ENABLING ADAPTIVE UV AND SOLAR-BASED DISINFECTION SYSTEMS TO REDUCE THE PERSISTENCE OF VIRAL PATHOGENS IN WASTEWATER FOR SUSTAINABLE REUSE

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Transport and Survival of Pathogens in Aquatic Environment: Water Reuse Application

http://www.water.ca.gov/recycling/2
Rotavirus: One of the Most Common Diarrhea Causing Viruses

Occurrence and Emission of Rotavirus to Surface Waters

Rotavirus Serotypes

• The capsid consists of VP2, VP6, and VP7
• VP6: 7 serological groups (A to G)
• VP7 (glycoprotein): 14 G serotypes
• VP4 (hemagglutinin protein): P serotypes

British Med Bull (2009) 90 (1)
Human and Animal Rotavirus Found in Lettuces from AZ

Aw, et al., International Journal of Food Microbiology, 223, 16 April 2016
Reuse of Treated Wastewater

Urbana-Champaign Wastewater Treatment Facility

Google earth image

Cortland Reclamation for Irrigation

Google earth image
Project Objectives

1. Determine the molecular mechanisms responsible for virus inactivation
2. Determine factors required for effective virus inactivation by natural sunlight and UVC
3. Develop pond and UVC design guidelines to achieve reliable virus inactivation and elucidate trade-offs across and within dimensions of sustainability.
Sunlight Is The Most Important Factor Causing Disinfection In Surface Water

2006; Davies-Colley et al. 1997, 1999; Da Silva, A.K, et al., 2008;

1. Direct UVB Damage

Causes direct effects on DNA by inducing dimerization of pyrimidine bases, leading to the formation of photo-products that may block DNA replication

Indirect UVA Damage:

2. Exogenous

Manuel A. et al., 2007.

Indirect photo-oxidation damage is caused by generating ROS that damage the genome, proteins, and/or lipids. ROS formation is catalyzed by endogenous sensitizers.

Indirect UVA Damage:
2. Exogenous
3. Endogenous

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2926726/figure/F2/
Experimental Setup

Xenon arc lamp with filters

![Xenon arc lamp with filters](image)

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**Experimental Setup**

- **UIUC Solar Simulator**
- **8/15/2012 at 12 pm (Sunny & Clear)**
- **3/8/2013 at 12 pm (Sunny & Clear)**
- **3/3/2013 at 12 pm (Cloudy)**

![Graph showing irradiance vs. wavelength](graph)
Hypotheses

1. The phenotypic and genotypic differences between virus strains can be used to identify the molecular mechanisms responsible for viral susceptibility to solar and UVC inactivation.

2. a) Photo-oxidation of viruses depends on the reactions between viruses and reactive radicals formed upon irradiation of wastewater, b) direct effect of UVC irradiation on viruses depends on the irradiation wavelength and the specific UV absorbance (SUVA) of the wastewater.

3. Human health risk and life cycle ecological impacts can simultaneously be reduced by leveraging solar disinfection.
Insignificant Endogenous Inactivation of Human and Porcine Rotavirus at 25°C

Human Rotavirus Is More Susceptible to Exogenous Inactivation by Direct Sunlight UVA than Porcine Rotavirus at 25°C

Indirect UV-A inactivation of Porcine Rotavirus is important at 40-50°C

Romero, et al. ES&T 2011
Inactivation of Human Rotavirus at 25°C Correlates with Inactivation of Porcine Rotavirus 50°C

\[
y = 0.61x + 0.06 \\
R^2 = 0.98 \\
p < 0.0001
\]

Reactive Oxygen Species in Natural Water

- **NOx**
  - •OH
  - Hydroxyl radical
  - *CO3^2-

- DOM
  - →^1DOM*
  - 3DOM*
  - triplet excited

- O2
  - ^1O2
  - singlet oxygen

- O2^-
  - H2O2
  - hydrogen peroxide

- •OH
  - Hydroxyl radical
Triplet DOM Can Inactivate Human Rotavirus

R² = 0.99
p = 0.004

3'-MAP (mg/L)

Human Rotavirus

k_{obs} (h^{-1})

3-Methoxyacetophenone
Triplet DOM* sensitizer
Singlet Oxygen $^{1}\text{O}_2$ Does Not Inactivate Human Rotaviruses

**L-Histidine: $^{1}\text{O}_2$ quencher**

<table>
<thead>
<tr>
<th>DOM solutions</th>
<th>$k_{\text{obs}<em>\text{quencher}}/k</em>{\text{obs}}$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blavet</td>
<td>1.0</td>
</tr>
<tr>
<td>SR95 HA</td>
<td>1.0</td>
</tr>
<tr>
<td>XAD8 EfDOM</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Human Rotavirus $k_{\text{obs}}$ (hr$^{-1}$)**

- Sensitizer-free buffer control;
- Blavet River DOM (20 mg C/L);
- HA SR95 DOM (20 mg C/L);
- XAD4 EfDOM (20 mg C/L);
- XAD8 EfDOM (20 mg C/L)

**$^{1}\text{O}_2$ Concentration (x10$^{-14}$ M)**

$p = 0.3$
Can H$_2$O$_2$ Inactivate Rotavirus?

- 0, 6, and 60 µM of H$_2$O$_2$ did not show significant inactivation of rotavirus at 50°C in the dark.
- The measured [H$_2$O$_2$] at 50°C in 20 mg/L TOC was ~ 6 µM after the 3 hrs of the experiment.
- Thus, H$_2$O$_2$ is not directly responsible for rotavirus inactivation at 50°C.
Need to look at OH radicals

\[
\begin{align*}
\text{NO}_x^{-} & \quad \text{DOM} \quad \rightarrow \quad 1\text{DOM}^{*} \\
\cdot\text{OH} & \quad \text{triplet excited} \\
\cdot\text{OH} & \quad \text{hydrogen peroxide} \\
\cdot\text{OH} & \quad \text{singlet oxygen}
\end{align*}
\]
Hydroxyl Radical Is Responsible for Inactivation of Human Rotavirus Wa: Hypothesis 2a Was Proven

Human Rotavirus Inactivation Rate Constant (h⁻¹)

Phenol first-order decay constant (h⁻¹)

pH=8.0 and 25°C

Indirect UVA Inactivation of Human Rotavirus In Waste Stabilization Pond Water

$pH=8.0$ and $25^\circ C$

$R^2 = 0.90$

$p = 0.0007$

Human Rotavirus $k_{obs}$ (h$^{-1}$)

Phenol $k_{exp}$ (h$^{-1}$)

- Buffer Control
- Pond 1
- Pond 2
- Pond 3
- Pond 4
- Pond 5
- Pond 6

http://erikaquiroz.com/map-of-arizona/
Inactivation Rate Constants Correlated with SUVA

Filled symbols: Porcine Rotavirus; Empty symbols: Human Rotavirus

\[ R^2 = 0.80 \quad p = 0.025 \]

\[ R^2 = 0.86 \quad p = 0.016 \]

Specific UV\(_{254nm}\) Absorbance

Rotavirus \( k_{obs} \) (h\(^{-1}\))

Sensitizer-free Buffer Control

Hydrophobic EfOM

Hydrophilic EfOM

Suwannee River NOM

Blavet River NOM

Buffer Control; Pond 1; Pond 2
Pond 3; Pond 4; Pond 5; Pond 6
Porcine Rotavirus Is More Resistant Than Human Rotavirus

### Significant Differences in Structural Protein Sequences of Human RV Wa and Porcine RV OSU

<table>
<thead>
<tr>
<th>Protein</th>
<th>% identity of OSU to Wa</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP1</td>
<td>97</td>
</tr>
<tr>
<td>VP2</td>
<td>97</td>
</tr>
<tr>
<td>VP3</td>
<td>94</td>
</tr>
<tr>
<td>VP4 *</td>
<td>69</td>
</tr>
<tr>
<td>VP5</td>
<td>75</td>
</tr>
<tr>
<td>VP6</td>
<td>93</td>
</tr>
<tr>
<td>VP7</td>
<td>79</td>
</tr>
</tbody>
</table>

Manuel A. et al., 2007.
Rotavirus Reassortment


Distribution of RV Genotypes Reported to the WHO Surveillance Network in 2010

John-T-Patton, Rotavirus diversity and evolution in the post vaccine world, Discovery Medicine, 2012
Studied Rotavirus Serotypes

<table>
<thead>
<tr>
<th>RV Glycoprotein</th>
<th>Strains</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Wa</td>
<td>Human</td>
</tr>
<tr>
<td>G2</td>
<td>S2</td>
<td>Human</td>
</tr>
<tr>
<td>G3</td>
<td>YO</td>
<td>Human</td>
</tr>
<tr>
<td>G4</td>
<td>ST3</td>
<td>Human</td>
</tr>
<tr>
<td>G5</td>
<td>OSU</td>
<td>Porcine</td>
</tr>
</tbody>
</table>

- **VP6**: 7 serological groups (A to G)
- **VP7** (glycoprotein): 14 G serotypes

Effects of Solar Irradiation and Temperature Treatments on the Life Cycle of Human Rotavirus Wa

Rotavirus Life Cycle

**Genome integrity**
- Targeted a portion of the NSP3 segment

**Binding ability**
- Tracked bound rotavirus to host cell receptors by quantifying the NSP3 gene by RT-PCR
- Assessed RNA synthesis by quantifying the levels of NSP3 gene copies generated inside the host by RT-PCR
- Conducted the infectivity assay

**RNA synthesis**

**Cell lysis**

Effects of Treatments on RV Life Cycle

![Diagram showing the relative contribution to the overall inactivation for different treatments.](image)

- **Direct genome damage**
- **Binding**
- **RNA synthesis**
- **Post RNA synthesis**

**Treatments**:
- UVA-vis
- UVA-vis + SRNOM
- Full spectrum
- Full spectrum + SRNOM
- 57 degrees C (dark)

**Relative contribution to the overall inactivation**

**References**:
Year 1 Summary

1. Hydroxyl radical produced by organic matter triggers rotavirus Wa inactivation (hypothesis 2a is proven for one strain).
2. Genetically different strains of rotavirus have different susceptibility toward solar disinfection.
3. When using solar disinfection, the decrease in RNA synthesis was responsible for approximately one-half of the decrease in infectivity, suggesting that other mechanisms, including posttranslational, contribute inactivation.
Year 2 and 3 Plan

1. Determine the molecular mechanisms responsible for inactivation of different RV strains;
2. Determine factors required for effective virus inactivation by UVC; and
3. Develop pond and UVC design guidelines to achieve reliable virus inactivation and elucidate trade-offs across and within dimensions of sustainability.
