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 toward various waterborne bacteria under different light sources. The inactivation mechanisms were systematically investigated. Reactive species (RSs), including electrons, holes, and reactive oxygen species (•O$_2^-$ and •OH), played major roles in inactivating bacteria. The antioxidant system of bacteria exhibited a self-protection capacity by eliminating the photogenerated 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 toward 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 cocatalysts 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 can not only provide sufficient active sites for reactions but also facilitate the separation of the photogenerated 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 CO2 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 log10 cfu/mL of Escherichia coli (E. coli) cells within 240 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 (MoS2) has been reported as a hydrophilic material with high surface energy.17 This property probably makes it desirable to combine with rape pollen and enhance its water dispersibility. Typically, the bandgap of MoS2 shifts from 1.9 to 1.29 eV by increasing the layers, which is favorable for light harvesting.18 In addition, few-layered MoS2 possesses more active edge sites, which can effectively promote its photocatalytic activity.19 For example, MoS2 with abundant active edge sites has been reported for enhanced photocatalytic hydrogen production.19 Cui et al. reported that the vertically aligned few-layered MoS2 could inactivate 5-log E. coli within 2 h under VL.20 The exfoliated MoS2 nanosheets exhibited antibacterial activity against both Staphylococcus aureus and Pseudomonas aeruginosa within 20 h.21 However, the preparation of these MoS2 catalysts 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.22 Xie et al. reported that the defective MoS2 with enhanced photocatalytic activity could be easily obtained by varying the concentration of precursors.22 However, the photocatalytic bacterial disinfection performance of the defect-rich MoS2 has not been investigated.

Herein, for the first time, we developed a hydrophilic biohybrid photocatalyst, MoS2-encapsulated rape pollen, by in situ anchoring of defective MoS2 nanosheets onto the rape pollen skeleton. The photocatalytic bacterial inactivation toward four common waterborne bacterial species (i.e., E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis) by the defective MoS2-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 the 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 utilization of earth-abundant biomaterials in a biohybrid photocatalysis for efficient wastewater treatment.

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, 1 mM of sodium molybdate dihydrate (Na2MoO4·2H2O) and 3 mM of thiourea were dissolved in 40 mL of DI water, then 0.03 g of the as-prepared rape pollen (see in the Supporting Information) was added into the solution. The mixed solution was sonicated for 2 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 several times and dried at 60 °C for 12 h. The product was denoted as PM1. Besides, the PM biohybrids with different loading amounts of defective MoS2 nanosheets were fabricated by varying the dosages of Na2MoO4·2H2O/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 two times and resuspended in the same volume of sterilized saline solution. Then 25 mg of catalysts was uniformly dispersed in 25 mL sterilized saline solution with a supplement of 300 μL bacterial suspension to achieve 107 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 18 h to count the viable cell. Besides, the different light spectra including full spectrum (without filter, 200 W/m²), simulated sunlight (Air Mass 1.5 filters, 200 W/m²), white LED light (400–840 nm, 100 W/m²) as well as the other three species of bacteria (P. aeruginosa, S. aureus, and B. subtilis) were used to further investigate the inactivation performance of the as-prepared catalysts. All the bacterial disinfection experiments were conducted in triplicate.

3. RESULTS AND DISCUSSION

3.1. Characterization of Materials. The X-ray diffraction (XRD) patterns (Figure 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 showed no obvious diffraction peaks, revealing its amorphous structure (Figure S1). The XRD patterns of PM biohybrid series are consistent with that of MoS2 (Figure 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 (Figure 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 biohybrid. However, further increment in the loading amount of MoS₂ decreased the surface area of biohybrids (PM4 and PM5).

The scanning electron microscopic (SEM) images (Figure 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 (Figure 1b), contributing to the light harvesting ability. The scanning transmission electron microscopic (STEM) images (Figure 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 (Figure 1e,f). Interestingly, even the microrods inside rape pollen were coated by MoS₂ layers (Figure 1g), greatly increasing the contact area between rape pollen and MoS₂. The elemental mappings (Figure 1i–k) of PM3 not only confirmed the coexistence of rape pollen and MoS₂ but also indicated the uniform distribution of MoS₂ on rape pollen.

The corresponding TEM images further verified the hollow structure of rape pollen (Figure 2a,b) and the uniform distribution of MoS₂ on rape pollen (Figure 2d). As shown in Figure 2c and Figure S4a, pure MoS₂ displayed a spherical structure formed by the self-assembled nanosheets, which was unfavorable for the exposure of active edges. After loading MoS₂ on rape pollen, more active edges were exposed (Figure 2e). In addition, the loaded MoS₂ displayed as flowerlike nanosheets (Figure 1e and 2d), which could improve the densities of active edges sites compared with the bulk MoS₂ with spherical morphology. It was reported that the active edges possessed higher catalytic activity than that of the in-plane surface, thus probably rendering the PM3 biohybrid with better bacterial disinfection performance. Moreover, the high-resolution TEM (HRTEM) images (Figure 2f) showed the well-defined lattice fringes within PM3. The interplanar space of 0.27 nm and the interlayer space of 0.62 nm coincided with the individual plane of (100) and (002), respectively. Besides, dislocations and slight rotations of lattice fringes exist within the (100) and (002) 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 deliscence of the surface, thus forming more active edges within the PM3 biohybrid. To further investigate the defective structure of the biohybrid, the ESR spectrum was recorded (Figure S5). Explicit ESR signal attributed to the unpaired electrons was 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 MoS2, suggesting the existence of sulfur defect.

Besides, the mass ratio of rape pollen to MoS2 in the PM3 biohybrid was evaluated through the thermogravimetric analysis (TGA). As shown in Figure 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 MoS2 to MoO3. Consequently, the mass ratio of rape pollen to MoS2 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 MoS2, implying that the PM3 biohybrid has a better dispersity in water than that of MoS2. The high PDI value of rape pollen even exceeded the detection limit, showing its poor dispersity in water. As confirmed by the Figure 3a,b, both PM3 and MoS2 were well-dispersed after 30 s of sonication, whereas rape pollen still severely aggregated and floated on water. Furthermore, the CA of PM3 was 4.5° (Figure 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° (Figure 3e). Compared with PM3, bulk MoS2 demonstrated a moderate hydrophobicity (CA 38.1°). This might be caused by the structural differences between bulk MoS2 and PM3 biohybrid. The bulk MoS2 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, MoS2 nanosheets are homogeneously located on the surface of pollen, leading to more exposed MoS2 surface and defects. The uniform distribution of MoS2 improves the densities of active edge sites. The combination of rape pollen with defective MoS2 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.22 In addition, the dispersive components, polar components, and surface energy of rape pollen, MoS2, 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 (CH2I2) 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_i \gamma_d / (\gamma_i + \gamma_d)] + \gamma_i \gamma_p / (\gamma_i + \gamma_p)]
\[(1 + \cos \theta_2)\gamma_2 = 4[\gamma_i \gamma_d / (\gamma_i + \gamma_d)] + \gamma_i \gamma_p / (\gamma_i + \gamma_p)]

where \(\gamma_d\) represents the dispersive components and \(\gamma_p\) indicates the polar components. \(\theta_1\) and \(\theta_2\) are the contact angle of the material in water and diiodomethane, respectively. The value of \(\gamma_1\) and \(\gamma_2\) are 72.8, 22.1, and 50.7 mJ/m² in water, and the values of \(\gamma_1\) and \(\gamma_2\) are 50.8, 44.1, and 6.7 mJ/m² in diiodomethane.

As shown in Table S3, the dispersive component (35.96 mJ/m²), polar component (40.14 mJ/m²) as well as surface energy values (76.1 mJ/m²) 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.

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 Figure S7a,b, the similar intensive signals at Mo 3d$_{3/2}$, Mo 3d$_{5/2}$, S 3d$_{3/2}$, S 2p$_{1/2}$, and 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 a high binding energy, whereas its O 1s spectra shifted to a low binding energy (Figure S7c,d), which were caused by the electron transfer and strong interaction between rape pollen and MoS$_2$ based on the previous reports.\textsuperscript{25,26} Similarly, the banding energy also slightly shifted in the spectra of Mo 3d and S 2p. The change of the peaks in 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$_2$.\textsuperscript{27} The light absorption capacity was shown in Figure S8, suggesting the as-prepared materials exhibited a broad light absorption range. The bandgap structures were calculated through the Tauc plot (Figure S9) based on UV–vis-DRS spectra,\textsuperscript{28} which were 1.45 and 1.84 eV for MoS$_2$ and rape pollen, respectively. The valence band maxima of MoS$_2$ and rape pollen were estimated to be 1.28 and 0.97 eV, respectively, according to the results of valence-band XPS spectra (Figure S10). Consequently, rape pollen and MoS$_2$ 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 first conducted to investigate the bacterial inactivation efficiency of defective PM biohybrids with different MoS$_2$ thickness as well as defect-free biohybrids, which were shown in Figure S11. The encapsulation of defect-free MoS$_2$ 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$_2$ coating on the rape pollen (Mo:S = 1:3),
there was a reduction of 1.7 log₁₀ cfu/mL of cell within 180 min. That meant about 97.85% of *E. coli* were inactivated by the combination of the biobloid. Interestingly, the bacterial inactivation efficiency decreased when further increasing the thickness of defective MoS₂ on pollen, among which the ratios of Mo:S were 1:4 and 1:5, respectively. Similar to our results, Xie et al. has also reported that the ultrathin MoS₂ assemblies possessed higher density of active sites compared with thicker MoS₂ nanosheet (Mo:S = 1:5), thus showing better hydrogen production efficiency.²² Therefore, after optimizing the molar ratio of Mo and S during the synthesis of defective PM, the photocatalytic performance of PM bioboids with different loading amounts of MoS₂ was investigated under VL irradiation (Figure 4a).

The bacterial cell density (10⁷ cfu/mL) remained unchanged within 180 min in the light control (without photocatalyst) and dark control (without irradiation) experiments (Figure S11–S12), indicating that both light irradiation (1,000 W/m²) and photocatalysts had no harmful effect on bacteria. As shown in Figure 4a, rape pollen exhibited a relatively low photocatalytic bacterial inactivation efficiency, which only inactivated 0.9 log₁₀ cfu/mL of the cell within 180 min. The defective MoS₂ showed a moderate bacterial inactivation efficiency with a complete bacterial inactivation within 180 min. Impressively, the PM bioboids (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 log₁₀ CFU/mL within 60 min under VL. The photocatalytic bacterial activity decreased when loading more defective MoS₂ on rape pollen (e.g., PM4 and PM5), which might be attributed to the light blocking by the extra thick MoS₂ layers and the stacking of the self-assembled MoS₂ spheres (Figure S4c). Also, abundant active edges were protruded from the PM3 bioboid (Figure 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 bioboid (Figure 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 bioboids made a minor contribution to the photocatalytic performance. It was induced that the synergetic effect of rape pollen and MoS₂ rather than the change in surface area mainly led to the significantly improved photocatalytic activity of the PM3 biobiod. On the other hand, the photothermal effect on bacteria inactivation was studied by removing the temperature control system (i.e., without using water bath). As shown in Figure S14a, a slightly enhanced bacterial inactivation was observed in the first 30 min without using a water circulation bath compared with that with the temperature control system. Nevertheless, they exhibited a similar photocatalytic disinfection efficiency. Under the same reaction condition, the suspension temperature increased rapidly in the first 45 min and then remained unchanged (Figure S14b). These results indicated that the photothermal effect was involved in the photocatalytic inactivation process but not a dominant factor.

Besides, the inactivation performance of PM3 bioboid was compared with some carbon-based and advanced materials under the same VL intensity (1000 W/m²) (Figure S15). Compared to the recently reported iodine-doped hydrothermal carbonation carbon (1-HTCC) and the widely studied C₅N₄, the photocatalytic inactivation of PM3 bioboid was much more outstanding. Moreover, graphite, carbon mesoporous, and red phosphorus (Red-P) exhibited poor bacterial inactivation efficiency, with a slight reduction of the bacterial density within 180 min. Apart from these carbon-based materials, the photocatalytic bacterial inactivation performance of PM3 is even better than that of the recently reported high-performing composite catalysts (Table S4). These results indicated that the PM3 bioboid 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.⁷ Meanwhile, the natural solar-driven photocatalysis was considered as a “green” and efficient water treatment method.² Thus, the photocatalytic inactivation performances of PM3 bioboid were further evaluated under artificial LED light and simulated natural light irradiation. As shown in Figure 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 bioboid is also substantially higher than that of the recently reported photocatalysts under similar reaction conditions (Table S5). This indicated the great potential of PM3 bioboid as a photocatalyst for indoor sterilization. In addition, the inactivation efficiency was greatly improved under the illumination of simulated sunlight (200 W/m²) (Figure 4b). The light control experiment demonstrated that the simulated sunlight had no photolysis on *E. coli* (Figure 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 efficiency of solar-driven bacterial inactivation efficiency was compared with that of the currently adopted disinfection methods (Table S6). We found that the solar-driven approach using our newly developed photocatalyst shows comparable or even higher efficiency than that of currently adopted water disinfection methods. This suggests that the PM3 bioboid can be used as a promising photocatalyst for water disinfection.

The photocatalytic performance of PM3 toward other waterborne bacteria such as *P. aeruginosa*, *S. aureus*, and *B. pumilus* were also investigated under VL irradiation (Figure 4c). The light and dark control experiments illustrated that both the PM3 bioboid and light intensity (1,000 W/m²) showed no harmful effects on bacteria (Figure S17). As displayed in Figure 4c, the *S. aureus* and *P. aeruginosa* were completely inactivated within 45 and 60 min, respectively. The disinfection rate of PM3 bioboid exceeds those of TiO₂ and MnO₂-based materials, as reported earlier.¹⁶ The disinfection rate of *B. pumilus* was relatively low, with a 2.15 log₁₀ 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.²⁸ 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 bioboid, the photocatalytic bacterial inactivation experiments were conducted in authentic water systems. As shown in Figure 4d, the photocatalytic inactivation efficiency of the PM3 bioboid 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 log₁₀ cfu/mL cell within 60 min. Similarly, a complete 7 log₁₀
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 log₁₀ CFU/mL cell densities within 150 min in effluent samples collected from Tai Po and Sha Tin wastewater treatment works. 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 five times (Figure 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 toward various waterborne bacteria with high stability. In addition, MoS₂ and rape pollen showed good biocompatibility and have been used for photothermal therapy (PTT) of cancer and drug delivery, respectively. Therefore, the practical utilization of the PM3 biohybrid might be extended to more fields.

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 (see in the Supporting Information). As shown in Figure 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 armed 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 MoS₂ 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 MoS2 (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 (Figure 3) provided sufficient contact areas between the biohybrid and cells. Meanwhile, the CB of rape pollen (CBM = −0.87 V vs NHE) was sufficiently negative to produce ample electrons, which could directly damage the cell by attacking cellular molecular structures and impairing the respiration chain.35 Also, the electron-induced reactive oxidative species (ROS) have great oxidative potential for inactivating E. coli cells in nonselective reactions and eventually led to cell death.33

Among the ROS, ⋅O2− demonstrated the most important role as the disinfection of E. coli was dramatically prohibited with the addition of TEMPO (Figure 5a). The leading bactericidal effect of ⋅O2− was probably because the redox potential for O2/⋅O2− (−0.33 V vs NHE) was thermodynamically favored by CB of rape pollen (0.87 V vs NHE) within the biohybrid. This further confirmed the Z-scheme charge transfer mode in the PM3 biohybrid because the CB of MoS2 (CBM = −0.14 V vs NHE) was not negative enough to produce O2/⋅O2−. To validate the Z-scheme mode within the PM3 biohybrid, the ESR analyses were carried out to detect the spin ⋅O2− produced by the individual catalysts and composite catalysts, respectively. As shown in Figure 5b, four significant characteristic peaks of DMPO−⋅O2− 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 MoS2 under VL irradiation. Meanwhile, Figure S19 showed that the DMPO−⋅O2− signals were not observed in the dark for all the photocatalysts, indicating that the ⋅O2− radicals cannot be generated without light irradiation. Consequently, all the above evidence indicated that the as-prepared PM3 biohybrid followed the Z-scheme electron transfer path rather than the traditional double-transfer mode. Otherwise, the photogenerated electrons would accumulate at the CB of MoS2 and thus lead to no production of ⋅O2− 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 MoS2. 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 ⋅O2− 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 (Figure 5a). It was noted that the generation of ⋅OH production was thermodynamically inhibited since the VB of MoS2 (1.28 V vs NHE) cannot directly convert H2O or OH− to ⋅OH (H2O/⋅OH = 1.99 eV vs NHE, OH−/⋅OH = 2.38 eV vs NHE). Nevertheless, ⋅OH could be produced through a multistep pathway,34 as shown in the following equations:

\[ \text{O}_2 + e^- \rightarrow \text{⋅O}_2^- \]  \hspace{1cm} (1)

\[ \text{H}^+ + \text{⋅O}_2^- \rightarrow \text{⋅HO}_2 \]  \hspace{1cm} (2)

\[ \text{⋅HO}_2 + \text{H}^+ + e^- \rightarrow \text{H}_2\text{O}_2 \]  \hspace{1cm} (3)

\[ \text{H}_2\text{O}_2 + e^- \rightarrow \text{⋅OH} + \text{OH}^- \]  \hspace{1cm} (4)

Compared with ⋅OH, ⋅O2− is easier to accumulate because its lifetime (τ1/2 ≈ 1 μs) is much longer than that of ⋅OH (τ1/2 ≈ 1 ns).34 This was another possible reason why ⋅O2− 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 H2O2 and ⋅O2− played negligible roles in this photocatalytic system. H2O2 and ⋅O2 radicals can be produced by the stepwise oxidation of H2O but accounted for a very small portion of the generated ROS and were hard to participate in the antibacterial process.

To further investigate the production of ⋅O2− and ⋅OH radicals by the PM3 biohybrid under VL irradiation, the ESR spin-trap spectra were recorded. Figure 5c,d showed that both signals of DMPO−⋅O2− and DMPO−⋅OH adducts were detected under VL irradiation, whereas there were no signals of ⋅O2− and ⋅OH in the dark. This indicated the formation of ⋅O2− 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 (⋅O2− and ⋅OH) were the dominant effective species for the inactivation of E. coli in the 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 Figure S20a,b, the signal intensities of ⋅O2− and ⋅OH produced by the PM3 biohybrid were higher than that of PM4 biohybrid under VL irradiation. There were no signals of ⋅O2− and ⋅OH detected in the dark (Figure S20c,d) for both PM3 and PM4 biohybrids, indicating that the ROS were generated under excitation of VL irradiation. Hence, the stronger signals of ⋅O2− and ⋅OH within the PM3 biohybrid can be more effective at inactivating the bacterial cells since ROS played an 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 Figure S21a, both PM3 and PM4 biohybrids exhibited dramatically enhanced photocurrents compared with bare rape pollen and MoS2, indicating that the combination of rape pollen and MoS2 could enhance the transfer efficiency of photoinduced electrons. The higher photocurrent of PM3 suggested a higher photogenerated carrier-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 MoS2 (Figure S21b). This indicated that the interfacial electrons and holes generated by the PM3 biohybrid could more easily transfer to the acceptors, resulting in the enhancement of photocatalytic performance. Moreover, the recombination rate of the photoinduced charge carriers was investigated by room-temperature photoluminescence (PL) spectra (Figure S21c). The weak and narrow PL emission peak of the PM3 and PM4 biohybrids revealed that the loading of defective MoS2 on pollen could effectively inhibit the recombination of electrons and holes,55,56 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 MoS2 loaded on rape pollen because the defects provide temporary trapping sites for electrons and shorten the diffusion distance of electrons and holes to surface.35 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.

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. First, the antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) were measured. The SOD can dismutase $\cdot$O$_2$ to O$_2$ and H$_2$O$_2$, and CAT can catalyze H$_2$O$_2$ to O$_2$ and H$_2$O.$^{38,39}$ Thus, the intracellular SOD and CAT act as scavengers for ROS and effectively protect the cell from the ROS-induced oxidative damage. As shown in Figure 6a, the activities of SOD and CAT increased first and then declined rapidly after 30 min during the inactivation process. The increment in the antioxidant enzyme activities indicated that 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 (Figure 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, which is selectively permeable and responsible for the energy-dependent reactions, such as ATP synthesis, ion transportation, and metabolism. Therefore, the inactivation patterns of membrane-bounded enzymes and metabolism during the photocatalytic inactivation process were investigated.

The membrane-bounded cell respiration was first evaluated through the reduction of 2,3,5- triphenyl tetrazolium (TTC) since the cytochrome in respiration chain could utilize the oxidized form of substrate such as O₂ and TTC as electron acceptors to produce energy. As shown in Figure 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 the respiration chain could no longer maintain the redox potential and produce protons for energy production. ATP is the main energy currency in the cell, which is synthesized through the oxidation of substrate driven by the proton electrodynamic force produced in the respiration chain. 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 (Figure 6b). Interestingly, the decline rate gradually became slow with an increasing reaction time. As the ATP could be temporarily used for membrane repair and transmembrane potential maintenance, 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 well coincided with the commencement of the antioxidative enzyme system at the initial stage (Figure 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 synthesize tiny amounts of ATP through substrate phosphorylation in a short time period after the damage of the 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 disintegration 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.

Lipid peroxidation (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 Figure 6c suggested the decrease of diffusion ability and the damage of the bilayer. However, the hydroperoxides were 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 a 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.\(^{50}\)

Protein carbonyl (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.\(^{51}\) The PC concentration increased with the prolonged irradiation time (Figure 6c), suggesting the protein oxidation by invaded ROS. Coincidently, the increasing leakage of \(K^+\) during the photocatalytic process further implied the damage of membrane integrity and the inactivation of the \(Na^+\)-\(K^+\) pump caused by the collapse of metabolism (Figure S22).\(^{52}\) 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.\(^{53}\) It was observed that DNA and RNA were rapidly released during the disinfection process (Figure 6d), illustrating the complete break of the genetic system.

In addition, the membrane destruction process was visually observed by SEM. As shown in Figure 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 (Figure 6i−l). The live bacteria with integrate 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 the complete destruction of membrane and dead bacterial cells.

The above evidence 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 \(\bullet O_2^-\) and \(\bullet OH\) under VL. Although E. coli cells could defend 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 of 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.

# **ASSOCIATED CONTENT**

## **Supporting Information**

The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acs.est.9b05627](https://pubs.acs.org/doi/10.1021/acs.est.9b05627).

Experimental details and results for pretreatment of rape pollen, synthesis of defect-free PM biohybrids, characterization of the materials, scavenger study, enzyme activity assay and biomolecule oxidation and leakage assay (PDF)

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### **Notes**

The authors declare no competing financial interest.

### **ACKNOWLEDGMENTS**

J.S. gratefully acknowledges the financial support from the National Natural Science Foundation of China (no. 21706224), the Science and Technology Innovation Commission of Shenzhen Municipality (ref: JCYJ2018307123906004), the Research Grants Council of Hong Kong (nos. CityU 21301817 and CityU 11215518 to J.S. and no. GRF14100115 to P.K.W.).

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