



AMERICAN  
WATER RESOURCES  
ASSOCIATION

# AWRA 2021 ANNUAL WATER RESOURCES CONFERENCE

November 8 -10, 2021  
Virtual



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# 2021 Annual Water Resources Conference

## **Virus- host interactions in anaerobic digester**

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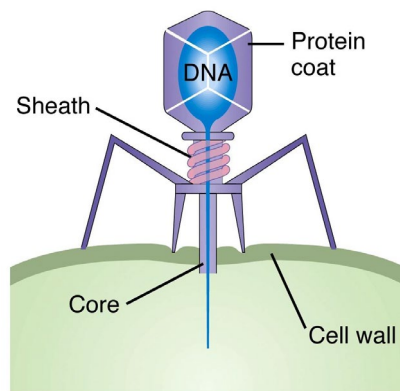
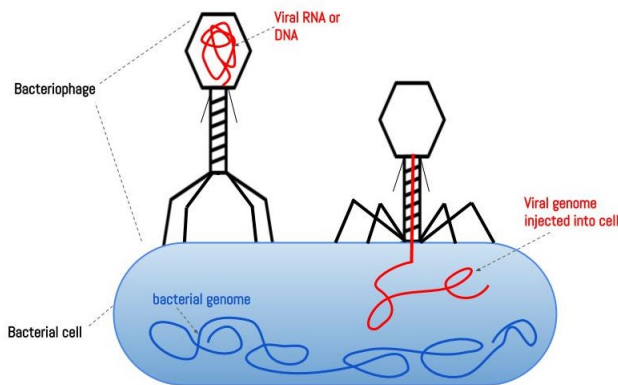
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November 9, 2021

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# Viruses

- Most abundant entities on the planet earth
- Ubiquitous
- **Obligate parasites**
- Made up of a core of DNA or RNA surrounded by a protein coat (capsid)
- Two distinct lifecycle: **Lytic** and **Lysogenic** lifecycle



[https://www.mun.ca/biology/scarr/4241smc\\_Bacteriophage.html](https://www.mun.ca/biology/scarr/4241smc_Bacteriophage.html)

## Viral infection

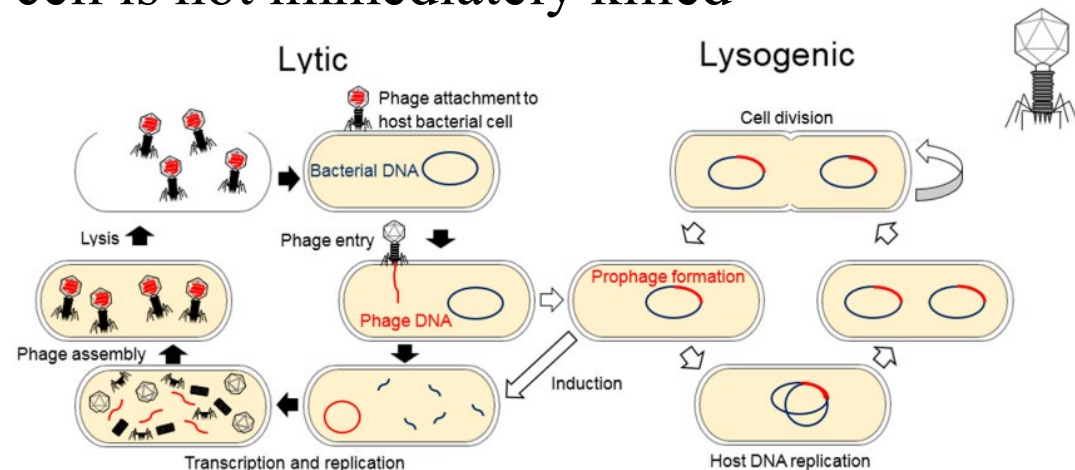
- First, bacteriophages attaches on surface of host cells
- The hollow core pierces the cell wall
- Contractile tail sheath injects the phage DNA into the host cell
- Hijacks the metabolism of the host cell

Two types of viral replication

1. **Lytic cycle (Virulent cycle):** Viral replication process rapidly kills the host cell

2. **Lysogenic cycle (Temperate cycle)**

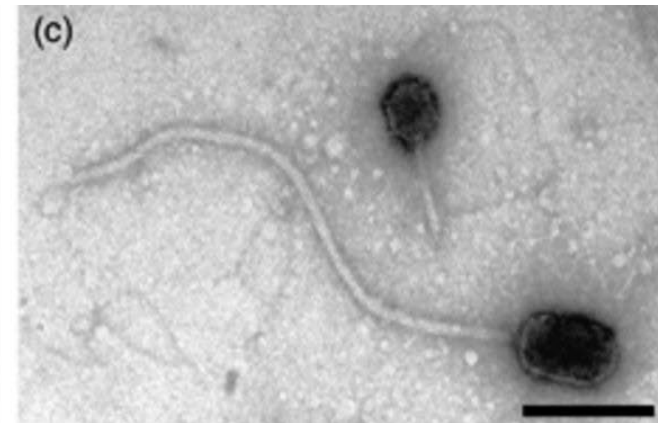
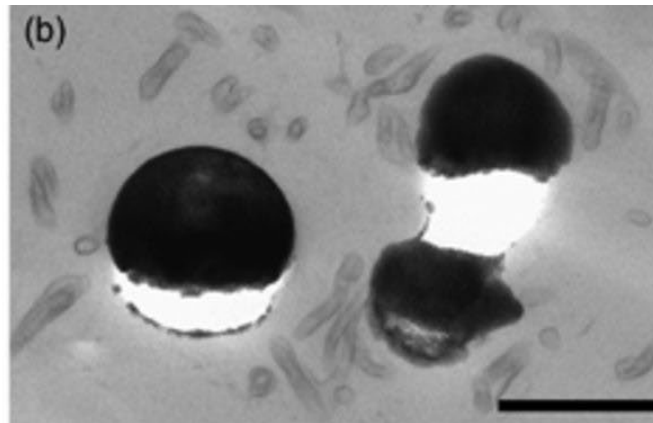
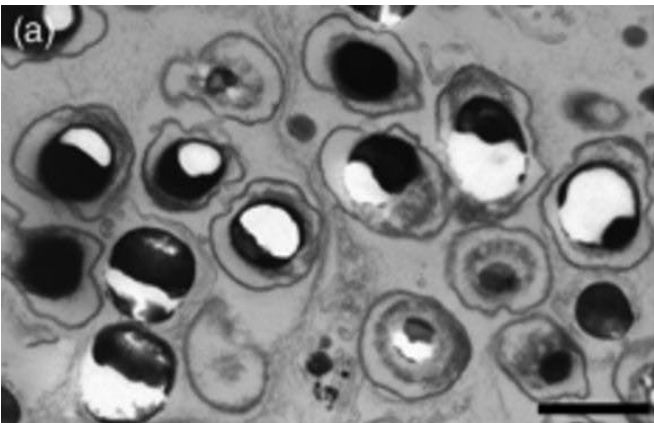
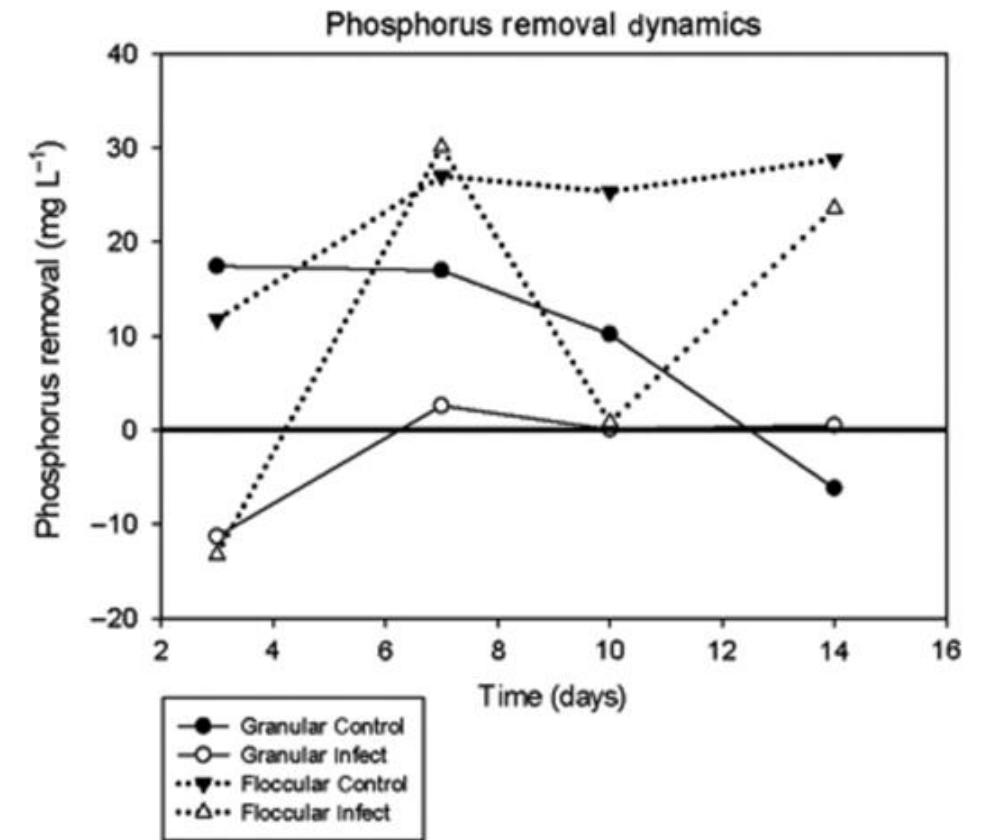
The host cell is not immediately killed



Batinovic, S., Wassef, F., Knowler, S. A., Rice, D. T., Stanton, C. R., Rose, J., ... & Franks, A. E. (2019). Bacteriophages in natural and artificial environments. *Pathogens*, 8(3), 100.

# Viruses in engineered bioreactors

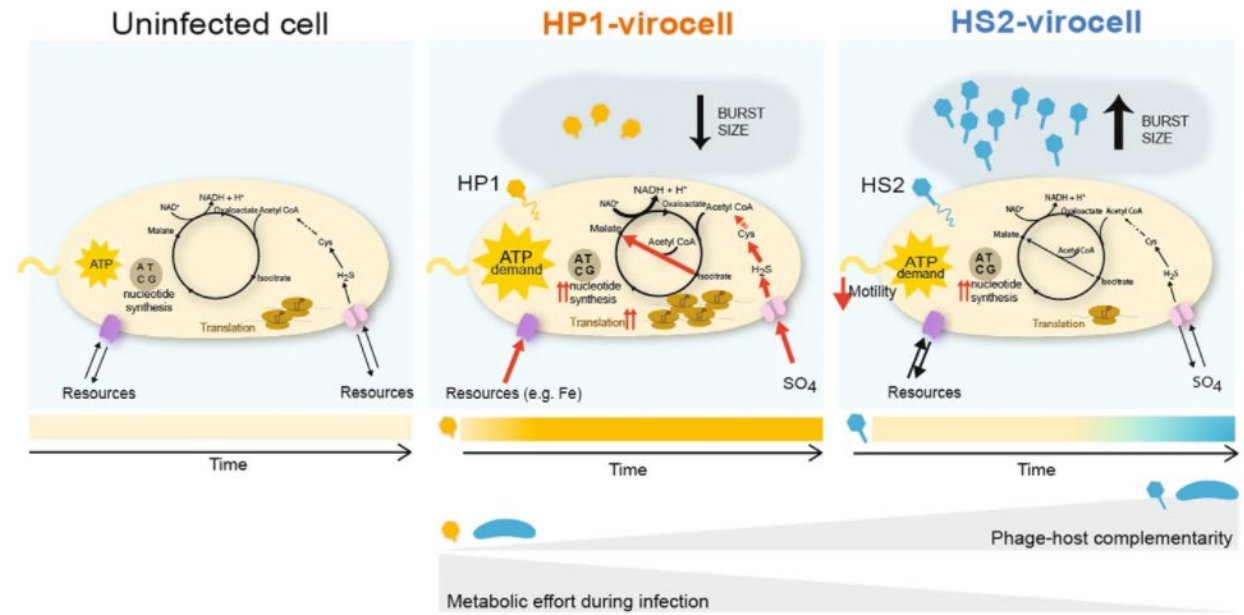
- Wastewater treatment plant contain highest concentration of viruses  $10^8$  to  $10^9$  phage-like particles per mL
- Drives microbial diversification
- maintains functional redundancy
- ensures performance stability in engineered bioreactors
- Failure of EBPR process through bacteriophage infection has been reported- **LYTIC INFECTION**





# Implication of viruses on host metabolism

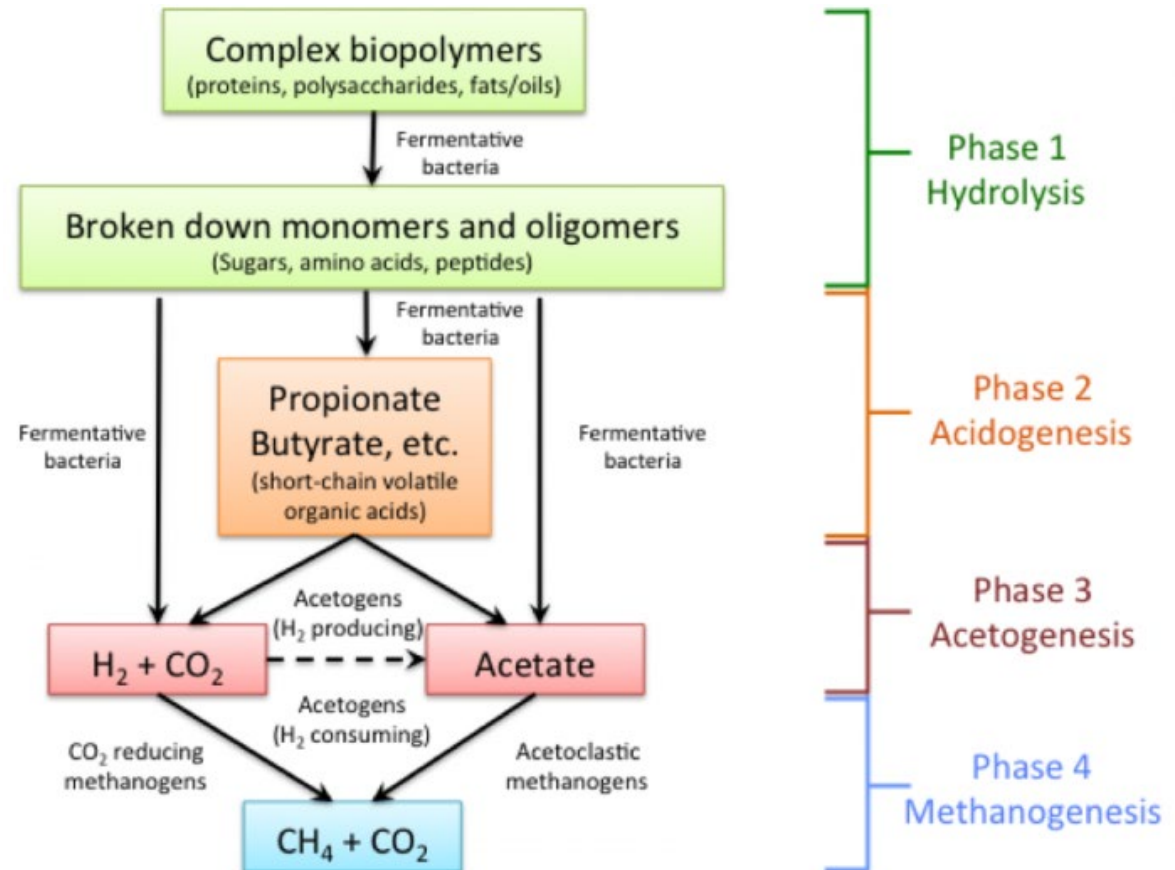
- Primary source of mortality
- Changes in flow of nutrients and microbial composition
- Phages constitute a repository of genetic information
- The genetic information is necessary for evolution and ecophysiology of host organism
- Phage infection can introduce new genetic information into host organism or viral progeny
- Hijacks bacterial and archaeal cellular machinery causing reprogramming of host metabolism
- Agents of horizontal gene transfer



Howard-Varona, C., Lindback, M. M., Bastien, G. E., Solonenko, N., Zayed, A. A., Jang, H., ... & Duhaime, M. B. (2020). Phage-specific metabolic reprogramming of virocells. *The ISME journal*, 14(4), 881-895.

# Viruses in anaerobic digesters

- Metabolic potential and lifestyle of phages not studied in detail- especially in complex engineering ecosystems such as anaerobic digester
- Diverse microbial communities in anaerobic digesters
- Interaction between microbes important for stability
- Viruses can cause nutrient regeneration, horizontal gene transfer
- Microbial abundance, composition, ecosystem function can be effected



# Anaerobic digesters

- Anaerobic digester hosts microbial community with diverse functional traits- hydrolysis, fermentation, acetogenesis, methanogenesis
- Produces renewable energy
- The syntrophic activity between the microbes enhances the stability of the process
- Understanding the microbial community dynamics and their interaction with viruses must be expanded
- Anaerobic digestion process can be mesophilic (35-40 °C) or thermophilic (55-60 °C)
- Samples from mesophilic and thermophilic anaerobic digesters were collected

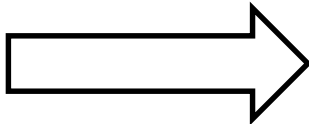




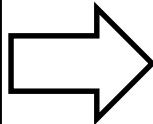
# Isolation of viral fraction from digested sludge



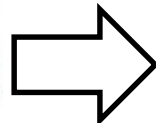
Sample collection



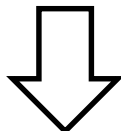
Sample  
+  
1% potassium  
citrate (3x  
volume)



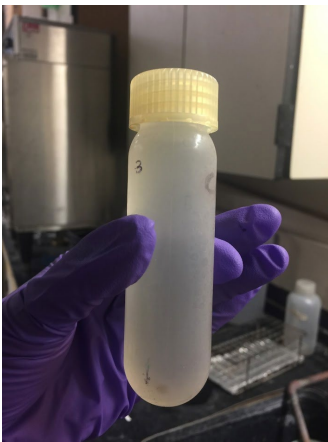
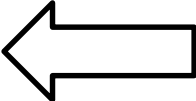
Centrifugation



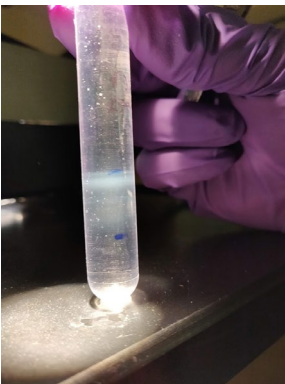
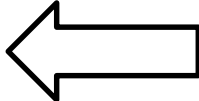
Sequential  
filtration



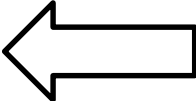
Centrifugation



Resuspension of  
pellet



Cesium chloride  
purification

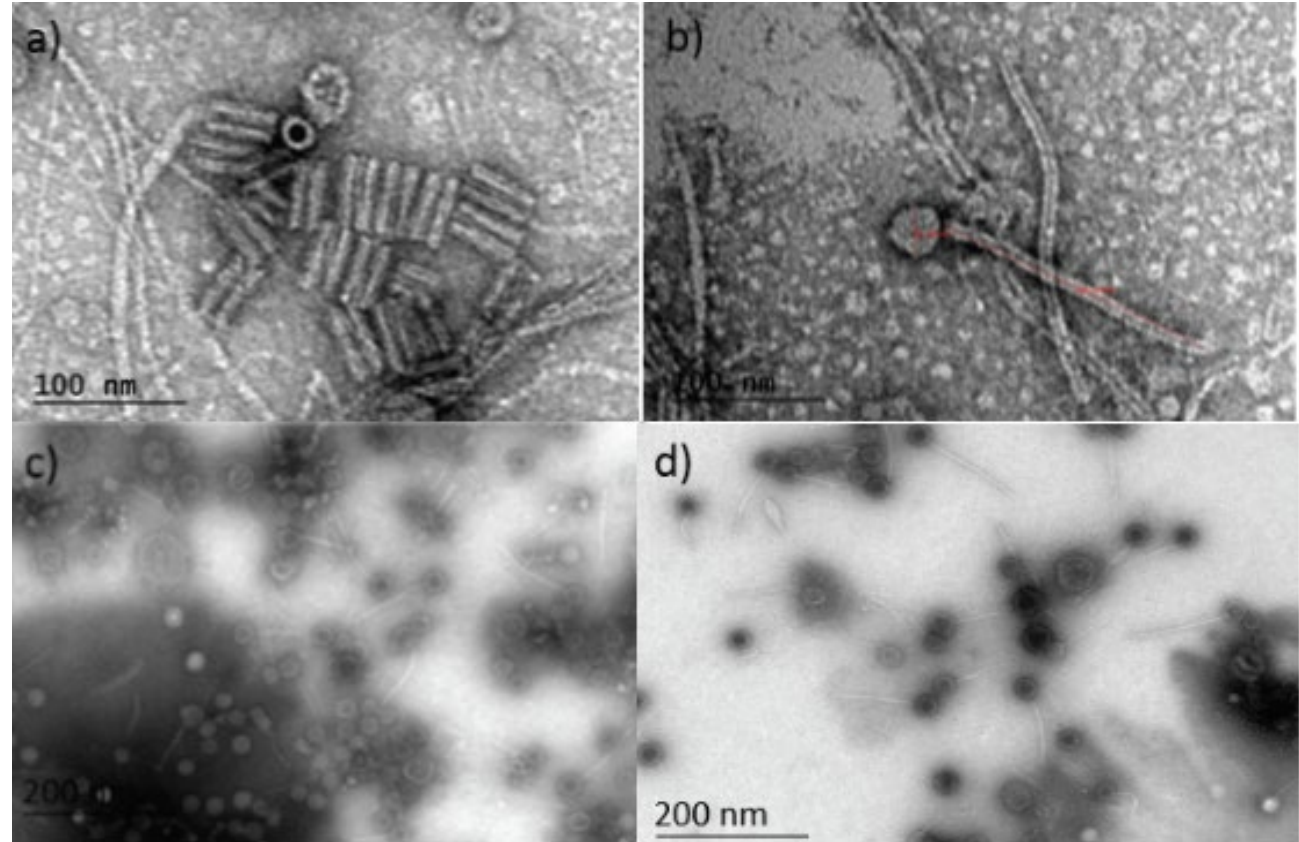


Transmission electron  
microscopy



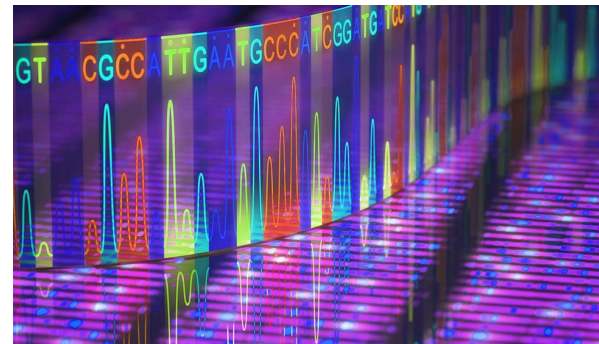
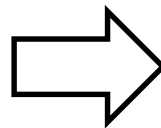
# Phages in anaerobic digester

- Viral fraction isolated and purified
- Morphological diversity of the viral community was observed
- To identify the metabolic potential, genomic DNA of the microbial and the viral fraction has been sequenced

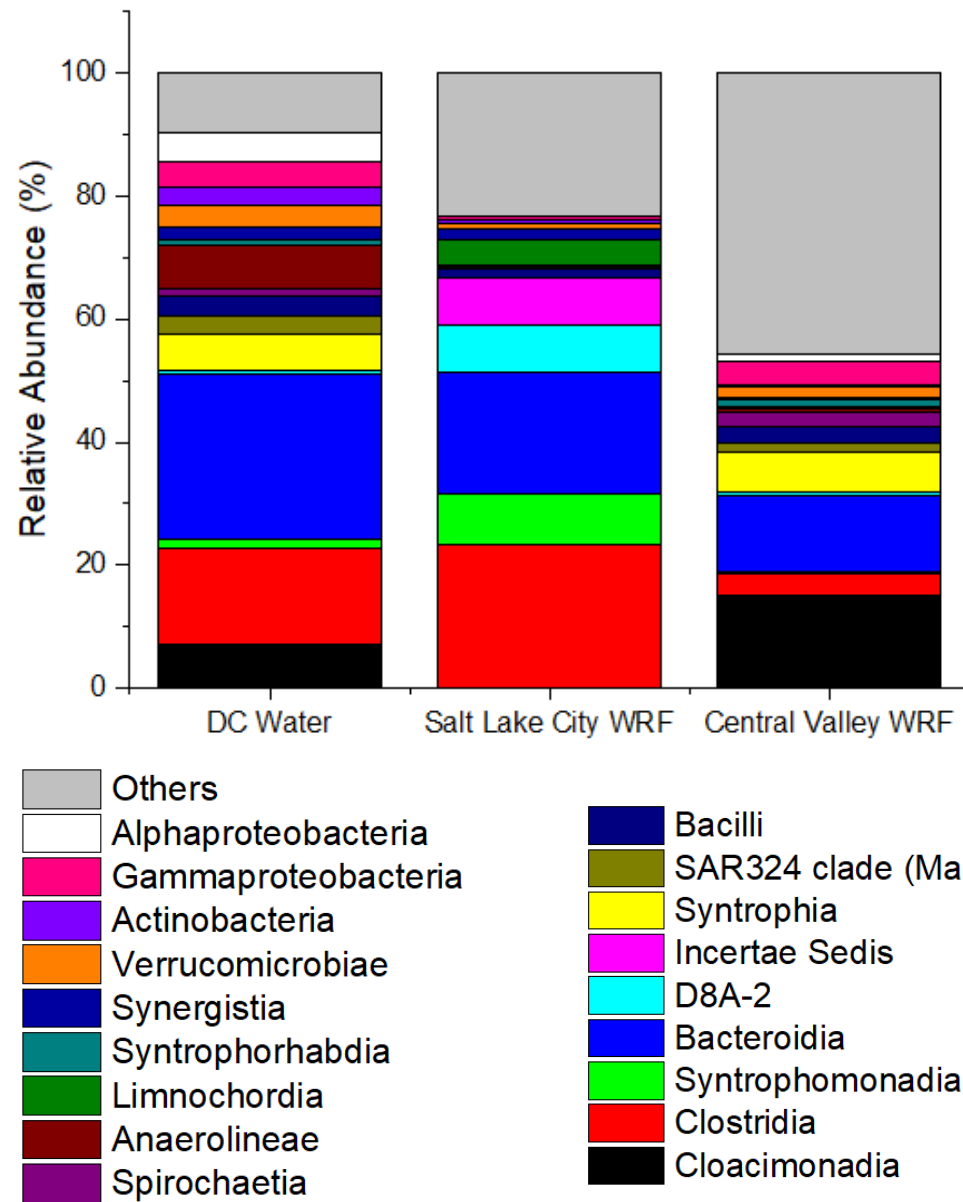


# Extraction of genomic content and sequencing

- Genomic DNA from sludge samples was extracted using DNeasy Powersoil kit (QIAGEN)
- Viral DNA will be extracted using Phage DNA isolation kit (Norgen Biotek Corp., Canada)
- Sequencing was performed on **Illumina NovaSeq** platform



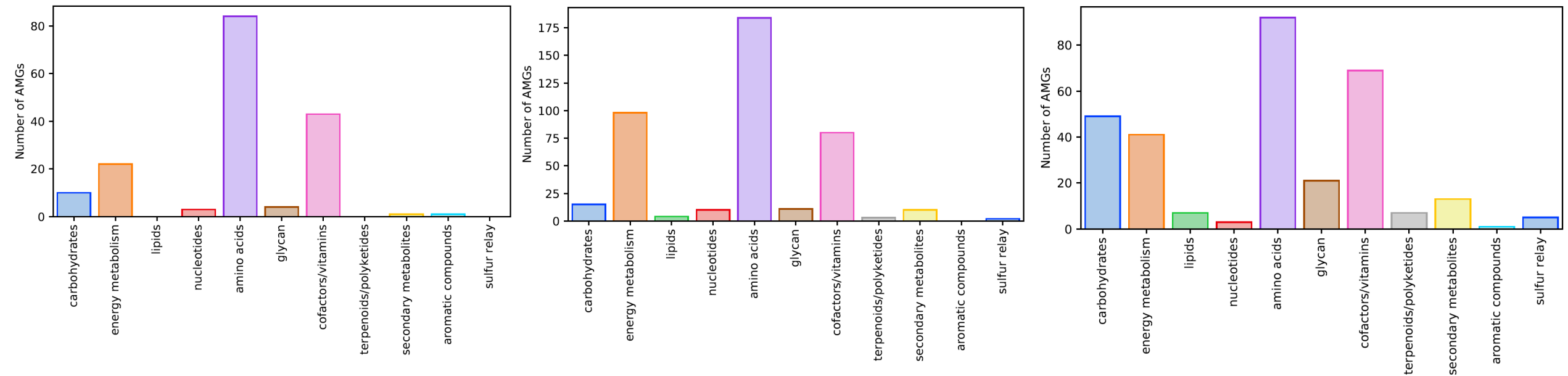
# Analysis of microbial community



- *Clostridia*, *Bacteroidia* dominant in all anaerobic digesters
- *Anaerolinea* identified in thermophilic digester
- Construction of metagenome assembled genomes will give idea about the metabolic pathways

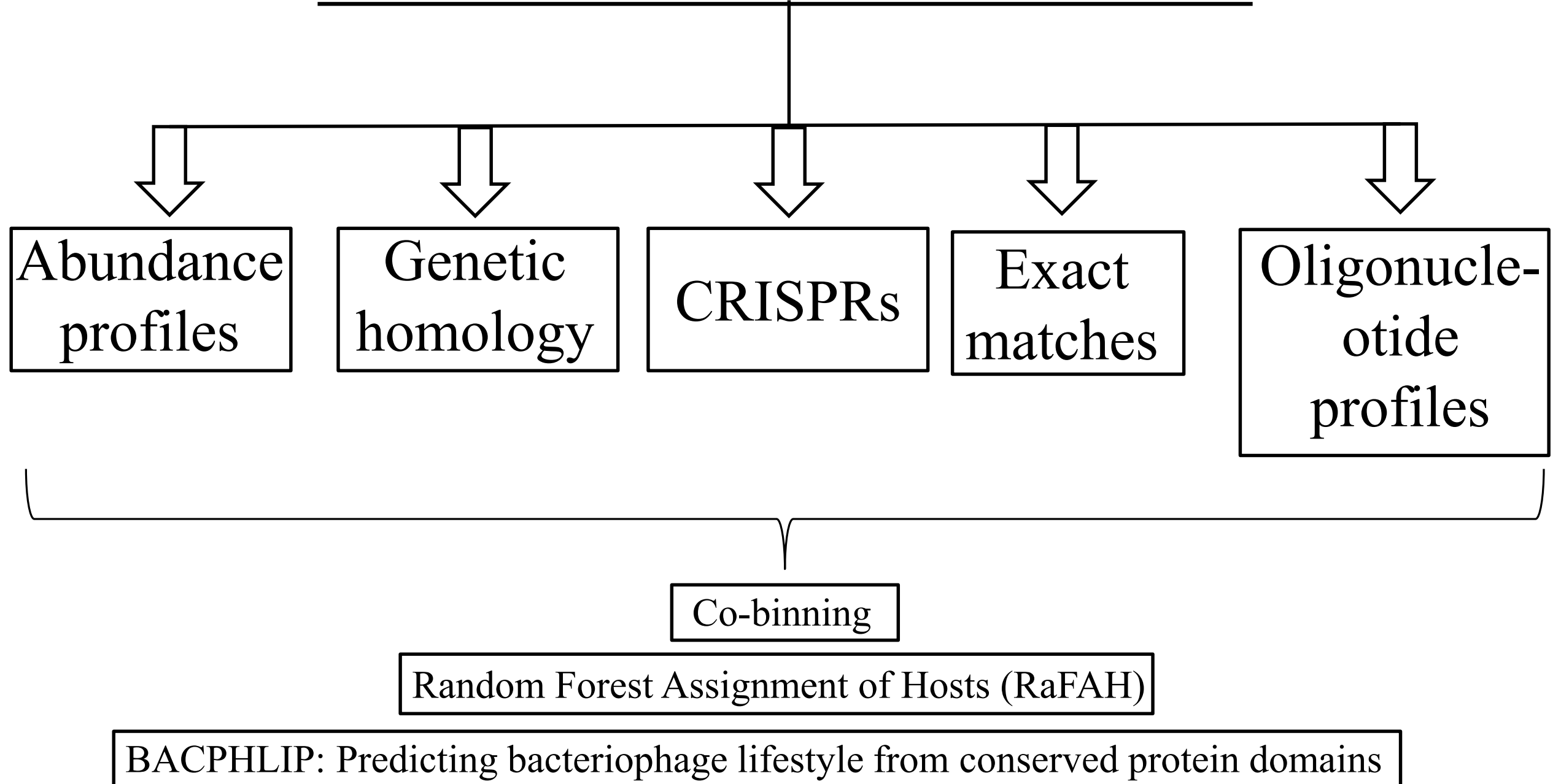
# Analysis of viral community

- A total of 2349, 4758, 3402 viral scaffolds identified in anaerobic digested sludge from DC-Water, SLCWRF, CVWRF
- Auxiliary metabolic genes for carbohydrate, energy metabolism was detected.
- lysine 2,3-aminomutase involved on lysine degradation detected





# Identification of virus-host association



# Conclusion

- Viruses can impact host metabolism
- Viruses in complex engineered ecosystems like anaerobic digesters not studied in detail
- This study investigated viruses in mesophilic and thermophilic digesters
- Auxiliary metabolic genes identified in viruses
- Virus- host association can provide more detail towards impact of virus on the reactor performance

# Thank you for participating!

## Contact us with questions:

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