

LOW-PRESSURE GASEOUS PLASMA FOR INACTIVATION OF VIRUSES IN WATER

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Doctoral Dissertation
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UPORABA NIZKOTLAČNE PLINSKE PLAZME ZA
INAKTIVACIJO VIRUSOV V VODI

Doktorska disertacija

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Abstract

Water scarcity is becoming a greater issue worldwide due to ever-increasing anthropogenic activity. A dedicated effort is required to recover and maintain the purity of our water resources and has created an environment for developing better technologies to tackle this issue. Low-pressure gaseous plasma, consisting of a complex mixture of reactive particles, ions, and neutrals and emitting powerful radiation, is a versatile, environmentally friendly tool, serving many purposes in various industries. Among other features, it can also produce intense vacuum ultraviolet (V-UV) radiation, serving as a potential sterilisation technique since the photons have enough energy to disrupt molecular bonds, causing the degradation of pollutants. The issue, however, is that V-UV is strongly absorbed by almost all substances, most notably the oxygen molecules in the atmosphere. Therefore, its application to treat any material should entail a unique system where the V-UV is actually allowed to interact with the desired substance.

The main objective of this study was to construct a low-pressure plasma system as a source of V-UV radiation and evaluate its potential to treat liquid samples for the purpose of disinfection. Bacteriophage MS2 served as a model contaminant for studying disinfection efficiency since viruses are difficult to remove from contaminated water.

The literature review revealed that this kind of water treatment has never been attempted in the past. We initiated our research by constructing a pulsed DC plasma system and carried out systematic characterization of operating parameters for producing V-UV radiation. A special sample chamber was constructed to treat liquid samples with low-pressure plasma radiation, physically separating the different pressure environments while still allowing the V-UV radiation to reach the sample. Through a series of different experimental setups and conditions, hydroxyl radicals ($\text{OH}^\bullet$) were shown to be the primary inactivation factors, and the presence of O_2 in the headspace of the sample greatly improved the inactivation efficiency due to the additional production of ROS ($\text{OH}^\bullet$, H_2O_2 , O_3).

Seeking additional information and improvement on the system, a radiofrequency inductively coupled low-pressure plasma system (RF ICP) was constructed and used for the same purpose. Similar conclusions were reached, namely that $\text{OH}^\bullet$ is the primary factor for virus inactivation, whose production is significantly enhanced by more intense V-UV radiation, along with O_2 being present in the headspace. Additionally, we subjected MS2 bacteriophage to V-UV treated water separately, whose poor inactivation demonstrated that short-lived ROS are primarily responsible for the observed inactivation. Expanding further, we utilised the RF ICP to treat other types of water pollutants, such as bacteria, antibiotics, and dyes, to showcase the method's general applicability towards all types of contamination.

To conclude our work, we made some calculations and compared the energy efficiency of our system against standard disinfection methods, which provided some promising prospects for this technique.

Povzetek

Pomanjkanje pitne vode zaradi vedno bolj intenzivnega človeškega delovanja predstavlja vedno večji problem v svetu. Potreba po obnovitvi in vzdrževanju neoporečnosti naših vodnih virov ustvarja okolje za razvoj boljših tehnologij za premagovanje te problematike. Nizkotlačna plazma, kompleksna zmes reaktivnih delcev, ionov, nevtralnih delcev in močnega sevanja, predstavlja vsestransko okolju prijazno metodo, ki je že našla uporabo v številnih industrijah. Med drugim jo lahko odlikuje tudi močno vakuumsko ultravijolično sevanje (V-UV), ki ga je moč uporabiti za namene sterilizacije, saj imajo ti fotoni dovolj energije za prekinitev molekulskih vezi, kar povzroči razgradnjo onesnažil. Težava je v tem, da V-UV močno absorbirajo praktično vse snovi, predvsem molekule kisika v atmosferi, zaradi česar je potrebno izdelati posebno napravo, ki omogoča obdelavo vzorca z V-UV sevanjem.

Glavni cilj te raziskave je ustvariti nizkotlačni plazemski sistem, ki omogoča obdelavo tekočih vzorcev z V-UV, ter preučiti njegovo delovanje za namen dezinfekcije. Bakteriofag MS2 je bil uporabljen kot modelno onesnažilo, saj virusi velik problem pri čiščenju vod.

Celovit pregled literature je razkril, da tovrsten pristop za čiščenje vod še ni bil preizkušen v preteklosti. Tako smo na začetku raziskave izdelali kapacitivno sklopljen plazemski sistem, napajan s pulznim enosmernim generatorjem, ter izvedli sistematske meritve, s čimer smo identificirali optimalne parametre za proizvodnjo močnega V-UV sevanja. Ločitev nizkotlačnega plazemskega sistema od atmosferskega, kjer se nahaja naš tekoči vzorec, smo dosegli z izgradnjo posebne komore, ki dovoljuje dostop sevanja do površine tekočine. Skozi sistematično izločanje dejavnikov smo pokazali, da so hidroksilni radikali ($\text{OH}^\bullet$) ključni dejavniki inaktivacije ter da se reaktivne kisikove ($\text{OH}^\bullet$, H_2O_2 , O_3) vrste tvorijo v večjih količinah, če je v obdelovalni komori prisoten kisik.

V želji po boljšem razumevanju in izboljšavi sistema smo izdelali še induktivno sklopljen plazemski sistem, napajan z radiofrekvenčnim generatorjem. Zaključki so bili podobni, namreč da so $\text{OH}^\bullet$ prvotno odgovorni za inaktivacijo virusa ter da je njihova produkcija povečana z bolj intenzivnim V-UV sevanjem, pogojeno s prisotnostjo kisika v komori. Poleg tega smo MS2 virus izpostavili še plazemsko obdelani vodi, čigar slaba inaktivacija je še dodatno potrdila ključno vlogo kratkoživečih reaktivnih kisikovih vrst za inaktivacijo virusa. Vsestransko uporabnost sistema za čiščenje vod smo pokazali z razgradnjo različnih onesnažil, kot so bakterije, antibiotiki in barvila.

V zaključku dela predložimo nekaj izračunov o energetske učinkovitosti sistema, kar poda obetavne možnosti tovrstne metode v primerjavi s standardnimi dezinfekcijskimi sredstvi.

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Abbreviations

AC	...	alternative current
AOP	...	advanced oxidation process
COD	...	chemical oxygen demand
CCP	...	capacitively coupled plasma
CPE	...	cytopathic effect
Ct	...	disinfectant contact time
DC	...	direct current
DOC	...	dissolved oxygen content
DNA	...	deoxyribonucleic acid
E	...	electric field
e_{aq}	...	solvated electron
EEDF	...	electron energy distribution function
ELISA	...	enzyme—linked immunosorbent assay
HTA	...	hydroxyterephthalic acid
$h\nu$	...	photon
ICP	...	inductively coupled plasma
MW	...	microwave frequency
NOM	...	natural organic matter
p	...	pressure
PMS	...	peroxymonosulphat
PCR	...	polymerase chain reaction
RF	...	radiofrequency
RO(N)S	...	reactive oxygen (and/or nitrogen) species
RNA	...	ribonucleic acid
TA	...	terephthalic acid
TEM	...	transmission electron microscopy
V—UV	...	vacuum ultraviolet radiation
α	...	ionisation degree

Chapter 1

Introduction

Clean and affordable drinking water is an essential resource for a healthy life, which is why it was recognised as a fundamental human right by the United Nations General Assembly in 2010 [1]. Nevertheless, more than 700 million people still lived in high water-stress conditions in 2020, described as either insufficient or inadequately sanitised water sources [2]. Changes in the availability of safe water are usually the result of increased human activity (urbanisation, industry, agriculture), climate change-related weather extremes (drought, flood, temperature extremes), or inappropriate allocation or sanitation of water resources [3]. The latter presents a technological challenge of providing safe water, combined with a socio-economic feature of administering affordable treatment technologies, serving anything from large urban areas down to remote rural settlements.

The largest health threat related to contaminated water are pathogenic microorganisms, which are responsible for over 500,000 preventable deaths of children under 9 years of age [4], [5]. This is why even the World Health Organization Guidelines for drinking Water Quality, generally regarded as the foundational document for establishing a safe water framework, put an emphasis on preventing microbial waterborne disease occurrence by establishing health-based targets and implementing water safety plans [4].

One of the most important water contaminants are viruses, ubiquitous parasites of all environments. Pathogenic waterborne viruses present a significant health risk since they are usually very stable and can survive outside their host for a long time, are difficult to remove and have a very low infectious dose (amount of exposure to particles to cause infection) [6]. The leading cause of gastroenteritis in humans, enteric viruses, for example, survive exposure to the gastrointestinal tract as well as environmental factors and may even withstand wastewater treatment facilities [7]. They are excreted in large numbers from infected individuals (10^9 particles per mL) and are an indicator of faecal-contaminated water or food [6].

Water disinfection methods, such as chemical treatment, ultraviolet (UV) radiation and ozone (O_3) that are integrated into water treatment schemes have greatly improved the health of consumers and the impact on the environment, however some deficiencies are present with each method [5]. Therefore, new technologies are still being explored to fill in the gaps and improve on the existing methods. Gaseous plasma, the fourth state of matter, is a complex mixture of reactive particles, electrons, neutrals, and radiation. In recent years, plasma-based technologies have replaced many harmful chemical treatment methods

and opened possibilities for several new, precise treatment processes. Water disinfection using plasma treatment is also being extensively researched since the reactive species produced are effective towards a broad range of pollutants, while on the other hand, requiring no chemical additives to achieve this effect. The issue in its use is the complexity of operation since many technological parameters (e.g., discharge power, reactor configuration, pressure, gas composition, also known as discharge parameters) and parameters of treated liquid (e.g., type of pollutant, temperature, pH, turbidity, dissolved oxygen) affect the disinfection outcome. Nevertheless, the treatment principle is similar to other Advanced Oxidation Processes (AOPs) in generating highly reactive oxygen species (ROS, e.g., $\text{OH}^\bullet$, O_3 , H_2O_2 , $\text{HO}_2^\bullet$, etc.) as well as germicidal UV radiation, to carry out the disinfection process. From this point of view, treatment optimisation should focus on producing the greatest amount of these agents and evaluating their effect on the target pollutant. Low-pressure plasma, while more challenging to implement for liquid water treatment, can be produced in a large volume while also being able to produce highly energetic vacuum-UV (V-UV) radiation, which can facilitate the production of the aforementioned (ROS). In this work, we constructed several low-pressure gaseous plasma systems for treating liquid water. We characterise their V-UV emission output, measure and optimise ROS production and implement the method for virus disinfection. Using bacteriophage MS2, a surrogate for many human pathogenic enteric viruses, as a primary target for assessing disinfection efficiency, we propose the underlying inactivation mechanisms. Lastly, we discuss energy efficiency and optimisation approaches for using this method on a larger scale, along with suggesting some directions for further research.

1.1 Viruses

The word virus originates from Latin and means *poison* [7]. Viruses present a distinct group of organisms, which have caused much controversy in the scientific community due to their variability in size, shape, genome structure and organisation, and overall interaction with their respective host(s). Their initial definition as “filterable disease agents” (i.e., infectious agents, too small for bacterial filters) was deemed unsuitable with the identification of giant (larger than bacteria) viruses in 2007 [8], and a more defined description of ‘fastidious parasites’ was bestowed upon them. In their bare essence, viruses are packets of genetic information that are unable to replicate on their own and, therefore, need to hijack their host’s replication machinery to reproduce. Their infection strategies, life cycles and relationships with their host are unique to each member, and as such, they present a large repertoire of molecular mechanisms to study and understand. They infect all kingdoms of life, shape host populations’ abundance, and drive evolutionary change; thus, they have a significant impact on the global ecology and human microbiome, the importance of which we are only now beginning to understand [9].

The practical need to classify viruses arose when a variety of new viruses were being discovered at a rapid pace [10]. In 1973, the International Committee on Taxonomy of Viruses (ICTV) was established to tackle the monumental task of developing a single, universal taxonomic scheme for all viruses. As of the last published report in 2023, the ICTV recognises over 14,000 species, divided into 314 families and 81 orders. To grasp the magnitude of change, imagine that only 10 years prior, the 2013 ICTV report recognised little more than 2,800 species, divided into 103 families and 7 orders [11]. After some initial

attempts, the current taxonomy groups viruses based on shared properties such as morphology, presence of envelope, genome composition, genome orientation (+/−), genome segmentation, gene sets and replication strategies, to name a few. No single characteristic is given higher priority, however, attributes for higher-level taxa are shared with all lower-level taxa. Before the advent of modern sequencing techniques, viruses were initially distinguished by their basic structure (shape), genome composition (double- or single-stranded DNA or RNA) and the presence or absence of an envelope [10]. Nowadays, information on the replication strategies, gene homology, and a detailed genome organisation are also included. Due to new methodologies for virus characterisation, the sheer amount of structural variability and the constant evolution of virus species, the ICTV report is a living, ever-changing document, open to re-classifications and establishment of new taxa.

A ‘packet of genetic information’ is a common and appropriate term for describing virions, the extracellular phase of the virus replication cycle. It stresses the fact that they cannot be considered living organisms due to the lack of any distinct feature of a living microbe, such as metabolism, self-replication, and the like. The description also provides a comment on the simplicity of their structure (Figure 1). Although the underlying molecular mechanisms are anything but, the overall virus structure can be considered simple. Regardless of which virus species, all viruses share the same basic structure, a polypeptide enclosure (capsid, composed of capsomeres) providing protection and transmission of its genetic material (DNA or RNA), with about 25% of viruses also having a host-derived lipid layer (envelope) with some proteins on its surface. The envelope is formed in the ‘budding’ process when the nucleocapsid initiates its release from the host cell. The lipids are host-derived, but the protruding proteins can be derived by either the host or virus code, and they aid in host infection [12].

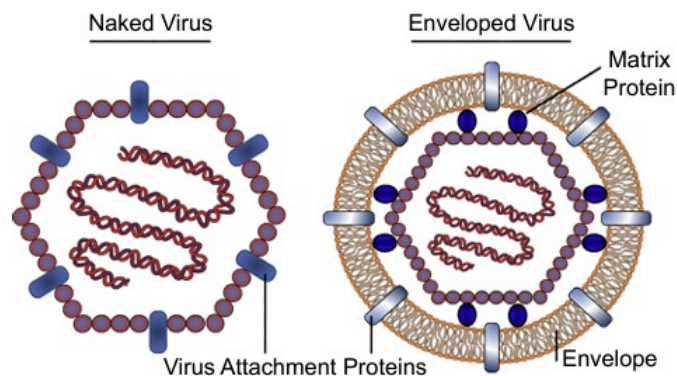


Figure 1: Basic components of non-enveloped (‘naked’) and enveloped viruses (adapted from [13]).

When studying viruses in natural water, with the exception of sewage, viral concentrations are usually too low to detect them without prior concentration of the sample. To concentrate viruses to a smaller volume (5–10 mL), large volumes need to be processed, sometimes even more than 100 L [14]. Several methods are available that concentrate viruses as though they were large proteins. Adsorption-elution techniques, in the form of charged membranes, are often used for concentrating viruses since they are low-cost and can process large volumes of water [14], [15]. The original sample is brought into contact with the solid matrix at specific pH and ionic conditions to facilitate virus adsorption, after which the virus is eluted from the matrix into a smaller, albeit sometimes still too large, volume [14]. Coagulation/flocculation of viruses with the addition of

chemical coagulants, such as polyethylene glycol, ferric chloride or skimmed milk, is the method of choice for complex water matrices (sewage, seawater, etc.). Despite being highly reproducible and low-cost, the concentration efficiency is not on par with other methods, so it is more appropriately used as a tool for controlling water safety [15], [16]. Ultrafiltration is the next method of choice to reduce the volume of samples. The selection of appropriate membrane cut-off allows for concentrating a wide range of viruses with good efficiency. However, the high equipment cost, and long operation times are the reason a pre-concentration step is desired [17]. Additionally, due to the small pores of the membranes, the method is limited by the membrane blockage by highly turbid waters [15]. Similarly, ultracentrifugation prides itself on being a universal technique for concentrating samples. A centrifugal force of over 100,000 g is applied to a sample, allowing for sedimentation of viral particles based on density [18]. The final sample volume is sufficiently low to be used in virus detection tests, but the low volumes of samples that can be processed, along with high costs, make ultracentrifugation a secondary concentration method of choice [14]. Other techniques, such as hydroextraction, metal oxide flocculation, coagulation, immunoaffinity, magnetic beads and similar, are seldom used but are described in detail by other authors [14], [17], [19].

Quantification and identification of viral particles are essential to study and understand their origin, host interaction, infectivity, life cycle, means of transmission, and how to design measures to prevent their adverse effects. Due to their incredibly small size (Figure 2), only some of the largest viruses, such as Poxviruses (220–450 nm), were initially observable by a light microscope, with a resolution limit of 300 nm [12]. With the invention of electron microscopy in the early 20th century, the ultrastructure of virions was finally accessible, revealing structural details and remarkable diversity of the virosphere. Even though the method is burdened by low sensitivity and cumbersome isolation and sample preparation procedure, it remains the go-to method for routine morphology determination, especially for non-culturable viruses [20]. The advent of X-ray crystallography enabled the visualisation of viral structure near the atomic level, providing essential information on the viral antigens and the aspect of their attachment and penetration into the host cell, which has instructed the design of new antiviral drugs [12].

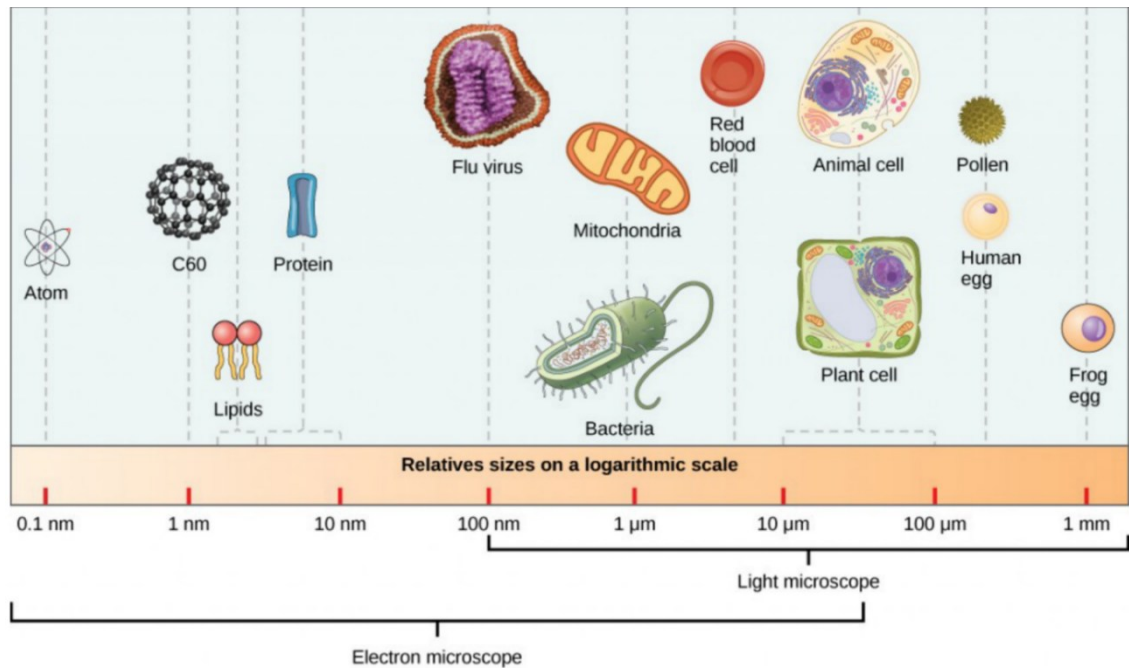


Figure 2: Relative sizes of bacteria, viruses, proteins, molecules, and atoms. The resolving powers of various techniques used in virology, light, and electron microscopy are indicated. Viruses span a broad range from that equal to some small bacteria to just under ribosome size (adapted from [21]).

Going further from physical observation of viral particles, several methods have been developed to study the morphology, molecular structure and life cycle (i.e. infection and multiplication) of viruses (Figure 3). Here, it is important to stress that, based on the detection method selected, significant differences in virus quantities are obtained due to the inherent differences in which factors are measured. For example, the number of viral particles counted by an electron microscope will always exceed the number of infectious particles counted in a sample by a plaque assay. This is to be expected, since all viral particles may not be infectious, due to e.g., unfavourable environmental conditions, random genome mutations, faulty assembly of virions or just a statistically unfavourable chances of virus-cell interactions [12]. Infectious viruses are identified by a cytopathic effect (CPE), a morphological change of the host cell, which indicates a successful infection [14].

When available, the plaque assay, the oldest and simplest quantitative test for viral infectivity, remains the golden standard for quantifying infectious particles in a sample [12]. There are several iterations of the method, but the underlying principle is the same: making serial dilutions of the virus-contaminated sample and exposing the dilutions to the appropriate host cell, usually on solid cell media on Petri dishes. After incubation, clear spots (plaques) are observed, which represent lysed cells due to the infection-replication-lysis life cycle of the studied virus [22]. Counting the number of plaques and knowing the dilution of the sample, a per-millilitre titre of *infectious* viral particles is calculated. When the virus does not propagate by a lytic cycle, where plaques are formed, a different approach to quantification must be employed, such as endpoint dilution assay, commonly referred to as tissue culture infectious dose (TCID₅₀). Here, the virus titre is calculated from a series of inoculated cell cultures and expressed as the dilution, producing a cytopathic effect (CPE) in 50% of the cultures [14], [22]. The infectivity assay, however, is often unavailable for most viruses, as it requires both the virus and its host to be culturable in a laboratory setting. Additionally, a detailed understanding of the appropriate conditions for infection to occur is required [14].

All these requirements lead to the development of methods that rely on the detection of specific viral particles rather than intact viruses, albeit in a semi-quantitative way. Several immunological assays are available that detect viral antigens using well-defined antibody-antigen interactions that enable detection of viral particles. Without going into too much detail, these methods utilise specific antigens that bind to viral antigens, resulting in a measurable change in the sample. The detection portion of these methods is the differentiating factor, as the result can either be a colour change (Enzyme-Linked Immuno Sorbent Assay; ELISA), radiation (radioimmunoassay), fluorescence (fluoroimmunoassay), agglutination, immunochromatography (Western blot) or similar. Perhaps the most known and versatile method for detecting different viruses, the ELISA method, still finds many applications today. Here, an immobilised antibody is used to bind virus particles to a solid substrate, after which an enzyme-linked antibody is bound to the virus. This enzyme serves as a catalyst, changing the colour of the added substrate, from which the virus titre can be determined [23]. Although these methods are rapid and can be very sensitive, they cannot distinguish between viable and non-viable organisms [12]. In recent decades, nucleic acid isolation and sequencing techniques have become reliable, rapid and extremely sensitive methods of identification and discovery of new viral species, as they provide a detailed genetic fingerprint of the virus. This has been largely possible through the development of the Polymerase Chain Reaction (PCR), which utilises the cell's enzymatic machinery (polymerase) to greatly multiply the target gene sequence, stringing together deoxynucleotide phosphate (dNTP) monomers, starting from the reverse and forward primer-pair sequences [24]. By also including a reverse transcriptase enzyme step, even RNA viruses can be sequenced and quantified [25]. The basic PCR, however, is not sufficient to provide quantitative information on the number of viruses present in a sample, as it only amplifies the target sequence without any information on the starting amount. An expansion of this method, called the quantitative PCR (qPCR), is available, where a fluorescently labelled probe is provided, which binds to the target sequence, producing a fluorescent signal when the sequence is transcribed. The intensity of the signal is amplified with each successive reaction cycle until it is strong enough to pass the threshold (C_t , reaction background) value, at which point the detection of the PCR product is recorded. When a sample with a known DNA concentration (reference sample) is included, the method can be reasonably quantitative [26].

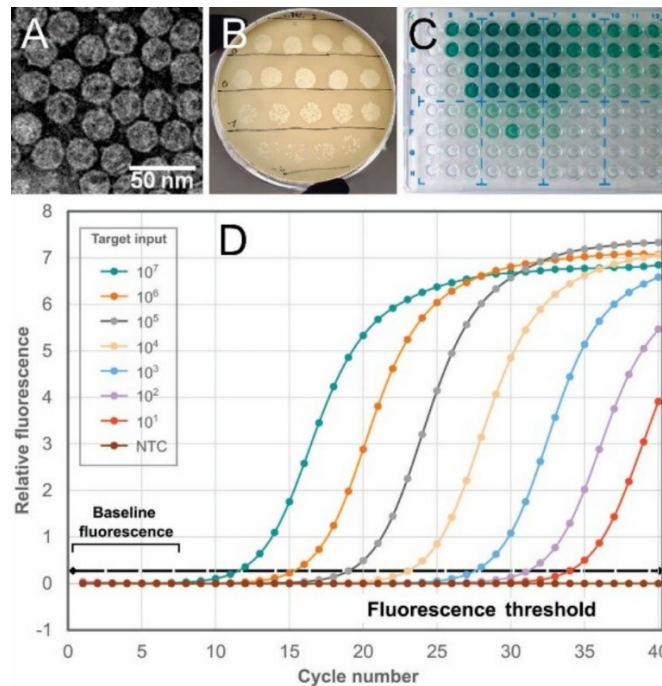


Figure 3: Exemplary results of commonly used virus detection and identification methods: A) Cryo-TEM picture of MS2 bacteriophage [27], B) Plaque assay of MS2 bacteriophage (own work), C) ELISA result [28], D) qPCR result [29].

Contamination of water sources is a global issue, and viruses present some of the most dangerous contaminants. About 1 in 4 people worldwide consume faecal-contaminated water containing pathogenic (disease-causing) organisms. Diarrhoea, the most common infection symptom, causes over 400.000 deaths per year globally from contaminated water consumption, around 18% of which is caused by viral infections [15]. Pathogenic viruses infecting mammals, animals and plants can be transported over long distances via water [30]. What's more, some tend to be more resistant than bacteria, and due to their small size, their removal is very challenging [30], [31]. Waterborne infections are associated with poor hygiene, inadequate sanitation, and the consumption of unsafe water, while also resulting in significant economic losses each year [32].

Enteric viruses have the ability to persist and replicate in the intestinal mucosa, from where they can cause disease symptoms [33]. Human enteric viruses, such as rotavirus, norovirus, adenovirus and enterovirus, enter the environment primarily through waste discharge from infected individuals and infect new hosts through the faecal-oral route [15]. They are host-specific and have a very low infectious dose (10–1000 particles), along with good environmental stability, making them the primary target for water disinfection [33], [34]. Consumption of contaminated water or food, inhalation of water droplets or exposure to contaminated recreational waters are the most common exposure scenarios [30], [35], [36], [37], [38], [39]. Infected individuals can expel between 10^5 – 10^{11} viral particles during a gastroenteritis episode, and 10^4 – 10^5 infectious particles per litre are commonly present in raw sewage, with 50–100 particles per litre found in WWTP effluents [37].

All major human pathogenic viruses, classified by the World Health Organization as a high health risk [4], are nonenveloped viruses (Table 1, Figure 4), with a great majority of them having a single-stranded RNA genome [12], [33]. They are associated with gastroenteritis, which can lead to diarrhoea and vomiting, as well as other symptoms such as fever, cramps, myocarditis, hepatitis, etc. Rotavirus alone is recognised as the most

critical life-threatening disease for children under 5 years of age [33].

Table 1: Water-transmitted pathogenic viruses, classified as moderate-high health risk (adapted from [4]).

Pathogen	Type species	Persistence in water supplies ^a	Relative infectivity ^b	Health significance ^c
<i>Adenoviridae</i>	Adenoviruses	Long	High	Moderate
<i>Astroviridae</i>	Astroviruses	Long	High	Moderate
<i>Caliciviridae</i>	Noroviruses, Sapoviruses	Long	High	High
<i>Hepeviridae</i>	Hepatitis E virus	Long	High	High
<i>Picornaviridae</i>	Enteroviruses, Parechoviruses, Hepatitis A virus	Long	High	High
<i>Reoviridae</i>	Rotaviruses	Long	High	High

^a long, over 1 month; ^b high, $1-10^8$ organisms can cause infection; ^c high severity of disease, including association with outbreaks.

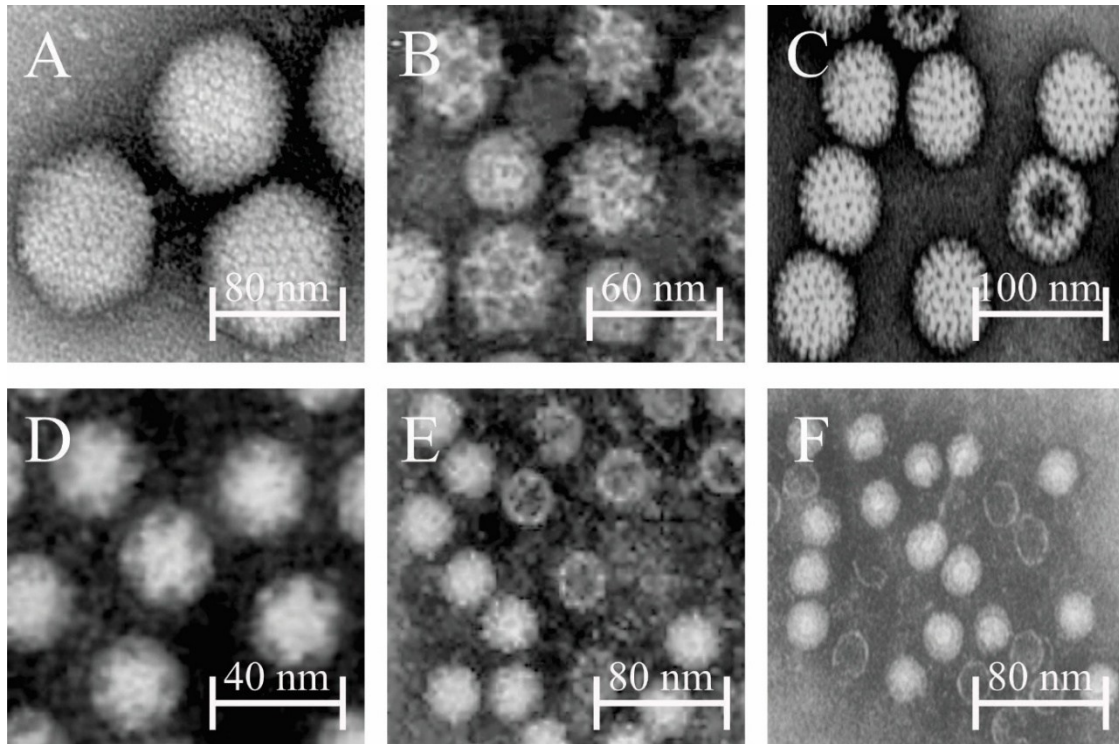


Figure 4: Scanning electron microscope (SEM) micrographs of human pathogenic viruses: A) Adenovirus, B) Norovirus, C) Rotavirus, D) Astrovirus, E) Hepatitis E virus, F) Hepatitis A virus (adapted from [12])

Understanding the environmental factors affecting virus survival can aid in developing tools to prevent their spread and reduce infections. Since viruses are not considered ‘living’ organisms, the term survival here refers to their ability to retain the ability to infect their host and produce infectious particles, thus completing their propagation (life) cycle. This can be achieved by either damaging their outer structures (capsid, envelope) or creating

unfavourable environmental conditions (pH, salinity, temperature) to prevent host infection or by damaging their genome, making them unable to produce infectious particles [40].

Factors affecting virus survival are numerous but can be either physical (temperature, sunlight, humidity), chemical (pH, surfactants, dissolved oxygen) or biological (microbial load, enzymatic activity, virus structure) [6]. The abundance of various virus types, infection cycles, genome organisation, capsid structure, presence/absence of envelope and a plethora of other structural features present in the virosphere also relate to different sensitivities of viruses towards various inactivation factors. Even within the same family of viruses, completely different sensitivities are observed. When three related bacteriophages (MS2, fr, and GA) were treated with different disinfectants (UV₂₅₄, ClO₂, ¹O₂, free chlorine), it was shown that minor differences in their composition, such as protein structure and genome composition (GC content), affected the disinfection outcome [41]. Similarly, Enriquez et al. compared the survival of Adenovirus (Adv) 40, 41, poliovirus type I and hepatitis A virus (HAV) in tap, sea or wastewater. The results show a remarkable difference in survival rates even between Adv 40 and Adv 41. When inoculated into tap water at 4 °C, the time to reach 99% inactivation (T_{99}) of Adv 40 was 92 days, while for Adv 41 it was 304 days, even though the viruses are closely related. A literature review of environmental conditions, such as temperature, salinity, etc., which resulted in 90% inactivation of the Phi6 virus, an enveloped virus surrogate, produced a time span between 24 minutes and 117 days [42], stemming from variation of the environmental conditions (temperature, relative humidity), composition of media, biological activity and other factors. At the same conditions, polio I and HAV displayed T_{99} of 41 and 56 days, respectively. These trends were similar when the temperature was raised to 15 °C or room temperature, while seawater produced an even greater discrepancy between Adv viruses and polio I. When inoculated into sewage, all viruses displayed similar survival rates, with increasing temperature being the main inactivation accelerator [43]. It was also shown that the type of host influences the survivability of viruses since Influenza A virus was more stable when grown in mammalian culture rather than the avian one, presumably due to the differences of the lipid envelope and respective glycoproteins derived from the host cell [44]. This makes comparison between different studies examining virus inactivation extremely challenging. Nevertheless, some general trends for virus inactivation can be imposed, such as increased temperature, sunlight exposure and intensity [45], presence of disinfecting substances [46], and intense biological activity [18], [42], [47]. Furthermore, non-enveloped viruses are usually more resistant than enveloped ones at similar conditions, although the latter are able to retain infectivity for a longer duration when frozen or exposed to low relative humidity environments [18], [46], [48], [49].

Most viruses have higher stability at lower temperatures since an increased temperature can cause protein and genome denaturation, preventing the adsorption to its host while also promoting the activity of antagonistic microbes [37], [50]. The latter can cause virus inactivation in aquatic environments by releasing proteolytic enzymes and cleaving viral proteins and genome.

Viruses in natural waters tend to form aggregates [51] or attach themselves to suspended solids (clay, organic material, algae, etc.) [37], which is one of the primary defence mechanisms of viruses. Promoted by environmental factors, such as low pH [18], [52], presence of divalent ions [53], [54], and their surface charge [6], aggregation or adsorption was found to provide protection against UV radiation [55], enzymatic activity [50], [54] and disinfectants [56]. Additionally, viruses can assimilate into biofilms, which provides protection against environmental stresses and enables the slow release of viral particles into the environment, presenting a secondary source of water contamination [6].

On the other hand, the microbes may release proteases and nucleases into the environment, which can act against viruses in the biofilm [54]. Viruses can also adsorb to organic matter and suspended solids, which greatly decreases the inactivation rates of waterborne viruses, possibly due to shielding effects (UV) or chemical inactivation of the applied disinfectants [35]. Viruses adsorbed to particles can settle to the bottom of the water column and accumulate in the sediment, where they are further shielded from inactivation. [37]. Ionic strength, i.e. salt concentration, can either decrease or increase virus inactivation by modulating the attraction and repulsion forces of surface proteins [46], [57], [58], [59]. However, seawater does not inhibit enteric viruses rapidly. Sewage-originating enteric viruses could be detected several kilometres off the coasts of Hawaii and have been reported to survive seawater exposure for over 100 days [60], [61]. What's more, they demonstrated that coliform bacteria, an indicator of faecal contamination of waters, could only last in seawater for some hours, showing that the correlation between faecal bacteria and viruses is not sufficient to use the former as an indicator for the latter [60]. Some viruses, such as Norovirus, cannot be cultured to evaluate their inactivation, which is why the United States Environmental Protection Agency (USEPA) recommends using coliphages as surrogates of human enteric viruses [39]. Bacteriophages (or phages) are commonly used as surrogates since they are safe to cultivate and share many similarities with human enteric viruses, such as size (27.5 nm), genome structure and morphology [62]. MS2 bacteriophage (Figure 5), a member of the *Fiersviridae* (formerly *Leviviridae*) family, is perhaps the most used surrogate for this purpose. It is a non-enveloped, F^+ specific virus, negatively charged over a wide pH range and displays high hydrophobicity [62]. It infects the bacteria *E. coli* (coliphages), another indicator organism for faecal contamination, which is also easily cultivatable and well-researched [63]. F^+ specific coliphages are a subgroup that infect the male, F pilus-expressing *E. coli* [39]. The infection of *E. coli* proceeds via adsorption to the bacterium pili, from where the genome is delivered inside the cell, while the capsid is left outside [64], [65]. The icosahedral capsid is composed of 180 coat proteins, each consisting of 129 amino acid residues with a molecular mass of 13.7 kDa [66]. Additionally, the capsid has one copy of the A protein (44 kDa, maturation protein), which is involved in virus assembly and host infection [66]. The positive sense single-stranded RNA genome is one of the smallest of any lifeform, consisting of only around 3500 nucleotides, which code for four proteins: maturation, coat, lysis and replicase enzyme [64]. MS2 is an appropriate surrogate for enteric viruses since it shares most of their features, including size and genome structure, is non-enveloped and highly resistant to UV [67] and chemical disinfection, making it a worst-case scenario viral indicator [45], [62], [68]. Its inactivation requires high oxidation power and proceeds via denaturation of capsid proteins [62], [68].

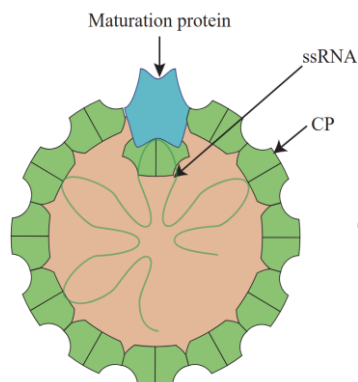


Figure 5: Schematic cross-section of MS2 bacteriophage (adapted from [69]). CP = coat protein, ssRNA = single-stranded RNA

1.2 Water treatment

Water quality criteria for producing sufficiently clean water, able to be discharged into the environment or used for other purposes, broadly address two types of water constituents (pollutants), dissolved and undissolved matter (Table 2). Dissolved matter entails gasses of environmental importance (e.g., NH_3 , H_2S , NO_2) and various soluble liquids and solids. Undissolved matter refers to precipitable, floating, or otherwise suspended particles (e.g., soil particles, plant residues, oils, clay minerals, etc.). Additionally, microorganisms are also grouped into this category [70]. At a quick glance, one can see the challenge of sufficiently removing the vastly different chemical and physical pollutants and producing sufficiently clean water at a large scale to prevent environmental degradation caused by anthropogenic activities.

Table 2: Chemicals and molecules found in water samples (adapted from [70]).

Compounds in water	
Natural	Anthropogenic
Cations: Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Al^{3+} , Fe^{2+} , Fe^{3+} , Mn^{2+} , NH_4^+ Anions: HCO_3^- , F^- , Cl^- , Br^- , NO_3^- , SO_4^{2-} Natural species: H_4SiO_4 , H_2CO_3 , O_2 , N_2 Humic substances	HPO_4^{2-} , H_2PO_4^- , pesticides, surfactants, heavy metals, drugs, alcohols, ethers, phenols, grease, aromatic compounds, etc.

An ideal water sanitation technique would be easily set up, operated and maintained, reasonably priced, and efficiently remove all types of pollutants while not causing damage to operators, consumers or the environment. It is also expected that this system would be scalable to address the needs of all scales, from modest consumers (households to rural areas) to large cities. Given the variety of requirements, it is commonly accepted that no single technique would be able to fulfil all criteria alone. Moreover, there remains the issue of adaptation or resistance of microbes to any single technique, which has already been observed for some common disinfection practices such as UV and chlorination [4]. Therefore, the water treatment plants and/or systems approach the purification in separate steps (levels), namely preliminary, primary, secondary, and, in some cases, tertiary treatment (Figure 6) [71]. Without going into too much detail, the preliminary treatment entails physical removal or processing of larger particles or floating substances (oils, grease) by a series of screens and grit chambers [71]. This step is sometimes combined with the primary treatment step, which again consists of the physical removal of suspended solids. This can take the form of a simple sedimentation step, or it can be improved by the addition of coagulants, which aid in the flocculation and subsequent sedimentation of suspended solids [72]. By this point in the process, we are left with a liquid containing few to zero suspended solids but loads of organic matter. The purpose of the secondary treatment step is to process the suspended organic matter, remove nutrients such as nitrogen and phosphorous, and reduce the Biological Oxygen Demand (BOD; oxygen consumption by microbial metabolic activity). Biological treatment is the method of choice for this step, where microbes carry out the removal of unwanted organic matter and nutrients, utilising them as an energy source for their growth. The process can be either anaerobic or aerobic by design, depending on the presence/addition of oxygen in the system. The most known example of an anaerobic digester is the septic tank, where the anaerobic conversion of organics to methane and carbon dioxide takes place. Although no oxygen addition, low energy requirements and a high degree of waste stabilisation are advantages of this systems, the process is hindered by the slow-growing microbes, high

temperature and pH sensitivity and inability to sufficiently remove ammonia [73]. Nevertheless, it may still be a part of a wastewater treatment facility for the treatment of sludge (sediment) discharged in the other stages [71] or as a standalone water treatment system for small rural communities [73]. The more common method for a large-scale water treatment plant is aerobic biological treatment, where air (containing oxygen) is supplied to the reactor, and the microbes are able to degrade organic matter and convert it into biomass, which is subsequently removed and treated. A major benefit of this approach is the reduction of BOD by the addition of oxygen, making the effluent safe to be discharged into the environment after secondary clarification and disinfection step. Tertiary or advanced treatment is the final stage before discharge, where water is treated to meet regulatory requirements before discharge. Here, excess nutrients such as nitrogen, phosphorous, heavy metals, inorganics, and contaminants of concern are excluded from the water by means of chemical precipitation, adsorption, filtration, and disinfection.

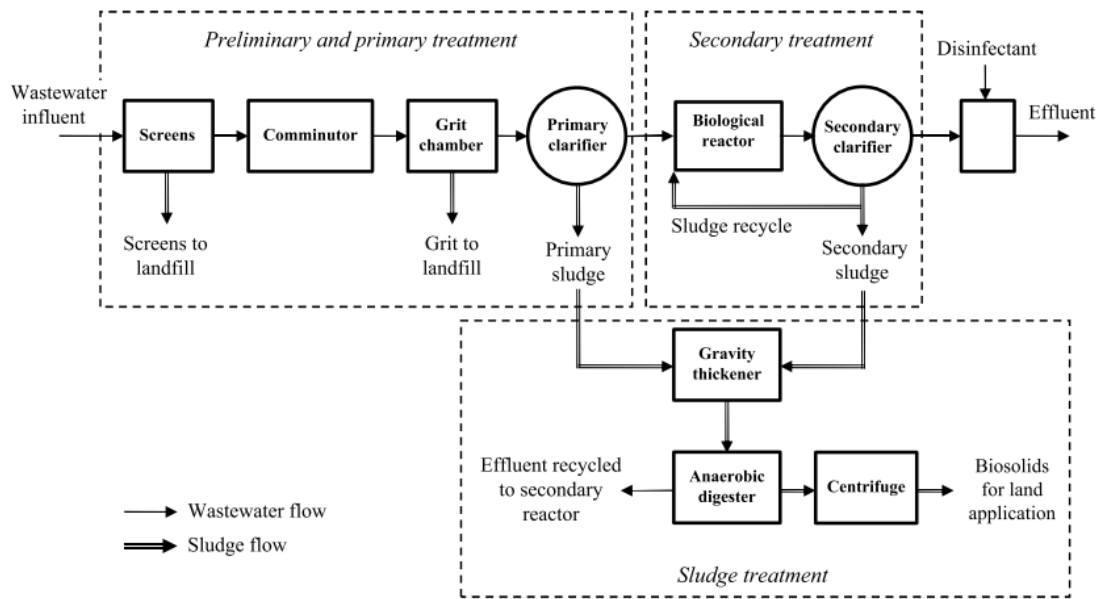


Figure 6: Flow diagram of a conventional wastewater treatment plant (adapted from [71]).

The practice of disinfection specifically refers to the removal or destruction of unwanted microorganisms – pathogens, to low enough levels to prevent health and safety issues for humans and animals. The goal is to reduce microbial populations to prevent them from causing environmental harm or health issues. This is achieved by physically removing them from the water source (e.g. filtration) or sufficiently damaging critical molecular structures to prevent microbes from multiplying, producing harmful compounds, or, in the case of viruses, preventing them from infecting their host (Figure 7). The first techniques of water disinfection date back to the ancient Greek civilisations in 4000 BCE, consisting of simple filtration and boiling procedures to improve the appearance (turbidity) and taste of water [74]. Similar practices were used throughout human history, up to the first large-scale municipal water treatment plants established in the 19th century [75]. Most disinfection practices involve the use of chlorine, as it cost-effectively removes the majority of pathogens while providing residual activity throughout the water transport systems [4]. Faecal water pollution presents the most significant health threat, as it can introduce pathogenic

microorganisms to water, facilitating their transport and infection of new hosts [4]. To prevent this, water sources are regularly monitored for the presence of *E. coli* and enterococci, indicators of faecal contamination; however, for viral contaminations, coliphages (MS2, Φ X174, GA, Q β , or SP) are preferably used [4], [39].

In untreated wastewater, virus genome copies can range from 10^5 – 10^7 copies per litre [76], [77]. Primary and secondary treatment steps usually remove between 10^3 – 10^4 viruses, which may not be sufficient to prevent contamination of natural water sources [77]. Without tertiary treatment, they are poorly reduced by wastewater treatment facilities and are thus discharged into the environment. Recreational use of such waters has inferred disease risk, and even tap water has been linked to gastrointestinal illnesses [7]. Even chlorination, the most frequently used disinfection method, was found to be relatively ineffective against Noroviruses, requiring a dose of 6.25 mg/L for 30 min [78].

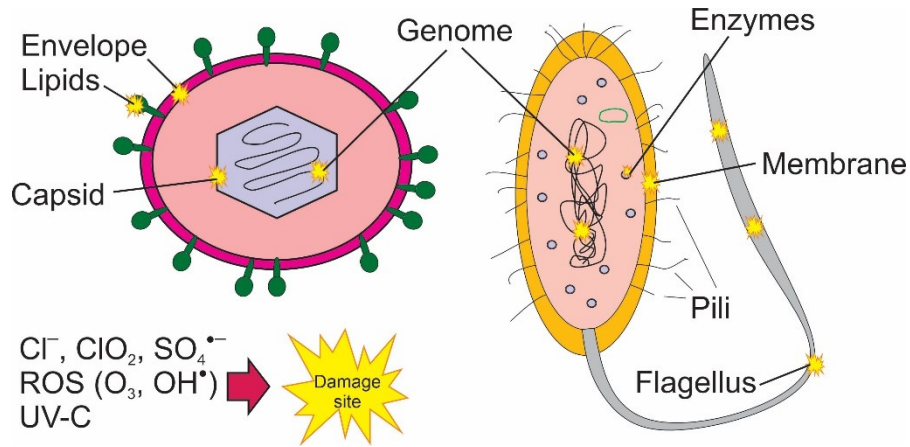


Figure 7: Disinfection targets for microbial inactivation.

For common disinfectants in use, an adequate disinfection dose for various pollutants is usually given as a Ct value, which is the product of contact time (t , min) and the concentration/dose of disinfectant (C , e.g. mg/L or mJ/cm² for UV (Table 3) [4], [79]). According to USEPA and Health Canada agencies, an efficient water treatment method reduces viral concentration by 99.99% ($4 \log_{10}$) [30]. For modelling virus inactivation, the USEPA assumes a second-order rate law (rate constant k), along with a constant concentration of disinfectant (Eq. 1). The latter usually is not the case in real-life applications, but it does provide a good estimation for evaluating disinfection efficiency [80]. Alternatively, the D-value (i.e., decimal reduction time, Eq. 2) is commonly used for expressing a logarithmic reduction (factor of 10) of a viable microbial population per unit of time. For example, if the initial concentration ($\log C_0$) of a microbe in the sample was reduced from 10^8 to a final concentration ($\log C_n$) of 10^2 within 5 minutes (t) of treatment, that would translate to a D-value of 0.83 min, meaning that 90% of microbes were inactivated within first 0.83 minutes (≈ 50 s) of treatment [81].

$$\frac{dN}{dt} = -kNC \quad \text{Eq. 1}$$

$$D_{90} = \frac{t}{\log C_0 - \log C_n} \quad \text{Eq. 2}$$

To study inactivation of microorganisms, survival (inactivation) curves are typically used (Figure 8), which show the reduction of surviving microbes based on the treatment (contact) time, with the number of organisms usually presented on a logarithmic scale. The curves display a typical concave shape with a lagging tail since the disinfectant becomes less efficient as the treatment progresses, with fewer interactions of disinfectant with ‘live targets’ as the inactivated organisms shield the viable ones [82]. Besides, the residual matter from the already-inactivated microorganisms competes with the viable microorganisms that still need to be inactivated.

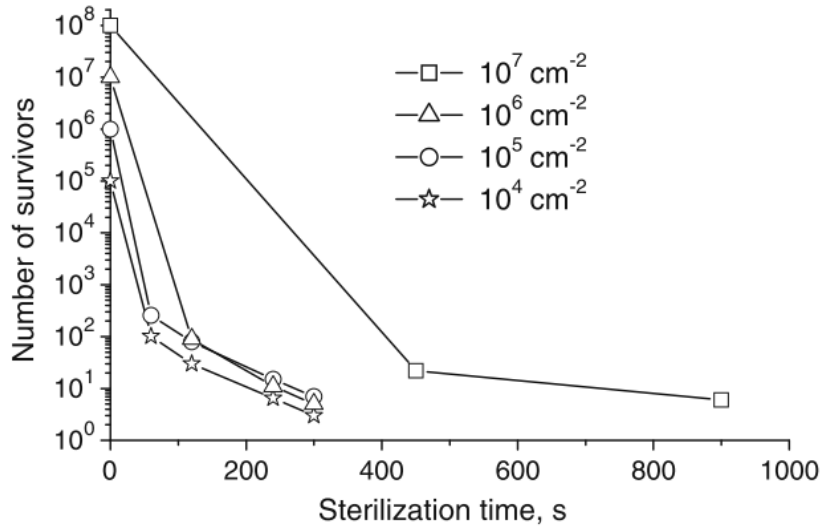


Figure 8: Inactivation curve example for plasma sterilisation of *B. subtilis* spores by a hollow cathode discharge plasma in air (adapted from [82])

Table 3: Range of Ct_{99} values (2- $\log_{10}$ microorganism reductions) for common primary disinfection methods (disinfectant concentration $\times$ treatment duration) (adapted from [4]).

Method Organism	Cl [min·mg/L]	ClO ₂ [min·mg /L]	O ₃ [min·mg/L]	UV [mJ/cm ²]*
Bacteria	0.04–0.08	0.02–0.3	0.02	0.65–230
Protozoa	25–245	100	0.5–40	<1–60
Viruses	2–30	2–30	0.006–0.2	7–186

*4 $\log_{10}$ reduction (range of $Ct_{99.99\%}$ values)

Present-day water treatment facilities have a variety of methods at their disposal for producing clean water, with some advantages and disadvantages associated with them (Table 4). They can be divided into thermal, physical or chemical methods (Figure 9) [36]. As already mentioned, the physical act of boiling water is one of the oldest and simplest

disinfection methods and is quite effective for inactivating any, up to the most extreme thermophilic microorganisms [83]. Heat destroys the cell's metabolism (enzymatic activity, disrupts membranes and cell walls and denatures the genome and proteins [84]. Viruses are primarily inactivated by capsid damage [40]. However, it requires filtration to remove suspended solids, does not improve the appearance (colour) and taste of water, and is extraordinarily energy-intensive on a large scale; therefore, it should only be used as a last resort for water purification purposes [79]. Right alongside heat treatment regarding simplicity is filtration, at least its earliest iterations, which were commonly filters consisting of readily available materials such as gravel and sand [85]. Nowadays, pressure membrane filtration techniques have largely replaced the classical filtration methods, although slow sand filtration still proves useful for smaller rural applications [86]. Depending on pore sizes, pressure-based filtration techniques can be separated into microfiltration (MF, 0.1–10 μm), ultrafiltration (UF, 0.01–0.1 μm), nanofiltration (NF, 0.001–0.1 μm), Reverse osmosis (RO, <0.001 μm). Usually applied after the coagulation/sedimentation step, microfiltration removes particles with a diameter larger than 0.1 μm , which is sufficiently large to remove most of pathogenic bacteria and protozoan parasites but cannot be relied on to remove smaller viruses [36], [40]. The slightly smaller pores of UF can already be sufficient for removing most viruses and macromolecules, while NF pore sizes are intended to remove other water constituents such as colour, total organic carbon, humic acids, etc. [36]. As a last-stage filtration technique, reverse osmosis membranes have the smallest pores (> 0.0001 μm). They rely on high pressures and diffusion to transport only the solvent through the membrane [36]. Micro and ultrafiltration are primarily responsible for solids removal, while nanofiltration and reverse osmosis are often used to prepare drinking water [71]. A considerable disadvantage of membrane filtration processes is the clogging and fouling of membrane pores, requiring frequent backwashing and eventually replacing the membranes, leading to significant management and cost increases [36].

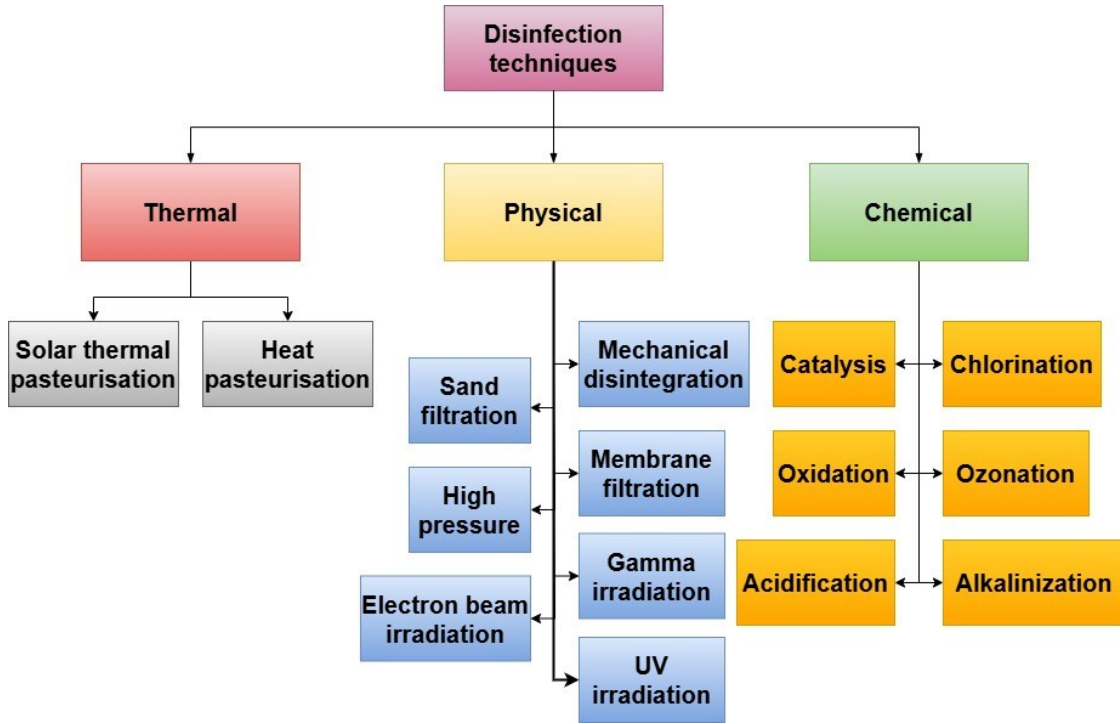


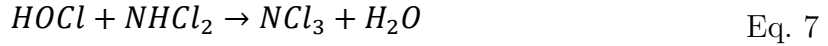
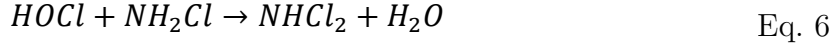
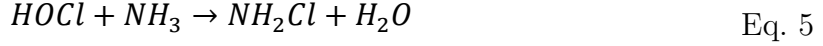
Figure 9: Common disinfection techniques (adapted from [36]).

Perhaps the most known and extensively used chemical disinfection method globally, chlorination, is also one of the oldest water disinfection methods, with the earliest reports of its use from the early 19th century [5]. Chlorine is a low-cost, effective disinfectant, simple to control and readily obtainable. When added to water, either in the form of a gas or as a hypochlorite salt, it undergoes hydrolysis, forming hydrochloric (HCl) and hypochlorous acid (HOCl) (Eq. 3), which further dissociates to hydrogen and hypochlorite ion (OCl⁻) at elevated pH (Eq. 4) [79]. Inactivation of microbes is achieved by several mechanisms, including reactions with membrane proteins, disrupting protein (enzyme) functions, inducing mutations, and producing additional toxic secondary compounds [36], [87]. Generally, it is less effective towards viruses than bacteria [88]. Typically added at the end of the disinfection process, it also improves odour, inhibits corrosion, reduces slime growth, and, most importantly, imparts residual antimicrobial activity, preventing contamination of water sources throughout the distribution and storage systems [71]. The major disadvantage of chlorination is the formation of by-products, specifically trihalomethanes and haloacetic acid species, formed by the reaction of chlorine with organic matter [89]. These compounds are classified as potentially carcinogenic and are the reason why reducing the use of chlorine is desired for water treatment [90].

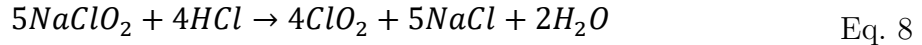


When hypochlorous acid (HOCl) reacts with ammonia (NH₃) in an aqueous environment, chloramines are generated, with monochloramine (NH₂Cl) being the most abundant chloramine at neutral pH (Eq. 5–7) [88]. Monochloramine disinfection was initially used as

an alternative to chlorination since it produces fewer disinfection by-products [5]. Due to its poor activity towards viruses, spore-forming microbes, and the longer contact times required, it is usually deployed as a secondary disinfectant meant for maintaining the quality of the treated water [38].



On the other hand, reacting sodium hypochlorite ($NaClO_2$) with hydrochloric acid (HCl) leads to the production of chlorine dioxide (ClO_2 , Eq. 8), a powerful oxidant, which is an even better disinfectant than chlorine in some instances [30]. ClO_2 reduces to chlorite at neutral pH, which reacts with microbial membranes and proteins, inhibiting metabolic processes and proper function [36], [88]. It is very unstable and needs to be generated on-site, requiring more maintenance and operational management [5], [91]. Additionally, overdosing of ClO_2 may result in organoleptic abnormalities or formation of harmful by-products like chlorite, chlorate and trihalomethanes (THM), therefore, it is usually used as a secondary disinfectant [5], [36].



Similar to boiling and filtration, exposing water to sunlight has been an ancient practice of improving the water's taste and smell [74]. A more targeted approach is commonly used in water disinfection steps, using the so-called germicidal UV-C radiation, which occupies the 200–280 nm range of the electromagnetic spectrum. Most commonly, it is produced by low-pressure mercury lamps, which emit 60% of their radiant power in the resonance line of 253.7 nm, making it an effective source for germicidal applications [92], [93]. Polynucleic acids inside organisms (DNA and RNA) strongly absorb germicidal radiation (Figure 10A) [94], leading to photochemical damage, which prevents proper replication, synthesis and function of the genetic material, resulting in microbial inactivation [79]. Some inactivation has also been attributed to protein damage [40]. The efficacy of the treatment depends on many factors, such as the emission spectrum of the UV source, radiation intensity, exposure time, etc. Genome structure also plays a role. The sensitivity of nucleic acids to UV photolysis was shown to progress by the following trend: ssDNA > ssRNA $\approx$ dsDNA > RNA, although the GC content also affects the photosensitivity [95]. For water and wastewater treatment, the total exposure of microorganisms to UV radiation is referred to as UV dose expressed in J/cm^2 , which encompasses total incident radiation from every direction times exposure time per unit area [96]. Viruses tend to be the most resistant microbes to UV disinfection but may still be inactivated, given sufficient radiation dose [79], [92], [97]. Compared to other forms of disinfection, UV disinfection produces no significant amount of harmful disinfection by-products and is relatively simple to operate (Figure 10B). However, it does have significantly higher energy requirements and is sensitive to temperature changes while also requiring the replacement of mercury lamps,

which are themselves an environmental burden if damaged or not disposed of properly. Furthermore, its activity is based on radiation reaching the contaminant, making it less applicable when the radiation pathway is obstructed by high turbidity [79], [93], [98]. It also has no residual disinfection capacity, meaning chemical disinfectants should still be used for storage and transportation purposes. Pure water is practically completely transparent to germicidal UV radiation.

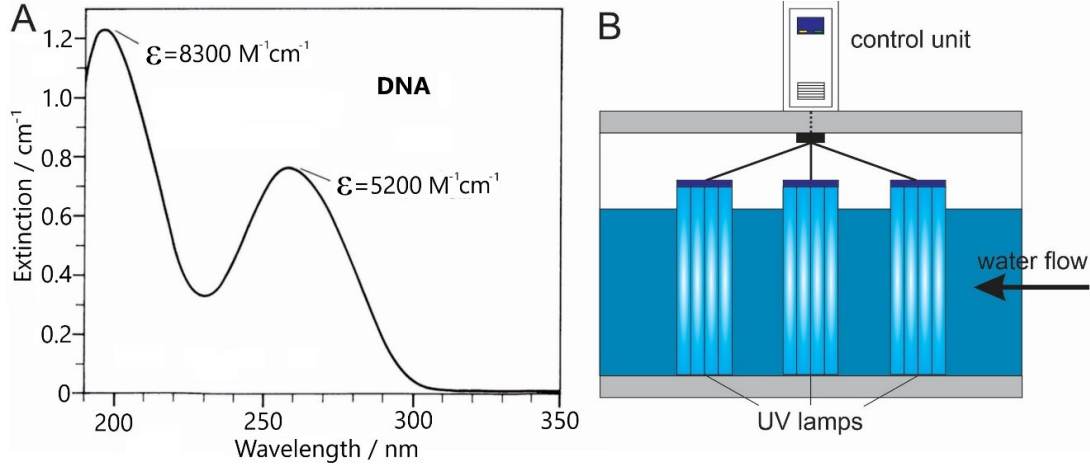
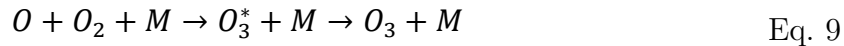


Figure 10: A) DNA absorption spectrum in the UV region (adapted from [94]), B) UV-C water treatment schematic.

Ozone gas is a powerful oxidant and has been used for disinfection, colour and odour removal for over a century [80]. Antimicrobial activity is achieved by the oxidation of organic matter, either directly by ozone or indirectly by its decomposition product, hydroxyl radical ($\text{OH}^\bullet$), with factors such as pH, temperature, and dissolved organic matter concentration being major drivers of ozone decay [80]. Similarly to chlorine, it may form some by-products, especially if bromine is present [99]. It is an unstable molecule, poorly soluble in water, therefore it needs to be generated on-site, usually by an atmospheric pressure corona plasma discharge, which dissociates oxygen molecules and re-forms them into ozone. A third collision partner, M , is needed (e.g., O_2 , O), with which the excess potential energy is shared but does not react chemically (Eq. 9) [100], [101]. An ‘ozone-contactor’ is required to provide the maximum mass transfer from gas-to-liquid and contact time for disinfection. As it is a toxic gas, the off gas from the contactor is fed to the ozone destruction chamber before release to the environment [87].



Ozone inactivates viruses by damaging both its capsid and genome, the primary mechanism apparently dependent on the virus type and applied disinfectant dose [40]. Generally speaking, ozone was found to be very efficient in virus inactivation, even more so than chlorine [38], with the majority of second-order constants (k , Eq. 1) being in the range of 10^3 – $10^4 \text{ mg}^{-1} \text{ min}^{-1} \text{ L}^{-1}$ at 20–25 °C. Mammalian viruses were found to be slightly more resistant than bacteriophages, which may slightly overestimate enteric virus inactivation when using bacteriophage MS2 as a surrogate [80]. Even though ozone is relatively efficient towards microbe inactivation (Table 3), it is somewhat selective towards certain types of pollutants, reacting preferentially with electron-rich organic moieties, such

as activated aromatic compounds (phenol, aniline), organosulfur compounds (cysteine, methionine) and deprotonated amines (NH^-), and reacting poorly with electron-drawing groups (e.g. COOH , NO_2) or single bonds [102], [103], [104]. A thorough review of ozone disinfection rates of waterborne viruses and their surrogates is available [80].

Table 4: Overview of standard water disinfection practices [71], [91]

Method	Advantages	Disadvantages
Coagulation/ sedimentation	Highly effective	- High coagulant doses needed - pH-driven process
*Filtration (MF, UF, NF, RO)	- Highly effective (depending on pore size) - Simple operation	- Efficiency depends on water quality - High costs of membrane replacements
UV radiation	- Highly effective - No by-product formation - Simple operation	- Hindered by high water turbidity - High cost of operation and maintenance - Viruses most resistant
Chlorination	- Low cost - Simple operation - Residual activity	- Formation of by-products - Environmentally dangerous - Worker exposure risks
Monochlor- amination	- Residual activity - No by-products formation	- Infective towards some microbes - Longer contact times required
Chlorine dioxide	- Highly effective - Low cost	- On-site generation required - Costly - Difficult to operate - High doses unsafe (by-products)
Ozonation	- Highly effective - Improves colour, taste and odour	- On-site generation required - Poor solubility in water - High energy costs and maintenance - By-product formation

* MF: Microfiltration ($0.1\text{--}10\ \mu\text{m}$), UF: Ultrafiltration ($0.01\text{--}0.1\ \mu\text{m}$), NF: Nanofiltration ($0.001\text{--}0.1\ \mu\text{m}$), RO: Reverse osmosis ($<0.001\ \mu\text{m}$)

1.3 Advanced oxidation processes

Initially defined as water treatment methods which generate hydroxyl radicals ($\text{OH}^\bullet$) as their primary purification agent [105], advanced oxidation processes (AOP) now also include techniques that generate other reactive oxygen species (ROS), such as hydroperoxyl radicals ($\text{HO}_2^\bullet$), sulphate radicals ($\text{SO}_4^{\bullet-}$), superoxide anion radicals ($\text{O}_2^{\bullet-}$) and singlet oxygen ($^1\text{O}_2$) (Table 6). Radicals are defined as atoms, molecules or ions with at least one

unpaired valence electron. They are involved in many natural processes, such as chemical bond cleavage, cellular signalling, etc. [106].

With a redox potential even higher than ozone (Table 5), $\text{OH}^\bullet$ are able to oxidise faster and less selectively than other treatments. Being a non-selective oxidant, which indiscriminately reacts with all and any matter, also prevents resistance development by microbes, given enough dose and contact time. Their lifetime is very short (20 ns, [107]), meaning they need to be generated *in-situ*, and there are several techniques on how to achieve that. The microbes are inactivated through the destruction of external structures (cell wall, envelope, capsid, proteins), immediately followed by the degradation of internal molecules (genome, enzymes, organelles) [108].

Table 5: Redox potential of commonly utilised water treatment oxidants [109], [110].

Oxidative species	Formula	Redox potential [V]
Hydroxyl radical	$\text{OH}^\bullet$	2.80
Sulphate radical	$(\text{SO}_4^{\bullet-})$	2.60
Ozone	O_3	2.07
Hydrogen peroxide	H_2O_2	1.78
Permanganate	MnO_4^-	1.69
Chlorine dioxide	ClO_2	1.56
Chlorine	Cl^-	1.36
Oxygen	O_2	1.23

The first processes, described as AOP by Glaze et al. in 1987, were combinations of $\text{O}_3/\text{H}_2\text{O}_2$, O_3/UV and $\text{H}_2\text{O}_2/\text{UV}$ [105]. It was observed that O_3 treatment performed much better at degrading pollutants when combined with either H_2O_2 or UV [111]. Nowadays, several more techniques are researched, such as Fenton processes, photocatalysis, electrochemical processes, sonolysis, persulfate-based AOP, and plasma-assisted AOPs. AOP methods can be separated into chemical, photochemical, radiation-induced, cavitation and electrochemical, based on the mechanism for generating ROS [107].

Hydrogen peroxide with UV radiation

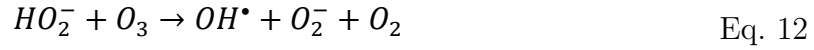
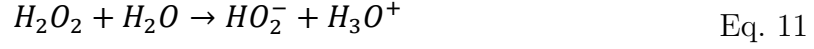
The homolytic cleavage of O—O bond of H_2O_2 by UV radiation (Eq. 10) can be considered the most straightforward path for producing $\text{OH}^\bullet$. A direct photolysis reaction of H_2O_2 by UV generates 2 $\text{OH}^\bullet$, having a quantum yield of 1 (each photon absorbed results in a photolysis reaction). However, since the molar extinction coefficient of H_2O_2 at 254 nm (mercury UV lamp emission output) is only $19.6 \text{ M}^{-1}\text{s}^{-1}$, compared to $3300 \text{ M}^{-1}\text{s}^{-1}$ for ozone, a high concentration of H_2O_2 is needed to achieve a high $\text{OH}^\bullet$ concentration [105].



Ozone with hydrogen peroxide

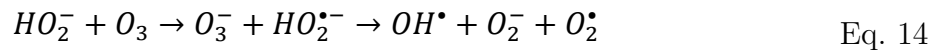
Combining ozone (O_3) with hydrogen peroxide (H_2O_2), colloquially named the ‘peroxone’ process, is one of the earliest and most frequently used advanced oxidation processes [91]. Since peroxide can act as both the initiator and scavenger of $\text{OH}^\bullet$ formation [105], the

O_3/H_2O_2 ratio should be carefully monitored to achieve the best results. For generating $OH^\bullet$ with the peroxone process, the generation of hydroperoxide anion (HO_2^-) from the reaction between H_2O_2 and water (Eq. 11), leads to the decomposition of O_3 with the said HO_2^- ($5.5 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$, Eq. 12). Since the decomposition of H_2O_2 is pH-dependent, the decomposition rate of O_3 increases with increasing pH [109].



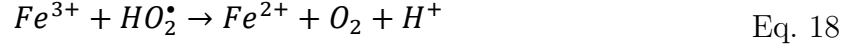
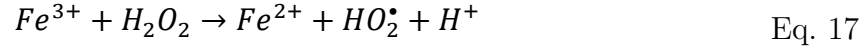
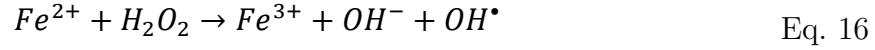
Ozone with UV radiation

Although both are potent disinfectants individually, ozone and UV radiation at 254 nm can be implemented simultaneously to provide a source of $OH^\bullet$. This may prove beneficial as both disinfectants are selective towards certain types of pollutants, whereas $OH^\bullet$ acts indiscriminately against all kinds of pollutants and molecules [112], [113]. The initial reaction of O_3 and UV_{254} generates H_2O_2 as an intermediate (Eq. 13), which can further decompose to $OH^\bullet$ by the UV radiation (see section above). Alternatively, $OH^\bullet$ can be obtained from the reaction of the HO_2^- with O_3 (Eq. 14). Lastly, at high pH, hydroxide (OH^-) can react with O_3 , leading to a series of reactions, some of which result in the production of $OH^\bullet$ (Eq. 15) [102]. The method was first explored by the company Houston Research Inc., which demonstrated that some compounds, not degraded by the two methods individually, were degraded when O_3/UV_{254} were applied together. The issue with this approach is the relatively poor quantum yield of $OH^\bullet$ production with UV_{254} , where only about one in ten photolysed ozone molecules actually generate an $OH^\bullet$ (Eq. 13), compared to H_2O_2 photolysis where all direct interaction with an energetic photon produces an $OH^\bullet$ (Eq. 10) [114].



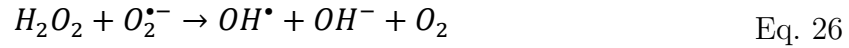
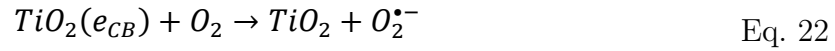
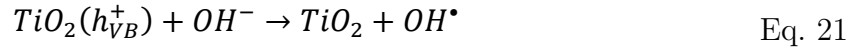
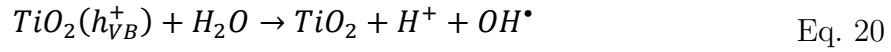
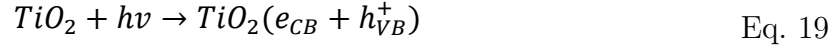
Fenton and Fenton-like processes

A Fenton reaction refers to H_2O_2 degradation to $OH^\bullet$ by Fe^{2+} catalyst under acidic (pH < 4) conditions (Eq. 16–18) [115]. Although the process is relatively easy to operate and displays a high degradation efficiency [108], its drawbacks are the requirements of acidic conditions for the reactions to take place, and the production of large amounts of iron-containing sludge in the water treatment plant. This results in high operational costs and the generation of secondary pollution, which requires further handling and disposal [116]. Ongoing research is attempting to circumvent these issues by utilising nanoscale transition metal oxide catalysts, which operate at neutral pH, replenish Fe^{2+} from Fe^{3+} quicker, or utilise other oxidants for the production of $OH^\bullet$ [116].



Photocatalysis

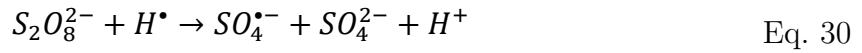
Photocatalytic water treatment utilises photon energy to excite electrons in a semiconductor catalyst, which subsequently produces $O_2^{\bullet-}$ from O_2 (aq) and $OH^\bullet$ from H_2O (Eq. 19–26) [108]. The most common catalyst in use is TiO_2 , which is usually applied as suspended particles or as part of an immobile substrate [68], which may perform an additional filtration function. The photoexcitation of TiO_2 produces electrons, excited to the conduction band (e_{CB}) as well as leaving holes with high oxidising ability in the valence band (h_{VB}^+), which then carry out the redox reactions with surrounding molecules on the catalyst surface.



Sulphate radical-based AOP

Peroxymonosulphate (HSO_5^-) or persulphate ($S_2O_8^{2-}$) salts are used to produce sulphate radical ($SO_4^{\bullet-}$), which has a very high oxidation potential (2.6 V [110], [117]) and displays high selectivity towards organic matter [107]. An external energy source in the form of UV radiation, heat or ultrasound is needed to activate the precursor salts into the active form (Eq. 27–31), and the final sulphate product requires additional polishing steps, making the method costly and complex to be implemented at large scale [107]. Nevertheless, a large-scale process was carried out by Rodriguez et al. [118], who compared tertiary treatment via UV-C radiation alone or with the addition of peroxymonosulfate (PMS), persulfate anions (PS) or H_2O_2 for the removal of micropollutants, while also performing a cost-efficiency analysis. The authors showed that the addition of either H_2O_2 or PMS improved

the removal efficiency of the process, compared to UV-C alone. However, the cost-efficiency was favoured by the addition of H_2O_2 on account of it being 20 times cheaper than PMS [118]. The USEPA regards $SO_4^{\bullet-}$ as a non-polluting, inert chemical with great promise for utilisation for wastewater or sludge treatment when economic feasibility is reached [110].



Catalytic ozonation

The drawback of ozonation is the poor solubility and mass transfer from gas to liquid phase and selective reactivity, preferring to react with specific amino acids (cysteine, histidine, methionine, tryptophan and tyrosine) [40], which makes it ineffective in the degradation of some contaminants. Accelerating its degradation to $OH^{\bullet}$ is desired in this sense and has usually been promoted by the addition of H_2O_2 or UV-C radiation [119]. An emerging field of catalytic ozonation is attempting to promote the degradation of ozone by introducing metal catalysts in either homogeneous form (Zn^{2+} , Mn^{2+} , Co^{2+}) or as heterogeneous metal oxides (TiO_2 , MnO_2) and metal-metal oxides ($Cu-TiO_2$, $Cu-Al_2O_3$,) [107]. While the addition of a catalyst definitely improves the utilisation of ozone and thus degradation efficiency, the research is still ongoing to find an efficient, reusable and cost-effective catalyst, which would justify its implementation on a large scale [107]. Recently, a large-scale study was carried out by He et al [120] using a heterogenous metal catalyst to purify bio-treated coking wastewater at a flow rate of $100 \text{ m}^3/\text{h}$. The authors reported successful 885-day operations with stable chemical oxygen demand (COD) removal efficiency but did encounter some difficulties due to catalyst deterioration, which was amended by air and water backflushing, giving evidence that such technologies may find their applications in the future [120].

Cavitation

In the process of cavitation, the rapid growth and subsequent collapse of microbubbles results in the release of large amounts of energy, which carry out pollutant degradation directly or indirectly by generating reactive particles through high-velocity interparticle collisions (Eq. 32–35) [121], [122]. While there are many different methods for producing the cavitation effect, hydrodynamic and acoustic cavitation are the main ones in the area of water disinfection due to efficient degradation of pollutants [123]. Acoustic cavitation or ultrasonication is the process of using low-density energy from ultrasonic waves (16–100 kHz) to produce microbubbles in the liquid, leading to high-density energy released upon the collapse of said bubbles. The supercritical region of the collapsed bubble may locally

exceed temperatures of over 5000 K and ultrahigh pressures (> 2000 atm), directly pyrolysing water molecules to produce ROS [108]. Despite being a green process with no by-product or secondary pollutant formation, its low energy efficiency and poor scalability currently make it unviable in large-scale installations [123]. The hydrodynamic cavitation effect is usually achieved by a physical constriction-expansion of the liquid flow (Venturi tube), producing microbubbles, which implode in the expansion phase, producing large amounts of energy and ROS to degrade contaminants [107], [124]. The hydrodynamic technique can be greatly improved by the addition of chemical oxidants while simultaneously improving the performance of said oxidants, which is why it is being considered as a pretreatment or a combined method with other disinfectants [107], [122], [125].

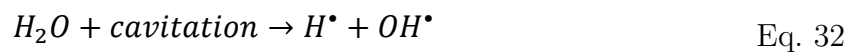


Table 6: Overview of Advanced Oxidation Processes

Method	Advantages	Disadvantages
Sulphate radical ($SO_4^{\bullet-}$)	• Longer lifetime than $OH^\bullet$ • Effective over a wide pH range (2.0–8.0)	• Sulphate ions as end product • Energy input required
Photocatalysis (TiO_2)	• Low cost • Environmentally friendly	• Low photoconversion efficiency • UV required
Catalytic ozonation	• Higher yield of $OH^\bullet$ than ozonation alone • Effective at any pH	• Costly catalysts, requires complex separation • By-products formation
Hydrodynamic cavitation	• Simple operation, low maintenance • Enhanced mass transfer	• May require additional oxidants (O_3, H_2O_2) • Large investment costs
Gamma radiation	• No additives required • No sludge production	• Energy and cost-intensive • Difficult and dangerous to operate
Electrochemical oxidation	• In-situ production of H_2O_2 • Simple operation • High degradation rates	• Costly electrodes • By-products formation

Fenton-like oxidation	• Low waste generation • High OH[•] production	• High cost • Poorly scalable
Plasma-assisted	• No chemicals required • A variety of reactive species produced	• Energy intensive • Difficult to operate

As it is apparent from the short overview, the concept of AOP has great potential to degrade even the toughest pollutants. However, no method is without its shortcomings, and more research is needed to fully understand and overcome these issues. In the following chapter, we discuss the plasma-assisted AOPs, which have some unique advantages over other systems, such as being an environmentally friendly technique, requiring no additional chemical inputs for its operation, and being a source of a plethora of chemically reactive species.

1.4 Gaseous plasma

Solid, liquid and gas are how we define the aggregate states in our daily lives, with a majority of the population having little knowledge of any other state of matter. However, over 99% of our observable universe is made from an entirely different form of matter named plasma [96]. In the simplest terms, plasma can be defined as ionised gas consisting of electrons, ions and neutrals in either their excited or fundamental states, with a total charge of zero [100]. The presence of free charged particles makes plasma electrically conductive [100]. Its unique chemical properties afford it the classification as the fourth state of matter. Due to the pressure of our atmosphere, we only observe it on Earth in the form of lightning, flame, Aurora Borealis, or artificially produced in controlled systems [126]. The study of plasma is a relatively young scientific field, being made possible with the invention of high-voltage direct current (DC) discharges in the early 1800s, while the term ‘plasma’ was actually coined in 1928 by Irving Langmuir [127]. Today, plasma is one of the key processing technologies, used in applications such as ozone generation, integrated circuit production, gas reformation, thin film deposition, plasma pyrolysis, wettability improvement, welding and many more [100], [128].

Gaseous plasmas can be artificially created by applying energy (e.g., thermal, radiation, electric field) to a gas. In the case of an electrical breakdown, an electric field is applied, accelerating free-flowing electrons, which collide with heavier particles (neutrals), exciting them and causing dissociation and ionisation. The electrons escaping from atoms in ionisation reactions are then also accelerated in the electric field, causing further collisions, eventually leading to gas breakdown and producing plasma [96], [126], [129]. Alternatively, excited atoms and molecules can de-excite spontaneously, emitting a photon in the process, which is why plasmas have a characteristic glow (glow discharge) based on the type of gas used [84]. Plasmas can be created in a wide range of pressures, electron temperatures and electron densities, which can span over 15 orders of magnitude (Figure 11) [96].

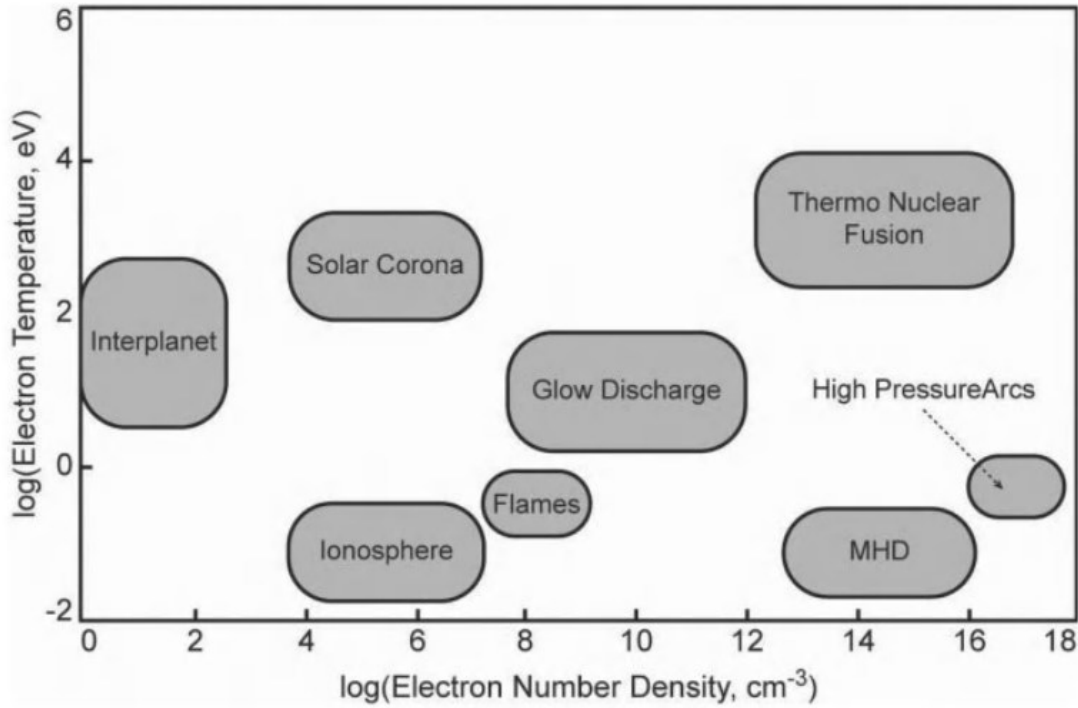


Figure 11: Plot of plasma temperatures and densities (adapted from [106]). MHD = Magnetohydrodynamic plasma

Gaseous plasma is a somewhat challenging research subject, as it is difficult to sustain a stable discharge, with many parameters affecting the discharge conditions. Additionally, due to the high kinetic temperature and energy densities, it is difficult to analyse, as the probes can get damaged, while simultaneously, even the insertion of a probe in the plasma discharge often interferes with the plasma characteristics [127]. Since the energetic particles interacting with each other and the surfaces of the reactor are described by non-linear, multi-body functions, a reduction of complexity via approximations leads to mathematical descriptions of plasma discharges or their rough estimates at best [127]. The collisions between the particles in the gas phase can be either binary or multi-bodied, producing charged particles, which can then further react with other particles. Depending on the nature of the colliding particles, collisions can be either elastic or inelastic. Elastic collisions are best described as the interaction of two billiard balls, where the total kinetic energy is the same before and after collision. On the other hand, when two particles collide inelastically, some kinetic energy is transformed into internal energy, producing particles with less total kinetic energy than before the interaction. Various outcomes of such inelastic reactions are presented in Table 7 [127]. Alternatively, super-elastic collisions, which usually occur at the collisions of three particles [129], will transform a part of the internal energy into the kinetic energy of the colliding particles.

Table 7: Principal kinds of collisions (adapted from [100], [127])

Electrons	
$e^- + A \rightarrow A^+ + 2e^-$	Ionisation
$e^- + A \rightarrow e^- + A^* \rightarrow e^- + A + h\nu$	Excitation
$A^* + B \rightarrow A + e^- + B^{*+}$	Penning ionisation
$e^- + A \rightarrow e^- + A$	Elastic scattering

$e^- + AB \rightarrow e^- + A + B$	Dissociation
$e^- + AB \rightarrow 2e^- + A^+ + B$	Dissociative ionisation
$e^- + AB \rightarrow A^- + B$	Dissociative attachment
$e^- + A^+ + B \rightarrow A + B$	Recombination
Ions	
$A^+ + B \rightarrow A + B^+$	Charge exchange
$A^+ + B \rightarrow A^+ + B$	Elastic scattering
$A^+ + B \rightarrow A^+ + B^+ + e^-$	Ionisation
$A^+ + B \rightarrow A^+ + B^* \rightarrow A^+ + B + h\nu$	Excitation
$A^+ + BC \rightarrow A^+ + B + C$	Dissociation
$A + BC \rightarrow C + AB$	Chemical reaction

Plasma discharges can be classified as hot (equilibrium, thermal) plasmas or cold (non-equilibrium), which is also the key factor separating their application for various purposes [100]. It refers to the degree of ionisation, with non-thermal plasmas always characterised as weakly ionised plasmas ($\chi < 1$) [100]. The ionisation degree (χ) refers to the content of charged particles compared to the total amount of particles present and can range from 10^{-8} to 1 [100]. The temperature is dependent on the average kinetic energies of plasma particles (ions, atoms and molecules in their ground and excited states), as well as their relevant degrees of freedom (translational, rotational, vibrational and electronic excitation energy states). The temperature difference relates to the kinetic energy difference between the electrons and heavier particles. This is proportional to the ratio between the electric field and pressure in the plasma chamber (E/p). When pressure is raised (i.e., more gas particles are present), collisions intensify, and elastic collisions impart higher temperatures to heavier particles, resulting in thermal plasma, with electrons and heavier particles having the same temperature in temporally stable plasmas [130]. This is useful when extremely high temperatures are required, for example, to melt ceramics or metals, where temperatures above 3000 K are required [127]. Examples of such plasmas are DC arcs or RF plasma torches, which are near or at thermodynamic equilibrium.

By contrast, in non-thermal (cold) plasma, the electrons have significantly higher temperature compared to heavier particles [84]. The electrons excite or ionise the heavier particles by inelastic collisions, which do not raise the heavier particles' temperature, resulting in plasma where temperatures can be as low as room temperature [126]. The reason for the coexistence of hot electrons and cold heavy particles in plasma is poor energy transfer at elastic collisions due to the conservation of energy and momentum at elastic collisions [129], [131]. Namely, the electron mass is at least 1000 times smaller than the mass of an atom, let alone heavier molecules. This is very useful for more precise or sensitive treatments, such as microelectronics manufacturing, sterilisation or carrying out chemical reactions [126]. Examples of non-equilibrium plasmas include corona or glow discharge plasmas [81]. Additionally, when flowing gas is used to sustain the plasma, some reactive species are transported with the gas flow, enabling the treatment of materials with a chemically reactive medium called the flowing afterglow, which contains significantly less charged particles and is mainly composed of radicals, molecules and neutral atoms in the ground or metastable excited state [84]. Throughout the years, a variety of gaseous

discharges have been developed that can be sustained at low temperatures, such as direct-current (DC) [132], radiofrequency (RF) and microwave (MW) discharges [133], powered under low current and low power conditions. Alternatively, they could be sustained by using a pulsed discharge [134], as in the case of corona and dielectric barrier discharges (DBD) [126].

Another classification of plasmas can be based on pressure, distinguishing between atmospheric and low-pressure plasmas (10^{-1} – 10^2 Pa). Atmospheric pressure plasmas have the advantage of not requiring expensive vacuum systems. Additionally, a higher density of neutral particles is produced locally, which enables higher reaction rates for certain processes. Non-thermal atmospheric pressure discharges are generated by corona discharges or gliding arcs in dielectric barrier discharges, jet or micro discharge configurations [100]. Thermal equilibrium cannot be achieved in low-pressure plasmas due to the low collision frequency, resulting in energy transfer from electron or excited atoms/molecules to molecules in the ground electronic state [100]. Low-pressure discharges are characterised by the large mean free path of the particles and, therefore, a low probability of two-body collisions and a symbolic frequency of three-body collisions [100]. The most widely known applications are the glow discharges (for example, neon advertising signs or fluorescent lamps), with an evacuated glass tube, with electrodes inside the discharge tube, connected to a relatively high voltage power supply [100], [127]. Nowadays, industrially used plasma systems are connected to either direct (DC) or alternating current (AC) power supplies, working in continuous or pulsed mode, or radiofrequency (RF) or microwave (MW) power supplies [100].

RF plasma discharges, with excitation frequencies usually at 13.56 MHz, are distinctly different from DC or AC current discharges in that the power from the generator is supplied to the plasma particles by the fast-alternating electrical field when the generator is capacitively coupled and induced electric and magnetic field when the electrodes are in the form of an inductively coupled coil [100], [127]. The crucial difference is in the heating of ions; if the frequency is low, the ions can follow the oscillations of the electric field, so they are accelerated the same as electrons. If the frequency is high, the massive ions cannot follow the electric field oscillations, so they do not gain kinetic energy from the electric field. This is very important because an energetic ion will transfer its kinetic energy to a slow neutral, thus causing heating [129], [131]. The power supply must also be connected to an impedance-matching network to minimise the reflected RF power by matching the generator's impedances to that of the plasma [100]. Although this entails additional cost (5–10 times as expensive per Watt as DC, as well as AC 50 Hz power supplies) in equipment and operational complexity, it does provide several advantages for industrial applications, such as less electron bombardment of electrodes, more flexibility of power coupling through the vessel walls and lower losses by recombination of radicals [127].

Gaseous plasma, a complex medium of chemically reactive particles and radiation, was first proposed as a sterilisation method in 1964 in a United States patent, where the inventors outlined its applicability to treat delicate materials, although the mechanism of inactivating microbes remained unexplored [135]. Several years later, in 1998, a sterilisation chamber for medical tools was designed, which used plasma as a sterilisation tool, where a cassette of H_2O_2 was released inside the chamber, followed by a plasma discharge inside the chamber by an applied RF electric field. However, this cannot be considered as purely

plasma sterilisation, as plasma was merely used for the destruction of the remaining hydrogen peroxide rather than sterilisation [136].

Plasma sterilisation in literature almost exclusively refers to cold-plasma sterilisation, where the gas temperature is much lower than hot plasmas since the heavier particles (neutrals and ions) have much lower temperatures than electrons [126], [129]. The prospect of using low-temperature plasma as a sterilisation technique was primarily to replace the standard sterilisation practices in medicine. Sensitive and delicate medical devices intended for reuse could not be sterilised by hot steam (autoclave), as the high temperature would damage the heat-sensitive materials, rendering the device unsuitable for further use. Additionally, some spores and bacterial metabolites (pyrogens) are not entirely destroyed by heat treatment [137]. Chemical sterilisation, using biocidal gases like hydrogen peroxide, formaldehyde, or ethylene oxide, is thus the method of choice to treat sensitive materials. However, these methods have their own drawbacks; for example, the handling and disposal of highly toxic and carcinogenic gases gives concerns about worker safety, requiring strict systems to be in place to prevent any accidents stemming from the sterilisation process. Furthermore, these toxic gases penetrate deeper into the bulk material, requiring lengthy outgassing procedures, which results in drawn-out sterilisation times [137].

From this perspective, low-temperature plasma offers several advantages as a sterilisation method since there are no chemical inputs required besides the treatment gas. The combination of chemically reactive species and V-UV and UV-C radiation rapidly and indiscriminately eradicates contaminants via physical and chemical processes. An additional benefit of plasma sterilisation is that the reactive chemical particles (radicals) can penetrate into the small cavities and complex shapes, making it practicable for various settings. Since gaseous plasma discharges can come in almost any shape or setup, the options to treat complex materials are nearly endless. For example, plasma discharges were able to be generated inside narrow glass and polytetrafluoroethylene tubes [137].

Plasma was initially being developed as a dry sterilisation technique, using bacterial spores as indicator organisms, since dormant bacterial spores are among the most robust organisms against standard sterilisation techniques [138], [139], [140]. Treating spores with atmospheric gaseous plasma was shown to damage their coat/inner membrane through the production of reactive oxygen and nitrogen species (RONS), resulting in a rapid decline in spore germination [138], [139], [140]. This may represent a limitation of atmospheric plasma sterilisation, as the sterilisation progresses in a layer-by-layer progression [84], which makes it less effective for stacked microbial structures, i.e., biofilms [141]. Furthermore, at atmospheric pressure, the radiation intensity in the germicidal UV region (< 280 nm) is very poor in molecular gases [106], [139], which is why low-pressure plasmas are better suited for microbial inactivation, despite greater equipment requirements and complexity of operations [106]. In the context of water disinfection, plasma discharges can be produced either far above the water surface, near the water surface or as an electrical discharge in a bubble/vapour (Figure 12) [142]. Some standard configurations include plasma jets, DBDs, gliding arc discharges or pulsed corona discharges. More information on these are provided elsewhere [126], [143].

Plasma can be used for water disinfection either through direct treatment or by using the flowing afterglow, where the plasma generation zone and the treatment chamber are spatially separated. Alternatively, plasma can be a source of germicidal radiation in the (V)UV spectrum, which can also be used for sterilisation purposes, although because it is efficiently absorbed by our atmosphere, it needs to be applied via specialised systems/setups [144].

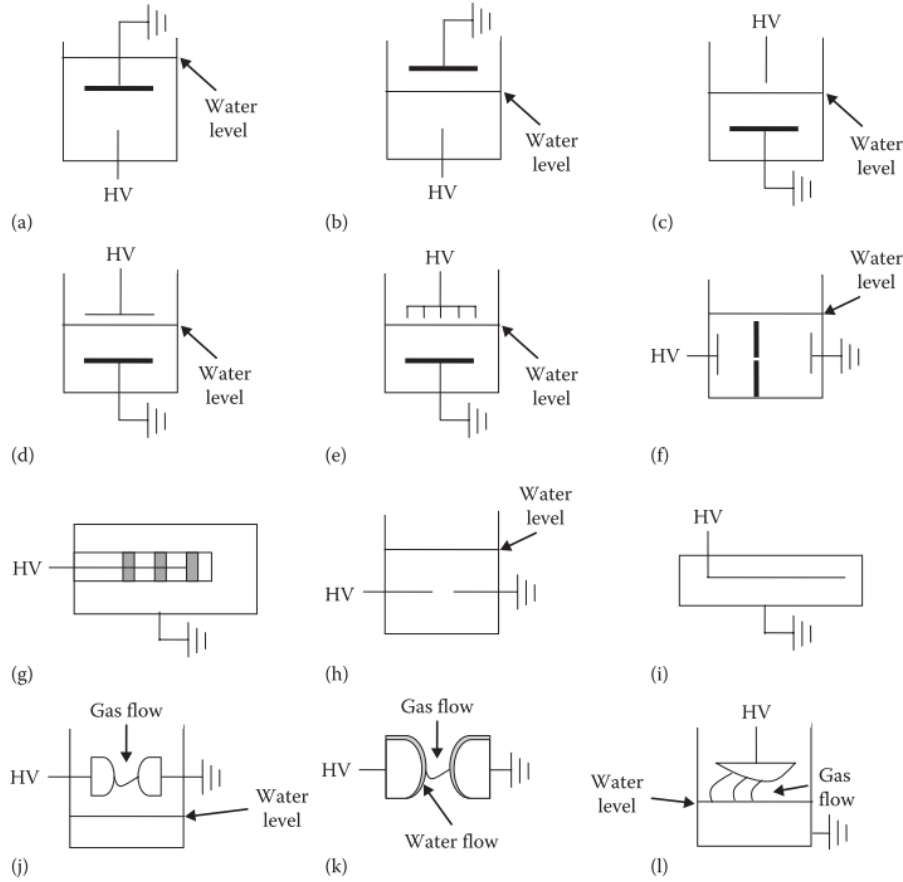


Figure 12: Reactor and electrode configurations for plasma water treatment: a) point-to-plate corona, b) point-to-plane with air gap liquid phase corona reactor, (c) single point-to-plane glow discharge reactor, (d) plane-to-plane glow discharge reactor, (e) multiple point-to-plane glow discharge reactor, (f) pinhole reactor, (g) ring electrode reactor, (h) point-to-point arc reactor, (i) wire-to-cylinder reactor, (j) gas-phase gliding arc over water surface, (k) gliding arc with water film, and (l) gliding arc to water surface (adapted from [126]).

1.4.1 Water disinfection with V-UV

The electromagnetic spectrum (Figure 13) encompasses all electromagnetic radiation, separating different types of radiation by frequency or wavelength. The average reader is mostly familiar with certain designations of the electromagnetic spectrum, which are separated by their wavelengths, in descending order: radiowave, microwave, infrared, visible light, ultraviolet, X-rays and gamma rays [145]. These designations have obtained their names based on the historical context of discovery or by the way they interact with matter, as there are no strict lines separating the types of radiation besides the wavelengths of their respective particles. Looking at Figure 13, it is immediately apparent that only a tiny fraction of the electromagnetic spectrum is visible to the human species, requiring various machines to detect the rest. This is not a coincidence since the higher energy photons, mainly coming from our Sun, are reflected or absorbed by the Earth's magnetosphere and atmosphere[144]. The radiation that passes our planet's 'shields' has lower energy, and the visible light is radiation that is reflected by matter and absorbed by the receptors in our eyes, allowing us to see and interact with our environment.

A photon, a massless particle travelling at the speed of light, is a quantum of

electromagnetic radiation [146]. When electrons are activated to a higher orbital state and subsequently return to their ground state, a photon is produced [147]. Another channel for photon production is Bremsstrahlung [145]. The properties of different photons are thus based on the difference between the energies of the upper and lower (or ground) excitation state of the atoms or molecules and are a product of their frequency (f) and wavelength (λ):

$$E = hf = \frac{hc}{\lambda} \quad \text{Eq. 36}$$

where c is the speed of light in vacuum ($c \approx 3 \times 10^8$ m/s) and h is the Planck's constant ($h = 4.14 \times 10^{-15}$ eV s). A practical unit for photon energy is an electron volt (eV) because the SI unit (Joule) is much larger ($1 \text{ J} = 6.24 \times 10^{18} \text{ eV}$). Since c and h are constants ($hc \approx 1240 \text{ eV nm}$), the energy of a photon can be directly obtained by knowing its wavelength. Inherent to a photon's wavelength is its ability to interact with matter. We utilise these properties for practical applications, where long-distance signals are sent by low-energy photons (long wavelength, radio waves), whereas ionising X-ray photons are used in medicine to observe structures beneath the surface. The term 'ionising' refers to a particle's ability to disrupt the structure of an atom by detaching electrons from an atom, producing ions or excited particles [146].

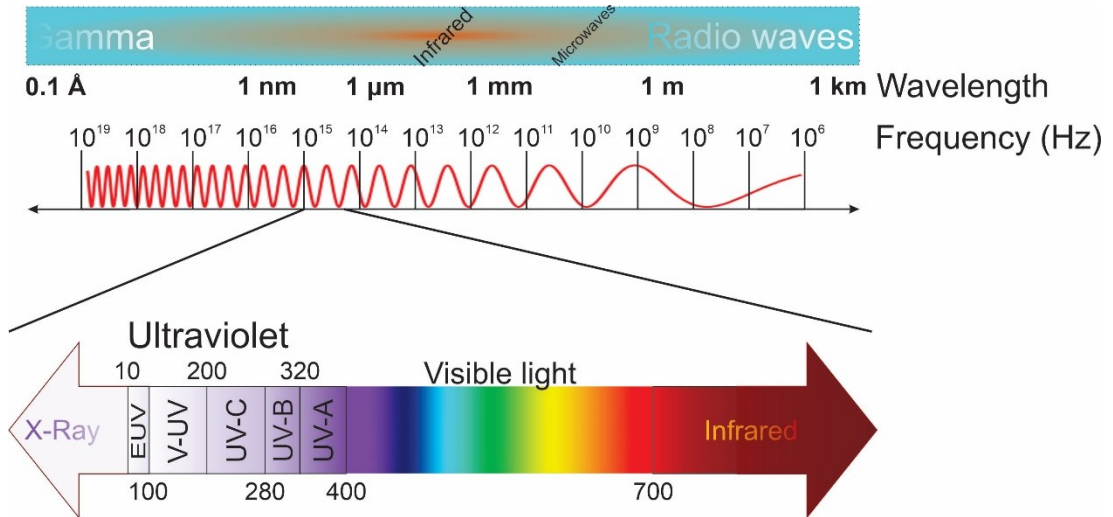


Figure 13: Electromagnetic spectrum

UV radiation is situated between 400 and 10 nm and presents the boundary between ionising and non-ionizing radiation since certain wavelengths can ionise certain particles quite well while being completely inactive towards others. The most known example of this is the UV-C radiation around 260 nm, which is strongly absorbed by the nucleotides in the DNA molecule, which we utilise for detection or sterilisation purposes of microbes [94]. Slightly longer wavelengths of UV-A (315–400 nm) are radiated in tanning beds, slightly burning our skin to cause the production of melanin, accompanying a darker shade of our skin [146]. Vacuum ultraviolet (V-UV) emission designation is reserved for photons with wavelengths from 200 to 100 nm (6.2–12.4 eV). Their energy is higher than the chemical bond strength of most materials, thus it is capable of producing photo-induced effects upon contact, such as dissociation and ionisation, even of gases in the atmosphere (CO_2 , O_2 , Ar, etc.) [148]. V-UV radiation has found considerable applications in materials processing, for example, in microelectronics manufacturing, dry etching, high-resolution lithography, wettability improvement, etc. [149] The benefit of using V-UV as a processing method is

the low-temperature conditions, which prevents the bulk modification of sensitive materials [150]. Further below resides the extreme-ultraviolet (E-UV) region, which is an arbitrary designation for radiation shorter than the H₂ Lyman-alpha line, from 121 nm down to the X-ray band of 10 nm [146].

To get a broad overview of plasma V-UV emission below 200 nm, a V-UV spectrometer is used, which also requires the implementation of cumbersome and expensive vacuum systems to avoid photon absorption during measurement. Alternatively, V-UV scintillators can be used to estimate the V-UV photon flux (Φ , photons $\times$ s⁻¹) reaching the sample. Sodium salicylate (NaSal) had previously been used as an *in-situ* fluorescence probe to measure V-UV flux produced by a low-pressure microwave plasma (Figure 14). Since quantum yield is constant over a broad spectral range (350–50 nm), the measured fluorescence provides good information about the V-UV particle flux in this spectral range [151].

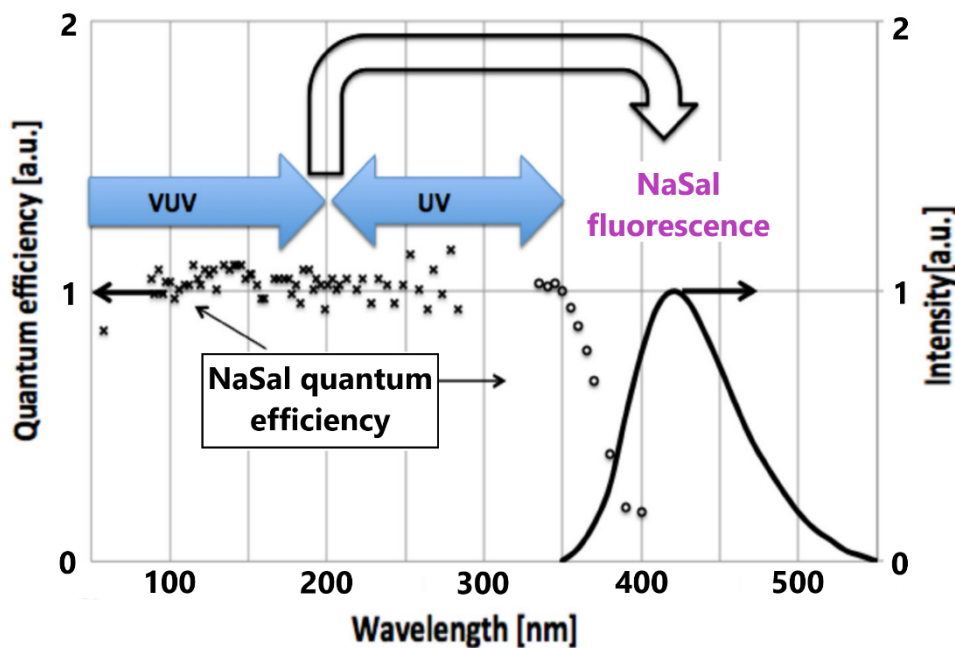


Figure 14: Measuring NaSal fluorescence to obtain information on V-UV photon flux to the sample (adapted from [151]).

Typical V-UV radiation sources are low-pressure mercury UV lamps, which have some emission at 185 nm. However, since germicidal radiation at 254 nm is desired, the lamps are usually encased in a quartz vessel doped with titanium dioxide to exclude lower wavelengths [147]. When high-purity quartz is used, the 185 nm line is available, emitting at about 10% radiant power efficiency compared to the 254 nm line [144]. Low-pressure (LP) mercury lamps are limited by the amount of electric input power they can efficiently convert to radiation (< 300 W), which is not a limitation for medium-pressure (MP) mercury lamps. These can be operated at much higher electrical input power (up to 30 kW), albeit with a reduced UV radiation output efficiency, greater heat generation and reduced lifetime [144].

Next are excimer lamps (excilamps), which produce UV or V-UV radiation (depending on the gas used) from excited molecular pairs (dimers, Eq. 37 and Eq. 38) of pure inert or inert-halogen gas mixtures (e.g. He^{*}₂, Ar^{*}₂, Xe^{*}₂, KrCl^{*}, XeCl^{*}, XeBr^{*}). Excimers are weakly bound excited states with no stable molecular ground state, emitting the binding

energy when passing into the ground state (Table 8) [100]. Usually generated in plasma sustained by a DBD or capacitive discharge, although several other variations exist, excilamps emit incoherent, narrow-band radiation in the UV or V-UV region with irradiances in the range of 1000 W/m^2 [144], [152]. Due to their long lifetimes and flexible geometries for producing V-UV radiation, they have found applications in the fields of surface sterilisation, functionalisation of synthetic polymers, photochemical synthesis of chemicals, as an ionising light source, and many other areas [153]. The main difference between excilamps and their UV counterparts is in their emission spectra, where the excilamps can be configured to emit about 80% of radiation in a narrow emission band (full width at half maximum $< 10 \text{ nm}$, Figure 15), valid mostly for halide exciplexes, which translates into high photon flux and relatively good electrical efficiency [93].

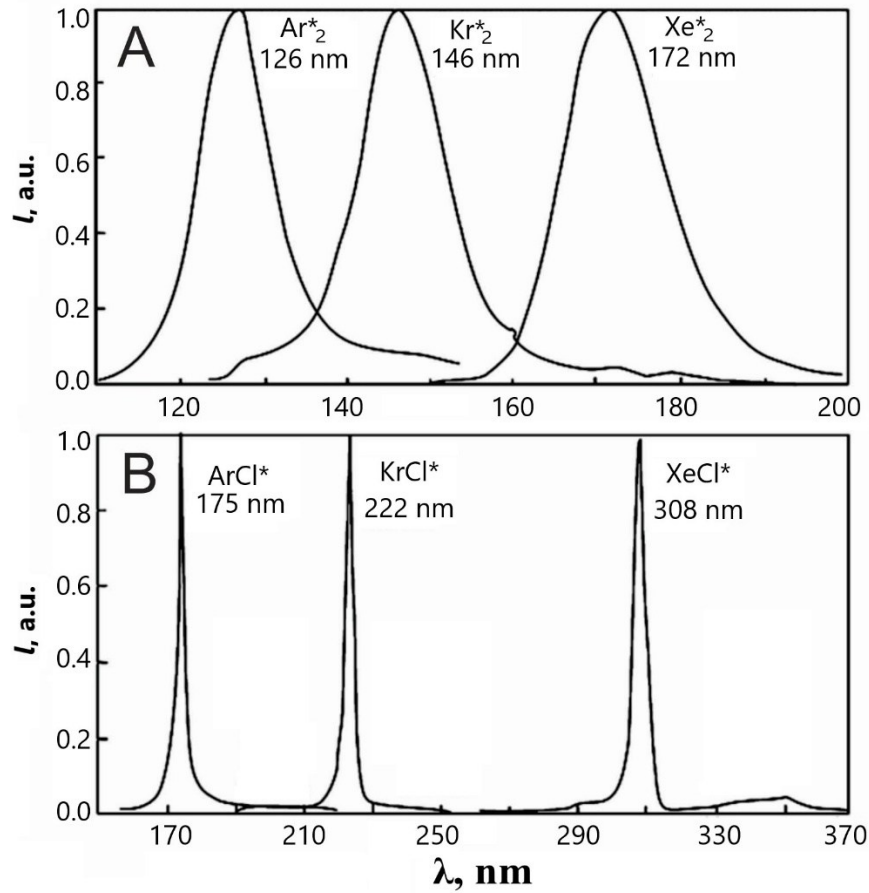


Figure 15: Emission spectra of: A) rare gas excimers and B) rare gas halide exciplexes (adapted from [93]).

Table 8: Excimer emissions on pure gases in the V-UV spectrum (adapted from [92]).

Excimer formation		R_2^*	Transition λ [nm]
$\text{R}^* + \text{R} + \text{R} \rightarrow \text{R}_2^* + \text{R}$	Eq. 37	Ar_2^*	126
		Kr_2^*	146
$\text{R}_2^* + \text{R} \rightarrow \text{R} + \text{R} + \text{R} + h\nu$	Eq. 38	Xe_2^*	172
		F_2^*	158

The concept of using excilamps for detoxifying and decontaminating water was initially demonstrated by Oppenländer et al., who used an Xe₂ excilamp flow-through reactor (Figure 16), to treat a solution of red dye Rhodamin B, producing a discoloured product after 3 minutes of treatment [154]. Local oxygen deficits occurred during the photo-mineralisation process through the scavenging of hydrogen atoms and carbon-centred radicals, prompting the investigators to improve the treatment through the addition of airflow through the solution [155]. Excimer lamps have been previously investigated for use in transfusion medicine to inactivate viruses within blood products. Parvovirus was inoculated into blood plasma and platelet suspensions, followed by irradiation treatment by XeBr (282 nm) and XeCl (308 nm) exciplex lamps [156]. Both treatments resulted in over 4-log₁₀ inactivation of viruses after 8 J/cm² radiant exposure 5 minutes, however the 282 nm treatment resulted in unacceptable damage to the treated blood sample. Nevertheless, the study shows how a narrow-band emission can serve as a tool for removing or modifying specific targets within complex suspensions.

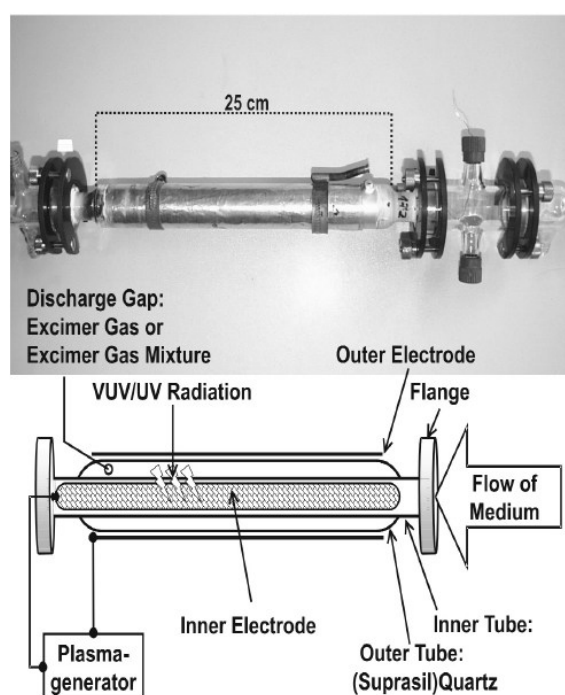


Figure 16: Typical flow-through excilamp setup for liquid treatment. (adapted from [153])

Plasma discharges can also be a source of powerful V-UV radiation if certain conditions are met. V-UV radiation is primarily generated by direct excitation from an electron-particle collision, exciting the particle into a higher energy state, followed by de-excitation and emission of a photon [157]. By modifying the setup in such a way that the treated medium is not directly exposed to plasma but only to V-UV radiation, one can overcome the obstacle of plasma particle recombination and overheating.

A thorough review of low-pressure plasma V-UV sources was published by Popović et al [158]. In their work, although the various systems cannot be reliably compared to each other due to the variety of factors influencing the outcome, the authors agree that gas, gas pressure and discharge power are the main factors affecting the V-UV radiation intensity [158].

1.4.2 Discharge parameters affecting plasma V-UV disinfection

Optimising a plasma discharge is a challenging task since many parameters are interconnected, where adjusting one parameter influences the rest (e.g. increasing the amount of particles raises the pressure). Thus, to suit the needs of water disinfection, it is more convenient to focus on optimising a single output parameter to optimise the efficiency. In this section, we discuss the relevant parameters of a gaseous discharge for V-UV generation in the context of water treatment.

1.4.2.1 Reactor configuration

The geometry and design of the plasma treatment reactor are the main factors influencing the efficiency of energy conversion to disinfection performance, as they dictate the contact time between the plasma reactive species and the treated medium. Batch or continuous flow modes are used to study plasma disinfection, as the former offers operational stability, whereas the latter provides the possibility of treating larger volumes of liquid [142], [159]. Naturally, the plasma volume cannot be increased indefinitely since a greater volume will require greater power input to achieve a stable discharge, which is further influenced by the material of the reactor [142]. To circumvent this, several reactors can be installed sequentially to increase the contact time, or the same reactor can treat the same liquid several times [100]. Additionally, water can be injected into the reactor by spraying or tangential flow, producing a rotating water column, increasing the surface-to-volume ratio and shortening the disinfection time [142], [159]. The produced $\text{OH}^\bullet$ from water homolysis reacts, by diffusion-controlled reaction rates, with organic substrates, which in turn consume the dissolved O_2 , leading to zones of oxygen depletion just below the surface of the irradiated volume [160]. With higher flow rates (Q or by mixing the solution, the zone of oxygen depletion reduces, thus enhancing the degradation rates of organic substrates [160].

The main issue with applying V-UV to liquid water is that it is absorbed by all materials, including regular borosilicate glass. For this purpose, high-purity windows are used, which are transparent to lower wavelengths. MgF_2 windows are usually used since they are transparent to V-UV radiation (above 112 nm). However, they are relatively costly and prone to degradation and fouling. Therefore, they may present a large portion of equipment cost [161].

1.4.2.2 Gas composition

Since the gas composition presents the ‘reservoir’ of the activated particles that are able to be produced by a gaseous discharge, this operational parameter has a great influence on the outcome of the treatment. Hydrogen plasma, for example, exhibits a very strong V-UV emission continuum [161] due to the Lyman (130–190 nm) and Werner (117–130 nm) series of molecular bands (Table 9) [98], [162]. The gas mixture may also comprise inert carrier gas (e.g. Ar), with an admixture of a gas providing the reactive component (e.g. O_2) [100]. It was observed early on that certain types of gases are more efficient at removing or inactivating robust organisms such as bacterial spores [84]. Generally speaking, oxygen-containing gas is more efficient at inactivating microbes in direct plasma treatment since it directly increases the production of germicidal reactive species, such as O_3 , $\text{OH}^\bullet$, O , $^1\text{O}_2$ and others [142]. When nitrogen is present in the discharge, nitrogen radicals ($\text{N}^\bullet$) are formed, which leads to production of NO_2 , NO_3 , HNO_2 or HNO_3 , decreasing the pH of the solution, and thus indirectly affecting the disinfection efficiency [142]. On the other hand,

the gas composition directly relates to the energy input required to achieve a discharge and the degree of ionisation and dissociation of plasma [129].

Table 9: Wavelength and transition probabilities of Balmer and Lyman series of the hydrogen atom. ‘1’ represents the ground state (adapted from [162]).

Transition ($k \rightarrow i$)	Wavelength (nm)	Lifetime of excited state (s^{-1})
$2 \rightarrow 1$	121.6	4.7×10^8
$3 \rightarrow 1$	102.6	5.6×10^7
$3 \rightarrow 2$	656.3	4.4×10^7
$4 \rightarrow 1$	97.3	1.3×10^7
$4 \rightarrow 2$	486.1	8.4×10^6
$5 \rightarrow 1$	95.0	4.1×10^6
$5 \rightarrow 2$	434.0	2.5×10^6
$6 \rightarrow 1$	93.9	1.6×10^6
$6 \rightarrow 2$	410.2	9.7×10^5

1.4.2.3 Pressure

The gas pressure of plasma dictates several discharge characteristics, including electron density, temperature, radiation emission profile, etc. [158]. Since its influence on the discharge is so profound, cold plasmas are often subdivided into low-pressure (0.15–1.5 Pa), medium-pressure (1.5–150 Pa) and atmospheric-pressure plasmas [84]. V-UV radiation is more readily available in low-pressure plasma discharges, as there is less quenching of excited states from ambient gases, although too low pressure decreases the probability of particle collisions, essential for the formation of excited states (Figure 17). Additionally, non-thermal low-pressure plasmas can be generated in much larger volumes, which is beneficial for large-scale applications such as water disinfection [163]. Several authors have demonstrated a more efficient spore reduction by low-pressure plasmas compared to their atmospheric pressure counterparts, presumably because of the lower amount of reactive species and UV radiation produced [164], [165], [166].

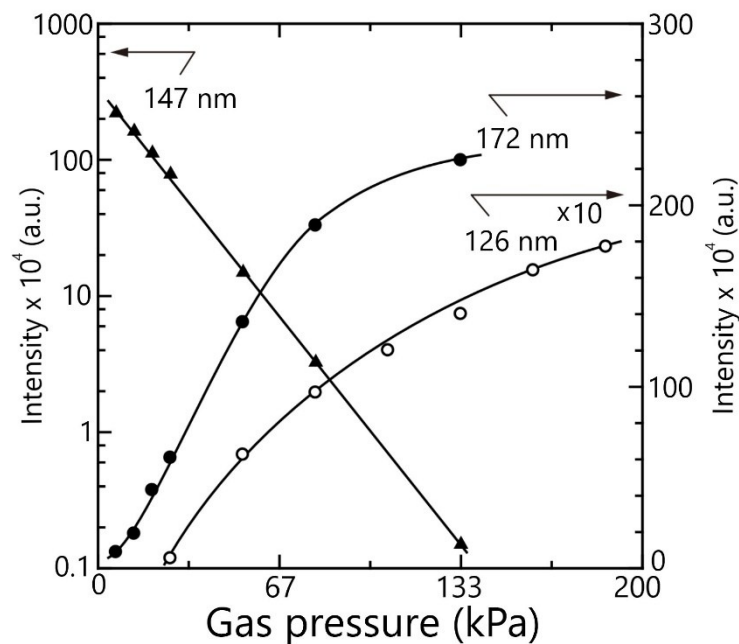


Figure 17: Gas pressure dependences of V-UV emission intensities from Xe^*_2 (172 nm) and Ar^*_2 (126 nm) excimers and Xe resonance line (147 nm) (adapted from [167]).

1.4.2.4 Power

In most plasma discharge systems, input power can be regulated by changing the voltage or the frequency of the generator. Input power directly affects the quantity of reactive species and radiation produced, which improves the pollutant degradation speed but not necessarily the efficiency since some energy is lost to heating [129]. At low pressure, the surface exothermic reactions prevail, so materials facing plasma are heated. At atmospheric pressure, gas is heated. Therefore, a careful balance between these two factors is required for optimal results [150].

1.4.2.5 Excitation frequency (f)

Although it has an appreciable effect on V-UV emission intensity (Figure 18A) excitation frequency is one of the least studied parameters regarding plasma disinfection, as it is technically challenging to modulate without changing the rest of the discharge parameters. While no references were found where this was examined in the context of virus decontamination of liquids, a recent examination by Yang et al. investigated the inactivation of food-pathogenic bacteria *L. monocytogenes* and *S. typhimurium*, exposed to atmospheric-pressure DBD plasma in water. Increasing the discharge frequency from 9 to 11 kHz resulted in an inactivation efficiency of 50% versus 89% for *L. monocytogenes* and 55% versus 92% for *S. typhimurium*, respectively [168].

Lerouge et al. treated *B. subtilis* spores with plasma generated with either RF (13.56 MHz) or MW (2.45 GHz) excitation frequency, applying the same amount of power, and obtained significantly less inactivation with the former. Greater excitation frequency led to higher V-UV intensity and reactive particle concentration; however, the authors attributed the enhanced inactivation rates to increased etching rates by reactive particles rather than V-UV radiation. Nevertheless, they acknowledged that the sterilisation effects are greatly influenced by the type of organism and operational parameters such as input

power, gas composition and pressure [98]. Similarly, Pollak et al. treated *B. atrophaeus* spores deposited on Teflon tubings with the Stripline high-frequency plasma device at two frequencies (700 and 2450 MHz, Figure 18B) and noted a markedly improved sterilisation at a higher frequency, which was attributed to greater UV/VUV emission [169].

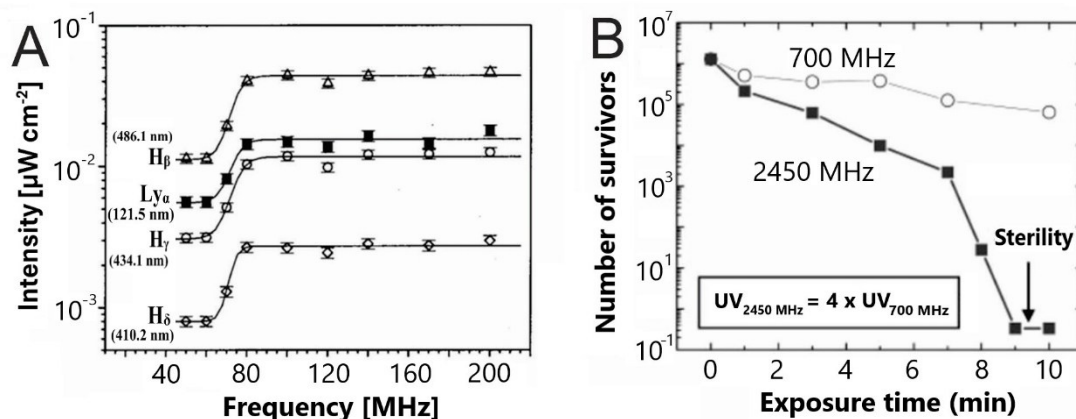


Figure 18: A) Frequency-dependent atomic hydrogen emission lines at $P = 50 \text{ W}$, $p = 66 \text{ Pa}$; and B) Inactivation curves of *B. atrophaeus* spores treated with argon plasma, operated at field frequencies of 700 or 2450 MHz (adapted from [141] and [169], respectively).

1.4.3 V-UV – liquid interactions

The research area studying radiation-induced chemical reactions is referred to as photochemistry. When V-UV radiation interacts with water molecules, a plethora of chemical reactions take place, which are influenced by a variety of parameters regarding both the V-UV source and the liquid composition. Since photons are the ones initiating the production of oxidising species (e.g. $\text{OH}^\bullet$), which then carry out oxidation reactions, the process is referred to as photo-initiated oxidation reactions. Water absorbs very efficiently at wavelengths below 190 nm, with a molar absorption coefficient (ϵ_λ) of $10 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 172 nm [160], [170], meaning the photolysis reactions are highly localised and limited to a thin layer (0.1 mm) of the irradiated volume (Figure 19). Water homolysis is the major reaction taking place between 140 and 190 nm, yielding the highly reactive $\text{OH}^\bullet$ (Eq. 39 and Eq. 40) as well as a hydrogen radical ($\text{H}^\bullet$) and a solvated electron (e_{aq}^- , free electron encased within water molecules), both of which are immediately trapped by dissolved O_2 , producing $\text{HO}_2^\bullet$ and $\text{O}_2^{\bullet-}$, respectively [70], [170]. These products then initiate a reaction cascade, each of them further influencing the end result downstream. All in all, a minimum of 25 reactions would need to be accounted for to describe the entire system of V-UV photolysis of pure water [170]. The excess energy after bond dissociation is primarily converted into kinetic or internal energy of the photoproducts, with the former being favoured for water molecules in the gas phase, while in the latter, the excess energy is mostly dissipated through interactions with other molecules, producing solvated products.

Little is known about the complex interactions between plasma-generated reactive species, their transfer through the gas/liquid phase into the bulk liquid, and their eventual interactions with the target pollutant. The short lifetime of reactive species ($< 1 \text{ ms}$), compared to their diffusion rate in water ($> 1 \text{ s}$), puts in question the efficiency of treating liquid surfaces with V-UV and their reactions with the target pollutants. Moreover, this explains the large importance of the distance between the plasma radiation source and the

treated liquid [171]. Considering the short diffusion distance of ionic species in air, it appears that ionic species have no impact on the treated liquids when the distances are above 0.1 cm [171].

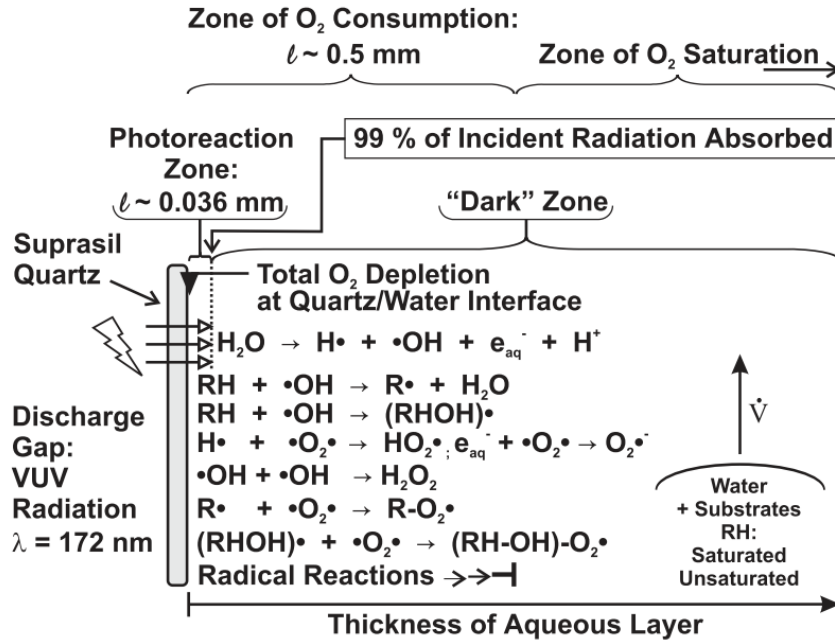
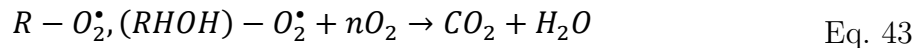
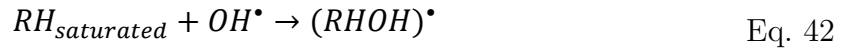
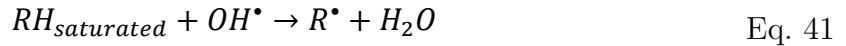
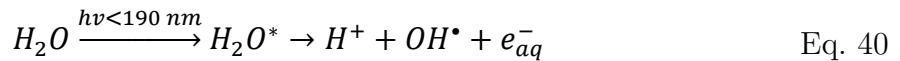
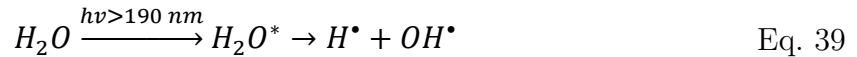


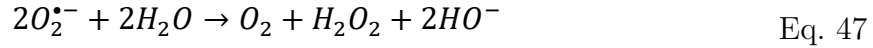
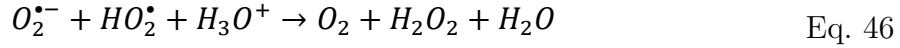
Figure 19: Absorption of V-UV radiation in a thin layer of water and the related reactions carried out (adapted from [160])

The highly unstable $\text{OH}^\bullet$ rapidly react with the pollutant, either by abstracting hydrogen (Eq. 41) or acting as an electrophile in the reaction (Eq. 42), eventually leading to complete mineralisation of the pollutant to CO_2 and H_2O in the presence of oxygen (Eq. 43) [155].



V-UV irradiation may also lead to H_2O_2 formation, a long-lasting germicidal product. Eq. 44–47 illustrate the reactions leading from radicals created by V-UV absorption in water to produce H_2O_2 , which could act as a residual disinfectant for water distribution systems, although the residual concentration may be too low to produce the desired effect [172], [173]. The H_2O_2 concentration is higher in the presence of oxygen due to a higher rate of perhydroxyl radical ($\text{HO}_2^\bullet$) production [172]. O_3 formation by V-UV reaction with water is negligible since it decomposes instantly, so its disinfection contribution can be

disregarded [113].



Besides being directly used to break molecular bonds and produce reactive species, V-UV can also be applied as a co-sterilisation method, in the same way as H_2O_2 /UV or O_3 /UV, since it promotes O_3 degradation more efficiently than UV, thereby promoting a better production of $OH^\bullet$ [174]. The utilisation efficiency of O_3 was 66% in a V-UV/ O_3 process, compared with 45% by UV/ O_3 when the pharmaceutical compound naproxen was treated in wastewater [174]. A V-UV/ O_3 flow-through reactor was able to efficiently degrade an antiviral drug, favipiravir, with $OH^\bullet$ being the primary degradation factor [113]. Ratpukdi et al. investigated the degradation of natural organic matter (NOM) by using either O_3 , UV, V-UV, O_3 /UV and O_3 /V-UV. They found that O_3 /V-UV was the best performing, capable of removing 71% of dissolved organic carbon (DOC) after 60 minutes of treatment, a two-fold greater efficiency than O_3 /UV treatment. One reason for this could be that while UV at 254 nm is optimally absorbed by ozone, leading to its photolysis, the resulting H_2O_2 that is produced (Eq. 13) has poor absorption at the same wavelength, presenting an underutilised source of $OH^\bullet$. V-UV radiation causes photolysis of H_2O_2 to $OH^\bullet$ while also providing additional $OH^\bullet$ from water photolysis, leading to an overall increase of $OH^\bullet$ production and subsequent NOM degradation [175]. The process was also pH-dependent, leading to a reduction of DOC at higher pH, presumably due to faster dissociation of $OH^\bullet$ to the anion radical ($O^{\bullet-}$); however, since several processes are affected by the change in pH, additional studies are needed to elucidate the exact mechanisms of these observations. V-UV/ O_3 pretreatment of greywater (household wastewater without toilet flushing) enhanced coagulation of organic compounds accelerated sedimentation and turbidity removal and could therefore be a useful pretreatment of wastewater in primary treatment steps [176].

Similarly, V-UV can be co-applied with common metal catalysts (Fe^{2+} , TiO_2), leading to increased production of ROS and subsequent degradation of pollutants [177], [178]. Combining VUV with chlorination results in the break of hypochlorous acid ($HOCl$), generating $OH^\bullet$ and chlorine radical ($Cl^\bullet$), not only improving the efficiency of pollutant degradation but introducing an entirely new set of degradation mechanisms [179].

1.4.4 Disinfection factors

Although it represents a complex physio-chemical process, the basic mechanisms of cold plasma sterilisation can be broken down into known antimicrobial factors: heat, reactive chemical species, and (V)UV radiation [165]. These factors are less or more important for the inactivation or disintegration of contaminants, depending on the environment and operation conditions of the plasma device.

1.4.4.1 Heat

Heat is a well-known sterilisation method, and standard heat-sterilisation devices (ovens, autoclaves) inactivate organisms by treating materials at temperatures above 120 °C for 20 minutes or so. Although cold plasma devices usually regard heat as an unwanted but unavoidable side-product, there are known synergies between heat applications with other disinfection technologies, such as UV and chemical reagents [180], [181], [182], [183]. Heat acts as an accelerator of chemical processes and, therefore, can shorten the treatment times required to achieve the desired effect [180]. However, heat is essentially excluded as a virus inactivation factor in low-temperature (< 50 °C) discharges, as well as in indirect, V-UV radiation-based methods.

1.4.4.2 Reactive chemical species

Interaction of reactive oxygen and nitrogen species (RONS) with the virus exterior leads to oxidation reactions, causing the erosion (etching) of molecules and biological structures, and results in the production of volatile compounds and oxidation products, such as CO₂ and H₂O. Chemically reactive species (e.g., NO, NO_x, OH•, H₂O₂, O₃, ¹O₂, O₂⁻, etc.) are first produced inside the air plasma discharge region, from where they are transported to the treated medium containing biological material. Ozone (O₃) and singlet oxygen (¹O₂) have been shown to cause breaks in genetic material and cause oxidation of nucleotide bases, with O₃ being more effective overall and ¹O₂ reacting mainly with guanine nucleotides [184]. OH• attack the outer structures of microbes, while some radicals (NO) participate in cellular signalling, disrupting the metabolic processes, leading to apoptosis of microbes [100]. If the microbe in question is a cell, some reactive species (NO, O₂⁻) will induce a cascade of intracellular signalling mechanisms, which interfere with normal cell functions, eventually resulting in cell apoptosis [96]. Viruses do not possess such intracellular machinery and will simply succumb to the degradation of proteins, lipids, and genetic material, leading to loss of function or outright degradation of the viral particle [106].

The diffusion rate of plasma active species in water is determined by their gas-phase concentration and aqueous solubility, as determined by Henry's law constants. Once the reactive species are in solution, their lifetimes are determined by their reactivity with other liquid constituents, acting as reaction partners. For example, OH•, due to its high reaction rate of 10⁷–10¹⁰ M⁻¹s⁻¹, has an extremely short lifetime in liquids, penetrating only about 100 nm below the gas-liquid interface [185]. Measuring and quantifying several reactions taking place simultaneously at a very thin layer in the gas/liquid interphase, with most of the reactants having a lifetime in the range of microseconds, makes this a challenging research area. Additionally, the multitude of reactions taking place simultaneously changes the water composition during treatment, producing local changes in temperature gradients, space charges, pH changes, etc. [171]. From the perspective of pH change during plasma treatment, Von Woedtke et al. provided some insight into the liquid behaviour. Distilled or NaCl solution were treated with atmospheric-pressure surface DBD plasma, with either air or argon as the discharge gas, with *E. coli* serving as a target microbe for disinfection. A decrease in pH was observed due to plasma treatment, and through a series of experiments, the decrease was attributed to nitrous acid (HNO₂). Inactivation of bacteria occurred when acidification of the solution took place, but not because of it, since inactivation was practically nil in an argon atmosphere. The authors attribute the inactivation to oxidising reactive species, such as nitrite radical (NO₂•), peroxyxynitrate (ONOO⁻), peroxyxynitrous acid (ONOOH) and hydrogen peroxide, but did not provide evidence for their conclusions [186]. Moreover, there is no mention of O₃ or OH•, two highly

potent oxidants known to be generated during an atmospheric air plasma discharge [137]. The pH decrease may, however, not be observed in a V-UV radiation treatment of water.

1.4.4.3 (V)UV radiation

During a plasma discharge, electrons collide with neutral molecules, exciting them, which releases photons upon relaxation to the ground state [142]. Some of these photons radiate in the germicidal UV-C region (254 nm or below), which are well-known to cause genome damage in microbes [136]. Additionally, UV photons can aid in the etching process initiated by RONS, acting synergistically and accelerating the degradation of microbes [165]. The germicidal radiation closely resembles the absorption spectra of pyrimidine bases (thymine and cytosine) of the genetic code, which tend to form dimers upon irradiation, leading to loss of function and inability to transcribe [147]. Furthermore, photochemical reactions have also led to structural changes and DNA-protein crosslinking when aromatic amino acids (tyrosine, tryptophan, phenylalanine and cysteine) have been subjected to UV-C radiation [147].

While in the germicidal UV-C region (220–280 nm), genome strands are broken through the excitation of DNA molecules, $\text{OH}^\bullet$, produced from V-UV water homolysis, is thought to be responsible for the genome damage of microbes [187]. Synergistic action was also observed when UV_{254} inactivation rate of bacteria in water was increased by 40% and when V-UV radiation at 185 nm, produced by high-pressure UV lamps, was co-applied [188]. A systematic study of bacterial spore inactivation with UV radiation, by applying 13 different wavelengths between 50 and 300 nm, revealed two main regions of inactivation: one in the V-UV (around 170 nm) and the other in the known germicidal region (220–270 nm) [189], coinciding with the observations made by Takakura et al. [187], regarding DNA damage in the V-UV region. Additionally, AOP pre-treatment of wastewaters containing non-biodegradable pollutants has been shown to improve the biological treatment of said pollutants further down the treatment process [160].

It was demonstrated by Moisan et al. [84] that in a dry sterilisation process, UV radiation is the primary inactivation agent up to the point where stacked spores prevent its penetration to those positioned beneath. Thus, in the second phase, the inactivation process is predominantly erosion-etching-based until sufficient quantities of biological material is removed for UV inactivation to take place once more (Figure 20) [84], [164]. Additionally, V-UV radiation can both etch organic material and produce reactive chemical species, which further disrupt biological materials, resulting in microbe inactivation [98]. While strong electric fields are generated and simultaneously applied to the treated medium, they are not powerful enough to cause meaningful inactivation of microorganisms [106]. V-UV radiation (between 100–200 nm), when coming into contact with polymers or organic bonds, excites the σ -bonds, such as C–C, C–H, C–O, leading to chain scissions, cross-linking, oxidation and other processes to occur [98], [149], [190]. V-UV has been shown to be strongly absorbed by DNA, presumably by the ribose-phosphate chain, rather than nucleotide bases [163], [191], [192]. However, since the water molecule strongly absorbs radiation below 190 nm wavelength, this mechanism is not expected to be a major contributor to inactivating microbes in a liquid.

When V-UV/UV-C radiation was applied to treat river water, complete disinfection of heterotrophic bacteria was achieved within 20 min of treatment. When peroxymonosulfate (PMS; HSO_5^-) was added, the effect was achieved twice as fast, showcasing the potential of combining VUV radiation with other disinfection techniques [193].

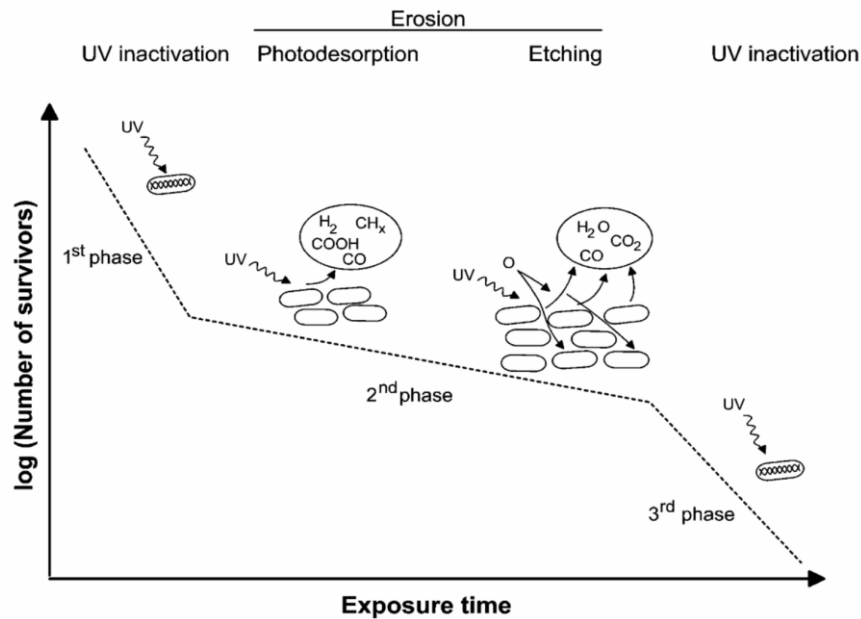


Figure 20: Graphical illustration of dry sterilisation by plasma treatment (adapted from [165]).

1.4.4.4 Liquid parameters

Besides plasma discharge parameters and the virus type, an entirely separate set of factors affecting disinfection efficiency is the composition of the liquid itself. Parameters such as heat [54], [194], [195], turbidity [7], [45], [67], [94], conductivity, soluble substances (salts, ions, enzymes, NOM) [39], [94], [194], dissolved gases [94], [196], pH [196], and many other have been previously shown to affect the disinfection efficiency of viruses in solution, to various degrees. While heat (temperature increase) has been proven many times over of being the most important environmental factor of virus inactivation [197], other parameters have not been proven or disproven to the same extent [77]. Conductivity, for example, long considered as one of the main factors affecting virus stability in natural waters [195], has also been shown to only have a negligible effect [54]. Salt content can either enhance or reduce the inactivation rate of viruses, depending on the type of virus and salt concentration or composition [39]. Photodegradation of haloacetaldehydes was reduced with elevated pH and dissolved oxygen, although rising pH led to increased photoreduction and elevated oxygen content resulted in increased photooxidation, separately [196]. NOM is a diverse mixture of carbon-containing organic compounds, which can either shield viruses from light exposure or increase the production of ROS, thus improving the inactivation of viruses [198]. All this is to show that, besides increasing the temperature, other water factors can affect disinfection efficiency positively, negatively, or not at all.

The results reported in the literature reviewed above were considered when constructing our experimental setups and performing virus inactivation research. The thesis consists of a review article and two original articles. The review article summarises the state of the art in the field of non-thermal plasma treatment of viruses in solution and also provides a meta-analysis of the results reported regarding plasma virus inactivation. The original scientific articles systematically characterise two different plasma systems regarding their capacity to produce V-UV radiation. This knowledge is then applied to study virus inactivation in liquid, as well as measure the production of key reactive species produced

in the liquid phase. Through a series of different experimental setups, applying various treatment regimes, modifying the treatment system, utilising ROS scavengers and curating the obtained results, we describe the mechanisms governing the inactivation of viruses in liquid and propose optimisation directions for further work.

1.5 Non-thermal plasma inactivation of viruses in water solutions

Beginning our journey to utilise low-pressure plasma for inactivating viruses in solution we conducted a thorough literature review. The proceeding article is the synopsis of this process, where we limit ourselves to publications concerning viruses placed into liquid and treated with a gaseous plasma technique. We thus excluded all instances where the virus sample was dried before plasma was ignited, however, we did include results from plasma-activated solutions, where the liquid was treated with plasma before the virus was mixed in. During our study, it became immediately apparent that comparing results from different authors was going to be challenging due to a variety of experimental parameters included in plasma treatment of solution. Conditions such as distance of plasma from the liquid surface, working gas, liquid composition, virus type, initial virus concentration, quantification method, etc., were all shown to have an impact on the treatment outcome, and even two studies using similar devices and conditions were hard to come by. Moreover, many authors failed to report their plasma operating conditions (e.g., input power, frequency, distance from liquid); therefore, we could not compare their results with other authors. When comparing inactivation results between different publications, we used the best-case scenario produced by each author for a particular setup, using virus inactivation as the only metric of efficiency. Since different quantification and inactivation methods for viruses exist, we transformed, where possible, the inactivation rates to D-values since this data was the most readily available data and is most relevant for these types of comparisons. For other treatment parameters (liquid volume, working gas, input power, plasma type, treatment time), we combined some of them to obtain a more insightful parameter for comparison (e.g., W/mL, W/initial concentration, kWh/m³) to find some correlation between the variety of reported results. The publication delves into these correlations, or lack thereof, and outlines the path forward for future research in this field.



Non-thermal plasma inactivation of viruses in water solutions

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ABSTRACT

Low-temperature (cold) gaseous plasma is a promising alternative for water treatment owing to its mild operating conditions, low environmental impact, and demonstrated effectiveness at degrading all types of organic molecules. It is being extensively researched as a novel tool for water treatment purposes, although it is still restricted to laboratory scale since disinfection mechanisms and operational parameters are numerous and inadequately understood. Different plasma treatment setups and approaches for disinfecting liquids are reviewed and critically evaluated. The original literature data is normalized and a meta-analysis is performed, providing insight into the major parameters affecting virus inactivation. The reported results differ significantly, but the virus inactivation efficiency (presented as D-value) is of the order of 10 s in all reports. The D-value does not depend much on the initial viral load although the authors probed a broad range from about 10^4 to 10^{11} PFU/mL. Different authors used plasma sustained at various powers. We found no correlation between the D-value and plasma power in the range of reported values, i.e., between 6 and 400 W/mL. The D-value increases with increasing energy consumption in the range between about 10 and a few 1000 kWh/m³. Inactivation curves of various treatment setups demonstrate the efficiency and disinfection kinetics with which cold plasma is able to destroy viruses in solutions. Although being a promising method for future applications, there is still an obvious lack of data to optimize the treatment parameters for large-scale applications.

1. Introduction

Viruses are tiny particles of various shapes and sizes (typically around 100 nm), containing genetic information. With an estimated number of around 10^{31} , viruses represent the most abundant “life” form on Earth, outnumbering the second-place bacteria (est. 10^{28}) by orders of magnitude [1]. Ubiquitous in nature, viruses reside in every and all natural water sources on Earth. While most of them are harmless for multicellular organisms, some viruses can infect and cause severe damage to animals, plants and humans. Vastly different in their morphology and means of replication they can be broadly separated into enveloped and non-enveloped viruses. Enveloped viruses have a host-derived lipid coating (envelope) which encompasses the entire nucleocapsid, while their counterparts lack this feature. The presence or absence of a lipid coating is usually a good predictor of their transmission and stability characteristics. Although not a definite rule, non-enveloped viruses tend to be more robust, surviving much lower

humidity conditions and infecting through surfaces, fomites and the like. Enveloped viruses require more stable surroundings, spreading primarily through respiratory droplets and are much more sensitive to sudden changes in pH, temperature or humidity. Another factor differentiating viruses from all other organism domains is their varying genome composition, which can be either DNA or RNA, single or double-stranded, circular or segmented, positive or negative-stranded [2].

Water serves as a transmission medium, through which many pathogens can spread and maintain their viability. Human viruses such as adenovirus, norovirus, poliovirus, and several others are known to remain infectious in water for several months [3]. The lack of adequate water sanitation and proper hygiene, leads to cases of acute gastroenteritis, caused primarily by pathogenic viruses such as norovirus, hepatitis E and A, and rotaviruses. Adding to the immense loss of human lives, is an estimated 260\$ billion global economic loss annually [4]. Consumption of contaminated fruit, vegetables or contact with an infected person is being recognized as a leading mode of virus

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transmission, and contaminated water sources have often been found as the primary transmission route [5]. Common methods for inactivating viruses in water can be separated into physical (e.g., heat, filtration), chemical (e.g., chlorination, ozonation, ammoniation), radiation (usually UV) or their combinations. When the primary mode of viral inactivation is via non-chemical oxidation, a method or a combination of methods are, termed “advanced oxidation processes” (AOP). However, all of these methods have flaws with either their cost, ease of application, effectiveness, health for consumers, or issues regarding environmental pollution [6].

Cold plasma is being considered an ecologically benign replacement or synergistic method for current water disinfection methods [7–10]. It is an effective way of disinfection without the use of toxic chemicals. Its disinfection capability has been demonstrated by many research groups utilizing several different devices and setups, and the attention given to this research area has been steadily increasing over the years. Compared to more common model pathogens like bacteria and fungi, viruses have received noticeably less attention regarding plasma disinfection. A reason for this may be the lack of appropriate model hosts for many important viral species, making the supply of sufficient virus material challenging to obtain, or prompting the use of appropriate virus surrogates (i.e., biochemically similar viruses). Moreover, diligent health and safety protocols should be followed, which further dissuades their research. It was not until the year 1998 that the first study demonstrating efficient plasma-based sterilization of viruses was published by Roberts et al. Their device (Sterrad 100 Sterilization System) caused between 2.5 log₁₀ and 5.5 log₁₀ (99.6 to 99.999 %) decrease of six different viral species. However, the Sterrad 100 can hardly be called a plasma sterilization device, since it utilizes hydrogen peroxide (H₂O₂) gas, a known virucidal agent, to treat dried virus samples before plasma is ignited for several minutes. In this case, plasma serves as a decomposing agent for the remaining H₂O₂ gas. The study disregarded the virucidal effect of H₂O₂ alone; therefore, the contribution of plasma was not known [11]. Since then, there have been many publications

demonstrating viral inactivation via different plasma configurations (dielectric barrier discharge (DBD) plasmas, plasma jets, in-liquid discharges), using a variety of process parameters (low-to-atmospheric pressure, with different process gas, input power, treatment duration, applied voltage and frequency, etc.). Various substrates such as glass, Si wafers, plastics, fruit, plants, and medical equipment have been subjected to such disinfection procedures, demonstrating the applicability of plasma sterilization across many different industries, reviewed elsewhere [12–16]. Since gaseous plasma inactivation of viruses in liquids proceeds by a different mechanism than on surfaces [17], and due to an increasing research focus in the field (Fig. 1), this review focuses on providing comprehensive state-of-the-art review of virus inactivation in solution. Moreover, a meta-analysis of the available data is performed to gain additional insight into the underlying mechanisms responsible for efficient virus inactivation in solution. The efficiency of any disinfection method is often reported as D-value, which is the time required to reduce the viral load in a sample by 1 log₁₀ (90 %) [18]. We utilize this factor in our analysis to make the comparison between different methods more straightforward, although it excludes some vital parameters which affect the outcome, such as initial viral load, treated volume, container dimensions etc.

2. Plasma for decontamination of viral solutions

Plasmas can be loosely described as an electrically neutral medium, comprising free electrons, positively or negatively charged ions, atoms, and molecules in their ground or excited states in the gas phase. Plasma in laboratories can be sustained in a broad range of pressures, but atmospheric pressure plasmas are usually applied to treat liquid samples. Some common plasma discharge setups include direct-current (DC) plasmas [19], capacitively coupled plasmas (CCP) [20], inductively coupled plasmas (ICP) [21], microwave (MW) [22], gliding arc [19], and plasmas sustained by high-impedance discharges such as dielectric barrier discharge (DBD) [23] or corona [24]. Two plasma types are most

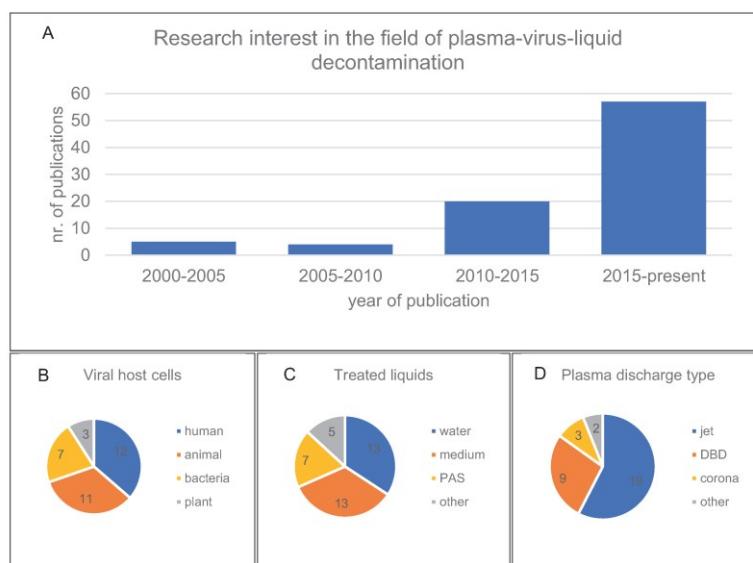


Fig. 1. A) Number of publications regarding plasma-based virus disinfection in liquids; Pie charts of plasma-based virus disinfection of liquids, based on B) Viral host, C) Treatment solution (PAS = plasma activated solution), D) Discharge configuration (DBD = dielectric barrier discharge). Source: Web of Science.

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often used for treating biological liquids directly due to the ease of application and ability to adjust several parameters, and they are plasma jet and DBD in a planar or torch configuration (Fig. 2A and B). Additionally, these types of plasma can also be used to treat liquid media and produce plasma-activated water, which is subsequently used to inactivate microbes, albeit less effectively than direct treatment.

2.1. Plasma jet

The distinguishing feature of a plasma jet discharge is the plasma “plume”, which is the glowing part of a gaseous discharge (Fig. 2C), transferred outside the discharge chamber via gas flow and ionization wave-head [25]. It is the most common plasma setup for treating biological samples, due to its mild conditions and operational flexibility. The treated samples are placed underneath the plasma plume at a certain distance, which affects the sterilization efficiency, since there is a larger amount of reactive particles and UV radiation in or closely adjacent to the plume than further away. Besides the total amount, the specific reactive species generated (e.g., atomic oxygen, ozone, hydroxyl radical, peroxyxynitrite, etc.) are perhaps more relevant to sterilization efficiency, and these are mainly dependent on the type of carrier gas used [17]. Furthermore, it is widely assumed that when treating liquids with a plasma jet, the plasma flow disturbs and mixes the liquid solution, causing more of the sample to come in contact with the reactive species, thus enhancing the method's effectiveness for disinfection. The drawback of this configuration is the rather small area which can be treated by the plume, resulting in most of the disinfection being carried out by plasma-generated reactive chemical species in solution [26]. The operational costs are marginal (only electricity) if plasma is sustained in the air. Otherwise, one should add the cost of gases. The gas flow is often several standard liter per minute because high gas flow rate causes the large reaction area between the water and the plasma [27].

The first report of a plasma jet being used for treating viral suspensions was provided by Brun and colleagues (2012) [28]. Using cold plasma jet, with helium as the working gas, they treated 200 μL of Phosphate buffered saline (PBS) suspensions of *Herpes simplex virus* type-1 (HSV-1) placed in 24-well tissue culture plates. Besides viruses, they also applied the treatment to bacterial and fungal cultures. Surprisingly, although the treatment effectively killed bacteria and fungi,

the treatment apparently did not affect the viral suspensions. This is the first and one of few reports indicating the inefficiency of plasma treatment for some viruses [28]. The low efficiency of disinfection may be attributed to using helium as a working gas, whose composition directly affects the type and abundance of reactive species produced. Namely, plasma sustained in pure helium is neither a source of chemically reactive species nor UV radiation. It is a source of vacuum ultraviolet (VUV) radiation, but water is not transparent for such energetic photons [29].

A year later, in 2013, Alshraideh and colleagues [30] investigated the addition of oxygen (0–1 vol%) to helium working gas, which greatly enhanced viral inactivation, until a saturation point was reached at 0.75 vol% of oxygen. A capillary DBD jet discharge, with a He + 1 vol% oxygen mixture, affected the inactivation negatively, supposedly because of molecular oxygen interfering with the discharge, resulting in lower production of reactive species. While the 1 $\log_{10}$ reduction of MS2 bacteriophage, suspended in 50 μL quarter strength Ringer's solution, treated with pure helium gas plasma, was reached within approx. 120 s, the same result was achieved within 60 s when 0.75 vol% oxygen was present [30].

The working gas is not the only discharge condition which affects virus inactivation efficiency. Wu et al. (2015) [31] treated 200 μL viral suspensions of MS2 bacteriophage with a plasma jet, while changing the discharge power or working gas. With these different treatment conditions, they demonstrated that inactivation efficiency increased proportionally with increasing discharge power. Furthermore, while room air worked better for lower discharge powers (24 W), Ar/O₂ mixture worked best at somewhat higher power (28 W). Optical emission spectroscopy (OES) showed an increased amount of atomic oxygen (O) at higher power levels. Damage to viral proteins, as well as RNA, was shown via SDS-PAGE (sodium dodecyl sulphate – polyacrylamide gel electrophoresis) and RT-PCR (real-time polymerase chain reaction), respectively [31].

By modulating plasma parameters of a plasma jet, and performing several diagnostic methods, Aboubakr and colleagues [32–34] were able to reveal several parameters playing a role in the Feline calicivirus (FCV) inactivation in various solutions. Their initial publication [32] evaluated the effect of plasma generation power, exposure time, and the distance of plasma plume from the sample, the effect of feed gas mixture, and the

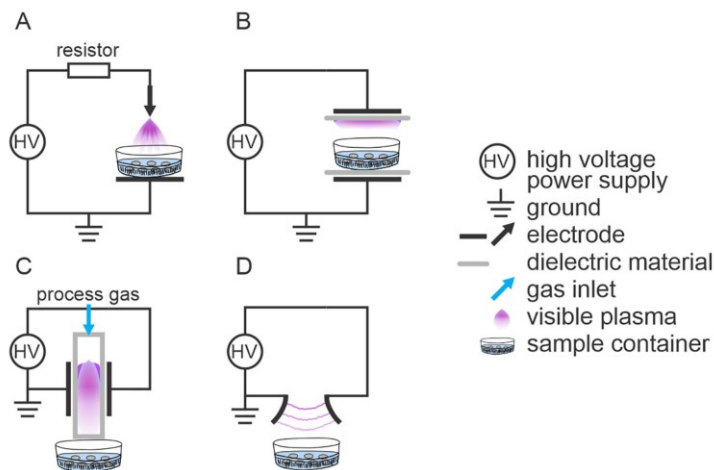


Fig. 2. Atmospheric plasma discharges used to treat liquid samples: A) corona, B) barrier, C) jet, D) gliding arc discharge.

effect of the medium composition on viral survival. They demonstrated that viral inactivation was enhanced by reducing the distance of the plasma plume from the sample, increasing generation power and/or extending the treatment time. More interesting findings are related to the various working gas mixtures used (Ar, Ar + 1 vol% O₂, Ar + 1 vol% dry air, and Ar + 0.27 vol% water vapor) and treated 100 µL medium compositions (sterilized double-distilled water, minimum essential medium [MEM], and NaCl-Tris-EDTA [NTE] buffer). Here, the Ar plasma with 0.27 vol% water vapor delivered the lowest inactivation efficiency, followed by Ar alone and Ar + 1 vol% air, while the best inactivation efficiency was achieved by Ar + 1 vol% O₂, reducing the viral population in 100 µL volume by 1 log₁₀ within 5 s. This agrees with a previous publication [30], where inert gas like He exhibited greater efficiency when oxygen, which produces more potent radicals, was present in the gas mixture. The distilled water resulted in the highest inactivation of viruses, followed by NTE buffer and MEM. While the greatest amount of hydrogen peroxide, often considered the main biocidal component of plasma-treated liquids, was generated in distilled water, its effect as the primary inactivation agent was excluded by separate experiments. Effects of pH and temperature were also excluded by the same approach. Thus, it had been postulated that short-lived reactive species performed the primary role in inactivation [33]. The lower protection from distilled water could be explained by the lack of scavenging compounds in the solution, which would prevent negative impacts of reactive species on the viral structure. The fact that MEM was supplemented with 8 vol% foetal bovine serum (FBS), FBS being a large radicals sink, would also agree with this hypothesis.

The same group demonstrated that the virucidal effect is determined by the generated reactive species, which are directly connected to the type of gas used. In the samples treated with Ar + 1 vol% of O₂ plasma, singlet oxygen was found to be the main inactivation agent, while peroxynitrite (ONOO⁻) produced the greatest inactivation result with Ar + 1 vol% air plasma. The latter is conditioned with the pH drop of the solution, which favours the protonated form. Ozone and nitrite were also found to contribute to the inactivation, while surprisingly, OH radicals supposedly did not significantly contribute to virucidal action. The effect of short-lived RONS (reactive oxygen and nitrogen species) was evaluated by the addition of radical scavengers to the solution, which react with the generated RONS, preventing damage to the microbes. By comparing capsid protein or genome damage of plasma-treated FCV, they were able to demonstrate that protein capsid damage precedes RNA damage, concluding that genome damage is secondary to protein damage in their CAP configuration. Furthermore, sequencing the genome of treated FCV showed no alterations to the code, proving that oxidation reactions are solely responsible for viral genome inactivation. Additionally, by using mass spectrometry to analyse capsid protein fragments, they were able to discern a number of specific amino acids modifications, which supposedly lead to capsid damage. It had been hypothesized that singlet oxygen primarily reacts with amino acids possessing double bonds or sulphur moieties. Histidine (His) 262 was identified as the most susceptible amino acid, leading to the formation of imidazolone/2-oxo-His, providing the first molecular basis for plasma-mediated capsid disruption. Other amino acids, such as tryptophan and methionine, were also affected [34].

A plasma jet system was also used for poultry vaccine preparation. To inactivate the Newcastle Disease virus and the Avian influenza virus, two of the most important pathogens affecting poultry, Wang and colleagues [35] treated the viral solution using a plasma jet. Operated in a Ar/O₂/N₂ gas mixture, they were able to sufficiently inactivate viral particles in solution, which were then used to formulate a vaccine for poultry. Based on their results, they concluded that the vaccines were as effective as the formaldehyde positive control, with no adverse side effects, proposing that non-thermal plasma can indeed be considered a novel tool for vaccine preparation [35].

Because plasma treatment systems come in various shapes, working conditions and treatment regimes, it is difficult to compare the

effectiveness of disinfection by different systems. Using a single system and modulating its parameters to treat a variety of microbes, can provide valuable insights on the plasma disinfection effectiveness on a variety of organisms. Takamatsu et al. [36] used their multi-gas plasma jet to treat liquid suspensions of bacteria, fungi, and viruses. While other parameters remained constant, the working gas was changed between Ar, O₂, N₂, CO₂ and synthetic air. CO₂ and N₂ performed best in inactivating *P. aeruginosa* and *S. aureus* (>6 log₁₀ in 60 s) and were thus used for further virus experiments. Two non-enveloped viruses were examined, namely feline calicivirus (FCV) and coxsackie virus (CSV). The plasma treatment lasted for 10 min or until >6 log₁₀ inactivation was achieved. Interestingly, while >6 log₁₀ inactivation was achieved for FCV in 5 and 3 min for CO₂ and N₂ gas, respectively, CSV exhibited greater resistance to the treatment, being inactivated by 6 log₁₀ after 10 min in N₂ gas plasma and by only 2 log₁₀ in CO₂ plasma. This demonstrates not only a large variety of inactivation due to the working gas, but also the variability in survival between different virus species. By supplementing the treated solution with radical scavengers, it was demonstrated that singlet oxygen (¹O₂) and hydroxyl radicals (OH) play a major role in viral inactivation, using CO₂ or N₂ working gas, respectively [36].

Plasma disinfection studies focus on inactivating pathogens before they infect their host. Amiran et al. [37] also investigated the effect of CAP as a post-infection treatment against single-cycle replicable-human immunodeficiency virus (SCR-HIV), an HIV surrogate. The HeLa host cell line was exposed to SCR-HIV and treated afterwards with a cold plasma jet, operated in helium, while also modulating the applied voltage and treatment duration. As expected, the infectivity decreased by raising the applied voltage and treatment time; however, the cytotoxic effect on the host cell increased proportionally, giving little reason to consider CAP as a post-infection treatment. Interestingly, treatment of HIV virions with plasma at 12 kV for 240 s reduced viral replication by 73.5 %, whereas treating the HeLa-virion mixture 6 h post-infection in the same conditions reduced viral replication by 78.2 %. However, this could be due to the increased cytotoxic effect of plasma-treated HeLa cells. Regardless, no treatment conditions produced >90 % replication reduction after 240 s of treatment [37].

Ensuring safe drinking water is an important driver in developing new water disinfection methods. All living things are vulnerable to viral infections, and water-mediated transfer is a common route for infection. Since agriculture consumes more potable water than any other industry [38], it is necessary to ensure that pathogens are excluded before administering a water source for agricultural or human use. Irrigation water is often collected in open gathering ponds and used on plants, which represents a potential source for viral spread. Moreover, with the increasingly popular indoor farming, water is recirculated through plants many times before it is cleaned; therefore, an infected plant can serve as an infection source for the whole system, releasing water-transmissible viruses into the circulating water stream through its roots [39]. Plant viruses are some of the more robust pathogens, and many of them can survive for prolonged periods of time in irrigation water [40]. Cold plasma jet has been investigated for plant virus inactivation in water systems by Filipić et al. [41,42]. By subjecting potato virus Y (PVY) and pepper mild mottle virus (PMMoV) to an Ar + 1 vol% O₂ plasma jet in a single-electrode configuration, viruses were successfully inactivated after only 1 to 5 min of treatment (in some cases even 1 min was enough), depending on the experimental setup. They verified genome and protein damage in both purified water samples as well as the infected homogenates, which was linked with the generation of RONS in the solution. Furthermore, they performed cyto- and genotoxicity studies of the treated water. The treated water was not found to produce significant adverse effects on the HepG2 cell culture in in-vitro tests, further demonstrating that cold plasma can be a useful tool in irrigation water treatment regimens [41,42]. Some details of experimental setups and results reported by cited authors who used plasma jets are presented in Table 1.

Table 1
Overview of plasma jet treatment of viruses in solution:

Virus	Host	Power source	Gas	Applied power [W]	Gap distance [mm]	Maximum treatment duration [min]	Medium	Treated volume [μ L]	D-value [s]	Genome damage	Protein damage	Inactivation agent	Reference
Non-enveloped, single-stranded RNA													
MS2 bacteriophage	<i>E. coli</i>	Haider PHK-2 k, 20 kHz, 6 kV	varying He/O ₂	N/A	10	9	QSRs	50	60	N/A	N/A	ROS	[30]
		10 kHz, 14 kV	air, Ar/O ₂ , He/O ₂	28	0	3	Water	200	30	Yes	Yes	ROS	[31]
Feline calicivirus	Animal	20 kHz with a duty cycle of 20 %	Ar, Ar/O ₂ , Ar/O ₂ , dry air, Ar/water	1–3	11.25 to 18.13	3	DW, MEM, NTE buffer	100	5	N/A	N/A	RONS	[32,34]
Coxsackie virus A7	Human	10 kV, 10 kHz	Ar	N/A	20	2	PRS	20	30	Yes	N/A	RONS	[43]
		16 kHz, 9 kV	N ₂ , CO ₂	10	6	10	PRS	200	N/A	N/A	N/A	RONS	[36]
		16 kHz, 9 kV	N ₂ , CO ₂	10	6	10	PRS	200	N/A	N/A	N/A	RONS	[36]
Potato virus Y	Plant	31 kHz	Ar/O ₂	3	0	180	Infected homogenate, or purified virus	10,000	N/A	Yes	N/A	ROS	[41]
Pepper mild mottle virus	Plant	31 kHz	Ar/O ₂	3	0	5	Water	10,000	180 (RNA deg.)	Yes	N/A	ROS	[42]
Tobacco mosaic virus	Plant	20 kV _{pk} , 8.3 kHz	Air	N/A	100	10	DW	40	30	Yes	No	N/A	[44]
Non-enveloped, double-stranded DNA													
Various adenovirus species	Human	N/A	N/A	N/A	15	2.5	DMEM	500	N/A	N/A	N/A	N/A	[45]
Enveloped, single-stranded RNA													
Herpes Simplex Virus-1	Human	6 kHz, 0.12 kV RF, 0.9 kV p-p	He	N/A	10	4	DMEM	100	>200	N/A	N/A	N/A	[37]
			He	N/A	2	5	PRS	200	a	No	yes	ROS	[28]
Newcastle disease virus	Animal	18 kV _{pk} , 10 kHz	Ar	N/A	N/A	2.5	DMEM	3000	N/A	Yes	N/A	N/A	[46]
			Ar/O ₂ /N ₂	N/A	100	4	Allantoic fluid	10,000	N/A	N/A	N/A	RONS	[35]

^a No inactivation observed; D-value = treatment time to achieve 1 log₁₀ inactivation; N/A = data not available; MEM = Minimal Essential Medium; DMEM = Dulbecco's Modified Eagle Medium; QSRs = Quarter Strength Ringer's Solution; RO(N/S) = Reactive Oxygen (and Nitrogen) Species; PRS = Phosphate Buffer Saline; DW = Distilled water, NTE = NaCl-Tris-EDTA.

2.2. Dielectric barrier discharges

Barrier discharges, also called “silent discharges”, are highly versatile setups for treating large sample surfaces. A barrier discharge is usually a simple two-electrode setup (Fig. 2b), often powered by AC voltage at or below atmospheric pressures. The electrodes can be embedded in a dielectric material (glass, quartz, ceramics). The high voltage power supply connected to electrodes produces a uniform plasma throughout the entire surface, hence the term dielectric barrier discharge (DBD). The discharge is often in the filament mode with stochastic electron streamers of short duration. Due to the uniformity of treatment on larger surfaces, they are commonly applied in textile, medical, electronics and environmental protection industries. They can be used for various purposes, e.g., to generate reactive agents (e.g., ozone), clean or etch surfaces, improve wettability and dyeability, and activate surfaces to improve adhesion. When operated at low pressure, they generate large amounts of vacuum ultraviolet (VUV) radiation, which can be beneficial for surface activation and sterilization. Owing to their mild operation conditions, low cost (usually, they operate in ambient atmosphere, so the only operating cost is electricity) and the fact that no harmful chemicals are required or produced, the sterilization aspect of DBDs has received more research attention in the past few decades [47].

In a low volume (20 μL) treatment, Yasuda et al. [48] demonstrated that DBD plasma primarily affects surface coat proteins in the earliest stages of inactivation. A plasma-inactivated λ phage was reconstituted through an in-vitro DNA packaging method, where the purified DNA from the treated virus suspension was mixed with a coat protein solution, producing infective bacteriophages. The reconstituted phages showed a higher infectivity rate than the control, indicating that DNA damage did not cause reduced infectivity. The SDS-PAGE analysis of protein degradation and agarose gel electrophoresis of DNA support these claims [48].

Zimmermann et al. [49] were the first to investigate viral inactivation in a solution, albeit using only 20 μL aliquots. In their study, using a surface micro discharge setup, they achieved over 6 $\log_{10}$ inactivation of Adenovirus 5, following a 240 s treatment regime. Among other things, they clearly demonstrated that common plasma-derived disinfection agents, UV radiation and ozone (O_3), are present in relatively small amounts during plasma treatment, and thus their contribution to viral inactivation is negligible. The central role in inactivation is attributed to reactive oxygen and nitrogen species produced in air discharge, which cause protein and DNA damage to non-enveloped viruses [49].

Shi and colleagues [50] used DBD plasma discharge to treat hepatitis B virus (HBV) infected serum of HBV patients. Although they have not determined the reduction of viral particles after treatment, their results provide assurance that plasma disinfection can reduce viral DNA in complex liquids such as saline-diluted HBV [50].

Different types of plasma produce different discharge conditions, which cause different inactivation efficiencies. This was demonstrated by Tanaka et al. [51], who compared inactivation efficiencies of atmospheric-pressure DBD versus corona discharge against bacteriophage ϕX174 . The noticeable discrepancies between them were that the DBD discharge initially provided slower inactivation until the 30-s mark. The phage infectivity decreased exponentially at that point, resulting in a 7 $\log_{10}$ inactivation after 60 s. On the other hand, the corona discharge produced a linear inactivation curve, eventually achieving 7 $\log_{10}$ inactivation after 90 s. Furthermore, they compared the inactivation efficiency of DBD plasma treating either “wet” or “dry” samples, where the latter was desiccated before the application of plasma. With a steep linear curve, the “wet” treatment reduced the number of viral particles by 10 $\log_{10}$ in 40 s, which is significantly better than the “dry” samples (3 $\log_{10}$ in 40 s). By measuring DNA activity and performing an SDS-PAGE analysis, they found that DNA damage is only the secondary inactivation mechanism, preceded by coat protein destruction [51].

Nayak et al. [47] reported a similar conclusion, who observed an

improved inactivation of wet versus dry FCV samples deposited on steel discs. Interestingly, modulating the distance between the sample and the plasma discharge (1–40 cm) did not affect the treatment efficiency significantly, leading to belief that long-lived species carry out the inactivation function. The same study also investigated the treatment of viral suspensions with a micro-discharge plasma operated in air. They found the inactivation of wet samples more efficient than the dry surface treatment. Moreover, the inactivation of viruses in liquid samples was positively correlated with a drop in pH, which could indicate RNS transfer into the bulk liquid to inactivate viruses. Plasma disinfection in solution seems to be limited by the mass transfer of reactive species into the liquid, which is important for designing a water treatment reactor, as the mixing of a liquid is minimal by using a DBD compared to a plasma jet [26]. The virucidal activity of plasma-activated solutions was also investigated and determined to be less efficient than direct plasma treatment [52].

A comprehensive study comparing direct plasma treatment to plasma-activated solution (PAS) was performed by Guo and colleagues in 2018 [53]. What's more, they used three different viruses (bacteriophage T4, MS2 and ϕ174) to account for the difference in susceptibility between different viruses. A DBD plasma device was operated in the air for direct treatment or, to promote the generation of reactive species, in Ar supplemented by 1 % artificial air (79 % N_2 , 21 % O_2) for PAS treatment. The authors show that, although both treatments were able to effectively inactivate all the viruses, the direct treatment performed slightly better. Through the use of radical scavengers, they identified singlet oxygen ($^1\text{O}_2$) and superoxide radical ($\text{O}_2^{\cdot-}$) as the primary inactivation for both treatment approaches. How scavengers of short-lived species prevent virus inactivation of PAS remains unexplained. Long-lived biocidal compounds, such as hydrogen peroxide, nitrite, and nitrate, have been identified in plasma-activated solutions. To evaluate their contribution to viral inactivation, these agents were compared separately to plasma-activated water (PAW). PAW had a markedly superior virucidal effect against all viruses and performed even better than the 1 %-formaldehyde solution used as the positive control. A 1 $\log_{10}$ reduction was achieved after <1 h of incubation time and neared the detection limit (reduction of approx. 10 $\log_{10}$) after 8 h, while 1 % formaldehyde reduced the viral infectivity by about 1–2 $\log_{10}$ during the same period. The treated solution retained its virucidal capacity even after 10 days of storage, which demonstrates the potential of PAW being used off-site. [53].

Hanbal et al. treated distilled water samples containing a plant virus Tobacco mosaic virus (TMV), using their DBD plasma [44]. Since the plasma was generated in a tube via DBD discharge, and subsequently administered to the samples by gas flow, the plasma is defined as a plasma jet. The treated samples showed reduced infectivity on the inoculated leaves after 30 s of treatment, and no infectivity after 5 min was observed. What is interesting is that contrary to the viral RNA, the coat protein subunit concentration was not being reduced with longer treatment times. The scanning electron microscope micrographs clearly show structural damage of the virus, which probably resulted in the viral genome being released into the solution. Based on the immunological and electrophoresis results, the viral coat protein appears intact, whereas the viral RNA seems mostly degraded after 1-min treatment. This puts into question whether a difference in sensitivities of proteins and viral genomes to the generated RONS plays a role in the viral inactivation mechanism. Nevertheless, these results seem promising for using plasma as a chemical-free method to disrupt plant viruses in water, a much-needed solution for the agronomy sector [44]. Similar findings were reported by Yamashiro et al. [43], who also evaluated the generation and role of various reactive species on viral inactivation. Using an atmospheric-pressure DBD plasma torch, they evaluated the inactivation mechanism of FCV. Through the use of radical scavengers, they demonstrated that singlet oxygen ($^1\text{O}_2$) and peroxyxynitrite (ONOO^-) were the main species responsible for viral inactivation. The latter was shown to be an important inactivation agent in acidic conditions. They

further excluded the contribution of heat and UV-A in the inactivation process and determined the time-dependent degradation of viral RNA [43]. Some details of experimental setups and results reported by cited authors who used a DBD discharge are presented in Table 2.

2.3. Other disinfection setups

Although the most appropriate for treating liquids, barrier and jet discharges are not the only plasma setups available in the field of water disinfection technologies. Some setups allow the plasma to be generated directly in liquid, as opposed to treating the liquid from a distance or submerging the plasma device into the treated liquid, where the discharge is still generated in the gas phase. One such discharge is streamer corona discharge and is named like that because streamer-shaped plasma discharges are formed at the tip of the first electrode, which are then transferred to the second electrode, which can be a pin or a plate, thus completing the circuit. While these streamers are transferred through the liquid, several physical and chemical processes take place, such as heating, UV radiation and formation of reactive radicals. Although such discharges require comparatively larger energy input than other discharges described, in 2010, Lee and colleagues [54] used a pin-to-plate streamer discharge in water to treat a 30 mL water suspension of MS2 bacteriophage. Although the applied power was relatively high (120 W), they achieved over 5 log₁₀ reduction of MS2 in 1 min. Additionally, they demonstrated that the inactivation efficiency was positively affected by higher charge storage capacitance while not being affected by water conductivity, pH (5.8 to 8.2) or temperature (4 °C to 37 °C) [54]. Another advantage of the corona discharge is its robustness, so the discharge is valid also for spraying contaminated liquid through the plasma, however this has not yet been attempted for virus inactivation [55].

El-Aragi [19] utilized a gliding arc discharge, which is a discharge generated between two diverging electrodes, pushed out by a gas flow, to treat heparinized blood samples containing the Hepatitis C virus. By either continuous or pulsed DC discharge, they were able to achieve 67 % and 62 % inactivation, respectively, within 2 min of treatment using nitrogen as process gas. Although it is not clear how the blood cells responded to treatment, it is evident that pulsed arc discharge has the potential to inactivate viruses even in complex biological liquids [19].

Optimization of plasma-based treatment can be a challenging task since many parameters of plasma processing affect the outcome. El-Kalliny et al. attempted to optimize the efficiency of CAP treatment by using the Response Surface Methodology (RSM) approach [56]. A point-electrode above the water surface was examined for viral inactivation, with the gap distance, input voltage and depth of water as parameters for optimization. Crystal violet dye (CV), an organic pollutant, was used to identify the optimal parameters of plasma treatment, which revealed that increased power input and lower plasma-to-liquid gap distance improved degradation efficiency. Surprisingly, a larger sample volume also improved CV removal efficiency up to a certain point. The authors provide no explanation for this finding; however, one could assume that a larger volume of water could provide a larger amount of oxidizing species, leading to increased removal rates. The generated models corresponded well with the experimental results obtained for the removal of an organic dye. These parameters were then used to study virus inactivation by treating bacteriophage ϕ X174 and rotavirus, achieving complete inactivation of ϕ X174 (5.5 log₁₀) after 1 min and 5 log₁₀ reduction of rotavirus after 10 min [56].

2.4. Plasma-activated solutions

Direct plasma treatment of viral suspensions exposes samples to a harsh environment of short-lived radicals, free electrons, highly energetic photons, excited molecules, atoms, positive and negative ions. By contrast, plasma-activated solutions (PAS) inactivate microbes through the action of rather long-lived oxidizing species, which remain in

Table 2
Overview of DBD experimental setups and results.

Virus	Host	Frequency, voltage	Gas	Applied power [W]	Gap distance [mm]	Maximum treatment duration [min]	Medium	Treated volume [L]	D-value [s]	Genome damage	Protein damage	Inactivation agent	Reference
Non-enveloped, single-stranded RNA													
Adenovirus	Human	4.7 kV _{pk-pk} at 10 kHz	Air	50 (0.5 W/cm ²)	12	4	PBS	20	N/A	N/A	N/A	ROS	[49]
MS2 bacteriophage	<i>E. coli</i>	N/A	Ar/	15	8	1.6	Water	8000	10	Yes	Yes	ROS	[53]
Feline calicivirus	Animal	20 kHz	O ₂	14.5	10–400	10	DW, NTE buffer	100	15	N/A	N/A	ROS	[52]
λ bacteriophage	<i>E. coli</i>	40 kV _{pk-pk} at 2 kHz	Air	8.7	N/A	N/A	Water	20	3	N/A	N/A	N/A	[48]
Hepatitis B	Human	60 kHz, 20 kV	Air	76 (1.2 W/cm ²)	3	0.6	HBV serum diluted with saline	100	N/A	Yes	Yes	N/A	[50]
Non-enveloped, single stranded DNA													
ϕ 174	<i>E. coli</i>	N/A	Ar/	15	8	1.6	Water	8000	10	Yes	Yes	ROS	[53]
ϕ 174	<i>E. coli</i>	14 kV, 2 kHz	O ₂	0.45 W/cm ²	2	0.6	TE buffer	50	3	Yes	Yes	N/A	[51]
Non-enveloped, double stranded DNA													
T4 Bacteriophage	<i>E. coli</i>	N/A	Ar/	15	8	1.6	Water	8000	10	Yes	Yes	ROS	[53]

D-value = treatment time to achieve 1 log₁₀ inactivation; N/A = data not available, PBS = Phosphate Buffer Saline; DW = Distilled water; (NTE) = (NaCl)-Tris-EDTA, HBV = Hepatitis B Virus; RO(MS) = Reactive Oxygen (and Nitrogen) species.

solution after plasma treatment. Given this difference in inactivation mechanisms, it is reasonable to distinguish PAS treatment as a separate category of plasma disinfection.

The appeal of using PAS for disinfection purposes lies in the ability to treat many different systems by using the same setup, while simultaneously excluding the need for plasma to be generated and used on site. However, it has been demonstrated that PAS gradually lose their virucidal capacity over time, with a sharp drop in effectivity observed after a few minutes [52,53]. The PAS biocidal effect also seems to be correlated with the pH of the solution [52].

Several factors play a role in the potency of PAS on viruses. Plasma treatment parameters such as gas composition, treatment duration, distance from liquid and applied power have all been shown to affect the antiviral potency of treated solutions [33,57,58]. Additionally, the composition of treated solutions also has a great effect on the type and amount of biocidal agents generated (e.g., H_2O_2), with pH being a good predictor on the main reactive species present and thus the inactivation mechanism [32,52,57,59].

PAS virucidal activity was first demonstrated when an air DBD plasma discharge was used to treat 1 mL of complete keratinocyte growth medium 2 (KGM-2), which was subsequently used to treat Herpes Simplex virus type 1 (HSV-1) infections of human corneal epithelial cells in vitro and ex-vivo [60]. Through an elaborate experimental design, the authors were able to produce convincing evidence of hindered HSV-1 infection and replication rates for 40 s plasma-treated medium. Moreover, they were able to show that plasma-treated solution did not produce detrimental tissue effects caused by either UV radiation or H_2O_2 exposure. Unfortunately, the information on pH and the type of active species generated were not provided, which would be useful to explain the inactivation mechanism [60]. Since the complete KGM-2 medium comprises a complex solution of nutrients, minerals, and growth factors, it is difficult to ascertain how the interactions of these compounds with a variety of species present in a plasma discharge contributed to the virucidal effect.

To discern the causal inactivation factors of PAS, a more straightforward approach is to treat liquids with known composition and evaluate the physiological and chemical changes of treated solutions. To this end, treating distilled water and NTE (NaCl-Tris-EDTA) buffer with an air DBD discharge gave insight into how this affects the chemical composition of the treated liquid. The drop in pH (from 6 to 3) of plasma-activated distilled water was positively correlated to the virucidal activity, which indicated the contribution of peroxynitrite chemistry to inactivation. Time-delay experiments also demonstrated that virucidal activity is better retained in non-buffered water than in buffered solution [52].

Since the composition of solution also plays a role in the potency of PAS, it is reasonable to assume that various compounds would be able to contribute to the viral inactivation of PAS positively. Since salt (NaCl) is present in the majority of water on Earth, it would be valuable to examine its contribution to antiviral efficiency in PAS. Su et al. [59] treated various solutions of water, water-NaCl (0.9 w/w %) and water- H_2O_2 (0.3 w/w %) with air DBD plasma and exposed Newcastle Disease Virus (NDV) to the treated solutions. Surprisingly there was no difference in the inactivation efficiency between the different solutions, even with different NDV-PAS ratios. Both viral RNA and protein concentration decrease was confirmed in treated samples, with RONS being credited as the inactivation agents [59].

Tsvetkov et al. [57] investigated PAS as a prophylactic measure against HSV-1 infection. Using an argon-fuelled plasma torch at 13 W input power to treat supportive DMEM medium, they reduced the HSV-1 infection rate up to 50 % in one experimental setup, which the authors claim is not a significant reduction. Meanwhile, direct plasma treatment of extracellular virions produced over 1.5 $\log_{10}$ reduction of virus titer after 300 s treatment time. Additionally, they examined the cytotoxicity of PAS, treated at various power inputs (20 W, 15 W or 13 W), on the Madin-Darby bovine kidney cell line. At 20 and 15 W of applied power,

the treated solutions produced a cytopathic effect (50CE) on the examined cell line, whereas no such effect was observed with the medium treated at 13 W [57].

PAW could also play a role in future pandemic aversions. With a similar procedure as in their previous study, Guo et al. [58] demonstrated the inability of SARS-CoV-2 A-protein binding to the HACE2 receptor after a brief treatment by PAW. The effect was observed after only 5 min of incubation with PAW, while after 10 min incubation, viral particles appeared to aggregate between themselves. This is promising for disinfection as the production of PAW requires no harmful chemicals [58]. Some details of experimental setups and results by cited authors who used PAS are presented in Table 3.

To summarize, different plasma setups require different equipment. The advantage of atmospheric-pressure plasmas is definitely easier usage, the downside being their production of ozone, which needs to be taken care of. Perhaps the most industrially used one is the corona discharge, which unfortunately requires quite high operating voltage of several 10 kV, which amounts to expensive equipment; it also produces a lot of ozone, which has to be properly disposed of. Dielectric barrier discharges are friendlier to work with, however they are effective only at the point of application and require bigger surface for any possibility of scale-up. From the practical point of view, perhaps atmospheric-pressure plasma jets are the easiest to operate, but are point-limited, meaning that for any scale-up one would require several setups, which again increases the cost.

3. Meta analysis of liquid disinfection setups

Low-temperature plasmas can be generated in various setups, with many parameters affecting the treatment characteristics. Although many publications give an insight into the primary inactivation factors, such as process gas, input power, gap distance, etc., they still differ significantly between various authors. Tables 1–3 summarize the experimental parameters and the main results. A meta-analysis was performed by correlating the reported treatment conditions with the inactivation efficiency (D-value), to identify any correlation between various setups and propose some guiding principles for plasma treatment optimization. The results are presented in Fig. 3A. The data are a representation of the “best” treatment conditions for a specific setup. The inactivation efficiencies of plasma-activated solutions are excluded, as they are not comparable to direct treatments in both effectiveness and mode of action. Furthermore, the variety of setups and treatment conditions make direct comparisons futile. When not available in the publication, the data for D-values were taken from the accompanying graph. Virus concentrations reported as tissue culture infectious dose 50 (TCID₅₀) have been recalculated to plaque-forming unit per millilitre (PFU/mL), by multiplying TCID₅₀ with the conversion factor 0.7, as is standard practice [61].

As can be observed from the multiple graphs shown in Fig. 3, the amount of data points available from the literature are too few to obtain relevant information on the optimal operational parameters. This is due to various methodologies used by the authors, where some only demonstrate the destruction of the virus or viral particles after a set treatment time, which is insufficient to determine the disinfection efficiency in terms of a D-value number. Moreover, some authors fail to provide the precise operational parameters used in their experiments, which makes the comparison of results difficult. The plot presented in Fig. 3A showcases the dispersion of data when an initial concentration of viruses is addressed. While many authors have reported a positive correlation between the amount of virus in the sample and inactivation efficiency, the plot suggests that this is not the only predictor of treatment efficiency. This is supported by literature, which shows that a combination of factors is more important than any one specific parameter. Interestingly, although many authors demonstrated an improved disinfection efficiency when more energy was supplied to the plasma system, an inverse correlation is seen on the plot shown in Fig. 3B,

Table 3
Overview of plasma-treated solution setups and results.

Virus	Host	Frequency/ voltage	Gas	Power [W]	Gap distance [mm]	Maximum treatment duration [min]	Medium	Treated volume [μL]	D- value [s]	Genome damage	Protein damage	Inact. agent	Ref.
Plasma jet, enveloped, single-stranded RNA													
Newcastle disease virus	Animal	20 kHz	Air	N/A	20	N/A	PAS: H ₂ O, 0.9 % NaCl, 0.3 % H ₂ O ₂ Medium and water	10 ⁴	N/A	Yes	Yes	N/A	[59]
Herpes simplex Virus-1 strain F	Human	MW 2.45-GHz electromag. Wave, AC 4.7 kV	Ar	20	N/A	N/A		100	300	N/A	N/A	N/A	[57]
DBD plasma, single-stranded RNA													
Herpes Simplex Virus-1	Human	+15.5-kV pulse amplitude, 1 kHz	Air	1.9 W/ cm ²	1	3	KGM-2	1000	25	N/A	N/A	N/A	[60]
Feline calicivirus	Animal	20 kHz, generated by an AC power source	Air	14.5	10	10	DW, NTE buffer	100	15	N/A	N/A	RONS	[52]
MS2 bacteriophage	<i>E. coli</i>	N/A	Ar/ O ₂	15	8	1.6	Water	8000	10	Yes	Yes	ROS	[53]
Pseudoviruses with SARS- CoV-2 S protein	—	23 kHz, 8.36 kV	Air	12.5	N/A	N/A	Deionized water	5000	N/A	N/A	N/A	N/A	[58]
DBD plasma, double-stranded DNA													
Bacteriophage T4	<i>E. coli</i>	N/A	Ar/ O ₂	15	8	up to 1.6	water	8000	10	Yes	Yes	ROS	[53]
φ174	<i>E. coli</i>	N/A	Ar/ O ₂	15	8	up to 1.6	water	8000	10	Yes	Yes	ROS	[53]

D-value = treatment time to achieve 1 log₁₀ inactivation; N/A = data not available; PAS = Plasma-activated solution, KGM-2 = complete keratinocyte growth medium 2, DW = Distilled water, NTE = NaCl-Tris-EDTA; RO(ONS) = Reactive Oxygen (and Nitrogen) Species.

further demonstrating that the disinfection efficiency is driven by a combination of factors, as explained above. Graphs in Fig. 3C and D are a representation of energy efficiency for treating a cubic meter of water when considering the virus type or plasma system, respectively. Besides no apparent correlation between the various setups, there is also an enormous amount of energy used for treating viral solutions for some setups, although this can be attributed to the small scale at which these lab-scale experiments are conducted. For comparison, the standard low-pressure UV systems use between 5 and 8 kW/m³ for water disinfection, showcasing the gap in energy consumption, which plasma treatment systems need to address [62].

Plotting all inactivation curves based on plasma discharge type (Fig. 4) allows us to observe two groups of inactivation efficiency. The majority of steep log-linear curves which demonstrate more efficient plasma disinfection systems are DBDs, which produce a more homogeneous discharge over a larger surface area. The other type represents a more gradual decline in virus titer over time, with a majority of these belonging to corona discharges.

Fig. 5 attempts to reveal any time-dependent disinfection performance when power per volume of treated solution is assessed. From the data available, we can observe that the input power alone does not necessarily correspond to improved efficiency after a certain point. The largest input powers are expected to have the best inactivation profile, which is true for the >30 W/mL results. The scattering of results below once again confirms that the sum of various treatment parameters is larger than the contribution of a single factor.

4. Conclusions and outlook

The available literature on the inactivation of viruses in water solution by cold gaseous plasma was presented, and the key results

summarized. Many authors varied the treatment time and reported the inactivation curves, which often followed the logarithmic decay of active viruses. The meta-analysis was performed in order to draw correlations between the processing parameters and the inactivation curves. We normalized the results reported by various authors to the D-value. The D-value does not depend much on the type of discharge used for sustaining gaseous plasma. Although details of particular experimental setups varied, the corona and dielectric barrier discharges were used by several authors, and many preferred the plasma jet. The meta-analysis reveals similar results for all these discharge configurations. The D-value does not depend on the initial viral load (at least in the range of probed values, i.e. between 3×10^3 and 1×10^{11} PFU/mL), because all authors reported values of a few seconds and only one article disclosed the D-value as large as one minute. The authors reported the discharge powers ranging from 6 to 435 W/mL. However, the meta-analysis did not reveal a correlation between the D-value and the discharge power normalized per volume of the treated liquid. A similar conclusion could be drawn for the D-value versus the discharge energy per liquid volume. The science of virus inactivation in water using gaseous plasma is still in its infancy and definitive correlations between the plasma parameters and inactivation efficiency are yet to be reported. Future research should focus on the detailed mechanisms of virus inactivation triggered by treatment with gaseous plasma. Several authors attributed the inactivation to reactive oxygen and nitrogen species formed in the liquid due to the plasma treatment. However, very few specified them, let alone measured their concentration versus the discharge parameters and the treatment time. Authors are also encouraged to provide more information pertaining to the major treatment parameters, which will enable more direct comparison between vastly different liquid treatment setups.

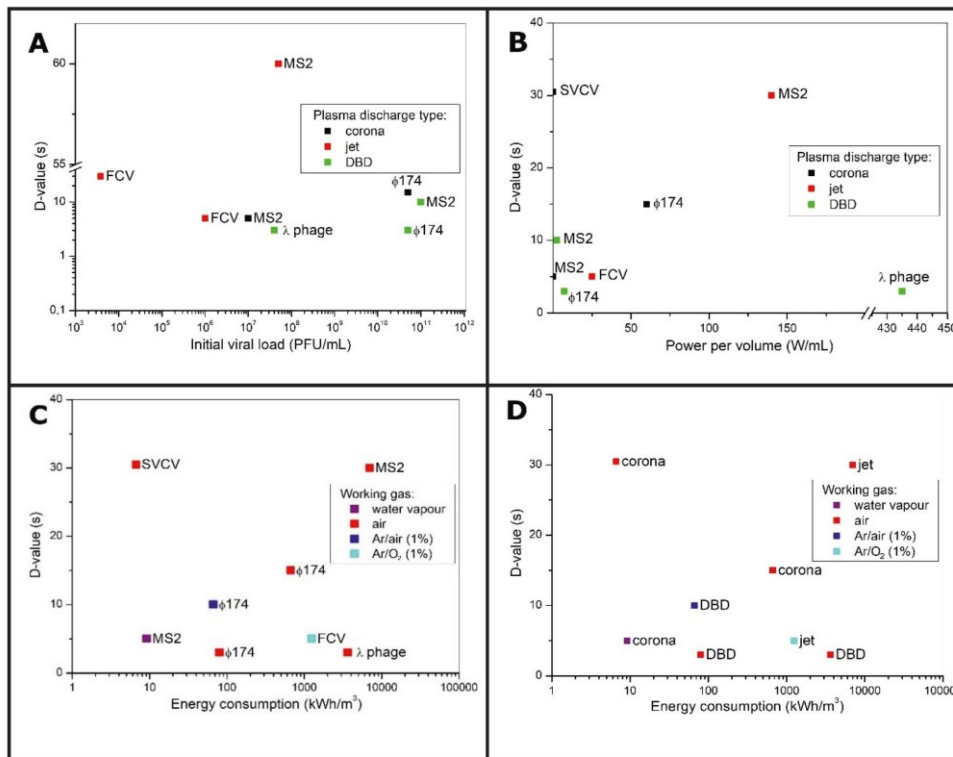


Fig. 3. Scatter plots of D-value in relation to the various inactivation factors: A) Initial viral load, B) Applied power per mL of solution, C) Energy consumption with inlaid virus type, D) Energy consumption with inlaid plasma discharge type.

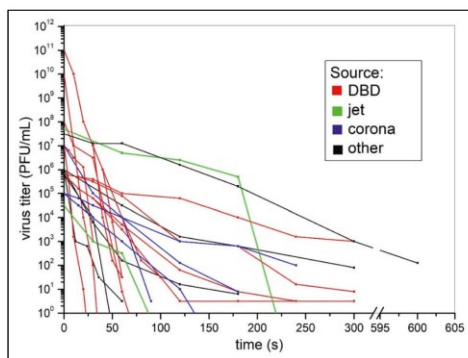


Fig. 4. Virus inactivation curves separated according to the plasma system.

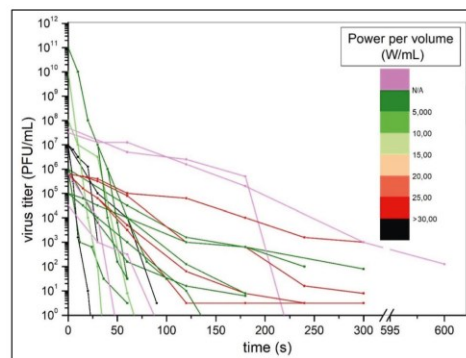


Fig. 5. Virus inactivation curves separated according to the amount of input power to treat an mL of virus solution.

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CRediT authorship contribution statement

Mark Zver: Conceptualization, Methodology, Data curation, Validation, Writing – original draft preparation, Investigation. Miran Mozetič: Conceptualization, Resources, Writing – review & editing. David Dobnik: Writing – review & editing. Rok Zaplotnik: Data curation, Writing – review & editing. Arjana Filipič: Writing – review & editing. Gregor Primc: Data curation, Resources, Conceptualization, Project administration, Funding acquisition, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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1.6 Hypotheses

Through a thorough literature review, we found no publications regarding low-pressure plasma as a V-UV radiation source for inactivating viruses in a solution. Nevertheless, a meta-analysis of available data of using (atmospheric pressure) gaseous plasma for inactivating viruses in solution revealed that: a) the variables affecting disinfection efficiency are numerous and physical (plasma discharge parameters), chemical (liquid composition, pH) and biological (e.g., virus type, initial concentration) in nature, and b) the reactive species produced by plasma are the primary inactivation factors, with the above-mentioned parameters affecting the primary chemical species produced.

To that end, we provide the following hypotheses:

1. Low-pressure plasma radiation can produce intense V-UV radiation and be applied to liquid water.
2. The produced V-UV radiation generates ROS ($\text{OH}^\bullet$, O_3 , H_2O_2) in a liquid.
3. ROS are produced in sufficient amounts to inactivate viruses in solution.
4. $\text{OH}^\bullet$ are the main inactivation factors of viruses.
5. Higher amounts of ROS are produced with greater V-UV intensity.

These hypotheses are investigated, and results are presented in the following original scientific papers. The investigations present a scientific as well as a technological challenge. Besides the above-stated objectives, we are also investigating what effect atmosphere composition, as well as the positioning of the liquid sample, has on ROS generation and subsequent virus inactivation. Furthermore, other types of water pollutants are also subjected to the treatment, and some initial energy efficiencies are presented and discussed.

Chapter 2

Low-Pressure Gaseous Plasma for Inactivating Viruses in Solution

To apply plasma-generated V-UV radiation to liquid samples, the two media need to be physically separated since they co-exist at two different pressures. Additionally, since V-UV is absorbed by regular borosilicate glass, the separating window has to be of great purity and of material that is at least partially transparent to the applied radiation. We chose a ceramic made of MgF_2 , which is transparent to V-UV radiation down to the wavelength of 112 nm [98], [190]. The plasma is generated at low pressure, and we evaluated the best working gas to produce the most radiation at a reasonable power input so as to not overheat the system. We tested several gases, such as ambient air, hydrogen, nitrogen, oxygen, carbon dioxide, argon, and helium (with radiation between 150–200 nm, which probably originates from impurities), among which hydrogen proved to be the best to fit into previously mentioned boundaries. Since the chamber containing the sample is at atmospheric pressure, this means that there is gas present above the treated liquid, which we could change. To allow for maximum V-UV transmission, N_2 was chosen, as it is mostly unaffected by radiation down to 150 nm [199]. On the other hand, since the goal is to produce ROS, and specifically $\text{OH}^\bullet$, we further evaluated the virus inactivation when either O_2 or air was introduced in the chamber since V-UV produced O_3 from oxygen, which can further react and produce $\text{OH}^\bullet$ [113]. Air was included in our investigation, as it would ease the practical application of such disinfection methods. Lastly, a series of different cover windows, separating the plasma radiation and liquid sample, were used, along with an $\text{OH}^\bullet$ scavenger, to elucidate the primary virus inactivation agent and understand how it is produced and reaches the sample. MS2 bacteriophage was chosen as a surrogate waterborne virus, with an F-specific *E. coli* bacteria as the virus' host for determining the MS2 infectivity post-treatment.

2.1 Capacitively coupled low-pressure plasma system

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Characterization of low-pressure gaseous plasma radiation and its usage for inactivation of waterborne viruses

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ABSTRACT

Water disinfection is a critical treatment step for removing harmful organisms from contaminated water sources. Vacuum ultra-violet (V-UV) radiation, generated by a low-pressure gaseous plasma discharge, consists of photons with high enough energy to break molecular bonds. In this work, we constructed and characterized a low-pressure capacitively coupled gaseous plasma and used it as a V-UV radiation source to inactivate MS2 bacteriophage, a surrogate for human enteric viruses. The treatment system allows for variation of gas composition inside the sample chamber and the modulation of V-UV radiation intensity. Both were used to separate the actual germicidal contribution of V-UV radiation from producing germicidal species by using virus inactivation to determine efficiency. OH^{*} radical production was determined through the terephthalic acid chemical probe, which showed that when air was present in the sample chamber, it resulted in the highest OH^{*} production and the best inactivation of MS2. Furthermore, we showcase that the OH^{*} production rate was wavelength dependent and that ozone, generated by plasma treatment in the gas phase, leads to hydroxy terephthalic acid degradation, allowing us to determine better the actual OH^{*} production rate with different treatment regimes. Lastly, by adding an OH^{*} scavenger to the liquid, we were able to elucidate it as the primary inactivation agent in this setup while also providing evidence of its production in the bulk liquid by the transport and subsequent decomposition of the longer-lasting ozone molecule. This study demonstrates, for the first time, the applicability of low-pressure plasma radiation as a water treatment method.

1. Introduction

In 2010, the United Nations General Assembly declared that access to safe and clean drinking water is a fundamental human right, equal to all other human rights [1]. Despite that, around 800 million people worldwide lacked access to safe drinking water sources in 2020 [2]. The term “safe” refers to water containing a sufficiently low quantity of physical, chemical, or microbial contaminants. The latter contaminants represent the most common health risks associated with the consumption of contaminated drinking water [3]. Viruses are among the smallest pathogenic microorganisms and often the most difficult to inactivate in water using common disinfection methods [4]. Human enteric viruses, including adenoviruses, astroviruses, enteroviruses, noroviruses, sapoviruses, hepatitis A and E, as well as rotaviruses, are frequently identified as the culprits behind waterborne disease outbreaks. Collectively,

they contribute to more than 1 million fatalities annually among children under the age of five [3,5]. Although they are obligatory parasites, they are particularly concerning because of their low infectious doses, ability to survive in the environment and difficulty of detection. [5]. Inactivation of viruses in water can be performed by various means, but at large scale, it is usually done by physical removal (membrane processes, coagulation, absorption), oxidation (chlorine, chlorine dioxide, ozone, Advanced Oxidation Processes (AOPs)), or ultraviolet radiation (UV) (for an overview see [6]). Although these techniques can remove or inactivate viruses in liquids, they have some drawbacks regarding efficiency, by-product formation, cost, and complexity of operations (for an overview, see [4]). AOPs are directed at generating large amounts of reactive oxygen species, primarily hydroxyl radical (OH^{*}), an even more potent oxidant than ozone, by chemical, photochemical, or electrochemical methods [7]. Despite their prohibitively high costs and

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complexity, AOPs have the additional benefit of being able to oxidize all types of organic contaminants, microbial or otherwise. This feature is making them increasingly relevant water treatment tools, especially as the amount of harmful substances in water systems increases [8].

Gaseous plasma, often called the fourth state of matter, is a form of ionized gas comprising a complex mixture of free electrons, ions, atoms, and molecules in their ground and excited states (some highly reactive) and electromagnetic radiation. We can differentiate plasmas based on temperature, pressure, working gas, etc. Nonequilibrium low-temperature (cold) plasma, which can be operated at a couple hundred degrees Celsius or down to room temperature, is preferred for treating biological samples. It can be generated at atmospheric or low pressures, however, only atmospheric pressure plasmas such as plasma jets [9–15], DBDs [16–19], corona discharges [20–22] and arc discharge [23] have been used to treat viruses in liquids, and their activity against a variety of different viruses has been demonstrated, including human [13,16,24–26], animal [12,13,22,27] and plant viruses [14,15,28], and bacteriophages [10,18–21,26]. Operational parameters such as process gas, input power, and distance from the sample have all been shown to contribute largely to disinfection efficiency. The general consensus is that the primary inactivation agents are reactive oxygen ($\text{O}^\bullet$, $\text{OH}^\bullet$, H_2O_2) and nitrogen species ($\text{ONOO}^\bullet$, NO , $\text{NO}_2^\bullet$), though their relative contribution to virus inactivation is still unclear, as it is mainly dependent on the treatment setup (for a detailed review see [29]).

To the best of our knowledge (also outlined in a recent review paper [29]), low-pressure plasma (LPP) discharge has not been used for treating viruses in a solution, which is to be expected since aqueous solutions evaporate in low-pressure environments, making such treatment setups very technically challenging. Nevertheless, LPP can be a source of highly energetic vacuum ultraviolet (V-UV) radiation, which occupies the 10–200 nm range of the electromagnetic spectrum. This radiation has enough energy to cause dissociation and excitation of molecules, in some instances leading to the production of the aforementioned $\text{OH}^\bullet$ radicals. The reactions involved in production of $\text{OH}^\bullet$ by plasma V-UV radiation of water are numerous and complex, with many interdependent conditions such as pH, temperature, liquid composition, V-UV radiation, etc. affecting the outcome. Broadly speaking, the initiation reactions can be distinguished by either direct V-UV interaction with water, resulting in homolysis or photolysis of water molecules upon impact (Equations 1 and 2) [30,31], or gas-phase interaction with gaseous oxygen molecules, producing excited atomic oxygen species (Equation 3), which can either interact with water molecules directly (Equation 4) or produce long-lasting secondary species (e.g., O_3 or H_2O_2), whose decomposition result in $\text{OH}^\bullet$ production (Equations 5–9). It has been proposed that $\text{OH}^\bullet$ in the bulk water solution primarily originates from ozone decomposition [32]. Readers are encouraged to look for more details in dedicated reviews of these processes [32–35]. This approach is similar to advanced oxidation processes, without the requirement for chemical additives such as hydrogen peroxide, and could, therefore, be a more sustainable approach for treating liquids.

Reaction	Equation	Ref.
$\text{H}_2\text{O} \xrightarrow{h\nu(185\text{nm})} \text{OH}^\bullet + \text{H}^\bullet$	(1)	[34]
$\text{H}_2\text{O} \xrightarrow{h\nu(185\text{nm})} \text{OH}^\bullet + \text{H}^\bullet + e_{\text{aq}}^-$	(2)	[34]
$\text{O}_2 \xrightarrow{h\nu(\leq 220)} 2\text{O}^\bullet$	(3)	[34]
$\text{O}^\bullet + \text{H}_2\text{O} \rightarrow 2\text{OH}^\bullet$	(4)	[36]
$\text{O}^\bullet + \text{O}_2 \rightarrow \text{O}_3$	(5)	[36]
$3\text{O}_3 + \text{H}_2\text{O} \xrightarrow{h\nu(220\text{nm})} 2\text{OH}^\bullet + 4\text{O}_2$	(6)	[35]
$\text{O}_3 + \text{HO}_2^\bullet \rightarrow \text{OH}^\bullet + \text{O}_2 + \text{O}_2$	(7)	[35]
$\text{O} + \text{H}_2\text{O}_2 \rightarrow \text{OH}^\bullet + \text{HO}_2^\bullet$	(8)	[36]
$\text{H}_2\text{O}_2 \xrightarrow{h\nu(220\text{nm})} 2\text{OH}^\bullet$	(9)	[34]

The main drawback of using $\text{OH}^\bullet$ radicals for water disinfection is their extremely short lifetime, which is in the order of 10 μs , requiring that

they be produced *in situ* [37]. Moreover, due to their low penetration depth in water (a few μm), their application to the liquid bulk via radiation is challenging [37]. V-UV radiation, produced by low-pressure mercury lamps or excimer lamps, has previously been shown to be efficient in degrading water contaminants such as antibiotics [38], herbicides [39], *Bacillus* spores [40], *Xanthomonas* sp. [41], natural organic matter [42], organic chemicals [43] and dyes [44], and there are even commercial devices for water purification (Wapure International GmbH, Germany). However, V-UV is efficiently absorbed by almost all substances, including air [33,38], therefore a specialized setup needed to be implemented to disinfect liquid water using LPP.

In this work, we present a proof-of-concept LPP setup for treating liquids at atmospheric pressure and use it to inactivate MS2 bacteriophages in water. This novel setup allows for the modulation of plasma working gas, radiation band wavelengths, and ambient gas in the sample chamber. Furthermore, by studying the degradation of a chemical probe, we were able to determine the $\text{OH}^\bullet$ production rate for various setups. MS2 bacteriophage is commonly used in water treatment trials as an enterovirus surrogate due to its similar size, shape, and genome composition, and it also serves as a bio dosimeter for evaluating UV radiation intensity [45].

2. Materials and methods

Since V-UV radiation is efficiently absorbed by almost everything, including oxygen molecules, the aim was to design a system where the path between the radiation source and the water surface would be composed of V-UV transparent constituents. First, to separate the liquid at atmospheric conditions and plasma radiation at low pressure, a unique sample chamber was constructed with a MgF_2 window, which is transparent to V-UV down to the wavelength of 120 nm (details below) [46]. Second, nitrogen gas is nearly transparent to V-UV radiation, at least down to wavelengths of 120 nm [47], which is why, in some instances, the sample chamber was filled with nitrogen gas before plasma treatment.

2.1. Low-pressure plasma irradiation device and sample chamber for treating liquids

The plasma device used in this study is a capacitively-coupled plasma (CCP) system (Fig. 1), composed of an upper electrode within a machinable ceramic (Macor®, Final Advanced Materials) holder and a lower stainless-steel mesh, encased in a Teflon holder, leaving the area below the mesh exposed for radiation treatment. The electrodes are connected to a pulsed DC power supply Plasmatec AP (J. Schneider Elektrotechnik, Germany), and the system is placed within a glass tube, from which the air was evacuated to a base pressure of 1 Pa with a two-stage rotary vane pump Trivac D40B (Leybold, Germany) as distinguished from Fig. 1. Pressure was measured with a capacitive absolute pressure transducer Baratron 722A (MKS Instruments, USA), and the process gas was leaked via precise needle valve EVN 116 (Pfeiffer Vacuum, Germany).

A special hermetically-tight sample chamber was constructed from aluminum to treat liquid samples at atmospheric pressure with V-UV, while the sample chamber itself was positioned in a low-pressure environment (Fig. 1) where the low-pressure gaseous plasma was generated. The sample chamber (Fig. 2a) consists of a holder and cover, which can be joined together to seal the content hermetically (air-tight). A glass petri dish ($\phi = 53$ mm) was placed inside the holder to hold the sample. On the top of the sample chamber, facing the plasma-sourced radiation, an MgF_2 window of thickness 3 mm and diameter 50 mm (Edmund Optics, USA) was positioned, allowing for the transmission of plasma radiation down to 120 nm [48]. Two valves placed on the sides of the sample chamber were used to exchange the ambient gas inside the sample chamber once it had been sealed. N_2 gas was used to allow V-UV radiation to reach the sample, while oxygen and air were used to provide

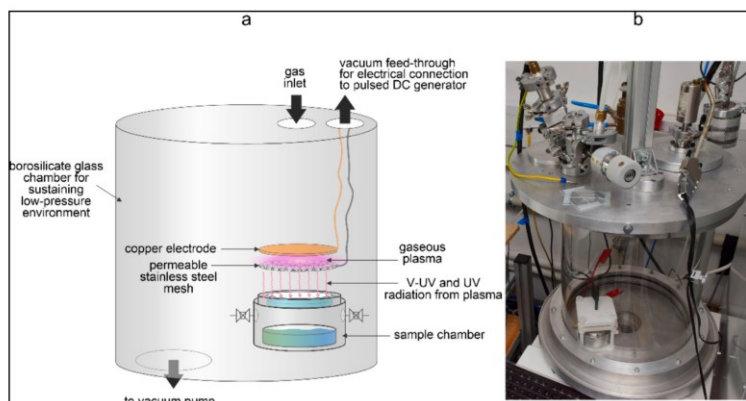


Fig. 1. a) A schematic of the capacitively coupled plasma discharge system embedded into a borosilicate chamber and b) its photograph.

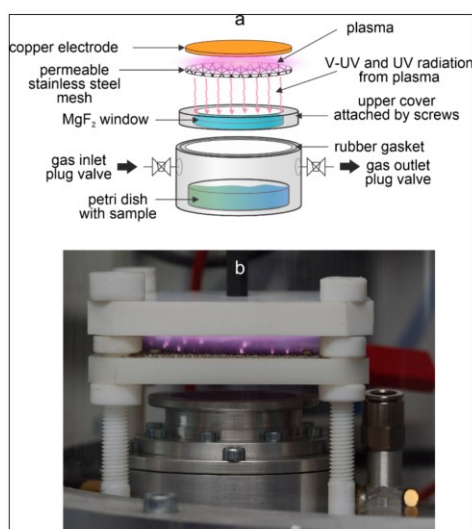


Fig. 2. a) Sample chamber, b) photograph of sample chamber with H_2 plasma during operation.

oxygen molecules in the sample chamber's headspace, which could be transformed into reactive oxygen species (ROS) by V-UV radiation [49,50]. The as-prepared sample chamber with liquid samples containing MS2 bacteriophage was then inserted into a specifically designed holder under the bottom electrode mesh to ensure an even radiation distribution (Fig. 2b).

2.2. V-UV spectroscopy

V-UV spectroscopy was used to measure emissions of the CCP plasma generated in various gasses (H_2 , He, N_2 , O_2 , air, Ar, and CO_2) at different discharge parameters in order to determine the optimal gas and

parameters for the treatment of viruses. Emission radiated from CCP plasmas was measured with VS7550 V-UV to NIR Mini-Spectrograph (Resonance LTD., Canada). It is a Czerny-Turner spectrometer that measures light intensity in the range from 104 to 1000 nm, where slit size, input $f\#$, order sorting filters, grating angle, and dark exposures can be controlled with VS7550 Spectrometer software. The spectrometer has a UHV MgF_2 window and a laminar flow purge system. In all measurements presented in this work, these parameters were used: a 100 μm slit, a 1200 L/mm grating, F3.2 aperture, -4° grating, and 1000 ms integration time. Some experiments required the V-UV intensity to be lowered, which we achieved by covering the MgF_2 window with either quartz or borosilicate glass (Fig. 3a). It can be seen that quartz glass weakly transmits only wavelengths above 150 nm, whereas borosilicate glass only transmits wavelengths above 300 nm.

2.3. Plasma treatment of viruses in water

To determine the virus inactivation potential of CCP radiation, before plasma treatment, the glass petri dish inside the sample chamber was filled with 2 mL of MilliQ water with bacteriophage MS2 (ATCC® 15597-B1™) at the initial concentration of $\sim 4.5 \log_{10}$ PFU/mL and sealed with the MgF_2 window cover. The water thickness was 1.1 mm. The gas inside the sample chamber was either air, N_2 , or O_2 . The latter two were introduced for some treatments by fitting a tube on the side valve and allowing the desired gases to flow through the sample chamber for 30 s. Nitrogen was chosen to enable the V-UV radiation to reach the sample freely, while O_2 was selected to evaluate the contribution of ozone produced during plasma irradiation. Air was used to assess the contribution of ozone and other RONS (reactive oxygen and nitrogen species) to inactivation efficiency, along with reducing the technical difficulty of the setup. After this time, the valves were closed on both sides, and the tube was subsequently removed. The sample chamber containing the prepared sample was then placed within the designated holder below the bottom mesh electrode of the plasma device. The distance between the electrodes was constantly kept at 8 mm, and the distance between the bottom electrode (radiation source) and the sample level was always 6 cm. The plasma was ignited using H_2 gas at 50 W and 500 Pa and was maintained at these conditions for 150 s. The control samples, 2 mL of MilliQ water with bacteriophage MS2 at the initial concentration of $\sim 4.5 \log_{10}$ PFU/mL, underwent the same procedure, except the plasma was not ignited. All treatment regimens were performed in triplicates, and virus inactivation was determined

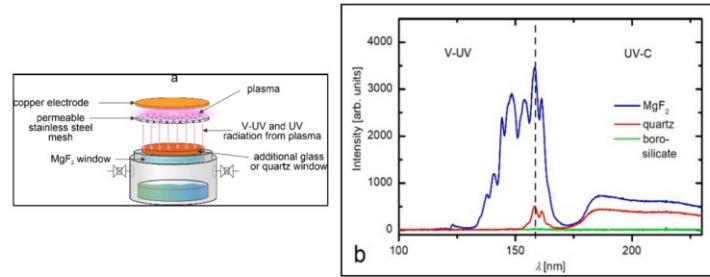


Fig. 3. a) Schematic representation of radiation modulation using different cover windows, b) radiation transmitted through three different windows for H₂ plasma at 500 Pa and 50 W.

using the spot-titre plaque assay, as described below.

2.4. Spot-titre plaque assay

For MS2 quantification, a modified version of the double-layer agar (DAL) method was used, previously described by Beck et al. [51]. All growth media referred to in this method consist of Trypticase Soy Broth (BD BBL™) medium, supplemented with 1.93 g/L MgCl₂·6H₂O (Sigma-Aldrich, USA) and 100 mg/L ampicillin sodium salt (Gibco™, USA), with varying concentrations (%) of bacteriological agar (Biolife, Agar Bios Special LL, USA). 100 μL of overnight *E. coli* CB390 culture, grown in a liquid medium, was inoculated into 5 mL of dissolved agar (0.7 %) medium, which was then mixed and poured over solid medium (1.5 %) plates. After 15 min, 10 μL droplets of virus sample dilutions were spotted on the plates (at least 4 per sample) and were placed lid-up in an incubator overnight at 37 °C. The number of plaques was counted the following day, and the number of infectious viruses was quantified by considering the sample dilutions using the following equations:

$$\text{Virus concentration} : \log(\bar{x} \times 10^{\log 4}) \quad (10)$$

$$\text{Logstdev} : \sqrt{\frac{1}{n} \times \sum (\log x_i - \log \bar{x})^2} \quad (11)$$

where $\bar{x}$ denotes the average of counted viruses, d the dilution step, n the number of measurements, and $\log 4$ represents the 1:4 dilution factor used for the experiment.

2.5. RONS measurement

The generation of OH* radicals in a solution was quantified by using a chemical probe, terephthalic acid (TA), which reacts with a single OH* radical in the solution, producing hydroxy terephthalic acid (HTA) [52]. HTA emits a fluorescent signal at 425 nm when excited at 318 nm. To prepare a 5 mM solution of TA (Sigma-Aldrich, USA), MilliQ water was preheated to 80 °C, and 1 M NaOH was used to adjust the pH to 11 before TA was added during mixing. The prepared solution was stored in the dark at room temperature until used. To measure the OH* radicals produced during plasma treatment, 2 mL of TA stock solution was placed in the glass petri dish inside the sample chamber and prepared in the same manner as for the virus inactivation experiments. The plasma treatment time was 0, 5, 15, 30, 60, 240, 420, 600, 900, or 1200 s. After treatment, the sample was transferred to a 2 mL Eppendorf tube, from which a 200 μL sample was placed in a 96-well plate (Brand, pureGrade™ S, Germany). Fluorescence measurements were performed with spectrometer Infinite 200 Pro (Tecan, Switzerland) with excitation and emission wavelengths of 318 nm and 425 nm, respectively, at a gain of 75. A calibration curve was prepared to quantify the amount of HTA

produced by making a dilution series of HTA (Sigma-Aldrich, USA) in MilliQ water. Prepared dilutions were transferred to the 96-well plate (Brand, pureGrade™ S, Germany), and fluorescence measurements were performed as described above. Additionally, to verify if the OH* radicals were responsible for the observed HTA signal, the 5 mM solution was supplemented with 200 mM D-mannitol (Sigma-Aldrich, USA) and treated with plasma with ambient air inside the sample chamber for 20 min.

Concentrations of long-lasting RONS (H₂O₂, O₃, and NO₂⁻) were measured after 10 min of water treatment with CCP plasma (500 Pa H₂, 50 W) in either air, nitrogen, or oxygen atmosphere, using the commercial water quality testing device, Palintest Photometer 7500BT (Palintest, UK).

2.6. HTA degradation

Since we observed a non-linear OH* production when oxygen was present inside the sample chamber, we assumed that there could be interference in measurement by the plasma-generated ozone. To verify this, we exposed HTA-containing water to either plasma treatment or merely exchanging the ambient gas with ozone. The rate of HTA degradation by CCP plasma was evaluated by separate experiments in which a 2 mL sample of HTA (274 μM) was subjected to CCP plasma for 0, 30, 120, 600, or 1200 s. Additionally, the HTA degradation rate was evaluated by filling the sample chamber with ozone, produced with an ozone generator OZ-AT10G (Ozonefac Ltd., China) at the rate of 40 mg/L for 30 s and incubated for the same duration. The amount of non-degraded HTA was quantified as described above.

2.7. Scavenger experiments

Scavenger experiments were performed using D-mannitol to determine the role of OH* radicals on virus inactivation. D-mannitol preferentially reacts with OH* radicals, with a reaction rate of $k = (1.8 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, thus preventing their interaction with other reactants [53].



Bacteriophage MS2 dilutions were prepared with either MilliQ water or MilliQ water supplemented with 200 mM D-mannitol and were treated for 150 s with CCP plasma, as described previously. The gas inside the sample chamber was ambient gas since it resulted in the highest production of OH* radicals during previous experiments. The control samples were treated in the same manner without igniting the plasma. All samples were treated in triplicates.

3. Results and discussion

3.1. Radiation characterization

Various gases and pressures were examined in our plasma system to determine the best discharge parameters and, consequently, the best plasma parameter (namely V-UV radiation intensity) to treat viruses in water. Fig. 4a and b present emission spectra of hydrogen, helium, nitrogen, oxygen, air, argon, and carbon dioxide plasma generated with the CCP system at the plasma power of 50 W at 500 Pa. As can be seen from these figures, plasma at the same pressure and discharge power emits completely different spectra when different gases are used. Here, we should stress again that only radiation emitted from the plasma was used for treatments, so only comparisons of intensities integrated over a range of wavelengths in the V-UV range are relevant. Therefore, the detailed description of the spectral features in these spectra is irrelevant for this study. However, more information on the spectral features of the V-UV generation in low-pressure plasmas can be found in a review article by Popovic et al. [54].

An energy of 5.17 eV is needed to dissociate water molecules to OH* and O [55]; therefore, only photons with a wavelength lower than 240 nm have enough energy to dissociate water. As quantum efficiency reaches 1 at 123.6 nm, lower wavelengths are better for the production of OH* [33] than higher wavelengths. Therefore, we compared only the emission of different plasmas in the V-UV region.

The spectra were integrated in the V-UV region at 50 W for the indicated gases at pressures from 100 to 900 Pa. In the case of carbon dioxide, the discharge could only be maintained up to 700 Pa, while in the case of helium, the discharge could be maintained from 250 Pa and

more. The integrated V-UV spectra versus pressure are presented in Fig. 4c. It can be seen that H₂ plasma is by far the best choice as a source of V-UV radiation at these discharge conditions. It emits over 2.5 times more V-UV radiation than the second-best, N₂ plasma. Similar findings, i.e., high V-UV intensity of plasma generated in these two gases, were also investigated in ICP plasma by Fantz et al. [56]. The pressure dependence shows a plateau of integrated V-UV intensity for almost all gases. For hydrogen plasma, there is even a weak maximum at approximately 500 Pa. The integrated V-UV intensities increase by increasing the discharge power, as shown in Fig. 4d. However, the plasma power of 50 W was chosen for all further experiments due to the overheating of the electrode holder system at higher powers, which would prevent performing experiments with longer treatment times. Our findings led to the use of hydrogen plasma generated with the plasma power of 50 W at 500 Pa for further research; hereafter, plasma ignited at these conditions is noted only as plasma.

3.2. Radiation and sample chamber gas-dependent virus inactivation

As presented in the experimental section, the plasma-emitted radiation was transmitted toward the sample through an MgF₂ window. To eliminate some of the V-UV radiation, the MgF₂ window was additionally covered with either quartz or regular borosilicate glass (Fig. 3a), which lowered the amount of V-UV radiation [46]. Fig. 3b presents spectra of the same hydrogen plasma measured under the MgF₂ window or a combination of MgF₂ windows and quartz or borosilicate glass. We used this setup to evaluate virus inactivation in solution by varying all three gases (N₂, O₂, and air) with all the radiation types (i.e., by using MgF₂, quartz, or borosilicate as cover windows). Based on previous

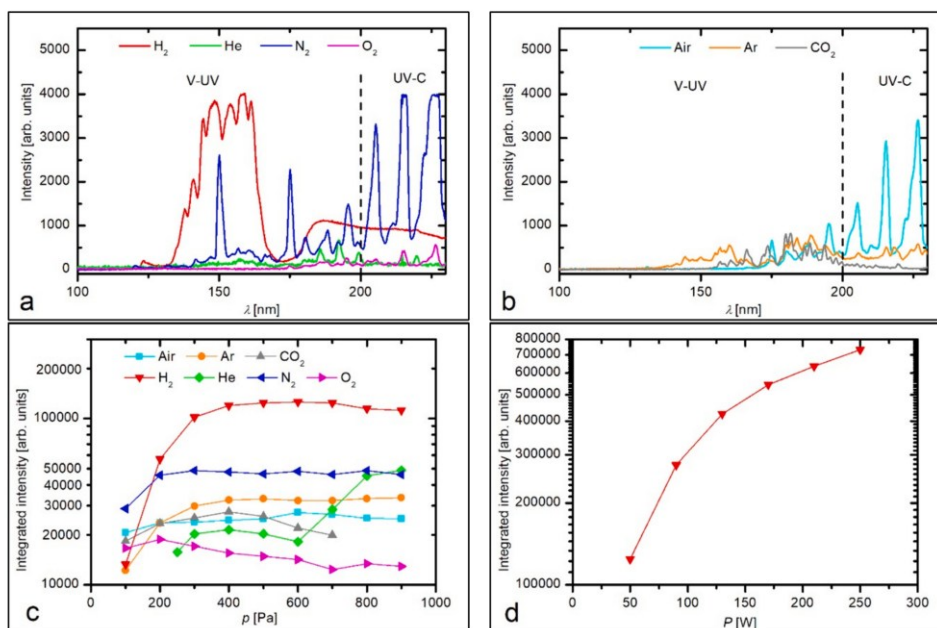


Fig. 4. Emission spectra of CCP plasmas generated at 500 Pa and 50 W for (a) hydrogen, helium, nitrogen, oxygen, and (b) air, argon, and carbon dioxide. Four reference wavelength ranges are marked: vacuum ultraviolet region (V-UV, 100–200 nm), ultraviolet C (UVC, 200–280 nm), ultraviolet B (UVB, 280–315 nm) and ultraviolet A region (UVA, 315–400 nm); (c) integrated intensity of the V-UV region of CCP plasmas of various gases generated at 50 W versus pressure, (d) V-UV radiation intensity at various plasma power inputs for hydrogen plasma at 500 Pa.

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trials, we chose the treatment time of 150 s to observe variations in viral inactivation at these conditions (Fig. 5). As can be seen, the viral inactivation was complete when oxygen or air were present in the sample chamber, covered with the MgF_2 window, whereas some viruses remained infectious with nitrogen inside the sample chamber. When covered with either quartz or borosilicate glass, none of the treatments produced complete viral inactivation, regardless of the gas inside the chamber. This demonstrates that V-UV radiation is necessary to cause bacteriophage inactivation. Additionally, it appears that ozone contributes significantly to virus inactivation, which is further supported by the fact that OH^* has a short lifetime and poor water penetration distance [37]. By contrast, when nitrogen was present in the sample chamber and irradiated with V-UV with an MgF_2 window, it only resulted in a $1 \log_{10}$ MS2 inactivation. Because no ROS is generated in the sample headspace, this inactivation results from direct inactivation by V-UV and RONS generated by V-UV interaction with water constituents. However, these results alone cannot determine whether virus inactivation is primarily caused by ozone or one of its decomposition products.

Although the literature on reactive oxygen species reacting with MS2 virus is scarce, some authors have evaluated the disinfection efficiency of microbes by plasma-activated water (PAW), compared to artificially created PAW just by mixing the measured concentrations of long-lasting RONS [19,57]. In all cases, the artificially created PAW performed worse than the direct plasma treatment, and by further experiments, it was confirmed that, indeed, the short-lived radicals were the main disinfection agents, although the primary disinfection agent varied based on the treatment setups.

3.3. RONS formation in V-UV irradiated water

Fluorescence measurements of HTA were performed to determine the amount of OH^* radicals produced in plasma-irradiated water. A calibration curve, presented in the Supplement, was prepared to convert fluorescence intensity into HTA concentration.

HTA concentration increased linearly with treatment time when the sample chamber was filled with pure nitrogen (Fig. 6a). The increase for the other two gases becomes linear after a prolonged time (approx. 100 s and 200 s for oxygen and nitrogen, respectively), but the increase was

much faster at a short treatment time. Air- and oxygen-filled sample chamber measurements could be fitted with a linear and exponential function:

$$y = a + b \times x - a \times e^{-\alpha x} \quad (13)$$

To demonstrate the specificity of OH^* -TA interaction, a point marked “mannitol + TA, air” is also displayed in Fig. 6a, where the TA solution was supplemented with 200 mM mannitol, and no HTA was detected after 20 min of treatment. This proves that, in our setup, only the OH^* -TA reaction can produce HTA, meaning that the density of OH^* radicals is directly linked to the HTA concentration. Therefore, all the discussions about HTA formation are also valid for OH^* formation. During the treatment, V-UV light dissociates water molecules into OH^* , which reacts with TA and forms HTA. However, besides the HTA formation, we assumed that a parallel HTA degradation process may also occur. In a nitrogen-filled sample chamber, HTA was not degraded (Fig. 6b), indicating that V-UV light is not the cause of HTA degradation. The plasma radiation is absorbed by oxygen, forming ozone when the sample chamber is filled with either air or pure oxygen [58]. One of the decomposition products of ozone is OH^* [32], which explains the greater OH^* concentration when either air or oxygen was present in the sample chamber. In a separate experiment, we proved that ozone was the main culprit for HTA degradation, presented in Fig. 6b. In this case, the HTA degradation follows an exponential decay, similar to the air- or oxygen-filled sample chamber irradiated with plasma radiation, with a slight difference: the HTA degradation of the ozone-filled sample chamber is even more pronounced because more ozone is produced with the ozone generator than in case of air- and oxygen-filled sample chamber. The exponential degradation of HTA due to ozone can explain the curves' shapes for HTA production presented in Fig. 6b. The convolution of a constant and an exponential decay is an integral of an exponential decay, i.e., a function (Equation (13)), which was used for fitting the HTA production curve in Fig. 6b. Furthermore, a deconvolution of HTA production and degradation curve fits gave a curve of HTA formation rate presented in Fig. 6c.

The deconvolution for a nitrogen-filled sample chamber gave a constant of 0.00434, which is exactly the slope of the linear curve presented in Fig. 6a. Because there is no HTA degradation in this case, the HTA is formed at a constant rate. There was a maximum HTA formation

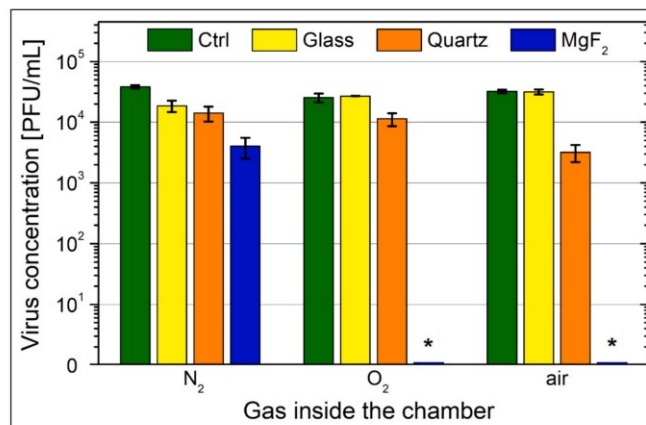


Fig. 5. Virus inactivation was achieved through 150-second treatment using H_2 plasma, ignited at 50 W and pressure of 500 Pa. Variations were made by altering the sample chamber gas and by covering the MgF_2 window with either quartz or borosilicate glass. The “*” symbol indicates that no plaques were present at those conditions.

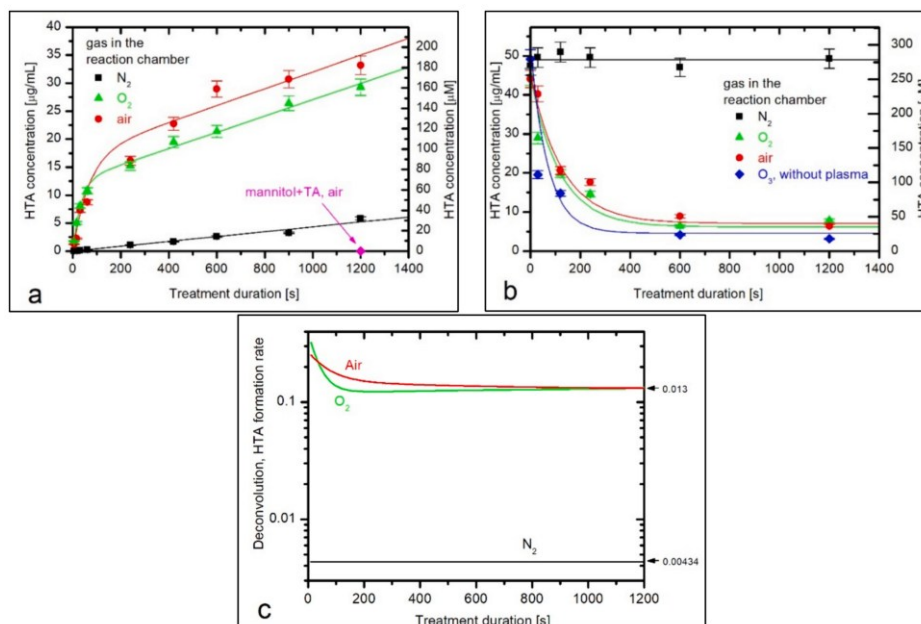


Fig. 6. a) Measurements of HTA production versus treatment time and its corresponding fits for different gases in the sample chamber, b) measurements of HTA degradation rate versus treatment time and its corresponding fits for different gases in the sample chamber, c) deconvolution of HTA production and degradation curves.

rate at the beginning of the treatment for the air- and pure-oxygen-filled sample chamber. In approximately 100 s, the HTA formation rate decreased to a constant value of 0.013. The HTA formation rate, with oxygen or air as the sample chamber gas, was much higher than when nitrogen was leaked in the sample chamber. Even though ozone caused HTA degradation, it decomposed to OH^{*}, which further promoted HTA formation [59]. Consequently, more HTA was produced when oxygen was present in the sample chamber.

Comparing disinfection results between different treatment regimes is often a futile task, as the treatment efficiencies vary based on initial virus concentration, water matrix, concentration of disinfectant, and contact time, to name just a few [29]. However, since MS2 is commonly used as a bio dosimeter of disinfection treatment efficiencies, there are some published results from which we can draw some conclusions as to whether the long-lasting RONS measured in our system may influence the MS2 inactivation. To that extent, we measured the concentrations of long-lasting RONS, specifically, H₂O₂, O₃, and NO₂⁻, using a commercial device for testing water quality parameters. The results are displayed in Table 1. Due to low quantities of analytes, only the results after 10 min of water treatment with CCP plasma (500 Pa H₂, 50 W) are displayed in either air, nitrogen, or oxygen atmosphere.

Table 1

Measured concentrations of H₂O₂, O₃, and NO₂⁻ in air, nitrogen, and oxygen atmosphere of the sample chamber.

Analyte	Air atmosphere	Nitrogen atmosphere	Oxygen atmosphere
H ₂ O ₂	0.1 mg/L	0.1 mg/L	0.04 mg/L
O ₃	0.04 mg/L	0.08 mg/L	0.01 mg/L
NO ₂	< 0 mg/L	5 mg/L	< 0 mg/L

Specifically, O₃ being a common stand-alone disinfectant for viruses in water, has a reported dose of 0.1–0.5 mg/L for 1 log₁₀ inactivation of MS2 in ultrapure water [60,61]. For H₂O₂, a 34 mg/L concentration was required to achieve a 1 log₁₀ inactivation of MS2, with a contact time of 30 min [60]. A combination of H₂O₂ and NO₂⁻ at concentrations of 17 mg/L and 3.45 mg/L, respectively, could only achieve 1 log₁₀ inactivation of MS2 [19]. Considering also results obtained in viral inactivation experiments (Fig. 5), where direct plasma treatment in nitrogen atmosphere inactivated only 1 log₁₀ of MS2, it is reasonable to assume that 5 mg/L of NO₂⁻ measured after treatment in nitrogen atmosphere would not have resulted in significant discrepancies compared to published results. Finally, the diminished inactivation of MS2 when mannitol, a scavenger of OH^{*}, was present in the treated liquid (Fig. 8) leads us to conclude that the measured long-lasting RONS concentrations are not high enough to impact the inactivation of MS2 bacteriophage significantly.

To further evaluate the impact of V-UV radiation and gas inside the sample chamber on OH^{*} radicals' production, we used the setup presented in Fig. 3a to treat 2 mL of TA solution for the maximum duration (20 min) and measured the produced HTA as previously described (Fig. 7). Using different cover windows (MgF₂, quartz, or borosilicate glass) between the V-UV radiation source and sample chamber reduces the amount of energetic photons interacting with molecules in the sample chamber, thus reducing the amount of generated OH^{*} radicals. As showcased in the OH^{*} quantification experiment (Fig. 2), nitrogen inside the sample chamber produces the lowest number of OH^{*} radicals. The absence of oxygen in the headspace hinders the formation of ROS, most of which have OH^{*} as a by-product when interacting with other molecules. When nitrogen is the only gas present in the headspace, not only is the production of reactive species heavily skewed towards RNS

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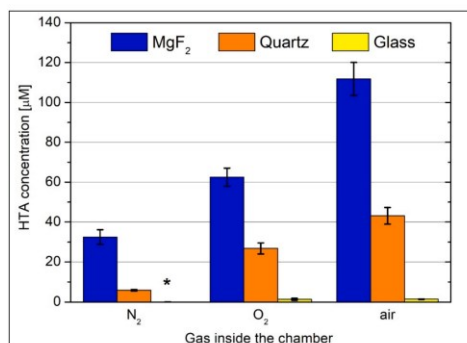


Fig. 7. OH* radicals' production after 20-min treatment for different cover windows and chamber gases. The "*" symbol indicates that the HTA concentration is too low to be visible in the graph.

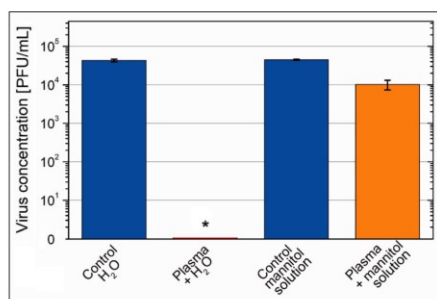


Fig. 8. Virus inactivation in water or mannitol solution with radiation from CCP hydrogen plasma at 500 Pa and plasma power of 50 W using an MgF₂ window. The "*" symbol indicates that no plaques were present at those conditions.

generation, but it also increases the interaction of RNS with OH* [58,62], which leaves less OH* to react with other molecules, in this case, terephthalic acid [63]. The majority of V-UV radiation is produced around the wavelength 150 nm (Fig. 4a), which is also the wavelength at which molecular oxygen absorbs the radiation optimally [64]. It is hypothesized that when pure oxygen is present in a sample chamber, the V-UV is entirely absorbed in the gas phase, which i) prevents V-UV from reaching the sample to cause water photolysis (Equations 1 and 2) and ii) reduces the radiation-initiated decomposition of O₃ and the production of OH* (Equation 6).

3.4. OH* inactivation of viruses in solution

To demonstrate the actual contribution of OH* radicals produced by V-UV radiation on viral inactivation, MilliQ water was supplemented with 200 mM of D-mannitol, which acts as a scavenger of OH* radicals, thus preventing their interaction with biological material. Fig. 8 shows virus concentrations for four samples: (i) only MilliQ water control, (ii) plasma-treated MilliQ water, (iii) D-mannitol in MilliQ water control, and plasma-treated D-mannitol in MilliQ water. As can be seen in Fig. 8, the presence of mannitol in the liquid significantly reduced the inactivation of viruses. The remaining observed antiviral activity could be attributed to other RONS generated during plasma irradiation, such as

¹O₂, O₃, H₂O₂, NO₂, or UV radiation [65], however this is outside the scope of this work. Although we show that O₃ is produced during V-UV irradiation of oxygen molecules above the water sample, the results in Fig. 8 demonstrate that the primary virus inactivation is carried out by OH*, which is generated *in situ* by the decomposition of O₃ transported into the bulk liquid.

Table 2 provides an overview of OH* radicals production within 20 min of treatment time and virus infectivity reduction after 150 s. In both cases, one can easily observe that air in the sample chamber resulted in the best OH* radicals production and virus inactivation. Therefore, air was the only gas evaluated for mannitol experiments. The % virus log reduction was calculated as follows:

$$\%red = \left(1 - 10^{\frac{B-A}{4}} \right) \times 100 \quad (14)$$

where A and B are the initial and recovered number of viruses, respectively.

4. Conclusion

We demonstrate that capacitively coupled H₂ plasma provides a good source of V-UV radiation. The radiation causes photolysis of water, leading to the production of reactive OH* radicals that can inactivate viruses in water. The amount of OH* produced can be quantified using the terephthalic acid chemical probe, although the production rate should be corrected for ozone-induced HTA degradation. The generated intensity of the V-UV radiation is sufficient to produce OH* radicals, and their production is significantly increased when oxygen-containing gas is present in the headspace of the sample chamber. This is due to ozone production, which decomposes in water, resulting in more OH* production. According to the United States Environmental Protection Agency, virus disinfection methods should reduce the concentration of viruses by 4 log₁₀ [66]. With our setup, we achieved it within 150 s of treatment. Finally, by adding a radical scavenger to the treated liquid, we demonstrate that the OH* radical, stemming from the produced ozone and transported to the bulk liquid, is indeed the primary inactivation agent of viruses in liquids. From a virus inactivation efficiency standpoint, these results are promising. Still, the large energy requirement for low-pressure plasma generation remains the main obstacle for low-pressure plasma to compete with current disinfection methods. Further research is also needed to evaluate the potential toxicity and formation of by-products, as pointed out by other authors [67]. In addition, the technique should be evaluated with other liquids, such as natural water or raw wastewater, to determine the inactivation capacity under more relevant conditions. Other plasma discharges, such as inductively coupled plasma, can produce even more intense V-UV radiation, but the energy requirements must be considered. Nevertheless, low-pressure plasma is an attractive advanced oxidation method for water treatment because it is environmentally friendly and has the potential to inactivate/degrade all organic contaminants.

CRediT authorship contribution statement

Mark Zver: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Rok Zaplotnik:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation. **Miran Mozetic:** Writing – original draft, Validation, Resources, Methodology. **Alenka Vesel:** Visualization, Investigation, Formal analysis, Data curation. **Arijana Filipić:** Writing – review & editing, Validation, Methodology. **David Dobnik:** Writing – review & editing, Supervision, Resources. **Gregor Primc:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Table 2

The amount of HTA produced in 20 min and virus infectivity reduction after 150 s, based on sample chamber gas and cover window type variations.

	HTA concentration [μM]				Virus reduction [%]			
	N ₂	O ₂	air	air + mannitol	N ₂	O ₂	air	air + mannitol
Glass	0.09 ± 0.012	1.38 ± 0.52	1.45 ± 0.12	N/A	51.46	0	1.38	N/A
Quartz	5.86 ± 0.36	26.80 ± 2.8	43.15 ± 4.2	N/A	63.23	55.47	90.04	N/A
MgF ₂	32.48 ± 3.7	62.50 ± 4.6	111.80 ± 8.3	0.23 ± 0.8	89.55	100	100	77.10

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Gregor Primc reports financial support was provided by Public Research Agency of the Republic of Slovenia. Mark Zver, Rok Zaplotnik, Miran Mozetic, Alenka Vesel, Arijana Filipi, David Dobnik reports financial support was provided by Public Research Agency of the Republic of Slovenia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seppur.2024.128691>.

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2.2 Inductively coupled low-pressure RF plasma system

Extending our understanding of the plasma disinfection system, we focused on implementing a more powerful radiofrequency inductively coupled plasma (RF ICP) discharge system, which produces a more intense V-UV emission in a more energy-efficient manner. Additionally, the system allows for a transition between E- and H-mode discharge regimes, the latter being more luminous than the former. These would be interesting to compare from an energy efficiency point of view. Since radiation intensity peak shifts with changing either gas pressure or discharge power, the system's pressure was kept constant when evaluating these two regimes. The mechanism of inactivation was assessed again through the use of a scavenger (D-mannitol), varying the chamber gas, implementing a radiation barrier and measuring the generated RONS. Furthermore, an experiment using plasma-treated (-activated) water was performed, where the MS2 bacteriophage was added after the water had been treated. Combining all obtained results, an energy efficiency calculation was performed to discern the optimal treatment regimen for practical applications. This was also compared to literature data. Lastly, commonly occurring water pollutants were subjected to the treatment system to demonstrate the disinfection effectiveness against a variety of contaminations.



Research article

Vacuum ultraviolet radiation from gaseous plasma for destruction of water contaminants

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ABSTRACT

Innovative technological solutions are needed for water decontamination to combat the diverse pollutants present in water systems, as no single optimal decontamination technique is appropriate for all circumstances. Vacuum-ultraviolet (V-UV) radiation is a source of energetic photons that break molecular bonds, producing a plethora of chemically reactive agents, most notably OH[•] radicals, which can cause the degradation of harmful pollutants. Low-pressure gaseous plasma is a good source of V-UV radiation; however, its application to liquid water poses challenges. We constructed an inductively coupled radiofrequency plasma to produce high-intensity V-UV radiation, which was applied to contaminated water via a V-UV-transparent window. Plasma was sustained in hydrogen, as it produces the highest V-UV intensity among all gases at selected discharge parameters. Bacteriophage MS2 was used as an indicator of microbial decontamination efficiency. Reactive oxygen and nitrogen species were measured at various treatment setups to quantify their effect on MS2 inactivation and elucidate the primary inactivation factors. At optimal conditions, the concentration of active virus dropped by 9 log₁₀ PFU/mL in 60 s. The optimal experimental setup was then used to treat bacteria *E. coli*, *S. aureus*, antibiotic tetracycline, and synthetic dye methylene blue as representatives of other types of pollutants, all of which were effectively removed/degraded within 10 min of treatment. A comparison of energy efficiency (EEO) to other disinfection setups was made for bacteriophage inactivation. With a low EEO value, we showcase the potential of this technique for further work in this field.

1. Introduction

Plasma is referred to as the fourth state of matter and is generated when sufficient energy is applied to a gas, causing ionisation of gas molecules, producing a highly reactive mixture of ions, electrons, radicals, atoms, and photons. Artificially generated gaseous plasma discharges are widely used in commercial applications such as semiconductor manufacturing (Kanarik, 2020), photovoltaics (Schlemm et al., 2005), surface treatment for adhesion improvement (Förster, 2022), thin layer deposition (Gosar et al., 2020), cutting or welding (Venkatramani, 2002), and much more. The high versatility of plasma treatment techniques stems from the various discharge setups (plasma jet, arc discharge, dielectric barrier discharge) and operating parameters (pressure, working gas, input power, frequency), which can produce a

variety of reactive particles for useful applications. In the case of radiofrequency (RF) plasma discharges, oscillation of free electrons is caused by the applied RF field, resulting in ionisation of atoms and subsequent gas breakdown or plasma discharge (Chabert et al., 2021). RF discharges can be capacitively or inductively coupled. The latter discharge generally displays significantly higher electron densities and is used in plasma propulsions, plasma lighting, or as a source of negative ions beams (Chabert et al., 2021). Inductively coupled plasmas are also characteristically able to transit between E-mode or H-mode, each with its unique plasma properties and discharge parameters. When plasma is sustained with a lower power input, the E-mode prevails, where the induced electric field is negligible compared to the voltage drop between the coil and the chamber wall (in our case, glass tube). In this case, plasma is characterised by low electron density, relatively high electron

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temperature, and weak light emission. When more power is absorbed, the plasma shifts into an inductive coupling regime, termed H-mode. The transition can be observed visually, as the H-mode discharge is significantly more luminous and has approximately two orders of magnitude higher electron density (Zaplotnik et al., 2011). To generate the highest possible amount of V-UV radiation for the purpose of water disinfection, the latter mode would be preferred, but one should also consider the required energy input as it relates to the operating costs of such treatments.

V-UV photons, with wavelengths between 100 and 200 nm, possess sufficient energy to break molecular bonds, which can be utilised for improving wettability (Vesel et al., 2023), sterilisation (D. Wang et al., 2010), gas and water treatment (M. C. Gonzalez and Braun, 1995; Ochiai et al., 2013). In water treatment applications, V-UV can directly interact and disrupt organic molecules, or it can act indirectly by generating $\text{OH}^\bullet$ (Eqs. (1) and (2)), which disrupt organic molecules via hydrogen abstraction, electrophilic addition, or electron transfer reactions (Eqs. (3)–(5)) (M. G. Gonzalez et al., 2004; Legrini et al., 1993). Water treatment processes utilising $\text{OH}^\bullet$ for pollutant degradation are called advanced oxidation processes (AOP).



In Equations (3)–(5), $\text{R}^\bullet$ and $\text{RX}^\bullet$ represent an organic substrate, $\text{R}^\bullet$ and $\text{RX}^\bullet$ an organic radical, while PhX and $\text{HOPhX}^\bullet$ present the nucleophile and the product of electrophilic addition, respectively. The amount and intensity of V-UV photons generated by a plasma discharge are dependent on many factors, such as working gas, operating pressures, input power etc. (Zaplotnik et al., 2011). V-UV radiation being efficiently absorbed by almost all material, including oxygen molecules in the air, means that specific setups using vacuum systems must be utilised to allow them to interact with the treated substance (D. Wang et al., 2010).

In the water purification application of V-UV radiation had been previously used for the degradation of pollutants such as natural organic matter (Buchanan et al., 2004), chemicals (W. Han et al., 2004; Imoberdorf and Mohseni, 2011; Krakkó et al., 2021, 2022; Ochiai et al., 2013), bacteria (Krakkó et al., 2021; Liu and Ogden, 2010; Ochiai et al., 2013), bacterial spores (Halfmann et al., 2007), and viruses (Moldgy et al., 2020; Ochiai et al., 2013). However, all authors used V-UV sources different from hydrogen plasma, such as medium-pressure mercury lamps, LED lights, or excimer lamps, with rather narrow waveband emissions, which can only interact with certain molecules. Some authors reported synergistic effects between different light sources (Matafonova and Batoev, 2022; Ramsay et al., 2000). Therefore, it would be reasonable to assume that a V-UV source emitting a richer radiation spectrum could more efficiently degrade a greater variety of contaminants from water.

In this article, we present a unique experimental setup with a radio-frequency inductively coupled plasma reactor (ICP), which can produce rather intense V-UV radiation in a range of wavelengths between about 150 and 200 nm (photon energy 6–8 eV) and applied it to liquid samples. We quantified the concentration of $\text{OH}^\bullet$ in polluted water and evaluated their contribution towards MS2 bacteriophage (MS2) inactivation by systematically excluding competing biocidal agents. The characterization of treatment was finalized by performing an energy efficiency comparison with similar methods.

Expanding further from bacteriophage treatment, Gram-negative bacteria *E. coli*, antibiotic tetracycline, and a massively produced synthetic dye methylene blue were also treated with the system. These were

selected as model representatives of waterborne bacterial disease outbreaks (M. N. V. Prasad and Grobelak, 2020), accumulating pharmaceutical contamination (Amangelsin et al., 2023), and harmful industrial runoff (Oladoye et al., 2022), respectively. By their rapid inactivation/degradation, we showcase the ability to treat different types of pollutants and provide a proof-of-concept to utilize such devices in practical applications.

2. Materials and methods

2.1. Inductively coupled low-pressure RF plasma system and sample treatment chamber

The plasma device used in this study is an inductively coupled plasma (ICP) system (Fig. 1) powered by an RF generator Cesar 1320



Fig. 1. A photograph of the plasma system with plasma in the H-mode.

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(Advanced Energy, USA), connected to a copper coil positioned around the centre of the discharge chamber. The input voltage and current waveforms were sine waves with a frequency of 13.56 MHz. For the parameters most frequently used in this research 50 W and 390 W of real power, the RMS voltages were 900 V_{RMS} and 1010 V_{RMS}, respectively. The discharge chamber consists of a borosilicate glass tube with a length and diameter of 30 and 4 cm, respectively. The tube was evacuated to a base pressure of 1 Pa with a two-stage rotary vane pump Trivac D40B (Leybold, Germany). Pressure was measured with a capacitive absolute pressure transducer Baratron 722A (MKS Instruments, USA), and the H₂ gas was leaked into the tube via mass flow controller Aera MFC FC-7700 (Advanced Energy, USA) (Fig. 2). Here, it should be stressed that the plasma generated in this system was used only as a source of radiation, especially V-UV radiation, and was not in contact with the polluted water. To apply V-UV radiation to liquid samples in atmospheric conditions, a special chamber was constructed, as described previously (Zver et al., 2025). Briefly, an aluminium chamber with a radiation-facing MgF₂ window, which is transparent to wavelengths down to about 110 nm, was utilised to contain a hermetically sealed glass petri dish, which contained the liquid sample at atmospheric pressure. The chamber also allows for the exchange of atmospheric gases via the two valves positioned on either side of the chamber and the use of an optional, non-hermetic radiation barrier. The barrier is used to obscure the treated sample from direct V-UV radiation. The radiation can produce reactive gaseous species above the barrier (headspace), which can subsequently diffuse to the liquid surface by passing through the gaps between the barrier and the sample chamber. Nitrogen does not absorb V-UV radiation down to around 110 nm (Tigrine et al., 2016), while oxygen present in the air does so efficiently (Watanabe et al., 1953), producing ozone in the process, which could aid in the disinfection process, while also enabling easier operation of the system from a practical application viewpoint. When required, the gas inside the chamber was exchanged with N₂ by fitting a tube on the side valve and allowing the desired gases to flow through the chamber for 30 s, after which the valves were closed on both sides, and the tube was subsequently removed. The chamber containing the prepared sample was then attached to the bottom of the ICP plasma system, and the treatment would proceed for up to 10 min. In some cases, the glass petri dish was non-hermetically covered with a radiation barrier (black-painted aluminium sheet), preventing direct

V-UV interaction with the liquid sample, while still allowing the reactive species to reach the liquid sample surface. In other cases, 200 mM of D-mannitol was added to act as a scavenger for OH[•], excluding them from interacting with contaminants. The distance between the MgF₂ window and the liquid surface was always 3 cm.

2.2. V-UV spectrometry

Emission radiated from ICP plasma was measured with VS7550 V-UV to NIR Mini-Spectrograph (Resonance Ltd., Canada). The device has a UHV MgF₂ window and a laminar flow purge system, through which a steady flow of N₂ gas was established to prevent radiation absorption by the ambient atmosphere. The spectrometer has a Czerny-Turner configuration, where slit size, input aperture (F#), order sorting filters, grating angle, and dark exposures can be controlled with VS7550 Spectrometer software. It allows light intensity measurements ranging from 104 to 1000 nm. In all measurements presented in this work, a 100 μm slit, a 1200 L/mm grating, F8.4 aperture, a -4° grating angle, and 6–500 ms integration time were used. The distance between the plasma-generation area and the V-UV spectrometer was always 30 cm. Based on the spectroscopic measurements of V-UV radiation (Fig. 4), the optimal V-UV radiation was emitted in ICP plasma with H₂ gas at approximately 20 Pa either in E-mode or H-mode, at a real power input of 50 W or 390 W, respectively.

2.3. Virus inactivation experiments and spot-titre plaque assay

For treating viruses in solution, 2 mL of Milli-Q (MQ) water with bacteriophage MS2 was placed in the glass petri dish (water layer thickness of 1.1 mm) inside the chamber with the initial concentration of 9 log₁₀ PFU/mL and sealed with the MgF₂ window cover. The contribution to virus inactivation from V-UV radiation, ozone, and OH[•] in solution were systematically excluded by using a non-hermetic radiation barrier, nitrogen atmosphere, and adding 200 mM of D-mannitol to the solution, respectively. The treatment conditions were as follows: plasma in either E-mode (50 W) or H-mode (390 W), plasma in H-mode + radiation barrier (B), and plasma in H-mode + 200 mM mannitol (M).

For MS2 quantification, a modified version of the double-layer agar (DAL) method, previously described by Beck et al. (N. K. Beck et al., 2009), was used. All growth media referred to in this method consist of Trypticase Soy Broth (BD BBL™) medium, supplemented with 1.93 g/L MgCl₂ × 6H₂O (Sigma-Aldrich, USA) and 100 mg/L ampicillin sodium salt (Gibco™, USA), with varying concentrations (%) of bacteriological agar (Biolife, Agar Bios Special LL, USA). An amount of 100 μL of overnight *E. coli* CB390 culture, grown in a liquid medium, was inoculated into 5 mL of dissolved agar (0.7 %) medium, which was then mixed and poured over solid medium (1.5 %) plates. After 15 min, 10-μL droplets of virus sample dilutions were spotted on the plates (at least 4 per sample) that were placed lid-up in an incubator overnight at 37 °C. The number of plaques was counted the following day, and the number of infectious viruses was quantified by considering the sample dilutions using the following equations:

$$\text{Virus concentration} : \log(\bar{x} \times 10^{d \times \log 4}) \quad (6)$$

$$\text{Log stdev} : \sqrt{\frac{1}{n} \times \sum (\log x_i - \log \bar{x})^2} \quad (7)$$

where $\bar{x}$ denotes the average of counted plaques, d the dilution step, n the number of measurements, and $\log 4$ represents the 4x dilution factor used for the experiment. All treatments were performed in triplicates. The control samples underwent the same procedure without the ignition of plasma.

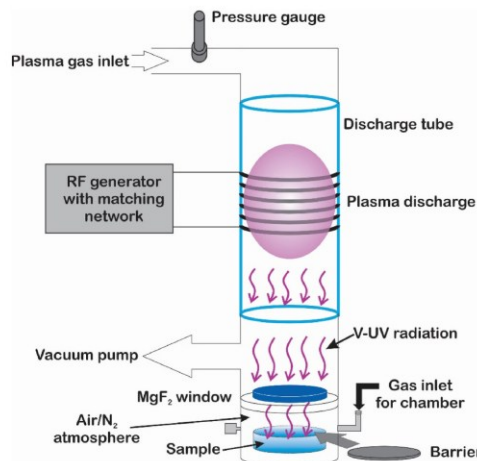


Fig. 2. Schematic presentation of the plasma treatment of samples.

2.4. Plasma-activated water MS2 disinfection

To evaluate the contribution of long-lived reactive species (H_2O_2 , O_3 , NO_2^-) to MS2 inactivation, an experiment was conducted by using plasma-activated (treated) water (PAW). PAW was prepared in the same manner as for the virus-inactivation experiments explained previously, without adding MS2 into the water. The water samples were all treated for the maximum duration of 10 min, after which 1 mL of the treated PAW was placed into a 1.5 mL tube, and MS2 bacteriophage was added to it at the initial concentration of $9 \log_{10}$ PFU/mL. After a 10-min incubation period, a dilution series was made, and the samples were spotted on agar plates, which was the same as described above. A one-way ANOVA analysis with a confidence interval of 0.05 was performed to reveal statistically different treatment outcomes. The liquids were treated with V-UV radiation in either nitrogen or air atmosphere inside the sample chamber. The treatment conditions were as follows: plasma in either E-mode (50 W) or H-mode (390 W), plasma in H-mode + radiation barrier (B), and plasma in H-mode + 200 mM mannitol (M). The quantity of long-lived reactive species (H_2O_2 , O_3) was measured with a commercial water quality testing device, Palintest Photometer 7500BT (Palintest, UK), using the appropriate testing reagents. The temperature and pH of the liquid remained consistent throughout the treatment procedure (25 °C, pH = 7), as measured by Palintest Multi-parameter pocket sensor (Palintest, UK).

2.5. $\text{OH}^\bullet$ radical quantification

A terephthalic acid (TA) assay was used to determine the amount of $\text{OH}^\bullet$ in water solution. TA reacts with $\text{OH}^\bullet$ in solution, producing 2-hydroxyterephthalic acid (HTA), which emits a fluorescent signal at 425 nm when excited with monochromatic laser radiation at 318 nm. A 5 mM solution of TA (Sigma-Aldrich, USA) was prepared by preheating MQ water to 80 °C and using 1 M NaOH to adjust the pH to 11 before adding TA during mixing. Additionally, to study the degradation profile of generated HTA, a 0.5 mM (100 mg/L) solution of HTA (Merck KGaA, Germany) was prepared by dilution with sterilised MQ water, and treating 2 mL samples, quantifying remaining HTA as described above. A glass petri dish containing 2-mL samples was placed inside the sample treatment chamber for treatments with V-UV radiation from plasma. A radiation barrier, N_2 gas, or mannitol was inserted when required and irradiated with plasma as described above for the indicated amount of time. After treatment, a 200 μL sample was transferred to a 96-well plate (Brand, pureGrade™ S, Germany), and fluorescence measurements were performed with a spectrometer Infinite 200 Pro (Tecan, Switzerland) at 75 gain. During the treatment of the TA solution, the HTA is formed, but at the same time it is also degraded. Therefore, in order to determine the actual production rate of the HTA and, consequently, of the $\text{OH}^\bullet$, a deconvolution had to be made between the measured HTA and HTA degradation curve (Zver et al., 2025). To do so, only HTA was treated to determine the degradation curve. The deconvoluted curve was then convoluted back with a constant, i.e., without a degradation. Because not every $\text{OH}^\bullet$ is used to form one HTA, the production rate of HTA is the lower value of the $\text{OH}^\bullet$ production rate.

2.6. Energy efficiency calculations

Besides disinfection effectiveness and consumer and environmental safety, a water treatment technique must also be cost-effective for implementation in practice (Bolton et al., 2001). Based on its working principle, plasma-generated V-UV irradiation is classified as an advanced oxidation process (AOP), relying on *in situ* generation of reactive oxygen and nitrogen species (RONS) to achieve disinfection of polluted water. Since electric energy represents the majority of operating costs for most AOPs, a standard approach to estimate the price-performance of AOPs is to calculate the energy efficiency per order (EEO, Equation (8)), defined as the energy, expressed in kWh, required

to reduce the concentration of a contaminant by one order of magnitude ($1 \log_{10}$) in a unit of volume (1 m^3).

$$\text{EEO} \left(\frac{\text{kWh}}{\text{m}^3 \cdot \text{order}} \right) = \frac{\text{Power input (kW)} \times \text{Treatment time (h)}}{\text{Volume (m}^3\text{)} \times 1 \log_{10}} \quad (8)$$

2.7. Pollutant degradation experiments

To evaluate the performance of the system towards other types of pollutants, the optimal treatment parameters, namely H-mode hydrogen plasma with air as the atmosphere in the sample chamber, were used to treat Gram-negative (G^-) *Escherichia coli* (EC, NCTC 12923, bioMérieux S.A., France) and Gram-positive (G^+) *Staphylococcus aureus* (SA, NCTC 10788, bioMérieux S.A., France) bacteria, as well as the antibiotic tetracycline (TETR, Sigma-Aldrich, 98.0–102.0% HPLC, USA) and an organic dye methylene blue (MB, Thermo Fisher Scientific, USA), for up to 10 min.

E. coli and *S. aureus* cultures were inoculated into liquid TSB medium and incubated in a shaker (200 rpm, 37 °C, 24h). The bacteria were harvested by three progressions of centrifugation (5 min, 5000 rpm) and washing with MQ water before being serially diluted, spread onto TSB plates, and incubated at 37 °C overnight. The following day, the bacteria were counted to prepare a sample solution of $\sim 8 \log_{10}$ bacteria for experiments with plasma-emitted V-UV radiation. The same counting method was utilised to quantify bacterial survival by plating 100 μL of sample dilutions on TSB agar plates, incubating them overnight at 37 °C, and subsequently enumerating the colonies.

For TETR degradation experiments, a 10 mg/L solution was prepared by dissolving TETR powder in MQ water. The solution was stirred overnight by a magnetic stirrer. As described above, 2-mL samples were placed in the glass petri dish and treated with plasma, followed by adding 100 μL of methanol to prevent further reactions. The samples were analysed by Liquid Chromatography (LC) using a Waters Acquity UPLC I-Class Plus System equipped with a photodiode array detector (PDA) at 25 °C. The reverse-phase column used was a Waters Acquity UPLC® BEH C18 (1.7 μm) 2.1 $\times$ 150 mm column. The equipment was operated in continuous mode using as mobile phase 70% of water/methanol/0.1 M oxalic acid with ratios of 80:10:10 (v/v) and 30% acetonitrile. The flow rate was 0.3 mL/min. Samples of 10 μL were injected and the retention time was 1.5 min. The limits of quantification and detection were 0.034 and 0.010 mg/L of TETR, respectively, at 357 nm.

MB was dissolved in MQ water at a concentration of 100 mg/L, treated with plasma as described above, and the degradation was measured by absorbance reduction at 664 nm.

For all pollutant treatments, 2-mL samples were placed in the glass petri dish and treated with plasma as described above. All experiments were conducted in triplicates for the indicated amount of time, and the average concentration and standard deviation were calculated.

3. Results and discussion

3.1. V-UV intensity

In our previous work, we confirmed that H_2 gas emits the most intense V-UV radiation (Zver et al., 2025). A typical spectrum of ICP hydrogen plasma used in this study is presented in Fig. 3. Intense radiation can be seen in wavelengths below 300 nm. The continuum above 190 nm arises from continuum a–b molecular transitions. However, the most intense radiation is due to the Lyman (B–X) and Werner (C–X) molecular band transitions. In order to compare radiation intensity at different discharge parameters, we integrated emitted light intensity in the V-UV region (from 100 to 200 nm).

Since plasma gas pressure and input power are the primary factors affecting plasma discharge characteristics, we modulated these two parameters to obtain the optimal conditions for highly intense V-UV

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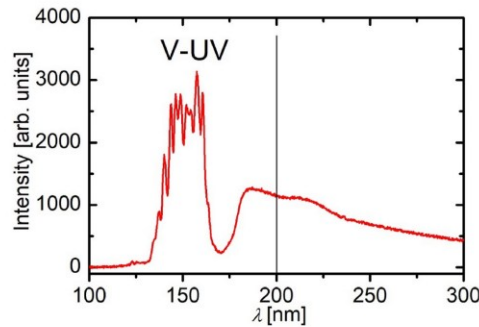
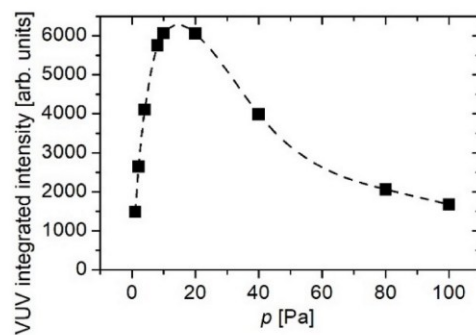
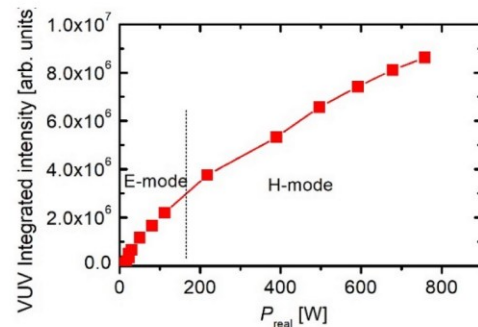


Fig. 3. A typical spectrum of ICP hydrogen plasma.

Fig. 4. Pressure-dependent V-UV intensity of H₂ plasma at 50 W real power.

radiation. At least 50 W was required to obtain a stable E-mode plasma, which we used as our starting point. We then incrementally increased the H₂ gas flow (pressure) and kept the pumping speed constant to identify the maximum V-UV intensity. As can be seen in Fig. 4, the maximum V-UV radiation from plasma generated at the absorbed real power of 50 W in an ICP plasma system was achieved at pressures around 20 Pa, which is explained by two contradictory effects. At low pressures, the electron density increases with increasing pressure because of the reduced loss of the charged particles at walls due to the increasing collision frequency and, thus, decreasing diffusion. The increasing electron density causes more excitations and, thus, more intensive radiation. At elevated pressure, on the other hand, the electron temperature starts decreasing with increasing pressure because of numerous collisions; hence, the lower electron temperature causes inefficient ionisation and excitation of radiative states. There are optimal conditions in between, evidently at the pressure of 20 Pa when using our experimental setup. When additional power is supplied to the system, the radiation increases with increasing power, and eventually, plasma discharge transforms from E-mode (predominant capacitive coupling) to H-mode (predominant inductive coupling), which characteristically produces more intense radiation. This can be seen in Fig. 5, where integrated V-UV radiation intensity is displayed versus the real RF power. Here, 'real RF power' denotes reflected power subtracted from forwarded power, where both values are measured by the RF generator.

For further treatments and experiments, 20 Pa and 50 W and 390 W of real RF power for E- and H-mode were used, because these were the

Fig. 5. Integrated V-UV radiation intensity of 20 Pa H₂ plasma versus the real power.

lowest powers where each mode was stable.

3.2. Virus inactivation

A comparison between E- and H-mode hydrogen plasma was performed to establish the power-dependent virus inactivation efficiency. These were performed with either air or N₂ inside the sample chamber to elucidate the contribution of direct V-UV water treatment (N₂ atmosphere) while excluding the contribution of any reactive oxygen species (air atmosphere) generated in the headspace of the sample treatment chamber.

As can be seen in Fig. 6, the presence of oxygen inside the sample chamber (because nitrogen was replaced with air) improves the overall bacteriophage inactivation efficiency when illuminated with V-UV radiation arising from hydrogen plasma in both E- and H-modes. Interestingly, hydrogen plasma in the E-mode with air atmosphere (black circles in Fig. 6) in the sample treatment chamber achieved total inactivation slightly faster than hydrogen plasma sustained in the H-mode in the nitrogen atmosphere (red squares in Fig. 6).

The MS2 inactivation shown in Fig. 6 could be explained by different efficiencies in OH• production in water when using nitrogen or air atmospheres in the sample treatment chamber. The contribution of various reactants was evaluated by systematically excluding the main inactivation agents, which are V-UV radiation, reactive oxygen species,

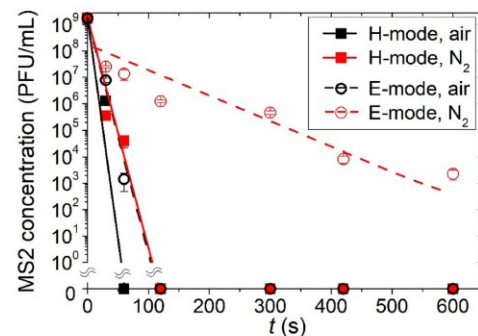


Fig. 6. MS2 bacteriophage inactivation by hydrogen ICP plasma ignited in H-mode (squares) and E-mode (circles) in either air (black colour) or nitrogen (red colour) atmosphere.

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and $\text{OH}^\bullet$, by utilising a non-hermetic radiation barrier, nitrogen atmosphere, and a mannitol solution, respectively. When air is exchanged for nitrogen (Fig. 7) in the sample chamber, there is a clear drop in inactivation efficiency, confirming the role of reactive oxygen species in virus inactivation. The virus inactivation was extremely slow or practically non-existent when a barrier was inserted into the nitrogen-containing chamber (Fig. 7 (a)). The radiation barrier was used to protect the water from direct V-UV radiation from plasma. The reactive species formed by plasma radiation in the sample's treatment chamber headspace were able to reach the liquid surface by passing through the sides of the barrier. Such an experiment removed the direct effect of V-UV plasma radiation on the water sample. When mannitol was added to the solution, the inactivation rate was reduced, but total inactivation was still achieved after 5 min of treatment, meaning that $\text{OH}^\bullet$ is not the only reactant responsible for virus inactivation.

However, treating the samples in an air atmosphere shows that other agents (not only $\text{OH}^\bullet$) are responsible for bacteriophage inactivation (Fig. 7 (b)). Most notable is the use of a V-UV barrier, where total inactivation was achieved after 7 min of treatment, even when the sample was completely obscured from the V-UV radiation. The addition of mannitol provided similar but slightly better results when compared to the nitrogen atmosphere in the sample's treatment chamber headspace. This indicates that when V-UV interacts with oxygen molecules in the sample's treatment chamber headspace, long-lasting reactive oxygen species are produced that can migrate to and react with the liquid, achieving virus inactivation without requiring direct irradiation of the polluted water with V-UV radiation. Therefore, oxygen inside the treatment chamber is beneficial to the overall plasma V-UV treatment performance.

3.3. PAW virus inactivation

Antimicrobial long-lived RONS, mainly H_2O_2 and O_3 (M. C. Gonzalez and Braun, 1995), are produced and dissolved in water during plasma V-UV irradiation. Their contribution to MS2 inactivation was evaluated by exposing MS2 bacteriophage to PAW. Both water treatment and virus exposure were set to 10 min. Additionally, the aforementioned RONS were measured using a photometric test.

For all the treatment conditions presented in Fig. 8, the measured NO_2 quantities were below the method's detection limit (>1.0 mg/L). For O_3 and H_2O_2 measurements, the only standouts were PAW treated in H-mode in an air atmosphere, which produced 1.6 ± 0.2 mg/L H_2O_2 , and PAW treated in H-mode with the addition of mannitol (M) with 2.27 ± 0.7 mg/L O_3 detected. All other samples contained less than 0.3 mg/L for both disinfectants. Mannitol is a scavenger of $\text{OH}^\bullet$, which is involved in upstream and downstream reactions for generating RONS (Goldstein and Czapski, 1984). Thus, providing a scavenger that reacts with $\text{OH}^\bullet$

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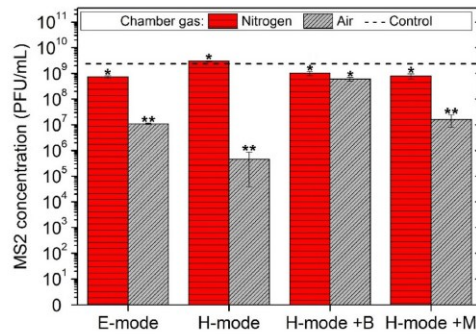


Fig. 8. MS2 bacteriophage exposed to plasma-activated water (produced by a 10-min plasma treatment) for 10 min; * and ** indicate statistically significant differences between treatments ($p < 0.05$).

and reduces the pool of reactants, which is expected to achieve less virus inactivation. Moreover, excluding the $\text{OH}^\bullet$ from the reaction mixture significantly improved the production of O_3 , indirectly aiding the inactivation efficiency.

Fig. 8 shows that only about $3 \log_{10}$ inactivation was achieved by treating MS2-polluted water with PAW prepared in H-mode plasma with air in the sample's treatment chamber headspace. An expected decrease in viral inactivation was observed at lower powers used to sustain hydrogen plasma (E-mode). When nitrogen was present in the sample's treatment chamber headspace, fewer reactive oxygen species were produced and dissolved in PAW, resulting in no inactivation of MS2. Since $\text{OH}^\bullet$ reacts with and degrades long-lived H_2O_2 and O_3 (Legrini et al., 1993; Reisz et al., 2003), adding mannitol to the water sample may raise their concentration in PAW. On the other hand, when a plasma radiation barrier was implemented to prevent direct V-UV interaction with water, no significant virus inactivation was observed, even with the presence of air in the chamber. This indicates that V-UV radiation increases the production of long-lived RONS in the liquid and can be achieved by a complex system of intermediate reactions between short- and long-lived RONS, indirectly affecting the production and transport of the latter to the liquid phase, reviewed by several authors (Anderson et al., 2016; Hoigné and Bader, 1976; Jiang et al., 2014; Legrini et al., 1993; Zhou et al., 2020).

Z.-C. Yang et al., 2024 reported that an O_3 concentration of 4.8 mg/L was required to inactivate $4 \log_{10}$ of MS2 in deionised water, while a H_2O_2 concentration of 850 mg/L was required for the same effect (Z.-C.

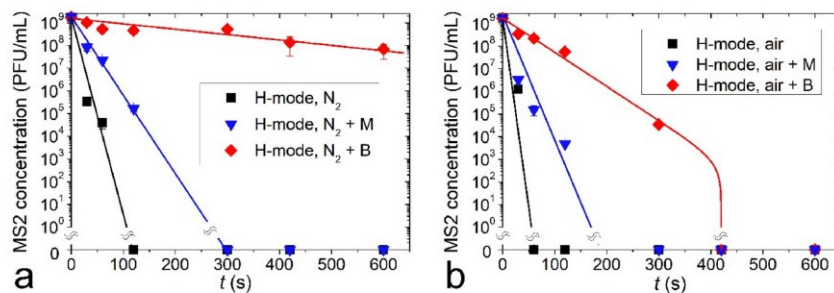


Fig. 7. MS2 bacteriophage inactivation in nitrogen (a) and air (b) atmosphere. B in the legend denotes that a radiation barrier was used, while M denotes that mannitol was used as an $\text{OH}^\bullet$ scavenger in the treated sample.

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Yang et al., 2024). A concentration of 0.1 mg/L O_3 caused an MS2 reduction by 1 $\log_{10}$ in 3 min exposure time, and the inactivation did not seem to continue after that time (J. Fang et al., 2014). However, the inactivation was markedly improved by additional UV exposure, which was explained by the co-generation of $OH^\bullet$ from O_3 interaction with UV. Similarly, less than 1 $\log_{10}$ inactivation was observed when 5 mg/L H_2O_2 was used to treat MS2, while a combined UV/ H_2O_2 process resulted in over 7 $\log_{10}$ inactivation, supposedly by the same effect (Sherchan et al., 2014). Due to a variety of factors affecting disinfection efficiency, it is difficult to compare results from different treatments directly; however, based on the published results, only the concentrations of O_3 for H-mode + mannitol (M) treatment are sufficient to explain the observed MS2 inactivation presented in Fig. 8. For clarity, a summary of key inactivation factors from various plasma treatments is presented in Fig. 9.

3.4. $OH^\bullet$ radical quantification

The results discussed above show that the major factor for virus inactivation in water treated by V-UV from hydrogen plasma are $OH^\bullet$ radicals. Therefore, we performed systematic measurements to quantify the concentration of $OH^\bullet$ in water samples by applying a TA assay. When $OH^\bullet$ is produced through a water homolysis reaction (Equation (1)) (M. G. Gonzalez et al., 2004), some react with TA to produce HTA, and the concentration of HTA can be measured with a spectrophotometer. However, HTA can also be degraded by the co-generated ozone when air is used in the sample's treatment chamber headspace (Zver et al., 2025), meaning that a degradation measurement is also needed to quantify generated HTA accurately. Fig. 10 shows the measured HTA signal when either TA or HTA solution was irradiated with V-UV from H_2 plasma, ignited at 50 W (E-mode) or 390 W (H-mode) at 20 Pa.

Again, the difference between E- and H-mode is immediately apparent since only the latter treatment caused total degradation of HTA within the designated treatment time. Knowing this, it is easier to interpret the curves of the TA irradiation experiments, which trend downwards after the initial upward progression. Using the results of our measurements (Fig. 10), one can estimate the $OH^\bullet$ production rate for each treatment condition. For that purpose, the formation and degradation curves presented in Fig. 10 were deconvoluted, and the

deconvoluted signal was then convoluted back with a constant response, i.e., without degradation. In this way, only an HTA formation rate without any degradation is determined. Fig. 11 presents the HTA formation rate curves during irradiation with V-UV arising from hydrogen plasma sustained in E-mode and H-mode in either air or nitrogen in the sample's treatment chamber headspace. The results correlate perfectly with the results of MS2 inactivation presented in Fig. 6. The most significant $OH^\bullet$ production rate was when H-mode plasma was used as the V-UV source and air was in the sample's treatment chamber headspace, whereas the lowest production rate was when E-mode plasma was used, and the sample's treatment chamber headspace was filled with nitrogen.

Previous studies reported $OH^\bullet$ production rates in the ranges of 10^{-8} – 10^{-10} M/s (Joshi et al., 1995; Kanazawa et al., 2011; Sahni and Locke, 2006), which is somewhat lower than production rates between 3.2 and $0.082 \pm 0.04 \times 10^{-6}$ M/s obtained from our setup. Here, we should stress again that the lines presented in Fig. 11 do not present the actual $OH^\bullet$ production rates but rather the HTA formation rates, which are related to $OH^\bullet$ production rate; therefore, they do not represent the total amount of $OH^\bullet$ produced. Nevertheless, the observed production rates still indicate a good performance of this setup regarding $OH^\bullet$ production.

3.5. Energy efficiency estimation

An extensive review comparing energy efficiency per order (EEO) estimations of different advanced oxidation processes was presented by Miklos et al. (2018), which led to categorisation into three groups based on median EEO values: <1 kWh/m³, 1–100 kWh/m³, and >100 kWh/m³. The most energy-efficient system was ozonation with 0.15 kWh/m³, followed by introducing UV treatment to the ozonation process at 0.2 kWh/m³, while plasma processes fell into the second group with around 3.3 kWh/m³ median EEO value. The decontamination efficiency was almost exclusively evaluating chemical contaminants, which behave differently from microbes. Nevertheless, it gives us something to compare the efficiency of our system. Here, it should be mentioned that some caveats are necessary to evaluate the applied V-UV radiation despite its sub-optimal application. Namely, even though the applied power of 50 W and 390 W were used to sustain the plasma in E- and H-mode, respectively, it must be stated that the radiation had dissipated in all directions (Fig. 1) of the discharge tube and only a small fraction of the plasma-emitted V-UV photons reached the sample. This fraction was calculated as a ratio between the solid angle of the plasma radiation impinging on the liquid in the sample chamber and the solid angle of the whole sphere. Approximately only 0.1 % of the plasma emitted V-UV light was actually used in these experiments, which was accounted for in the EEO calculation. Various plasma systems that used MS2 as a water decontamination target are also presented in Table 1, providing the most objective comparison between plasma discharges. The amount of V-UV light used for water treatment requires many optimisations, but the estimations in Table 1 indicate that the overall efficiency could be economical after optimisation of the treatment system. Furthermore, low-pressure plasmas can be sustained in large volumes at the specific power (power per unit volume of plasma) as low as about 1 W/liter (Gosar et al., 2020) so upscaling the experimental results reported in this scientific article looks feasible.

It can be seen that the best-performing methods (O_3 , O_3/H_2O_2 , UV lamps) also have applications in large water treatment setups. Relatively high EEO values are calculated for atmospheric-pressure plasma discharges, which is explained by a lower production of RONS, V-UV, and UV, as a large portion of energy is lost to heating the gas (Tendero et al., 2006). On the other hand, the low-pressure ICP system studied in this article achieves quite promising EEO values, with the E-mode discharge with an air atmosphere being the most energy-efficient technique among those probed in our experiments. The simultaneous application of powerful UV and V-UV radiation, along with a plethora of RONS generated due to the oxygen in the atmosphere, is sufficient for 9 $\log_{10}$

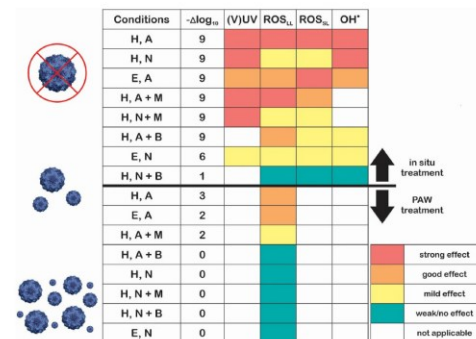


Fig. 9. Visual representation of major factors affecting MS2 inactivation with plasma treatment. (V)UV denotes V-UV and UV radiation, ROS_{UL} denotes long-lived ROS (O_3 , H_2O_2); ROS_{SL} denotes short-lived ROS (O , 1O_2 , O_2), excluding $OH^\bullet$; H denotes plasma in the H-mode; E denotes plasma in the E-mode; A denotes air used in the sample's treatment chamber headspace, while N denotes nitrogen; M denotes use of $OH^\bullet$ scavenger mannitol; B denotes the use of a non-hermetic radiation barrier; $-\Delta\log_{10}$ denotes a logarithmic change in virus infectivity.

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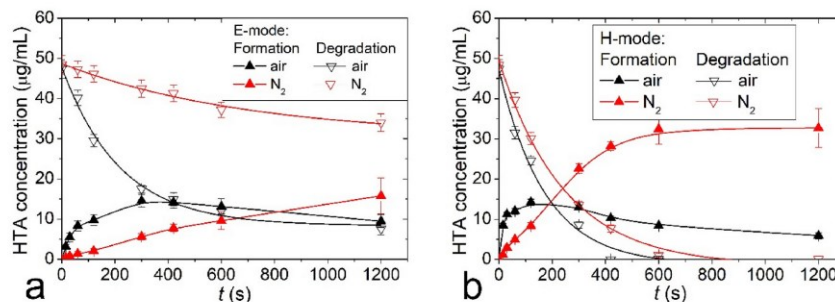


Fig. 10. HTA formation (upward triangles) and degradation (downward triangles) after plasma irradiation in E-mode (a) or H-mode (b) in either air (black line) or nitrogen (red line) atmosphere in the sample treatment chamber headspace.

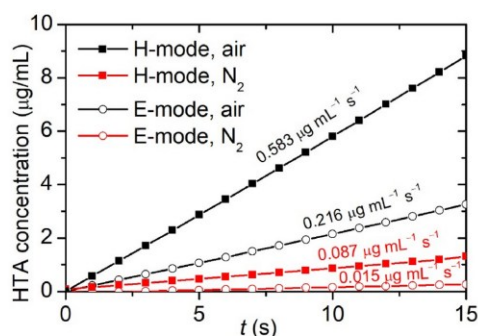


Fig. 11. HTA formation during E-mode and H-mode plasma irradiation treated in either air or nitrogen atmosphere.

Table 1

Energy efficiency per order for MS2 inactivation in water. Ozonation and O_3/H_2O_2 added for 'best-in-class' comparison.

System	EEO (kWh/m ³ /order)	Reference
Ozonation	0.15	Miklos et al. (2018)
O_3/H_2O_2	0.2	Miklos et al. (2018)
Plasma jet	1166.67	(Y. Wu et al., 2015)
Streamer corona discharge	311.11	Lee et al. (2011)
Dielectric barrier discharge	5.21	Guo et al. (2018)
UV-C LED 260/280 nm	2.022	(S. E. Beck et al., 2017)
Low-pressure UV lamp	0.028	(S. E. Beck et al., 2017)
Medium-pressure UV lamp	0.06	(S. E. Beck et al., 2017)
KrCl excimer lamp 222 nm	0.05	Hull and Linden (2018)
ICP plasma, E-mode, N_2	0.66	This work
ICP plasma, E-mode, air	0.07	This work
ICP plasma, H-mode, N_2	0.57	This work
ICP plasma, H-mode, air	0.30	This work

inactivation of MS2 within a minute of treatment time, requiring nearly eight times less energy input than the treatment with plasma in the H-mode. Again, it needs to be stated that the values calculated in this article only represent the 0.1 % of energy applied to the sample itself, and many optimisations of the technology still need to be done.

3.6. General aspects of pollutant degradation using V-UV radiation from hydrogen plasma

In the preceding sections, we proved the degradation efficiency of viruses when using V-UV radiation from hydrogen plasma and explained the mechanisms involved. The method, however, is applicable for destroying many other organic pollutants in water since the $OH^\bullet$ readily interact with any organic matter, causing the formation of radicals (Equation (3)) and irreversible oxidation by forming organic radicals (Equation (4)). In the following section, we provide a proof of concept for three other types of pollutants, including two types of bacteria, *E. coli* and *S. aureus*, the antibiotic tetracycline, and the industrial dye methylene blue.

Viruses are challenging to remove from water systems because of their small size, while bacteria can grow, replicate, form biofilms, repair damage, and adapt to their environment, meaning they may be more persistent in water systems when the disinfection method is not destructive enough (Lan et al., 2019). Gram-negative (G^-) *E. coli* and Gram-positive (G^+) *S. aureus* are standard strains for evaluating water disinfection performance (Duque-Sarango et al., 2023; Makarova et al., 2017).

Besides biological pollutants, chemical contamination of water also presents an issue with current water treatment techniques. Persistent chemicals with biological activity, like endocrine disruption, toxicity, antibiotic resistance, inflammation, and the like, can pose harmful consequences if not properly eliminated. TETR and MB are both persistent chemical pollutants that need to be removed from water by physical, chemical, or biological means due to their negative environmental impact (Slokar and Le Marechal, 1998). Standard wastewater treatment practices are sometimes inefficient or can result in large amounts of sludge (Robinson et al., 2001; W. Xu et al., 2007). Moreover, incomplete mineralisation can lead to the formation of some toxic byproducts (Ouzar and Kim, 2022; J. Wu et al., 2010), however there are some inconsistencies in the literature regarding byproducts toxicity of TC treatment with plasma (C. Fang et al., 2022; Ouzar and Kim, 2022). In detail, Ouzar et al. proposed that some byproducts may be more mutagenic than TC itself, while Fang et al. reported negligible biological toxicity of TC degradation byproducts. The effect of this can not be attributed to residual TC, since the study reporting biotoxicity had lower remaining TC concentrations after treatment than the other one (4.7 mg/L and 7 mg/L, respectively). Both studies used a software-based prediction approach (T.E.S.T.), while Fang et al. also evaluated toxicity towards bacteria *E. coli*, which showed no significant adverse effects. Here, H_2O_2 had been removed from the solution before carrying out the test, which may explain these discrepancies.

An ideal water treatment method should be able to degrade various types of pollutants, whether biological or chemical, as they are usually

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present simultaneously in wastewater. Therefore, we conducted additional treatments of common wastewater pollutants to gain more insight into the effectiveness of the ICP plasma treatment method. The results are summarised in Fig. 12. Although the contaminants differ significantly, the trend is very similar: the treatment with V-UV radiation arising from hydrogen plasma in the H-mode efficiently destroys all these organic pollutants.

Different research teams used various experimental systems to evaluate the inactivation/degradation of bacteria, antibiotics, and organic dyes, so the results are not comparable, but the key difference between our approach and the brief state of the current knowledge as presented below is in the range of wavelengths used for UV and/or V-UV-assisted degradation of organic pollutants.

A broad subdivision of bacterial species to either G^- or G^+ stems from the latter having a thicker peptidoglycan cell wall (20–80 nm), allowing them to better retain the crystal violet staining dye (Coico, 2006). Since RONS initially interact and cause damage to the cell walls of microbes (Chen et al., 2021), it is reasonable to assume that G^- bacteria would be more susceptible to RONS disinfection techniques. This was indeed demonstrated by Kühn et al., using a UVA + TiO_2 disinfection method for producing $\text{OH}^\bullet$, and noting that the disinfection efficiency of various bacteria was correlated with the thickness of their respective cell walls (Kühn et al., 2003). Plasma-based techniques have previously been used to disinfect *E. coli*- or *S. aureus*-contaminated solutions, albeit on a small scale. Ma et al. used an atmospheric pressure DBD plasma to treat 50 μL of *E. coli* and *S. aureus* bacteria suspensions, achieving $>6 \log_{10}$ inactivation within 7 and 10 s, respectively (Ma et al., 2008). Similarly, Han et al. attempted to demonstrate the difference between direct versus indirect DBD plasma treatment. Interestingly, they obtained better inactivation of both *E. coli* and *S. aureus* with indirect rather than direct plasma treatment, which they attributed to long-lived RONS generated by the plasma discharge (L. Han et al., 2016).

Tetracycline, one of the most prolific antibiotics in human and veterinary medicine, is regularly detected in water systems due to its overuse, stability, and run-off to the environment (L. Xu et al., 2021), which may result in adverse effects on the biosphere (Amangelsin et al., 2023; Shutter and Akhondji, 2024). Standard treatment practices such as ozonation (J. Wu et al., 2010), UV treatment (Yuan et al., 2011) or their combination (F. Yang et al., 2024) have been shown to degrade TETR in wastewater, although the large-scale implementation of these methods has some disadvantages related to cost and/or efficiency (Antos et al., 2024). Research has been focused on developing new techniques for its expulsion from water systems. AOPs have been shown to efficiently degrade TETR only when O_3 was included in the treatment setup,

pointing to a strong correlation between TETR degradation and the presence of $\text{OH}^\bullet$, obtained from O_3 decomposition (Luu and Lee, 2014). Moreover, it was demonstrated by the same authors that the toxicity towards *E. coli* was lowered significantly after treatment, while the same was not observed in the *V. fischeri* bioluminescence assay, suggesting more research needs to be done to evaluate the generation of harmful by-products. Previously, atmospheric-pressure plasma treatment (He et al., 2014; Tang et al., 2018) and V-UV radiation (Krakkó et al., 2022; Yao et al., 2017) have been evaluated as methods for eliminating TETR from water. He et al. used a corona discharge in combination with a TiO_2 nano-catalyst, achieving an 85 % degradation after 24 min of treatment (He et al., 2014). Although the addition of a catalyst improved the degradation efficiency, a greater effect was demonstrated by increasing the plasma input power. On the other hand, 93 % of TETR was degraded by the dielectric barrier discharge plasma within 20 min of treatment (Tang et al., 2018). In both studies, it was postulated that the $\text{OH}^\bullet$ played a pivotal role in degrading TETR. V-UV radiation effectively degraded TETR from 650 mL solution within 10 min of treatment. Krakkó et al. demonstrated an increased degradation using UV/V-UV treatment of aqueous TETR, achieving complete degradation of 22.2 mg/L within 5 min. Additionally, possible degradation pathways and reactions were proposed, which aids in further optimisation of similar systems (Krakkó et al., 2022). A combined UV/V-UV process with adding a Fe (II) catalyst was able to degrade approximately 98 % of 8.8 mg/L of TETR within 30 min of treatment. With the 4-W cold-cathode low-pressure mercury lamp, the technique, according to the calculated EEO, was deemed cost-effective with 8.46 kWh/m³ per order. The principal degradation agent was found to be the $\text{OH}^\bullet$ (Yao et al., 2017).

Paper and textile industries generate large amounts of wastewater, which are difficult to purify due to the amount and robustness of some pollutants. MB, a common dye in these industries, is a model compound for studying degradation kinetics and efficiency. Plasma has been employed several times to degrade this pollutant, with varying results. Although the authors are in general agreement that ROSs are the primary degradation factors, it is not as conclusive which is the primary one, as it varies between different setups. H_2O_2 was deemed the main degradation factor for alternating current- and microwave-excited atmospheric pressure plasma jets (Chandana et al., 2015; García et al., 2017), however, authors in both publications used pure argon gas for plasma generation, which is not ideal for ROS production besides H_2O_2 (Aboubakr et al., 2016; Bruggeman et al., 2016). When air or oxygen was supplied to the plasma gas mixture, a greater efficiency was achieved, which was attributed to a greater amount of ROS produced (B. Wang et al., 2017; L. Wu et al., 2019). A degradation pathway was proposed by Wu et al., who suspect that high energy electron, ozone, and hydroxyl radical are the primary main degradation agents for their atmospheric pressure DBD plasma reactor, however, they do not claim to have any V-UV radiation present, therefore these degradation mechanisms are not necessarily applicable to our system (L. Wu et al., 2019). One of the best reports of plasma MB degradation in water utilised an atmospheric-pressure dielectric barrier discharge air plasma. Large amounts ($>50 \text{ mg/L}$) of H_2O_2 were generated, which were further converted to $\text{OH}^\bullet$ by the addition of a Fe (II), resulting in around 67 g/kWh degradation rate (Manoj Kumar Reddy et al., 2013).

4. Conclusion

Inductively coupled low-pressure plasma was used as a source of V-UV radiation in the range of wavelengths between about 150 and 200 nm. It is a highly versatile source of V-UV photons, which can produce various outcomes in a water treatment regime. Treatment parameters such as input power and the presence of oxygen at the gas-water interface have a profound effect on the efficiency of pollutant degradation, as showcased by an increase of $\text{OH}^\bullet$ generation, and pollutant degradation efficiency. With direct V-UV radiation in an oxygen atmosphere, the treatment was able to degrade 8 $\log_{10}$ of MS2 bacteriophage, 8 $\log_{10}$ of

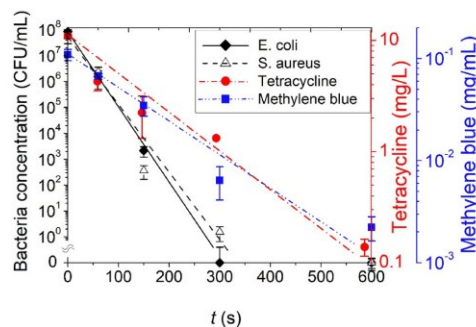


Fig. 12. V-UV treatment of common water pollutants, with plasma in the H-mode, air in the sample's treatment chamber headspace.

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E. coli and *S. Aureus* bacteria, as well as 10 mg/L of antibiotic tetracycline and 100 mg/L of industrial dye methylene blue, within 10 min of treatment time, all of which were remarkably concentrated, to demonstrate the potential of this method to treat highly polluted waters. Even though the energy of V-UV photons is large enough to disrupt the structure of viruses, causing their inactivation, it is the presence of oxygen at the gas-water interface that has the most significant effect on the overall production of $\text{OH}^\bullet$ and, subsequently, the inactivation of viruses. The experiments utilising a barrier for V-UV radiation showcase that some inactivation also occurs without direct interaction between the V-UV radiation and water. The insertion of the V-UV radiation barrier clearly showed that the gaseous radicals, created upon irradiation of air with V-UV photons, enter the water and contribute to virus inactivation, but the effect is smaller compared to the inactivation by $\text{OH}^\bullet$. The short- and long-lived RONS are thus inter-connected, being generated in either gas or liquid phase, and simultaneously carry out pollutant degradation, which would be interesting to investigate in future works. Some experiments were performed by adding the $\text{OH}^\bullet$ scavenger mannitol into the polluted water, and the results proved that the radicals play a prominent but not exclusive role in virus inactivation. However, the synergistic action of all the inactivation agents provides the best outcome, as long as the virus inactivation is the merit. In certain setups (E-mode, air), the EEO value ($0.07 \text{ kWh/m}^3/\text{order}$) even outperformed some of the established water treatment methods, such as ozonation and UV-C irradiation (0.15 and $2 \text{ kWh/m}^3/\text{order}$, respectively), indicating that there is potential for this technique to be used as a water treatment method, especially when considering the range of different types of pollutants being effectively removed. Building on this proof-of-concept study, further work should evaluate the level of mineralisation, i.e., the amount of pollutant that is completely oxidised to CO_2 and H_2O , since degradation by-products may also pose health concerns when treated water is consumed or discharged. On a similar note, toxicological studies of treated water would be required, as harmful long-lived RONS may remain in liquid and cause harmful effects if not removed after treatment. One of the primary benefits of utilising V-UV in water treatment is its indiscriminate effectiveness against biological and chemical pollutants. However, its low penetration depth does necessitate a carefully designed setup to treat larger volumes of water, while optimizing energy efficiency could make this technique a valuable asset to the water treatment arsenal.

CRedit authorship contribution statement

Mark Zver: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rok Zaplotnik:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Miran Mozetič:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Alenka Vesel:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Arijana Filipić:** Writing – review & editing, Validation, Methodology. **David Dobnik:** Writing – review & editing, Validation, Supervision, Project administration. **Belisa Alcantara Marinho:** Writing – review & editing, Validation, Methodology, Investigation. **Gregor Primc:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mark Zver, Rok Zaplotnik, Miran Mozetič, Alenka Vesel, Arijana Filipić, David Dobnik, Belisa Alcantara Marinho reports financial support was provided by Slovenian Research and Innovation Agency. Gregor Primc reports financial support was provided by Slovenian Research and

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Data availability

Data will be made available on request.

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Chapter 3

Conclusions

Virus inactivation in water using low-pressure plasma radiation was investigated in this work. Starting with an extensive literature review of plasma treatment of viruses in liquid and performing a meta-analysis of the available data, it was revealed that the treatment efficiency is case-specific, with several factors affecting the final result. A major deficiency in reporting the treatment parameters was also observed, which made much of the available data unusable. This prompted us to be more diligent in characterising our systems and reporting the operational parameters. What was also revealed was that the reactive oxygen species were primarily responsible for inactivating microbes in solution, which we decided was worthy to investigate and include in our system setup. Through several technical iterations, we managed to design and construct a capacitively (CCP) and an inductively coupled (ICP) plasma system for producing a stable low-pressure plasma discharge as a V-UV source. Additionally, a specialised treatment chamber was constructed, where the sample could be present in a controlled atmosphere. Our initial focus was the CCP system, where a variety of working gases were tested while changing the discharge power and pressure. Using a V-UV spectrometer, we analysed the emission profiles and identified hydrogen gas as the most appropriate for producing V-UV radiation. The pressure of the system was kept at a constant to avoid changing the radiation intensity peaks. The spectrometer measurements were performed with three different types of cover windows, confirming that the MgF_2 ceramic, which was also the cover window used for inactivation experiments, was transparent to V-UV radiation emitted by H_2 plasma in the range between about 135 and 165 nm. Thus, our first objective to produce stable low-pressure plasma V-UV radiation and apply it to a liquid sample was achieved. The generation of $\text{OH}^\bullet$ in liquid was confirmed by using terephthalic acid (TA) as an $\text{OH}^\bullet$ quantification probe. By changing the cover window from MgF_2 to quartz or borosilicate, we demonstrated that V-UV was responsible for the production of $\text{OH}^\bullet$ in the liquid. Several insights were obtained using either N_2 , O_2 or air as the ambient gas in the treatment chamber. The N_2 gas, being the most transparent to V-UV radiation in the selected range of photon energies, produced the least amount of $\text{OH}^\bullet$ in the liquid. In contrast, O_2 and air produced significantly more at comparable conditions. This indicated that the V-UV photolysis reaction is not the primary source of the $\text{OH}^\bullet$ in liquid. It would seem that the majority of $\text{OH}^\bullet$ in liquid are the product of reactions taking place in the gas phase above the liquid sample and then transported to the liquid. Furthermore, observing the non-linear slopes of hydroxyterephthalic acid (HTA) production when either O_2 or air were present prompted us to investigate further and realise that the co-generated ozone degrades the HTA molecule. By investigating the degradation rates of HTA and performing calculations, we were able to account for the degradation and produce a linear HTA production rate. Although this does not necessarily reflect the actual $\text{OH}^\bullet$ formation rate, since not every

$\text{OH}^\bullet$ reacts with TA, it does provide a valuable quantification correction to compare the $\text{OH}^\bullet$ production rates at various treatment conditions.

With the system characterised, and the $\text{OH}^\bullet$ formation understood, we could proceed with virus inactivation experiments. By performing the inactivation experiments with different cover windows (MgF_2 , quartz or borosilicate glass) and varying the ambient gas inside the chamber (N_2 , O_2 , air), it was seen that only when the MgF_2 was used in combination with an oxygen-containing gas (O_2 or air) in the treatment chamber, that total virus inactivation was achieved (approx. $5 \log_{10}$) within a treatment time of 150 seconds. This additionally confirms that: a) V-UV alone is not responsible for virus inactivation, and b) ROS are primarily produced by V-UV radiation in the gas phase above the sample. Expanding further, we added D-mannitol to the liquid as an $\text{OH}^\bullet$ scavenger when we confirmed that it does not affect the virus viability while effectively inhibiting HTA formation ($\text{OH}^\bullet$ production) in the liquid. When the scavenger was present, the virus inactivation was almost non-existent, while total inactivation was seen in the absence of the scavenger. H_2O_2 , O_3 and NO_2^- in the liquid were also measured, and while they were detected in almost all setups, their concentrations were relatively low ($< 1 \text{ mg/L}$). Combining these two observations, we concluded that the $\text{OH}^\bullet$ is primarily responsible for the inactivation of viruses in water, using the pulsed DC plasma as a V-UV radiation source.

Although a great tool to generate and study V-UV radiation treatment, the pulsed DC system has some downsides when practical applications are considered. The treatment duration and/or the power input need to be reduced since some cathode effects were observed at higher power inputs, producing localised arcs, despite using advanced arc-protection, which would damage the system. To exclude this issue and also attempt to use a more energy-efficient system, an inductively coupled plasma (ICP) system powered by a radiofrequency (RF) generator was constructed. Here, it is also possible to modulate between the capacitively coupled E-mode and inductively coupled H-mode discharge by varying input power. It was immediately observed that the system produces more V-UV radiation compared to the pulsed DC system at the same power input at lower pressures. It was also observed that the HTA signal rapidly decreased after the initial uptick when oxygen was present in the treatment chamber, which we could now correct by applying the same methodology described previously. This allowed us to calculate $\text{OH}^\bullet$ formation rates for various treatment conditions. A faster production of $\text{OH}^\bullet$ translated into faster virus inactivation, while the negligible amount of other RONS again confirmed the primary role of $\text{OH}^\bullet$ as the main inactivation factor. Interestingly, when a physical radiation barrier was inserted into the treatment chamber, virus inactivation still occurred, albeit more slowly, when air was present in the chamber. If the same experiment was performed while exchanging the gas with N_2 , virus inactivation was virtually non-existent, achieving only $1 \log_{10}$ reduction in 10 minutes of treatment time. Placing mannitol in the liquid reduced the rate of virus inactivation. However, in contrast to the pulsed DC capacitively coupled plasma system, the virus inactivation was complete, even in an N_2 atmosphere, showcasing the more significant role of V-UV radiation in the inactivation. Further cementing the short-lived reactive species as the primary inactivation factors, a plasma-irradiated water experiment was performed. Here, the water was treated separately with plasma radiation and subsequently inoculated with viruses. Following an incubation period of 10 minutes, the viruses were quantified. Although some reduction in virus numbers was observed, none of the treatments produced a complete inactivation ($9 \log_{10}$). Gathering all treatment data, an energy efficiency calculation was performed to compare the treatment with standard disinfection methods such as O_3 and UV. As the treatment system is not yet optimised for the practical treatment of samples, it was necessary to include some caveats to only account for the radiation actually interacting with the sample. Interestingly, this revealed that in

some conditions, the treatment performed as well as in some standard disinfection systems, although we understand that a lot more optimisations would need to be done for this to be economically viable. Lastly, to expand on the proposed benefits of such systems, an assortment of various pollutants (bacteria, dyes, antibiotics) were treated with the inductively coupled plasma system, showing complete inactivation/degradation within 10 minutes of treatment time. This proves the indiscriminate action of the treatment of different pollutant types. Further research should be directed towards scaling up the system to treat larger volumes while also improving upon the amount of radiation transferred to the actual liquid treated. One of the major limiting factors for economical scale-up is the lack of a low-cost V-UV transparent material, which is the crucial component for delivering the germicidal radiation to the treated medium. On the other hand, the degradation products should be carefully examined to ensure that no harmful by-products are formed and that the treated liquid is safe for further use or discharge into the environment.

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Biography

Mark Zver was born in 1993 in Ljubljana. After completing his high school education at gimnazija Ledina in 2012, he continued his education at the Biotechnical faculty of University of Ljubljana. In 2015 he obtained a bachelor's degree (BSc) by presenting his thesis titled: *“Pregled pristopov preprečevanja in zdravljenja okužbe z virusom HIV”*. Shortly thereafter, he enrolled to a Biotechnology Master's programme (MSc) at the Aalborg University (Denmark), where he earned a degree, by presenting his work on potato genome editing in the thesis titled: *“Design of Cas9/single-guide RNA gene knock-out system and gene expression study of cold-induced sweetening in Solanum tuberosum”*. Throughout his undergraduate career, Mark has been actively involved in an international group of technical students (BEST), through which he obtained many valuable skills in communication, leadership and problem solving. Additionally, he actively participated in a youth society of his hometown, organizing community events and concerts for the municipality. After returning to Slovenia, he was employed by a Slovenian industrial biotechnology company, Acies Bio, where he worked as a researcher and business developer for 3 years, obtaining valuable insight into the commercial aspects of biotechnological operations.

In October of 2020, Mark began his doctoral studies as a young researcher at Jožef Stefan International Postgraduate School (IPS) by enrolling in the Sensor technologies program. Working at the Department of Surface Engineering (F4), he was tasked with carrying out bio-chemical analyses of plasma treatment of various materials, specializing on virus inactivation. Moreover, during his employment he successfully designed, constructed and registered a level 2 biosafety laboratory at the department, where he established workflows for a variety of biological tests, practicable for the department's interdisciplinary projects. He was also a co-inventor on a patent for a virucidal textile material, for which he also received a reward for best presentation at the 14th IPSSC conference. While enrolled at the IPS, he actively participated and even served as the Student Council president for two non-consecutive years, organizing social and educational events for students, as well as promoting their interests to the school's governing bodies. In his four years of working at the F4 department, Mark gained extensive experience with various plasma systems and diagnostics, as well as knowledge of carrying out several microbe inactivation studies, along with performing water analysis techniques.