The Antibacterial Activities of MoS_2 Nanosheets towards Multi-Drug Resistant Bacteria

Chemical Communications

Supporting Information

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Materials and Methods

Materials and chemicals. MoS_2 bulk powder, n-butyllithium, furfural alcohol (FFA), 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT), potassium phosphate (K_2HPO_4 and KH_2PO_4) obtained from commercial sources and used without further purification. All other chemicals were used as purchased and dissolved in deionized (DI) water from a milli-Q water purification system.

Solar irradiation. Solar simulator (Abet Technology Sun 3000, CT, USA), was used for all solar experiments. The solar intensity was set to be 1 sun. The UV-vis spectrum of MoS_2 nanosheets was monitored upon solar irradiation. Size change was evaluated by TEM, AFM and DLS measurement.

Preparation of Bacterial Solutions. For all experiments, utensils were autoclaved for sterility at 120℃ for at least 20 minutes. *E. coli* was grown in a Luria Bertani medium (LB) and cultured overnight under 37℃ at 125 rpm. The cultures were then diluted in a fresh autoclaved LB medium and cultured to log phase. Bacteria were harvested by centrifuging (5000 rpm for 2 mins), and then were washed with sterilized 0.9% NaCl solution. The supernatant was discarded and the remaining bacteria were resuspended in 0.9% NaCl solution, and diluted with a concentration of 10^6 ~10^7 CFU/mL.

Antibacterial activities of MoS_2 nanosheets. MoS_2 nanosheets with certain concentration were added and mixed with *E. coli* suspension. After 3-hr incubation under 37℃ at 125 rpm, the mixture solution was diluted and spread on LB agar plates by using glass spreader, which should always be sterilized by alcohol fire and cooled down before used. Then the agar plates were incubated under 37℃ overnight before colony counting. Inhibition effects of radical scavengers on solar disinfection of MoS2 nanosheets were also evaluated using furfural alcohol (FFA), superoxide dismutase (SOD) and 4-chlorobenzoic acid (*p*CBA) for $^1O_2$, $O_2^{\cdot-}$ and ·OH, specifically to conform the role of ROS in the sterilization process.

Preparation of MoS_2 nanosheets with different sizes. Chemically exfoliated MoS_2 nanosheets was prepared by lithium ion intercalation followed by forced hydration. [1] Specially, bulk MoS_2 was incubated in Li-containing organic solvent (e.g. n-butyllithium in hexane), where Li-intercalated MoS_2 with weakened interlayer attractions generated. Followed by ultra-sonication, uniformly

stable mono-layered MoS_2 suspension was formed by the reaction between Li_xMoS_2 with water. To prepare MoS_2 with different size distributions, gradient centrifugation method was used. After obtaining chemically exfoliated MoS_2 suspension using lithium ion intercalation method, the suspension was centrifuged at 5000 rpm for 30 min. The precipitate and the supernatant were collected separately, and the supernatant was centrifuged at 8000 rpm for 30 min. The precipitate and the supernatant were again collected separately. Finally, the supernatant was centrifuged at 12000 rpm for 45 min and the precipitate was gathered. Those precipitates were re-dispersed in DI water for experiments.

ROS detection. Reactive oxygen species (ROS) were detected by using specific chemical probes that selectively react with each ROS at near diffusion-limited rates. Only one probe was used at a time, as for superoxide anion ($O_2^{\cdot -}$) require that they be added before incubation, as the measured response is due to accumulative ROS production. $O_2^{\cdot -}$ measurement: XTT was employed as a scavenger for detecting superoxide anion ($O_2^{\cdot -}$), as the reaction between XTT and $O_2^{\cdot -}$ produces a pink soluble reduced product that absorbs light at 470 nm. MoS_2 and XTT were mixed in a 5 mM phosphate buffered solution under pH 7 and the light absorbance at 470 nm was monitored over time. Samples were filtered by 0.22 μm filter to remove MoS_2. Buffers were prepared with phosphate salts (i.e., KH_2PO_4 and K_2HPO_4). $^1O_2$ measurement: FFA was used as the probe to detect the formation of $^1O_2$. The FFA concentration at specific time was determined by HPLC analysis with the UV/Vis detector at 219 nm. The mobile phase was 50% methanol and 50% water at a flow rate of 1 mL/min. H_2O_2 measurement: H_2O_2 concentrations were measured by DPD/HRP method as previously reported. [2]

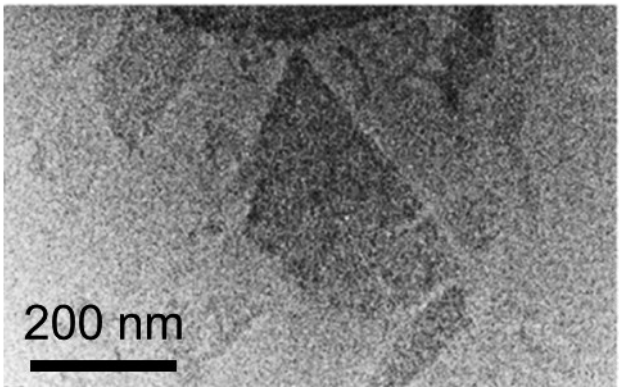

Figure S1. Characteristic of as-prepared chemically exfoliated MoS_2 nanosheets. Representative TEM micrograph of MoS_2 nanosheets on silicon.

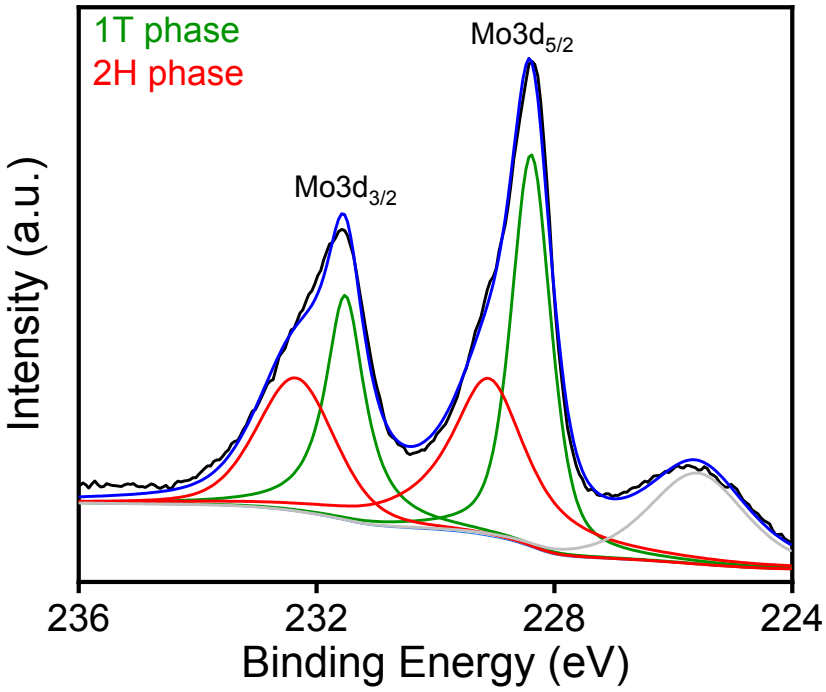

Figure S2. XPS measurement of MoS_2 nanosheets.

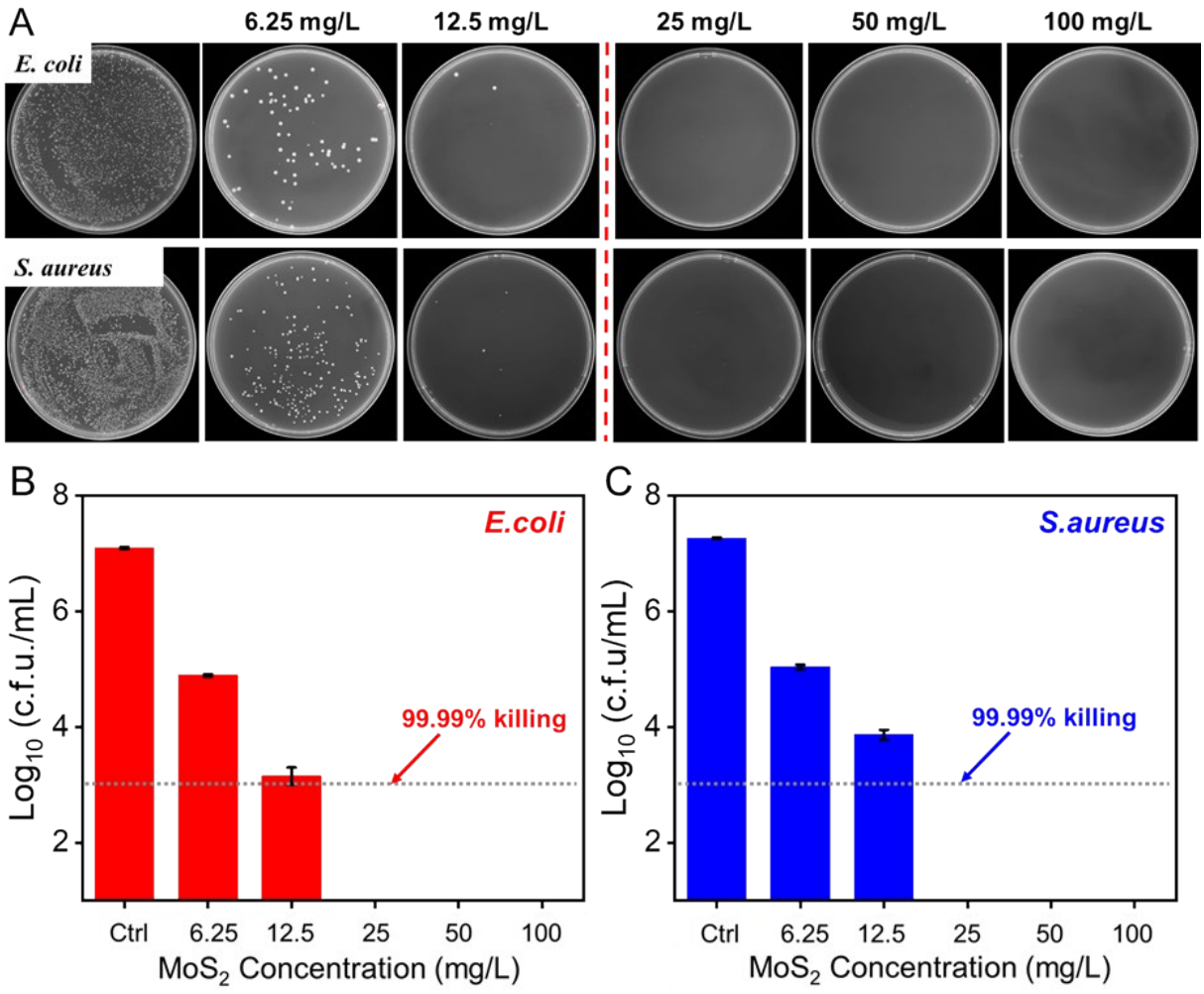

Figure S3. Solar disinfection by MoS_2 nanosheets as a function of concentration. (A) Colony-forming ability of *E.coli* and *S.aureus* after treating with di□erent concentrations of MoS_2 for 30 min. (B) Solar disinfection performance of *E.coli* after treating MoS_2 with different concentration. (C) Solar disinfection performance of *S.aureus* after treating MoS_2 with different concentration.

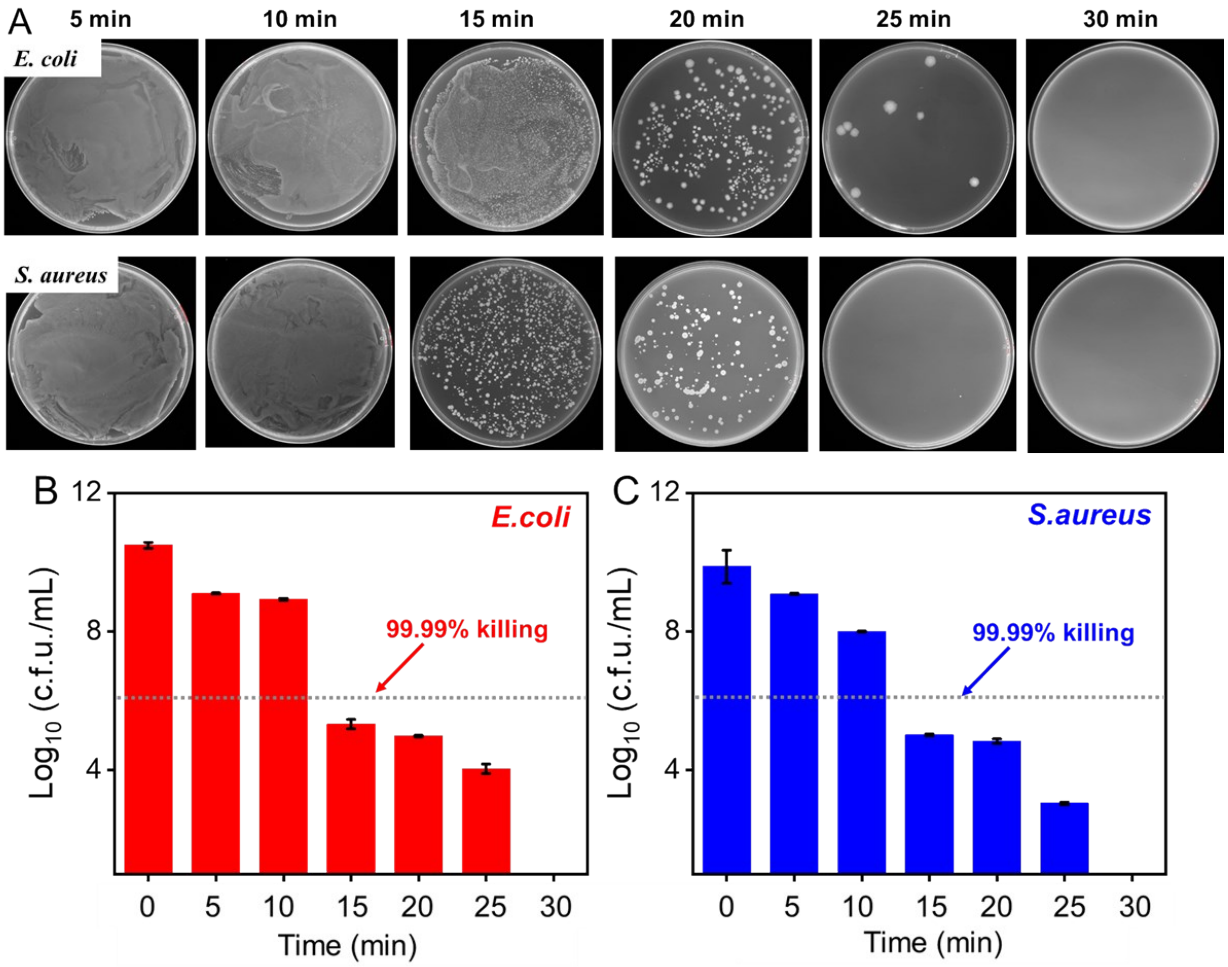

Figure S4. Solar disinfection by MoS_2 nanosheets as a function of time. (A) Colony-forming ability of *E.coli* and *S.aureus* after treating with 25 mg/L MoS_2 with di□erent time. (B) Solar disinfection performance of *E.coli* after treating MoS_2 with different time. (C) Solar disinfection performance of *S.aureus* after treating MoS_2 with different time.

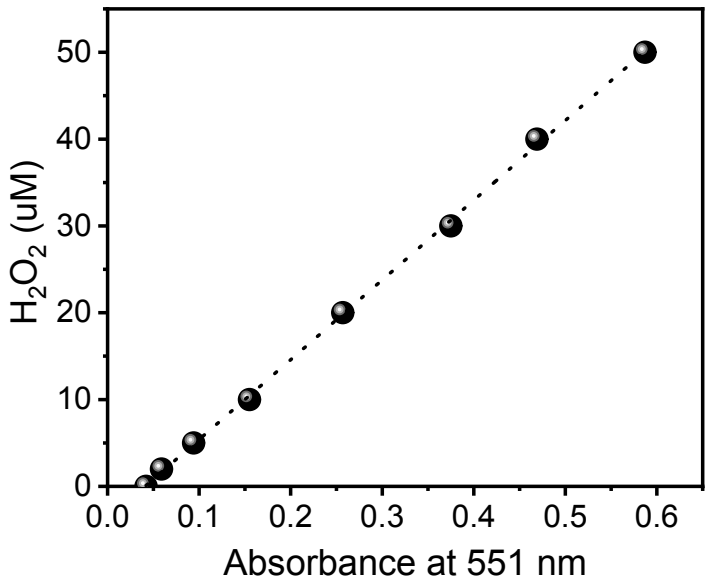

Figure S5. Standard Curve of H_2O_2 measurement by DPD/HRP method.

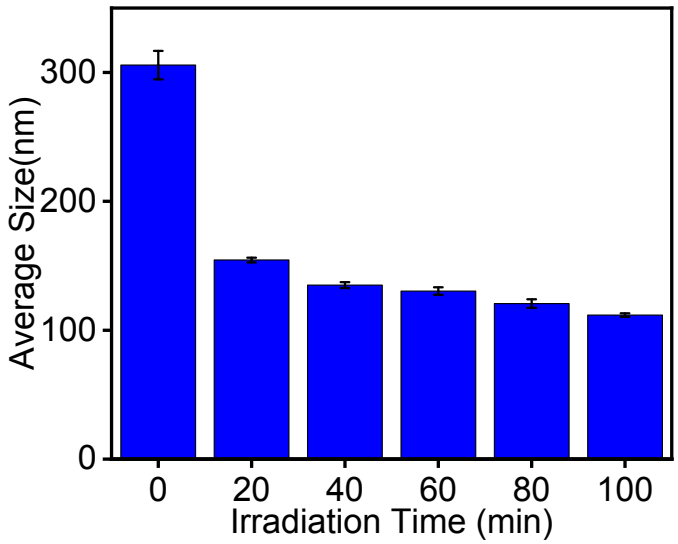

Figure S6. DLS measurement of MoS_2 size as a function of solar irradiation time.

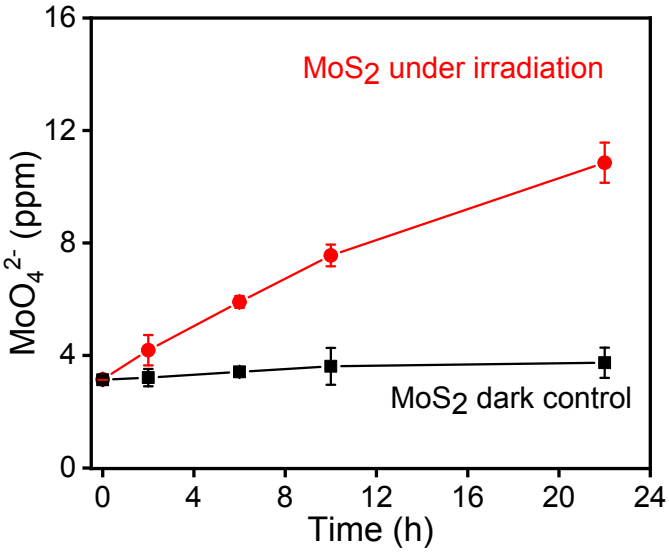

Figure S7. MoO_4^{2-} generation of MoS_2 under irradiation and for the dark control samples.

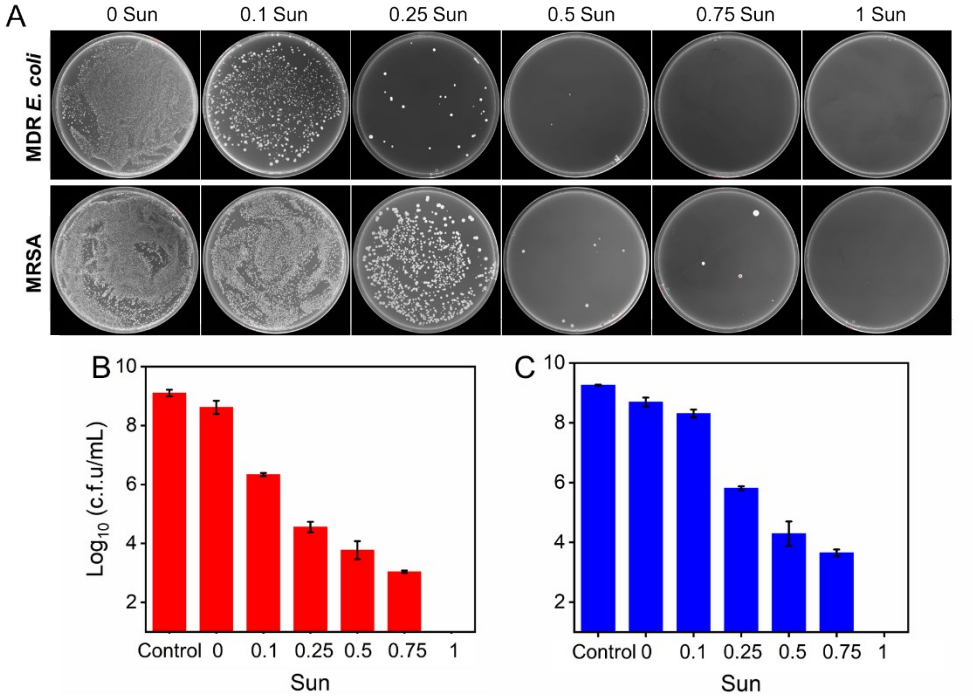

Figure S8. Combat of multi-drug resistant bacteria by solar disinfection using MoS_2 nanosheets as a function of solar intensity. (A) Colony-forming ability of MDR *E.coli* and MRSA after the treatment of MoS_2 under different solar intensity. (B) Solar disinfection performance of MoS_2 toward MDR *E.coli* under different solar intensity. (C) Solar disinfection performance of MoS_2 toward MRSA under different solar intensity.

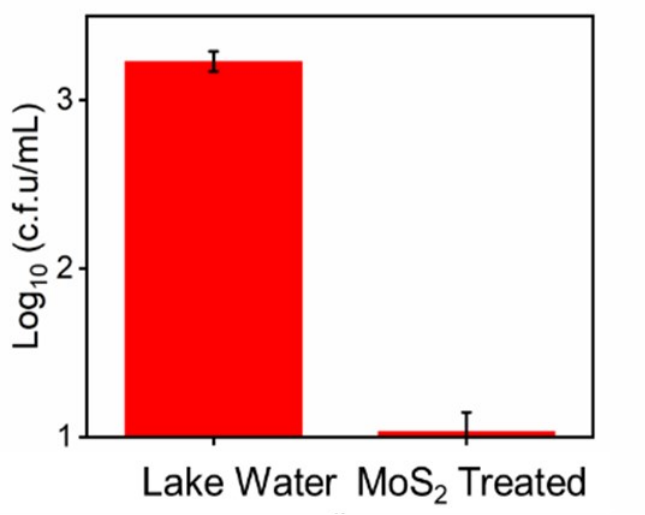

Figure S9. Solar disinfection of lake water by MoS_2.

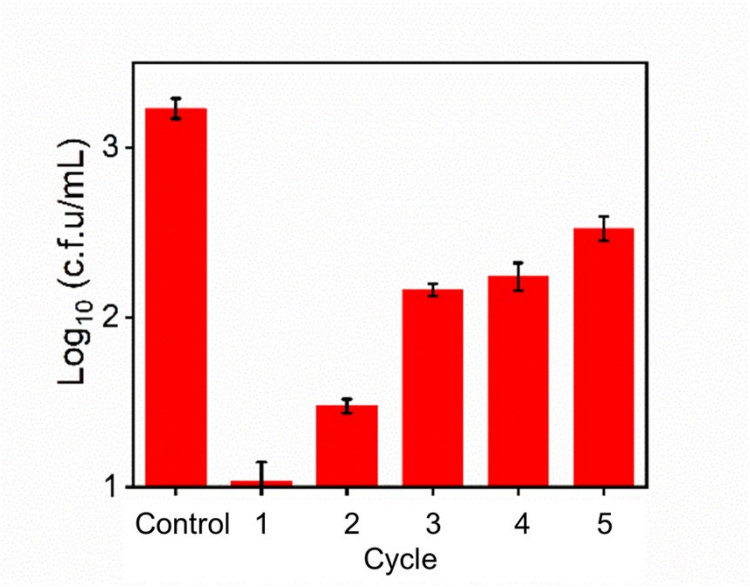

Figure S10. Solar disinfection of lake water by MoS_2 for 5 cycles.

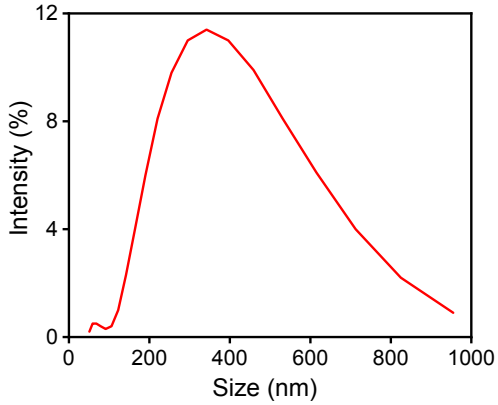

Figure S11. DLS measurement of MoS_2.

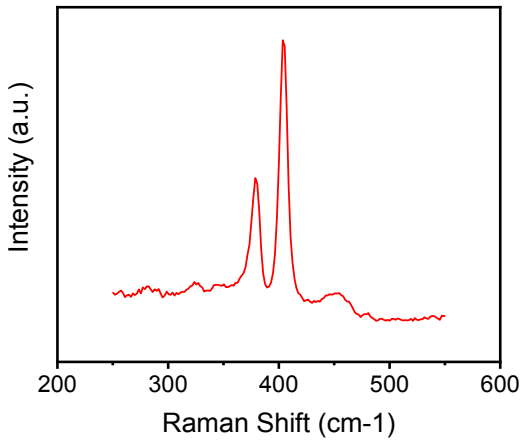

Figure S12. Raman spectra of MoS_2.

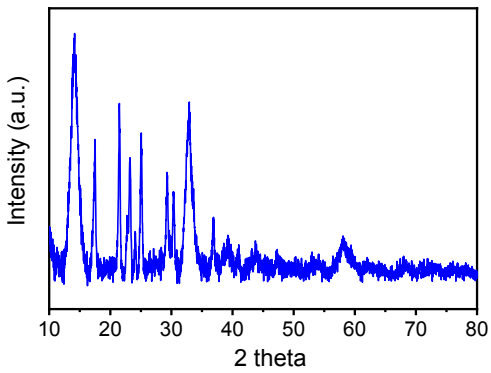

Figure S13. XRD patterns of MoS_2.

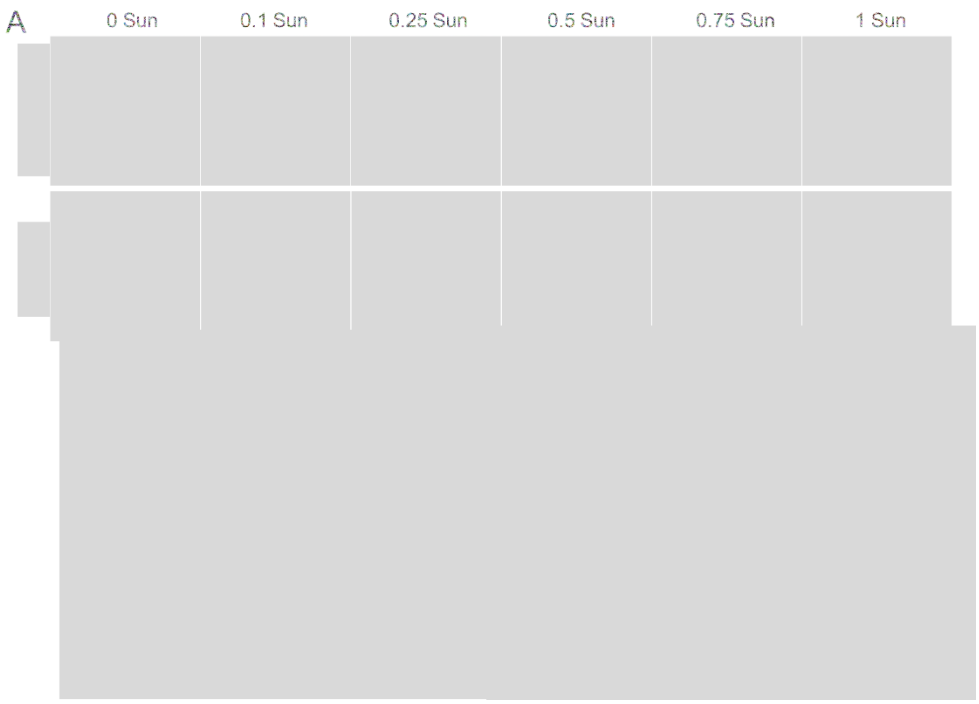

Figure S14. Combat of multi-drug resistant bacteria by solar disinfection using MoS_2 nanosheets as a function of solar intensity. (A) Colony-forming ability of MDR *E.coli* and MRSA after the treatment of MoS_2 under different solar intensity. (B) Solar disinfection performance of MoS_2 toward MDR *E.coli* under different solar intensity. (C) Solar disinfection performance of MoS_2 toward MRSA under different solar intensity.

Table S1. Summary of antibacterial activity MoS_2 in existing studies and our study.

Nano-material	**Synthesis Method**	**With Light or not?**	**Micro organism**	**Inhibition Rate**	**Proposed Mechanism**	**Reference**
MoS_2	Li-intercalation	No	*E. coli*	5 mg/L, 38.9% (2 hr) 80 mg/L, 91.8% (2 hr)	membrane & oxidation stress	3
MoS_2	thiol ligand functionalized MoS_2	No	*P. aeruginosa*	minimum inhibitory concentration: ＞15 ppm [Mo]	GSH oxidation & strong attachment	4
MoS_2	exfoliation in NMP	No	*E. coli*	52% (3 h)	oxidative stress & GSH oxidation	5
MoS_2	few-layered vertically aligned MoS_2	Yes	*E. coli*	99.999%, under sunlight (1 h)	photocatalytic ROS generation	6
MoS_2	**Li-intercalation**	**Yes**	***E. coli,* *S. aureus,* MDR *E. coli,* MRSA**	**99.9999%, 30 min**	**ROS generation & size decrease**	**this work**

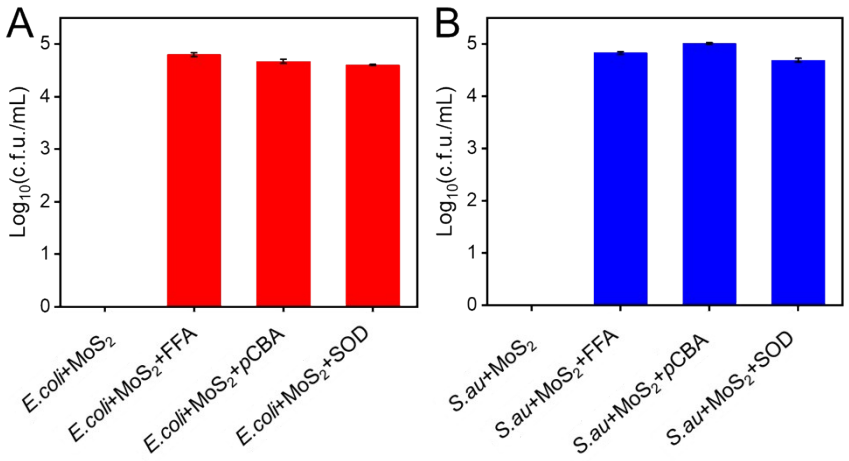

Figure S15. Inhibition effect of radical scavengers on solar disinfection of MoS_2 nanosheets. (A) Inhibition effects of radical scavengers (FFA for $^1O_2$, pCBA for ·OH and SOD for $O_2^{\cdot-}$) towards *E.coli* on solar disinfection of MoS_2. (B) Inhibition effects of radical scavengers (FFA for $^1O_2$, pCBA for ·OH and SOD for $O_2^{\cdot-}$) towards *S.au* on solar disinfection of MoS_2. The concentration of FFA, *p*CBA and SOD are 1mM, 1mM and 40 U/mL, respectively.

References

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