Polychromatic UV Dose Determination V.1

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Water TEAM

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DISCLAIMER

ABSTRACT

For polychromatic UV light sources, UV irradiance throughout the sample depth can be calculated by changing variables the “Germicidal Fluence (UV Dose) Calculations for a Low Pressure UV Lamp” obtained from Bolton Photosciences Inc. (Edmonton, Alberta, Canada) modified to account for polychromatic UV radiation sources (Linden and Darby, 1997). Because UV LEDs are polychromatic, the sensitivity of the radiometer (calibration data supplied by International Light) must be adjusted to the relative lamp emission of the LED (Bolton et al., 2015).

PROTOCOL CITATION

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KEYWORDS

UV dose, UV disinfection, polychromatic, UV LED, collimated beam

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PARENT PROTOCOLS

In steps of

Photorepair Fluence Response Protocol

MATERIALS TEXT

Materials

- Sterile phosphate buffered solution, 1X
- Microorganism working solution
- Agar plates for microorganism enumeration
- Microcentrifuge tubes, autoclaved

Equipment

- UV Dose Determination spreadsheet modified for polychromatic light sources
- Shaker incubator
- Vortex
- Centrifuge
- UV LED device or other UV light source
- Laptop with software for running radiometers and for calculating UV doses
- Spectroradiometer (e.g. Ocean Optics UV-VIS Spectrophotoradiometer)
- UV-VIS (e.g. Agilent Cary 4000)
- Radiometer (e.g. International Light Technologies ILT5000) with detector (e.g. SED 240W photodetector)
- Quartz cuvette
- P10, P100, P1000 pipets and tips, autoclaved
- 35 mm Petri dishes (60 mm or 100 mm may also be used) for UV exposures
- 100 mm Petri dishes for microorganism enumeration
- Magnetic stir plate
- Miniature magnetic stir bars
- Lab jacks
- Ruler
- Red light working lamp

SAFETY WARNINGS

Follow safety precautions for working with UV light sources. Wear PPE such as purple nitrile gloves, face shield, goggles, and lab coat. Put up signage to warn other lab personnel of potential UV light exposure.

DISCLAIMER:

BEFORE STARTING

Sanitize bench top surfaces with 70% ethanol.

Introduction

1 The purpose of this protocol is to document the steps used for determination of UV doses for polychromatic UV sources such as UV LEDs and medium pressure mercury lamps. The method is modified from Bolton and Linden (2003). Gather equipment and materials. Sterilize materials and prepare agar for enumerating microorganisms.

2 **UV Dose Determination Spreadsheet.** The example loaded is for UV LEDs but other polychromatic light sources can be added to and used in this spreadsheet. Append the date of the experiment and other details as appropriate.

LED 265 nm Fluence Calculations DATE.xlsx

Microorganism preparation 4h

3 Prepare microorganism cultures for UV exposure experiment.

For this document, *E. coli* K-12 was used as the microorganism. You may have different microorganisms and preparation steps.

3.1 Bacterial culture: Add **200 µL** of *E. coli* K-12 cryostock to **20 mL** sterile Tryptic Soy Broth. Incubate **180 rpm, 37°C** for **04:00:00** . 4h

3.2 Remove culture from incubator and transfer to 50 mL centrifuge tubes. Centrifuge **5000 rpm, 00:10:00** . 10m

Make sure to balance the tube containing the bacterial culture with a 50 mL tube filled with DI water of the same mass (within 0.1 grams).

3.3 Decant and discard supernatant. Transfer sterile 1X Phosphate Buffered Saline (PBS) solution to the bacterial cells to balance the tubes. Vortex to mix.

3.4 Centrifuge **5000 rpm, 00:10:00** . Decant and discard supernatant. Suspend cells in 20 mL fresh 1X PBS. 10m

Note: The expected *E. coli* K-12 concentration should be 10^8 CFU/mL in the re-suspended solution (20 mL). This solution will be diluted in 1X PBS to prepare the working stock for UV exposures.

3.5 Dilute the *E. coli* re-suspension into the desired volume of sterile 1X PBS to create working stock for UV exposures.

Note: Calculate the volume of the *E. coli* re-suspension to add to fresh 1X PBS using the equation: $C_AV_A = C_BV_B$, where C = concentration, V = volume, A = re-suspension solution, and B = working stock. Solve for V_A.

3.6 Take absorbance scan from 200-800 nm of working stock using UV-VIS Spectrophotometer.

A quartz cuvette must be used to take absorbance scans for UV dose determinations. Be careful not to handle the quartz cuvette with bare hands. Wear gloves. Only use lint-free wipes to dry the cuvette surfaces.

1. Turn on UV-VIS Spectrophotometer. Warm up lamps at least 10 minutes or longer (e.g. turn on before removing culture from incubator).
2. Blank using distilled water.
3. Take absorbance scan of distilled water. Empty cuvette.
4. Rinse cuvette three times with working stock.
5. Take absorbance scans for at least duplicate samples of working stock.
6. Export absorbance scan spectra to flash drive.

4 **Additional Cell Preparation:** If using bacterial cells, aliquot working stock into as many 15 30s

or 50 mL centrifuge tubes as needed for the number of UV exposures that you will perform. Sonicate for **00:00:30** .

Note: Sonicating disaggregates cells and may prevent clumping of cells during UV exposures and plating.

UV Dose Determination

5 Determine UV exposures by importing UV absorbance scan data into a UV Dose Determination spreadsheet.

Before your experiment, ensure that you have measured the UV lamp emission spectra with a UV-VIS spectroradiometer (NIST calibrated preferred). You may choose to interpolate emission spectra values to obtain integer wavelengths for the UV Dose Determination spreadsheet. Interpolation can be performed in Microsoft Excel or other computational software (e.g. in R Studio with 'approx' function).

Warm up your UV source before starting the experiment.

5.1 Select UV doses for constructing UV dose response curve.

It is recommended that between 6 to 7 unique UV doses are selected.

5.2 Set the distance between the UV source and the surface of the sample. Enter the height in the UV Dose Determination spreadsheet.

Note: When setting the height of the UV lamp, remember to account for the sample depth.

Tip: Adjustable lab jacks will help with adjusting the height of the UV source, sample, or radiometer.

5.3 Set up radiometer.

Ensure you use the proper wavelength sensitivity factor when measuring irradiance of different wavelengths. Sensitivity factors should be provided by the manufacturer during recalibrations.

Obtain the Petri Factor by measuring the irradiance at the plane level that the sample surface will be at. Take measurements in a 0.5 cm grid in both the x- and y-direction starting from the center of the Petri dish. Take enough measurements in all directions so that the exterior of the Petri dish falls within the outer most measurements.

Enter measurements in the UV Dose Determination Spreadsheet in the **Determination of the "Petri Factor"** Section.

For example, for a 35 mm Petri dish, take at least 4 measurements spaced at 0.5 cm intervals in each direction to cover a total of 40 mm (>35 mm) per x- and y-direction.

5.4 Follow steps in the **Instructions and Notes** Section of the UV Dose Determination spreadsheet.

Performing UV Exposures 16h

6 Perform UV exposures by transferring samples to a sterile Petri dish, adding a stir bar, and placing on the magnetic stir plate. Expose the sample to UV irradiation for the calculated exposure times.

Magnetic stirrer rotation should be set so that the stir bar does not create a vortex in the sample, which would disrupt the sample surface plane.

Use a shutter to control the duration of exposure time.

The lowest exposure time is recommended to be > 30 seconds to minimize effect of timing uncertainty and errors on UV dose delivered when using hand-operated shutters.

6.1 Collect samples after exposures. Dilute and plate samples. Record the volume used for plating each dilution. Plating may be done with spot plating or spread plating. After samples are dried, incubate plates. 16h

E. coli: **37 °C** **16:00:00** (time may vary based on growth media)

Optional: Save samples for further molecular analyses such as DNA extraction or RNA extraction. Record the volume saved.

6.2 Calculation concentrations in non-irradiated and irradiated samples to determine log inactivation. Construct UV dose response curve by plotting log inactivation vs. UV dose.