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RESEARCH ARTICLE

Improved water quality as a source of nutrient water by nanobubble engineering of various gases: Preliminary study for potato aeroponic systems

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ABSTRACT

Bacterial wilt (*Ralstonia solanacearum*, RS) is a major constraint in aeroponic potato (*Solanum tuberosum* L.) seed production. While hydrogen peroxide (H_2O_2) is a common low-cost sterilizer in aeroponic, nanobubbles (NB) offer a superior alternative by simultaneously sterilizing and enhancing its physicochemical water quality. This study aimed to test the use of NB to produce high-quality nutrient solutions for aeroponics. The first stage tested three water types, namely distilled water, water contaminated with RS at 10^4 CFU mL^{-1} (RS4) and at 10^8 CFU mL^{-1} (RS8), with and without H_2O_2. The second stage tested four water types, namely distilled water, garden water, RS4, and RS8, as controls and after NB treatment using air, O_2, or O_3 for 10, 20, or 30 min. The observed variables were RS population, temperature, pH, dissolved oxygen (DO), salinity, conductivity, particle size, and zeta potential. Data were analyzed using ANOVA, orthogonal contrast, Duncan test, Pearson correlation, and principal component analysis (PCA). The results showed five clusters, with PCA 1 and 2 representing 69.9% of total variation. The NB-O_2 not only inhibited RS growth effectively but also improved water quality by increasing DO to 18.77-22.58 µmol mol^{-1} and reducing particle size, while maintaining suitable pH and temperature closer to the range required for potato nutrient water. Therefore NB-O_2 is a promising preliminary source-water treatment, but validation in aeroponic system with potato plants is still required.

Key words: Aeroponics, gas, nanobubble, potato, *Ralstonia solanacearum*, *Solanum tuberosum*, water quality.

INTRODUCTION

Water for nutrients in hydroponic artificial systems must be sterile or free of pathogens for plants (Mamun and Islam, 2025), because this soilless artificial system relies heavily on nutrients brought into the system (Saldinger et al., 2023; Mamun and Islam, 2025). Similar to aeroponics as an example of a hydroponic system for potato (*Solanum tuberosum* L.) seed production (Dianawati et al., 2013), this system experiences the main obstacle of withering attack caused by bacteria *Ralstonia solanacearum* (RS) (Dianawati et al., 2024; 2025). This RS bacteria can be carried by nutrient water, so that it can cause crop failure in the aeroponic system (Dianawati et al., 2024). This failure occurs because the recirculation of water in the aeroponic system runs continuously, so that exposure to contaminated water by RS occurs repeatedly (Saldinger et al., 2023; Dianawati et al., 2025). So far, the easiest and cheapest water sterilization in aeroponic systems is to use hydrogen peroxide (H_2O_2) (Dianawati et al., 2025).

The innovation of water sterilization using nanobubbles (NB) is currently developing with significant progress due to its unique existence, behavior, and characteristics (Jia et al., 2023), so that the potential is developed in aeroponic systems. The NB is a small gas bubble in a liquid with a diameter of ≤ 0.2 µm (Ebina et al., 2013), which will produce reactive oxygen species (ROS), so that it can inactivate microorganisms (Marui, 2013). The smaller the bubble size, the higher the oxygen pressure value in the water, thus increasing the dissolved oxygen (DO) in the water to a higher level compared to micro-bubbles (10-50 mm in diameter) (Ebina et al., 2013). In addition, NB exhibits several outstanding characteristics in aqueous solutions, including higher solubility, high zeta potential, outstanding stability to coalescence, large surface area, and high energy release through bubble disintegration (Nirmalkar et al., 2018). Increased DO due to the use of NB can promote root and plant growth (Wang et al., 2021; Mamun and Islam, 2025), because DO is a critical indicator for maintaining healthy roots (Deboer et al., 2024; Mamun and Islam, 2025), especially in hydroponic systems (Saldinger et al., 2023).

The addition of other gases in NB can suppress pathogenic bacteria in the water (Domingos et al., 2021). The NB can be produced from a variety of gases, such as oxygen, ozone, nitrogen, and carbon dioxide (Dien et al., 2022). Thus, the testing of free air gases, O_2, and O_3 in NB makes it possible to study because the source of the gas is readily available. In addition, the effectiveness of NB with various gases against the attack of RS bacteria that attack potato plants and its suitability as a source of nutrient water physically-chemically in aeroponic systems is not known for sure yet. Based on Meegoda et al. (2018) stated that the type of gas plays an important role in the solubility and reactivity of NB in producing dissolved oxygen. The purpose of this study was to test the use of NB with various types of gas engineering and its application time for later produce quality water as a source of potato nutrient water in the aeroponic system.

MATERIALS AND METHODS

The research was carried out at the KST Samaun Samadikun Laboratory, National Research and Innovation Agency, Bandung, West Java, Indonesia. There were two stages of testing in this study, namely H_2O_2 testing on various types of water and nanobubbles (NB) engineering testing with various gases and application times on various types of water.

The first test was a two-factor factorial experiment arranged in a randomized complete block design with six replicates. The factors were water type and H_2O_2 treatment. The water types were distilled water, water contaminated with *Ralstonia solanacearum* (RS) at 10^4 CFU mL^{-1} (RS4), and water contaminated with RS at 10^8 CFU mL^{-1} (RS8). The H_2O_2 factor consisted of untreated water and water treated with 3% H_2O_2 at 2 mL L^{-1}. The observed variable was the RS population (CFU mL^{-1}). The data was analyzed with ANOVA and then followed by an orthogonal contrast test with the SAS 9.1 program, version 9.4 (SAS Institute, Cary, North Carolina, USA) to see the differences between treatment groups.

The second test used a single factorial treatment factor that was randomly arranged in three replicates. The treatment consisted of 40 treatments, namely four controls of four types of water and 36 interactions between the types of gases and the time of their application to different types of water. The type of water used was the same as the first test and garden water was added. The types of gases tested for NB were air, O_2, and O_3 with application times of 10, 20, and 30 min. The observed variables were RS population, temperature, pH, dissolved oxygen (DO), salinity, and conductivity. For particle size and potential zeta, the treatment tested was only at a NB application time of 30 min. The data were analyzed with ANOVA and then followed by the Duncan test and the Pearson correlation test using the SAS 9.1 version 9.4 program to see the differences between treatments. In addition, the analysis was continued with Principal Component Analysis (PCA) to obtain biplot images in cluster groupings with the RStudio 2026 program version 4.4.3. (R Foundation for Statistical Computing, Vienna, Austria). The packages used were gplots, ggplot2, factoMiner, factoextra, corrplot, and cluster.

Distilled water was obtained from a water purification system (Milli-QDirect-Q 3 UV Remote Water Purification System, Merck, Darmstadt, Germany). Bacteria were isolated from the stems of tomato plants infected with RS in Manoko Village (6°52'54.997" S, 107°36'41.113" E; 1300 m a.s.l.), Lembang District, West Bandung Regency. The garden water was obtained from groundwater wells around potato (*Solanum tuberosum* L.) plantations with the same location as the sampling of RS bacteria. Distilled water was inoculated RS bacteria by 10^4 CFU mL^{-1} for RS4 and by 10^8 CFU mL^{-1} for RS8.

The nozzle NB generator and the 12 V submersible water bilge pump mini-immersion pump were placed in the aquarium for NB testing. The aquarium (50 × 15 × 20 cm) was filled by water with volume of 8 L in each treatment. The NB generator system was made according to the scheme in Alam et al. (2022). The NB generators without O_2/O_3 generators for NB-air treatment (Figure 1a) or connected with O_2 generators for NB-O_2 treatment (Figure 1b) or connected with O_2 and O_3 generators for NB-O_3 treatment (Figure 1c) with a speed rate of 1 m^3 h^{-1}. Measurement of the RS population by means of serial dilution according to the method of Dianawati et al. (2024). Temperature, pH, DO, conductivity, and salinity were measured using a multimeter (WA-2017SD, Lutron, Coopersburg, Pennsylvania, USA). The calibration of the tool followed the method of (Alam et al., 2022). The ZetaSizer Nano ZS particle size analyzer (Malvern Instruments, Malvern, Worcestershire, UK) was used to measure particle size based on the dynamic light scattering (DLS) method and measure potential zetas based on the electrophoretic light scattering (ELS) method (Alam et al., 2022).

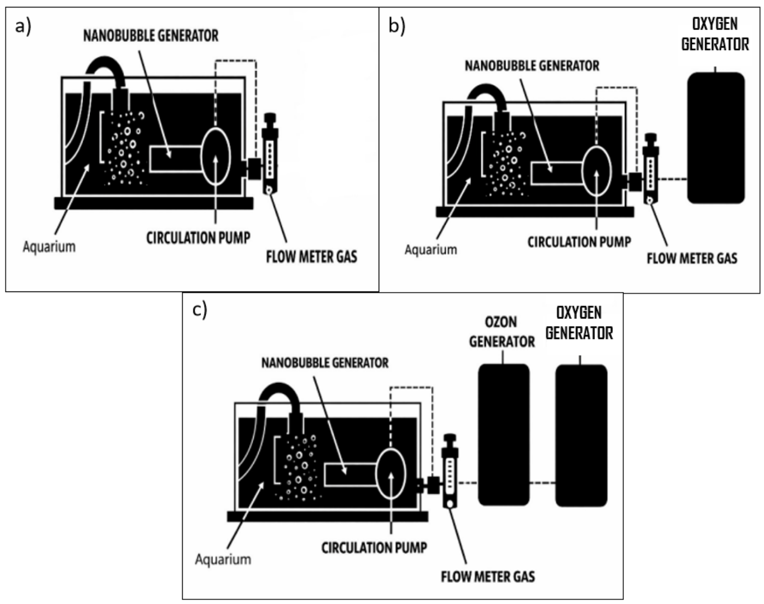

Figure 1. Scheme of nanobubble (NB) generator in each treatment: NB-air (a), NB-O_2 (b), NB-O_3 (c).

RESULTS

H_2O_2 effectiveness testing

The H_2O_2 treatment group had a lower RS population than the group without H_2O_2 (Table 1). If not given H_2O_2, the highest RS population was RS8 (115.15 × 10^2 CFU mL^{-1}), followed by RS4 (36.17 × 10^2 CFU mL^{-1}), and distilled water (22.67 × 10^2 CFU mL^{-1}). These results indicated that RS was still detected in distilled water even without the addition of H_2O_2, so the distillation method did not completely guarantee the absence of RS. Furthermore, RS inoculation in this study was shown to increase the RS population, with the highest number found in the RS8 treatment (Table 1). If H_2O_2 was given to distilled water, the RS population of 22.67 × 10^2 CFU mL^{-1} could be reduced to zero (Table 1). Meanwhile, in water contaminated with RS4 and RS8, the adding of H_2O_2 could not kill the RS and there was still a population of RS with nonsignificant difference between RS4 and RS8, but still lower than without the adding of H_2O_2 (Table 1).

Table 1. Orthogonal contrast test of different types of water with hydrogen peroxide (H_2O_2). RS4/8: Water contaminated by *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/by RS 10^8 CFU mL^{-1}; CFU: colony forming unit. *Numbers showed noticeable differences using orthogonal contrast test ($p < 0.05$). [1]Result of log transformation (x + 4) with coefficient variance (CV) 24.19%.

Group comparison	*Ralstonia solanacearum* population[1] (10^2 CFU mL^{-1})
A. Non H_2O_2 *vs*. H_2O_2	58.11 > 12.78*
• In the non H_2O_2 group	
- Distillated water *vs*. contaminated water by RS4 and RS8	22.67 < 75.83*
- Contaminated water by RS4 *vs*. RS8	36.17 < 115.50*
• In the H_2O_2 group	
- Distillated water *vs*. contaminated water by RS4 and RS8	0.00 < 19.17*
- Contaminated water by RS4 *vs*. RS8	15.67 *vs*. 22.67
B. Non H_2O *vs*. H_2O_2 in each type of water	
• Distillated	22.67 < 0.00*
• Contaminated by RS4	36.17 < 15.67*
• Contaminated by RS8	115.50 < 22.67*

Nanobubble engineering with different types of gases and application times in different types of water

Water quality was evaluated based on biological, physical, and chemical properties. The biological properties of water in this study were evaluated from the RS population (10^2 CFU mL^{-1}). The RS population in RS4 and RS8 control water showed the highest compared to control of garden water and control of distilled water (Table 2). This result showed that the RS inoculation treatment in this study successfully infected the distilled water so that the RS population increased and could be used for next NB testing. Meanwhile control of garden water had a higher RS population than control of distilled water, but lower than control of RS4 and RS8 (Table 2). Furthermore, the use of NB in distilled water with various gases and NB application times did not affect the RS population (Table 2). The use of NB in garden water with a relatively moderate RS population in control, might suppress the RS population across all NB treatments (Table 2). Meanwhile, the use of NB in RS4 and RS8 could suppress the RS population only in the treatment of NB-O_2 and NB-O_3 at various application times. In RS4, NB-O_2 for 30 min could suppress the RS population to zero (Table 2). Although there was a decrease in the RS population in both RS4 and RS8 due to the use of NB-air, the RS population was still high at 10 to 30 min (Table 2). Thus NB-air showed lower effectiveness when the RS population was high (RS4 and RS8), so it took a minimum of 20 min to apply.

Table 2. *Ralstonia solanacearum* (RS) population (×10^2 CFU mL^{-1}) in different types of water with nanobubble (NB) engineering. RS4/8: Water contaminated by *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/by RS 10^8 CFU mL^{-1}; CFU: colony forming unit. Numbers followed by the same letters in columns and rows showed significant differences from the Duncan test ($p < 0.05$). Result of log transformation (x + 4) with coefficient variance (CV) 29.07%.

		Types of gas								
		NB-Air			NB-O_2			NB-O_3		
Types of water	Control	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Distillated	7.5^{g}	3.5^{g}	0.0^{g}	0.0^{g}	0.0^{g}	0^{g}	0.0^{g}	0.0^{g}	0.0^{g}	0.0^{g}
Garden	54.0^{cde}	10.5^{fg}	4.0^{g}	0.0^{g}	14.0^{fg}	5^{g}	3.5^{g}	3.0^{g}	0.5^{g}	3.5^{g}
RS4	145.7^{a}	64.0^{cd}	22.3^{fg}	29.5^{efg}	13.5^{fg}	3.67^{g}	0.0^{g}	10.7^{fg}	1.7^{g}	0.1^{g}
RS8	117.0^{a}	77.5^{c}	32.0^{efg}	41.3^{dfe}	27.7^{efg}	2.17^{g}	0.8^{g}	27.3^{efg}	10.7^{g}	2.9^{g}

The physical property of water in this study was shown from the temperature of the water produced after being given NB engineering treatment. Garden water had the coldest temperature compared to distilled water, RS4 and RS8 at the controls before being treated with NB (Table 3). This result was because garden water came from nature and did not receive any physical treatment such as distillation treatment or RS inoculation. The use of NB with different types of water and gases increased the water temperature in line with the increasing application time of

NB. The highest temperature in various types of water was obtained when using NB-O_3 of 27.3-28.4 °C (Table 3). However, the water temperature due to the use of NB-O_2 and NB-air for 30 min did not differ significantly (Table 3).

The chemical properties of the water in this study were shown from the pH, DO, salinity, and conductivity values of the tested water (Table 4). The use of NB-O_3 at various times showed the highest pH of 14 compared to NB-O_2 and NB-air. Meanwhile, high pH in NB-O_2 was found only in garden water (Table 4). The use of NB-O_2 and NB-O_3 could increase the DO to close to 20 μmol mol^{-1} or even more, whereas the DO of NB-air was not significantly different from the controls (Table 4). However, increased application time of NB-O_2 and NB-O_3 could decrease DO especially in RS4 and RS8. Control of garden water had high salinity and conductivity compared to the other three types of control water and the use of NB-O_2 and NB-O_3 in control of garden water could increase salinity and conductivity (Table 4).

Table 3. Temperature (°C) at different types of water with nanobubble (NB) engineering. RS4/8: Water contaminated by *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/by RS 10^8 CFU mL^{-1}; CFU: colony forming unit. Numbers followed by the same letters in columns and rows showed significant differences from the Duncan test ($p < 0.05$); coefficient variance (CV) 2.98%.

Types of water	Control	Types of gas								
		NB-Air			NB-O_2			NB-O_3		
		10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
Distillated	22.1^{st}	22.2^{rst}	23.7^{n-q}	24.3^{j-p}	23.9^{l-q}	24.7^{l-o}	25.3^{g-k}	25.0^{hm}	26.2^{d-h}	27.3^{a-e}
Garden	21.7^{u}	22.0^{st}	24.2^{k-p}	24.7^{j-p}	23.3^{o-s}	24.3^{j-p}	25.3^{g-l}	26.0^{e-i}	26.9^{b-f}	27.8^{abc}
RS4	22.9^{p-t}	23.8^{m-q}	24.6^{l-o}	25.5^{g-k}	23.5^{o-r}	24.5^{j-o}	25.2^{g-m}	25.8^{f-j}	26.9^{b-f}	27.9^{ab}
RS8	22.6^{q-t}	23.5^{o-r}	24.4^{j-p}	25.3^{g-l}	23.5^{o-r}	24.4^{j-p}	25.3^{g-k}	26.5^{c-g}	27.5^{a-d}	28.4^{a}

Table 4. pH, dissolved oxygen, salinity, and conductivity in different types of water with nanobubble (NB) engineering. RS4/8: Water contaminated by *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/by RS 10^8 CFU mL^{-1}; CFU: colony forming unit; CV: coefficient variance. Numbers followed by the same letters in columns and rows showed significant differences from the Duncan's test ($p < 0.05$).

Types of water	Control	Types of gas								
		NB-Air			NB-O_2			NB-O_3		
		10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min
pH (CV: 3.87%)										
Distillated	5.8^{hi}	5.9^{f-i}	5.9^{ghi}	5.8^{i}	6.1^{e-i}	6.0^{f-i}	6.4^{d-h}	13.9^{a}	14.0^{a}	14.0^{a}
Garden	6.8^{cd}	6.6^{def}	6.6^{d-g}	6.7^{de}	7.4^{bc}	7.7^{b}	7.8^{b}	14.0^{a}	14.0^{a}	14.0^{a}
RS4	5.6^{i}	5.7^{hi}	5.9^{ghi}	6.1^{e-i}	6.7^{de}	6.8^{cd}	6.9^{cd}	14.0^{a}	14.0^{a}	14.0^{a}
RS8	5.8^{hi}	6.1^{e-i}	5.9^{hi}	6.2^{e-i}	6.6^{def}	6.8^{cd}	6.9^{cd}	14.0^{a}	14.0^{a}	14.0^{a}
Dissolved oxygen (μmol mol^{-1}) (CV: 5.63%)										
Distillated	6.8^{l}	4.0^{m}	4.0^{m}	4.1^{m}	21.1^{c-i}	21.9^{b-h}	20.8^{e-i}	20.5^{f-i}	22.1^{a-f}	20.3^{hij}
Garden	6.2^{l}	7.2^{l}	7.6^{l}	7.6^{l}	20.1^{ij}	19.9^{ijk}	18.8^{jk}	23.6^{a}	22.1^{a-f}	18.6^{k}
RS4	7.0^{l}	6.8^{l}	7.0^{l}	6.9^{l}	22.4^{a-e}	21.1^{c-i}	20.4^{ghi}	23.4^{ab}	22.6^{abc}	21.4^{c-i}
RS8	7.9^{l}	7.2^{l}	6.9^{l}	6.8^{l}	22.6^{a-d}	22.0^{a-g}	20.9^{d-i}	22.7^{abc}	22.0^{a-g}	21.3^{c-i}
Salinity (10^{-2} %) (CV: 33.31%)										
Distillated	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	1.0^{b}	0.0^{c}	1.0^{b}	2.0^{a}
Garden	1.0^{b}	0.0^{c}	0.0^{c}	0.0^{c}	2.0^{a}	2.0^{a}	2.0^{a}	1.0^{b}	2.0^{a}	2.0^{a}
RS4	0.0^{c}	1.0^{b}	0.3^{bc}	0.7^{bc}	0.3^{bc}	0.0^{c}	0.3^{bc}	0.7^{bc}	0.7^{bc}	1.0^{b}
RS8	0.0^{c}	0.7^{bc}	0.7^{bc}	0.7^{bc}	0.3^{bc}	0.3^{bc}	0.0^{c}	0.7^{bc}	1.0^{b}	1.0^{b}
Conductivity (μS cm^{-1}) (CV: 13.05%)										
Distillated	1.4^{h}	1.5^{h}	1.4^{h}	1.5^{h}	2.6^{h}	2.3^{h}	2.7^{h}	4.0^{h}	7.6^{h}	10.0^{h}
Garden	257.3^{f}	517.0^{a}	312.7^{e}	165.0^{g}	405.3^{bc}	322.3^{e}	409.7^{b}	383.0^{d}	388.7^{cd}	313.7^{e}
RS4	1.7^{h}	3.2^{h}	3.2^{h}	3.0^{h}	2.6^{h}	2.7^{h}	3.1^{h}	3.7^{h}	5.4^{h}	6.3^{h}
RS8	2.0^{h}	2.6^{h}	2.9^{h}	2.8^{h}	2.8^{h}	2.7^{h}	2.8^{h}	5.4^{h}	8.2^{h}	7.1^{h}

Correlation between variables

An increase in pH could increase the temperature by 73% and DO by 65% (Table 5). This result showed the high interconnected of these three variables. Meanwhile, the RS population was significantly negatively affected by DO, temperature, and pH, but with low values, less than 50% (Table 5). Thus, an increase in pH, DO, and temperature, could cause the RS population to decrease, but pH, DO, and temperature, were not the main factors causing the decline in RS.

Table 5. Correlation between observation variables. *Differences between variables ($p < 0.05$).

Observation variables	pH	Temperature	Conductivity	Dissolved oxygen	Salinity
Ralstonia solanacearum population	-30*	-30*	-15	-41*	-14
pH		73*	10	62*	41*
Temperature			-9	57*	42*
Conductivity				3	45*
Dissolved oxygen					30*

Principal component analysis

There were five clusters with a PCA value of 1 and 2 of 69.9% (Figures 2 and 3). Temperature as a physical property formed a vector adjacent to the chemical properties of pH and DO and lead to the right side (Dimension 1 positive). This result indicated a very strong positive correlation between the three variables. In contrast, the RS population vector points to the lower left side (Dimension 1 negative) and formed an angle of almost 180° or in opposite directions to the pH, DO, and temperature groups. This visually proved that the increase in pH, DO, and temperature values was inversely proportional to the RS population. Meanwhile, conductivity and salinity formed separate groups leading to the top of the graph (Dimension 2 positive) (Figure 2).

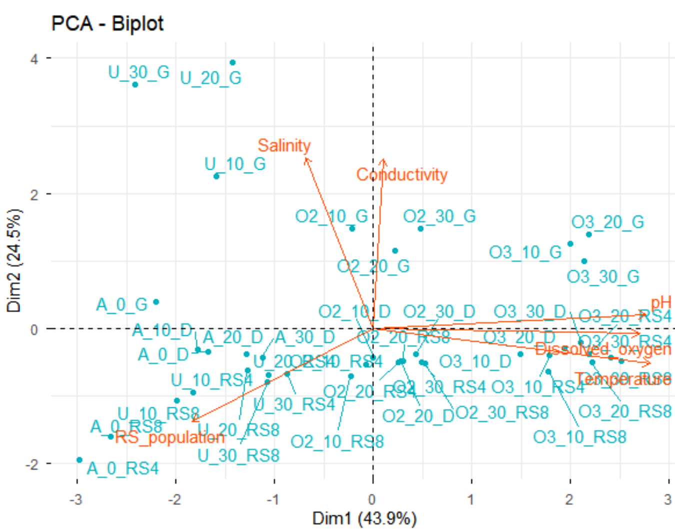

Figure 2. PCA biplot with various nanobubble treatments and observation variables: U/O_2/O_3: gasses of air/O_2/O_3; 0/10/20/30: nanobubble application time (min); D/G/RS4/RS8: types of water (distillated/garden/contaminated with *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/contaminated RS 10^8 CFU mL^{-1}.

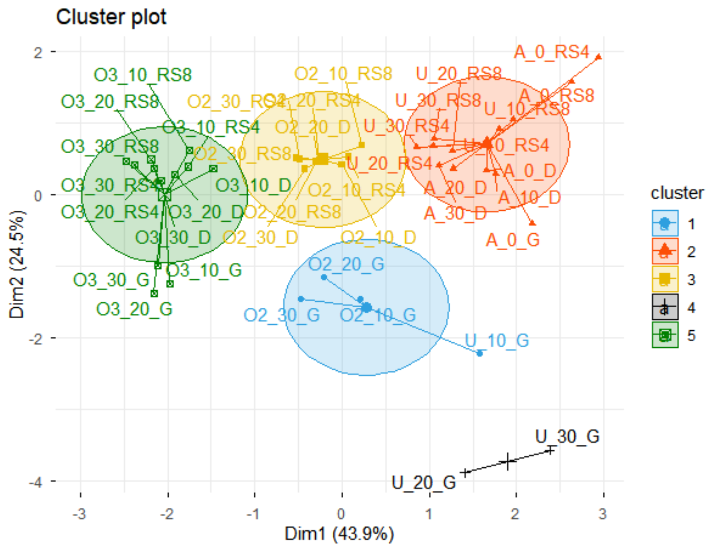

Figure 3. PCA biplot with five clusters with various nanobubble treatments: U/O_2/O_3: gasses of air/O_2/O_3; 0/10/20/30: nanobubble application time (min); D/G/RS4/RS8: types of water (distillated/garden/contaminated with *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/contaminated RS 10^8 CFU mL^{-1}.

Garden water had a distinctive characteristic of high conductivity values, but it was then divided into two clusters, namely the first and fourth clusters (Figure 3). The first cluster had a lower conductivity, namely garden water with NB-air at 20 and 30 min compared to the fourth cluster, namely garden water with NB-air at 10 min and NB-O_2 at various application times. The fifth cluster was NB-O_3 at various application times on different types of water. This fifth cluster appeared to have a high pH, DO, and temperature, but a low RS population (Figure 3). The next cluster was non-garden water and non-NB-O_3 clusters. All control water and NB-air with different types of water except garden water, were included in the second cluster. This cluster had a special characteristic, namely a high RS population with low pH, DO, and temperature (Figure 3). While the third cluster was a medium cluster, namely NB-O_2 with various application times in various types of water except garden water with low pH, temperature, and RS population characteristics, but high DO (Figure 3).

DISCUSSION

Hydrogen peroxide (H_2O_2) is commonly used as a water sterilizer for hydroponic and aeroponic nutrients (Lau and Mattson, 2021; Dianawati et al., 2025). This is because H_2O_2 has antibacterial activity that can suppress various diseases including *Ralstonia solanacearum* (RS) both in the greenhouse and in the field (Hong et al., 2013). As a powerful oxidizing compound, H_2O_2 could damage the integrity of cell membranes through the process of lipid peroxidation and cause damage to essential macromolecules such as DNA and proteins (Heller and Tudzynski, 2011). This was shown by the declining RS population in this study in all types of water after H_2O_2 was applied (Table 1). Exposure to H_2O_2 could inhibit the production of extracellular enzymes that bacteria normally use to degrade their hosts, thus drastically lowering the virulence of those bacteria (Nanda et al., 2010). However, the use of H_2O_2 in nutrient tubs often caused precipitation due to its properties as strong oxidizers that trigger destructive chemical reactions (Perez-Sena et al., 2024). The H_2O_2 tended to damage the chelates bonds of microelements, especially Fe, so that the mineral was detached, oxidized, and turns into solid, insoluble particles at the bottom of the tub (Winterbourn, 1995). This caused water quality to decrease because important nutrients became locked and could no longer be absorbed by plant roots even though the electroconductivity values on the measuring instrument looked normal. It was therefore necessary to look for other alternatives that could sterilize water in aeroponic systems, but also did not degrade water quality.

The use of oxygen (NB-O_2) and ozone nanobubbles (NB-O_3) for 10-30 min and NB-air for 20-30 min on distilled water showed potential as an alternative preliminary disinfection approach because RS populations reached zero, similar to the H_2O_2 treatment (Tables 1 and 2). Even with a high RS population (10^8 CFU mL^{-1}; RS8), the use of NB-O_2 and NB-O_3 for 30 min in this study showed that RS population was 0.83 and 2.92 (10^{-2} CFU mL^{-1}) respectively (Table 2) lower than the RS population with H_2O_2 of 22.67 (10^{-2} CFU mL^{-1}) (Table 1). The inability of H_2O_2 to suppress RS attacks was also found in the study of Hong et al. (2013), who reported that reactive oxygen species (ROS) produced from H_2O_2 could not overcome RS attacks in high RS attack conditions. Meanwhile, NB could reportedly kill bacteria through a combination of physical and chemical mechanisms that damage the cellular structure of cells. When the NB burst, highly reactive hydroxyl radicals and oxygen radicals were produced. These radicals would oxidize the bacterial cell membrane, resulting in bacterial cell death (Ci et al., 2025; Lu et al., 2025). This result was in accordance with the research of Yokoyama et al. (2021), which reported that NB could delay the initial attack and suppress the development of RS in tomatoes at all RS inoculation concentrations. The advantage of NB as a water sterilizer compared to H_2O_2 was the stability of NB in water, so that the effect of NB on damaging bacterial cell membranes was more consistent than H_2O_2, especially at RS8 concentrations (Tables 1 and 2). The NB had characteristics residence time longer in water and a high contact surface area, allowing these oxidizing gases to interact more intensively with bacteria in the water (Nanda et al., 2010). The stability of NB could be seen from the high potential zeta of NB both air, O_2, and O_3 from -8.72 to -28.72 mV (Table 6). A high absolute value of potential zeta correlated with a higher colloidal stability (Jia et al., 2023). The negative charge of NB could also prevent NB from merging into large bubbles, thus remaining stable in water (Ebina et al., 2013). Alam et al. (2022) reported stable NB-O_2 for up to 10 mo compared to stable NB-air for only 5 mo.

Table 6. Particle size and zeta potential in different types of water with nanobubbles (NB) of various gases for 30 min. RS4/8: Water contaminated by *Ralstonia solanacearum* (RS) 10^4 CFU mL^{-1}/by RS 10^8 CFU mL^{-1}. Numbers followed by the same letters in columns and rows showed significant differences from the Duncan test ($p < 0.05$).

	Size of particle (nm)			Zeta potential (mV)		
	NB-air	NB-O_2	NB-O_3	NB-Air	NB-O_2	NB-O_3
Distillated	476.3^{a-d}	57.6^{f}	95.8^{f}	-8.7^{a}	-10.3^{ab}	-23.5^{ef}
Garden	352.4^{cd}	308.9^{de}	97.8^{f}	-13.9^{abc}	-14.5^{abc}	-13.0^{abc}
RS4	616.4^{a}	357.3^{cd}	413.7^{cd}	-20.8^{de}	-23.8^{ef}	-17.3^{cd}
RS8	576.6^{ab}	333.2^{abc}	148.6^{ef}	-28.7^{f}	-23.7^{ef}	-16.0^{cd}
CV, %		29.13			17.4	

Apart from being a water sterilizer, NB for hydroponic systems is expected to increase plant growth through improving water quality especially increasing of DO value. However, the present study did not evaluate nutrient-solution performance, root responses, plant growth, or yield. Therefore, the results should be interpreted as laboratory evidence of changes in biological and physicochemical water indicators. The results of this study showed that there were five clusters in response to the treatment interactions tested against all observed variables (Figure 3). The fifth cluster with a low RS population, but high temperature, pH, and dissolved oxygen (DO) were all NB-O_3 treatments. This fifth cluster was in contrast to the second cluster, namely all control water and NB-air non-garden water. Garden water treatment with high conductivity values was divided into two clusters, namely the first and fourth clusters, while non-garden water NB-O_2 was incorporated into the third cluster with low pH, temperature, and population characteristics, but the DO remained high (Figures 2 and 3).

The fifth cluster, namely NB-O_3 at different times of application on different types of water, showed a low RS population with high pH, temperature, and DO (Figures 2 and 3; Tables 2, 3, and 4). Saijai et al. (2019) reported that the use of O_3 in NB could suppress the development of bacteria compared to without O_3. This was because O_3 was known as a powerful oxidizer for sterilizing water (Domingos et al., 2021). Once dissolved in water, O_3 rapidly decomposed, forming hydroxyl radicals with higher oxidation potential (Wang and Xu, 2012). This oxidation reaction would attract electrons on the cell membrane, so that the integrity of the membrane was damaged and bacteria die (Nanda et al., 2016; Saijai et al., 2019). Meanwhile, in terms of water quality, the oxygen produced by NB-O_3 in this study increased to around 18.57-23.57 µmol mol^{-1}, but pH and temperature also increased (Tables 3 and 4), as did the high correlation values between pH, temperature, and DO (Table 5). In addition, NB-O_3 consistently has smaller particle sizes in all types of water, but high potential zeta only in distilled water (Table 6). Smaller particles would ensure a wider surface to absorb more oxygen and a high potential zeta would guarantee the stability of the NB (Meegoda et al., 2018), so that the DO of NB-O_3 was the highest (Table 4). Alam et al. (2022) reported that the higher the pH, the higher the pH, the potential zeta value increased, while the particle size decreased.

The increase in water temperature with the longer application time of NB-O_3 (25-28 °C) (Table 3) occurs due to thermodynamics, the NB implosion released local heat energy that contributed to the rise in water temperature, where these higher temperatures accelerated the rate of O_3 oxidation reactions (Petrov and van Breusegem, 2012). This was in accordance with the research of Jhunkeaw et al. (2021), which reported an increase in temperature at NB-O_3 by 1.9-2.6 °C in the first 10 min, and by 4.3-4.7 °C after the third 10 min. Meanwhile, the high pH value in this study which reached 14 (Table 4) showed a very saturated concentration of OH in the water. Several studies reported that the use of NB-O_3 caused a stable pH around 8 (Saijai et al., 2019; Jhunkeaw et al., 2021). This was because NB-O_3 tended to be more stable at higher pH due to the greater accumulation of OH at the gas-liquid interface (Meegoda et al., 2018). The NB would not last long at low pH due to the lack of OH ions needed to form an electrical double layer around the bubble surface (Nirmalkar et al., 2018; Wang et al., 2025). The high pH of NB-O_3 indicated a strong alkaline condition, which was biologically lethal to most microorganisms, including bacteria such as RS. Thus, the effectiveness of RS control at high concentrations (RS8) through this technology was not only caused by the oxidative action of ozone gas, but also the result of the synergy of changes in the physical-chemical environment of water in the form of high pH that was deadly to water-borne microorganisms (Sneh et al., 2013). However, the higher the pH and temperature of NB-O_3 in this study, not only would kill the RS bacteria, but also the potato plant, so it could not be used in aeroponic systems. Potato plants need a pH of 5.5-6.5 (Popescu, 2020), while the nutrient water temperature was no more than 25 °C or optimal at 18-21 °C (Dianawati et al., 2025). In addition to the weakness of high temperature and high pH, NB-O_3 had low stability in terms of potential zeta values in high electrolyte conditions (garden water) and microbial contamination (RS4 and RS8) (Table 6). Nirmalkar et al. (2018) stated that one of the causes of NB would easily disappear or become unstable if their surface charge was neutralized by ions or other materials. Therefore, NB-O_3-treated water should not be recommended for direct use in potato aeroponic systems based on the present data. If NB-O_3 is used, it should be considered only as a pre-treatment in source-water reservoirs before entering the aeroponic system, not in the aeroponic system, to ensure no contamination of the RS.

Garden water had a distinctive character, namely its high conductivity in Cluster 1 and Cluster 4 (Table 4, Figures 2 and 3), because the content of dissolved ions was higher from various water sources, both rainwater and water flowing around the garden. The NB-O_2 and NB-O_3 in garden water (cluster 1) could cause not only their high conductivity as NB-air (cluster 4), but also higher salinity than NB-air (Table 4). This indicated that the aeration process of NB-O_2 and NB-O_3 in garden water triggered the release or ionization of dissolved particles in water rather than NB-air (Meegoda et al., 2018). The high conductivity and salinity of garden water was certainly not suitable for use as a source of nutrient water in aeroponics or other hydroponic systems, although the use of NB, both air, O_2 and O_3 could suppress the RS population (Table 2) and the particle size decreased in NB-O_3 (Table 6). This was because the conductivity and salinity range for hydroponics should be 1.0 to 2.5 mS cm^{-1} so that nutrients in the artificial system could be absorbed directly by the plant (Singh and Bruce, 2016). In addition, high electrolytes could neutralize the NB charge (Deboer et al., 2024), so that there was no difference in potential zeta between NB treatments in garden water (Table 6). However, this study could prove that NB in garden water with high conductivity could still suppress the RS population.

Use of NB-air in all types of water except garden water and all control water treatment (Cluster 2) indicated a pH and temperature that was normal for plant growth, i.e., pH was around 5.6-6.1, while the water temperature ranged from 21.7-25.9 °C was lower than other treatments (Tables 3 and 4). However, this treatment had a large particle size (Table 6), low DO (Table 3) and a high RS population, especially when the RS inoculation was high in RS4 and RS8 (Table 2). Thus NB-air appeared to be less effective than other gases O_2 and O_3. This result was because the composition of the air was dominated by nitrogen gas, so the partial pressure of oxygen was not strong enough to increase the concentration of DO beyond its natural saturation point (Yaparatne et al., 2022). Some studies have also reported lower DO of NB-air compared to NB-O_3 and NB-O_2 as well (Meegoda et al., 2018; Dien et al., 2022).

Meanwhile, the use of NB-O_2 with its various application times in non-garden water (cluster 3), had a low RS population like NB-O_3, but had a pH and temperature not as high as NB-O_3 (Tables 3 and 4), and the particle size was between NB-air and NB-O_3 (Table 6), so it had a DO of more than 18 µmol mol^{-1} (Table 4). The DO was a critical indicator for maintaining healthy roots (Mamun and Islam, 2025), so water with high DO in a closed hydroponic system was needed to improve water and nutrient absorption, photosynthesis ability, and plant resistance to biotic and biotic stress (Wang et al., 2021). This showed that this cluster could be applied in aeroponic systems. Fauziah et al. (2026) reported that increasing DO from 5 to 6.5 µmol mol^{-1} by NB could increase the fruit number and weight of tomatoes in drip irrigation system. However, the temperature produced by NB-O_2 in this study was still quite high at 23-25 °C. Even its temperature in short application was lower than longer application (Table 3). Saijai et al. (2019) reported that the longer the use of NB could increase the temperature by up to 30 °C. Thus, lowering the temperature lower than 25 °C could be the next research topic of this study. This was because high temperatures were not only undesirable for plants, but also NB at high temperatures become unstable (Meegoda et al., 2018). As stated by Deboer et al. (2024), the increase in temperature and pH with the longer the use of NB-O_2 and NB-O_3 could be a challenge for their application in the agricultural world.

CONCLUSIONS

To support smart farming, one generator can be used multifunctionally to be more efficient in aeroponic systems, namely as a sterilizer for *Ralstonia solanacearum* (RS) and improve water quality so that potato production can increase. Nanobubbles (NB) of O_2 and O_3 are potential as an alternative water sterilizer in high contamination of RS. However, NB-O_2 not only inhibited RS growth effectively but also improved water quality by increasing dissolved oxygen to 18.77-22.58 µmol mol^{-1} and reducing particle size, while maintaining suitable pH and temperature closer to the range required for potato nutrient water. Therefore NB-O_2 is a promising preliminary source-water treatment, but validation in aeroponic system with potato plants is still required. The increase in temperature and pH with the longer the use of nanobubble of O_2 and O_3 could be a challenge for their application in the agricultural world.

Author contribution

Conceptualization & Methodology: M.D., K.K.H., H.S.A. Software, validation, formal analysis, & data curation: M.D., K.K.H., H.S.A., B.S., I.C., H.H., A.N. Writing-original draft: M.D., K.K.H., I.C., B.S., E.S.H., Y.H., Writing-review & editing: M.D., K.K.H., H.S.A., B.S., I.C., H.H., A.G., E.S.H., Y.H., N.G., I.B.R., A.K.K., A.A., P.E.R.P. Visualization: M.D., B.S. Supervision: M.D., K.K.H., E.S.H. Funding acquisition: M.D. All co-authors reviewed the final version and approved the manuscript before submission.

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