Disinfection applications of ozone micro- and nanobubbles

Petroula Seridou and Nicolas Kalogerakis

Micro- and nanobubbles (MNBs) are microscopic gas bodies sized at micro (<100 μm) and nanoscale (<1 μm), that have a long lifetime in aqueous solutions and large specific surface area due to their small size. Recently, scientific interest has been focused on ozone micro- and nanobubbles (OMNBs) used in disinfection processes since research findings support the idea that ozone micro and nanosized bubbles can significantly improve the disinfection capacity and the residual activity of ozone. The aim of this critical review is to present recent studies which investigate the feasibility of ozone-based disinfection processes by exploiting the strong oxidizing ability of ozone and the noteworthy longevity of MNBs in aqueous solutions. Properties of MNBs and generation techniques are briefly discussed besides the monitoring methods for their characterization in terms of size and number. In this critical review, we provide recent research related to the application of OMNBs in disinfection of drinking water, as well as in aquaculture, agriculture, and wastewater treatment. Finally, research gaps and limitations of this technology are highlighted and directions for future studies are suggested.

Environmental significance

Bacterial contamination and subsequent infections are recognized as a major threat to human health and there is dire need to prevent the waterborne diseases to ensure water safety. Moreover, attention must be paid on the occurrence and fate of trace organic compounds that have become an emerging concern, since conventional wastewater treatment plants (WWTPs) have not been designed for their elimination leading to their discharge to natural water bodies. Within the context of upgrading the water and wastewater treatment processes, the development of new disinfection technologies is addressed, with a view to provide high quality water at the least possible cost to the consumers. By utilizing the higher gaseous ozone half-life time (3 days versus 20 min at 20 °C) and the remarkable properties of ultra fine bubbles, the ozone delivery by MNBs has been found to improve the disinfection capacity and the residual concentration. In this regard, the application of OMNBs technology is paving the way to novel integrated and highly efficient disinfection systems.

1. Introduction

Nowadays, the mass production of wastewater derived from increasing population and industrialization is of major concern since it poses a remarkable threat to existing water resources. Consequently, reclamation and reuse of wastewater are extremely important to meet the human needs arising from inadequate water supplies. However, the core problem of reclaimed water is that it may contain different types of resistant pathogens and persistent organic compounds.1 The microbiological quality plays a crucial role for any potential reuse options, and hence, the presence and persistence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG) after tertiary treatment is considered an issue of great importance regarding public health.2–6 In order to prevent the dispersal of ARB, several treatment strategies have been tested and their inactivation efficiency was evaluated,7–9 however, most of these studies have not been conducted in real drinking water and/or wastewater revealing a considerable risk arising from the reduced disinfection ability compared to non-resistant bacteria.10 Emerging organic contaminants (EOCs) consist of a large and relatively new group of compounds covering complex synthetic or naturally occurring molecules or even any microorganism, not commonly monitored in the environment.11 These chemical compounds are classified as endocrine disrupting chemicals (EDCs), pharmaceutically active compounds (PhACs) and personal care products (PCPs), which even in low concentrations (from ng L−1 to μg L−1) may have detrimental ecological and human health effects.12–14 Moreover, in the last couple of decades, it is well documented that the effluent of WWTPs is the major pathway to aquatic environment,15–19 since they are poorly removed by the conventional activated sludge treatment.20 The emergence of new contaminants in effluent wastewater streams has led to the development of advanced technologies in order to achieve an efficient degradation of these emerging contaminants.21–23
Critical review

MNBS technology is novel and vitally important owing to the ability to generate highly reactive free radicals. In general, microbubbles (MBs) and nanobubbles (NBs) are microscopic gaseous bodies sized with diameters from tens of nanometres to several tens of micrometres. Since the majority of commercially available generators produce gas-carrying bubbles with a diameter within micro- and nano-range, a significant amount of research has been conducted on the use of MNBS technology.

Ozonation is recognized as a favourable treatment method since ozone is an extremely powerful oxidant and is used to inactivate pathogenic microorganisms for the prevention of waterborne diseases spread to users and the environment. Furthermore, ozone in aqueous solution auto-decomposes quickly and is converted to oxygen resulting in no harmful residues. However, this is also the main limitation of this method as ozone dissolved in water is unstable and short-lived and hence, the residual action in a drinking water network is very limited. Air MNBS are used to improve gas-liquid contacting and achieve increased effectiveness and enhanced mass transfer compared to conventional aeration including the use of ozone/air mixtures for more efficient ozonation.

The attribute of micro and nanobubbles to ozonation has stimulated widespread interest, and hence, a growing body of literature has investigated the effect of combined micro- and nanobubbles technology and ozonation in many fields of engineering and wastewater treatment. Despite the considerable progress in academic studies related to MNBS, there are limited comprehensive reviews that focus on the ozonation technology applied to disinfection as shown in Table 1. In this review, we summarize recent research findings regarding the application of ozone micro- and nanobubbles technology on disinfection and thus assist researchers who wish to become involved in this fast-expanding field.

2. Nanobubbles–microbubbles

2.1 Fundamental properties

According to Temesgen et al., there is no clear definition in terms of diameter size of MNBS. A proposed categorization is that MBs and NBs are in size scale at 10–100 μm and less than a micron, respectively even though in many studies MBs are classified less than 50 μm and NBs less than 200 nm. In this critical review, based on the majority of existing studies, we define MBs less than 100 μm and NBs less than 1 μm, according to Fig. 1.

As seen in Fig. 1, bubbles have different properties based on their size. In particular, large bubbles, known as millibubbles or macrobubbles (MaBs) rise rapidly and directly to the liquid surface, where they burst out. Compared to ordinary large bubbles, microbubbles have several interesting features such as longevity in aqueous solutions due to low rising velocity, large gas–liquid interfacial area and the most important the generation of hydroxyl radicals by their collapse providing an oxidation ability, which makes the dissolution easier. So far, a number of researchers have recognized the significance of these properties and they have employed MBs technology in various applications. In particular, the striking property of MBs, high surface area per unit volume has been used for degradation of organic pollutants and water disinfection. Nevertheless, they have been found to be unstable for a long period of time (~min), rising slowly to the liquid surface.

Smaller bubbles than MBs, classified as nanobubbles display noteworthy stability resulting in high stagnation times. NBs can remain stable in aqueous solution for a longer period of time.

Table 1 Published reviews on the application of ozone micro- and nanobubbles (OMNBs) technology

<table>
<thead>
<tr>
<th>Title</th>
<th>Content</th>
<th>Ref./year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications of Ozone Micro- and Nanobubble Technologies in Water and Wastewater Treatment: Review</td>
<td>The use of ozone MNBS • For disinfection in water and wastewater • For oxidation of organic and inorganic pollutants • For colour removal of water and wastewater treatment • To control water pollution in other areas The future of ozone MNBS</td>
<td>Tekle, Kim and Lee, 2017</td>
</tr>
<tr>
<td>Micro and nanobubble technologies as a new horizon for water-treatment techniques: A review</td>
<td>Applications of MBs and NBs in water-treatment technology • Disinfection with ozone microbubbles (OMBs) Properties and generation of microbubbles; applications in ozone treatment</td>
<td>Temesgen et al., 2017</td>
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<tr>
<td>A Review of Microbubble and its Applications in Ozonation</td>
<td>Application of microbubble ozonation for degradation of contaminants • Dyestuff • Pharmaceuticals • Other organic compounds (phenols, heterocyclic organic compounds, nitro aromatic compounds, etc.) Challenges and future prospects of microbubble ozonation • Microbubble generation methods • Reactor configuration</td>
<td>Shangguan et al., 2018</td>
</tr>
<tr>
<td>Microbubbles and their application to ozonation in water treatment: A critical review exploring their benefit and future application</td>
<td></td>
<td>John et al., 2020</td>
</tr>
</tbody>
</table>
long period of time (weeks), due to their negligible buoyancy and excellent stability against coalescence.\textsuperscript{50,51} Considering their unique characteristics, they improve the mass transfer and oxidation ability, simply because the gas/liquid contact area is increased.\textsuperscript{52} Moreover, the gas solubility and chemical reactions at the gas–liquid interface are remarkably enhanced.\textsuperscript{48,51}

The degree of nanobubbles stability is associated with the absolute value of zeta potential, which is presented in detail in the “Monitoring methods” section. More recent evidence\textsuperscript{53} highlights that the generation of smaller and more stable nanobubbles is achieved in solutions of high pH, low temperature and low salt concentrations. Another study by Hewage et al. demonstrated the stability of nanobubbles for one week in solutions of different electrolytes at a low concentration (0.001 M), confirming that the neutral and high pH values under low valency cation adsorption leads to negative charged bubbles.\textsuperscript{54} The highest negative charge of bulk nanobubbles and therefore their stability was also reported in alkaline solutions by Michailidi et al. In the case of oxygen and air nanobubbles, the magnitude of negative zeta potential increases as pH increases.\textsuperscript{55}

Thus far, a number of studies have reported that they have widely applied NBs in water treatment, aquaculture, agricultural cultivation, health preservation, mineral flotation\textsuperscript{56} and in removing organic pollutants in wastewater treatment.\textsuperscript{37,58} It is crucial to note that in relevant scientific literature, there is remarkable growth in microbubbles and nanobubbles-related citations and publications over the last 20 years as presented in Fig. 2.\textsuperscript{59}

However, there is still considerable controversy surrounding the existence and the stability of bulk NBs. In order to ascertain that the stable detected nanoentities are gas-filled domains and not impurities or nanodroplets, many analytical experimental techniques have been employed.\textsuperscript{60-65}

Even though, there is considerable discussion in the literature on whether NBs can exist or are thermodynamically stable, it has been demonstrated that the Young–Laplace equation is valid even at nanoscale.\textsuperscript{66} More precisely, the

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**Fig. 1** Range of bubbles sizes and corresponding major properties. Adapted from ref. 37 with permission from Elsevier, copyright 2017.

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**Fig. 2** (a) Annual number of publications for nanobubbles, (b) annual number of publications for microbubbles. Reproduced from ref. 59 with permission from Elsevier, copyright 2021.
pressure inside the gas cavities is defined in relation to the diameter of bubbles in accordance to the thermodynamic calculation based on Young–Laplace:

\[ P_{in} = P_{out} + \frac{2\gamma}{r} \]

where, \( P_{in} \) is the internal pressure inside a gaseous bubble (N m\(^{-2}\)), \( P_{out} \) is the pressure of bulk liquid (N m\(^{-2}\)), \( \gamma \) is the surface tension (mN m\(^{-1}\)) and \( r \) is the radius of bubbles (nm). It is estimated that a radius of NBs equal to 100 nm can result in an internal pressure \( 1.5 \times 10^6 \) N m\(^{-2}\) when the surface tension is 72 mN m\(^{-1}\) and the atmospheric pressure in the surrounding water is \( 10^5 \) N m\(^{-2}\).67

Hence, the inner pressure of the bubble increases when the size decreases which is expected to lead to a rapid dissolution and disappearance within seconds. Prior study describes the lifetime (\( t_b \)) of a bubble according to the following equation:

\[ t_b = \frac{Kd_a^2}{12RTD} \]

where, \( K \) is the Henry’s law constant (J mol\(^{-1}\)), \( d_a \) is the bubble diameter at \( t = 0 \) (nm), \( R \) is the gas constant (J K\(^{-1}\) mol\(^{-1}\)), \( T \) is the temperature (K) and \( D \) is the diffusion constant (m\(^2\) s\(^{-1}\)). For instance, a nanobubble with a diameter 100 nm should exist for only 10 \( \mu \)s.60,68 Surprisingly, this is not the case with nanosized gas cavities which can stay in aqueous solutions for prolonged periods of time (up to 12 months) compared to larger bubbles.48

In order to explain the longevity of nanobubbles, Ohgaki et al. proposed that the surfaces of nanobubbles contain strong hydrogen bonds at the gas–liquid interface similar to those found in ice and dehydrated gas. This ameliorates the stability of NBs as it decreases the gas diffusion in liquid, which contributes to kinetic balance against high internal pressure.49 Another possible explanation for the stability of NBs is that it may be dependent on the selective adsorption of anions at the interface that could result in electrostatic repulsive forces, leading to balance the compressive force from surface tension. Hence, a non-contact between gas molecules inside the NBs and the bulk liquid is created due to balance of these forces from the surface tension.69

2.3 Monitoring methods

Several methods have been reported in the literature for the measurement of the size distribution of MNBs.65,66,67,68 The size detection of bubbles has become a crucial issue in classification of ultratine bubbles due to the fact that it is complex to distinguish the gas bubbles from other colloidal dispersions such as oil nanodroplets or nanoparticles. Undoubtedly, there is a need for the development of techniques with higher level of sensitivity and spatial resolution. Until now, most researchers have utilized mostly dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), both based on scattering and diffraction of laser on the micro- and nanobubbles.77

**Light scattering technique.** The light scattering method is a simple and easy monitoring method based on Tyndall effect.55,78 More precisely, as a light beam passes through a colloid, the light scatters and reflects light, making the beam visible.79 Hence, as the nanobubbles do not rise quickly they can be illuminated by a laser beam and can be viewed with bare eyes while in a clean solution no laser beam can be detected.80 It is an ideal method for simple detection in clear water.

**Nanoparticle tracking analysis (NTA).** Nanoparticle tracking analysis offers direct and real-time visualisation of nanoparticles in liquids and size determination within the size range \( \sim 10 \) to 1000 nm.81 This technique captures the movement of each scattering object with dark field microscopy and their sizes are derived from the analysis of the particles trajectories. It should be highlighted that this technique can also provide adequate information about the particle concentration,82 which is fundamental for the estimation of the micro/nanobubbles generator performance. The main advantage of this technique is that it can record individual particles providing higher resolution and visual
information, and thus some kinetic processes can be observed, such as aggregation phenomena.\textsuperscript{83} However, the main drawback is the analysis of particles with low refractive indices (RI) compared to the background, as it becomes somewhat challenging due to low light scattering intensity.\textsuperscript{82}

**Dynamic light scattering (DLS).** Dynamic light scattering is among the most widely used methods to measure the size distribution of micro- and nanobubbles, typically ranging from 0.5 nm to 6 \( \mu \)m. A laser beam illuminates the sample and the fluctuations of the scattered light are detected by a photon detector at a scattering angle \( \theta \). The particles follow the Brownian motion, with the larger giving greater scattering but slower fluctuations. Analysis of the intensity fluctuations can provide the particle size distribution.\textsuperscript{67} The results obtained by light scattering alone may be misleading as a result of the high sensitivity to nano-sized contaminants. Therefore, it is recommended the combination of this technique with acoustic-based flow cytometry in order to ascertain the existence of nanobubbles instead of particles.\textsuperscript{84}

A study conducted by Gnyawali \textit{et al.} demonstrated that the acoustic flow cytometer can be used in order to detect individual NBs using high-frequency ultrasound and photoacoustic waves since the amplitude of the detected ultrasound backscatter signal is dependent on the NBs size.\textsuperscript{85}

**Zeta potential.** Another method of gas bubbles detection that is often used is the measurement of the zeta potential value. Nanobubbles have strong electron affinity and that is identified by a high magnitude of zeta potential ranging from 10 to 50 mV in absolute values. The measurement of zeta potential shows high negative values in most studies verifying that NBs in solution are normally negatively charged.\textsuperscript{53,86,87} This can be illustrated by the preferential adsorption of hydroxide ions (OH\textsuperscript{−}) at the gas-liquid interface,\textsuperscript{88} which results in electrostatic repulsive forces leading to balance the compressive force from surface tension. Thus, aggregation and coalescence of NBs are prevented.\textsuperscript{89}

Other methods employed for monitoring of nanobubbles are resonant mass measurement (RMM),\textsuperscript{90} electron microscopy\textsuperscript{91} and electrical sensing zone method.\textsuperscript{92}

### 3. Ozone

Ozone has been applied for primary disinfection in drinking water treatment since the beginning of the 20th century and its use is becoming gradually more common. It is an unstable trioxegen molecule and therefore it must be generated on-site. As it is a very strong oxidant among other commonly used disinfectants (free chlorine, chlorine dioxide and UV light), it provides an excellent inactivation capacity against waterborne pathogens including bacteria, viruses, protozoa and endospores.\textsuperscript{93} Disinfection parameters such as ozone concentration and contact time are very important for the design of disinfection systems and depend strongly on the operating temperature. Moreover, the rate of inactivation of microorganisms by ozone depends on the type of organism and can vary by about four orders of magnitude. Moreover, other factors that influence the disinfection efficiency are the dissolved organic carbon (DOC), pH and bromide concentration.

### 3.1 Health risks of ozone

In waters containing significant concentrations of bromide, the required ozone exposures for a certain degree of inactivation may lead to high levels of bromate, which is a carcinogen for humans.\textsuperscript{84} Thus, in many applications bromate formation may be the limiting factor, and measures have to be taken to comply with the drinking water standard.\textsuperscript{95} According to a study conducted by Rice \textit{et al.}, in order to meet the requirements for an efficient microbial disinfection in drinking water treatment, the usual ozone dosage is 1.5 to 2 mg L\textsuperscript{−1}, while for viral inactivation, a residual ozone concentration of 0.4 mg L\textsuperscript{−1} should be detected at least 4 min after the initial ozone dosage.\textsuperscript{96}

Ingestion of drinking water treated by ozone poses no danger since ozone is short-lived and all the concentration present in water will decline to zero when reaching the consumer through the distribution system. However, there is a significant risk though the direct exposure to ozone; inhalation since it is very corrosive. Exposure to ozone at levels below 1 ppm for 10 min is asymptomatic. More severe exposures (1.5 to 2 ppm of ozone for 2 h) produce acute symptoms, such as dryness of mouth and throat, chest pains, coughing etc.\textsuperscript{96}

### 3.2 Ozone disinfection mechanism

Ozone can react with microbes and contaminants in two different ways, directly and indirectly. Direct reactions involve ozone molecules and are very specific. On the other hand, the indirect reaction involves free hydroxyl radicals (OH\textsuperscript{•}) produced by the ozone decomposition in water and are more reactive (\( E^0 = 2.80 \) V) and less selective than ozone (\( E^0 = 2.07 \) V). The pH of water is a vital factor in ozone decomposition, because of the fact that hydroxyl ions can initiate the reactions that take place. The direct ozonation dominates when pH < 4, while the indirect pathway prevails above pH 10. In waters with pH = 7, both direct and indirect ozone reactions can be important and they should be taken into account in the process of treatment design.\textsuperscript{97} The mechanism and kinetics of the basic reactions regarding the ozone decomposition was under investigation by many researchers.\textsuperscript{98} The interpretation of the processes is based on the following reactions in alkaline medium proposed by Tomiyasu \textit{et al.}\textsuperscript{99} In acidic medium, the sequence of reactions taking place are also listed in Table 2.\textsuperscript{100}

An important reaction is the first one in Table 2, where ozone reacts with OH\textsuperscript{−} and hence, it is greatly dependent on pH. At alkaline pH, eqn (5) describes the generation of HO\textsuperscript{•}. Higher concentration of hydroxyl ions leads to the increased generation of HO\textsubscript{2}\textsuperscript{−}, O\textsubscript{2}\textsuperscript{−}, O\textsubscript{3}\textsuperscript{−} and HO\textsuperscript{•}. At 7 \(< \) pH < 9, the generation of hydroxyl radicals is slow corresponding to the rate constant of the reaction (5) \((20–30 \text{s}^{-1})\). The propagation
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3.3 Ozone interaction with microorganisms

Ozone even in low concentrations (0.01 ppm) is effective against bacteria due to its high oxidation potential. There is limited information in the literature concerning the inactivation mechanisms of microorganisms by ozone. The bactericidal efficiency lies on the fact that there are many ozone reactions with chemicals of high biological importance. First of all, it is suggested that ozone attacks the glycoproteins and glycolipids in the cell membrane resulting in rupture of the cell. In addition, another bactericidal activity is the oxidation of the sulphydryl groups of certain enzymes which results in disruption of cellular enzymatic activity and loss of function. Moreover, ozone attacks the purine and pyrimidine bases of nucleic acids leading to DNA damage.

The proposed mechanism for the inactivation of E. coli proceeds in the following order of viability indicators:29

I. Direct oxidation/destruction of the cell wall with leakage of cellular constituents outside of the cell.

II. Reactions with radical by-products of ozone decomposition entering the cell.

III. Damage to constituents of the nucleic acids (purines and pyrimidines).

IV. Breakage of carbon–nitrogen bonds leading to depolymerization and to cell wall disintegration causing cell lysis.

The antimicrobial capacity of ozone includes not only bacteria, but also molds, viruses, and protozoa. Ozone can react with numerous organic compounds and generate radical species such as hydroxyl radical that have more oxidative potential. Both HO$_2$ and the HO$^+$ radicals are highly reactive and play a fundamental role in the disinfection process. After the direct protoplasmic oxidation of bacteria, the free radicals produced react with the nucleic acids and provoke a sufficient damage, and incontrovertibly achieve inactivation.103

3.4 Properties of ozone micro- and nanobubbles

One factor credited for the stability of MNBs in aqueous solutions is their zeta potential. High zeta potential values prevent the bubbles from coalescence by increasing the repulsive electrostatic force.50 In the case of OMNBs, the long term stability has a strong effect on dissolved ozone concentration and consequently on enhanced disinfection efficiency. It should be noted that small diameter with high specific area and low rising velocity increases the mass transfer rate and the ozone reactivity to target contaminants.104 The main factors that have a great impact on OMNBs are the following:

**Temperature.** The temperature is considered a crucial factor that can influence the stability of OMNBs. A recent study by Hewage et al. investigated the effect of temperature on the size of ozone nanobubbles and the zeta potential. They reported elevated temperatures resulted in an increase of diameter and a decrease of the zeta potential. The size was in the range of 100–300 nm, and the negative zeta potential values were within the range of −25 to −14 mV. To elucidate the fact that temperature is inversely proportion to zeta potential, the adsorbed ions at the gas–liquid interface should be taken into account since in high temperature they decrease owing to higher mobility.105

**pH.** The aforementioned studies have emphasized the strong impact of solution pH on zeta potential and specifically suggested that NBs produced in water at a high pH value exhibit small diameter and high zeta potential.53 In the case of OMNBs, the same trend was confirmed by another study where they investigated the values of zeta potential over a range of pH conditions.106 It was reported that the zeta potential value increased in absolute values as the pH values increased. Specifically, at pH = 2, 4.5, 7.5 and 8, zeta potential values were found to be 9.92, 2.35, −32.34, −37.55, respectively.106 Another research study produced similar results. The zeta potential of OMNBs in deionized water was approximately −33 mV at pH = 8 and above −20 at pH = 7.107 Hence, it is clear from these results that at high pH the stability of OMNBs is greater, mainly due to increased adsorbed OH$^-$ ions at the interface. However, since ozone decomposes more quickly at high pH,108 in order to achieve the same levels of ORP, a greater amount of bubbles is required at higher pH.109

**Salt concentration.** The generation of ozone nanobubbles under various salt concentrations (0.01, 0.1 and 1 M) showed that increasing sodium chloride (NaCl) concentration

<table>
<thead>
<tr>
<th>No</th>
<th>Reactions</th>
<th>Rate constant</th>
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<tbody>
<tr>
<td>In alkaline medium</td>
<td>$O_3 + OH^- \rightarrow O_2 + HO_2^-$</td>
<td>$k_1 = 40 M^{-1} s^{-1}$</td>
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<tr>
<td>2</td>
<td>$O_3 + HO_2^- \rightarrow O_2^- + O_2$</td>
<td>$k_2 = 2.2 \times 10^6 M^{-1} s^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>$HO_3^- + OH^- \rightarrow O_2 + H_2O$</td>
<td>$pK = 4.8$</td>
</tr>
<tr>
<td>4</td>
<td>$O_2^- + O_3 \rightarrow O_3 + O_2$</td>
<td>$k_4 = 1.6 \times 10^3 M^{-1} s^{-1}$</td>
</tr>
<tr>
<td>5</td>
<td>$O_3^- + H_2O \rightarrow HO^+ + O_2 + OH^-$</td>
<td>$k_5 = 20-30 s^{-1}$</td>
</tr>
<tr>
<td>6</td>
<td>$O_3^- + HO^- \rightarrow O_2 + HO_2$</td>
<td>$k_6 = 6 \times 10^9 M^{-1} s^{-1}$</td>
</tr>
<tr>
<td>7</td>
<td>$O_3^- + HO^- \rightarrow O_2 + OH^-$</td>
<td>$k_7 = 2.5 \times 10^9 M^{-1} s^{-1}$</td>
</tr>
<tr>
<td>8</td>
<td>$O_3 + HO^- \rightarrow HO_2 + O_2$</td>
<td>$k_8 = 3 \times 10^9 M^{-1} s^{-1}$</td>
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</table>

In acidic medium

<table>
<thead>
<tr>
<th>No</th>
<th>Reactions</th>
<th>Rate constant</th>
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<tbody>
<tr>
<td>9</td>
<td>$O_3 \rightarrow O + O_2$</td>
<td>$k_9$</td>
</tr>
<tr>
<td>10</td>
<td>$O + H_2O \rightarrow 2HO^-$</td>
<td>$k_{10}$</td>
</tr>
<tr>
<td>11</td>
<td>$HO^+ + O_3 \rightarrow HO_3^- + O_2$</td>
<td>$k_{11} = 1.1 \times 10^8 M^{-1} s^{-1}$</td>
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<tr>
<td>12</td>
<td>$HO_2^- + O_3 \rightarrow HO_2 + O_2$</td>
<td>$k_{12} &lt; 10^4 M^{-1} s^{-1}$</td>
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resulted in a decrease in the magnitude of zeta potential with a slight increase in diameter.\(^{53}\) It is noted that the values of the zeta potential were negative in all cases. Another experiment focused on the effect of salinity on the stability of OMNBs in terms of zeta potential and size distribution showed that OMNBs are stable under various salinity levels, since they remained negatively charged. Specifically, the salinity caused a reduction in negative zeta potential when no obvious effect on the diameter of OMNBs was observed.\(^{30}\)

**Hydroxyl radicals.** Hydroxyl radicals exhibit microbicidal activity, and as such, their generation should be taken into consideration in order to provide some insight into the observed disinfection efficiency. Takahashi et al. reported that the generation of free radicals occurs by the micro- and nanobubbles collapse thanks to the high density of ions in the gas-liquid interface and they concluded that ozone microbubbles generate hydroxyl radicals under strong acidic conditions.\(^{110}\) Several studies, for instance,\(^{106,107,111}\) have proven that hydroxyl radicals existed in water containing ozone microbubbles using fluorescence intensity. It is noted that the capacity for generating free radicals is of high importance as hydroxyl radicals are strong oxidants and not selective, and thus the oxidation processes can be accelerated.\(^{106}\)

### 3.5 Ozone dissolution with micro- and nanobubbles

Even though conventional ozonation is widely used for ozone dissolution in aqueous phase, the main drawback is the high amount of escaping ozone gas resulting in a high level of gas consumption. When microbubbles is used for ozonation, the degradation of trace organic compounds were found to be efficiently enhanced since the solubility of ozone in water is increased.\(^{112}\) Several research studies suggest an association between bubble size diameter and the enhancement of ozone solubilization rate in the aqueous phase. Table 3 lists a number of existing studies, which have examined the comparison of ozone dissolution between macro and MNBs. All the available information about the experimental conditions is provided. A notable increase in peak value of dissolved ozone concentration was reported more recently by Hu and Xia,\(^{30}\) as the ozone level for OMNBs was 10.09 mg L\(^{-1}\) compared to macrobubbles which provided a very low ozone concentration.

#### Table 3 Comparison of ozone dissolution between ozone macrobubbles (OMaBs) and ozone micro- and nanobubbles (OMNBs)

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Size</th>
<th>Flow rate (L min(^{-1}))</th>
<th>Ozone conc. (mg L(^{-1})) or rate (g h(^{-1}))</th>
<th>Time (min)</th>
<th>Volume (L)</th>
<th>Temp. (°C)/pH</th>
<th>Type of water</th>
<th>Peak concentration (mg L(^{-1})) OMaBs vs. OMNBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>Micro/nano (32–460 nm, 4.55 × 10(^{3}) bubbles per mL)</td>
<td>4 L min(^{-1})</td>
<td>50 mg L(^{-1})</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td>Deionized</td>
<td>0.64 vs. 10.09</td>
</tr>
<tr>
<td>113</td>
<td>Micro (&lt;50 µm)</td>
<td>2.5 L min(^{-1})</td>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>De-chlorinated</td>
<td>3-fold lower-1.58 ppm</td>
</tr>
<tr>
<td>114</td>
<td>Micro (peak at 15 µm)</td>
<td>1 L min(^{-1})</td>
<td>50 mg L(^{-1})</td>
<td>30</td>
<td>5</td>
<td>Ambient</td>
<td>Distilled</td>
<td>5.5 vs. 8.3</td>
</tr>
<tr>
<td>115</td>
<td>Micro/nano</td>
<td>25 g h(^{-1})</td>
<td>5</td>
<td>20</td>
<td>20 ± 1/6</td>
<td>Distilled</td>
<td>0.65 vs. 2.16</td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>Micro (5–25 µM = 50%)</td>
<td>0.61–0.72 g h(^{-1})</td>
<td>5</td>
<td>20</td>
<td>10/7</td>
<td>Ultrapure</td>
<td>0.50 vs. 1.67</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Nano</td>
<td>0.5 L min(^{-1})</td>
<td>11 mg L(^{-1})</td>
<td>3</td>
<td>20</td>
<td>17.4 ± 1.2</td>
<td>Distilled</td>
<td>1.74 vs. 3.91</td>
</tr>
<tr>
<td>117</td>
<td>Micro/nano (3.38 µm, 2.41 × 10(^{3}) bubbles per mL)</td>
<td>0.5 L min(^{-1})</td>
<td>5 g h(^{-1})</td>
<td>14</td>
<td>3</td>
<td>20/8</td>
<td>Wastewater from acrylic fiber manufacturing industry</td>
<td>8.4 vs. 9.6</td>
</tr>
<tr>
<td>111</td>
<td>Micro (&lt;58 µm, 2.9 × 10(^{3}) counts per mL)</td>
<td>0.5 L min(^{-1})</td>
<td></td>
<td>10</td>
<td>20</td>
<td>18 ± 2</td>
<td>Deionized</td>
<td>∼8 vs. 13</td>
</tr>
<tr>
<td>119</td>
<td>Micro</td>
<td>0.2 L min(^{-1})</td>
<td>36 mg L(^{-1})</td>
<td>40</td>
<td>8</td>
<td>20</td>
<td>Tap</td>
<td>∼4 vs. 11</td>
</tr>
<tr>
<td>120</td>
<td>Ultra-fine (0.5–3 µm)</td>
<td>30 mL min(^{-1})</td>
<td></td>
<td>10</td>
<td>1</td>
<td>25</td>
<td>Distilled</td>
<td>3.5 vs. 8.3</td>
</tr>
<tr>
<td>121</td>
<td>Micro</td>
<td>3–4 mg L(^{-1})</td>
<td>12</td>
<td>80</td>
<td>20/7</td>
<td>Secondary treated sewage water</td>
<td>2.49 vs. 4.00</td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>Nano (133.7 nm, 5.25 × 10(^{7}) particles per mL)</td>
<td>7</td>
<td></td>
<td>25/7</td>
<td>Synthetic semi-conductor wastewater containing TMAH</td>
<td>∼1 vs. 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>Micro</td>
<td>5</td>
<td></td>
<td>20</td>
<td>Tap</td>
<td>3.5 vs. 5.3 (reached in 2 min)</td>
<td>7.9 vs. 13.4</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>Nano (580 nm, 2.16 × 10(^{5}) particles per mL)</td>
<td>0.5 L min(^{-1})</td>
<td>38 mg L(^{-1})</td>
<td>30</td>
<td></td>
<td>Deionized</td>
<td>0.82 ppm</td>
<td></td>
</tr>
</tbody>
</table>

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**Environmental Science: Nano**

Critical review

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value (0.64 mg L\(^{-1}\)) within a generation time 30 min. Kobayashi et al.\(^{113}\) noted that aqueous dissolved ozone concentration is higher when the water is treated with microbubbles compared to macrobubbles. In 5 min ozonation with microbubbles, the concentration of ozone reached 1.58, 1.24 and 0.82 ppm at 15 °C, 25 °C and 30 °C, respectively. On the other hand, when macrobubble ozonation was applied, the concentration was found 3-fold and 4-fold lower at 15 °C and 25 °C, respectively and no ozone was detected at the highest temperature. Another comparison of ozone microbubbles and normal bubbles demonstrated that the dissolved ozone concentration was approximately 2.5 times higher than that obtained by ordinary bubbling.\(^{114}\) More recent evidence\(^ {115}\) showed that ozone dissolution using micro- and nanobubbles was approximately 50% higher after 5 min-aeration compared to a classical mixing pump with larger bubbles. The findings of another study confirm the observation that bubbles with smaller diameter can enhance the dissolution of gaseous ozone into the aqueous phase.\(^ {116}\) In fact, the concentration of dissolved ozone by the regular method of ozone delivery was found to be 0.5 mg L\(^{-1}\) at 20 °C, when microbubble ozonation could reach the value of 1.67 mg L\(^{-1}\) in the presence of para-chlorobenzoic acid (pCBA).\(^ {116}\) An increase in ozone concentration with nanobubbles was also reported in the study conducted by Batagoda et al.\(^ {104}\) where the initial dissolved ozone concentration was 52.79 mg L\(^{-1}\), higher than 48.28 mg L\(^{-1}\) found with ozone macrobubbles. Fan et al.\(^ {117}\) illustrated that the concentration of dissolved ozone after MNBS aeration was 3.54 mg L\(^{-1}\) in 25 min while after the millibubbles ozonation the ozone reached only 1.74 mg L\(^{-1}\) in 30 min. The most striking result to emerge from this study is that the ozone solubility was calculated about 4 times higher in 5% acetic acid solutions after OMNBs aeration reaching the ozone value of 15.26 mg L\(^{-1}\). It is well documented that acetic acid is considered an ozone stabilizer due to non-reactivity with it and thus, it can be beneficial to the ozonation process.\(^ {115}\) Further confirmation is given by another research study,\(^ {118}\) where the saturated ozone concentration with microbubble ozonation reached the value of 9.6 mg L\(^{-1}\) within 7 min and was found to be enhanced since the macrobubble ozonation achieved a lower dissolved ozone concentration at longer time period. This can be elucidated by the fact that ozone mass-transfer coefficient was 2.2 times higher than that of the conventional ozonation process.\(^ {118}\) Research findings from two other studies corroborate with the previous result as the augmentation of total mass transfer in microbubbles ozonation was also proved for simulated dyestuff wastewater treatment (1.8 times higher) and for landfill leachate pre-treatment (1.5 times higher).\(^ {111,119}\) Similar results were reported by Wang, Lin and Liao,\(^ {120}\) a team which recently explored experimentally the raise in ozone dissolved concentration, when ultrafine bubbles are used. Within 10 min, the maximum dissolved ozone concentration reached the value of 8.3 and 3.5 mg L\(^{-1}\), during ozonation with MNBS and MaBS, respectively. The most recent evidence confirms once more the higher dissolved ozone concentration of 4 mg L\(^{-1}\) in microbubbles ozonation instead of 2.49 mg L\(^{-1}\).\(^ {121}\) In three test fluids, pure water, tap water and phosphate buffered saline, the ozone dissolution velocity (mg L\(^{-1}\) min\(^{-1}\)) was found higher by 1.5, 1.6 and 2.7 times when ozone injected by microbubble generator instead of porous diffuser.\(^ {122}\) In the course of the ozonation of synthetic semiconductor wastewater containing tetramethyl ammonium hydroxide within 7 min, the gas transfer to water by nanobubbles (1.67 mg L\(^{-1}\) min\(^{-1}\)) was 9.8 times faster than that of macro-ozone (0.17 mg L\(^{-1}\) min\(^{-1}\)). Consistently, the ozone dissolution was found once again 1.5 times higher by ozone microbubbles injection in tap water.\(^ {124}\) Finally, a group of researchers in 2021 has investigated the ozone mass transfer coefficient with nanobubble aeration and compared it with macrobubble aeration. Their findings are in line with all the previous results. In fact the volumetric mass transfer coefficient (K\(_{\text{L}}\)a) was estimated 0.179 min\(^{-1}\), reaching the peak value of ozone concentration of 13.4 mg L\(^{-1}\), while in macrobubble aeration the volumetric mass transfer coefficient was 4.7 times lower (0.038 min\(^{-1}\)) with a dissolved ozone concentration up to 7.9 mg L\(^{-1}\).\(^ {125}\) These findings demonstrate the strong effect of MNBS to ozone solubilization. In general, it can be concluded that the use of MNBS in ozonation leads to a more efficient process as the ozone utilization efficiency is higher.

### 3.6 Ozone decomposition rate

The half-life time of ozone in gas phase is much higher than in aqueous phase. In more detail, at 20 °C the gaseous ozone will be degraded in 3 days, in contrast the degradation of dissolved ozone in water will take place within only 20 minutes.\(^ {126,127}\) Due to its low utilization efficiency, nanobubbles technology is gradually used for ozone application in a more efficient way. However, there are very limited research studies that investigated the comparison of the half-life times between OMNBs and macrobubbles owing to the fact that the academic interest is focused on the study of ozone solubility and mass-transfer. Hu and Xia\(^ {128}\) have also investigated the half-life time of dissolved ozone with and without the use of MNBS and their results demonstrated that the average lifespan for the MNB system was 10.51 min, whereas that for macrobubbles system was only 0.70 min for 30 minutes generation time. A 2007 research study observed that a longer half-life was found when a microbubble generator injected ozone in tap water instead of a porous diffuser (1.6 times longer at 19.2 °C).\(^ {122}\) The lifespan when ozone delivered through nanobubbles in water was greater than conventional ozone bubbles. In fact, ozone is retained in water approximately four times longer than using a sandstone diffuser.\(^ {104}\) In another research study,\(^ {117}\) ozone decomposition was investigated when OMNBs were present in various concentrations of acetic acid and in water alone. In this case, the results showed that the average half-lives of ozone were longer by 1.39, 2.04 and 3.52 times in 0.5, 3 and 5% acetic acid solutions, respectively. The evidence from this study points
towards the idea that acetic acid can further enhance the longevity of ozone in water apart from MNBs. Remarkably in a very recent study, it was shown that half-life of ozone generated by nanobubbles was found to be 23 times higher than that of macro-ozone. The ozone lifespan was investigated in nanobubble and macrobubble aeration groups and was found to be 3.50 h and 1.75 h in the latter. In the presence of hydroxypropyl-β-cyclodextrin (HPβCD), which is used as an ozone stabilizer, the ozone half-life time were 2.8, 4.3, 9.3 and 2.2 times higher than those estimated from the microbubbles aeration under different HPβCD:O₃ molar ratios (1:1, 3:1, 5:1 and 10:1, respectively). The results so far confirmed that the utilization of MNBs can extend the ozone half-life. Moreover, it can be concluded that the addition of an ozone stabilizer can further intensify the ozone lifespan and can be utilized to strengthen the ozonation process.

4. Application of ozone-based macro- and nanobubble technology in disinfection

4.1 Antimicrobial and disinfection process

Bacterial contamination and subsequent infections are recognized as being a major threat to human health and there is an urgent need to inactivate pathogenic organisms and prevent the waterborne diseases spread to users and the environment. In this regard, the development of novel technologies based on the application of OMNBs is of paramount importance.

Furuichi et al. reported that ONBs water deactivates both Gram-positive and Gram-negative bacteria while this approach does not show any cytotoxicity against human gingival fibroblasts, unlike conventional mouth wash. Dissolved ozone concentration of 1.5 mg L⁻¹ provided a sufficient bactericidal activity for periodontal pathogens. Specifically, the inactivation of the bacterial cells (S. aureus:2.4 × 10⁸ CFU mL⁻¹, S. sanguinis:1.5 × 10⁸, K. pneumoniae:7.6 × 10⁶ CFU mL⁻¹ and E. coli:1.6 × 10⁹ CFU mL⁻¹) was >99.99% since the viable bacteria were below detection limit (<10 CFU mL⁻¹). For P. gingivalis cells with initial bacterial concentration 7.0 × 10⁷ CFU mL⁻¹, the percentage of killed bacteria was higher than 99.99%, while the disinfection activity was deteriorated in case of S. mutans with initial bacterial concentration 1.7 × 10⁸ CFU mL⁻¹, since it reached a maximum disinfection of 94.69% within three minutes. Another study for the evaluation of the bactericidal activity against periodontal pathogenic bacteria (P. gingivalis and A. actinomycetemcomitans) reported that ONBs water with concentration 1.5 mg L⁻¹ was capable to reduce the numbers of colony forming units (CFU mL⁻¹) below the limit of detection (<10 CFU mL⁻¹) after only 0.5 min of exposure, providing evidence that it is not cytotoxic to cells of human oral tissues.

As it is mentioned before, ozone is highly unstable and this is a problem posed in terms of storing ozone aqueous solutions. This issue was explored by Seki et al. implementing ONBs technology for the storage of ozone. It was found that such an approach produces good efficiency in storage as the microbicidal activity was adequate for different set time periods. ONBs stored at 4 °C retained more than 90% of ozone after a week and more than 65% after a month. Moreover, the residual concentration of ozone stored at 4 °C for 1 year was adequate to kill one of the most resistant bacteria, M. smegmatis, within 15 min; even though E. coli was not entirely killed even after a 60 min exposure.

4.2 Drinking water disinfection

A common strategy used to ensure safety in drinking water is ozonation. The rapid decomposition of ozone in water and the low residual concentration are the main drawbacks of this process. Utilizing NBs serves as a more efficient alternative to drinking water disinfection as the decomposition of ozone in water is decelerated and the ozone dosage required against contaminants or pathogens is reduced thanks to a greater dissolution. Sumikura et al. found that the ozone dose was lower when OMNBs were used instead of the conventional ozonation with macrobubbles providing the same inactivation rate of target pathogen E. coli.

One of the most crucial parameters of conventional ozonation is the cost effectiveness of installation. A recently conducted cost–benefit analysis indicated that the installation of a ONBs generator is beneficial for existing water treatment plants as the total cost would be four times less and could save 375$k per year.

Another important parameter is the effect of inlet ozone gas concentration on the removal rate. This issue has been investigated on the log reduction of B. subtilis using ONBs water with concentration 1.5 mg L⁻¹, when the initial gas concentration was 40 mg L⁻¹. It was found that the Kₐ was almost been doubled from inlet gas concentration 40 mg L⁻¹ to 140 mg L⁻¹, while the Sauter mean diameter was decreased from 75.7 μm to 49.7 μm, respectively. Combination of ozonation and hydrodynamic cavitation showed the best performance in disinfection of E. coli with an initial bacterial concentration of approximately 10⁹ CFU mL⁻¹ was decreased to zero within 45 min whereas for the same ozone concentration using only ozonation without cavitation, the bacterial concentration reached zero after 60 minutes.

Summarizing, the higher mass transfer leading to lower ozone dosage renders the use of OMNBs a promising and an efficient technology in terms of cost and disinfecting capacity.

4.3 Disinfection of wastewater treatment plant effluents

Apart from the importance of disinfection, attention must also be paid on the occurrence and fate of trace organic compounds in wastewater effluents as a consequence of ozonation due to their highly unstable nature. This is a problem posed in terms of ozone storage and transportation. To overcome this issue, ONBs technology was explored by Seki et al. implementing ONBs technology for the storage of ozone.
compounds that are considered of emerging concern. It is of major importance to eliminate these pollutants as they can be discharged to water bodies and induce adverse and undesirable effects onto humans, living organisms and environment even at low concentrations. As demonstrated in literature, the ozone amounts required for PPCPs oxidation may lead to a partial disinfection, hence it is crucial to highlight the influence of emerging contaminants on the ozone disinfection capacity.

In a study on wastewater treatment, an analysis on the deactivation of faecal and total coliforms in domestic wastewater in Peru indicated that through applying air–ozone micro–nanobubbles, it was obtained 99.58% for faecal coliforms and 99.01% for total coliforms. Lee et al. investigated the degradation of pharmaceuticals compounds by a microbubble ozonation process and showed that it was markedly enhanced by the decrease in diameter of the ozonated bubbles. It was found that the residual concentrations of the selected pharmaceuticals compounds, including 17α-ethinylestradiol (EE2), ibuprofen (IBU) and atenolol (ATE) were estimated at 0.61, 0.75 and 0.77, respectively, when treated with microbubbles and differ significantly from ozone millibubbles treatment, where the residual concentrations were found to be 0.79, 0.88 and 0.87.

Another investigation on the degradation of phosphates in water showed that the introduction of microbubble ozonation improved significantly the removal efficiency against Streptococcus agalactiae and Aeromonas veronii in fresh water which are considered pathogenic fish bacteria. Three consecutive ozone treatments (10 min exposure at ONBs at 15 min intervals) were tested. The first 10 min treatment reduced the bacterial load of S. agalactiae and A. veronii 26 and 48 fold or 96.11% and 97.92%, respectively. The next two 10 min ONBs treatment reduced further the bacteria load in water reaching higher than 99.9% reduction for both pathogenic bacteria. In water taken from a Nile tilapia-cultured tank (initial bacterial concentration: 8.18 × 10^5 CFU mL^-1) with the presence of organic matter the disinfection efficacy of ozone nanobubbles was reduced and reached the 59.63% after the first treatment and the other two treatments were required to reach the 99.29%. The loss in the disinfection capacity can be illustrated by the fact that the presence of the organic matter led to the rapid ozone oxidation and degradation.

In another research study, the disinfection of Vibrio parahaemolyticus at a concentration 10^6 CFU mL^-1 in 15% saline water was studied. At the end of the experiment, the bacterial concentration (CFU mL^-1) was estimated 2.3 × 10^1, 2.2 × 10^0 and 0 CFU mL^-1 for 2-, 4- and 6-minute ONBs exposure, respectively. The results of the oxidation–reduction potential (ORP) showed that the initial ORP value, which was 240 mV rose to 830 ± 70 mV after six minutes operation and remained stable at over 900 mV as the nanobubbles generator continued working for ten more minutes.

Thanh Dien et al. reported that even though, the bacterial concentration was high (~2 × 10^6 CFU mL^-1), multiple ONBs treatments in the first two days reduced the bacteria between 15.9% and 35.6% of total bacterial load in water, while bacterial concentration increased from 13.1% to 27.9% in the

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### Table 4: Applications of ozone micro- and nanobubbles (OMNBs) in aquaculture

<table>
<thead>
<tr>
<th>Target microorganism</th>
<th>Bacterial conc. (CFU mL^-1)</th>
<th>Type of water</th>
<th>ORP</th>
<th>Time (min)</th>
<th>Disinfection efficiency with NBs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. agalactiae</em></td>
<td>3.45 × 10^6</td>
<td>Dechlorinated tap water</td>
<td>834 ± 22 mV</td>
<td>10</td>
<td>96.11%</td>
<td>140</td>
</tr>
<tr>
<td><em>A. veroni</em></td>
<td>1.65 × 10^6</td>
<td>15% saline water</td>
<td>830 ± 70 mV</td>
<td>6</td>
<td>100%</td>
<td>141</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>1.8 × 10^5</td>
<td>Artificial sea water</td>
<td>960 mV (~3.5 mg O_2 per L)</td>
<td>5</td>
<td>100%</td>
<td>143</td>
</tr>
</tbody>
</table>
untreated control. ONBs sea water at 960 mV ORP was used to carry out disinfection experiments against V. parahaemolyticus EMS/AHPND strain. From these results it is clear that ONBs treatment provide a high disinfection efficiency, since after 1 min incubation over 99.99% of tested bacteria were killed and after 5 min or longer incubation the sterilization efficiency was 100%.

Apart from the ozone disinfection efficiency, Kurita in 2017 demonstrated the killing effect of cavitation treatment on small planktonic crustaceans that can cause detrimental problems in invertebrate aquaculture tanks through predatory damage or competition for food resources with the aquaculture species. The residual ozone concentration is of high importance since it has been found that concentrations within the range 0.01–0.1 mg L
−1 can be highly toxic to fish in fresh- and seawater. There is significant difference between the ozone reaction with saline and freshwater in terms of disinfection. The presence of bromide ion (Br−) in seawater results in the formation of brominated compounds like bromate (BrO3−) by ozone oxidation, which is toxic to aquatic organisms. On the other hand, in fresh water ozone decomposes to oxygen elevating the levels of dissolved oxygen in the system, which may also have detrimental effects on fish if it is very high. In terms of ORP, several studies suggest that the levels in the range from 300 to 425 mV can ensure the safety of fish, crustaceans and molluscs. Summarizing, in order to apply a safe ozone disinfection system, the lethal limits, which depend on the cultured species and the type of water, have to be determined and not exceeded during operation.

A study from Jhunkeaw et al. suggested that a single 10 min exposure to ONBs with an ozone level 860 ± 42 mV is safe for Nile tilapia in fresh water. Even though no mortality was observed after receiving the second and the third consecutive ONBs treatments, the increased exposure caused damage in the gill filaments. However in another study, they set up a modified recirculation system to reduce direct exposure to the fish, in order to avoid any alterations in exposed fish. In this case, juvenile Nile tilapia did not exhibit any abnormalities in behaviour or mortality by the application of multiple ONBs treatments. ONBs in seawater containing ozone dose at 3.5 mg L
−1 and 960 mV ORP was proven to be toxic to shrimp, therefore a twofold dilution of ozonated seawater was suggested as shrimp survival and excellent inactivation activity was observed. An additional study in the literature regarding the exposure of Nile tilapia (Oreochromis niloticus) to ozone nanobubbles noted that innate immunity genes involved in the systematic frontline defence system were stimulated. In all examined organs, these genes expressed an upregulation very fast within 15 min – post ozone nanobubbles treatment and lasted from 12 to 24 h in the gills, the head kidney and the spleen. It was thus concluded that based on the efficient stimulation of the genes by ONBs treatment, a protection to cultivated animals from potential pathogenic infections can be provided. In addition, any possible negative effect of the ultrafine bubbles in cavitation treatment on two juvenile sea cucumbers (Apostichopus japonicus) and sea urchins (Strongylocentrotus intermedius) was evaluated and it was found that all individuals were intact and unjured four days after exposure to ozone nanobubbles.

Experimental results provide a basis for the application of ozone nanobubbles in aquaculture since it is efficient for reducing pathogenic bacteria (Table 4). Future studies should aim to replicate results in a larger scale and further explore the efficiency to prevent disease outbreaks. The safety of using ONBs is a core issue and should be investigated in more detail in order to gain a better understanding of the toxicity to fish, which depends upon species and the life stage.

4.5 Agriculture

The effect of ozone ultra-fine bubbles on washing fresh vegetables was tested and when acidic electrolyzed water containing ozone ultra-fine bubbles and strong mechanical action combined, the lowest viable bacterial count was recorded among other treatments including sodium hypochlorite. The disinfection efficiency of F. oxysporum f. sp. melonis spores was tested and the results confirmed that ozone microbubbles exhibited higher disinfection efficiency than macrobubbles (Table 5). In addition, spores treated with OMBs showed surface injury after 30 s and wavy deformation of cell membrane was observed after 180 s, which may be caused by the generation of hydroxyl radicals penetrating into the spores. Two phytopathogens, Fusarium oxysporum f. sp. melonis and Pectobacterium carotovorum subsp. carotovorum have been investigated and the results suggest that ozone-rich microbubbles showed higher disinfection activity than the millibubbles over the same period of application. It is reported that the number of these two phytopathogens decreased rapidly thanks to elevated initial ozone concentration (3 logs at 0.33 min). At the same ozone level, they concluded that OMBs provided higher disinfecting activity against both pathogens. Micro and nanobubbles technology was also implemented to tackle tomato airborne disease. The results highlighted that the inactivation activity against Alternaria solani Sorauer conidia was reduced by 2 logs when ozone concentration of 1.6 mg L
−1 was applied. In the case of Cladosporium fulvum conidia it was found that one log reduction was achieved when 1.8 mg L
−1 of ozone was used. This level of ozone application did not affect tomato growth. The study by Kwack et al. have verified that using ozone microbubbles for seed sterilization is the most feasible treatment since the germination and growth of...
Critical review

Table 5  Applications of ozone micro- and nanobubbles (OMNBs) in agriculture

<table>
<thead>
<tr>
<th>Target microorganism</th>
<th>Bacterial conc. (CFU mL⁻¹)</th>
<th>Ozone conc. (mg L⁻¹)</th>
<th>Disinfection efficiency with OMNBs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. oxysporum f. sp. melonis</td>
<td>1 × 10⁵−1 × 10⁶</td>
<td>1.5 ppm (15 °C)</td>
<td>The number of surviving spores reached the detection limit in 45 s with OMNBs instead of 60 s with OMaBs</td>
<td>149</td>
</tr>
<tr>
<td>F. oxysporum f. sp. melonis</td>
<td>~1 × 10³</td>
<td>0.1 ppm (20 °C)</td>
<td>2.6 logs of surviving cells with OMNBs instead of 2.9 logs with OMaBs after 180 s</td>
<td>113</td>
</tr>
<tr>
<td>P. carotovorum subsp. carotovorum</td>
<td></td>
<td></td>
<td>2.5 logs of surviving cells with OMNBs instead of 2.9 logs with OMaBs after 180 s</td>
<td></td>
</tr>
<tr>
<td>Alternaria solani Sorauer conidia</td>
<td>1 × 10⁵</td>
<td>1.6 ppm</td>
<td>2 logs reduction</td>
<td>115</td>
</tr>
<tr>
<td>Cladosporium fulvum conidia</td>
<td>1 × 10⁵</td>
<td>1.8 ppm</td>
<td>1 log reduction</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>1 × 10⁵–1 × 10⁷ (CFU g⁻¹)</td>
<td>1 ppm (30 °C)</td>
<td>2.6 logs reduction</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 5 Applications of ozone micro- and nanobubbles (OMNBs) in agriculture

alfalfa sprouts have not been negatively affected. Another study provides additional support into the superiority of OMNBs over other sanitizers such as sodium hypochlorite. After washing with OMNBs at 1 mg L⁻¹ for 7 minutes, the bacterial reduction of S. typhimurium was the highest reaching the value of 2.6 log CFU g⁻¹ or 99.8%, converting into percentage. Increasing attention has been given to the removal of persistent, highly toxic and accumulative pesticides which are extensively used in agriculture. The degradation of fluopyram is more efficient with OMNBs, among different treatment methods. More specifically, the half-life of fluopyram in ozonated water was found to be 6.1 times higher compared to OMNBs treatment and whereas in MBs treatment (without ozone) was only 1.3 times higher. The removal of fenitrothion in three kinds of vegetables (lettuce, cherry tomatoes and strawberries) was investigated and was found to be higher when OMNBs generated by decompression compared to OMNBs generated by gas-water circulation were used. This can be explained by the creation of a larger number of smaller OMNBs by the former, yielding a higher efficiency of fenitrothion degradation as the infiltration of smaller OMNBs into vegetables is easier.

5. Research limitations and needs

Although MNBs technology is widely used in various applications, there are still gaps in our understanding of the behaviour of NBs that need further investigation which is however outside the scope of this review. This critical review has tried to cover most of the important research conducted in the field of disinfection using OMNBs in order to get a better insight on the correlation of ozone dose-exposure time and microorganism viability, and highlight the major advantages of utilizing these MNBs-based processes. Besides the increasing number of research studies in this field there all still research limitations that are encountered in the implementation of this technology. In particular, the challenges that need to be addressed regarding the OMNBs are:

- Even though there are many comprehensive studies carried out in water treatment regarding the inactivation of various microorganisms, this is not the situation for ballast water treatment. There are some investigations about the generation of nanobubbles under different salt concentrations, however, there is no literature about the disinfection capacity of OMNBs in real seawater. The sodium chloride present in seawater reacts quickly with ozone generating a mixture of oxidants which kill microbial pathogens. In addition, it is important to examine the inactivation efficiency when OMNBs are used in the presence of bromide in order to estimate the concentration of by-products derived from the reaction between the ozone and the bromide and compare with that created in a typical ozonation.

- One of the objectives of this review was also to gather knowledge regarding the degradation of organic pollutants present in wastewater treatment plants by OMNBs since their existence may influence the disinfecting potential in tertiary treatment. However, the formation of oxidation by-products is an issue of greater importance since they can be more resistant towards ozone and a higher level of disinfectant may be required. As reported in the literature, a carbamazepine by-product was highly persistent and the ozone level had to be elevated up to 15 mg L⁻¹, when only 5 mg L⁻¹ of ozone was sufficient to remove the target compound. Hence, we must bear in mind the detection and identification of emerging by-products in order to evaluate in an accurate manner the efficiency of OMNBs in eliminating organic compounds.

- A general limitation is that all studies have been performed in laboratory or small pilot scale (up to 50 L). It would be helpful to examine the upscaling of this process in the field and at industrial scale. Moreover, in this case it is important to mention that a cost/benefit analysis should also be conducted, since an ozonation system is often portrayed by high energy requirements.

- The characterization of MNBs with high resolution has been to some extent achieved; however, there is a chance the size measurement to be misleading as the gas cavities cannot be distinguished among nanodroplets and impurities derived from the equipment or present in the water. It is worth mentioning that most studies have investigated the use of NBs on ultrapure water and hence, typical drinking water or wastewater matrices may influence the NBs size and entangle the measurement of number concentration thanks to the existence of other colloids. There are several MNBs.
generators available commercially but without providing a detailed description concerning the size distribution and concentration of the generated bubbles.\textsuperscript{35} For that reason a standard measurement protocol should be established in order to ensure the correct characterization of MNBs.

Finally, although theoretical models for micro-nano-bubbles mass transfer and stability\textsuperscript{154,155} have been developed they should be extended in order to simulate the reactions with microorganisms or micropollutants for a given number of OMNBs and their size distribution taking into consideration potential ozonation by-products. Such a model would be valuable for the optimal design of ozone-based disinfection systems, specifically in the case that existing treatment plants will be retrofitted by the installation of MNBs technology.

Conclusions

Even though several researchers have expressed doubts about the existence and the stability of MNBs, many studies have proven that their application by different types of gas can enhance process efficiency compared to conventional aeration since the results so far have been very encouraging. The OMNBs technology can reduce the operation and maintenance cost of an ozonation system since it can overcome at least partially the serious weakness which is the limited residual disinfection capacity and the low solubility of ozone leading to the requirement of a high ozone dose. Apart from the reduced construction and operational cost, there is another positive aspect concerning the environment. The chemical dosage is lower due to the excellent mass transfer and hence, it can be considered more eco-friendly than conventional ozonation. Further work needs to be carried out to standardize the selection of a OMNBs system in accordance with the disinfection needs in wastewater treatment and reuse, taking into account the economic and environmental impact.

Author contributions

P. S.: investigation, validation, writing first draft and revising.
N. K.: supervision, conceptualization, reviewing and editing.

Conflicts of interest

There are no conflicts to declare.

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