MOLYBDENUM DISULFIDE 2-D NANOSHEETS TOXICOLOGY TO THE ENVIRONMENTAL SURFACES: SOIL BACTERIA SURVIVABILITY ANALYSIS

A Thesis

by

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ABSTRACT

2-D nanomaterials have received much attention as building blocks in energy-related devices and applications. The increasing demand for those nanomaterials has brought along environmental concerns at the same time. Due to their unique thermal, mechanical, and interfacial properties, such nanomaterials constitute unknown principles in the context of nanosafety, nanotoxicity, and the environmental effects to the entire ecosystem of the earth. The research to know the science of those nanomaterials is required, as disposal of such chemicals can be toxic to the environment. In this thesis, the concise mechanism of surface adhesion of 2-D nanomaterials to soil bacteria cells will be reviewed. In addition, from the relationship between microorganisms and the entire soil environment system, we can predict the environmental impacts of the mass disposal of those nanomaterials systemically. Experiments were conducted to investigate the influence of 2-D nanosheets of Molybdenum Disulfide (MoS_2) to the survivability of soil bacteria dissolved in deionized water. Gram positive bacteria, *Bacillus cereus* will be released to the several different concentrations of MoS_2 nanosheets in a 24-hour timeline, to examine the cytotoxicity of these nanoparticles in pure water. The entire procedure can be set as a standard to assess the toxicity of any other energy-related 2-D nanomaterials to the other gram positive and negative bacteria for further research.

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Contributors

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NOMENCLATURE

AFM	Atomic Force Microscopy
CBN	Carbon-Based Nanomaterial
CFU	Colony Forming Unit
DI	Deionized
DLS	Dynamic Light Scattering
LPE	Liquid-Phase Exfoliation
MoS_2	Molybdenum Disulfide
NMP	N-Methyl-Pyrrolidone
OD	Optical Density (UV-Vis)
PBS	Phosphate-buffered Saline
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscopy
TMD	Transition Metal Dichalcogenide
TSA	Trypticase Soy Agar
TSB	Tryptic Soy Broth
UV-Vis	Ultraviolet – Visible Spectroscopy

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1. INTRODUCTION

For the development of modern energy-related technologies, we need to understand the transport of energy by-products and related contaminants released to the environments. Those transports of materials interact with environmental surfaces such as cell, soil, and the atmosphere. With the recent development of nanotechnology, many kinds of nanomaterials were introduced to enhance the performance of energy-related devices. Especially for the energy converting modules such as photovoltaic cells, novel nanomaterials like Molybdenum Disulfide 2-D nanosheets have become the key upgrading component for the technical modification. However, these nanomaterials are so tiny that tracking and observing the transports and surface interactions of those are a bit difficult. It is not known whether or how much those are harmful to the environment compared to the other conventional materials that scientists have already researched before. Hence, the investigation of nanosafety and nanotoxicity is now in demand. To preserve and protect the environment and public safety, the research of predicting the aftermath of new technology is highly appreciated. In this thesis, the preparation of MoS_2 in the form of 2-D nanosheets, its industrial applications, cytotoxicity, environmental aspects after the disposal, the concept of bacteria growth pattern, and gram positive soil bacteria survivability experiments will be studied.

1.1 Exfoliation of Molybdenum Disulfide (MoS_2)

For the preparation of 2-D nanomaterials, liquid-phase exfoliation (LPE) method is one of the most popular method creating the solution of 2-D nanosheets (Ciesielski et al., 2014). It is able to produce large scale of graphene nanosheets from Graphite by exfoliating those in liquid-phase with using organic solvents such as N-methyl-pyrrolidone (Hernandez, Yenny, et al., 2008). Transition Metal Dichalcogenide (TMD) such as Boron Nitride, MoS_2, WS_2, and TaS_2 can be generally prepared in the form of 2-D nanosheets by using LPE method (Coleman, Jonathan N., et al. 2011).

Molybdenum Disulfide (MoS_2) belongs to Transition Metal Dichalcogenide (TMD), which has monolayer of transition metal bound by two surrounding layer of chalcogen (S, Se, or Te) like a sandwich. Those layers attract each other with Van Der Waals force to sustain the multi-layered structures (Chng et al., 2014). Multi-layered MoS_2 has interlayer spacing of approximately 6.5Å (A Nayak Pradeep et al., 2016). Those materials have strong in-plane bonding and weak Van Der Waals forces to form a 2-D structure if totally exfoliated. With applying the sonicating techniques, the production of 2-D nanosheets became available in a large scale. Imaging those 2-D nanosheets can be conducted with Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Atomic Force Microscopy (AFM). TEM and SEM can take transparent images of nanomaterials, whereas AFM can characterize the dimension of nanomaterials. Also, using Dynamic Light Scattering (DLS) can estimate the particle size distributions and the mobility of the nanomaterials in the solution before and after the exfoliation (Forsberg V et al., 2016).

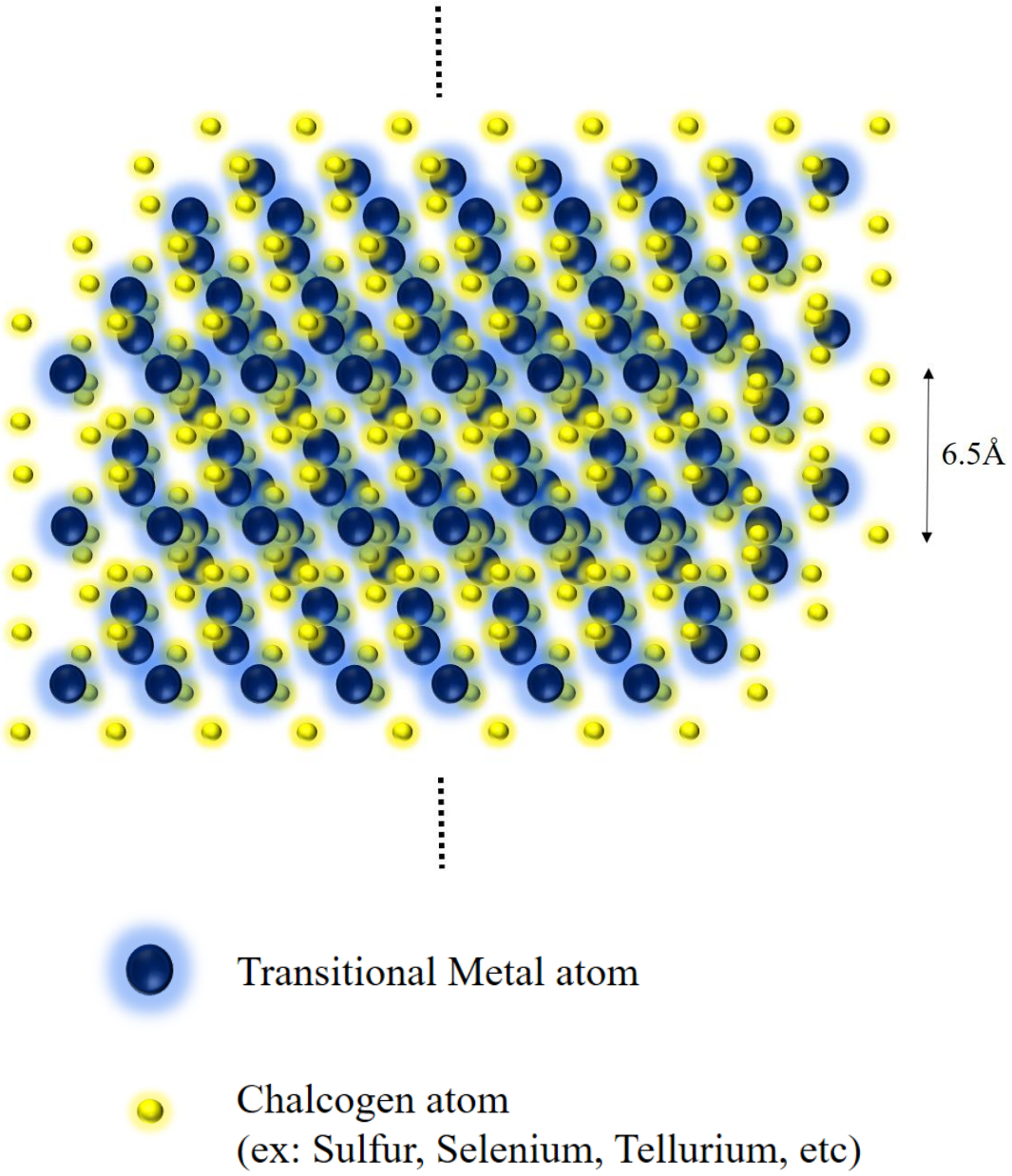


Figure 1. Multi-layered structure of Transition Metal Dichalcogenide (TMD)

Sonication exfoliates the multilayer of the nanomaterials into a 2-D nanosheet layers. Sonication makes shear forces inside the solution, breaking the cavitation of bubbles that can peel off the multilayer nanomaterials. For the solvents, purely distilled water or the organic solutions can generally be used. Some studies have shown that specific nanomaterial has exceeding rate of exfoliation when dissolved in organic solvent. MoS_2 can be exfoliated efficiently in the solvent of N-methyl-2-pyrrolidone (NMP) with a trace water (Gupta et al., 2016). The crystalline structure of exfoliated MoS_2 nanosheets forms a unique hexagonal pattern. If it is well exfoliated, a high-resolution microscope image will show TMDs having a defect-free honeycomb structure (Ataca, et al. 2012).

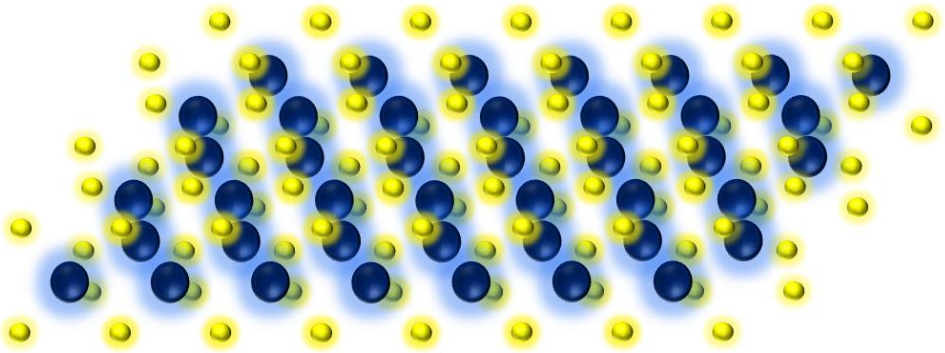


Figure 2. Mono-layered structure of Transition Metal Dichalcogenide (TMD)

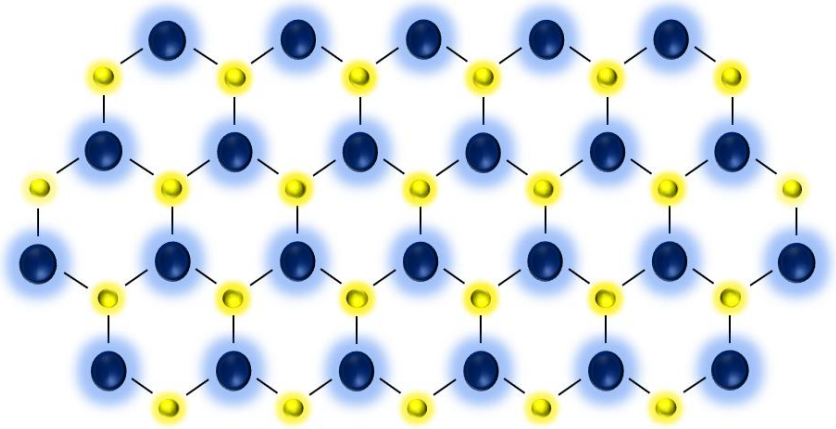


Figure 3. Honeycomb structure of Transition Metal Dichalcogenide (TMD)

1.2 The applications of MoS_2 and MoS_2 2-D nanosheets

MoS_2 can be applied to a variety of technologies such as lubricant, catalysis, electronics, photonics, and photovoltaics. Especially when utilized as 2-D nanomaterials, MoS_2 has additional features that have not shown before when it is in the form of bulky materials (Liu et al., 2015). Before nanosheets were utilized, MoS_2 particles with the size of 1 ~ 100 micron were commonly used as a dry lubricant. When added to plastics, MoS_2 forms a composite with enhanced durability and reduced friction (Clauss et al., 2012).

In addition, MoS_2 works as a co-catalyst for desulfurization in the chemical plant. For instance, the process of hydrodesulfurization at petrochemical plant employs MoS_2 as a catalyst. By doping additional metal elements like cobalt or nickel, catalytic reaction becomes even more effective. They are also a good catalyst for hydrogen evolution from the operation of the electrolysis of water (Laursen, A. B. et al., 2012). In addition, MoS_2 can be used to boost the process of hydrogenation or hydrogenolysis for organic synthesis (Laursen et al., 2012).

When MoS_2 becomes 2-D nanomaterials, it can cover the surface dimension to make the area become photoelectrocatalytic since chemical activity and surface area can

sharply increase in nanoscale particles. Moreover, Polymers such as Nylon, Teflon, Vespel can be used in the combination of MoS_2. MoS_2 nanosheets can encapsulate the conjugated polymers to make electrically conductive composites, also improving the strength and reducing the friction (Bissessur et al., 1994). The band gap of monolayer MoS_2 increases as it has a strong photoluminescence compared to a bulk scale of MoS_2. As a catalytic agent at the active site on the surface area, MoS_2 has a great potential to upgrade the performance of photovoltaic cell in the future (Splendiani et al., 2010).

This photoluminescence property of MoS_2 has an advantage against Graphene 2-D nanosheets. Graphene has been one of the most popular nanomaterial because it is chemically durable and very conductive for the heat and electricity. However, the bandgap of Graphene is very slight in spite of its form as 2-D nanosheets, considering that generally the band gap can increase when the material is scattered in a nanoscale. Compared to Graphene bandgap of 0.15 eV, monolayer of MoS_2 has much higher bandgap of 1.8 eV. This characteristic is very useful when fabricating a semiconductor. Monolayer of MoS_2 has reasonable cost and advantageous property to build nanoelectronic devices such as transistors. For instance, Field-Effect Transistor (FET) biosensor fundamentally require some bandgap to avoid leakage and maintain sensitivity, and MoS_2 has shown more advantageous and flexible material for the circuit than Graphene (Radisavljevic et al., 2011).

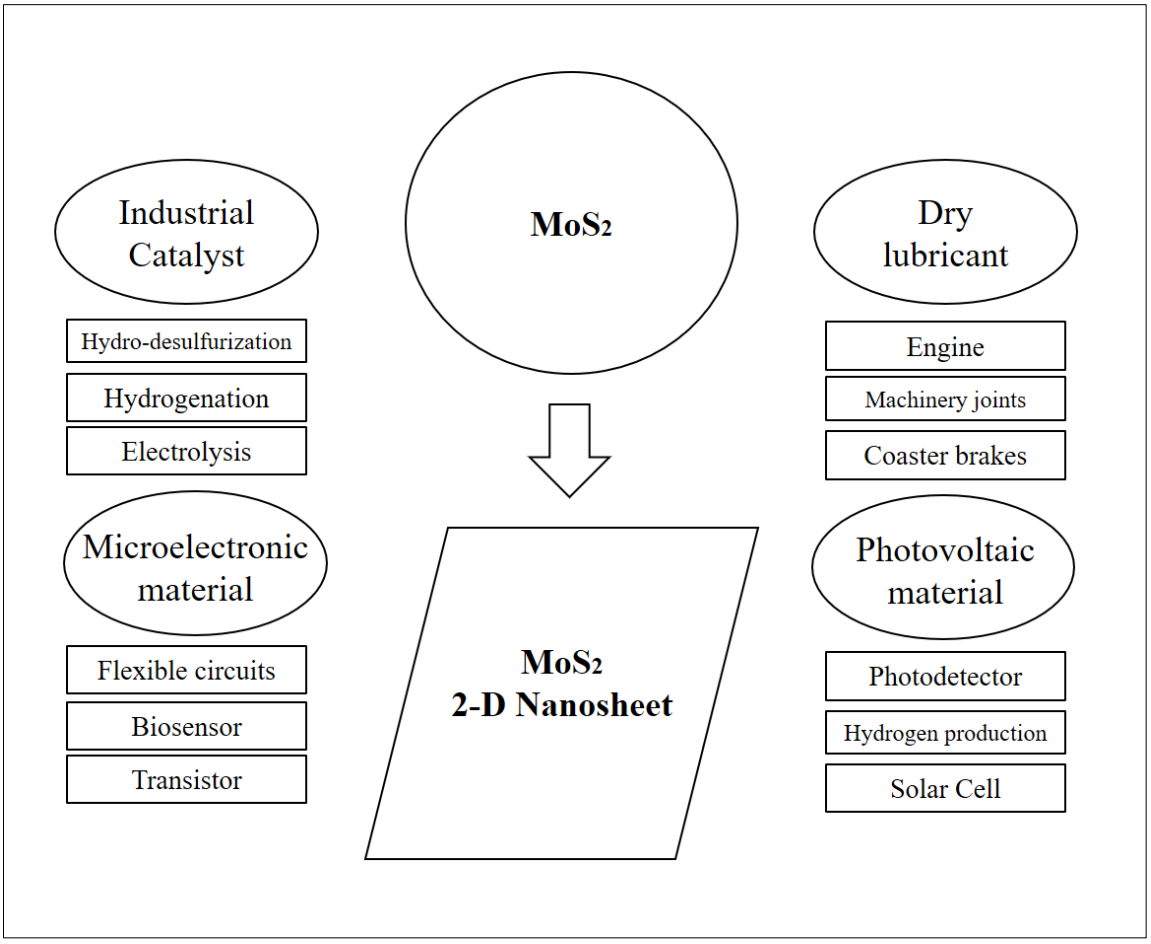


Figure 4. Applications of MoS_2 and MoS_2 2-D nanosheets

1.3 Toxicity of nanomaterials

Nanomaterials have chemically reactive ultrahigh surface area (Liu et al., 2015). In addition, the size of those materials allow them to invade into cell membranes to contact several important organelles inside the cytoplasm. The release of such chemicals are highly deprecated as those transport can harm the environments and public health. In other words, the living things can directly contact or uptake the nanomaterial from external substances anytime. Our lungs when we respire, our intestinal barrier when we consume, nanomaterial can transport through the wall of cells by the air, water or any other possible form of medium. As lung and intestines are developed to absorb tiny molecules inside our body, nanomaterials can easily enter those channels. There have been some investigations on the rats and mice whether carbon nanotube shows a sign of nanotoxicity (Hoet et al., 2004). The cytotoxicity of carbon nanotubes was revealed to have similar symptoms of inhaling asbestos, such as asbestosis, lung cancer, and malignant mesothelioma of the pleura. Carbon-based nanomaterials (CBNs) have been used as one of the most attractive building blocks as they can be reformed to a variety of different forms. Fullerenes, single- or multiple-walled carbon nanotubes, nanofibers, graphene sheets are the examples of CBNs. Those have been already in mass production. In addition, combustion streams of any fossil fuels contain nanoscale carbon particles and those are released to everyday life. The release of toxic nanoparticles has shown obvious clues of occurring severe inhibition of cell proliferation and cell death (Magrez et al., 2006).

Hence, knowing the cytotoxicity of the nanomaterials which are recently produced in a mass scale is highly required (Andre E. Nel et al., 2009). The increased supply of 2-D nanosheets may occur the exposure of tiny nanoparticles to the air, water and the soil. When exfoliated, the chemical compounds tend to have more reactivity as it have become nanoscale materials with high surface area. For instance, MoS_2 has shown significantly higher toxicity when it has been well-exfoliated (Chng et al., 2014). Furthermore, there is an actual case that scientists revealed that man-made nanoparticles

such as ZnO, TiO_2, and CuO have a toxicity to few bacteria samples such as *V. fischeri* and *T.platyurus* (Heinlaan et al., 2008). In sum, we have to consider that an increase of industrial nanoparticles will bring about the damage to the ecosystem as well as to public health. It is required that we have to prepare for the unexpected consequences derived from the development of nanotechnology (Anthony Seaton et al., 2009).

1.4 Physicochemical interactions between nanomaterials and environmental surfaces

The study of biophysicochemical interactions between the nanoparticles and the cell has helped understanding the cytotoxic mechanism in the molecular level. Nanoparticles can interact with cells directly and they are nearly as small as the size of the hole at the cell membranes. 0D and 1D nanostructures can be simply diffused inside the cell as they are smaller than the size of the hole at the membranes. However, 2D and 3D nanostructures can adsorb onto the boundary of the cell membranes, as they are not tiny enough to be diffused inside the cell. To explain the interaction between the nanoparticles and cell surfaces, important physicochemical characteristics, such as chemical composition, surface functionalization, shape, angle of curvature, porosity, surface crystallinity, heterogeneity, roughness, hydrophobicity, hydrophilicity, effective surface charge (zeta potential), particle aggregation, state of dispersion, stability/biodegradability, dissolution characteristics, hydration, valence of the surface layer, ionic strength, pH, temperature, pressure, presence of other molecules, detergents can be all mentioned to analyze the microscopic phenomena (Andre E. Nel et al., 2009).

In terms of the surface interfacial physiology, the charge of cell surfaces can be measured as Zeta potential parameter to estimate the electrophoretic mobility of cells in a semi-electric field. For hydrophobic 2D nanomaterials inside the water solution, as they are in the form of the sheets mostly not dissolved, they have a high-energy interfacial region that is very unstable. Over the time, those suspended sheets in a dispersion starts to adhere upon each other, or any other surfaces that can decrease the interfacial regions. As the particles have higher electrical surface charges, this characteristic can induce more rapid electrostatic adhesion between different charge particles or the repulsion upon similarly charged particles inside the colloidal solution. Colloids with high Zeta potential will be stabilized in a dynamic dispersion, while colloids with low Zeta potential will make coagulation and flocculation as time goes on (Greenwood et al., 1999), (Hanaor et al., 2011).

Bacterial cell surfaces usually have negative electrostatic charge that is induced from ionized phosphoryl, amino, and carboxylate substituents on the cell membrane. Particularly, outer cell envelope of the boundary is exposed to the extracellular environment that can interact with any particles from the outside. These kinds of characteristics play an important role for immunological patterns and cell growth & division. Bacerial Zeta potential can be estimated by electrophoretic mobility in an electric field. Gram positive bacteria has thick peptidoglycan cell wall, and it has surface electronegativity induced by phosphoryl group with teichoic and teichuronic acid residues, and unsubstituted carboxylate groups. Gram negative bacteria has no exposure to the extracellular environment of peptidoglycan cell wall, but it has phosphoryl and 2-keto-3-deoxyoctonate carboxylate groups of lipopolysaccharide attached at the outer membrane, inducing electronegative charge on the cell surface (Wilson et al., 2001).

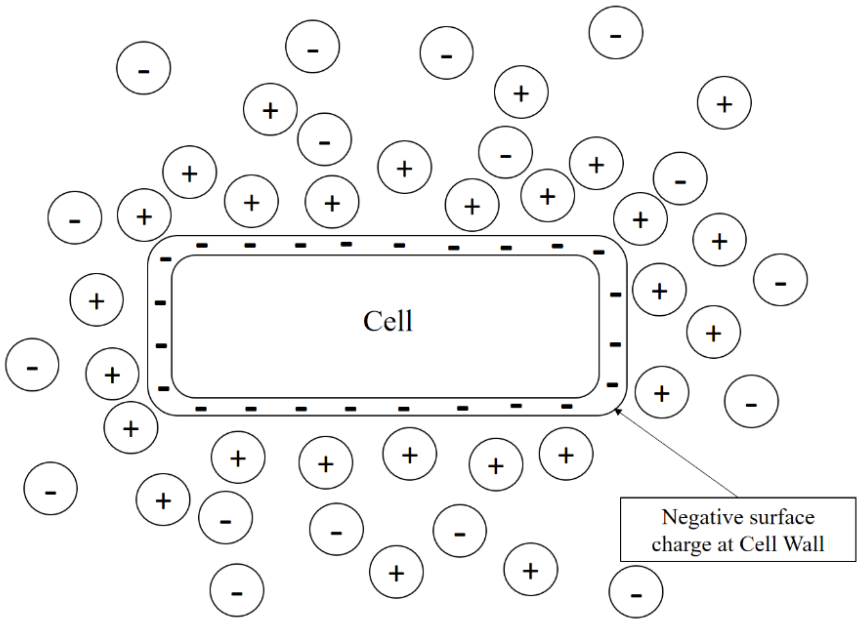


Figure 5. Ionic distribution near negative charged cell surface

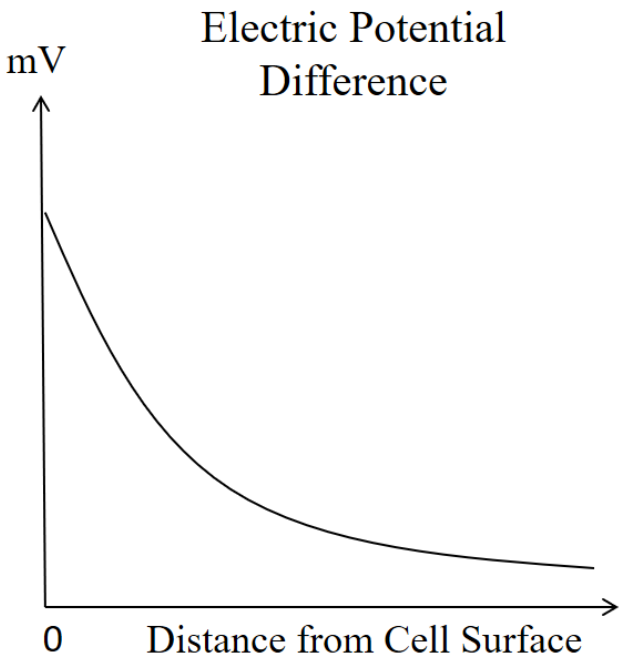


Figure 6. Electric potential difference depending on the distance from cell surface

1.5 Bacteria growth pattern

The number of bacteria grow exponentially when it is provided with abundant nutrition. The Bacterial growth modeling have described the logarithm of the relative population size [$y = \ln(\frac{N}{N_0})$] against time (t). There are three phases of the growth curve, with three important parameters. The maximum specific growth rate, μ_m is a tangent at the inflection point. The lag time, λ is a x-axis intercept of tangent. The asymptote [$A = \ln(\frac{N_\infty}{N_0})$] is the maximum number of bacteria increased. As time passes, the curve start to decline and we call this the death phase (Zwietering et al., 1990).

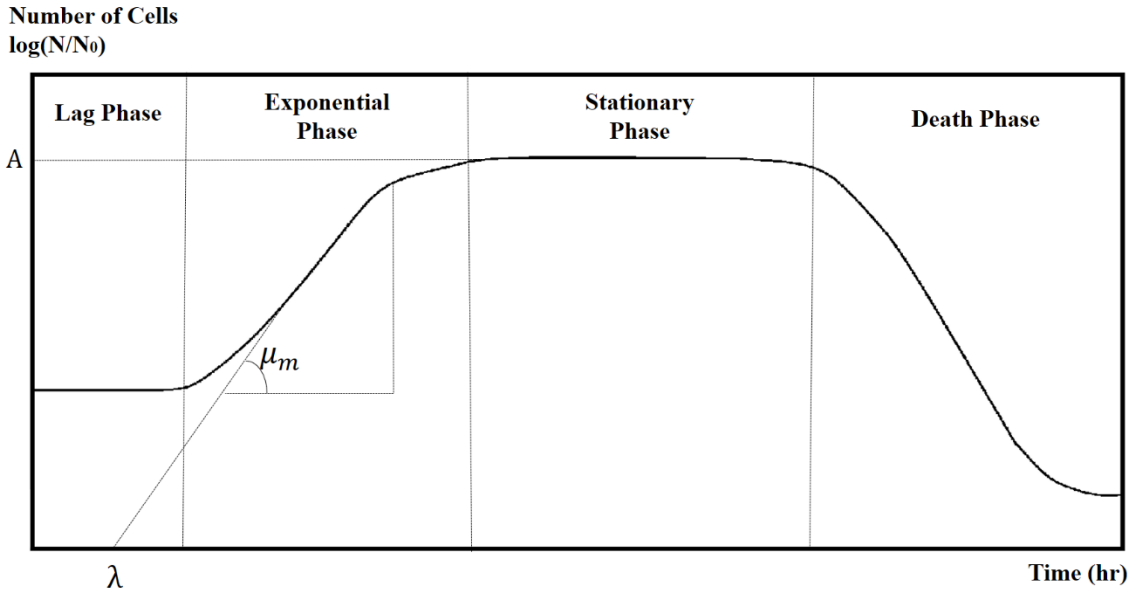


Figure 7. Bacteria growth curve

When we incubate bacteria culture, we transfer diluted inoculum from overgrown medium to fresh medium. Fresh medium starts the life cycle again from Lag phase, Exponential phase, enter Stationary phase, and end with Death phase. Wherever bacteria are observed overgrown is during or after the Stationary phase. The growth of the colony is not consistent as the age, size and the compositions of medium dynamically change with time. For the growth rates, bacteria usually has doubling time from 25 minutes to 2

hours. Back to early days of bacteriology when there was no spectrophotometer, the main method to measure bacterial growth was counting the number of grown colonies. For instance, researchers have found that there was no observed growth for at least 1 hour, and this phase is the classic lag phase. The length of each phase is dependent on the species of bacteria, the quantity of medium, and the other environmental circumstances (Cooper et al., 2012).

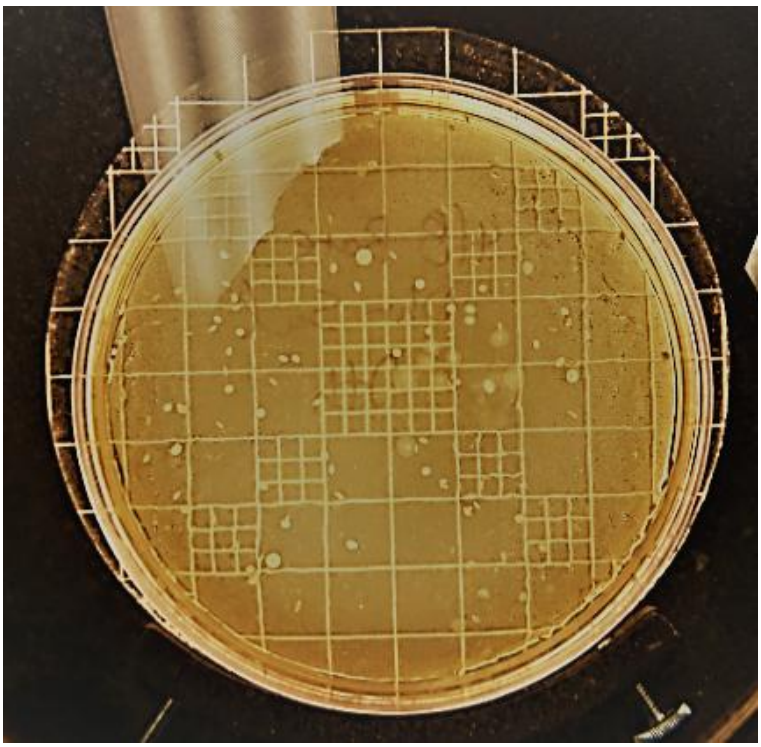


Figure 8. Grown bacteria colonies (*Bacillus cereus*) on the Petri dish

2. LITERATURE REVIEW

To evaluate the survivability of bacteria inside the sterile water and phosphate-buffered saline (PBS), 148 kinds of bacteria at the stationary-phase were incubated in rich agar media and then transferred to the solution. Most of Gram negative bacteria tested survived equally during the experiment for at least 30 weeks, whereas Gram positive bacteria declined more rapidly in water than in PBS (Liao et al., 2003). 3 kinds of Probiotics were packed with uncoated calcium alginate beads, and coated ones with material, chitosan, sodium alginate, and poly-L-lysine in combination with alginates. Among those, Chitosan-coated alginate beads provided the best protection for probiotics. (Krasaekoopt et al., 2004). Silver nanoparticles have shown antimicrobial activity against E.coli and possibly, against to all Gram negative bacteria. To observe the particles and bacteria in a nanoscale, SEM and TEM were used. The research has found that silver nanoparticles cause the formation of pits in the cell wall accumulate on the cell surface. This facilitates a significant increase of permeability of the cell wall, resulting a death of cell. These are nontoxic to human, and cost-effective materials that can be applied to antimicrobial purpose (Sondi et al., 2004). One of TMDs, Molybdenum Disulfide has exhibited the stronger toxicity to human lung carcinoma epithelial cell line A549 when it was more exfoliated in an organic solvent. There are few different organic solvents which have different ability of exfoliating MoS_2, t-Bu-Li solvent has shown the most cytotoxic result among Me-Li, n-Bu-Li, and t-Bu-Li solvents. MTT assay and WST-8 assay were used to measure the viability of cells (Chng et al., 2014). Also, Pour Plate method have been used in many other researches. For instance, to count the survivability of soil bacteria, soil extract agar with 0.2 percent yeast extract, asparagine-mannitol agar, nutrient agar were poured to the sample and incubated at 30℃, examined daily for 14 days (Chen et al., 1972). For counting the number of grown colonies of bacteria, fluorescence microscopy can be also used as well (Lindahl 1995). For imaging purpose, SEM was used to determine morphology of bacteria or the materials such as fresh vegetables, characterize the physical changes of the surface of

bacteria after some treatment, and observe attaching behavior of bacteria or the materials (Zhang et al., 2013). To inhibit food pathogens, oxygen plasma sanitization experiment on few vegetable surfaces were conducted, and Polyethylene glycol was coated on the vegetable to prevent the adhesion of bacteria. Right after oxygen plasma treatment, this makes the outermost surfaces to be hydrophilic, so the coating can be added after the treatment for hindering the attachment of bacteria on the food (Zhang et al., 2014). Basically bacteria has an attaching behavior on a flat surface, so the modification of surface to become hydrophobic can help inhibiting bacteria contamination. Hydrophobic nanoporous silica materials were synthesized and coated on the quartz to control the hydrophobicity. Also, AFM image has assisted to show the change of thickness on the nanocoated surface (Oh et al., 2016). These kinds of nanocoating techniques can be applied to the industry such as the products which requires the high quality of antimicrobial surface. Gloves made of latex, nitrile, and polyethylene can be nanocoated with fluorinated silica nanoparticles to improve the hydrophobicity and anti-adhesive character. Bacterial experiment has shown around 1-2 log improvement of bacteria decrease in comparison of bare gloves (Oh et al., 2016).

3. RESEARCH OBJECTIVE

Upgraded performance of the microscope allowed us to observe a lot more tiny scale of scientific phenomena. To see and control the nanoscale chemicals and microscale bacterial cell became available nowadays. However, as these kinds of experiments include invisible region from the naked eyes, there are always risks that researchers have to undertake. As preliminary researches has proven, tiny particles usually show more reactive characteristics that might be harmful to the living things. In addition, predicting the effects of those became a lot more difficult as we are not clearly aware how to handle and prevent the proliferation of those particles. Hence, the research of nanosafety has a clear goal to protect the people and the environment.

This research will produce 2-D nanomaterial and verify its toxicity by conducting bacteria survivability experiment. From the preparation of the nanomaterials to the disposal of the biological wastes through the experiment, the entire process observes and controls the micro- and nanoscale region. To observe, SEM and AFM microscope will be used to obtain the image of bacteria and 2-D nanomaterials. In addition, Zetasizer will be used to verify the size of nanomaterials with Dynamic Light Scattering (DLS) technology, in order to examine whether MoS_2 is well exfoliated and became nanoscale particle. Zetasizer equipment will also measure Zeta potential of nanoparticles, gram positive and negative bacteria, as those data are related to its physical property and dynamic patterns. Zeta potential data will help understanding the interactions at cell surfaces, such as assisted adsorption of nanomaterials at the cell membrane induced by the surface charge.

Next, bacterial experiment will be conducted to analyze the cytotoxicity of 2-D nanomaterials. There will be 5 different concentrations of chemical solutions from pure DI water (0M of chemical input) to almost maximum dispersion of 2-D nanomaterials dissolved in DI water. The research will try to incubate the bacteria, and transfer to Trypticase Soy Agar (TSA) to observe the growth of bacteria from different chemical concentrations. Pour Plate method will be used to count the exponential number of

growth of bacteria. We expect the result of less survivability of bacteria in higher concentration of chemical solution. A cell is the smallest unit of the living thing, and bacteria is Prokaryote which consists of one single cell as an independent living thing. By observing the cytotoxicity upon bacteria, the result will show the evidence of understanding the phenomena of the other complex living things interacting with toxic chemicals. Both Gram positive and negative bacteria will be experimented, since the structure of the cell wall of those are different.

By analyzing the survivability data of the 24-hour timeline with different concentrations, we can systemically predict what is going to happen after the mass disposal of nanomaterials to the environment. Especially to the soil environment, as the extended research will cover both Gram positive and negative soil bacteria, *Bacillus cereus* as Gram positive and *Pseudomonas fluorescens* as Gram negative specimen. Those soil bacteria are beneficial for the ecosystem of the soil. Hence, the cytotoxicity upon those will cause a threat to the ecosystem. Also, those can be possibly harmful to the people and the other living things as well, as cytotoxic character of the nanomaterials will act quite similarly to them.

4. METHODOLOGY

4.1 Exfoliation of nanomaterials with sonication

CAS No. 41827 Molybdenum (IV) Disulfide, 99% (metals basis) powder was purchased from Alfa Aesor. To get the AFM image of 2-D nanosheets, MoS_2 was prepared in the concentration of 0.02M (3.2 mg/ml). Solution containing MoS_2 was washed by using Centrifuge for twice, 15 minute, 4000 rpm to get rid of the nano-size dust particles. Next, Probe Sonicator (Ultrasonic cell crusher, Ningbo Haishu Sklon Electronic Instrument Co., Ltd., SYCLON) was used to exfoliate MoS_2 (50℃, time 30mintute, on 3second, off 1second). Sonication ran for 1 hour (30 minutes × 2 times) in total. During the Sonication, the vial of 20 ml volume was used and placed inside the ice bath to stabilize the low temperature.

Figure 9. Centrifuge in operation (4000 rpm)

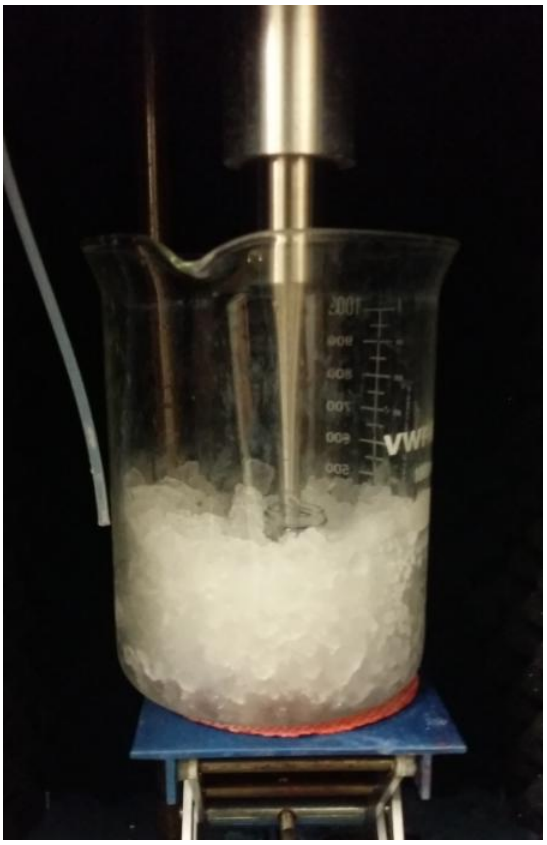


Figure 10. Probe sonication of MoS_2 inside the ice bath

Figure 11. Exfoliated MoS_2 in DI water (20ml vial)

4.2 Characterization of nanomaterials

Zetasizer Nano ZS90 was used for measuring the size of the nanoparticle. DLS beam hits MoS_2 nanoparticle dispersed inside the water and take out size and intensity data. Those data are gathered to produce a cumulative plot of particle size distribution as well as the intensity of the particles. Zeta average size is expressed with the intensity based harmonic mean of the particle size.

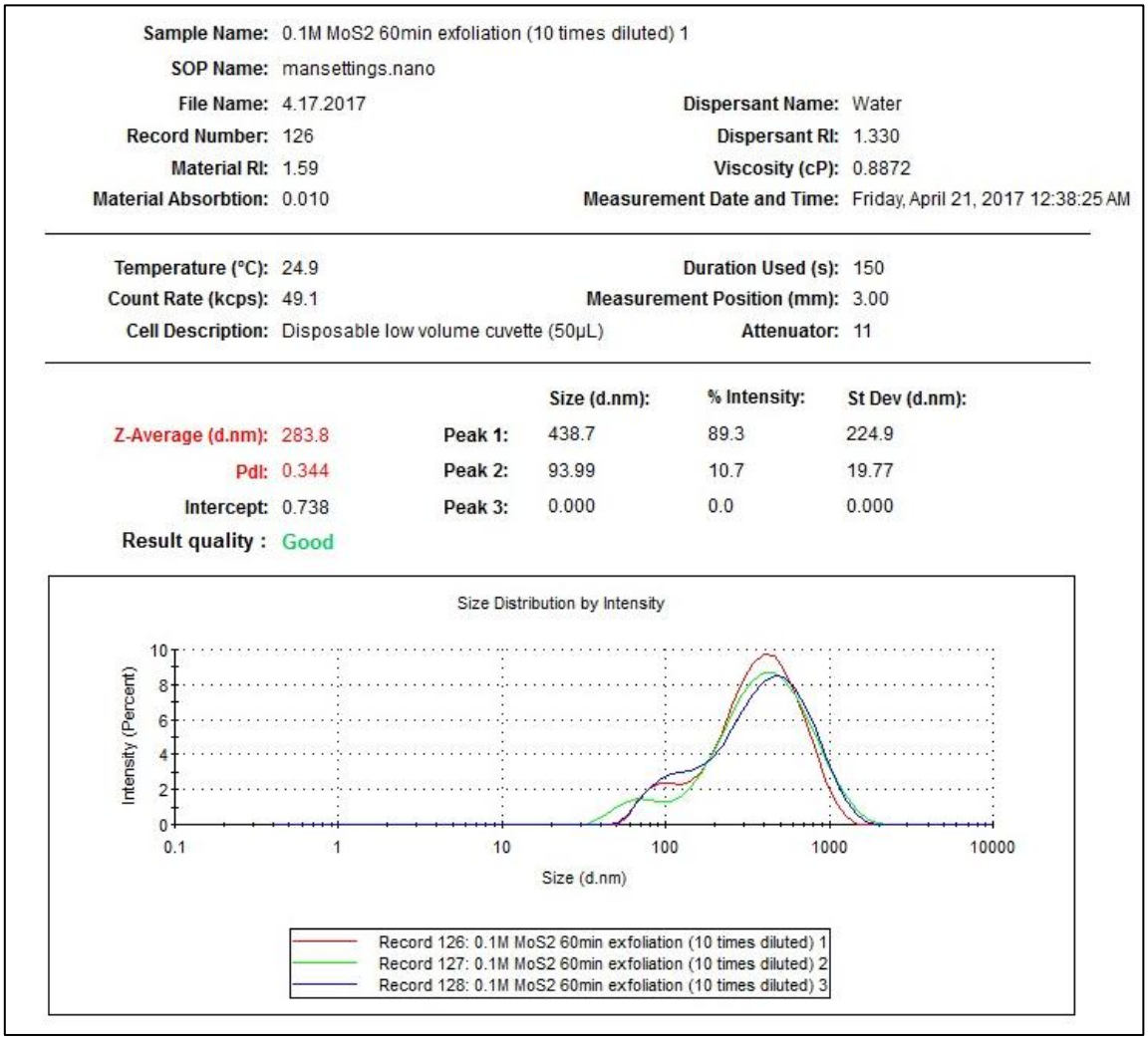


Figure 12. Zeta-size data: 0.1M MoS_2 in a DI water exfoliated for 60 minutes

For the characterization of nanomaterials, AFM image of MoS_2 was taken after the exfoliation. One drop of exfoliated solution of MoS_2 was placed on the polished silicon wafer plate, and was dried at the oven (50℃) for over 24 hours to fix the nanoparticles. To polish the silicon wafer plate, Piranha solution with a 3:1 mixture of Sulfuric Acid and Hydrogen Peroxide was used and then washed with DI water.

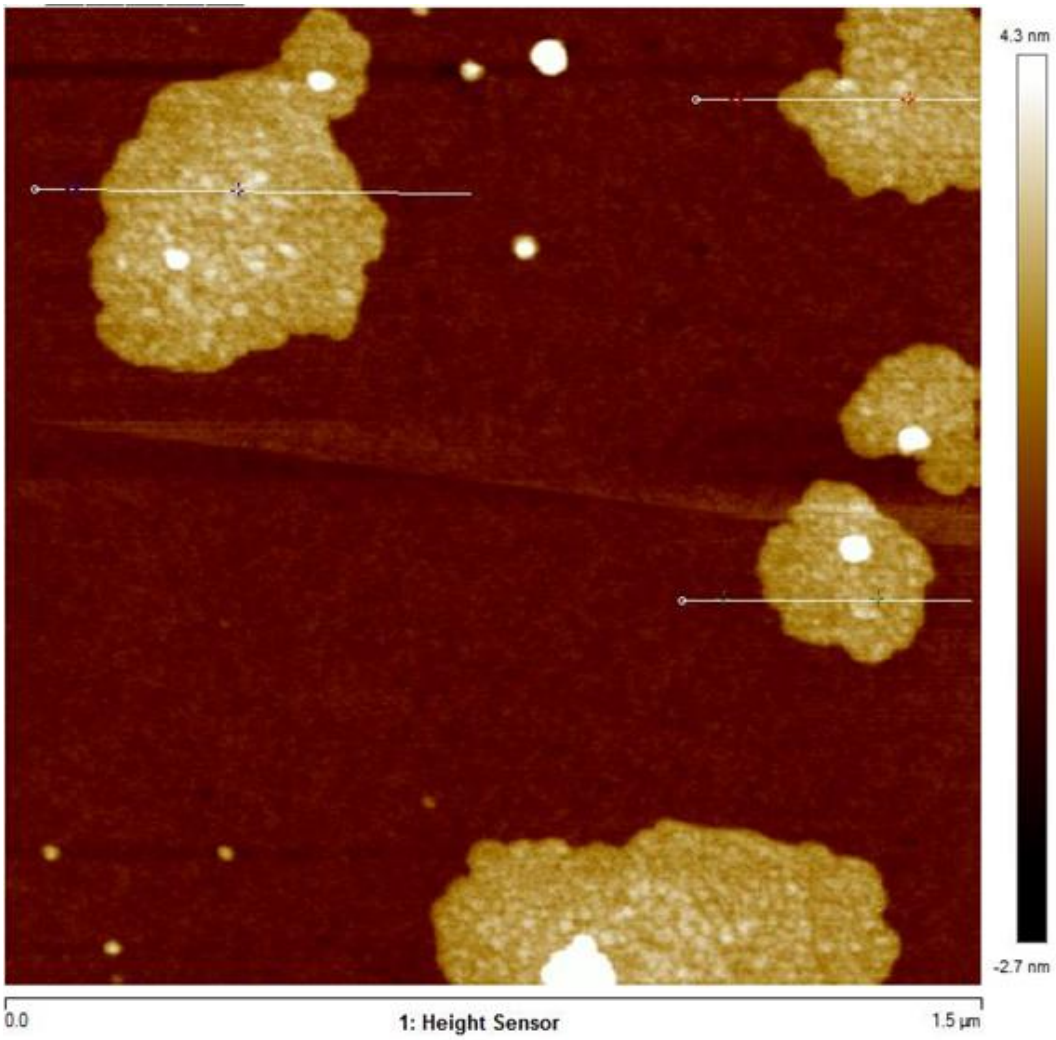


Figure 13. 2-D nanosheets of MoS_2 on 1.5µm × 1.5µm region (AFM)

4.3 Soil bacteria selection

Bacillus cereus was selected for Gram positive soil bacteria. SEM image will be taken to identify we have used the right strains of bacteria. *Bacillus cereus* is a large, 1 × 3-4 μm rod-shaped aerobic bacteria. This grows optimally at temperatures between 20 ~ 40℃ and has strong survivability at any wide range of environmental conditions (Vilain et al., 2006). In terms of ecology, it interacts with several microorganisms around the roots of plants. As soil bacteria, it assists the growth of the plant by inhibiting the disease of plants caused by other pathogens. However, when it comes to pathology this is pathogenic and causes food poisoning (Jensen et al., 2003).

4.4 Preparing and clearing bacterial experiment

For growing soil bacteria, Tryptic Soy Broth (TSB, Soybean-Casein Digest Medium, Catalog # 211825, Bacto) powder was used as medium in 30g/L DI water. Also, they are incubated with the temperature of 36℃ to maximize the growth rate. For the suspension before conducting Pour Plate method, Peptone (Catalog# 211677, Bacto) was used as a temporary medium in 1g/L DI water. Tryptic Soy Agar (TSA, Soybean-Casein Digest Agar Medium, Catalog# 236920, Difco) powder was used as a medium in 40g/L DI water to grow a colony of bacteria during Pour Plate method procedure. All of the liquid materials used for bacteria experiments should be treated at Autoclave with "Liquid 20" procedure. TSB, TSA, Peptone water, DI water, all of these should be sterilized before used. At the Falcon tube, bacteria are suspended inside TSB to be grown for 1st day as 1st transfer. Next day, 1st transfer is suspended inside another TSB to be grown as 2nd transfer. 1st transfer is discarded in this period. Third day, 2nd transfer is suspended inside another TSB to be grown as 3rd transfer. The rest of 2nd transfer solution can be used for the experiment. At the same time, the new 1st transfer is made. Fourth day, 3rd transfer can be used for the experiment, the new 1st transfer is suspended to another TSB to be grown as new 2nd transfer. Again, 1st transfer solution is discarded in this period. The procedure above can be repeated everyday to have fresh bacteria suspended in TSB that can be used for the experiment. Before using bacteria sample solution, it needs to be

treated inside the centrifuge for 15 minutes, 4000 rpm to get rid of TSB liquid. After the centrifuge process, there is a solid precipitate at the bottom, we suspend those with DI water, and treat inside the centrifuge again in the same way twice for the washing. For the last time, peptone water is replaced with DI water in the end. However, to take an image of SEM, the sample should be suspended in DI water in the end. For discarding the wastes, all disposable equipments that has potentially contacted bacteria surface, and solution with bacteria should be sterilized by the Autoclave with "Waste" procedure.

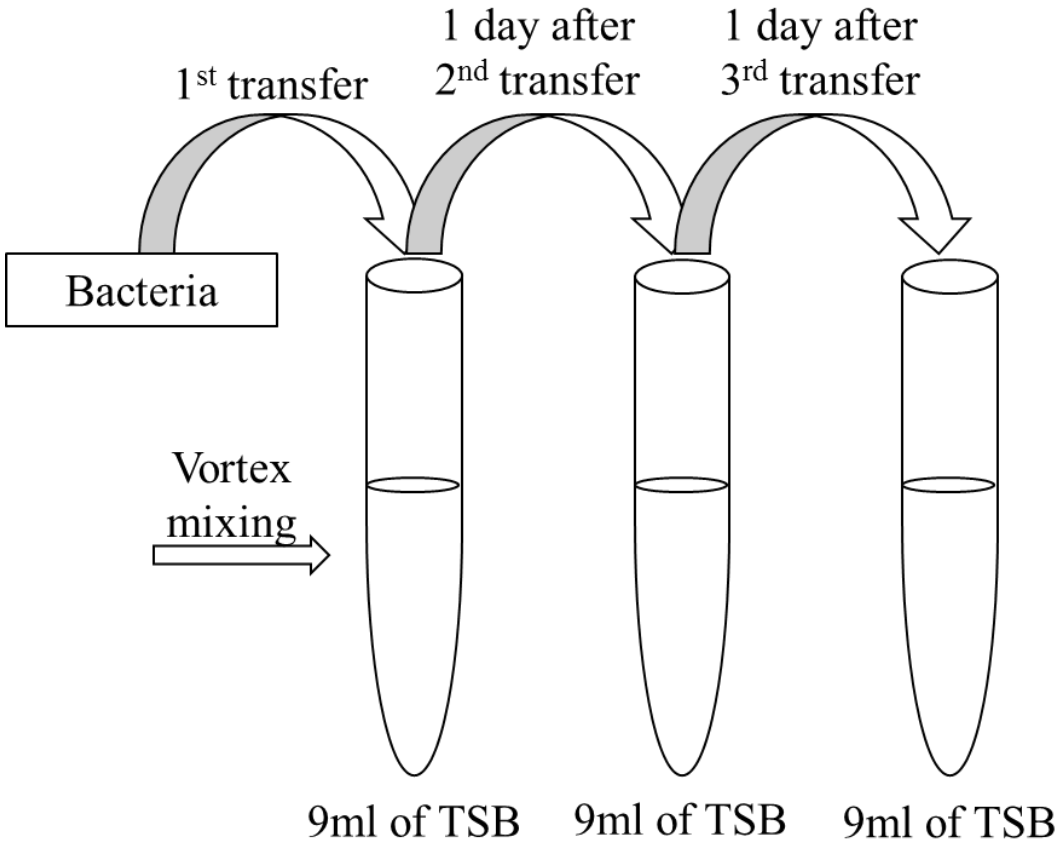


Figure 14. Bacteria transfer

4.5 Soil bacteria survivability experiment

Timelines which consist of 0, 2, 4, 8, 12, 24 hours after mixing the chemicals with bacteria solution were set to gradually plot the influence of toxic chemicals to the survivability of bacteria. For the concentration of the chemicals, 0M, 0.005M, 0.01M, 0.02M, 0.04M, 0.05M were selected after pretreatment experiments to find the lethal concentration range. MoS_2 dissolved in a water was exfoliated for an hour, and added to bacteria sample solution and swirled strongly with vortex equipment. To count fully grown numbers of colonies on TSA plate, Pour Plate method was used. Before conducting Pour Plate method, dilution of log scales are conducted several times to divide the region of concentrations. 1ml from original inoculation is transferred to 9ml of Peptone water tube. Vortex can assist swirling the mixture of these. Everytime the dilution proceeds, the sample concentration is divided by 10 times (1 log difference). After 5 times of dilution, the number of colony counted on the plate will be multiplied with 10^5, which means the dilution of 5 log difference.

To conduct Pour Plate method, 1ml from a sample was dropped on the center of Petri dish, using a sterile pipette. After, 39~43℃ of molten TSA was poured into the Petri dish minimum enough to fill the bottom of the plate. Petri dish was swirled 5 times of each to clockwise and counterclockwise to evenly disperse the sample drop inside the molten TSA. Petri dish was cooled in a room temperature for 30 minutes with a cover ceiling, to wait TSA become coagulated. Next, Petri dish was placed upside down at the incubator oven at 37℃ for 24~48 hours, in order to avoid the drop of water produced at the ceiling of Petri dish cover. After the incubation, the number of colonies represent a “Colony Forming Unit (CFU)”.

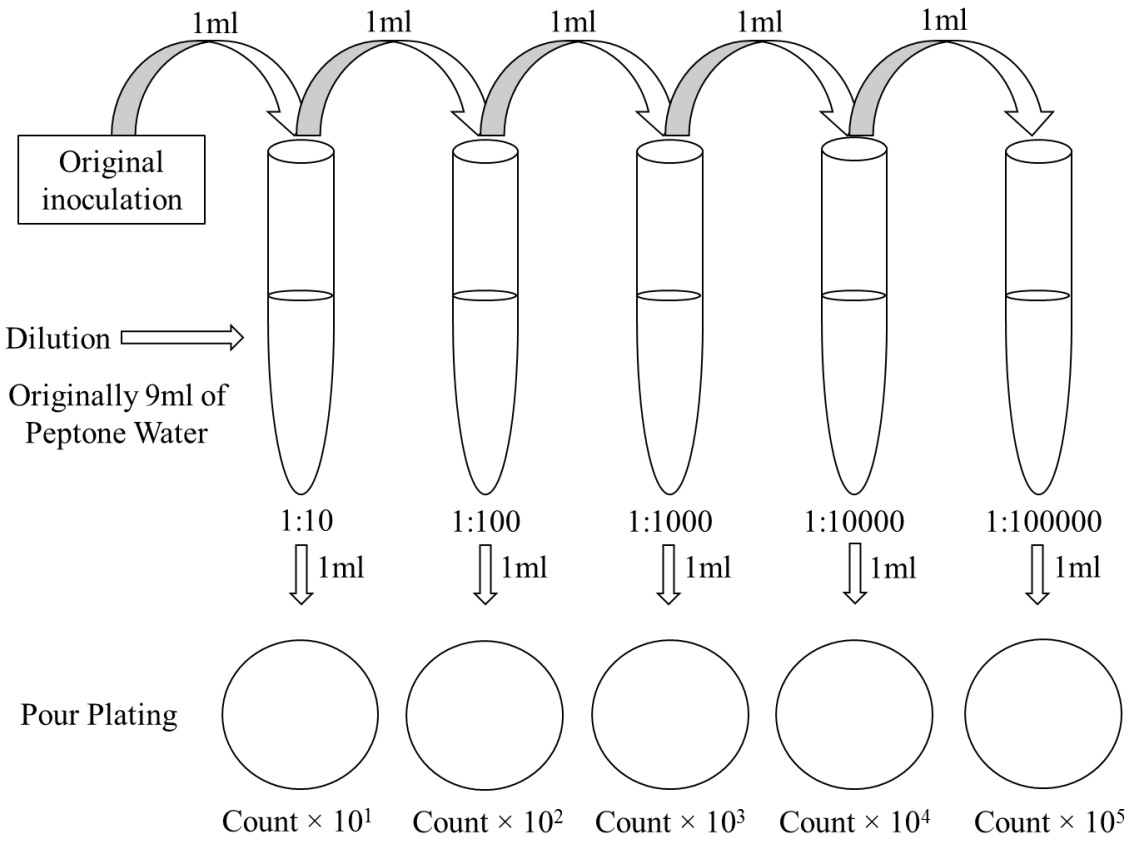


Figure 15. Dilution for Pour Plate Method

5. RESULTS

5.1 2-D nanomaterial characterization

5.1.1 DLS & SEM

The Zeta-Average is the intensity weighted mean of the nanoparticle measured by DLS. Zeta-Average has decreased sharply between 0 ~ 30 minutes of exfoliation. Between 30 ~ 120 minutes of exfoliation, we can identify the size distribution becomes broader and the intensity decreases greatly. More exfoliation beyond 120 minutes makes the counting rate very low that DLS can't measure the data. MoS_2 can be exfoliated between 1 ~2 hours and mostly they break into smaller 2-D nanosheets.

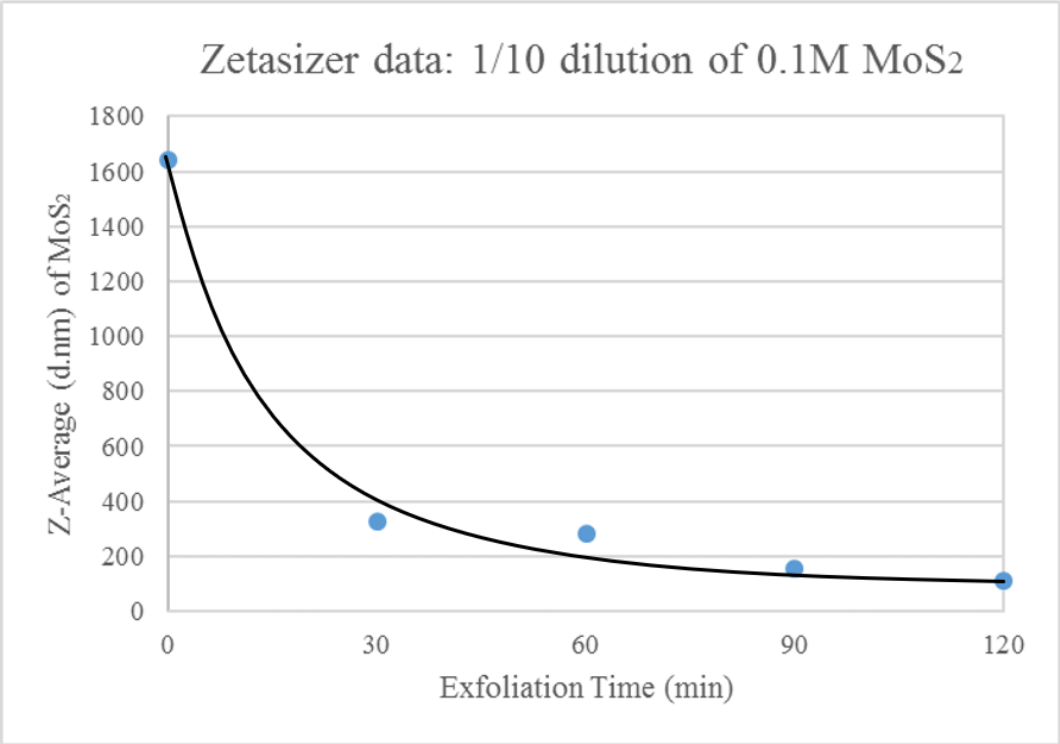


Figure 16. Zeta-Average (d.nm) of MoS_2 upon exfoliation

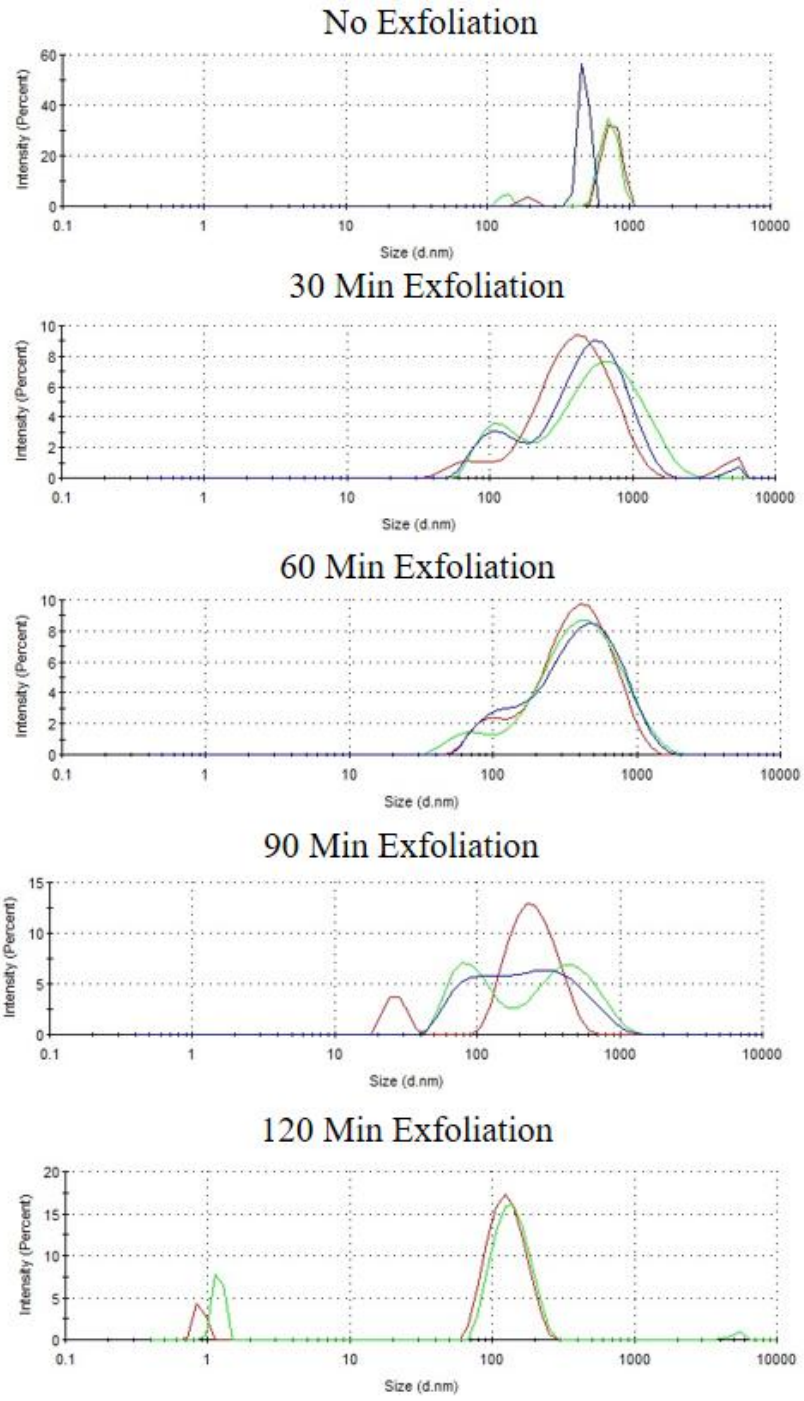


Figure 17. Zeta-Average (d.nm) of MoS_2 cumulative plots

5.1.2 AFM image

AFM result includes the thickness data. We have found nanosheet regions around 1.5 µm × 1.5 µm, but the thickness around 5 nm. If the gap length between the layers of nanosheets is 6.5Å, the sheets may include 3~5 multilayers.

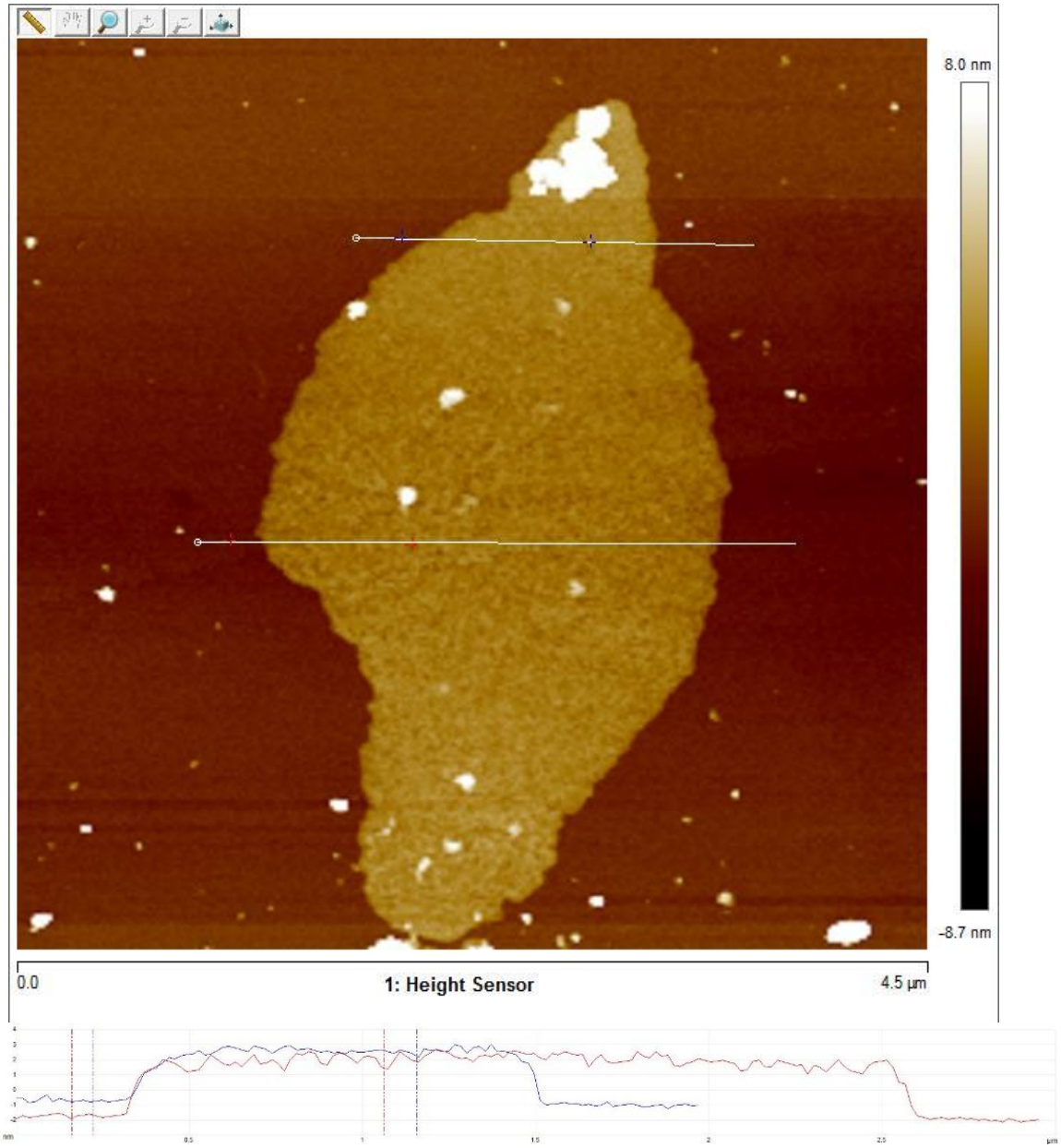


Figure 18. MoS_2 2-D nanosheet

5.2 Bacteria image and surface charge

5.2.1 SEM image

Through SEM, rod shaped bare *Bacillus cereus* was observed. Fully grown cells have 1~2 long flagella around the body. They usually duplicate the number with the division to the longer axis. Figure 22. Shows the process of division, that 3 ~ 4 cells are seen all connected to the direction of the longer axis.

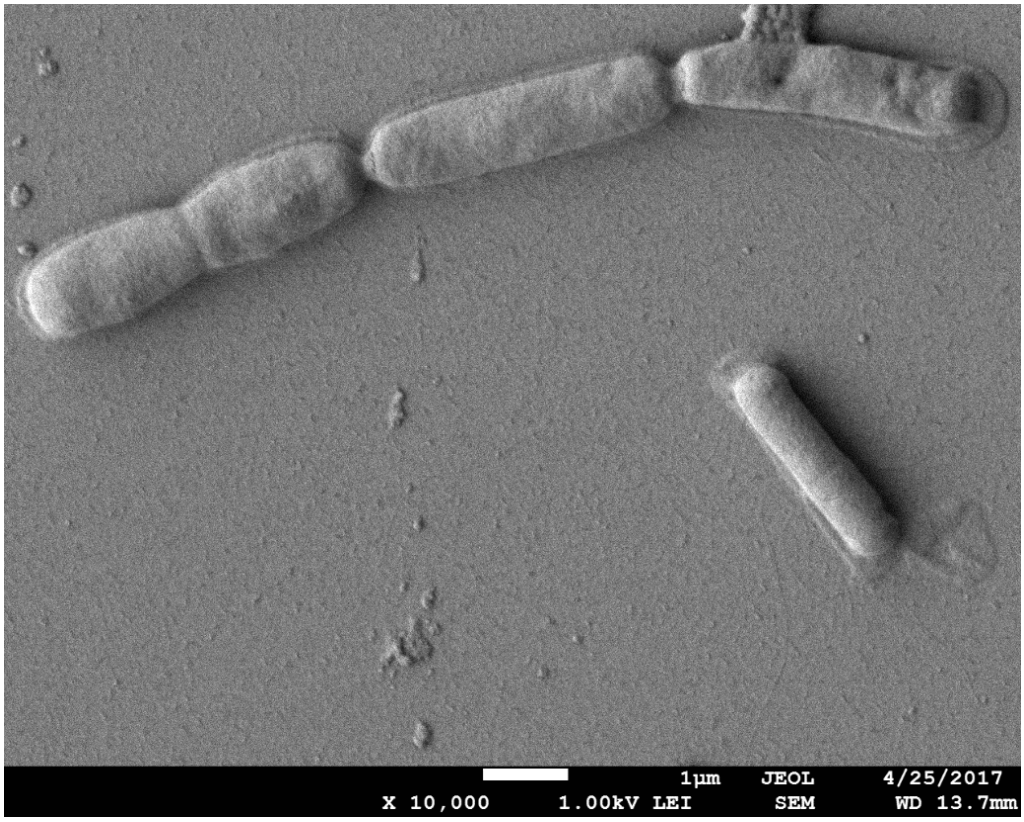


Figure 19. Bare *Bacillus cereus* (SEM image)

5.2.2 Zeta Potential

Bacillus cereus has -33.3mV, MoS_2 solution has -31.1mV of Zeta-potential. The data had very clear cumulative plot. MoS_2 showed broader and not uniform cumulation, and this might be due to the broad size distribution.

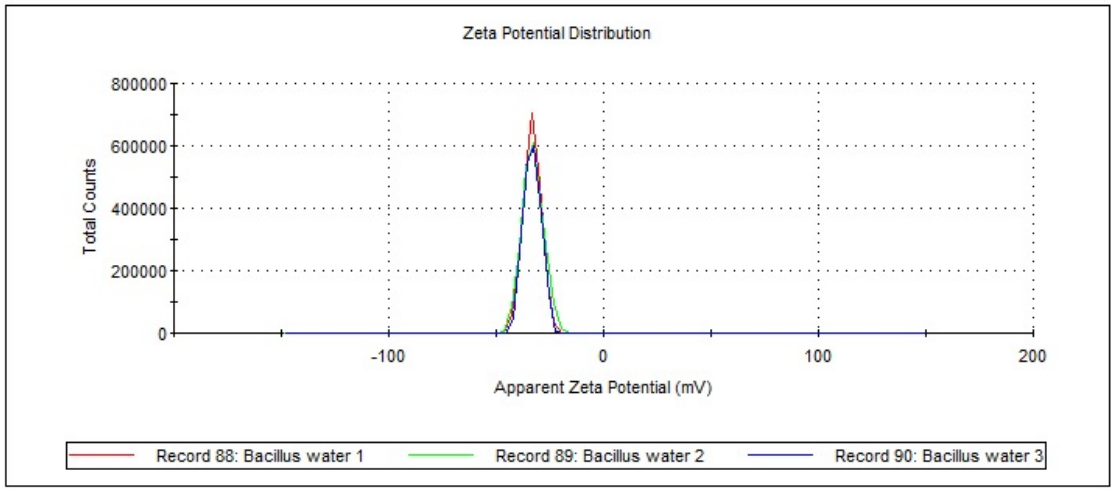


Figure 20. *Bacillus cereus* Zeta-potential cumulative plot

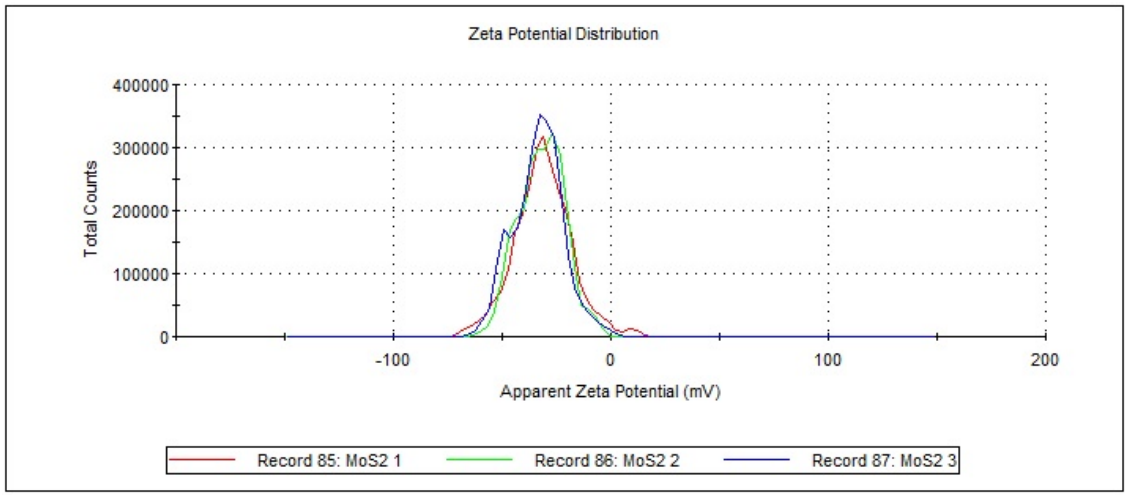


Figure 21. MoS_2 Zeta-potential cumulative plot

5.3 Pour Plate result

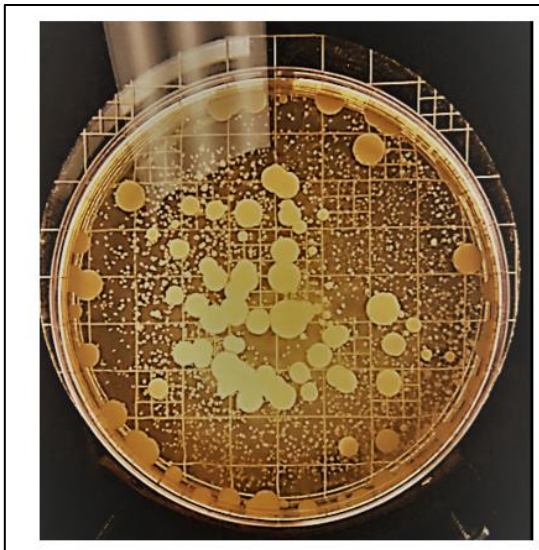
Based on Pour Plate experiment, we counted the number of colony grown on TSA petri dish. Both arithmetic scale of CFU in average of Pour Plate experiment (2 ~ 4 repetition) and logarithmic scale of CFU in average of Pour Plate experiment (2 ~ 4 repetition) are arranged below. Each timeline shows the length of treatment after mixing bacteria and MoS_2 solution. After treated in TSA, they were incubated for 24~48 hours in order to make it fully grown and able to count the marginal number of CFU on the petri dish. Around 20~200 CFU is appropriate to be counted. More or less is uncountable.

Table 1. Arithmetic counting average of *Bacillus cereus* colonies (Pour Plate)

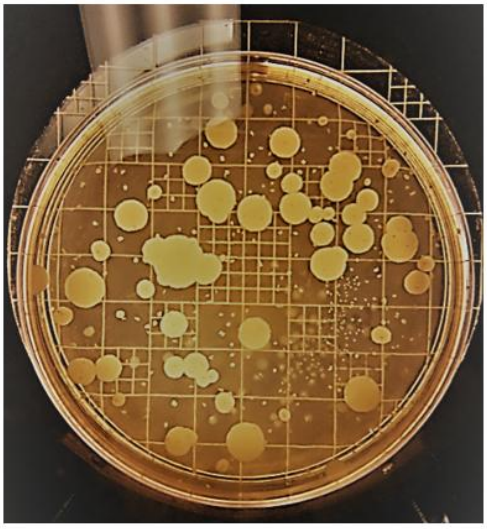
Bacillus cereus (CFU)	Hour					Arithmetic Average (1 ~ 4 try)
Concentration	0	2	4	8	12	24
0M	4.87E+06	1.18E+06	1.10E+06	3.29E+06	4.23E+06	3.49E+06
0.005M	3.83E+06	2.56E+05	2.60E+05	6.65E+05	1.26E+06	2.73E+06
0.01M	2.50E+06	1.53E+05	8.48E+04	3.04E+04	1.15E+05	9.43E+05
0.02M	2.35E+06	1.18E+05	2.50E+04	7.13E+03	2.99E+03	2.19E+05
0.04M	8.20E+05	1.12E+05	2.24E+04	3.93E+03	1.78E+03	1.31E+03
0.05M	5.97E+05	5.97E+04	7.50E+03	2.47E+03	1.01E+03	6.00E+02

Table 2. Logarithmic counting average of *Bacillus cereus* colonies (Pour Plate)

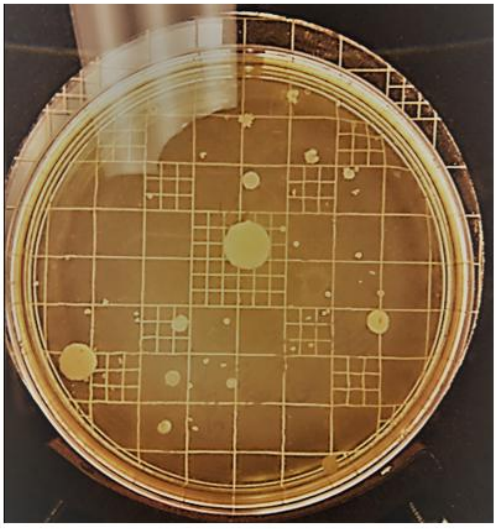
Bacillus cereus Log(CFU)	Hour					Logarithmic Average (1 ~ 4 try)
Concentration	0	2	4	8	12	24
0M	6.69	6.07	6.04	6.52	6.63	6.54
0.005M	6.58	5.41	5.41	5.82	6.10	6.44
0.01M	6.40	5.18	4.93	4.48	5.06	5.97
0.02M	6.37	5.07	4.40	3.85	3.48	5.34
0.04M	5.91	5.05	4.35	3.59	3.25	3.12
0.05M	5.78	4.78	3.88	3.39	3.00	2.78



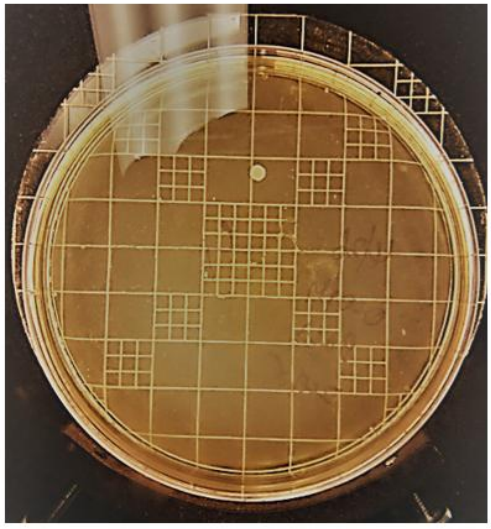
Bacillus cereus treated in 0.01M MoS_2 for 24 hours 2^{nd} dilution (1:100)



Bacillus cereus treated in 0.01M MoS_2 for 24 hours 3^{rd} dilution (1:1000)



Bacillus cereus treated in 0.01M MoS_2 for 24 hours 4^{th} dilution (1:10000)



Bacillus cereus treated in 0.01M MoS_2 for 24 hours 5^{th} dilution (1:100000)

Figure 22. *Bacillus cereus* grown on TSA media Petri dish

5.4 Soil bacteria survivability plot

The plot contains the data of timeline of 24 hours and concentrations of 0M, 0.005M, 0.01M, 0.02M. 0.04M, 0.05M MoS_2 solution. Basically the survivability of bacteria decreased when the concentration is higher, also the time of treatment is longer at the same time. Lower concentration has shown steady growth of bacteria throughout 24-hour timeline.

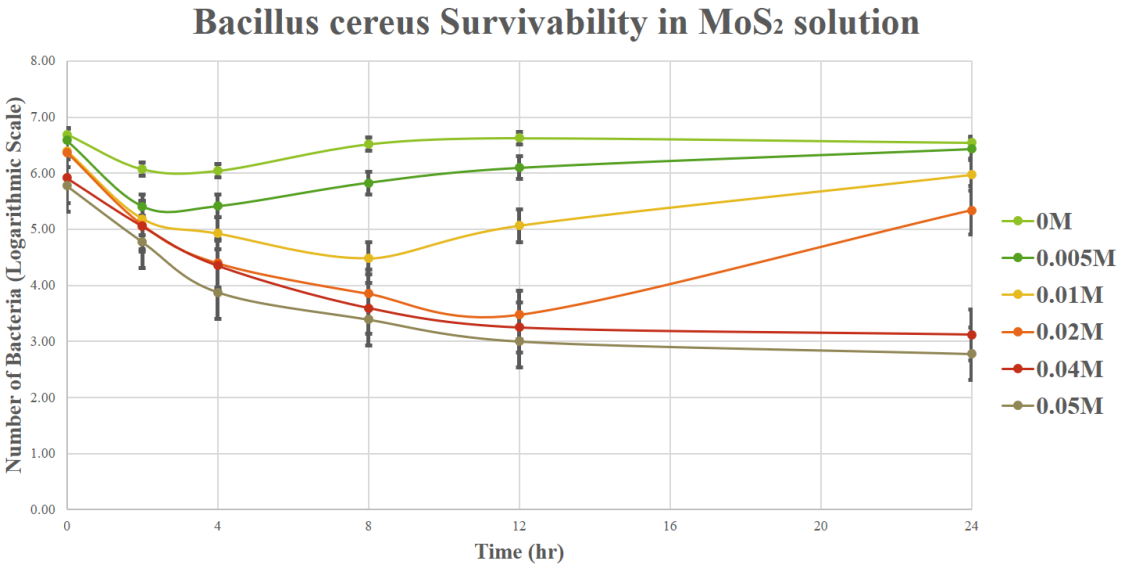


Figure 23. *Bacillus cereus* survivability plot (24-hour timeline) in MoS_2 solution (0 ~ 0.05M) concentrations

6. DISCUSSIONS

6.1 Exfoliation of nanomaterials

The bulk powder of MoS_2 can be dissolved with a concentration below 0.1668M based on the reference. As the concentration of 26.7 ± 0.7 mg/ml has been said to be the highest concentration in aqueous solution of 45 vol% ethanol/water combination with the support of grinding MoS_2 in NMP (Yao et al., 2013). MoS_2 is known as insoluble material in water (Haynes et al., 2011). However, several researches has shown the dissolved MoS_2 in water with the picture of black colored solution. When it is well dispersed, MoS_2 solution shows dark gray-green color as well, after most of the particles precipitate on the bottom. We can conclude this has very low solubility in water. The highest concentration for this research dissolved in water was set to 0.1M (16 mg/ml). Higher concentration range than 16mg/ml does not dissolve MoS_2 anymore, make a precipitation on the bottom. As MoS_2 aggregates in a solution because of weak Van Der Waals force, too high concentration of MoS_2 can make small 2-D sheets to gather and make the bulk structure. The vial of 20 ml volume was used and placed inside the ice bath to stabilize the low temperature. As MoS_2 breaks down with sonication, MoS_2 solution may receive the mechanical energy and get heated. Ice bath can stabilize the temperature of the vial around 5℃. Actually, the rise of the temperature may occur unwanted dynamics of aggregation inside the solution of MoS_2.

According to the DLS Zeta-average data, 1 ~ 2 hour was on the range that most of the particles have Zeta-average less than 1000 nm. We observed SEM image of 1 hour exfoliated MoS_2. SEM image has shown different size distributions of MoS_2. When they are exfoliated and swirled by the vortex, they might be dispersed due to the negative surface charge. However, after the solvent dries, weak Van Der Waals force governs and MoS_2 starts to aggregate as shown in the image. Small particles tend to work as an adhesives between bigger nanosheets. Over 2 hours of exfoliation didn't show DLS data due to low count rates. We could see some cracks of 2-D nanosheets with AFM image of 2 hour exfoliation of MoS_2. We can conclude 1~2 hours of exfoliation is enough for

making 2-D nanosheets. Taking too much time for the exfoliation may break the structures of 2-D dimension into smaller 0-D particles.

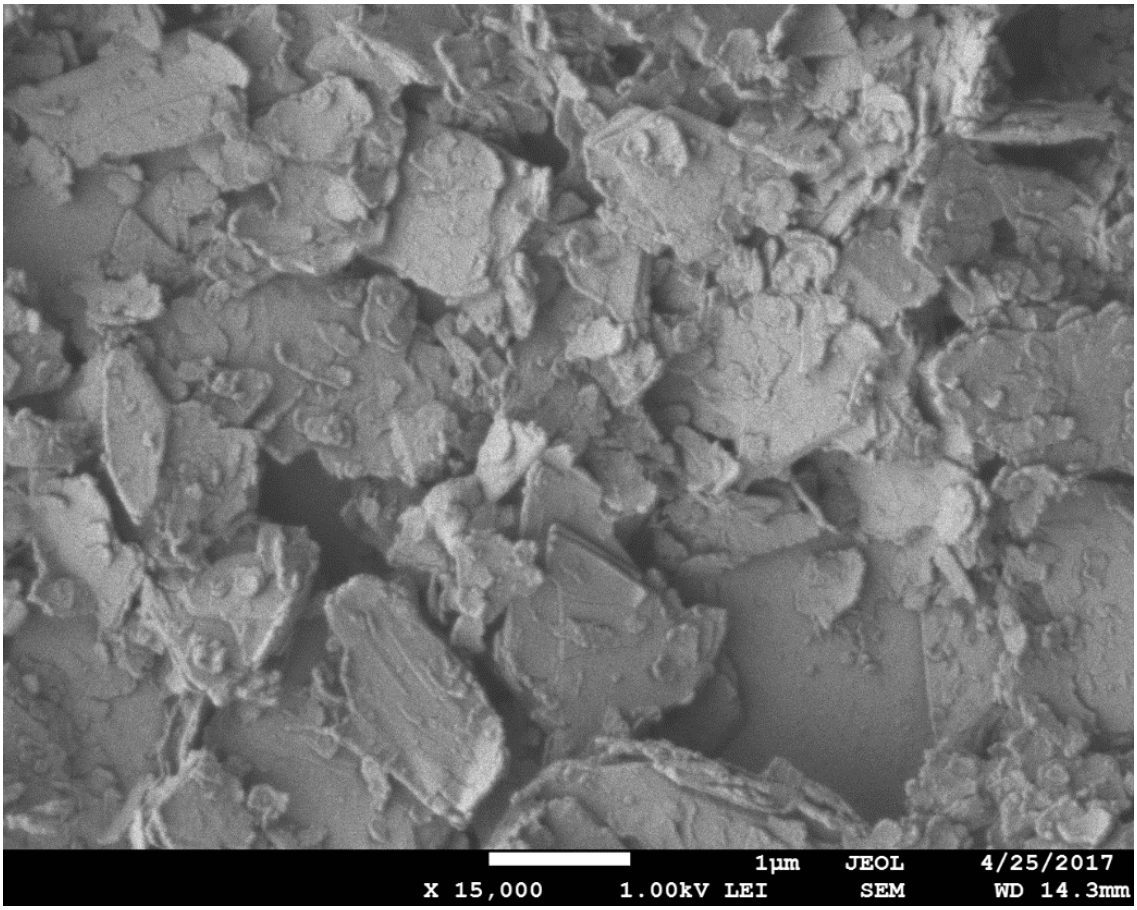


Figure 24. SEM image of MoS_2

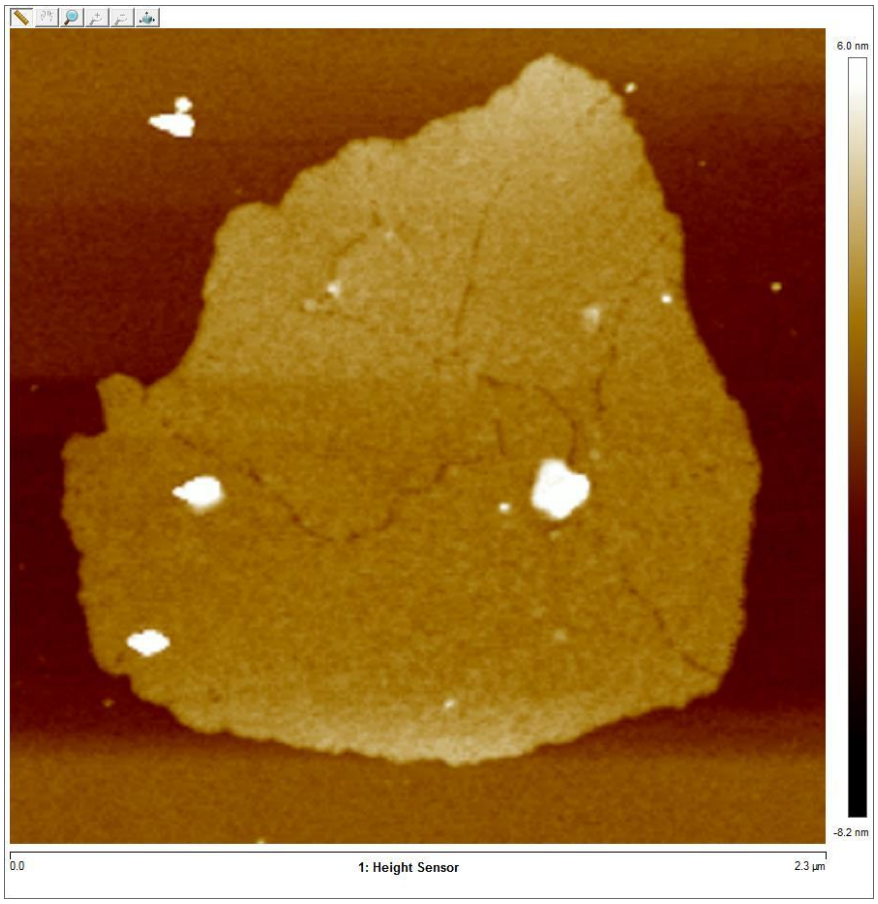


Figure 25. Cracked MoS_2 nanosheet

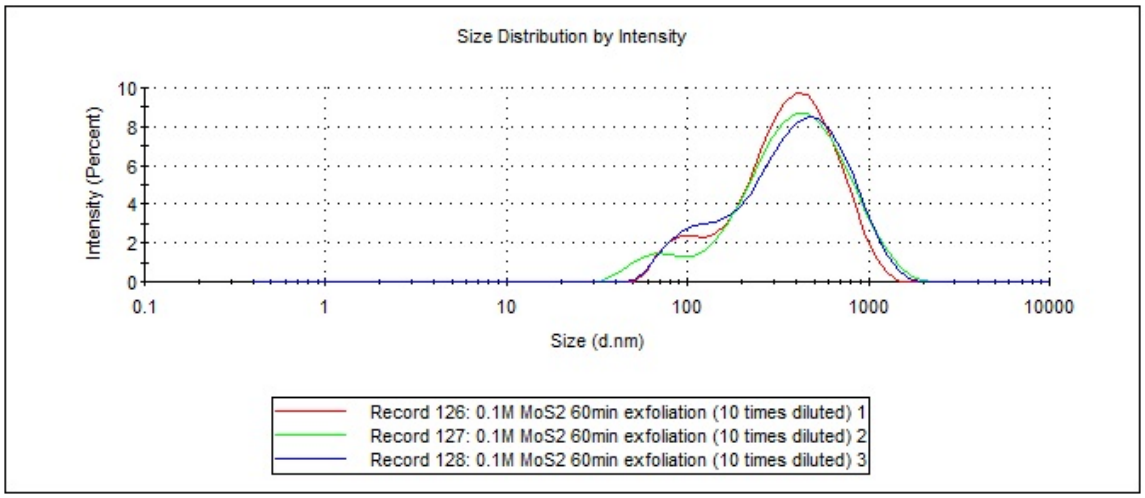


Figure 26. Size distribution of MoS_2 after 1 hour exfoliation

6.2 Soil bacteria survivability

Bacteria showed less surviving behavior when the concentration of nanomaterials got higher. From 0~0.01M it was not significantly toxic, but after 0.02M, it showed intensive toxicity and prevented 99% of the growth compared to the bare solution. At 0.05M MoS_2 solution, 99.99% of the growth was hindered compared to the bare solution.

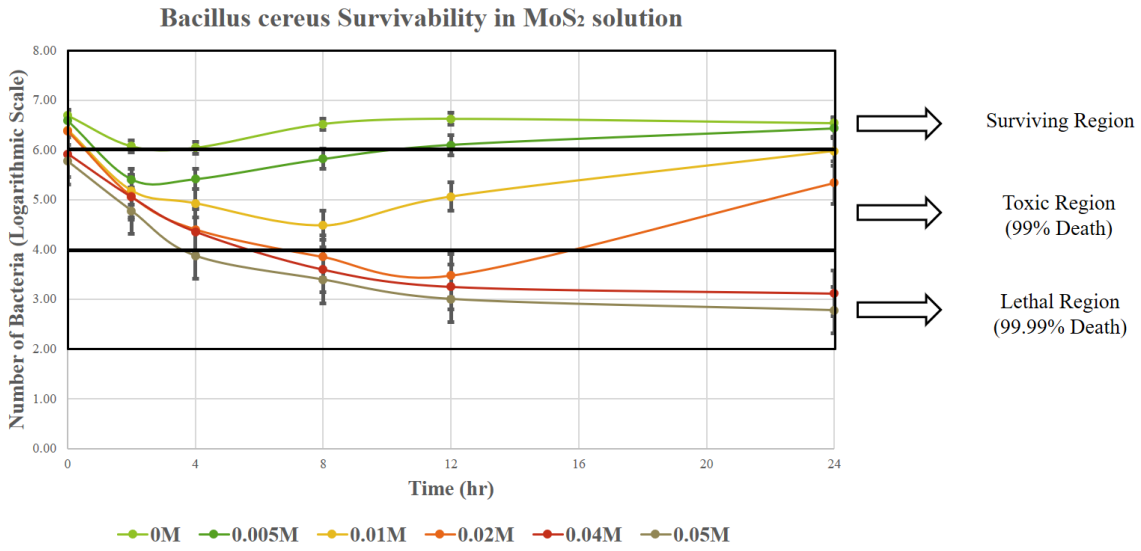


Figure 27. *Bacillus cereus* survivability plot (24-hour timeline) in MoS_2 solution (0 ~ 0.05M) concentrations

Bacteria counting has many uncertainties. The control of biological experiment has high rate of uncertainties because it has less repeatability due to bio-diversity. Not every single bacteria is identical. They are in all different condition and that may occur the errors. To decrease the uncertainty, Pour Plate data were measured at least 3 times and most of the data has shown less than 1 log difference of CFU scale. The reason of error basically comes from the external effects such as temperature, nutrition concentration, condition of oxygen concentration, particle sizes of the drug, and the time difference for even for the few minutes. Hence, it is reasonable we should count the number of CFU at least 3 times to verify the survivability.

6.3 Cytotoxicity of MoS_2

Bare Bacillus has no wrinkled surface on the cell body. However, as it deforms in the process of death, it shows wrinkles and the adhesion of nanomaterial on the surface. As the concentration of the solution increases, SEM showed more aggregation of MoS_2 and the number of *Bacillus cereus* significantly decreased on the image. We could observe aggregation of nanomaterials and bacteria, and the adhesive character of MoS_2.

Bacillus cereus has -33.3mV, MoS_2 solution has -31.1mV of Zeta-Potential. Zeta-Potential around from ±30 to ±40 usually shows moderate stability. So those bacteria and nanosheets will be dispersed in a solution, but also they will aggregate because of weak Van Der Waals force. As solution dries, the aggregation governs and they will all stick together. Bacteria cells are buried by MoS_2 nanosheets as TMDs are inorganic materials that have higher density and are heavier than bacterial cell. We could see broken pieces of cell structures around the debris of MoS_2. MoS_2 has a shape of sheet, while the debris of cell has round shape that can be distinguished. If industrial MoS_2 nanosheets are released to the soil environment, the habitat of *Bare Bacillus* will be damaged and it will cause the harmful effects to the near environments as well. Furthermore, the other bacteria, or even other plant cells could be damaged by MoS_2 because of its adhesive behavior. This may hinder the transport of nutrients and water, also cause the deformation of the cell structures of the living things after the adhesion of MoS_2 nanosheets.

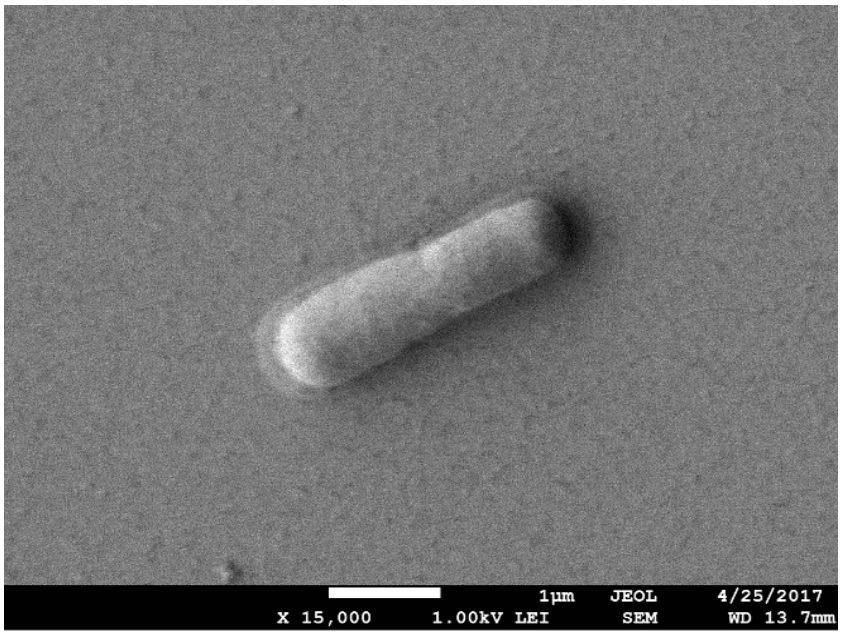


Figure 28. Bare *Bacillus cereus*

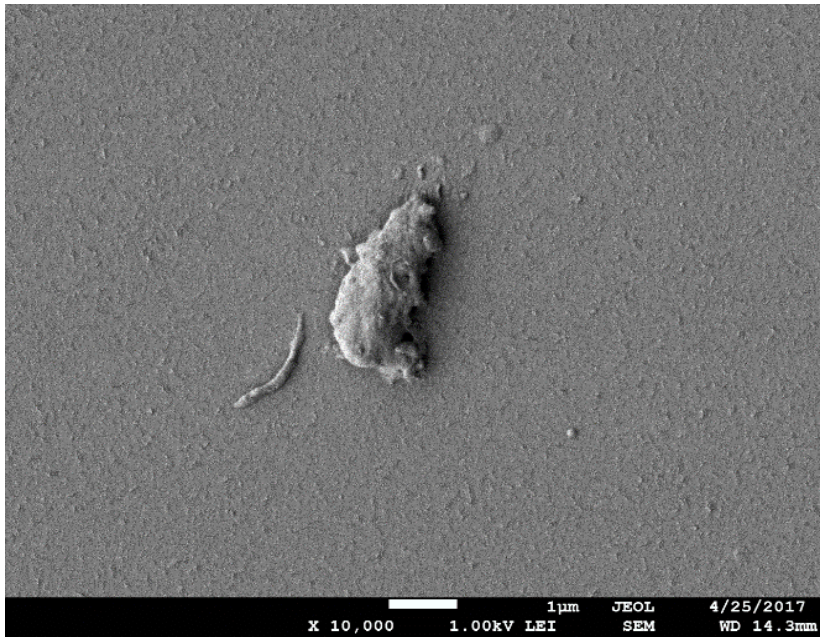


Figure 29. *Bacillus cereus* treated in 0.05M MoS_2 solution

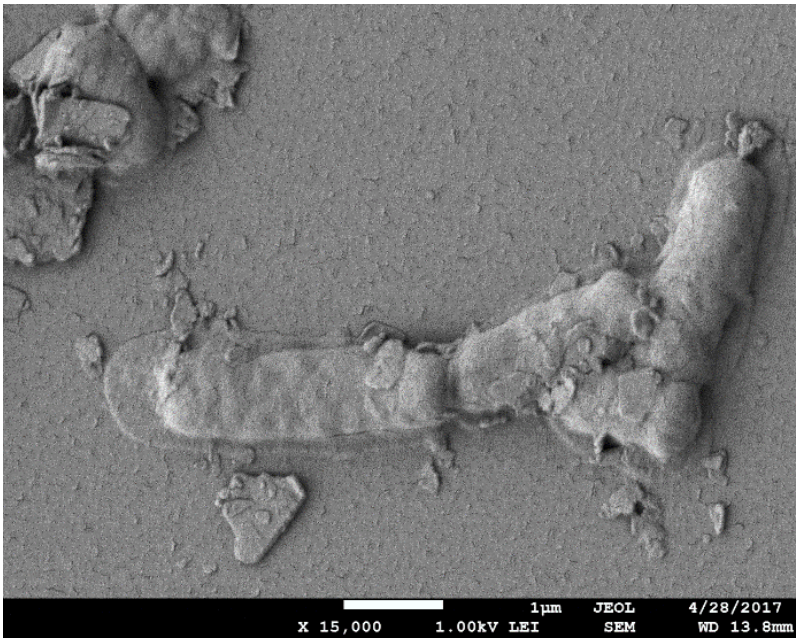


Figure 30. *Bacillus cereus* treated in 0.01M MoS_2 solution

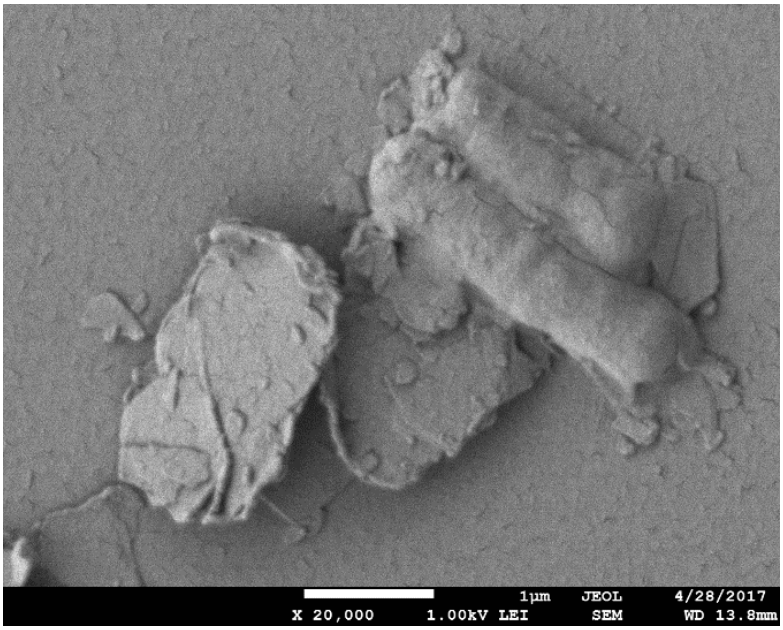


Figure 31. *Bacillus cereus* treated in 0.01M MoS_2 solution

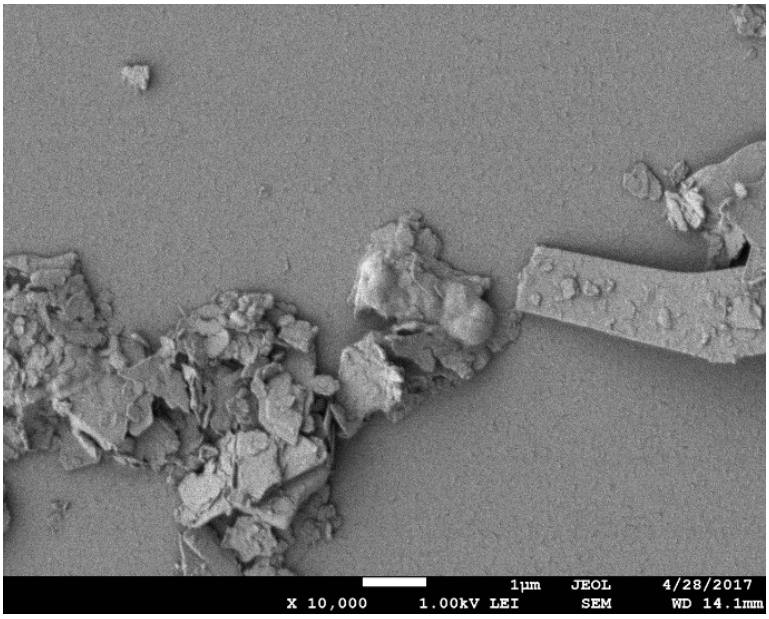


Figure 32. *Bacillus cereus* treated in 0.02M MoS_2 solution

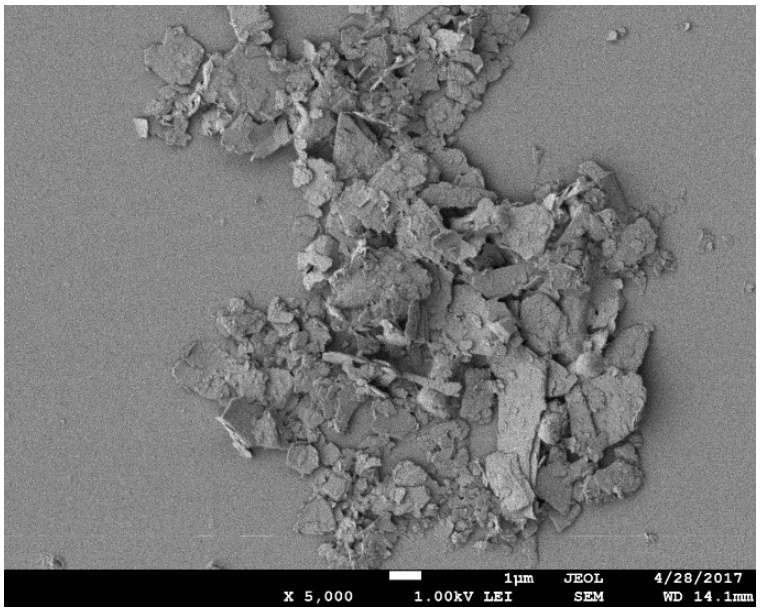


Figure 33. *Bacillus cereus* treated in 0.02M MoS_2 solution

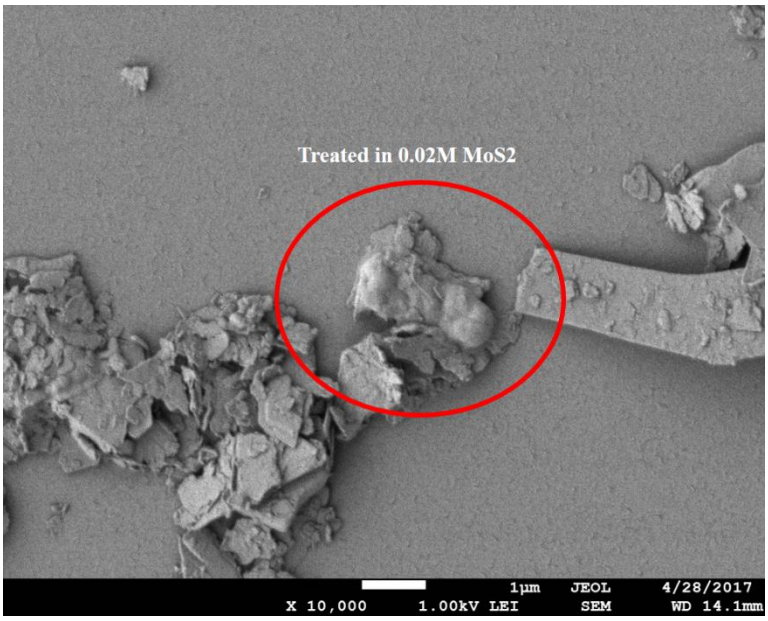


Figure 34. *Bacillus cereus* treated in 0.02M MoS_2 solution

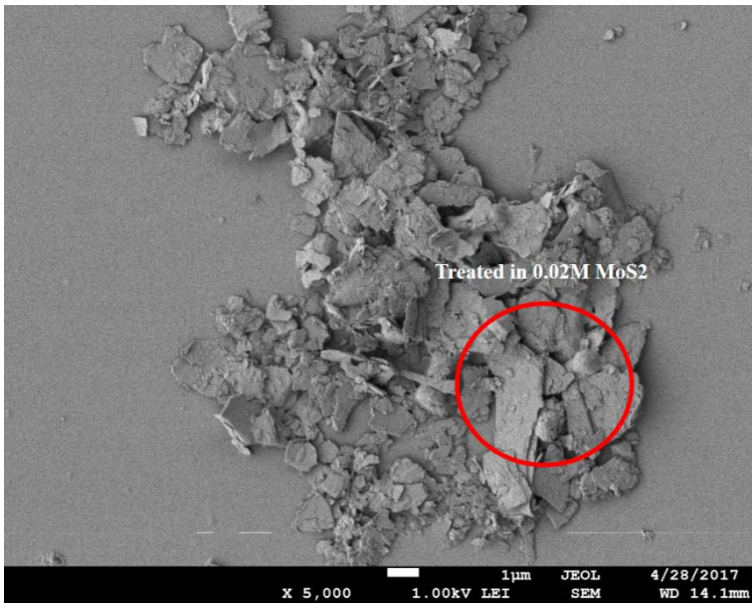


Figure 35. *Bacillus cereus* treated in 0.02M MoS_2 solution

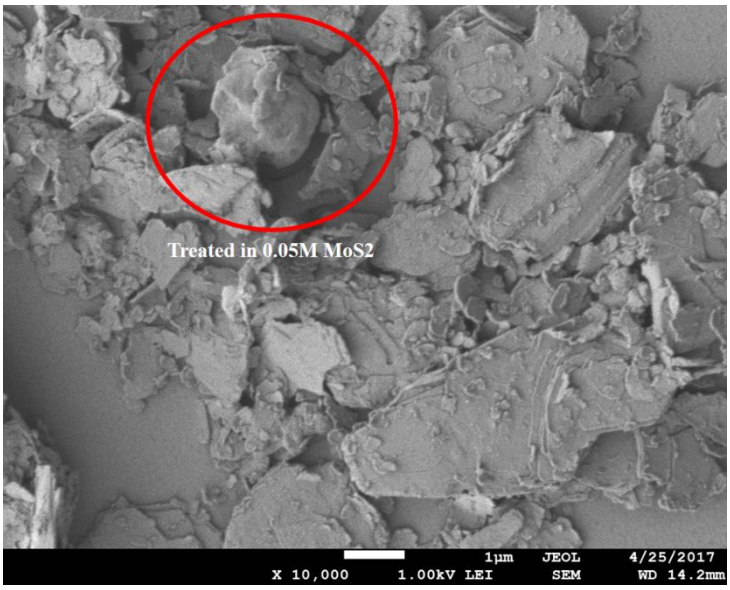


Figure 36. *Bacillus cereus* treated in 0.05M MoS_2 solution

Table 3. Zeta-Potential and the stability behavior of the colloid [1]

Zeta-Potential [mV]	**Stability behavior of the colloid**
from 0 to ±5,	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

[1] https://en.wikipedia.org/wiki/Zeta_potential

7. CONCLUSION

Throughout this research we have done the observation and control of nano-scale materials and Micro-scale cells. MoS_2 2-D nanosheets were prepared by exfoliation and characterization using probe sonicator and AFM image. Also, SEM image has shown the shape of the nanomaterials, DLS Zeta-sizer gave the size distribution data with a cumulative plot of x-axis (size) and y-axis (intensity). *Bacillus cereus* was grown and treated in a incubator, and transferred to 0 ~ 0.05M range of nano-toxic solution. DLS Zeta-potential of both *Bacillus cereus* and MoS_2 were measured. Also, SEM image gave the shape of the cell as well as showed adhesive behavior between nanomaterials and the cells. We verified the toxicity of MoS_2 2-D nanosheets, and the survivability of *Bacillus cereus* based on the Pour Plate data and SEM image. We concluded that MoS_2 is significantly toxic if it is over 0.04M. SEM image has shown the adhesive behavior of MoS_2 to bacterial cells and the clue of inducing a deformation of the structure. As demand of MoS_2 in the industry will continuously increase, we should also consider its toxicity when it is released to the soil environment. This can harm the plants and beneficial microorganisms in the long run. In conclusion, industrial 2-D nanomaterial may show adhesive behavior to the cell surface and it can be eventually harmful to the survival of cell. It is required that industrial 2-D nanomaterials should be separated and disposed properly in order not to be released recklessly to the environment.

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APPENDIX

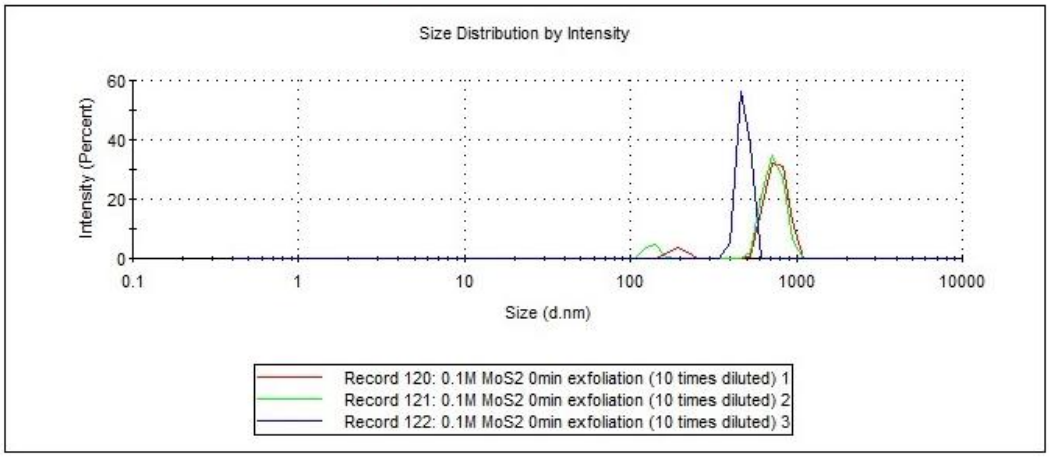
Zeta-Average, Zeta-Potential Data

Sample Name: 0.1M MoS2 0min exfoliation (10 times diluted) 1
SOP Name: mansettings.nano
File Name: 4.17.2017
Record Number: 120
Material RI: 1.59
Material Absorbtion: 0.010
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Thursday, April 20, 2017 10:24:56 PM

Temperature (°C): 25.0
Count Rate (kcps): 193.3
Cell Description: Disposable low volume cuvette (50µL)
Duration Used (s): 60
Measurement Position (mm): 3.00
Attenuator: 9

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	1643	Peak 1:	765.6	92.6	106.5
PdI:	1.000	Peak 2:	194.1	7.4	20.14
Intercept:	1.08	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

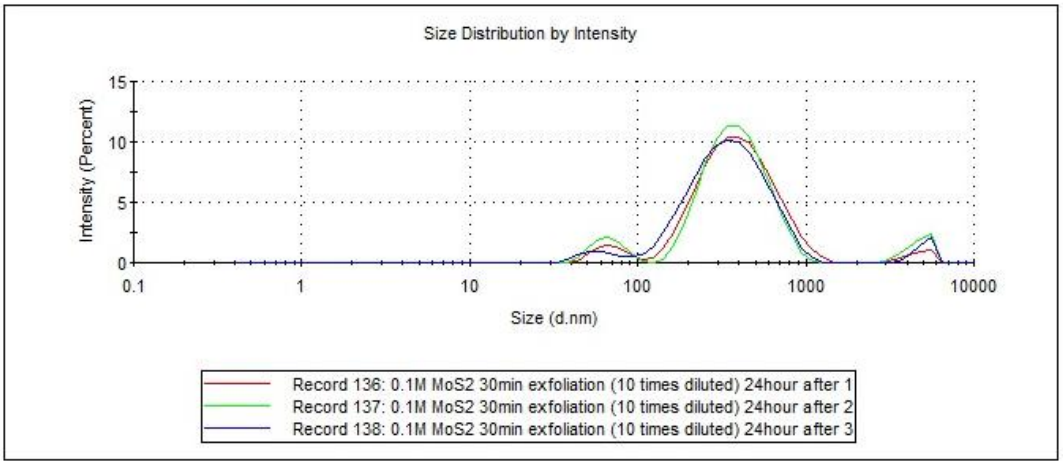


Sample Name:	0.1M MoS2 30min exfoliation (10 times diluted) 24hour after 1		
SOP Name:	mansettings.nano		
File Name:	4.21.2017 (2).dts	**Dispersant Name:**	Water
Record Number:	136	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, April 21, 2017 10:30:20 PM

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	195.7	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	313.4	**Peak 1:**	429.8	91.1	210.5
PdI:	0.357	**Peak 2:**	70.07	5.8	15.40
Intercept:	0.787	**Peak 3:**	4624	3.2	818.8

Result quality : Good

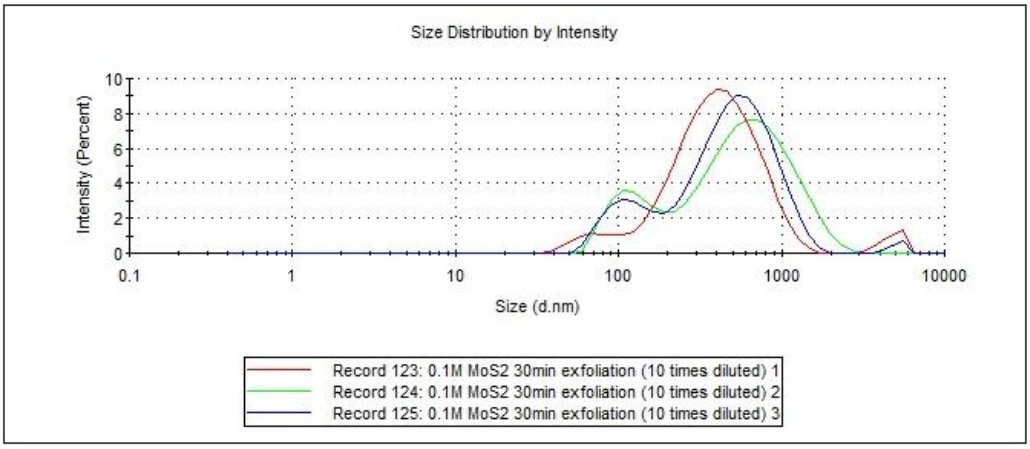


Sample Name:	0.1M MoS2 30min exfoliation (10 times diluted) 1		
SOP Name:	mansettings.nano		
File Name:	4.17.2017	**Dispersant Name:**	Water
Record Number:	123	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Thursday, April 20, 2017 11:58:53 PM

Temperature (°C):	25.0	**Duration Used (s):**	80
Count Rate (kcps):	150.8	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	325.0	**Peak 1:**	453.8	91.1	246.6
PdI:	0.382	**Peak 2:**	68.68	5.3	15.26
Intercept:	0.770	**Peak 3:**	4762	3.6	747.8

Result quality : Good

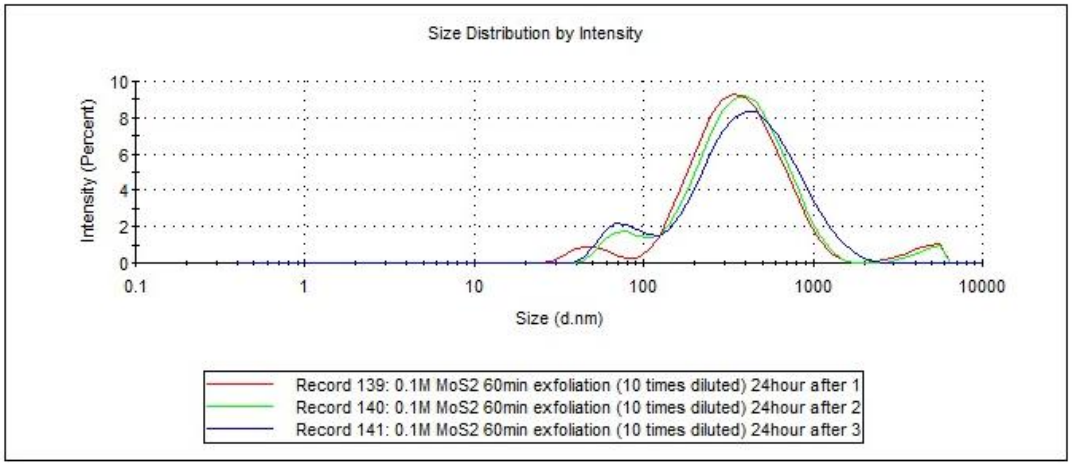


Sample Name:	0.1M MoS2 60min exfoliation (10 times diluted) 24hour after 1		
SOP Name:	mansettings.nano		
File Name:	4.21.2017 (2).dts	**Dispersant Name:**	Water
Record Number:	139	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, April 21, 2017 10:42:20 PM

Temperature (°C):	25.0	**Duration Used (s):**	110
Count Rate (kcps):	78.3	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	290.1	**Peak 1:**	410.5	91.8	228.6
PdI:	0.383	**Peak 2:**	50.21	4.3	12.84
Intercept:	0.740	**Peak 3:**	4423	3.9	914.2

Result quality : Good

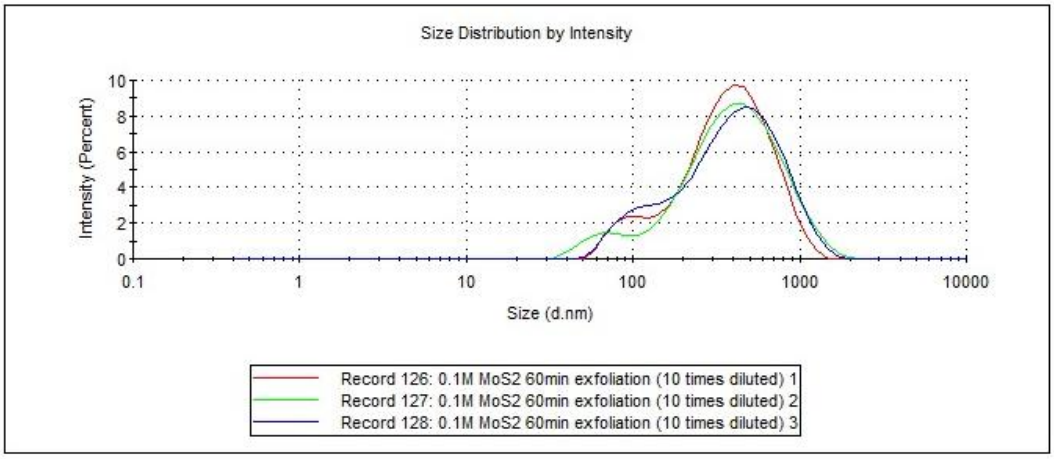


Sample Name:	0.1M MoS2 60min exfoliation (10 times diluted) 1		
SOP Name:	mansettings.nano		
File Name:	4.17.2017	**Dispersant Name:**	Water
Record Number:	126	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, April 21, 2017 12:38:25 AM

Temperature (°C):	24.9	**Duration Used (s):**	150
Count Rate (kcps):	49.1	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	283.8	**Peak 1:**	438.7	89.3	224.9
PdI:	0.344	**Peak 2:**	93.99	10.7	19.77
Intercept:	0.738	**Peak 3:**	0.000	0.0	0.000

Result quality : Good

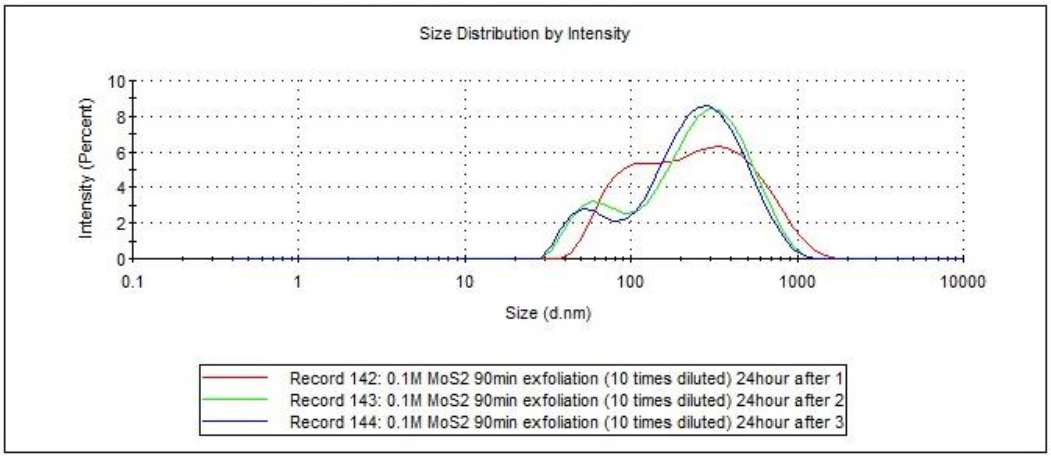


Sample Name:	0.1M MoS2 90min exfoliation (10 times diluted) 24hour after 1		
SOP Name:	mansettings.nano		
File Name:	4.21.2017 (2).dts	**Dispersant Name:**	Water
Record Number:	142	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, April 21, 2017 11:10:02 PM

Temperature (°C):	25.0	**Duration Used (s):**	500
Count Rate (kcps):	7.8	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	170.8	**Peak 1:**	396.4	68.3	237.4
PdI:	0.407	**Peak 2:**	98.22	31.7	28.13
Intercept:	0.678	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

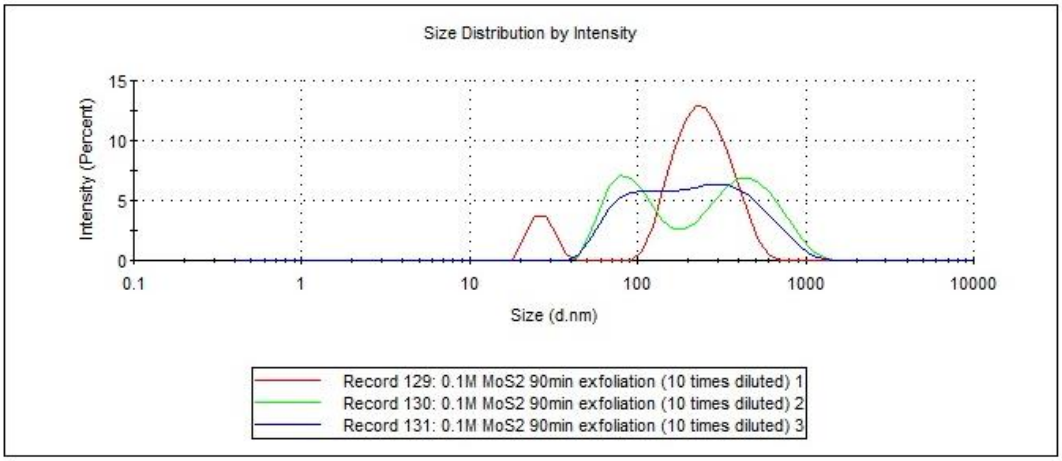


Sample Name:	0.1M MoS2 90min exfoliation (10 times diluted) 1		
SOP Name:	mansettings.nano		
File Name:	4.17.2017	**Dispersant Name:**	Water
Record Number:	129	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, April 21, 2017 1:22:17 AM

Temperature (°C):	25.0	**Duration Used (s):**	500
Count Rate (kcps):	2.8	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	153.2	**Peak 1:**	257.8	88.2	96.29
PdI:	0.418	**Peak 2:**	27.03	11.8	4.352
Intercept:	0.582	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

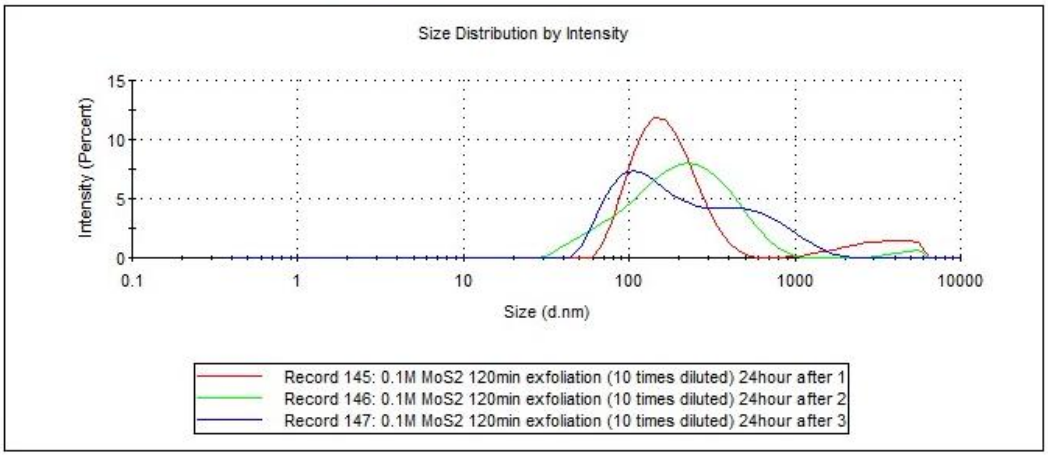


Sample Name:	0.1M MoS2 120min exfoliation (10 times diluted) 24hour after 1		
SOP Name:	mansettings.nano		
File Name:	4.21.2017 (2).dts	**Dispersant Name:**	Water
Record Number:	145	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Saturday, April 22, 2017 12:04:17 AM

Temperature (°C):	24.9	**Duration Used (s):**	500
Count Rate (kcps):	2.9	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	148.2	**Peak 1:**	174.5	87.8	74.45
PdI:	0.416	**Peak 2:**	3306	12.2	1305
Intercept:	0.650	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report

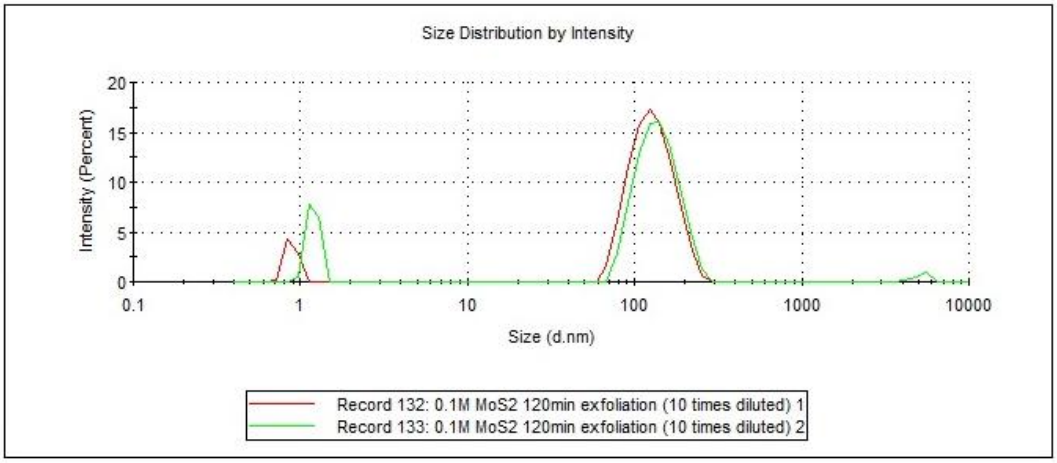


Sample Name:	0.1M MoS2 120min exfoliation (10 times diluted) 1		
SOP Name:	mansettings.nano		
File Name:	4.17.2017	**Dispersant Name:**	Water
Record Number:	132	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Friday, April 21, 2017 2:16:05 AM

Temperature (°C):	25.1	**Duration Used (s):**	500
Count Rate (kcps):	1.8	**Measurement Position (mm):**	3.00
Cell Description:	Disposable low volume cuvette (50µL)	**Attenuator:**	11

			Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	109.3	**Peak 1:**	130.4	91.1	37.52
PdI:	0.378	**Peak 2:**	0.8811	7.3	0.06989
Intercept:	0.582	**Peak 3:**	5241	1.6	448.1

Result quality : Refer to quality report

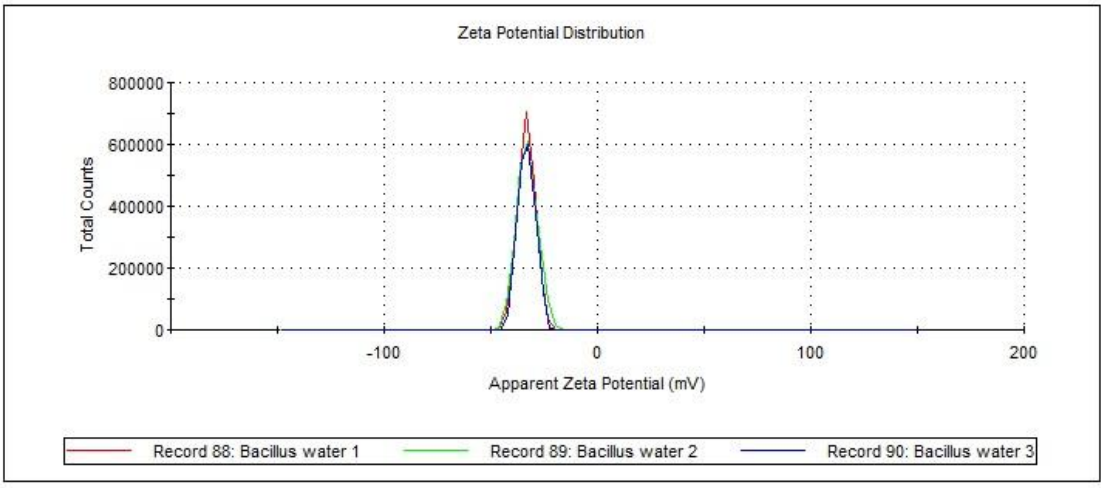


Sample Name:	Bacillus water 1		
SOP Name:	mansettings.nano		
File Name:	4.26.2017	**Dispersant Name:**	Water
Record Number:	88	**Dispersant RI:**	1.330
Date and Time:	Wednesday, April 26, 2017 2:15:59 PM	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	710.0	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	5

			Mean (mV)	**Area (%)**	**St Dev (mV)**
Zeta Potential (mV):	-33.3	**Peak 1:**	-33.3	100.0	4.10
Zeta Deviation (mV):	4.10	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0124	**Peak 3:**	0.00	0.0	0.00

Result quality : Good



Sample Name:	MoS2 1		
SOP Name:	mansettings.nano		
File Name:	JUN.dts	**Dispersant Name:**	Water
Record Number:	85	**Dispersant RI:**	1.330
Date and Time:	Wednesday, April 26, 2017 2:05:34 PM	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

Temperature (°C):	25.1	**Zeta Runs:**	12
Count Rate (kcps):	281.8	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	8

			Mean (mV)	**Area (%)**	**St Dev (mV)**
Zeta Potential (mV):	-31.1	**Peak 1:**	-31.5	98.8	12.7
Zeta Deviation (mV):	13.3	**Peak 2:**	9.54	1.2	2.90
Conductivity (mS/cm):	0.0249	**Peak 3:**	0.00	0.0	0.00

Result quality : See result quality report