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BioMoSS: Biocidal MoS_2 for Disinfection of Spacecraft Potable Water Systems

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It has been reported in the literature that few-layered, vertically-aligned molybdenum disulfide (FLV-MoS_2) can produce biocidal agents for water disinfection with great effectiveness under visible light excitation. The nano-structure, with limited layers and small domain size, increases the band gap of the MoS_2 in the visible region such that visible light can be used to drive the production of reactive oxygen species (ROS) for biocidal control. This paper will present the BioMoSS (biocidal MoS_2) for space applications concept and present initial results of the investigation into turning the FLV-MoS_2 into a viable biocidal system.

Nomenclature

Ag^+	=	silver(I) ion
CFU	=	colony forming unit
cm^{-1}	=	reciprocal centimeter
E. coli	=	*Escherichia coli*
FLV-MoS_2	=	few-layer, vertically-aligned molybdenum disulfide
H_2O_2	=	hydrogen peroxide
LED	=	light emitting diode
µM	=	micromolar
µL	=	microliter
mM	=	millimole
mL	=	milliliter
MoS_2	=	molybdenum disulfide
mTorr	=	milliTorr
nm	=	nanometer
ROS	=	reactive oxygen species
RCF	=	relative centrifugal force
SCCM	=	standard cubic centimeter
UV	=	ultraviolet

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I. Introduction

WITH renewed interest in human exploration beyond low-Earth orbit, there has been a concomitant demand for technological advances in environmental control and life support systems. The opposing requirements of dramatically increased duration/reliability and reduced system mass/complexity require novel solutions in such systems. Of key importance is the recycling, storage, and residual disinfection of crew potable water. Traditional means of water disinfection employ minute additions of biocidal agents (e.g. Iodine, Ag^+, bleach, etc.) by means of chemical dosing or electrodissolution of metal. While the biocidal efficacies of such agents are well proven, they also have there own drawbacks. Numerous potential alternatives of interest are reported in the literature, including the FLV-MoS_2 upon which the Biocidal MoS_2 (BioMoSS) system is based.

In the past several years, increasing attention has been given to the photoelectrochemical production of biocidal agents at the water/semiconductor interface. Through the reduction of dissolved oxygen and oxidation of water, photo-excited semiconductors with appropriate band structures and surface chemistry produce what are known as reactive oxygen species (ROSs), including hydrogen peroxide (H_2O_2), hydroxyl radicals, superoxide radicals, and singlet oxygen. At and inside the cell membrane, these ROSs can inactivate microbes through several mechanisms. While the half-lifes of most ROSs are very short, H_2O_2 can persist for hours or more, depending on the impurities in the water, possibly allowing for some residual biocidal activity.

One of the most promising materials for this application was described in two papers by the Cui Group at Stanford.[1,2] Shown schematically in Figure 1, few-layer vertically aligned molybdenum disulfide (FLV-MoS_2) is a nanomaterial grown by the sulfurization of thin Mo metal films.[2] MoS_2 is a layered material, with strong covalent intralayer bonding and weak van der Waals interlayer bonding. Under appropriate growth conditions, sulfurization results in small, vertically aligned grains, rather than basally-oriented stacked planes. This has been explained by the relative facility of S diffusion in the van der Waals gap (between the layers)[1] to that across basal planes at elevated temperature and the relaxation of stress, generated by volume expansion, perpendicular to the free surface of the film.[3]

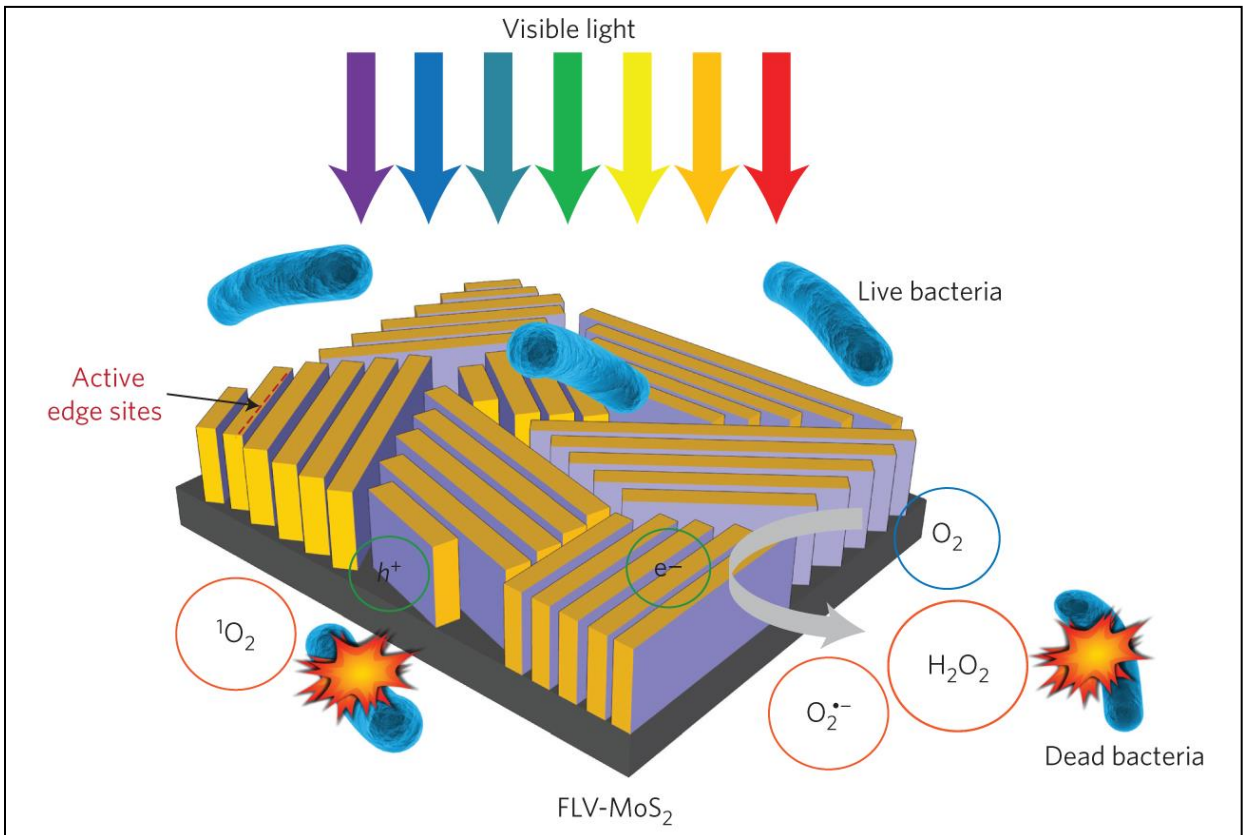

Figure 1. Schematic of visible light disinfection process with FLV-MoS_2 (reproduced from Ref. 2 with permission). © Springer Nature

This results in a widened band-gap (still in the visible range) capable of driving electrochemical ROS generation and a high density of exposed (high energy) dangling bonds. The combination of electrochemically compatible band structure and high surface activity make FLV-MoS_2 a highly effective material for the inactivation of microbes in water, with reported kill efficacy of >99.999% with 60 minutes of exposure to visible light (with intensity similar to that of sunlight).[2] Additionally, upon decorating the top of the FLV-MoS_2 with copper, they were able to decrease the disinfection time to 20 minutes.

While reports of FLV-MoS_2 and other ROS producing semiconductor materials promise very high biocidal efficacy, a deep understanding of the mechanisms involved is still lacking. Many argue that H_2O_2 is the key agent in many of these systems, but countering evidence suggests effectively null biocidal efficacy at the observed bulk concentrations (<10 μM) in *E. coli*. Interestingly, at medium concentrations (10 mM), below normally effective levels it has been shown that there is a synergistic effect of H_2O_2 and blue light, explained in the literature as up-regulation of intracellular Fe^{2+} (and thus hydroxyl radical concentration) production.[4] Regardless, it is likely that some combination of synergistic effects from various ROS species, visible light exposure, and interactions near or at the semiconductor-water interface plays an important role in the biocidal action of such systems.

Most previous work has tested the biocidal efficacy of FLV-MoS_2 and other similar materials in static conditions such as in beakers or other similar configurations. The goal of the BioMoSS project is to translate these results into a flow cell configuration with conditions similar to those found in the humidity condensate and potable water systems of a manned spacecraft. While it is beyond the scope of this work to delve too deeply into the problem of chemical/biological mechanisms, we hope to find confirmatory evidence of this material's unusual biocidal efficacy and examine film longevity and disinfection performance with humidity condensate *ersatz*.

II. BioMoSS System Concept

The current method of biocidal control of potable water is through the addition of iodine (a resupply item) to the water bus. The use of iodine also has a substantial ESM cost associated with its removal from the water to make it potable. Alternatively, NASA is looking into using Ag^+ as a replacement to iodine. Ag^+, although it removes much of the resupply and ESM cost associated with the use of iodine, has issues with its loss from solution meaning that the water will need to be continually redosed with Ag^+. Additionally, there are still several technical issues with the dosing of Ag^+ reliably into solution. Finally, the use of a single biocidal method of disinfection has the potential of producing a resistant organism to that method.

The BioMoSS System, is envisioned to operate off of a finite number of BioMoSS Subsystem Units (BSUs). Each BSU is a small, stand-alone flow cell which will have no moving parts and operates off of a FLV-MoS_2 coupon illuminated by a small bank of LEDs. The BSUs will be strategically placed to optimize protection of the water system, resulting in a system which will have a high reliability, low resupply, and low ESM cost. For dormancy, the light banks could be left on to provide continued protection. If a single LED should fail, it would only minimally diminish the BSUs effectiveness. The power required for the operation of the BioMoSS would be supplied continuously by solar panals.

III. Materials and Methods

A.FLV-MoS_2 Synthesis

The 1st generation process employed in the early work is described here. Modifications to improve process integrity and parameters are described in the Results and Discussion section below. FLV-MoS_2 is grown similarly to as described in References 1-3 and 5. A Mo thin film (~ 10-20 nm) is sputtered (using a 99.9% Mo source, Sigma-Aldrich) on a fused quartz substrate (17 mm x 25 mm) with an IBS/e Ion Beam Sputterer (South Bay Technology) at a rate of ~ 0.2 nm/min and a system base pressure of 6×10^{-6} Torr. Sulfurization was done in a 2.54 cm quartz tube furnace (Lindbergh Blue M) under vacuum, with a base pressure of 17 Torr. 1 g of sulfur powder (99.998%, Sigma-Aldrich) is placed in a quartz boat centered in the insulated edge of the tube furnace, such that S vapor is carried through the tube to the sample at the heating center. Ar gas (UHP, Matheson) is flown through the system at a rate of 95 SCCM, giving a system pressure of approximately 21 Torr at the tube inlet. The system is purged of residual air and water vapor for 60 min. by the Ar flow, and then the temperature at the heating center was ramped linearly to 550 °C in 20 min. and held there for 10 min. Heating is then stopped, the top of the tube furnace is opened, and a natural cool down is allowed.

B. Growth and Suspension of E. Coli

Key to the investigation of biocidal systems is the culturing and enumeration of bacteria. For biocidal efficacy experiments, a culture of K12 *E. coli* is grown from frozen laboratory stock. An individual colony is taken by streaking and inoculated in LB Lennox broth overnight at 37 °C to a measured optical density at 600 nm of ~ 3 (SpectraMax Plus^{384}). The culture is then centrifuged to a pellet and washed in deionized water (900 RCF for 5 minutes, two times) to remove the nutrient broth, and then diluted by 10^3 in deionized water. This produces a suspension with ~ 10^6 CFU (colony forming units)/mL in deionized water. All water and glassware employed are sterilized by autoclaving in steam to prevent spurious results.

C. Photobiocidal Efficacy Test: Visible Light Exposure

The FLV-MoS_2 substrate is placed film-side up at the bottom of a 50 mL Pyrex beaker, which is then filled with 25 mL of the ~ 10^6 K12 *E. coli* suspension. The substrate-containing beaker and a control beaker containing the suspension but no substrate are illuminated from above using a homemade illumination system employing an LED flood lamp (Cree) inside of a biological safety cabinet. The distance from the light source to the FLV-MoS_2 is adjusted to ensure greater than solar intensity in the visible wavelengths. The spectral intensity of the incident light at the MoS_2 is measured using a portable spectral light meter (Gigahertz-Optik, MSC15). As the light source does not produce significant ultraviolet (UV) radiation, no filter is necessary to prevent UV based biocidal activity in our tests.

D. Photobiocidal Efficacy Test: Post-Exposure Enumeration

The concentration of viable bacteria (CFU) in the control (exposed and unexposed to the LED flood lamp light) and FLV-MoS_2 systems after light exposure (two and four hours) are enumerated in triplicate by the single plate-serial dilution spotting method.[6] The liquid samples are poured into tubes and well-mixed. Aliquots are drawn from each tube and serially-diluted with deionized water in sterile 96-well plates, and each dilution is deposited as a series of microdroplets (total volume 20 µL/dilution) onto a section of an LB agar plate and incubated at 37 °C overnight. The resultant colonies are than counted manually. Alternatively, H_2O_2 production in deionized water can be measured colorimetrically using a modified ferrous oxidation in xylenol orange 1 assay.[7]

IV. Results and Discussion

A. FLV-MOS_2 Systhesis

Data from preliminary Raman microscopy experiments (Figure 2) suggest that FLV-MoS_2 was successfully synthesized with the first generation CVD system. The relative intensities of the $E^1{}_2g$ (383 cm^{-1}) and A^1g (408 cm^{-1}) bands of the Raman spectrum indicate the alignment of the MoS_2, with weaker $E^1{}_2g$ relative intensity corresponding to more vertical character. The BioMoSS FLV-MoS_2 spectrum agrees well with that found in Ref. 1 and a freshly exposed mineral MoS_2 edge.

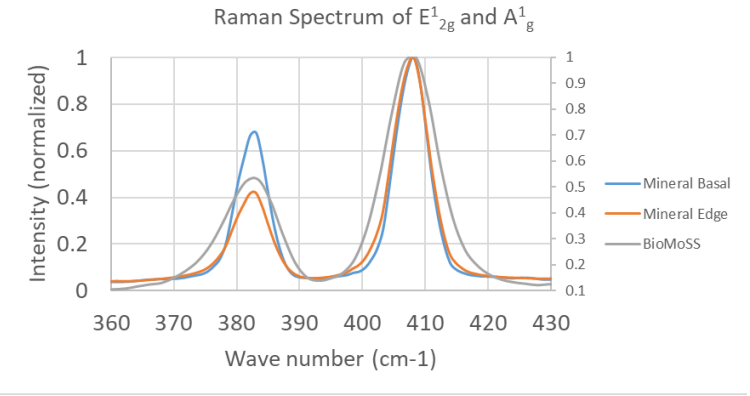

Figure 2. Raman microscopy data from our initial FLV-MoS_2 (BioMoSS) and a MoS_2 mineral reference.

However, early testing of the synthesized material did not show significant biocidal efficacy (>1 log kill) or H_2O_2 production. This is likely due to a combination of incomplete sulfurization, incorrect domain geometry, and a high fraction of horizontal domains. The pump and sulfur trap employed in the preliminary work were unable to attain the base/process pressures specified in the literature. In addition, the membrane filter, used to protect the pump from sulfur intrusion, may have introduced atmospheric gas into the heating center of the tube, regardless of Ar gas flow. Semiconductors are extremely sensitive to doping, and the photoelectrochemical activity of the material was likely diminished significantly. In addition, the excessive pressure may have changed the reaction kinetics of the sulfurization, perhaps changing domain size or other structural properties.

To rectify these problems, we have identified the probable causes listed above and have improved our process to avoid them. We have redesigned our vacuum system for the CVD apparatus, employing a scroll pump with a base pressure two orders of magnitude lower (100 mTorr) than the piston pump used previously. In addition, we have substituted a liquid nitrogen cold trap for the membrane trap in order to protect the pump from sulfur vapor and reduce gas intrusion from the atmosphere. The current CVD system is pictured in Figure 3. These changes allow a process pressure of $\lesssim$1 Torr, with negligible atmospheric intrusion. We have identified a commercial thin film deposition service, which can deposit Mo films with the techniques found in the literature (electron-beam evaporation, plasma sputtering), rather than ion beam sputtering, and avoid possible contamination in the IBS/e deposition system.

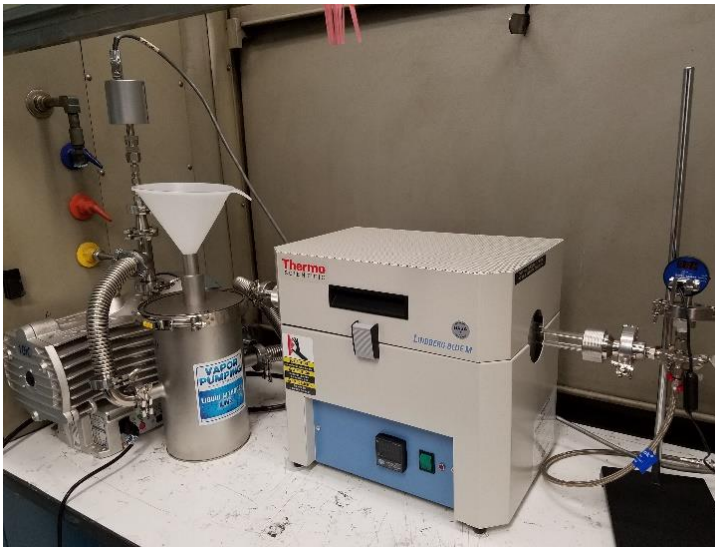

Figure 3. Current CVD/Sulfurization system with improved pump, trap, and instrumentation.

The transmission spectra of the BioMoSS film synthesized in an improved process, prestine Mo film, and literature FLV-MoS_2 data on transparent substrates are shown in Figure 4, High sulfurization completion and similar absorption characteristics are found in the literature and this work, while the variance in magnitude is largely due to difference in film thicknesses. A high-resolution transmission electron micrograph of a thin film containing partial FLV-MoS_2 synthesized in an improved process is shown in Figure 5. Importantly, the layer thickness and length of the vertical domains produced appears greater than that found in the literature.[1-4] Additionally, a significant fraction of the film is not oriented vertically, reducing photoabsorption efficiency and surface activity.

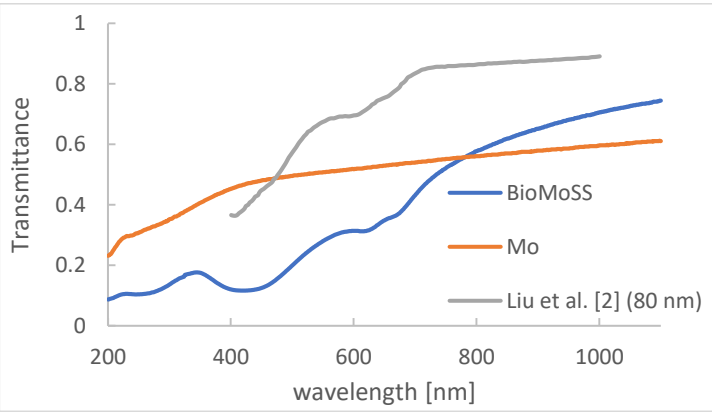

Figure 4. Comparison of the UV-vis spectrum of reported in the literature compared to that of a molybdenum thin film and BioMoSS chip grown in the CVD reactor shown in Figure 3.

B. Photobiocidal Efficacy Test

Our LED-based light exposure system functioned as desired. Figure 6 shows the spectrum incident on the FLV-MoS_2 in our experiments and the incident solar spectral irradiance during a random sunny day at Ames Research Center, for comparison, as measured by our light meter. One important change that may be required in future work is the mechanical agitation of the liquid samples to induce circulation during light exposure.

As mentioned above, our 1st generation FLV-MoS_2 did not show significant biocidal efficacy (>1 log kill) or H_2O_2 production (>0.5 μM in 4 hours) as quantified by the techniques described above. However the enumeration technique works as expected. Figure 7 shows an example of the CFU enumeration in culture dishes for a BioMoSS chip made in an improved process. The concentration of viable bacteria is found by multiplying the colony count in a section with appropriate density by 5×10^{x}, where x is the dilituion factor. An inactivation of ~ 1 log is observed vs. the light exposed control suspension.

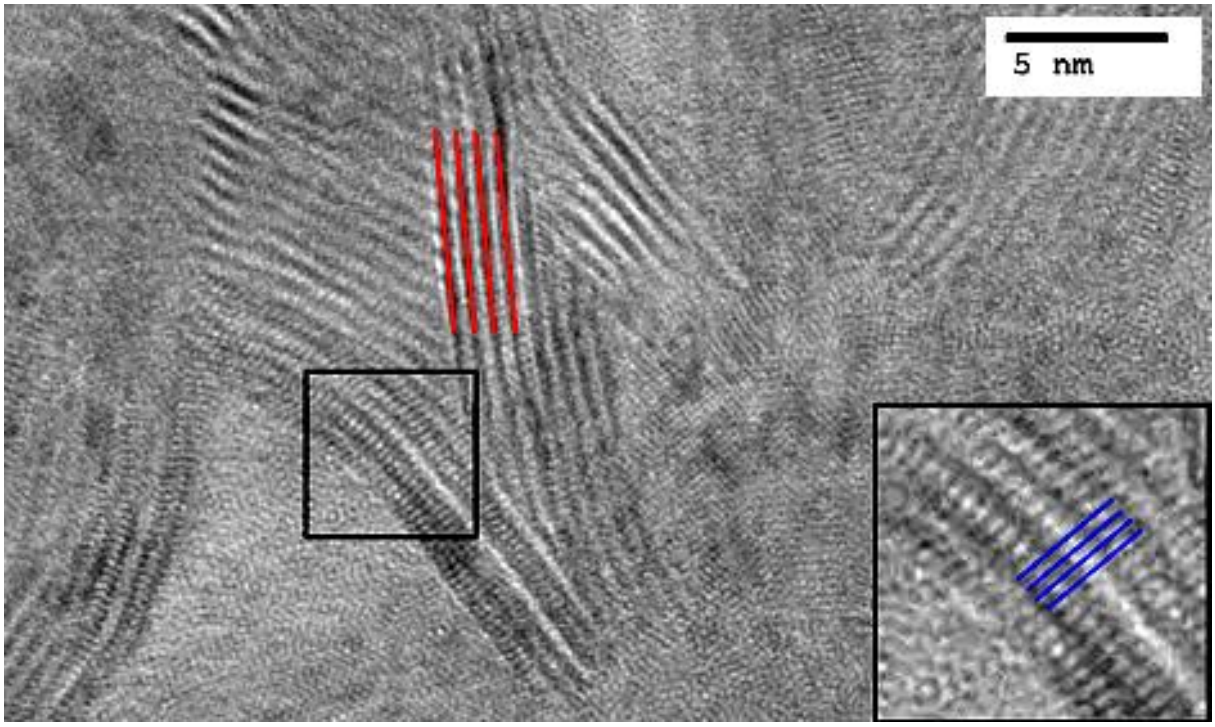

Figure 5. High-resolution transmission electron micrograph showing the grain structure of a partially vertical MoS_2 thin film. The red lines correspond to the (002) basal lattice fringes, which indicate vertical alignment of domains within the film. The blue lines (within the insert) correspond to the (100) lattice fringes. The vertical and non-vertical domains can easily be identified in the image, demonstrating the utility of this technique for film characterization.

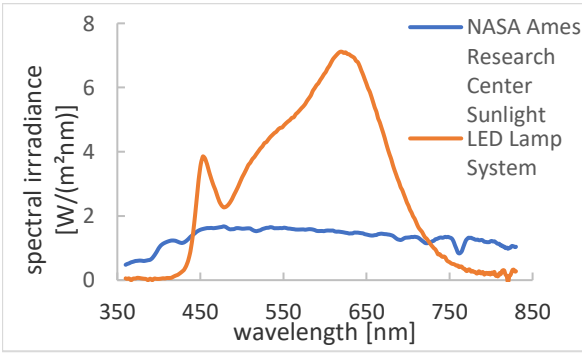

Figure 6. Spectral irradiance from LED lamp system and sunlight.

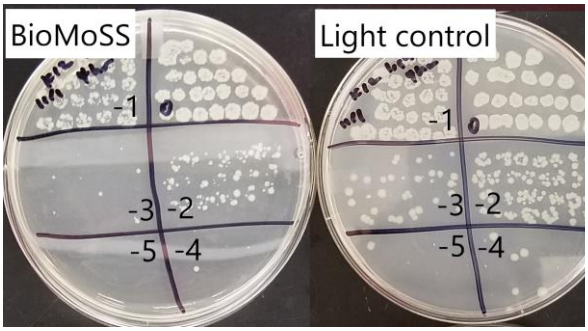

Figure 7. Enumeration of *E. Coli* suspensions after 4 hour visible light exposure. The numbers indicates the exponent *x* for the dilution factor 10^x in the respective section.

V. Conclusions and Future Work

In the near-term we will continue to synthesize the material with the improved processes and conduct further biocidal efficacy and H_2O_2 production tests. By varying the processing parameters (heating profile, pressure, sulfur position, etc.), we hope to produce MoS_2 with near perfect verticality and appropriate domain size / layer thickness.

After a successful *E. coli* inactivation , we will characterize the ROS production and microbe inactivation kinetics, and then move on to the rational design and testing of flow cells in a humidity condensate system analogue. The addition of surface catalysts such as copper, which have been reported to increase inactivation kinetics significantly, may also be investigated.

Acknowledgments

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