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Review

Can ozone inactivate SARS-CoV-2? A review of mechanisms and performance on viruses

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ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic has challenged societies around the globe. Technologies based on ozone, a powerful oxidant, have been evaluated to inactivate this virus in aerosols and fomites. However, the high data diversity hinders the possibility of establishing a common ground for determining best practices for the use of these technologies. Furthermore, there is a lack of consensus regarding which are the main mechanisms of ozone virus inactivation. This critical review examined the most relevant information available regarding ozone application in gas-phase for different viruses inactivation (including recent publications dealing with SARS-CoV-2), and pointed towards envelope alteration as the main reaction pathway for enveloped viruses, such as is the case of SARS-CoV-2. It could also be concluded that gaseous ozone can be indeed an effective disinfectant, successfully inactivating viruses such us influenza A H1N1, MERS-CoV, SARS-CoV-1 or even SARS-CoV-2 in aerosols or fomites. In reviewed works, low ozone exposures, just around 0.1–0.4 mg L^{-1} min, achieve about 4 log_{10} of inactivation in aerosols, while exposures between 1 and 4 mg L^{-1} min may be needed to guarantee an inactivation of 3–4 log_{10} in different fomites. Although further studies are required, ozone is an effective candidate to be used against SARS-CoV-2 or other viruses in surfaces and indoor locations.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a novel coronavirus, has recently emerged from China and quickly spread around the world. SARS-CoV-2 is a member of the Coronaviridae Orthocoronavirinae family and, as its relatives, mainly causes infections in the respiratory and gastrointestinal tracts (Rey and Lok, 2018). The World Health Organization declared derived disease (COVID-19) outbreak a pandemic on March 11, 2020 (World Health Organization, 2020a). This infectious disease was still hitting the world when this review was written, and the precedents suggested it might not be the last. During the first twenty years of this millennium the appearance of other serious, well-known, global outbreaks of the acute respiratory syndrome coronavirus (SARS-CoV-1) (2002–2003), Middle East respiratory syndrome coronavirus (MERS-CoV) (2013) and influenza swine flu pandemic (H1N1 influenza A virus) (2009–2010) have been warning about the possibility of new and more severe future outbreaks.

Apart from direct contact, and like other coronaviruses such as Human Coronavirus (HCoV), MERS-CoV and SARS-CoV (Kutter et al., 2018), for SARS-CoV-2 liquid droplets (short range) and aerosols (long range), generated when coughing, sneezing, speaking, singing or breathing heavily, were supposed to be the main spread paths in humans (World Health Organization, 2020b). In the case of aerosols (< 5 μm particles), SARS-CoV-2 can remain viable, suspended in indoor scarcely ventilated premises, for at least 3 h with a reduction of fifty-percent tissue culture infective dose ($TCID_{50}$, a parameter that quantifies the amount of virus to kill or produce a cytopathic effect in 50% of inoculated tissue culture cells) just from $10^{3.5}$ to $10^{2.7}$ per liter of air in that time (van Doremalen et al., 2020). Few papers confirm however the viability of viruses in aerial samples, mainly due to sampling limitations, such is the case of the work recently performed in a hospital room with COVID-19 patients (Lednicky et al., 2020).

Meanwhile, short-range droplets (≥ 5 μm), called Flügge drops, remain in suspension for a limited time and do not disperse beyond 1.5 or 2 m from the emitter. Surfaces contaminated with these drops (fomites) have also the potential to infect by touching those surfaces followed by eyes, nose and mouth contact. In fact SARS-CoV-2 particles were still found on household and hospital related solid surfaces, and

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remained stable in most of them, $TCID_{50} > 10^{1.5}$ per mL, after 7 days (Liu et al., 2021). However, it has been stated recently that there is low risk of SARS-CoV-2 transmission through this path in real-life conditions (Mondelli et al., 2021), thanks to standard cleaning procedures and precautions in healthcare facilities. Nevertheless, those fomites were an important concern at the first peak of the pandemic regarding the contamination of protective sanitary material (masks, face shields, etc.) whose supply was specially jeopardized those days, and brand new protocols for cleaning and reuse were abruptly put in place.

According to all this, the need for safe and validated technologies, capable of ensuring the disinfection of air environments, room surfaces and sanitary materials, has become evident against the current pandemic or future events. In this sense, ozone (O_3) can be a valid option. This well-known oxidant has already demonstrated its virucidal capacity in aqueous media (Farooq and Akhlaque, 1983; Hirneisen et al., 2011; Wolf et al., 2018). The most important operation parameter in O_3 disinfection is the ozone exposure, also known as the concentration per time (CT) value (mg L^{-1} min), which in its most general form represents the time-integrated ozone concentration. The temperature of the medium has strong influence as well (Khadre et al., 2001; Roy et al., 1982; von Sonntag and von Gunten, 2012; Wickramanayake and Sproul, 1988; Wolf et al., 2018). When ozone is applied in gas-phase, humidity is also a key parameter, requiring high relative humidity conditions to obtain significant inactivation of target microorganisms (Blanchard et al., 2020; Dubuis et al., 2020; Hudson et al., 2009; Li and Wang, 2003; Sato et al., 1990; Tseng and Li, 2008, 2006). Chemical composition of the surface to be treated and its shape and texture could also be important factors.

At the time this publication was submitted, only two very recent, concise works were found in literature where O_3 was applied for the disinfection of SARS-CoV-2 (Clavo et al., 2020; Yano et al., 2020). Also, a paper reviewing the use of ozone for indoor air treatment against viruses was published (Alimohammadi and Naderi, 2020). Related to this topic, another publication concluded also that COVID-19 transmission was negatively impacted by higher tropospheric ozone level and lower relative humidity (Yao et al., 2020). All these works point to an effective application of ozone as disinfectant of SARS-CoV-2 but they were still inconclusive.

Furthermore, the mechanisms for SARS-CoV-2 ozone inactivation, such in the case of most other viruses, were not clearly elucidated. Moreover, ozone effectiveness or optimal operation parameters in the gas-phase were not proven irrefutably yet, given the diversity of conditions and results reported in the literature. In fact, ozone was not included by then as a virucidal agent either by the Spanish Health Ministry or by the US Environmental Protection Agency (Ministerio de Sanidad (Spain), 2020; United States Environmental Protection Agency, 2020).

Thus, the objective of this article is to carry out a critical review of all the published works related to O_3 gas-phase applications in order to discern the effectiveness of this method as a virucidal agent, and, if possible, to establish application conditions that could allow the elimination of SARS-CoV-2. It will start shedding light to the –known to date– main potential mechanisms accountable for O_3 virucidal activity. It will follow with a deep literature review regarding works on the application of gaseous ozone. Finally, those aspects on which conducting more research would be necessary are highlighted. With all the information gathered, it is expected that this review could guide consumers, manufacturers, researchers and policy makers towards an effective and responsible use of this powerful oxidant to address current SARS-CoV-2 or other future pandemics.

2. Materials and methods

2.1. Search for publications of viral inactivation by ozone in gas-phase

For the critical review of ozone gas virucidal inactivation mechanisms and applications, Scopus, Google Scholar and Web of Science were used as the main repositories to find the published references about the topic. Different combinations of the following main terms were used: ozone, disinfection, inactivation, virus, mechanisms, kinetics, gas and air. More than 200 publications were analyzed. To filter the retrieved sources, only those works using ozone in gas-phase and for virus inactivation were considered. Accordingly, all the works where O_3 was supplied in aqueous solution, where bacteria were the main target, or where ozone was used as a medical therapy, were discarded. Three of the works considered in this review have been recently published as preprint (Blanchard et al., 2020; Dave et al., 2020; Lee et al., 2020), as a direct response to the current COVID-19 pandemic. Since they present a rigorous scientific approach and due to the interest of their topic, they have been included. Concerning the review of mechanisms of viral inactivation by ozone, only studies in which mechanistic interpretations appear supported by experimental results were included.

2.2. Classification and presentation of the results

To summarize and present the selected works on ozone gas applications, two tables were created. Table 1 presents all the papers reporting virus inactivation in aerosols (6 publications). Table 2 displays those investigations focused on the disinfection on surfaces or fomites (14 publications). Only the lowest ozone concentration values that achieved an inactivation of 3 log_{10} or more are included. In case that inactivation was not reached, the closest value is included. The extended versions of Tables 1 and 2 with all the data collected can be consulted in the Supplementary Material, as Table S1 and Table S2. Supplementary tables include extra experiments and information about the experimental conditions, as the volume injected or the initial virus concentration.

A great variety of viruses has been used as target specimens in the reviewed works, up to 29 different species. The information about the main characteristics of the virus listed are included in Table 3. Three large groups of virus types predominate in the table: norovirus, bacteriophage viruses and viruses responsible for respiratory diseases, kindred to the worldwide pandemics of this millennium mentioned in the introduction: influenza A virus H1N1, MERS-CoV, SARS-CoV and SARS-CoV-2. Regarding the experimental settings found, most authors worked with sealed chambers, at room temperature and controlled relative humidity, even when the disinfection of airborne particles was the purpose of the work. Different models of ozonators (O_3 generators) were used, with corona discharge technology being the preferred one. A good number of studies indicated the ozone concentration during the experiments. However, several of them did not specify if this value was constant during all the experiments, if the ozone evolved along the process, or if it was a mean value.

3. Virus inactivation by ozone

3.1. Mechanisms of viral inactivation by ozone

Ozone is a powerful oxidizing agent with many industrial applications for water and air treatment among others (Martinelli et al., 2017; Wei et al., 2017). Even though the lethality of O_3 against viruses is widely known, the understanding of the inactivation mechanisms still remains limited. Most of this knowledge comes from studies dealing with virus disinfection by ozone in water, although it might be expected that it can be extrapolated to the disinfection in other media such as aerosols or solid surfaces, as similar principles may apply (Wigginton et al., 2012).

Inactivation of viruses by ozone is the result of reactions between this oxidant and the biomolecules constituting the essential structures, external and internal, of the target organism. Other oxidant species generated through ozone reactions, such as hydroxyl radical and singlet oxygen (von Sonntag and von Gunten, 2012), may also contribute to

Table 1
Shortlist of results for reduction of viruses in **aerosols** in contact with ozone in gas phase. It is listed, for each publication and experimental condition, the lowest CT that achieves at least 3 log_{10} inactivation or infectious ratio (IR[a,b]). If only lower values below 3 log_{10} were reached, the highest one is included. RH refers to Relative Humidity, CT is the ozone concentration multiplied by the contact time, T means temperature, (b.d) "below detection" and MMAD is the mass median aerodynamic diameter. The extended version of the table can be found in the Supplementary Material (Table S2).

INACTIVATION OF VIRUS IN AEROSOLS BY GAS OZONE									
Virus	Enveloped?	[O_3] (ppm)	Time (min)	CT[c] (mg L^{-1} min)	Medium (virus suport)	log_{10} reduction or IR[a,b]	RH %	T (°C)	Ref.
Feline calicivirus	NO	20			PBS	2.6			(Hudson et al., 2009)
MS2	NO	200	0.017	0.007	PBS	5.0	–		(Kekez and Sattar, 1997)
MS2	NO	9000	0.017	0.294	PBS+ 10% Bovine serum	3.0			(Kekez and Sattar, 1997)
MS2	NO	11,500	0.017	0.376	PBS + 25% Bovine Serum	3.0			(Kekez and Sattar, 1997)
MS2 HER462	NO	1.13	10	0.023	1.24 μm MMAD, buffer+Antifoam A	3.9[a]	85	19	(Dubuis et al., 2020)
MS2 HER462	NO	1.13	70	0.158	1.27 μm MMAD, buffer+Antifoam A	> 4 (b.d.)[a]	55	19	(Dubuis et al., 2020)
MS2 HER462	NO	1.13	70	0.158	1.10 μm MMAD, buffer+Antifoam A	> 4 (b.d.)[a]	20	19	(Dubuis et al., 2020)
MS2 ATCC 15597-B1	NO	2.30	0.307	0.0014	Deionized water, 0.5–3 μm diameter	2.0	85		(Tseng and Li, 2006)
MS2 ATCC 15597-B1	NO	2.90	0.307	0.0017	Deionized water, 0.5–3 μm diameter	2.0	55		(Tseng and Li, 2006)
Murine Norovirus-1 PTA-5935	NO	0.23	70	0.032	1.24 μm MMAD, buffer+Antifoam A	3.0[a]	85	19	(Dubuis et al., 2020)
Murine Norovirus-1 PTA-5935	NO	0.23	70	0.032	1.10 μm MMAD, buffer+Antifoam A	0.0[a]	20	19	(Dubuis et al., 2020)
PR772 HER221	NO	1.13	40	0.091	1.24 μm MMAD, buffer+Antifoam A	> 4 (b.d.)[a]	85	19	(Dubuis et al., 2020)
PR772 HER221	NO	1.13	70	0.158	1.27 μm MMAD, buffer+Antifoam A	> 4 (b.d.)[a]	55	19	(Dubuis et al., 2020)
PR772 HER221	NO	1.13	70	0.158	1.10 μm MMAD, buffer+Antifoam A	1.1[a]	20	19	(Dubuis et al., 2020)
T7 ATCC 11303-B1	NO	3.50	0.307	0.0021	Deionized water, 0.5–3 μm diameter	2.0	85		(Tseng and Li, 2006)
T7 ATCC 11303-B1	NO	5.12	0.307	0.0031	Deionized water, 0.5–3 μm diameter	2.0	55		(Tseng and Li, 2006)
φ x174	NO	0.04–0.11	35	0.0027–0.0076	Distilled water	3.0	70		(de Mik and de Groot, 1977)
φ x174 HER-036	NO	1.80	6	0.0219	Water buffer, pH 7.5	2.0[b]	80	11–22	(Vyskocil et al., 2020)
φ x174 HER-036	NO	0.3–1.8	6	0.0219–0.0036	Water buffer, pH 7.5	< 1.0[b]	40	11–22	(Vyskocil et al., 2020)
φ X174 ATCC 13706-B1	NO	1.60	0.307	0.0010	Deionized water	2.0	85		(Tseng and Li, 2006)
φ X174 ATCC 13706-B1	NO	1.90	0.307	0.0011	Deionized water	2.0	55		(Tseng and Li, 2006)
φ X174 HER36	NO	1.13	10	0.023	1.24 μm MMAD, buffer+Antifoam A	3,8[a]	85	19	(Dubuis et al., 2020)
φ X174 HER36	NO	1.13	40	0.091	1.27 μm MMAD, buffer+Antifoam A	2,8[a]	55	19	(Dubuis et al., 2020)
φ X174 HER36	NO	1.13	70	0.158	1.10 μm MMAD, buffer+Antifoam A	0,8[a]	20	19	(Dubuis et al., 2020)
φ6 ATCC 21781-B1	YES	1.20	0.307	0.0007	Deionized water + Tween 80, 0.5–3 mm diameter	2.0	85		(Tseng and Li, 2006)
φ6 ATCC 21781-B1	YES	1.43	0.307	0.0009	Deionized water + Tween 80, 0.5–3 mm diameter	2.0	55		(Tseng and Li, 2006)
φ6 HER102	YES	1.13	40	0.091	1.24 μm MMAD, buffer+Antifoam A	> 4 (b.d.)[a]	85	19	(Dubuis et al., 2020)
φ6 HER102	YES	1.13	70	0.158	1.27 μm MMAD, buffer+Antifoam A	1,6[a]	55	19	(Dubuis et al., 2020)

[a] This value refers to Relative Infectious ratios (RIR) and it is calculated by dividing mean culture counts (PFU mL^{-1}) with mean qPCR values (genomes mL^{-1}) and then normalized as proposed by Dubuis et al. (2020).

[b] This value refers to Corrected Infectious ratios (CIR) and it is calculated by dividing mean culture counts (PFU mL^{-1}) with mean qPCR values (genomes mL^{-1}) and then corrected by calculating the infectious ratio at each time point divided by the infectious ratio at time point 0 as proposed by Vyskocil et al. (2020).

[c] CT calculated considering pressure = 1 atm and temperature the one shown in column "T". If T was not provided, it was considered 25 °C.

some extent to the inactivation of viruses during this process. Ozone can react with the nucleic acids of the viral genome, following molecular diffusion through the external structures towards the nucleic material. The external structures are the capsid proteins and, only for some species, the proteins and lipids of the viral envelope, such as in the case of SARS-CoV-2. Some authors reported that the knowledge about the composition of the virus helps in providing indications on the most probably sites of attack, particularly for some non-enveloped viruses such as Poliovirus 1 (Wigginton and Kohn, 2012), the bacteriophage MS2 and enteric adenoviruses (Mayer et al., 2015). These works suggest

Table 2
Shortlist of results for reduction of viruses on **surfaces** in contact with ozone in gas phase. It is listed, for each publication and experimental condition, the lowest CT that achieves at least 3 log_{10} reduction. If only lower inactivation values were reached, the highest one is included. RH refers to Relative Humidity, CT is the ozone concentration multiplied by the contact time, T means temperature and (b.d) "below detection". The extended version of the table can be found in the Supplementary Material (Table S2).

INACTIVATION OF VIRUS ON SURFACES BY GAS OZONE									
Virus	Enveloped?	$[O_3]$ [ppm]	Time [min]	CT[d] (mg L^{-1} min)	Medium (virus suport)	log_{10} Reduction	RH %	T [°C]	Ref.
Adenovirus (Ad 3,11)	NO	20[a]	60	1.8	undetermined	3.0	40–95	room	(Hudson et al., 2009)
Feline calicivirus	NO	20[a]	60	1.8	polystyrene	0.6	38	room	(Hudson et al., 2009)
Feline calicivirus	NO	20[a]	60	1.8	polystyrene	3.9	40–95	room	(Hudson et al., 2009)
Feline calicivirus	NO	20[a]	< 60	1.8	plastic surface	3.7	> 70%	< 23	(Hudson et al., 2007)
Feline calicivirus	NO	20[a]	< 60	1.800	Fabric surface	3.0	> 70%	< 23	(Hudson et al., 2007)
Feline calicivirus	NO	20[a]	< 60	1.800	cotton surface	3.0	> 70%	< 23	(Hudson et al., 2007)
Feline calicivirus	NO	20[a]	< 60	1.800	carpet surface	4.0	> 70%	< 23	(Hudson et al., 2007)
Feline calicivirus – 2280	NO	20	18	0.706	glass surface	4.0 (b.d.)	80	room	(Cannon et al., 2013)
HCoV-229E Coronavirus	YES	120	1	0.235	face mask	3.0 (b.d.)	–	–	(Lee et al., 2020)[c]
Hepatitis A HM175/18 f	NO	5	3	0.030	raspberries (surface)	0.6	52	17	(Brié et al., 2018)
Herpes simplex-1, BC-CDC	YES	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
Herpes simplex-1, BC-CDC	YES	28[a]	60	2.500	glass surface	2.0	40	20	(Hudson et al., 2009)
Infectious bovine rhinotracheitis virus	YES	0.64	3960	4.780	aqueous layer	3.7		37	(Bolton et al., 1982)
Infectious canine hepatitis virus	NO	0.64	3960	4.780	aqueous layer	1.7		37	(Bolton et al., 1982)
Influenza A (WSN strain)	YES	0.64	1440	1.738	aqueous layer	3.0		37	(Bolton et al., 1982)
Influenza A H1N1 (A/PR/8/34)	YES	10	210	4.120	polystyrene petri dish	4.0	65	23–29	(Tanaka et al., 2009)
Influenza A H1N1 (A/PR/8/34)	YES	20	150	5.886	polystyrene petri dish	5.0	65	23–29	(Tanaka et al., 2009)
Influenza A H1N1 (A/PR/8/34)	YES	20	600	23.54	glass petri dish	5.0	65	23–29	(Tanaka et al., 2009)
Influenza A/WSN/33 H1/N1	YES	20	18	0.706	Tyvek ®	3.0	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	18	0.706	N95 Resp.	4.0 (b.d.)	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	face mask	3.0	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	face mask	2.4	40	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	Tyvek ®	1.4	40	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	N95 Resp.	1.0	40	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	50	40	3.924	face mask	3.5	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	50	40	3.924	Tyvek ®	3.5	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	50	40	3.924	N95 Resp.	3.5	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	5	0.196	Tyvek	3.4	53	48	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	bunny suit	2.8	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	PAPR Plastic	3.3	80	24	(Blanchard et al., 2020)[c]
Influenza A/WSN/33 H1/N1	YES	20	90	3.531	PAPR Fabric	3.3	80	24	(Blanchard et al., 2020)[c]
Influenza H3N2	YES	20[a]	60	1.800	polystyrene	2.6	70	room	(Hudson et al., 2009)
Influenza H3N2	YES	20[a]	60	1.800	polystyrene	0.1	38	room	(Hudson et al., 2009)
Influenza H3N2	YES	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
MS2, ATCC 15597-B1	NO	0.6–1.2	< 80	0.105	gelatin	2.0	85		(Tseng and Li, 2008)
MS2, ATCC 15597-B1	NO	0.6–1.2	< 80	0.200	gelatin	2.0	55		

(*continued on next page*)

Table 2 (*continued*)

INACTIVATION OF VIRUS ON SURFACES BY GAS OZONE									
Virus	Enveloped?	[O_3] [ppm]	Time [min]	*CT*[d] (mg L^{-1} min)	Medium (virus suport)	log_{10} Reduction	RH %	T [°C]	Ref.
									(Tseng and Li, 2008)
Murine coronavirus	YES	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
Murine Hepatitis Virus	YES	300	60	35.315	glass (dry s.)	3.0	80	20–23	(Sato et al., 1990)
murine norovirus	NO	20	18	0.706	glass surface	4.0 (b.d.)	80	room	(Cannon et al., 2013)
Murine Norovirus-1	NO	41	10	0.800	1 mL water in weighting boats	4.1		25	(Predmore et al., 2015)
Murine Norovirus-1	NO	41	10	0.800	strawberries	3.3		25	(Predmore et al., 2015)
Murine Norovirus-1	NO	41	40	3.200	strawberries (inside)	1.5		25	(Predmore et al., 2015)
Murine Norovirus-1	NO	41	30	2.400	lettuce	2.7		25	(Predmore et al., 2015)
Murine Norovirus-1 S99	NO	3.0	1	0.006	raspberries	3.3 (b.d.)	52	17	(Brié et al., 2018)
Murine Norovirus-1 S99	NO	1.0	3	0.006	raspberries	1.8	52	17	(Brié et al., 2018)
P22 bacteriophage ATCC® 19585 -B1™	NO?	25	150	7.357	N95 Resp.	6.4		room	(Dave et al., 2020)[c]
Poliovirus	NO	0–28[a]	60	2.500	glass surface	2.0	40	20	(Hudson et al., 2009)
Poliovirus	NO	0–20[a]	60	1.800	polystyrene	2.9	70	room	(Hudson et al., 2009)
Poliovirus	NO	0–20[a]	60	1.800	polystyrene	0.0	38	room	(Hudson et al., 2009)
Poliovirus	NO	0–20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
Poliovirus type I Sabin vaccine	NO	0.64	3960	4.780	aqueous layer	0.0		37	(Bolton et al., 1982)
Reo type 3 virus	NO	300	240	141.25	plastic (wet s.)	3.0	80	20–23	(Sato et al., 1990)
Respiratory syncytial virus A2	YES	20	40	1.570	Tyvek ®	4.0 (b.d.)	80	24	(Blanchard et al., 2020)[c]
Respiratory syncytial virus A2	YES	20	40	1.570	N95 Resp.	4.0 (b.d.)	80	24	(Blanchard et al., 2020)[c]
Rhinovirus 1A and 14	NO	28[a]	60	2.500	glass surface	2.0	40	20	(Hudson et al., 2009)
Rhinovirus 1A and 14	NO	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
SARS-CoV-2	YES	10000	0.500	9.810	PPE gown	[b] RNA undetected	53–65	22	(Clavo et al., 2020)
SARS-CoV-2	YES	4000	0.500	3.924	face mask	[b] RNA undetected	53–65	22	(Clavo et al., 2020)
SARS-CoV-2	YES	4–6.5	30	0.324	PPE gown	[b] RNA undetected	99	22	(Clavo et al., 2020)
SARS-CoV-2	YES	4–6.5	50	0.540	face mask	[b] RNA detected	99	22	(Clavo et al., 2020)
SARS-CoV-2 (JPN/TY/WK-521)	YES	6	55	0.647	stainless steel	3.3	60–80	25	(Yano et al., 2020)
Sendai virus	YES	200	30	11.77	glass (dry s.)	3.7	80	20–22	(Sato et al., 1990)
Sendai virus	YES	200	180	70.62	glass (dry s.)	0.9	50	20–23	(Sato et al., 1990)
Sendai virus	YES	300	60	35.31	plastic (wet s.)	3.0	80	20–23	(Sato et al., 1990)
Sindbis virus	NO	20[a]	60	1.800	plastic	3.8	40–95	room	(Hudson et al., 2009)
T7, ATCC 11303-B1	NO	0.6–1.2	< 120	0.190	gelatin	2.0	85		(Tseng and Li, 2008)
T7, ATCC 11303-B1	NO	0.6–1.2	< 120	0.230	gelatin	2.0	55		(Tseng and Li, 2008)
Theilers' Murine encephalomielitis virus	NO	100	180	35.31	glass (dry s.)	3.2	80	20–23	(Sato et al., 1990)
Theilers' Murine encephalomielitis virus	NO	200	180	70.62	glass (dry s.)	3.5 (b.d.)	80	20–23	(Sato et al., 1990)
Theilers' Murine encephalomielitis virus	NO	300	180	105.94	glass (dry s.)	4.2	80	20–23	(Sato et al., 1990)
Theilers' Murine encephalomielitis virus	NO	300	120	70.62	plastic (wet s.)	3.0	80	20–22	(Sato et al., 1990)
Tulane virus	NO	40.8	40	3.200	1 mL water in weighting boats	4.2		25	(Predmore et al., 2015)
Tulane virus	NO	40.8	30	2.400	strawberries	4.2		25	(Predmore et al., 2015)
Tulane virus	NO	40.8	40	3.200	strawberries (inside)	1.8		25	(Predmore et al., 2015)
Tulane virus	NO	40.8	10	0.800	lettuce	2.3		25	(Predmore et al., 2015)

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Table 2 (*continued*)

INACTIVATION OF VIRUS ON SURFACES BY GAS OZONE									
Virus	Enveloped?	[O_3] [ppm]	Time [min]	CT[d] (mg L^{-1} min)	Medium (virus suport)	log_{10} Reduction	RH %	T [°C]	Ref.
Vaccinia virus	YES	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
Vesicular stomatitis virus	YES	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
Vesicular stomatitis virus	YES	0,64	1320	1.593	aqueous layer	4.0		37	(Bolton et al., 1982)
Yellow fever virus	YES	20[a]	60	1.800	undetermined	3.0	40–95	room	(Hudson et al., 2009)
φ X174 ATCC 13706-B1	NO	0.6–1.2	< 40	0.066	gelatin	2.0	55		(Tseng and Li, 2008)
φ X174 ATCC 13706-B1	NO	0.6–1.2	< 40	0.053	gelatin	2.0	85		(Tseng and Li, 2008)
φ6 ATCC 21781-B1	YES	0.6–1.2	< 40	0.059	gelatin	2.0	55		(Tseng and Li, 2008)
φ6 ATCC 21781-B1	YES	0.6–1.2	< 40	0.050	gelatin	2.0	85		(Tseng and Li, 2008)

[a] [O_3] starting at zero, ozonator was switched on at t = 0 and [O_3] was increased for about 15' during the experiment up to the desired concentration value, showed in the table.
[b] Virus presence was determined by RT-PCR so quantification was not possible and only virus detection or not detection is provided.
[c] These references refer to preprints articles and have not been per-reviewed yet.
[d] CT calculated considering pressure = 1 atm and temperature the one shown in column "T". If T was not provided, it was considered 25 °C

that viruses are expected to be inactivated by O_3 mainly by protein capsid damage rather than genome degradation. However, there is strong experimental evidence that Poliovirus 1 inactivation by ozone mainly takes place through genome damage (Jiang et al., 2019; Roy et al., 1981) after diffusion of the ozone molecules through the capsid (Roy et al., 1982, 1981). This indicates that virus component information alone cannot provide a real description of the mechanisms of viral inactivation. Besides composition, other factors should be taken into account. The influence of higher levels of molecular organization (e.g., the virus structure) is one of them, as it determines the orientation of the biomolecules and thus oxidant accessibility to the potential viral targets (Wigginton and Kohn, 2012). Besides, ensuing alterations of the components do not necessary lead to viral inactivation. For example, it was noted that two different capsid proteins of Poliovirus 1 were modified upon ozonation, but the observed viral inactivation was instead mainly attributed to genome damage (Roy et al., 1981), since the modified proteins did not take part in the virus-cell attachment stage, a fundamental step in the viral life cycle. Therefore, it can be concluded that these three factors, composition, structure and function, dictate the mechanisms of virus disinfection by ozone. Any difference in any of these factors, even if viruses that belong to the same species, and only differ in their strain, may lead to different disinfection mechanisms and therefore different susceptibility to ozone (Hirneisen et al., 2010; Meister et al., 2018; Roy et al., 1982; Sigstam et al., 2013; Torii et al., 2020; Wigginton and Kohn, 2012; Wolf et al., 2018). In addition, the contribution of secondary reactive species generated as a consequence of direct ozone reactions with chemical subunits, and the potential for viral damage repair (by taking advantage of host repair enzymes, for example), may also play a role in the inactivation mechanism (Kim et al., 1980; Mayer et al., 2015; Shinriki et al., 1988; von Sonntag and von Gunten, 2012).

During the last five decades, several research groups have attempted to experimentally determine the ozone inactivation mechanisms of several viruses. In this regard, most of the available mechanistic information come from studies with non-enveloped viruses. Pioneering works back in the seventies concluded that ozone attack to Poliovirus 2 (non-enveloped, single stranded (ss) RNA), damages the viral capsid (Riesser et al., 1976), together with a reduction of the ability of treated virus to penetrate host cells (Cronholm et al., 1976). The effect of ozone on the virus genome has also been found as responsible for viruses inactivation. de Mik and de Groot (1977) found that the bacteriophage ΦX174 (non-enveloped, ssDNA) was mainly inactivated via capsid damage, even though genome damage should also contribute to some extent. The loss of viability of bacteriophage f2 (non-enveloped, ssRNA), on its part, was suggested to occur by initial disruption of the attachment ability followed by a break of the capsid and release and damage of the genome (Kim et al., 1980). Similarly, the bacteriophage T4 (non-enveloped, dsDNA) (Sproul et al., 1982) and Poliovirus 1 (non-enveloped, ssRNA) (de Mik and de Groot, 1977; Martinelli et al., 2017; Wei et al., 2017), were found to be inactivated by a combination of both capsid and genome damage. All these experimental data agreed with the hypothesis that inactivation of this bacteriophage was rate-limited by diffusion of the ozone molecules through the capsid to the nucleic material (Roy et al., 1982, 1981). Some works bring more details on the inactivation mechanisms of non-enveloped viruses by ozone. Of special interest are some examples from the eighties with the tobacco mosaic virus (non-enveloped, ssRNA), which demonstrated that inactivation of this species took place preferentially by damaging the guanine moieties of its RNA (Shinriki et al., 1981). They showed also how degraded moieties cross-linked with both unaltered and altered amino acids on the viral capsid, disrupting this way the virus ability of uncoating and causing inactivation (Shinriki et al., 1988). In another work Norwalk Virus, Poliovirus 1 and the bacteriophage MS2 (all of them non-enveloped, ssRNA) inactivation was compared with genome damage measured by PCR and suggested that inactivation occurred very fast because of RNA alterations (Shin and Sobsey, 1998). On the other hand, other works studying Murine Norovirus, Tulane Virus (both non-enveloped and ssRNA) and Hepatitis A Virus (non-enveloped, ssRNA), demonstrated that damage to the capsid proteins hindered virus-host attachment, meanwhile RNA, although slightly altered, remained intact (Predmore et al., 2015; Wells et al., 1991). A work by Torrey et al. (2019) reported for the first time the use of transfection, a tool for the determination of the true genome functionality, instead of the classical PCR-based methods which do not provide an exact picture of it (Lim et al., 2010; Young et al., 2020). Its application to Echovirus 11 (non-enveloped, ssRNA) genome after ozone treatment allowed to 100% correlate its inactivation to alterations in the nucleic material (Torrey et al., 2019). Precisely in an attempt of improving PCR-based methods for the assessment of virus infectivity, Young et al. (2020) designed a PCR test to assess the damage of the whole genome and compared it to the inactivation of Coxsackievirus B5 and Echovirus 11 (both non-enveloped, ssRNA). They found that genome decay was about 6 times faster than viral inactivation, which suggested that more that multiple ozone reactions are required to cause inactivation events.

Table 3
list of viruses used in the 19 publications listed in Tables 1 and 2. Information about genome and particle size was obtained at (International Committee on Taxonomy of Viruses, 2020) and (Swiss Institute of Bioinformatics, 2020).

General virus designation	acronym	family	Enveloped?	times used in listed works	Significance	Genome	Particle size	References
Adenovirus	AV	Adenoviridae	NO	1	Representative adenoviruses	dsDNA 26–48 kB	icosahedral, 70–90 nm	(Hudson et al., 2009)
Coronavirus: HCoV-229E	HCoV	coronaviridiae	YES	1	Surrogate for SARS virus	ssRNA (+), 26–32 kB	pleomorphic, spherical or bacilliform 75–220 nm	(Lee et al., 2020)
Coronavirus: Murine	MCV	coronaviridiae	YES	1	Surrogate for SARS virus	ssRNA (+), 26–32 kB	pleomorphic, spherical or bacilliform 75–220 nm	(Hudson et al., 2009)
feline calicivirus	FCV	Caliciviridae	NO	3	Surrogate for human norovirus	ssRNA (+) 7.4–8.3 kB	icosahedral, 27–40 nm	(Cannon et al., 2013; Hudson et al., 2009)
Hepatitis A	VHA	Picornaviridae	NO	1	Relevant human pathogen	ssRNA (+) 6.7–10.1 kB	30–32 nm icosahedral virions comprising 60 protomers	(Brié et al., 2018)
Herpes simplex virus	HSV	Herpesviridae	YES	1	Representative herpes virus	dsDNA	150–200 nm in diameter, containing a T = 16 icosahedral capsid and glycoprotein spikes	(Hudson et al., 2009)
Infectious bovine rhinotracheitis virus	IBR	Herpesviridae	YES	1	Representative herpes virus	dsDNA	150–200 nm in diameter, containing a T = 16 icosahedral capsid and glycoprotein spikes	(Bolton et al., 1982)
Infectious canine hepatitis virus	ICH	Adenoviridae	NO	1	First adenovirus discovered	dsDNA 26–48 kB	icosahedral, 70–90 nm	(Bolton et al., 1982)
influenza A Virus	IAV	Orthomyxoviridae	YES	6	Representative human and aviar influenza virus	ssRNA (-) 10–14.6 kB	spherical or pleomorphic, 80–120 nm	(Blanchard et al., 2020; Bolton et al., 1982; Hudson et al., 2009; Kekez and Sattar, 1997; Tanaka et al., 2009)
levivirus MS2	MS2	Leviviridae	NO	4	Phage virus, well-known widely used model for norovirus	ssRNA (+) 3.6 kb	icosahedral and spherical geometry, and T = 3 symmetry. 26 nm approx.	(Dubuis et al., 2020; Kekez and Sattar, 1997; Tseng and Li, 2008, 2006)
Murine Hepatitis Virus	MHV	coronaviridiae	YES	1	Representative betacoronavirus, possible surrogate for SARS virus	ssRNA (+), 26–32 kB	pleomorphic, spherical or bacilliform 75–220 nm	(Sato et al., 1990)
Murine norovirus	MNV	Caliciviridae	NO	5	Surrogate for human norovirus	ssRNA (+) 7.4–8.3 kB	icosahedral, 27–40 nm	(Brié et al., 2018; Cannon et al., 2013; Dubuis et al., 2020; Hudson et al., 2007; Predmore et al., 2015)
P22	P22	Podoviridae	NO	1	Phage virus, well-known widely used	dsDNA 40–42 kB	head-tail structure, with a T = 7 laevo icosahedral symmetry. 60–65 m	(Dave et al., 2020)
Poliovirus	PV	Picornaviridae	NO	2	Enteric virus, causative of polio	ssRNA (+) 6.7–10.1 kB	30–32 nm icosahedral virions comprising 60 protomers	(Bolton et al., 1982; Hudson et al., 2009)
PR772	PR772	Tectiviridae	NO	1	Phage virus, model for adenovirus	linear dsDNA, 15 kb	icosahedral virion with a pseudo T = 25 symmetry.66–80 nm with apical spikes of 20 nm.	(Dubuis et al., 2020)
Reo type 3 virus	RT3	Reoviridae	NO	1		dsRNA virus, 18.2–30.5 kB	icosahedral virion, multilayer capsid structure, 60–80 nm	(Sato et al., 1990)
Respiratory syncytial virus A2	RSV	Pneumoviridae	YES	1	Human pathogen	ssRNA (-) 13–15 kB	spherical and filamentous virions, 150 nm	(Blanchard et al., 2020)
Rhinovirus types 1A & 14	RV	Picornaviridae	NO	1	Common cold virus, enterovirus	ssRNA (+) 6.7–10.1 kB	30–32 nm icosahedral virions comprising 60 protomers	(Hudson et al., 2009)
SARS-CoV-2		Coronaviridiae	YES	2	COVID19 responsible	ssRNA (+), 29.9 kB	spherical, some pleomorphism, 60–140 nm	(Clavo et al., 2020), (Yano et al., 2020)
Sendai virus	HVJ	Paramyxoviridae	yes	1				(Sato et al., 1990)

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Table 3 (*continued*)

General virus designation	acronym	family	Enveloped?	times used in listed works	Significance	Genome	Particle size	References
					Representative respirovirus	ssRNA (-) virus 15 kb	pleomorphic, but usually spherical or filamentous, > 150 nm	
Sindbis virus	SINV	Togaviridae	YES	1	Surrogate for hepatitis C virus	ssRNA (+) 10–12 kB	spherical, icosahedral with 80 spikes, 65–70 nm	(Hudson et al., 2009)
T7	T7	Autographiviridae	NO	2	phage virus, well-known widely used	dsDNA, 40–42 kB	icosahedral and Head-tail geometries, T = 7 symmetry. 60 nm	(Tseng and Li, 2008, 2006)
Theilers' Murine encephalomielitis virus	TMEV	Picornaviridae	NO	1	Murine model for studying encephalomyelitis	ssRNA (+) 6.7–10.1 kB	30–32 nm icosahedral virions comprising 60 protomers	(Sato et al., 1990)
Tulane virus	TV	Caliciviridae	NO	1	Surrogate for human norovirus	ssRNA (+) 7.4–8.3 kB	icosahedral, 27–40 nm	(Predmore et al., 2015)
Vaccinia virus	VV	Poxviridae	YES	1	Representative pox virus	dsDNA, 130–375 kB	brick-shaped or ovoid virion, 220–450 nm long and 140–260 nm wide	(Hudson et al., 2009)
Vesicular stomatitis virus	VSV	Rhabdoviridae	YES	3	Representative of Rhabdovirus	ssRNA (-) 11–15 kB	bullet shaped, 180 nm long and 75 nm wide	(Bolton et al., 1982; Hudson et al., 2009; Kekez and Sattar, 1997)
Yellow fever virus	YFV	Flaviviridae	YES	1	Surrogate for hepatitis C virus	ssRNA (+), 9–13 kB	spherical, 50 nm	(Hudson et al., 2009)
φ6	Φ6	Cystoviridae	YES	3	Well-known virus, phage with lipid membrane, influenza's surrogate	dsRNA 13.5 kB	spherical, 80 nm. Double icosahedral capsid, with spikes	(Dubuis et al., 2020; Tseng and Li, 2008, 2006)
φX174	φX175	Microviridae	NO	5	Common bacteriophage virus used, first DNA based genome to be sequenced	ssDNA (+) 4.4–6.1 kB	round shape, T = 1 icosahedral symmetry, 30 nm	(de Mik and de Groot, 1977; Dubuis et al., 2020; Tseng and Li, 2008, 2006; Vyskocil et al., 2020)

Although scarce, there are a few works addressing ozone inactivation mechanisms in enveloped viruses. Wells et al. (1991) studied the ozone inactivation of the Human Immunodeficiency Virus (enveloped, ssRNA) and suggested viral particle disruption, reverse transcriptase inactivation and perturbation of the ability of the virus to attach to the host cell as the probable causes for inactivation. In a more recent study conducted by Murray et al. (2008), several enveloped viruses were subjected to ozonation and malondialdehyde was detected in the medium during the process. Being that species a common byproduct of lipid and protein peroxidation, it was concluded that enveloped viruses are inactivated by membrane or capsid damage. Microscopy images also revealed disruption in the morphology of these structures.

Taking into account all this information, it can be inferred that inactivation of non-enveloped viruses by O_3 is the result of genome and/or capsid alterations at varying weightings, which in most cases still need to be experimentally clarified. Regarding enveloped species, the few available studies point to alterations in the membrane lipids and proteins as the most probable causes for virus inactivation (Murray et al., 2008; Wells et al., 1991), being in general less resistant than non-enveloped viruses to oxidation (Bolton et al., 1982; Hall and Sobsey, 1993; Herbold et al., 1989; Hudson et al., 2009; Murray et al., 2008; Sato et al., 1990; Tseng and Li, 2008, 2006). This would mean that viruses such as the SARS-CoV-2, which has a lipidic envelope containing several proteins taking part of different essential functions, would be rapidly inactivated by the attack of ozone on these viral components. Based on quantum chemical calculations, a recent study provides theoretical insights on the exact proteinic and lipidic subunits of the SARS-CoV-2 envelope potentially attacked by O_3 (Tizaoui, 2020). Another 2020 study, on the basis of experiments with model systems, also concludes that degradation and destabilization of the phospholipid compounds of the viral envelope may be a significant route for coronaviruses inactivation (Cataldo and Ori, 2020). A schematic representation of the mentioned inactivation mechanisms for enveloped and non-enveloped viruses is displayed in Fig. 1.

3.2. Main results and comparison of references

19 works have been found to fulfill the described requirements in their study of the inactivation of viruses in aerosols and fomites by means of ozone in gas-phase. This number was expected to be higher due to the relevant interest of the topic and the well-known effectivity of O_3 as disinfectant in aqueous phase (Hirneisen et al., 2010; Khadre et al., 2001). Table 1 and Table 2 summarize the main results of these publications. The papers working with viruses present on **aerosols** (Table 1) focused on confirming the ozone efficacy as general disinfectant and determining the optimal application conditions (Dubuis et al., 2020; Tseng and Li, 2006; Vyskocil et al., 2020) and defining the ozone attack mechanism (de Mik and de Groot, 1977). Noteworthy was the work carried out by Dubuis et al. (2020), who proposed using ozone at low concentration for disinfecting air ducts or rooms. Authors studied the effectiveness of ozone for inactivating four types of phages and a norovirus at low ozone doses and different humidity. They achieved up to 3–4 $\log_{10}$ reduction in all viruses tested with exposures between 0.023 and 0.158 mg L^{-1} min. Similar work was done by Tseng and Li (2006), who studied the effects of ozone concentration, contact time, different capsid architecture and relative humidity on inactivating four airborne bacteriophage viruses. Authors found that ozone was effective for all the tested viruses. Survival fraction of viruses decreased exponentially with ozone dose, being for 90% of inactivation 2 times higher than that for 99%. For all viruses, the required ozone dose at 85% relative humidity (RH) was lower than that at 55%.

It is noticeable that, in aerosol disinfection, most of the evaluated viruses are model bacteriophages and very few human pathogens or their surrogates (i.e., representative similar species) have been tested.

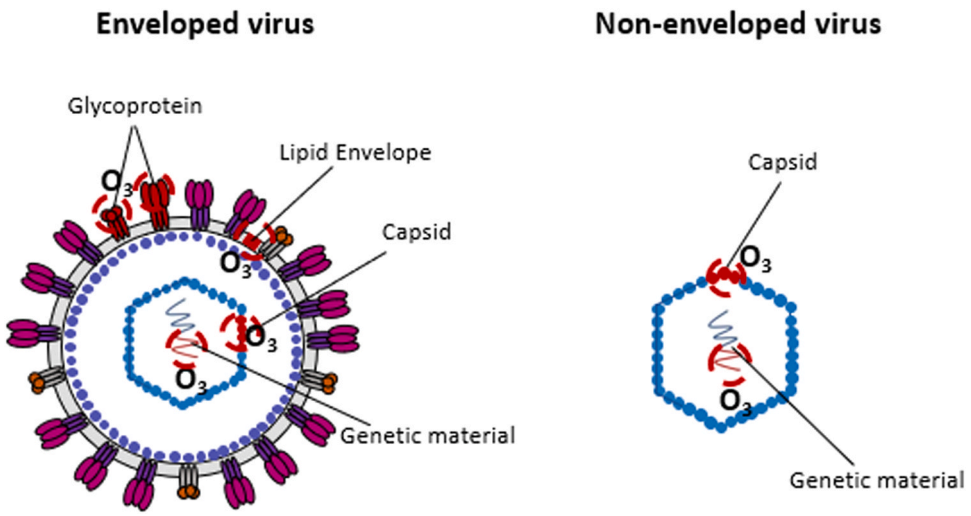

Fig. 1. Inactivation mechanisms of enveloped and non-enveloped viruses by ozone.

Regarding to this medium, most publications worked with deionized water or just adding some buffer. Only one reported publication (Kekez and Sattar, 1997) studied the effect of the nature of aerosols. Authors worked nebulizing virus in water and in water with bovine serum up to 25% in volume. They found that with this last media, the required CT for achieving 3 $\log_{10}$ inactivation was much higher (from 0.007 to 0.376 mg L^{-1} min). It would be necessary to carry out more research in these two topics.

At the time the present review was written, only two studies were found that directly worked with SARS-CoV-2 (Table 2). Clavo et al. (2020), tested the ozone capacity for eliminating SARS-CoV-2 from PPE gown and face masks at different ozone concentration, time and humidity. Authors determined the presence of virus by RT-PCR so it was only possible to determine the presence or absence of virus genetic material but not to quantify the virus inactivation. They concluded that ozonation was a viable technology for disinfecting PPE since with CT values of 9.8 mg L^{-1} min (or lower for 99% of relative humidity) virus genetic material was not even detected. Other work by Yano et al. (2020) presented a brief study that reflected that 6 ppm of ozone and 55 min could inactivate up to 3.3 $\log_{10}$. Both studies concluded that ozone could satisfactorily remove SARS-CoV-2.

Among the rest of works focused on the study of the efficacy of ozone for inactivating viruses from surfaces or fomites (Table 2), three of them have been also recently published as a response to the COVID-19 sanitary emergency, studying the disinfection of personal protective equipment (PPE) for its safe reuse (Blanchard et al., 2020; Dave et al., 2020; Lee et al., 2020). Different types of viruses (most of them enveloped and close to SARS-CoV-2) were tested on different PPE: face masks (Blanchard et al., 2020; Lee et al., 2020), Tyvek fabric (employed in disposable gowns and PAPR hoods) (Blanchard et al., 2020) and N95 face respirators (Blanchard et al., 2020; Dave et al., 2020). Additionally, the authors of the three reports examined if the materials had undergone changes after ozone treatment and in all cases their protective function was unaffected, being only the elastic straps altered after treatment. However, due to the wide range of different PPE and materials, extensive study of these issues should be carried out.

Of special interest was the type of study published by Hudson et al. (2007, 2009). The authors carried out disinfection tests in an office, a hotel room and a cruise cabin using a gas ozone mobile commercial apparatus. In those cases, after the treatment, leaving the room with O_3 concentrations below the dangerous exposure limit for people is absolutely crucial due to the ozone toxicity. The Short-Term Exposure Limit (STEL) is considered 0.30 ppm (0.60 mg m^3) while the value for Transitional Limit (TWA) is 0.10 ppm (0.20 mg m^3) (OSHA). Further works like this one are required to ensure the efficacy of different ozonators to disinfect rooms and indoor premises from a practical point of view.

Finally, there were also two publications which dealt with the disinfection of food surfaces (Brié et al., 2018; Predmore et al., 2015). Actually, several works in literature have been found focusing on the direct disinfection of food using ozone. Nevertheless, most of them applied ozone in the aqueous phase and are not included in this review (Arévalo Camargo et al., 2019; Crowe et al., 2012; Hirneisen et al., 2011; Ianni et al., 2019; Kim et al., 1999; Mahapatra et al., 2005; Naito and Takahara, 2006; Nayak et al., 2020; Sproul et al., 1982).

The results of the studies summarized in Tables 1 and 2 proved ozonation as an effective process against the viruses tested, achieving in several studies inactivation near to 3 $\log_{10}$ when optimal conditions of humidity were applied. Only Infectious Canine Hepatitis (ICH) was not inactivated satisfactorily, as reported in just one publication (Bolton et al., 1982). Most of the works with a limited virus inactivation (2 $\log_{10}$ or less) are those in which the CT values were significantly lower than in the rest of the studies. That is the situation of the works by Brié et al. (2018), Tseng and Li (2006, 2008) or Vyskocil et al. (2020), in which the exposure to ozone was approximately one order of magnitude lower than the average value tested successfully by the rest of authors. It is therefore very likely that the disinfection values in those cases would increase significantly by increasing the contact time or the ozone concentration. Despite these examples, it can be concluded that gas-phase ozonation is a viable technology for disinfection of airborne aerosols and fomites.

Regarding the virus experimentally tested and gathered in Table 3, it is worth noting that at the time this review was written there were already two publications in which ozone was tested against the responsible agent for current pandemic, that is, SARS-CoV-2 and it was proven effective (Clavo et al., 2020; Yano et al., 2020). Furthermore, there were also three works (Blanchard et al., 2020; Hudson et al., 2009; Lee et al., 2020) which proposed 4 different surrogates for this species (influenza A virus, respiratory syncytial virus, murine coronavirus and HCoV-229E) against which ozone was again operative. Likewise, there was a fourth study (Sato et al., 1990) with murine hepatitis virus, which is a strain of murine coronavirus and probably the most studied coronavirus prior to the discovery of SARS-CoV, pointing also towards gas-phase O_3 positive inactivation. Therefore, and according to current evidences, it could be concluded that gaseous ozone is effective for disinfecting SARS-CoV-2. Besides, one of the main characteristics of SARS-CoV-2 and its surrogates, the enveloping capsid, seems to be damaged in all the presented studies by gaseous O_3, this enables to conclude that this maybe the main mechanism for inactivation, rather

than genome alterations, as it was also pointed out in literature (Brié et al., 2018; Lee et al., 2020).

3.3. Operation parameters applied for inactivation of viruses

An important aspect for a successful application of ozone as virucidal agent is to determine the optimal operation conditions to achieve a satisfactory inactivation of viruses. Some of the listed publications studied the role of CT, relative humidity and virus matrix, which affect the process the most, as previously mentioned. These conditions are listed in Tables 1 and 2.

Figs. 2 and 3 show CT value versus the virus inactivation in aerosols (from 4 publications (de Mik and de Groot, 1977; Dubuis et al., 2020; Kekez and Sattar, 1997; Tseng and Li, 2006)) and on surfaces (from 10 publications: (Blanchard et al., 2020; Cannon et al., 2013; Dave et al., 2020; Hudson et al., 2007, 2009; Lee et al., 2020; Predmore et al., 2015; Tanaka et al., 2009; Tseng and Li, 2008; Yano et al., 2020)) according to full data in Tables S1 and S2 respectively. Only those experiments with a RH between 65%–95% (near optimal conditions) and inactivation quantification by plaque forming units (PFU) assay or the tissue culture infective dose have been included. In Table 2, data corresponding to tests in which viruses were suspended on aqueous solutions have been discarded. As mentioned before, results based on the quantitative polymerase chain reaction (qPCR) were discarded since this technique does not measure infectivity and may underestimate the effectiveness of the virucidal agent (Hudson et al., 2007).

Regarding to the inactivation of viruses in aerosols, Fig. 2 shows how very low CT values, above 0.1–0.4 mg L^{-1} min, achieve about 3–4 log_{10} of virus reduction. Furthermore, different works results are quite consistent, and a relatively small diversity of experimental data is observed, in spite of the different experimental conditions. Fig. 2 distinguishes also between disinfection of enveloped and non-enveloped viruses. No difference is observed in the disinfection capacity of ozone to eliminate both types of virus. This fact is quite remarkable since, as it has been previously commented, it is generally accepted that enveloped viruses are more sensitive to chemical treatments. It is noteworthy since, as stated previously, even small differences in the virus conformation can lead to important different sensitivities to ozone. A possible explanation is that both types of virus actually present a different sensitivity to ozone (as expected and cited in literature) but this difference is not large enough and is masked by the other aspects that can generate

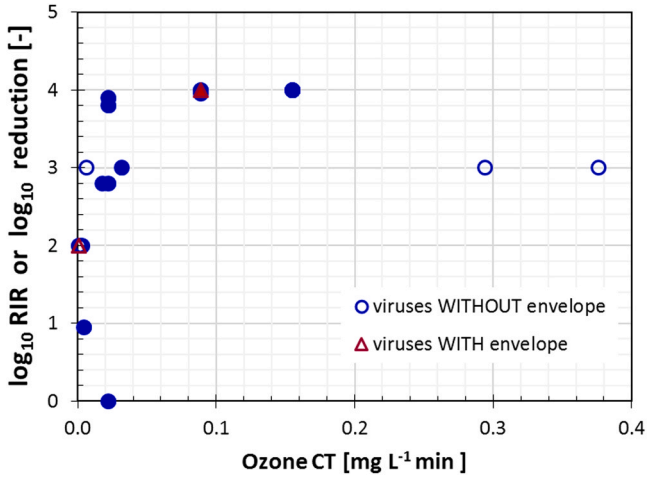

Fig. 2. CT value vs log_{10} virus reduction for aerosols disinfection (empty dots) or vs log_{10} Relative Infectious Ratio (RIR) (solid dots). RIR was calculated by dividing mean culture counts (PFU mL^{-1}) with mean qPCR values (genomes mL^{-1}) and normalized as proposed by Dubuis et al. (2020). Figure shows those experiments from Table S1 in Supplementary Material (extended version), with a relative humidity between 65%– 95% (26 points from 4 publications).

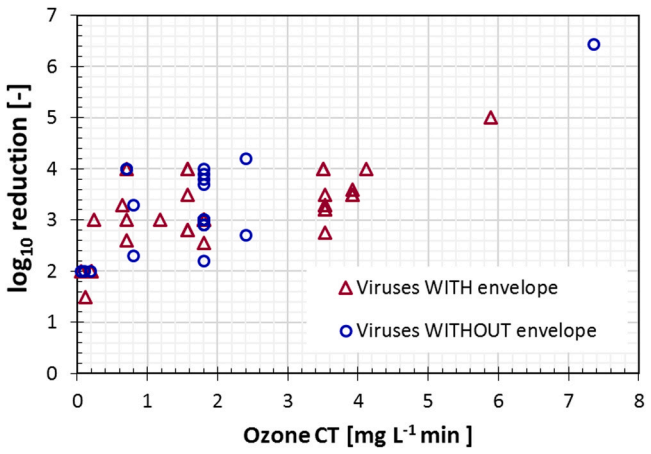

Fig. 3. *CT value* vs *log_{10}* virus reduction for surfaces disinfection. It shows those experiments from Table S2 in Supplementary Material (extended version), with a relative humidity higher than 65% and virus deposited in solid matrixes and moderate ozone exposure (CT < 10 mg L^{-1} min) (59 points from 10 publications).

diversity in the results: different experimental devices and conditions, volume or initial virus concentration inoculated, suspension medium, etc. All these factors can influence the ability or rate of ozone to penetrate into a particle, influencing in the CT value. Due to the small amount of data concerning gas-phase studies, further research should be carried out to clarify this point.

In Fig. 3, the required values of CT for the disinfection of surfaces present a greater diversity. Despite this, it seems that by increasing the CT values, the effectiveness of the process increases. Although it is difficult to be conclusive and propose a narrow threshold of CT values that ensures a general inactivation of viruses, it seems that between 1 and 4 mg L^{-1} min may guarantee an inactivation of 3–4 log_{10}. Again, surprisingly the effectivity of ozone against enveloped or non-enveloped viruses seems to be similar, despite the great diversity observed and in spite of colleting much more data about viruses with envelope than in the previous case. Thus, same reasons pointed out for aerosol disinfection can be also valid here and further research is recommended to clarify this point.

When Figs. 2 and 3 are compared, an important difference is observed, together with significant differences in data variability: CT values required for achieving a 3–4 log_{10} disinfection of surfaces seems to be almost one order of magnitude higher than the CT value required for achieving the same disinfection level in aerosols. In the case of surfaces disinfection, the access of ozone molecules to viruses is more difficult and depends on a higher number of variables: CT values, room humidity, the type of material, as well as surface properties such as roughness, sample shape, position, thickness and moisture. Conversely, the key variables in aerosol disinfection studies are mainly three: CT value, ambient humidity and the droplet size. Thus, the environment and accessibility to viruses is probably much more homogenous between the studies working with aerosols. Furthermore, the surface area of aerosols exposed to ozone should be much higher. Ozone may access and attack those viruses contained in those small liquid particles in an easier way than those in hard surfaces, requiring a lower CT value. These facts could explain both main differences pointed between Figs. 2 and 3. However, the low number of experiments found, especially with aerosols, makes it difficult to present these conclusions categorically. Again, further research is needed to confirm these trends.

Another key value to consider and presented in the tables is the relative humidity. Several authors have studied it and the conclusions are quite homogeneous and can be quickly corroborated in Table 2: best inactivation rates are achieved at relative humidity between 70% and 90%, at room temperature. Lower humidity requires higher oxidant

exposure values and, in several studies, it is even impossible to achieve acceptable disinfection rates at humidity below 50–40% (Blanchard et al., 2020; Dubuis et al., 2020; Hudson et al., 2009; Sato et al., 1990; Tseng and Li, 2008, 2006; Vyskocil et al., 2020). This optimal RH are easy to achieve in a closed chamber. However, for the disinfection of ventilation ducts or rooms, produce such high and steady RH values may be difficult and may lead to mold growth or even damage to buildings, so it would be necessary to work at a compromise value.

Some reported publications also tested the effect of the type of materials (glass, plastics, textiles, gelatin, food or PPEs) that supports viruses on the disinfection. Ozone has proved to be effective with all the assayed materials although with considerable diversity in the results and the CT required. For instance, Cannon et al. (2013) studied the inactivation on glass of two viruses as surrogates for norovirus and concluded that low O_3 exposures (0.706 mg L^{-1} min) were required to obtain about 5 log_{10} reduction. Similar results were found by Hudson et al. (2009) in a study with more than 12 viruses tested on the same surface. Nevertheless, Tanaka et al. (2009) and Sato et al. (1990), who inactivated various types of RNA virus, reported the requirement of much higher ozone exposures than other authors. The inactivation of viruses on polystyrene surface was studied by Tanaka et al. (2009) and Hudson et al. (2009), obtaining good viral reductions in both cases with low O_3 exposure. Plastic surface was also investigated in the works by Sato et al. (1990) and Hudson et al. (2009). Large differences of O_3 exposure to attain similar levels of viral inactivation were found between these two publications. Therefore, with the current data is difficult to justify the observed diversity and extract definitive conclusions. As mentioned along the text, diversity may be due to different aspects as the relative resistance of target organisms, the matrix that supports the virus or differences in the experimental set-up and ozonation procedure. More systematic research should be conducted to answer this question.

4. Conclusions

In response to the current COVID-19 pandemic or potential future outbreaks, the present paper has reviewed existing publications focused on the application of gaseous ozone as a virucidal agent.

The route of inactivation of viruses by ozone has been extensively studied, although the mechanism remains unclear for non-enveloped viruses. According to the reported data, the inactivation of non-enveloped viruses is due to the damage caused by ozone in the capsid and/or the genome, at varying proportions, which in most cases still need to be experimentally clarified. For enveloped viruses, although only a few papers have studied the mechanisms of inactivation, there is greater agreement and the most common inactivation mechanisms proposed are the alterations that ozone generates in the lipids and proteins present in the membrane of these viruses.

Regarding the application of the ozone, 14 publications that study the disinfection of viruses on surfaces and 6 publications that evaluate the disinfection of airborne viruses have been found.

The published results show that ozone can be an effective disinfectant in the gas-phase, successfully inactivating (more than 3 log_{10}) up to 28 different viruses of the 29 tested, including SARS-CoV-2, according to 2 works which tested it satisfactorily. Ozone has proven to disinfect airborne viruses at very low concentrations, inactivating about 3–4 log_{10} from 0.1 to 0.4 mg L^{-1} min. Surface virus inactivation requires CT values an order of magnitude higher than airborne virus. Furthermore, when applied to surfaces, the results among publications are much more dispersed; placing the general disinfection threshold in CTs between 1 and 4 mg L^{-1} min. Relative humidity has turned out to be another key variable of the process. Many of the reviewed papers conclude that optimal process conditions require RH between 70% and 90%. Regarding the surfaces types, ozone has demonstrated to be effective in all the tested materials (plastic, glass, textile, gelatin…), although with important differences, depending on the study, about the required CTs. In the experimental data analyzed, no significant differences have been observed in the inactivation of enveloped and non-enveloped viruses by ozone.

According to all of above, ozonation appears to be a valid technology for the inactivation of viruses in fomites and suspended in the air, included SARS-CoV-2. However, the uncertainties in front of a widespread use of ozone gas for virus inactivation are still significant. Based on the results listed, the following aspects should be further investigated: the understanding of the inactivation mechanisms, the optimal application conditions, the effect of the matrices and media in the virus inactivation process, and the effects and damages on materials exposed to ozone. Likewise, it is necessary to carry out further research to test this technology in real applications and environments, especially for aerosols disinfection.

CRediT authorship contribution statement

Bernardí Bayarri: Conceptualization, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Alberto Cruz-Alcalde:** Investigation, Writing - original draft, Writing - review & editing. **Núria López-Vinent:** Investigation, Writing - original draft, Writing - review & editing, Visualization. **María M. Micó:** Investigation, Writing - original draft, Writing - review & editing. **Carme Sans:** Conceptualization, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.125658.

References

Alimohammadi, M., Naderi, M., 2020. Effectiveness of ozone gas on airborne virus inactivation in enclosed spaces: a review study. Ozone Sci. Eng. 1–11. https://doi.org/10.1080/01919512.2020.1822149.

Arévalo Camargo, J., Murray, K., Warriner, K., Lubitz, W., 2019. Characterization of efficacy and flow in a commercial scale forced air ozone reactor for decontamination of apples. LWT 113, 108325. https://doi.org/10.1016/j.lwt.2019.108325.

Blanchard, E.L., Lawrence, J.D., Noble, J.A., Xu, M., Joo, T., Ng, N.L., Schmidt, B.E., Santangelo, P.J., Finn, M.G.G., 2020. Enveloped virus inactivation on personal protective equipment by exposure to ozone, 2020.05.23.20111435 medRxiv. https://doi.org/10.1101/2020.05.23.20111435.

Bolton, D.C., Zee, Y.C., Osebold, J.W., 1982. The biological effects of ozone on representative members of five groups of animal viruses. Environ. Res. 27, 476–484. https://doi.org/10.1016/0013-9351(82)90102-5.

Brié, A., Boudaud, N., Mssihid, A., Loutreul, J., Bertrand, I., Gantzer, C., 2018. Inactivation of murine norovirus and hepatitis A virus on fresh raspberries by gaseous ozone treatment. Food Microbiol. 70, 1–6. https://doi.org/10.1016/j.fm.2017.08.010.

Cannon, J.L., Kotwal, G., Wang, Q., 2013. Inactivation of norovirus surrogates after exposure to atmospheric ozone. Ozone Sci. Eng. 35, 217–219. https://doi.org/10.1080/01919512.2013.771953.

Cataldo, F., Ori, O., 2020. On the action of ozone on phospholipids, a model compound of the external envelope of pericapsidic viruses like coronavirus. Part 1. Ozone Sci. Eng. 42, 478–491. https://doi.org/10.1080/01919512.2020.1816448.

Clavo, B., Córdoba-Lanús, E., Rodríguez-Esparragón, F., Cazorla-Rivero, S.E., García-Pérez, O., Piñero, J.E., Villar, J., Blanco, A., Torres-Ascensión, C., Martín-Barrasa, J. L., González-Martin, J.M., Serrano-Aguilar, P., Lorenzo-Morales, J., 2020. Effects of

ozone treatment on personal protective equipment contaminated with sars-cov-2. Antioxidants 9, 1–10. https://doi.org/10.3390/antiox9121222.
Cronholm, L.S., Mccammon, J.R., Fleischman, M., Reisser, V., 1976. Enteric Virus Survival in Package Plants and the Upgrading of the Small Treatment Plants Using Ozone.
Crowe, K.M., Skonberg, D., Bushway, A., Baxter, S., 2012. Application of ozone sprays as a strategy to improve the microbial safety and quality of salmon fillets. Food Control 25, 464–468. https://doi.org/10.1016/j.foodcont.2011.11.021.
Dave, N., Pascavis, K.S., Patterson, J.M., Kozicki, M., Wallace, D.W., Chowdhury, A., Abbaszadegan, M., Alum, A., Herckes, P., Zhang, Z., Chang, J., Ewell, C., Smith, T., Naufel, M., 2020. Characterization of a novel, low-cost, scalable ozone gas system for sterilization of N95 respirators and other COVID-19 related use cases, 2020.06.24.20139469 medRxiv. https://doi.org/10.1101/2020.06.24.20139469.
Dubuis, M.-E.E., Dumont-Leblond, N., Laliberté, C., Veillette, M., Turgeon, N., Jean, J., Duchaine, C., 2020. Ozone efficacy for the control of airborne viruses: bacteriophage and norovirus models. PLoS One 15, e0231164. https://doi.org/10.1371/journal.pone.0231164.
Farooq, S., Akhlaque, S., 1983. Comparative response of mixed cultures of bacteria and virus to ozonation. Water Res. 17, 809–812. https://doi.org/10.1016/0043-1354(83)90076-3.
Hall, R.M., Sobsey, M.D., 1993. Inactivation of hepatitis A virus and MS2 by ozone and ozone-hydrogen peroxide in buffered water. Water Sci. Technol. 27, 371–378. https://doi.org/10.2166/wst.1993.0377.
Herbold, K., Flehmig, B., Botzenhart, K., 1989. Comparison of ozone inactivation, in flowing water, of hepatitis A virus, poliovirus 1, and indicator organisms. Appl. Environ. Microbiol. 55, 2949–2953. https://doi.org/10.1128/aem.55.11.2949-2953.1989.
Hirneisen, K.A., Black, E.P., Cascarino, J.L., Fino, V.R., Hoover, D.G., Kniel, K.E., 2010. Viral inactivation in foods: a review of traditional and novel food-processing technologies. Compr. Rev. Food Sci. Food Saf. 9, 3–20. https://doi.org/10.1111/j.1541-4337.2009.00092.x.
Hirneisen, K.A., Markland, S.M., Kniel, K.E., 2011. Ozone inactivation of norovirus surrogates on fresh produce. J. Food Prot. 74, 836–839. https://doi.org/10.4315/0362-028X.JFP-10-438.
Hudson, J.B., Sharma, M., Petric, M., 2007. Inactivation of norovirus by ozone gas in conditions relevant to healthcare. J. Hosp. Infect. 66, 40–45. https://doi.org/10.1016/j.jhin.2006.12.021.
Hudson, J.B., Sharma, M., Vimalanathan, S., 2009. Development of a practical method for using ozone gas as a virus decontaminating agent. Ozone Sci. Eng. 31, 216–223. https://doi.org/10.1080/01919510902747969.
Ianni, A., Grotta, L., Martino, G., 2019. Feeding influences the oxidative stability of poultry meat treated with ozone. Asian-Australas. J. Anim. Sci. 32, 874–880. https://doi.org/10.5713/ajas.18.0520.
International Committee on Taxonomy of Viruses, 2020. The 10th ICTV Report on Virus Classification and Taxon Nomenclature [WWW Document]. URL https://talk.ictvonline.org/. Accessed 12 March 2020.
Jiang, H.J., Chen, N., Shen, Z.Q., Yin, J., Qiu, Z.G., Miao, J., Yang, Z.W., Shi, D.Y., Wang, H.R., Wang, X.W., Li, J.W., Yang, D., Jin, M., 2019. Inactivation of poliovirus by ozone and the impact of ozone on the viral genome. Biomed. Environ. Sci. 32, 324–333. https://doi.org/10.3967/bes2019.044.
Kekez, M.M., Sattar, S.A., 1997. A new ozone-based method for virus inactivation: preliminary study. Phys. Med. Biol. 42, 2027–2039. https://doi.org/10.1088/0031-9155/42/11/002.
Khadre, M.A., Yousef, A.E., Kim, J.G., 2001. Microbiological aspects of ozone applications in food: a review. J. Food Sci. 66, 1242–1252. https://doi.org/10.1111/j.1365-2621.2001.tb15196.x.
Kim, C.K., Gentile, D.M., Sproul, O.J., 1980. Mechanism of ozone inactivation of bacteriophage f2. Appl. Environ. Microbiol. 39, 210–218. https://doi.org/10.1128/aem.39.1.210-218.1980.
Kim, J.G., Yousef, A.E., Dave, S., 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. 62, 1071–1087. https://doi.org/10.4315/0362-028x-62.9.1071.
Kutter, J.S., Spronken, M.I., Fraaij, P.L., Fouchier, R.A., Herfst, S., 2018. Transmission routes of respiratory viruses among humans. Curr. Opin. Virol. 28, 142–151. https://doi.org/10.1016/j.coviro.2018.01.001.
Lednicky, J.A., Lauzardo, M., Fan, Z.H., Jutla, A., Tilly, T.B., Gangwar, M., Usmani, M., Shankar, S.N., Mohamed, K., Eiguren-Fernandez, A., Stephenson, C.J., Alam, M.M., Elbadry, M.A., Loeb, J.C., Subramaniam, K., Waltzek, T.B., Cherabuddi, K., Morris, J. G., Wu, C.-Y., 2020. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. Int. J. Infect. Dis. 100, 476–482. https://doi.org/10.1016/j.ijid.2020.09.025.
Lee, J., Bong, C., Bae, P.K., Abafog, A.T., Baek, S.H., Shin, Y.-B., Park, M.S., Park, S., 2020. Fast and easy disinfection of coronavirus-contaminated face masks using ozone gas produced by a dielectric barrier discharge plasma generator, 2020.04.26.20080317 medRxiv. https://doi.org/10.1101/2020.04.26.20080317.
Li, C.-S.S., Wang, Y.-C.C., 2003. Surface germicidal effects of ozone for microorganisms. Am. Ind. Hyg. Assoc. J. 64, 533–537. https://doi.org/10.1080/15428110308984851.
Lim, M.Y., Kim, J.M., Lee, J.E., Ko, G., 2010. Characterization of ozone disinfection of murine norovirus. Appl. Environ. Microbiol. 76, 1120–1124. https://doi.org/10.1128/AEM.01955-09.
Liu, Y., Li, T., Deng, Y., Liu, S., Zhang, D., Li, H., Wang, X., Jia, L., Han, J., Bei, Z., Li, L., Li, J., 2021. Stability of SARS-CoV-2 on environmental surfaces and in human excreta. J. Hosp. Infect. 107, 105–107. https://doi.org/10.1016/j.jhin.2020.10.021.
Mahapatra, A.K., Muthukumarappan, K., Julson, J.L., 2005. Applications of ozone, bacteriocins and irradiation in food processing: a review. Crit. Rev. Food Sci. Nutr. 45, 447–461. https://doi.org/10.1080/10408390591034454.
Martinelli, M., Giovannangeli, F., Rotunno, S., Trombetta, C.M., Montomoli, E., 2017. Water and air ozone treatment as an alternative sanitizing technology. J. Prev. Med. Hyg. 58, E48–E52.
Mayer, B.K., Yang, Y., Gerrity, D.W., Abbaszadegan, M., 2015. The impact of capsid proteins on virus removal and inactivation during water treatment processes. Microbiol. Insights 8s2, MBI.S31441. https://doi.org/10.4137/mbi.s31441.
Meister, S., Verbyla, M.E., Klinger, M., Kohn, T., 2018. Variability in disinfection resistance between currently circulating enterovirus B serotypes and strains. Environ. Sci. Technol. 52, 3696–3705. https://doi.org/10.1021/acs.est.8b00851.
de Mik, G., de Groot, I., 1977. Mechanisms of inactivation of bacteriophage σX174 and its DNA in aerosols by ozone and ozonized cyclohexene. J. Hyg. 78, 199–211. https://doi.org/10.1017/S0022172400056096.
Ministerio de Sanidad (Spain), 2020. Productos Virucidas Autorizados en España.
Mondelli, M.U., Colaneri, M., Seminari, E.M., Baldanti, F., Bruno, R., 2021. Low risk of SARS-CoV-2 transmission by fomites in real-life conditions. Lancet Infect. Dis. https://doi.org/10.1016/S1473-3099(20)30678-2.
Murray, B.K., Ohmine, S., Tomer, D.P., Jensen, K.J., Johnson, F.B., Kirsi, J.J., Robison, R. A., O'Neill, K.L., 2008. Virion disruption by ozone-mediated reactive oxygen species. J. Virol. Methods 153, 74–177. https://doi.org/10.1016/j.jviromet.2008.06.004.
Naito, S., Takahara, H., 2006. Ozone contribution in food industry in Japan. Ozone Sci. Eng. 28, 425–429. https://doi.org/10.1080/01919510600987347.
Nayak, S.L., Sethi, S., Sharma, R.R., Sharma, R.M., Singh, S., Singh, D., 2020. Aqueous ozone controls decay and maintains quality attributes of strawberry (Fragaria × ananassa Duch.). J. Food Sci. Technol. 57, 319–326. https://doi.org/10.1007/s13197-019-04063-3.
OSHA Occupational Chemical Database | Occupational Safety and Health Administration [WWW Document], n.d. URL https://www.osha.gov/chemicaldata/chemResult.html?RecNo=9. Accessed 2 January 2021.
Predmore, A., Sanglay, G., Li, J., Lee, K., 2015. Control of human norovirus surrogates in fresh foods by gaseous ozone and a proposed mechanism of inactivation. Food Microbiol. 50, 118–125. https://doi.org/10.1016/j.fm.2015.04.004.
Rey, F.A., Lok, S.M., 2018. Common features of enveloped viruses and implications for immunogen design for next-generation vaccines. Cell 172, 1319–1334. https://doi.org/10.1016/j.cell.2018.02.054.
Riesser, V.M., J.R. Perrich, Silver, B.B., McCammon, J.R., 1976. Possible mechanism of poliovirus inactivation by ozone, in: Forum on Ozone Disinfection, Proceedings of the International Ozone Institute, International Ozone Institute. Syracuse (NY), pp. 186–192.
Roy, D., Wong, P.K.Y., Engelbrecht, R.S., Chian, E.S.K., 1981. Mechanism of enteroviral inactivation by ozone. Appl. Environ. Microbiol. 41, 718–723.
Roy, D., Englebrecht, R.S., Chian, E.S.K., 1982. Comparative inactivation of six enteroviruses by ozone. J. Am. Water Work. Assoc. 74, 660–664. https://doi.org/10.1002/j.1551-8833.1982.tb05034.x.
Sato, H., Wananabe, Y., Miyata, H., 1990. Virucidal effect of ozone treatment of laboratory animal viruses. Jikken Dobutsu 39, 223–229. https://doi.org/10.1538/expanim1978.39.2_223.
Shin, G.A., Sobsey, M.D., 1998. Reduction of norwalk virus, poliovirus 1 and coliphage MS2 by monochloramine disinfection of water. Water Sci. Technol. 38, 151–154. https://doi.org/10.2166/wst.1998.0528.
Shinriki, N., Ishizaki, K., Ikehata, A., Yoshizaki, T., Nomura, A., Miura, K., Mizuno, Y., 1981. Degradation of yeast RNA, yeast phenylalanine tRNA and tobacco mosaic virus RNA. Biochim. Biophys. Acta 655, 323–328.
Shinriki, N., Ishizaki, K., Yoshizaki, T., Miura, K., Ueda, T., 1988. Mechanism of inactivation of tobacco mosaic virus with ozone. Water Res. 22, 933–938. https://doi.org/10.1016/0043-1354(88)90031-0.
Sigstam, T., Gannon, G., Cascella, M., Pecson, B.M., Wigginton, K.R., Kohn, T., 2013. Subtle differences in virus composition affect disinfection kinetics and mechanisms. Appl. Environ. Microbiol. 79, 3455–3467. https://doi.org/10.1128/AEM.00663-13.
von Sonntag, C., von Gunten, U., 2012. Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications. IWA Publishing, London (UK). https://doi.org/10.2166/9781780400839.
Sproul, O.J., Pfister, R.M., Kim, C.K., 1982. The Mechanism of Ozone Inactivation of Water Borne Viruses 14, 303–314.
Swiss Institute of Bioinformatics, 2020. ViralZone Bioinformatics [WWW Document]. URL https://viralzone.expasy.org/. Accessed 12 April 2020.
Tanaka, H., Sakurai, M., Ishii, K., Matsuzawa, Y., 2009. Inactivation of influenza virus by ozone gas. IHI Eng. Rev. 42, 108–111.
Tizaoui, C., 2020. Ozone: a potential oxidant for COVID-19 virus (SARS-CoV-2). Ozone Sci. Eng. 42, 378–385. https://doi.org/10.1080/01919512.2020.1795614.
Torii, S., Itamochi, M., Katayama, H., 2020. Inactivation kinetics of waterborne virus by ozone determined by a continuous quench flow system. Water Res. 186, 116291 https://doi.org/10.1016/j.watres.2020.116291.
Torrey, J., von Gunten, U., Kohn, T., 2019. Differences in viral disinfection mechanisms as revealed by quantitative transfection of echovirus 11 genomes. Appl. Environ. Microbiol. 85, 1–14. https://doi.org/10.1128/AEM.00961-19.
Tseng, C.-C., Li, C.-S., 2006. Ozone for inactivation of aerosolized bacteriophages. Aerosol Sci. Technol. 40, 683–689. https://doi.org/10.1080/02786820600796590.
Tseng, C.-C., Li, C.-S., 2008. Inactivation of surface viruses by gaseous ozone. J. Environ. Health 70, 56–62.
United States Environmental Protection Agency, 2020. List N: Disinfectants for Use Against SARS-CoV-2 (COVID-19) [WWW Document]. URL https://www.epa.gov/pesticide-registration/list-n-disinfectants-use-against-sars-cov-2-covid-19. Accessed 9 March 2020.

van Doremalen, N., Bushmaker, T., Morris, D.H., Holbrook, M.G., Gamble, A., Williamson, B.N., Tamin, A., Harcourt, J.L., Thornburg, N.J., Gerber, S.I., Lloyd-Smith, J.O., de Wit, E., Munster, V.J., 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N. Engl. J. Med. 382, 1564–1567. https://doi.org/10.1056/NEJMc2004973.

Vyskocil, J.M., Turgeon, N., Turgeon, J.-G., Duchaine, C., 2020. Ozone treatment in a wind tunnel for the reduction of airborne viruses in swine buildings. Aerosol Sci. Technol. 1–8. https://doi.org/10.1080/02786826.2020.1790495.

Wei, C., Zhang, F., Hu, Y., Feng, C., Wu, H., 2017. Ozonation in water treatment: the generation, basic properties of ozone and its practical application. Rev. Chem. Eng. 33, 49–89. https://doi.org/10.1515/revce-2016-0008.

Wells, K.H., Latino, J., Gavalchin, J., Poiesz, B.J., 1991. Inactivation of human immunodeficiency virus type 1 by ozone in vitro. Blood 78, 1882–1890. https://doi.org/10.1182/blood.v78.7.1882.1882.

Wickramanayake, G.B., Sproul, O.J., 1988. Ozone concentration and temperature effects on disinfection kinetics. Ozone Sci. Eng. 10, 123–135. https://doi.org/10.1080/01919518808552161.

Wigginton, K.R., Kohn, T., 2012. Virus disinfection mechanisms: the role of virus composition, structure, and function. Curr. Opin. Virol. 2, 84–89. https://doi.org/10.1016/j.coviro.2011.11.003.

Wigginton, K.R., Pecson, B.M., Sigstam, T., Bosshard, F., Kohn, T., 2012. Virus inactivation mechanisms: impact of disinfectants on virus function and structural integrity. Environ. Sci. Technol. 46, 12069–12078. https://doi.org/10.1021/es3029473.

Wolf, C., Von Gunten, U., Kohn, T., 2018. Kinetics of inactivation of waterborne enteric viruses by ozone. Environ. Sci. Technol. 52, 2170–2177. https://doi.org/10.1021/acs.est.7b05111.

World Health Organization, 2020b. Coronavirus Disease (COVID-19): How is it Transmitted?

World Health Organization, 2020a. Timeline: WHO's COVID-19 Response [WWW Document]. URL https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactive-timeline. Accessed 9 August 2020.

Yano, H., Nakano, R., Suzuki, Y., Nakano, A., Kasahara, K., Hosoi, H., 2020. Inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by gaseous ozone treatment. J. Hosp. Infect. 106, 837–838. https://doi.org/10.1016/j.jhin.2020.10.004.

Yao, M., Zhang, L., Ma, J., Zhou, L., 2020. On airborne transmission and control of SARS-Cov-2. Sci. Total Environ. 731, 139178 https://doi.org/10.1016/j.scitotenv.2020.139178.

Young, S., Torrey, J., Bachmann, V., Kohn, T., 2020. Relationship between inactivation and genome damage of human enteroviruses upon treatment by UV254, free chlorine, and ozone. Food Environ. Virol. 12, 20–27. https://doi.org/10.1007/s12560-019-09411-2.