World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Application of Coliphage in treatment of wastewater

Aakash Dayanand Saini, Huda Nisar Shaikh and Pratibha Jinesh Shah *

Department of Microbiology, Kishinchand Chellaram college, Mumbai-400 020, India.

World Journal of Biology Pharmacy and Health Sciences, 2024, 20(03), 396-400

Publication history: Received on 02 November 2024; revised on 15 December 2024; accepted on 18 December 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.20.3.1014

Abstract

Phages have diverse applications in the domains of therapeutics, diagnostics, bioremediation, and biotechnology. Coliphages are a group of viruses that infect coliform bacteria. In the last decade, coliphages have gained significant attention for their application in sewage water treatment as a tool to reduce coliform load. The main objective of this study was to treat sewage water with coliphage and evaluate it for reduction in coliform count. In the current study, isolation, enrichment, and enumeration of coliphage was carried out from a sewage sample. The titre of the enriched phage lysate was estimated to be 4.89 x 10^7 pfu/ml. The pre-treated sewage sample was quantitated for coliform count by the Most probable number (MPN) method, and the count was found to be >2400/100 ml. Post-treatment, the MPN of the phage-treated sewage sample was found to be reduced. After eight hours of incubation, an 80-fold reduction (30 org/100 ml) in MPN count was observed for the treated sewage sample. Chemical oxygen demand (COD) of pretreated and post-treated sewage samples was estimated, and the efficiency of the treatment was calculated to be 50.01%. The current study suggests the application of bacteriophage as a complementary tool for the reduction of coliform in wastewater along with other treatment procedures.

Keywords: Coliphages; Most probable number (MPN); Chemical oxidation demand (COD); Sewage water treatment activity

1. Introduction

Coliforms in sewage water or any wastewater are a signal of faecal contamination and the potential presence of pathogenic microorganisms. These bacteria, including *Escherichia coli*, are found in the intestines of warm-blooded animals and are often excreted in large numbers in faeces. When present in wastewater, coliforms indicate that the water is contaminated with faecal matter, which can harbour harmful pathogens such as viruses, bacteria, and parasites that pose serious health risks. These pathogens can cause diseases like gastroenteritis, cholera, dysentery, and hepatitis. Additionally, high coliform levels in wastewater can complicate the treatment process, necessitating more rigorous and costly treatment measures to ensure compliance with disposal standards [1, 2].

Coliphages are emerging as a promising tool for reducing coliform loads in sewage and wastewater. These viruses naturally prey on coliforms, significantly diminishing their populations and thereby enhancing the overall efficacy of sewage treatment processes. The application of coliphages in wastewater treatment offers a significant advantage, as they replicate within the bacterial cells, amplifying their impact [3]. Employing coliphages in wastewater management could be a cost-effective and environmentally sustainable practice, reducing the need for chemical disinfectants that may have adverse effects on aquatic ecosystems. By integrating coliphages into conventional wastewater treatment systems, we may achieve more efficient and eco-friendly wastewater treatment systems [4,5].

* Corresponding author: Pratibha Shah

2. Materials and Methods

2.1. Sewage sample collection

The current study aims to isolate coliphage from the most promising source, i.e., sewage water**.** Sewage water was collected from Priyadarshini Park in Mumbai, in a sterile and clean container. The collected sewage water was filtered through a membrane of 0.45 µm. The bacteria-free filtrate was used as the source of bacteriophage [6].

2.2. Detection of coliphage and enrichment

A log-phase culture of *Escherichia coli* was prepared and spread evenly onto sterile nutrient agar plates using a sterile cotton swab. Lysate containing phage was then spotted onto the nutrient agar plates. To screen for phage-mediated lysis of coliforms, the plates were incubated at 37 ℃ for 24 hours, and observation of plaques confirmed the presence of coliphage [6]. The host specificity was checked with *Klebsiella* and S*taphylococcus,* species similarly. After the host confirmation, the lysate was enriched as per the standard protocol described by Beheshti et al. [6].

2.3. Enumeration of coliphage by plaque assay

The phage lysate was diluted in sterile saline solution (pH 7) through 10-fold serial dilutions. Then, 0.1 mL of the diluted lysate was mixed with 0.3 mL of a 4-hour-old culture of *Escherichia coli* (host bacterium) in a sterile tube and incubated for 20 minutes to allow for phage adsorption. Following the incubation period, 7 mL of molten sterile soft agar was added to the tube containing the lysate-bacteria mixture, thoroughly mixed, and overlaid onto basal nutrient agar plates. After solidification, the plates were incubated at 37 ℃ for 24 hours to allow plaque formation [7].

2.4. Optimisation of conditions for propagation of phage

For thermal stability analysis, 5 mL of phage lysate was aliquoted into five sterile test tubes, and the tubes were subjected to temperatures of 37 ℃, 50 ℃, 60 ℃, 70 ℃, and 80 ℃ for 30 minutes each. Following this treatment, the tubes were allowed to cool to room temperature, and the viability of each lysate was assessed using spot assay, as described above.

For pH stability evaluation, the pH of sterile nutrient broth was adjusted to 1, 3, 5, 7, 9 and 13. One ml of phage lysate was added to each pH-adjusted broth and incubated at 37 ℃ for 1 hour. Spot assay, as described above, was conducted to detect phage presence [7].

2.5. Estimation of coliform count (MPN method)

Water samples were inoculated into double and single-strength Lauryl tryptose broth (LTB) tubes, with varying volumes, and incubated at 37 37 ℃ for 24 to 48 hours, as per standard protocol. MPN analysis of the pre- and post-treated sewage samples was performed. For phage treatment of sewage samples, purified coliphage was added to sample, followed by incubation at 37 37 ℃ for various time regimes. MPN analysis was conducted at regular intervals post-treatment (2, 4, 8, and 24 hours) to assess changes in coliform count in the sewage sample [6].

2.6. Chemical Oxygen Demand (COD) estimation

Sewage samples were titrated with potassium dichromate and ferrous ammonium sulphate to determine COD, as per standard protocol [8]. Titrations were performed in triplicate, data were recorded, and COD was determined for pre- and post-treated sewage samples, along with the efficiency of treatment [8].

3. Result and Discussion

The use of bacteriophages in controlling bacterial populations has been applied in medicine, agriculture, and food industries but has been poorly investigated for wastewater treatment. Raghu et al. have discussed several roles that bacteriophages play in the environment and their role in biofilm control and water treatment [9]. In another study by Zhang and Hu, there was an application of a combined approach of chlorination and phage therapy for biological control of biofilms created by *P. aeruginosa* on various surfaces. They reported that their combined method could eliminate the bacterial biofilm by up to 95%-97% in two days [10]. There are a few reports suggesting that the normal presence of bacteriophages in sewage could be useful in wastewater treatment [11].

In this investigation, the coliphage was isolated from sewage water samples collected from Priyadarshani Park, Mumbai, and *Escherichia coli* was found to be the host organism (fig. 1). After 24 hours of incubation, plaque was observed indicating the presence of phage. Host analysis revealed a narrow host range for the isolated phage, indicating their specific affinity for *E. coli*. This monovalent characteristic suggests that the isolated phage may not effectively target a broader range of bacterial species, potentially limiting their applicability in wastewater treatment settings targeting diverse microbial populations.

Figure 1 Detection of coliphage in enriched lysate by Spot assay

Further, the phage lysate was enriched and enumerated, and the phage titre of the lysate was calculated as 4.89 x 10^7 pfu/ml (fig. 2).

Figure 2 Enumeration of phage by plaque assay

The phage was further evaluated for differential stability of temperature and pH under varying conditions. The isolated phage exhibited a wide temperature tolerance range of 20-70 ℃ alongside resilience to both acidic and alkaline pH levels, within the range of 5 to 9 (table 1).

Table 1 Stability of coliphage at different pH and temperatures

pH	Result	Temperature	Result
1	-	20 ℃	+
3	+	37 ℃	+
5	+	50 ℃	+
7	+	60 ℃	-
9	+	70 ℃	-
13	-	80 ℃	-

Key: (+) plaque observe, (-) no plaque observed

Quantification of coliforms in pre-treated sewage sample was performed using the Most Probable Number (MPN) method. The result indicated high counts exceeding 2400 per 100 ml in the sewage water samples, signifying faecal contamination. Post-treatment, the MPN of the phage-treated sewage sample was found to be reduced. After two hours

of treatment of the sewage sample with coliphage, a 10-fold reduction (240 org/100 ml) was observed. After four hours of treatment, an 18-fold reduction (130 org/100 ml), and after eight hours of incubation, an 80-fold reduction (30 org/100ml), in MPN count was observed for the treated sewage sample (table 2). Thus, it was observed that there was more reduction in coliform count up to eight hours, indicating that the increased duration of exposure resulted in reduction of coliform in sewage water (fig. 3). However, no reduction in coliform count was observed after 24 hours of exposure of the sewage sample to the phage. These results signalled for evaluation of the bacterial growth curve in the presence of coliphage. The subsequent increase after 24 hours could stem from bacterial regrowth, phage decay, or bacterial resistance mechanisms.

Table 2 Enumeration of coliforms in sewage sample before and after treatment with coliphage by MPN method

Incubation time (hours)	Number of Positive tubes Before phage treatment	Number of Positive tubes After phage treatment	Inference org/100ml
2	5-5-5	5-5-0	240
4	5-5-5	5-4-0	130
8	5-5-5	5-1-0	30
24	5-5-5	5-5-5	2400

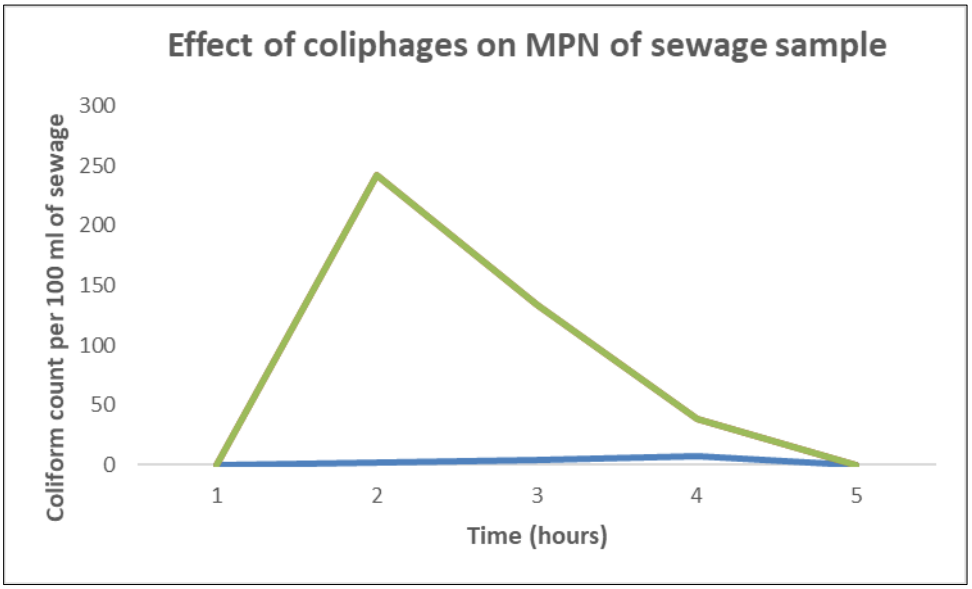

Figure 3 Effect of time on coliform count due to coliphage exposure by MPN method (for 3 hours)

Chemical Oxygen Demand (COD) for untreated sewage water was found to be 53.34 mg/L, and the COD for the sewage sample treated with phage was determined to be 26.66 mg/L. Thus, COD analysis revealed a treatment efficiency of 50.01%, indicating the effectiveness of phage treatment in reducing organic pollutants in the sewage samples. This finding underscores the potential of phage-based treatment approaches in wastewater management.

The findings of this investigation are in accordance with another study conducted in Iran by Beheshti et al. In that study, isolation and identification of two novel lytic myovirus and podovirus from the Zayandehrood River in Isfahan was performed, and they observed a reduction in coliform's population of Isfahan municipal wastewater. They suggested that the use of lytic coliphages can cause the reduction of coliform population in sewage and can serve as an effective alternative for costly equipment of the old wastewater treatment plants [6].

Overall, the results of this study provide valuable insights into the behaviour and efficacy of isolated coliphages in wastewater treatment, paving the way for further research and optimisation of phage-based wastewater treatment strategies.

4. Conclusion

The treatment of wastewater before its release into the environment is a general practice in high-income countries; outbreaks of waterborne infectious diseases are still relatively frequent. The current study was designed with the aim of reducing the coliform count in the sewage water. The results suggest the application of coliphages as a complementary tool for the reduction of the coliform load in sewage water, along with other standard methods, such as the active sludge process. However, the effectiveness of biological control of coliforms by bacteriophages should be monitored at a wastewater treatment plant.

Compliance with ethical standards

Acknowledgement

This research was carried out under the Science Honors Program, and the authors are grateful to the Head of the Department of Microbiology, Kishinchand Chellaram College, Mumbai.

Disclosure of conflict of interest

The authors declare that they have no competing interests with whomsoever.

References

[1] Shivajirao PA. Treatment of distillery wastewater using membrane technologies *Int. J. Adv. Eng. Res. Stud*. 2012,1(3);275-283.

[2] Baggi F, Demarta A, Peduzzi R. Persistence of viral pathogens and bacteriophages during sewage treatment: lack of coorelation with indicator bacteria. *Res Microbiol*. 2001;152(8): 743-51.

[3] Jofre J, Lucena F, Blanch AR. Coliphages as a Complementary Tool to Improve the Management of Urban Wastewater Treatments and Minimize Health Risks in Receiving Waters. Water. 2021; 13(8):1110. https://doi.org/10.3390/w13081110

[4] Bonde GJ. Bacterial indicators of water pollution. *Adv. Aquatic Microbiol*. 1977,1:273-364

[5] Suttle CA. Viruses: unlocking the greatest biodiversity on Earth. *Genome*. 2013;56(10):542-4. doi: 10.1139/gen-2013-0152.

[6] Beheshti MK, Soleimani DA, Salmanizadeh S. Isolation and Identification of Two Novel Escherichia coli Bacteriophages and Their Application in Wastewater Treatment and Coliform's Phage Therapy. *Jundishapur J Microbiol*. 2015,21;8(3):e14945. doi: 10.5812/jjm.14945.

[7] Van TR, Kropinski AM. Bacteriophage enrichment from water and soil. *Methods Mol Biol.* 2009;501:15-21. doi: 10.1007/978-1-60327-164-6_2.

[8] Muhaimin M, Prayoga R, Eniati E. Determination of chemical oxygen demand (COD) concentration in domestic wastewater using UV-Vis Spectrophotometry method based on the effect of reflux time and preservation time. *Stannum : Jurnal Sains Dan Terapan Kimia*, 2022. 4(1), 13-18. https://doi.org/10.33019/jstk.v4i1.2866

[9] Raghu HV, Gaare M, Manjunatha BM, Mishra S, Sawale P. Beneficial face of bacteriophages: Applications in food processing. *Int J Qual Res.* 2012;6(2):101–8.

[10] Zhang Y, Hu Z. Combined treatment of *Pseudomonas aeruginosa* biofilms with bacteriophages and chlorine. *Biotechnol Bioeng.* 2013;110(1):286–95. doi: 10.1002/bit.24630.

[11] Runa V, Wenk J, Bengtsson S, Jones BV, Lanham AB. Bacteriophages in Biological Wastewater Treatment Systems: Occurrence, Characterization, and Function. *Front. Microbiol.* 2021,12:730071. doi: 10.3389/fmicb.2021.730071