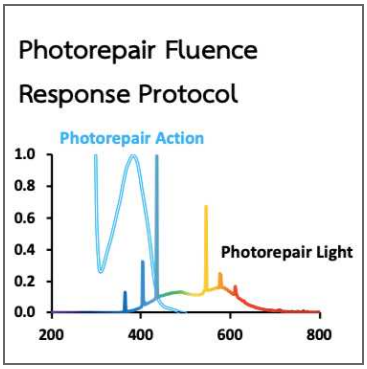

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We use this protocol and it's working

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Photorepair Fluence Response Protocol

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ABSTRACT

Photorepair light exposures are performed on microorganism samples following UV light exposure (i.e. UV disinfection) to determine microorganism regrowth and repair kinetics. Photorepair is a light-dependent, enzyme mediated DNA repair process in bacteria that may reverse UV-induced DNA damage and decrease UV disinfection efficacy. Previously, photorepair kinetics were determined on a time-basis (i.e. exposure times). However, time-based photorepair determinations are not standardized. A photorepair fluence response protocol was developed here according to a standardized protocol proposed by Bohrerova and Linden (2007). When using this protocol, we request that you cite the original reference.

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PROTOCOL integer ID: 63358

Keywords: photorepair fluence, UV disinfection

MATERIALS

- UV light source
- Photorepair light source (e.g., 60 W Red LED)
- Laptop with software for running radiometers and for calculating UV doses
- Spectroradiometer, calibrated with calibration files (e.g., Ocean Insight HDX)
- UV-VIS (e.g. Agilent Cary 4000)
- Radiometer with detector, calibrated with calibration files (e.g. ILT 5000 Radiometer)
- Quartz cuvette (e.g. 1 cm, 5 cm, 10 cm path length)
- Sample containers (e.g. plasticware, glassware, and sterile for microbial samples)
- Magnetic stir plate(s)
- Miniature magnetic stir bars, sterilized
- Lab jacks
- Ruler
- Red light working lamp (not emitting between 300-500 nm, for experiments where photorepair of bacteria is a concern)

SAFETY WARNINGS

UV exposure risk! Follow safety precautions for working with UV light sources. Wear PPE such as nitrile gloves, face shield, goggles, and lab coat. Put up signage to warn other lab personnel of potential UV light exposure. Work in biosafety cabinets according to the safety levels required for the microorganisms to be used in experiments.

BEFORE START INSTRUCTIONS

For microbial samples, sanitize bench top surfaces with 70% ethanol.

Introduction and Pre-Experiment

1 The photorepair fluence response protocol is based on Bohrerova and Linden (2007).

CITATION

Zuzana Bohrerova, Karl G. Linden (2007). https://doi.org/10.1016/j.watres.2007.03.015. Water Research.

LINK

https://doi.org/10.1016/j.watres.2007.03.015

To perform the photorepair fluence response experiment, you will first need to perform a UV

fluence response experiment after the references below. Photorepair experiments and sample handling must be performed in a dark environment. Minimal red light may be used for visibility. This protocol assumes that the user has already performed the UV fluence response experiment. The Polychromatic Fluence (UV Dose) Response Protocol, linked below, can be used for performing the UV exposures.

Protocol

NAME

Polychromatic Fluence (UV Dose) Response Determination

CREATED BY

Daniel Ma

PREVIEW

Note

Note that "UV dose" and "UV fluence" are interchangeable terms in quasi-collimated beam experiments.

Before the Photorepair Experiment

1.1 **UV Irradiation**: Perform the UV fluence response experiment to prepare the UV irradiated samples for the photorepair fluence response experiment. Experiments must be performed in a dark environment with minimal red light for visibility.

Note

Use a pre-determined sample volume for the UV exposure that will permit you to split the sample into two equal volumes and to collect the desired number of samples at pre-determined time intervals.

Experimental decision making will determine how much sample to perform the UV fluence response exposure with...

- How many intervals to collect for photorepair exposures? (e.g. after 15, 30, 60, 120 minutes)
- For immediate culturing, an example volume to collect: 0.1 mL for enumeration
- For later extraction and molecular analyses, some example volumes to collect: 0.5 mL for nucleic acid analysis, 1 mL for protein analysis, 1.5 mL for both

The volume at the start of the photorepair and dark repair incubations should be sufficient to permit sampling from all the determined sampling intervals.

After UV irradiation: Immediately remove sample from UV exposure. Keep samples in the dark and covered. Perform sample handling and plating in the dark with only red light for visibility. Take sample for microorganism enumeration and molecular analyses. Dilute samples and plate. Set aside samples for molecular analysis and store at -80°C.

Transfer the desired of the irradiated sample for photorepair and the remaining sample for dark repair into two identical, sterile Petri dishes (60 mm dishes).

Preparing the Photorepair Apparatus

2 Before performing the photorepair fluence response experiment, set up the photorepair fluence response experiment apparatus. A light source emitting the desired photorepair light (minimum 300-500 nm emission spectrum) should be suspended above a magnetic stirrer and dish containing the sample.

1. Warm up the photorepair light source (e.g. 30 minutes before performing exposures).
2. Start up spectroradiometer and acquisition software.
3. If your spectroradiometer has not been set up to obtain spectral irradiance measurements, set it up, load calibration data (if necessary), and obtain background reading as part of the set up.
4. Obtain spectra irradiance measurements at the surface of the water sample (Figure 1).

Note

The spectroradiometer should be maintained at a perpendicular angle to the light source.

2.1 Use the Photorepair Fluence spreadsheet to determine the photorepair fluence: PhotorepairFluence_Calculation.xlsx. The contents of each sheet are summarized in Table 1.

	A	B
	Sheet Name	Description
	Fluence Calculations	Primary sheet where fluence calculations are performed. Values must be entered by user for volume, diameter, water path length, distance from light source to sample, weighting factor, and radiometer reading.
	Absorbance	UV-VIS spectrophotometer measurements for the microorganism working stock solution is imported into this sheet.
	Reflection Factor	Pre-calculated reflection factor based on personal communications with Professor Jim Bolton (2016).
	Weighting	Sheet that accounts for the relative lamp emission of the photorepair lamp, radiometer factor, sample absorbance, Beer Lambert law, photolyase absorbance, and weighting factors to calculate the average intensity.
	Lamp Spectra	Sheet that depicts the lamp spectra, average wavelength, and peak wavelength.
	Irradiance Interpolation	Sheet that performs interpolation function in Excel to output spectral irradiance values for integer wavelengths so that values can be used in the Weighting tab.
	Photolyase Spect Interpolation	Sheet that performs interpolation function in Excel to output photolyase action spectra values for integer wavelengths so that values can be used in the Weighting tab.

Table 1. Description of contents for the sheets included in PhotorepairFluence_Calculations.xlsx

Note

In this spreadsheet, the *E. coli* photolyase action spectra is used as the weighting spectra. Using other microorganisms for photorepair will require obtaining action spectra for photolyase in that microorganism.

Data for the photolyase action spectra was digitized from Payne and Sancar (1990):

CITATION

Gillian Payne, Aziz Sancar (1990). Absolute Action Spectrum of E-FADH2 and E-FADH2-MTHF Forms of Escherichia coli DNA Photolyase. Biochemistry.

LINK

10.1021/bi00485a021

2.2 **Optional**: To compensate for the change in sample distance from the light source with each subsequent volume withdrawal for sample analysis by calculating the height differential between sampling events, and measuring the light intensity at the center of the dish at the expected level at the start of the experiment to account for the changes in fluence calculation. The change in distance can be calculated using the volume withdrawn and the geometry of the dish (e.g. inner diameter and surface area). The number of measurements will be determined by the number of sampling events that should be pre-determined at the start of the experiment.

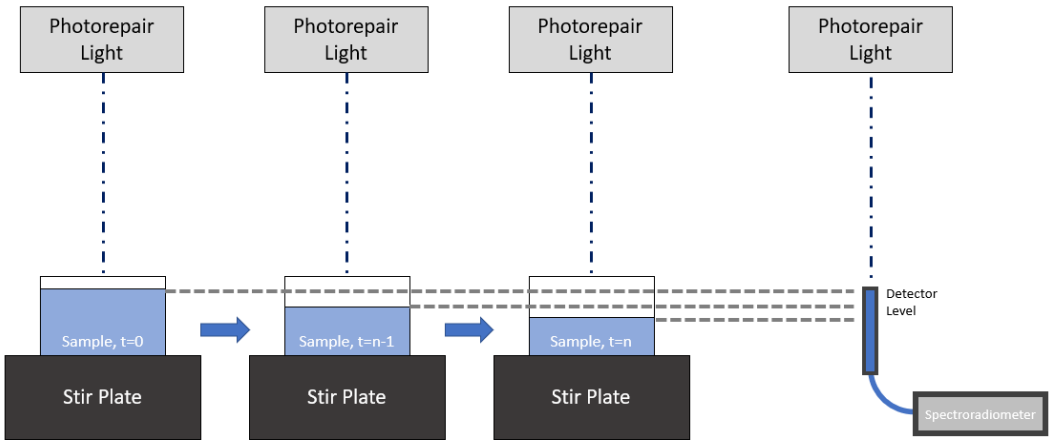

Figure 1. Spectral irradiance measurements may be taken at the plane of the water surface level after each sampling event.

2.3 **Photorepair Irradiance Example Data**: Measurements obtained using the method described in Figure 1 are shown in this example: PhotorepairIrradiance.xlsx . A summary of the contents of this spreadsheet is provided in Table 2 below.

	A	B
	Sheet Name	Description
	Plot	Shows a plot of all spectral irradiance measurements obtained at different distances from the light source
	RawData	Contains all the raw measurements, including background measurements (i.e., dark controls with the cap on the spectroradiometer), and measurements taken at different distances
	SpecIrr_BkgdCorrected	Spectral irradiance data with background corrections (i.e., background spectra subtracted from light spectra measurements)
	Tabs named 12 to 12.35	Each tab has the data for measurements obtained at the various heights (units in cm). The sheets interpolate the measurements to integer wavelength values which can then be used in the Photorepair Fluence Calculation spreadsheet. The total irradiance from 320-475 nm is calculated in cell M2 as the sum of the spectral irradiance values between 320-475 nm.

Table 2. Description of contents for the sheets included in PhotorepairIrradiance.xlsx

Performing the Photorepair Fluence Response Experiment

3 After obtaining measurements (**optional**: for each height required), export spectral irradiance data.

Note

You can determine the change in sample height after removing sample by using the volume of a cylinder formula for given volume and a known Petri dish diameter.

Note

Prepare: Determine the number of sample tubes you will need for collecting samples. Label the tubes with sample information, such as: UV source, UV fluence, and time interval, light or dark repair, etc.

Additionally: Prepare 1X PBS dilution tubes ahead of time for dilution and enumeration.

4 **Photorepair**: Set the distance from the sample surface to the light source. Place the sample for photorepair exposure under the photorepair light at the pre-determined distance. Stir the sample

continuously with the magnetic stirrer.

Dark Repair: Place the dark repair sample on magnetic stirrer in a dark environment.

Note

Work in a dark environment with minimal red light for visibility to prevent any unwanted photorepair. Check that the red light does not emit any light in the photorepair action spectra (300-500 nm) using a spectroradiometer.

Note

Users may opt to use a quartz cover to prevent evaporation for prolonged exposures.

5 **Sampling**: After each time interval, remove the pre-determined sampling volume from the photorepair and dark repair samples.

Handling: Transfer samples to empty, sterile microcentrifuge tubes.

Dilute and plate organisms immediately: Perform serial dilutions with 1X PBS of samples and plate. Incubate plates under the desired growing conditions for the microorganism. Incubation times will vary per microorganism.

Note

Sample Preservation: Save samples for later molecular analyses at -80°C.

Tip: Keep samples organized by UV source, UV dose, and time interval. Maintain a record of what samples have been collected and where they are stored.

Post-Experiment

5.1 **Optional if users account for the height adjustment and spectra measurements at different heights**: After the experiment, calculate the photorepair fluence for each time interval. Use the irradiance measurements taken at each height for the corresponding time interval to account for the differences in light intensity at different distances from the lamp:

PhotorepairFluence_Post-Calculation.xlsx

5.2 Perform enumeration of microorganism and calculate concentrations. The reference list below provides some sample equations used in the literature for determining repair kinetics.

Percent (Photo)reactivation

CITATION

Karl G. Lindenauer, Jeannie L. Darby (1994). Ultraviolet disinfection of wastewater: Effect of dose on subsequent photoreactivation. Water Research.
LINK
https://doi.org/10.1016/0043-1354(94)90087-6

Survival Ratio

CITATION

E. Nebot Sanz, I. Salcedo Davila, J.A. Andrade Balao, J.M Quiroga Alonso (2007). Modelling of reactivation after UV disinfection: Effect of UV-C dose on subsequent photoreactivation and dark repair. Water Research.
LINK
https://doi.org/10.1016/j.watres.2007.04.008

5.3 Perform molecular analyses or molecular processing such as DNA extraction or protein extraction and perform further desired downstream molecular quantification methods, such as examples referenced below.

ATP Quantification

CITATION

Chao Yang, Wenjun Sun, Xiuwei Ao (2019). Bacterial inactivation, DNA damage, and faster ATP degradation induced by ultraviolet disinfection. Frontiers of Environmental Science & Engineering volume.
LINK
https://doi.org/10.1007/s11783-019-1192-6

Cyclobutane Pyrimidine Dimer ELISA for DNA Damage

CITATION

Natalie M. Hull, Mythili R. Isola, Brian Petri, Po-Shun Chan, Karl G. Linden (2017). Algal DNA Repair Kinetics Support Culture-Based Enumeration for Validation of Ultraviolet Disinfection Ballast Water Treatment Systems. Environ. Sci. Technol. Lett..

LINK

https://pubs.acs.org/doi/full/10.1021/acs.estlett.7b00076

Endonuclease Sensitive Site (ESS) Assay for DNA Damage

CITATION

Oguma K, Katayama H, Mitani H, Morita S, Hirata T, Ohgaki S (2001). Determination of pyrimidine dimers in Escherichia coli and Cryptosporidium parvum during UV light inactivation, photoreactivation, and dark repair..

LINK

https://doi.org/

CITATION

Zuzana Bohrerova, Karl G. Linden (2006). Assessment of DNA damage and repair in Mycobacterium terrae after exposure to UV irradiation. Journal of Applied Microbiology.

LINK

https://doi.org/10.1111/j.1365-2672.2006.03023.x

Quantitative Polymerase Chain Reaction for DNA Damage

CITATION

Sara E. Beck, Roberto A. Rodriguez, Karl G. Linden, Thomas M. Hargy, Thomas C. Larason, Harold B. Wright (2013). Wavelength Dependent UV Inactivation and DNA Damage of Adenovirus as Measured by Cell Culture Infectivity and Long Range Quantitative PCR. Environ. Sci. Technol..

LINK

https://doi.org/10.1021/es403850b

Quantification of Protein Damage

CITATION

Beck SE, Rodriguez RA, Linden KG, Hargy TM, Larason TC, Wright HB (2014). Wavelength dependent UV inactivation and DNA damage of adenovirus as measured by cell culture infectivity and long range quantitative PCR..

LINK

https://doi.org/10.1021/es403850b