Surface Disinfection on Non-porous Coupons using TiO_2 with UV

by

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ABSTRACT

The ongoing COVID pandemic has opened the doors for the development of effective surface disinfection technologies. UV technology is one of the most effective technique to be used in combination with different photocatalytic agents such as Titanium Dioxide (TiO_2) for microbial inactivation. There are many bacteria and viruses which have the potential to infect humans via surface-oral/inhalation pathway. Thus, it is important to evaluate the effectiveness of these techniques used to inactivate microorganisms to minimize environmental transmission. UV light directly acts on bacteria and viruses by damaging their nucleic acids and protein structures. TiO_2 acts as a photocatalyst, generates hydroxyl radicals under UV, leading to enhanced inactivation efficacy. This study focuses on the impact of UVC light at 254 nm wavelength in combination with spray formulations with TiO_2 for the inactivation of *E. coli* (exposure times of 1, 5 and 10 minutes) and bacteriophages P22 (exposure times of 5 and 10 minutes) and MS2 (exposure times of 1 and 5 minutes). This study includes tests that explored the long-lasting impact of spray formulations on non-porous surface. Minimal inactivation of ~ 0.15 log inactivation of *E. coli* was resulted using TiO_2 alone but when UV was added to the procedure on average 3 log inactivation was achieved. It was noted that MS2 was found to be more susceptible to UV as compared to P22 due to its higher inactivation rate. The spray formulation homogeneity is a critical factor in consistent microbial inactivation. In addition, the UV intensity of the handheld device is an important factor for total disinfection. However, the combined spray formulation and UV technology is an effective method of surface disinfection.

DEDICATION

I would like to dedicate my thesis to my father, mother, brother, sister-in-law, husband, and friends. Without their unconditional support and love, I would not have reached this stage of my career.

ACKNOWLEDGMENTS

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CHAPTER 1

BACKGROUND

1.1 Introduction

Covid-19 pandemic and its etiological agent transmission have demonstrated the importance of surface disinfection and sanitization. As reported in a study by Buonanno et al., the recommended approach to control the viral transmission is to inactivate them in a short span of their production (Buonanno et al., 2020). It is critical to minimize or eliminate the environmental transmission of disease-causing microorganisms. As UV irradiation directly impacts microorganisms (Kowalski, 2009), its application on surface disinfection has been widely studied. When a catalytic oxidant is added to the disinfection process, the overall impact of UV radiation increases, which in turn increase the inactivation efficiency. This study focuses on the application of titanium dioxide (TiO_2) and 254 nm UV to measure the efficacy of the inactivation of *E. coli* (exposure times of 1, 5 and 10 minutes), bacteriophages MS2 (exposure times of 1 and 5 minutes) and P22 (exposure times of 5 and 10 minutes). The high-intensity UVC light can potentially damage eye and skin severely (Trevisan et al., 2006), therefore a handheld UV device was used for the inactivation experiments. Due to difficulties and costs of using coronavirus, *E. coli* and bacteriophages MS2 and P22 were selected to assess the performance efficiency of the combined TiO_2 and UV technologies. *E. coli* was selected for the early performance evaluation of the technology due to its similarity with other common waterborne bacterial pathogen such as *Salmonella, Shigella* (Cho et al., 2004). MS2 and P22 were used as surrogate bacteriophages for RNA and DNA viruses, respectively. The selected bacterial and viral surrogates provide a reasonable assessment

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of the applicability of the technology for the inactivation of Covid-19 on non-porous surfaces. In addition, the study evaluated the efficacy of TiO_2 separately and combined with UV for surface disinfection. TiO_2 was added to a spray formulation which acted as a catalyst to increase the overall log inactivation of the selected microorganisms.

1.2 Study Objectives

The main objective of the study was:

- To evaluate the performance of a TiO_2 based disinfectant formulation separately and combined with UVC for surface disinfection.

The specific objectives were:

➢ To evaluate microbial inactivation efficacy of different spray formulations.

➢ To study long-lasting impact of TiO_2 for surface disinfection on non-porous surfaces.

To evaluate the effect of change in UV dosage over time on microbial inactivation.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of Surface Disinfection

Environmental surfaces may play a major role in the transmission of bacteria and viruses such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Clostridium difficile*, norovirus, rotavirus, and rhinovirus, causing several outbreaks (Rutala & Weber, 2004). Microbial survival time on the surface and infectious dose are contributing factors to the spread of infection. The study conducted by Gebel et al., 2013 mentions that bacteria and viruses such as *C. difficile* spores, VRE, MRSA have a survival time of ~4-5 months on the surface. Moreover, low concentration of some microbes can spread infection, i.e., infectious dose for norovirus is one, which means that a single viral particle has the potential to infect (Gebel, 2013). Thus, regular cleaning and surface disinfection are of great importance.

As per the Centers for Disease Control (CDC) directed guidelines for disinfection and sterilization in healthcare facilities, 2008, three processes are used to inactivate microbes, Cleaning, Disinfection and Sterilization. Cleaning is the first step to remove inorganic and organic matters which may hinder the impact of the other two processes. As per the CDC guidelines, disinfection may kill many or all microorganisms present on the inanimate surfaces except resilient microbial spores. Whereas, sterilization is sporicidal, which means it kills all the microorganisms including microbial spores. There are different categories of disinfectants based on their level of disinfection capacity. Low-level disinfectants eliminate mostly all vegetative bacteria and to some extent fungi and viruses within the elapsed time of 10 minutes. Intermediate-level disinfectants damage

mostly all forms of microbes including mycobacteria but are not biocidal for bacterial spores. High-level disinfectants may destroy all the microbes except large bacterial spores. Moreover, there are two types of disinfectants that can be used to kill microbes. Chemical disinfectants such as alcohol, hydrogen peroxide, formaldehyde, etc. and miscellaneous inactivating agents such as metals, UV radiation, pasteurization, etc (CDC, 2008).

Disinfectants should be used correctly, in appropriate proportion to avoid any additional cost. In addition, immunocompromised personnel may get airway diseases with the use of some disinfectants, which can be prevented by avoiding the use of such disinfectants or keeping the personnel out of that area. Use of proper protective clothing and ventilation is advisable while using some of the disinfectants such as formaldehyde, chlorine, etc (CDC, 2008).

Environmental Protection Agency (EPA) has considered disinfectants and sterilizers as part of antimicrobial products in the section of pesticide registration. Based on terminologies mentioned on EPA, disinfectants eliminate many forms of microbes except their spores on inanimate surfaces, whereas sterilizers kill microbes up to certain levels which are considered as safe in the regulations. Disinfectants need to follow stringent EPA rules as compared to sanitizers. EPA divides disinfectants into two categories: Hospital type disinfectants and general use disinfectants. The former type is used to disinfect medical instruments, floors, and other inanimate surfaces, whereas the latter one is used in the houses, swimming pools, etc. There is major two types of sanitizers: Food contact products and non-food contact products. The former is used in food industry to keep food free from microbes and the latter is used mainly to sanitize

carpet, laundry, etc (EPA, 2020). Based on the product performance test guidelines for sanitizers, 2012, a successful sanitizer should eliminate >= 99.9 % bacteria, which is 3-log reduction within 5 minutes (EPA, 2012) and a successful disinfectant should achieve minimum 5 log bacterial inactivation within 10 minutes (EPA).

2.2 UV-induced Photocatalic Reaction

The review done by Bono et al., 2021 discusses two major mechanisms by which UV damages microorganisms: When nucleic acids (NAs) and proteins present in bacteria and viruses absorb UV photons, photo-induced reactions take place which led to microbial cell damage and when reactive oxygen species (ROS) are formed due to UV irradiation of powerful oxidant materials or photosensitive molecules which cause photo oxidation. Based on wavelength ranges, UV is divided into three categories – UV-A, UV-B and UV-C. The wavelength range for UV-A is 315-400 nm, UV-B is 280-315 nm and UV-C is 100-280 nm. Among these three, UV-B and UV-C are most effective as DNA and RNA contain monosaccharides and nucleobases which have maximum absorption peak at 200 and 265 nm, respectively. UV-A is less effective in inactivating microbes. When a microbial cell is exposed to UV light, structural damages to NAs and the replication/transcription/translation sites may occur due to generation of photoproducts. In case of viral disinfection, UV light hinders genome replication, which leads to their inactivation. UV irradiation promotes photoreactions between and within NAs which cause linkage of two adjacent pyrimidine bases that accelerate the production of photo dimers such as cyclobutene pyrimidine dimers (CPDs) and pyrimidine-6,4-pyrimidone (6,4PP). CPDs and 6,4PP photo dimers are responsible for the bending of double helix by 7-9º and 44º, respectively, which eventually hinders replication. Shorter NA sequences

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are known to have greater probability of getting damaged by UV light. In some instances, photoreaction may also take place due to the presence of purine base adenine (A). This purine base may bond with another A or T while subjected to UV irradiation (Bono et al., 2021).

However, microorganisms may repair themselves by photoreaction while exposed to UV alone (Kebbi et al., 2020). Thus, UV light is often used in combination with photocatalysts to increase its efficiency in inactivating the microbes by avoiding photoreaction (Bono et al., 2021). Studies done by Gerrity et al.,2008 and Ryu et al., 2008 also indicate that the requirement of UV dose is decreased for viral inactivation and protozoan parasite *Cryptosporidium parvum* when used in combination with strong oxidants such as TiO_2. It is a white colored photocatalyst, widely used in foods and pharmaceutical industries. Rutile, brookite and anatase are three forms of TiO_2. Among these three forms, anatase is very effective in the disinfection process (Bonetta et al., 2013). Since Rutile form has lower density and narrower band gap, it acts as a good UV blocker, whereas anatase can be used as an effective catalyst (Yang and Zhu, 2004). When TiO_2 is exposed to UV light, electron (e^-) and holes are generated. Their reaction along with the microbial type decide the efficacy of disinfection. The study conducted by Bono et al., also shows the mechanism behind formation of superoxide anion ($O_2 \bullet$) and hydroxyl radical ($\bullet OH$) by photocatalysis. When TiO_2 is subjected to UV irradiation, positive holes (h+) get generated in valence band (VB) due to migration of e^- from VB to conduction band (CB) of TiO_2. This is represented in equation (1) (Bono et al., 2021).

$TiO_2 + h\nu \rightarrow e_{cb}^- + h_{vb}^+$ ……………… (1)
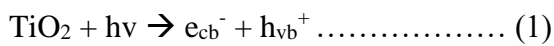
The generation of h+ and shifting of e$^-$ from VB to CB is a continuous process during photocatalysis. Since the reaction between h+ and e$^-$ is very fast, unused h+ and e$^-$ will recombine with each other which hinders the overall inactivation. When water (H_2O) or oxygen (O_2) encounter excited h+ and e$^-$, H_2O uses h+ to create •OH radicals and O_2 uses e$^-$ to form O_2• radicals. The stoichiometric reaction can be seen in equation (2) (Bono et al., 2021).

$$O_2 + e_{cb}^- \rightarrow O_2^-•$$

$$h_{vb}^+ + H_2O \rightarrow •OH + Haq+ \ldots\ldots\ldots\ldots (2)$$
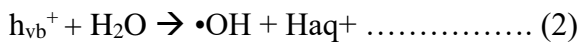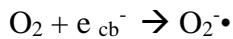
As OH radical is very short-lived radical with half-life of 10^{-9} seconds (Sies, 1994), it is important to use OH radical within short period of its generation for high inactivation.

When bacteria are subjected to TiO_2 and UV, damage to cell wall and intracellular compounds followed by cell death take place due to generated reactive oxygen species (ROS). Inactivation of viruses take place by damage to viral proteins and envelope followed by NA leakage due to photocatalytic reactions (Bono et al., 2021).

Although inactivation of microbes using different technologies found promising, there are many factors which impact the overall microbial inactivation. A review done by Guo et al., summarizes the influence of some factors on inactivation such as heat, relative humidity, etc. The review shows that high inactivation is achieved in the moist environment as compared to dry environment. Since high humidity is an indication of high H_2O molecules which react with h+ during photocatalysis to produce OH radicals, high OH radical production is obtained in presence of high humidity resulting in high inactivation (Guo et al., 2015). UV-induced photocatalysis is widely used in water treatment plants to mitigate the formation of carcinogenic disinfectant by-products

(DBPs) such as halo acetic acid (HAA) and total trihalomethane (TTHM). However, when the wastewater is subjected to limited photocatalysis, increase in DBP formation was observed due to incomplete oxidation. It is advisable to use high energy photocatalysis to avoid DBP formation, although the energy intensive process is not economically viable in high flow water treatment plants (Mayer et al., 2014).

2.3 Bacteriophage P22 and MS2

P22 is double-stranded DNA virus and a member of the *Podoviridae* family which consists of viruses with short tails. P22 was earlier known as PLT 22 and is widely used in the studies related to bacterial genetics. Since bacteriophages require a host to infect and grow, P22 starts attaching to the lipopolysaccharide (LPS) of the host cell *Salmonella* which allows it to penetrate through the outer surface due to digestion of O antigen and tight bond occurs (Byl et al., 2000).

Male-specific (MS2) is a single stranded RNA and infects F^+ pili which acts as a viral receptor, generated by *E. coli*. MS2 is a short single-stranded RNA genome surrounded by a protein capsid (Beck et al., 2016). The protein capsid consists of coat protein dimers and a single copy of A protein, but the exact location of A protein is not clear (Kuzmanovic et al., 2003).

Since MS2 has small size and simple composition, it is widely used as a model organism for viral replication, translation, infection, etc (Kuzmanovic et al., 2003). Also, since it is easy to grow and harmless to humans, it is used as a quantitative marker to check the effectiveness of antiviral and antiseptic agents.

CHAPTER 3

MATERIALS AND METHODS

3.1 Surface Disinfection

Surface disinfection experiments were carried out under laboratory conditions to check the effect of different formulations of TiO_2 spray and 254 nm UV for the inactivation of interested microbes. A UV lamp (266.66 mm X 37.8 mm X 41.84 mm) (CureUV, Florida, USA) was used as a source of UVC light with strength >= 2750 $\mu W/cm^2$, and total six spray formulation bottles (Lot 1 to Lot 6) of TiO_2 were used for all the experiments. Both UV lamp and spray formulations were provided by a company to check their microbial inactivation efficacy. Two 5 inches stands were used to support the UV lamp for *E. coli* and bacteriophages experiments. To avoid any contamination; all the experiments were carried out in a laboratory hood.

Stock culture of *E. coli* (ATCC strain 25922), bacteriophage P22 (ATCC strain 19585-B1) and MS2 (ATCC strain 15597- B1) were used. The host for P22 and MS2 were selected as *Salmonella enterica* (ATCC strain 19585) and *E. coli* (ATCC strain 15597), respectively. To culture the microorganisms; brilliant agar media (Sigma®) for *E. coli* and Tryptic Soy Agar for both P22 and MS2 were prepared and used.

The UV lamp with its cover and configuration is shown in Figure 3A (1).
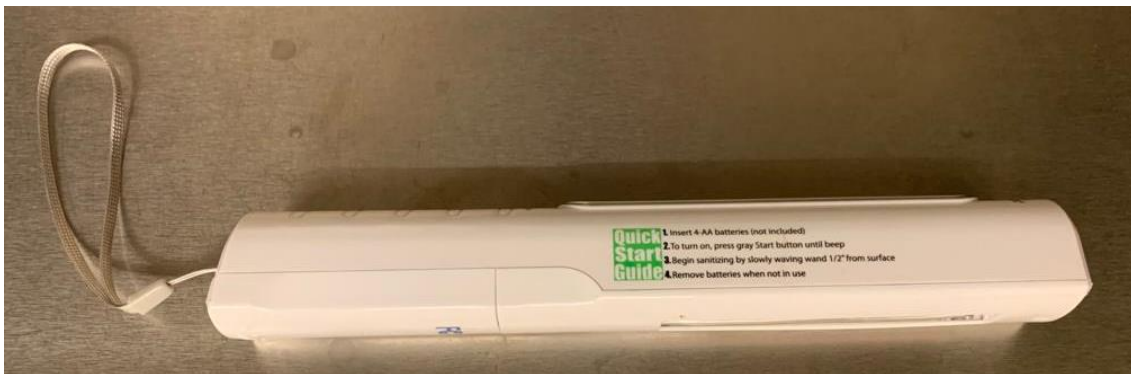
Figure 3A (1): UV Lamp - From Top to Bottom (UV Lamp Cover and UV Lamp)

The UV lamp had configurations as below:

Dimensions-266.66 x 37.8 x 41.84 mm, UVC

UV Strength >= 2750 μW/cm^2 = 825 mJ/cm^2 (5 mins)

An experimental set-up of UV lamp on 5-inches stands facing microbial sample is shown

in Figure 3A (2).
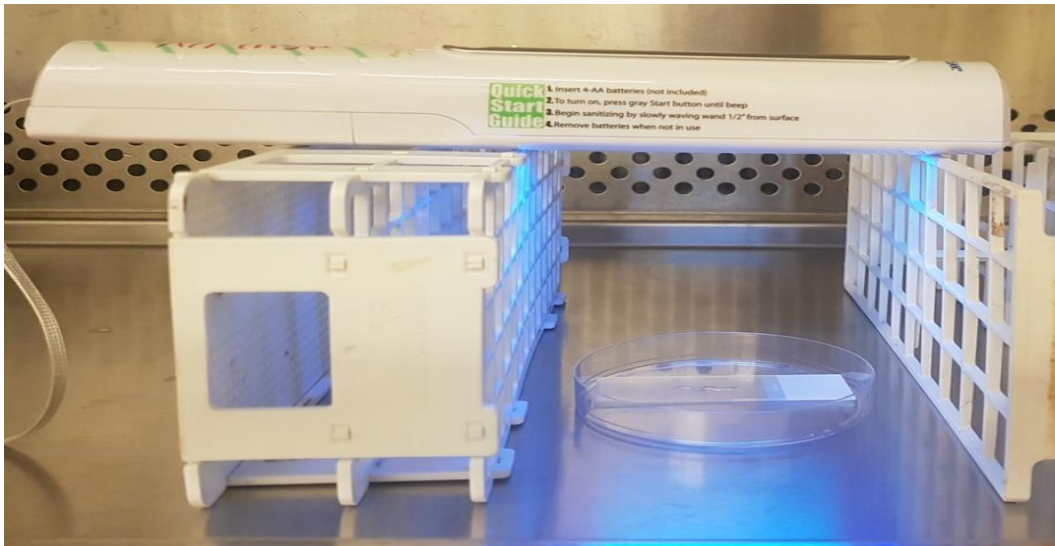
Figure 3A (2): Application of Spray and UV Light on Microbial Sample

The spray formulation bottles had different nozzles and had different homogeneity when sprayed on the petri dishes, which had an overall impact on inactivation. Figure 3B shows different spread of spray formulations on petri dishes.

Figure 3B: Spray Formulations (Right to left – Lot 1 to 6)

Figure 3C shows different spread of various spray formulations when applied on non-porous coupons.

Figure 3C: Different Homogeneity of Spray Formulations
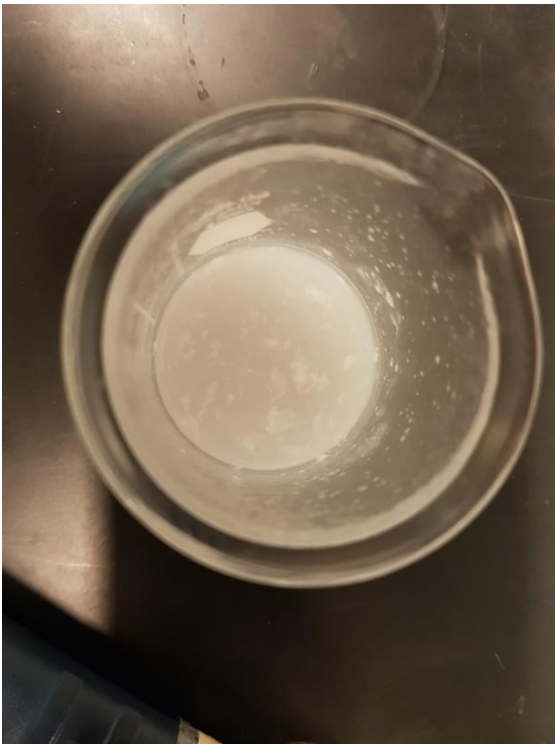
Figure 3D: Non-homogenous TiO$_2$ Spray Formulation

Figure 3D shows that larger TiO_2 particles may wrap up around the microbial surface and hinder UV penetration, which can contribute towards lower inactivation.

The samples containing 50 microliters (µL) of *E. coli* and 20 µL of bacteriophages (P22 and MS2) were deposited onto non-porous coupons and the samples were allowed to dry in the incubator at 37° C for 45 minutes. After incubation period, the dried samples were taken to the biosafety-hood to perform experiments. To check the efficiency of spray formulation to inactive the microbes, first Lot-1 to 5 were applied on *E. coli* and experiments were performed using spray formulations separately and combine with UV. Based on the higher log inactivation and better consistency, Lot-5 was selected for all experiments of bacteriophages.

3.2 Material Preparation for Experiments

Procedure to prepare brilliant media for *E. coli*

Brilliant agar media which is a selective media for *E. coli*, was prepared and used for all the experiments of *E. coli*. To prepare brilliant media, 500 ml of deionized (DI) water was measured using a graduated cylinder and transferred into a volumetric flask. The agar media was weighed to 10.14 gm and added to the DI water. For complete mixing and heating, the flask was kept on a hot plate at 100° C with a magnetic stirrer (level 6). Once the mixture boiled, it was shifted to the autoclave at 121° C for 20 mins with a warm liquid setting. After 20 minutes of sterilization in the autoclave, it was safely taken to the hot water bath which was set at 50° C and was kept there for 60 minutes before being used for experiments.

Procedure to prepare tryptic soy agar for P22 and MS2

Tryptic Soy Agar (TSA) was prepared and used for both bacteriophages. 500 ml of DI water was measured and taken into a volumetric flask for heating. TSA media was weighed to 19.25 gm and poured into the flask. The flask was then kept on a hot plate at 100º C with a magnetic stirrer (Level 6) and the mixture was completely stirred till it got boiled. Once it got completely dissolved, the flask was shifted to autoclave for further heating at 121º C for 20 minutes. After 20 minutes of sterilization in the autoclave, it was incubated for 60 minutes in the water bath set at 50º C before using it for pour-plate method.

Procedure to prepare stock culture of *E. coli* and *Salmonella*

Pure culture of frozen *E. coli* (ATCC strain 25992) and *Salmonella enterica* (ATCC strain 19585) was put in the 37º C incubator to completely thaw. Once thawed, the culture was streak plated on a TSA sterile petri dish and incubated at 37º C for at least 24 hours allowing for colonies to fully develop. After colony formation, the TSA plate was kept in the 4º C refrigerator as a monthly plate for preparation of experimental cultures. To grow overnight culture, a colony was taken from the monthly plate and added in the fresh 5 ml TSB and was kept in the 37º C incubator overnight. The overnight culture was then used to start a fresh 5 ml TSB centrifuge tube every day for new generation. The generations used for all the experiments were between second and fifth generations.

3.3 Surface Inactivation of *E. coli* on Non-porous Coupons using TiO_2 and UV

To check the inactivation efficacy of spray formulations (Lot-1 to 6), 50 µL of *E. coli* (ATCC strain 25992) was pipetted onto nonporous coupons and were put in the incubator to dry for 45 minutes. Once dried, the coupons were taken in the biosafety-hood to perform the experiments. Two pumps of spray formulations were applied on the coupons containing 50 µL dried bacterial samples. Once sprayed, depending on the treatment, UV light was applied for exposure times of 1 and 5 mins. The samples were then taken into a 20 ml neutralizing buffer tubes to make sure that the impact of inactivation had stopped after the specified exposure time for accurate results. Control sample without any disinfection treatment was used for each experiment to calculate initial concentration. All the samples were further serially diluted by adding 0.5 ml of sample containing neutralizing buffer into 4.5 ml of Phosphate Buffered Saline (PBS). Once serially diluted, the samples were plated using pour-plate method by pouring 1ml of sample onto sterilized petri dishes and adding 15 ml of brilliant media which was kept in the hot water bath at 50º C in the plates. For proper mixing, the petri dishes were swirled/rotated in a clockwise direction for several times for even distribution of the colonies. All the petri dishes were kept outside until they solidified and then shifted to the incubator at 37º C for 24 hours for bacteria to grow. After 24-48 hours the plates were taken out from the incubator and the colonies were counted.

The initial and final concentration have been obtained by averaging the concentration of bacterial colonies per ml of neutralizing buffer (Elution buffer) for control plates (CFU/ml) and concentration of bacterial colonies per ml of neutralizing buffer (Elution buffer) for samples (CFU/ml) respectively. As lot-5 was considered for all the

experiments of MS2 and P22, examples of initial and final concentration obtained for lot-5 is as below.

Initial concentration = 1636000 CFU/ml = 1.63 x 10^5 CFU/ml

Final concentration = 200 CFU/ml = 2.00 x 10^2 CFU/ml

The same calculations were conducted for all other Lots.

3.4 Surface Inactivation of Bacteriophages (P22 and MS2) on Non-porous Coupons using TiO$_2$ and UV

The steps followed for preparing samples of bacteriophages on non-porous surface were same as *E. coli.* After performing experiments on *E. coli*, it was found that Lot-5 worked the best among all six spray formulations due to higher log inactivation power and homogeneity and was used for all experiments conducted on P22 and MS2. After preparing serial dilutions, the samples were taken near the water bath where TSA top agar tubes were kept at 50º C for an hour. Sterile petri dishes with TSA media were taken out from 4º C and kept in the biosafety-hood to acclimate at room temperature. Once tempered, the petri dishes were taken to the platform near the hot water bath. 1 ml of host cell (*Salmonella* for P22 and *E. coli* for MS2) was added to each 5 ml TSA top agar (0.7%) tubes. After mixing the tubes properly, 1 ml of bacteriophage sample was added to them. The tubes were then poured onto TSA media plates and mixed well before putting them in the incubator at 37º C for 24 hours. After 24 hours, the plates were taken out and plaques were counted to calculate the inactivation.

3.5 Long-lasting Impact of Spray Formulation on Non-porous Coupons

It is very important to know the duration of spray formulation impact can stay on the surface after its application. To check the long-lasting impact of spray formulation on non-porous coupons, two pumps of Lot-5 was sprayed on glass coupons kept in the petri dishes. The petri dishes were kept outside on the surface with their lids to avoid any contamination. 30 minutes of drying time was provided to spray formulation and elapsed spray time were 24 and 48 hours as shown in Figure 3E. After 30 minutes, 24 and 48 hours, one set of coupons were used for the experiment directly after the elapsed spray time and another set of coupons were wiped using a weight of ~ 100 grams for five times (back and forth) before putting MS2 sample on them. Control sample was used to calculate initial concentration without any disinfection treatment. The sample consisting of 20 µL of MS2 was applied to the coupons and the impact of UV lamp along with TiO_2 for exposure time of 5 minutes was analyzed with the same procedure mentioned in the section 3.4.
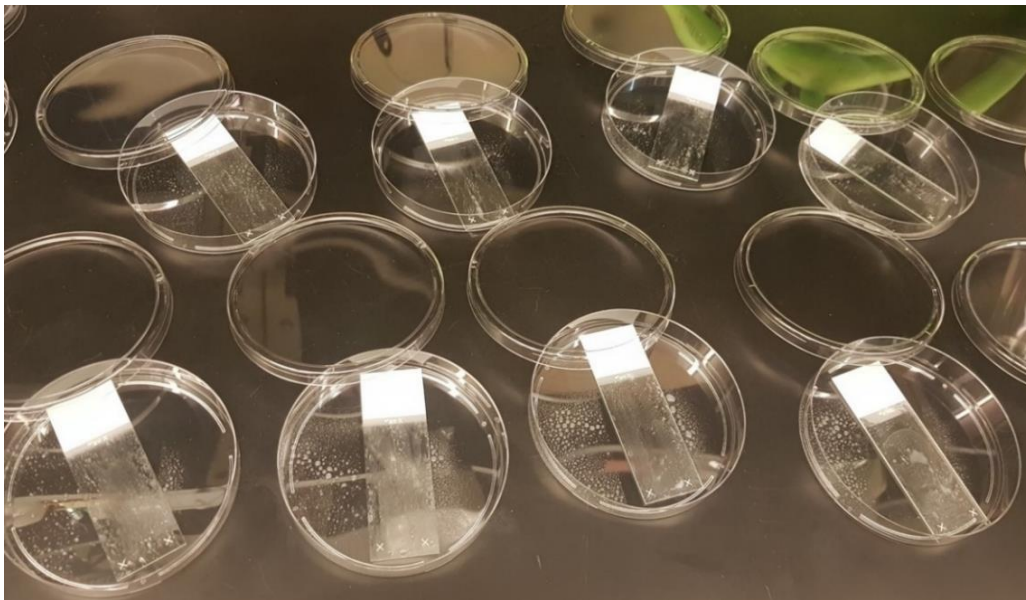
Figure 3E: Drying of Lot-5 on Non-porous Coupons for 30 minutes, 24 and 48 hours

3.6 Measurement of UV Intensity

Every UV lamp has a certain age after which it needs to be replaced. To check the UV lamp age, UV intensity of two UV lamps with same configurations were measured after 15 minutes time interval. Among the two lamps, one lamp was used for ~ 3 hours. The intensity of both the lamps were measured and compared several times after approximately 15 minutes of usage. UV intensity was measured using a detector of spectrophotometer (Avantes, Louisville, CO) with Avaspec-2048 L and calibration wavelength range of 200-1100 nm. The intensity was measured by keeping the detector at the center of the UV lamp and at 5 inches from the center where the samples were kept, to check how much intensity was reaching to the samples. The readings were generated using AvaSoft-8.11 full software after warming up both the UV lamps for almost 15 minutes. Figures 3F and 3G show the set-up for UV intensity experiments.
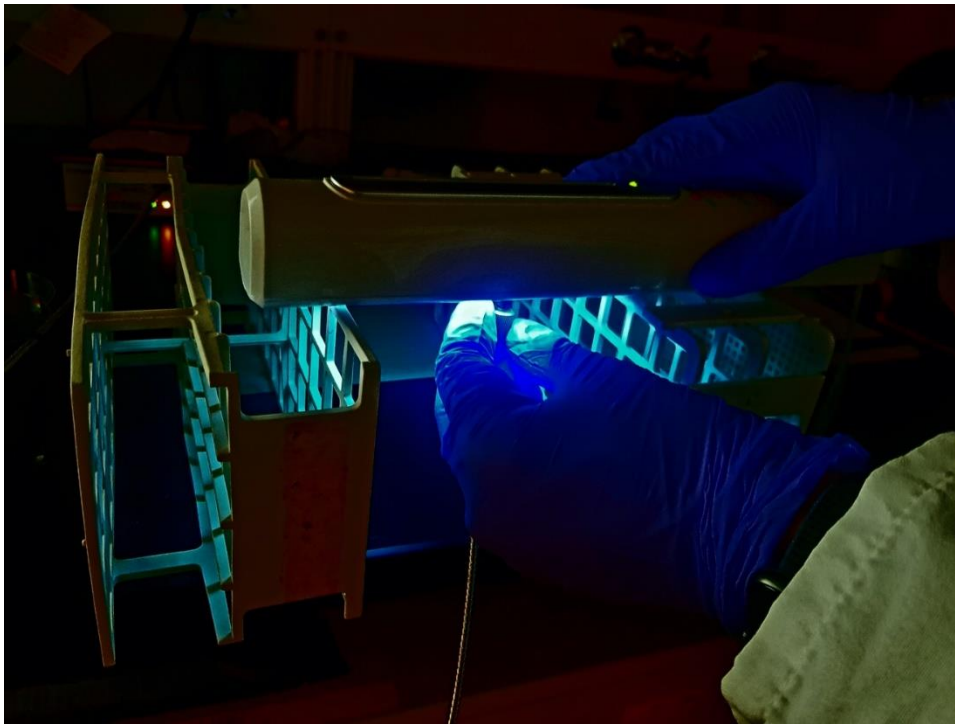
Figure 3F: Measurement of UV Intensity at the Source
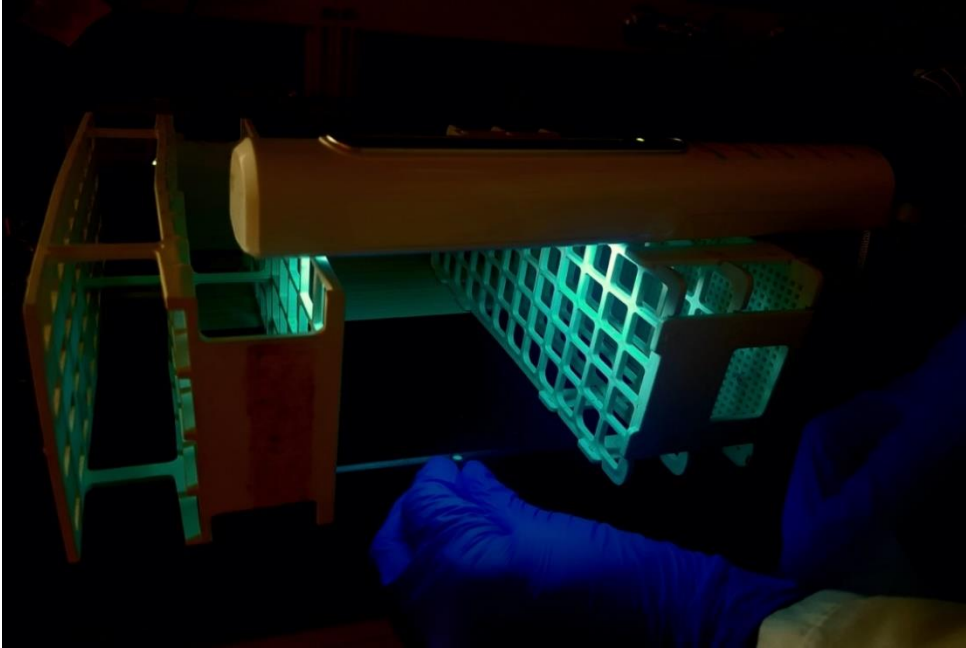
Figure 3G: Measurement of UV Intensity at Distance of 5 inches

UV dosage was calculated by converting UV intensity measured using spectrophotoradiometer (I) from $\mu W/cm^2$ to mW/cm^2 and then multiplying it with exposure time in seconds(s). mW/cm^2.s gave the UV dosage in mJ/cm^2.

Thus, UV dosage $(mJ/cm^2) = (I\ (\mu W/cm^2) / 1000)$ X Time (s)

To convert μW into mW, the intensity was divided by 1000 for unit balance on both the sides.

For Lot-5, the UV intensity measured was 130 $\mu W/cm^2$ at 5 inches from the UVC light surface where the samples were kept for experiments, and the exposure time was 5 minutes. UV dosage was calculated as below:

UV dosage = (130 / 1000) X 5 x 60 = 39 mJ/cm^2

The same calculations were done for all other Lots in respect to different UVC exposure time.

3.7 Measurement of Spray Formulation Turbidity

To measure the spray formulation turbidity, all the spray formulation bottles were shaken well and 1 ml from each spray formulation were added in 9 ml of DI water to make serial dilutions. 1000-fold dilution was done using all the spray formulations. Turbidity was measured using spectrophotometer with 600 nm UV wavelength. Initially, DI water was taken in a covet as blank sample. After setting the value of turbidity to zero, turbidity of six spray formulations were measured.

3.8 Measurement of UV Absorption Capacity

To measure the UV absorption capacity of six spray formulations, all the spray formulation bottles were shaken well and 1 ml from each spray formulation was added in 9 ml of DI water to make serial dilutions. Due to different TiO_2 concentration in spray formulations, turbidity was measured and set to 5 NTU prior to measuring UV absorption capacity. The absorption was measured using spectrophotometer with 254 nm UV wavelength. Initially, DI water was taken in a covet as blank sample. After setting the value of absorbance to zero, sample readings were measured.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Log Inactivation of *E. coli* on Non-porous Coupons using TiO_2 and UV

The log inactivation of *E. coli* on non-porous coupons was evaluated with spray formulation separately and combined with UV at different exposure times, distance from UV source and UV dosages. The results are presented in Table 4A, and all the calculations are attached in Appendix-A.

At first, the experiments were conducted using spray formulation Lots-1 and 2. However, as the log inactivation was less than 1, all the additional experiments were conducted with combined UV exposure. To obtain microbial inactivation using both the treatments, log inactivation was calculated using the following formula.

Log inactivation = $Log_{10}(A/B)$

OR

Log inactivation = $Log_{10}A - Log_{10}B$

A= Colony forming units per milliliter (CFU/ml) of bacteria before treatment

B= Colony forming units per milliliter (CFU/ml) of bacteria after treatment

Table 4A: Log Inactivation of *E. coli* on Non-porous Surface using TiO₂ and UV

Spray Formulation Lot	Application	Before Treatment (CFU/ml)	After Treatment (CFU/ml)	Exposure Time (min)	Distance from UV lamp (inches)	UV Dosage (mJ/cm^2)	Log Inactivation
1	Spray	6.6×10^4	1.2×10^4	1	-	-	0.80
	Spray						
	Spray+UV	2.2×10^3	1.5×10^2	1	1	7.8	1.43
	UV	4.6×10^2	<1	1	5	7.8	>2.67
	Spray	6.5×10^2	4.5×10^2	3	-	-	0.15
2	Spray	2.2×10^5	2.5×10^4	5	-	-	0.92
	Spray+UV	2.2×10^5	5.0×10^1		5	39	3.53
3	Spray+UV	2.7×10^3	1.9×10^3	10	5	78	0.22
3 (diluted)	Spray+UV (10 X)	2.9×10^3	1.7×10^3	5	5	39	0.23
	Spray+UV (100 X)	2.9×10^3	<1				>3.48
4	Spray+UV	1.6×10^5	2.1×10^3	5	5	39	2.15
5	Spray+UV	1.6×10^5	2.0×10^2	5	5	39	**3.08**
6	Spray+UV	1.6×10^5	3.7×10^3	5	5	39	2.45

It is noticed that with higher exposure time for Lot-3 as compared to Lot-2 (10 minutes for Lot-3 against 5 minutes for Lot-2), 0.22 log inactivation was achieved with Lot-3 versus 3.53 log inactivation with Lot-2 (Table 4A). This could happen due to non-homogeneity of spray formulation. As presented in other studies, the lower bacterial inactivation could be the result of scattering effect of TiO_2 particles. Since TiO_2 particle size measured to be on the order of thousands nanometer, they are large enough to form layers on bacterial cells, resulting in limited UV light penetration for the inactivation of *E. coli* (Benabbou et al., 2007). In addition, holes in valence band and electrons in conduction band in TiO_2 particles are produced due to UV-induced photocatalysis. They would recombine with TiO_2 particles if not consumed by bacteria (Benabbou et al., 2007). Also, it was observed that UV played a pivot role in bacterial inactivation since >2.67 log inactivation was achieved using UV alone versus 1.43 log using UV in combination with Lot-1. The study conducted by Zarif, 2017 also observes that higher log inactivation for *E. coli* was achieved when exposed to UV alone versus when exposed to UV in combination with TiO_2. The study also mentions that as the concentration of TiO_2 increases, the log inactivation of *E. coli* decreases (Zarif, 2017).

Based on the results obtained from the six spray formulations, it was decided to use Lot-5 for the remaining experiments due to its better homogeneity resulting in higher log inactivation.

4.2 Log Inactivation of P22 on Non-porous Coupons using TiO_2 and UV

Lot-5 combine with UV was used to conduct all the experiments for P22 inactivation on non-porous coupons for exposure times of 5 and 10 minutes to evaluate the efficacy of the combined treatment.

Table 4B: Log Inactivation of P22 on Non-porous Surface using TiO_2 and UV

Spray Formulation Lot	Application	Before Treatment (PFU/ml)	After Treatment (PFU/ml)	Exposure Time (min)	Distance from UV source (inches)	UV Dosage (mJ/cm^2)	Log Inactivation
5	Spray+ UV	9.0×10^3	2.5×10^1	5	5	39	2.57
		2.9×10^3	1.3×10^2			<39	1.35
		7.5×10^2	0	10		<39	>2.88

It is evident that UV intensity played a vital role in P22 inactivation. Due to a drop in UV intensity due to malfunctioning of UV device, lower log inactivation was observed for exposure time of 5 minutes due to decrease in UV intensity (Table 4B). This shows that lower UV intensity will result in lower UV dosage and would impact the overall log inactivation. The study done by Liu and Zhang also shows that higher UV intensity leads to higher microbial inactivation (Liu and Zhang, 2006).

4.3 Log Inactivation of MS2 on Non-porous Coupons using TiO_2 and UV

The first set of runs were conducted using both Lot-5 and 254 nm UV for exposure time of 5 minutes and a higher log inactivation of 4.45 log was achieved. Due to higher log

inactivation, further experiments were conducted for exposure time of 1 minute instead of 10 minutes to check the efficacy of spray formulation combined with UV. With a UV dosage of 14.4 mJ/cm^2 and exposure time of 1 minute, 3.25 log inactivation was observed for MS2 against UV dosage of 39 mJ/cm^2 and exposure time of 5 minutes for P22. This indicates that MS2 is more susceptible to UV than P22. Since, MS2 is a single stranded RNA and P22 is a double stranded DNA, higher inactivation of MS2 was found with lower UV dosage (Tseng, 2007).

The results of MS2 experiments are summarized in Table 4C.

Table 4C: Log Inactivation of MS2 on Non-porous Surface using TiO₂ and UV

Spray Formulation Lot	Application	Before Treatment (PFU/ml)	After Treatment (PFU/ml)	Exposure Time (min)	Distance from UV source (inches)	UV Dosage (mJ/cm^2)	Log Inactivation
5	Spray+ UV	6.0 x 10^5	1.03 x 10^3	1	5	14.4	3.25
		1.1 x 10^6	4.7 x 10^1	5		75	4.45

4.4 Long-lasting Impact of TiO₂ on MS2

The results obtained from long-lasting impact of TiO₂ Lot-5 spray formulation with UV dosage of 72 mJ/cm^2 on MS2 inactivation are shown in Table- 4D. It was observed that after 30 minutes of drying time, maximum log inactivation was obtained. This result was consistent with UV dosage of 75 mJ/cm^2 without any drying time. However, the inactivation decreased with the increase in drying time.

Table 4D: Long Lasting Impact of TiO$_2$ on MS2 Inactivation

Spray Formulation Lot	Application	Exposure Time (min)	Distance from UV source (inches)	UV Dosage (mJ/cm^2)	Drying Time	Log Inactivation
5	Spray+UV	5	5	72	30 mins	4.30
	Spray					<1
	Spray+UV				24 Hr	4.24
	Spray					<1
	Spray+UV				48 Hr	3.84
	Spray					<1

4.5 UV Lamp Intensity

UV intensity for both the lamps were measured at UV source and 5 inches from the source after 15 minutes of warm up time before each experiment. Total four experiments were conducted as described in section 3.6. The results obtained are mentioned in Table 4E.

Table 4E: Changes in UV Lamp Intensity Over Time

UV Dosage (mJ/cm^2) with 5 mins exposure time				
Warm-up time (minutes)	**At the source**		**5 inches from the lamp**	
	New lamp	**After 3 Hrs.**	**New lamp**	**After 3 Hrs.**
15	2550	1500	75	39
15	3120	1200	72	28.5
15	2730	1260	64.8	24
15	2005	1110	54	22.5

The results indicate that the UV dosage decreases with increase in the lamp hours. Both the lamps came with same initial intensity. In can be inferred that UV dosage of new lamp is double than one with three hours usage (Table 4E). This shows that UV intensity has a great impact on microbial inactivation. The results obtained by Rincon and Pulgarin, 2003 also indicate that microbial inactivation increases with the increase in light intensity. The study also shows that bacterial inactivation is highly dependent on UV light intensity in the absence of TiO$_2$ (Rincon and Pulgarin, 2003). Figures 4A and 4B shows two graphs comparing UV dosage of both the lamps at UV source and 5 inches from the UV lamp.
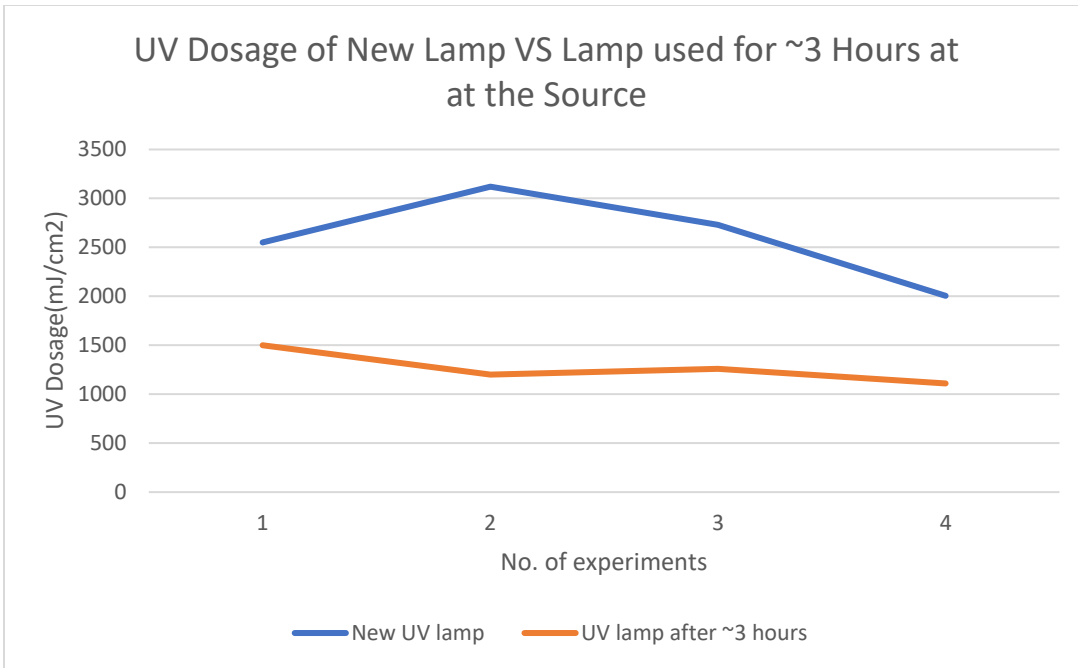
Figure 4A: UV Dosage of New Lamp vs Lamp used for ~3 Hours at the Source
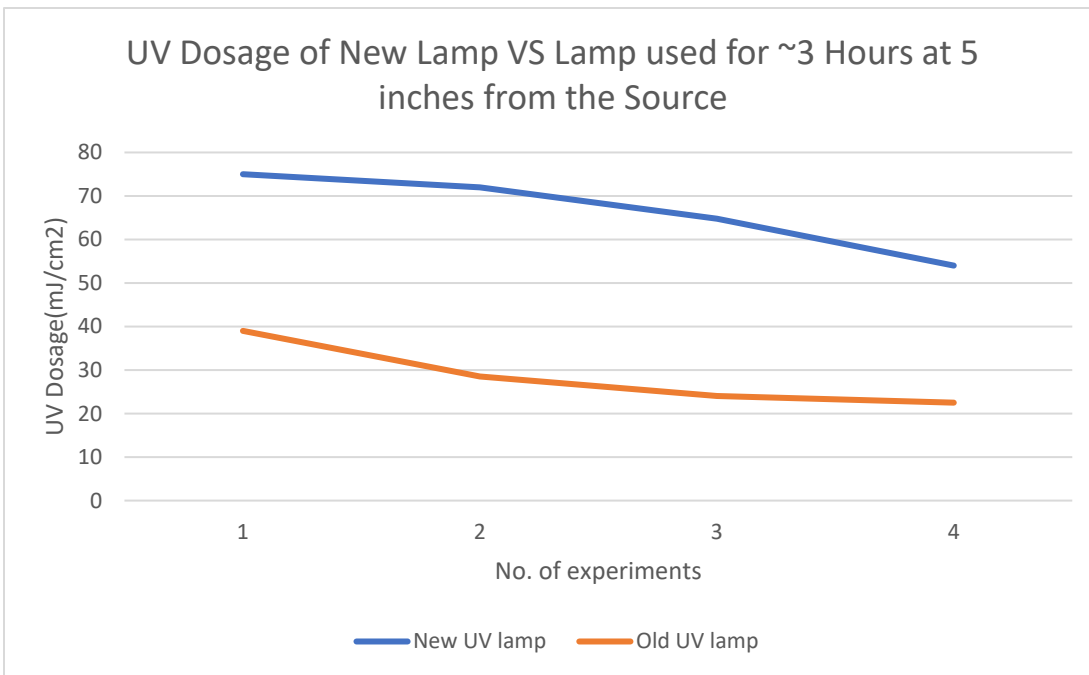

Figure 4B: UV Dosage of New Lamp vs Lamp used for ~3 Hours at 5 inches from the Source

The Figures 4A and 4B show that the trend of UV dosage of new and used lamp is almost similar, declining in a similar way. This indicates that with increase in exposure time, the dosage deceases, which results in lower inactivation. Due to drop in UV dosage over time, it is advisable to have a battery indicator in the UV lamp which can notify consumers once certain battery level is achieved.

4.6 Impact of Turbidity (Milky) of Spray Formulation on Microbial Log Inactivation

Due to lower inactivation found using some of the spray formulations combine with UV, impact of turbidity was measured to evaluate its role in microbial inactivation.

The results for six spray formulations with respective bacterial inactivation and turbidity are shown in Table 4F.

Table 4F: Impact of Turbidity (Milky) of Spray Formulation on Microbial Log Inactivation

Spray-formulation	Log Inactivation	Turbidity (NTU)
Lot-1	1.43	16
Lot-2	3.53	31
Lot-3	0.22	17
Lot-4	2.15	7
Lot-5	3.08	5
Lot-6	2.45	3
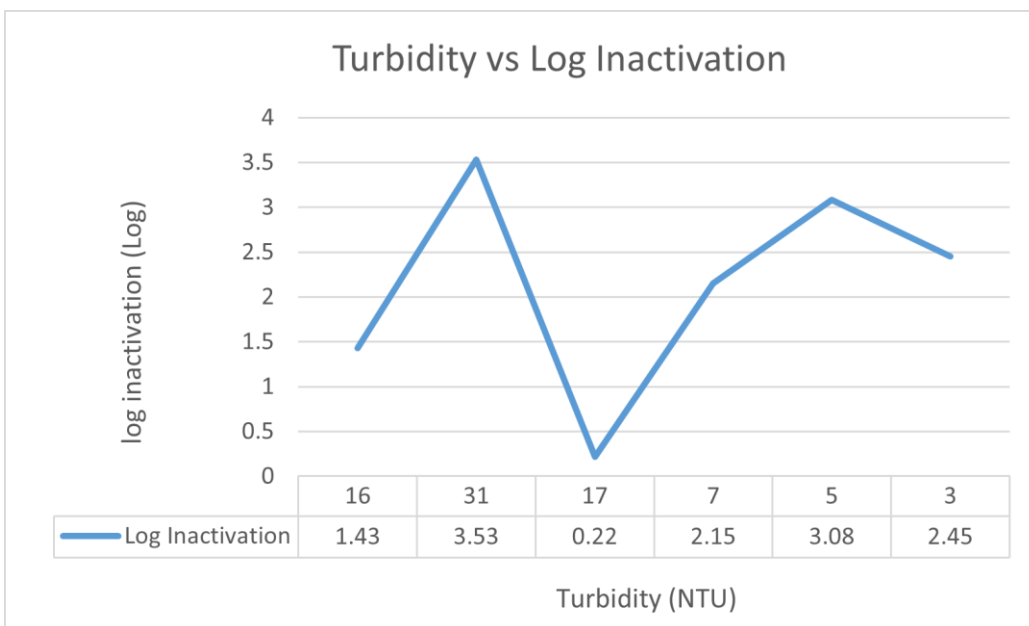
Figure 4C: Relation Between Turbidity and Log Inactivation

Although the turbidity measured for spray formulations after 1000-fold dilution for Lot-1 and 3 were similar, Lot-1 gave higher *E. coli* inactivation of 1.43 log versus 0.22 log achieved by Lot-3 (Figure 4C) and the maximum bacterial inactivation was achieved using Lot-5 with turbidity of 5 NTU. This indicates that turbidity of the entire spray formulation may not have a direct impact on the bacterial inactivation. In this study, the non-homogeneity of spray formulation on non-porous coupons was found to be the contributing factor for low *E. coli* inactivation. However, some of the studies indicate that high turbidity of TiO_2 leads to low *E. coli* inactivation (Gerrity, 2008 and Zarif, 2017).

4.7 Measurement of UV Absorption Capacity of Spray Formulations

The results for six spray formulations with respective UV absorption capacity are shown in Table 4G.

Table 4G: UV Absorption Capacity of Spray Formulations

Spray-formulation	Log Inactivation	UV absorption capacity
Lot-1	1.43	0.009
Lot-2	3.53	0.014
Lot-3	0.22	0.019
Lot-4	2.15	0.024
Lot-5	3.08	0.025
Lot-6	2.45	0.021

The results shown in Table 4G indicate that even though UV absorption capacity of Lot-3 was higher than Lot-1 and 2, lower log inactivation was achieved using Lot-3. Whereas, UV absorption capacities of Lot- 4, 5, and 6 were almost similar but higher inactivation of 3.08 log was achieved using Lot-5. This demonstrates that there are other factors may contribute to the overall inactivation.

CHAPTER 5

CONCLUSIONS

5.1 Study Outcome

The following can be concluded from the study.

A high log inactivation of *E. coli* was achieved i.e., on the order of 3 $\log_{10}$ inactivation using spray formulation combined with UV. Using spray formulation alone resulted in only ~0.15 $\log_{10}$ inactivation. This indicates that UV plays a major role in microbial inactivation. However, homogeneity of spray formulation also plays a vital role in achieving higher microbial inactivation. Therefore, an optimized TiO_2 concentration must be used to enhance the overall surface disinfection.

Unexpected lower log inactivation was noticed due to malfunctioning of the device. Therefore, it is critical to incorporate control strategy to make sure of proper UV device performance.

Log inactivation of MS2 was higher as compared to P22. This was expected and in line with reports by other researchers regarding higher susceptibility of MS2 to UV irradiation.

Results from long-lasting impact of spray formulation indicate that there is a decrease in log inactivation with increase in elapsed spray time.

Overall, homogeneity of spray formulation and UV dosage of a handheld UV device are major combined factors in achieving desired microbial inactivation. The spray formulation homogeneity is a critical factor in consistent microbial inactivation. In addition, the UV intensity of the handheld device is an important factor for the total

disinfection. However, the combined spray formulation and UV technology is an effective method of surface disinfection.

5.2 Future Work

Based on the lesson learned during the experiments, improvements can be made to increase inactivation efficacy. Factors such as optimization of TiO_2 concentration, different exposure time and the distance of the UV device to the surface should be considered in the future experimental plan. These factors may have a significant impact on the overall microbial inactivation. In addition, homogenous spray formulations should be used to avoid TiO_2 particle clumping. There could be less UV penetration as TiO_2 particles may cause masking of microorganism.

REFERENCES

Beck, S. E., Rodriguez, R. A., Hawkins, M. A., Hargy, T. M., Larason, T. C., & Linden, K. G. (2016). Comparison of UV-induced inactivation and RNA damage in MS2 phage across the germicidal UV spectrum. *Applied and environmental microbiology*, *82*(5), 1468-1474.

Benabbou, A. K., Derriche, Z., Felix, C., Lejeune, P., & Guillard, C. (2007). Photocatalytic inactivation of Escherischia coli: Effect of concentration of TiO_2 and microorganism, nature, and intensity of UV irradiation. *Applied Catalysis B: Environmental*, *76*(3-4), 257-263.

Bono, N., Ponti, F., Punta, C., & Candiani, G. (2021). Effect of UV irradiation and TiO_2-photocatalysis on airborne bacteria and viruses: an overview. *Materials*, *14*(5), 1075.

Buonanno, M., Welch, D., Shuryak, I., & Brenner, D. J. (2020). Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-67211-2

Byl, C. V., & Kropinski, A. M. (2000). Sequence of the genome of Salmonella bacteriophage P22. *Journal of bacteriology*, *182*(22), 6472-6481.

Cho, M., Chung, H., Choi, W., & Yoon, J. (2005). Different inactivation behaviors of MS-2 phage and Escherichia coli in TiO$_2$ photocatalytic disinfection. *Applied and environmental microbiology*, *71*(1), 270-275.

EPA (2012), Product Performance Test Guidelines OCSPP 810.2300: Sanitizers for Use on Hard Surfaces—Efficacy Data Recommendations [EPA 712-C-07-091]

Gebel, J., Exner, M., French, G., Chartier, Y., Christiansen, B., Gemein, S., ... & Sonntag, H. G. (2013). The role of surface disinfection in infection prevention. *GMS hygiene and infection control*, *8*(1).

Gerrity, D., Ryu, H., Crittenden, J., & Abbaszadegan, M. (2008). Photocatalytic inactivation of viruses using titanium dioxide nanoparticles and low-pressure UV light. *Journal of Environmental Science and Health, Part A*, *43*(11), 1261-1270.

Gerrity, D. W. (2008). *Photocatalytic disinfection with titanium dioxide and low-pressure UV light*. Arizona State University.

Guo, J., Huang, K., & Wang, J. (2015). Bactericidal effect of various non-thermal plasma agents and the influence of experimental conditions in microbial inactivation: A review. *Food Control*, *50*, 482-490.

Introduction Disinfection & Sterilization Guidelines | Guidelines Library | Infection Control | CDC. (2008). CDC. Retrieved July 5, 2021, from https://www.cdc.gov/infectioncontrol/guidelines/disinfection/introduction.html

Kebbi, Y., Muhammad, A. I., Sant'Ana, A. S., do Prado-Silva, L., Liu, D., & Ding, T. (2020). Recent advances on the application of UV-LED technology for microbial inactivation: Progress and mechanism. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3501-3527.

Kowalski, W. (2010). *Ultraviolet germicidal irradiation handbook: UVGI for air and surface disinfection*. Springer science & business media.

Kuzmanovic, D. A., Elashvili, I., Wick, C., O'Connell, C., & Krueger, S. (2003). Bacteriophage MS2: molecular weight and spatial distribution of the protein and RNA components by small-angle neutron scattering and virus counting. *Structure*, *11*(11), 1339-1348.

Liu, W. J., & Zhang, Y. J. (2006). Effects of UV intensity and water turbidity on microbial indicator inactivation. *Journal of Environmental Sciences*, *18*(4), 650-653.

Mayer, B. K., Daugherty, E., & Abbaszadegan, M. (2014). Disinfection byproduct formation resulting from settled, filtered, and finished water treated by titanium dioxide photocatalysis. *Chemosphere*, *117*, 72-78.

Perez, G. L., Huynh, B., Slater, M., & Maloy, S. (2009). Transport of phage P22 DNA across the cytoplasmic membrane. *Journal of bacteriology*, *191*(1), 135-140.

Rincón, A. G., & Pulgarin, C. (2003). Photocatalytical inactivation of E. coli: effect of (continuous–intermittent) light intensity and of (suspended–fixed) TiO_2 concentration. *Applied Catalysis B: Environmental*, *44*(3), 263-284.

Rutala, W. A., & Weber, D. J. (2004). The benefits of surface disinfection. *American journal of infection control*, *32*(4), 226-231.

Ryu, H., Gerrity, D., Crittenden, J. C., & Abbaszadegan, M. (2008). Photocatalytic inactivation of Cryptosporidium parvum with TiO_2 and low-pressure ultraviolet irradiation. *Water research*, *42*(6-7), 1523-1530.

Tseng, C. C., & Li, C. S. (2007). Inactivation of viruses on surfaces by ultraviolet germicidal irradiation. *Journal of occupational and environmental hygiene*, *4*(6), 400-405.

Trevisan, A., Piovesan, S., Leonardi, A., Bertocco, M., Nicolosi, P., Pelizzo, M. G., & Angelini, A. (2006). Unusual high exposure to ultraviolet-C radiation. *Photochemistry and photobiology*, *82*(4), 1077-1079.

Sies, H. (1994). Strategies of antioxidant defense. *EJB Reviews 1993*, 101-107.

What are Antimicrobial Pesticides? (2021, March 3). US EPA.

https://www.epa.gov/pesticide-registration/what-are-antimicrobial-pesticides

Yang, H., Zhu, S., & Pan, N. (2004). Studying the mechanisms of titanium dioxide as

ultraviolet-blocking additive for films and fabrics by an improved scheme. *Journal of*

Applied Polymer Science, *92*(5), 3201-3210.

Zarif, M. N. (2017). *Inactivation of Bacteria and Viruses in Water Using Ultraviolet*

Light and Advanced Oxidation Processes in a Bench-scale and Two Pilot-scale Systems.

Arizona State University.

APPENDIX A

LOG INACTIVATION CALCULATION FOR MICROBES

Lot -1 for *E. coli*

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray	A1	10	24.5	245	0.95
Spray	A2	100	0.5	50	1.64
Spray	B1	10	180	1800	0.09
Spray	B2	100	4.5	450	0.69
				Average	0.84
Spray+UV	C1	10	3	30	1.87
Spray+UV	C2	100	0.5	50	1.64
				Average	1.43
UV	D1	10	1	10	2.34
Control	G2	100	22	2200	

Lot-2 for *E. coli*

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray	A1	10	2336	23360	0.98
Spray	A2	100	312	31200	0.86
				Average	0.84
Spray+UV	B1	10	4.5	45	3.70
Spray+UV	B2	100	1	100	3.35
				Average	3.53
Control	G2	100	2328	232800	5.35
Control	G3	1000	217	217000	

Lot-3 for *E. coli*

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray+UV (10-fold)	A1	10	158.5	1585	0.28
Spray+UV (10-fold)	A2	100	19.5	1950	0.19
				Average	0.23
Spray+UV (100-fold)	B1	10	0	0	>3.48
Spray+UV (100-fold)	B2	100	0	0	
Control	G1	10	274	2740	3.48
Control	G2	100	32.5	3250	

Lot-4 to Lot-6 for *E. coli*

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray+UV (Lot-4)	A	1	7	7	2.39
Spray+UV (Lot-4)	A1	10	0	0	0
				Average	2.39
Spray+UV (Lot-5)	B	1	1	1	>3.24
Spray+UV (Lot-5)	B1	10	0	0	
Spray+UV (Lot-6)	C	1	1	1	>3.24
Spray+UV (Lot-6)	C1	10	0	0	
Control	G	1	1728	34560	3.24
Control	G1	10	28.5	285	

Lot-4 to Lot-6 for *E. coli*

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray+UV (Lot-4)	A2	100	20.5	3.31	1.90
Spray UV (Lot-5)	B2	100	2	2.30	2.91
Spray+ UV (Lot-6)	C2	100	37	3.57	1.65
Control	G2	100	1636	5.21	

Lot-5 for P22 inactivation for exposure time 5 minutes

Treatment	Sample	Dilution Factor	Avg PFU/plate	PFU/ml	Log Inactivation
Spray+ UV	A1	10	2	20	2.65
Spray+UV	B1	10	3	30	2.48
Control	G2	100	90	9000	

Lot-5 for P22 inactivation for exposure time 5 minutes

Treatment	Sample	Dilution Factor	Avg PFU/plate	PFU/ml	Log Inactivation
Spray+ UV	A1	10	20	200	2.46
Spray+UV	B1	10	13	130	1.35
Control	G2	100	29	2900	

Lot-5 for P22 inactivation for exposure time 10 minutes

Treatment	Sample	Dilution Factor	Avg PFU/plate	PFU/ml	Log Inactivation
Spray+ UV	A1	10	0	0	>2.88
Spray+UV	B1	10	0	0	
Control	G1	10	75	750	

Lot-5 for MS2 inactivation for exposure time of 5 minutes

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray+ UV	A	1	121	121	4.09
Spray+UV	B	1	22	22	4.83
Control	G4	10000	148	1480000	
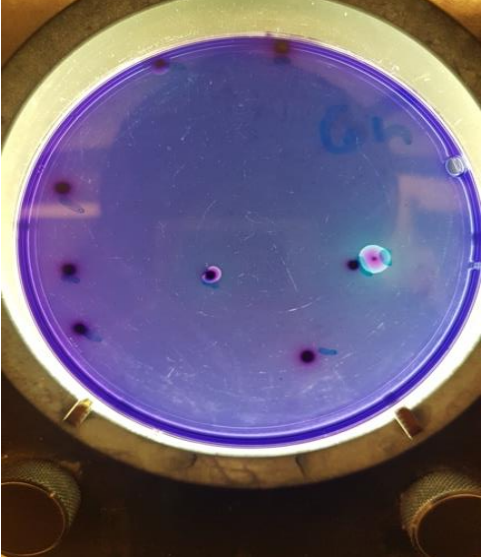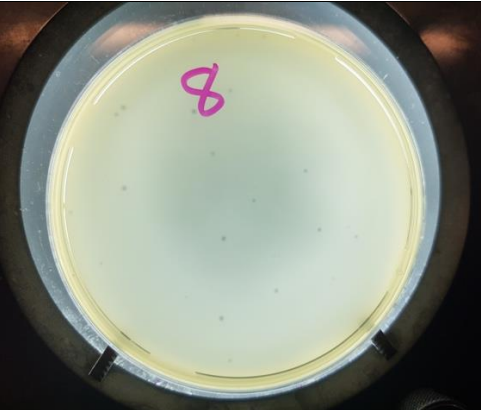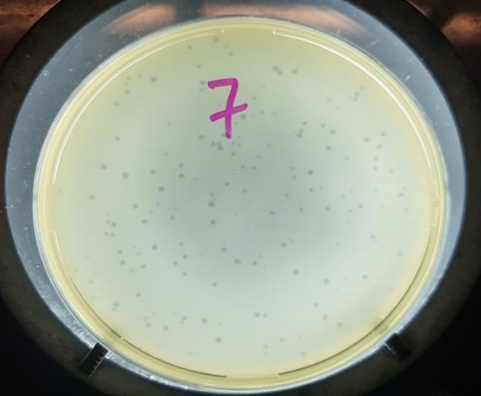
Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray+UV	A	1	24	24	4.43
Control	G4	10000	65	650000	

Lot-5 for MS2 inactivation for exposure time of 1 minute

Treatment	Sample	Dilution Factor	Avg CFU/plate	CFU/ml	Log Inactivation
Spray+ UV	A	1	115	115	3.69
Spray+UV	B1	10	91	910	2.79
				Average	3.25
Control	G4	10000	56	560000	

APPENDIX B

E. coli, P22 AND MS2 ON MEDIA PLATES

E. coli on Brilliant Plate	
P22 on TSA Plate	
MS2 on TSA Plate	