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Impacts of oxygen and ozone nanobubbles on bacteriophage in aquaculture system

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ABSTRACT

Injection of ozone nanobubbles into water reduces bacterial load, improves dissolved oxygen, and modulates the fish innate immune system. Little is known about the effect that nanobubble treatment has on the concentration of viruses in water. This study, investigated the disinfection impact of oxygen and ozone nanobubbles (NB-O_2 and NB-O_3) on an *Aeromonas hydrophila*-specific phage, pAh6.2TG, a virus lab model. After 5-, 10- and 15-min treatments with NB-O_2, the concentration of phage remained the same, while the same treatment with NB-O_3 eradicated 99.99 to 100% of the phage in the water. Since this phage has been shown to control bacterial infections in fish, we further investigated whether NB-O_2 improved the adherence of the phage to the body surface of the fish (i.e. skin mucus, and gills) and phage penetration into fish internal organs, specifically the liver. Nile tilapia, *Oreochromis niloticus* were used as experimental fish in this study. The results indicated that the number of phages adhered to the skin mucus and gills in NB-O_2 treatment group was 1.07 to 15.0 times higher than in the untreated control group without gas nanobubbles. The phage uptake into fish liver after NB-O_2 treatment increased 1.29 to 4.75 fold compared to untreated control. These findings suggested a plausible application of NB-O_2 treatment for improving efficacy of phage therapy in aquaculture. On the other hand, NB-O_3 application may be useful for disinfection of harmful viruses in culture water, but the application would need to be omitted during phage treatment. This study provides preliminary information on potential applications of nanobubble technology in aquaculture to reduce viral load in the water.

1. Introduction

Aquaculture is one of the fastest-growing food industries, and it plays a crucial role in global food security and nutrition, particularly in low- and middle-income countries (LMICs) (FAO, 2020; Hicks et al., 2019; Naylor et al., 2021; Webb et al., 2020). However, aquaculture sectors have faced increasing challenges with infectious diseases including antimicrobial resistance (AMR) in microorganisms (Stentiford et al., 2020; Stentiford et al., 2017). Thus, research efforts on non-chemical approaches to controlling pathogens, such as the use of nanobubbles (NBs) have increased in recent years to reduce the risk of AMR and address production losses caused by the emergence of pathogenic AMR

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bacterial strains (Hoelzer et al., 2018; Reverter et al., 2020; Watts et al., 2017).

Nanobubbles are bubbles less than 100 nm in diameter. They can be created with different gases, and have neutral buoyancy, which enables them to have a lengthy residence time in water (Agarwal et al., 2011; Tsuge, 2014). In aquaculture, oxygen nanobubbles (NB-O_2) are commonly used for improving dissolved oxygen (DO) and promoting the growth of aquatic animals (Mahasri et al., 2018; Mauladani et al., 2020; Rahmawati et al., 2020). Recent studies have indicated that ozone nanobubbles (NB-O_3) have potential to reduce pathogenic bacteria, improve DO in water, and modulate the immune systems of fish against bacterial infection (Dien et al., 2021b; Imaizumi et al., 2018; Jhunkeaw et al., 2021; Linh et al., 2021; Nghia et al., 2021). Hitherto, the effect of NB-O_2 and/or NB-O_3 on aquatic viruses remains uninvestigated.

Lytic bacteriophages (also known as phages) are viruses that infect and kill bacteria (Kutateladze and Adamia, 2010). Due to the high similarity to animal virus properties, phages have been considered as models for studies related to the survival of viruses under different environmental conditions and to evaluate the efficacy of disinfection methods (Grabow, 2001; Pinon and Vialette, 2018). On the other hand, phage therapy could also be used as a natural strategy to control bacteria to replace or supplement chemotherapy in aquaculture (Angulo et al., 2019; Culot et al., 2019; Rao and Lalitha, 2015; Richards, 2014). Several studies have revealed that the increase of phage binding to the mucosal layer of the host improved protection against bacterial infections (Almeida et al., 2019; Barr et al., 2013; Barr et al., 2015; Dabrowska et al., 2005). Given the properties of nanobubbles, we hypothesized that depending on the gas used this technology could either destroy phages or enhance their uptake into fish, which might improve their therapeutic function against bacterial diseases.

The effects of NB-O_2 and NB-O_3 treatments on phage concentration in water were explored in this study. Subsequently, we investigated whether NB-O_2 treatment could improve adherence of phages to fish body surfaces and their uptake into the fish.

2. Materials and methods

2.1. Bacteria, phage, and nanobubble system

The bacterial isolate, *Aeromonas hydrophila* BT14 and the *Chaseviridae* phage pAh6.2TG (Genbank accession no. MZ336020.1) used in this study were isolated and characterized in our previous studies (Dien et al., 2021a; Dien et al., 2021b). Prior to phage propagation, *A. hydrophila* BT14 was cultured in 15 mL of Tryptic Soy Broth (TSB; Becton Dickerson, USA) at 28 °C for 24 h. Then, 100 μL of phage (10^8 PFU/mL) was mixed with 100 μL of bacteria in 3 mL of TSB supplemented with 0.5% agar. The mixture was propagated on Tryptic Soy Agar (TSA; Becton Dickerson, USA) incubated at 28 °C for 16 h. Subsequently, a total of 3 mL of SM buffer (100 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris-HCl, pH 7.5) was added to each plate and kept at 4 °C for 24 h. The solution of phage in SM was then collected and centrifuged at 10,000 ×*g*, 4 °C for 15 min. The supernatant was filtered through a 0.2 μm filter. The filtrate was harvested and enumerated by a phage plaque assay as indicated previously (Dien et al., 2021a). Nanobubble system (Model: aQua+075MO, AquaPro Solutions Private Limited Company, Singapore) was set up as previously described (Jhunkeaw et al., 2021) with oxygen input of 2 L/min.

2.2. Effect of oxygen and ozone nanobubbles on phage concentration in water

To evaluate the effect of oxygen (NB-O_2) and ozone (NB-O_3) nanobubbles on phage concentration in water, three groups with 2 replicates each were used (Fig. 1A): group 1 (control group) used normal aeration,

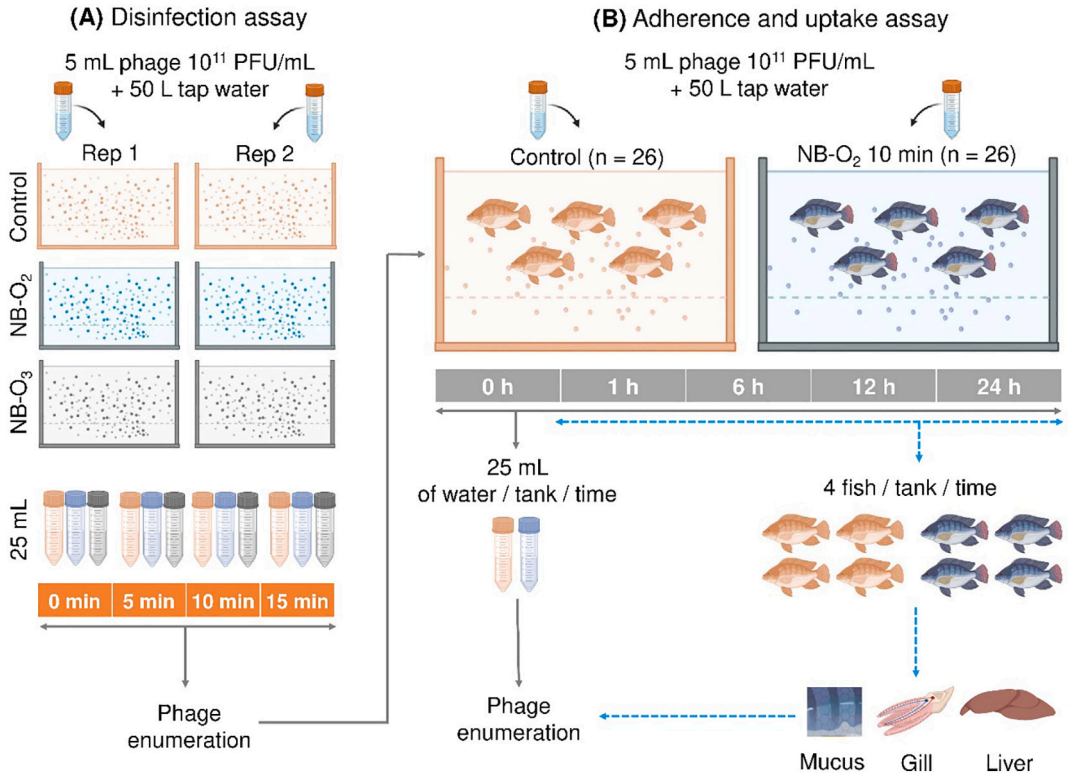


Fig. 1. Experimental design of disinfection assay (A) and adherence and uptake assay (B). Effects of oxygen and ozone nanobubbles (NB-O_2 and NB-O_3) on disinfection of phage as a viral model in water compared to the control group with normal aeration were investigated in (A) in a time course manner. Application of NB-O_2 to improve phage adherence (on tilapia mucus and gills) and uptake (in liver) was assessed in (B) compared to the control group with normal aeration. Phage enumeration from water of both treated tanks was also carried out.

while groups 2 and 3 were exposed to NB-O_2 and NB-O_3, respectively for 15 min prior to phage addition. Each group used 100 L-fiberglass tanks contained 50 L dechlorinated tap water. A total of 5 mL of phage pAh6.2TG (approx. 10^{11} PFU/mL) was added to each experimental tank to get a final concentration of 10^6 PFU/mL. A volume of 25 mL of water (a mixture of 5 mL from each tank corners and 5 mL from the middle of the tank) was sampled at 0, 5, 10, and 15 min. One mL of each collected water sample was centrifuged at 4 °C, 10,000 ×g, for 5 min. The supernatant was used for phage enumeration using a serial dilution plaque assay method with some modifications as described in Kropinski et al. (2009). Water parameters including temperature, pH, DO, and oxidation reduction potential (ORP) were measured before and during treatment using a multi-parameter meter (YSI Professional Plus, YSI Incorporated, USA) (Fig. S1). Ozone was measured at a few times points (0, 2, 4, 6, 8, 10 min) during the study using a K-7434 Ozone Vacu-vials Kit (Oxidation Technologies, USA). We plotted the dissolved ozone concentration (mg/L) vs. ORP (mV) measurements (Fig. S2) to extrapolate the concentration of O_3 when it could not be measured due to time restraints.

2.3. Effects of oxygen nanobubbles on adherence and uptake of phage to fish

2.3.1. Experimental fish

Healthy Nile tilapia (4.68 ± 0.67 g) was obtained from a tilapia hatchery (Department of Fisheries, Thailand) with no history of disease outbreaks. Fish were acclimated for 14 days at 28 ± 1.0 °C, and fed twice daily with commercial tilapia feed (30% crude-protein) at the rate of about 3% of their body weight. Before starting the experiments, 10 fish were randomly selected for bacterial isolation and confirmed to be free of *A. hydrophila* and phage pAh6.2TG. The experimental animal protocols used in this study were approved by Chulalongkorn University (no. CU-IACUC 2031006).

2.3.2. Adherence and uptake of phage to fish

To investigate the effects of NB-O_2 on adherence and uptake of phage to fish, a total of 52 Nile tilapia were randomly divided into two groups (Fig. 1B): group 1 (control group) was exposed to normal aeration while group 2 was treated with NB-O_2 for 10 min and later supplied with normal aeration throughout the remainder of the experimental period (24 h). Phage pAh6.2TG (5 mL) was added to each tank after their treatment with aeration or nanobubbles to make a final concentration of phage of approx.10^6 PFU/mL. In order to investigate the effect of NB-O_2 treatment on the concentration of phage in rearing water, a volume of 25 mL water collected from both groups was sampled at 0 min, 1, 6, 12, and 24 h post-treatment for phage enumeration.

At 1, 6, 12, and 24 h post NB-O_2 treatment, 4 fish from each group were collected for phage enumeration in their mucus, gills, and liver (Fig. 1B). In order to collect skin mucus, no anesthesia was given to the fish and the fish were placed in tanks with phage-free dechlorinated tap water for 10 min before being transferred to a plastic bag containing 1 mL of SM buffer and gently rubbed for 30 s. The fish were removed from the bags and mucus was collected into 1.5 mL microtubes. The mucus samples were then centrifuged at 4,000 ×*g* for 15 min. The supernatant from the mucus samples was then aliquoted into sterile tubes and used for phage enumeration by the method described in Kropinski et al. (2009). After the mucus collection, the fish were euthanized by immersion in clove oil (1 g/L water) prior to necropsy. A total of 0.1 g of the gills and liver were collected from each fish, transferred into 1.5 mL microtubes containing 0.2 mL of SM buffer. Individual fish tissues were separately homogenized by a tissue grinder with suitable pestles. The samples were then combined with 700 μL of SM buffer and centrifuged at 10,000 ×*g*, for 5 min. The obtained supernatant was used for phage enumeration as described above. The phage adherence was measured using phage titers in mucus (PFU/mL) and gill (PFU/g), while phage uptake was evaluated by comparing phage titer (PFU/g) in the liver. Behavioral abnormality and mortality were recorded over a 7-day period for the remaining fish (10 fish/tank) to assess the safety of NB-O_2 treatment.

2.4. Statistical analysis

Phage titers in the water of the control group, NB-O_2, and NB-O_3 treatment groups at 0 min were analyzed by Kruskal-Wallis Test, *p*-values of 0.05 or less were considered statistically significant levels. Phage titers in the water at 5, 10, 15 min of the control group and NB-O_2 treatment group; phage titers between control and NB-O_2 treatment groups in mucus, gill, and liver were analyzed using a Mann-Whitney *U* Test, *p*-values of 0.05 or less were considered statistically significant levels. All statistical analyses were performed using SPSS Software ver22.0 (IBM Corp., USA).

3. Results

3.1. Ozone nanobubbles kill bacteriophage while oxygen nanobubbles do not

The changes of the phage concentration in the control and nanobubble treatment groups are shown in Fig. 2. Before treatment (0 min), concentration of phage pAh6.2TG in control, NB-O_2, and NB-O_3 treatment groups were not significantly different, Kruskal-Wallis test: $H(2) = 0.857$, $p = 0.651$ at $2.63 \times 10^6 \pm 2.75 \times 10^5$, $2.53 \times 10^6 \pm 2.75 \times 10^5$, and $2.45 \times 10^6 \pm 1.50 \times 10^5$ PFU/mL, respectively. However, after 5 min of treatment, phage titers in control and NB-O_2 treatment groups remained at the same level, while 99.99% of phages in the NB-O_3 treatment group were destroyed (from $2.45 \times 10^6 \pm 1.50 \times 10^5$ to 8.75 ± 1.25 PFU/mL). A slight reduction was recorded in the control and NB-O_2 treatment groups at 10 and 15 min post-treatment (Fig. 2). However, this was not statistically significant compared to control group ($p = 0.439$ and $p = 0.121$, respectively). By contrast, phages pAh6.2TG could not be detected after 10 and 15 min of NB-O_3 treatment (Fig. 2).

With respect to water parameters, temperature was slightly increased (Fig. S1). In the control group, the temperature was 33 ± 1.34 °C at 0 min to 32.8 ± 1.27 °C at 15 min post treatment, while the values of 32.7 ± 0.85 to 34.5 ± 0.42 °C and 31.4 ± 0.07 to 33.6 ± 0.07 °C were recorded in NB-O_2 and NB-O_3 treatments, respectively (Fig. S1A).

The DO in NB-O_2 and NB-O_3 treatment groups also increased steadily from 3.45 ± 0.84 and 3.5 ± 0.36 mg/L at 0 min to 27.68 ± 0.12 and 23.84 ± 1.1 mg/L at 15 min, respectively. DO in the control group remained at the same level during treatment period (Fig. 3A). ORP in the control and NB-O_2 treatment groups were stable during the treatments, while this value in the NB-O_3 treatment group increased from 310.45 ± 32.31 mV at 0 min to 829.9 ± 60.53 mV at 5 min, and 941.55 ± 11.1 mV at 15 min. Fluctuation of ORP levels in NB-O_3 treatment group and its conversion to concentrations of dissolved ozone is shown in Fig. 3B-C. The pH values at 0 min in the control, NB-O_2, and NB-O_3 groups were 7.38 ± 0.63, 7.37 ± 0.09, and 7.43 ± 0.72, respectively. At 15 min, these values were 7.77 ± 0.21, 7.27 ± 0.05, and 7.69 ± 0.08, respectively (Fig. S1B).

3.2. Oxygen nanobubbles improve adherence of bacteriophage on fish and uptake into fish

The number of phages adhered to fish mucus, and gills at 1, 6, 12 and 24 h post treatment in both NB-O_2 and control groups are shown in Table 1. Overall, the concentration of phage titers from fish mucus and gills was higher in NB-O_2 treatment group. In mucus, phage adherence was 1.25, 1.88 and 2.36 fold higher than that of control after 6, 12 and 24 h post treatment, respectively. In fish gills, phage titers in NB-O_2 treatment group were 1.07 to 1.76 fold higher than in the control group at the first 12 h post treatment, but after 24 h, there was a 15 fold higher level of phage adhered to the gill in NB-O_2 treatment group (7500 ±

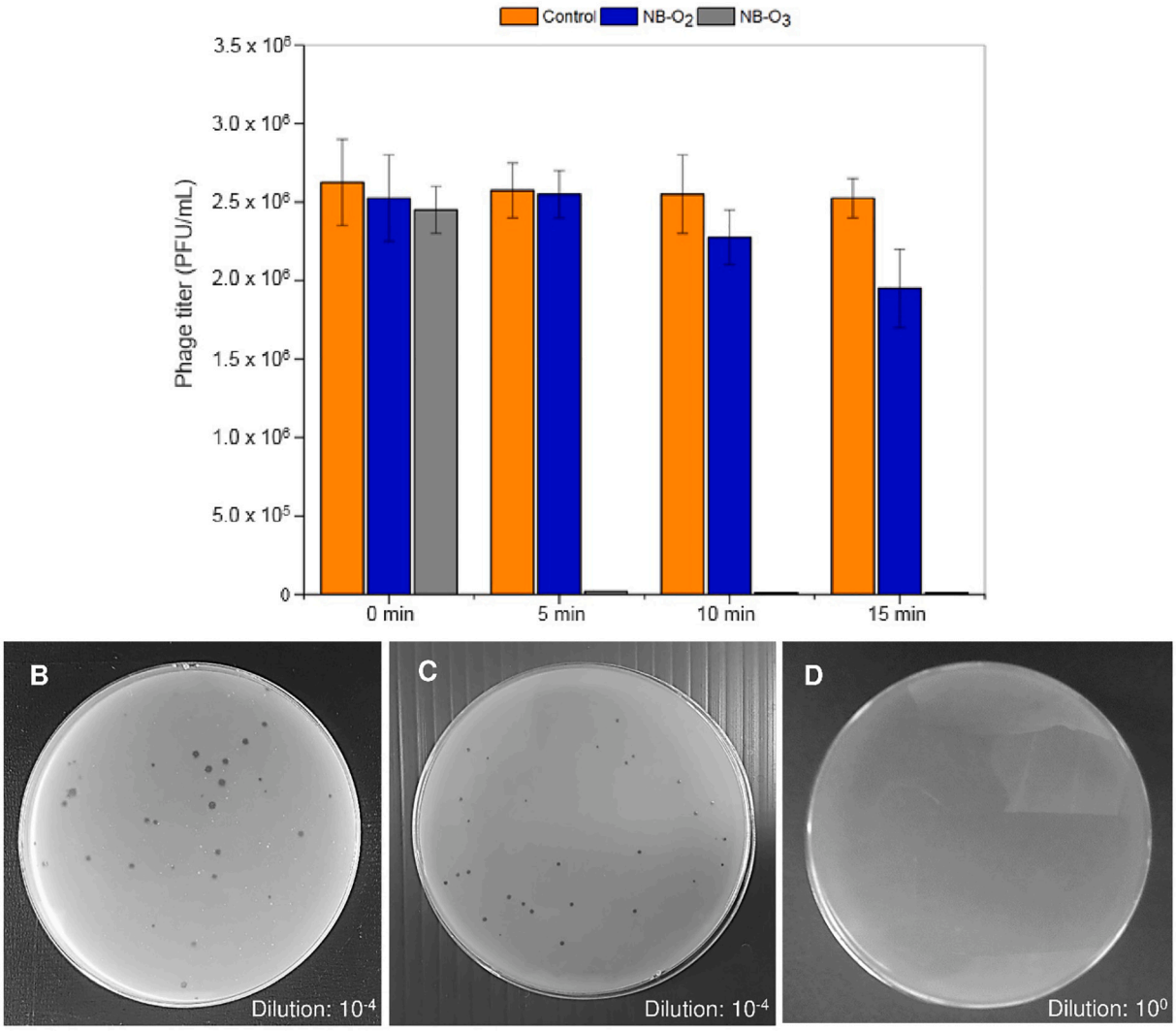


Fig. 2. Effect of oxygen (NB-O_2) and ozone (NB-O_3) nanobubbles on phage titer in water (A). Values of pAh6.2TG phage titer (PFU/mL) are mean ± a standard error of the mean (SEM) (n = 2). Representative enumeration results of phage pAh6.2TG in control (B), NB-O_2 (C), and NB-O_3 (D) after 10 min post treatment.

4402 PFU/g) compared to the control group (500 ± 204 PFU/g) with p = *0.028* (Table 1).

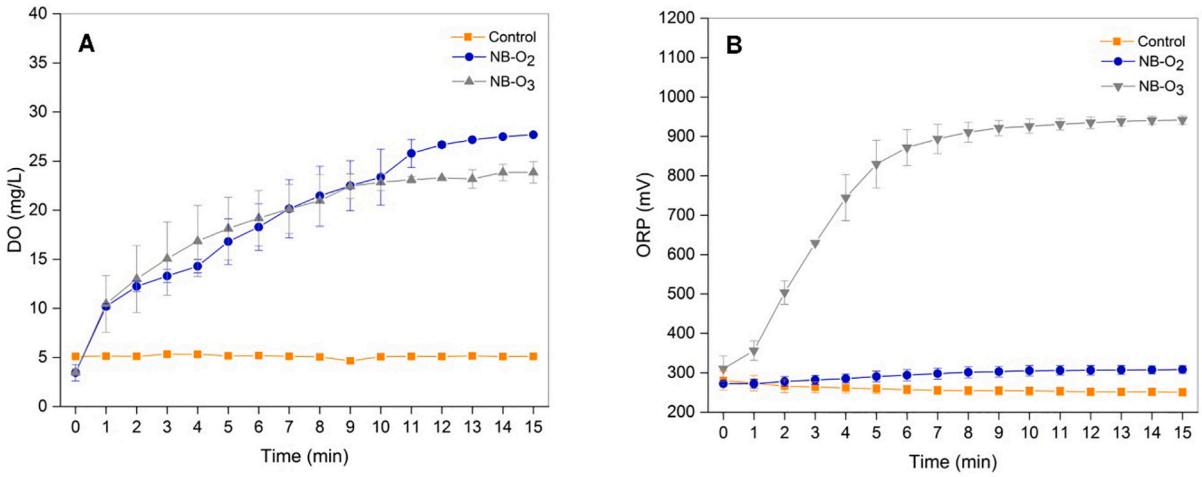
The concentration of phages in the fish liver of NB-O_2 and control treated groups is illustrated in Table 1. After 1 h exposure to NB-O_2, phage the titer in liver of the treatment group was 212 ± 90 PFU/g and no phage was detected in the control group (p = *0.014*). At 6 h post-treatment, phage titers in the liver were 512 ± 356 and 662 ± 313 PFU/g in control and NB-O_2 treatment groups (p = *0.663*), respectively (1.29 fold difference). However, phage titer in NB-O_2 treatment group after 12 h and 24 h were 4.75 and 4.0 fold higher than that of the control group, respectively (Table 1). The differences in phage concentration were statistically significant at 12 and 24 h post-treatment (p = *0.027* and p = *0.034*, respectively).

No fish mortality or behavioral abnormalities were observed during and 7-day post-treatment in either the control or the NB-O_2 groups.

4. Discussion

Disinfection of NB-O_3 against pathogenic bacteria has previously been investigated in both marine and freshwater. Imaizumi et al. (2018) reported that most *Vibrio parahaemolyticus* were killed after 1 min incubation in NB-O_3 seawater. Nghia et al. (2021) also demonstrated that treatment with NB-O_3 for 6 min inactivated 100% of the *V. parahaemolyticus* in laboratory experiments. In addition, Jhunkeaw et al. (2021) demonstrated that 10 min of NB-O_3 treatment in freshwater reduced 96.11 and 97.92% of *Streptococcus agalactiae* and *Aeromonas veronii*, respectively. However, there has not been any study on the effects of nanobubbles (either NB-O_2 or NB-O_3) on the concentration of viruses in water. In this study, we discovered that NB-O_3 treatment effectively eradicated phages in water after 5 to 10 min treatments, with corresponding ORP levels ranging from 829.9 ± 60.53 to 925.75 ± 18.46 mV, which was equivalent to dissolve ozone levels of 1.09 ± 0.15 to 1.33 ± 0.55 mg/L (Fig. 3C). Although we did not evaluate pathogenic fish viruses specifically, disinfection of bacteriophage with NB-O_3 implied that this technology may be effective for fish viruses commonly infecting tilapia aquaculture systems such as tilapia lake virus, (TiLV) (Jansen et al., 2019), infectious spleen and kidney necrosis virus (ISKNV) (Machimbirike et al., 2019; Ramírez-Paredes et al., 2020), and nervous necrosis virus (NNV) (Keawcharoen et al., 2015; Machimbirike et al., 2019). However, further investigation is needed to confirm whether NB-O_3 treatment is effective in eradicating these specific viral pathogens in water.

Previous studies suggested that ozone inactivates virus by damage the viral capsid proteins of non-enveloped viruses or oxidizing the viral envelope, and destroying its normal structure of the enveloped viruses (Kim et al., 1980; Mayer et al., 2015; Sproul et al., 1982; Tizaoui, 2020;



C

Time (min)	ORP (mV)	Ozone (mg/L)	Time (min)	ORP (mV)	Ozone (mg/L)
0	310.45 ± 32.31	0	8	910.75 ± 25.53	1.29 ± 0.06
1	356.25 ± 24.82	0	9	921.35 ± 19.45	1.32 ± 0.05
2	503.85 ± 29.91	0.27 ± 0.07	10	925.75 ± 18.46	1.33 ± 0.05
3	629.3 ± 3.39	0.59 ± 0.01	11	930.9 ± 14.71	1.34 ± 0.04
4	744.55 ± 58.48	0.87 ± 0.15	12	935 ± 14.85	1.35 ± 0.04
5	829.9 ± 60.53	1.09 ± 0.15	13	938.05 ± 12.52	1.36 ± 0.03
6	871.55 ± 45.75	1.19 ± 0.11	14	940.5 ± 10.18	1.36 ± 0.03
7	893.4 ± 37.05	1.25 ± 0.09	15	941.55 ± 11.1	1.37 ± 0.03

Fig. 3. Measurement of DO (A), ORP (B) during 15 min treatment of NB-O_2, NB-O_3 with 2 L/min oxygen input in tap water and conversation of dissolved ozone concentration (mg/L) from ORP (C). Values of water parameters are mean ± standard deviation (SD) (n = 2).

Table 1
Phage concentration in fish organs after NB-O_2 treatment.

Organ	Experimental group	Phage concentration[#]			
		1 h	6 h	12 h	24 h
Mucus	Control	26,875 ± 10,166	33,500 ± 7,309	68,750 ± 16,007	25,375 ± 9,562
	NB-O_2	25,125 ± 16,085	41,750 ± 15,682	74,500 ± 19,002	59,875 ± 22,935
	Fold change^{+}	−1.07	+1.25	+1.08	+2.36
Gills	Control	5,025 ± 1,546	6,375 ± 5,047	4,375 ± 2,164	500 ± 204
	NB-O_2	5,362 ± 2,416	11,250 ± 4,191	5,500 ± 1,568	7,500 ± 4,402*
	Fold change^{+}	+1.07	+1.76	+1.26	+15
Liver	Control	0	512 ± 356	50 ± 20	62 ± 12
	NB-O_2	212 ± 90*	662 ± 313	237 ± 51*	250 ± 134*
	Fold change^{+}	NC	+1.29	+4.75	+4.0

NC: Not calculate; Fold change^{+}: fold change in NB-O_2 treatment group compared to control group.
Comparision of phage titer between control and NB-O_2 treatment groups was performed by Mann-Whitney *U* Test, *p*-values of 0.05 or less were considered statistically significant. "*" indicates statistically significant difference between groups ($p < 0.05$), without "*" means not significant.
[#] PFU/mL ± SE for mucus, PFU/g ± SE for gill and liver.

Wigginton and Kohn, 2012). Ozone also caused oxidation on genomic components of the virus after diffusion through the capsid, which led to the loss of viral infectivity and replication (Jiang et al., 2019; Roy et al., 1981; Sproul et al., 1982).

The findings in this study also suggest that NB-O_3 should not be used during phage therapy due to the disinfection property of ozone. However, phage treatment may be considered after NB-O_3 treatment since ozone was shown to degrade relatively quickly after the cessation of these treatments in other studies (Jhunkeaw et al., 2021).

After identifying that 10 min NB-O_2 treatment is not harmful to bacteriophages, we then explored whether NB-O_2 technology improved adherence and uptake of phage on the mucosal surface and internal organs of fish. Increased concentration of phage pAh6.2TG in mucus, gills, and liver indicated that NB-O_2 technology could be combined with bacteriophage therapy to improve efficacy or uptake. Barr et al. (2013) revealed the increased concentration of the lytic phage on mucosal surfaces provided an antimicrobial defense that limited mucosal bacteria. The sub-diffusive motion of phage on the mucosal surface enhanced bacterial encounter rates for phages, especially when bacterial concentration is low (Barr et al., 2015). Another study by Almeida et al. (2019) also showed that the binding of phage FLC-2 and T4 on the rainbow trout (*Oncorhynchus mykiss*) skin mucosal provided protection against *Flavobacterium columnare* infection. Pretreatment with a single dose of phage cocktails (FLC-2 and T4) for 1 day before *F. columnare* challenge delayed the disease onset and improved fish survival by 25%.

Phages have been investigated for treating bacterial infections in aquaculture system (Angulo et al., 2019; Culot et al., 2019; Ninawe et al., 2020). They have been found to reduce the densities bacteria sufficiently to enable the fish immune system to fight off infections (Levin and Bull, 2004). However, phages must reach the sites of infection to be effective. Nakai and Park (2002) suggested that phages could penetrate into the fish body, via the skin and gills. Thus, the uptake of phage in internal organs is a critical factor that contributes to efficacy of phage therapy.

The enhancement of phage-mucin protein interaction and the improvement of phage diffusion across the mucus by NB-O_2 activity possibly explains the higher adherence and uptake of phage in fish

compared to control group. Most phages have an overall negative charge (Anany et al., 2011; Hosseinidoust et al., 2014; Van Voorthuizen et al., 2001), which permits them to adhere to the glycan component of mucin through weak binding interactions with the Hoc capsid proteins (Barr, 2017). However, mucus also contains a high density of negatively charged glycoproteins and oligosaccharides (Crater and Carrier, 2010). Consequently, the binding of phages on the mucus layer may be less effective due to the electrostatic repulsion between negative charge components (Esteban et al., 2016). NB-O_2 also have a negative charge with zeta potential about −34 to −45 mV (Ushida et al., 2012; Ushikubo et al., 2010). It is possible that the electrostatic repulsion between high densities of NB-O_2 in water and negative charge glycoproteins, oligosaccharides on the mucus layer may facilitate the binding of phages and mucosal glycan. Moreover, free-adherence phages with negative zeta potential can move easily within the mucus layer (Crater and Carrier, 2010; Pangua et al., 2021). The electrostatic repulsive forces between phage and NB-O_2 may facilitate free-phage diffusion across the mucus layer. It is also possible that the NB-O_2 treatment increases the permeability of the gills facilitating the entry of phages into the host.

In this study, the higher uptake of phage pAh6.2TG in the fish liver after NB-O_2 treatment compared to the control group may improve the efficacy of phage therapy against bacterial infections in fish. This should be evaluated with laboratory challenge tests. There is a necessity for further studies to investigate the mechanism of NB-O_2 to enhance phage adherence to mucus and uptake of phages into fish organs.

Although this study revealed the potential application of NB-O_2 in improving phage adherence and subsequent uptake into the liver, one of the limitations of this study was the small sample size and short duration of the phage study. Further future studies should explore the function of these phages under NB-O_2 by challenging fish with bacteria to gain a better understanding of the mechanism and potential benefits of this promising technology for disease prevention and mitigation. Increasing technical replicates would be beneficial in the future to confirm the results of this study.

In conclusion, using nanobubbles in aquaculture could improve pathogen control in several ways. Using phage as a virus model, this study demonstrated that NB-O_3 treatment was effective at eradicating viruses in water. This opens a novel application for this technology as one of the biosecurity measures that could be used to prevent viral diseases in aquaculture. In addition, this study also discovered that NB-O_2 treatment improved the adherence and uptake of phage in fish, which may improve the success of phage therapy in aquaculture. From the author's point of view, NB-O_2 and NB-O_3 treatments are promising and potential practical for application in hatcheries or indoor recirculation systems.

Disclaimers

The views expressed herein do not necessarily represent those of IDRC or its Board of Governors.

Data availability statement

The data that support the findings of this study are available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.737894.

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