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Poultry slaughterhouse wastewater treatment using nanobubble technology

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ABSTRACT

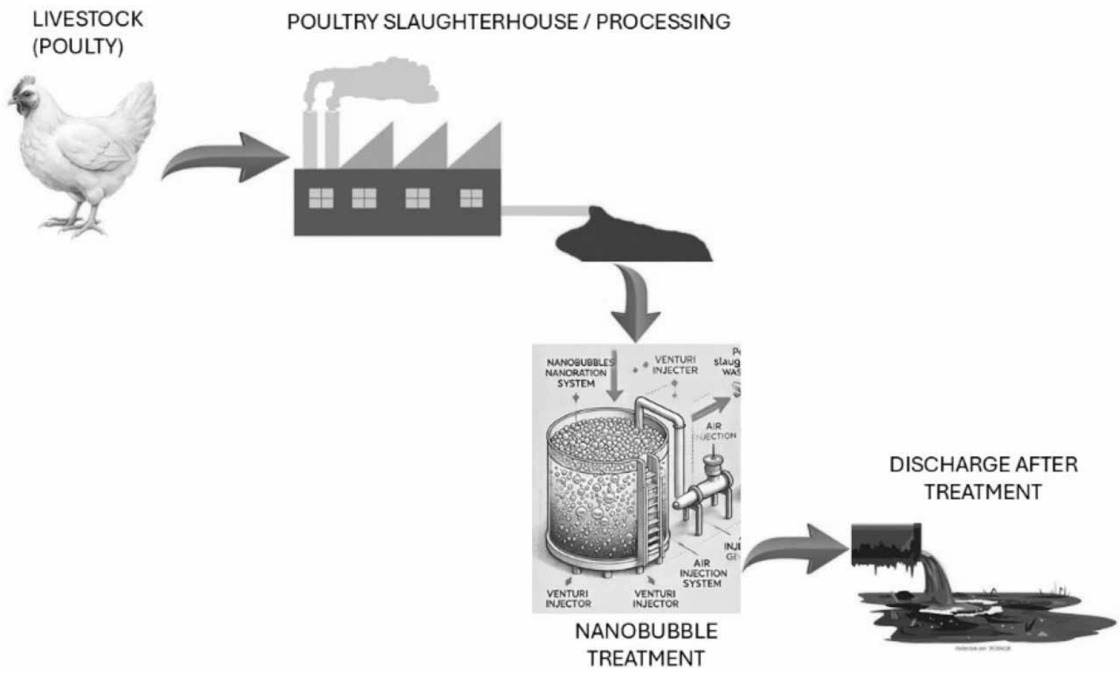
This study investigates the enhancement of aerobic treatment of poultry slaughterhouse wastewater (PSW) using nanobubble (NB) technology. Three aeration methods, i.e. air-NB, ozone-NB, and air-NB combined with Ecoflush enzymes were evaluated for removing chemical oxygen demand (COD), total suspended solids (TSS), NH_3-N, total nitrogen, and fats, oil, and grease (FOG). Air-NB and ozone-NB achieved over 80% COD removal within 2 h, while NBs with Ecoflush enzymes initially showed lower removal rates but reached 99.5% FOG removal after 6 h. TSS removal efficiency remained steady across all methods after 4 h, with ozone-NBs performing best. Ammonia removal was most effective with NBs and Ecoflush enzymes, achieving 99% removal after 6 h. Both ozone-treated NBs and NBs with Ecoflush enzymes showed notably high FOG removal. The findings demonstrate that NBs can enhance mass transfer in wastewater treatment, making them an effective method for improving pollutant degradation in PSW.

Key words: aeration systems, chemical oxygen demand, nanobubble, poultry slaughterhouse wastewater

HIGHLIGHTS

- Nanobubble (NB) technology effectively treats poultry slaughterhouse wastewater.
- Air-NB and ozone-NB achieved over 80% chemical oxygen demand removal in 2 h of aeration.
- Air-NBs combined with Ecoflush enzymes achieve 99.2% ammonia removal.
- Ozone-NBs achieved 99.5% fats, oil, and grease removal.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Poultry slaughterhouses release substantial volumes of wastewater into the environment because of their extensive use of freshwater for ongoing activities such as meat cutting and rinsing. This poultry slaughterhouse wastewater (PSW) is highly contaminated, featuring organic matter measured by biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and total suspended solids (TSS). Additionally, it contains high levels of nitrogen and phosphorus components, encompassing substances such as blood, fats, oil and grease (FOG), and proteins (Basitere *et al.* 2019; Ng *et al.* 2022; Teo *et al.* 2023). Improper discharge of inadequately treated PSW poses a substantial risk of contaminating freshwater sources. This poses potential environmental and health hazards, including river deoxygenation, groundwater pollution, eutrophication, and the potential spread of waterborne diseases. Therefore, it is extremely essential to undertake on-site treatment of PSW (Kothari *et al.* 2024), thereby avoiding or reducing environmental contamination and facilitating the potential reintegration of treated wastewater into plant operations, particularly surface cleaning (Reilly *et al.* 2019; dos Santos Pereira *et al.* 2024).

Biological treatment methods, including aerobic and anaerobic processes, have been widely adopted for managing the challenges associated with treating PSW (Philipp *et al.* 2021). Aeration systems, recognized as one of the oldest and simplest methods, have been extensively used to treat PSW (Gutu *et al.* 2021; Philipp *et al.* 2021; dos Santos Pereira *et al.* 2024). These methods are particularly preferred for handling wastewater concentration of pollutants from industrial effluents, agricultural runoff, or wastewater from urban sources, due to their easy construction and operation, as highlighted by Musa & Idrus (2021) and Ng *et al.* (2022).

Table 1 shows typical conventional aerobic processes for the treatment of PSW. These processes achieved between 72.2 and 94.7% COD removal. However, conventional aeration methods have their drawbacks, including the build-up of significant sludge, substantial energy consumption for aeration, vulnerability to high organic loads, and extended hydraulic retention times (Ahmadi *et al.* 2022). Moreover, conventional aeration methods have faced difficulties due to their limited oxygen transfer efficiency, typically ranging from 6 to 10%.

To address these challenges, significant attention has been directed towards technologies aimed at enhancing aerobic processes through the optimisation of aeration methods and gas diffusion, with a specific emphasis on controlling bubble sizes (Sakr *et al.* 2022). Nanobubble (NB) technology has emerged as a promising advancement in the domain of wastewater treatment. NBs are tiny gas cavities with diameters smaller than 1 μm according to International Organization for Standardisation (2017). Compared to ordinary bubbles, NBs possess distinct characteristics. Their small size and high surface tension enhance the liquid–gas contact area, facilitating

Table 1 | PSW organic and nutrient removal using conventional treatment methods

Process	Achievement	Disadvantages	References
Treatment of PSW using aerobic moving bed biofilm reactor	Achieved a COD removal efficiency of 94.7%, a TDS removal efficiency of 61.4%, a NO_3^- removal efficiency of 71.7%, and a PO_4^{3-} removal efficiency of 62.9% over a retention period of 7 h	Increasing organic load strength can reduce the removal efficiency of pollutants in the MBBR system, affecting overall treatment performance	Baddour *et al.* (2016)
GAS–SBR in treating chicken slaughterhouse wastewater	2 h aeration time led to removal efficiencies: 72.8% for COD, 72.2% for BOD, and 59.8% for TSS. Extending aeration to 4 h resulted in a removal efficiency of 65.8% for total ammonia	The values of COD and BOD did not meet the quality standard Regulation of the Minister of Environment	Oktafani *et al.* (2019)
Constructed wetlands for removal of COD, TSS, TDS, BOD5, nitrate, and phosphate from SWW	The removal rates for phosphate, COD, BOD, nitrate, TDS, and TSS were 77.5, 93.3, 68.0, 71.3, and 88.7%, respectively	The mean concentrations of some parameters in the effluent failed to comply with the maximum permissible limits required for the safe discharge of industrial wastewater into inland water bodies	Keerthana & Thivyatharsan (2018)
Biological pre-treatment of PSW with enzymes (Ecoflush) using macro bubbles	The bio-delipidation capabilities of the pre-treatment unit were assessed with an average FOG removal rate of 80.0%, while the removal rates for TSS and COD reached 38.0 and 56.0%, respectively	The resultant effluent exceeded the set standard for effluent discharge	Dyosile *et al.* (2021)

physical adsorption, chemical reactions, and mass transport at the gas–liquid interface (An *et al.* 2019; Shi 2022). Due to their low buoyancy, NBs ascend to the surface more slowly, prolonging their presence in the liquid phase (Kalogerakis *et al.* 2021). Moreover, they enhance gas mass transfer efficiency by reducing bubble size and increasing internal pressure (Azevedo *et al.* 2019).

NBs have proven to be highly useful in wastewater treatment. For instance, air-NBs have proven effective in treating various types of wastewater. In their study, Leyva & Flores (2018) demonstrated a reduction of 79.0% in TSS and 85.0% in COD in sugar sector wastewater in under 90 min. Another study by Reyes & Flores (2017) reported a 66.21% removal efficiency for total coliforms. Additionally, Wang & Zhang (2017) investigated the integration of NB into a deep subsurface wastewater infiltration system, achieving removal percentages of 95.1, 98.5, and 99.9% for COD, NH_4^+-N, and total phosphorus; respectively.

While NB technology has been applied to other high-organic-load wastewater streams such as sugar industry effluent (Leyva & Flores 2018) and synthetic wastewater (Van Leeuwen *et al.* 2009), its application to PSW remains unexplored. Conventional methods such as aerobic reactors and constructed wetlands have achieved pollutant removal rates of up to 94.7% for COD and 93.3% for TSS (Keerthana & Thivyatharsan 2018), but these methods face limitations, including high sludge production and prolonged hydraulic retention times (Ahmadi *et al.* 2022). This study demonstrates the potential of NB technology for PSW treatment using air-NBs, ozone-NBs, and air-NBs combined with enzymes. Unlike previous studies, this research provides a comparative analysis of three NB-based systems, elucidating the specific advantages of each approach.

2. MATERIALS AND METHODS

2.1. PSW source

The PSW was collected from a local poultry abattoir in Cape Town, South Africa. The wastewater resulted from diverse activities, including slaughtering, feather removal, evisceration, trimming, carcass washing, deboning, chilling, packaging, and the cleaning of facilities and equipment. The collection point was a stream situated between the abattoir and the equalization tank. The raw wastewater was placed in 25 L polyethylene containers, which were then stored in a refrigerator at 4 °C. The composition of the PSW used in this study is presented in Table 2. It was important to note that the composition of poultry PSW can vary significantly depending on several factors. These include the specific practices and methods employed at the slaughterhouse, the variations in the slaughtering process, and the timing of wastewater sample collection. Factors such as the scale of operations, the types of poultry processed, and the cleaning protocols in place also contribute to the variability. The time of sample collection, whether during peak processing periods or after cleaning activities, can lead to differences in the concentration and types of pollutants present in the wastewater.

Table 2 | Composition of PSW vs. the South African water act general discharge limits

Parameter	PSW composition from literature globlly (Yaakob *et al.* 2018; Njoya *et al.* 2019; Ngobeni *et al.* 2022)	PSW composition for this study	General discharge limits as set in the SA National Water Act 36 of 1998 of Republic of South Africa
pH at 25 °C	6.1– 8.0	6.7–7.1	5.5–7.5
COD (mg/L)	2,133–12,490	2,000–3,600	75
TSS (mg/L)	405–8,319	516–718	25
FOGs (mg/L)	280–1,668	100–226	2.5
Ammonia as N (NH_3-N) (mg/L)	160–274	59–127	6
Nitrates as N (mg/L)	50–840	–	15
Nitrites as N (mg/L)	40–700	–	15
Total phosphates as P (mg/L)	15–200	19–95	10

The PSW composition in this study aligns with the ranges reported in previous studies from internationally published papers. However, except for pH, all measured parameters exceed the general discharge limits established by the South African National Water Act of 1998. This highlights a significant requirement for the treatment of PSW to mitigate the environmental impact associated with PSW discharge, ensuring compliance with regulatory standards, and protecting the environment.

2.2. Experimental setup

The study focuses on evaluating different NB aeration methods for treating PSW in three phases:

- Phase 1: Treatment with air-nanobubbles (air-NBs)
- Phase 2: Treatment with ozone-nanobubbles(ozone-NBs)
- Phase 3: Treatment with air-NBs combined with enzymes (Ecoflush)

Each phase consisted of a 200 L polyethylene tank filled with PSW. Prior to aeration using the NB generator (MK3), the PSW underwent screening using three Madison test sieves of 100 μm to eliminate suspended solids. The MK3 NB generator, connected to the bottom of the tank for continuous circulation, was equipped with a nozzle for simultaneous air/ozone injection and NB generation. The PSW was aerated for 6 h to allow the breakdown of organics and nutrients, with samples collected from the bottom of the aeration tank at 2 h intervals.

The NB generator used in this study was an MK3 nanobubbler™ from Fine Bubble Technology (Pty) Ltd, based in Porterville, South Africa. The MK3 NanoBubbler™ has been tested and proven to produce 220,000,000 NBs/mL with an average size of 76 nm (range 10–300 nm) in diameter (Fine Bubble Technologies 2024).

2.2.1. Phase 1: treatment of PSW using air-NB

The MK3 NanoBubbler™ uses the venturi method to generate NBs and comprises a porous nanofilm membrane venturi tube directly connected to a water supply source. It is inserted into a pipe-shaped body with a vent, mounted at the centre of a pipeline conduit of a water-supplying pipe, and connected to the vent through a hose for air injection. The generator includes a nano-bubble water generating unit with multiple vortex formation units and pipe couplers positioned on both sides of the nano-bubble generator.

2.2.2. Phase 2: treatment of PSW using ozone-NB

An ozone generator 0Z–3G was used to produce ozone and the ozone was injected into the gas inlet of the venturi of the MK3 NB. The OZ-3G ozone generator uses a fan-cooled corona discharge (CD) tube to produce ozone from various pressurized sources such as compressed air, bottled oxygen, or an oxygen generator. It is equipped with a light-duty air compressor for self-generated compressed air, ensuring high ozone concentration with low energy consumption.

The ozone generator and air compressor feature switches and light-emitting diode (LED) status indicator lights. Ozone discharge concentration is adjustable from 0 to 100% using a dial potentiometer, regulating the current of the CD tube, as indicated on a 0–500 mA ammeter. The unit has electrical protection through an externally accessible fuse.

2.2.3. Phase 3: treatment of PSW with air-NB in combination with enzymes

A volume of 500 mL Ecoflush^{TM} (Mavu Biotechnologies Pty Ltd SA) was added to the 200 L of raw PSW. The mixture was aerated for 6 h using the MK3 NB generator to allow the activation of the enzymes and biodegradation of FOG. NB aeration ensured a consistent and sufficient provision of dissolved oxygen (DO), promoting optimal proliferation of aerobic bacteria in the Ecoflush^{TM}.

Ecoflush is a bioremediation agent commercially produced by Ergofito and distributed in South Africa through Mavu Biotechnologies. It is a blend of natural components and bacteria. It remains inactive until exposed to a nutrient-rich organic source, such as PSW, which serves as a substrate. Once activated, it primarily generates enzymes for the hydrolysis of FOG. The natural ingredients in Ecoflush are sourced from glaucids and essential amino acids, forming potent decomposing agents that stimulate specific bacteria to produce enzymes naturally. These enzymes have the capability to break down the hydrocarbon chains present in FOG.

2.3. Sampling and analysis

For each phase, PSW samples were collected from the sampling point at the bottom of the aeration tank at 2 h intervals. The following parameters were analysed at a South African National Accreditation System accredited laboratory (Bemlab, Somerset West, South Africa): COD, TSS, ammonia-nitrogen (NH_3-N), total nitrogen (total-N), as well as FOG. Furthermore, pH, temperature, and DO were measured at 1 h intervals using a multi-parameter.

3. RESULTS AND DISCUSSION

3.1. Introduction

Wastewater treatment for PSW is challenging due to high levels of organics and nutrients. NB aeration technologies offer innovative solutions by improving mass transfer, oxidation, and biological activity. This section presents and discusses the performance of three NB-based treatments: air-NB treatment (Phase 1), ozone-NB treatment (Phase 2), and air-NB treatment combined with enzymes (Ecoflush) (Phase 3) in treating PSW. First, the performance of each phase is analysed individually in removing pollutants.

The analysis is presented in two parts. First, the individual performance of each treatment phase is evaluated, highlighting their strengths and limitations in addressing specific pollutants. Second, a comparative assessment of the three phases is conducted, providing insights into their relative efficiency and suitability for treating PSW. This comprehensive evaluation aims to identify the most effective method for optimizing wastewater treatment in this context.

3.2. Performance of air-NB treatment

Understanding the removal mechanisms of organics and nutrients by air-NB is crucial to direct future technology development and implementation. In Phase 1 (Figure 1(a)), the performance of air-NB in treating PSW was evaluated, and the results are presented in Figure 2(a). The nutrient and organics removal rates after 6 h of air-NB were 90.0% for COD, 88.6% for total-N, 78.8% for TSS, 44.0% for FOG, and 40.2% for NH_3-N. Notably, a COD removal efficiency of 84% was achieved within just 2 h. Conversely, 78.7% TSS removal efficiency was achieved after 4 h, although air-NB achieved above 78% removal for COD, TSS, and total-N. The removal rate was below 45% for FOG and NH_3-N. The high total-N removal relative to the low NH_3N can be attributed to the simultaneous nitrification and denitrification, which is a biological process that converts ammonia to nitrite, followed by nitrite conversion to nitrate, then the nitrate is reduced to nitrogen gas (Luo *et al.* 2022). On the other hand, ionised ammonium (NH_4) reacts with hydroxyls (OH^-) to form unionised ammonia (NH_3) which increases the concentration of ammonia (Purwono *et al.* 2017).

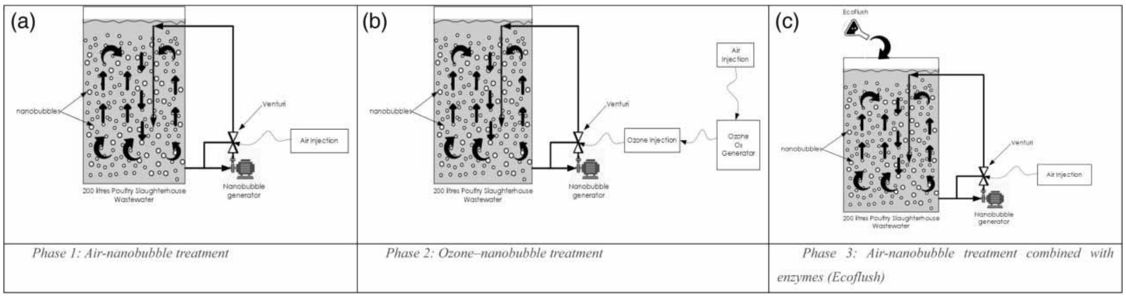


Figure 1 | Experimental setup used for PSW treatment using nanobubbles (a) air-NB treatment setup (Phase 1); (b) ozone-NB treatment setup (Phase 2); and (c) Air-NB treatment combined with Ecoflush enzyme solution (Phase 3). All treatments were conducted using the MK3 nanobubbler connected to a 200 L tank containing raw PSW. (a) Phase 1: Air-NB treatment. (b) Phase 2: Ozone-NB treatment. (c) Phase 3: Air-NB treatment combined with enzymes (Ecoflush).

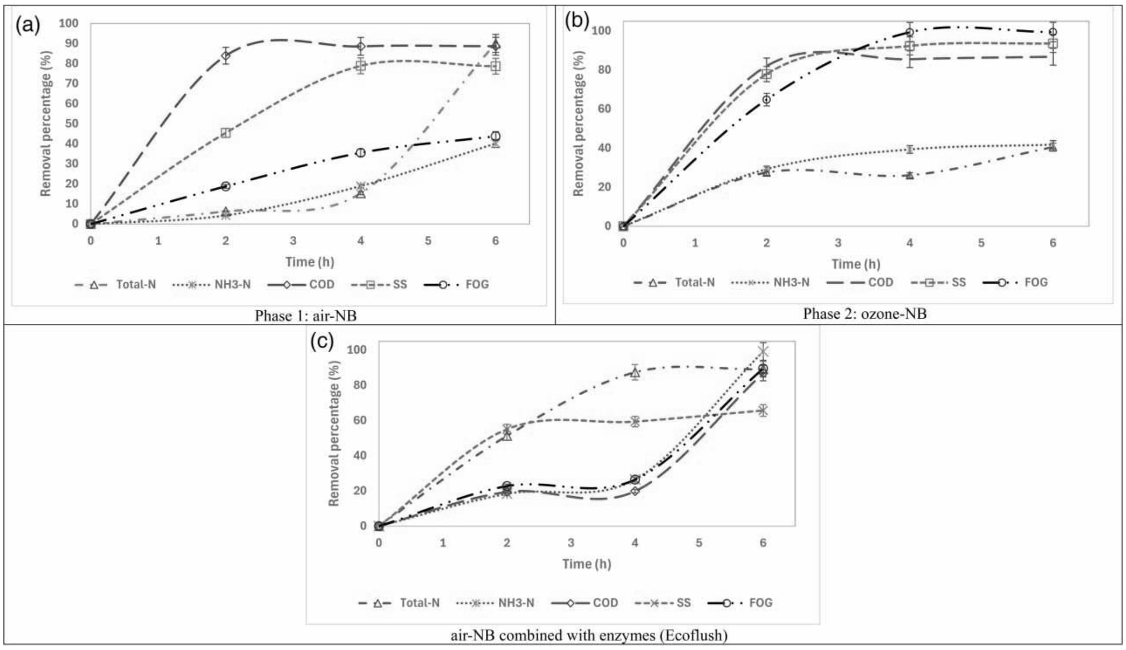


Figure 2 | Pollutant removal efficiencies after 6 h of treatment using three NB-based methods. (a) Air-NB aeration; (b) ozone-enriched NB treatment; and (c) air-NB treatment combined with Ecoflush enzymes. Parameters measured include COD, TSS, NH_3-N, total nitrogen, and FOG. (a) Phase 1: air-NB. (b) Phase 2: ozone-NB. (c) air-NB combined with enzymes (Ecoflush).

Ahmed *et al.* (2023) generated NBs by injecting gases such as oxygen and air through a ceramic membrane to enhance secondary effluent (municipal wastewater) treatment. They reported that air-NB injection resulted in a 28% reduction in TSS, a 26% decrease in COD, a 43% decrease in BOD_5, an 11% decrease in organic nitrogen, and a 96% decrease in NH_3-N after 2 h of aeration. Similarly, Oktafani *et al.* (2019) investigated the effect of aeration on chicken slaughterhouses to assess organics removal using the granular activated sludge–sequencing batch reactor (GAS–SBR) system. The findings showed that after 2 h of aeration, the removal of COD, and BOD was 72.8%. Extending the aeration period to 4 h resulted in a total NH_3-N removal of 65.8%.

The results in this study after 6 h of air-NB of PSW achieved higher COD reduction (24.2% higher) compared to the conventional aeration GAS–SBR chicken wastewater treatment by Ahmed *et al.* (2023). This is attributed to the high surface area of NBs, facilitating enhanced mass transfer and subsequent oxidation of pollutants combined with their ability to release hydroxyl radicals, which can interact directly and non-selectively with organic pollutants. Lastly, the increased contact between bubble surfaces and contaminants contributes to this effect (Fan *et al.* 2019).

In Table 3, the results of air-NB treatment after 6 h compared to the standard discharge limits outlined in the National Water Act 36 of 1998 show that only total-N met the discharge standards. NH_3-N, COD, TSS, and FOG

Table 3 | NB treatment of PSW vs. discharge limits

	PSW composition after 6 h						
	Phase 1 air-NB treatment		Phase 2 ozone-NB treatment		Phase 3 air-NB treatment combined with enzymes (Ecoflush)		General discharge limits as set in the National Water Act 36 of 1998
	Initial	After	Initial	After	Initial	After	
pH	6.8	7.6	7.1	7.5	6.7	5.4	5.5–7.5
Total nitrogen (mg/L)	100	10.0	145	87.0	116	13.0	15.0
NH_3-N (mg/L)	72.9	43.6	127	74.7	59.1	0.5	6.0
COD (mg/L)	2,000	228.0	2,200	292.0	3,600	472.0	75.0
TSS (mg/L)	516	109.0	637	41.0	718	246.0	25.0
FOG (mg/L)	100	56.0	226	1.0	122	12.0	2.5

are still above the required discharge standards and may require extended aeration times of more than 6 h to further reduce their levels and meet the discharge standards. Further exploration into extending the aeration duration is necessary.

3.3. Performance of ozone-NB in treating PSW

Ozone has been applied to remediate organics, ammonia, and disinfection in water and wastewater treatment since the 1970s (Shangguan *et al.* 2018; Sakr *et al.* 2022). Despite its capabilities in decomposing organics and inactivating microorganisms, the broader utilization of ozone is constrained by challenges such as low mass transfer efficiency, limited saturation solubility, and a short half-life. These constraints often result in reduced reaction efficiency and underutilization of ozone in water treatment (Andinet *et al.* 2016). To address these limitations, the application of NB technology is explored to enhance the ozonation process in water and wastewater treatment (Shangguan *et al.* 2018; Xia & Hu 2018). In Phase 2 (Figure 1(b)), ozone-NBs were used to treat PSW, and the removal rates for COD, TSS, and FOG were 86.7, 93.5, and 99.5%, respectively, after 6 h. For total-N and NH_3-N, ozone-NB treatment achieved 40.0 and 41.2%, respectively. The low removal rates of total-N and NH_3-N can be attributed to several factors. The process of nitrogen produces intermediate compounds such as nitrites and nitrates, which contribute to total nitrogen (Luo *et al.* 2022). Additionally, high pH and alkalinity can reduce ozone effectiveness, while competing reactions with other contaminants lower ozone availability for nitrogen removal (Ma *et al.* 2023; Chen *et al.* 2024). The pH of ozone-NB treatment increased from 7.2 to 7.5. Further optimisation of the pH is required for the removal of ammonia and total-N by ozone-NB.

These results exhibit similarities to previous studies. For instance, Van Leeuwen *et al.* (2009) treated synthetic wastewater containing methylene blue using ozonation during the process. They reported an average COD removal of about 80.5% in the ozonated and 79.6% in the un-ozonated control. Similarly, Wu *et al.* (2022) utilized an integrated approach combining NB and ozone oxidation to enhance ammonia (NH_3-N) removal from wastewater. They found that ammonia concentration decreased slightly to 910 mg/L with a removal efficiency of 44.2% in the control group. In contrast, the NB treatment group saw a faster decrease, reaching 277 mg/L with a removal efficiency of 82.5% in 30 min due to the NB's ability to slowly release gas into the water.

The results of ozone-NB treatment presented in Table 3 are compared to the standard discharge limits outlined in the National Water Act 36 of 1998 of Republic of South Africa (1998), only FOG met the discharge standards. NH_3-N, COD, TSS, and total-N may require extended aeration time (more than 6 h) to further reduce their levels and meet the discharge standards. Further exploration into extending the aeration duration is necessary.

3.4. Performance of air-NB in combination with enzymes (ecoflush) in treating PSW

Enzymes are used in the hydrolysis of fats and greases in wastewater such as PSW (Affes *et al.* 2017). The enzymatic approach enhances the performance of microorganisms in subsequent biological treatment processes, as indicated by Jamie *et al.* (2016). Ecoflush, a blend of natural components and bacteria, remains inactive until exposed to a nutrient-rich organic source, such as PSW, which serves as a substrate. Once activated, it primarily generates enzymes for the hydrolysis of FOGs (Mdladla *et al.* 2021; Meyo *et al.* 2021). Figure 2(c), demonstrates that the removal rates of COD, TSS, FOG, NH_3-N, and total-N were 86.8, 65.7, 88.8, 99.2, and 88.8%, respectively. It can be noted that NH_3-N, which could not be removed efficiently by air-NB (40.2% removal) and ozone-NB (41.2% removal) after 6 h of aeration, achieved a much higher removal rate (99.2%) when air-NB was combined with an enzyme such as Ecoflush. Enzymes combined with NBs achieve higher ammonia removal rates compared to air-NB aeration alone due to several factors. Enzymes specifically target and break down ammonia more efficiently than microbial processes alone (Liu & Smith 2021). NBs enhance oxygen transfer, supporting both enzyme activity and aerobic microbial processes involved in nitrification (An *et al.* 2019; Azevedo *et al.* 2019; Shi 2022). The combination of enzymes and NBs creates a synergistic effect, optimizing conditions for enzyme action and microbial degradation.

Dlamini *et al.* (2021) used enzymes (Ecoflush) to treat PSW using conventional aeration (macro bubbles), the treatment resulted in an average removal rate of 80 $\pm$ 6.3% for FOG, 38 $\pm$ 8.4% for COD, and 56 $\pm$ 7.2% for TSS after 24 h of aeration, which pale in comparison to air-NB with the same concentration of enzymes (Ecoflush). Similarly, Ngobeni *et al.* (2022) reported removal rates of FOG by 85–99%, and COD by 20–50% using Ecoflush with macrobubbles after 24 h of aeration treating PSW. These results highlight the superior performance of air-NB combined with Ecoflush compared to Ecoflush with macro bubbles aeration.

The findings presented in Table 3 are compared to the standard discharge limits outlined in the National Water Act 36 of 1998 of Republic of South Africa (1998). After 6 h of aeration, NH_3-N and total-N levels complied with the specified limits. However, COD, TSS, and FOG still exceeded the discharge limits. Further exploration into extending the aeration duration is necessary to comply with the discharge standards for COD, TSS, and FOG.

3.5. Performance comparison of the three aeration methods

Understanding the impact of NB aeration on the removal of pollutants is essential for understanding its mechanisms and potential applications in wastewater treatment. Therefore, analysing the removal efficiencies of parameters such as COD, TSS, ammonia, total-N, and FOG was essential in assessing the effectiveness of NBs. The images depicting the PSW before and after 6 h of aeration for each phase are presented in Figure 3. It is evident that ozone-NB treatment reduced colouration in PSW within 6 h. However, neither air-NB treatment alone nor in combination with Ecoflush successfully removed the colour. In the case of air-NB with Ecoflush, the resulting product adopted the colour of the thick brown Ecoflush solution.

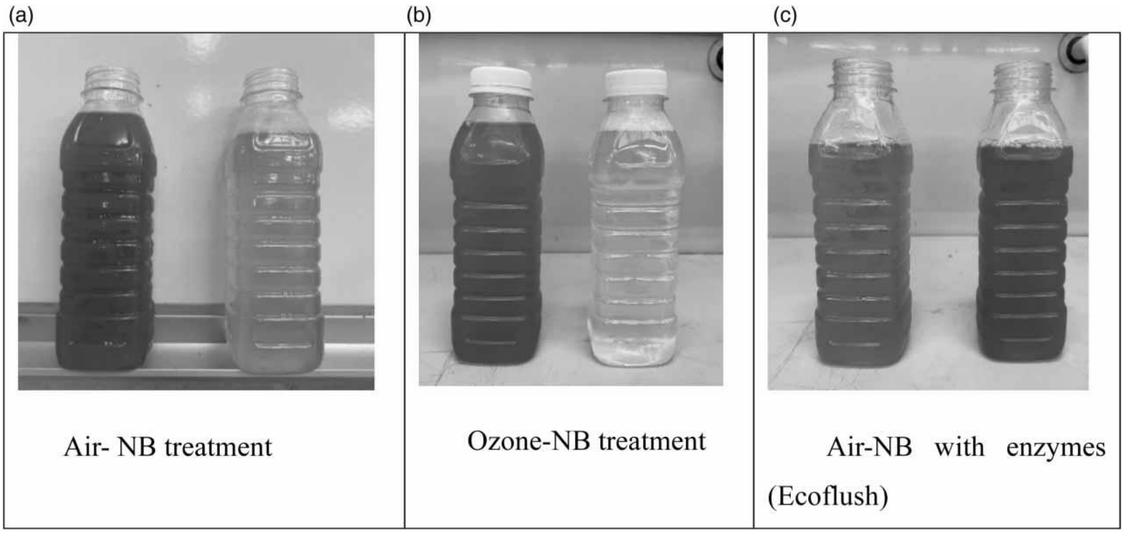


Figure 3 | Visual changes in PSW after 6 h of treatment under different NB-based systems. (a) Air-NB aeration; (b) ozone-NB treatment showing noticeable colour reduction; and (c) air-NB with Ecoflush, which retained the colour of the Ecoflush solution. Images demonstrate visual changes in clarity and colour as indicators of treatment efficacy. (a) Air-NB treatment. (b) Ozone-NB treatment. (c) Air-NB with enzymes (Ecoflush).

3.5.1. Change in pH and DO during treatment

Figure 4 illustrates the impact of various types of aeration methods on the pH levels of PSW over a 6 h period. Initially, the pH of the PSW sample varied between 6.7 and 7.1. The findings demonstrate that air-NB and ozone-NB led to an increase in the solution's pH during aeration, likely due to the removal of dissolved CO_2 or carbonic

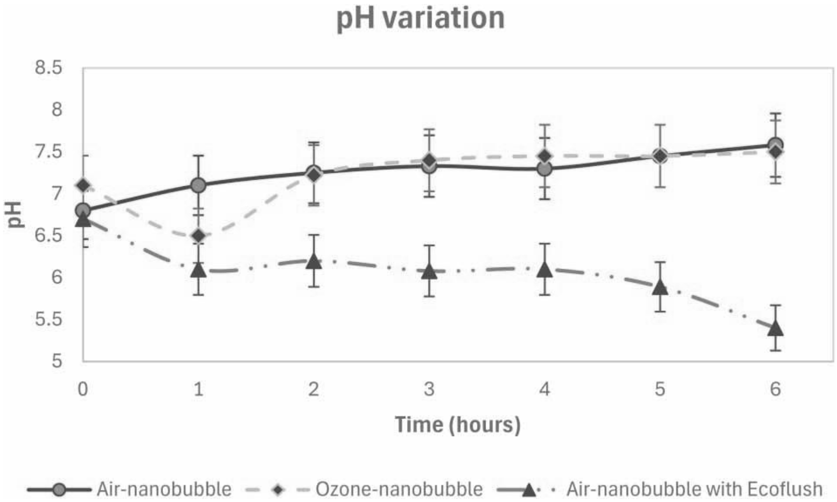


Figure 4 | pH variation in PSW during 6 h of NB aeration.

acids (Zang *et al.* 2011). This correlation can be attributed to the relationship between oxidation-reduction characteristics and the acidity of PSW, where higher oxygen concentrations result in reduced acidity (Zang *et al.* 2011). For air-NB the increased pH from 6.8 to 7.6 while for ozone-NB the pH increased from 7.1 to 7.5. However, air-NB when combined with Ecoflush led to a decrease in pH from 6.7 to 5.4. This decrease in pH can be attributed to the acidity of Ecoflush solution. Additionally, Figure 5 depicts the DO concentrations of air-NB, ozone-NB, and air-NB with Ecoflush in PSW over time. The original DO level in the sample was 9.5 mg/L. Results indicate a consistent increase in DO levels across all types of aeration methods. Ozone-NB exhibited the highest increase in DO, reaching the saturation limit of 14.5 mg/L after 6 h, indicative of efficient gas–liquid mass transfer. Conversely, continuous injection of air-NBs and air-NB combined with Ecoflush for 6 h resulted in DO levels recorded at 13.5 and 13.0 mg/L. The oxygen concentration within ozone-NB surpassed that of air-NB and that of air-NB combined with Ecoflush due to Henry's law, which states that the gas concentration in a liquid is directly proportional to the gas partial pressure (Sakr *et al.* 2022). Consequently, NBs demonstrate superior effectiveness in enhancing the DO concentration of PSW.

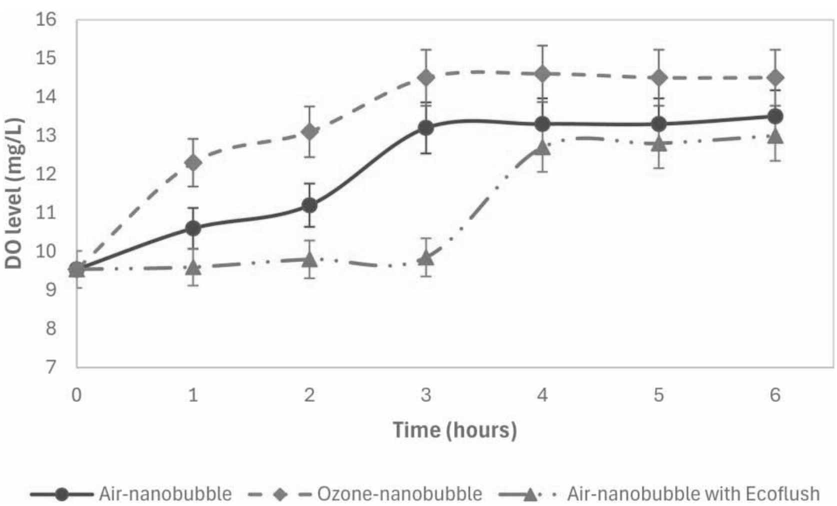


Figure 5 | Variation in DO concentration over 6 h of NB aeration.

3.5.2. COD removal

COD serves as a standard parameter to gauge water pollution levels, with its analysis revealing changes in water-soluble oxygen content and indicating the ease or difficulty of decomposition. The heightened COD levels observed in PSW stem from the significant presence of organic waste, including residual organs, blood, and unused chicken parts (Basitere *et al.* 2019).

Figure 6 illustrates the percentage of COD removal over a 6 h period for three aeration methods: NB aeration, ozone-treated NBs, and NB with Ecoflush. NB aeration and ozone-treated NBs achieved over 80% COD removal within 2 h, while NB with Ecoflush only removed 20% of COD during the same timeframe. As time elapsed, the efficiency of COD removal increased for all three methods, reaching an 86% removal efficiency. Comparing these results to those of conventional aeration methods, NB aeration demonstrated a higher COD removal rate. Oktafani *et al.* (2019) utilized the GAS–SBR to assess COD removal after 6 h and noted that the GAS–SBR system attained its highest efficiency in removing COD after 2 h of aeration, achieving removal rates of 72.83%. This superior COD removal efficiency by NBs can be attributed to the enhanced degradation of organic matter in the activated sludge induced by NBs (Agarwal *et al.* 2011).

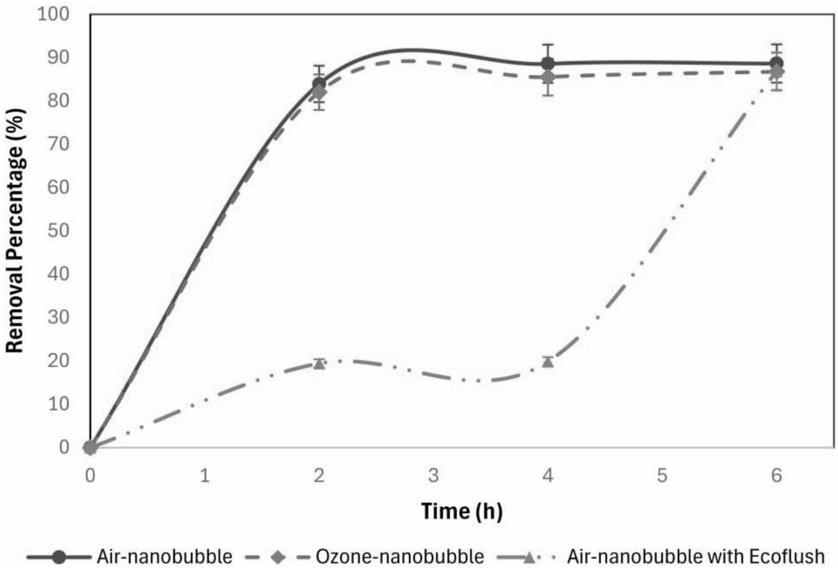


Figure 6 | COD removal efficiency over time for different NB-based treatments.

Air-NB and ozone-NB exhibited the most significant treatment effect on PSW, leading to 88.6 and 86.7% reduction in COD after 4 h of treatment, compared to air-NB combined with Ecoflush (19.4%). This is attributed to the higher concentration of DO in NB, facilitating enhanced mass transfer and subsequent oxidation of pollutants. Furthermore, the collapse of NBs can release hydroxyl radicals, which can interact directly and non-selectively with organic pollutants. Lastly, the increased contact between bubble surfaces and contaminants contributes to this effect (Fan *et al.* 2019).

It was observed that the COD removal by air-NB and ozone-NB reached a plateau after 2 h of aeration due to several factors. The plateau can be attributed to the system reaching a saturation point where available oxygen is fully utilized. The depletion of easily degradable substrates and the formation of biofilms, which limit oxygen and nutrient diffusion, can also contribute to the observed plateau in COD reduction (Yaparatne *et al.* 2022).

The COD removal by Ecoflush combined with NB was delayed and slow during the first 4 h due to several factors. First, enzymes require time to activate and catalyse the breakdown of organic matter, and there may be initial diffusion limitations of nanobubbles and enzymes throughout the wastewater (Liu & Smith 2021). Additionally, inhibitory substances such as FOG in PSW can temporarily reduce enzyme activity, and the system needs time to reach an equilibrium state for optimal COD removal (Povis & Pérez 2023).

3.5.3. TSS removal

Analysing the distribution of TSS serves as a common approach to evaluating water quality. Elevated TSS levels indicate increased pollution, obstructing light penetration into the water and disrupting photosynthesis. Additionally, TSS can absorb solar thermal energy, potentially elevating water temperature and subsequently reducing DO levels.

The data presented in Figure 7 illustrates how the duration of aeration influences TSS removal. The TSS removal rates were 65.7, 78.9, and 93.5% for NB with Ecoflush, NB aeration, and ozone-treated NBs, respectively. All three aeration methods followed a similar trend, achieving over 45% removal efficiency after 2 h of aeration. Notably, after 4 h, the removal efficiency of all three methods remained consistent.

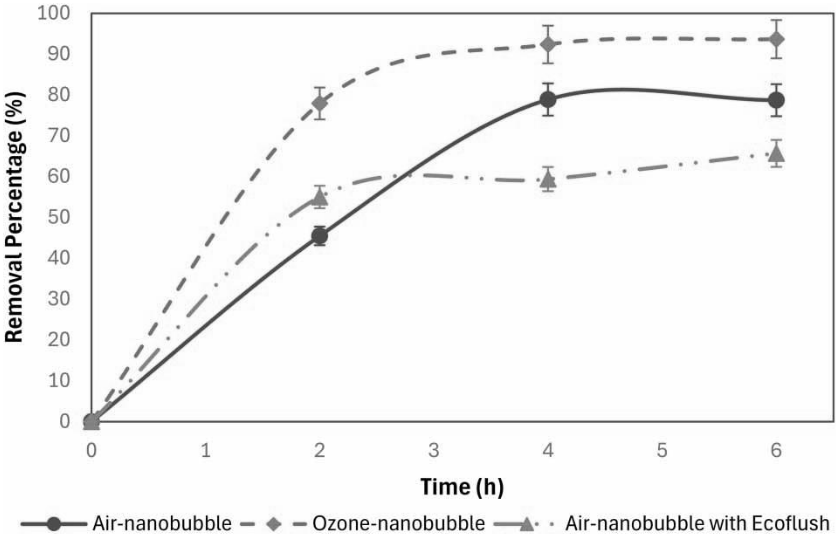


Figure 7 | TSS removal over 6 h for different treatments.

The performance of ozone-NBs significantly reduced the TSS content of the PSW by 93.5%, marking the most substantial decrease compared to air-NBs, which achieved a reduction of only 78.9 and 65.7% when combined with Ecoflush. This highlights the practical effectiveness of NBs in TSS reduction. This is attributed to the NBs' capability to broaden the range of flotation particle sizes, enhance particle surface hydrophobicity, and improve froth flotation efficiency (Wu *et al.* 2021). This technique has been widely recognized for its efficacy in TSS removal due to the similar size and opposing charge of bubbles and suspended particles, facilitating enhanced collisions and adherence. Additionally, the organic nature of these solids enables further oxidation with ozone-NBs, contributing to their effectiveness in reducing TSS content (Kyzas *et al.* 2021). The findings of this study align closely with previous research. For instance, Rameshkumar *et al.* (2019) examined the impact of ionization-induced NBs on domestic wastewater treatment, noting a complete reduction of TSS by nearly 100%.

3.5.4. Ammonia removal

Ammonia compounds pose significant risks to aquatic life, often stemming from sources like urine, faeces, and the microbial decomposition of organic matter in both natural and industrial water bodies. Even at concentrations as low as 1 mg/L, ammonia can diminish oxygen levels in water, posing a severe threat to aquatic organisms and potentially resulting in fatalities.

Figure 8 illustrates the trend of ammonia removal over a period of up to 6 h. When combined with Ecoflush, air-NBs achieved the highest removal rate (99%) after 6 h, whereas air-NB aeration alone and ozone-NB only reached 40.2 and 41.2% removal over the same timeframe. Initially, the removal efficiency of NB combined with Ecoflush was low up to 4 h, but it substantially increased to 99% after 6 h of aeration. This delay in efficacy can be attributed to the activation time required by Ecoflush enzymes before they commence breaking down pollutants like ammonia in PSW. Enzyme activation time is the period during which enzymes undergo changes and biochemical interactions that allow them to become fully active and initiate their catalytic processes (Sahal *et al.* 2023). The activation of enzymes in wastewater treatment begins when the enzyme binds to its specific substrate, forming an enzyme-substrate complex. This complex allows the enzyme to catalyse the conversion of the substrate into a product.

The outcomes of this study exhibit similarities to prior research accomplishments. For instance, Atkinson *et al.* (2019) introduced NBs into a wastewater treatment facility in Missouri, witnessing a rapid reduction in organics and turbidity, although with a significant decline in ammonia removal. Furthermore, Wang *et al.* (2020) explored

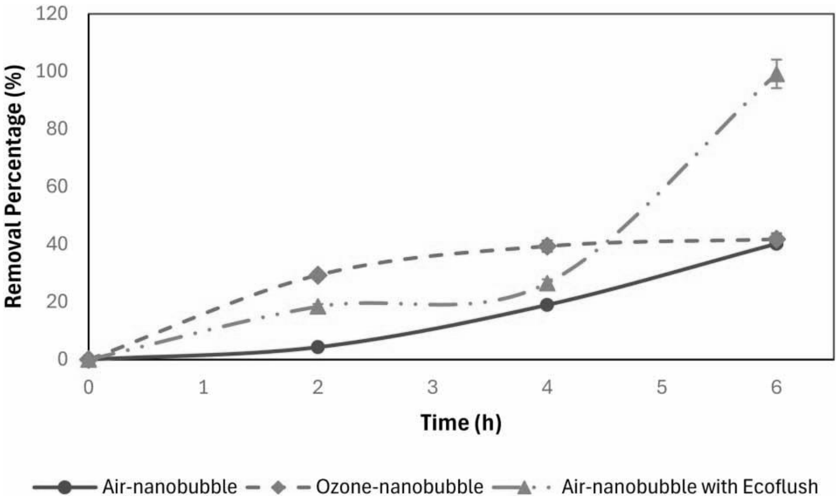


Figure 8 | Ammonia-nitrogen (NH_3-N) removal efficiency over time FOG removal.

the oxygenation and concurrent regulation of nitrogen and phosphorus release at the sediment-water interface using oxygen-NB modified material, resulting in notable reductions in NH3-N, and total nitrogen (TN) by 96.4, 51.1, and 24.9%, respectively.

The high concentration of FOG in PSW can impede the efficiency of pollutant removal within the biological treatment system (Musa & Idrus 2021). This is because fats have a lower density than water, hindering the transfer of mass from the solid to the liquid phase (Long *et al.* 2012; Sultana *et al.* 2022). As shown in Figure 9, both ozone-NB and air-NB combined with Ecoflush achieved FOG removal efficiencies of over 90%, whereas NB aeration alone achieved only 44.0% removal efficiency after 6 h of aeration. The enzymes present in the Ecoflush primarily target FOGs, as evidenced by the graph in Figure 9, where FOGs exhibited the highest removal efficiency, nearing 100%. It is worth noting that air-NB combined with Ecoflush enzymes requires a longer time to achieve a high FOG removal of 90%. This delay is due to the activation time required by the enzymes, which consume oxygen before breaking down the FOG in PSW. The introduction of ozone-NBs into the PSW led to a significant decrease in FOG by 99.5% after 6 h, demonstrating notable efficacy. Similarly, air-NBs combined with Ecoflush also decreased the FOG by 90.2% after 6 h. However, the use of air-NBs resulted in a smaller reduction, decreasing the FOG by 44.0% after 6 h. The enhancement in aeration and flotation efficiency underscores the effectiveness of NBs in treating wastewater containing FOG. Gas flotation relies on the attachment of

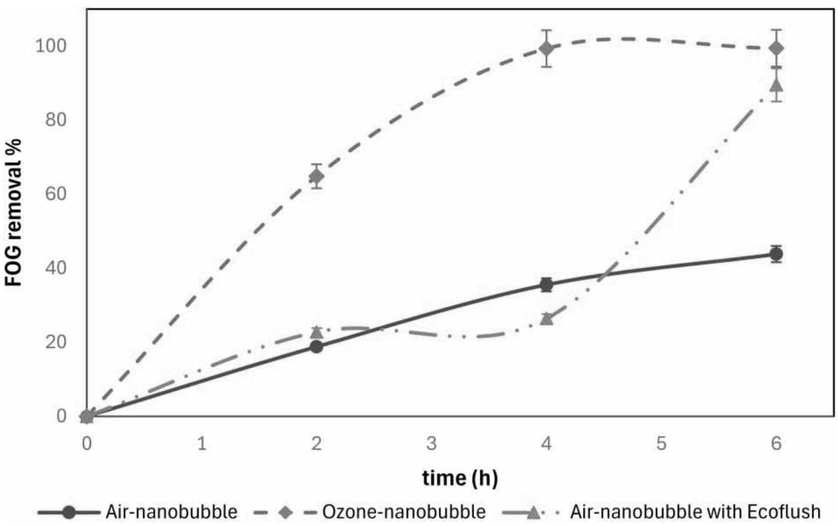


Figure 9 | FOG removal efficiency after 6 h treatment.

gas bubbles to oil droplets to form lighter aggregates that ascend to the surface of wastewater, explaining the rationale behind ozone-NBs' capability in this aspect.

4. RECOMMENDATIONS FOR FUTURE STUDIES

This study demonstrates the potential of NB technology in the treatment PSW, particularly in achieving high removal efficiencies of ammonia, COD, TSS, and FOG. To advance the practical application and scalability of this technology, several key areas merit further investigation. Future research should focus on optimising aeration duration to ensure complete pollutant removal. While significant reductions were achieved within 6 hours, certain parameters – especially COD and TSS – remained above discharge limits. Investigating extended aeration times, along with the effects on additional contaminants such as nitrates and nitrites, could provide deeper insights into treatment efficiency. Integrating NB technology with other treatment processes offers a promising avenue for performance enhancement. Hybrid systems incorporating biological reactors, advanced oxidation processes, or membrane technologies may overcome current limitations, such as incomplete degradation or excessive sludge generation, by leveraging complementary mechanisms. Assessing the long-term performance and sustainability of NB systems under real-world conditions is crucial. Pilot- and full-scale studies are needed to evaluate operational reliability, energy consumption, and cost-effectiveness, thereby supporting industrial-scale implementation. Finally, mechanistic studies on the interaction between NB and enzymatic agents – such as Ecoflush – could clarify the synergistic effects observed in this study, particularly for ammonia and FOG removal. Elucidating the activation pathways and enhancement of enzymatic activity in the presence of nanobubbles may inform the development of more effective enzyme-assisted NB treatments.

5. CONCLUSIONS

This study demonstrates the efficacy of NB technology in the treatment of PSW, particularly in the removal of pollutants such as COD, TSS, ammonia, and FOG. The results indicate that air-NB aeration and ozone-treated NBs achieved impressive COD removal rates of over 80% within just 2 h, highlighting their rapid pollutant removal capabilities. Furthermore, the study shows that NBs combined with Ecoflush enzymes, although initially slower in pollutant removal, can achieve high removal rates after 4–6 h of aeration, particularly for ammonia and FOG. The steady TSS removal efficiency across all methods after 4 h underscores the reliability of NB technology in TSS reduction. Additionally, the study highlights the importance of enzyme treatments like Ecoflush in enhancing FOG removal efficiency. Overall, the findings suggest that NB technology, especially when coupled with enzyme treatments, holds promise for efficient and sustainable PSW treatment.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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