Evidence of OH· radicals effect for indoor air and surfaces disinfection in a harmless for humans method.

Abstract

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An advanced harmless environmental sanitation method for the elimination of pathogenic microorganisms and volatile organic compounds (VOC's) in large air spaces and surfaces are advanced oxidation processes (POA) based on hydroxyl radicals (OH•) in sufficient concentrations to perform biocidal functions on pathogenic microorganisms and degrade airborne organic compounds to mineral forms or harmless organic compounds.

It is a technology recognized as clean and safe and is generally carried out through solar radiation as a process initiator with photocatalyst material. The problem presented in the photocatalysis methods is its low speed, the generation of toxic degradation intermediates, deactivation of the material and the need for UV irradiation.

The increased airborne spread of pathogenic microorganisms has raised serious concerns about its threat to environmental security. However, there is no effective method to quickly eliminate these harmful microorganisms in a large airspace. Compared to conventional disinfectants, OH• radical, based oxidation processes have excellent advantages.

Keywords: *hydroxyl radicals (OH•); disinfection; VOC's, advanced oxidation process, infectious diseases, ozone, terpenes.*

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Introduction

The development of human societies around the world has generated at the same time a very serious environmental damage that threatens human health and the survival of animals and plants due to a higher incidence of infectious diseases.

The atmosphere does not have a native microbiota but is a rapid and global means of dispersal for many types of microorganisms. The history of aerobiology has demonstrated until the last century the fundamental role that respiratory air pollution plays in the development of epidemics such as cholera, influenza and Legionella [1]. This respiratory air pollution together with the ease of mobility of human beings around the world has generated in this new century extremely serious respiratory syndromes for survival.

Between November 2002 and July 2003, severe acute respiratory syndrome (SARS) spread rapidly from China to 37 other countries around the world, causing 775 human deaths with an economic loss of $ 40 billion [2].

In early 2009, a new strain of H1N1 of porcine origin spread worldwide from Mexico. H1N1 was declared a flu pandemic by the World Health Organization (WHO), causing around 17,000 human deaths in early 2010[3].

In 2012 a new episode of coronavirus emerged, the MERS-CoV (Middle East Respiratory Syndrome coronavirus). The emergence of SARS-CoV in 2002 and MERS-CoV in 2012 has changed the perspective of the Coronoviridae family since the pneumonias they have caused (SARS and MERS) have mortality figures of 10% and 30%, which are elevated compared to the rest of the viruses in the family [4].

In December 2019, a third new coronavirus named SARS-COV2 (Severe acute respiratory syndrome coronavirus 2) emerged in the last 17 years in Wuhan Hubei province, China [5], was named COVID-19 in February 2020 and declared a pandemic by the World Organization of Health (WHO). Therefore, it is very important to develop a fast and efficient method for the elimination of pathogenic microorganisms in large air spaces.

An advanced harmless environmental sanitation method for the elimination of pathogenic microorganisms and volatile organic compounds (VOC's) in large air spaces and surfaces are advanced oxidation processes (POA) based on hydroxyl radicals (OH•) in sufficient concentrations to perform biocidal functions on pathogenic microorganisms and degrade airborne organic compounds to mineral forms or harmless organic compounds [6].

The results of different studies show that OH• radicals rapidly destroy different microorganisms with a concentration of 0.8 mg/L and a spray density of 21 $\mu L/m^2$ in 4 seconds [7]. Vital and essential cellular morphological changes in pathogenic microorganisms are also observed under a microscope when exposed to a fatal dose of OH• radicals

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The increased airborne spread of pathogenic microorganisms has raised serious concerns about its threat to environmental security. However, there is no effective method to quickly eliminate these harmful microorganisms in a large airspace. Compared to conventional disinfectants, OH• radical, based oxidation processes have excellent advantages.

Currently, chlorine, alkali, and alkali-alcohol-amine are the three main types of chemical disinfectants that are widely used to eliminate microbial contamination. However, some drawbacks are as follows: Selectivity, a type of chemical disinfectant, can only selectively kill one or more types of pathogenic microorganisms. Its processing time is long, in a range of 0.5 - 1 hour due to the low chemical reaction rate and its very high lethal dosage value, which could reach 9% (v / v), which implies secondary contamination severe by the remaining chlorine intermediates. Finally, its lethal processing is limited to the surface of the objects, making it impossible to apply in large air spaces [8,9].

Compared to previous chemical disinfectants, advanced OH• radical, based oxidation technology has several advantages: 1) Absence of selectivity, they can kill any pathogenic microorganism in low lethal doses due to its strong oxidative character, with an oxidation potential of 2.8 V, slightly less than that of fluorine (3.03 V). 2) Processing time of OH•radicals it is very short, several seconds. The reason is that the chemical reaction rate of OH• radicals is greater than 10^9 L $mol^{-1}second^{-1}$, which is 10^7 times greater than that of other oxidants such as O_3, H_2O_2, Cl_2, etc. 3) As a green oxidant, OH• radicals decompose into H_2O and O_2 without any residual oxidants after their biochemical reactions [10,11].

Formation of OH• radicals

Oxygen is an essential molecule for life, but given its high reactivity, it also becomes a toxic element that gives rise to the so-called *oxygen paradox*. Oxygen is a basically oxidizing molecule. The following concentration of pollutants is generally found in "clean" outdoor air (without sources of pollution): carbon dioxide, 320 ppm; ozone, 0.02 ppm, carbon monoxide, 0.12 ppm, nitric oxide, 0.003 ppm, and nitrogen dioxide, 0.001 ppm. However, these values increase significantly in urban air [12].

The OH• radical is the most important natural oxidant in tropospheric chemistry. It is often called the "detergent" in the atmosphere since it reacts with many pollutants, initiating the process of depurifying of them, it also plays an important role in the elimination of greenhouse gases such as carbon dioxide, methane and ozone. The characteristics that make the Advanced Oxidation Processes (POA) attractive are among others, that with them the contaminant is destroyed, not concentrated or transferred to the environment, a total or almost total mineralization of organic pollutants is achieved, that is, they have application in the destruction of the vast majority of organic compounds, especially in non-biodegradable compounds such as organochlorines, PCBs, PAHs, etc. It is a clean and safe technology and, in some processes, solar radiation can be used as the initiator of the process.

The main problem for rapidly eliminating pathogenic microorganisms in large air space is how to produce the OH• radicals with high concentration and large production. Currently, the main methods are Fenton catalysis, photocatalysis, ozone, as well as their collaborative effects [13-17]. However, these technologies have some serious disadvantages: 1) The OH• radical are obtained in a low concentration and low amount of production, so that the whole biochemical reaction time is long, in the range of 15 - 360 min. 2) The above-mentioned technologies are only applied to the smallscale experiments or applications. 3) A large number of chemical reagents such as H_2O_2, TiO_2, Fe^{2+}, and so on are necessary in the process of OH• production, resulting in its high cost as well as one safety problem. 4) In order to increase the OH• radicals production, several kinds of technologies are collaborated together resulting in large-volume accessory equipments such as the bubble tower, rotating packed bed, and so on.

In previous studies, the production of a large number of OH• radicals has been reported by ionization and dissociation of O2 in air and H2O in the gaseous state using a physical method of strong electric field discharge. In this way, OH• radicals have been successfully used in the treatment of ship's ballast water and red-tide in the ocean [18,19].

Titanium oxide is the reference as a photocatalyst material currently, given its high activity, relative stability, low cost and low toxicity. However, there are problems to be solved such as the low rate of photocatalysis, generation of toxic degradation intermediates, deactivation of the material and the need for UV irradiation as its band gap is not coupled with sunlight [20].

Development of a new advanced oxidation process for the decontamination of air and surfaces.

Given the described scenario, a challenge for safe and effective technological development is generated in the decontamination of air and surfaces. The technological objective is based on the milestone of achieving a technology capable of producing OH• radicals in sufficient quantities by means of an innovative system that ensures their efficacy and safety for human beings. The

Wadu02© system is a device by means of which an oxygen reactive species (ERO) such as hydrogen peroxide (H_2O_2), or a terpene such as d-limonene, is evaporated. This evaporation will react with an internal ozone emission below a concentration of 0.050 ppm (0.1 mg / m^3), the ozone exposure limit established in the regulations issued by the WHO in the environmental limit values (VLA) of the year 2000 for the general public in exposures of up to 8 hours [21], taken as an international benchmark of safety in ozone emission and thus obtain a constant and non-damaging production of OH• radicals.

Wadu02's ozone emissions were evaluated through testing of household electrostatic air cleaners in an external laboratory [22] under the Electrostatic air cleaners standard, SUN - UL 867 and tested with a Teledyne® ozone calibrator and monitor, at temperature and humidity controlled by Vaisala® transducer and flow meter. The ozone emission of the Wadu02® device was certified in active mode and night mode in parameters less than 0.020ppm (0.012 - 0.015 ppm without filters and 0.015 -0.016 ppm with filters, respectively) results lower than all international standards regarding safety in prolonged exposures to ozone.

The production of OH• radicals through the oxidation of H_2O_2, under controlled conditions, was evaluated according to the oxidative functionality of the Wadu02® device and with a comparison made with liquid hydrogen peroxides in the purity ranges of 0.25% to 0.75%, aided by the colorimetric reaction performed on a potassium iodide test strip. The results indicate that the average oxidative capacity of a H_2O_2 at 0.5% purity is equivalent to the oxidative capacity offered by the Wadu02® model devices, with a maximum production of 0.9 mg/m^3 (0.64 ppm), which is approximately 64.2% of the current workplace exposure limit (WEL) adjusted to 1.4 mg / m3 (1ppm) [23,24].

The threshold concentration for the acute irritant effects of hydrogen peroxide gas in the respiratory tract is 10 mg/m^3 (equivalent to 7 ppm) in humans; while the corresponding values for the skin are 20mg/m^3. Regarding its prolonged exposure, hydrogen peroxide has not been found to cause teratogenic or carcinogenic effects in humans. Mutagenic or chromosomal effects have also not been observed.

It was also verified, as an alternative to the high natural reactivity that hydrogen peroxide presents, the substitution of the cartridge load with aromatic essences extracted from flowers and plants for the biocidal role that terpenes have for their antiviral and antibacterial properties and was analyzed the process of advanced oxidation under the same conditions of low ozone emission (less than 0.02ppm) with the Wadu02 model, to compare the proven efficacy with hydrogen peroxide.

Limonene is one of the most abundant monoterpenes in nature, present in essential oils extracted from the peel of citrus fruits, including the essential oils of orange and tangerine. This monoterpene is susceptible to oxidation to generate compounds with higher added value [25].

Terpenes are hydrocarbons that consist of more than one unit of isoprene with five carbons that are present in essential oils. Monoterpenes, most terpenes, along with sesquiterpenes and diterpenes, comprise the majority of essential oils. Due to the low molecular weight and high volatility of monoterpenes and sesquiterpenes, the use of essential oils in indoor environments can increase the levels of volatile organic compounds (VOCs) [26].

Terpenes contain one or more C=C double bonds, which interact easily with strong oxidants such as ozone, hydroxyl radicals [27-29], and nitrate radicals. Ozone is a common indoor pollutant, the general levels of which are distributed approximately 20 to 40 ppb [30,31]. The use of office machines, such as copy machines, printers, and fax machines, also elevates indoor ozone concentrations [32]. The VOCs emitted through the evaporation of indoor terpene-based products may interact with ozone and generate secondary air pollutants, mostly formaldehyde and suspended particulates [33-37]. Secondary organic aerosols generated by the interaction of terpenes and ozone consist of fine and ultrafine particles [38-40]. Consequently, prior evaluations of total limonene consumption were performed on the Wadu02® device to obtain controlled and safe evaporation.

The total consumption of d-limonene in the Wadu02® products was determined to be of the order of 0.4 g/24 h. According to the functionality of this device and the average evaporation of the measurements recorded in the laboratory, Wadu02® products emit a cloud containing d-limonene with a concentration of approximately 1.84 ppm, which in a room of $60m^2$ ($180m^3$) can give rise to a maximum concentration with a value less than 2ppb. This concentration is significantly lower than the Swedish and German OEL levels [41] (occupational exposure limits) which are 27ppm and 10ppm, respectively.

Toxicology

For the evaluation and analysis of the amounts of formaldehyde, which can be generated directly from the reaction of ozone with the structural units of C=C bonds, reports indicating that the proportions of formaldehyde formed by this mechanism during ozone-initiated reactions with terpenes represent only a small percentage of reactions to ozone were evaluated [33,34].

The main mechanism that forms formaldehyde is initiated by the reaction of ozone with the functional group C = C to generate ozonide. Subsequently, the ozonide decomposes into a carbonyl and an energy-rich (bi-radical) Criegee intermediate. Both products participate in various additional oxidation reactions to form highly reactive species such as hydroxyl radicals and stable products. These stable products can be ketones and carboxylic acids if the process has taken place in an oxidizing medium or aldehydes and ketones when the process has taken place in a reducing medium.

The forming of stable carbonyls with low molecular weights, including formaldehyde, acetaldehyde, acetone, and propionaldehyde, were observed during the gas-phase reactions of ozone with terpenes [31,33,36]. Reactive hydroxyl radicals generated from ozone reactions with terpenes played a vital role in forming indoor formaldehyde.

Reactive hydroxyl radicals generated from ozone reactions with terpenes played a vital role in forming indoor formaldehyde. Several studies have indicated that indoor OH• radicals concentrations generated by ozone reactions with unsaturated compounds were higher than those outdoors at midday or night [42-44]. OH• radicals were responsible for 56~70% of indoor formaldehyde in reactions between ozone and 23 VOCs and ozone and terpenes [45]. Therefore, a new objective is the evaluation of the reactions of OH• radicals by means of terpenes and the possible contribution to obtain high levels of formaldehyde indoors and potential effects on indoor air quality [46].

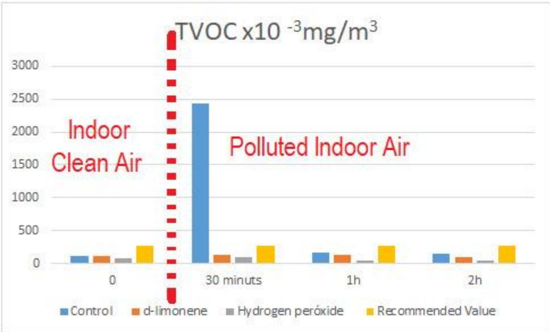
Security test

Once it was verified that the total consumption of limonene and hydrogen peroxide does not exceed limits that are considered teratogenic and carcinogenic in humans and that the emission of ozone is less than international regulations, the effectiveness in reducing formaldehydes was evaluated in the advanced oxidation of limonene with Wadu02® using the SPS-KACA002-132: 2016 test method under controlled temperature and humidity conditions (21 ± 1) °C (45 ± 5)% RH with d-limonene in cartridge and with d- limonene in a gel [47]. The results indicate that the reduction of formaldehyde in ozonolysis reactions with emissions less than 0.020 ppm and with low emission concentrations of d-limonene with an evaporation of 0.4 g / 24 h equivalent to 1.84 ppm is significant and reaches values of 19% with gel and 41% with liquid limonene cartridge.

These results show that, despite the high reactivity of d-limonene with ozone for the formation of formaldehyde, the controlled emission of ozone below 0.02ppm and the evaporation of limonene below 2ppb in a space of $60m^2$ is a safe and harmless reaction.

To validate this hypothesis, a series of experimental tests were carried out to determine the reduction of particles and air pollutants emitted by the burning of an incense stick during a 2-hour exposure to a Wadu02® air purifier, using loaded cartridges with d-limonene and H_2O_2 in a 225.72 m3 (6.6 X 6 X 5.7) volume controlled chamber [48].

Five air quality lectures were performed, under different conditions: the first lecture was determined by the initial air quality in the room, without any exposition to incense or air purifiers. The second lecture was taken after 2 hours since a half of incense stick was burned; the third lecture was determined when the initial air quality was reached and then, a half of an incense stick was burned with the presence of hydrogen peroxide cartridge air purifier for two hours. The fourth lecture was taken by the same conditions that third lecture but, in this case, with a D-limonene cartridge air purifier presence; and finally the fifth lecture was determined by the same conditions that third and fourth lectures but, this time, with the presence of both air purifiers (D-limonene and hydrogen peroxide cartridges).



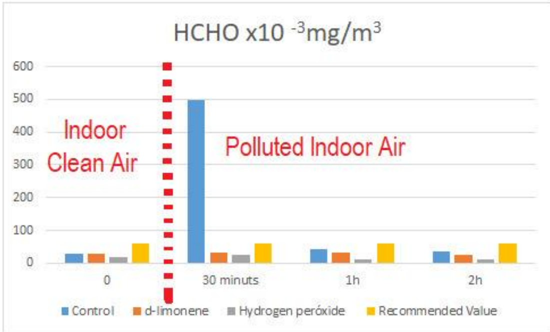


Figure 1. Air quality lectures before and after contaminating the room with the combustion of an incense stick, comparison of the safety and efficacy of Wellis in the elimination of VOCS and formaldehyde with hydrogen peroxide and limonene.

The study shows that under these conditions; burning an incense stick generates poor air quality an average of 30 minutes after the start of combustion; with a tendency to regularize after one hour, to return to the initial conditions two hours after the start of combustion, as the particles emitted dispersed in the air space of the room. However, the formaldehyde and VOC's readings are higher than the control reading, reflecting a risk of prolonged exposure.

The results in the presence of Wadu02® purifiers regardless of the cartridge content (d-limonene or H_2O_2), maintain the initial air quality from the first half hour of exposure; significantly reducing the values of particle matters, formaldehyde and VOCs. The efficacy in terms of the reduction of formaldehyde and VOCs according to the use of d-limonene or H_2O_2 to carry out the emission of OH• radicals is not significant, although H_2O_2 presents more efficient values.

The results make it possible to declare that; the operation of the Wadu02® air purifier, based on the emission of ozone in low concentrations (< 20ppb) and the evaporation of standardized amounts of d-limonene or H_2O_2 from the cartridge, for the execution of the advanced oxidation process; they are safe, harmless and effective in reducing suspended particles, VOCs and formaldehyde.

Application of OH• radicals as a broad spectrum biocide

Free radicals and ions cause irreversible alterations in macromolecules (proteins, membrane or DNA) as a consequence of the movement of electrons, which is why they have a morbid effect. Reactive Oxygen and Nitrogen Species (RONS) are the most unstable, reactive and those that first react with others. Within this group, the OH• radicals is the species with a more ephemeral half-life due to its high reactivity, and will therefore be the most dangerous [49].

The efficacy of the concentration of OH• radicals in the elimination of pathogenic microorganisms was studied. Under conditions of spray density of 21µL/cm^2 and at processing times of 4 seconds, a dramatic decrease in survival cells has been reported for *S. Marcescens* at concentrations just above 0.15mg/L and almost entirely in concentrations of 0.41mg/L. In *B. subtilis*, the levels were practically undetectable at concentrations of 0.5 mg/L, whereas in *bacillus* spores the reduction was significant at levels of 0.3mg/L and practically entirely at maximum concentrations of 0.8mg/L [50].

The biocidal function of OH• radicals is based on the advanced oxidation process. This cellular stress mechanism is generated by the phenomenon of "respiratory explosion" through a cascade of reactions by the release of reactive oxygen species [51-52]:

1) The OH• radicals is the most reactive species in biology. (half-life 100 picoseconds)

2) Hydrogen peroxide can pass through biological membranes.

3) The hypochlorite ion modifies and degrades all biological molecules. It is a product of the respiratory explosion (similar to the mitochondrial).

The main effects of these reactive forms occur on the membranes, lipids and sulfhydryl bonds of DNA proteins and nucleotides [53], producing:

- Lipid peroxidation, the resulting peroxides of which initiate a catalytic chain reaction leading to further loss of unsaturated fatty acids and extensive membrane damage.
- Production of cross links between proteins, through the formation of disulfide bonds.
- Mutations in the genetic material of the pathogenic microorganism.

During the cellular oxidation process, unsaturated chains are easily attacked by OH• radicals. The peroxidation of the fatty acids in the membranes generates peroxyl radicals (ROO•), decreasing their functionality. These radicals have a lower reactivity than the OH• radicals and, therefore, their half-life is somewhat longer.

The presence of damage to the cell caused by oxidative stress provokes an antioxidant response in the cell: they try to pass the electrons from one species to another until they inactivate the radicals and restore their stability. On the other hand, these interactions can generate cascades, spreading the damage [54].

Lipid oxidation. Biological membranes are made up of unsaturated fatty acid chains and are easily oxidized. OH• radicals attack the double bonds of these structures and leave an unpaired electron in the chain that will bind to an oxygen (O_2) molecule to re-stabilize, giving rise to a peroxyl radical. The formation of peroxide radical modifies its functionality irreversibly, since it changes its spatial distribution causing instability in the membrane [55].

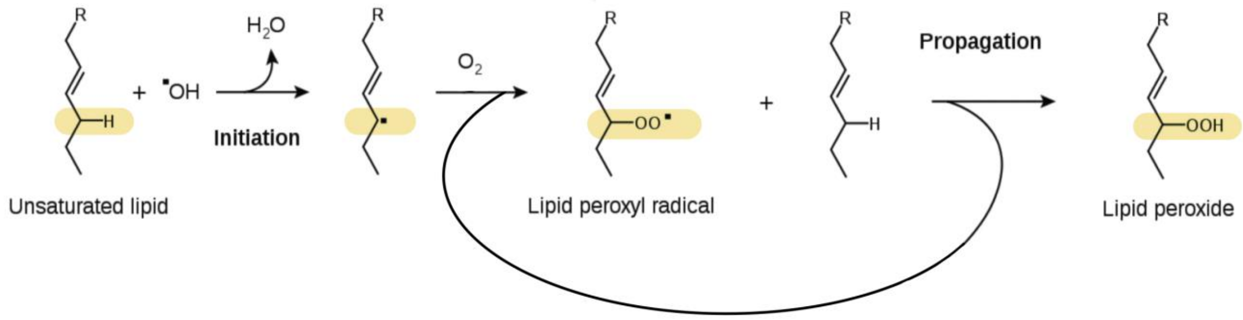


Figure 2. •OH radicals attack the bonds of these structures and generate a chain with an unpaired electron, which will react with an oxygen (O_2) molecule to re-stabilize. As a product, peroxyl radicals appear in the membrane, which acts as positive feedback, further increasing damage to the membrane.

- ***Direct protein oxidation (produced by RONS) or indirect (produced by lipid peroxidation)***

Free radicals cause changes in the molecular structure of amino acids by modifying their charge. This can end up breaking the polypeptide chain, fragmenting the protein.

Peroxyl radicals give rise to substances with aldehyde groups, highly reactive species that establish covalent between amino side groups of proteins. The Cross-linking, intra and interprotein. Finally there is loss of conformation and formation of protein aggregates, and therefore, the decrease / inhibition of the correct functioning of the protein.

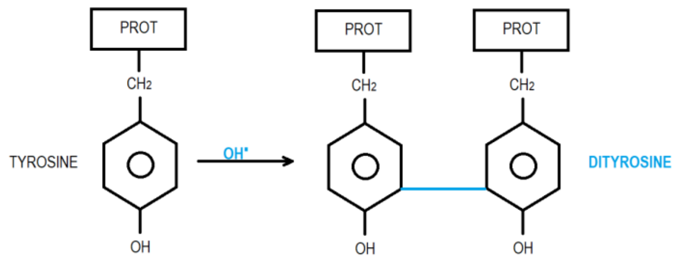


Figure 3. The OH• radicals forms irreversible covalent bridges between two tyrosines, giving the end product dityrosine. This is not recognized by the kinases of the signaling pathways, so the information that the Tyr had to transmit is lost. In addition, this structure is not degradable so that functional proteins accumulate with intra and intermolecular bridges.

Morphological changes of microorganisms.

The study by Bai et al 2012 [50] verified the morphological changes of *B. subtilis* and *Bacillus* spores with treatment with OH• radicals under microscopic observation in *B. Subtilis, S. Marcescens* and *Bacillus* spores.

It was clearly observed that *B. subtilis* in the form of intact cane and evenly distributed cytoplasm after treatment with OH• radicals, greatly lost the integrity of the membrane. In comparison, the *Bacillus* spore cells features a tough, multi-layered outer coating that makes it impossible to quickly kill *Bacillus* spores with conventional chemical disinfectants such as chlorine, alkali, and alkali-alcohol-amine. However, after treatment with OH• radicals, the *bacillus* spores also ruptured and the round-shaped cells disappeared. Consequently, *Bacillus* spores require a higher concentration of OH• radicals scavenging, spray density - dispersion and time. Concentration, dispersion spray density and processing time are the three important parameters for the destructive effect of OH• radicals on microorganisms.

The demonstration that the effect of OH• radicals on a microorganism will be with greater biocidal efficacy according to its more superficial structure collected in previous studies determines the need to recognize the morphological characteristics of pathogenic microorganisms from their outer layer to the interior of the specific cell [56,57].

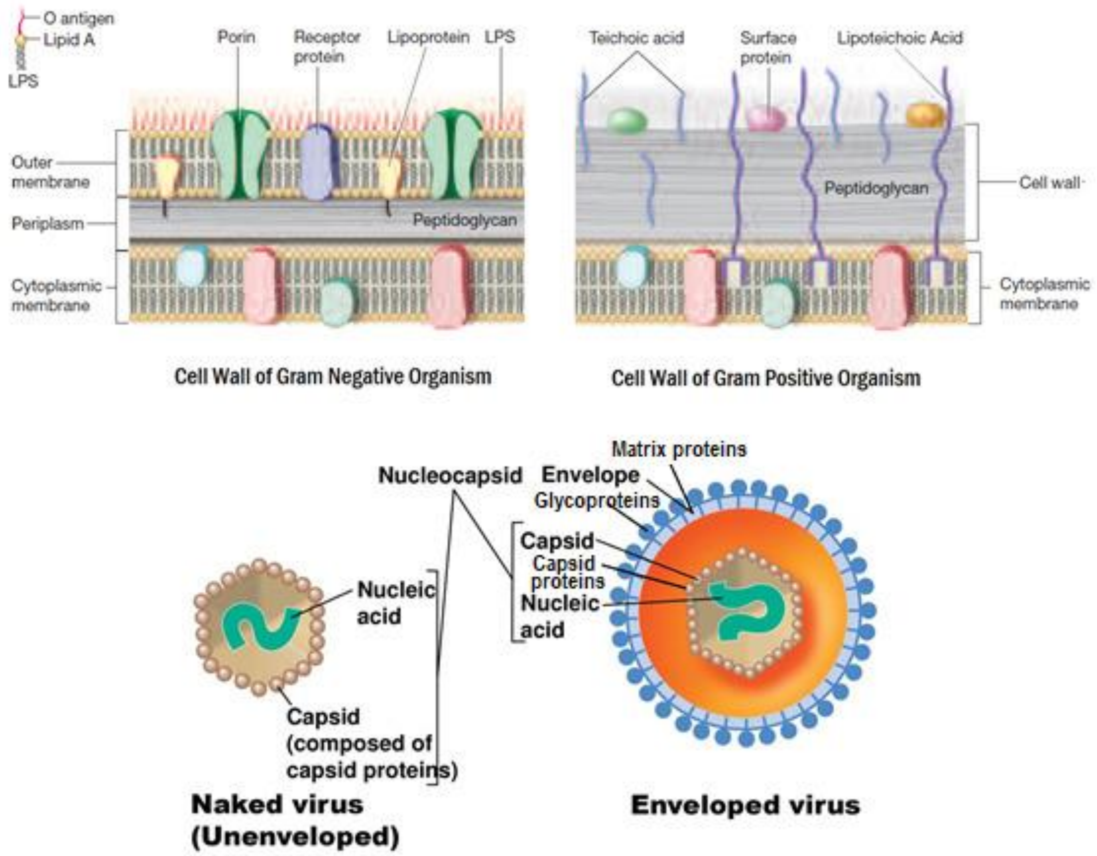


Figure 4.cellular envelopes according to the type and morphology of viruses and bacteria. Adapted from Pearson education, Inc ©2015 & laboratoryinfo.com

Some microorganisms have been able to reverse this oxidative process through super oxide dismutase (SOD), a family of three metalloenzymes (FeSOD, MnSOD and CuZnSOD) with a high capacity to interact with oxidants, neutralizing them and reducing oxidative damage.

As is the case with MnSOD that *Escherichia coli* synthesizes [58] after exposure to oxygen and is induced by the presence of superoxide radicals. Both SOD and catalase activity have been detected in the cytosol of microbial cells and in the periplasmic space (located between the plasma membrane and the cell wall) of the bacteria. Likewise, a protective role has been demonstrated against ROS generated in the catalase respiratory burst in *Staphylococcus aureus.* [59-61]

In recent years, they have been developed by different laboratories, external certifiers and university research centers; various studies to check the biocidal efficacy of the Wadu02®, in the presence of pathogenic microorganisms in different spaces.

The results are reflected in the following table, in which we can show that, in the case of gram + and gram - bacteria the reduction reaches an average of 99.9% on average in the first hour of exposure to the advanced oxidation process in air and surfaces.

In the case of viruses, the results are observed depending on the conditions of relative humidity and the morphology of the virus. The efficacy results of Wadu02® in no-enveloped viruses indicates that in humid conditions the efficacy is less than in dry environments, averages of 99% are reached. While in enveloped viruses, humidity favors the advanced oxidation process and virus elimination than in dry environments.

Report on stability and disinfection of 2019-nCoV

The 2019-nCoV is a new strain of coronavirus that was first detected in Wuhan City (China) in december 2019. The number of infected patients has grown rapidly in recent weeks, becoming a serious public health concern. The transmission of the virus occur mainly via respiratory droplets produced by an infected person that can land in the mouths or nose of people nearby or possibly be inhaled into de lungs. Coronaviruses are a large family of viruses that are common in many different species of animals, including camels, cattle, cats, and bats. Rarely, animal coronaviruses can infect people and then spread between people such as with MERS, SARS, and now with 2019-nCoV [62]

The new coronavirus has been classified as a *Betacoronavirus*, like MERS and SARs, both of which have their origins in bats. Coronaviruses are in the subfamily *Coronavirinae* in the family *Coronaviridae*, in the order *Nidovirales*. They are divided in 4 subgenders *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus* and Gammacoronavirus [63].

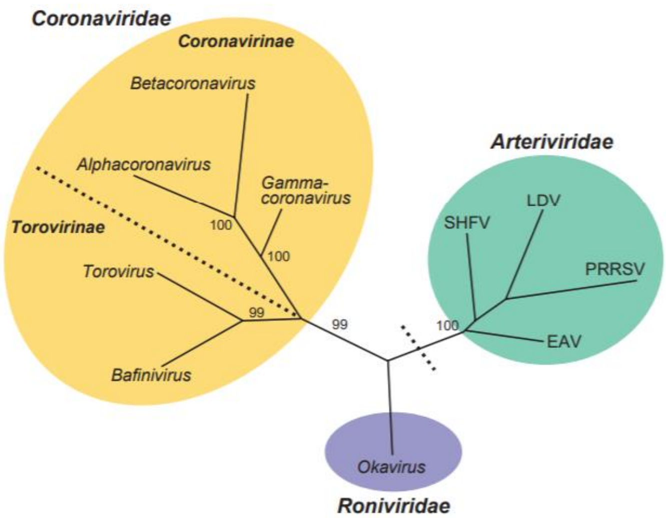


Figure 5. ***Nidovirus*** **phylogeny. The Nidoviral order consists of three families: *Coronavirinae, Roniviridae and Arteriviridae* from International Committee on Taxonomy of Viruses, 2012 ©.**

Based on the genetic material, these viruses are included in group IV of the Baltimore classification, as the viral particle contains only a single linear single stranded RNA strand of positive polarity. Therefore, the genetic material itself acts as a messenger RNA since they share the positive polarity, when translated, the

RNA polymerase and the different structural proteins that form the capsid are synthesized [64].

Its diameter is around 60-200 nm, they present a nucleocapsid with helical symmetry and a lipid sheath that derives from the membrane of the previously infected host cell and contains glycoproteins and surface antigens. From the lipid sheath the characteristic projections of this genus arise forming a solar corona around it that is visible under a microscope and gives the family its name. Despite what might be expected, having an envelope implies that the virus is sensitive to different factors and external agents such as heat, lipid solvents, non-ionic detergents, formaldehyde and oxidizing agents and UV irradiation [63].

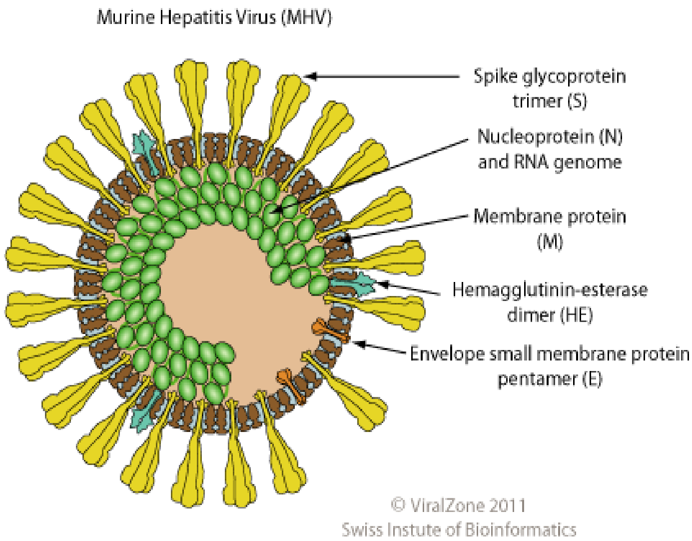


Figure 6. Structural proteins of *coronavirus* (from ViralZone©)

As can be seen in figure 6, the coronavirus capsid consists of the following structural proteins:

The Spike glycoprotein (S) protrudes from the outer envelope of the virus forming the "corona" visible under a microscope. Its function is to stick to the proteins found on the surface of the cell that will be infected. In some cases, the S protein causes the infected cell to fuse with other adjacent cells, thus favoring the spread of the virus. The Envelope protein (E) is responsible for the formation of new viral particles and their release from the infected cell, being necessary for virus diffusion. The Membrane protein (M) is attached to the inner part of the virus membrane, and causes this membrane to bend, determining the spherical shape of the virions. M also interacts with the nucleocapsid formed by the RNA of the virus and the N protein. And finally, the Nucleocapsid protein (N) is phosphorylated and binds to the viral genome during assembly.

They are viruses distributed worldwide due to their genetic diversity, their short incubation periods and the high mutation rate they present. The combination of these factors allows the pathogen to infect not just animals but also humans.

It is well known that ozone, at concentrations above 100ppm and high humidity rates, is an effective disinfection treatment, and specially for RNA-viruses with or without envelope [65-67]. However, high ozone concentrations may be harmful to coexist in habitable urban enviroments. Reactive oxygen species (ROS) including OH• radicals, hydrogen peroxide (H_2O_2) and ozone O_3 have been reported to enhance disinfection efficiencies of several microorganisms [68,69].

According to the results obtained with Wadu02® in viruses with similar structures (RSV) to Covid-19, we can expect that the efficacy of the device will have an expected elimination result of an average of 99 to 92% depending on the relative humidity conditions.

Table1.- Wadu02© biocide efficacy Test result with limonene

Pathogen	Table 1. Test result (limonene)			Documented testing
	Means of dispersion	Exposure	effectivenes s %	
Bacillus subtilis	Surface	1 h	99,4	Bacillus, Esch, Staph - KNU
	Air	20 min	99,6	Bacillus, Esch, Staph - KNU
Staphylococcus aureus	Surface	1 h	52,3	Bacillus, Esch, Staph - KNU
	Surface	4 h	99,9	Esch, Pseudo, Staphy- KCL
	Air	1 h	99,9	Bacillus, Esch, Staph - KNU
Staphylococcus aureus resistente (MRSA)	Surface	4 h	99,9	Salm, Kleb, MRSA - KCL
	Air	4 h	99,9	MRSA - KCL
Pseudomonas aeruginosa	Surface	4 h	99,9	Esch, Pseudo, Staphy- KCL
Enterobacter species: Salmonella	Surface	4 h	99,9	Salm, Kleb, MRSA - KCL
Enterobacter species: Klebsiella	Surface	4 h	99,9	Salm, Kleb, MRSA - KCL
	Air	4 h	99,9	Klebsiella - KCL
Enterobacter species: Escherichia coli	Surface	1 h	99,9	Bacillus, Esch, Staph - KNU
	Surface	4 h	99,9	Esch, Pseudo, Staphy- KCL

Pathogen	Table 1. Test result (limonene) Means of dispersion	Exposure	effectiveness %	Documented testing
	Air	20 min	99,9	Bacillus, Esch, Staph - KNU
Influenza Virus (Enveloped)	Wet	30 min	86	Influenza A - UB
	Dry	30 min	38	Influenza A - UB
VRS Respiratory Syncytial Virus (Enveloped)	Wet	2 h	99	VRS - UB
	Dry	2 h	92	VRS - UB
Rotavirus (naked)	Wet	2 h	37	RoV - UB
	Dry	2 h	99	RoV - UB

Conclusions

The results show that the use of OH• radicals in the advanced oxidation process produced by the Wadu02® purifier, is a new safe and effective method to quickly eliminate pathogenic microorganisms in large air spaces and surfaces.

The application of OH• radicals in different studies, has shown that their application in advanced oxidation processes, standardized as a safety measure carried out by Wadu02®; they are safe, innocuous and effective in the control of pathogenic microorganisms, elimination of suspended particles, formaldehyde and VOCs.

The evidence on the efficacy of OH• radicals as a biocide shows us that their use is endorsed for being a strong oxidant, capable of eliminating microorganisms in low concentrations (0.8 mg/L) equivalent to 10 thousandths of the dose of conventional chemical disinfectants. Its spray density - dispersion is 22ml/cm^2 representing one thousandth of other disinfectants, its constant high reaction rate 10^9L/mol • sec in the processing of OH• radicals is shorter than 4 seconds, which is one thousandth of chemical disinfectants. Finally, the damage that has been observed to pathogens under a microscope is irreversible.

Basing our homeostatic state on the correct functioning of our internal antioxidant system and the experimental demonstration of the use of OH• radicals effectively in the disinfection of air and surfaces, we can issue a safety statement on the use of Wadu02® technology to achieve safe, effective and harmless advanced oxidation processes in humans in the purification and decontamination processes of air and surfaces.

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