Review Article

Bacteriophages: Their Possible Application to Wastewater Treatment Systems

Raghad Shubbar Jaafar*, Satar Aziz Gmais, Omar Abdulameer Al-Badran

Biological Development Department, Marine Science Center, University of Basrah, Basrah, Iraq.

Abstract | Recently, considerable challenges have emerged in environmental and public health due to microbial contamination of wastewater systems and rise of microbial resistance. Despite the effectiveness of traditional treatment methods, they often lack the ability to selectively target pathogenic bacteria and may lead to secondary contamination or development of resistance among the contaminating microorganisms. Currently, the use of bacteriophages has gained increased attention as a promising approach in bioremediation and various environmental applications. The present work aims to shed light on the potential application of bacteriophages in managing wastewater systems by exploring their specificity, self-replicating nature, and environmental safety as key advantages. The study begins by discussing the methods used for phage isolation and characterization, referencing previous studies that have documented their activity against common wastewater pathogens, and comparing their effectiveness to conventional treatment approaches. In addition, we examine the limitations of phage applications, including narrow host range, potential for resistance development, and regulatory considerations. Finally, the study presents a future outlook on phage-related scientific research, their practical implementation, and integration of phage-based technologies into sustainable wastewater management strategies.

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***Correspondence** | Raghad Shubbar Jaafar, Biological Development Department, Marine Science Center, University of Basrah, Basrah, Iraq; **Email:** raghad.jaafar@uobasrah.edu.iq
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Introduction

By the year 2050, the global population is expected to reach 9.7 billion, which will lead to a significant increase in municipal and household wastes (Kaza *et al*., 2018). Recently, there has been growing global concern about the accumulation of such wastes, particularly in urban areas, due to their negative impact on both environment and public health. As a result, wastewater treatment has become a serious environmental challenge. The discharge of raw or inadequately treated wastewater can promote the development of harmful pathogens, including antibiotic-resistant bacteria (Yao *et al*., 2021). A key indicator of fecal bacterial contamination is the presence of *Escherichia coli*, which frequently occurs in wastewater and poses a major threat to water quality and public health. The conventional methods for wastewater treatment include chemical sterilization, waste dumping, and burning, which have come under wide scrutiny due to their environmental effects, lack of efficiency in handling antibiotic-resistant microbial strains, and high operational costs. These treatment methods often lead to the formation of harmful byproducts that contaminate the water sources. Meanwhile, physical methods such as incineration create air pollution and contribute to greenhouse gas emissions (Kumawat *et al*., 2017). Economically, these techniques are not cumbersome and also non-sustainable, particularly in resource-constrained settings (Kaza *et al*., 2018). Therefore, an alternative process, which is innovative, cost-effective, and ecofriendly has become paramount. The most promising biological solution to this problem is the use of bacteriophages, as they act as natural predators of harmful bacteria. Phages have promising features, mainly they can replicate independently and exhibit high specificity, enabling them to target and lyse bacteria without harming the nontarget organisms or the surrounding environment. As a result, it can be used in wastewater treatment plants as a biocontrol agent against pathogenic bacteria, with many previous studies confirming their effectiveness in reducing bacterial loads in contaminated water (Jassim *et al* 2016; Shivaram *et al*., 2023; Ragab *et al*., 2024). Bacteriophages differ from conventional broad-spectrum antibiotics in that they possess high host specificity. While antibiotics may disrupt the natural microbial communities and cause ecological imbalances, phages selectively target pathogenic bacteria, reducing the risk of unintended environmental impact. Due to the intense microbial activity and rapid organic matter decomposition, landfill sites represent rich environments that support diverse bacterial and phage populations, making them valuable sources for phages isolation (Reisoglu and Aydin, 2023). Leachate fluids from landfills have been recognized as vital sources for isolating beneficial lytic phages capable of targeting a wide range of bacterial pathogens, including *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. Several previous studies have documented that bacteriophages isolated from landfill sites can considerably reduce bacterial contamination in wastewater, serving as a sustainable and low-cost alternative to the traditional treatment methods (El-Shibiny and El-Sahhar, 2017; Padmesh *et al*., 2024). Phages have a unique ability to multiply on their own when their bacterial hosts are present. This means that they can maintain their antimicrobial activity without needing constant external inputs such as chemicals or synthetic agents (Abozahra *et al*., 2025). Because of this self-replicating behaviour, phage's offer a cost-effective and environmentally sustainable approach to wastewater treatment, especially in areas that lack access to advanced treatment infrastructures. By lowering the levels of harmful bacteria in wastewater, phages not only help improve the water quality but also reduce the environmental impact typically associated with conventional treatment methods. In light of these benefits, the current study aims to isolate bacteriophages from landfill leachates and evaluate their effectiveness in controlling pathogenic bacteria in wastewater treatment. By exploring phage-based solutions, this study could provide valuable insights into sustainable and biologically-based alternatives for wastewater management, which minimize environmental damage, reduce treatment costs, and improve public health outcomes.

An overview about the bacteriophage characterization

Bacteriophages are a type of bacterial viruses and represent the most prevalent biological entities on Earth. Their global abundance is estimated to exceed 10^{31} entities, outnumbering bacteria by approximately tenfold (Dion *et al*., 2020). In microbial communities, phages play an essential role in shaping community structure, managing horizontal gene transfer, and influencing global biogeochemical cycles. They are diverse microorganisms found throughout various ecosystems, including marine and freshwater environments, soil, and artificial settings such as wastewater treatment plants and landfills (Howard-Varona *et al*., 2017; Clokie *et al*., 2021). A bacteriophage consists of either a single-stranded or double-stranded DNA or RNA, enclosed within a protein shell called a capsid. Some phages possess a lipid envelope derived from the host membrane; however, the majority especially the tailed phages (Caudoviricetes) are non-enveloped (Ackermann, 2009). Phage structures vary widely, including isometric (icosahedral), filamentous, and complex forms with either motile or non-motile tails, which play a crucial role in host recognition and genome insertion (d'Acapito *et al*., 2025).

Phage replication is generally classified into two life cycles: The lytic and the lysogenic cycles. In the lytic cycle, the phage utilizes the host's biosynthetic machinery to produce new virions, ultimately leading to host cell lysis and the release of progeny phages. This stage is the core for exploits in phage therapy and bacterial control. On the other hand, latent phages can enter the lysogenic cycle, integrating their genome into the host chromosome as a prophage, where they can remain inactive and replicate passively within the host until stimulation triggers a switch to the lytic phase (Touchon *et al*., 2016; Howard-Varona *et al*., 2017).

The host specificity of a phage is one of its remarkable characteristics, often targeting specific strains within a bacterial species. This feature arises from the molecular interaction between phage receptor-binding proteins and structures on the bacterial surface, such as lipopolysaccharides, teichoic acids, or membrane proteins (Koskella and Meaden, 2013). Even though challenges related to such phage specificity arise during broad-spectrum applications; however, it provides remarkable benefits in targeted approaches, such as controlling pathogenic bacteria in wastewater without harming the beneficial microbial populations (Palma and Qi, 2024).

In addition, phages express rapid proliferation kinetics, a high mutation rate, and considerable genetic flexibility, enabling them to adapt quickly to evolving bacterial hosts. These features have been widely harnessed in environmental and clinical applications (Wilcox, 2017). In wastewater treatment, phages are being investigated as biocontrol agents against antibiotic-resistant bacteria and as tools to enhance the stability of microbial communities (Bolsan *et al*., 2022). Phages have become attractive agents in biotechnological applications for several reasons, mainly their ability to survive and remain viable in harsh environments such as sewage, sludge, and landfill leachate (Selcuk and Ozbek 2024). Recently, bacteriophages have been recognized as valuable tools in modern microbiology, with substantial potential in diagnostics, bioremediation, biosensors, and phage-based disinfection strategies.

Bacteriophage isolation techniques

The landfill region is considered as one of the most dynamic and microbially rich environments, as a result of the accumulation of organic wastes, leachate, and a variety of bacterial populations that serve as host origins for bacteriophages. These habitats are largely identified as promising reservoirs for isolating the phages with potential applications in bioremediation and wastewater treatment (Clokie *et al*., 2021; Olawade *et al*., 2024). A combination of microbial classification and molecular techniques is essential for the isolation of bacteriophages from various landfill environments. Among these methods, the enrichment technique remains the most widely used approach. It involves cultivating samples from the landfill with specific host bacteria under optimal conditions to promote phage replication (Artawinata *et al*., 2023). To remove solids and bacterial debris during the pre-treatment step, samples typically undergo centrifugation followed by filtration using a 0.22 µm filter to retain the viral particles (Shahzad *et al*., 2021). After the incubation period, the supernatant is tested using the double-layer agar (DLA) technique, which allows observation of lytic activity *via* plaque formation (Lingga *et al*., 2020).

In contrast, direct isolation techniques involve culturing raw, non-filtered samples immediately on bacterial lawns. Although less sensitive, this method is important for identifying phages that naturally occur in high abundance (Strange *et al*., 2021). Furthermore, various molecular techniques are now applied, including quantitative polymerase chain reaction (qPCR), metagenomic sequencing, and viral metaviromics (Shkoporov and Hill, 2019; Džunková *et al*., 2019). These tools enable the scientists to analyze phage communities in complex environments and identify promising new candidates for targeted applications. Furthermore, a previous study has relied on bioinformatics-guided isolation, where metagenomic data from landfill viromes guide the selection of host strains and enhance the success rate of culturable phage recovery (Strange *et al*., 2021). All in all, combining traditional culturing methods with high-throughput molecular and computational approaches offers a reliable strategy for isolating effective bacteriophages from landfill environments, particularly for applications in wastewater management.

Implementation of bacteriophages in wastewater treatment

Recently, bacteriophages application in wastewater treatment as biocontrol agents has become of more interest, as a result of the increasing prevalence of multidrug- resistant (MDR) bacteria in wastewater environments. Bacteriophages are viruses that specifically infect and lyse particular bacterial hosts without disrupting the essential microbial community. These properties make them highly valuable in wastewater treatment processes (Anomaly, 2020; Bolsan *et al*., 2022). Several previous studies have demonstrated the efficiency of bacteriophages in reducing bacterial populations such as *E. coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Shigella* spp. in both raw sewage and treated effluents (Méndez *et al*., 2020; Lin *et al*., 2022; Alseth *et al*., 2025). In addition, bacteriophages have been applied to treat bacteria found in biofilms commonly present in wastewater pipelines, which are often resistant to antibiotics and disinfectants (Weiling *et al*., 2010). Studies have indicated the ability of bacteriophages to reduce biofilm formation by *Pseudomonas aeruginosa*, highlighting their potential roles in controlling membrane biofouling in membrane bioreactors (MBRs). In their study, Silva *et al*. (2014) used a phage cocktail in anaerobic sludge digesters to manage sulfate-reducing bacteria and mitigate hydrogen sulphide production; a major cause of bad smile and corrosion in treatment plants.

Phages also represent an effective approach for targeting fecal indicator bacteria (FIB), including *E. coli* and *Enterococcus* spp, which frequently exhibit resistance to the standard disinfection methods such as chlorination and ultraviolet treatment (Omran *et al*., 2024). Santiago-Rodriguez *et al*. (2015) reported that the coliphages could be used as alternative indicators for fecal contamination and phage-based treatment technique in wastewater recycle process. Moreover, bacteriophage management has shown promise in the third step of wastewater treatment, sharing to effluent polishing, and enhancing the bacteriological safety of recycled water (Fan *et al*., 2023; Hong *et al*., 2025).

More applications have recently been applied related to adapt the phages in wastewater systems (Ragab *et al*., 2024). The alginate or chitosan-based micro-capsulation technique has been applied to protect the phage from the environmental stresses (*i.e*., temperature, pH, and ultraviolet (UV) irradiation), which allows it stay active in the sludge systems (Colavecchio *et al*., 2017). Additionally, genomic analysis of phages and their bacterial hosts allows the design of broad-spectrum phage cocktails to target diverse pathogens across several treatment stages (Clokie *et al*., 2021).

The efficiency of bacteriophages in wastewater treatment compared to the conventional treatment techniques

The traditional wastewater treatment methods used for microbial inactivation include chlorination, ozonation, UV irradiation, and advanced oxidation processes. These methods are effective in reducing bacterial loads; however, they have limitations in treating both biofilm-associated bacteria and MDR strains. For instance, chlorination is one of the most commonly used disinfection methods, characterized by its low cost; however, it may lead to the formation of harmful and carcinogenic byproducts, such as trihalomethanes and haloacetic acid (Guo *et al*., 2023). Furthermore, prolonged exposure to these chemicals may lead to the development of resistant bacterial strains and damage native strains that play a role in the treatment process (Guo *et al*., 2023). On the other hand, bacteriophage treatment strategies provide a biocontrol activity, as they are highly specific to their targets and leave the native bacteria unharmed (Bisen *et al*., 2024). A previous study conducted by Montenegro-Díaz *et al*. (2022) revealed that phage treatment followed by traditional chlorination effectively enhanced the removal of biofilm-forming *E. coli* on stainless steel surfaces. This combination treatment was able to remove up to 90% of the biofilm mass, in contrast to the individual chlorination method, which achieved an efficiency of approximately 60%. Similarly, Fernandes *et al*. (2024) reported that the use of bacteriophages reduced *P. aeruginosa* biofilm formation by more than 70% within 2h, compared to 4h required for biofilm reduction when using benzalkonium chloride.

Moreover, phage-based treatment methods help limit the dissemination of drug-resistant bacteria in wastewater systems and prevent their release into natural water bodies; thereby, reducing the risk of wider environmental spread (Pallavali, and Choi, 2023). Phages can selectively target antimicrobial-resistant (AMR) pathogens such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterococcus faecalis*, commonly isolated from hospital and industrial effluents (Hong *et al*., 2025). These approaches can support the global efforts to reduce reliance on antibiotics for controlling pathogenic bacteria, helping to curb the emergence and spread of MDR strains.

The limitations of phage application and challenges

Despite the wide-range use of bacteriophages in wastewater treatment, their application still faces many critical challenges and limitations. One obstacle is the limited host range of bacteriophages, which reduces their ability to effectively target bacteria in wastewater environments (Koskella and Meaden, 2013). In addition, wastewater contains high concentrations of suspended soil particles, organic substances, and bacterial biofilms, all of which impede the interaction between phages and bacteria, reducing the overall sterilization efficiency (Liu *et al*., 2021; Shivaram *et al*., 2023). Fluctuations in environmental factors such as pH, temperature, salinity, and UV substantially affect phage viability and stability *in situ*, often leading to their decreased occurrence and effectiveness in open or dynamic treatment systems (Artawinata and Waturangi, 2023). Several bacterial resistance mechanisms against phages, including receptor modification and CRISPR-Cas adaptive immunity, reflect challenges similar to those associated with antibiotic resistance and highlight the importance of optimizing phage cocktails (Seed, 2015).

From a practical standpoint, the expansion of phage applications faces challenges from both technical and economical perspectives. These challenges are further intensified by the lack of standardized techniques for the isolation, purification, and quality assurance of phages, which are considered as essential elements required for industrial phage application (João *et al*., 2021). There is a scarcity of environmental data on the use of bacteriophages in various applications, necessitating environmental risk assessments and long-term monitoring to ensure biosafety and environmental sustainability (Puxty and Millard, 2023).

Advances in bacteriophage applications and future perspectives

The application of bacteriophages in wastewater treatment has progressed from a conceptual idea to a promising technological approach, particularly in response to the rise of antibiotic-resistant bacteria and the limitations of conventional chemical and biological treatment methods. Recent studies have confirmed the superiority of bacteriophage applications in removing microbial pollutants from wastewater compared to conventional methods, owing to their ability to specifically target pathogenic bacteria while preserving beneficial microbial communities that are essential for pollutant degradation (Liu *et al*., 2021). The development and application of phage cocktails in wastewater treatment represent a significant advancement, as they can target a broad spectrum of bacterial strains, reduce the likelihood of resistance development, and minimize the effort required to treat heterogeneous wastewater (Singh, 2023). Manipulation of genetic engineering to construct synthetic bacteriophages has enhanced phage capabilities in several areas, including increasing lytic activity, expanding the host range, and improving the ability to penetrate biofilms. Genetic engineering can be applied to design engineered phages that express enzymes responsible for biofilm degradation, such as depolymerases or endolysins, which are more effective in breaking down bacterial biofilms; a major barrier in conventional wastewater treatment methods (Strathdee *et al*., 2023). Recently, nanotechnology has played a significant role in producing encapsulated bacteriophages, enabling their functionality under extreme conditions, including pH fluctuations and exposure to UV radiation**.** This allows for their safe and controlled release within the wastewater treatment systems (Palma and Qi, 2024). Scientific advancements, particularly in omics technologies; most notably metagenomics and transcriptomics, have played a pivotal role in enhancing our understanding of the mechanisms governing the interactions between bacteria and bacteriophages. They also provide critical insights into the potential environmental impacts of bacteriophage applications within the complex microbial communities of wastewater. Practical application of these advanced tools contributes remarkably to optimizing the selection of suitable bacteriophages, enabling the specific prediction of their bacterial hosts, and facilitating the development of more effective application protocols tailored to specific wastewater treatment systems (Harshitha *et al*., 2022; Ragab *et al*., 2024). For the large-scale application of bacteriophages technology, it is essential to address several environmental challenges, including fluctuations in temperature, pH, and organic load. Additionally, there is a need to adopt standardized protocols for phage production and efficacy of evaluation. Furthermore, ensuring the environmental safety of widespread phage application requires a rigorous scientific framework supported by long-term field studies to monitor ecological impacts and potential shifts in the bacterial community dynamics (Sarat *et al*., 2023; Delgado Vela and Al-Faliti, 2023). Despite the challenges facing the application of bacteriophages, recent scientific researches confirm their potential for practical manipulation in wastewater treatment. This approach is considered ecofriendly and holds great promise for achieving long-term environmental sustainability goals.

Conclusions and Recommendations

All evidence obtained from the new research studies confirm that the use of bacteriophages in wastewater treatment is considered as a promising and environmentally safe alternative for wastewater treatment. This is related to their high specificity, independent-replicating ability, and selective targeting of the pathogenic bacteria, mitigating the risk of secondary pollution and MDR development. Although phage application has shown effectiveness in most studies; however, their application still encounters many challenges such as limited host range, potential resistance development, and regulatory constraints. Therefore, progress of scientific research is fundamental to develop isolation and application methods and improve encouraging regulatory schemes, emphasizing the incorporation of this technique into renewable wastewater management. In light of these findings, it is recommended to focus the future researches on developing broad-spectrum phage cocktails and standardized application protocols. This will allow establishment of monitoring strategies to manage resistance, integrating phage treatment with conventional wastewater systems for synergistic effects, conducting environmental risk assessments to ensure ecological safety, and promoting collaboration among scientists, policymakers, and environmental agencies to build supportive regulatory frameworks and validate the technology through large-scale pilot studies.

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Novelty Statement

The current study deals with using bacteriophages as a sustainable, eco-friendly, and safe alternative for wastewater treatment, due to their efficacy in selectively targeting of pathogenic bacteria and potential to reduce MRD. It further highlights practical strategies used for phage application, integration into existing treatment systems, and addressing current challenges such as host range limitations and regulatory barriers.

Author's Contribution

RSJ: Conceptualization, data curation, project administration, resources, supervision, writing original draft, writing review and editing.
SAG: Data curation, resources, writing review and editing.
OAA: Formal analysis, software.

Ethical approval
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