Supporting Information

Photoresponsive MoS_2 and WS_2 microflakes as mobile biocide agents

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Experimental Section

Reagents and Materials. S. aureus lenticular discs (cat. CRM06571M), *E. coli* lyophilized cells (cat. EC11303), CV (cat. C0775), methanol (cat. 34860), molybdenum (IV) sulfide (cat. 804169) and tungsten (IV) sulfide (cat. 790583) were purchased from Sigma-Aldrich (Spain). Ethanol absolute (cat. ET0005005P) was provided by Scharlau (Spain). Phosphate buffer saline (cat. 28372) was purchased from Thermo Scientific. 24 and 96 multi-well cell culture sterile plates were purchased from Corning (Spain). Cell proliferation kit I (MTT) (cat. 11465007001) was purchased from Merck. LIVE/DEAD Bacterial Viability Kit (cat. L7007) and "SYPRO Ruby Protein Gel Stain" was purchased from Invitrogen (Spain). Penicillin-Streptomycin Antibiotic (cat. 15140-148) was purchased from Gibco™. LB medium was purchased from Universidad de Alcala cell culture center. Milli Q water was obtained using a Millipak Express Filter (cat. MPGP04001) and Vent Filter (cat. TANKMPK01) which were purchased from Merck Millipore (Germany).

Equipment. An ultrasonic processor (VCX 130, Vibra-cell Sonics®) was used to synthesize the microflakes. To capture the videos, an inverted Nikon Eclipse Instrument Inc.Ti–S/L100 optical microscope, coupled with a Zyla sCMOS camera, was used. The microscope is equipped with a xenon arc lamp light source system (Sutter instrument company, LB-LS/30) attached and an FTIC (480 nm), and a G-2A (535 nm) filter cubes (Nikon) to promote microflakes movement. Two SEM, JSM-IT500 (JEOL) and Hitachi TM1000 (Hitachi) were utilized to capture pictures of the biofilms and the microflakes. TEM images were captured with a Zeiss EM10C microscope. A Synergy HTX microplate reader (BioTek S1LFA) was used for optical density and LIVE/DEAD Bacterial Viability Kit measurement. The images were processed using the NIS AR software (Nikon).

Photophoretic micromotors synthesis. To synthesize the MoS_2 or WS_2 microflakes, 7.5 mg of molybdenum (IV) sulfide or tungsten (IV) sulfide, respectively, were added to 10 mL of milli-Q water and sonicated by the ultrasonic processor for 3 hours. Finally, they were washed 3 times with ultrapure water.

Photophoretic micromotor actuation. To induce the photophoretic motion of the microflakes, samples containing the corresponding microflakes were placed in a 96-well plate and irradiated with a xenon arc lamp in an inverted microscope setup. Fast time-lapse images were recorded using a high-resolution monochromatic camera.

Amperometric measurements. To assess the generation of reactive oxygen species by the optical actuation of semiconductor MoS_2 and WS_2-based micromotors, 50 µL of microflakes solution was placed on commercial ITO screen-printed electrodes (DropSens) and this sample was mounted in the inverted microscope setup. The transient photocurrent was measured at -0.6 V vs Ag reference while irradiating the sample transversally. Phosphate buffer saline was employed as a reference electrode.

Temperature measurements. The temperature was measured while the solutions containing micromotors were irradiated by the inverted microscope's excitation light source in a 96-well plate. To record the temperature, a K-type thermocouple was immersed in the solution and fixed during the experiment while the readings were recorded.

Biofilm culture procedure. S. aureus or *E. coli* biofilms were grown in 96 multi-well cell culture plates with sterile LB medium at 37ºC overnight. Next, the LB medium was replaced, followed by another incubation step for 24 h. The biofilm was then ready for further experiments. This assay was performed in "Centro de Apoyo a la Investigación en Medicina/Biología, Unidad de Cultivos Celulares" of the University of Alcala following all the ethical legislation and mandatory health measurement.

Biofilm removal experiments. To remove biofilm by photophoretic microflakes, firstly, the LB medium was removed carefully, avoiding sticking the biofilm of each well. Secondly, they were washed once with 0.01 M PBS, and then, the microflakes were added, followed by light irradiation with the Xe arc lamp for 20 min. After that, the flakes were removed carefully, the wells were washed with 0.01 M PBS and finally, the remaining biofilm was fixed with 96% ethanol for 15 min. To quantify the remained biofilm, 0.1% crystal violet solution was added to the fixed biofilm, followed by a washing step with 0.01 M PBS. After that, the well was filled with methanol to resolubilize crystal violet and it was pipetted to a clean well to avoid possible interferences. All the results were compared with a triplicate blank (0% biofilm) and a triplicate positive control (100% biofilm).

Antibiotic assay of bacterial development. E. coli and *S. aureus* were cultured overnight at 37ºC under shaking without penicillin/streptomycin and with penicillin/streptomycin 1:10, 1:100, 1:1000, and 1:10.000 dilutions. Finally, the OD_{600} of 300µL of bacterial suspensions treated with each dilution and of LB medium without bacteria were measured in the plate reader. The measurements were performed in triplicate.

Protein biofilm removal stain (SYPRO Ruby). The well where the biofilm was grown and treated was cleaned with water. After that, we fixed the biofilm with a solution containing 50% methanol and 7% acetic acid for 15 min. Next, the biofilm was cleaned with milli-Q water. SYPRO Ruby solution was added and incubated during the g for 30 seconds at 80ºC, followed by 5 minutes of shaking at 50 ºC. Finally, the biofilm was washed with a solution containing 10% methanol and 7% acetic acid for 15 min and twice with milli-Q water. To visualize the protein biofilm stain, we used the inverted optical microscope with a QDLP-C filter cube (450-610 nm).

Viability of the remaining bacteria in the unremoved biofilm. To measure the viability of remaining bacteria in the biofilm after removal treatment, the LIVE/DEAD BacLight staining test was used. Firstly, equal amounts of components "A" (Syto-9 dye) and "B" (propidium iodide) were mixed by vigorous stirring. Secondly, the mixture was added to a bacterial biofilm suspension (3 µL of components "A" and "B" per mL of bacterial suspension) and gently mixed for 15 min in the dark. Optical fluorescence microscopy images of the stained biofilms were taken using a FITC (467-498 nm) filter to image live bacteria or a G-2A (510-560 nm) filter to image dead bacteria. Finally, the mix of biofilm suspension and LIVE/DEAD kit was measured in the microplate reader using 360 ± 40 nm as the excitation filter and 528 nm (live) and 620 nm (dead) as the emission filter. All the measurements were performed in triplicate. Biofilms treated with sodium hypochlorite 0,1% were used as a blank (0% live bacteria from 5 minutes treated well and 2 washed to eliminate sodium hypochlorite), and untreated biofilms as a positive control (100% live bacteria from an untreated well and 2 washed to be as the treated well) a 1:1 mix of the blank and positive control was also used as an internal control (50% live bacteria).

Supporting Figures

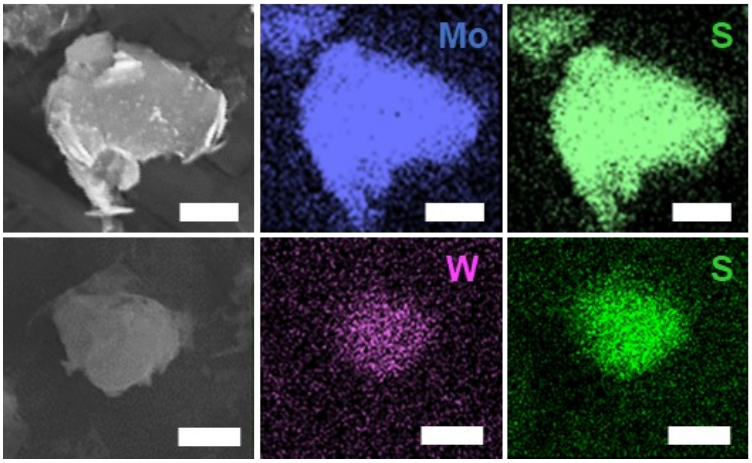

Figure S1. SEM/EDX and Raman characterization of the MoS_2 and WS_2 microflakes. Scale bars, 1 μm.

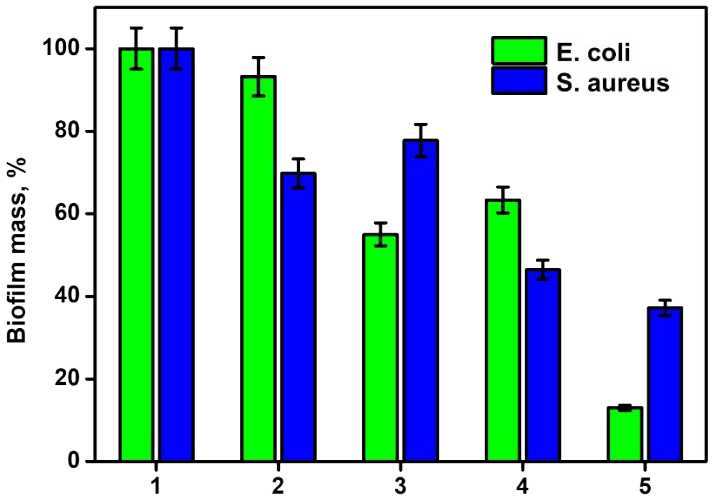

Figure S2. Biofilm mass of *E. coli* and *S. aureus* under different treatments. For each biofilm the optimal conditions were used (MoS_2 and 535 nm irradiation for *E. coli* and WS_2 and 480 nm irradiation for *S. aureus*): 1) Control biofilm, 2) Biofilm grown in presence of micro flakes, 3) Biofilm treated with microflakes without irradiation, 4) irradiated non-motile TMD, and 5) irradiated microflakes.

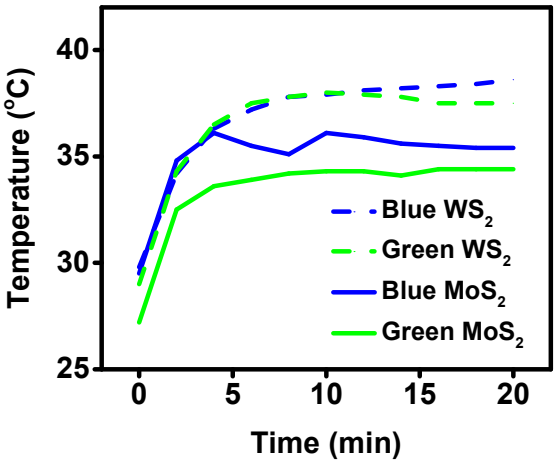

Figure S3. Evolution of temperature in control wells containing TMDs microflakes under blue and green irradiation.

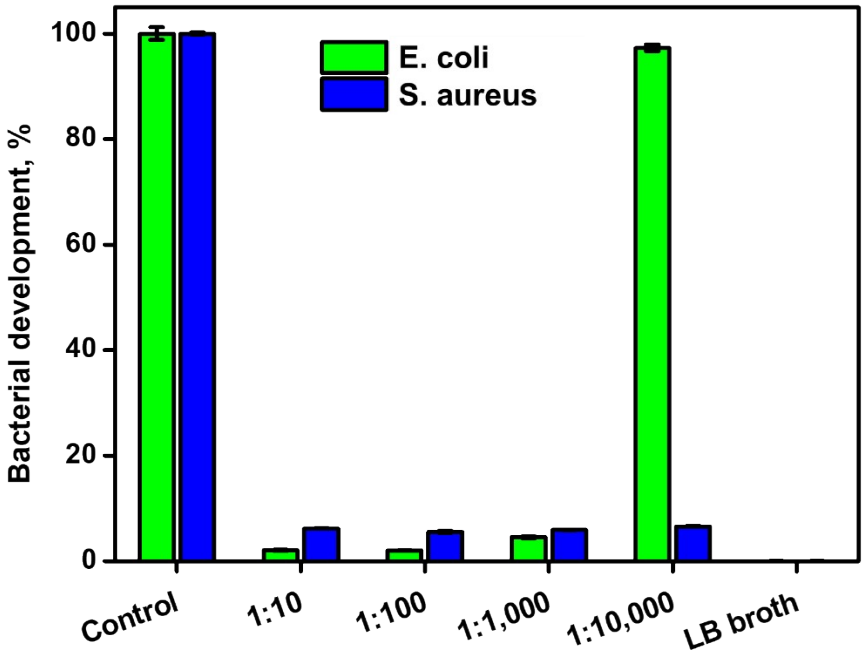

Figure S4. Effect on bacterial development of *E. coli* and *S. aureus* in presence of different dilutions of penicillin/streptomycin mixtures and LB broth without bacteria.