PERIODICUM BIOLOGORUM
VOL. 126, No 3–4, 105–116, 2024
DOI: 10.18054/pb.v126i3-4.33428

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Review article

Nanobubble technology: An ultrafine solution to emerging challenges in aquaculture

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Keywords: aquaculture; dissolved oxygen; disinfection; nanobubbles; and wastewater remediation

Received October 31, 2024
Revised February 3, 2025
Accepted February 4, 2025

Abstract

Aquaculture stands out as a highly promising practice within sustainable agriculture, especially when compared to other livestock production sectors. However, it faces significant challenges, including water pollution, fluctuations in dissolved oxygen levels, and the prevalence of both chronic and acute diseases. To address these issues, researchers in the aquaculture sector have been exploring and implementing innovative solutions. One of the most notable advancements is the use of nanobubble technology, which has already been applied in numerous aquaculture farms to mitigate these challenges. Nanobubbles (NBs) with a diameter of less than 200 nm have shown promising results in improving water quality in aquaculture systems, increasing dissolved oxygen availability, and improving the overall health of cultured organisms. This review will emphasize various practical applications of nanobubble technology in different areas of aquaculture, including their properties and characterization methods, and discuss the hotspots of current research to help shape the future of intensive aquaculture. It will also uncover the nanobubble technology application in water and wastewater treatment processes, such as disinfection, sterilization, and detoxification. Additionally, the review will discuss the application of nanobubble technology in improving the growth performance and health of aquatic organisms, including its roles in aeration, water quality management, nutrient, drug delivery, monitoring, algae control, and functional feeding strategies. However, extensive research and standardization to be optimized for the application of nanobubbles in culture systems to understand their chronic effects on growth and overall production of fishes.

INTRODUCTION

Nanobubble technology is an emerging and promising field with diverse applications in aquaculture. The nanobubbles are tiny water bubbles, often measuring less than 200 nanometers in diameter *(1, 2)*. The International Organization for Standardization (ISO) defines a nanobubble as a nanoscopic gaseous domain in a medium enclosed within an interface *(3)*. The nanobubble with a diameter of less than 200 nm can randomly drift in a medium, which is known as Brownian motion, this allows them to stay suspended in liquids for a prolonged period and modify the typical properties of water. The development and application of nanobubbles in aquaculture have generated considerable interest due to their exceptional gas solubility, prolonged stability, and the potential to enhance various facets of aquaculture practices. These have also proven to be versatile in furthering the effectiveness of disease treatment at both cellular and molecular levels *(4)*. Nanobubble technology serves as a means to augment the dissolved oxygen levels within the culture water. In addition to that, this technology extends beyond oxygen augmentation, where it has a pivotal role in the orchestrated management of both organic and inorganic waste matrices within the culture system. By facilitating the supply of dissolved oxygen to the water, nanobubbles provide a valuable resource of oxygen for the microbial decomposition of the organic waste, thus aiding in waste processing and management. Nanobubbles excel in efficiently dissolving gases like oxygen and ozone into water, enhancing the oxygen levels in aquatic environments and effectively eliminating excess toxic gases *(5)*. Fundamentally, there is still debate in science over the existence of nanobubbles *(6-8)*, whereas from the industrial perspective, due to its stability and longevity, these nanobubbles bubbles have advantages in many fields like aquaculture *(2)*, wastewater treatment *(9)*, hydroponics *(10, 11)*, fuel *(12)*, and medical applications *(13)*.

CHARACTERISTICS OF NANOBUBBLE

The term "nanobubbles" was proposed by Parker et al. (1994) which represents gas-filled pockets having a height of greater than 10 nm and less than 100 nm *(9)*, They look spherical in shape, and the radius of the contact line measures between 50 and 500 nm *(14)*. Nanobubbles were not new to the field as they have existed a way back from the 1950s starting from the Epstein–Plesset theory. The first image of nanobubbles was captured by Atomic Force Microscopy in 2000 *(14)*. The use of nanobubble in various fields starting from agriculture to medicine has gained importance as they possess enormous features within it. The key distinctive characteristics that make nanobubble to be applied in various fields are as follows,

Small size

Nanobubbles typically measure less than 100 nm in diameter (14), whereas microbubbles have a size of 10 to 100 µm in diameter *(15)* and regular bubbles have a size range between 1 to 2 mm in diameter *(16)*. The nanobubbles have the wider application of being smaller in size. In medicine, the nanobubbles are used for their stronger penetration power, longer half-life in blood circulation, aggregation-enhanced imaging, and increased surface area, effectively improved the drug loading capacity as reported by Jin et al. (2022) *(17)*. It also has been used in ultrasonography imaging of tumors *(18)*. In addition to that the nanobubbles had greater oxygen pressure holding capacity which results in holding dissolved oxygen for a longer time in the water bodies *(11)*. Similar results were obtained in the study of Baram et al. (2022), where the nanobubbles increased surface area and stronger penetration power delivered the water containing oxygen molecules to the plants which facilitated more dissolved oxygen to the plants and the increased surface area of nanobubble with water facilitated the water to penetrate down the soil and improved the water absorption efficiency of the roots of the plants *(19)*.

Gas solubility

The gas solubility of the nanobubbles in the water is determined by the zeta potential of each gas held inside the nanobubbles *(20)*. Usually, the ozone nanobubbles had 13 times more solubility than oxygen nanobubbles and held the largest diameter. Whereas nitrogen nanobubble had a lower solubility than others and held the smallest diameter *(6)*. A study by Imaizumi et al. (2018) reported that, due to the higher ozone solubility potential inside the nanobubbles, the ozone nanobubble has greater application in aquaculture in inhibiting the growth of *Vibrio parahaemolyticus* causing acute hepato pancreatic necrosis disease (AHPND) without inducing toxicity to the shrimps (21). These ozone nanobubbles were also effective in oxidizing the organic pollutants present in the environment (22).

Stability

The stability of the nanobubbles depends on the gases inside their cavities, the zeta potential of the gas, and the pH of the solution. The gases with increased zeta potential increase the hydrogen bonds around the bubbles which will increase the stability of nanobubbles for a longer time. The zeta potential of the gases varies according to the pH of the solution, where the increase in the number of Hydroxyl ions leads to an increase in the Zeta potential of the gas which determines the stability and solubility of the gases, whereas the increase in the hydrogen ions decreases the pH of the solution and reduces the zeta potential which affects the gas stability and solubility of the gases inside the solution *(20)*. The nanobubbles were stable and retained their size in the neutral and alkaline solution. Higher negative zeta potential values create stable nanobubbles unlike the nanobubbles present in the acidic solution having positive zeta potential values and are unstable *(20)*. The higher negative potential and free radicals around the nanobubble is an added advantage as it makes the nanobubbles undergo chemical reactions with organic compounds and heavy metals present in the wastewater resulting in the treatment of wastewater *(23)*.

Surface area and electrostatic charges

The nanobubbles had a high surface area and high electrostatic energy on the active sites which enabled to germination of bactericidal effect over the pathogens by disrupting their external structures *(24)*. It has also been used in the froth floatation process for the separation of minerals from the other impurities due to their larger surface area with inherent greater binding ability and stronger stability to avoid burst in the froth floatation process as reported by Zang and Seddon (2016) *(25)*. The

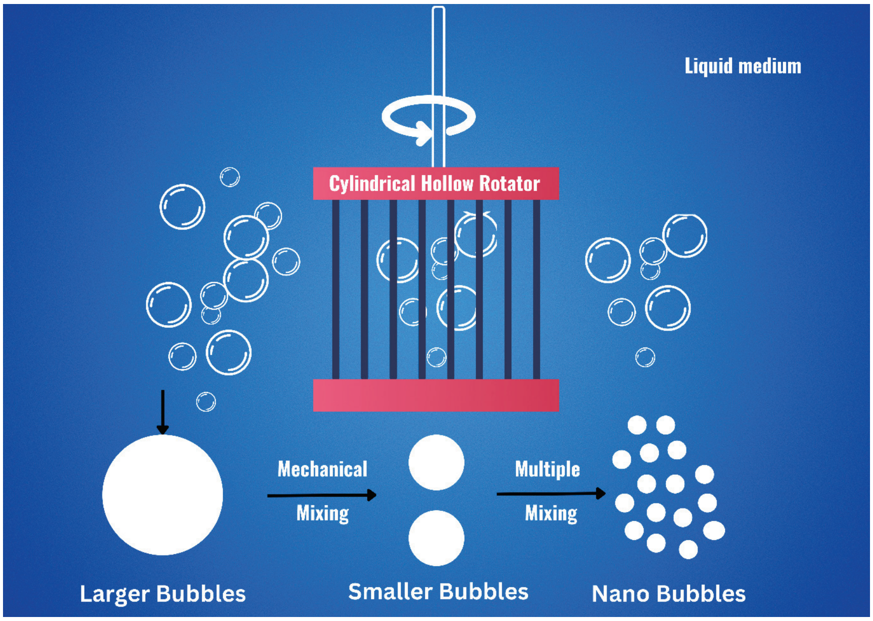

Figure 1. *Mechanical stirring method of nanobubble preparation.*

surface of nanobubbles is very effective in removing contaminants from the environment due to greater absorption area *(26, 27)*.

PRODUCTION OF NANOBUBBLES

Generally, the nanobubbles were categorized into two different forms; surface nano bubbles (SNB) i.e., the nanobubbles attached to the surface, or bulk nano bubbles (BNB) i.e., the nanobubbles freely suspended in the medium *(28)*. The production of these nanobubbles involves different processes and methods which are described as follows.

Bulk nano bubbles (BNB)

The bulk nano bubbles are described as gas-filled spherical cavities that usually range less than 1000 nm and are prepared by adjusting the gas pressure, the ultrasonic intensity, or stirring capacity. These nanobubbles are prepared through different methods as described as follows.

Mechanical stirring method

The bulk nano bubble is prepared by mechanical agitation which involves subjecting a liquid medium containing surfactant against the cylindrical hollow rotor through a mechanized mechanism. Due to the high shear, intense collision and turbulence, and hydrodynamic cavitation during agitation resulted in developing intense interactions between the gas and liquid medium leading to bubble production. The continuous shearing of the bubbles drastically reduces to a smaller size after multiple cycles leading to the formation of bulk nano bubbles (BNB) *(29)*. These bubbles had a greater longevity and stability of maintaining structural integrity for 90 days *(30)*. A schematic diagram of the mechanical stirring method for nanobubble preparation is sketched in Figure 1.

Nanoscale pore membrane method

The nanoscale pore membrane method involves injecting the gas at high pressure inside the nanoporous membrane contained in the liquid medium resulting in the formation of bulk nanobubbles. In the Initial step, the gas containing nanobubbles was injected into the liquid medium which was equal to the porous membrane, when the water flow rate inside the nanoporous membrane increased causing the bulk nanobubble to expand which resulted in the formation of bulk nanobubbles more than the actual diameter of the porous membrane. The lesser the flow rate and the drag force, the smaller the size of the bulk nano bubble *(31)*. Different nanoporous membranes have been used for the production of bulk nano bubbles including shirous glass porous membrane *(32)*, tube ceramic membrane *(33)*, membrane-based physical ceiving method *(34)*. In the study conducted by Kukizaki et al. (2006), a shirous glass porous membrane was used for the production of nanobubbles where the process involves passing the gas at higher pressure inside a sodium dodecyl sulfate solution at a concentration ranging from 0.05 to 0.5% *(32)*. The gas-containing solution was introduced into the shirous glass nanoporous membrane at a bubble point pressure ratio of 1.1-2.0 resulting in the formation of a stable nanobubble having an average diameter of 360-720 nm. Hence the size of the nanobubble was 8.6 times larger than that of the pore, showing that the size of the bubble directly depends on the size of the pore of the membrane and the drag force of the medium flows inside the membrane. Similar results were obtained in the study of Ahmed et al. (2018), where the size of the nanobubble depends on the injecting air at different pressures into the water through the tube ceramic membrane *(33)*. However, at the same injection pressure, the resultant size of the nanobubble formed through the nanoporous membrane was similar to the nanobubble formed in the earlier study conducted by *(32)*. In the membrane-based physical sieving method of Zhang et al. (2022), it is estimated to produce bulk nanobubbles of controllable size by controlling the gas filtration rate and the quality of the membrane *(34)*. However, the result showed that not only that the 3-step membrane used in the membrane-based physical sieving method reduce the larger bubble to a smaller bubble but also fused the smaller bubbles to form larger bulk nanobubbles.

Microfluidic method

The microfluidic method of producing bulk nanobubbles was developed by Xu et al. (2021), which employs a gaseous mixture of bubbles that passes through the liquid medium it exerts a viscous force over it resulting in the formation of microbubbles *(35)*. Some of the gases present in the microbubbles dissolve in the aqueous phase which eventually shrinks and forms the bulk nanobubbles. The mechanism involves the water-soluble nitrogen and water-insoluble perfluorocarbon as the gaseous phase of the microfluidic bubble generator. During the operation, the liquid medium exerts a viscous force over the microbubbles and the microbubble shrinks through the dissolution of water-soluble nitrogen resulting in the formation of bulk nanobubbles of the desired size (Figure 2). The most significant advantage of using the method is the size and uniformity of bulk nanobubbles generated through this method can be controlled.

Acoustic cavitation method

The acoustic cavitation method involves the induction of negative pressure inside the liquid medium through the propeller operated under high speed or through the high-intensity sound waves generated from the ultrasound processor. The result of this method develops the micro and bulk nanobubble with tiny gas nuclei *(36)*. This method of bulk nanobubbles production was developed by Nirmalkar et al. (2019) *(37)*. Through this experiment, it was found that the bulk nanobubbles existed only in water not in organic solvents and the bulk nanobubbles extorted high electrostatic charge and stabilized by absorbing hydroxyl ions on their surface through ionization of water which was uncertain in the pure organic solvent where the ionization is unfeasible.

Hydrodynamic cavitation method

The hydrodynamic cavitation method is used instead of the acoustic cavitation method and the results were similar to the acoustic cavitation method in the generation of bulk nanobubbles. The most significant advantages of employing this method are low cost, high efficiency, and scalability. This method was developed by Alam et al. (2022), which employs a two-chambered swirling jet nozzle, which generates bulk nanobubbles in a saturated or supersaturated liquid medium flowing inside a circulation system by altering the flow velocity of

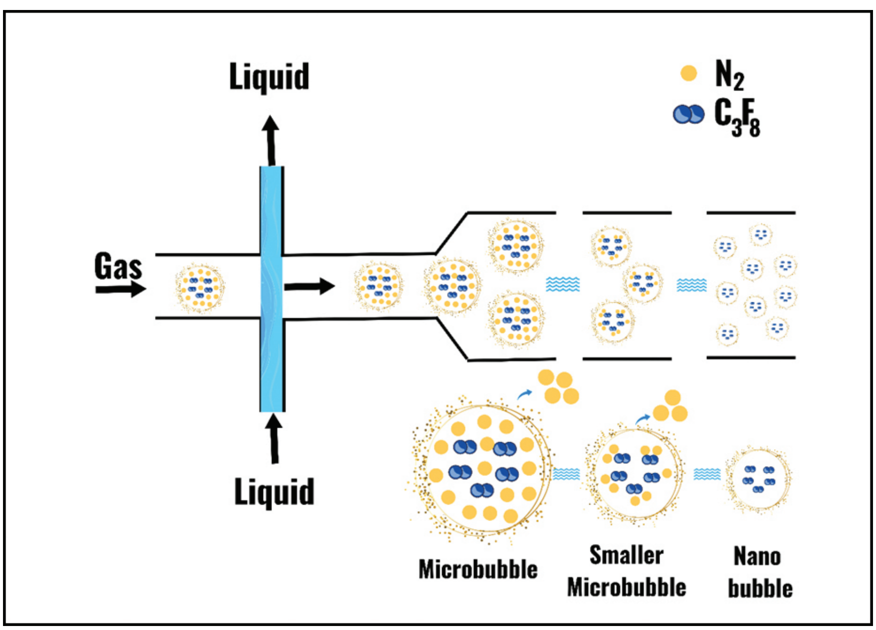

Figure 2. *A schematic diagram of the microfluidic method of bulk nanobubble.*

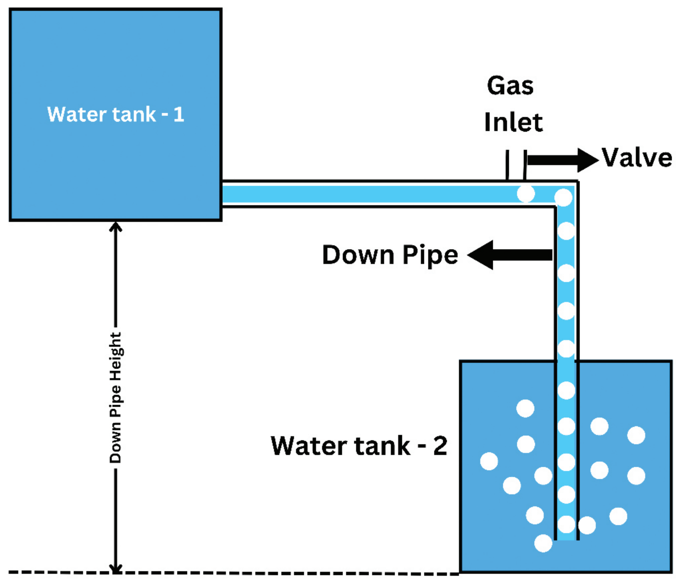

Figure 3. *A schematic diagram of the hydraulic air compression method.*

the liquid and causing pressure fluctuation in the liquid to generate the bulk nanobubbles *(38)*. The resulting outcome leads to the generation of bulk nanobubbles less than 200 nm diameter. In another study by Wu et al. (2022), he fabricated a laboratory scale vortex type micro-nanobubble generator by altering the design and the geometric parameters on the flow field structure *(24)*. Subsequently, through this method, the generation of bulk nanobubbles of 301 nm diameter has been achieved.

Dissolved gas release method

The bulk nanobubble prepared by employing the dissolved gas release method involves increasing the pressure of the gas to dissolve it in the liquid medium before reducing the pressure of the gas to allow the gas molecules to precipitate and form bulk nanobubbles *(39)*. In this method, the size of the gas molecule is directly proportional to the solubility of the gas but the diameter is inversely proportional to the solubility of the gas in the liquid medium *(6)*. A study by Wang et al. (2021) employed CO_2 gas to generate the bulk nanobubble via the dissolved gas release method *(40)*. However, it is found that the solubility of CO_2 gas with or without hydrates in pure water depends on the temperature of the medium, and the outcome of the study resulted in the generation of bulk nanobubbles by operating the equipment for 28.47 minutes and by maintaining 2.87% gas-liquid ratio with an optimum water temperature of 25.52^{0}C.

Periodic pressure variation method

The goal of this method is to produce a bulk nanobubble by adjusting the pressure periodically in the gas-liquid medium to control its gas solubility and precipitation *(41)*. An experimental setup has been developed by Wang et al. (2019) to produce a stable nitrogen bulk nanobubble by constantly adjusting the pressure and longer exposure of liquid to the pressure resulting in the formation of bulk nanobubbles *(42)*.

Hydraulic air compression method

The hydraulic air compression method is one of the most reliable methods for the production of bulk nanobubbles due to its high efficiency, scalability, and mass production. This method was initially developed by Yang et al. (2022), which employs the production of bulk nanobubbles based on compressing the air through hydraulic pressure flow, where the concentration depends on the height of the outlet pipe (Figure 3) *(43)*.

Advantages and disadvantage of different methods used for bulk nanobubble preparation

Bulk nanobubbles are produced through various production methods. However, each production methods has its own merits and demerits which emphasized its importance. Hence, the advantages and disadvantages were listed in the Table 1.

Surface nano bubbles (SNB)

The surface nano bubbles (SNB) are the small bubbles that form at the solid and liquid interface. The stability and generation of surface nanobubbles depend on three factors such as dissolution of gas, liquid-surface tension, and the hydrophilic or hydrophobic nature of the surface. These nanobubbles are prepared through different methods as described as follows.

Table 1. *Advantages and disadvantages of different methods used for bulk nano bubble preparation.*

Methods	Advantages	Disadvantages
Mechanical stirring method	The method of implication is simple and feasible to implement	The production of bulk nanobubbles is much less
Nanoscale pore Membrane method	In this method, the size of the bulk nanobubble and the concentration can be controlled.	It requires different pore-size membranes to produce a desired size of bulk nanobubble and the efficiency decreases if there is any blockage in the membrane.
Microfluidic method	The production of bulk nanobubble, its size, and its distribution are under control. The high degree of automation offers significant performance in the production of bulk nanobubbles.	The fabrication of microfluidic equipment and its accessories is very complex.
Acoustic cavitation method	The method is very simple and efficient in the rapid generation of bulk nanobubbles.	The fabrication of equipment and the generation of ultrasound is complex. The control over the bulk nanobubble size and concentration is very limited.
Hydrodynamic cavitation method	The method is very reliable, has less cost, high scalability, and high efficiency in the production of bulk nanobubbles.	The production efficiency depends on the flow rate of gas and the pressure.
Dissolved gas release method	The method of application is very easy to implement at low cost.	The control of the bubble size and its distribution is very difficult.
Periodic pressure variation method	This method generates uniform size by controlling the pressure and period.	Only very few bulk nanobubbles can be generated.
Hydraulic air compression method	The bulk nanobubbles can be produced with high efficiency on a large scale at low cost.	The control of the size and the concentration is limited.

Aqueous solution electrolysis method

This method involves the electrolysis of hydrogen and oxygen to generate surface nanobubbles when the concentration of molecules reaches the critical concentration for nucleation. In this method, highly ordered pyrolytic graphite (HOPG) is used as conductive material which acts as an anode to generate oxygen surface nanobubbles. The size and concentration of the surface nanobubbles depend on the voltage and duration of the electrolysis of the instrument. The size of the surface nanobubble was inversely proportional to the voltage *(44)*. In this method, the production of oxygen was lower than the generation of hydrogen surface nanobubbles and it is monitored by AFM Cantilever (Figure 4).

Cold water method

The cold water method is a simple relative approach that uses heated graphite and cold water for the produc-

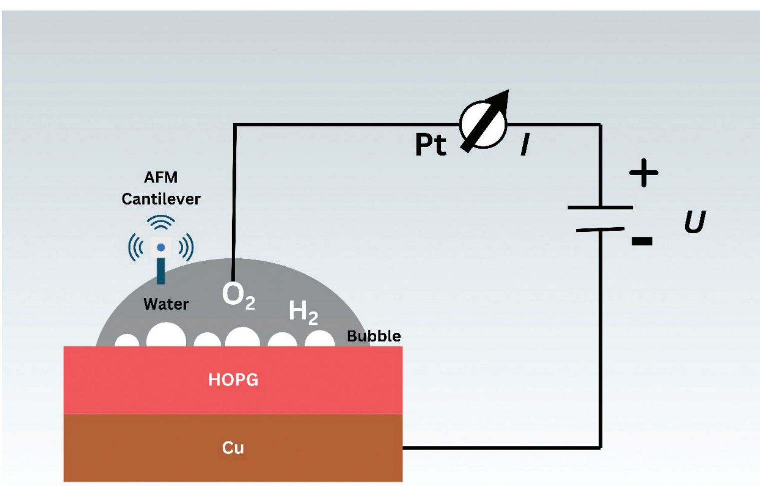

Figure 4. *A schematic representation of the aqueous solution electrolysis method.*

Table 2. *Advantages and disadvantages of different methods used for surface nano bubble preparation.*

Methods	Advantages	Disadvantages
Aqueous solution electrolysis method	This method allows complete control over the production of surface nanobubbles.	This method required specialized electrolysis equipment for surface nanobubble production.
Cold water method	It is a very simple and easily accessible method and it does not require complex equipment for the production of surface nano-bubbles.	The control over the diameter of the surface nanobubble and the stability was uncertain.
Solvent exchange method	This method was feasible to implement and does not involve specific costs.	The control over the diameter of the surface nanobubble and the concentration was uncertain.
Depressurization method	The most reliable method for the production of surface nanobubbles and there is much control over the stability and production	The produced surface nanobubble stability was uncertain.

tion of surface nanobubbles. Initially, the graphite was maintained in pure cold water below 4^0C for 12 hours and baked the graphite for two hours at 40^0C – 80^0C in the oven. During the experiment, the pure water was randomly distributed over the surface of HOPG. It was observed that the surface nanobubbles were formed over the surface of heated graphite which was detected through Atomic Force Microscopy (AFM). It is clear that with the HOPG temperature increase, the formation of surface nano bubble gets denser and the formed surface nano bubbles had good stability and longevity for over 5 days *(45)*.

Solvent exchange method

The solvent exchange method can be conducted with ethanol-water substitution (46) or with sodium chloride – water substitution *(47)*. This method involves the substitution of liquid with low gas solubility with liquid with high gas solubility. This method was subsequently performed by Qiu et al. (2017), who found that a massive generation of surface nanobubbles in his module which was detected by a nanoparticle tracking analyzer *(48)*. It was observed that the higher gas solubility was directly proportional to the production of surface nanobubbles.

Depressurization method

In this depressurization method, the pressure exerted on the liquid was altered which in turn alters the solubility of the gas in the liquid and leads to the generation of surface nanobubbles. the concept behind the production involves the reduction of pressure for a short time in turn decreases the solubility of gas causing precipitation and formation of surface nanobubbles *(42)*. However, an experiment conducted by Fang et al. (2018) showed that dripping unsaturated pure water over the HOPG surface and reducing the pressure resulted in the formation of surface nano bubbles which were detected by AFM *(49)*. The extension of depressurization time to 20 minutes resulted in no surface nanobubbles formation and reported that the surface nanobubbles escaped through the liquid.

Advantages and disadvantage of different methods used for surface nanobubble preparation

Surface nanobubbles are produced through various production methods. However, each production methods has its own merits and demerits which emphasized its importance. Hence, the advantages and disadvantages were listed in the Table 2.

Cost-effective production of nanobubbles

Advancements in nanobubble technology focus on cost-effective and energy-efficient production methods for industrial applications *(50)*. Researchers are developing durable, low-cost porous membranes with precise pore sizes and engineering nozzles to enhance fluid shear forces and turbulence, maximizing nanobubble yield while minimizing energy consumption. Renewable energy sources, such as solar and wind, are being integrated to power nanobubble generators, reducing operational costs and carbon emissions *(51)*. Computational fluid dynamics (CFD) and machine learning are utilized to model and optimize production processes, improving efficiency and scalability *(40, 42)*. Compact, modular reactor designs enable continuous and large-scale nanobubble generation, facilitating seamless industrial integration. The use of low-cost industrial gases, such as CO_2 or nitrogen, is being explored to reduce dependence on expensive oxygen inputs *(38)*. Additionally, energy recovery and recycling mechanisms are incorporated into systems to further cut operational expenses. These innovations aim to make nanobubble technology viable for diverse industries while promoting environmental sustainability *(4, 52)*.

APPLICATION OF NANOBUBBLES IN AQUACULTURE

Presently there are different types of nanobubbles used in aquaculture such as oxygen nanobubbles, ozone nanobubbles, and air nanobubbles. These nanobubbles were generated through the above-mentioned methods.

Oxygen nanobubbles

Oxygen nanobubbles are mainly used to improve the dissolved oxygen and oxidative redox potential. In the field of aquaculture, these nanobubbles can efficiently deliver dissolved oxygen to the water which maintains optimal oxygen levels throughout the culture and ensures the overall health and growth of aquatic organisms, particularly in intensive aquaculture systems or during times of diurnal fluctuations of dissolved oxygen *(47, 48)*. In the study of Rahmawati et al. (2021), oxygen nanobubbles were used in the culture of White leg shrimp in indoor raceway ponds which enhanced the dissolved oxygen, growth, and survival rates of white leg shrimp compared to the control *(53)*. A similar study conducted by Mahasri et al. (2018) reported that a nanobubble generator enhanced the dissolved oxygen levels in the fish culture tanks from 6.5 ppm to 25 ppm *(54)*. In turn, it improved the water quality, and appetite and decreased the feed conversion ratio which resulted in the higher growth rate and survival of fish. Similar results were obtained in the study by Ebina et al. (2013), where the dissolved oxygen was increased through the nanobubbles aerator from 7.7 ppm to 31.7 ppm in the culture tank of Ayu fish and Rainbow trout which enhanced the growth and survival of fish *(11)*. Nowadays, these oxygen nanobubbles have been used in the transportation of live fish for a longer distance which decreases the stress and mortality rate of the fish due to the longevity and stability of nanobubbles in holding oxygen for a longer period compared to the conventional transportation method (34, 55, 56). A study conducted by Lima et al. (2020) on the transportation of pirarucu fingerlings using oxygen nanobubbles and the outcomes of the study stated that by using oxygen nanobubbles the stocking density of the fish can be increased fourfold per liter than the average stocking density used in the conventional transportation method *(55)*. In addition to that, these oxygen-loaded nanobubbles could disrupt the formation of harmful algae which is detrimental to the aquatic ecosystems *(57)*. Groundwater is a significant water source for aquaculture particularly for intensive aquaculture systems *(58)*, However, this water has low dissolved oxygen, salinity, low pH, and high carbon dioxide concentrations *(59)*. Hence to remediate this the application of oxygen nanobubble has demonstrated significant promise due to its large surface area, extended stagnation, high oxygen transfer efficiency, negatively charged surface, and other characteristics. The findings of Li et al. (2013), stated that salinity was significantly affected by the oxygen nanobubble's performance; thus, when using nanobubble technology to improve bioremediation, groundwater salinity needs to be carefully taken into account *(60)*. The findings also imply that speeding up the oxygen transfer process will significantly improve bioremediation.

Ozone nanobubble

Nanobubbles offer a unique potential for precise drug delivery, transporting medications, vaccines, or disease control agents directly to targeted organisms. This focused approach to drug delivery not only enhances the effectiveness of treatments but also minimizes the use of chemicals in aquatic environments. In the case of tilapia, utilizing ozone nanobubbles has proven highly beneficial. They boost the survivability of the fish and combat pathogenic bacteria such as *Streptococcus agalactiae* and *Aeromonas vernoi*. Remarkably, it has been observed that within a brief span of 10 to 30 minutes of treatment, the bacterial load experiences a substantial reduction, amounting to approximately 99.9% *(61)*. The findings of Dien et al. (2022) stated that the utilization of nano bubble-O_3 presents a potentially effective non-antibiotic approach for addressing bacterial diseases, encompassing multidrug-resistant (MDR) bacterial strains, where the Ozone nanobubble has increased the survivability of *Oreochromis niloticus* against the multidrug-resistant *Aeromonas hydrophila* (62). This approach holds substantial potential for implementation within the realm of recirculation aquaculture systems (RAS). In the study of Linh et al. (2021), employing ozone nanobubbles can trigger the innate immune response, activating the non-specific defense mechanism in fish, which further helps in increasing the survival rate of fish when faced with subsequent bacterial infections *(63)*. According to Tasaki et al. (2019), the application of ozone nanobubbles helps in lowering waterborne bacterial infections and also helps in diminishing the likelihood of mycobacteriosis, thereby increasing the survival rate of Siamese fighting fish (*Betta splendens*) when exposed to *Mycobacterium chelona*, which is a multidrug-resistant bacteria *(64)*. Utilizing nanobubble has been found to boost the effectiveness of vaccines. When subjects are pre-treated with nano bubbles before vaccination, it enhances the uptake of antigens. Furthermore, nano bubbles serve as immunostimulants by augmenting innate immunity, leading to improved expression of genes related to the immune system which helps in increasing the survival rate of fish when faced with subsequent bacterial infections *(65)*. Nanobubble technology enables fish farmers to reduce the use of chemicals and antibiotics by naturally improving water quality and controlling pathogens. This not only lowers operational costs but also minimizes environmental impact, contributing to more sustainable aquaculture practices. In addition to that, the ozone nanobubble can deliver beneficial microorganisms directly to the gut of aquatic organisms by offering protective efficacy to probiotics such as *Lactobacillus rhamnosus* and *Lactobacillus plantarum* with an RPS value of 66.7% (66) and 64% (67). This promotes a healthy gut microbiome, improves digestion, and enhances the overall immunity of aquatic organisms, reducing the risk of disease outbreaks. These ozone nanobubbles have been tested in the wastewater treatment and disinfection process *(68)* which was elucidated in the study of Terfasa (2017), where the application of ozone nanobubble for the breakdown of aerobic waste in wastewater treatment was very high compared to traditional bubbles *(69)*.

Air nanobubble

The application of air nanobubble for increasing the growth and survival of fish is an emerging insight in aquaculture which has been significantly proven by Ebina et al. (2013), where the application of air nanobubble in the culture system of sweet fish (Ayu fish) has significantly increased the growth from 3.0 kg to 10.2 kg in compared to 6.4 kg increase in standard water *(11)*. Similarly, observations were noticed in the rainbow trout where its total weight showed notable enhancement, rising from 50.0 to 148 kg in air nanobubble water within six weeks, in comparison to standard water which has increased the weight up to 129.5 kg from 50 kg (11). Hence, it proves that NBs have the potential to improve the growth of the culture species and reduce the FCR.

SWOT ANALYSIS

A strategic planning method called SWOT analysis is used to assess technology's strengths, weaknesses, opportunities, and threats. A SWOT analysis of nannobubble technology is depicted in Figure 5.

ENVIRONMENTAL CONCERNS DUE TO THE APPLICATION OF NANOBUBBLE TECHNOLOGY

Even though nanobubble technology has the bioremediating capability and various advantages it possesses environmental concerns, where the application of this technology can enhance gas dissolution in water, which can alter microbial communities and disrupt aquatic ecosystems by favoring aerobic over anaerobic species *(70)*. When combined with chemicals like ozone or hydrogen peroxide, nanobubbles may cause oxidative stress and produce harmful byproducts that affect aquatic organisms. The application of ozone nanobubble produces additional unwanted hazardous chemicals (also known as ozone-produced oxidants), such as bromine (Br_2) and bromate (BrO_2^-), which are extremely corrosive to the equipment when in contact with seawater *(27, 71)*. Excessive oxygenation could lead to supersaturation, resulting in gas bubble disease in marine life. Additionally, the use of nanomaterials with nanobubbles poses risks due to the uncertain environmental fate of these particles, which may be toxic or bioaccumulated in food chains. These nanobubbles can also modify water chemistry, such as pH, redox potential, and composition, impacting overall ecosystem health *(45, 72)*. These potential environmental risks highlight the need for careful application and regulation to minimize unintended ecological consequences *(73)*.

CONCLUSION

Nanobubble technology can be expensive, especially in large-scale applications, which may limit its adoption in specific industries or regions. Producing and stabilizing nanobubbles in large quantities can be technically chal-

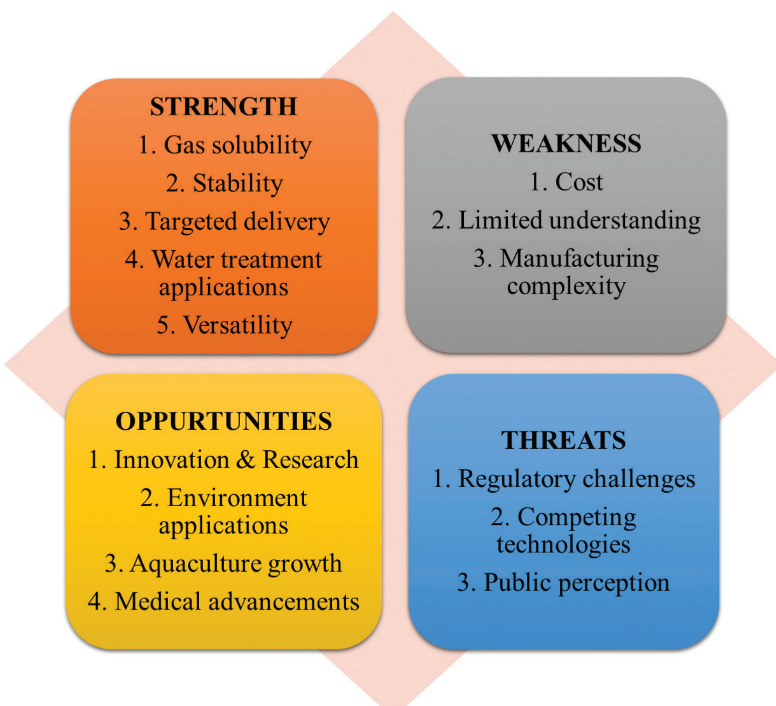

Figure 5. *SWOT analysis of nanobubble technology.*

lenging, and maintaining their stability over extended periods may require advanced equipment and techniques. Continued research and development efforts can lead to discoveries, improved methods for nanobubble production, and a better understanding of their potential applications. Nanobubbles could have applications in environmental remediation and pollution control, offering a sustainable approach to water treatment and ecosystem restoration. In the medical field, nanobubbles can revolutionize drug delivery, medical imaging, and other targeted therapies. The use of Nanobubbles in various applications might face regulatory hurdles due to potential safety and environmental concerns, slowing down their widespread adoption. Public perception and acceptance of nanobubble technology might be influenced by concerns related to nanotechnology, environmental impact, and potential unknown risks. As nanobubble technology develops, there could be challenges related to patent infringement and protecting intellectual property rights. Addressing these challenges and conducting further research is crucial to realizing the full potential of nanobubble technology in aquaculture and other industries. Nanobubble technology in aquaculture is highly advisable as it is a chemical-free approach. However, it is a very new to field, it requires further research to explore Nanobubbles' potentiality and consistency in improving the water quality and disease control. As research and development efforts continue, nanobubble technology will likely undergo further refinement and optimization, leading to improved nanobubble generation, stability, and targeted delivery methods. By responsibly harnessing the capabilities of nanobubbles, we can unlock new opportunities to address pressing global challenges and contribute to more sustainable and efficient practices in aquaculture and beyond.

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