Effects of Natural Organic Matter and Algae on the Survival of Coliphage

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- Research Objectives
- Materials and Methods
- Results
Background

- Surrogate Viruses
  - Similar morphology, origin
  - Used as model virus to study enteric viruses
  - Easy to culture
  - Male specific coliphage, somatic coliphage, etc
Survival and Persistence

- Inactivation mechanism
  - DNA/RNA damage
  - Capsid damage
- Environmental factors
  - Temperature
  - Sunlight
  - Salinity
  - Water matrix
  - Sediment
  - Indigenous microorganisms

- Direct photolysis
- Endogenous photooxidation
- Exogenous photooxidation
Natural Organic Matter and Algae

- Natural Organic Matter (NOM)
  - Photosensitizer (Canonica, Jans et al. 1995)
  - Increase light attenuation (Bricaud, Morel et al. 1981)

- Algae
  - Morphology and structure
  - Affects photolysis of chemicals (Zepp and Schlotzhauer 1983)
  - Antiviral capacity (Hudson, Kim et al. 1998)
  - Increase light attenuation (Kirk 1975)
Research Objective

- To study the overall effects of natural organic matter on survival of coliphage
- To study the effects of algae isolated from Singapore reservoirs on the survival of coliphage
- To establish predictive model to quantitatively estimate coliphage inactivation based on these two parameters
Materials and Method

- **Materials:**
  
somatic coliphage phiX174, E.Coli CN-13 (host bacteria),
microcystis (isolated from Kranji Reservoir, Singapore)
Suwannee River NOM

- **Experiment conditions:**
  
simulated sunlight intensity: 450W/m²
temperature: 30 °C
Volume: 10ml
Experiment Design

Effects of Natural Organic Matter on Somatic Coliphage Inactivation

- To Identify active ROS
- K vs. [ROS]
- K=f(NOM, I, z)

Effects of Microcystis on Somatic Coliphage Inactivation

- Adsorption
- Inactivation
Effects of Natural Organic Matter

- Somatic coliphage inactivation followed pseudo first order under simulated sunlight
- NOM does not have significant effect on somatic coliphage inactivation rate in dark condition
- NOM (10ppm) enhanced somatic coliphage inactivation under simulated sunlight

- Light intensity: 450W/m²
- Temperature: 30 °C
- NOM concentration: 10ppm
- Duration: 2 h
Quencher Experiment

➢ Rationale:

- light → NOM → ROS → Virus inactivation

Quencher
Quencher Experiment

- Light intensity: 450W/m²
- Temperature: 30 °C
- NOM concentration: 10ppm
- Duration: 2 h

- \( \cdot \text{OH} \) and \( \cdot \text{O}_2 \) may cause exogenous photooxidation of somatic coliphage

### Table

<table>
<thead>
<tr>
<th>Quencher</th>
<th>2,4-hexadienoic acid (50mM)</th>
<th>Catalase (200u/ml)</th>
<th>SOD (2U/ml)</th>
<th>Sodium Formate (50mM)</th>
<th>L-Histidine (20mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>*NOM</td>
<td>Peroxide</td>
<td>Superoxide</td>
<td>Hydroxyl radical</td>
<td>Singlet Oxygen</td>
</tr>
</tbody>
</table>
Inactivation Rate

Constant was found to be linearly correlated to 

\[ [\cdot \text{OH}] \]

\[ K(h^{-1}) = 2^{13}[\cdot \text{OH}](M^{-1}) + 0.6082, R^2=0.8527 \]

No correlation was found between inactivation rate constant and \( ^1\text{O}_2 \)
Effects of Different NOM concentration

- Inactivation followed pseudo first order reaction
- NOM(11ppm) sample had highest inactivation rate
- Some samples had reduced inactivation rate than control experiment

- Light intensity: 450W/m²
- Temperature: 30 °C
- Duration: 2 h
Quantitative Assessment

Virus inactivation follows pseudo-first order inactivation

\[ \frac{dN}{dt} = -K_1 N \]  \hspace{1cm} (1)

\[ K_1 = K_i + K_d \]  \hspace{1cm} (2)

Where \( K_i \) is pseudo-first order inactivation rate constant \((\text{h}^{-1})\)

\( K_i \) is indirect inactivation rate constant

\( K_d \) is direct inactivation rate constant

\[ \ln \left( \frac{N_t}{N_0} \right) = -K_1 t, \quad \log_{10} \left( \frac{N_t}{N_0} \right) = -\left( \frac{K_1}{\ln(10)} \right) t \]

\[ \log_{10} \left( \frac{N_t}{N_0} \right) = -K_2 t \]

\[ K_1 = \ln(10)K_2, \quad K_2 = K_i' + K_d' \]  \hspace{1cm} (3)
Quantitative Assessment (Cont’d)

For indirect inactivation

\[ K_i' = K_r [ROS]_{ss}^a \] (4)

Where value of \(a\) was found in experiment, \((a=1, \text{ROS: } \cdot \text{OH})\)

Assume,

\[ [ROS]_{ss} = b[NOM][I] \] (5)

Light attenuation caused by light absorbing substance \((\text{Lee and Rast 1997})\) can be expressed as follows,

\[ [I] = [I_o] e^{-\mu[S]} \] (6)

For this study,

\[ [I] = [I_o] e^{-c[NOM]} \] (7)

For equation (4), Substitute \([ROS]\), Obtain

\[ K_i' = K_{ir} [NOM][I_o] e^{-c[NOM]} \] (8)
Quantitative Assessment (Cont’d)

For direct inactivation

\[ K'_{d} = K_{e}[I] (10) \]
\[ K'_{d} = K_{e}[I_{o}]e^{-c*[NOM]} (11) \]

For \( I_{o} = 450 \) W/m²

\[ K'_{d} = K_{n}e^{-c*[NOM]} (12) \]

Therefore,

\[ K_{2} = K_{m}[NOM]e^{-c*[NOM]} + K_{n}e^{-c*[NOM]} \]

Unknown parameters \( K_{m}, K_{n}, \) and \( C \)
Quantitative Assessment (Cont’d)

Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Km</td>
<td>.129</td>
<td>.022</td>
<td>.077 - .181</td>
</tr>
<tr>
<td>Kn</td>
<td>.975</td>
<td>.087</td>
<td>.771 - 1.180</td>
</tr>
<tr>
<td>C</td>
<td>.041</td>
<td>.004</td>
<td>.031 - .050</td>
</tr>
</tbody>
</table>

\[
K = \frac{1}{450} \left\{ K_m [NOM] e^{-\alpha c * [NOM] Z} + K_n e^{-\alpha c * [NOM] Z} \right\}
\]
Effects of Microcystis on Coliphage Removal from Water Column

Experiment Design:

- **Purified Microcystis culture**
  - Dark (Adsorption) (8hr)
    - Coliphage (+) Microcystis (-)
      - Coliphage (+) Microcystis (+) (OD=0.21 at 678nm)
      - Coliphage (+) Microcystis (-)
    - Coliphage (+) Microcystis (+) (OD=0.47 at 678nm)
    - Coliphage (+) Microcystis (+) (OD=0.16 at 678nm)

Half of the volume of all samples were filtered through 0.22µm membrane to differentiate the total viable coliphage in the sample and coliphage that were not associated with algae cells.
Effects of Algae-Adsorption

A. Total viable somatic coliphage without microcystis

B. Total viable somatic coliphage with microcystis

C. Suspended viable somatic coliphage without microcystis

D. Suspended viable somatic coliphage with microcystis
Effects of Algae-Inactivation

- Total viable somatic coliphage with microcystis at high density
- Suspended viable somatic coliphage with microcystis at high density
Conclusion

- Natural organic matter could either enhance coliphage inactivation or reduce coliphage inactivation in the presence of sunlight at different concentrations.
- The effects of natural organic matter on coliphage inactivation at fixed sunlight intensity at depth $Z$ can be expressed as

$$K = \frac{1}{450} \{ K_m [NOM] e^{-\alpha c* [NOM]Z} + K_n e^{-\alpha c* [NOM]Z} \}$$

- Microcystis isolated from Singapore reservoir was found not affecting coliphage removal from water environment, either from adsorption or inactivation in preliminary study.
Acknowledgement

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Thank you!