Journal of Environmental Management 401 (2026) 128879

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Screening of antiviral activity in freshwater and marine microalgae: Inactivation capacity over enveloped and non-enveloped viruses

Antonio Leon-Vaz [a,b,*], Lucía Tejero-Álvarez [a,b], Pedro Antonio García-Encina [a,b], Raúl Muñoz [a,b], Andrés Felipe Torres-Franco [a,b,**]

[a] Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina, s/n, 47011, Valladolid, Spain
[b] Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina. s/n, 47011, Valladolid, Spain

ARTICLE INFO

Keywords:
Bacteriophage
Chlorella vulgaris
MS2
Phi6
PhiX174
Thalassiosira weissflogii
Viral inactivation

ABSTRACT

New viruses are one of the major health challenges that human society is facing during this century. One of the most promising solutions is searching for natural organisms or molecules with antiviral capacity, with emerging microalgae as a promising solution in recent years. Thus, in this work, the antiviral capacity of ten species of freshwater and marine microalgae was tested against three enveloped and non-enveloped bacteriophages (PhiX174, MS2 and Phi6), showing inhibition efficiencies ranging from 40 to 80% compared with control infections. Moreover, the PCA analysis revealed the influence of the microalgal cell wall on the different bacteriophages' inactivation capacity. Finally, the inactivation of the three bacteriophages in liquid cultures was studied using the microalgae *Chlorella vulgaris* and *Thalassiosira weissflogii*. These microalgae inactivated 99.999% of the MS2 and Phi6, and 99.9% of PhiX174 after 72 h of cultivation, respectively. Additionally, T_{90} values ranging from 7 to 12 h for PhiX174, 3 to 12 h for MS2 and 2.5 to 3 h for Phi6 were achieved by the two tested microalgae. These results highlight the potential of microalgae for the inactivation of viruses in wastewaters and as an outstanding source of antivirals.

1. Introduction

Healthcare is one of the highest concerns nowadays worldwide. New viruses are one of the major health challenges human society are facing during this XXI century. These new viral diseases have emerged in different geographical areas as a result of the propagation of the Ebola virus, West Nile virus or coronaviruses (CoV), caused or probably caused by zoonoses (Dhama et al., 2020). The most recent striking example is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, which quickly spread throughout the world in 2020, causing more than 556 million confirmed cases and over 6.3 million deaths in the period 2019-2022 (Gasch-Illescas et al., 2023). In this context, searching for new organisms and molecules able to inactivate viruses can be an excellent approach to deal with this current healthcare issue and in preparation for future potential new threats.

Microalgae are a heterogeneous group of photosynthetic organisms with a wide range of metabolisms and applications. These microorganisms capture higher amounts of CO_2 than C4 plants, being able to fix about 50 gigatons of CO_2 per year worldwide (Ashour et al., 2024; Hong, 2022). Moreover, microalgae produce biomass in this process, which render them as an outstanding biotechnological resource (Liu et al., 2024; Ugya et al., 2024). In recent decades, many species of microalgae have been investigated for their ability to produce different bioactive compounds, such as polyunsaturated fatty acids (PUFA), including docosahexaenoic acid, eicosapentaenoic acid, or linolenic acid (Cañavate, 2019; Maciel et al., 2024); carotenoids such as fucoxanthin, astaxanthin or β-carotene (Mapelli-Brahm et al., 2023); or polysaccharides (Sen et al., 2025), which can be used in cosmetic, pharmaceutical or aquaculture industries. The ability to rapidly produce high concentrations of these compounds points to microalgae as a potential source of bioactive products.

Different microalgae have been considered in the pharmaceutical and biomedical sector due to their anti-inflammatory, antioxidant or antibacterial metabolites. For instance, the microalga *Microchloropsis*

* Corresponding author. Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina, s/n, 47011 Valladolid, Spain.
** Corresponding author. Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina, s/n, 47011, Valladolid, Spain.
E-mail addresses: antonio.leon@uva.es (A. Leon-Vaz), lucia.tejero@uva.es (L. Tejero-Álvarez), pedroantonio.garcia@uva.es (P.A. García-Encina), raul.munoz.torre@uva.es (R. Muñoz), andresfelipe.torres@uva.es (A.F. Torres-Franco).

https://doi.org/10.1016/j.jenvman.2026.128879
Received 23 September 2025; Received in revised form 25 January 2026; Accepted 3 February 2026
Available online 9 February 2026

gaditana has been described as a natural antimicrobial source against the bacterium *Piscirickettsia salmonis* (Díaz et al., 2025). Moreover, microalgae have been recently proposed as a potential platform for antimicrobial peptides expression (Wang et al., 2025). Furthermore, the antiviral capacity of different microalgal extracts has been investigated in recent years. Hernández-Urcera et al. (2024) demonstrated the antiviral capacity against the spring viraemia of carp virus using DMSO and CH_2Cl_2 extracts of 33 microalgal strains. In addition, different microalgal metabolites have also been proposed as potential antiviral compounds. Chlorophylls and lutein-enriched extracts from *Tetraselmis* sp. strains obtained high inactivation values against Zika and vaccinia virus infections (Kang et al., 2024b; Kim et al., 2023). On the other hand, different polysaccharides and lectines from microalgae have been postulated as antiviral molecules due to their capacity to envelope the infective proteins of viruses (Osathanunkul et al., 2025). However, most of the studies focused on antiviral capacity of microalgae were performed with microalgal extracts.

Although consortia of microalgal species have been recently described as a potential inactivation platform of different RNA viruses and bacteria in wastewater (Torres-Franco et al., 2025), the number of works assessing the antiviral capacity of axenic species is scarce, and more knowledge is necessary to unravel the mechanisms that microalgae use to inactivate viruses. Bacteriophages, such as the enveloped Phi6, or the non-enveloped MS2 and PhiX174, have traditionally been used to assess viral viability in wastewater treatment, as they pose no biosafety risks and are common surrogates of broad variety of viruses, including those of sanitary concern (Torres-Franco et al., 2025; Yang et al., 2022). Additionally, viral inactivation mechanisms were also studied in these bacteriophages (Zhu and Ye, 2025).

Thus, the aim of this work is to test the antiviral capacity of ten species of freshwater and marine microalgae belonging to 6 different *phyla* and their supernatants against three different bacteriophages. These viruses are the single-strain DNA (ssDNA) virus PhiX174, and two RNA viruses (Phi6 and MS2). Phi6 is an enveloped double-strain RNA (dsRNA), while MS2 has single-strain RNA genomes (ssRNA). Moreover, the kinetics of decay against these bacteriophages were also investigated for the best-performing strains.

2. Materials and methods

2.1. Microalgal strains and growth conditions

For this work, 10 microalgal species (Table 1) were selected from 6 phyla in the AlgaBase classification (Guiry and Guiry, 2022). The selection of this heterogeneous group of microalgae was performed to evaluate variability in viral inactivation across freshwater and marine microalgae.

Table 1
Microalgal strains used in this work.

Species	Code	Phylum
Arthrospira platensis	SAG 21.99	Cyanobacteria
Coelastrella sp.	ISP-0125	Chlorophyta
Chlorella vulgaris	SAG 211-11b	Chlorophyta
Dunaliella salina	SAG 19-3	Chlorophyta
Tetrasemis chuii	CCMM 03/0201	Chlorophyta
Porphyridium cruentum	CCMM 08/0101	Rhodophyta
Isochrysis galbana	CCMM 05/0401	Haptophyta
Phaeodactylum tricornutum	CCMM 07/0402	Bacillariophyta
Thalassiosira weissflogii	CCMM 07/0602	Bacillariophyta
Nannochloropsis gaditana	CCMM 04/0201	Eustigmatophyta

The freshwater and marine microalgae *Arthrospira platensis*, *Chlorella vulgaris* and the marine microalga *Dunaliella salina* were acquired from SAG Culture Collection of Algae (Germany), while the marine species *Tetrasemis chuii*, *Porphyridium cruentum*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Thalassiosira weissflogii* and *Nannochloropsis gaditana* were acquired from the Marine Microalgal Culture Collection of the Institute of Marine Sciences of Andalusia (ICMAN-CSIC, Spain). Finally, the freshwater strain *Coelastrella* sp. was isolated and identified at the Institute of Sustainable Processes (ISP, Spain).

Freshwater microalgae were cultured in SK medium as described in León-Vaz et al. (2025). *A. platensis* was cultured in Zarrouk's medium (Chen et al., 2020). Marine microalgae were cultured in modified Guillard F/2 medium as described in León-Vaz et al. (2023). All strains were cultivated at 25 °C, under continuous light irradiation (200 μmol m^{-2} s^{-1}) and agitation (240 rpm) in closed glass bottles with butyl septa and aluminium caps. These light and temperature conditions were previously reported as favourable for the heterogeneous group of species listed in Table 1 (León-Vaz et al., 2023; Maltsev et al., 2021; Singh and Singh, 2015). The headspace of the gas-tight bottles was supplemented with 15-20% (v/v) of CO_2 as a carbon source, and the initial pH was adjusted to 8.1 in marine strains and 7.0 in freshwater strains.

In the screening experiments, microalgae were inoculated at an initial optical density at 600 nm (OD_{600}) of 0.2 from cultures grown under the above-described conditions for 4 days (end of exponential phase). The algal biomass of the inocula was concentrated until an OD_{600} of 5.0 and then resuspended in SM buffer (5.84 g L^{-1} NaCl, 0.96 g L^{-1} $MgSO_2$ and 6.06 g L^{-1} Tris-HCl) for phages at pH 7.5.

2.2. Bacteriophages and host strains

The bacteriophages used in this work were the ssDNA virus PhiX174 (DMS-4497), the ssRNA virus MS2 (DMS-13767) and the dsRNA virus Phi6 (DMS-21518); and their respective hosts *E. coli* (DMS-4860 and DMS-5695) and *Pseudomonas* sp. (DMS-21482). All of them were acquired from the German Collection of Microorganisms and Cell Cultures (DSMZ). Stock solutions of the three bacteriophages were achieved by applying the same procedure described in Torres-Franco et al. (2024), obtaining concentrations of 2.1×10^{-9}, 1.5×10^{-11}, and 1.7×10^{-10} PFU mL^{-1} for PhiX174, MS2 and Phi6, respectively.

2.3. Screening of the antiviral capacity of freshwater and marine microalgae

Screening experiments were performed using the double agar technique (Fedorenko et al., 2020). For this experiment, the inactivation capacity of PhiX174, MS2 and Phi6 was studied using the biomass and supernatant of cultures of the microalgal strains described in Table 1. In this experiment, 200 μL of the host bacterium were mixed with 100 μL of virus stock solution and 100 μL of the microalgal biomass or supernatant without cells obtained as described in section 2.1. This mixture was added to 5 mL of 0.6% agar medium, previously prepared at 45-50 °C and spread in 2.5% agar plates. Finally, double agar plates were incubated at 25 °C and 37 °C for *Pseudomonas* sp., or *E. coli* experiments, respectively, for 16 h in dark conditions. Bacteriophage titers were reported as the mean and standard deviation of the triplicates of each microalgal culture in PFU mL^{-1}.

2.4. Bacteriophage decay kinetics

Biodegradation of PhiX174, MS2, Phi6 and control experiments in bottles were conducted for 72 h. The selected microalgal strains (*Chlorella vulgaris* and *Thalassiosra weissflogii*) were cultured in 100 mL flasks under the conditions previously described in light and dark conditions, using an initial OD_{600} of 0.5-0.6. Additionally, a negative control without algal biomass was included in the experiments under the same conditions. Samples from the liquid cultures were taken at 0, 12, 24, 48 and 72 h using sterile needles, filtered through sterile 0.22 μm filters and stored under sterile conditions at 4 °C. Bacteriophage decay was calculated using the double agar technique as described in Torres-Franco et al. (2024). In this work, first-order linear regression models were applied to all cases (Eq. (1)):

$$\log \frac{C_t}{C_0} = \log a + kt \quad (1)$$

Where C_t and C_0 correspond to PFU measured at time t and time 0, respectively, and k corresponds to the decay rate. Moreover, OD_{600}, pH, and Q_y values were monitored every 24 h along the experiment using 2 mL samples of the cultivation broth, just as the composition of the headspace in order to add CO_2 when needed to control the O_2/CO_2 balance.

2.5. Analytical procedures

CO_2 and O_2 were determined by GC coupled to a thermal conductivity detector (TCD) using a Bruker 430 GC-TCD (Bruker Corporation, USA). The compounds were separated in a CP-Molsieve 5A and in a CP-PoraBOND columns. The injector was maintained at 150 °C, and the columns were held at 45 °C for 5 min, with a helium flux of 3.5 mL min^{-1}. The temperature of the TCD detector was 200 °C. The pH measurements were carried out using a pH-meter Basic 20 (Crison, Spain), and the OD_{600} was measured using a UV mini 1240 spectrophotometer (Shimadzu, Japan). Q_y values were determined using an AquaPen-C system (Photon Systems Instruments, Czech Republic).

2.6. Statistical analysis

All the experiments were carried out using biological triplicates and the results were represented as the mean value ± standard deviation. Along with the screening, one-way analysis of variance (ANOVA), followed by a post hoc Duncan Multiple Range Test (DMRT) test, was applied to identify significant differences between control and algal/supernatant treated infections, which were considered for values with $p < 0.05$. A Pearson correlation test was applied to survival data to assess the correlation between the antiviral capacity of the microalgal biomass and supernatants against the three viruses tested. Principal component analysis (PCA) was applied to evaluate the variability in the antiviral profile in order to find axes in a multivariate space that best separate a priori established groups. Statistical analyses were performed using IBM SPSS Statistics v29.0 software (Armonk, NY, USA).

3. Results and discussion

3.1. Screening of the antiviral capacity of freshwater and marine microalgae

The screening of the antiviral potential of 10 microalgae belonging to 6 *phyla* showed varying inhibition levels against each of the assessed bacteriophages. To the best of the authors' knowledge, no previous works assessed the antiviral potential of *Coelastrella* sp., *D. salina, I. galbana, P. tricornutum and T. weissflogii.* The exposure of the bacteriophages to microalgae biomass (Fig. 1) during host infection on plates resulted in significant differences ($p < 0.05$) between the control and algal-treated conditions in all the MS2 infections and most of the PhiX174 and Phi6 infections, except for *Coelastrella* sp. in PhiX174 and *T. chuii* and *T. weissflogii* in Phi6. Compared with the control plates, the least inhibited bacteriophage was PhiX174 (Fig. 1A), with a lower survival of 52% in the presence of *C. vulgaris* biomass. MS2 and Phi6 survival was reduced to 31% and 23% when *Coelastrella* sp. and *T. weissflogii* biomass were added to MS2 and Phi6 plates, respectively.

The higher resistance to inhibition of PhiX174 reported in this work with the different microalgal strains (mean 29% of inhibition) than for MS2 (mean 45% of inhibition) and Phi6 (mean 36% of inhibition) was in agreement with previous studies, reporting lower inactivation for PhiX174 than Phi6 and MS2 when exposed to different coagulants, such as alum, poly-aluminium chloride, ferric chloride, or nanoscale zero-valent iron particles (Cheng et al., 2022; Maneein et al., 2024). In addition, the higher survival in water matrices of non-enveloped,

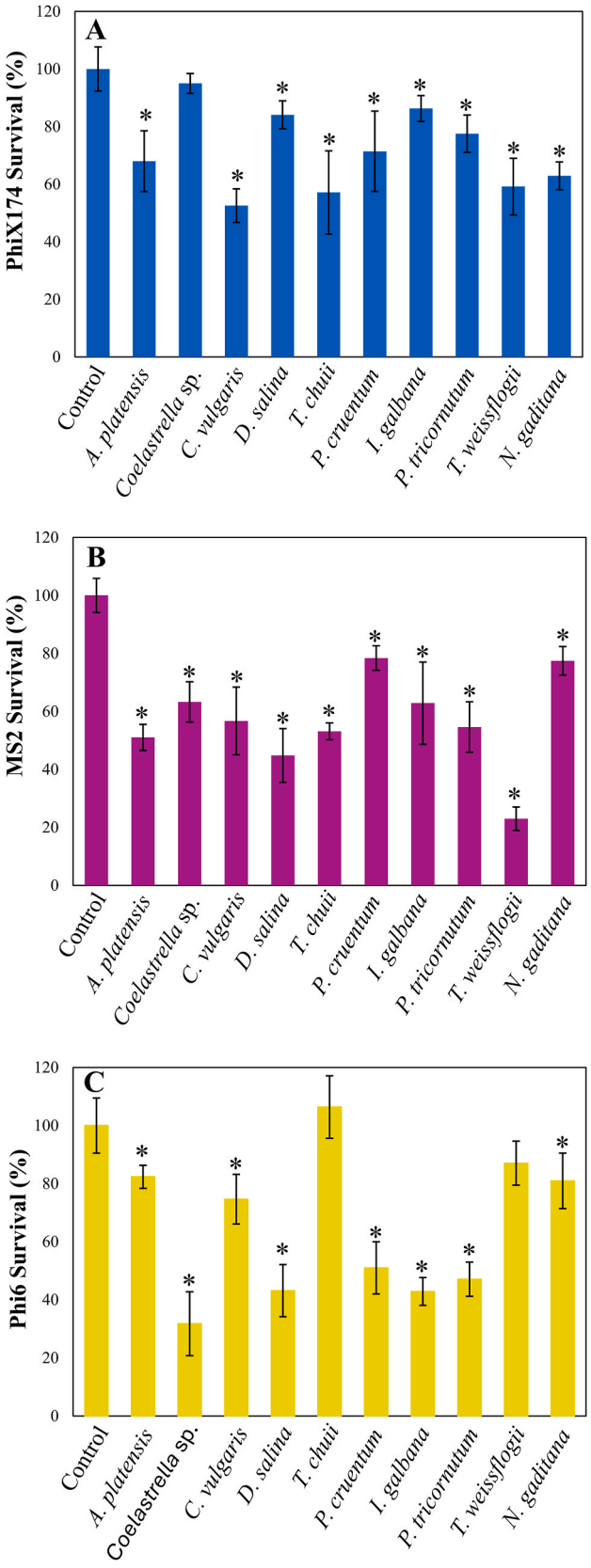

Fig. 1. Inhibition of the infection capacity of PhiX174 (A), MS2 (B) and Phi6 (C) using the biomass of the different microalgal strains tested. 100% of survival corresponds to 3.9×10^{-9}, 6.6×10^{-11}, and 7.2×10^{-9} PFU mL^{-1} for PhiX174, MS2 and Phi6, respectively.

compared to enveloped viruses, such as PhiX174 and Phi6, respectively, is well documented (Fedorenko et al., 2020; Lin et al., 2020). Although there is a trend for RNA viruses to be more resistant to antiviral compounds than DNA viruses due to their mutation capacity and different repair mechanisms (Durmuş and Ülgen, 2017), recent studies have demonstrated that human adenovirus 5 (DNA virus) exhibits longer T_{90} values (7.8-8.06 days) than other RNA viruses, such as enterovirus A71 (3.9-5.9 days) or respiratory syncytial virus A2 (4.7-6.4 days) in water samples, suggesting also a virus-dependent inactivation (Kevill et al., 2025). In this context, other factors, such as the higher stability and higher capacity to deal with oxidative damage of the DNA chain, could explain the higher resistance of PhiX174 than MS2 observed herein (Zhang et al., 2010).

3.2. Screening of the antiviral capacity of supernatants

The inactivation capacity of the supernatant from the tested microalgae cultures was also assessed since many microalgal species can secrete extracellular bioactive compounds. The results are presented in Fig. 2. An additional negative control using the corresponding culture medium was also performed to confirm that viral inhibition was not significantly attributable to culture media.

Overall, the inhibitory effect of supernatants (Fig. 2) from both freshwater and marine microalgae followed a similar trend to that of the bulk microalgal biomass (Fig. 1). All tested supernatants significantly inhibited the replication of MS2 and Phi6 ($p < 0.05$). However, only supernatants from *Coelastrella* sp., *C. vulgaris*, *D. salina*, and *T. chuii* demonstrated inhibitory activity against PhiX174. In the case of MS2 infections (Fig. 2B), the inhibition ranged from 14% with *T. chuii* to 54% with *Coelastrella* sp. supernatants. In the infections with Phi6 (Fig. 2C), the highest inhibition (94%) was observed in the presence of *P. cruentum* culture supernatants, while the lowest inhibition efficiencies of 29% were detected when the supernatants of *A. platensis*, *T. chuii* and *I. galbana* were added to plates.

These results suggest that potential extracellular compounds secreted by microalgae can significantly inactivate RNA viruses such as MS2 and Phi6. Notably, the supernatant of *P. cruentum* exhibited a strong antiviral effect against Phi6, which can be related to *Porphyridium* exopolysaccharides (EPS). These EPS have previously been reported to exhibit antiviral activity against other enveloped RNA viruses, such as SARS-CoV-2 or viral haemorrhagic septicaemia virus (VHSV) (Ben Hilma et al., 2022; Parra-Riofrio et al., 2023). Moreover, *P. cruentum* is well known for releasing high concentrations of extracellular high-molecular-weight polysaccharides into the culture medium (Decamp et al., 2023), which could be responsible for the high Phi6 inactivation capacity observed herein. These EPS are typically composed of glucose, with a higher proportion of this monomer than in other strains previously studied (Table S1) (Cristofoli et al., 2023). Moreover, red microalgae have been reported to produce higher amounts of alanine as a free amino acid (Table S1) than the other phylogenetic groups (León-Vaz et al., 2023), which may also contribute to the inactivation of Phi6 infectivity (Mosu et al., 2024).

On the other hand, PhiX174 survival efficiencies were significantly lower ($p < 0.05$) than control infections only in the supernatants of the cultures of the four chlorophytes strains i.e., *Coelastrella* sp., *C. vulgaris*, *D. salina* and *T. chuii*, with inhibition values of 23%, 33%, 38% and 21% of those in the control plates, respectively (Fig. 2A). It has been reported that green algae, such as *C. vulgaris*, *Parachlorella kessleri* or *Neochloris oleoabundans* can produce extracellular metabolites, such as EPS, small peptides, free amino acids, lipids or alkaloids, which have bioactive properties (Deore et al., 2022; Li et al., 2020). As described in Table S1, previous studies have reported that EPS produced by green microalgae are mainly composed of galactose (Toshkova-Yotova et al., 2024; Zhang et al., 2019), which is not usual in other microalgal groups (Brezeştean et al., 2021; Toucheteau et al., 2023), and could contribute to PhiX174 inactivation.

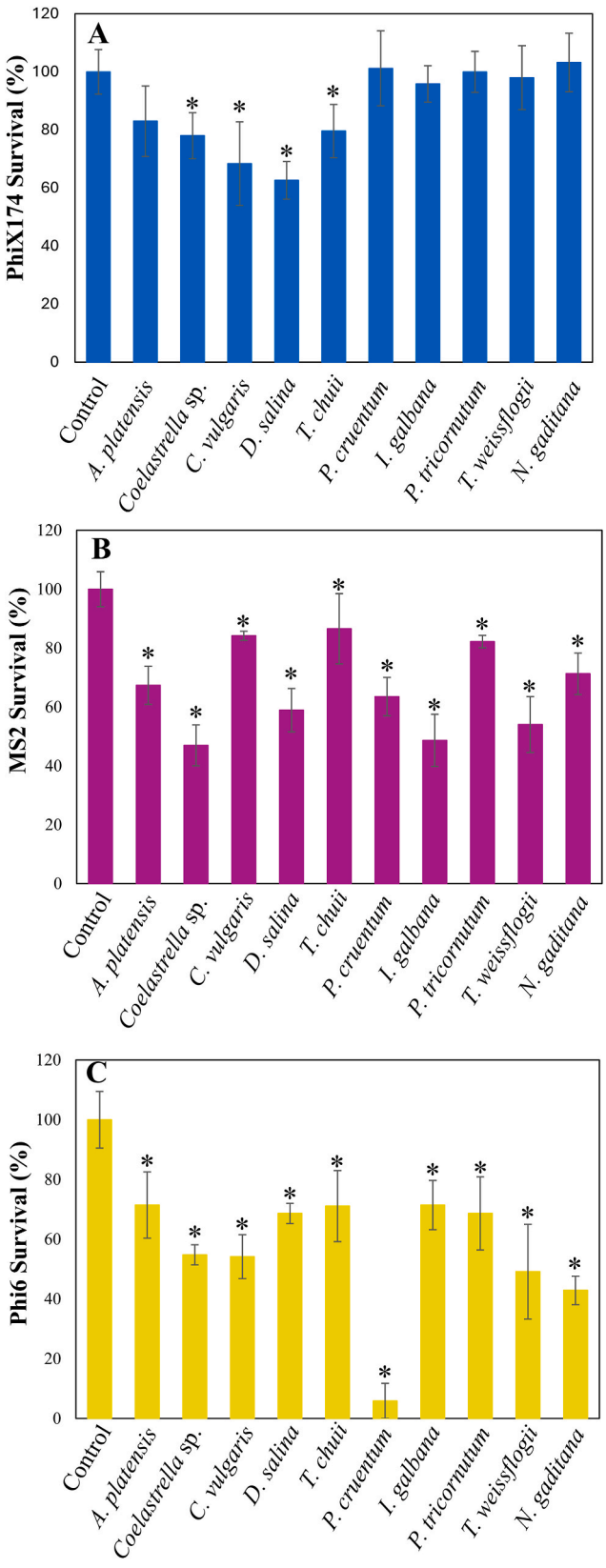

Fig. 2. Inhibition of the infection capacity of PhiX174 (A), MS2 (B) and Phi6 (C) using the supernatant of the different microalgal strains tested. 100% of survival corresponds to 3.9×10^{-9}, 6.6×10^{-11}, and 7.2×10^{-9} PFU mL^{-1} for PhiX174, MS2 and Phi6, respectively.

Further studies are necessary to identify the specific compounds, including EPS, produced by *P. cruentum, Coelastrella* sp., *C. vulgaris, D. salina*, and *T. chuii*, and their capacity to inhibit non-enveloped and enveloped viruses.

3.3. Antiviral distribution patterns in the tested microalgae

Multivariate analysis suggested the possible occurrence of patterns in the inactivation capacity of the studied bacteriophages. The PCA applied to the set of variables (percentage of inhibition after the incubation with microalgal biomass/supernatant) indicated strong sample arrangement, with 96% of total variation explained by the two main axes in the algal biomass case (Fig. 3A). In the first axis (left), the biomass of five strains was strongly related to PhiX174 inactivation, suggesting a mild clustering pattern (Fig. 3A). Although these five species (*T. chuii*, *T. weissflogii*, *A. platensis*, *C. vulgaris* and *N. gaditana*) share no phylogenetic group, they are well known for having a resistant cell wall composed mainly of polysaccharides, which can be more selective in the secretion of different high molecular weight molecules, mainly small molecules, such as EPS, small proteins or secondary metabolites (Alhattab et al., 2019; Chen et al., 2020; Kotzsch et al., 2016).

The other five samples were distributed on the right side of axis 1 (Fig. 3A), four of them (*I. galbana*, *P. tricornutum*, *D. salina* and *Coelastrella* sp.) forming a cluster. Among them, two of these strains (*I. galbana*, and *D. salina*) are known for the absence of a cell wall (Alhattab et al., 2019; Sun et al., 2019). Moreover, *P. tricornutum* has one of the least silicified cell walls of diatoms, being less rigid than other diatom species (Song et al., 2020). Similarly, some *Coelastrella* species have been reported to have smooth cell walls (Shetty et al., 2021). These four species can be clustered together because of their non-resistant cell walls, which are likely to favour Phi6 inactivation (Fig. 3A). Additionally, *P. cruentum* was not included in these two clusters due to its high capacity to inactivate the three bacteriophages at relatively similar mild levels.

These preliminary results suggest that cell wall type and composition can be related to the inactivation capacity of different viruses by microalgae. In this context, PhiX174 attaches to lipopolysaccharides in the outer membrane of *E. coli*, which serve as receptors (Feige and Stirm, 1976), whereas Phi6 infection occurs via membrane fusion with *Pseudomonas* outer membrane (Bamford et al., 1987). Consequently, Fig. 3 suggests that higher PhiX174 inactivation could be mediated by preferential adsorption on microalgae cell walls with high concentrations of polysaccharides, while Phi6 could be more easily adsorbed and inactivated by microalgae with a membranous cell wall. On the other hand, MS2 infection is produced by adsorption with a specific F-pilus from

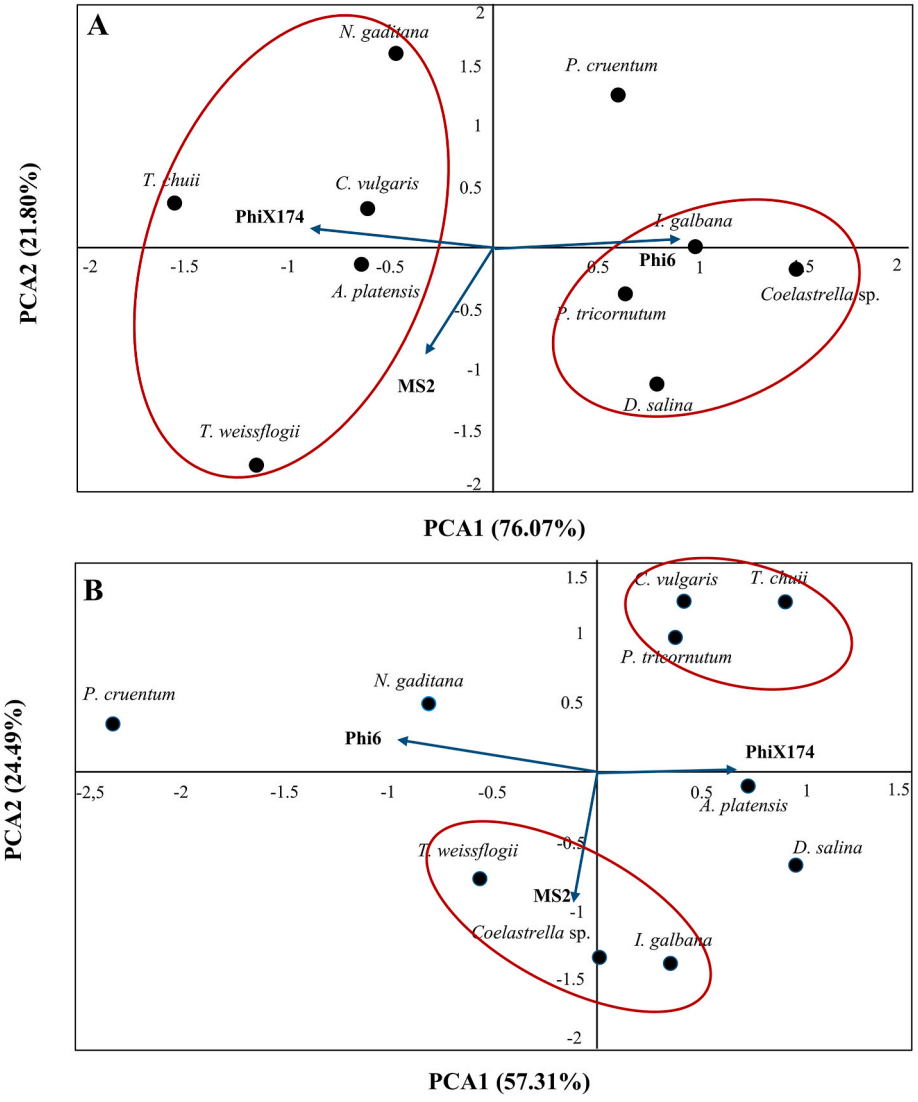

Fig. 3. Principal component analysis of the inactivation capacity of viruses using the biomass (A) and the supernatant (B) of the different microalgae selected in the present study.

E. coli, which does not have any similarity in microalgae wall structures (Meng et al., 2019), explaining the lack of association of this bacteriophage with the above-described clusters and an inactivation mechanism that seems to be less dependent on partitioning to microalgae cell walls. Further studies with a higher number of microalgal strains are needed to confirm these trends.

A PCA analysis of the capacity of the microalgal supernatants to inactivate the target viruses was also performed (Fig. 3B). In this case, axes 1 and 2 explained 82% of the total variation. Despite the low significance indicated by the KMO and Barlett test, this PCA suggests two clusters characterized by their capacity to inactivate MS2, with a higher inhibition caused by *T. weissflogii* compared to *C. vulgaris*. This trend of higher inactivation capacity could be attributed to a better interaction with the negatively charged viral capsids of MS2 by acidic EPS associated with the siliceous frustule of *T. weissflogii* (Bhaskar and Bhosle, 2005; Passow, 2002) that could remained in the supernatant after biomass separation. In contrast, comparatively inert cellulose-rich cell walls, which secret lower concentrations of reactive EPS, may limit the antiviral effect against MS2 of supernatants of *C. vulgaris* and *T. chuii* cultures. Moreover, the higher inactivation of MS2 compared to PhiX174 when exposed to *T. weissflogii* supernatant is aligned with an easier adsorption onto its anionic EPS matrix of the higher negatively charged capsid of MS2, promoting higher destabilization (Zhang et al., 2010). However, further investigation of the EPS composition of these strains, as along with the screening of more microalgae strains is needed to confirm this trend.

A correlation analysis was also performed to confirm the results obtained in the PCA analysis (Table 2). This analysis revealed a strong, significant negative correlation ($R^2 = -0.883$) between the type of microalgae that inhibits replication in PhiX174 and in Phi6, reinforcing the hypothesis that the most efficient strains against PhiX174 infections showed only a limited performance against Phi6 infections. More specifically, *T. weissflogii*, which showed one of the highest inhibition capacities against PhiX174 (59% of survival, compared with control), resulted in no significant differences compared to control plates when tested against Phi6 (87% of survival, Fig. 1). Besides the above-described effect of microalgal cell wall in PhiX174 and Phi6 inhibition based on their infection mechanisms (Bamford et al., 1987; Feige and Stirm, 1976), Table 2 reinforces the divergences on the predominant effect of microalgae against these two viruses, likely based also on the non-enveloped ssDNA and enveloped dsRNA structures of PhiX174 and Phi6 bacteriophages, respectively (Karczewska et al., 2023).

In addition, a significant negative correlation ($R^2 = -0.673$) between microalgal biomass inhibiting PhiX174 infections and supernatants inhibiting MS2 infections was also identified (Table 2). Although *C. vulgaris* and *T. chuii*, which were the two best-performing microalgal biomass against PhiX174, with low survival rates of 52 and 57%, these strains resulted in the highest survival rates when MS2 was incubated in their supernatants (84 and 86% of survival, respectively). In this sense, MS2 seemed to be more affected by microalgae with the capacity to excrete EPS, many of them with reported antioxidant and anticancer activity (Toshkova-Yotova et al., 2024; Tsotsouli et al., 2025). That was the case of *Coelastrella* sp., *I. galbana*, *T. weissflogii* and *D. salina,* which were the best-performing supernatants against MS2 infections (Fig. 2B). Besides the specific interactions between virus and EPS, a high oxidation of soluble MS2 and PhiX174 could occur from ROS formed in the photosynthetic culture.

Table 2
Multiple regression analysis using Pearson correlation to calculate the inactivation capacity of different viruses using microalgal biomass and supernatants (N = 10). Significant correlations ($p < 0.05$) are marked by asterisk.

		MS2 B	Phi6 B	PhiX174 S	MS2 S	Phi6 S
PhiX174	**R^2**	0.192	−0.883*	0.060	−0.673*	0.155
Biomass	**Sig**	0.595	<0.001	0.869	0.033	0.668
MS2 Biomass	**R^2**		−0.288	0.420	0.094	−0.462
	Sig		0.276	0.441	0.796	0.179
Phi6 Biomass	**R^2**			0.046	0.555	0.091
	Sig			0.900	0.096	0.802
PhiX174	**R^2**				−0.091	−0.442
Supernatant	**Sig**				0.802	0.225
MS2	**R^2**					0.114
Supernatant	**Sig**					0.753
Phi6	**R^2**					
Supernatant	**Sig**					

Overall, the correlations above identified suggest that the inhibitory effect against viruses is not directly related to the phylogenetic group of microalgae but to microalgal cell wall composition and, in particular, to its interactions with bacteriophages. Furthermore, other bioactivities studied in microalgae have been demonstrated to be non-dependent on different phylogenetic groups. For instance, Hernández-Urcera et al. (2024) demonstrated that antiviral, anti-inflammatory and cytotoxic activities of different microalgal extracts were not related to microalgal phyla. Similar results were reported by Maadane et al. (2015) on the antioxidant activity of nine microalgal strains, showing that most of the microalgal bioactivities were related to the species instead of phylogenetic groups.

3.4. Kinetics of viruses inactivation by Chlorella vulgaris and Thalassiosira weissflogii liquid cultures

Among all the microalgae tested in this study, *C. vulgaris* (a freshwater species) and *T. weissflogii* (a marine species) were selected to evaluate the kinetics of decay of the three tested bacteriophages in liquid cultures due to their efficient inactivation against PhiX174, MS2 and Phi6. The effect of light during virus inactivation was discriminated using cultures in dark and light conditions. Furthermore, the negative control culture included under each of these conditions demonstrated that viral inactivation was mainly produced by microalgal growth and activity (Fig. 4).

The highest survival among the tested bacteriophages in microalgal cultures was observed in PhiX174 (Fig. 4A). Under light conditions, *C. vulgaris* and *T. weissflogii* achieved comparable inactivation levels, reducing PhiX174 concentrations in the supernatants by 3.3 and 3.0 $\log_{10}$ units after 72 h, respectively. In contrast, under dark conditions, *C. vulgaris* demonstrated higher inactivation capacity than *T. weissflogii*, with reductions of 2.1 vs. 1.4 $\log_{10}$ units, respectively. Negative controls (without microalgae) showed only minimal reductions of 0.4 (dark) and 1.0 (light conditions) $\log_{10}$ units (Fig. 4A) during the same 72 h period. These results suggest that microalgae were mainly responsible for the viral inactivation due to intense photosynthetic activity in both cultures, confirmed by the consistent increases in headspace oxygen concentration, pH and Q_y values through the cultivation time (Fig. S1). In this sense, the highly oxidative environment mediated the higher inactivation capacity of PhiX174 under light conditions, as was previously hypothesized in literature (Fang et al., 2014; Torres-Franco et al., 2025).

Nonetheless, a higher relative contribution of photosynthetic activity seemed to occur for *T. weissflogii* than for *C. vulgaris*, for which non-photosynthetic-mediated inactivation showed relatively higher shares (54.25 and 38.31%, Table S2). Both microalgae maintained similar values of biomass and oxygen concentrations in the headspace under dark conditions (Fig. S1), in which the inactivation of PhiX174 by C. *vulgaris* cultures was higher (2.1 $\log_{10}$) than that of *T. weissflogii* (1.4 $\log_{10}$) (Fig. 4A). Overall, PhiX174 inactivation in the cultures supernatant under both light and dark conditions was consistent with the screening results (Figs. 1–3), with higher inactivation in the presence of biomass from *C. vulgaris* than from *T. weissflogii*.

The highest resistance to inactivation of PhiX174 compared with MS2 or Phi6 was also verified by its lower decay in the negative controls. Furthermore, these bacteriophages experienced similar reductions (between 0.3 and 2.2 $\log_{10}$ units) under light control conditions than under dark control conditions (0 to 2.3 $\log_{10}$ units) (Fig. 4A, B and C).

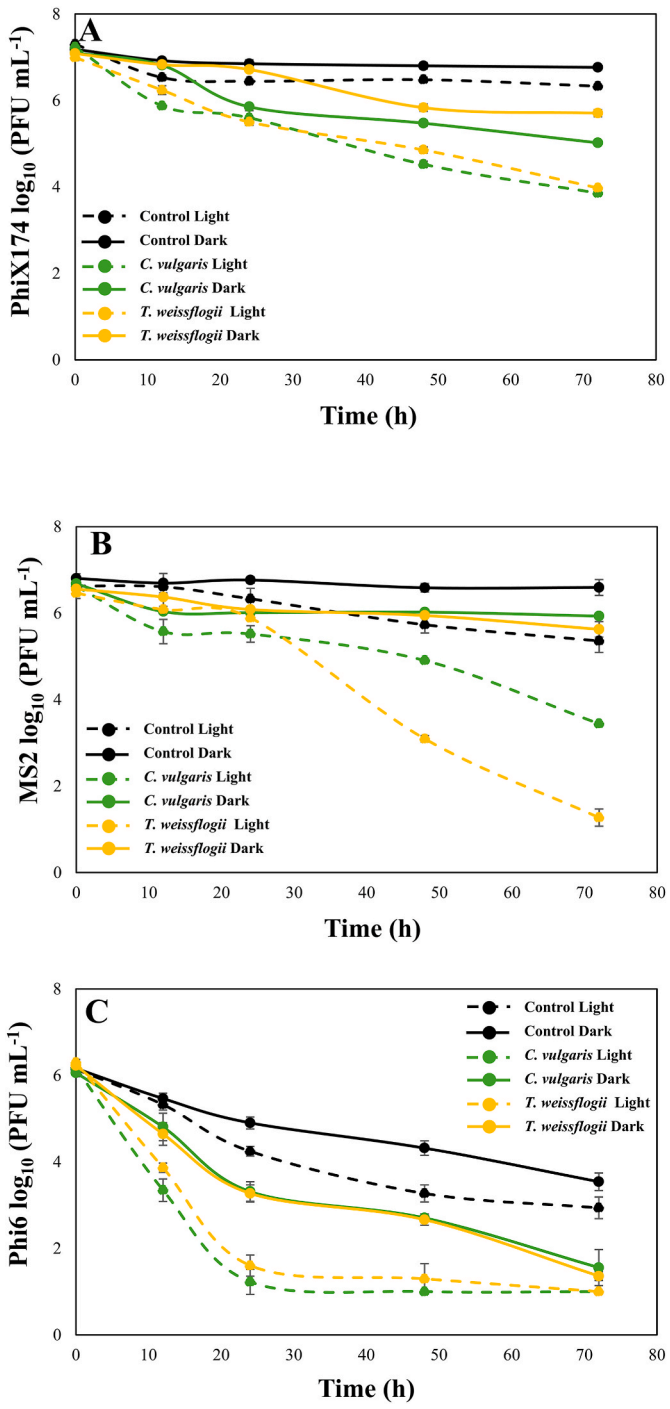

Fig. 4. Time course of the average log_{10}-units reduction in the concentration of PhiX174 (A), MS2 (B) and Phi6 (C) in the control cultures under light (continuous black line) and dark (dashed black line) conditions, *C. vulgaris* cultures under light (continuous green line) and dark (dashed green line) conditions, and *T. weissflogii* cultures under light (continuous yellow line) and dark (dashed yellow line) conditions.

Relatively similar results in control cultures were reported by Yang et al. (2024) under mesophilic and thermophilic anaerobic digestion, where PhiX174 showed lower reductions than Phi6 and T4, and similar to MS2 after 20 and 7 days of exposure, respectively.

Regarding MS2 inactivation, *T. weissflogii* supported higher values MS2 inactivation than *C. vulgaris*, with reductions of 5.2 log_{10} units and 3.2 log_{10} units, respectively, under light conditions after 72 h (Fig. 4B). These results confirmed that *T. weissflogii* was more effective for MS2 inactivation under illuminated conditions, which agrees with the screening results (Figs. 1 and 2), where both biomass and supernatant analysis showed high MS2 inactivation values associated to *T. weissflogii*, likely due to the higher interaction of the cell wall or EPS with MS2 capsid. Indeed, different molecules synthetized by *T. weissflogii*, such as small proteins, EPS or pigments (Kim et al., 2023; Vasilakis et al., 2025) (e.g fucoxanthin, a carotenoid produced by marine diatoms) were previously identified as an antiviral compound against the ssRNA Zika virus (Kang et al., 2024a).

Furthermore, inactivation of MS2 under dark cultivations conditions for *C. vulgaris* and *T. weissflogii* were 0.8 and 0.9 log_{10} units, respectively (Fig. 4B), similar values to those in the abiotic dark control (0.2 log_{10} units). In this sense, MS2 inactivation seemed to depend mainly on oxidation and photosynthetically induced processes (Table S2). Indeed, previous work demonstrated that the presence of ROS or oxidative agents, such as chlorine or ozone, could inactivate MS2 bacteriophage with high efficiency (Fang et al., 2014; Shang et al., 2007). Moreover, the permeable nature of MS2 capsid increases sensitivity to ROS species (Jahan et al., 2025; Majiya et al., 2018).

Inactivation of Phi6 was observed under all tested conditions, including negative controls (Fig. 4C). However, the presence of microalgae significantly enhanced the decay rates. *C. vulgaris* inactivated Phi6 below the limit of detection within 48 h, while *T. weissflogii* reached the same level of inactivation after 72 h under light conditions, with both microalgae showing reductions of 5.2 log_{10} units (Fig. 4C). Under dark conditions, the inactivation capacity was similar in both microalgal species, with reductions of 4.5 and 4.9 log_{10} units for *C. vulgaris* and *T. weissflogii* after 72 h of cultivation, respectively. Interestingly, this reduction in Phi6 inactivation capacity recorded in the negative control assay under light conditions (3.2 log_{10} units) was lower than that recorded in the microalgae cultured in the dark, suggesting that microalgal metabolites and adsorption and inactivation under light or dark cultivation conditions may also play a key role in Phi6 inactivation (Table S2). In this regard, pH values below the isoelectric point of Phi6 (6.9), achieved during cultivation of *C. vulgaris* and *T. weissflogii* in dark conditions, could favour higher aggregation in these treatments, ultimately yielding higher relative contributions than photosynthetic cultures (Table S2), in which higher inactivations were enhanced by background light. Indeed, Phi6 showed higher photosensitivity than PhiX174 and MS2, as supported by the higher inactivation in the light control than in the dark control (Fig. 4). Previous studies indicated a greater contribution of solids partitioning to microalgae-mediated inactivation and higher photosensitivity in Phi6 than in other non-enveloped bacteriophages, such as MS2 (Torres-Franco et al., 2024, 2025)

Inactivation decay kinetics were further evaluated by estimating the decay rates and T_{90} values for the three bacteriophages in negative controls, as well as in *C. vulgaris* and *T. weissflogii* liquid cultures. First-order linear regression models were applied to all cases, demonstrating good fit in most cases (Table 3). The only R^2 values lower than 0.9 were observed in the PhiX174 negative controls (light and dark), and in MS2 control and *C. vulgaris* cultures in dark conditions, due to limited viral inactivation after the first 12 h of experiment (Fig. 4; Table 3). Despite these exceptions, the results from most of the microalgal cultures fit well with first-order inactivation kinetics. Previous studies have reported this model as a robust model for viral decay processes, especially for MS2 and Phi6 inactivation (Calgua et al., 2014; Dean and Mitchell, 2022; French et al., 2023; Wu et al., 2019). However, other models, such as biphasic or non-linear models, have also been applied with high robustness in similar contexts (Dean and Mitchell, 2022; Torres-Franco et al., 2025).

The ability of the studied microalgae to inactivate the three tested bacteriophages was further supported by the higher *k* rates and the lower T_{90} values compared to the control cultures. The most pronounced differences were observed for PhiX174 under light conditions, where both microalgae exhibited *k* values of -0.007 h^{-1} in contrast with

Table 3
Decay parameters (k rates, R^2, T_{90} and significance values) of PhiX174, MS2 and Phi6 in the control, *C. vulgaris* and *T. weissflogii* assays under light and dark conditions.

Virus	Culture	k (h^{-1})	R^2	T_{90} (h)	Sig.
PhiX174	**Control light**	−0.0004	0.529	119	0.164
	Control dark	−0.0003	0.689	247	0.078
	***C. vulgaris* light**	−0.007	0.972	7.02	0.002
	***C. vulgaris* dark**	−0.005	0.936	19.43	0.007
	***T. weissflogii* light**	−0.007	0.989	12.75	<0.001
	***T. weissflogii* dark**	−0.003	0.939	32.21	0.007
MS2	**Control light**	−0.004	0.993	40.02	0.003
	Control dark	−0.0004	0.753	247	0.216
	***C. vulgaris* light**	−0.008	0.923	11.93	0.009
	***C. vulgaris* dark**	−0.001	0.479	90.70	0.195
	***T. weissflogii* light**	−0.027	0.955	3.39	0.011
	***T. weissflogii* dark**	−0.002	0.956	47.87	0.003
Phi6	**Control light**	−0.011	0.953	14.38	0.004
	Control dark	−0.007	0.990	22.55	<0.001
	***C. vulgaris* light**	−0.061	0.993	2.55	0.090
	***C. vulgaris* dark**	−0.018	0.973	8.26	0.002
	***T. weissflogii* light**	−0.031	0.999	2.95	0.067
	***T. weissflogii* dark**	−0.020	0.970	7.27	0.002

$-0.0004\ h^{-1}$ for the negative controls. Correspondingly, the T_{90} values of 7.02 and 12.75 h observed respectively for *C. vulgaris* and *T. weissflogii* were significantly shorter than the 119 and 247 h recorded in the negative controls (Table 3). Similar trends were observed for MS2 under light conditions, with T_{90} values of 11.93 and 3.39 h for *C. vulgaris* and *T. weissflogii,* respectively, The results in negative controls agree with similar results reported by Torres-Franco et al. (2024), with T_{90} values for Phi6 from 3 to 14 h, and for MS2 from 32 to 66 h in synthetic wastewater. It is also noteworthy the low T_{90} values that *T. weissflogii* showed for MS2 virus under light conditions (3.39 h), which is one fourth lower than *C. vulgaris* (11.93 h) and previous studies with other microalgae, such as *Nannochloropsis salina* or a consortium (Torres-Franco et al., 2024; Unnithan et al., 2014). These results suggest that this microalgae possess different mechanisms, such as a particular silicified cell wall, able to inactivate this virus faster than other strains with cell walls mainly composed of carbohydrates, such as *Chlorella* or *Nannochloropsis* strains. However, further studies are needed to unravel this mechanism.

4. Conclusions

This study demonstrated the high capacity of 10 different microalgae to inactivate three DNA and RNA bacteriophages providing, for the first time, results on the antiviral potential of *Coelastrella* sp., *D. salina, I. galbana, P. tricornutum and T. weissflogii*. The findings in the screening performed in this work demonstrated that the type of cell walls in microalgae can be related to their antiviral activity, although specific mechanisms require further investigation. Moreover, the two selected microalgal strains, *Chlorella vulgaris* and *Thalassiosira weissflogii* could inactivate 99.999% of MS2 and Phi6 viruses and 99.9% of PhiX174 in liquid cultures after 72 h, which supports that microalgae can inactivate different viruses with high efficiency likely based on oxidative conditions and interaction with microalgal cell wall and exo-compounds. The outcomes of this study and other investigations on the topic can be important for future developments in the field of pharmaceutics and wastewater reclamation processes.

CRediT authorship contribution statement

Antonio Leon-Vaz: Writing – original draft, Resources, Methodology, Conceptualization. **Lucía Tejero-Álvarez:** Methodology. **Pedro Antonio García-Encina:** Writing – review & editing, Supervision, Resources. **Raúl Muñoz:** Writing – review & editing, Supervision, Resources, Conceptualization. **Andrés Felipe Torres-Franco:** Writing – review & editing, Methodology, Conceptualization.

Funding

This work was supported by Spanish MICIU/AEI/10.13039/501100011033 (research grant n°: JDC2022-049636-I) and European Union NextGenerationEU/PRTR, and by the Department of Education of the Regional Government of Castilla y León and co-financed by the European Union through the European Regional Development Fund (ERDF) (References: CLU-2025-2-06, UIC320 and UIC379).

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.

Acknowledgments

We thank Prof. Viktoriia Komarysta from the Karazin Kharkiv National University and ISP, for kindly providing the *Coelastrella* sp. strain.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2026.128879.

Data availability

Data will be made available on request.

References

Alhattab, M., Kermanshahi-Pour, A., Brooks, M.S.L., 2019. Microalgae disruption techniques for product recovery: influence of cell wall composition. J. Appl. Phycol. 31, 61–88. https://doi.org/10.1007/s10811-018-1560-9.

Ashour, M., Mansour, A.T., Alkhamis, Y.A., Elshobary, M., 2024. Usage of Chlorella and diverse microalgae for CO 2 capture - towards a bioenergy revolution. Front. Bioeng. Biotechnol. 12, 1–20. https://doi.org/10.3389/fbioe.2024.1387519.

Bamford, D.H., Romantschuk, M., Somerharjul, P.J., 1987. Membrane fusion in prokaryotes: bacteriophage Phi6 membrane fuses with the Pseudomonas syringae outer membrane Dennis. EMBO J. 6, 1467–1473. https://doi.org/10.1002/j.1460-2075.1987.tb02388.x.

Ben Hilma, H., Farhat, A., Akermi, S., Khemakhem, B., Ben Halima, Y., Michaud, P., Fendri, I., Abdelkafi, S., 2022. In silico evidence of antiviral activity against SARS-CoV-2 main protease of oligosaccharides from Porphyridium sp. Sci. Total Environ. 836, 155580. https://doi.org/10.1016/j.scitotenv.2022.155580.

Bhaskar, P.V., Bhosle, N.B., 2005. Microbial extracellular polymeric substances in marine biogeochemical processes. Curr. Sci. 88, 45–53.

Brezeştean, I., Bocăneală, M., Gherman, A.M.R., Porav, S.A., Kacsó, I., Rakosy-Tican, E., Dina, N.E., 2021. Spectroscopic investigation of exopolysaccharides purified from Arthrospira plantensis cultures as potential bioresources. J. Mol. Struct. 1246. https://doi.org/10.1016/j.molstruc.2021.131228.

Calgua, B., Carratalà, A., Guerrero-Latorre, L., de Abreu Corrêa, A., Kohn, T., Sommer, R., Girones, R., 2014. UVC inactivation of dsDNA and ssRNA viruses in water: UV fluences and a qPCR-Based approach to evaluate decay on viral infectivity. Food Environ. Virol. 6, 260–268. https://doi.org/10.1007/s12560-014-9157-1.

Cañavate, J.P., 2019. Advancing assessment of marine phytoplankton community structure and nutritional value from fatty acid profiles of cultured microalgae. Rev. Aquacult. 11, 527–549. https://doi.org/10.1111/raq.12244.

Chen, W., Xu, J., Yu, Q., Yuan, Z., Kong, X., Sun, Y., Wang, Z., Zhuang, X., Zhang, Y., Guo, Y., 2020. Structural insights reveal the effective Spirulina platensis cell wall dissociation methods for multi-output recovery. Bioresour. Technol. 300, 122628. https://doi.org/10.1016/j.biortech.2019.122628.

Cheng, R., Zhang, Y., Zhang, T., Hou, F., Cao, X., Shi, L., Jiang, P., Zheng, X., Wang, J., 2022. The inactivation of bacteriophages MS2 and PhiX174 by nanoscale zero-valent iron: resistance difference and mechanisms. Front. Environ. Sci. Eng. 16, 108. https://doi.org/10.1007/s11783-022-1529-4.

Cristofoli, N.L., Lima, A.R., Rosa da Costa, A.M., Evtyugin, D., Silva, C., Varela, J., Vieira, M.C., 2023. Structural characterization of exopolysaccharides obtained from Porphyridium cruentum exhausted culture medium. Food Bioprod. Process. 138, 162–171. https://doi.org/10.1016/j.fbp.2023.02.001.

Dean, K., Mitchell, J., 2022. Identifying water quality and environmental factors that influence indicator and pathogen decay in natural surface waters. Water Res. 211, 118051. https://doi.org/10.1016/j.watres.2022.118051.

Decamp, A., Martineau, E., Grizeau, D., Pruvost, J., Gonçalves, O., 2023. Effects of the salinity on the biosynthesis of the polysaccharides of the marine microalgae Porphyridium cruentum. Algal Res. 71, 103089. https://doi.org/10.1016/j.algal.2023.103089.

Deore, P., Barlow, C.K., Schittenhelm, R.B., Beardall, J., Noronha, S., 2022. Profiling of grazed cultures of the chlorophyte alga Dunaliella tertiolecta using an untargeted LC–MS approach. J. Phycol. 58, 568–581. https://doi.org/10.1111/jpy.13254.

Dhama, K., Khan, S., Tiwari, R., Sircar, S., Bhat, S., Malik, Y.S., Singh, K.P., Chaicumpa, W., Bonilla-Aldana, D.K., Rodríguez-Morales, A.J., 2020. Coronavirus disease 2019–COVID-19. Clin. Microbiol. Rev. 33 e00028-20.

Díaz, N., Muñoz, S., Medina, A., Riquelme, C., Lozano-Muñoz, I., 2025. Microchloropsis gaditana as a natural antimicrobial with a one health approach to food safety in farmed salmon. Life 15, 455. https://doi.org/10.3390/life15030455.

Durmuş, S., Ülgen, K., 2017. Comparative interactomics for virus–human protein–protein interactions: DNA viruses versus RNA viruses. FEBS Open Bio 7, 96–107. https://doi.org/10.1002/2211-5463.12167.

Fang, J., Liu, H., Shang, C., Zeng, M., Ni, M., Liu, W., 2014. E. coli and bacteriophage MS2 disinfection by UV, ozone and the combined UV and ozone processes. Front. Environ. Sci. Eng. 8, 547–552. https://doi.org/10.1007/s11783-013-0620-2.

Fedorenko, A., Grinberg, M., Orevi, T., Kashtan, N., 2020. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS - CoV - 2) in evaporated saliva microdroplets deposited on glass surfaces. Sci. Rep. 10, 22419. https://doi.org/10.1038/s41598-020-79625-z.

Feige, U., Stirm, S., 1976. On the structure of Escherichia coli C cell wall lipopolysaccharide core and on its øX174 receptor region. Biochem. Biophys. Res. Commun. 71, 566–573. https://doi.org/10.1016/0006-291X(76)90824-X.

French, A.J., Longest, A.K., Pan, J., Vikesland, P.J., Duggal, N.K., Marr, L.C., Lakdawala, S.S., 2023. Environmental stability of enveloped viruses is impacted by initial volume and evaporation kinetics of droplets. mBio 14. https://doi.org/10.1128/mbio.03452-22.

Gasch-Illescas, A., Calle-Serrano, M., Vallejo-Vaz, A.J., Praena-Fernández, J.M., Guerrero, J.A., Calderón, E.J., Pollán, M., Medrano, F.J., 2023. Impact of the first wave of the COVID-19 pandemic on non-COVID inpatient care in southern Spain. Sci. Rep. 13, 1–10. https://doi.org/10.1038/s41598-023-28831-6.

Guiry, M., Guiry, G., 2022. Algaebase [WWW Document]. World-wide Electron. Publ. Natl. Univ. Ireland. Galway. Retrieved from.

Hernández-Urcera, J., Romero, A., Cruz, P., Vasconcelos, V., Figueras, A., Novoa, B., Rodríguez, F., 2024. Screening of microalgae for bioactivity with antiviral, antibacterial, anti-inflammatory and anti-cancer assays. Biology (Basel). 13, 255. https://doi.org/10.3390/biology13040255.

Hong, W.Y., 2022. A techno-economic review on carbon capture , utilisation and storage systems for achieving a net-zero CO 2 emissions future. Carbon Capture Sci. Technol. 3, 100044. https://doi.org/10.1016/j.ccst.2022.100044.

Jahan, S., Pruvost, J., Cogne, G., Titica, M., Fallowfield, H., 2025. Inactivation of an indicator virus during microalgae-based wastewater treatment. J. Appl. Phycol. 37, 1593–1606. https://doi.org/10.1007/s10811-024-03435-3.

Kang, N., Kim, E.A., Park, A., Heo, S.Y., Heo, J.H., Heo, S.J., 2024a. Antiviral potential of fucoxanthin, an edible carotenoid purified from Sargassum siliquastrum, against zika virus. Mar. Drugs 22, 247. https://doi.org/10.3390/md22060247.

Kang, N., Kim, E.A., Park, A., Heo, S.Y., Heo, J.H., Lee, W.K., Ryu, Y.K., Heo, S.J., 2024b. Antiviral activity of chlorophyll extracts from Tetraselmis sp., a marine microalga, against zika virus infection. Mar. Drugs 22, 397. https://doi.org/10.3390/md22090397.

Karczewska, M., Strzelecki, P., Szalewska-Pałasz, A., Nowicki, D., 2023. How to tackle bacteriophages : the review of approaches with mechanistic insight. Int. J. Mol. Sci. 24, 4447. https://doi.org/10.3390/ijms24054447.

Kevill, J.L., Herridge, K., Li, X., Farkas, K., Malham, S.K., Robins, P., Jones, D.L., 2025. Comparative impact of sunlight and salinity on human pathogenic virus survival in river, estuarine, and marine water microcosms. Water Res. 278, 123411. https://doi.org/10.1016/J.WATRES.2025.123411.

Kim, E.A., Kang, N., Heo, S.Y., Oh, J.Y., Lee, S.H., Cha, S.H., Kim, W.K., Heo, S.J., 2023. Antioxidant, antiviral, and anti-inflammatory activities of lutein-enriched extract of Tetraselmis species. Mar. Drugs 21, 369. https://doi.org/10.3390/md21070369.

Kotzsch, A., Pawolski, D., Milentyev, A., Shevchenko, Anna, Scheffel, A., Poulsen, N., Shevchenko, Andrej, Kröger, N., 2016. Biochemical composition and assembly of biosilica-associated insoluble organic matrices from the diatom Thalassiosira pseudonana. J. Biol. Chem. 291, 4982–4997. https://doi.org/10.1074/jbc.M115.706440.

León-Vaz, A., Giráldez, I., Moreno-Garrido, I., Varela, J., Vigara, J., León, R., Cañavate, J. P., 2023. Amino acids profile of 56 species of microalgae reveals that free amino acids allow to distinguish between phylogenetic groups. Algal Res. 74. https://doi.org/10.1016/j.algal.2023.103181.

León-Vaz, A., Torres-Franco, A.F., García-Encina, P.A., Muñoz, R., 2025. Developing a microalgal-bacterial consortium for the removal of organic pollutants from petrochemical industry. J. Water Process Eng. 73, 107663. https://doi.org/10.1016/j.jwpe.2025.107663.

Li, Y., Wang, C., Liu, H., Su, J., Lan, C.Q., Zhong, M., Hu, X., 2020. Production, isolation and bioactive estimation of extracellular polysaccharides of green microalga Neochloris oleoabundans. Algal Res. 48, 101883. https://doi.org/10.1016/j.algal.2020.101883.

Lin, K., Schulte, C.R., Marr, L.C., 2020. Survival of MS2 and Φ 6 viruses in droplets as a function of relative humidity , pH , and salt , protein , and surfactant concentrations. PLoS One 15, e0243505. https://doi.org/10.1371/journal.pone.0243505.

Liu, X., Bian, Z., Hu, S., Dickinson, C.F., Benjamin, M.M., Jia, J., Tian, Y., Place, A., Hanna, G.S., Luesch, H., Croot, P., Reddy, M.M., Thomas, O.P., Hardiman, G., Puglisi, M.P., Yang, M., Zhong, Z., Lemasters, J.J., Korte, J.E., Waters, A.L., Heltzel, C.E., Williamson, R.T., Strangman, W.K., Valeriote, F., Tius, M.A., Ditullio, G.R., Ferreira, D., Alekseyenko, A., Wang, S., Hamann, M.T., Wang, X., 2024. The chemistry of phytoplankton. Chem. Rev. 124, 13099–13177. https://doi.org/10.1021/acs.chemrev.4c00177.

Maadane, A., Merghoub, N., Ainane, T., El, H., 2015. Antioxidant activity of some Moroccan marine microalgae : Pufa profiles , carotenoids and phenolic content Antioxidant activity of some Moroccan marine microalgae : Pufa profiles , carotenoids and phenolic content. J. Biotechnol. 215, 13–19. https://doi.org/10.1016/j.jbiotec.2015.06.400.

Maciel, F., Madureira, L., Geada, P., Teixeira, J.A., Silva, J., Vicente, A.A., 2024. The potential of Pavlovophyceae species as a source of valuable carotenoids and polyunsaturated fatty acids for human consumption. Biotechnol. Adv. 74, 108381. https://doi.org/10.1016/j.biotechadv.2024.108381.

Majiya, H., Adeyemi, O.O., Stonehouse, N.J., Millner, P., 2018. Photodynamic inactivation of bacteriophage MS2: the A-protein is the target of virus inactivation. J. Photochem. Photobiol. B Biol. 178, 404–411. https://doi.org/10.1016/j.jphotobiol.2017.11.032.

Maltsev, Y., Maltseva, K., Kulikovskiy, M., Maltseva, S., 2021. Influence of light conditions on microalgae growth and content of lipids, carotenoids, and fatty acid composition. Biology (Basel). 10, 1–24. https://doi.org/10.3390/biology10101060.

Maneein, S., Sangsanont, J., Limpiyakorn, T., Sirikanchana, K., Rattanakul, S., 2024. The coagulation process for enveloped and non-enveloped virus removal in turbid water: removal efficiencies, mechanisms and its application to SARS-CoV-2 Omicron BA.2. Sci. Total Environ. 931, 172945. https://doi.org/10.1016/j.scitotenv.2024.172945.

Mapelli-Brahm, P., Gómez-Villegas, P., Gonda, M.L., León-Vaz, A., León, R., Mildenberger, J., Rebours, C., Saravia, V., Vero, S., Vila, E., Meléndez-Martínez, A.J., 2023. Microalgae, seaweeds and aquatic bacteria, archaea, and yeasts: sources of carotenoids with potential antioxidant and anti-inflammatory health-promoting actions in the sustainability era. Mar. Drugs 21, 340. https://doi.org/10.3390/md21060340.

Meng, R., Jiang, M., Cui, Z., Chang, J., Yang, K., Jakana, J., Yu, X., Wang, Z., Hu, B., Zhang, J., 2019. Structural basis for the adsorption of a single-stranded RNA bacteriophage. Nat. Commun. 10, 3130. https://doi.org/10.1038/s41467-019-11126-8.

Mosu, N., Yasukochi, M., Nakajima, S., Nakamura, K., Ogata, M., Iguchi, K., Kanno, K., Ishikawa, T., Sugita, K., Murakami, H., Kuramochi, K., Saito, T., Takeda, S., Watashi, K., Fujino, K., Kamisuki, S., 2024. Isolation, structural determination, and antiviral activities of a novel alanine-conjugated polyketide from talaromyces sp. J. Antibiot. (Tokyo). 77, 499–505. https://doi.org/10.1038/s41429-024-00740-4.

Osathanunkul, M., Thanaporn, S., Karapetsi, L., Nteve, G.M., Pratsinakis, E., Stefanidou, E., Lagiotis, G., Avramidou, E., Zorxzobokou, L., Tsintzou, G., Athanasiou, A., Mpelai, S., Constandinidis, C., Pantiora, P., Merino, M., Mullor, J.L., Dobrovic, L., Cerasino, L., Ogawa, T., Tsaousi, M., Rodrigues, A.M.C., Cardoso, H., Pires, R., Figuereido, D., Costa, I.F., Anjos, C., Labrou, N.E., Madesis, P., 2025. Diversity of bioactive compounds in microalgae : key classes and functional applications. Mar. Drugs 23, 222. https://doi.org/10.3390/md23060222.

Parra-Riofrio, G., Moreno, P., García-Rosado, E., Alonso, M.C., Uribe-Tapia, E., Abdala-Díaz, R.T., Bejar, J., 2023. Tetraselmis suecica and Porphyridium cruentum exopolysaccharides show anti-VHSV activity on RTG-2 cells. Aquac. Int. 31, 3145–3157. https://doi.org/10.1007/s10499-023-01202-8.

Passow, U., 2002. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. 55, 287–333. https://doi.org/10.1016/S0079-6611(02)00138-6.

Sen, S., Tiwari, O.N., Arya, R.K., Bhowmick, T.K., Gayen, K., 2025. New insights on microbial extracellular polysaccharides: production, biological activity, and applications. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-025-06802-3.

Shang, C., Cheung, L.M., Liu, W., 2007. MS2 coliphage inactivation with UV irradiation and free chlorine/monochloramine. Environ. Eng. Sci. 24, 1321–1332. https://doi.org/10.1089/ees.2006.0261.

Shetty, P., Farkas, A., Pap, B., Hupp, B., Ördög, V., Bíró, T., Varga, T., Maróti, G., 2021. Comparative and phylogenomic analysis of nuclear and organelle genes in cryptic Coelastrella vacuolata MACC-549 green algae. Algal Res. 58, 102380. https://doi.org/10.1016/j.algal.2021.102380.

Singh, S.P., Singh, P., 2015. Effect of temperature and light on the growth of algae species: a review. Renew. Sustain. Energy Rev. 50, 431–444. https://doi.org/10.1016/j.rser.2015.05.024.

Song, Z., Lye, G.J., Parker, B.M., 2020. Morphological and biochemical changes in Phaeodactylum tricornutum triggered by culture media: implications for industrial exploitation. Algal Res. 47, 101822. https://doi.org/10.1016/j.algal.2020.101822.

Sun, Z., Wang, X., Liu, J., 2019. Screening of Isochrysis strains for simultaneous production of docosahexaenoic acid and fucoxanthin. Algal Res. 41, 101545. https://doi.org/10.1016/j.algal.2019.101545.

Torres-Franco, A.F., Leroy-Freitas, D., García-Encina, P.A., Muñoz, R., 2025. Viral RNA reduction from wastewaters using microalgae-based treatments: elucidating the effect of light and zero-valent iron nanoparticles. Bioresour. Technol. 427, 132389. https://doi.org/10.1016/j.biortech.2025.132389.

Torres-Franco, A.F., Leroy-Freitas, D., Martínez-Fraile, C., Rodríguez, E., García-Encina, P.A., Muñoz, R., 2024. Partitioning and inactivation of enveloped and nonenveloped viruses in activated sludge, anaerobic and microalgae-based wastewater treatment systems. Water Res. 248, 120834. https://doi.org/10.1016/j.watres.2023.120834.

Toshkova-Yotova, T., Sulikovska, I., Djeliova, V., Petrova, Z., Ognyanov, M., Denev, P., Toshkova, R., Georgieva, A., 2024. Exopolysaccharides from the green microalga strain Coelastrella sp. BGV—Isolation, characterization, and assessment of anticancer potential. Curr. Issues Mol. Biol. 46, 10312–10334. https://doi.org/10.3390/cimb46090614.

Toucheteau, C., Deffains, V., Gaignard, C., Rihouey, C., Laroche, C., Pierre, G., Lépine, O., Probert, I., Le Cerf, D., Michaud, P., Arnaudin-Fruitier, I., Bridiau, N., Maugard, T., 2023. Role of some structural features in EPS from microalgae stimulating collagen production by human dermal fibroblasts. Bioengineered 14, 2254027. https://doi.org/10.1080/21655979.2023.2254027.

Tsotsouli, K., Didos, S., Koukaras, K., Argiriou, A., 2025. Mixotrophic cultivation of Dunaliella tertiolecta in cheese whey effluents to enhance biomass and exopolysaccharides (EPS) production: biochemical and functional insights. Mar. Drugs 23, 120. https://doi.org/10.3390/md23030120.

Ugya, A.Y., Sheng, Y., Chen, H., Wang, Q., 2024. Microalgal bioengineering : a futuristic tool for carbon capture. Results Eng. 24, 102990. https://doi.org/10.1016/j.rineng.2024.102990.

Unnithan, V.V., Unc, A., Smith, G.B., 2014. Role of Nannochloropsis salina for the recovery and persistence of MS2 virus in wastewater. Algal Res. 4, 70–75. https://doi.org/10.1016/j.algal.2013.11.004.

Vasilakis, G., Marka, S., Ntzouvaras, A., Zografaki, M.E., Kyriakopoulou, E., Kalliampakou, K.I., Bekiaris, G., Korakidis, E., Papageorgiou, N., Christofi, S., Vassilaki, N., Moschopoulou, G., Tzovenis, I., Economou-Amilli, A., Papanikolaou, S., Flemetakis, E., 2025. Wound healing, antioxidant, and antiviral properties of bioactive polysaccharides of microalgae strains isolated from Greek coastal Lagoons. Mar. Drugs 23, 77. https://doi.org/10.3390/md23020077.

Wang, Q., Ma, Y., Sun, F., Wang, K., Ma, J., Zhu, B., Cao, K., Shao, Y., 2025. Development and research progress of microalgae as a production platform for antimicrobial peptides. J. Appl. Phycol. https://doi.org/10.1007/s10811-025-03472-6.

Wu, X., Tang, A., Bi, X., Nguyen, T.H., Yuan, B., 2019. Influence of algal organic matter of Microcystis aeruginosa on ferrate decay and MS2 bacteriophage inactivation. Chemosphere 236, 124727. https://doi.org/10.1016/j.chemosphere.2019.124727.

Yang, W., Cai, C., Dai, X., 2022. Interactions between virus surrogates and sewage sludge vary by viral analyte: recovery, persistence, and sorption. Water Res. 210, 117995. https://doi.org/10.1016/j.watres.2021.117995.

Yang, W., Cai, C., Wang, S., Wang, X., Dai, X., 2024. Unveiling the inactivation mechanisms of different viruses in sludge anaerobic digestion based on factors identification and damage analysis. Bioresour. Technol. 413, 131541. https://doi.org/10.1016/j.biortech.2024.131541.

Zhang, H., Zhang, J., Zhao, B., Zhang, C., 2010. Removal of bacteriophages MS2 and phiX174 from aqueous solutions using a red soil. J. Hazard. Mater. 180, 640–647. https://doi.org/10.1016/j.jhazmat.2010.04.084.

Zhang, J., Liu, L., Chen, F., 2019. Production and characterization of exopolysaccharides from Chlorella zofingiensis and Chlorella vulgaris with anti-colorectal cancer activity. Int. J. Biol. Macromol. 134, 976–983. https://doi.org/10.1016/j.ijbiomac.2019.05.117.

Zhu, C., Ye, Y., 2025. Reactivity of viral proteins with free chlorine: structural insights and implications for virus inactivation. Environ. Sci. Technol. 59, 17188–17197. https://doi.org/10.1021/acs.est.5c01689.