Using the coliphage naturally present in secondary effluent as a means for monitoring the removal of virus in advanced wastewater treatment

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All the results are from San Diego’s Water Repurification Research Project
In potable reuse, microbiological contaminants are the most important. 

... and in California, the Department of Health Services has designated viruses as the target micro-organism because viruses are the smallest of the pathogens, because they are poorly understood, and because they are the most difficult to remove.
Much of San Diego’s proposed treatment train depended on physical processes for virus removal (membranes).

- So a monitoring parameter was needed that would provide direct confirmation of performance.
- But since physical removal was involved we did not have to prove that enteric viruses were inactivated, just that they had been removed.
Coliphage were an early candidate

There was an established literature on coliphage which demonstrated that:

» suitable analytical methods are well-established

» coliphage are abundant in secondary effluent
Comparision of Coliphage with Bacterial Indicators and Enteric Viruses During Treatment
(Source: Rose, etal, 1996)
Comparision of Coliphage with Bacterial Indicators and Enteric Viruses During Treatment
(Source: Rose, et al., 1996)

Coliphage are at higher levels in secondary effluent than are enteric viruses.
This is not to say that coliphage are a more important risk. They are not even pathogens. Rather it is just to say that there are lots of coliphage in 2\textsuperscript{0} effluent (10\textsuperscript{4} to 10\textsuperscript{3} org/L), so they are relatively easy to find.
Coliphage is a general term used to describe the variety of bacterioviruses (bacteriophage) that attack coliform.

For our purposes today coliphage can be classified as either “F⁺-Specific” or “Somatic”.
A look at the coliform host

- To understand this way of classifying of phage it is first useful to review some background on the *E. coli*, their host organism

- *E. coli* can also classified into two groups:
  - Those that have “pili”
  - Those that do not have “pili”
A photomicrograph of E. Coli as we usually see them

"we have no pili"
An *E. coli* with extensive “pili”
The “pili” on *E. coli* are formally named, “fimbriae.” An *E. coli* with extensive “pili.”
An *E. coli* with extensive “pili”

*E. coli* use their fimbriae for attaching to surfaces
An *E. coli* with extensive “pili”

*E. coli* also use their fimbriae and to transfer plasmids. As a result, *E. coli* with fimbriae are often referred to as “male” *E. coli*.
Returning to forms of coliphage

Coliphage also come in two, related, forms:

» Those that attack *E. coli* through the “pili” … referred to as “F\(^+\)-specific phage” or “male-specific phage”

» Those that attack *E. coli* through the cell wall … referred to as somatic phage

Let’s look at some examples of these
SEM Photomicrograph with MS2 coliphage
MS2 is the most "famous" $F^+$ specific coliphage
SEM Photomicrograph with MS2 coliphage

100 nm
3D Rendering of T-4, a somatic coliphage
3D Rendering of T-4, a somatic coliphage

TEM Photo of T4, attacking a cell wall

Cell Wall
3D Rendering of T-4, a somatic coliphage

TEM Photo of T4, attacking a cell wall

100 nm
3D Rendering of T-4, a somatic coliphage

TEM Photo of T4, attacking a cell wall
## Comparison of Size Between Common Coli Phage & Viruses Associated with Human Diseases

<table>
<thead>
<tr>
<th>Human</th>
<th>Viruses</th>
<th>E. Coli</th>
<th>Phage</th>
</tr>
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<tbody>
<tr>
<td>Virus</td>
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**Conclusions:**
- MS coliphage are as small as any enteric virus
- Somatic phage are a bit larger
A summary of what we found at San Diego

- We had done a lot of work earlier, using seeded MS2 to trace virus removal in unit processes.
- We decided to look at the coliphage indigenous to the wastewater, using the same assay as was used for the earlier MS2 tracer work -
  - Double Agar Layer (DAL) method
  - a C3000 host (measures both MS and somatic)
Another Siderbar: the Double Agar Layer (DAL) method.

- M. Adams, *Bacteriophages*, 1959
  - Coliform “lawn” is grown on agar
  - “lawn” is exposed to sample
  - coliphage adsorb to the “lawn”
  - sample is drained off
  - “lawn” is covered with more agar (double agar)
  - plaques are counted after 24 hrs incubation
  - standard sample volume is 0.1 mL, 1 mL is o.k.
  - In clean water the City was able to get down to 1 pfu/100ml using many plates
San Diego used this method to monitor the levels of coliphage at their pilot plant at the Aqua 2000 Research Center.
Schematic of the Pilot-Scale Train
(Aqua 2000 Research Center)

Hyacinth Pond Effluent → Coagulation/Filtration → Microfiltration → Ultrafiltration

RO A → waste, permeate
RO B → waste, permeate
RO C → waste, permeate
RO D → waste, permeate
Schematic of the Pilot-Scale Train
(Aqua 2000 Research Center)

Hyacinth Pond Effluent

Coagulation/Filtration

Microfiltration

Ultrafiltration

RO A waste

RO B waste

RO C waste

RO D permeate

RO A permeate

RO B permeate

RO C permeate

RO C waste
Schematic of the Pilot-Scale Train
(Aqua 2000 Research Center)
Monitoring of coliphage in San Diego system with the DAL method - MF

7 months of coliphage data

- PFU/100mL

- Percent less than or equal to
Monitoring of coliphage in San Diego system with the DAL method - MF

Pond Effluent ~ $10^{4.5}$ PFU/100 mL

7 months of coliphage data

- Pond Effluent
- Settled
- Filtered
- MF Effluent
Monitoring of coliphage in San Diego system with the DAL method - MF

After Coagulation/Settling ~ $10^{3.9}$ PFU/100 mL

7 months of coliphage data
Monitoring of coliphage in San Diego system with the DAL method - MF

After Filtration ~ $10^{3.3}$ PFU/100 mL

7 months of coliphage data
Monitoring of coliphage in San Diego system with the DAL method - MF

After MF ~ 10^0 or 1 PFU/100mL

7 months of coliphage data
Monitoring of coliphage in San Diego system with the DAL method - MF/RO

[7 mos data at Aqua 2000]

After RO, all measurements N.D.
Success with the DAL method led to new ambitions

- the work demonstrated that coliphage were present in large numbers and suggested that their removal might be used as a means of demonstrating removal of enteric viruses
- But the technique also had some flaws:
  - The detection limit wasn’t quite low enough
  - The assay time (24 hrs) was too long
  - The method detected both somatic and F\(^+\) specific phage, but it was the removal of the F\(^+\) specific phage that was particularly important
San Diego set out to find improved methods.

- Several PCR procedures were examined but rejected:
  - too time-consuming
  - too complicated to execute
- The Membrane Filter Method (MFM), developed by Sobsey at UNC Chapel Hill, was found to be promising.
- The City also used the method with two hosts:
  - Famp, a special host cell developed to amplify male specific coliphage … and
  - C3000, the host that had been used before
The UNC Membrane Filter Method

- M. Sobsey, 1990 - UNC Chapel Hill
- Method uses a 0.45 μm membrane (like coliform)
- Again, coliform “lawn” is grown on agar
- Sample is filtered through membrane
- The phage adsorb to the membrane
- Membrane is placed face-down on “lawn”
- Plaques are counted after 24 hrs incubation
- Standard sample volume is 100 mL, 1L o.k.
MFM and DAL plates side by side
MFM and DAL plates side by side
MFM and DAL plates side by side
MFM vs DAL

San Diego compared the MFM and DAL methods in secondary effluent before and after coagulation and filtration.
Comparing coliphage as reported by MFM versus DAL:

Hyacinth Pond Effluent
C3000 Host

Conclusion: DAL is more sensitive in secondary effluent
Comparing coliphage as reported by MFM versus DAL:

Coagulated, Filtered Pond Effluent

C3000 Host

Conclusion: MFM is as-or-more sensitive in filtered effluent
MFM Method is also very fast
[Looking only at positives for each host]

80% of the positives show up in the first six hours
Summary of Observations:

- Indigenous coliphage are present in secondary effluent at fairly high levels.
- There are robust, well-developed methods available to measure coliphage in water.
- The DAL method was successfully used to achieve a detection limit of 1PFU/100 mL.
- The MFM method shows potential to extend that detection limit further.
Conclusions:

- Monitoring for native coliphage can be an effective method for demonstrating the removal of enteric viruses from secondary effluent.
- Monitoring for native coliphage can be accomplished with methods comparable in cost and complexity to those used to monitor the coliform organisms.
- Using the MFM method with Famp media, it is likely that positives will appear in a six hour test (e.g. same day).
Returning to forms of coliphage

- All *E. coli* have a cell wall
- But only the “male” *E. coli* have pili
- So Somatic phage can attack all *E. Coli*, but MS phage cannot
- As a result, special cell lines of “all male” *E. coli* can be used to distinguish between them