Bacteriophages in Wastewater Treatment: Can They Be an Approach to Optimize the Anammox-based Processes?

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Abstract

In this paper, we explore the applications of bacteriophages and the advantages of using these viruses to elucidate the possibility of applying them to control substrate competition organisms in anammox-based processes. The partial nitrication/anammox offers economic and structural advantages compared to conventional nitrogen removal processes. However, the appearance of substrate competing bacteria is typical, as is the case of nitrite-oxidizing bacteria (NOB), which tend to grow unrestrainedly in the system, converting all ammonia to nitrate, globally impacting the system once substrate availability is compromised. Thus, control measures to prevent this substrate competition are vital for process efficiency. We used a literature review depicting the main applications of bacteriophages in wastewater treatment plants. Initial studies demonstrated bacteriophages have great potential to be applied in anammox-based processes, such as partial nitrication/anammox (PN/A) acting to combat NOB, a valuable economic and environmental tool to improve the efficiency of nitrogen removal processes. Although these viruses have already been studied in various applications to optimize treatment plant processes, technology transfer remains a challenge due to the limitations of the technique and the complexity of biological systems. The research development focused on application strategies in conjunction with molecular biology techniques can expand this study area, enabling the discovery of bacteria and bacteriophages.

1. Introduction

Bacteriophages are viruses that parasitize bacteria (Kwiatek et al. 2020). They are considered the most abundant beings in the biosphere. It is estimated that there are $10^{31}$ bacteriophage particles distributed in the most diverse environments of the planet, and this number is ten-fold higher than bacterial populations (Mann 2005). In natural aquatic environments, such as the oceans, bacteriophages have been related to vital functions, promoting the formation of microbial communities, acting in the biogeochemical cycles, and gene transference (Liu et al., 2021).

Bacteriophages mostly have two primary forms of replication: lytic and lysogenic. Virulent bacteriophages adopt a lytic replication cycle. Viral reproduction occurs inside the bacterium, causing its lysis and destruction by releasing new viral particles (virions) with the potential to bind to new bacterial cells. In the lysogenic cycle, its genetic material is integrated into the host genome and remains indefinitely as a prophage. This material replicates concomitantly with the host bacterium's genome duplication (Doss et al., 2017).

Are estimated bacteriophage concentrations at approximately $10^8$-$10^9$ particles mL$^{-1}$ in biological wastewater treatment systems. The presence of bacteria is also abundant in these systems (Du et al. 2021). Although bacteria are the key players in these systems, some species can be problematic, causing competition with the target organisms harming the process globally. There are still no practical solutions for this. Therefore, bacteriophages may be viable alternatives (Mathieu et al. 2019). These viruses have been showing potential to control environmental problems arising from the wastewater process, such as:
reducing foam formation caused by microorganisms; dehydration capacity and sludge digestibility; fighting pathogenic bacteria; and reducing competition between unwanted bacteria and functionally important microbial populations (Withey et al., 2005; Kim et al., 2019; Tagliaferri et al., 2019; Nielsen and Singleton, 2021; Runa et al., 2021).

Compared to other procedures for wastewater treatment, the use of bacteriophages has some advantages: 1- They are host-specific and do not harm beneficial bacteria; 2- They are environmentally sustainable due to their non-toxic nature; 3- They have high efficiency since they can quickly lyse the target bacteria; and 4 - They are self-replicating meaning dosage reposition is less necessary than with chemical antimicrobial agents (Ji et al., 2021).

The PN/A process combines anaerobic ammonium oxidation (anammox) and partial nitrification and has been extensively studied as an alternative to conventional nitrogen removal processes due to its advantages (Zhu et al. 2008). To ensure the PN/A process's effectiveness, it is necessary to suppress the activity of nitrite-oxidizing bacteria (NOB). Conventionally, this is done by controlling the operational conditions. However, operational strategies can affect the subsequent process and hinder the anammox performance. In this sense, due to the ability of bacteriophages to infect specific bacteria without affecting the process, their use to achieve a successful partial nitrification process becomes an appealing approach.

The application of bacteriophages in wastewater treatment plants is still recent and little explored. There are many gaps in the literature considering bacteriophages as a feasible approach to optimize the PN/A. In this sense, this review summarizes the literature recently reported on the use of bacteriophages in wastewater treatment processes. The use of bacteriophages was critically analyzed, given the limitations and technological challenges so that these viruses can be used to optimize the PN/A process.

2. Bacteriophages As Bacterial Control Agents In Wastewater Treatment Plants

The potential of using bacteriophages in bacterial growth control in wastewater treatment plants has been increasingly explored (Fig. 1). When performing the search for topics "phage" OR "bacteriophage" AND "wastewater treatment" in the Web of Science database, were obtained 806 publications, were about 40% of which published in the last five years. The increase in the number of publications in recent years indicates the growing interest in using bacteriophages to improve wastewater treatment systems and technological advances related to the technique(Mathieu et al. 2019; Sohail et al. 2020), which have enabled more and more applications outside the laboratory.

Analyzing the keywords used in the 806 publications through the VOSviewer software, it is possible to observe some trends and applications already being studied. It is possible to observe that bacteriophages in treatment plants are strongly linked to bacteria such as Escherichia coli (Beheshti Maal et al. 2015)
and related to disinfection inactivation, sewage, and wastewater. In recent years, words such as bacteriophage therapy and biocontrol have gained prominence.

The use of these viruses for disinfection treatment systems has been expanded in recent years, concomitantly with the increased use of antibiotics in various sectors of society. The emergency in finding solutions to mitigate the problem of antibiotic resistance has done the research with bacteriophages more frequent in areas that focus on disinfection and lysis of undesirable bacteria compared to the application of this technology in an open ecosystem as is the case of effluent treatment plants. Thus, it is necessary to seek efficient methodologies of application appropriate to the reality of the systems we want to apply this virus.

The use of bacteriophages must be accurately characterized for biology and genetic characteristics. The application of lytic bacteriophages, when not well defined, may trigger undesirable virulence factors, such as an adaptive response (Howard-Varona et al., 2017).

Considering the increasing advancement of bacteriophage-related research, we will now discuss the typical applications of bacteriophages in wastewater treatment plants and their potential to control unwanted bacteria in PN/A systems.

### 2.1. Control of foam and sludge forming bacteria

Among the wastewater treatment processes (WWTP), the activated sludge system is one of the most used due to its simplicity and low cost; also, it can be applied to industrial and agricultural effluents (Kamali et al. 2022). Although this process can efficiently treat wastewaters with a high pollutant load, it has the disadvantage of generating foam due to the excessive proliferation of filamentous bacteria (Gnida et al. 2018). This is a common problem in many WWTP, and the main genera associated with are Microthrix, Gordonia, Nocardia, Corynebacteria, Dietzia, Rhodococcus, and Skermania (Liu et al., 2015). The excessive growth of filamentous bacteria directly affects the aerobic granules present in the system, generating several operational problems and affecting nutrient removal efficiency. Among these problems are the reduced oxygen transfer, odor problems, and reduced biomass in the system due to washing out (Collivignarelli et al., 2020; di Bella and Torregrossa, 2013).

The strategies to control these organisms commonly involve chlorine, ozone, iron, and aluminum salt lysis these microorganisms (Henriet et al. 2017). However, the application of the chemicals can be challenging since they can inhibit the bacteria responsible for the process, affecting the removal of nutrients and organic matter and causing the breakdown of flocs (Mamais D et al. 2011). In a study developed by Liu et al., 2015, isolating four bacteriophages from an activated sludge system treating wastewater was possible. A cocktail using GordTnk2, Gmala 1, GordDuk1, and Gsput1 significantly reduced the genus Gordonia, resulting in a process ten times more satisfactory than reactors without no treatments. Another successful example is Petrovski et al., 2011 who isolated a bacteriophage from a WWTP in Australia. GTE7, as it was named, was tested against 65 strains of mycolate, causing the lysis of 3 species of Gordonia and 2 species of Nocardia, characterizing the GTE7 as polyvalent.
*Sphaerotilus natans* is a filamentous bacterium known for the harmful effects it causes in the WWTP. The bacteriophage called SnaR1 was isolated from a WWTP to control this species. The authors tested the efficacy of SnaR1 in different multiplicities of infection (MOI). For an MOI of 10, there was an 83% decrease in the growth of *S. natans* DSM 6575 after 24 h of infection. The results obtained by the authors are proof of the principle that bacteriophages can play an essential role in controlling the overgrowth of filamentous bacteria (Ferreira et al. 2021).

Three bacteriophages for *Nocardioforms* (NOC1, NOC2, NOC3) were isolated from a laboratory-scale reactor, and after isolation, the application of bacteriophages in bench-scale reactors resulted in a significant reduction in foam. The authors performed several tests on the isolated bacteriophages, and the NOC2 bacteriophage was considered stable after exposure to high temperature and pH, emphasizing that the bacteriophage can withstand seasonal/operational fluctuations. Studies in this area are still limited. However, the control of filamentous and foam-forming bacteria in wastewater may be controlled using a biological method rather than a physical or chemical method (Khaimar et al., 2016).

### 2.2. Pathogen reduction

Pathogenic microorganisms are present in large quantities in WWTP, *E. coli*, *Salmonella spp*, *Staphylococcus aureus*, and *Campylobacter jejuni* are the most common. These bacteria represent a risk to bodies receiving these wastewaters' environmental health (Liu et al. 2021). As efficient and specific bacteria predators, bacteriophages can act, enabling the reuse, recovery, and safe disposal of treated wastewaters to the environment, including the opportunity to return to the source waters (Mathieu et al. 2019).

With the increasing use of antibiotics in the most diverse sectors of society, the dispersion of resistance genes has already been detected in biological WWTP (Luo et al. 2020). The use of bacteriophages can be an alternative to control and eliminate resistant *S. aureus* and *E. coli*. A study pointed out that polyvalent bacteriophages added to an activated sludge system thrived and suppressed multidrug-resistant *E. coli* NDM-1 strains without generating disturbances in the overall heterotrophic bacteria population (Yu et al., 2017).

Other studies reported bacteriophages as control agents of pathogenic bacteria. The bacteriophage AS1 isolated from sewage wastewater was tested against *E. coli* S2, showing lytic activity against this bacterium (Ullah et al. 2022). Another study showed the isolation of two new bacteriophages from Myoviridae and Podoviridae families, acting against *E. coli* SBSWF27, a native strain of *E. coli* present in a municipal WWTP Isfahan (Iran). They also showed positive effects in controlling the *E. coli* strain PTCC1399. Application of the bacteriophage and municipal wastewater treatment resulted in a 22-fold decrease in bacteria (most probable number method) from 2,400 to 110 after two hours of incubation (Beheshti Maal et al., 2015).

To aim *Salmonella spp.* bacteria, three distinct bacteriophages designated sww65, sww275, and sww297 were isolated from wastewater (Turki et al. 2012). The bacteriophage cocktail showed promise in
reducing *Salmonella* both in vitro and in wastewater and other Enterobacteriaceae members. Other bacteriophages isolated to combat *Salmonella* strains were isolated from the sewage wastewater. Five bacteriophages’ strains were applied against *Salmonella enterica* serotype *Typhimurium*, removal results higher than 2 logarithmic units in 4 hours were obtained for this bacterial strain compared to the control group (Heringa et al., 2010).

The use of bacteriophages against methicillin-resistant *S. aureus* was demonstrated by Tan et al., 2020, where ten bacteriophages were isolated from raw sewage wastewater. Two of them, named ΦNUSA-1 and ΦNUSA-10 from the Myoviridae and Siphoviridae family, respectively, exhibited a broad host range, showing more than 80% in controlling the resistant strains tested. In the study of Gautam et al., 2018 a bacteriophage was isolated from the Guheswori wastewater treatment plant (Nepal). The bacteriophage was able to infect *E. coli, Salmonella Typhi*, and *Enterococcus faecalis*. Therefore, wastewater plants are potential sources of bacteriophages isolation for application in the combat and control of pathogenic bacteria.

### 2.3. Improving sludge dewatering

The activated sludge process presents a challenge to managing the amount of sludge produced, making the process economically and environmentally more expensive (Luo et al. 2019). The treatment and disposal of excess sludge represent up to 60% of the total operating cost in wastewater treatment plants from the activated sludge process (Kim et al. 2016). The discharge of filamentous bacteria causes excessive sludge volume and the excessive accumulation of high viscosity extracellular polysaccharide substances (EPS) secreted by bacteria of the genus *Zoogloea* and *Thauerea* (Yang et al. 2017). EPS generates more interstitial water retention present in the sludge, causing low sedimentation capacity due to the large size of the floc, influencing the stability and dehydration of the sludge (Sheng et al., 2010).

Some bacteriophages can act by degrading the bacterial exopolysaccharide capsid to bind to primary receptor sites on the bacterium’s outer membrane. This process is mediated by polysaccharide depolymerase (PDE) enzymes secreted during bacteriophage binding to the bacterial host (Harper et al. 2014). This characteristic has sparked interest in its application to control biofilm formation, triggered by high EPS rates in the system (Tait et al. 2002). These organisms can be applied to treat biofilm-causing bacteria in sewage treatment plants since studies have already shown satisfactory results in other applications. The use of lytic bacteriophage was first demonstrated in the study by Hughes et al., 1998 the lytic bacteriophage SF153b was isolated from sewage and applied to combat two strains of *Enterobacter agglomerans*, EPS-producing bacteria.

In an urban wastewater treatment plant in Poland, about 48 *Proteus mirabilis* specific bacteriophages were obtained, with the ability to lyse the biofilm depending on the bacterial strain (Maszewska et al. 2016). Another excellent example of bacterial degradation associated with biofilm formation was described by Adnan et al., 2020 where bacteriophage MA-1 exhibited activity against *Pseudomonas aeruginosa* – 2995, *P. aeruginosa* – 2949, *P. aeruginosa* – 3007, *P aeruginosa* – 3098, *P aeruginosa* – 3117, and *P. aeruginosa* – 3088. Satisfactory results were also found by Bhattacharjee et al., 2015 where
the lytic bacteriophage DTP1, isolated from a wastewater treatment system, was efficient in combating the bacterium *Delftia tsuruhatensis*, reducing the biofilm formation up to 70%.

Although there are successful applications of bacteriophages for biofilm degradation, in some cases, the excess of filamentous bacteria can hamper bacteriophage diffusion and penetration. In addition, the target bacteria are often protected by non-host bacteria, making it more difficult to spread bacteriophages (Ferriol-González and Domingo-Calap, 2020). Polyvalent bacteriophages can be an alternative to reduce this problem since they can attack a wide range of bacteria with low adsorption rates (Yu et al. 2017b). Another strategy is the enzymes blocking the cell-to-cell communication active in biofilm formation (Pei and Lamas-Samanamud, 2014). Also, synthetic biology is a tool that has been studied to facilitate the penetration of bacteriophage bacteria. Good results were already presented by Lu and Collins, 2007, where T7 bacteriophage expressing DspB (polysaccharide depolymerase) reduced the biofilm by 97.997%, being more efficient than wild bacteriophage.

These characteristics can be selected over time or introduced via genetic modification in bacteriophages. The magnetic nanomaterial applied to bacteriophages can also disrupt biofilm matrices, as they facilitate the bacteriophage-bacteria bond due to the magnetic field generated. The use of bacteriophages shows promise; however, one must optimize ways to create the bond between virus and host, making the application efficient (Li et al., 2017).

### 2.4 Application of bacteriophage in PN/A process

Although the PN/A process has several advantages, such as reduction of aeration costs, low sludge production (Ma et al. 2016), and removal of high nitrogen (Zhu et al. 2008); the competition for substrate between the NOB and the anammox bacteria is a significant obstacle regarding the technological application of the process. This competition occurs because anammox bacteria oxidize \( \text{NH}_4^+ \) to \( \text{N}_2 \) using \( \text{NO}_2^- \) as an electron acceptor. However, NOB also uses \( \text{NO}_2^- \) as a substrate in conventional nitrification processes, oxidizing it to nitrate (Strous et al. 1998; Zhang et al. 2019). Therefore, for anammox bacteria to prevail, only partial nitrification of \( \text{NH}_4^+ \) must occur, preventing the oxidation of \( \text{NO}_2^- \) to \( \text{NO}_3^- \). Thus, for a successful implementation of the PN/A process, the inhibition or wash out of NOB is necessary (Ma et al., 2015).

Generally, operational parameters such as temperature, pH, dissolved oxygen (DO), and solids retention time (SRT) are used as a strategy to suppress NOB activity (de Prá et al. 2016; Hewawasam et al. 2017). However, altering these parameters can impair the activity of the bacteria of interest. In this sense, bacteriophages are exciting and promising, as the specificity of bacteriophages allows biological control of unwanted bacteria without adversely affecting other groups of microorganisms.

Among the NOBs, the *Nitrobacter* and *Nitrospira* represent important groups that compete for substrate in PN/A systems (Koch et al. 2015). Some studies have been developed to reduce these populations in activated sludge systems. Choi et al., 2010 used a strain of *Nitrosospira multiformis*, whose genome contained two prophages. These were activated under certain stress situations, such as exposure to
chemicals, low pH, and high temperature. After undergoing these processes, it was observed that bacteriophage replication occurred and led to an increased abundance of the virus and consequently to a decrease in the number of *N. multiformis* and lower nitrification activity.

The use of molecular biology tools, such as high-throughput sequencing, makes it possible to discover viral metagenomes and bacterial 16S rRNA genes. In this way, it was possible to characterize bacterial and bacteriophage communities present in the sludge of an activated sludge system that treats domestic wastewater. The nitrifying bacteria of the *Nitrosomonadaceae* and *Nitrospiraceae* families decreased significantly when a higher abundance of viral contigs was observed, implying that bacteriophage-mediated lysis may reduce nitrifying in the treatment plant (Liu et al., 2017).

Other studies have found potential bacteriophages in wastewater treatment systems. In the study by Hantula et al., 1991, 49 viruses and their respective hosts were identified, which were present in the aeration system of a municipal sewage treatment plant. In the study done by Wu and Liu, 2009, using the epifluorescence microscopy technique, it was possible to observe many indigenous viruses in different parts of the municipal wastewater treatment system, including morphotypes like bacteriophages. Shapiro et al., 2010, also presented results in which the bacterial community was affected by the bacteriophages present in a full-scale membrane bioreactor treating industrial wastewater.

A bacteriophage was used to remove *P. aeruginosa* in wastewater filtration systems and negatively affect the nitrogen removal process. Although the ammonia concentration was below the detection limit, the NO$_2^-$ concentration in the effluent increased substantially after bacteriophage dosing, indicating that NOB species are more sensitive to changes caused by bacteriophage treatment AOB species. This study suggests that a specific bacteriophage for nitrifying species may not be needed, rather than a polyvalent bacteriophage as it is sufficient to reduce NOB activity and improve de PN/A process (Zhang et al., 2013).

The biota of a nitrification reactor is vast. In a study developed by Brown et al., 2019), the presence of viruses was observed to affect the AOB communities, directly impacting the concentration of ammonia present in the system. The ability of viruses to affect the AOB is an indication that in nitrification systems, there is the presence of bacteriophages and is an indication that it may be possible to find a bacteriophage capable of inactivating NOB to favor partial nitrification.

### 3. Bacteriophages: Limitations Of Use

Although the study and application of bacteriophages in wastewater treatment systems have gained prominence in recent years, there are still few reports of the use of bacteriophages *in situ*, as shown in Table 1. It is still challenging to determine the bacteriophage infection outside laboratory conditions because the biological treatment systems have a complex microbiota with many potential hosts, which may not correspond to the environment reproduced *in vitro* (Liu et al. 2021). Although bacteriophages are known for their specificity, many can be polyvalent, showing interspecies and even inter border infectivity, making it difficult to predict their behavior in a complex environment (Yu et al. 2016).
It is necessary to understand how the insertion of bacteriophages *in situ* conditions can interfere in the microbiota, not disrupting the synergy and balance between the different populations of microorganisms present in wastewater treatment systems. The mechanisms of action and infection of bacteriophages under the target bacteria are already well known. Still, their activity at the ecosystem level and their performance under distinct bacterial communities have not been widely studied (Howard-Varona et al. 2017).

**Table 1: Use and applications of bacteriophages in combating operational problems in effluent treatment plants.**
<table>
<thead>
<tr>
<th>Problem</th>
<th>Bacteria</th>
<th>Test type</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus removal process failure</td>
<td>Candidatus’Accumulibacter phosphatis</td>
<td>In situ</td>
<td>Supernatant from a biological reactor with failure in the phosphorus removal process – direct in the reactor;</td>
<td>(Barr et al. 2010)</td>
</tr>
<tr>
<td>Phosphorus removal process failure</td>
<td>Candidatus Accumulibacter</td>
<td>In situ</td>
<td>Supernatant from an enhanced biological phosphorus removal (EBPR) – direct in the reactor</td>
<td>(Motlagh et al. 2015)</td>
</tr>
<tr>
<td>Phosphorus removal process failure</td>
<td>Microlunatus phosphovorus</td>
<td>In vitro</td>
<td>Supernatants of activated sludge processes - Plaque formation - PYA medium</td>
<td>(Lee et al. 2006)</td>
</tr>
<tr>
<td>Biomass bulking</td>
<td>Haliscomenobacter</td>
<td>In vitro</td>
<td>Mixed liquor- Biomass sample from a full-scale wastewater treatment plant – Plaque formation</td>
<td>(Kotay et al. 2011)</td>
</tr>
<tr>
<td>Biomass bulking</td>
<td>Tetrarphaera jenkinsii</td>
<td>In vitro</td>
<td>Activated sludge plant – Plaque formation</td>
<td>(Petrovski et al. 2012)</td>
</tr>
<tr>
<td>Foaming in wastewater treatment</td>
<td>Rhodococcus</td>
<td>In vitro</td>
<td>Wastewater treatment plants – Plaque formation</td>
<td>(Petrovski et al. 2013)</td>
</tr>
<tr>
<td>Foaming in wastewater treatment</td>
<td>Gordonia terrae, Rhodococcus globulus, Rhodococcus erythropolis, Rhodococcus erythropolis, Nocardia otitidiscaviarum, and Nocardia brasiliensis</td>
<td>In vitro</td>
<td>Wastewater treatment plants- Plaque formation</td>
<td>(Petrovski et al. 2011b)</td>
</tr>
<tr>
<td>Foaming in wastewater treatment</td>
<td>Skermania piniformis</td>
<td>In vitro</td>
<td>Activated sludge sample – Plaque formation</td>
<td>(Dyson et al. 2016)</td>
</tr>
<tr>
<td>Foaming in wastewater treatment</td>
<td>Tsukamurella inchnonensis</td>
<td>In vitro</td>
<td>Isolated and subsequently purified from an activated sludge sample -Plaque formation</td>
<td>(Dyson et al. 2015)</td>
</tr>
<tr>
<td>Pathogenic bacteria</td>
<td>Acinetobacter johnsonii</td>
<td>In vitro</td>
<td>Bulking sludge – Plaque formation</td>
<td>(Fan et al. 2019)</td>
</tr>
</tbody>
</table>
### Problem

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Test Type</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pathogenic and biofilm-forming bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>In vitro</em></td>
<td>Crude sewage from a sewage treatment plan – Plaque formation</td>
<td>(Kwiatek et al., 2017)</td>
</tr>
<tr>
<td><strong>Pathogenic bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em></td>
<td><em>In vitro</em></td>
<td>Poultry slaughterhouse wastewater – Plaque formation</td>
<td>(Sonalika et al., 2020)</td>
</tr>
<tr>
<td><strong>Pathogenic bacteria</strong></td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td><em>In vitro</em></td>
<td>Urban sewage – Plaque formation</td>
<td>(Costa et al. 2020)</td>
</tr>
</tbody>
</table>

Bacteriophage replication *in situ* depends on its ability to survive in adverse situations and protect its genome. For replication to occur, it needs to freely access the host to initiate the infection process making the bacteria a progeny-forming unit (Principi et al., 2019). The infection efficiency can be influenced by several factors, among them: physical-chemical conditions (e.g., pH, temperature, presence of some ions); the presence of physical barriers that make access to the target difficult; viral concentration (bacteriophage x bacteria ratio); specificity in lysing and infecting the target bacteria; and bacterial resistance. (Ly-Chatain, 2014).

During viral infection, bacteria can develop defense mechanisms against the invading organism, limiting the use of bacteriophages. Bacteria can change or lose their receptors, expelling substances that prevent the adhesion of the bacteriophage to the host and inhibiting the replication and release of the virus (Seed et al., 2012). The biofilm mechanism expressed by some bacterial species affects the adsorption, penetration, diffusion, and proliferation of bacteriophages. Furthermore, bacteria can recognize bacteriophage-specific nucleic acid and destroy them, preventing infections (Amankwah et al. 2021). This and other mechanisms that confer antibacterial resistance can decrease using a bacteriophage cocktail (Principi et al., 2019).

However, cocktails are not a guarantee of success in all cases. Thus, considering the different factors that interfere in the efficiency of using these viruses, the importance of developing methods that validate the effectiveness of bacteriophage treatment when applied in microbial control is evident. Therefore, improving and developing strategies to predict the bacteriophage-host relationship and the bacteriophage-environment relationship is vital.

### 4. Technological Challenges And Future Perspectives

Thus, bacteriophages have great potential to be applied in PN/A processes and can be a valuable economic and environmental tool to improve the efficiency of nitrogen removal processes. However, although bacteriophages have already been studied in several applications to optimize treatment plant processes, technology transfer is still a challenge due to the limitations of the technique. However, despite the apparent difficulties of applied microbiology, the challenge has also not yet been overcome with engineering tools alone (control of operating parameters).
Many bacteria can still be studied and fought with bacteriophages, so the development of new research allied with molecular biology techniques can help track bacteria and bacteriophages, allowing the expansion of this research area. Furthermore, it is necessary to intensify and demystify the fear of using viruses as biological control tools, focusing on the fact that it is possible to use these organisms in a very safe condition.

Declarations

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Bruno Venturin: Conceptualization, Investigation, Data Curation, Visualization

Naiana Cristine Gabiatti: Conceptualization, Visualization, Writing - Original Draft, Writing - Review & Editing, Funding acquisition.

Marcelo Bortoli: Conceptualization, Visualization, Writing - Review & Editing.

Airton Kunz: Conceptualization, Visualization, Funding acquisition.

Marina Celant De Prá: Writing - Review & Editing, Project administration, Funding acquisition.
Data availability: All data generated or analyzed during this study are included in this article.

References


64. Sohail HA, Coffey A, Debrowska K et al (2020) Bacteriophages: Emerging Applications in Medicine, Food, and Biotechnology. PHAGE 1:75–82. https://doi.org/10.1089/phage.2020.29004.has


Figures
Figure 1

Publications related to "phage" OR "bacteriophage" AND "wastewater treatment" by year

Figure 2

Mainly keywords related to "bacteriophages" and "wastewater treatment".