ASSESSMENT OF NANOBUBBLE AERATION PERFORMANCE IN TREATING POULTRY SLAUGHTERHOUSE WASTEWATER

by

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DECLARATION

I, **EPHRAIM VUNDIKANWA KASKOTE**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **_Date_** 06/11/2024

ABSTRACT

Due to their simplicity and effectiveness, biological treatment methods, including aerobic and anaerobic processes, are widely used for treating medium to high-strength wastewater, such as poultry slaughterhouse wastewater (PSW). Conventional aeration methods, although effective in removing organics and nutrients from wastewater, have drawbacks such as high sludge production, substantial energy consumption, and low oxygen transfer efficiency. To overcome these challenges, technologies such as nanobubble (NB) technology have been developed to enhance aerobic processes by optimising aeration methods and gas diffusion. NBs, with diameters less than 200 nm, have emerged as a promising alternative due to their ability to enhance the efficiency of aeration and reduce sludge production. NBs possess unique properties that contribute to physical, chemical, and biological processes in water and wastewater treatment.

This research investigates the performance of NB technology in the application and enhancement of aerobic treatment of PSW. Three NB aeration methods were evaluated: air-NBs, ozone-NBs, and air-NBs combined with Ecoflush enzymes. These methods were tested for their effectiveness in removing chemical oxygen demand (COD), total suspended solids (TSS), ammonia (NH_3-N), total nitrogen, and fats, oils, and grease (FOG) over a period of 6 h.

Air-NB and ozone-NB aeration methods demonstrated high efficiency in COD removal, achieving over 80% removal within just 2 h of treatment. In contrast, NBs combined with Ecoflush enzymes exhibited initially lower COD removal rates (20%) in the first 4 h but ultimately achieved 86.8% removal of COD and 99.5% removal of FOG after 6 h of aeration. TSS removal efficiency remained consistent across all aeration methods after 4 h, with the ozone-NB method showing the highest removal efficiency. Ammonia removal was most effective when using NBs combined with Ecoflush enzymes, reaching 99% removal after 6 h of treatment. Both the ozone-NBs and NBs combined with Ecoflush enzymes showed high FOG removal capabilities. These findings highlight that nanobubbles can significantly enhance mass transfer in wastewater treatment processes, providing an effective method for improving the degradation of pollutants in PSW.

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DEDICATION

To my mother,

Kahambu Gentine

Thank you for your prayer and support.

To my late father,

Kambale Jonathan

You left an indelible mark on my life, and I am forever grateful.

And to my late sister,

Eugenie Kaskote

Your absence has left a void that cannot be filled.

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LAYOUT OF THESIS

This thesis is organized into the following four chapters:

- **Chapter 1**: Introduces the research problem, justifies the study, elaborates on the hypotheses, states the study's aims and objectives, discusses its relevance, and outlines its scope.
- **Chapter 2**: Offers an overview of the characteristics of poultry slaughterhouse wastewater (PSW), and the conventional treatment methods for PSW. It also provides an overview of Nanobubble (NB) technology, the generation methods for NBs and its applications in wastewater treatment methods, as well as the potential application of NB to PSW treatment.
- **Chapter 3**: Presents the methodology and experimental results of the study. It specifies the operating conditions, the sampling, analytical methods, and presents the results of the performance of the individual PSW aeration systems, including a detailed discussion.
- **Chapter 4**: Concludes the study with overall findings and provides recommendations for future research.

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GLOSSARY

Activated Sludge	The biomass produced in wastewater by the growth of organisms in the presence of organic matter (Metcalf & Eddy *et al.*, 2003).
Aerobic	Conditions where oxygen acts as an electron donor for biochemical reactions (Gerardi, 2003).
Anaerobic	Conditions where a biochemical process occurs in complete absence of oxygen (Gerardi, 2003).
Biochemical Oxygen Demand (BOD)	The amount of oxygen required or consumed for the decomposition of microbial reactions within wastewater.
Chemical Oxygen Demand (COD)	The amount of oxygen required to chemically oxidise substances in wastewater (Judd, 2011).
Eco-flush	A blend of naturally occurring environmental microorganisms, including aerobic, anaerobic, nitrifying, and sulphur-oxidizing bacteria, along with fungi and enzymes, all maintained within a polymeric vehicle of natural origin (Ergofito, 2024).
Electrical Conductivity	The measure of the ability of a solution to conduct electricity.
Hydraulic Retention Time (HRT)	A measure of the average length of time that a soluble compound remains in a bioreactor
Microbubbles (MB)	Tiny bubbles with a diameter of 10–100 μm (Agarwal *et al.*, 2011).
Nanobubbles (NB)	Tiny bubbles with a diameter less than 1000 nm (Rameshkumara *et al.*, 2019).
Total Suspended Solids (TSS)	The total number of particles that are in suspension in water/wastewater.
Total Dissolved Solids (TDS)	The combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionised or micro-granular suspended form.
Turbidity	An expression of the optical property of a liquid medium and its ability to transmit light. This is a measure of relative sample clarity and not colour.

LIST OF ABBREVIATIONS

Abbreviation	**Definition**
BOD	Biochemical oxygen demand
CoCT	City of Cape Town
COD	Chemical oxygen demand
DAF	Dissolved air flotation
DEGBR	Downflow expanded granular bed reactor
DWA	Department of Water Affairs
EC	Electrocoagulation
EGSB	Expanded granular sludge bed reactor
FOG	Fats, oil, and grease
MBR	Membrane bioreactor
N	Nitrogen
OLR	Organic loading rate
P	Phosphorous
pH	Potential of hydrogen
PSW	Poultry slaughterhouse wastewater
RO	Reverse osmosis
SA	South Africa
SGBR	Static Granular Sludge Bed Reactor
TDS	Total dissolved solids
TOC	Total organic carbon
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge bed
WWTP	Wastewater treatment plant

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1. Background of the research problem

Poultry slaughterhouses generate substantial volumes of wastewater as a consequence of their continuous operations reliant on abundant freshwater. This wastewater is characterised by high organic materials, fats, oils, and grease (FOG), and nutrients such as phosphorus and ammonia (Chollom *et al*., 2019). Additionally, it contains traces of inorganic substances that can negatively impact the environment and human health. The discharge of wastewater from poultry slaughterhouses into rivers holds the potential to induce eutrophication, leading to a rapid reduction in dissolved oxygen levels, subsequently leading to the decline of aquatic life (Musa & Idrus, 2021).

It is crucial to undertake on-site treatment of wastewater from poultry slaughterhouses, thereby avoiding environmental contamination and enabling the potential reintegration of water into plant operations, particularly surface cleaning, as underscored by Reilly *et al*. (2019). Biological treatment methods, including both aerobic and anaerobic processes, are widely adopted and promising strategies for effectively managing the complexities of poultry slaughterhouse wastewater (PSW), according to Besharati Fard *et al.* (2019) and Philipp *et al*. (2021) as they offer advantages such as reduced chemical usage, lower operational costs, and the ability to enhance the degradation of organic matter and nutrients.

Among the range of wastewater treatment techniques, aeration systems, which constitute one of the oldest and simplest forms of PSW treatment, have found extensive application in agricultural sectors such as piggeries, tanneries, and abattoirs, as explained by Iqbal *et al*. (2021). These approaches are particularly preferred for dealing with moderately to heavily concentrated wastewater due to their fundamental simplicity in terms of construction and operation, as highlighted by McCabe *et al*. (2013) and Musa & Idrus (2021). However, these conventional methods present disadvantages and drawbacks, including the production of large amounts of sludge, extensive energy required for aeration, sensitivity to high organic loading rates, and longer hydraulic retention times (HRT) (Ahmadi *et al*., 2022).

Hence, there arises a compelling necessity to explore alternative treatment methods, with a focus on technologies such as nanobubble (NB) technology to enhance the aeration process and reduce sludge production. NBs, characterised by their smaller size with diameters below 200 nm, present distinct advantages in terms of enhancing diffusion and mass transfer processes within environmental applications (Sakr *et al*., 2022). Notably, their extended residence in aqueous solutions, enabled by slow buoyancy, coupled with a substantial gas-liquid interface, sets them apart from ordinary bubbles. An outstanding characteristic of NBs lies in their capacity to generate hydroxyl radicals when they burst, which leads to enhanced oxidation potential and increased dissolution efficiency, as observed by Rameshkumara *et al.* (2019) and Etchepare *et al.* (2017a).

The emergence of NB technology signifies an advancement aimed at enhancing aeration systems, primarily with the intention of reducing sludge production and energy consumption. This, in turn, increases the efficiency of pollutants removal from wastewater, as noted by Ahmadi *et al.* (2022). NB technology is applied to improve the aeration process, and particular attention has been given to optimising gas diffusion through the manipulation of bubble sizes, an important factor in enhancing aerobic processes (Tekile *et al.*, 2017).

Considering the recent advancements in wastewater treatment, a compelling need arises for an investigation into the efficacy and effectiveness of NBs in PSW treatment. This analysis would aid in unearthing novel opportunities for its application. Consequently, the aim of this study was to explore the performance of NBs in the treatment of PSW, encompassing methodologies such as aeration and advanced oxidation. It is anticipated that the use of NBs could be a promising effective treatment technology for PSW.

1.2. Motivation for the research study

The wastewater generated by poultry slaughterhouses is characterized by high levels of organic matter, including chemical oxygen demand (COD) and biological oxygen demand (BOD). It also contains suspended solids, colloidal matter such as total suspended solids (TSS), fats, oils, and grease (FOG), and nutrients such as nitrogen and phosphorus. Due to the prevalence of these pollutants, the wastewater fails to meet industrial discharge standards. Consequently, it cannot be released into the environment without proper treatment, as it poses a risk of polluting natural water sources and harming aquatic life.

In South Africa, a common practice involves poultry slaughterhouses subjecting their wastewater to preliminary treatment before discharging it into municipal sewage systems, where further treatment occurs. Nevertheless, this initial treatment often falls short of meeting the rigorous discharge standards of South Africa and can lead to escalated discharge expenses due to municipal effluent penalty charges. Alternately, some slaughterhouses opt for on-site treatment to mitigate pollution loads, aiming to adhere to legislative requirements before discharge (Chollom *et al.*, 2019). In either approach, wastewater management is linked to cost implications. Hence, there is an urgent need for the poultry slaughterhouses to adopt advanced technologies that have emerged in recent years, with the objective of attaining more stringent emission standards while minimising operational expenses. In this context, NBs have emerged as a potential technology, offering an improved and effective wastewater treatment technology (Xiaoli *et al.*, 2017; Shi, 2022).

1.3. Statement of the research problem

The low efficiency of conventional aeration techniques for treating medium to high-strength wastewater such as PSW and the excessive production of sludge are significant concerns in aerobic wastewater treatment systems. To overcome these limitations, there is a need to explore efficient techniques that enhance the degradation of organic matter in the wastewater and its oxidation ability for practical application.

1.4. Research questions

Throughout this study, the investigation addresses the following inquiries:

1) How does NB technology perform in terms of removing organic matter from PSW?
2) To what extent does the incorporation of Ozone enhance the performance of NB technology in the removal of organic matter from PSW?
3) To what degree does the introduction of Ecoflush contribute to the enhancement of NB technology's efficiency in the removal of organic matter from PSW?

1.5. Research Hypotheses

1) The application of NB technology to enhance aeration will result in an improved degradation of organic matter in high-strength wastewater, specifically PSW. NBs, due to their extended residence in water, are anticipated to facilitate more efficient oxygen diffusion, consequently enhancing overall treatment efficiency.
2) The concurrent application of ozone and NB will lead to an enhanced oxidation and degradation of organic matter in PSW. Capitalising on their expansive surface area, swift mass transfer rates, and enduring presence in water, NBs are expected to boost the stability of ozone, thereby significantly amplifying the oxidation process for organic matter in PSW.
3) The combination of the Ecoflush bioremediation agent with NB technology will contribute to an elevated degradation of FOGs, and organic matter within SW.

1.6. Research Aim and Objectives

This study aimed to examine the efficiency of NB technology in treating PSW. The study was structured into distinct phases:

1) Phase 1: Assess the treatment performance of air-NB aeration in the removal of organic matter and nutrients from PSW.
2) Phase 2: Assess the treatment performance of the ozone-NB aeration in terms of removing organic matter and nutrients from PSW.

3) Phase 3: Investigate the treatment performance of the air-NB aeration combined with Ecoflush, focusing on the removal of organic matter and nutrients from PSW.

1.7. Significance of the Study

This study holds significant importance as it will furnish insights into the novel NB technology, shedding light on its effectiveness and performance concerning the removal of organic matter and nutrients from PSW. Additionally, the potential implementation of this technology for PSW treatment could provide notable advantages for poultry slaughterhouses, including the potential reduction of discharge costs and penalties. This research stands to contribute significantly to the knowledge base in the field of wastewater treatment, paving the way for more effective practices in managing wastewater from poultry slaughterhouses.

NB technology has emerged as the potential solution to the low efficiency of aeration and high sludge production in aeration systems (Khan *et al*., 2020; Wu *et al*., 2021). Recent studies indicate that NBs have the ability to substantially improve degradation efficiencies for different types of organic contaminants in wastewater (Fan *et al*., 2021). However, there is still a lack of information about the comparison of the removal efficiency of pollutants in wastewater treatment systems using conventional aeration to NB aeration. Hence, this research work aims to investigate the efficiency of aeration using NBs in treating PSW.

1.8. Scope of the Study

The following aspects are excluded from the scope of this study:

1) Characterization of NBs: This study does not cover the characterization of nanobubbles (NBs).
2) Determination of Bubble Sizes: The NB generation unit's certification provides information regarding the range of bubble sizes produced. Given the anticipated consistency of bubble sizes generated by the unit, the determination of bubble size is not within the scope of this study.
3) Post-Treatment of SW: The study does not delve into the post-treatment of PSW.
4) Interactions of Microbial Agents: The interactions involving microbial agents within the aerobic process fall outside the scope of this study.
5) Economic study: cost and financial sustainability are excluded from this study.

CHAPTER 2

LITERATURE REVIEW

This chapter was published as

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CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Water stands as a vital element crucial for all forms of life, playing a central role in sustainable development. Its significance extends to socio-economic prosperity, the well-being of ecosystems, and the very survival of humans. The escalating demand for water has underscored the urgency of effective water management. Simultaneously, in-adequate wastewater treatment practices in certain regions have exacerbated the improper discharge of wastewater into the environment, contributing to the pollution of natural water resources. Consequently, global efforts have increasingly shifted from merely dis-posing of wastewater to emphasising water reuse and recycling, driving advancements in wastewater treatment technologies capable of recycling and reusing wastewater (Bustillo-Lecompte & Mehrvar, 2015).

Industries such as poultry slaughterhouses significantly contribute to freshwater consumption. The high demand for poultry meat consequently amplifies freshwater consumption by poultry processing plants (Hilares *et al*., 2021). Poultry processing plants release substantial volumes of wastewater into the environment due to their extensive use of freshwater for ongoing activities such as meat cutting and rinsing. This wastewater is highly contaminated, featuring organic matter measured by biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Moreover, it contains elevated levels of nitrogen and phosphorus components, encompassing substances such as blood, fats, oil, grease, and proteins (Basitere *et al*., 2019) The attributes of poultry slaughterhouse wastewater (PSW) as well as guidelines for effluent discharge as set by the South African national water act 36 of 1998, are outlined in

Table 2.*1*. It is essential to treat PSW to meet or fall below the specified standard limits since the parameters of untreated PSW substantially exceed the acceptable thresh-olds established in the National Water Act 36 of 1998.

Moreover, the 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 (Njoya *et al*., 2019).

Table 2.1: Characteristics of Poultry Slaughterhouse Wastewater vs Discharge Limits

Parameter	Significance	PSW (Basitere *et al.*, 2019)	General discharge limit (National Water Act 36 of 1998)
pH at 25ºC	Measure of acidity and basicity	6.3 – 7.3	5.5 – 7.5
COD (mg/L)	Measure of the amount of oxygen needed to oxidize organic and inorganic matter	5 126 ± 2 534	75
TSS (mg/L)	Measure of particles in wastewater	1 654 ± 1 695	25
FOGs (mg/L)	Measure of fats, oils and grease.	715 ± 506	2.5
Ammonium as N (mg/L)	Nutrient source for irrigation	216 ± 56	6
Nitrates as N (mg/L)	Nutrient source for irrigation	3.33 – 4.45	15
Nitrites as N (mg/L)	Nutrient source for irrigation	-	15
Total phosphates as P (mg/L)	Nutrient source for irrigation	-	10

Typically, PSW conventional treatment approaches involves physical, chemical, and biological methods. However, these traditional techniques encounter challenges such as the absence of nutrient recovery, frequent reliance on chemical cleaning agents, and the deterioration of valuable compounds within the wastewater. Consequently, alternative methods, including nanobubble technology, are being investigated for PSW treatment. This literature review identifies the potential application of nanobubble technology for advanced PSW treatment.

2.2. Materials and Methods used for literature review

In this study, a systematic methodology is followed in conducting the literature review, adhering to transparent and explanatory practices with the objective of ensuring the scientific rigor and value of its findings. It follows the crucial steps recommended by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Page *et al*., 2021).

2.2.1. Information Sources and Search

In adherence to the systematic review principles, an exhaustive search was undertaken to identify all relevant articles published until January 2024. This search spanned two electronic databases as primary sources (Scopus and ScienceDirect). The search string was constructed using keywords such as "poultry*", "wastewater", "treatment" and "nanobubble".

2.2.2. Selection of studies

The process followed during the selection of studies is depicted in the flowchart illustrated in Figure 2.1. In the initial phase, the search yielded 606 records from the databases, including 16 documents that were over 10 years old. Following an initial screening of abstracts, 300 articles were eliminated due to their lack of relevance to wastewater treatment. A comprehensive review of the full texts of the remaining 290 papers resulted in the exclusion of 187 studies that did not feature case studies.

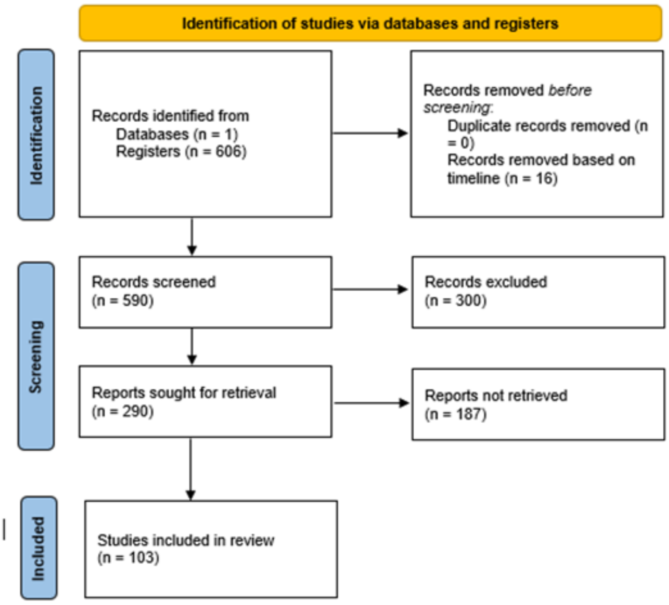

Figure 2.1. Flowchart of study selection based on PRISMA.

Crucial details from the selected articles, including author names, publication year, title, abstract, keywords, and source, were extracted from the databases and organized in a format compatible with Microsoft Excel worksheets. The compiled data underwent analysis using VOSviewer to conduct bibliometric mapping, unveiling significant themes within the research field of nanobubble technology and poultry wastewater treatment. VOSviewer aided in visualising interconnections among the gathered data, clustering them based on keywords that appeared at least four times.

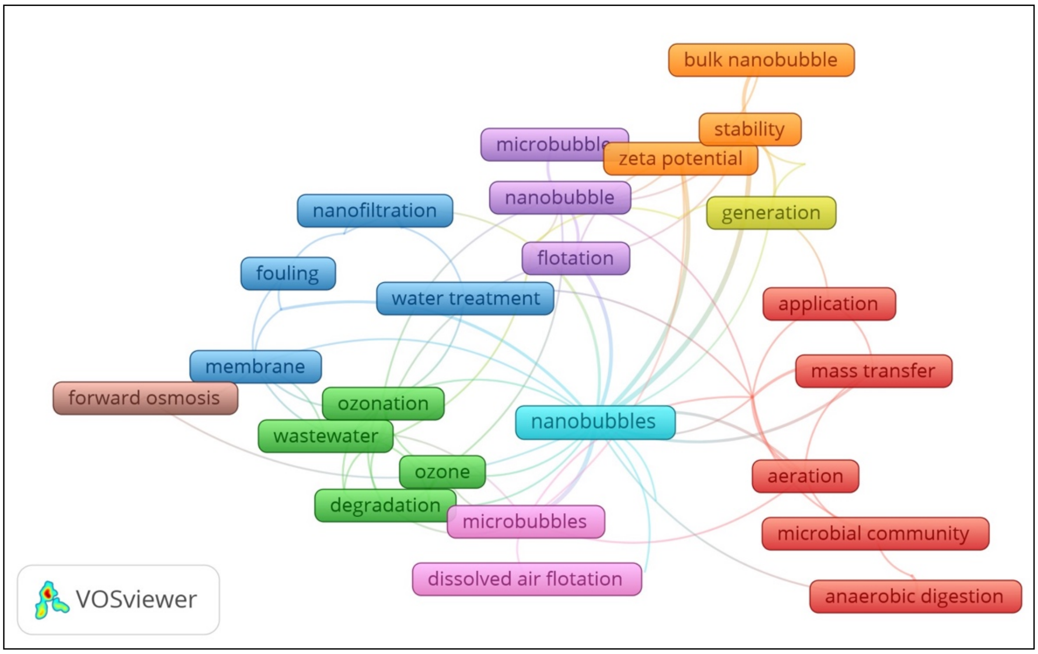

Figure 2.2. Co-occurrence analysis of the authors' keywords.

Figure 2.2 displays a co-occurrence analysis of keywords, visually depicting the relationships among terms based on their frequency and organising them into distinct color-coded clusters. In this analysis, blue nodes group keywords associated with "water treatment," linked to membrane processes such as nanofiltration. The purple cluster encompasses keywords related to flotation processes. The red nodes cluster terms related to aeration processes, connected to microbial community, flotation, and dissolved air flotation, indicating a growing interest of NB aeration in biological process. The orange cluster underscores the properties of NBs, mostly their stability and the seta potential. The green cluster relates to advanced oxidation processes (AOP), emphasising the use of NB and ozone as oxidants. The presence of the keyword "generation" in this context indicates the different generation methods for NBs.

2.3. Nanobubble technology

2.3.1. Bubble Size

Numerous researchers have categorized bubbles based on their sizes, distinguishing nanobubbles (NBs) (with a diameter of <200 nm), fine bubbles (FBs) (with a diameter of 200 nm−10 µm), microbubbles (MBs) (with a diameter of ≤50 µm), and macro bubbles (with a diameter of 2−5 mm) (Agarwal *et al*., 2011; Rameshkumara *et al*., 2019; Kim & Kwak, 2017). ISO 20480-1 (2017), on the other hand, classifies bubbles based on their volume equivalent to diameter. In this classification, bubble sizes are de-noted as micro, fine, and ultrafine bubbles/nanobubbles, covering the ranges of 1 to 100 µm, less than 100 µm, and less than 1 µm, respectively [Kim & Kwak, 2017; Gurung *et al*., 2016).

2.3.2. Fundamental properties of Nanobubbles

Table 2.2 outlines the specific properties of various bubbles based on their diameters. The unique properties of NBs facilitate the enhancement of chemical reactions and physical adsorption by improving mass transfer efficiency at the liquid–gas interface (Gurung *et al*., 2016). According to Phan *et al.* (2020), the high stagnation of NBs in the liquid phase contributes to increased gas dissolution above supersaturation in water.

Table 2.2: Properties of different bubbles according to their size (Sakr et al., 2022)

Bubble property	Macrobubbles	Microbubbles	Nanobubbles
Zeta potential	Low	High	Higher
Free radicals	Low	High	Higher
Mass transfer efficiency	Low	High	Higher
Bubble stability	Unstable	Stable	Stable
Rising velocity	Fast	Slow	Slower
Rising time	Short	Long	Very long
Oxygen transfer process	Inefficient	Efficient	Efficient
Internal pressure	Low	High	Higher

2.3.2.1. Negative zeta potential

According to Gurung *et al*. (2016), zeta potential (ZP) refers to the electric potential exhibited by suspended particles such as gas bubbles, which may result in either attraction or repulsion between them. It arises at the interface between the particles and the liquid medium. The level of colloidal dispersion stability can be gauged by the value of ZP. A higher absolute ZP value signifies that the solution or dispersion is more stable, as it possesses a greater resistance to agglomeration, as per Shangguan *et al*., (2018). Typically, bubbles present in distilled water carry a negative charge owing to the presence of hydroxyl ions (OH^-) adsorbed at the interface between the gas and liquid phases (Bui *et al*., 2019), which contributes to the stability of the bubbles and influences various physicochemical interactions, such as aggregation and coalescence, in aqueous systems.

The ZP is influenced by various factors, including viscosity, bulk solution density, electrolyte concentration, chemical surfactants, pH, and temperature. When gas flow rates are controlled, and sufficient energy or pressure is provided, bubbles exhibit high ZP values, regardless of the gas type (Meegoda *et al.*, 2018). Takahashi *et al.* (2007b) highlighted the significance of adsorbed OH^- and H^+ in influencing the charge at the gas–water interface. The electrostatic force leads to the attraction of electrolyte ions to the interface, generating an electrical double layer.

2.3.2.2. Ability to generate free radicals.

The collapse of NBs induced by ultrasonic waves triggers the generation of free radicals, particularly hydroxyl radicals (OH^-), known for their potent oxidising capabilities (Serizawa, 2017). This generation of free radicals is a primary reason for employing NBs in the oxidative treatment of wastewater. The collapse process leads to an increase in the ZP, contributing to the formation of free radicals. The proposed mechanisms encompass the entrapment of excess ions at the NB interface, the abrupt disappearance of the gas–liquid inter-face during bubble collapse leading to a pronounced environmental alteration, and the generation of free radicals attributed to an instantaneous high density of ions (Xiong *et al.*, 2018)

In other terms, the collapse of bubbles results in the elimination of the gas–liquid interface. During the self-pressurising process of NBs, a remarkably high concentration of charged ions accumulates at the interface, aiming to dissolve, and this accumulation, upon dissolution, rapidly releases the chemical energy responsible for generating hydroxyl radicals (OH^-) (Zhang *et al.*, 2020). The stability of cavitation bubbles is indicated by the efficiencies of free radical formation through the thermal decomposition of water in a hot spot. The presence of MBs in the solution can impact the formation of free radicals through ultra-sound, potentially enhancing the generation of hydroxyl radicals (OH^-) (Masuda *et al.*, 2015).

2.3.2.3. Gas mass transfer

Enhancing the efficiency of mass transfer between gas and liquid is achievable with NBs since the mass transfer rate of a gas is dependent on the mass transfer area of gas–liquid phases. NBs offer a significantly higher mass transfer area, leading to the potential for the gas dissolution rate in water to reach a supersaturated state (Zhang *et al.*, 2020). The reduction in bubble radius, coupled with an increase in internal pressure, contributes to an increased mass transfer rate of NBs to the surrounding liquid (Li *et al.*, 2013). Additionally, the diffusion rate of gas moving from a higher-pressure region to a lower-pressure region is directly proportional to the pressure gradient (Li *et al.*, 2014). Consequently, utilising smaller bubble sizes could augment the efficiency of gas transfer.

The success of aerobic biodegradation relies on dissolved oxygen (DO) as the electron acceptor and the ability to deliver oxygen to microorganisms. Consequently, the efficacy of aeration may be constrained by the rate at which oxygen transfers from gas to liquid and the oxygen consumption rate by microbes. Employing smaller-sized bubbles, such as MBs, has the potential to enhance gas transfer efficiency and augment beneficial reactions in water treatment (Atkinson *et al*., 2019). Liu and Tang (2019) verified that micro-nanobubbles (MNBs) can enhance the DO levels and oxygen mass transfer rate, leading to an accelerated removal of pollutants. They found out that in comparison to air MBs, the rate at which DO is transported by oxygen NBs was approximately 125 times quicker during the highest DO, near-ly three times greater, and the increased endurance of DO was 16 times longer than that of air MBs. The choice of gas has a notable impact on stagnation time, with oxygen bubbles, encompassing both macrobubbles and MBs, displaying residence times at least four times longer than air bubbles (Liu and Tang, 2019).

2.3.2.4. Stability of NBs

The stability of NBs is determined by the duration they persist in a solution (Atkinson *et al*., 2019; Zhang *et al*., 2020). Enhanced stability is advantageous as it prolongs the period for gas mass transfer into the water, ranging from 60 min to several months for NBs (Atkinson *et al*., 2019). The minimal rising velocity, attributed to Brownian motion, and the subdued buoyancy forces contribute to the increased stability of these bubbles. By maintaining higher pH levels with an abundance of OH^- ions, stable and smaller NBs with elevated ZP values can be generated (Meegoda *et al*., 2018). Azevedo *et al*. (2019) explained that the stability of NBs can be attributed to various mechanisms, encompassing those expounded in gas density theory, liquid height theory, Knudsen gas theory, and line tension theory. Moreover, dynamic equilibrium model and surface forces play roles in the comprehensive stability of NBs.

The presence of an electrically charged interface between the liquid and gas induces repulsion forces that impede bubble coalescence, thereby contributing the stability of nanobubbles (NBs). Furthermore, the high concentration of dissolved gas in the water facilitates the preservation of a minimal concentration gradient between the gas and the liquid.

2.3.3. Generation of Nanobubbles

NBs can be produced in a liquid by adjusting gas pressure, ultrasonic intensity, or stirring intensity. Most popular methods for generating NBs include mechanical stirring, gas dissolution release, pressure variation, and cavitation. Additionally, methods such as microfluidic systems and nano-porous membranes can be employed for the preparation of NBs. These methods are reviewed in the sections 2.3.2.1 to 2.3.2.6 and are summarised in Table 2.3.

Table 2.3: Summary of NB generation methods

Method	Principle	Advantages	Disadvantages	References
Mechanical Stirring	Iterative rotational stirring facilitates bubble formation due to shear forces and turbulence.	Rapid generation, stability for an extended period.	Limited control over size distribution.	Senthilkumar *et al.*, 2021
Venturi-based Bubble Generation	Utilizes converging and diverging flow to induce pressure changes, leading to bubble formation.	Simple design, controllable bubble size with divergent angle and liquid flow rate.	Limited uniformity in bubble size.	Li *et al.*, 2017; Zhao *et al.*, 2017
Porous Membrane Method	Compressed gas introduced through membrane pores into a liquid phase, generating bubbles on the membrane surface.	Controlled bubble size by adjusting membrane pore size and liquid flow velocity.	The influence of membrane properties on bubble size needs consideration.	Zhang *et al.*, 2022
Acoustic Cavitation	Induces local negative pressure in liquid through high-speed propeller rotation or high-intensity sound waves, forming micro- and nano-scale bubbles.	High energy efficiency, scalability.	Potential for bubble coalescence and fusion, sensitivity to organic solvents.	Ferrari, 2017; Nirmalkar *et al.*, 2019
Microfluidics-based Approach	Regulates the flow of mixed gas and liquid in microfluidic chips, resulting in the formation of microbubbles that evolve into nanobubbles.	Precise control over size and uniformity, adjustable by gas ratio.	Requires specialized equipment, complexity in setup.	Yi-Qiang *et al.*, 2018
Hydrodynamic Cavitation	Alters flow velocity to induce cavitation, causing pressure fluctuations and generating nanobubbles.	High energy efficiency, low cost, scalability.	Requires optimization for specific applications.	Zheng *et al.*, 2022

2.3.2.1. Mechanical Stirring Method

The generation of NBs through mechanical stirring entails subjecting a liquid phase with surfactants to repeated rotational stirring using a mechanical system. This process induces high shear forces, intense turbulence, collision effects, and hydrodynamic cavitation, fostering interactions between the gas and liquid phases and resulting in bubble formation. These bubbles, undergoing multiple agitation cycles, undergo continuous shearing, leading to the gradual creation of smaller bubbles and the eventual formation of NBs (Li *et al.*, 2014).

In experiments conducted by Etchepare *et al.* (2017b) on NB preparation through the mechanical stirring method, they used a pump and circular column under varying pressures and air–liquid interfacial tensions. They found that this technique could swiftly produce stable NBs that maintained their stability for over 60 days. Additionally, Senthilkumar *et al.* (2021) generated NBs using mechanical stirring in heat transfer oil. The produced NBs had diameters of less than 200 nm, contributing to improvements in the thermal conductivity and viscosity of the heat transfer oil.

2.3.2.2. Venturi-based generation

The Venturi bubble generator comprises three main elements: a narrowing entrance, a central throat, and an expanding outflow (Zhao *et al.*, 2017). In this procedure, gas is introduced into the venturi tube simultaneously with water, either through the narrowing entrance (Parmar & Majumder, 2013) or at the throat section (Li *et al.*, 2017).

The mechanisms of bubble generation in a venturi-type bubble generator were explored by Zhao *et al.* (2017) and they observed a pressure decrease in the throat region leading to an increase in bubble velocity. Subsequently, the air bubbles undergo swift deceleration as they enter the widening outflow section, experiencing pressure recovery. The difference in flow velocities between the liquid phase and air bubbles creates a shock wave characterized by intense shear forces, causing the deformation of bubbles and the trans-formation of large bubbles into numerous smaller ones (Zhao *et al.*, 2017).

The divergent angle plays a crucial role in determining the performance of a venturi-type bubble generator as demonstrated by Agarwal *et al.* (2011) and Li *et al.* (2017). They found that an increase in the divergent angle results in a reduction of bubble size produced. Additionally, Huang *et al.* (2020) highlighted that the liquid flow rate is a key parameter influencing bubble size and distribution. A more uniform bubble size distribution can be achieved by increasing the liquid flow rate.

2.3.2.3. Porous membrane method

Porous membrane bubble generation involves introducing compressed gas from the outside of the membrane through its pores, while a liquid phase flows inside the membrane, generating shear force to create bubbles on the membrane surface (Ulatowski and Sobieszuk, 2020).

Kukizaki & Goto (2006) utilized Shirasu Porous Glass (SPG) nanoporous membranes to generate NBs. The SPG membrane, developed by SPG Corporation in Japan in 1981, is an inorganic membrane with uniform and adjustable micropore sizes. In their experimental setup, compressed air was introduced into a solution of sodium dodecyl sulfate with concentrations from 0.05 to 0.5 w%. The solution underwent filtration through a SPG membrane with a transmembrane/bubble point pressure ratio of 1.1 – 2.0. Consistently mono-disperse MNBs were prepared, with diameters from 360 to 720 nm. The resulting NBs had an average diameter 8.6 times larger than that of the membrane pore, and their size remained relatively unaffected by air velocity or liquid surface tension. Consequently, the size of the NBs could be effectively manipulated by changing the pore size of the membrane.

Zhang *et al*. (2022) introduced a membrane-based physical sieving approach for producing controllable-sized NBs. The objective of this technique is to regulate the size distribution of the produced NBs through the manipulation of gas filtration rate and the characteristics of the membrane. Experimental sieving of NBs was carried out using three types of membranes, revealing that the membrane not only had the capability to break down larger bubbles into smaller ones but also facilitated the merging of small bubbles into larger ones during the filtration of bulk nanobubbles (BNBs).

2.3.2.4. Acoustic cavitation method

The acoustic cavitation technique involves inducing localized negative pressure in the liquid through either high-speed propeller rotation or generating negative pressure half-cycles using intense sound waves. This process leads to the formation of micro- and nano-scale bubbles near small gas nuclei (Ferrari, 2017). In their experiments on NB generation using the acoustic cavitation method, Nirmalkar *et al.* (2019) revealed the presence of NBs in pure water but not in organic solvents. The disappearance of NBs occurred at a specific ratio of organic solvent to water. This occurrence is ascribed to the electrostatic charge on the NBs' surface, which stabilizes them through the adsorption of hydroxyl ions produced via water's autoionization. In contrast, pure organic solvents lack auto-ionization.

2.3.2.5. Microfluidic Method

In microfluidics method, control over the flow of a combined gas and liquid is achieved using microfluidic chips (Yi-Qiang *et al.*, 2018). A mixture of gases is introduced through a gas inlet, and as it moves through the liquid phase, it experiences viscous forces from the liquid, leading to the generation of MBs. Within these MBs, a portion of the gas dissolves into the aqueous phase and subsequently contracts, giving rise to the formation of non-spherical bubbles (NBs).

This approach involves utilising a gas mixture containing water-soluble nitrogen and water-insoluble perfluorocarbon (PFC) as the gaseous component in the microfluidic bubble generator. Initially, monodisperse MBs are generated, and as the water-soluble nitrogen dissolves, these microbubbles gradually contract, ultimately forming NBs of a specific size. The degree of bubble contraction can be finely tuned by adjusting the ratio of water-soluble nitrogen to water-insoluble PFC. A notable advantage of this approach lies in its precise control over the size and uniformity of the resulting nanobubbles (Xu *et al*., 2021).

2.3.2.6. Hydrodynamic Cavitation Method

The hydrodynamic cavitation technique involves inducing cavitation in a medium by modifying the flow velocity, leading to pressure fluctuations, similar to the effects achieved through acoustic cavitation methods (Zheng *et al*., 2022). Consequently, hydrodynamic cavitation can serve as an alternative to acoustic cavitation for NB generation. Alam *et al*. (2021) performed an investigation on NB generation through hydrodynamic cavitation. The out-comes indicated the successful production of NBs with diameters below 200 nm, and these nanobubbles displayed a negative charge in water. In another study, Wu *et al*. (2022a) optimised the cavitation reactor by using numerical simulation to analyse the influence of different geometric parameters on the flow field structure. They determined the most effective design and went on to build a laboratory-scale MNB generator with a vortex-type configuration.

2.3.4. Application of Nanobubbles in wastewater treatment

NBs have proven to be highly useful in wastewater treatment, especially in key processes including enhanced oxidation, flotation, disinfection, and aeration. Table 2.4 provides an overview of the uses of NBs in wastewater treatment technologies. These bubble-based technologies have been extensively studied to enhance pollutant removal efficiency while concurrently achieving goals such as facility downsizing, reduced operation time, and lowered operation and maintenance costs for water treatment plants (Temesgen *et al*., 2017).

Gases such as air, oxygen, and nitrogen, ozone-NBs, have been employed for decomposing different compounds. In aerobic biodegradation processes, the use of small-sized NBs proves effective in delivering oxygen to inaccessible regions, enhancing the aerobic biodegradation of substances such as phenanthrene, as demonstrated with saponin-based MNB suspensions (Khuntia *et al*., 2012; Kaushik and Chel, 2014). Additionally, both aerobic and anaerobic reactor microbial activity can be boosted through the application of air and nitrogen NBs in submerged membrane bioreactors. Furthermore, the catalysation of chemical reactions and the improvement of detoxification in chemical treatment processes have been achieved using NBs (Kaushik and Chel, 2014). These advancements contribute to the overall efficiency and sustainability of water and wastewater treatment methodologies. The following section reviews the application of NB in floatation, aeration, ozone oxidation as well as membrane technology.

Table 2.4: Application of NB in wastewater treatment technologies

Application	**Research focus**	**Results and achievements**	**Reference**
Aeration	Investigation of nanobubble effects on aeration	Improved oxygen transfer efficiency, enhanced DO content, and accelerated pollutant removal.	Liu & Tang (2019)
Floatation	Evaluation of nanobubble impact on froth flotation	Reduction in bubble rising velocity, improved froth flotation conditions for coarse particles.	Etchepare *et al.* (2017a)
Membrane technology	Application of nanobubbles in membrane processes	Improved permeability, reduced fouling, and enhanced efficiency in various membrane technologies.	Dayaranthne *et al*. (2019)
Ozone Oxidation	Use of nanobubbles in ozone treatment	Increased stability, generation of hydroxyl radicals (OH), and improved oxidative efficiency.	Zheng *et al*. (2015)

2.3.2.1. Nanobubbles in flotation technology

Flotation is commonly recognized as the most consistent and feasible separation technique for eliminating suspensions containing FOG, combined with low-density organic suspended solids and colloids (Kim *et al*., 2020). This separation method operates on the adsorption of gas bubbles, as they rise, onto the surface of finely suspended particles. The adsorption reduces the specific gravity of the particles, causing contaminants to ascend to the surface and boosting their upflow velocity (Nazari *et al*., 2018). This technique is frequently employed to separate extremely fine particles from the solution that lack a significant settling rate.

DAF and induced air flotation (IAF) stand out as widely available flotation techniques. DAF involves the creation of bubbles by reducing the pressure of water already saturated with air above atmospheric pressure (Fonseca *et al*., 2017). Conversely, IAF relies on the mechanical means to generate bubbles, combining a high-speed mechanical agitator with an air injection system (Saththasivam *et al*., 2016; Prakash *et al*., 2018). Other commercial separation techniques based on flotation include electro-flotation, nozzle flotation, column flotation, centrifugal flotation, jet flotation, and cavitation air flotation.

The study conducted by Lee *et al*. (2020) proved the effective removal of micro-sized oils (less than 20 μm), that may not be efficiently eliminated with ordinary bubbles, was successfully achieved through the integration of DAF coupled with a selectively adjustable NB slit nozzle (ranging from 1 to 100 μm). The suspended solids containing oil contaminants were eliminated at a remarkable rate of 95% for COD with a simultaneous 95% recovery. Xiao *et al*. (2018) conducted a study to explore the role of NBs in wastewater treatment, specifically targeting the precipitation of styryl phosphoric acid lead particles and the recovery of organic phosphine. The research revealed that NBs played a crucial role in inhibiting the crystallization of styryl phosphoric acid lead precipitation, resulting in a sediment flotation recovery of less than 20%. However, upon completion of the crystallization process, the precipitated particles experienced flocculation facilitated by NBs, leading to a substantial improvement in flotation recovery, reaching 90%.

Additionally, Etchepare *et al*. (2017b) highlighted the significant potential of NBs in wastewater treatment, particularly in achieving high overall oil removal efficiency. The study revealed that combining flocculation with 5 mg/L Dismulgan, followed by flotation with both MBs and NBs at a saturation pressure of 5 bars, resulted in oil removal efficiency exceeding 99%. Furthermore, the study highlighted the ability of NBs to improve overall oil removal efficiency; during the NB conditioning stage following flocculation with 1 mg/L of Dismulgan, an increase in flotation efficiency from 73% to 84% was achieved.

In terms of economic considerations, the expenses associated with coagulation-flocculation using NB flotation technology were found to be more economical compared to conventional methods. The conclusion drawn was that this treatment method proves to be cost-effective for refining wastewater treatment, both chemically and mechanically. This economic efficiency is attributed to the negatively charged nature of NB in conjunction with the coagulation and flocculation process, especially with the application of Poly Aluminum Chloride as a coagulant (Temesgen *et al.*, 2017).

2.3.2.2. Nanobubbles in aeration

Aeration is a crucial process in aerobic wastewater treatment, constituting 45–75% of the overall plant energy cost, making it the most substantial portion of the expenses. Effective control of the energy cost is possible by managing DO, a key parameter due to its influence on biological processes (Drewnowski *et al.*, 2019). Aeration is used to biologically treat contaminated water by supplying oxygen to bacteria, facilitating the breakdown of organic substances.

Efficient removal of organic pollutants in wastewater is achievable through traditional activated sludge or the aerated lagoon treatment process. These methods involve aerobic microorganisms with high metabolic kinetics, enabling rapid degradation of organic pollutants in the presence of sufficient oxygen. However, as reported by Huggins *et al.* (2013), sludge disposal and treatment constitute a significant portion (60%) of the total operational cost. The utilization of NBs is, therefore, characterized by a reduction in sludge production and an enhancement in oxygen transfer efficiency within sequencing batch reactor systems. This is accomplished by boosting the number of active bacteria within the floc mass, resulting in a faster and more intense breakdown of organic compounds when compared to aeration with ordinary bubbles.

Air NBs have demonstrated notable effectiveness in treating both domestic and industrial wastewater from diverse origins. For example, Leyva & Flores (2018) treated wastewater from the sugar sector with air NBs exhibited a reduction of 79% in total suspended solids (TSS) and 85% in chemical oxygen demand (COD) in under 90 min. Furthermore, Reyes and Flores (2017) reported that the application of air NBs led to a removal efficiency of 66.21% for total coliforms in wastewater.

Wang and Zhang (2017) investigated the incorporation of fine bubble aeration into a deep subsurface wastewater infiltration system to assess nitrogen removal and its mechanisms. The combined system effectively treated wastewater, achieving removal percentages of 95,12%, 98,52%, and 99,98% for COD, NH_4^+-N and total phosphorus; respectively. The incorporation of fine bubble aeration not only improved the nitrogen removal capacity but also minimised the necessity for temperature adaptation in the deep soil infiltration system. Moreover, the reduction in wastewater COD contributed to a lowered demand for infiltration bed depth.

In contrast to traditional bubbles, NBs significantly enhanced both mass transfer and degradation in wastewater treatment. In a study conducted by Yao *et al*. (2016), municipal wastewater was artificially recreated and subjected to treatment in aerobic activated sludge systems using NBs. The outcomes of this approach were compared with those obtained using conventional bubble aerators. Notably, the rates of COD removal were considerably superior to those achieved with traditional bubbles. Specifically, there was a 2.04-fold increase at an initial COD concentration of 200 mg/L, a 5.9-fold increase at an initial COD concentration of 400 mg/L, and a 3.26-fold increase at an initial COD concentration of 600 mg/L. Furthermore, the investigation conducted by Xiao & Xu (2020) underscored the substantial energy-saving potential, amounting to nearly 80%, associated with the utilization of NB aeration.

2.3.2.3. Physiochemical treatment with Nanobubbles

NBs have been used in physicochemical wastewater treatment techniques, such as adsorption, membrane filtration, and ion exchange, to enhance the treatment process and obtain high removal efficiencies.

Dayaranthne *et al*. (2019) investigated the use of MNBs on the RO membrane surface to manage scaling development without the need for additional chemicals. Air MNBs demonstrated superior performance as a chemical-free method for inhibiting scale compared to the use of antiscalants. Experimental results showed that, over four days of continuous operation with MNBs, permeate flux reductions were 86.5% and 83.0% with $Ca\text{-}CO_3$ and $CaSO_4$ feed solutions, respectively. Without MNBs, the permeate flux decreased even more, declining to 63.5% with $CaCO_3$ and 55.8% with $CaSO_4$.

In another study by Dayarathne *et al*. (2017), the use of MNBs proved effective in achieving a 100% recovery of permeate flux and enhancing the cleaning in place of RO membranes in an environmentally friendly approach. The outcomes demonstrated a substantial increase in permeate flux by 24.62% and a solute rejection of 0.8%, attributed to the disruption of the layer triggered by MNBs. This approach contributes to cost reduction in the overall process by eliminating the necessity for restarting the process. Furthermore, the use of air NBs is regarded as an environmentally sustainable method in ceramic membrane filtration processes. Ghadimkhani *et al*. (2016) demonstrated the successful unclogging of membrane pores by applying air NBs in comprehensive pilot and bench-scale investigations targeting resistance to fouling. The results proved the reinstatement of permeate flux to its original values through the utilization of NBs.

2.3.2.4. Advanced oxidation with ozone-Nanobubbles

Ozone, a potent disinfectant widely used in water treatment, exhibits efficacy by binding to bacterial cell walls, rendering them inactive. 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 (Hung, 2016). To address these limitations, the application of NB technology has been explored to enhance the ozonation process in water treatment (Andinet *et al.*, 2016; Shangguan *et al.*, 2018). Leveraging their substantial surface area, rapid mass transfer rates, and prolonged stability in water, NBs contribute to bolstering ozone stability, thereby significantly improving the overall efficiency of the ozonation process.

In the context of wastewater treatment, ozone MNBs have proven effective in addressing both real and synthetic wastewater contaminated with organic pollutants, showcasing notable efficacy across bubble sizes ranging from 20 µm to 1000 nm. Xia and Hu (2018) reported that the aeration of ozone NBs successfully reduced sludge organic compounds, resulting in a decrease in mixed liquor suspended solids (MLSS) from 53.5% to 31.4% and a decline in oil content from 77.5% to 51.7%. However, this reduction was accompanied by an increase in chemical oxygen demand (COD) by approximately 221% and NH_4^+ by 26%. The incorporation of MBs and catalysts in the process offers a potential cost reduction in sludge management. The treatment efficacy was influenced by pH, with maximum efficiency observed at pH = 5. Under these conditions, the COD removal rate exceeded 63% after 14 h. Additionally, Menendez & Flores (2018) treated hospital wastewater containing organic contaminants with ozone-air MNBs, resulting in a substantial decrease in the initial concentrations of the samples and high COD efficiency (92.51% for the first sample and 87.9% for the second sample).

In comparison to traditional ozone techniques, the utilization of ozone NBs has demonstrated enhanced efficiency, varying from 1.3 to 19 times, in terms of volumetric ozone gas mass transfer across the gas-liquid interface as reported by Achar *et al.* (2020). Additionally, the oxidation process was found to be more rapid at pH = 6 compared to pH = 7, emphasising the significance of pH control (Achar *et al.*, 2020). Another study by Jabesa & Ghosh (2016) corroborated the effectiveness of employing high ozone generation rates and elevated pH levels in conjunction with ozone NBs in a pilot plant system for the removal of highly water-soluble and toxic diethyl phthalate.

MNBs have demonstrated superior performance compared to macrobubbles, highlighting the effectiveness of smaller-sized bubbles in wastewater treatment processes. A study conducted by Zheng *et al.* (2015) involved the comparison of ozonation using MNBs and macrobubbles for treating wastewater from acrylic fibre manufacturing. The results highlighted that MNBs enhanced the removal of organics, facilitating improved ozonation and biodegradability by accelerating the degradation of alkanes, aromatic compounds, and biorefractory organic compounds. In the MNB-ozonation process, the removal efficiencies for COD, NH_3-N, and UV-254 in wastewater were 42%, 21%, and 42%, respectively. Notably, these rates surpassed those achieved by macrobubble ozonation by 25%, 9%, and 35%, respectively, at an equivalent ozone dose of 5 g/h.

In a separate investigation by Chu *et al*. (2008) involving textile wastewater, a comparison was made between the MB system and a bubble contractor. The findings revealed that the MB system exhibited a faster decolorization rate, with a 20% higher removal efficiency of COD compared to the bubble contactor. Additionally, the MB system achieved very high ozone utilization, as evidenced by a significantly lower concentration of off-gas ozone compared to the bubble contactor. When comparing the time required for 80% removal of colour, the ozone MB system demonstrated a shorter duration of 140 minutes compared to the conventional bubbles, which took 280 minutes.

From previous research, it is evident that ozone MNBs prove highly effective in pollutant reduction, disinfection, and enhancing biodegradability in wastewater treatment. The reduction in bubble size contributes to an improved treatment process by allowing for higher ozone inlet concentrations and better ozone utilization. However, the optimal ozonation rate is contingent on various factors, including process conditions, the nature of pollutants, and the source of wastewater.

2.3.5. Degradation mechanism of pollutants by nanobubbles

The primary mechanism for removing pollutants from wastewater by the NB-based AOP involves the generation of reactive oxygen species (ROS) such as hydroxyl radicals (OH^-) and superoxide radicals (O_2^-). These ROS are generated at the NB-water interface. When NBs collapse, they release significant energy that leads to the formation of ROS (Wang B *et al*., 2024). The ROS attack the pollutants, leading to their degradation. Hydroxyl radicals are identified as the most effective ROS in this process. The mechanisms of degradation involve the adsorption of pollutants onto the NB surface, followed by their oxidation through these ROS. This process is facilitated by the collapse of NBs, which generates localized high temperatures and pressures, enhancing the formation of ROS (Wang *et al*., 2024a; Wang *et al*., 2024b).

Since NBs produce OH- radicals and generate shear stress, enabling them to degrade pollutants and sterilize bacteria, NB technology can be used to remove organic pollutants and microorganisms from PSW. In PSW treatment processes, NBs can be used in flotation to eliminate SS due to their strong adsorption capability, in biological and aerobic treatment to enhance DO levels and biological activity because of their high mass transfer efficiency, and in AOPs to degrade organic pollutants.

2.3.6. Factors affecting pollutant removal by nanobubbles

The efficiency of pollutant removal by NBs can be influenced by a range of factors. These include the pH level, the temperature, the initial concentration of pollutants as well as the salinity and ions in PSW. Each of these variables can significantly impact the overall removal efficiency (Yu *et al*., 2017).

(a) Effect of pH: The degradation of organic pollutants by NBs is influenced by pH levels. Research indicates that acidic conditions enhance the degradation of certain pollutants by NBs, while other studies suggest that an alkaline environment is more effective for different pollutants (Wang T *et al*., 2024). For example, NBs best degrade methyl orange, phenol, and rhodamine B under acidic conditions (Yu *et al*., 2017). Conversely, pollutants such as alachlor, benzothiophene, and diethyl phthalate are more effectively degraded by NBs in alkaline conditions (Yu *et al*., 2017; Khuntia *et al*., 2015). This variation is due to the impact of pH on the free radicals produced by NBs and the physical and chemical properties of the pollutants themselves (Wang B *et al*., 2024). Thus, the degradation of organic pollutants by NBs involves the dual influence of these factors, which should be comprehensively considered.

The pH of PSW can fluctuate, potentially impacting the effectiveness of AOPs. The quality and pH of PSW is affected by the quality of water used during slaughtering, the type of operation during wastewater collection, the sampling methods used by the individuals involved, and the specific cleaning and sanitising procedures of the abattoir (Njoya *et al*., 2019; Gutu *et al*., 2021). The pH of PSW was reported to vary between 4.9 – 8.1 with a mean of 6.5 (Bustillo-Lecompte *et al*., 2016; Oktafani *et al*., 2019). To evaluate how pH influences the degradation process, a study needs to be conducted with NBs across various pH levels.

(b) Effect of temperature: Temperature also plays a significant role in the generation of ROS species by NBs and conversely affect the degradation of pollutants. Yu *et al*. (2017) found that in alkaline NB solution, the concentration of ROS species initially increased and then decreased as the temperature rose, displaying a parabolic trend with a peak concentration at 65°C. This phenomenon was attributed to the combined effects of temperature on oxygen reactivity, diffusion coefficient, and DO concentration, where ROS levels followed the same trend. In another study, Wang *et al*. (2024b) investigated the impact of temperature on the degradation of rhodamine B (RhB) using cavitation-induced and rotating jets. Their findings showed that the degradation efficiency of rhodamine B improved as the temperature increased from 20°C to 40°C, but decreased when the temperature rose further from 40°C to 60°C.

These findings demonstrate that temperature has a dual effect on pollutants removal efficiency by NBs. As the equilibrium vapor pressure increases with temperature, the formation of NBs is promoted, which aids in the generation of OH^- and the degradation of organic matter. However, excessively high temperatures cause water vapor to fill the cavitation bubbles, reducing bubble collapse, which hinders the generation of ·OH and the degradation of organic matter (Wang *et al*., 2024b).

(c) Effect of initial concentrations of pollutants: Ahmadi *et al*. (2022) assessed the impact of different initial COD concentrations (400, 600, and 800 mg/L) on removal efficiency in a NB aeration system. They found that the removal efficiency decreased as the pollutants' concentration (i.e., COD) increased. This decline was attributed to a shortage of DO in the wastewater, which is essential for the oxidation process. Enhancing the oxygen content in the wastewater is crucial. Factors such as the bacterial growth curve, the existing phase, and the sludge volume index (SVI) are highly influential. Similarly, Wang *et al*. (2024b) investigated the effect of initial concentrations of RhB (0.1, 1, and 10 mg/L) on their removal efficiency by NBs. The results showed that at a high initial concentration of RhB, the degradation of intermediates (by-products) may compete for the consumption of ROS with the parent RhB compound, leading to a slower reaction rate.

(d) Effect of salinity and other ions: Various constituents in PSW, such as ions, salinity, hardness, and alkalinity, can pose significant challenges for ROS-based AOPs in degrading organic pollutants from the wastewater (Ahmadi *et al*., 2022). Some studies have highlighted the impact of foreign ions on the stability of nanobubbles (Yu *et al*., 2021). However, Wang et al. (2024b) examined the removal efficiency of RhB in the presence of 300 mg/L of background ions, including Ca^{2+}, Mg^{2+}, HCO_3^-, and Cl^-. Their findings showed that oxygen nanobubbles can achieve a removal efficiency of RhB exceeding 92% even in the presence of the background ions. They concluded that the background ions have a negligible impact on degradation by oxygen nanobubbles.

2.4. Conventional Treatment of Poultry Slaughterhouse Wastewater

The choice of a technology relies on the characteristics of the wastewater, the existing technology options, and adherence to regulations governing the discharge of wastewater and industrial effluents. Conventional treatment for PSW consists of preliminary, primary, and secondary treatments. After preliminary treatment, several combined treatment approaches are possible, with the most prevalent combination being physicochemical treatment as the primary method, followed by biological treatment as the secondary step.

Anaerobic treatment is commonly employed due to its effectiveness in treating wastewater with elevated organic concentrations. However, achieving complete degradation of organic matter in PSW is not attainable with anaerobic treatment alone. Consequently, it is recommended not to use either anaerobic or aerobic processes as the sole treatment method. Combining anaerobic and aerobic processes is proposed as a strategy to minimize the overall cost compared to relying solely on aerobic processes, which incur high expenses for aeration and sludge disposal due to elevated chemical oxygen demand (COD) levels (Cao & Mehrvar, 2011; Gutu *et al.*, 2021).

2.4.1. Preliminary treatment

The purpose of preliminary treatment is to remove suspended solids and Fats, Oils & Grease (FOG) from PSW, protecting wastewater equipment from fouling, clogging, and jamming. In the NB treatment of PSW, it is essential to eliminate suspended solids from the wastewater to avoid damage to the NB generator. Furthermore, proper sizing of the screening equipment is crucial to prevent frequent clogging and blockages of the sieve, which would otherwise necessitate extensive manual efforts for screen cleaning.

The most common unit operations for preliminary treatment include screeners, sieves, and strainers. Therefore, large solids with a 10 – 30 mm diameter are retained while the wastewater passes through. Other preliminary treatment methods include homogenisation, equalisation, and flotation, among other systems such as catch basins and settlers (Bustillo-Lecompte *et al.*, 2016; Musa & Idrus, 2021).

Mesh screening, being the most common, has been proven effective. In the study done by Rusten *et al*. (2017), pilot-scale mesh rotating belt sieves (RBS) demonstrated over 40% removal of total suspended solids (TSS) and 30% removal of chemical oxygen demand (COD) using a 350 micron belt at high sieve rates up to 160 m^3/m^2-h.

2.4.2. Primary treatment

After preliminary treatment, it is essential to subject the effluent to additional treatments to eliminate pollutants, including organic compounds and nutrients, which may not have been effectively removed during the initial treatment. An effective primary wastewater treatment method is Dissolved Air Flotation (DAF), which proves practical for reducing Fats, Oils, and Grease (FOG), Total Suspended Solids (TSS), and Biochemical Oxygen Demand (BOD) (Bhatia *et al.*, 2020).

Table 2.*5* outlines the most commonly employed physicochemical treatment methods.

Table 2.5: Primary wastewater treatment methods

Treatment Method	**Description**	**Advantages**	**Disadvantages**	**References**
DAF	Introduction of air to facilitate the separation of FOG, and solid materials from wastewater.	75% removal for FOG, BOD and TSS.	High operational and maintenance costs.	(Musa & Idrus, 2021; de Nardi *et al*., 2011)
Chemical Coagulation / Flocculation	Addition of chemicals to induce particle aggregation for easier removal.	Effective in treating colloidal and fine particles.	Chemical cost and sludge generation.	Teh *et al*. (2016)
Equalization Tanks	Balancing and smoothing flow variations and pollutant concentrations before entering treatment processes.	Reduces shock loads to downstream processes.	Requires additional space and monitoring.	Fotso (2021)
Primary Filtration	Physical filtration of suspended solids using barriers like sand or cloth.	Effective for fine particle removal.	Regular maintenance and clogging issues.	Gidstedt *et al*. (2022)

2.4.3. Secondary treatment

Preliminary and Primary treatments usually do not achieve complete treatment of SW to the satisfaction levels specified by regulations. Therefore, secondary treatment is introduced to eliminate the remaining soluble organic compounds left after primary treatment. In the treatment of SW, biological treatment serves as a secondary step to decrease the concentration of Biochemical Oxygen Demand (BOD) and other soluble compounds subsequent to primary treatment (Musa & Idrus, 2021). In contrast to primary treatment, biological treatment utilizes microorganisms to eliminate organic substances from wastewater. Various technologies fall under biological processes, which can be broadly categorized into anaerobic and aerobic treatment methods (Bustillo-LeCompte *et al*., 2015; Gutu *et al*., 2021; Philipp *et al*., 2021). The following section explores both aerobic and anaerobic treatment methods, along with the prospective utilization of NB in these processes.

2.4.3.1. Anaerobic treatment

Anaerobic digestion (AD) is a biological process that occurs in the absence of oxygen, whereby microorganisms break down organic matter, resulting in the production of carbon dioxide (CO_2) and methane (CH_4). Anaerobic digestion comprises hydrolysis, acidogenesis, acetogenesis, and methanogenesis stages, where a diverse array of microorganisms, including bacteria and archaea, facilitate the decomposition of complex organic compounds in the absence of oxygen. The degradation process is highly dependent on the activity rates of various bacteria (Aziz *et al*., 2019). Within anaerobic treatment, organic compounds undergo breakdown into methane, water, and carbon dioxide through the actions of anaerobic bacteria in an oxygen-deprived environment.

The up-flow anaerobic sludge blanket (UASB) reactor is the most common anaerobic digester for treatment of PSW. In studies conducted by Musa *et al*. (2018) at various organic loading rates (OLR), the UASB reactor exhibited effective COD removal, achieving 90% removal at an OLR of 0.4 g/L/d. The removal percentages were sustained at 70%, 65%, and below 50% for OLRs of 3, 10, and 15 g/L/d, respectively.

In a different approach, Loganath and Mazumder (2018) employed a hybrid UASB with polypropylene media as surfaces for attached growth, resulting in enhanced removal efficiency for total organic carbon (TOC) and total suspended solids (TSS). The hybrid UASB achieved remarkable removal rates, with 95% efficiency for TOC at a loading rate of 7 kg TOC/m^3·d and a hydraulic retention time (HRT) of 10 h. Furthermore, removal efficiencies for TOC and TSS were as high as 96% and 98%, respectively.

Other commonly used anaerobic digesters include anaerobic filters, anaerobic baffled reactors (ABR), expanded granular sludge beds (EGSB), sequencing batch reactors (SBR), and the downflow expanded granular bed reactors (DEGBR) and static granular bed reactor (SGBR).

Table 2.***6*** summarizes the significant results, advantages, and disadvantages of different anaerobic digesters treating PSW.

Table 2.6: Achievement of common anaerobic digesters

Anaerobic digester	**Achievement**	**Advantages**	**Disadvantages**	**References**
DEGBR and SGBR	attained a 95% reduction in BOD5, COD, and FOG on days of optimal performance for both reactors.	The DEGBR consistently exhibited more substantial biogas production compared to the SGBR.	The SGBR required over 50 days to achieve a 95% removal of FOG, while the DEGBR accomplished this in 14 days.	Loganath and Mazumder (2018)
UASB	Approximately 90% COD removal was achieved at an organic loading rate (OLR) of 0.4 g/L.d, resulting in a biogas production of 5 L/d.	VFAs concentration remained low, and HRT of 1 day proved effective in removing more than 70% of COD.	COD removal decreased to less than 50% with an increase in the loading rate to 15 g/L/d.	Musa *et al.* (2018)
SGBR integrated with a single-stage nitrification-denitrification (SND) bioreactor and an ultrafiltration membrane.	Average removal efficiencies of 91% for COD, 51% for orthophosphate, 97% for TSS, and 52% for TDS were attained over a 52-day period.	ufMMs operated in the dead-end filtration mode demonstrated an additional reduction of 65% for COD and 54% for TSS on average.	The final effluent did not meet the standards for industrial wastewater for PO43− and NH4+-N.	Rinquest *et al*. (2019)
EGSB coupled with Membrane Bioreactor (MBR)	Overall system efficiency exceeded 97% for TSS and COD removal and 97.5% removal efficiency for FOG.	The EGSB's performance was not affected by varied organic loading rates (OLR), emphasising its robustness under different conditions.	FOG removal fluctuated and did not show a consistent improvement	Meyo *et al*. (2021)

Currently, the application of NB with AD is being explored by researchers in the field of wastewater treatment (Hou *et al.*, 2021; Fan *et al.*, 2020a; Fan *et al.*, 2020b; Yang *et al.*, 2019; Yang *et al.*, 2020). Recent studies have demonstrated the creation of NB-infused waters with various gases, serving as additives in AD batch systems. The unique characteristics of NB, such as enhanced gas solubility and the promotion of electrostatic interactions, can influence the physicochemical properties of liquids (Atkinson *et al.*, 2019).

The presence of NBs has shown potential in improving substrate digestibility by generating reactive oxygen species (ROS), thereby facilitating the oxidation of organic materials (Wang *et al.*, 2020b). Moreover, NBs, particularly those containing air and oxygen, can induce microaerobic conditions, improving the performance of the AD process by enhancing facultative bacterial activity and methanogenesis stage (Nguyen & Khanal, 2018; Nguyen *et al.*, 2019).

In a study conducted by Hou *et al.* (2021), the impact of NB with nitrogen and NB with air on a two-stage anaerobic digestion (AD) of food waste was investigated. In the initial stage, both nitrogen-NB and air-NB resulted in greater hydrogen production, demonstrating increases of around 23.7% and 39.9%, respectively, in comparison to deionized water. In the subsequent stage, nitrogen-NB and air-NB contributed to increased methane production by 15.2% and 24.7%, respectively, compared to deionized water.

2.4.3.2. Aerobic treatment

Aerobic digestion utilizes oxygen to decompose organic substances and pollutants, converting them into environmentally less harmful compounds as methane, carbon dioxide and water. The oxygen requirements as well as duration of this treatment are influenced by the organic content of PSW. Typically, aerobic digestion is implemented as the final step for nutrient removal when combining it with anaerobic treatments for sludge purification. Advantages of aerobic treatment include low odour generation, rapid biological growth, adaptability to changes in temperature and loading rates without requiring elevated operation temperatures (Hamawand *et al.*, 2017).

Instead of relying solely on an aerobic process, research has explored the integration of aerobic and anaerobic methods for wastewater treatment. Svierzoski *et al.* (2020) investigated the treatment of wastewater derived from cattle slaughterhouses in the northern region of Brazil (state of Rondônia). They used a two-stage anoxic-aerobic biological system followed by UV-C disinfection to improve nitrogen and organic matter removal. Through the addition of external COD in the form of ethanol, they achieved a maximum total nitrogen removal of 90% with a load of 0.28 kg of Nitrogen/m3/d.

Palomares-Rodríguez *et al*. (2017) provided economic and energy-related justification for combining aerobic and anaerobic treatment. Their proposal demonstrated a 76% reduction in energy requirements and a 30% decrease in environmental impact.

While aerobic processes prove efficient in breaking down organic pollutants in wastewater, the major drawback remains the excessive production and disposal of sludge. However, the use of NB provides an alternative by reducing sludge production and improving oxygen transfer efficiency in aerobic systems. This is achieved by increasing the count of active bacteria within the floc mass, resulting in faster and more intense breakdown of organic compounds compared to aeration with fine bubbles. The exploration of NB application in aerobic processes is detailed in section2.3.2.2.

2.4.4. Nanobubble application prospect for PSW treatment

NBs have demonstrated promising outcomes in various wastewater applications such as flotation, aeration/oxidation, membrane processes and ozone oxidation enhancement. However, no attention has been given to the application of small-sized bubbles in slaughterhouse wastewater treatment. There is a need for further exploration in this area to integrate NBs into PSW treatment methods. This approach has the potential to offer a sustainable and chemical-free treatment method, enhancing energy efficiency in the process. Hence, this systematic review was conducted with the objective of identifying the gap in the NB application in wastewater treatment and proposing the application of NB in PSW treatment technologies. This section discusses the potential application of NB in PSW treatment.

Despite the existence of abundant literature on the treatment of PSW and the individual applications of NB technology, this review highlights a significant gap in studies focusing on the integration of NB technology specifically for treating PSW. Consequently, this section provides a concise overview of the current treatment methods and technologies for PSW that could be integrated with NB technology. By doing so, the review seeks to underscore the need for more comprehensive research in this area and to draw attention to the potential benefits of combining NB technology with existing PSW treatment methods, highlighting how this innovative approach could enhance treatment efficiency and effectiveness. Therefore, this overview encourages researchers to investigate and enhance the application of NBs in PSW treatment, aiming to address and close the current knowledge gap.

2.4.4.1. Nanobubble Aeration with Enzymes

PSW typically contains substantial amounts of FOG, hindering its effectiveness in biological treatment (Bustillo-Lecompte and Mehvar, 2015). The primary issue arises from the excessive presence of fats and greases, leading to various problems. Firstly, these substances can accumulate on the sludge surface, diminishing the transfer rates of solution substrate to biomass and oxygen to aerobic microorganisms. Secondly, they can inhibit sludge activity and the development of filamentous microbes, affecting the sediment of the sludge and causing biomass losses through bioreactor outflows (Musa and Idrus, 2021). Hence, a pretreatment process becomes essential to hydrolyse fats and greases and enhance the efficiency of subsequent biological treatment of PSW.

Enzymes are used in the hydrolysis of fats and greases in wastewaters such as PSW. Enzymes function as biocatalysts and have demonstrated efficacy in breaking down and transforming complex triglycerides into simpler free fatty acids (FFAs) (Affes *et al*., 2017). This enzymatic approach enhances the performance of microorganisms in subsequent biological treatment processes, as indicated by Jamie *et al*. (2016). Eco-flush, a bioremediation agent commercially produced by Ergofito and distributed in South Africa through Mavu Bio-technologies, 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 FOGs (Ergofito, 2024; Meyo *et al*., 2021). The natural ingredients in eco-flush are sourced from glaucids and essential amino acids, forming potent decomposing agents that stimulate specific bacteria to naturally produce enzymes. These enzymes have the capability to break down the hydrocarbon chains present in FOG.

A research investigation conducted by Mdladla *et al*. (2021) involved the application of an Ecoflush bioremediation agent for pre-treatment, revealing removal percentages ranging from 50 to 96% for TSS, 30 to 76% for COD, and 48 to 96% for FOG prior to anaerobic treatment with an EGSB reactor. Similarly, Dyosile *et al*. (2021) conducted a study on pre-treating PSW, resulting in removal of FOG up to 80%, along with TSS and COD average removal rates of 38% and 56%, respectively, prior to introducing PSW into the anaerobic digester. These studies represent a few of the limited reports on the application of the Ecoflush reagent. The noted efficacy in removal underscores the considerable promise of bioremediation technology as a pre-treatment step for high-fat content wastewater like PSW.

Aeration is required to induce the production of enzymes necessary for breaking down FOG. The utilization of NBs can enhance the oxygen transfer efficiency of enzymatic treatment of PSW. This is achieved by increasing the number of active bacteria, resulting in the acceleration and intensification of hydrolysis of fats and greases in wastewater.

2.4.4.2. Nanobubble aeration with Ozone

The efficiency of ozone in treating wastewater contaminated with organics is constrained by its slow dissolution rate and rapid decomposition in the aqueous phase. NBs present a novel approach to extend the reactivity of ozone in the aqueous phase, thereby expediting the treatment of contaminants. Nano Ozone bubbles, as discussed earlier in this review, exhibit longer lifespans and higher specific areas compared to ordinary bubbles. This characteristic enables them to efficiently eradicate pathogens, highlighting significant potential for treatment of wastewater such as PSW. The treatment efficiency of ozone-NBs requires investigation. It is hypothesized that ozone-NBs, with their remediation efficiency of organics-contaminated wastewater will present impressive or significant results in treating PSW.

2.4.4.3. Aerobic treatment of PSW with nanobubbles

In aerobic systems, aerobic bacteria are responsible for removing organic materials in the presence of oxygen. Aerobic treatment is typically employed for final decontamination and nutrient removal following physicochemical or anaerobic methods (Gutu *et al.*, 2021). Common configurations for the aerobic treatment of PSW include activated sludge (AS), rotating biological contactors (RBCs), aerobic sequencing batch reactors (SBR), and the moving bed biofilm reactor (MBBR). These aerobic systems have been widely used for the treatment of PSW due to their simplicity to operate and excellent removal efficiencies of pollutants (Drewnowski *et al.*, 2019). For instance, Koide *et al.* (2018) found that ASBR, operating in 6-hr cycles, achieved removal efficiencies of 95% for COD, 98% for TP, and 97% for TN. 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. Their 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 ammonia removal of 65.8%.

However, the production of sludge and the high energy requirements for aeration make their operation costly and less viable (Huggins *et al.*, 2013). Therefore, these aerobic systems could be integrated with NB technology for the treatment of PSW to reduce the sludge production and high energy requirement for aeration. As discussed in section 2.3.2.2, NBs enhance wastewater aeration by significantly improving oxygen transfer efficiency due to their high surface area-to-volume ratio and prolonged stability in water.

2.5. Literature review summary and recommendations

This literature review has highlighted the efficacy of NB technology in wastewater treatment, capitalising on distinctive bubble characteristics such as small size, slow rising velocity, negatively charged ZP, stability, and the ability to generate free radicals. NB applications in wastewater treatment demonstrate heightened mass transfer rates, facilitating efficient treatment with air, oxygen, and ozone. Numerous studies across different wastewater sources validate the enhanced mass transfer achieved through stabilized small-sized bubbles and have been proven successful in removing a spectrum of contaminants in wastewater. However, some wastewaters, like poultry wastewater, necessitate further investigation into their treatment using NB technology. NBs could potentially be applied with enzymes, ozone, or aeration for enhanced poultry wastewater treatment. These treatment methods require further investigation to study their efficacy. Furthermore, NBs present an environmentally friendly approach to wastewater treatment through the generation of free radicals, offering potential replacements for current expensive treatment processes. A comprehensive examination of related costs and energy consumption is essential for a thorough understanding of the wastewater treatment process. Overall, NBs exhibit great potential for novel applications and continued exploration.

Based on the conclusions drawn and the information provided in the literature, the following suggestions for future research are proposed:

a. Exploration of novel applications: This review highlighted the effectiveness of NB technology in various wastewater treatment processes, including flotation, aeration, physicochemical treatment, and ozone oxidation. Future studies can explore novel applications of NBs in treating specific types of wastewaters, such as PSW.
b. Optimisation of operating conditions: Research is needed to optimise the operating conditions such as pH, temperature, DO, aeration time and pollutants levels in PSW on the NB performance in treating PSW. Understanding the influence of these parameters on treatment efficiency and energy consumption can lead to more sustainable and cost-effective treatment solutions for PSW.
c. Integration with advanced treatment methods: NB technology can be integrated with other advanced treatment methods, such as membrane filtration, floatation, and advanced oxidation processes. Future studies can investigate the synergistic effects of combining NBs with these techniques to enhance pollutant removal efficiency.
d. Economic assessments: Future studies should include comprehensive assessments of NB technology compared to conventional treatment methods. Evaluating factors such as energy consumption and chemical usage can help identify the economic benefits of NB-based treatment for PSW.

By addressing these perspectives in future studies and developments, researchers can advance the knowledge and application of NB technology for PSW treatment, ultimately contributing to improved water quality, environmental sustainability, and public health.

CHAPTER 3

METHODOLOGY, RESULTS AND DISCUSSION

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CHAPTER 3: METHODOLOGY, RESULTS AND DISCUSSION

3.1. 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 & 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 (Philipp *et al.* 2021; Gutu *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 3.1 shows typical conventional aerobic processes for 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%.

Table 3.1: Poultry slaughterhouse wastewater treatment using conventional 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 and Thivyatharsan, 2018.
Biological pre-treatment of PSW with Enzymes (Ecoflush) using macrobubbles	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.

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). 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 enhances the liquid-gas contact area, facilitating 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 wastewaters. In their study, Leyva and Flores (2018) demonstrated a reduction of 79.0% in total suspended solids (TSS) and 85.0% in COD in sugar sector wastewater in under 90 min. Another study by Reyes and Flores (2017) reported a 66.21% removal efficiency for total coliforms. Additionally, Wang and 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.

Despite the unique properties of NBs and their promising outcomes in various wastewater applications such as flotation, aeration/oxidation, membrane processes and ozone oxidation enhancement, no attention has been given to the application of NBs in the treatment of PSW. Therefore, this study focused on the integration of NB technology to enhance pollutant removal from PSW. The primary aim was to assess the effectiveness of NB aeration in treating PSW.

3.2. Materials and methods

3.2.1. Poultry Slaughterhouse Wastewater 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 are presented in Table 3.2. The PSW composition in this study aligns with the ranges reported in previous studies. 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.

Table 3.2: Composition of poultry slaughterhouse wastewater vs the South African Water Act General Discharge limits

Parameter	PSW composition from literature (Ngobeni *et al.,* 2022; Njoya *et al.,* 2019; Yaakob *et al.,* 2018)	PSW composition for this study	General discharge limits as set in the SA National Water Act 36 of 1998
pH at 25ºC	6.1– 8.0	6.7 – 7.1	5.5 – 7.5
COD (mg/L)	2133 – 12490	2000 – 3600	75
TSS (mg/L)	405 – 8319	516 – 718	25
FOGs (mg/L)	280 – 1668	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

3.2.2. Experimental setup

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

a. Phase 1: Treatment with air-nanobubbles (air-NB)
b. Phase 2: Treatment with ozone-nanobubbles (ozone-NB)
c. Phase 3: Treatment with air-nanobubbles (air-NB) 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 3 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 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^{TM} 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).

3.2.2.1. Phase 1: Treatment of PSW using air-nanobubble

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. Figure 3.1 illustrates the experimental setup of the MK3 NanoBubbler™ system using air-NBs for PSW treatment.

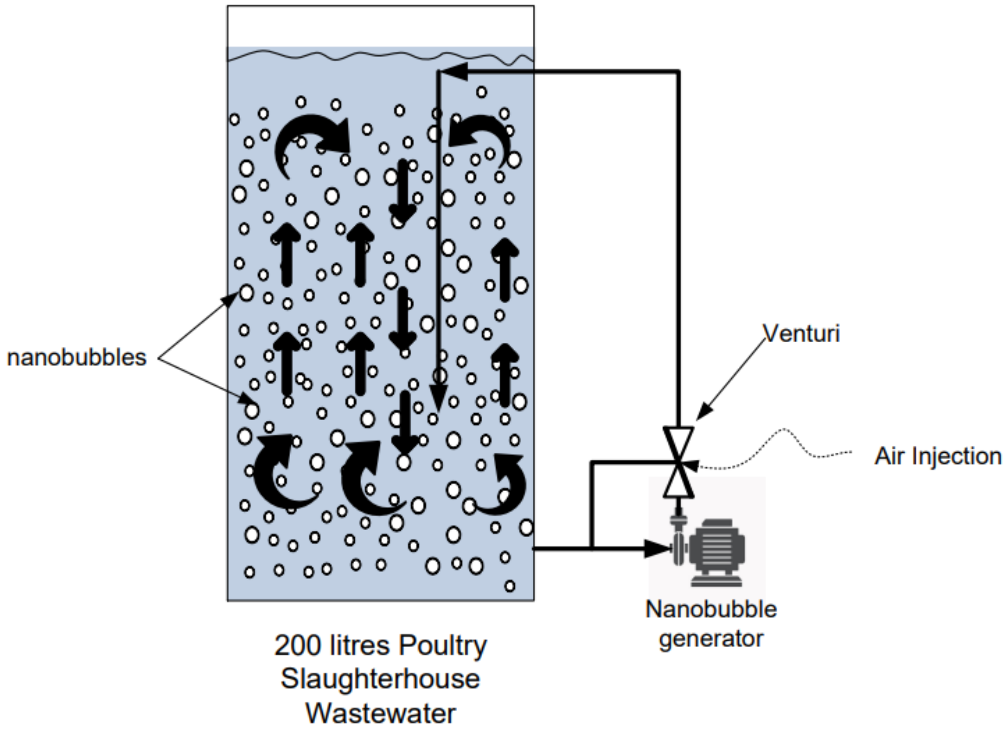

Figure 3.1: Phase 1: Air-nanobubble treatment.

3.2.2.2. Phase 2: Treatment of PSW using ozone-nanobubble.

An ozone generator 0Z–3G was used to produce ozone and the ozone was injected at 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 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. Figure 3.2 illustrates the experimental setup of the MK3 NanoBubbler™ system using ozone-NBs for PSW treatment.

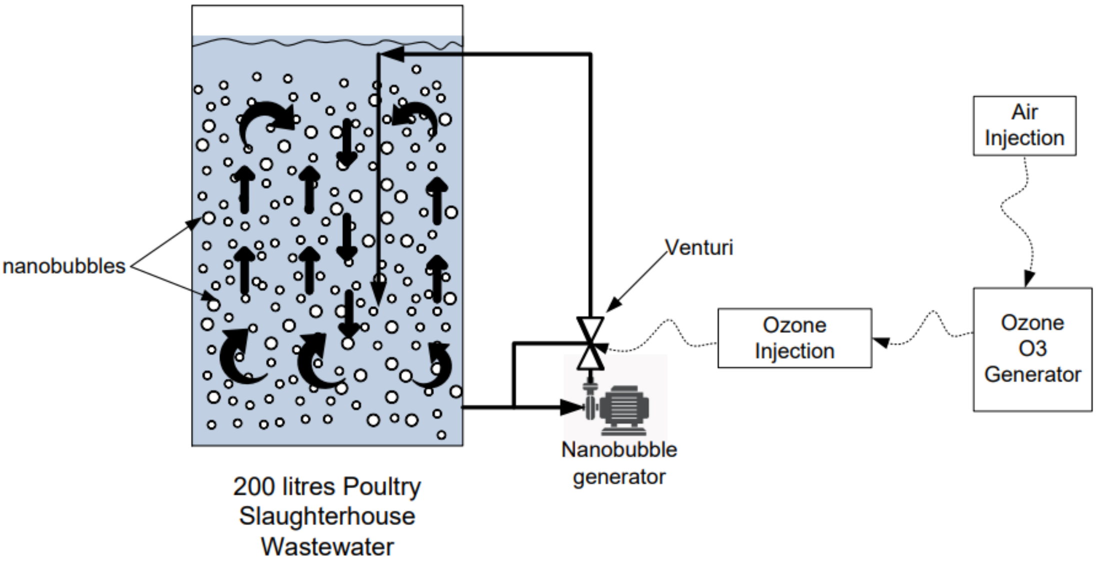

Figure 3.2: Phase 2: Ozone–nanobubble treatment

3.2.2.3. Phase 3: Treatment of PSW with air-nanobubble 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, promoting optimal proliferation of aerobic bacteria in the Ecoflush^{TM}.

Eco-flush 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.

Figure 3.3 illustrates the experimental setup of the MK3 NanoBubbler™ system using air-NBs in combination with Enzymes for PSW treatment.

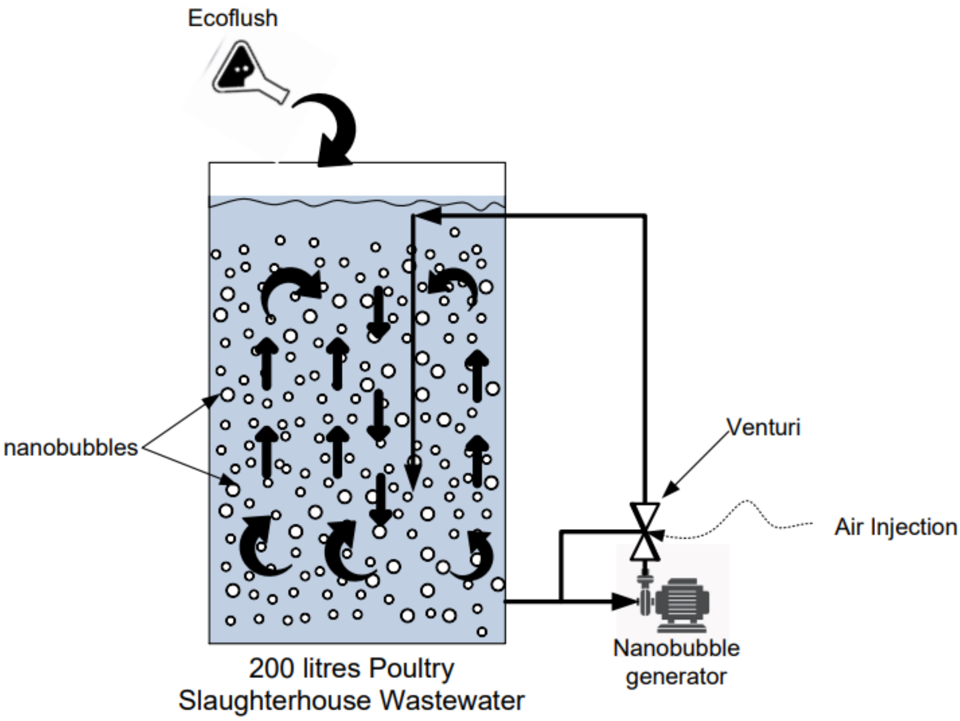

Figure 3.3: Phase 3: Air-nanobubble treatment combined with enzymes

3.2.3. Sampling and analysis

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

3.3. Results and discussion

3.3.1. Performance of air-nanobubble treatment

Understanding the removal mechanisms of organics and nutrients by air-NB is crucial to direct future technology development and implementation. In this phase 1, the performance of air-NB in treating PSW was evaluated, and the results are presented in Figure 3.4. 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_3-N 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 increased the concentration of ammonia (Purwono *et al.* 2017).

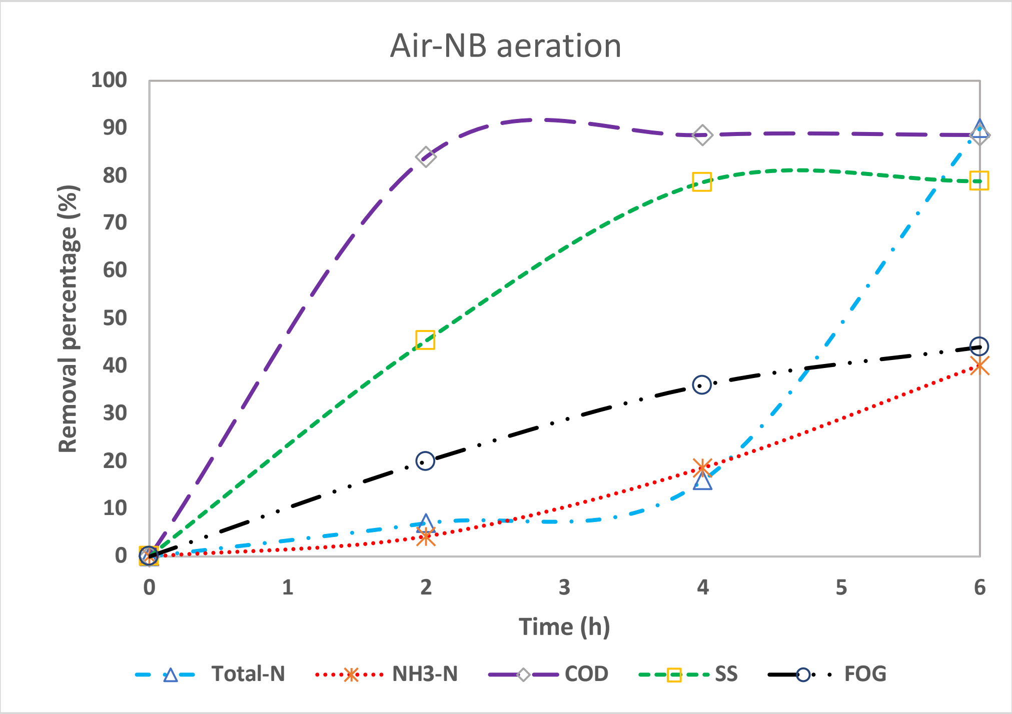

Figure 3.4: Performance of Air-nanobubble

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 28% reduction in TSS, 26% decrease in COD, 43% decrease in BOD_5, 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 3.3, the results of air-NB treatment after 6 h compared to the standard discharge limits outlined in the National Water Act 36 of 1998, shows that only total-N met the discharge standards. NH_3-N, COD, TSS, and FOG 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.

3.3.2. 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 (Sakr *et al.* 2022; Shangguan *et al.* 2018). 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 and Hu, 2018). In this phase 2, 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 (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 removal of ammonia and total-N by ozone-NB.

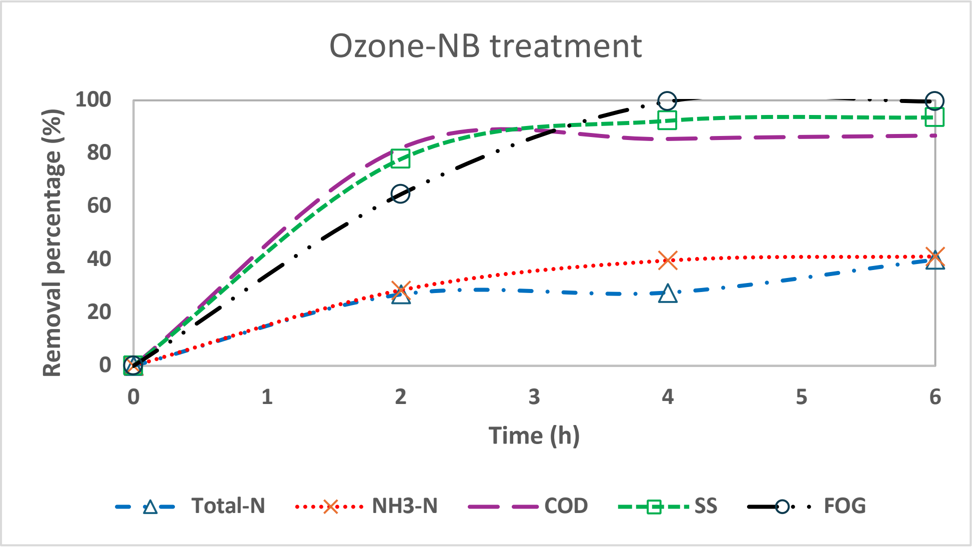

Figure 3.5: Performance of ozone-nanobubble

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.* (2022b) 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 3.3 are compared to the standard discharge limits outlined in the National Water Act 36 of 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.

3.3.3. Performance of air-NB in combination with enzymes (Ecoflush) in treating PSW

Enzymes are used in the hydrolysis of fats and greases in wastewaters 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 (Meyo *et al.* 2021; Mdladla *et al.* 2021). Figure 3.6, 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 enzyme such as Ecoflush. Enzymes combined with NBs achieved 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; Shi 2022; Azevedo *et al.* 2019). The combination of enzymes and NBs creates a synergistic effect, optimising conditions for enzyme action and microbial degradation.

Dlamini *et al.* (2021) used enzymes (Ecoflush) to treat PSW using conventional aeration (macrobubbles). The treatment resulted in an average removal rate of 80 ± 6.3% for FOG, 38 ± 8.4% for COD, and 56 ± 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 to 99%, COD by 20 to 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 macrobubbles aeration.

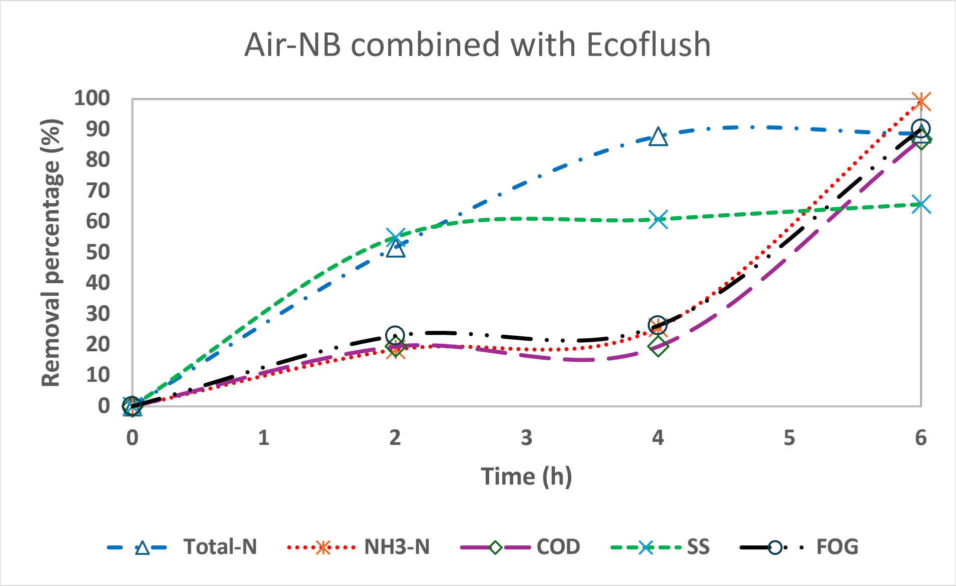

Figure 3.6: Performance of air-nanobubble combined with Ecoflush

The findings presented in Table 3.3 are compared to the standard discharge limits outlined in the National Water Act 36 of 1998. After a 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.

Table 3.3: Nanobubble treatment of PSW vs Discharge limits

	PSW composition after 6 h			General discharge limits as set in the National Water Act 36 of 1998
	Phase 1	Phase 2	Phase 3	
pH	7.6	7.5	5.4	5.5 – 7.5
Total Nitrogen (mg/L)	10.0	87.0	13.0	15.0
NH_3-N (mg/L)	43.6	74.7	0.5	6.0
COD (mg/L)	228.0	292.0	472.0	75.0
TSS (mg/L)	109.0	41.0	246.0	25.0
FOG (mg/L)	56.0	1.0	12.0	2.5

3.3.4. Performance Comparison of the 3 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 3.7. It is evident that ozone-NB treatment reduced coloration 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.

(a): Air- nanobubble treatment

(b): Ozone-nanobubble treatment

(c): Air-nanobubble with enzymes (Ecoflush)

Figure 3.7: PSW before and after 6 h of treatment for (a) air-NB; (b) ozone-NB; and (c) air-NB with enzyme

3.3.4.1. Change in pH and dissolved oxygen during treatment.

Figure 3.8 illustrates the impact of various types of aeration methods on the pH levels of PSW over 6 h periods. 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 acids (Zang *et al.,* 2011). This correlation can be attributed to the relationship between oxidation-reduction characteristics and 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 the Ecoflush solution. Additionally, Figure 3.9 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 mg/L 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.

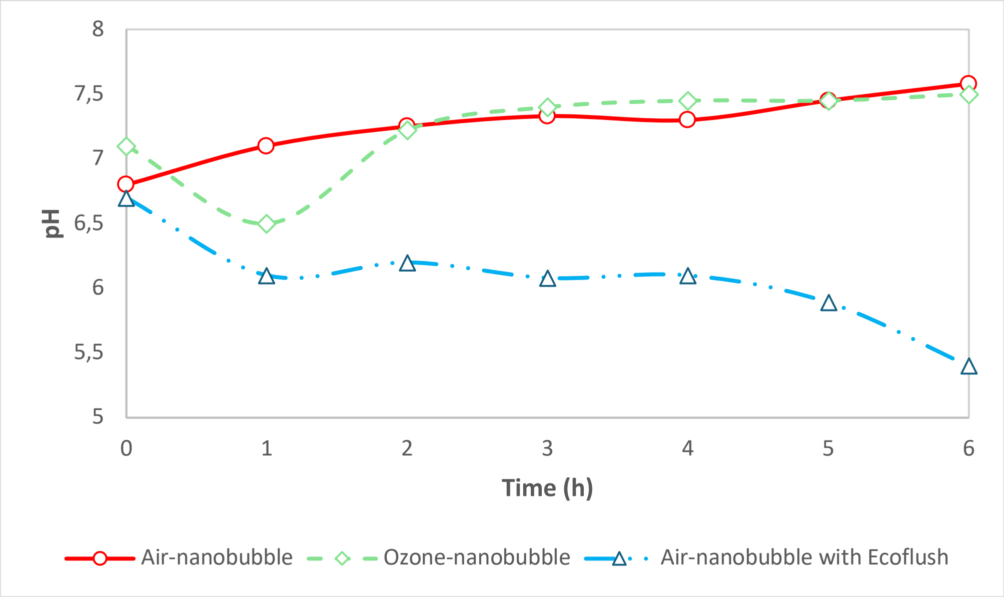

Figure 3.8: pH variation

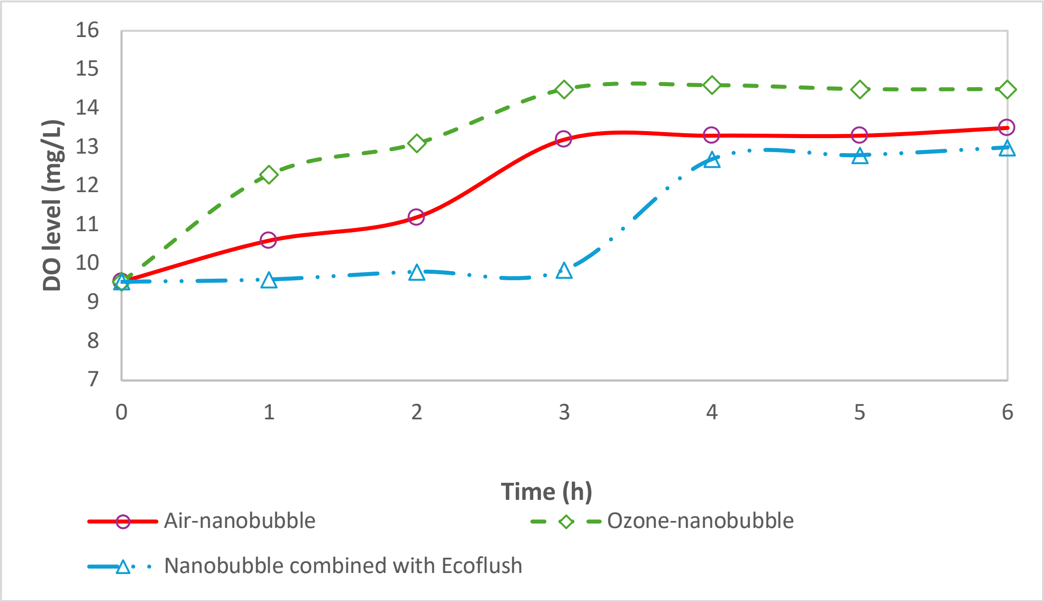

Figure 3.9: Dissolved oxygen variation.

3.3.4.2. Chemical Oxygen Demand (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 3.10 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 Granular Activated Sludge - Sequencing Batch Reactor (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).

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. Firstly 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).

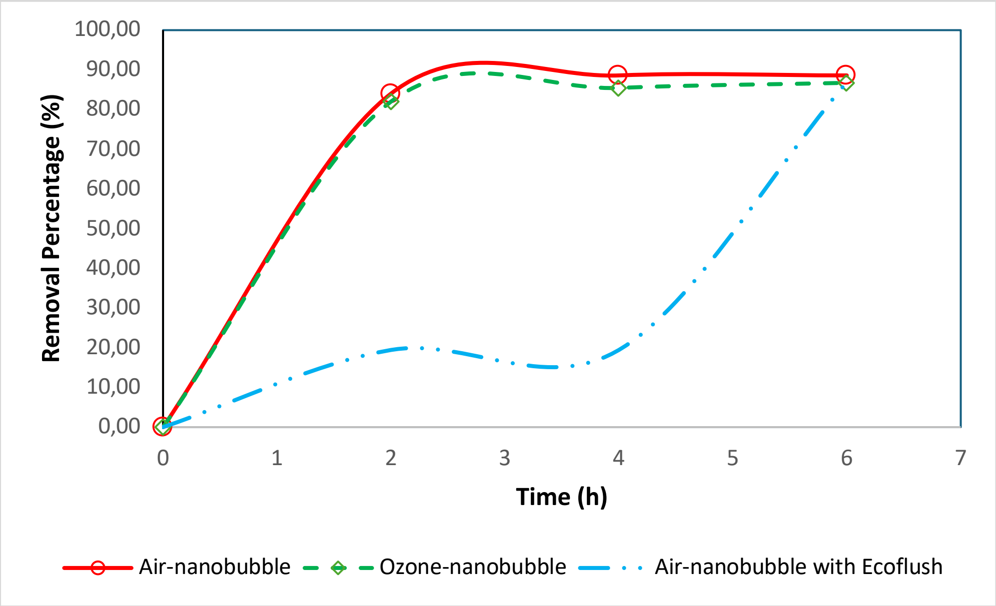

Figure 3.10: COD removal for the 3 aeration methods

3.3.4.3. Total Suspended Solids (TSS) removal

Analysing the distribution of TSS serves as a common approach to evaluate 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 dissolved oxygen levels.

The data presented in Figure 3.11 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.

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%.

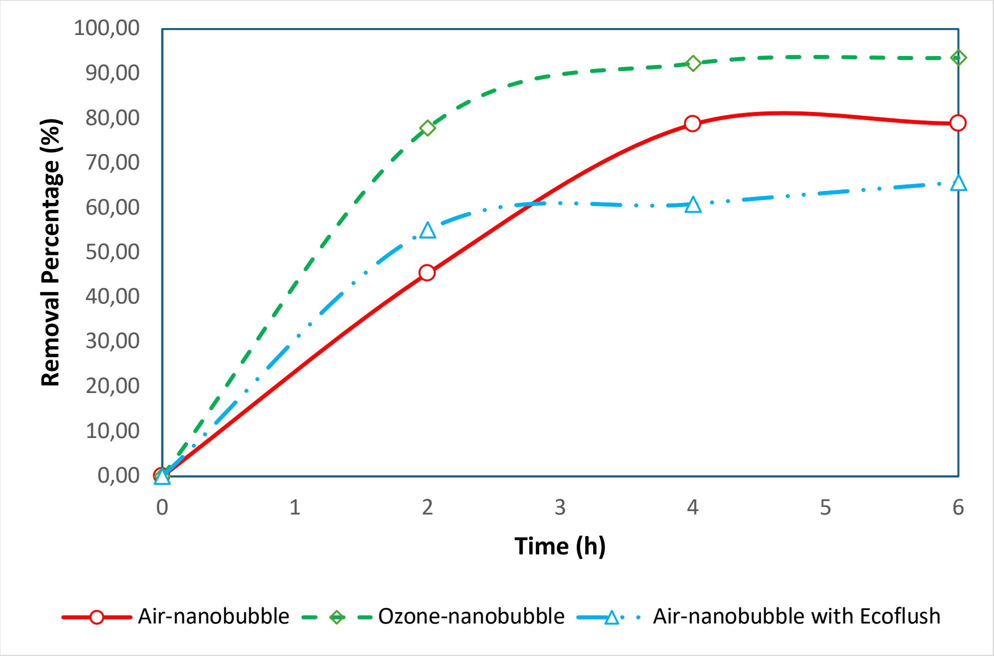

Figure 3.11: Total Suspended Solids removal for the 3 aeration methods

3.3.4.4. 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 3.12 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.

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.* (2020a) explored the oxygenation and concurrent regulation of nitrogen and phosphorus release at the sediment-water interface using oxygen-NB modified material (ONBMM), resulting in notable reductions in NH_3-N, and TN by 96.4%, 51.1%, and 24.9%, respectively.

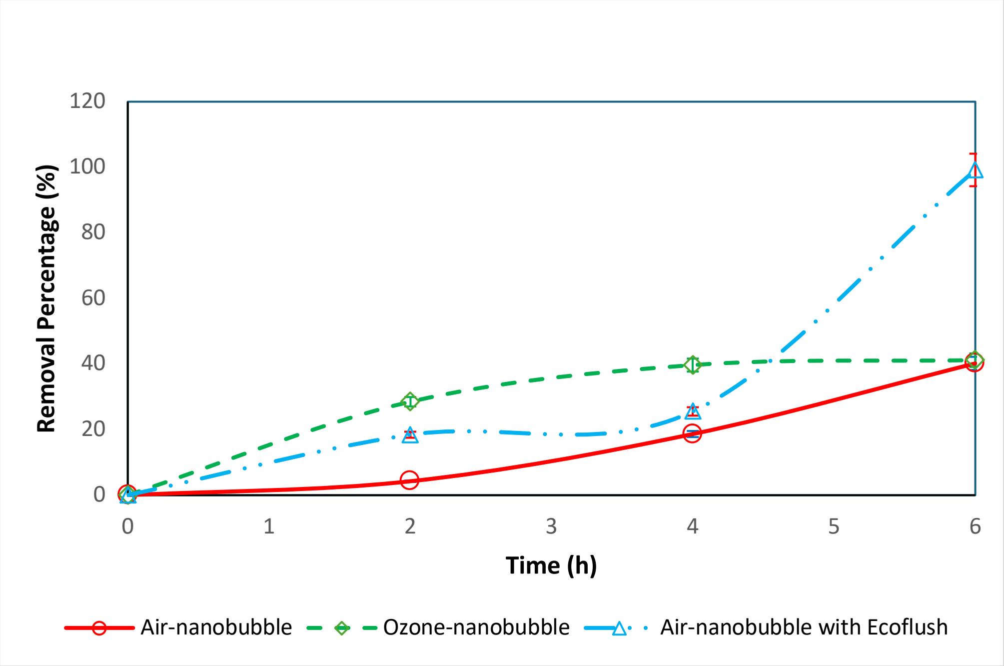

Figure 3.12: Ammonia removal for the 3 aeration methods

3.3.4.5. FOG removal

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 3.*13*, 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 3.13, 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 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.

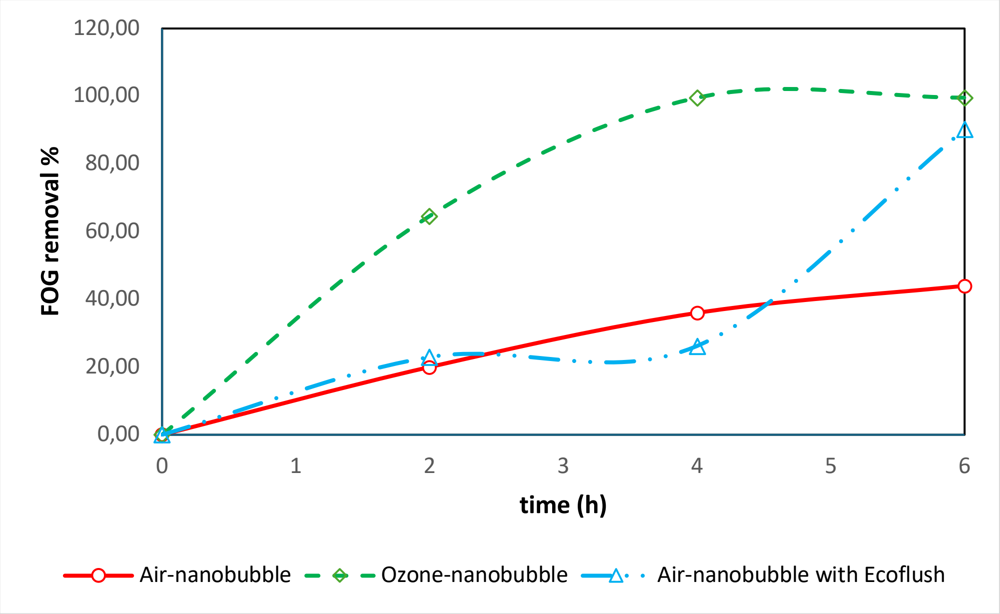

Figure 3.13: FOG removal for the 3 aeration methods

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

This study demonstrates the efficacy of NB technology treating of PSW, particularly in removing pollutants such as COD, TSS, NH_3-N and FOG. In the first phase, air-NB treatment achieved high removal rates for COD (90.0%), TSS (78.8%), and total-N (78.8%) after 6 h of aeration, with COD and TSS showing notable improvements within 2 h. Despite these successes, the removal rates for FOG (44.0%) and NH_3-N (40.2%) were relatively low, necessitating further optimization. The high removal efficiency for total-N, compared to NH_3-N, is due to simultaneous nitrification and denitrification processes.

In the second phase, ozone-NBs aeration resulted in significant removal rates of 86.7% for COD, 93.5% for TSS, and 99.5% for FOG. However, the low removal efficiency of 40.0% for total-N and 41.2% for NH_3-N was attributed to the production of intermediate nitrogen compounds and competing reactions in high pH environments. Nonetheless, only FOG met the discharge standards set by the National Water Act 36 of 1998, indicating the need for extended aeration to meet regulatory requirements. In the third phase, combining air-NB with enzymes (Ecoflush) resulted in higher removal rates for NH_3-N and FOG, significantly outperforming traditional conventional (macrobubble) aeration methods.

Comparing the three aeration methods, ozone-NBs were most effective in reducing TSS, achieving a 93.5% removal rate, while air-NB combined with Ecoflush was most effective for NH_3-N, reaching a 99% removal rate after 6 hr. Notably, the pH and dissolved oxygen (DO) levels varied across the treatments, with ozone-NB treatment showing the highest increase in DO, indicative of efficient gas-liquid mass transfer. However, further optimization of pH is necessary for improved nitrogen removal in ozone-NB treatments.

Overall, the findings suggest that NB technology, particularly when coupled with enzyme treatments, holds significant promise for efficient PSW treatment. The superior performance of NBs indicates their potential to improve the removal of organic compounds and nutrients from wastewater. However, due to the complexity of pollutants and varying environmental conditions, further research is necessary to ensure successful application in PSW treatment plants.

4.2. Recommendations and future studies

To optimise the treatment of PSW using NB technology, it is recommended to extend and optimise the aeration time for NBs to enhance the removal rates of FOG and ammonia. Integrating NBs with ozone and enzyme treatments (such as Ecoflush) should be pursued for improved pollutant removal, particularly for ammonia and FOG. A thorough scalability study and cost-benefit analysis are essential for large-scale applications, evaluating operational costs and potential savings. Hybrid treatment systems combining NBs with conventional methods can leverage the strengths of both technologies.

Future studies should focus on:

- Long-term performance evaluations to assess sustainability and consistency.
- The effects of different gas compositions in nanobubbles on treatment efficiency.
- The impact of nanobubble treatment on microbial community dynamics.
- Developing guidelines for the practical implementation of NB technology in various wastewater treatment scenarios.

In conclusion, NB technology, with its promising pollutant removal capabilities and potential for sustainable wastewater treatment, warrants further investigation and development for broader application in the industry.

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APPENDICES

RAW DATA

		Air-nanobubble with Ecoflush			
		Raw PSW	After 2 h of aeration	After 4 h of aeration	After 6 h of aeration
Sample	**Unit**	EF-Raw PSW	EF-Treated PSW 01	EF-Treated PSW 02	EF-Treated PSW 03
Time	**(h)**	0	2	4	6
pH		6,7	6,2	6,1	5,4

DO	**Mg/L**	9.5	9.8	12.7	13
Total Nitrogen	Conc (mg/L)	116	56	14	13
	Removal %	**0**	**51,72**	**87,93**	**88,79**
NH3-N	Conc (mg/L)	59,1	48,2	44	0,48
	Removal %	**0**	**18,44**	**25,55**	**99,19**
COD	Conc (mg/L)	3600	2900	2900	472
	Removal %	**0**	**19,44**	**19,44**	**86,89**
TSS	Conc (mg/L)	718	323	281	246
	Removal %	**0**	**55,01**	**60,86**	**65,74**
FOG	Conc (mg/L)	122	94	90	12
	Removal %	**0**	**22,95**	**26,23**	**90,16**

		Air-nanobubble			
		Raw PSW	After 2 h of aeration	After 4 h of aeration	After 6 h of aeration
Sample		NB-Raw PSW	NB-Treated PSW 01	NB-Treated PSW 02	NB-Treated PSW 03
Time (h)		0	2	4	6
pH		**6,8**	**7,25**	**7,3**	**7,58**
DO	**mg/L**	**9.5**	**11.2**	**13.3**	**13.5**
Total Nitrogen	Conc (mg/L)	100	93	84	10
	Removal %	**0**	**7,00**	**16,00**	**90,00**
NH3-N	Conc (mg/L)	72,9	69,8	59,3	43,6
	Removal %	**0**	**4,25**	**18,66**	**40,19**
COD	Conc (mg/L)	2000,00	320,00	228,00	228,00
	Removal %	**0,00**	**84,00**	**88,60**	**88,60**
TSS	Conc (mg/L)	516,00	282,00	110,00	109,00
	Removal %	**0,00**	**45,35**	**78,68**	**78,88**
FOG	Conc (mg/L)	100,00	80,00	64,00	56,00
	Removal %	**0,00**	**20,00**	**36,00**	**44,00**

		Ozone-nanobubble			
		Raw PSW	After 2 h of aeration	After 4 h of aeration	After 6 h of aeration
Sample		OZ-Raw PSW	OZ-Treated PSW 01	OZ-Treated PSW 02	OZ-Treated PSW 03
Time (h)		0	2	4	6
pH	**mg/L**	**7,1**	**7,22**	**7,45**	**7,5**
DO		**9.5**	**13.1**	**14.6**	**14.5**
Total Nitrogen	Conc (mg/L)	145	106	105	87
NH3-N	**Removal %**	**0**	**26,90**	**27,59**	**40,00**
	Conc (mg/L)	127	90,8	76,6	74,7
	Removal %	**0**	**28,50**	**39,69**	**41,18**
COD	Conc (mg/L)	2200	396	320	292
	Removal %	**0**	**82,00**	**85,45**	**86,73**
TSS	Conc (mg/L)	637	141	49	41

	Removal %	**0**	**77,86**	**92,31**	**93,56**
FOG	Conc (mg/L)	226	80	1	1
	Removal %	**0**	**64,60**	**99,56**	**99,56**