Photocatalytic Bacterial Inactivation by a Rape Pollen-MoS$_2$
Biohybrid Catalyst: Synergetic Effects and Inactivation Mechanisms

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Abstract

A novel and efficient 3D biohybrid photocatalyst, defective MoS$_2$ nanosheets encapsulated carbonized rape pollen, was fabricated and applied to water disinfection. The rape pollen-MoS$_2$ (PM) biohybrid showed excellent dispersibility, high stability, and efficient charge-carrier separation and migration ability, resulting in the highly enhanced photocatalytic inactivation performance towards various waterborne bacteria under different light sources. The inactivation mechanisms were systematically investigated. Reactive species (RSs), including electrons, holes, and reactive oxygen species ($\bullet$O$_2^-$ and $\bullet$OH), played major roles in inactivating bacteria. The antioxidant system of bacteria exhibited a self-protection capacity by eliminating the photo-generated RSs from PM biohybrid at the early stage of inactivation. With the accumulation of RSs, the cell membrane and membrane-associated functions were destroyed, as suggested by the collapse of cell envelope and subsequent loss of cell respiration and ATP synthesis capacity. The microscopic images further confirmed the destruction of the bacterial membrane. After losing the membrane barrier, the oxidation of cytoplasmic proteins and lipids caused by invaded RSs occurred readily. Finally, the leakage of DNA and RNA announced the irreversible death of bacteria. These results indicated that the bacterial inactivation began with the membrane rupture, followed by the oxidation and leakage of intracellular substances. This work not only provided a new insight into the combination of semiconductors with earth-abundant biomaterials for fabricating high-performance photocatalysts, but also revealed the underlying mechanisms of photocatalytic bacterial inactivation in depth.
1. Introduction

The ever-increasing global challenges towards water security and shortage emphasize the importance of developing efficient and low-cost disinfection techniques. However, traditional water disinfection techniques, such as ozonation, chlorination and ultraviolet irradiation, suffer from unavoidable drawbacks of recolonization, secondary pollution, and/or energy-intensive operation.\(^1\) To address these issues, the semiconductor-based photocatalytic water disinfection has been extensively studied, presenting a “green”, cost-effective, and promising strategy.\(^2,3\)

Owing to their earth-abundance, good stability and non-toxicity\(^4\), the carbonaceous materials, such as hydrothermal carbonation carbon (HTCC)\(^5\), graphene\(^6\), graphene carbon nitrides (g-C\(_3\)N\(_4\))\(^7\), and carbon nanotubes\(^8\), have been widely explored as catalysts or co-catalysts for photocatalysis. More recently, it has been reported that rape pollen, as a kind of plant-based material, showed high surface areas and a 3D porous network structure.\(^9\) The unique structure of rape pollen cannot only provide sufficient active sites for reactions, but also facilitate the separation of the photo-generated holes and electrons by decreasing their diffusion length.\(^10,11\) In addition, rape pollen possesses good light harvesting ability and high mechanical/chemical resistances due to its 3D carbon skeletons.\(^12,13\) More importantly, rape pollen is widely available and the rape pollen-derived materials can be easily obtained with low cost. This overcomes the disadvantages of conventional carbon-based photocatalysts prepared via complicated procedures with expensive chemical precursors.\(^9,14\) However, studies on the photocatalytic applications of rape pollen are quite limited. Very recently, the acid-treated rape pollen was utilized for visible light (VL) photocatalytic CO\(_2\) reduction in a solid-gas system.\(^9\) Unfortunately, bare rape pollen is highly hydrophobic, displaying obvious aggregation and poor dispersion in water, which remarkably inhibited its liquid-phase applications.\(^15\) It has been reported that after 60-min ultrasonic dispersion treatment, the rape pollen can only inactivate 7 log\(_{10}\) cfu/ml of \(E.\) coli cells within 180 min under VL irradiation.\(^16\) Hence, a smart design of rape pollen-based photocatalysts with good hydrophilicity and enhanced photocatalytic activity is desirable for water disinfection.
Molybdenum disulfide (MoS$_2$) has been reported as a hydrophilic material with high surface energy.\textsuperscript{17} This property probably makes it desirable to combine with rape pollen and enhance its water dispersibility. Typically, the bandgap of MoS$_2$ shifts from 1.9 to 1.29 eV by increasing the layers, which is favorable for light harvesting.\textsuperscript{18} In addition, few-layered MoS$_2$ possesses more active edge sites, which can effectively promote its photocatalytic activity.\textsuperscript{19} For example, MoS$_2$ with abundant active edge sites has been reported for enhanced photocatalytic hydrogen production.\textsuperscript{19} Cui \textit{et al.} reported that the vertically aligned few-layered MoS$_2$ could inactivate 5-log \textit{Escherichia coli} (\textit{E. coli}) within 2 h under VL.\textsuperscript{20} The exfoliated MoS$_2$ nanosheets exhibited antibacterial activity against both \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa} within 20 h.\textsuperscript{21} However, the preparation of these MoS$_2$ faces challenges of manipulation complexity, poor scalability and limited bacterial disinfection rate. The defect engineering can directly lead to the cracking of crystal and subsequently increase the accessible active edge sites. The defects in photocatalysts can also improve the light-harvesting capacity and charge separation efficiency by narrowing the bandgap and accelerating the charge-transfer.\textsuperscript{3} Xie \textit{et al.} reported that the defective MoS$_2$ with enhanced photocatalytic activity could be easily obtained by varying the concentration of precursors.\textsuperscript{22} However, the photocatalytic bacterial disinfection performance of the defect-rich MoS$_2$ has not been investigated.

Herein, for the first time, we developed a hydrophilic biohybrid photocatalyst, MoS$_2$ encapsulated rape pollen, by in-situ anchoring of defective MoS$_2$ nanosheets onto the rape pollen skeleton. The photocatalytic bacterial inactivation towards four common waterborne bacterial species (i.e. \textit{E. coli}, \textit{Pseudomonas aeruginosa}, \textit{Staphylococcus aureus}, and \textit{Bacillus pumilus}) by the defective MoS$_2$-rape pollen (PM) biohybrids were investigated. In order to gain an in-depth understanding of the inactivation pathway and the underlying mechanisms, the presence and roles of reactive species (RSs) were evaluated. Furthermore, the defense and damage of bacterial antioxidative enzyme system, the destruction of cell membrane, the loss of membrane-associated functions, and the final biomolecules leakage during the photocatalytic treatment process over PM were examined. This work would shed light on the

2. Experimental Section

2.1 Synthesis of Defective PM Biohybrids

A series of defective PM biohybrids were prepared by a facile hydrothermal treatment method. Typically, 1mM of sodium molybdate dihydrate (Na$_2$MoO$_4$•2H$_2$O) and 3mM of thiourea were dissolved in 40 mL of DI water, then 0.03 g of the as-prepared rape pollen (see in Supporting Information) was added into the solution. The mixed solution was sonicated for seconds and stirred for 1 h, then transferred to a 100-mL Teflon-lined stainless-steel autoclave and held at 200 °C for 24 h. After that, the black precipitate was washed with DI water and ethanol for several times and dried at 60 °C for 12 h. The product was denoted as PM1. Besides, the PM biohybrids with different loading amount of defective MoS$_2$ nanosheets were fabricated by varying the dosages of Na$_2$MoO$_4$•2H$_2$O / thiourea to 2/6, 4/12, 6/18, and 8/24 mM, respectively. The products were accordingly denoted as PM2, PM3, PM4 and PM5, respectively.

2.2 Photocatalytic Bacterial Inactivation

The photocatalytic bacterial inactivation activity and mechanisms were investigated by using *E. coli* K-12 as the model bacterium. The bacterial cells were harvested by centrifugation (13,000 rpm, 1 min) after incubation at 37 °C for 16 h. The cell pellets were washed with sterilized saline (0.9% NaCl) for twice times and resuspended in same volume of sterilized saline solution. Then 25 mg of catalysts was uniformly dispersed in 25 mL sterilized saline solution with supplement of 300 µL bacterial suspension to achieve $10^7$ cfu/mL of final cell density. The mixture was then illuminated by a 300-W Xenon lamp (PLS-SXE300C, Beijing Perfect Light Technology Co., Ltd. China) equipped with a 420 nm cutoff filter. At fixed time intervals, 0.5 mL of samples were collected and diluted to spread at Nutrient Agar (Lab M, Lancashire, U.K.) plates. The agar plates were incubated at 37 °C for 18h to count the viable cell. Besides, the different light spectra including full spectrum (without filter, 200 W/m$^2$), simulated sunlight (Air Mass 1.5 filters, 200 W/m$^2$), white LED light (400-840 nm, 100 W/m$^2$) as
well as the other three species of bacteria (P. aeruginosa, S. aureus and B. pumilus) were used to further investigate the inactivation performance of the as-prepared catalysts. All the bacterial disinfection experiments were conducted in triplicate.

2.3 Scavenger Study

In order to study the bacterial disinfection mechanisms of PM biohybrids, different scavengers were used to quench the corresponding RSs produced during the photocatalytic process. The addition concentration of scavengers were optimized before the experiments, which were 0.05 mM Cr (VI) for electrons (e\textsuperscript{-}), 0.5 mM sodium oxalate for holes (h\textsuperscript{+}), 2 mM isopropanol for hydroxyl radicals (•OH), 2 mM TEMPOL for superoxide radicals (•O\textsubscript{2}\textsuperscript{-}), 0.2 mM Fe(II)-EDTA for hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), 1mM L-histidine for singlet oxygen (\textsuperscript{1}O\textsubscript{2}). Besides, 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was used to trap the signals of •O\textsubscript{2}\textsuperscript{-} and •OH with the electron spin resonance (ESR) spin-trap technique under visible light irradiation and in the dark.

2.4 Enzyme Activity Assay

The cellular respiration ability was determined spectrophotometrically at 484 nm using 2,3,5-triphenyl tetrazolium (TTC) as substrate. The ATP synthesis ability was evaluated with ATP Detection Assay Kit-Luminescence (Cayman 700410, USA). The antioxidant enzymes containing superoxide dismutase (SOD) activity and catalase (CAT) activity were assayed using the Superoxide Dismutase Assay Kit (Cayman 706002, USA) and the Catalase Assay Kit (Cayman 707002, USA), respectively.

2.5 Biomolecule Oxidation and Leakage Assay

The oxidation of protein and lipid in cell were monitored by qualifying the levels of protein carbonyl (PC) and lipid peroxidation (LPO), which were evaluated using the Protein Carbonyl Colorimetric Assay Kit (Cayman 10005020, USA) and Lipid Hydroperoxide Assay Kit (Cayman 705003, USA), respectively. The released potassium (K\textsuperscript{+}) in the reaction mixture was determined by a polarized Zeeman atomic absorption spectrophotometer (AAS) (Hitachi Z-2300, Japan). The DNA and RNA leakage were measured with a Nanodrop spectrophotometer (Thermo Nanodrop One, USA).

The direct membrane destruction was monitored by SEM. Aliquots of sample
collected at given time intervals were firstly centrifuged and prefixed using 5% glutaraldehyde solution for 4 h. Then, the mixture solution was transferred to polylysine coated FTO after washing with phosphate buffer solution (PBS, 0.1 M, pH 7). The samples were dehydrated in the gradient ethanol and subjected to the critical-point drying (LADD 28002, USA). Finally, the cell membrane was observed with a SEM (FEI Quanta 400 F, USA) after gold-sputtering.

3. Results and Discussion

3.1 Characterization of Materials

The X-ray diffraction (XRD) patterns (Fig. S1) showed that a pure phase of MoS2 was successfully synthesized. The peaks were well indexed to 2H-MoS2 (PDF card No.:37-1492). Bare rape pollen displayed no obvious diffraction peaks, revealing its amorphous structure (Fig. S1). The XRD patterns of PM biohybrid series are consistent with that of MoS2 (Fig. S1-S2), indicating that the MoS2 structure remained unchanged after being coupled with rape pollen. The N2 adsorption-desorption isotherms of rape pollen, MoS2 and PM3 biohybrid indicated the presence of mesoporous and microporous structures (Fig. S3). As shown in Table S1, the surface areas of rape pollen, MoS2, and PM3 biohybrid are 4.21, 5.54 and 7.79 m2/g, respectively, indicating that encapsulation of MoS2 on pollen enhanced the surface area of the biohybrid. However, further increases of the loading amount of MoS2 on rape pollen decreased the surface area of biohybrids (PM4 and PM5).

The scanning electron microscopic (SEM) images (Fig. 1a) indicated that the rape pollen has an elliptical 3D porous network structure. Besides, the well distributed and abundant pore channels within the rape pollen structure were observed (Fig. 1b), contributing to the light harvesting ability. The scanning transmission electron microscopic (STEM) images (Fig.1c-d) further revealed the uniform distribution of channels within the hollow structure of rape pollen. As for the PM3 biohybrid, the MoS2 nanosheets were homogeneously grown on the carbon skeleton of rape pollen (Fig. 1e-f). Interestingly, even the micro-rods inside rape pollen were coated by MoS2 layers (Fig. 1g), greatly increasing the contact area between rape pollen and MoS2. The
elemental mappings (Fig. 1i-k) of PM3 not only confirmed the coexistence of rape pollen and MoS$_2$, but also indicated the uniform distribution of MoS$_2$ on rape pollen.

**Figure 1.** Characterization of the as-prepared catalyst. (a-b) SEM images of rape pollen, (c-d) STEM of rape pollen, SEM images of the (e) external surface, (f) internal surface and (g) inside micro-rods of PM3, elemental mappings of PM3 for (h) C, (i) N, (j) Mo, and (k) S. (Scale bar: (a), (c) and (d): 10 μm; (b), (e) and (f): 2 μm; (g) 1μm, (h)-(k): 0.5 μm )

The corresponding TEM images further verified the hollow structure of rape pollen (Fig. 2a-b) and the uniform distribution of MoS$_2$ on rape pollen (Fig. 2d). As shown in Fig. 2c and Fig. S4a, pure MoS$_2$ displayed a spherical structure formed by the self-assembled nanosheets, which was unfavorable for the exposure of active edges.
After loading MoS$_2$ on rape pollen, more active edges were exposed (Fig. 2e). In addition, the loaded MoS$_2$ displayed as lamellar flower-like nanosheets (Fig. 1e and 2d), which could improve the densities of active edges sites compared with the bulk MoS$_2$ with spherical morphology. It was reported that the active edges possessed higher catalytic activity than that of the in-plane surface$^{23}$, thus probably rendering the PM3 biohybrid with better bacterial disinfection performance. Moreover, the high resolution TEM (HRTEM) images (Fig. 2f) showed the well-defined lattice fringes within PM3. The interplanar space of 0.27 nm and the interlayer space of 0.62 nm were coincided with the individual plane of (100) and (002), respectively.$^{22}$ Besides, many dislocations and slight rotations of lattice fringes exist within the (100) planes, suggesting the presence of defects and distorted atomic arrangement on the basal plane. In addition to the defects, the deformed atomic alignment could also result in the dehiscence of the surface, thus forming more active edges within the PM3 biohybrid. To further investigate the defective structure of the biohybrid, the ESR spectra were recorded (Fig. S5). Explicit ESR signals attributed to the unpaired electrons were observed, further affirming the defective structure of the PM3 biohybrid. The X-ray photoelectron spectroscopy (XPS) was applied to investigate the atomic ratio of the PM3 biohybrid to confirm the origination of unpaired electrons and the defect type. According to the XPS results, the atomic ratio of S/Mo was 1.76 for PM3 biohybrid, which was lower than the theoretical value of bare MoS$_2$, suggesting the existence of sulfur defect. Besides, the mass ratio of rape pollen to MoS$_2$ in the PM3 biohybrid was evaluated through the thermogravimetric analysis (TGA). As shown in Fig. S6, the evaporation of adsorbed water occurred below 200 °C, whereas the decomposition of rape pollen occurred at 200-400 °C. The remarkable mass loss was observed in the temperature range of 700-800 °C, which was ascribed to the oxidation of MoS$_2$ to Mo$_2$O$_3$. Consequently, the mass ratio of rape pollen to MoS$_2$ within PM3 biohybrid was estimated to be 0.124.
The dispersity and hydrophilicity of the as-prepared materials were evaluated by the polydispersity index (PDI) obtained from dynamic light scattering (DLS) and the contact angle (CA) measurements, respectively. As shown in the Table S2, the PDI value of PM3 biohybrid was slightly lower than that of MoS$_2$, implying that the PM3 biohybrid has a better dispersity in water than that of MoS$_2$. The high PDI value of rape pollen even exceeded the detection limit, showing its poor dispersity in water. As confirmed by the Fig. 3a-b, both PM3 and MoS$_2$ were well dispersed after 30 seconds of sonication, whereas rape pollen still severely aggregated and floated on water. Furthermore, the CA of PM3 was 4.5° (Fig. 3c), far less than 10°, illustrating its superhydrophilic (SHL) property. On the contrary, rape pollen showed a highly hydrophobicity with the CA of 132.6° (Fig. 3e). Compared with PM3, bulk MoS$_2$ demonstrated a moderate hydrophobicity (CA 38.1°). This might be caused by the structural differences between bulk MoS$_2$ and PM3 biohybrid. The bulk MoS$_2$ exhibited...
a microsphere morphology because of its continuous growth and self-assembly, which
was easier to form a well-ordered surface and limited the exposure of the defect-
induced active sites. As for the PM3 biohybrid, MoS$_2$ nanosheets are homogenously
located on the surface of pollen, leading to more exposed MoS$_2$ surface and defects.
The uniform distribution of MoS$_2$ improves the densities of active edges sites. The
combination of rape pollen with defective MoS$_2$ also enhances the spatial separation
and migration of electrons and holes. These together promoted the production of
hydrophilic ROS absorbed on the surface of PM3 biohybrid and enhance the polarity
for better dispersing in water. In addition, the dispersive components, polar
components and surface energy of rape pollen, MoS$_2$ and PM3 were calculated to
further investigate the dispersity and hydrophilicity of the catalysts. In addition to the
contact angle with water, the contact angle was measured with diiodomethane (CH$_2$I$_2$)
for all samples. The surface free energy and polarity were calculated according to the
Harmonic mean equations, as shown below:

\[
(1 + \cos \theta_1)\gamma_1 = 4[\gamma_1^d \gamma_1^d / (\gamma_1^d + \gamma_1^d) + \gamma_1^p \gamma_1^p / (\gamma_1^p + \gamma_1^p)]
\]

\[
(1 + \cos \theta_2)\gamma_2 = 4[\gamma_2^d \gamma_2^d / (\gamma_2^d + \gamma_2^d) + \gamma_2^p \gamma_2^p / (\gamma_2^p + \gamma_2^p)]
\]

Where $\gamma_1^d$ represents the dispersive components and $\gamma_1^p$ indicates the polar
components. $\theta_1$ and $\theta_2$ are the contact angle of the material in water and
diodomethane, respectively. The value of $\gamma_1^d$, $\gamma_1^p$ and $\gamma_1^p$ are 72.8, 22.1 and 50.7
mJ/m$^2$ in water, and the values of $\gamma_2^d$, $\gamma_2^d$ and $\gamma_2^p$ are 50.8, 44.1 and 6.7 mJ/m$^2$ in
diodomethane.

As shown in the Table S3, the dispersive component (35.96 mJ/m$^2$), polar
component (40.14 mJ/m$^2$) as well as surface energy values (76.1 mJ/m$^2$) of PM3
biohybrid were higher than those of rape pollen and MoS$_2$, which together contributed
to the excellent dispersity and hydrophilicity of the PM3 biohybrid. These properties of
PM3 would favor the contact between photocatalysts and bacterial targets, thus
promoting the photocatalytic disinfection performance in a liquid-phase reaction.
Figure 3. Photograph of the catalyst suspension (a) before sonication and (b) after ultrasonication for 30 s; Contact angle of (c) PM3 biohybrid, (d) MoS$_2$, and (e) rape pollen.

The elemental composition and band structure of the as-prepared catalysts were measured by XPS and UV-Vis diffuse reflectance spectroscopy (DRS) spectra, respectively. As presented in Fig. S7a-b, the similar intensive signals at Mo 3d$_{3/2}$, Mo 3d$_{5/2}$, S 3d$_{3/2}$, S 2p$_{1/2}$, S 2p$_{3/2}$ peaks suggested the dominant existences of Mo$^{4+}$ and S$^{2-}$. Compared with pristine rape pollen, the C 1s spectra of pollen-MoS$_2$ biohybrid shifted to the high binding energy, whereas its O 1s spectrum shifted to the low binding energy, which were caused by the electron transfer and strong interaction between rape pollen and MoS$_2$ based on the previous reports.$^{25,26}$ Similarly, the banding energy also slightly shifted in the spectra of Mo 3d and S 2p. The change of the peaks within the spectra of C 1s and O 1s between rape pollen and pollen-MoS$_2$ biohybrid might be due to the covalent binding of functional groups in rape pollen with MoS$_2$. The light absorption capacity was shown in Fig. S8, suggesting the as-prepared materials exhibited a broad light absorption range. The bandgap structures were calculated through the Tauc plot.
(Fig. S9) based on UV-vis spectra\textsuperscript{28}, which were 1.45 eV and 1.84 eV for MoS\textsubscript{2} and rape pollen, respectively. The valence band maximum of MoS\textsubscript{2} and rape pollen were estimated to be 1.28 eV and 0.97 eV, respectively, according to the results of valance-band XPS spectra (Fig. S10). Consequently, rape pollen and MoS\textsubscript{2} showed well-aligned straddling band structures, which might favor the charge-carrier transfer and enhance the photocatalytic activity.

### 3.2 Photocatalytic Bacterial Inactivation Efficiency

A gram-negative bacterium, \textit{E. coli}, was chosen to evaluate the photocatalytic activity of the PM biohybrid catalysts. The optimization experiments were firstly conducted to investigate the bacterial inactivation efficiency of defective PM biohybrids with different MoS\textsubscript{2} thickness as well as defect-free biohybrids, which was shown in Figure S11. The encapsulation of defect-free MoS\textsubscript{2} on the rape pollen showed no obvious bacterial inactivation effect as the cell density remained almost unchanged within 180 min. However, with the introduction of the defective MoS\textsubscript{2} coating on the rape pollen (Mo:S = 1:3), there was a reduction of 1.7 log\textsubscript{10} cfu/mL of cell within 180 min. That meant about 97.85\% of \textit{E. coli} were inactivated by the combination of the biohybrid. Interestingly, the bacterial inactivation efficiency decreased when further increased the thickness of defective MoS\textsubscript{2} on pollen, among which the ratios of Mo:S were 1:4 and 1:5, respectively. Similar to our results, Xie \textit{et al.} has also reported that the ultrathin MoS\textsubscript{2} assembles possessed higher density of active sites compared with thicker MoS\textsubscript{2} nanosheet (Mo:S = 1:5), thus showing better hydrogen production efficiency.\textsuperscript{22} Therefore, after optimizing the molar ratio of Mo and S during the synthesis of defective PM, the photocatalytic performance of PM biohybrids with different loading amount of MoS\textsubscript{2} was investigated under VL irradiation (Fig. 4a).

The bacterial cell density (10\textsuperscript{7} cfu/mL) remained unchanged within 180 min in the light control (without photocatalyst) and dark control (without irradiation) experiments (Fig. S11-S12), indicating that both light irradiation (1,000 W/m\textsuperscript{2}) and photocatalysts had no harmful effect on bacteria. As shown in Fig. 4a, rape pollen exhibited a relatively low photocatalytic bacterial inactivation efficiency, which only inactivated 0.9 log\textsubscript{10}
cfu/mL of cell within 180 min. The defective MoS$_2$ showed a moderate bacterial inactivation efficiency with a complete bacterial inactivation within 180 min. Impressively, the PM biohybrids (except PM1) exhibited an obviously enhanced photocatalytic bacterial inactivation performance. Among them, PM3 showed the highest bacterial inactivation efficiency, with a rapid bacterial inactivation of 7 log$_{10}$ cfu/mL within 60 min under VL. The photocatalytic bacterial activity decreased when loading more defective MoS$_2$ on rape pollen (e.g., PM4 and PM5), which might be attributed to the light blocking by the extra thick MoS$_2$ layers and the stacking of the self-assembled MoS$_2$ spheres (Fig. S4c). Also, abundant active edges were protruded from the PM3 biohybrid (Fig. 2e), resulting in the better exposure of defect-induced active sites, which thereby contributed to bacterial inactivation. Furthermore, the normalized bacterial inactivation efficiency of the as-prepared catalysts by the surface areas also indicated the best inactivation efficiency of PM3 biohybrid (Fig. S13). The results showed that photocatalytic bacterial efficiency was almost the same before and after the normalization. This revealed that the increased surface area of PM biohybrids made a minor contribution to the photocatalytic performance. It was induced that the synergetic effect of rape pollen and MoS$_2$ rather than the changed surface area mainly led to the significantly improved photocatalytic activity of the PM biohybrids. On the other hand, the photothermal effect on bacteria inactivation was studied without using water bath. As shown in Fig. S14a, a slightly enhanced bacterial inactivation was observed in the first 30 min in the system without using water circulation bath than that with the temperature control system. Nevertheless, they exhibited a similar photocatalytic disinfection efficiency. Under same reaction condition, the suspension temperature increased rapidly in the first 45 min and then remained unchanged (Fig. S14b). These results indicated that the photothermal effect was involved in the photocatalytic inactivation process, but it was not a dominant factor.

Besides, the inactivation performance of PM3 biohybrid was compared with some carbon-based and advanced materials under the same VL intensity (1000 W/m$^2$) (Fig. S15). Compared to the recently reported iodine doped hydrothermal carbonation carbon (I-HTCC) and the widely studied C$_3$N$_4$, the photocatalytic inactivation of PM3
biohybrid was much more outstanding. Moreover, graphite, carbon mesoporous and red phosphorous (Red-P) exhibited poor bacterial inactivation efficiency, with a slight reduction of the bacterial density within 180 min. Besides these materials, the photocatalytic bacterial inactivation performance of PM3 is even better than those of the recently reported composite catalysts (Table S4). These results indicated that the PM3 biohybrid is an excellent photocatalyst for bacterial inactivation under VL.

Owing to the high energy-efficiency and long-life span, LED lamp were widely used in our daily life and considered as the next generation of light source. Meanwhile, the natural solar-driven photocatalysis was considered as a “green” and efficient water treatment method. Thus, the photocatalytic inactivation performances of PM3 biohybrid were further evaluated under artificial LED light and simulated natural light irradiation. As shown in Fig. 4b, a complete 7-log₁₀ cell inactivation was achieved within 150 min under white LED light (100 W/m²) with low photoexcitation energy. By comparison, the inactivation efficiency of PM3 biohybrid is also higher than the recently reported photocatalysts under similar reaction conditions (Table S5). This indicated the great potential of PM3 biohybrid as a photocatalyst for indoor sterilization.

In addition, the inactivation efficiency was greatly improved under the illumination of simulated sunlight (200 W/m²) (Fig. 4b). The light control experiment demonstrated that the simulated sunlight had no photolysis on E. coli (Fig. S16b). Under the same intensity of sunlight, a complete inactivation of 7-log E. coli cells was achieved by PM3 within 45 min. Besides, the solar-driven bacterial inactivation efficiency was compared with the currently adopted disinfection methods (Table S6). Such efficiency is comparable and even better than those of currently adopted water disinfection methods. This suggests that the PM3 biohybrid can be used as a promising photocatalyst for water disinfection.

The photocatalytic performance of PM3 towards other waterborne bacteria such as P. aeruginosa, S. aureus and B. pumilus were also investigated under VL irradiation (Fig. 4c). The light and dark control experiments illustrated that both the PM3 biohybrid and light intensity (1,000 W/m²) showed no harmful effects on bacteria (Fig. S17). As displayed in Fig. 4c, the S. aureus and P. aeruginosa were completely inactivated within
45 min and 60 min, respectively. The disinfection rate of PM3 biohybrid exceeds those of TiO$_2$ and MnO$_2$ based materials, as reported earlier.$^{16}$ The disinfection rate of $B.\ pumilus$ was relatively low, with a 2.15 log$_{10}$ cfu/mL reduction within 60 min, which was probably caused by the protection effect of its thick peptidoglycan cell wall and its strong sporulating ability.$^{29}$ However, it was satisfactory because about 99.9% of $B.\ pumilus$ cells were inactivated within a short time period (15 min).

To evaluate the environmental application of the newly developed PM3 biohybrid, the photocatalytic bacterial inactivation experiments were conducted in authentic water systems. As shown in Fig. 4d, the photocatalytic inactivation efficiency of the PM3 biohybrid in surface water collected from The Chinese University of Hong Kong (CUHK) stream was close to that in saline solution, with a total inactivation of 7 log$_{10}$ cfu/ml cell within 60 min. Similarly, a complete 7 log$_{10}$ cfu/ml cell inactivation was achieved within 90 min by the PM3 biohybrid in the Tai Po river sample. The bacterial inactivation efficiency in the secondarily treated sewage effluent samples decreased compared with that in surface water samples. It was observed that the complete reduction of 7 log$_{10}$ cfu/ml cell densities within 150 min in effluents samples collected from Tai Po and Sha Tin wastewater treatment works, respectively. The decline inactivation efficiency was attributed to the presence of organic components in the effluent, which competed with bacterial cells for RSs, leading to a relatively long time to produce sufficient RSs to completely inactivate bacterial cells. As shown in Table S7, the concentration of total carbon (TC), total organic carbon (TOC) and total nitrogen (TN) in the secondary wastewater effluents were much higher than those in the surface water. However, the photocatalytic inactivation performance in effluents is satisfactory because it is even better than that performed in saline solution or ultrapure water, as reported previously in Table S4. Thus, the PM3 biohybrid shows a great potential in authentic water disinfection.

To further investigate the photostability of the PM3 biohybrid, the VL-driven photocatalytic bacterial inactivation experiment was repeated for five times (Fig. S18). The PM3 biohybrid still worked efficiently after five cycles, confirming its good stability and reusability. In summary, the PM3 biohybrid possesses a broad-spectrum
photocatalytic inactivation ability towards various waterborne bacteria with high stability. In addition, MoS$_2$ and rape pollen showed good biocompatibility and have been used for photothermal therapy (PTT) of cancer and drug delivery, respectively $^{30,31}$ Therefore, the practical utilization of the PM3 biohybrid might be extended to more fields.

Figure 4. (a) Photocatalytic inactivation of $E$. coli by the as-prepared catalysts under VL irradiation (1,000 W/m$^2$), (b) photocatalytic inactivation of $E$. coli by PM3 under xenon lamp full-spectra irradiation (200 W/m$^2$), simulated sunlight (200 W/m$^2$), and white LED light (100 W/m$^2$), (c) photocatalytic inactivation towards $P$. aeruginosa, $S$. aureus, and $B$. pumilus by PM3 under VL irradiation (1,000 W/m$^2$), (d) photocatalytic inactivation of $E$. coli in authentic water samples by the PM3 biohybrid under VL irradiation (1,000 W/m$^2$).

3.3 Photocatalytic Bacterial Mechanisms

The scavenger studies were conducted to investigate the photocatalytic bacterial mechanisms by analyzing the contributions of the specific RSs. Before the experiments, the applied scavenger concentrations were optimized to ensure the maximum
scavenging effect but with no toxicity to bacteria. As shown in Fig. 5a, the addition of sodium oxalate significantly inhibited the photocatalytic bacterial inactivation process, indicating that holes were strongly involved in the reaction. Besides, the important role of photoinduced electrons were affirmed by the prominent reduction of inactivation efficiency after adding Cr (IV) in the suspension. The significant role of electrons and holes suggested a possible Z-scheme charge carriers migration mode in the PM3 biohybrid, which meant that the electrons generated from the conduction band (CB) of MoS$_2$ would transfer to the valence band (VB) of rape pollen caused by the internal electric fields, maintaining the strong reduction ability of electrons and the strong oxidation ability of holes. Therefore, the VB of MoS$_2$ (VBM = 1.28 V vs NHE) within the PM3 hybrid was positive enough to produce abundant holes under VL irradiation. As holes were mainly accessible on the surface of catalysts, the good dispersibility and hydrophilicity of the PM3 biohybrid (Fig. 3) provided sufficient contact areas between the biohybrid and cells. Meanwhile, the CB of rape pollen (CBM = -0.87V vs NHE) was sufficiently negative to produce ample electrons, which could directly damage the cell by attacking cellular biological molecules and impairing respiration chain. Also, the electron-induced reactive oxidative species (ROS) have great oxidative potential for inactivating *E. coli* cells in nonselective reactions and eventually lead to cell death. Among the ROS, •O$_2^-$ demonstrated the most important role as the disinfection of *E. coli* was dramatically prohibited with the addition of TEMPOL (Fig. 5a). The leading bactericidal effect of •O$_2^-$ was probably because the redox potential for O$_2$/•O$_2^-$ (-0.33V vs NHE) was thermodynamically favored by CB of rape pollen (0.87V vs NHE) within the biohybrid. This further confirmed the Z-scheme charge transfer mode in the PM3 biohybrid because the CB of MoS$_2$ (CBM = -0.14 V vs NHE) was not negative enough to produce O$_2$/•O$_2^-$. To validate the Z-scheme mode within the PM3 biohybrid, the ESR analyses were carried out to detect the spin •O$_2^-$ produced by the individual catalysts and composite catalysts, respectively. As shown in Fig. 5b, four significant characteristic peaks of DMPO•O$_2^-$ signals with identical intensity were observed in the ESR spectra of PM3 biohybrid under VL irradiation, which were higher than that of rape pollen. However, no signal was detected for MoS$_2$ under VL irradiation. As Fig.
S19 showed that the DMPO-•O_2^- signals were not observed in the dark for all the photocatalysts, indicating that the •O_2^- radicals cannot be generated without light irradiation. Consequently, all the above evidences indicated that the as-prepared PM3 biohybrid followed the Z-scheme electron transfer path rather than the traditional double-transfer mode. Otherwise, the photo-generated electrons would accumulate at the CB of MoS_2 and thus lead to no production of •O_2^- due to its much lower reducibility within the double-transfer mode. The unique Z-scheme of the PM3 biohybrid can maximize the overpotential by utilizing the high CB of rape pollen and low VB of MoS_2. Under the Z-scheme mode, the photogenerated electrons would accumulate at the CB of rape pollen, leading to a strong reduction ability of rape pollen and production of enough •O_2^- radicals, as demonstrated in Scheme S1. Besides, •OH played a moderate role in the antibacterial process, as revealed by the moderately decreased bacterial inactivation activity after adding isopropanol (Fig. 5a). It was noted that the generation of •OH production was thermodynamically inhibited since the VB of MoS_2 (1.28 V vs NHE) cannot directly convert H_2O or OH^- to •OH (H_2O/ •OH=1.99 eV vs NHE, OH^-/ •OH=2.38 eV vs NHE). Nevertheless, •OH could be produced through a multistep pathway, as shown in the following equations:

\[
\begin{align*}
O_2 + e^- &\rightarrow •O_2^- \quad (1) \\
H^+ + •O_2^- &\rightarrow •HO_2 \quad (2) \\
•HO_2 + H^+ + e^- &\rightarrow H_2O_2 \quad (3) \\
H_2O_2 + e^- &\rightarrow •OH + OH^- \quad (4)
\end{align*}
\]

Compared with •OH, •O_2^- is more easily to accumulate because its lifetimes (t_{1/2} ≈ 1 µs) is much longer than that of •OH (t_{1/2} ≈ 1 ns). This was another possible reason why •O_2^- was more reactive for the bacterial inactivation. On the contrary, the disinfection efficiency almost remained unchanged with the addition of Fe-EDTA and L-histidine, indicating that H_2O_2 and ^1O_2 played negligible roles in this photocatalytic system. H_2O_2 and ^1O_2 radicals can be produced by the stepwise oxidation of H_2O, but accounted for a very small portion of the generated ROS and were hardly to participated
in the antibacterial process.

To further investigate the production of •O$_2^-$ and •OH radicals by the PM3 biohybrid under VL irradiation, the ESR spin-trap spectra were recorded. Fig. 5c-d showed that both signals of DMPO-•O$_2^-$ and DMPO-•OH adducts were detected under VL irradiation, whereas there were no signals of •O$_2^-$ and •OH in dark. This indicated the formation of •O$_2^-$ and •OH during the VL-driven bacterial inactivation process. The above results illustrated that the photogenerated holes and electrons as well as the derived ROS (•O$_2^-$ and •OH) were the dominant effective species for the inactivation of £. coli in present system. In this respect, the generation ROS by PM4 biohybrid that showed the best bacterial inactivation efficiency among the rest PM biohybrids series except for PM3, was further investigated as a comparison to analyze the excellent photocatalytic inactivation performance of PM3 biohybrid. As shown in Fig. S20a-b, the signal intensities of •O$_2^-$ and •OH produced by the PM3 biohybrid were higher than that of PM4 biohybrid under VL irradiation. There were no signals of •O$_2^-$ and •OH detected in the dark (Fig. S20c-d) for both PM3 and PM4 biohybrids, indicating that the ROS were generated under excitation of VL irradiation. Hence, the stronger signals of •O$_2^-$ and •OH within the PM3 biohybrid can be more effectively to inactivate the bacterial cells since ROS played important role in the bacterial inactivation process.

The photoelectrochemical experiments were conducted to further study the mechanisms of the promoted photocatalytic activity of the PM3 biohybrid. As demonstrated in Fig. S21a, both PM3 and PM4 biohybrids exhibited dramatically enhanced photocurrents compared with bare rape pollen and MoS$_2$, indicating that the combination of rape pollen and MoS$_2$ could enhance the transfer efficiency of photoinduced electrons. The higher photocurrent of PM3 suggested that a higher photogenerated charger-carrier density. The electrochemical impedance spectroscopy Nyquist plots reflected that the PM3 biohybrid had a smaller semicircular arc than those of PM4 biohybrid, rape pollen and MoS$_2$ (Fig. S21b). This indicated that the interfacial electrons and holes generated by the PM3 biohybrid could more easily transfer to acceptors, resulting in the enhancement of photocatalytic performance. Moreover, the recombination rate of the photoinduced charge carriers were investigated by room-
temperature photoluminescence (PL) spectra (Fig. S21c). The weak and narrow PL emission peak of the PM3 and PM4 biohybrids revealed that the loading of defective MoS$_2$ on pollen could effectively inhibit the recombination of electrons and holes$^{35,36}$, which was favorable for the better utilization of charge carriers and thus accelerated the inactivation process. On the other hand, the better charge carriers separation ability of PM biohybrids can be attributed by the defective structure of MoS$_2$ loaded on rape pollen, because the defects provide temporary trapping sites for electrons and shorten the diffusion distance of electrons and holes to surface.$^{37}$ Thus the encapsulation of defective MoS$_2$ on rape pollen can enhance the electron transfer and suppress the recombination of charge carriers, and eventually favor the photocatalytic performance of bacterial inactivation.

**Figure 5.** (a) VL-driven photocatalytic inactivation of *E. coli* by PM3 biohybrid with different scavengers, ESR spectra of (b) superoxide radicals of MoS$_2$, rape pollen and PM3 biohybrid under VL irradiation, (c) superoxide radicals of PM3 biohybrid and (d) hydroxyl radicals of PM3 biohybrid under VL irradiation and in the dark.
3.4 Bacterial Cell Damage Process

To better understand the process of bacterial cell damage induced by the PM3 biohybrid, an in-depth investigation of the bacterial cell damage processes was conducted. Firstly, the antioxidant enzymes including SOD and CAT were measured. The SOD can dismutase \( \cdot \text{O}_2^- \) to \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \), and CAT can catalyze \( \text{H}_2\text{O}_2 \) to \( \text{O}_2 \) and \( \text{H}_2\text{O} \).\(^{38, 39}\) Thus, the intracellular SOD and CAT act as scavengers for ROS and effectively protect cell from the ROS-induced oxidative damage. As shown in Fig. 6a, the activities of SOD and CAT increased firstly and then declined rapidly after 30 min during the inactivation process. The increment in the antioxidant enzyme activities indicated that \( \text{E. coli} \) cells initiated a self-protection behavior when facing the oxidative damage of ROS. This might also explain the “shoulder” stage of bacterial disinfection in the first 30 min (Fig. 4a). But with the accumulation of ROS, these two enzymes were destroyed, and the cell defense capacity was overwhelmed.

After destruction of the oxidative defense system, attacks started from the bacterial membrane. The bacterial cell membranes mainly consist of lipids and proteins, exhibiting as the phospholipid bilayer structure including inward- and outward-facing leaflets\(^ {40} \), which is selectively permeable and responsible for the energy-dependent reactions, such as ATP synthesis, ion transportation and metabolism.\(^ {41} \) Therefore, the inactivation patterns of membrane-bounded enzymes and metabolism during the photocatalytic inactivation process were investigated.

The membrane-bounded cell respiration was firstly evaluated through the reduction of 2,3,5-TTC since the cytochrome in respiration chain could utilize the oxidized form of substrate such as \( \text{O}_2 \) and TTC as electron acceptors to produce energy.\(^ {16} \) As shown in Fig. 6b, the respiratory ability decreased drastically with increasing irradiation time, which was caused by the short-circuiting of the electron transport chains with the breaks of membrane conformation. The final loss of the respiration ability implied that the destruction of respiration chain could no longer maintain the redox potential and produce protons for energy production. ATP is the main energy currency in cell, which is synthetized through the oxidation of substrate driven by the proton electrodynamic force produced in respiration chain.\(^ {42} \) Accordingly, the synthesis of ATP was monitored
to reveal the damage of cell membrane and metabolism. Like cell respiration, the ATP level descended with the prolonged reaction time (Fig. 6b). Interestingly, the decline rate gradually became slow with increasing reaction time. As the ATP could be temporarily used for membrane repair and transmembrane potential maintenance, thus the rapid decrease of ATP at the beginning was probably attributed to the energy consumption for self-repair until the cell was completely inactivated. This phenomenon was well coincided with the commencement of the antioxidative enzyme system at the initial stage (Fig. 6a). Compared with the trend of cell respiration, the slower drop of ATP level was observed since E. coli was facultative anaerobe bacteria and could synthetize tiny amounts of ATP through substrate phosphorylation in a short time period after the damage of respiration chain.

The damaged membrane-bounded bacterial energy metabolism system (ATP synthesis ability and cell respiration chain) were not able to maintain the membrane potential, which would accompany with the compartmentation of bacterial membranes. The above results illustrated the damage of bacterial membranes at the energy metabolism functional level. The membrane damage process was also explored on its structural level to reveal how RSs acted on bacterial membranes to cause membrane disintegration. The RSs including holes and electrons-induced ROS can oxidize the membrane-bounded lipids and proteins, destroy the conformational structures and physicochemical properties of these molecules, resulting in the dysfunction and disintegrate of the membranes. Thus, the oxidation of the cellular building blocks such as proteins and lipids were investigated to reflect the disintegration of membranes and the final bacterial decomposition process.

LPO is an indicator of hydroperoxides produced by the oxidation of saturated and unsaturated lipids by ROS. Since lipids are responsible for the fluidity and permeability of membrane bilayer, the increased LPO level in Fig. 6c suggested the decrease of diffusion ability and the damage of bilayer. However, the hydroperoxides were highly reactive and unstable, which can decompose to a range of products such as epoxides, ketones, acids, and aldehydes under conditions of high level of RSs. Besides, the transition metal ions can accelerate the decomposition of lipid peroxides by
chelation. The molybdenum in the PM3 biohybrids may boost the degradation of lipid peroxides in interfacial reaction during later reaction period. Thus the decline of the LPO after 30 min in the experiment was caused by the decomposition of the LPO. The hydrogen atom from lipids has one proton and a single electron, which is easily attacked by the ROS. So when a radical gives one electron to a nonradical lipid, the nonradical molecules becomes a radical, proceeding as chain reactions that one radical begets another. The lipids would undergo molecular rearrangement and further attack membrane proteins when subjected to oxidation chain reaction, leading to the fragmentation of the phospholipid bilayer, impairment of membrane-bounded functions and increment of nonspecific permeability.

PC is the most commonly used marker of protein oxidation, which is formed by the attack of the ROSs to transform the side-chain amine groups on several amino acids (i.e., lysine, arginine, proline, or histidine) of proteins into carbonyls. The PC concentration increased with the prolonged irradiation time (Fig. 6c), suggesting the protein oxidation by invaded ROS. Coincidentally, the increasing leakage of K⁺ during the photocatalytic process further implied the damage of membrane integrity and the inactivation of Na⁺-K⁺ pump caused by the collapse of metabolism (Fig. S22). However, only the loss of the genetic materials is lethal to the bacteria because they can regrow as a nonculturable state even without the metabolism system. It was observed that DNA and RNA were rapidly released during the disinfection process (Fig. 6d), illustrating the complete break of the genetic system.

In addition, the membrane destruction process was visually observed by SEM. As shown in Fig. 6e-h, the bacterial cells displayed a well-preserved morphology with smooth surface before the photocatalytic treatment. After being treated for 20 min, the membrane became rough and contained some pits, suggesting the initial damage of cell envelope caused by the PM3 biohybrid. Hollows and decomposition of cell membrane were observed with the prolonged photocatalytic period, indicating that the severe membrane destruction occurred. Finally, the cells were thoroughly destroyed with disorganized morphology after 60 min treatment of the PM3 biohybrid. Moreover, the bacterial membrane integrity was intuitively monitored by optical microscopy by using
the BacLight Bacterial Viability Kit (Fig. 6i-l). The live bacteria with intact membrane emit the green fluorescence, whereas the dead ones with damaged membrane emit red fluorescence. At the initial time, the untreated cells with intact membranes presented dense green fluorescence signals. However, the red fluorescence signals increased with the prolonged photocatalytic treatment, indicating the damage of membrane during the photocatalytic inactivation process. All the fluorescence signals turned to red at 60 min, revealing that the completely destruction of membrane and the death of bacterial cells.

**Figure 6.** (a) SOD and CAT activity, (b) ATP synthesis and cell respiration, (c) lipid peroxidation and protein carbonyl, and (d) the leakage of DNA and RNA during bacterial destruction process. SEM and microscopy images of *E. coli* cells after the photocatalytic treatment by PM3 for (e) and (i) 0 min, (f) and (j) 20 min, (g) and (k) 40 min, and (h) and (l) 60 min under VL irradiation (1,000...
The above evidences demonstrated the photocatalytic inactivation pathway of *E. coli* by PM3 biohybrid under VL illumination, which was schematically proposed in Scheme 1. The PM3 biohybrid generated abundant and effective electrons-hole pairs and their induced ROS such as •O$_2^-$ and •OH under VL. Although *E. coli* cells could defense against a minor amount of ROS through their antioxidant system at the early inactivation stage, a series of nonselective attacks caused by ROS occurred after destroying the cell self-protection system. The cell membrane was the primary target of ROS, and the membrane integrity and functions (e.g., cell respiration and metabolism) were gradually lost, resulting in the molecular rearrangement and fragmentation phospholipid bilayer. The destruction of the membrane allowed for the oxidation and leakage of the cytoplasmic substances (e.g., K$^+$, lipids, proteins, and DNA/RNA), ultimately resulting in the cell death.

**Scheme 1.** The proposed bacterial inactivation mechanism by the PM3 biohybrid under VL irradiation.

**4. Conclusion**

In summary, the as-prepared defective MoS$_2$ encapsulated rape pollen follows a Z-scheme charge transfer pathway, which showed excellent and stable broad-spectrum photocatalytic bacterial inactivation performance towards various waterborne bacteria.
Moreover, the significantly improved dispersity and hydrophilicity of PM3 biohybrid further enhanced the contact between photocatalysts and bacterial targets, thus promoting the photocatalytic disinfection performance in the liquid-phase reaction. The mechanism study indicated that the electrons, holes, •O₂⁻, and •OH radicals collectively contributed to the superior photocatalytic antibacterial performance of PM3 biohybrid. Especially, after the destruction of antioxidant enzyme system, the cell membrane associated metabolism activities were totally overwhelmed with the deactivation of ATP synthesis and cell respiration capacity under the attack of ROS. Meanwhile, the oxidation of membrane-bounded lipids and proteins led to the disintegration of cell membranes, resulting in the leakage of the cytoplasmic substances and genetic materials and the irreversible cell death. Combined with good biocompatibility and wide availability of the precursors, the PM biohybrid works as a promising photocatalysts for authentic water disinfection. In addition, the recovery and the practical environmental remediation application of the PM biohybrid would be explored in future research.

Author Contributions

K.X. and T.W. contributed equally to the work.

Conflicts of Interest

The authors declare no conflict of interest.

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