Ozonated nanobubbles - A potential hospital wastewater treatment
during the COVID-19 outbreak in Indonesia to eradicate the
persistent SARS-CoV-2 in HWWs?

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Abstract

Background: Indonesia has a very limited capacity in dealing with medical wastes treatment, mainly
HWWs, enhancing threats to larger SARS-CoV-2 outbreaks. Aims: This article was aimed to
investigate the potential of ozonated nanobubbles as a single treatment of HWWs during this
pandemic by emphasizing its potency to eradicate persistent SARS-CoV-2. Settings and Design: We
conducted a scoping literature review as filed by Arskey and O’Malley. Methods and Material: We
explored the Google scholar search engine, as well as nature and science direct databases using
combined keywords related to our topic. We did a qualitative and quantitative assessment to conclude.
We selected 115 articles most matched with our topic, whilst we obtained 422 articles in total.
Results: We found that several treated wastewaters were still contaminated by SARS-CoV-2. More scientists
were starting to worry about the possibility of SARS-CoV-2 being transmitted via the fecal-oral route.
Ozone efficiently killed the bacteria that are structurally more complex and rigid than viruses by
generating another reactive species named nitric oxide (NO). Several studies exhibited the
mechanistic pathway used by ozone to lead to SARS-CoV-2 functionally loss. But, conventional
ozonation resulted in inefficient treatment leading to high treatment cost due to dramatically lack of

ozone dissolved in HWWs. **Conclusions:** Ozonated nanobubbles improved the distribution, concentration, and lifetime of ozone in HWWs which allows oxidation and disinfection to take place much longer and thoroughgoing. Thus intensifying ozone exposure to persistent microbes including SARS-CoV-2 in HWWs rising its potency to be developed as a single technology to treat HWWs.

**Keywords:** Ozonated nanobubbles, ozone, ozonation, SARS-CoV-2, HWWs and HWWs treatment.

**Key Messages:**

Several studies had reviewed the potential of ozone to disinfect SARS-CoV-2 in HWWs by performing a biochemical killing pathway. However, we had not found an article quantitatively examining the potential of ozonated nanobubbles by considering the distribution and solubility of the produced ozone. This article was intended to provide an overview of the downstream of ozonated nanobubbles as a single technology to deal with HWWs.


**Introduction**

Among numerous kinds of medical wastes, hospital wastewaters (HWWs) has the most complex structure. HWWs contain a variety of toxic or persistent substances such as pharmaceuticals, radionuclides, solvents, and disinfectants for any medical purposes (1–3). It has so much (4 to 150 times higher) micropollutant contaminants than urban wastewater (UWW) (4). It is also considered as major reservoirs of pathogenic and antibiotic-resistant bacteria (4,5). The development of antibiotic-resistant genes (ARGs) against more than one drug (multidrug resistance) usually occur in HWWs system as the result of the constant exposure of bacteria to antibiotics (6,7). HWWs remains a serious problem that highly impacts on the environment and human health quality until nowadays as how conventional approaches are not efficient in the removal of active pharmaceuticals and pathogenic microbes in hospital effluents (8,9). Moreover, since it was first reported, severe acute coronavirus (SARS-CoV)-2 was detected in the stool of a patient suffering from COVID-19 (10). Numerous studies then had been conducted to investigate SARS-CoV-2 in sewage yielding positive results of the presence of SARS-CoV-2 in wastewater streamlines (11). Some researchers then wonder whether this virus might be transmitted through oral-fecal pathways from one individual to another (12,13). Thus, the presence of SARS-CoV-2 in HWWs takes a big concern regarding its treatment during this pandemic. The Ministry of Health of the Republic of Indonesia via a webinar stated that the handling of medical...
waste during the pandemic refers to standardized handling procedures. Unfortunately, just 1,279 out of a total of 12,893 health care facilities carry out the standardized waste treatment and around 15 instances provide services of hazardous wastes treatment in Indonesia. As a result, an 70,432 out of 294.66 tons of medical waste cannot be handled each day. Hence, properly efficient techniques and methods are needed to treat HWWs.

Ozone is gaining a lot of attention due to its ability to perform oxidation and disinfection functions in water and wastewater treatment(14). Other properties of ozone are its ability to act as a nonselective yet green oxidizer \([E^o = 2.08 \text{ V}]\), a promising property of environmentally friendly wastewaters treatment technology(15). The mechanism of ozone utilization in wastewater treatment is then called as ozonation. Ozonation is conducted by the dissolution of ozone in wastewater, yet this mechanism resulted in low concentration of ozone in water \((1.0 \times 10^{-6} \text{ mol/m}^3 \cdot \text{Pa} \text{ to } 1.3 \times 10^{-4} \text{ mol/m}^3 \cdot \text{Pa})\) (16,17) which leads to inefficiency of ozone utilization for treating wastewater due to their different phase(18,19). Micro and nano bubble technologies were proven to increase ozone solubility and lifetime in both liquid and the aqueous phase(20). Ozone solubility and lifetime may be proportional to the size of bubbles produced by bubbles aerator(20,21). Some researches had been conducted to study the treatment of wastewaters including artificial HWWs using ozonated nanobubbles(22,23), and all of them performed overwhelming results mainly in pharmaceuticals degradation and microbes elimination. Ozonated microbubbles were shown to remove >90% of 33 different pharmaceutical compounds at the concentration of 6.5 mg/L(22). Another study indicated that the ultrafine bubble ozonation process degraded and mineralized tetracycline up to 99.5% and 40% respectively with 60-min treatment(23) and in the degradation of antimicrobial activity of ofloxacin after atmospheric cold plasma treatment (24). The number of \(\text{Escherichia coli}\), and \(\text{Pseudomonas fluorescens}\), \(\text{Salmonella typhi}\), and \(\text{Klebsiella pneumoniae}\) were reduced after the ozonation process(25). \(\text{B. Antrachis}\) was also been reduced to up to 6log\(_{10}\) colony after exposure using 9,800 ppm ozone(26). Some articles were published to exhibit the potential of ozone to deal with SARS-CoV-2 might exist in HWWs. However, to our knowledge, no articles were analyzing the potential of ozonated nanobubbles to perform HWWs treatment during this current situation. Thus, this article was designed to scope the possibilities of ozonated nanobubbles as a single method dealing with HWWs by focusing on the possibility of ozonated nanobubbles to eradicate the persistent SARS-CoV-2 in HWWs by scoping literature approach promoted by Arksey and O'Malley (27). We resulted in 115 articles that were then reviewed and found that ozonated microbubbles pose a highly potential feature to be developed further to overcome the problems of HWWs treatment mainly in Indonesia during this pandemic.
Subjects and Methods

This article was designed to scope our incoming research. We conducted a literature study approach as to how of Arksey and O’Malley framework (27). We explored for a peer-reviewed article via Google Scholar search engine as well as nature and ScienceDirect databases. We used keywords related to with SARS-CoV-2, hospital wastewater, ozone-based wastewater treatment, and ozone-organic compounds interaction. This exploration resulted in 422 articles related to those keywords. We did secondary selection based on a tighter term as such persistent SARS-CoV-2 in wastewaters, hospital wastewaters profiles, and schematic interaction of ozone and SARS-CoV-2. We finally resulted in 115 articles in the final as shown by the diagram previewed by figure 1. This review was done both qualitatively and quantitatively to obtain the most appropriate analysis of the possibility to develop ozonated nanobubbles as a promising technology to treat HWWs in general and persistent SARS-CoV-2 in particular. We focused on the investigation of how ozone induces microbial inactivation through several pathways to project possible mechanism of SARS-CoV-2 – ozone interactions in HWWs and explored how nanobubbles enhance those mechanisms.

Figure 1. The diagram of articles selection

Current Hospital Wastewaters (HWWs) Hazards during Pandemic

SARS-CoV-2 Contamination in Hospital Wastewaters

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a virus from the coronaviridae family that causes an outbreak of pneumonia coronavirus disease 2019 (COVID-19)(28). The Coronaviridae study group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) tentatively named the virus as SARS-CoV-2, based on phylogeny analysis(29). SARS-CoV-2 has about 79% and 50% genetic similarities to SARS-CoV and MERS-CoV, which were the cause of the SARS and MERS outbreaks in the early twenty-first century(28). SARS-CoV-2 has a
major similarity to SARS-CoV in terms of the structure of the receptor-binding domain (RBD)(28,30). Coronaviruses express glycoprotein spikes (S), which are protruding protein on the surface of the viral membrane, aside from the envelope (E), membrane (M), and nucleocapsid (N) proteins(31–33). These spike proteins are the mediators that allow Coronaviruses to sticks, joins and invades the target cells(34–36). RBD SARS-CoV-2 is located in the S1 subunit, which enables SARS-CoV-2 to recognize, attach and bind covalently to the peptidase domain (PD) of angiotensin-converting enzyme 2 (ACE2) receptor(34,37,38). This human ACE2 (hACE2) receptor was known to be expressed by epithelial cells lining the arteries and veins found in all organs of the human body(39). In fact, the ACE2 receptor was known to be abundantly expressed by the epithelial cells lining the surface of the alveoli and small intestine(40), enabling both SARS-CoV and SARS-CoV-2 to infect the deep gastrointestinal (GI) tract. Whereas, MERS-CoV might infect the GI via the dipeptidyl peptidase 4 receptor(41). Unfortunately, SARS-CoV-2 utilizes ACE-2 receptor of epithelial cells on GI surfaces with higher binding affinity and more efficient than SARS-CoV did (38). A study stated that SARS-CoV-2 has a binding power to ACE2 receptors lining small intestines up to 10 – 20 times greater than SARS-CoV(37).

Table 1: Viral loads and Ct values of SARS-CoV-2 in positive detection using real-time RT-PCR of fecal sample

<table>
<thead>
<tr>
<th>No.</th>
<th>Reference</th>
<th>Patient Number</th>
<th>SARS-CoV-2 Load (copies/mL)</th>
<th>Ct Value</th>
<th>Gene Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wolfel et al. (54)</td>
<td>9</td>
<td>$10^3$ – $10^7$</td>
<td>$&gt;10^7$</td>
<td>ORF1ab N gene S gene</td>
</tr>
<tr>
<td>2.</td>
<td>Zhang et al. (55)</td>
<td>23</td>
<td>5,623</td>
<td>$10^{5.8}$</td>
<td>~25 to 43</td>
</tr>
<tr>
<td>3.</td>
<td>Cheung et al. (56)</td>
<td>59</td>
<td>$10^{3.4}$ – $10^{7.6}$</td>
<td>$10^{4.7}$</td>
<td>31.4±5.1</td>
</tr>
<tr>
<td>4.</td>
<td>Wang et al. (57)</td>
<td>153</td>
<td>&lt;$2.6\times10^4$</td>
<td>30.33±8.12, 26.85±11.42, 28.42±6.79</td>
<td>RdRp N gene E gene ORF1ab N gene</td>
</tr>
<tr>
<td>5.</td>
<td>Wu et al. (50)</td>
<td>41</td>
<td>2x$10^3$ – 2x$10^7$</td>
<td>23 to 37</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Xu et al. (58)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Previous studies about SARS and MERS outbreaks already stated that, both SARS and MERS seemed to show enteric symptoms aside from respiratory symptoms (13,42). SARS outbreak indicated that 16–73% of patients with SARS underwent diarrhea within the first week of illness (43). A study revealed that viral RNA of SARS-CoV was detected in patient stool since the 5th-day onwards (44). In addition, this study stated that the viral RNA was still presented in the feces of a small number of patients even after 30 days of illness (44). Whereas during the MERS-CoV outbreak, an estimated quarter of the total patients with MERS showed gastrointestinal (GI) symptoms such as diarrhea or...
abdominal pain (45). Other patients initially presented fever and GI symptoms before the subsequent manifestation of a more severe respiratory syndrome (46). Around 2–10% (47,48) or 12% (49) of patients with COVID-19 manifested GI symptoms such as diarrhea, abdominal pain, and vomiting. Abdominal pain was reported to occur more frequently in patients admitted to the intensive care unit (47). As to how several confirmed cases that some stool samples of COVID-19 patients test resulted ‘positive’ for SARS-CoV-2, even when their respiratory tract samples already showed ‘negative’ for SARS-CoV-2 (11,50). The other one was the anal swab examination of an 8-years old girl with asymptomatic COVID-19 in Wuhan, China, which constantly resulted in positive for SARS-CoV-2 for up to 42 days since the first anal swab was carried out (51). The other rectal swab of 8 out of 10 children brought out a ‘positive’ result for SARS-CoV-2 using real-time reverse transcription PCR (rRT-PCR) in China(52). These series of cases opened up the possibility that feces contain SARS-CoV-2(53) adding a threat to environmental surveillance.

Live SARS-CoV-2 had been isolated in feces and urine of several patients with COVID-19 (47,55). Additionally, Zhang et al. Also suggested that the mean of SARS-CoV-2 viral loads were much higher in the fecal samples (5,623/ml) than those in respiratory samples (2,535/ml) (55). Table 1 serves various amounts of SARS-CoV-2 loads detected in the fecal sample. SARS-CoV-2 shedding was also observed almost in 40.5% of patients with confirmed SARS-CoV-2 infection (49). SARS-CoV-2 shedding in a particular amount from the urine or the feces of COVID-19 patients into wastewater could be the main cause of SARS-CoV-2 contamination in sewage(59), which in turn will be concentrated in the wastewater treatment plant (WWTP). Aside from its presence in feces or urine, SARS-CoV-2 was also detected by rRT-PCR in the blood of patients suffering from COVID-19 (47).

The respiratory fluid (such as sputum) of patients with COVID-19 might be a source of SARS-CoV-2 contaminants in the HWWs, considering that SARS-CoV-2 can effectively infect the upper respiratory tract, which SARS-CoV and MERS-CoV cannot(40). Another possible source of SARS-CoV-2 contamination in HWWs was the residues produced by the microbiology laboratory, which plays a role in examining the specimens of COVID-19 patients. SARS-CoV which is a very close relative of SARS-CoV-2 was found in the sewage and HWWs (60), and so does SARS-CoV-2 (11). Thus the high possibility of SARS-CoV-2 existence in both untreated and treated wastewaters, including HWWs, gives us an additional alert to become more aware of HWWs treatment processes. Moreover, a study involving encapsulated viruses such as MHV and Φ6 which was known as the projection of coronavirus, found that nearly 26% of the total viruses could be absorbed into solids present in the wastewaters (61).
Recommended treatment procedures of Hospital Wastewaters (HWWs) in Indonesia

Hospital wastewaters (HWWs) are the wastewater from various hospital activities, namely medical activities (specific discharge) and non-medical (domestic discharge) activities. The water consumed by hospitals is in a constant amount, which is about 200 – 1,200 L in developed countries and 200 – 400 L in developing countries every day(4). The number of wastewaters generated by the hospital varies greatly, depending on the characteristics of the hospital itself. In general, HWWs could be said to be similar to municipal wastewaters (MWWs)(62). Therefore several countries treated both of HWWs and MWWs in the same WWTP installation(63,64). However, the characteristics of these wastewaters remained different, even after they had passed a specific pre-treatment before being discharged into sewers or water bodies (4,65–68). HWWs was still a serious hazard to human health and environmental sustainability, considering the various macro and micro contaminants in HWWs (Table 2), rendering HWWs to be 5 - 15 times more toxic than urban wastewaters (UWWs)(69).

Table 2: HWWs effluent characteristics based on conventional parameters, hazardous chemical compounds, and biological agents contained therein

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Range of Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (μS/cm)</td>
<td>300 – 1,000</td>
<td>(77,78)</td>
</tr>
<tr>
<td>pH</td>
<td>6 – 9</td>
<td>(79,80)</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>850 – 950</td>
<td>(77,81)</td>
</tr>
<tr>
<td>Fat and oil (mg/L)</td>
<td>50 – 120</td>
<td>(81)</td>
</tr>
<tr>
<td>Chlorides (mg/L)</td>
<td>80 – 400</td>
<td>(78,82)</td>
</tr>
<tr>
<td>Total N (mg.N/L)</td>
<td>60 – 98</td>
<td>(80,83)</td>
</tr>
<tr>
<td>NH₄ (mg.NH₄/L)</td>
<td>10 – 68</td>
<td>(78,84)</td>
</tr>
<tr>
<td>Nitrite (mg.NO₂/L)</td>
<td>0.1 – 0.58</td>
<td>(85)</td>
</tr>
<tr>
<td>Nitrate (mg.NO₃/L)</td>
<td>1 – 2</td>
<td>(86)</td>
</tr>
<tr>
<td>Phosphate (mg.P-PO₄/L)</td>
<td>6 – 19</td>
<td>(78,81,85)</td>
</tr>
<tr>
<td>Suspended solids (mg/L)</td>
<td>120 – 400</td>
<td>(78)</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>1350 – 2480</td>
<td>(67)</td>
</tr>
<tr>
<td>Dissolved COD (mg/L)</td>
<td>380 – 700</td>
<td>(69)</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>120 – 130</td>
<td>(69)</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>31 – 180</td>
<td>(88)</td>
</tr>
<tr>
<td>BOD₅/COD (biodegradability index)</td>
<td>0.3 – 0.4</td>
<td>(89)</td>
</tr>
<tr>
<td>AOX (μg/L)</td>
<td>550 – 10,000</td>
<td>(90)</td>
</tr>
<tr>
<td>E. coli (MPN/100 mL)</td>
<td>10⁵ – 10⁶</td>
<td>(18,88)</td>
</tr>
<tr>
<td>Enterococci (MPN/100 mL)</td>
<td>10⁵ – 10⁶</td>
<td>(88)</td>
</tr>
<tr>
<td>Fecal coliform (MPN/100 mL)</td>
<td>10⁵ – 10⁶</td>
<td>(88)</td>
</tr>
<tr>
<td>Total coliform (MPN/100 mL)</td>
<td>10⁵ – 10⁶</td>
<td>(83)</td>
</tr>
<tr>
<td>Streptococci (MPN/100 mL)</td>
<td>10⁵ – 10⁶</td>
<td>(83)</td>
</tr>
<tr>
<td>EC₅₀ (Daphnia), TU (MPN/100 mL)</td>
<td>9.8 – 117</td>
<td>(91)</td>
</tr>
<tr>
<td>Total surfactants (mg/L)</td>
<td>4 – 8</td>
<td>(81)</td>
</tr>
<tr>
<td>Total disinfectants (mg/L)</td>
<td>2 – 200</td>
<td>(78,90)</td>
</tr>
<tr>
<td>Total detergents (mg/L)</td>
<td>3 – 7.2</td>
<td>(64)</td>
</tr>
<tr>
<td>Benzalkonium chloride (μg/L)</td>
<td>49</td>
<td>(64)</td>
</tr>
<tr>
<td>BAC_C12 (μg/L)</td>
<td>34</td>
<td>(64)</td>
</tr>
<tr>
<td>Antibiotics (μg/L)</td>
<td>30 – 200</td>
<td>(78)</td>
</tr>
<tr>
<td>Antinfammatories (μg/L)</td>
<td>5 – 1,500</td>
<td>(78)</td>
</tr>
<tr>
<td>Lipid regulators (μg/L)</td>
<td>1 – 10</td>
<td>(78)</td>
</tr>
<tr>
<td>Cytostatic agents (μg/L)</td>
<td>5 – 50</td>
<td>(78)</td>
</tr>
<tr>
<td>ICM (μg/L)</td>
<td>0.2 – 2,600</td>
<td>(78)</td>
</tr>
<tr>
<td>Psychiatric drugs (ng/L)</td>
<td>128 – 1123</td>
<td>(2)</td>
</tr>
<tr>
<td>X-ray contrast media (μg/L)</td>
<td>0.461 – 1,400</td>
<td>(92)</td>
</tr>
<tr>
<td>Beta-blockers (μg/L)</td>
<td>0.4 – 25</td>
<td>(78)</td>
</tr>
</tbody>
</table>
Unfortunately, the HWWs effluent was being discharged into the same sewers used to drain the surrounding urban and municipal wastewaters until today\(^\text{64}\). This practice was still conducted by many developing countries, including Indonesia\(^\text{70,71}\). In Indonesia, only 36\% of total hospitals have an installed WWTP, which means another 64\% of total hospitals might directly discharge their wastewaters into receiving water bodies \(^\text{71}\). Even during this pandemic, Indonesia still has a very limited capacity and ability to treat medical wastes, including HWWs.

In addition to hazardous chemical compounds, several HWWs also contain physical agents such as \(^\text{131}\)I, \(^\text{125}\)I, \(^\text{133}\)Xe, \(^\text{60}\)Co, \(^\text{18}\)F, phosphorus-32, strontium-89, and yttrium-90 where about 90\% of the total radioisotope administered to patients was concentrated in HWWs in the form of human excreta\(^\text{72}\). Another threat was coming from the fact that some infectious particles such as prions, viroids, and toxins were also found in HWWs. HWWs could be an ideal environment for several microbes to survive or even develop. However, biotechnological methods commonly applied to treat HWWs were reported to enhance the emergence of antimicrobials resistant bacterial, considering their limited ability to degrade complex compounds constructing antibiotics\(^\text{73,74}\). These improperly degraded antimicrobials compounds led to the inhibition of the growth of susceptible bacteria, thereby underlying the development of antimicrobials resistance mechanism, arising an abundance of resistant microbes existing in HWWs. Discharged resistant bacteria into the environment might act as a vector carrying a transmissible gene or as a reservoir of antibiotic-resistance genes (ARGs)\(^\text{75}\). Several studies had shown that the emergence of resistant microbes in the water environment mainly was due to the HWWs effluent\(^\text{76}\). Currently, the detected SARS-CoV-2 in wastewaters added those previous hazard of hospital wastewaters.

According to the Ministry of Health of the Republic of Indonesia, final disinfection efforts of SARS-CoV-2 in the HWWs were carried out through chlorination after a series of standardized treatments. This recommendation aligns with methods filed by WHO (free chlorine \(>0.5\) mg/L more than 30 minutes) and China Centers for Disease Control and Prevention (free chlorine \(<6.5\) mg/L up to at least 1.5 hours). However, the recent research elicited that disinfection using sodium hypochlorite resulted in a strikingly high level of SARS-CoV-2 residues \([(0.5 – 18.7) \times 10^3 \text{ copies/mL}] inside Wuchang Cabin Hospital septic tank in China\) even after suggested chlorination procedures were applied \(^\text{93}\). Unfortunately, these suggestions seemed to be not efficient in killing persistent SARS-CoV-2 in HWWs and arose ecological effect as the consequence of highly sodium hypochlorite concentration and by-products toxicity. Fortunately, the advanced oxidation process (AOP) was known to solve the problems of HWWs treatment through a chain of random oxidation. However, most of the AOP techniques applied in HWWs treatment were generally still combined with several conventional techniques, such as membrane bioreactor or conventional activated sludges, and still results in a lack
of effectiveness in pharmaceutical and microbial removal(94,95), as well as an increase in the treatment costs of HWWs. Ozonation was the most widely used technique among other AOP techniques, which is a technique injecting ozone into liquids, in this case, HWWs. Ozonation might need low doses of ozone \((2 - 15 \text{ mg/L})\)(96), while chlorination at least needed more than 30 mg/L in order to inactivate 90% antibiotic resistant bacteria and antibiotic resistance gene (ARG), which is impracticable(97). In addition, ozone posed a unique biocide effect trend where its biocidal activity was constantly presented to still have about 50% of the initial concentration. Meanwhile, other disinfection methods might show a slowing downtrend with a lower concentration and higher exposure time (98). However, ozonation only produced a small amount of dissolved ozone in the liquid(16,17), limiting the efficiency of the oxidation process. Thus, conventional ozonation tends to consume higher energy, time, and costs(18,19). This would be further discussed below.

**Ozonated Nanobubbles**

Ozonated nanobubbles are a combination of ozonation techniques and nanobubbles technology. Conventional ozonation techniques had been widely applied as a secondary or even tertiary HWWs treatment in hospital WWTP installations. Ozonation is carried out by continuously injecting ozone into the HWWs in an attempt to break down complex compounds into simpler molecules through the advanced oxidation process (AOP). Conventional ozonation techniques have been reported to degrade the complex compounds constructing antibiotics(99). Recent studies utilized cold plasma to degrade ofloxacin and ciprofloxacin for 25 minutes, had shown that conventional ozonation broke down ofloxacin and ciprofloxacin for up to 88 - 92% and 75 - 89% respectively at 70 kV and 80 kV(24). Ozone is a strong oxidizing agent \((E^o = 2.08 \text{ V})\) that can react with organic and non-organic compounds. The mechanism of oxidation by ozone takes place in two ways, namely direct and indirect pathways. The direct pathway involves the interaction between dissolved ozone and complex compounds, while the indirect pathway occurs through interactions involving reactive oxygen species (ROS) including hydroxyl groups \([\text{OH} \ (E^o = 2.80 \text{ V})]\), atomic oxygen \([\text{O}^* \ (E^o = 2.42 \text{ V})]\), singlet oxygen \((^1\text{O}_2)\), superoxide anion \((\text{O}_2^-)\), and hydrogen peroxide \([\text{H}_2\text{O}_2 \ (E^o = 1.78 \text{ V})]\) (100). In the acidic wastewaters (pH <4), most of the oxidation reactions directly involve dissolved ozone as shown by Equation (1) below:

\[
3\text{O}_3 + 2\text{OH}^- + 2\text{H}^+ \rightarrow 2\text{OH} + 4\text{O}_2
\]  

(1)

If the wastewaters are alkaline (pH > 9), the majority of the oxidation reactions occur indirectly by involving hydroxyl groups as follows:

\[
\text{O}_3 + \text{OH}^- \rightarrow \text{O}_2 + \text{H}_2\text{O}_2^-
\]  

(2)

\[
\text{O}_3 + \text{HO}_2^- \rightarrow \text{HO}_2 + \text{O}_3^-
\]  

(3)
\[ \text{HO}_2 \rightarrow H^+ + \text{O}_2^- \quad (4) \]
\[ \text{O}_2^- + \text{O}_3 \rightarrow \text{O}_2 + \text{O}_3^- \quad (5) \]
\[ \text{O}_3^- + H^+ \rightarrow \text{HO}_3 \quad (6) \]
\[ \text{HO}_3 \rightarrow \text{OH} \cdot + \text{O}_2 \quad (7) \]

However, in the alkaline wastewaters, most of the oxidation takes place according to the equation:
\[ \text{HO} + \text{O}_3 \rightarrow \text{HO}_2 \cdot + \text{O}_2 \quad (9) \]

As it is known that the rate of degradation during ozonation will increase with increasing pH(99), since basically pH may promote the ozone decomposition into free radicals as shown by Equation (2) to Equation (7). The reaction on Equation (9) rapidly generates hydroperoxyl radicals (E° = 1.65 V), which tends to decrease the oxidation capability of ozone due to its presence actually reduces the proportion of hydroxyl. Its strong oxidizing ability allows ozone to induce damage to cell walls and microbial genetic material, making ozone a promising chemical disinfectant agent even since the mid-1900s(101). The role of ozone as a disinfecting agent had been studied by several scientists, among which ozone had been shown to kill microbes that were resistant to chlorine including spore-producing microbes. This study was conducted by Ding et al. (2019), which used a sample of drinking water treated by a WWTP in South China (102). Research conducted by Ding et al. resulted in 99.9% inactivation of Bacillus cereus spores, and more than 75% of Bacillus cereus DNA material was damaged by 1.5 mg/L of ozone (102). Several studies on the efficiency of ozonation in killing microbes had been summarized by Gomes et al. (2019) (103) and Scholtz et al. (2015) (104), and showed that all viruses can be killed by ozone (96).

Figure 2. The classification of bubbles currently used
Some researches had been conducted to test the effect of virucidal ozone. Alshraieedeh et al. used a plasma jet to generate ozone which then was used to disinfect the contamination of M2bacteriophage (which constituted the microbe substituting norovirus attaching human) on the surface of objects. The research found that the ozone exposure could eliminate M2 bacteriophage up to 3 log_{10} (99.9%) only in 3 minutes and >7 log_{10}after 9 minutes(105). Venezia et al. once had tested the activity of virucidal plasma non-thermal towards bacteria, fungus, and some enveloped and non-enveloped viruses such as adenovirus, poliovirus, parainfluenza, and herpes simplex type 2. Based on the experiment by Venezia et al., non-thermal plasma exposure for 10 minutes could inactivate the entire virus included (inoculum: 10^4 - 10^6 cfu)(106). An instrument of cold oxygen plasma (COP) is recognized for its ability to reduce airborne virus infecting human respiratory to 6.5; 3.; and 4 log_{10}each for human parainfluenza virus type 3 (hPIV-3), respiratory syncytial virus (RSV), and influenza virus A (H5N2)(107). Unfortunately, most experiments conducted involved ozone and solvent media such as gas, which makes the process of disinfection more effective than ozonation. Recalling that during the process of conventional ozonation, experts found some obstacles such as the low dissolved ozone leading to the ineffectiveness of disinfection. The mechanism took by the ozone to induce inactivation towards the microbe, however, in general is the same without being influenced by the solvent medium phase.

Ozonation in HWWs containing organic compounds will produce many reactive nitrogen species (RNS) such as nitric oxide (NO), which then increases the ability of ozone to kill bacteria(108). The mechanism of ozone in killing microbes is not much different from the mechanism of microbial inactivation by chlorination, considering that they are both chemical oxidizers in which ozone tends to kill microbes by destroying the bonds between C - C, C - O, and C - N found in the peptidoglycan compounds constructing the microbial cell walls(109). ROS easily causes protein denaturation when interacting with the membrane, causing the microbial membrane to rupture(101). The destruction of the fat structure making up the membrane results in the deregulation of the mass transport through the membrane. On the other hand, charged particles which are also accumulative derivatives of ozone, cause cell wall or cell membrane damage through electrostatic disturbance mechanisms. The accumulation of charged particles adhering to the surface of the cell membrane disrupts the electrostatic equilibrium, which if it exceeds the electrostatic strength tensile, may break the bonds of the atoms constituting the cell membrane. ROS and RNS can also lower intracellular pH after diffusing into the virus, causing viral inactivation due to the absence of pH homeostasis(110). OH- dan H2O* were found to break the bonds between the C - N and C - C peptides respectively(111). Furthermore, these two radicals could also cause damage to the protein structure on the N-terminal. Also, ROS and RNS might oxidize amino acids, nucleic acids, and fatty acids, which are important compounds.
making up genetic material and microbial membranes. ROS and RNS will oxidize nucleic acids to 8-hydroxy-2 deoxyguanosine and amino acids to 2-oxo-histidine(110). The interaction of ROs and RNS with cell membranes induces damage to genetic material (DNA and/or RNA) through the formation of pores or gaps in the membrane arising after ROS and RNS oxidation. However, Yasuda et al. had ever conducted an experiment involving λ phage to determine DNA resistance under cold plasma exposure. Yasuda et al. found that the resistance curve of repackaged-DNA of λ phage tends to get bigger ($D_0 = 3s$ and $D_f = 25s$)(112). This indicates that DNA oxidation plays a hard role in viral inactivation.

Also, Xu et al. (2002) stated that the dose of ozone required to kill microbes in wastewater highly depends on the characteristics of the wastewater itself(96), which was also confirmed by Tachikawa et al., where the effectiveness of ozonation removal of biofilm strongly depended on the biofilm structure(98). However, conventional ozonation methods still present a lot of drawbacks. The most important is conventional ozonation provides a measly amount of dissolved ozone in water(16,17). Conventional injection of ozone as a gas phase into a liquid phase may produce macro bubbles that quickly burst on the surface of the water. Thus, the mass transfer of ozone from the bubble to the liquid tends to be quite small, as the contact time between the ozone bubbles and the water is relatively short. In fact, the mass transfer of ozone from bubbles to water is one key to the effectiveness of HWWs ozonation. Furthermore, the inefficiency of HWWs treatment through conventional ozonation causes the HWWs treatment cost to increase, which in turn makes conventional ozonation less comparative than other disinfection methods such as chlorination, which generally offer affordable disinfectant solutions. Another problem that may need to be considered is the fact of the ozone odors and easily irritating property, especially to mucus membranes. Therefore, conventional ozonation in which the majority of ozone gas bubbles burst on the surface of the water, highly increase this vulnerability. Fortunately, hospital WWTP installations are closed systems. Thus, the risk of irritation might be ruled out. One way to increase the contact time between ozone bubbles and water substances is to reduce the size of the bubbles. Since the early 2000s, scientists had developed nanobubbles technology which is now widely used in various fields, one of which is the wastewaters treatment. It was said that nanobubbles bring many advantages, especially in increasing the efficiency of the existing wastewaters treatment methods or enabling the emergence of new technology, more environmentally friendly technologies without putting the quality of treated water aside. Several experiments had been conducted to verify the benefits of ozonated micro-nano bubbles in increasing the effectiveness of ozonation in dyestuff wastewaters (113), coke wastewaters (114), and acrylic fiber wastewaters (115).
The characteristic of nanobubbles is 'longevity', meaning they may last for long periods and form large contact angles in the water (Figure 2)(116). This property arises since the nanobubbles have almost no buoyancy. According to Demangeat's (2015) experiment, nanobubbles (<5 μm in diameters) tend to not rise towards the water surface. On the other hand, macro bubbles (>1 mm in diameters) will rise rapidly and burst on the surface of the water(117). Oxygen nanobubbles were reported to generate OH radicals after being observed with a fluorescent probe(118). Furthermore, Atkinson et al. suggested that in general, the generation of nanobubbles with input gas does not mineralize organic compounds (O₂ and air), indirectly causes reactivity which was thought coming from OH, O₂⁻, and ^1O₂(119). This property allows the enhancement of the oxidation reaction during ozonation as Fan et al. (2019) showed that secondary effluent treatments using micro-nano bubbles technology generated OH radical(120). Some other properties of nanobubbles that enhance wastewaters treatment were summarized in Figure 3. Fan et al. (2020) had confirmed that micro-nano bubbles increase the solubility and mass transfer coefficient of ozone both in deionized water and in acetic acid (HAc) solution. This experiment resulted in the ozone lifetime was increased up to 1.39 - 3.52 times longer after injected into the micro-nano bubbles system [more dominated by millibubbles (1.93 ± 0.26 mm) than micro-nano bubbles (3.38 ± 0.73 μm) population](121). So far, the combination of the ozonation technique with nanobubbles technology has not been developed much. Most of the researches still focus on the mechanism occurring during ozonation with the help of a micro-nano
bubbles generator. Optimization studies on ozonated micro-nano bubbles technology are deemed necessary considering the previous research showed that micro-nano bubbles powerfully increase the effectiveness of conventional ozonation which is considered less efficient in terms of energy and costs consuming.

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