition in the luminous and nonluminous batches. Two experiments were carried to illustrate the influence of photothermal conversion. In the first method, two cuvettes containing starch concentration (10 g/L, 2 mL) were placed at white and dark positions of a traditional Chinese Tai-chi diagram to vividly represent luminous and nonluminous treatment. Solar light was immediately supplied when polypyrrole-coated *Asp* solution (10 mg/mL, 20 μL) was added. As a result of the chromogenic

reaction with starch, I_2/KI solution (0.2 g/L, 0.2 mL) was introduced as color developing reagent to monitor the degree of starch decomposition at different hydrolysis time. Optical images of the solutions at different time (0 s, 150 s, 300 s, 450 s, 600 s, 750 s, 900 s) were characterized to exhibit the hydrolysis stages. In the second method, I_2/KI (0.2 g/L, 0.2 mL) were added into both luminous and nonluminous batches before light illumination. Two solutions are initially mazarine as a result of chromogenic reaction between starch and iodine. The phenomenon of gradual fading is indicative of starch hydrolysis. And time-dependent images at 0 s, 60 s, 120 s, 180 s, 240 s, and 300 s, were captured to visualize the starch decomposition at different stages. It is intriguing to find that the fading phenomenon was accelerated in this method compared with the first one, which may be due to the photothermal effect of I_2/KI.

Recycling ability of photothermal catalysis.

A three-round experiment was conducted to assess the repeated regulation of cellular metabolism based on photothermal conversion. Two cuvettes were settled at the dark and luminous poles to stand nonluminous and luminous batches separately. The initial solution in both cuvettes were composed of starch solution (10 g/L) and standard starch-iodine solution (0.2 g/L). Immediately the standard polypyrrole-coated *Asp* solution (10 mg/mL) was added into the cuvettes, solar light illumination was implemented to trigger the first round. After 5 min hydrolysis, the luminous batch faded into transparent while the nonluminous almost kept mazarine. Then, 2 mL of the starch solution (10 g/L) was added into the cuvette to start the second round and received another 5 min hydrolysis. The third round was carried samely. The recyclable

photothermal catalysis was verified by the successful fading phenomenon in the second-round and third-round tests with addition of new starch-iodine complex. Specifically, residual starch concentration in the three-round tests with solar illumination is 0.34, 0.89, 1.18 g/L, respectively, while it is 8.93, 8.42, 8.00 g/L without solar illumination. These results confirmed the reliable bioactivity of polypyrrole-coated fungus cells after multiple photothermal treatment, offering great promise for the repeated microorganism-based catalysis.

2. Supplementary Figures

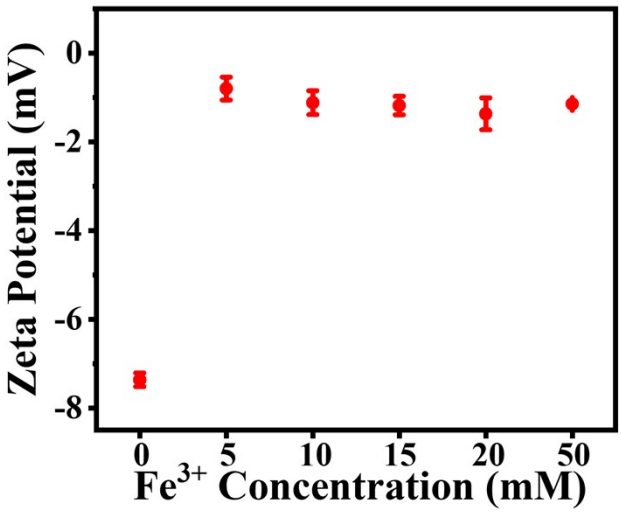

Fig. S1. The zeta potential of *Asp* cells before or after absorbing Fe^{3+} with different concentration: 5 mM, 10mM, 15mM, 20mM, 50mM.

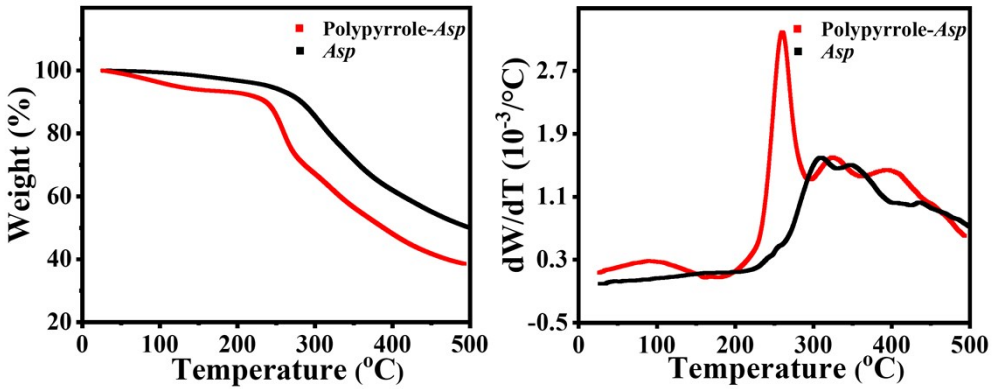

Fig. S2. (a) TGA and (b) DTG characterizations of the *Asp* and polypyrrole-coated *Asp*.

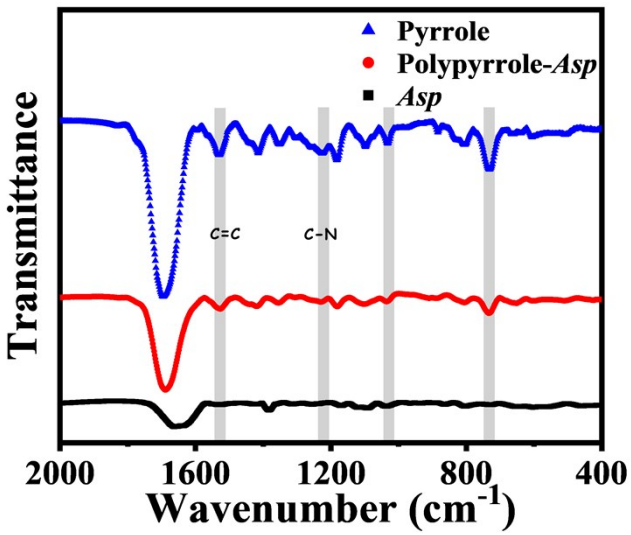

Fig. S3. FT-IR spectra of the pyrrole, *Asp* and polypyrrole-coated *Asp*. Characteristic peaks at 1247 cm^{-1} and 1530 cm^{-1} are assigned to stretching vibration of C–N and C=C bonds of polypyrrole, respectively.

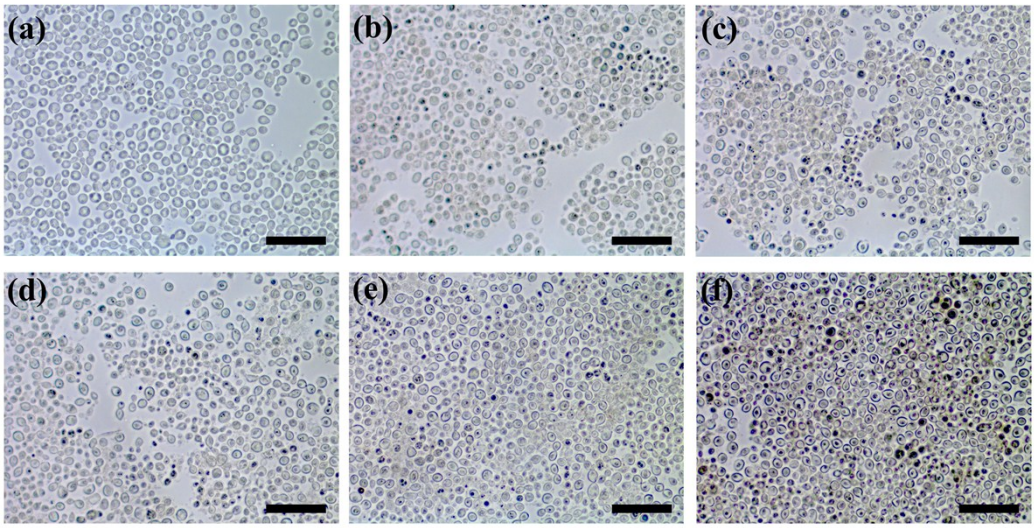

Fig. S4. Microscope images of the *Asp* and polypyrrole-coated *Asp* mad by $FeCl_3$ with different concentration. (a) 0 mM, (b) 5 mM, (c) 10 mM, (d) 15 mM, (e) 20 mM, (f) 50 mM. Scale bar is 50 μm.

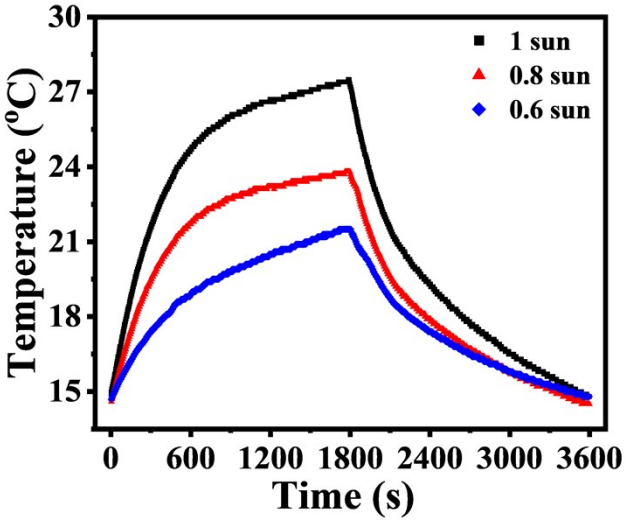

Fig. S5. The time-dependent temperature change of the neat *Asp* solution under solar illumination. Power density was set as 1.0 (black line), 0.8 (red line) and 0.6 sun (blue line), respectively.

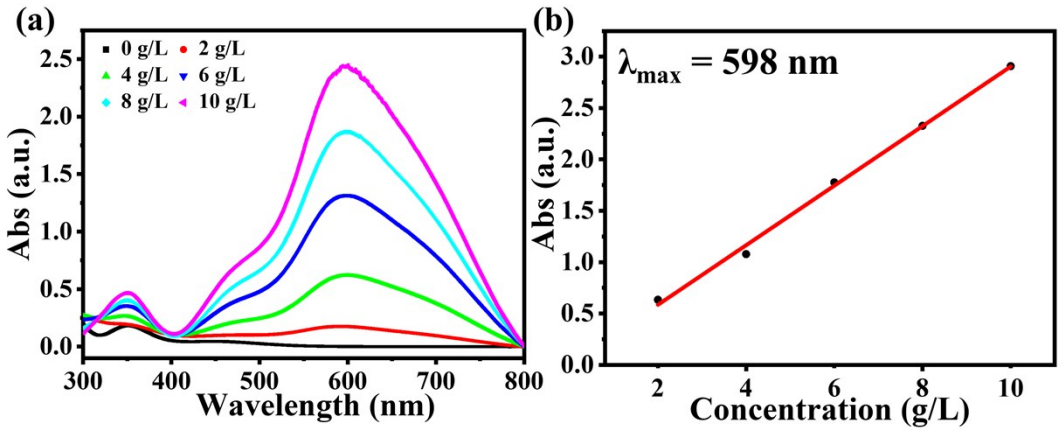

Fig. S6. (a) UV-vis/NIR absorption spectra of the iodine-starch solution with starch concentration: 0, 2, 4, 6, 8, 10 g/L. (b) Calibration curve of the iodine-starch solution. The specific absorption wavelength was set at 598 nm.

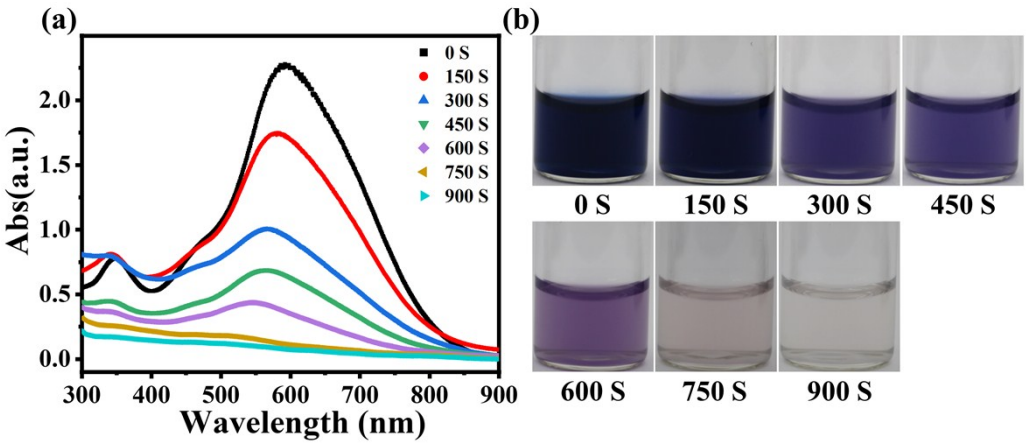

Fig. S7. (a) UV-vis/NIR absorption spectra and (b) corresponding optical images of starch-iodine solutions at different decomposition time.

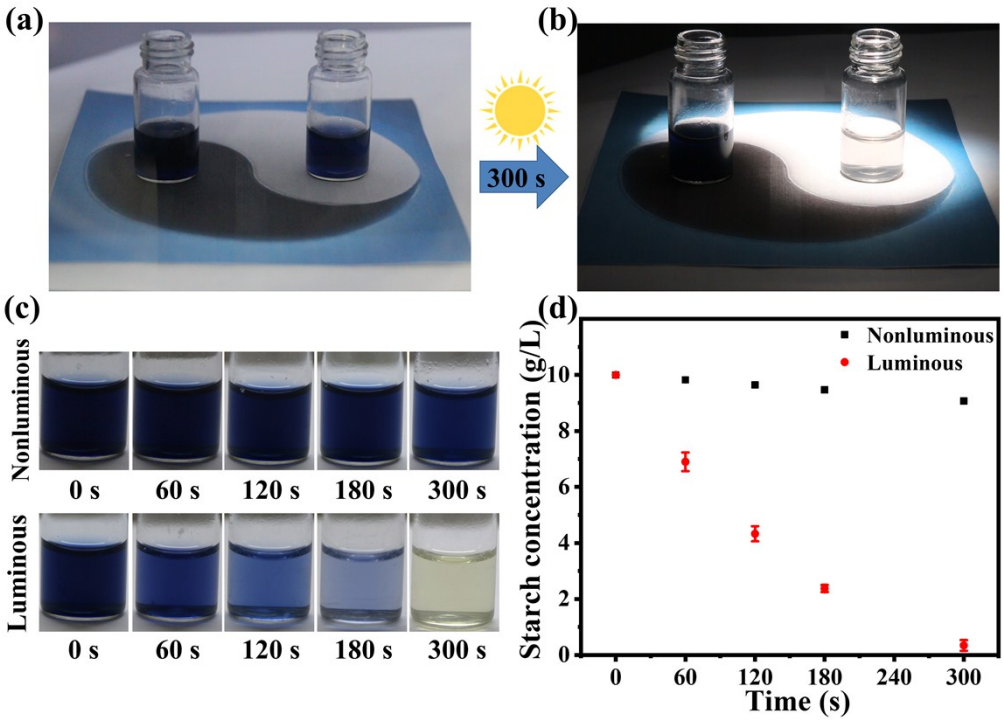

Fig. S8. Photothermal effect on the starch hydrolysis in the presence of polypyrrole-coated *Asp* cells. (a, b) Optical images of the mixture solutions containing starch, I_2/KI complex, and polypyrrole-coated *Asp* cells, before and after solar illumination for 5 min. (c) Optical images to demonstrate the color change and (d) time-dependent

starch concentration change in luminous or nonluminous batches. The power density is set as 0.6 sun in order to ensure the high cell viability.

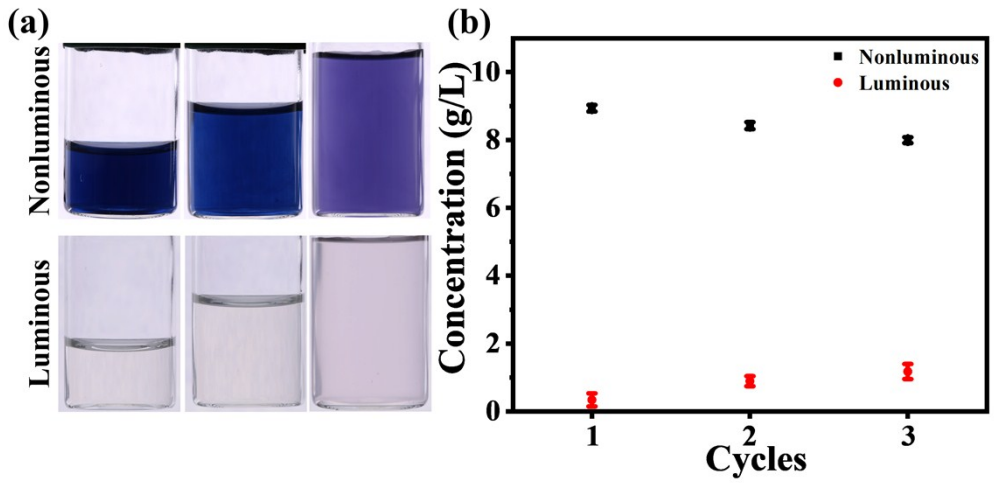

Fig. S9. Repeated photothermal treatment on fungus-based starch decomposition. (a) Optical images and (b) statistical results of residual iodine-starch solutions in three rounds.

Table S1. Cell viability of polypyrrole-coated *Asp* hybrid at different oxidant concentration.

Oxidant Concentration (mmol/L)	Relative cell viability (%)	Standard Deviation (%)
0	100	0
5	96.0	1.1
10	93.5	1.0
15	84.8	1.9
20	37.1	6.3
50	8.3	4.1

3. References for the Supporting Information

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