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Going Viral: Emerging Opportunities for Phage-Based Bacterial Control in Water Treatment and Reuse

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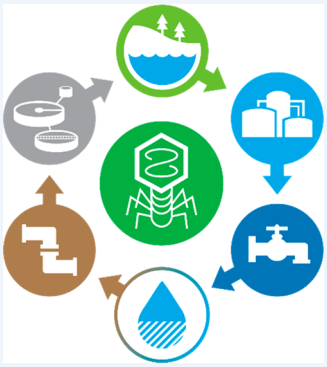
CONSPECTUS: Water security to protect human lives and support sustainable development is one of the greatest global challenges of this century. While a myriad of water pollutants can impact public health, the greatest threat arises from pathogenic bacteria that can be harbored in different components of water treatment, distribution, and reuse systems. Bacterial biofilms can also promote water infrastructure corrosion and biofouling, which substantially increase the cost and complexity of many critical operations.



Conventional disinfection and microbial control approaches are often insufficient to keep up with the increasing complexity and renewed relevance of this pressing challenge. For example, common disinfectants cannot easily penetrate and eradicate biofilms, and are also relatively ineffective against resistant microorganisms. The use of chemical disinfectants is also curtailed by regulations aimed at minimizing the formation of harmful disinfection byproducts. Furthermore, disinfectants cannot be used to kill problematic bacteria in biological treatment processes without upsetting system performance. This underscores the need for novel, more precise, and more sustainable microbial control technologies.

Bacteriophages (phages), which are viruses that exclusively infect bacteria, are the most abundant (and perhaps the most underutilized) biological resource on Earth, and hold great promise for targeting problematic bacteria. Although phages should not replace broad-spectrum disinfectants in drinking water treatment, they offer great potential for applications where selective targeting of problematic bacteria is warranted and antimicrobial chemicals are either relatively ineffective or their use would result in unintended detrimental consequences. Promising applications for phage-based biocontrol include selectively suppressing bulking and foaming bacteria that hinder activated sludge clarification, mitigating proliferation of antibiotic resistant strains in biological wastewater treatment systems where broad-spectrum antimicrobials would impair pollutant biodegradation, and complementing biofilm eradication efforts to delay corrosion and biofouling. Phages could also mitigate harmful cyanobacteria blooms that produce toxins in source waters, and could also serve as substitutes for the prophylactic use of antibiotics and biocides in animal agriculture to reduce their discharge to source waters and the associated selective pressure for resistant bacteria. Here, we consider the phage life cycle and its implications for bacterial control, and elaborate on the biochemical basis of such potential application niches in the water supply and reuse cycle. We also discuss potential technological barriers for phage-based bacterial control and suggest strategies and research needs to overcome them.

1. INTRODUCTION

Water security is a grand challenge inextricably linked to global health and economic development (Table 1). While a myriad of water contaminants can cause disease, by far the greatest threat arises from waterborne pathogenic microorganisms. Approximately 1.8 million people die each year due to water-related illnesses that are a result of unsafe drinking water or poor sanitation.[1] Some waterborne bacteria can also cause major problems for industry and public infrastructure by exacerbating corrosion and biofouling,[2] both of which can significantly increase the cost and complexity of many critical operations. As the cost of upgrading and replacing aged water infrastructure in the United States alone would likely exceed $ 1.7 trillion over the next three decades,[3] minimizing water quality and infrastructure degradation due to microbial processes could provide substantial economic benefits.

Because of its broad relevance to both public health and industry, microbial control in water systems has been a central

Table 1. Microbial Contamination of Water: Broad Impacts

In the 20th century, improved water disinfection and sanitation increased life expectancies in the US from 49 to 78 years. However, waterborne disease is rising again due to the aging water infrastructure.[79]
Improvements in water quality could lower the global child mortality rate, preventing up to 1.5 million child deaths/year.[80]
The cost of microbially induced corrosion and biofouling to the US water infrastructure is more than $ 7 billion annually.[81,82]

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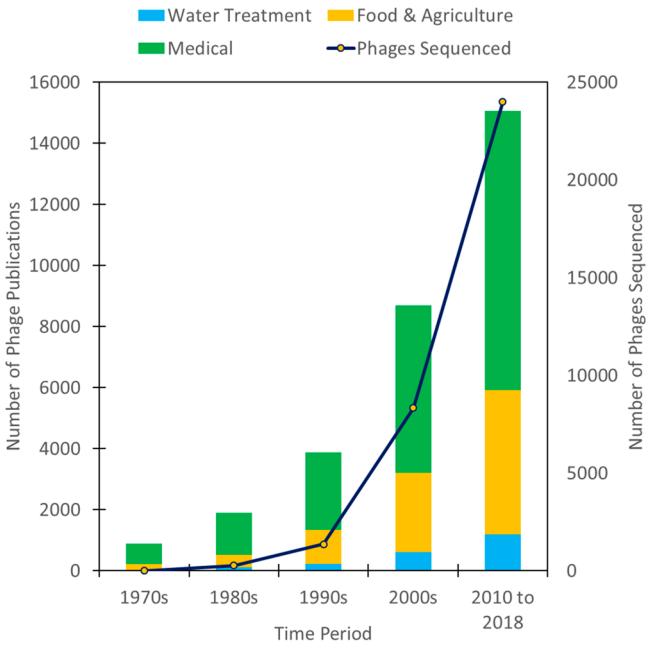

Figure 1. Exponential increase in phage application-related publications and number of phages sequenced over past decades. NCBI PubMed results for the number of yearly publications on phage and NCBI Nucleotides results for the number of yearly phage sequences from 1970 to 2018.

challenge for centuries. This has resulted in numerous antibacterial agents and processes that are widely used today in both urban and rural settings. However, several converging factors are pushing the limits of conventional disinfection and microbial control, including human population densification, a more stringent water quality regulatory environment, a shifting climate exacerbating freshwater scarcity, the widespread proliferation of antimicrobial resistance, and a growing need for water reuse. Conventional disinfectants are becoming increasingly ineffective against some microorganisms,[4,5] while their use is being curtailed by regulations. Not only do commonly used disinfectants produce carcinogenic byproducts, but their poor specificity can harm benign organisms, leading to unintended detrimental effects on biological water or wastewater treatment processes. Thus, there is a critical need for more precise and sustainable microbial control technologies to both replace and augment existing approaches.

Inspired by the medicinal use of bacteriophages (phages) to treat pathogenic bacterial infections (i.e., "phage therapy"),[6] there is growing interest in the use of phages as selective, self-replicating bacterial control agents for water and wastewater treatment (Figure 1).[7] At a total population of about 10^{31}, phages are by far the most abundant (and perhaps the most underutilized) biological entities on Earth.[8,9] In this Account, we discuss their potential application for bacterial control in water supply and reuse systems, consider potential technological barriers and strategies to overcome them, and highlight specific niches where phages may be of great benefit.

2. BACTERIOPHAGE LIFE CYCLE AND IMPLICATIONS FOR BACTERIAL CONTROL

Phages are viruses that exclusively infect bacteria and that can be harnessed for bacterial control and gene delivery applications.[9] Because of their host specificity, phages offer the potential to control problematic bacteria without significantly impacting other members of the microbial community.[10] Since their discovery in the early 20th century, phages have been used for selective control of several important medical and agricultural pathogens (Figure 2). However, their use was mostly supplanted by the discovery of antibiotics, which were easier and more practical to implement for broader spectrum antimicrobial effects. Recently, there has been a resurgence of interest in phages due to both the widespread proliferation of multidrug resistant bacteria, and the realization that broad-spectrum antimicrobials can negatively impact beneficial functions of microbial communities.[11] Furthermore, unlike antibiotics or biocides, whose concentration decreases with time after dosage, phages may continue to replicate and infect the target bacteria, eventually disappearing with their hosts in a typical predator–prey relationship.[6] In addition to directly eliminating susceptible bacteria, phages can suppress their hosts' metabolic repertoire, fitness, and competitive capability.[12,13]

Phages are diverse in size; ranging from 20 to 200 nm, though some can exceed 200 nm.[14] Most known phages consist of a protein capsid, a highly condensed nucleic acid core, and a thin tail with tail fibers. Their high specificity toward particular hosts is due to proteins at the tip of the tail fibers, which selectively bind to receptors on the bacterial surface prior to the phage injecting its genetic material through the host membrane.[15] Almost all bacterial surface components such as flagella, pili, capsules, transport proteins, and lipopolysaccharides can serve as phage receptors.[15] While most characterized phages are species- or strain-specific, some phages exhibit a relatively broad host range (i.e., polyvalent phages) by virtue of relaxed receptor binding specificity, or their ability to recognize and attach to multiple receptors on different bacterial species and genera.[16] Furthermore, host

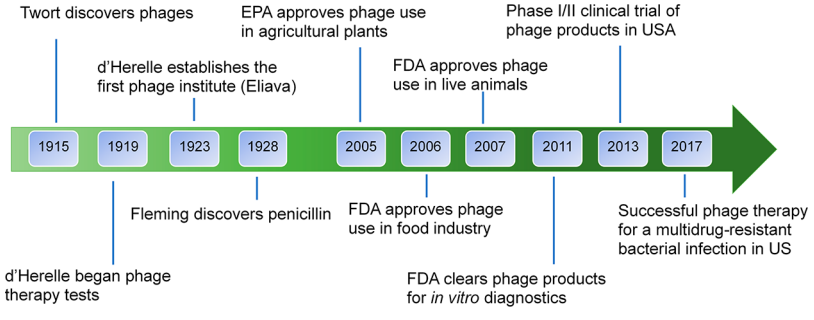

Figure 2. Historical landmarks in phage research and applications.

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range can be extended by tail-bound depolymerases that degrade extracellular polymeric substances (EPS) or the lipid-polysaccharide layer of many bacteria, facilitating phage access to otherwise occluded receptors for subsequent infection.[17]

Phages primarily bind to host receptors via noncovalent interactions, such as hydrogen bonds,[18] electrostatic attraction,[19] hydrophobic interactions,[20] and van der Waals forces (Figure 3). Thus, water chemistry and environmental factors

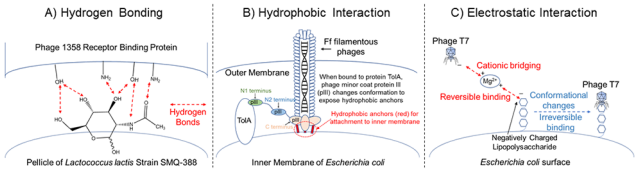

Figure 3. Examples of phage attachment by hydrogen bonds, hydrophobic interactions, and cation bridges. (A) Phage 1358 adsorbs to *Lactoccous lactis* strain SMQ-388 via hydrogen bonds. Adsorption of this phage to other strains is prevented by steric hindrance near the binding site caused by oligosaccharide substitutions, resulting in strain-specific host recognition. (B) Binding of phage protein pIII to *E. coli* transport protein TolA cause conformational changes exposing phage hydrophobic anchors on the C terminus for attachment to inner membrane. Adapted with permission from ref 20. Copyright 2006 Elsevier. (C) Phage T7 reversibly binds to lipopolysaccharide on *E. coli* via cationic bridges, then undergoes conformational changes to become irreversibly bound.

(e.g., salinity or temperature) could affect such interactions and (under phage-specific suboptimal conditions) hinder phage absorption and infectivity.[21] Furthermore, at pH lower than the phage isoelectric point, electrostatic repulsion between phage particles is overcome by van der Waals attraction forces and hydrophobic interactions, causing phages to aggregate and decrease infection efficiency.[22] The presence of certain ions can also affect the efficacy of phage-based bacterial control. For example, negatively charged phage particles can aggregate and precipitate with positively charged iron oxyhydroxides.[23] Additionally, divalent cations such as Ca^{2+} and Mg^{2+} enhance adsorption of many phage species (Figure 3C), while trivalent cations such as Al^{3+} and Fe^{3+} may coagulate and destabilize phages.[24] Thus, it is important to consider the characteristics of each phage in the context of the aqueous environment it is being consider for implementation.

After phage DNA is injected into the host, phage replication proceeds through one of four generalized life cycle strategies (Figure 4A): (1) nontemperate lytic, (2) nontemperate chronic, (3) temperate lytic, and (4) temperate chronic.[25] Nontemperate lytic phages immediately begin replication and rapidly lyse their host, making them suitable for microbial control applications. In contrast, temperate (also called lysogenic) phages enter the lysogenic cycle where the phage genome is inserted into the host genome and when the host replicates, the phage genome is replicated with it. This makes temperate phages more suitable for gene delivery applications (e.g., to augment pollutant biodegradation capabilities[26]). Temperate phages are typically induced to enter the lytic cycle by an environmental stimulus (e.g., mitomycin C or ultraviolet light[27]). However, it was recently discovered that phages can also respond to bacterial quorum sensing molecules, allowing them to enter the lytic cycle when a

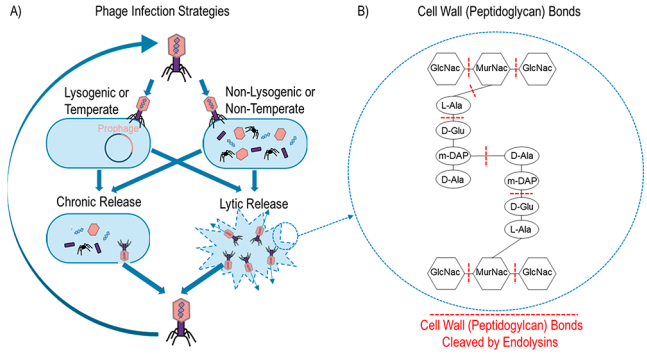

Figure 4. Phage life cycle and infection strategies. (A) Nonlysogenic or nontemperate phages immediately begin replication to lyse the host and release phage progeny. Lysogenic or temperate phages insert the phage genome into the host genome and replicate it when the host replicates. Lytic release involves phage progeny assembled inside the host and released by host lysis. Chronic release involves phage progeny assembled and released continually without host lysis. (Note that lytic release is more common than chronic release.) (B) Phage endolysins can degrade the cell wall by cleaving peptidoglycan bonds marked with a dotted red line.

sufficient cell density is present to support productive replication.[28]

Most characterized phages follow a lytic release strategy where phage progeny is released via host lysis by phage enzymes such as holin, endolysin, and spanin. Holins form holes in the cell membrane, allowing endolysins to enter the periplasm and degrade the cell wall by hydrolyzing the glycosidic or peptide bonds in peptidoglycan (Figure 4B).[29] Spanins complete the process by fusing the inner and outer cell membrane to form holes through which phage progeny are released.[30] A less common strategy is chronic release where the phage progeny are assembled and released over long intervals without host lysis. Chronic phage might be useful for applications that require persistent gene expression by the transduced bacteria.

3. OPPORTUNITIES FOR PHAGE-BASED BACTERIAL CONTROL IN WATER TREATMENT AND REUSE

Bacteria are present at every stage of the water supply and reuse cycle in both urban and rural settings. Depending on their type, concentration, location, and niche, bacteria can be beneficial (e.g., biodegrade pollutants) or problematic (e.g., cause disease and corrosion). Phages have the (unexploited) potential to enhance some beneficial bacterial processes (e.g., serve as transduction vectors to enhance horizontal transfer of catabolic genes to degrade recalcitrant pollutants).[31] Due to their high specificity in attaching to their hosts, some phages could also serve as components in sensors to detect harmful bacteria.[32] However, the most readily implementable application for phages in water systems is as antimicrobial agents where selective bacterial targeting is warranted and broader spectrum antimicrobials are either marginally effective or result in unwanted consequences (e.g., impairing a biological treatment process). Phages cannot match the broad-spectrum capabilities of antimicrobial chemicals (which led to their rapid replacement by antibiotics (Figure 2)) and are thus not recommended for drinking water disinfection.

Phages can control pathogenic and other problematic bacteria through (1) selective lysis of target strains, (2) decreasing target population fitness by selecting for slower-

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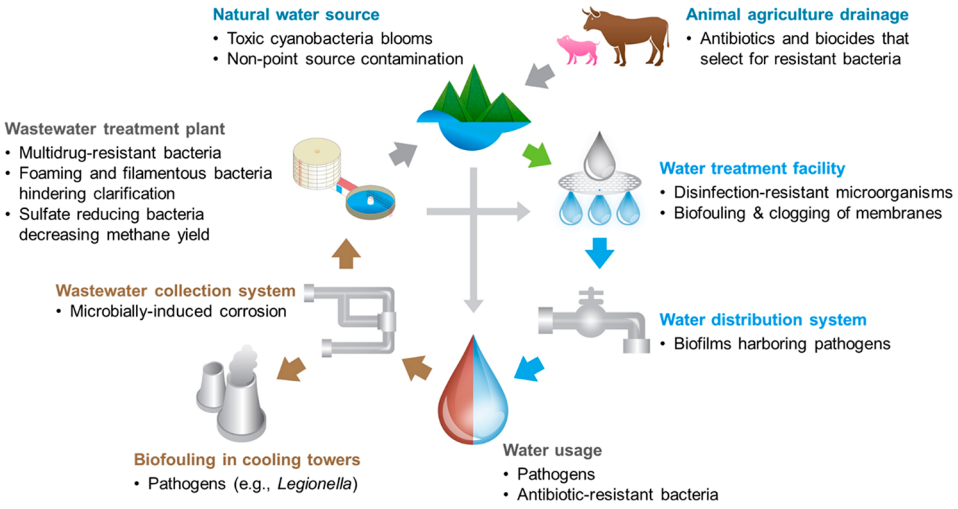

Figure 5. Opportunities for phage-based bacterial control in the water supply and reuse cycle.

growing phage-resistant bacteria that are more susceptible to biocides or to competitive exclusion, (3) eradicating biofilms that typically protect the target bacteria, and (4) supplementing or replacing antibiotics and biocides to minimize their incidental or accidental discharge to the environment and mitigate their associated selective pressure for resistant bacteria in water systems.

Let us consider opportunities for phage-based bacterial control in the water supply and reuse cycle, starting with an example of selective lysis of problematic strains in surface drinking water sources. Toxic cyanobacterial blooms can threaten drinking water supplies and conventional treatment processes (e.g., sand filtration, coagulation and flocculation) are inefficient at eliminating cyanotoxins.[33] Accordingly, cyanobacterial populations (including the very toxic *Microcystis aeruginosa*) could be controlled in situ by the addition of cyanophages (i.e., phages specifically infecting cyanobacteria) without modification to treatment facilities. Phages could also contribute indirectly to minimize selective pressure for problematic bacteria in source waters. For example, phage use in animal agriculture to treat infectious diseases would curtail the use of antibiotics and associated drainage to source waters.[34] This would mitigate the selective pressure exerted by residual (sublethal) antibiotic concentrations for antibiotic resistant bacteria (ARB), and their potential propagation in water systems.

As an example of decreasing target population fitness, phages can also select for slower-growing problematic bacteria with increased sensitivity to antibiotics. Bacteria can develop phage resistance by decreasing expression of phage receptors on their cell surface, but this typically reduces cell fitness.[35] This occurs because phage receptors include proteins that contribute to host metabolism, such as protein BtuB for vitamin B12 uptake, protein LamB for diffusion of sugars, and protein FhuA and TonB for ferrichrome transport.[15] Similarly, some phage receptors occur on antibiotic efflux pumps that contribute to antibiotic resistance (such as TolC in *Escherichia coli*[36] or OprM in *Pseudomonas aeruginosa*[37]) and phage resistance comes at the cost of deficient efflux pumps, which increases sensitivity to antibiotics.

Phages could also enhance biofilm and biofouling control in drinking water treatment and distribution systems or in industrial or power plant cooling towers. Biofilms, the preferred lifestyle of bacteria, serve as refuges for pathogens (e.g., *Legionella pneumophila*) and ARB, and protect them against disinfectants. Biofilms may also accelerate water infrastructure corrosion.[2] Mutation frequencies and horizontal gene transfer for bacteria in biofilms can be significantly higher than in suspended bacteria,[38] which accelerates development of resistance against antibiotics and disinfectants. Accordingly, antibiotic minimal inhibitory concentrations (MICs) may be up to 1000-fold higher for bacteria within biofilms relative to planktonic bacteria.[39] There is a need for cost-effective approaches to eradicate biofilms while avoiding undesirable unintended consequences (e.g., damage to biofouled water filtration membranes by cleansing chemicals). Thus, phages that target structural core bacteria that maintain biofilm integrity (i.e., "keystone" species) would be very useful for biofouling control.[40] Phages that express depolymerase enzymes to breakdown exopolysaccharide compounds that constitute the biofilm matrix would also be valuable. Examples of these include phage K1F which produces endosialidases to degrade the polysaccharide capsule of *Escherichia coli* by cleaving α2,8 linkages in sialic acid polymers,[41] and phage H4489A which has hyaluron lyases that degrade the hyaluronan capsule of *Streptococcus pyogenes*.[42]

Phages could also enhance wastewater treatment for reuse, resource recovery, or safe discharge to the environment, including return to source waters (Figure 5). Phages could target pathogens and ARB in wastewater treatment plants (WWTPs).[10] Recent studies have shown that some WWTPs serve as breeding grounds and point sources for environmental dissemination of antibiotic resistant genes (ARGs) and multidrug resistant "superbugs".[43] One strategy to alleviate this problem would be to use phages that target species that commonly harbor multidrug resistance genes, such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and some *E. coli* strains.[44] For example, polyvalent (broad-host-range) phages added at 10^7 pfu/mL to activated sludge microcosms thrived and successfully suppressed multidrug-resistant *E. coli* NDM-1 without upsetting overall heterotrophic activity.[10] Additionally, lytic phages that bind to receptors on antibiotic efflux pumps or other proteins responsible for antibiotic resistance could be useful for targeting ARB.

Another challenge that could be addressed by phages is the presence of sulfate-reducing bacteria (SRB) in anaerobic

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Table 2. Barriers to Phage Biocontrol and Overcoming Strategies

barrier	overcoming strategy
unknown target bacteria	use metagenomics and other novel molecular based technologies that link phylogeny to function (epicPCR and meta3C)[52,53]
narrow phage-host range	use phage cocktails and/or polyvalent phages for broader host coverage[10]
phage-resistant bacteria	use phage cocktails, phage training, or phages in combination with disinfectants and other antimicrobials[34,57−59]
bacteria protected by biofilm	use phages with biofilm disrupting or quorum-quenching enzymes; enhance biofilm penetration by conjugating phages to magnetic nanoparticles[62−64]
insufficient host density	use polyvalent phages which can target alternative hosts to maintain titer when target host density is low[10]
phage inactivation and decay	use protective shells such as alginates and bacteria spores[68,69]

digesters. SRB can outcompete methanogens for hydrogen utilization, which decreases methane yields.[45] SRB also produce toxic sulfides that contribute to corrosion and odor problems. Accordingly, SRB could be suppressed in digesters by the injection of lytic phages, which would enhance resource recovery and infrastructure longevity.

WWTPs also face operational problems associated with overgrowth of foaming bacteria (e.g., *Gordonia* spp. and *Nocardia* spp.) and filamentous bulking bacteria (e.g., *Microthrix* spp. and *Nocardia* spp.) which hinder sludge settling and treated wastewater clarification.[46] Successful foaming stabilization has been demonstrated in laboratory-scale experiments using polyvalent bacteriophage GTE7 lysing both *Gordonia* and *Nocardia* species.[47] Batch reactors inoculated with a lytic phage isolated from wastewater mixed liquor (i.e., phage HHY from the Myoviridae family, MOI = 0.001) showed 54% removal of the bulking bacteria *Haliscomenobacter hydrossis*, resulting in faster sludge settling velocity and 33% lower sludge volume index.[48] However, application of phages as long-term solution against bulking and foaming problems remains to be demonstrated at the field scale.

4. BARRIERS AND POTENTIAL SOLUTIONS TO ENABLE PHAGE-BASED MICROBIAL CONTROL

To achieve successful microbial control, sufficient infective phages should be delivered where problematic bacteria occur. These target bacteria are diverse and could exist in sludge flocs, microcolonies, and biofilms, which are not easily accessible. Current major challenges facing phage application in water systems include (1) not knowing which bacterial species to target (e.g., thousands of different species could be carrying antibiotic resistant genes or be responsible for microbially induced corrosion), (2) narrow phage host ranges requiring different specific phages for each bacterial strain, (3) development of phage resistance by target bacteria, (4) biofilms that represent a diffusion barrier hindering phage penetration, (5) insufficient host concentration to ensure that phage growth rates exceed decay rates, and (6) phage inactivation in the environment (Table 2).

Discerning and Identifying Target Bacteria

Considering that phages have limited infective spectra, accurate discernment and phylogenetic identification of the problematic bacteria is essential to match the right phages.[49] Similarly, to mitigate microbially induced corrosion, prior knowledge of the bacterial species involved in sulfate reduction and iron oxidization is required for appropriate phage isolation.[50] Fortunately, recent improvement of culture-independent, long-read gene sequencing approaches (e.g., nanopore sequencer) have facilitated analyzing nearly the full-length of 16S rDNA and assigning bacterial taxonomic classification down to the species level.[51] Emerging techniques include metagenomics and chromosome conformation capture (meta3C) to identify species within mixed communities without any prior isolation or knowledge of the community composition,[52] and Emulsion, Paired Isolation, and Concatenation PCR (epicPCR),[53] which links functional genes and phylogenetic markers and allows massively parallel-sequencing of single cells to identify problematic bacteria.

Narrow Phage Host Ranges

Phage specificity can be a disadvantage when the target bacteria include numerous taxonomically distant species that require different phages. In addition, narrow host range restricts phage large-scale production because of complex (occupationally hazardous) production and separation of phages that target pathogenic bacteria.[54] Phages with narrow host ranges also have low replication rates in environments with low target host concentrations and may face extinction if decay rates exceed production rates. However, high-throughput approaches have been established for preferential isolation of polyvalent phages.[55] Successful polyvalent phage isolation depends on three factors: samples with diverse bacterial and phage communities, nonbiased phage pool preparation, and sequential screening and enrichment with multiple hosts. Such broad host-range phages, whose production can be surrogated using nonpathogenic and fast-growing hosts,[10] are more resilient to environmental stresses and able to treat multiple bacterial species simultaneously.[56] This not only improves their efficacy within diverse microbial communities, but also decreases the size and complexity of phage banks, which are need for practical implementation of phage-based bacterial control.

Bacterial Phage Resistance

Target bacteria may defend against phages by preventing phage adsorption, blocking phage genome entry, destroying phage genomes, and aborting phage life cycles.[35] Phage cocktails consist of multiple phage types that target different bacterial receptors and avoid different resistance mechanisms, thus delaying or even preventing the regrowth of phage-resistant bacteria after treatment.[57] Directed phage training with wild-type and consecutively isolated phage-resistant variants as hosts also hinders selection and regrowth of phage-resistant variants.[58] Combinations of (or rotations with) compatible antimicrobial chemicals (e.g., biocides and bacteriocins) with phage biocontrol hold promise for enhanced microbial control because the frequency of simultaneously developing resistance to both bactericidal agents is much lower than that for just resistance against phages or disinfectants.[34,59]

Physical Barriers and Heterogeneity within Biofilms

Problematic bacteria could exist in biofilms, whose matrix hinders phage diffusion and penetration. Target bacteria are also shielded by surrounding nonhost bacteria that do not

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propagate phages. These challenges can be overcome by utilizing polyvalent phages[60] with low adsorption rates[61] that can use a broad range of bacteria (which otherwise represent a diffusion barrier to narrow-host range phages or chemical disinfectants) to proliferate and propagate within the biofilm. Phage-associated depolymerases that degrade the biofilm matrix[62] or quorum-quenching enzymes that disrupt cell-to-cell communication critical to biofilm formation[63] also contribute to biofilm eradication. Such traits can be selected for when isolating natural phages or introduced into phages via genome engineering. Phage-magnetic-nanomaterial conjugates could also be used to disrupt biofilm matrices as they facilitate penetration under a weak magnetic field into otherwise inaccessible host cells.[64] Phages conjugated with amino functionalized superparamagnetic nanomaterials (e.g., nano-magnetite) also ensure that phage tail fibers are exposed to the hosts for easier infection.

Insufficient Host Densities

Many reported failures in phage therapy and biocontrol can be attributed to low concentrations of the target host that are insufficient to support phage replication at rates that exceed decay.[65] This may be overcome by using high initial phage concentrations, which may not be practical for large-scale systems. A more practical approach would be to use polyvalent phages that can target both the problematic bacteria as well as one or more highly abundant members of the community. This not only helps increase phage titers, but also provides alternative replication options when resistant populations emerge. Alternatively, benign production hosts can be added simultaneously with phages to enhance phage abundance (by continuous productive infection) and offset phage decay.[10]

Phage Activity Loss in the Environment

Phages may be damaged by various environmental stresses or adsorbed before reaching the target bacteria.[66] Phage inactivation mainly results from either structural protein damage, core nucleic acid destruction, or in some cases envelope loss.[67] Encapsulation of phage with protective materials (e.g., alginate-chitosan) and in liposomes can improve phage resistance to environmental stresses.[68] Bacterial spores, the most resilient biological entity, can also serve as phage-genome protective shells against various stressors, including extreme temperatures and pH, desiccation and UV radiation.[69] This also facilitates long-term unrefrigerated storage and shipment to for application in rural areas and other locations where they can be easily activated upon exposure to some amino acids.[69] Because there are widely documented inverse relationships between phage host range and decay rate,[70] polyvalent phages are recommended for phage applications in relatively harsh environments.

5. RESEARCH NEEDS AND OUTLOOK FOR PHAGE-BASED BIOCONTROL IN WATER SYSTEMS

While phage-based microbial control could significantly alleviate various bacteria-related problems in water treatment and reuse, several research needs must be met to fulfill this potential. Perhaps foremost is the need to better understand water-related microbiomes and how their structure impacts water quality and related infrastructure. This is critical to identify bacterial targets for a given outcome. As discussed above, novel metagenomic approaches can be helpful in this regard, allowing species- and strain-level characterization, which is needed for both target and phage selection. Additionally, such data can be interrogated using network analysis to determine co-occurrence probabilities of species and to identify keystone species.[71] Understanding these microbial interactions is important for avoiding unintended consequences and for achieving desired functional outcomes with the least intervention.

Using phages in combination with other antimicrobials (concurrently or *in tandem*) presents many exciting opportunities. As most research on phages has focused on medical applications, a substantial body of literature exists which has repeatedly demonstrated synergistic activity between phages and antibiotics.[34,72] While there are also reports of synergy between phages and some chemical disinfectants,[73] more systematic studies are needed to optimize such approaches for microbial control in different water systems, and understanding potential unintended effects.

Phage efficacy can also be enhanced via genome engineering, or by functionalization through conjugation to various effectors. For example, phages have been engineered to produce enzymes that degrade extracellular polymeric substances and help break up biofilms,[62] or to deliver targeted nucleases (e.g., CRISPR-Cas systems) to selectively kill ARB.[74] The potential use of phages as gene delivery (bioaugmentation) vectors is also intriguing. However, phage genome engineering is tedious, and the methods are often not applicable outside of a few well-characterized phages. To overcome this challenge, some researchers are attempting to develop universal phage chassis with plug-and-play parts that can be easily swapped out to alter characteristics such as host range.[75] Nevertheless, it is not clear how this might accelerate phage applications over the use of conventional selection strategies, and the environmental release of engineered phages presents a significant regulatory barrier as unintended consequences such as disruption of microbial ecosystem services and associated ecological imbalances remain to be systematically explored.

Finally, it is important to recognize that phages are not implemented in the same manner as other microbial control agents and will require new infrastructure and practices. To facilitate widespread scale up and economical use, phage banks need to be created for the most important and likely bacterial targets in the water treatment and reuse cycle, and methods for rapid microbiome characterization need to be standardized. It will also be important for operators to understand the factors that can impact the efficacy of phage-based microbial control, and how they should respond to changing conditions. Thus, education and training programs will also be needed for the successful application of phages.

While much research remains to be completed before the widespread adoption of phage technology, the future appears promising. Because phages are harmless to mammalian and plant cells, the U.S. Environmental Protection Agency (USEPA) has approved the use of certain phage preparations against plant pathogens and the U.S. Food and Drug Administration (USFDA) has approved the use of phage formulas in meat and poultry products, granting certain phages Generally Recognized as Safe (GRAS) status.[76,77] Health agencies in Israel, Canada, Switzerland, Australia, New Zealand, and the European Union have also approved use of phage product on foods.[78] This bodes well for the use of naturally occurring phages in water systems. Overall, phages offer great potential to supplement or replace some biocides and disinfectants for selective microbial control of problematic

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bacteria and may also hold promise for microbiome engineering. Improving fundamental understanding of their physiological and ecological constraints will thus likely enhance cost-effective microbial control in both urban and rural water systems.

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