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A mini-review on new disinfection alternative: bacteriophages and pathogen removal potential from water and wastewater

Mohsen Sadani[a], Mohammad Mehdi Golbini Mofrad[b,*], Ghasem Kiani Feizabadi[c], Mostafa Hadei[d], Fatemeh Yousefian[e]

[a]*Department of Environmental Health Engineering, School of Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran, email: mohazesfahan@yahoo.com*
[b]*Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, email: mgolbini@yahoo.com*
[c]*Environment Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, and Department of Environmental Health Engineering, School of Health, Semnan University of Medical Sciences, Semnan, Iran, email: ghasem_kia@yahoo.com*
[d]*Sabzevar University of Medical Sciences, Sabzevar, Iran, email: mostafa.hadei@gmail.com*
[e]*Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, email: hadis.naderi@yahoo.com*

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ABSTRACT

Disinfection is a practice that inactivates and destroys pathogenic organisms. The conventional disinfectants for water and treated wastewater effluents have defects such as dangerous disinfection by-products, the resistance of bacteria and the related biofilms to disinfectant, high costs, no residual disinfecting action, and high risks involved in producing, transporting, and handling a large amount of chlorine and ozone. Accordingly, investigating new disinfection alternatives has been a necessity. Bacteriophages are used to treat a bacterial infection, which is known as phage therapy. In the recent decades, some studies revealed the role of phages in water and wastewater treatment, especially disinfection. In addition, the abundance of phages specific to enteric bacterial pathogens in natural water bodies is disclosed in many studies. This review discusses the phages specified to fecal coliform and other waterborne bacteria, the main advantages for applying the phages to reduce pathogens, restrictions of disinfection using phages, and the prospective applications of phages in order to improve the design and operation of the treatment plants.

Keywords: Disinfection; Bacteriophage; Pathogen; Water; Wastewater

1. Introduction

Human societies require water for drinking, sanitation, cleaning, and other applications; therefore, inadequate access to clean and safe water is one of the most prevalent problems affecting people throughout the world [1–3]. Microorganism has an important effect on quality of treated water produced from water reuse [4,5]. Microbial pathogens are one of the major health risks related to water and wastewaters [6], and waterborne diseases have been carried through microorganisms such as bacteria, viruses, and protozoa [7]. Therefore, water and wastewater disinfection are one of the most important measures applied in the treatment system or treatment plant [8]. However, disinfection of pathogens in water and wastewater continues to be an essential obstacle to diminish the risks of pathogen exposure and waterborne infectious diseases of humans and animals [9]. Disinfection is a practice that inactivates and destroys pathogenic organisms, especially bacteria such as *Escherichia coli* pathogenic strains,

* Corresponding author.

Campylobacter, *Vibrio cholera*e, *Salmonella*, and *Shigella* in water by applying physical approaches, and chemicals, thereby preventing waterborne diseases. The disinfectants used for chemical disinfection of water are chlorine (Cl_2), chlorine dioxide (ClO_2), chloramine, hypochlorite (OCl^-), iodine, ozone, metals (such as copper and silver), soaps, detergents, several acids, and bases; with chlorine being the most commonly used disinfectant. The most generally physical disinfection is ultraviolet (UV) radiation, gamma rays and heat, filtration, ultrasound waves, and finally hydrogen peroxide with UV. Moreover, chemical coagulation can be applied as a physicochemical or electrochemical technique [10–12]. In general, disinfections by using UV light, chlorination, and ozonation are the most commonly used strategies for water and wastewater [13]. The undesired side reactions of the disinfectants with substances present in the water is a common defect that leads to disinfection by-products, which are sometimes dangerous. In addition, there are high risks involved in producing, transporting, and handling a large amount of chlorine and ozone. The main defect of the mentioned physical disinfection approaches is the absence of a residual effect. In this regard, these approaches are only effective in the immediate surroundings of their operating devices and have no lasting residual in water and wastewater that result in microbial regrowth into effluent reservoirs and pipelines after treatment [14,15]. Besides, disinfection cost limits applying some disinfection approaches; for example, UV light disinfection for destroying and inactivating pathogens needs higher UV doses and consequently energy [9]. Also, the related cost for ozone disinfection (equipment and production), no residual disinfecting action, and measurement difficulties are some considerable disadvantages of these approaches [12,15]. Bacterial resistance to disinfectants of some bacteria (e.g., *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa* strains) [16–19] and biofilm-associated bacteria have been reported as being up to 3,000 times more than that of free chlorine, probably due to the exopolysaccharide produced by microorganisms [20]. Therefore, there is always a constant need for investigating the newer disinfection strategies or approaches aimed to overcome the defects of other disinfectants. One of the new disinfection strategies is using bacteriophages as a biological disinfectant.

Bacteriophages or phages were first discovered in the early 20th century by Twort in 1915 and by Herelle in 1917 [21]. Phages are viruses that were identified as epizootic infections of prokaryotes used to treat a bacterial infection, known as phage therapy. Phage therapy might be effective against a broad range of human infections caused by members of the genera *Staphylococcus*, *Salmonella*, *Klebsiella*, *Escherichia*, *Proteus,* or *Pseudomonas*. Overall, in the recent decades, some studies have revealed the role of phages in water and wastewater treatment especially disinfection [22–25]. For the first time, Marks and Sharp [26] represented that the waters of the Jamuna and Ganges rivers in India could kill many kinds of bacteria adsorbed on the host cells and their entry is mediated by specific receptors such as carbohydrates, proteins, and lipopolysaccharides (LPSs) existing on the surface of the host cell. The used phages for phage therapy and disinfection are lytic, which disrupt bacterial metabolism and introduces lyse to the bacteria [27]. Phages have been applied to treat bacterial infections, especially in Eastern Europe, and have been shown to decrease biofilm formation. In the late 1960s, the World Health Organization (WHO) set up an internal trial of phage therapy for cholera in countries such as Pakistan, and Bangladesh supported and supervised by the National Institution of Health. The results showed that the high doses of anticholera phage were able to kill bacteria in vivo even if they were not able to complete many cycles of replication and amplification [28]. Phages occur abundantly in the natural water bodies including lakes, rivers, and ponds, as they are ubiquitous in nature. In addition, the abundance of phages specific to enteric bacterial pathogens in the natural water bodies is disclosed in many studies. For instance, somatic coliphages, F specific phages, and *Bacteroides fragilis* phages were detected in different water environments as indicators of fecal contamination and tracers of the effectiveness of microbial removal by wastewater treatment systems [29–32]. Phages are used as indicators or tracers for the presence of bacteria in wastewater treatment systems.

This paper reviews some types of pathogenic bacteria strains in water and wastewater and the associated phages, and also, the feasibility of using phages to control these bacteria, and highlights critical study needs to realize phages-enabled disinfection and microbial control. Hence, we will discuss the restrictions of applying phages as a disinfectant in water and wastewater treatment systems or plants. To this end, we searched keywords such as disinfection, bacteriophage, pathogen, water, and wastewater in Google Scholar and scientific data banks such as Scopus, Web of Science, PubMed, and Science Direct.

2. Phages of fecal origin bacteria

Fecal coliform bacteria are regarded as the sole indicator of fecal contamination of water around more than 70 years [33,34]. Among these bacteria, the *E. coli* as a primary indicator is applied directly as the thermotolerant coliform limit and the amount of that is considered as a threshold to measure the efficacy of the disinfection process [15].

Many studies indicate applications of phages in coliform bacteria removal from aqueous environments. Abdulla et al. [22] researched the role of phages in the engineered wetland for domestic wastewater treatment. The results of this work showed that the addition of a mixture of phages isolated from raw sewage resulted in the removal of 37% of fecal coliforms, while the addition of high titer of coliphages resulted in the removal of 34%. Elshayeb [25] studied and determined the existence of phages in wastewater of Soba Stabilization Station by isolating and identifying methods for their activities against *E. coli* and *S. aureus* isolated from the anaerobic, facultative, and maturation ponds. This study showed that phages naturally exist where their hosts are present and they naturally destroyed bacteria that aided to recover from the polluted environment. Periasamy and Sundaram [35] assessed the possibilities of phages for pathogen removal from hospital wastewater. According to these authors, the total heterotrophic and total coliform population ranged from 1.6×10^5 to 8.3×10^6/mL and from 1.2×10^3 to 1.6×10^3/100 mL of sample, respectively. The higher frequencies of antibiotic resistant *E. coli*, *Pseudomonas* sp., *Streptococcus* sp., and *Bacillus* spp. observed in all sites demonstrate the extent of pollution. All samples had specific phages against *E. coli* with none of

them having phages against microbial type culture collection culture. *E. coli* specific phage was isolated and the population of phage required for effective killing of *E. coli* was standardized as 3×10^4 PFU/mL of lysate. The inoculation resulted in 100% removal of the pathogen from sewage water within 14 h of incubation. Higgins et al. [36] evaluated the use of a specific phage treatment to reduce *Salmonella* in poultry products and reported that all phage treatments reduced ($P < 0.05$) frequency of *Salmonella* recovery as compared with controls. Also, sufficient concentrations of an appropriate phage, or a phage mixture, can significantly reduce recoverable *Salmonella* from carcass rinses.

Regardless of these investigations, phages did not have a proper yield in wastewater treatment plants (WWTPs). Elsewhere, Yasunori et al. [37] investigated the fate of coliphage in a wastewater treatment plant in the central part of Japan from March to December 2001 and observed no phages in treated wastewater; because the aerobic treatment using activated sludge and/or the addition of flocculants such as powder activated carbon was effective for the removal of coliphage. In another work, Tait et al. [38] investigated the ability of phages and their associated saccharide depolymerases to control enteric biofilm formation and showed that phage and bacteria can coexist stably within a biofilm; thus, phage would provide poor tools for the control of biofilm formation. Altogether, it is suggested that combined treatment with phage polysaccharide depolymerases and disinfectant may supply an alternative control strategy. The studies of coliform reduction using phages will not be limited only to water and wastewater, as Sharma et al. [39] determined the efficiency of a mixture of three *E. coli O157:H7*-specific phages (ECP-100) in reducing the number of viable *E. coli O157:H7* on contaminated fresh-cut iceberg lettuce and cantaloupe. At the end, it was found that populations on cut cantaloupes treated with ECP-100 on days 2, 5, and 7 (0.77, 1.28, and 0.96 log CFU/mL) stored at 4°C were significantly lower than those of cut cantaloupes treated with the control (3.34, 3.23, and 4.09 log CFU/mL). Also, Kudva et al. [40] isolated *E. coli O157* antigen-specific phages and tested their ability to lyse laboratory cultures of *E. coli O157:H7*. A total of 53 bovine or ovine fecal samples were enriched for phage, and five of which were found to contain lytic phages that grow on *E. coli O157:H7*. Among these phages, three of them designated as KH1, KH4, and KH5, were evaluated. At 37°C or 4°C, a mixture of these three *O157*-specific phages lysed all the *E. coli O157* cultures tested but none of the non-*O157 E. coli* or non-*E. coli* cultures tested. Later, it is observed that virulent *O157* antigen-specific phages could play a role in biocontrol of *E. coli O157:H7* in animals and fresh foods without compromising the viability of other normal flora or food quality. In another foodborne diseases study, Sillankorva et al. [41] described isolation and characterization of a lytic phage capable to infect a variety of *Pseudomonas fluorescens* strains isolated from Portuguese and United States dairy industries. It is concluded that the isolated T7-like phage, phage ΦIBB-PF7A, are fast and efficient in lysing different *P. fluorescens* strains and may be good candidates to be used as sanitation agents to control the prevalence of spoilage-causing *P. fluorescence* strains in dairy and food-related environments.

There are few reports suggesting that phages may be active components of activated sludge systems [42,43]. Specific phages for *Salmonella* group of organisms were isolated from swine effluent lagoons and were characterized by McLaughlin et al. [44]. Faruque et al. [45] detected and isolated *Shigella* specific phage from surface water samples from Bangladesh that specifically lyses strains of *Shigella dysenteriae* type 1. The results of this study indicate that phage SF-9 may have epidemiological applications in tracing the presence of *S. dysenteriae* type 1 in environmental waters. Kęsik-Szeloch et al. [46] worked on characterizing the biology of novel lytic phages infecting multidrug-resistant *Klebsiella pneumoniae*. The samples for this research were collected from aquatic environments such as conventional WWTP, irrigated fields, cesspool holding tank, roadside ditch, home well, and excavation pond in Poland. These samples were taken from sewage, mechanical treated sewage, biological treated sewage, and environmental water. The selected 32 isolated phages belonged to families *Myoviridae*, *Siphoviridae*, and *Podoviridae*. Also, their host range was characterized against 254 clinical *Enterobacteriaceae* strains including multidrug-resistant *Klebsiella* isolates producing extended-spectrum beta-lactamases. Next, it was seen that no other antirestriction mechanisms exists; that is, atypical nucleotides (hmC or glucosyl hmC). In this connection, *Myoviridae* phage KP27 encodes an unknown antirestriction mechanism that needs further investigation. Tartera and Jofre [47] observed phages active against *B. fragilis* in sewage-polluted waters. This researcher tested 12 strains of different *Bacteroides* species for their efficiency of phage detection from sewage and investigated the host range of several isolated phages. The results indicated that there was a high degree of strain specificity. Then, by applying *B. fragilis* HSP 40 as the host, proved to be the most efficient for the detection of phages, feces from humans and several animal species and raw sewage, river water, water from lagoons, seawater, groundwater, and sediments were tested for the presence of phages that were active against *B. fragilis* HSP 40. Phages were detected in feces of 10% of the human fecal samples tested but never detected in feces of the other animal species studied. These phages were B40-1 to B40-27, B2151-1, and B2151-2. Moreover, phages were only recovered from sewage and sewage-polluted samples of waters and sediments, but not from non-polluted samples. Camprubí et al. [48] investigated isolation and characterization of phage FC3-10 from *Klebsiella* spp. on a rough mutant (strain KT707, chemotype Rd) of *K. pneumoniae C3*. The phage receptor for this phage was shown to be the low-molecular-mass LPS fraction (LPS-core oligosaccharides), specifically the heptose content of the LPS inner-core. In the following, it was noticed that the spontaneous phage-resistant mutants from different *Klebsiella* strains were deep-rough LPS mutants or encapsulated rever-trans from unencapsulated mutant strains. Verthé et al. [49] assessed stability and activity of an *Enterobacter aerogenes*-specific phage under simulated gastrointestinal conditions. In this regard, a phage designed as UZ1 with lytic activity against a clinically import strain (BE1) of *E. aerogenes* was isolated from hospital sewage. Chaudhry et al. [50] characterized a virulent phage LK1 specific to *Citrobacter freundii* isolated from sewage water. *C. freundii* is a worldwide emerging nosocomial pathogen with escalating incidence of multidrug resistance. This bacterium exists in natural environments, especially in healthcare settings and is difficult

to eradicate. This phage has a pH range of 5.0–6.0 and the lack of thermal stability as their viability decreases to 0% at 65°C. Using LK1 to infect six other clinically isolated pathogenic strains, a relatively narrow host range was observed. LK1 was capable of eliciting efficient lysis of *C. freundii*, revealing its potential as a non-toxic sanitizer for controlling *C. freundii* infection and contamination in both hospital and other public environments. Zhao et al. [51] characterized phiCFP-1, a virulent phage specific for *C. freundii*. This phage was isolated and characterized by its ability to lyse the multidrug-resistant clinical *C. freundii* strain P10159. Additionally, based on genome content and organization, this phage was categorized as a classic T7-related phage, which is known to have linear genomes with direct terminal repeats. Jamal et al. [52] characterized new *Myoviridae* phage WZ1 against multidrug-resistant *S. dysenteriae*. This phage isolated from wastewater was found to inhibit the growth of *S. dysenteriae*. Phage WZ1 showed a maximum stability at 37°C and was stable up to 65°C but was totally inactive at 70°C. Moreover, pH stability increased from low to high and was totally inactive at pH 3 while the maximum stability was observed at an optimal pH of 7. Phage WZ1 adsorption rate to the host bacterium was significantly enhanced by the addition of $CaCl_2$. Finally, it was concluded that phage WZ1 is a very promising candidate for phage therapy and other applications such as phage typing. Carson et al. [53] researched the use of lytic phages in the prevention and eradication of biofilms of *Proteus mirabilis* and *E. coli*. This researcher applied *E. coli* T4 phage ATCC 11303-B4 and a coli–Proteus phage isolated from a commercially available phage preparation. The prevention of biofilm formation on Foley catheter biomaterials following impregnation of hydrogel-coated catheter sections with a lytic phage has also been investigated in this work. The results showed an approximate 90% reduction in both *P. mirabilis* and *E. coli* biofilm formation on phage-treated catheters compared with untreated controls. Day [54], through studying bacterial sensitivity to phage in the aquatic environment, disclosed that phage P1 with a temperate infective cycle can be effective against *E. coli* and other enteric bacteria.

3. Other waterborne bacteria specific phages

Some waterborne pathogens bacteria (rather than being coliform) such as *V. cholerae*, *Clostridium botulinum*, *Mycobacterium*, *Leptospira*, *Bacillus anthracis*, *Legionella*, *Staphylococcus*, *Listeria monocytogenes* and *Campylobacter jejuni* are important in health issues. According to Alisky et al. [55], the majority of these bacteria can be controlled and removed by applying phages as antimicrobial agents. Zhilenkov et al. [56] demonstrated the ability of flagellum-specific *Proteus vulgaris* phage PV22 to interact with *C. jejuni* flagella in culture. Later on, this researcher illustrated that a phage that productively infects *P. vulgaris* is able to bind *C. jejuni*. Moreover performing a spot test, the growth of *C. jejuni* is reduced relative to control bacteria in the region of phage application. There are two potential interesting applications of this effect. First, it may be possible to test phage PV22 as an antimicrobial to decrease *C. jejuni* colonization of the chicken intestine. Second, the phage could potentially be utilized for investigating biogenesis of *C. jejuni* flagella. Letchumanan et al. [57] reviewed phage application in controlling *Vibrio* species and found that phages have the ability to control luminous vibriosis among *Vibrio* species. Also, phages have great potential as biocontrol agents to control and inhibit virulence of *Vibrio* species isolated from both clinical and environmental samples. Moreover, this study represented that lytic phage as VP4B can be useful in significant growth inhibition of pathogenic *Vibrio harveyi* and biological control of *Vibrio* diseases in mariculture. In 2006, US Food and Drug Administration (FDA) approved the use of commercial phage cocktail ListShield™ targeting *L. monocytogenes* [58]. Faruque and Mekalanos [59] studied pathogenicity, islands, and phages in *V. cholerae* evolution and reported that the phages VPIΦ, KSF-1Φ, and CTXΦ are effective against *V. cholerae*. Synnott et al. [60] isolated and characterized novel *S. aureus* phages with wide host ranges and potent lytic capabilities. The host ranges of 52 phages isolated from sewage influent were determined by performing spot tests with the 15 *S. aureus* isolates, and two phages were subsequently chosen for further analysis. SA039 had the widest host range, producing clear plaques on 13 of the 15 isolates (87%), while SA012 produced clear plaques on 8 isolates (53%) as the only phage that could so on a non-mastitic *S. aureus* strain. Bielke et al. [61] observed *Salmonella* host range of phages that infect multiple genera. This researcher isolated WHR phages from a common phage source (wastewater). Not all phages were as host specific as the types used for bacterial typing. Moreover, these types of WHR phages were detected as potential candidates for the treatment of bacterial infections.

Overall, the related diseases based on Ahiwale's study [13] and phages specific to total mentioned bacteria based on the conducted studies are given in Table 1.

Since phages are among the typical members of microbial ecosystem of the gastrointestinal tract of animals and humans [75], the majority of phages indicated in Table 1 can be commonly isolated from community wastewater streams (municipal and slaughterhouse wastewater) and animal feces. Therefore, Santos et al. [67] disclosed that phage PVP-SE1 isolated from a German wastewater plant presents a high potential value as a biocontrol agent and as a diagnostic tool, even compared with the well-studied typing phage Felix 01, due to its broad lytic spectrum against different *Salmonella* strains.

4. Phage applying and restrictions

There are some cases of phage applying to reduce pathogens in water and wastewater as environmental and health approach. For instance, application of phages to selectively remove *P. aeruginosa* in water and wastewater filtration systems was evaluated by Zhang et al. [76]. Phages for this research were isolated from wastewater by the double layer agar method. The obtained results suggest that phage treatment can selectively remove pathogenic bacteria with minimal impact on beneficial organisms from attached growth systems for effluent quality improvement. In another study, Goldman et al. [77] showed inhibition of biofilm formation on ultra filtration (UF) membrane using specific phages and the potential use of specific lytic phages to prevent UF membrane biofouling. Additionally, future application of specific phages in other membrane processes such as nanofiltration and reverse osmosis, that encounter less bacterial species diversity, can be successful. However, different environmental

Table 1
List of major waterborne pathogens, the related possible diseases (or symptoms) and phages

Pathogen	Disease (or symptoms)	Applied bacteriophages
E. coli (enteropathogenic strains)	Hemorrhagic colitis	M2, ECP-100, DC22
Klebsiella	Nosocomial infection, pneumonia	FC3-10, *Myoviridae* phage KP27 encodes
Shigella	Bacillary dysentery	SF9, WZ1
Pseudomonas	Otitis externa, skin infections	T7-like phage, phage ΦIBB-PF7A δ, I, and 001A [62]
Staphylococcus aureus	Wound, ear, and skin infections	MR11φ [63], SA039 ΦH5, ΦA72, and cocktail of these lytic phages [64]
Mycobacterium	Swimming pool granuloma, hypersensitivity, pneumonitis, leprosy, tuberculosis	CU 14A [65]
Salmonella spp.	Typhoid, salmonellosis	Cocktail of phages designed as sww65, sww275, and sww297 [66], PVP-SE1 [67] WHR (with wide-host-range)
Leptospira spp.	Hemorrhagic jaundice, aseptic meningitis, leptospirosis (Weil's disease)	LE1, LE3, LE4 [68]
Vibrio cholerae	Cholera	Phage cocktail (ATCC – B1, B2, B3, B4, B5), lysogenic filamentous phage as CTXΦ VPA, KSF-1Φ, and VPIΦ phages
Legionella	Legionellosis (Pontiac fever and Legionnaire's disease)	*Myoviridae* family as ΦLP6, ΦLPJ1, ΦLPJ5, ΦLPJ6, and cocktail of these [69]
Clostridium perfringens	Gastroenteritis (food poisoning)	ϕ3626, ΦCP39O, ΦCPV1, and ΦCP26F [70–72]
Listeria monocytogenes	Listeriosis	P100, A511 [73], and phage cocktail ListShield™
Streptococcus faecalis	Endocarditis, septicemia, urinary tract infections, meningitis, and other infections in humans	Q69 [74]
Bacteroides fragilis	Endogenous infections in humans, intra-abdominal, diabetic foot and obstetric–gynecologic tract, endocarditis	B40-1 to B40-27, B2151-1, and B2151-2
Enterobacter aerogenes	Bacteremia, lower respiratory tract infections, skin and soft tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, central nervous system infections, ophthalmic infections, bone and joint infections	Designed as UZ1
Campylobacter jejuni	Campylobacteriosis	PV22

physicochemical factors such as acidity, temperature, salinity, and ions influence the incidence, viability, and storage of phages and can deactivate a phage through damage of its structural elements (head, tail, and envelope), lipid loss, and/or DNA structural changes [78,79]. Besides, there are other potential restrictions to applying phages for phage therapy especially disinfection that has been mentioned in some studies (Table 2). One of these restrictions is host specificity through which host range for bacteriophages is influenced by the specificity of interaction between phage attachment structures and host cell surface receptors. Host range for aquatic phages is usually supposed to be narrow [80]. However, *Cyanophages* unusually show a broad host range [81].

Phages are omnipresent in nature since their abundance in the aquatic environment is ranging from 10^4 mL^{-1} to excess of 10^8 mL^{-1} [82]. Therefore, the restrictions cannot prevent developing disinfection or pathogen reduction studies based on phages and their applications with this purpose. Phages are simple and inexpensive to isolate, produce, and store. They may also be applicable for utilization in technologically less developed regions. The bacteria can simply mutate and become resistant to an individual phage. Thus, using a single phage for control is risky [83].

Successful phage treatment of wastewater bacterial pathogens depends on the prevalence and diversity of pathogen groups in the wastewater. Also, it is almost impractical to produce phage targets for all pathogenic serotypes as a high diversity of *E. coli* and *Salmonella* exists in the wastewater. However, since the biological and non-biological essentially reduce the numbers of pathogenic bacteria, the additional reduction potential of the plenty as semi-disinfection will be provided for specific and dominant pathogenic bacteria

Table 2
Comparison of different disinfectants with phages in water and wastewater effluent disinfection

Disinfectant	Advantages	Disadvantages
Ozone [8,12,88]	(1) Powerful disinfection action; easy removal of spores, bacteria, viruses, and cysts (2) Destruction of color (3) Produced on site and hence avoids problems associated with transport and storage of dangerous chemicals (4) Little evidence of production of potentially dangerous breakdown products	(1) Expensive (2) No residual disinfecting action (3) Measurement difficulties (4) Toxicity of reactant (5) Low solubility under operational conditions (6) Elevated skill levels required for operation
UV [15,88,89]	(1) Effective against many microbe types (2) No chemical by-products or toxics (3) It is a physical process that eliminates any effect from the need to generate, handle, transport, or store toxic/hazardous or corrosive chemicals (4) UV does not increase the TDS of the treated water unlike chlorination/dechlorination (5) User-friendly and safe for operator	(1) Penetration capacity through water limited (2) Color, turbidity, and organics efficiency (3) No residual effect (4) UV harmful to eyes, skin
Chlorine dioxide (ClO_2) [1,15,88–90]	(1) Strong oxidant, long residual (2) Effective against many microbes (3) More effective than chlorine at short contact (4) Not reactive with ammonia or aromatic organics to yield THMs[a] (5) Forms chlorinated organics less readily than chlorine	(1) High cost (2) Produced by-products or halogenated disinfection products (e.g., THMs and HAAs[b]) when producing excess chlorine (3) Corrosive
Electrochemical and photocatalysis processes [1,14,88]	(1) No chemicals: no disinfectant removal needed, fewer impacts on water quality (e.g., from disinfection by-products), and lower safety risks posed by chemicals (2) It can degrade organic contaminants that being microbial shelters (3) Production of free chlorine from the chloride content of water and electrochemical ozone	(1) No residual disinfectant (2) Lack of basic long-term information (deactivation of the catalyst, and practical applicability at high flow) (3) Probably ineffective in low-transmittance waters (4) Complicated electrochemical ozone production system
Ultrasonic [88,89]	(1) Effective against many microbe types (2) Aids hardness removal (3) No chemicals: no disinfectant removal needed, fewer impacts on water quality (e.g., from disinfection by-products), and lower safety risks posed by chemicals (4) It works better in waters with higher solids contents	(1) Thick films of water attenuate sound and reduce effectiveness (2) Expensive (3) Difficult to control
Phages [83, 91]	(1) Phages are self-replicating and self-limiting; they replicate only as long as the host bacterium is present in the environment, but are quickly degraded in its absence (2) Phages are natural components of the biosphere; they can readily be isolated from wherever bacteria are present, including soil, water, plants, and animals (3) Phages are non-toxic to the eukaryotic cell. Thus, they can be used in situations where chemical control is not allowed owing to legal regulations (4) Phages are specific or highly discriminatory, eliminating only target bacteria without damaging other, possibly beneficial (5) Phage preparations are fairly easy and inexpensive to produce and can be stored at 4°C for months without significant reduction in titer (6) No chemicals	(1) Mechanisms for effective phage delivery need to be developed (2) Dosages and formulations may need optimization (3) No single phage can protect against all strains of each coliform bacteria (4) Lysogenic phages need to be avoided (5) Potential restrictions mentioned in Table 3

[a]Trihalomethanes.
[b]Haloacetic acids.

strains such as *E. coli O157* by applying the related phages (e.g., phage DC22 for *E. coli O157* strains) [84]. Water fluoridation, is the controlled addition of fluoride to the water supply and water treatment plants (WTPs), with the aim of reducing the prevalence of dental caries [85]. Applying phages and phage-encoded antibacterial enzymes into mouthwash solutions can reduce and prevent dental decay and oral diseases with dental plaques removal, and it is favorable for the removal or reduction of fluoride into this solutions and even drinking waters; as a result, the cost of adding fluoride as fluoridation to drinking waters in WTPs would be declined. Many phage therapies related patents exist for dental decay and mouth diseases control, with some of them mentioned by Lad [86]. For example, Fischetti and Loomis [87] presented a patent of employing bacterial phage associated lysing enzymes for treating bacterial infections of the mouth and teeth. Also, the large-scale production of phages can be provided easily in the laboratory based on these patents when phage resource is considered.

According to Letchumanan et al. [57], phages especially vibriophage can be isolated from environments such as wastewater treatment ponds or facilities, natural pools, etc. Also, these phages can be successfully applied in aquaculture and hatchery waters for the removal of *Vibrio* pathogens (e.g., *Vibrio parahaemolyticus* contamination). This application is well depicted in Fig. 1 and is a clear example of the application of biological disinfection without chemicals or other approaches. The advantages for this phage applying can be (1) reducing the plasmid mediated multidrug-resistant pathogen, (2) specificity, (3) antibiofilm, and (4) virulence inhibition. The other advantages, as well as disadvantages in comparing with other conventional disinfection approaches for water and wastewater effluents, are presented in Table 2. Overall, potential restrictions to apply phages in water and wastewater disinfection are considered in Table 3. As shown in the table, specificity is a limitation here.

Besides, phages improve wastewater treatment processes by influencing microbial populations in wastewater to control foam-causing microorganisms in activated sludge systems and remove pathogenic bacteria [106]. As a result, the quality of secondary clarifier effluent or disinfection influent

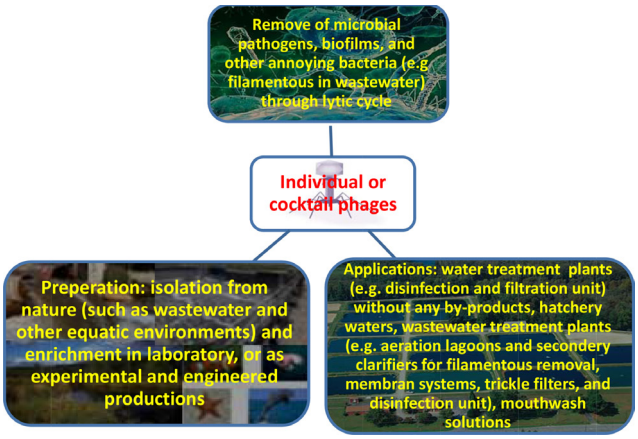

Fig. 1. Diagram of possible phage therapy role in water and wastewater treatment, and aquaculture: from detection and isolation to applications.

in WWTP s and consequently the efficiency of disinfectant would be enhanced. It is an indirect impact on disinfection of the treated wastewater effluent in treatment plants. In this regard, a review study entitled as "the role of phages in membrane-based water and wastewater treatment processes" carried out by Wu et al. [107] well explains the phage-based method in biofouling control.

5. Phage cocktails

The problem of bacterial resistance to infection, which is a well-documented phenomenon associated with phage predation, is resolved using a number of different phages in combination with each other as a phage cocktail [108,109]. Phage cocktails not only potentially provide a means to circumvent resistance to a single phage but also allow the treatment of multiple pathogens simultaneously [110]. The phage cocktails application has well been pointed out by different studies [109,111,112]. For instance, Perera et al. [113] observed that commercially phage cocktails such as ListShield™ significantly reduce or eliminate *L. monocytogenes* contamination on lettuce, apples, cheese, smoked salmon, and frozen foods. Also, Jensen et al. [114] isolated a phage from environmental waters by enriching it with two different bacterial host species (as compared with a single host). Using this method, they found a phage with lytic activity against *Sphaerotilus natans*, *P. aeruginosa* (multiple strains), *E. coli*, *Shigella flexneri*, *P. vulgaris*, and *Rhodospirillum rubrum*. Hence, it was revealed that the limitation of natural host specificity could overcome by designing phage cocktails with a wide range of activity using one or more of the approaches described. Turki et al. [66] evaluated a cocktail of three bacteriophages for the biocontrol of *Salmonella* spp. of wastewater. In this study, phages infecting *Salmonella* spp. were isolated from wastewater and evaluated for (1) their potential to lyse environmental *Salmonella* strains in vitro at different multiplicity of infections (MOIs) and temperatures, and (2) to control the wastewater bacterial community. In this work, three distinct phages (sww65, sww275, and sww297) were obtained from wastewater. Challenge tests were performed at 37, and 30°C with the infection of the *Salmonella* cultures with individual phage, a mixture of two phages, and a cocktail of three phages at MOIs of 10^0, 10^2, and 10^4 PFU/CFU. At 30°C and 37°C a cocktail of three phages reduced all the *Salmonella* cultures tested. Moreover, the dynamic monitoring of *Salmonella* community during wastewater treatment was performed using PCR detection of virulence gene invA. The results correlated with the ERIC-PCR fingerprints and suggested that *Salmonella* community was affected by the phage treatment. Indeed, in wastewater, phages reduced *Salmonella* and other members of the *Enterobacteriaceae*. These results indicated that dynamic changes are closely related with the process of treatment. The introduction of wide host range phages in wastewater can have a potential impact on the dynamics of the microbial communities, manifested by the reduction or the elimination of microbial species.

6. Conclusions

The main advantages of applying phages to reduce pathogens are that they are (1) non-toxic to eukaryote cells, (2) ubiquitous in aquatic environments and simple isolation

Table 3
Potential restrictions to apply phages in water and wastewater disinfection

Restrictions	Description	Possible solution
Host specificity [13,27]	Phages are host specific and a particular phage preparation cannot be used to control different types of microorganisms [13]	(1) Use of polyvalent phages (2) Phage cocktails; phage targeted toward dominant pathogen strains only
Phage isolation and production [13,23]	The specific phage can be both isolated and enriched to produce sufficient numbers for the phage therapy application [92]. Isolation and enrichment are two important steps in the large-scale production of phages. Phage Enrichment involves mixing environmental samples (source of phages) and specific host strain in enrichment media with overnight incubation [93]	(1) Further research into identification of bacteria responsible for process failure (2) Isolation of alternative host (3) Use multiple host methods of enrichment where possible
Host resistance [13,83]	Bacteria can easily mutate and become resistant to an individual phage [83,94]. Experimental studies have shown that pseudolysogeny (a transient immunity to infection induced by infected hosts) can be one of the reasons for this behavior [95–97]	(1) Disinfection or reduction cycles with different phage (2) Use of phage cocktails
Decay and loss of infectivity [13]	Removal of viruses during activated sludge treatment occurs by viral adsorption to sludge flocs [37]	(1) High phage dosing (2) Isolation of phage from wastewater environment which is adapted to survive conditions (3) Further research on conditions affecting phage survival in wastewater/sludge (4) Timing of application of treatment
Transduction [23]	Transfer of genetic material between bacterial cells can appear through temperate transducing phages. Transduction arises when host DNA is mistakenly packaged into the phage capsid during production of the viral progeny [98,99]	(1) Carefully select lytic phages (2) Screen for genetic homology with known lysogenic genes (3) Select narrow host range phage for highly virulent strains
Stability of phages [79]	As mentioned above, unfavorable physicochemical factors such as pH [100] (low acidity can reduce the efficiency of phages as Leverentz et al. [101] was observed), low and high temperature [34,102], exposure to sunlight radiation and in particular UV, desiccation, leaching [83] salinity, and ions [103,104] affect growth and reproduction and may reduce phage populations	(1) Phage inoculation should coincide with a bacterial population density sufficient to support phage replication [105] (2) Loss of phage infectivity needs to be fulfilled reapplying phage preparations constantly (3) It could constrain the practicality of some phage treatments

from wastewater, (3) capable of removing or reducing chemicals as disinfectant in WWTPs, (4) safe to beneficial bacteria because of their specificity, (5) capable of simple preparation and storage, (6) the remove of fluoridation from drinking waters in WTPs by sinking culture of applying phages that being useful for mouth and dental biofilm removal in latest water consumption point as home, schools, etc., and (7) antibiofilm. Eliminating restrictions such as specificity and narrow host ranges will be realized using bacteriophage cocktails that are the spontaneous use of several phages. Also, the use of filamentous bacteria specific phages in aeration lagoons and secondary settling section will improve the quality and efficiency of final effluent disinfection as indirect in a WWTPs. The pH ranges close to neutral probably is favorable for applying them in the environment. Hence, almost all reviewed studies showed that some bacteria (e.g. *E. coli*, *S. aureus*, *Salmonella*, *Clostridium perfringens*, *Vibrio*, and *Legionella*) have several phages, while other waterborne bacteria (e.g., and *Streptococcus faecalis*, *Mycobacterium*, *B. anthracis*) have a few or even no specific phages. Overall, it is necessary to conduct some experimental investigations on specific phages on these bacteria as in vitro. Besides, to reduce restrictions, it is required to conduct further researches on stability, host specificity, transduction, host range, and other limitations. Another noteworthy point is that it is likely that the use of phages along with chemical and non-chemical disinfectants in water and wastewater disinfection in treatment plants would probably decrease the amount of this disinfection. However, prospective studies for developing of identification, isolation, and production of fecal coliforms and other waterborne bacteria for a demonstration of this review are needed.

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