

Thesis Report On

**The Efficacy of Nanobubble Generator Technology for Microbial Inactivation in Urban
Lake Water: A Comparative Study of Contamination Profiles**

By

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Thesis report submitted to the Mathematics and Natural Science Department in partial fulfillment
of the requirements for the degree of
Biotechnology

Department of Mathematics and Natural Science
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Declaration

It is hereby declared that

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2. The report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Presence in Water Samples Focusing Inside and Outside Dhaka District” submitted by

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Ethics Statement

This research on the application of nano-bubble technology prototype for waterborne pathogen degradation in Dhaka city's polluted urban waters was conducted with careful consideration of ethical and environmental responsibilities. All experimental procedures were designed to ensure that no harm was caused to the surrounding ecosystems or local communities during water sampling and treatment. The study was primarily focused on environmental water samples, and every precaution was taken to minimize disturbances to natural habitats. Collected data has been handled with integrity, ensuring accuracy, transparency, and the avoidance of any fabrication or manipulation. By adhering to these ethical standards, this research contributes valuable insights into sustainable water purification approaches while safeguarding both human and environmental rights.

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Abstract

The current growing situation of water contamination crisis is the pressing issue for worldwide and most prominently in Bangladesh. Requires the urgency of affordable and efficient purification systems. This research focuses on development of a multi-staged Nano-bubble (NB) technology with a promising solution by generating ultra-fine bubbles less than 250nm capable of releasing reactive oxygen species (ROS), degrading pollutants, and destroying pathogenic microbes in treating polluted surface water collected from Hatirjheel, Banani lake and Aftabnagar Lake water in Dhaka, Bangladesh.

The Assembly of NanoBubble generator begins by choosing high-performing components: a water pump, air compressor, a custom made nano bubble injector, and a nanobubble diffuser (100–500 nm pores). A microcontroller is connected to the flow and water pressure to track system performance. PWM motor drivers and solenoid valves manage air and water flow. The dynamic PID control code changes dynamically control inputs for optimal bubble formation. The system is then mounted with pressure-rated piping and securely fastened. During integration, code is uploaded into the controller, and the setup is calibrated. Air-water ratios are optimized to enhance nanobubble dissolution. Polluted water (10 L) from each water sample was treated for 1 hour, and microbial analysis was performed using Luria Agar (LA), Nutrient Agar (NA), and selective media such as EMB, MacConkey, TCBS and XLD. Dynamic Light Scattering (DLS) analysis was conducted to determine bubble size distribution and stability.

To check the effectiveness of the Nano-bubble generator prototype on microbial population, biological tests were conducted on both treated and untreated water samples from Hatirjheel, Dhaka. In 10 L of water, the generator ran for 1 hour, then treated and untreated samples were diluted in saline to 10^{-4} and 100 μ L from each diluted sample were plated on Luria Agar (LA), Nutrient Agar (NA) media and with dilution at 10^0 , 100 μ L was spread plated on EMB, MacConkey, and XLD media. After 24 hours of incubation, the untreated sample yielded $2.5\text{--}3.5 \times 10^6$ CFU/mL in LA and $7.9\text{--}9.9 \times 10^6$ CFU/mL in NA, and *E. coli*, *Salmonella*, and *Shigella* were identified, showing heavy to moderate growth. In comparison, the treated sample result showed a **significant reduction**, with $0.8\text{--}0.9 \times 10^6$ CFU/mL in LA and $2.8\text{--}3.8 \times 10^6$ CFU/mL in NA and in selective media, noticeably reduced or no growth of pathogens were detected. This represents a maximum **68% reduction in microbial load in LA** and **71.7% reduction in NA**, indicating the potential of nanobubbles inhibiting dangerous pathogens.

Our findings demonstrate the high efficiency in producing nano-sized bubbles with more than 75% of bubbles under 250 nm and significantly lowering microbial contamination in polluted surface water and efficiency of this Nano bubble generator can filter polluted water better than the classic techniques used in Bangladesh.

Keywords: Nano-enabled water purification, Nano bubbles, Reactive oxygen species, Electrostatic adsorption

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List of Acronyms

PCR	Polymerase Chain Reaction
MSA	Mannitol Salt Agar
PDI	Polydispersity Index
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose Agar
DLS	Dynamic Light Scattering
TSI	Triple Sugar Iron
UV	Ultraviolet
ROS	Reactive Oxygen Species
DO	Dissolved Oxygen
CFU	Colony Forming Unit

Glossary

- **Polymerase Chain Reaction (PCR):** A molecular biology technique used to amplify specific DNA fragments, allowing detection and identification of microorganisms through targeted genetic markers.
- **Mannitol Salt Agar (MSA):** A selective and differential culture medium designed to isolate *Staphylococcus* species based on their ability to ferment mannitol in a high-salt environment.
- **Polydispersity Index (PDI):** A dimensionless measure representing the distribution uniformity of particle sizes within a sample; lower values indicate more homogeneous nanoparticles.
- **Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar:** A selective medium used for isolating *Vibrio* species, particularly *Vibrio cholerae* and *Vibrio parahaemolyticus*, from environmental or clinical samples.
- **Dynamic Light Scattering (DLS):** An analytical technique used to determine particle size distribution and zeta potential of Nano bubbles or nanoparticles based on light scattering patterns.
- **Triple Sugar Iron (TSI) Test:** A differential biochemical test used to identify bacteria based on carbohydrate fermentation and hydrogen sulfide production in a slanted agar medium.
- **Reactive Oxygen Species (ROS):** Highly reactive oxygen-containing molecules (e.g., hydroxyl radicals, superoxide anions) generated during Nano bubble collapse, contributing to microbial inactivation.
- **Dissolved Oxygen (DO):** The concentration of oxygen molecules dissolved in water, crucial for evaluating water quality and microbial activity.
- **Colony Forming Unit (CFU):** A measure used to estimate the number of viable microorganisms in a sample, based on colonies formed on agar plates.
- **Venturi Injector:** A device that creates a pressure differential by constricting fluid flow through a narrow passage, facilitating gas–liquid mixing for Nano bubble generation.
- **Zeta Potential:** An electro kinetic potential indicating the stability of colloidal or nanoparticle suspensions; higher absolute values suggest greater stability.
- **Hydrodynamic Diameter:** The effective diameter of a particle in suspension, representing its diffusion behavior as measured by DLS.
- **Laminar Flow Cabinet:** A sterile workspace used in microbiological and molecular laboratories to prevent contamination during sample handling and media preparation.

Chapter 1

Introduction

1.1 Water Quality of Dhaka City and Rural Bangladesh

Several water bodies in Dhaka City including Hatirjheel, Banani Lake, Aftabnagar Lake, Dhanmondi Lake, Crescent Lake, Gulshan Lake, Uttara Lake, and Ramna Lake, are highly contaminated. Untreated sewage, industrial effluents, and solid waste are the primary reasons leading to the elevated levels of organic matter, ammonia, and chemical pollutants in these lakes. More importantly, they are the closest to harmful microorganisms that can seriously cause harm to the local's health who are mostly exposed to these waters that are contaminated with pathogens like *Shigella*, *Salmonella*, *E. Coli*, *Vibrio cholerae* and *fecal coliforms*. In Bangladesh's rural areas, the water crisis is even worse. Rural populations are especially susceptible to waterborne illnesses because of the frequent overabundance of heavy metals, microbial contamination, and chemical pollutants in many rural water sources, such as ponds, rivers, and tube wells, according to numerous Department of Environment (DoE) surveys.

In order to address and overcome these obstacles, this study looks into the design and implementation of a nanobubble generator, as well as its physicochemical properties, microbial degradation effectiveness, and profile identification of contaminated water. The research further evaluates the effectiveness of nanobubble treatment on water samples from Dhaka's urban lakes: Hatirjheel, Banani Lake, and Aftabnagar Lake focusing on microbial reduction and comparative analysis of pathogen degradation.

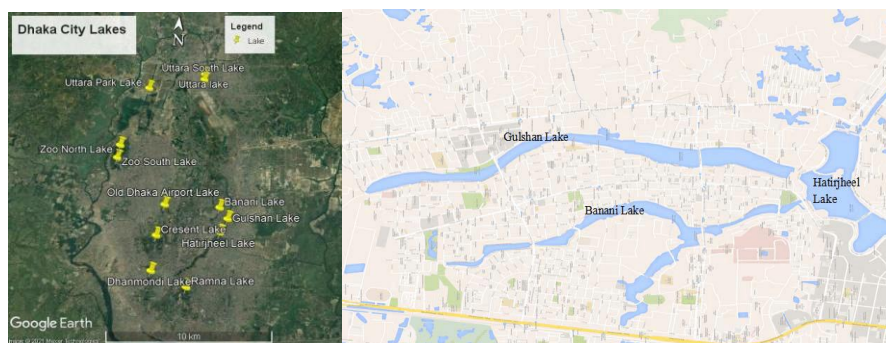


Fig 01: Map of Dhaka city water resources and lakes

1.2 Contamination of Surface and Groundwater in Dhaka

Surface and groundwater resources of Bangladesh are highly affected by toxic heavy metals like chromium and arsenic as well as pesticides and pathogenic microorganisms. Waterborne diseases caused by these contaminants pose a serious public health risk, particularly among children. Domestic and industrial effluents, agricultural runoff, and untreated hospital waste are the major contributors to water pollution. For instance, the concentrations of lead (Pb) are 0.98 mg/L which is significantly higher than the permissible limit of 0.10 mg/L have been reported in the Barisal municipality area. Most of the Dhaka's urban lakes, including Hatirjheel, Banani Lake, and Aftabnagar Lake, are experiencing extreme water quality deterioration due to various pollutants. Furthermore, a study by Huda et al. (2025) demonstrated the range of Chemical Oxygen Demand (COD) values in these lakes from 45 mg/L to 64 mg/L, indicating high levels of organic pollution. The Biological Oxygen Demand (BOD) values in Hatirjheel and Gulshan lakes vary from 1 to 20 mg/L, surpassing the permissible limit of 0.2 mg/L set by the Environmental Conservation Rules (ECR) of Bangladesh. Additionally, heavy metal concentrations in these water bodies have been found to exceed safe limits; lead (Pb) levels range from 1.8 to 3.5 µg/L, and nickel (Ni) levels range from 4.5 to 6.9 µg/L, as reported by Huda et al. (2025). These elevated concentrations pose serious health risks to the urban population.

Another pressing issue for water is microbial contamination. A study by Pasha et al. (2017) assessed the heavy presence of *coliform* bacteria while testing the water quality of Hatirjheel Lake. This presence of fecal coliforms indicates contamination from human and animal waste, posing significant public health risks.

1.3 Microbial Contamination of Surface Water in Dhaka City

Microbial contamination is one of the major problems in Dhaka's water bodies. That involves Hatirjheel, Banani Lake, and Aftabnagar Lake as well. Many research has shown the presence of diverse harmful microorganisms in these waters, causing serious public health threats. Total viable bacteria and fungi levels of up to 2.0×10^6 CFU/mL and 3.4×10^2 CFU/mL was found in water samples from Hatirjheel Lake demonstrated in a study by Miah et al. (2016). Specific pathogens such as *Klebsiella spp.* (3.0×10^2 CFU/mL), *Salmonella spp.* (2.0×10^2 CFU/mL), *Pseudomonas spp.* (8.3×10^2 CFU/mL), and *Vibrio spp.* (6.0×10^2 CFU/mL) were also found in these samples.

These findings show that Hatirjheel Lake has a high level of microbial contamination, posing possible risks of waterborne diseases. Likewise, Shathi et al. (2024) reported the presence of *coliforms*, *E. coli*, and *Salmonella spp.* in samples from Banani Lake and Aftabnagar Lake, indicating fecal pollution and associated health concerns.

1.4 Pathogenic Bacteria in Polluted Water

Fecal coliforms are widely recognized as indicators of contamination from human or animal waste. Their presence suggests that disease-causing organisms such as *Salmonella*, *Shigella*, various viruses, and parasites may also be present. The World Health Organization (WHO) recommends that drinking water contain less than one colony-forming unit (CFU) of fecal coliforms per 100 mL ideally none at all. However, water from tube wells and surface sources like ponds often contains microbial contaminants. A survey of 207 tube-well samples from Comilla, Brahmanbaria, and Sirajgonj showed that 41% were positive for *coliforms*, and 13% contained *Escherichia coli*. Another investigation found that four out of five pond samples were contaminated with fecal coliforms, with concentrations ranging from 1.63×10^4 to 6.48×10^5 CFU/mL.

The polluted lakes and water bodies in Dhaka provide favorable conditions for the growth of waterborne pathogens. *E. coli*, *Salmonella*, *Vibrio*, and *Shigella* are often detected in untreated surface water and inadequately treated drinking water (*Characterization of Vibrio spp.*, 2022). These microorganisms indicate fecal pollution and are linked to outbreaks of gastrointestinal and other infectious diseases. The growing antimicrobial resistance among these pathogens further complicates public health challenges, as it diminishes the effectiveness of standard treatments.

1.5.1 *Escherichia coli*

Escherichia coli is a rod-shaped bacterium belonging to the Enterobacteriaceae family. Although many strains are harmless and naturally found in the intestines of humans and animals but certain pathogenic types can lead to severe illnesses such as hemolytic uremic syndrome, a condition that may result in kidney failure or even death. Among these, Shiga toxin-producing *E. coli* (STEC), also referred to as verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC), releases toxins that damage the intestinal lining, causing diarrhea and contaminating both food and water supplies. In Bangladesh, infections from pathogenic *E. coli* remain a major public health

issue, contributing to diarrheal diseases that affect around 26% of children under the age of five each year (WHO, 2022). Some strains require only a very small infectious dose to cause illness, making outbreaks from contaminated water sources more likely (Ahmed *et al.*, 2019).

1.5.2 *Vibrio cholerae*

Vibrio species are the gram-negative, another rod-shaped bacteria found in both freshwater and marine environments. The common harmful strains are *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio alginolyticus*. Typically, humans get infected by these bacteria via contaminated water or by eating undercooked seafood. *V. cholerae* is the main cause of cholera and causes severe diarrhea by contaminating or growing in common freshwater sources. It spreads through direct contact or by drinking contaminated water. Other non-cholera *Vibrio* species (*V. parahaemolyticus* and *V. vulnificus*) can cause vibriosis leading to gastroenteritis or systemic infections (Baker-Austin *et al.*, 2018). Moreover, cholera affects about 1.3 million people each year Globally and Bangladesh is one of the most affected countries among others (WHO, 2022).

1.5.3 *Klebsiella spp.*

Klebsiella spp. are gram-negative, non-motile, rod-shaped bacteria belonging to the *Enterobacteriaceae* family. These encapsulated opportunistic pathogens are commonly found in soil, water, and on the skin and mucosal surfaces of humans and animals. The genus includes several clinically important species, such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Klebsiella variicola*, which are associated with infections like pneumonia, urinary tract infections, septicemia, and wound infections. According to the World Health Organization (WHO, 2024), *Klebsiella pneumoniae* is among the major causes of antimicrobial-resistant infections, especially in hospital environments and intensive care units. Recent research has reported an increasing presence of *Klebsiella spp.* in contaminated water sources and healthcare facilities, with multidrug-resistant strains emerging as a serious global health issue (Rahman *et al.*, 2023). The persistence of *Klebsiella spp.* in both clinical and community settings underscores its growing threat to public health and emphasizes the urgent need for effective surveillance and control measures.

1.6.1 Fundamentals of Nanobubble

Nanobubbles are tiny gas cavities in a liquid, usually smaller than 200 nanometers in diameter. Their small size leads to differences in stability, movement, and behavior compared to regular bubbles. They have low buoyancy, allowing them to stay suspended in liquids for extended periods, which improves their interaction with the liquid phase. In water treatment systems, the longer residence time and increased surface area of nanobubbles enhance gas-liquid contact. Takahashi et al. (2007) showed that using nanobubbles significantly increases the oxygen transfer rate in water by extending gas-liquid contact time and increasing surface area.

1.6.2 Physicochemical properties of Nanobubbles

Nanobubbles have specific measurable physicochemical properties that influence their stability and behavior in water. Typical zeta (ζ)-potentials for oxygen/air nanobubbles at near-neutral pH are negative, often ranging from about -15 to -35 mV, though literature reports values from roughly -4 to -62 mV, depending on the measurement method, type of gas, salinity, and pH. This negative charge leads to electrostatic repulsion, which reduces bubble merging and helps keep smaller bubble populations stable. Research and extended-DLVO modeling indicate that internal pressure, surface tension, and the ion-rich interfacial layer contribute to their long lifetimes. Controlled studies have documented stable nanobubble populations in deionized or low-ionic media for days, with some reports indicating stability for up to 60 days for N_2/O_2 /air nanobubbles in low-salt conditions. Higher ionic strength or specific gas types, like CO_2 , can decrease this stability.

1.6.3 Functionalities of Nanobubbles

Because of their physicochemical traits, nanobubbles offer several functional benefits in water treatment applications: Firstly, they enhance gas transfer. Due to their very small size and high surface area-to-volume ratio, nanobubbles increase the interfacial contact between gas and liquid, promoting more efficient dissolution of gases. For example, in an experimental study of nanobubble aeration, the oxygen transfer into water was found to be six times higher compared to conventional aeration methods, and soluble Chemical Oxygen Demand (COD) removal increased by $\sim 10\%$. Additionally, nanobubbles have demonstrable, quantified benefits in water- and

wastewater-treatment contexts: systematic reviews and case studies report wide but consistently positive ranges for pollutant removal — Biological Oxygen Demand (BOD)₅ reductions typically reported between ~69% and 100% and Chemical Oxygen Demand (COD) reductions between ~68% and 99% depending on process configuration (aeration, flotation, advanced oxidation) and influent characteristics; individual case studies report Chemical Oxygen Demand (COD) removals up to ~85–95% in optimized systems. Nanobubble-assisted aeration/oxidation has also shown substantial improvements in nutrient removal (e.g., phosphorus removal ~54–60% and very high nitrogen removal efficiencies under some configurations). Microbial inactivation results are gas- and process-dependent: ozone-nanobubbles (NB-O₃) have produced near-complete bacterial reductions (reports of 99.99% reduction within short contact times in lab trials), while air/O₂ nanobubbles often yield appreciable but more modest bacterial reductions (for some matrices reductions of 1–2 log or 70–90% have been reported, whereas other studies — especially with complex biofilms or produce-wash applications — report lower single-pass reductions unless combined with oxidants or surfactants). Meta-analyses and recent reviews therefore emphasize that Nano bubbles can deliver very high BOD/COD and pathogen removals in many real systems, but performance is highly sensitive to gas type (air, O₂, O₃), contact time, water chemistry, and whether nanobubbles are combined with other treatment steps.

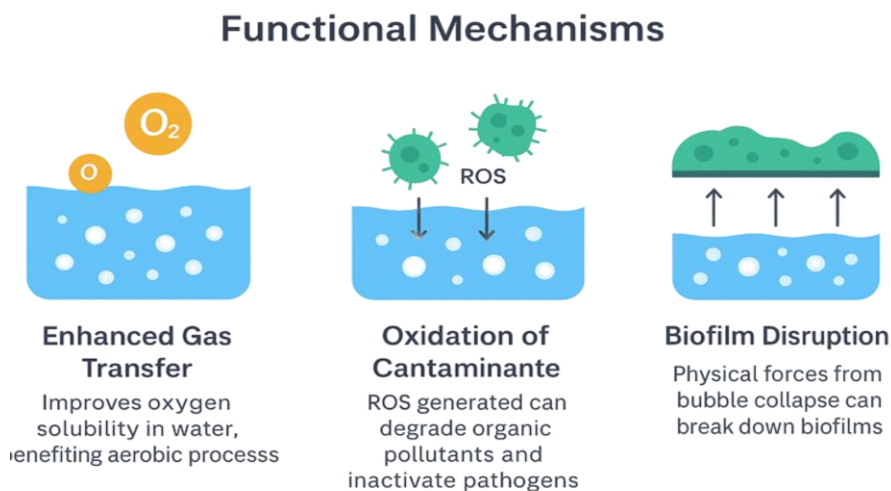


Fig 02: Nano-bubble mechanism in water purification

1.6.4 Nano-Bubble Generator System Architecture

The proposed water treatment system integrates UV-C sterilization with Venturi-based bubble generation in a solar-powered configuration designed for off-grid operation. The system comprises five primary subsystems: power generation and storage, UV pre-treatment, Venturi bubble generation, sensor monitoring, and automated control. Unlike conventional single-mechanism purification, this hybrid approach combines photochemical disinfection with oxygen-enhanced reactive species generation to address both pathogenic and chemical contamination.

1.6.5 Operating Principle

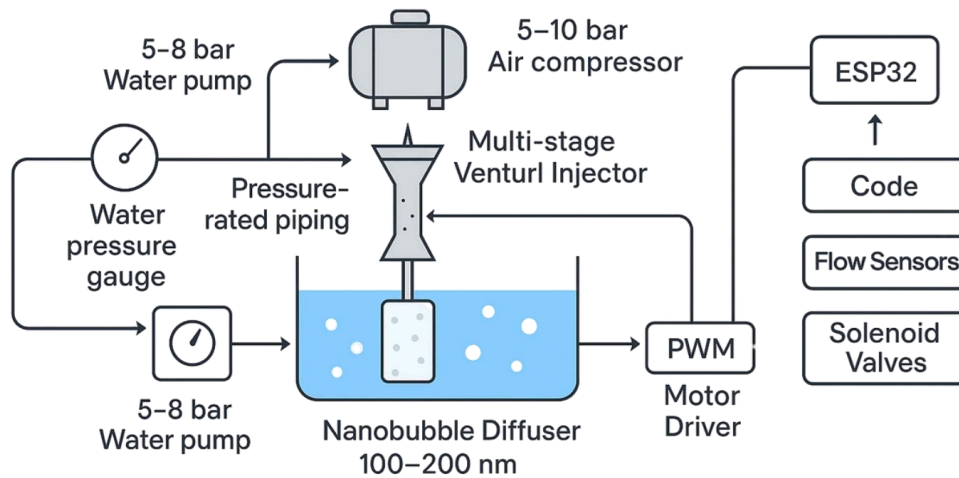


Fig 03: Diagram of Nanobubble generator prototype design

The treatment system works in two main stages. First, the contaminated water enters a UV-C chamber, where it is exposed to a 13-watt germicidal lamp operating at a wavelength of 254 nm. This ultraviolet light acts as the primary disinfectant, destroying microorganisms by causing thymine dimers to form in their DNA, which prevents them from reproducing. After the UV exposure, the water moves into a second chamber containing a Venturi injector with a throat diameter of 6.68 mm and a convergence angle of roughly 15°. In this section, air and water streams mix, generating a wide range of bubble sizes. The Venturi effect produces strong localized zones—around 100 μm in size—where micro and nanobubbles form due to cavitation and shear forces. These extremely small bubbles increase the level of dissolved oxygen in the water and extend the period during which oxidative degradation of residual contaminants can occur.

1.7 Objectives

1. Specifically, the main goal of this study was to compare the isolated bacteria from nanobubble generator treated and direct water samples without any treatment, collected from three different but highly populated places near the lakes of the Dhaka district.
2. To be more precise, the study was intended to check the levels of disease causing bacterial species and their corresponding decline rates after treatment, i.e., *Escherichia coli*, *Vibrio cholerae*, *Klebsiella spp.*, *Salmonella spp.*, etc. in the water sources of the selected areas.
3. Another major priority of this work was to study the physicochemical stability and surface charge behavior of the nanobubbles produced based on the Dynamic Light Scattering (DLS) method. Hydrodynamic diameter, size distribution, and zeta potential of nanobubbles produced for surface water treatment were measured by the Malvern Particle Analyzer machine. Through monitoring these parameters, the study aims at giving an account of stability, dispersibility, and the role of electrostatic repulsion in prolonging the lifetime of nanobubbles in suspension.
4. Lastly, differences in pH & dissolved oxygen level (DO) between the Nanobubble generator-treated and untreated water samples were observed in this research.

Chapter 2

Materials and Methods

2.1 Study Place:

The research project's laboratory work took place in the Biotechnology, Microbiology, Molecular Biology Laboratory, and EEE laboratory which is part of the Mathematics and Natural Sciences Department and EEE Department of BRAC University.

2.2 Study Duration:

The study project spanned from January 2025 through October 2025.

2.3.1 Nanobubble Generator Construction Methodology

Venturi Injector Design and Fluid Dynamics

The Venturi injector represents the core nano bubble formation mechanism. The device features a converging-diverging nozzle geometry with the following specifications: inlet diameter 34.35 mm, minimum throat diameter 6.68 mm, and outlet diameter 24.19 mm. DLS analysis by a Malvern Panalytical ZS XPLOER showed that this geometry indeed creates a bimodal bubble size distribution with a Z-average diameter of 1093 nm and polydispersity index of 0.7813. The primary size peak occurs at 215.7 nm (intensity-weighted), representing the population of fine bubbles around the nano-micro boundary, whereas a secondary peak at 35.8 nm suggests the presence of a smaller fraction of true nanobubbles. The high polydispersity reflects the stochastic nature of bubbles in turbulent flow conditions, where pressure fluctuations and variable shear rates produce bubbles spanning three orders of magnitude—from 10 to more than 1000 nm. The pressure differential across the Venturi throat can be estimated using Bernoulli's equation for incompressible flow:

$$\Delta P = \frac{1}{2} \rho (v_t^2 - v_i^2)$$

ΔP = pressure difference between two points in the fluid (Pa)

ρ = fluid density (kg/m³)

v_t = velocity at the target or downstream point (m/s)

v_i = velocity at the initial or upstream point (m/s)

where ρ is the density of water (1000 kg/m³), v_t is the velocity at the Venturi throat, and v_i is the velocity at the inlet.

Given the pump specificity (0.65 MP_a operating pressure, 5 L/min flow rate), the throat velocity can be approximated as:

$$v_t = Q/A_t = (5 \times 10^{-3} / 60) / \pi (3.34 \times 10^{-3})^2 / 4 = 23.8 \text{ m/s}$$

The Reynolds number in the throat region is:

$$Re = \rho v_t D_t / \mu = 1000 \times 23.8 \times 6.68 \times 10^{-3} / 8.9 \times 10^{-4} \approx 1.79 \times 10^5$$

This highly turbulent flow ($Re \gg 4000$) creates the shear forces necessary for bubble fragmentation, though the relatively large throat diameter limits the minimum achievable bubble size compared to designs with sub-2 mm throats reported in literature.

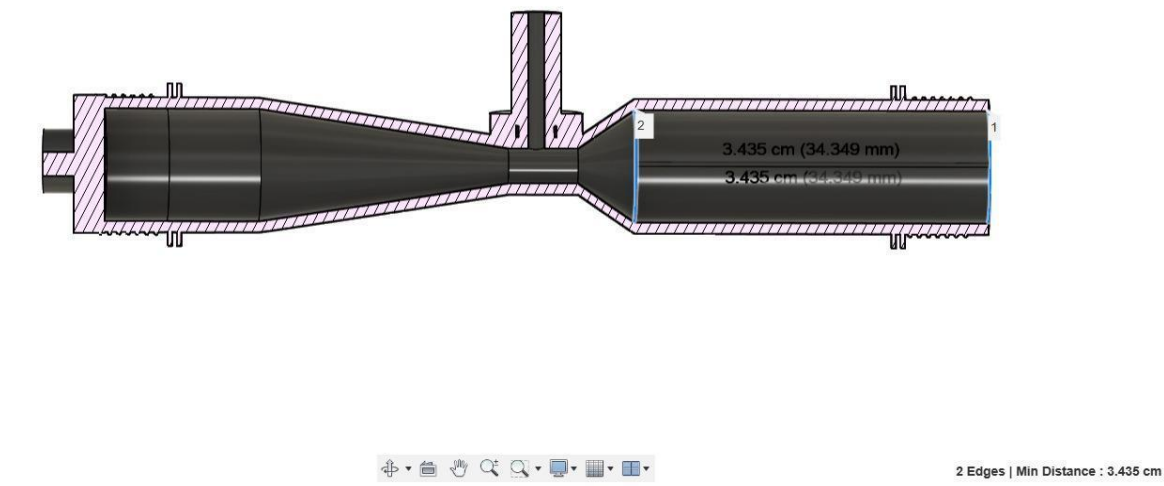


Fig 2.1: Venturi injector 3D design

2.3.2 Power System Design and Energy Analysis

The system operates on a 12 V DC architecture powered by a 40 W monocrystalline solar panel. Energy flows through a 20 A solar charge controller to a 12 V, 12 A_h sealed lead-acid battery, providing 144 W_h of stored energy:

$$E_{\text{bat}} = V \times C = 12\text{V} \times 12\text{A}_h = 144\text{W}_h$$

where:

- E_{bat} = battery energy capacity
- V = voltage (12 V)
- C = capacity (12 A_h)

The primary electrical loads comprise: (1) DC water pump: 72 W (12 V, 6 A), (2) UV lamp via DC-AC inverter: 13 W, (3) ESP32 microcontroller and sensors: 7 W, yielding total system power consumption:

$$P_{\text{total}} = P_{\text{pump}} + P_{\text{UV}} + P_{\text{ctrl}} = 72 + 13 + 7 = 92 \text{ W}$$

The battery allows for about 1.57 hours of runtime under continuous operation:

$$T_{\text{runtime}} = E_{\text{bat}} \div P_{\text{total}}$$

$$t_{\text{runtime}} = 144 \div 92 = 1.57 \text{ hour} \approx 94.2 \text{ minutes}$$

Almost a full 70% conversion efficiency are showed within 5 hours of maximum sunlight and 40 watt of solar panel is able to generate 140 Wh everyday, which is enough to support almost 1.5 operational per charge-discharge cycle. Full battery recharge requires:

$$t_{\text{charge}} = 144 / (40 \times 0.7) = 5.14 \text{ h}$$

The pump hydraulic power output, based on measured operating conditions (0.65 MP_a, 5 L/min), is calculated as:

$$\text{Hydraulic Power; } (P_h) = \Delta P \times Q = (0.65 \times 10^6 \text{ Pa}) \times (5 / (1000 \times 60) \text{ m}^3/\text{s}) = 54.2 \text{ W}$$

With an estimated pump efficiency of 75%, the electrical-to-hydraulic conversion yields:

$$\eta_{\text{pump}} = P_h / P_{\text{elec}} = 54.2 / 72 = 0.75$$

This efficiency is consistent with small centrifugal pumps in this power range.

2.3.3 Sequential Treatment Control Logic

The two-stage treatment process is driven by an ESP32 microcontroller operating on sensor feedback (Espressif Systems, dual-core 240 MHz). The following sequence is implemented through controlled algorithm:

1. UV Pre-Treatment Phase: Activated DC pump draws water into the UV chamber, and the water level inside the chamber is balanced by a sensor mounted in it that detects the completion of filling. Once the sensor threshold is exceeded, immediately 60-second UV gets activated-indicating sufficient water volume for UV exposure-to ensure a minimum 30 mJ/cm² germicidal dose.
2. Venturi Oxidation period: When the oxidation is done, the controller opens the solinoidal velbe pushing water to the venturi section and continuous turns a 6 volt DC air pump. The flow rate of the air is 0.5 L/minute. The pressurized air-water mixed with and passes through the throat of the venturi injector and creates pressurized bubbles. Then the nano-bubble mixed water pass through the 1.2 cm nozzle to collect and reserve.
3. Water Quality Monitoring: pH from 0-14, resolution ± 0.1 ; temperature by DS18B20, with an accuracy of $\pm 0.5^\circ\text{C}$; and humidity sensors log continuously to the internal memory of the ESP32 and broadcast via Wi-Fi to a cloud dashboard for remote monitoring.

The control firmware implements pulse-width modulation of pump speed to provide flow rate adjustment between 2-8 L/min depending on requirements related to water quality and the state of charge of the battery.

2.3.4 Bubble Characterization Methodology

Bubble size distributions were measured by dynamic light scattering (Malvern Panalytical ZS XPLOER, $\lambda = 633$ nm) at 25 °C. Samples were collected immediately after Venturi treatment and transferred to disposable polystyrene cuvettes for analysis. The instrument determines the translational diffusion coefficient, D , from the time-dependent intensity autocorrelation function produced by Brownian motion; the Stokes–Einstein relation,

$$D = kBT / (3\pi\mu D_h); D_h = \text{Hydrodynamic diameter}$$

$D = kBT / (3\pi\mu D_h)$, where D_h is the hydrodynamic diameter, k_B is Boltzmann's constant (1.380649×10^{-23} J/K), T is absolute temperature, μ is dynamic viscosity, and D is the translational diffusion coefficient derived from the correlation function.

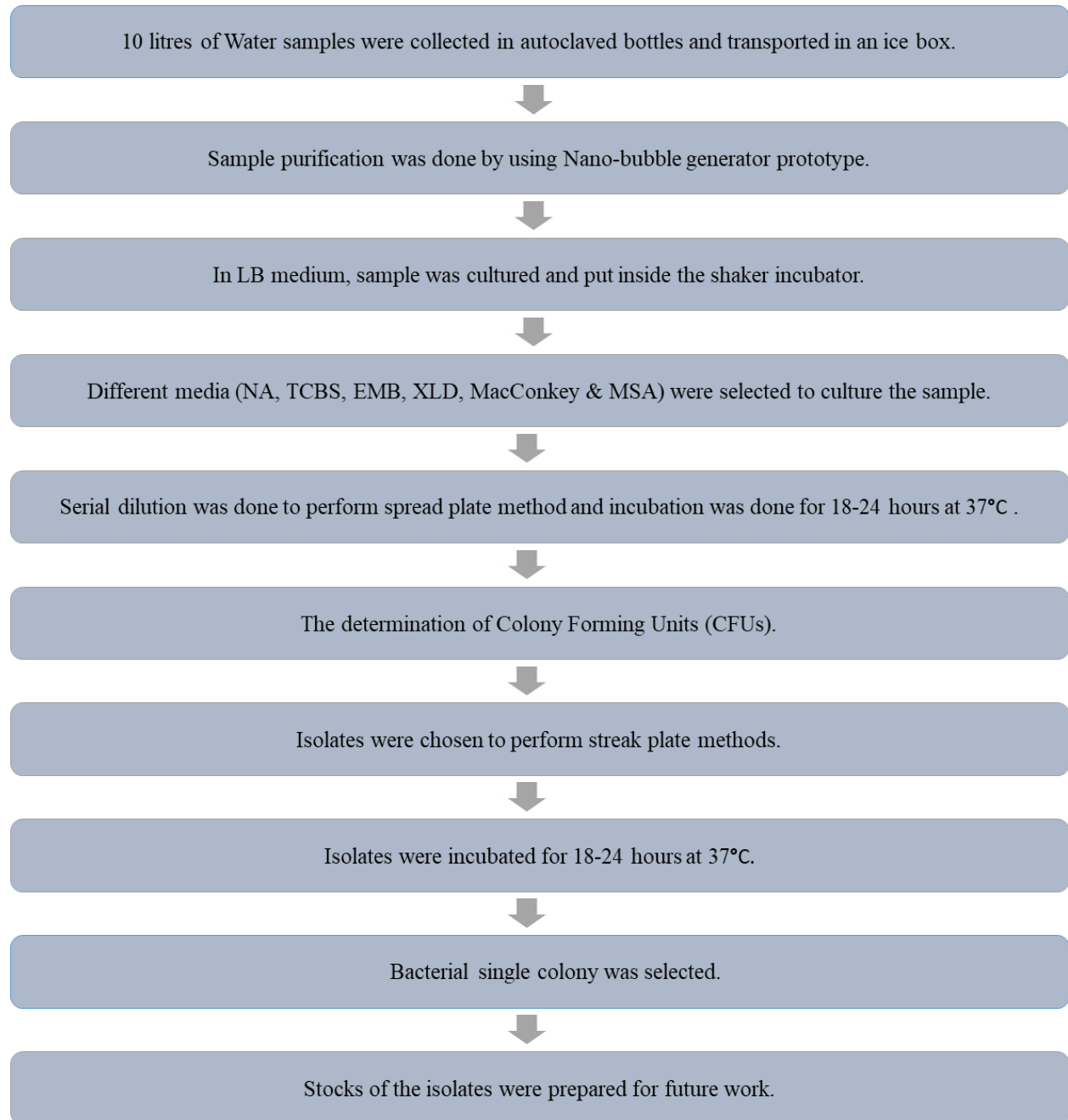
2.3.5

2.3.5 System Integration and Deployment Configuration

The system is designed to be suitable for outdoor conditions, with more weather-resistant components, having an IP54 rating, fully suitable for field deployment. Solar panels are mounted on a hinged frame at a 23° angle, optimized for Bangladesh's latitude but able to be adjusted seasonally for maximum sunlight capture. All fluid connections utilize food-grade PVC tubing with a 12 mm inner diameter capable of supporting up to 1.5 MPa of pressure. The UV treatment chamber consists of a cylindrical quartz sleeve, 10 cm long and 3 cm in diameter, housing the germicidal lamp. This sleeve provides both electrical insulation and very good UV transparency, with over 90% of the 254 nm UV light passing through.

Floating deployments have the system sitting atop a buoyant platform made from recycled HDPE barrels, with the Venturi outlet submerged such that the bubbles are efficiently dissolved into the water. In this configuration, additional sensors including a dissolved oxygen probe and GPS module enable real-time water quality monitoring, making it possible to track treatment effectiveness across large water bodies.

2.4.1 Microbial Experiment Workflow



2.4.2 Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) is a non-invasive machine based technique that is used to determine the hydrodynamic diameter of particles or bubbles in nanoscale size dispersed in liquid. Through Brownian motion more light scatters by nano-particles due to its faster movements and the temporal fluctuations of the scattered intensity were auto correlated to extract the translational diffusion coefficient and then converted to a hydrodynamic diameter by following the Stokes–Einstein equation. DLS was chosen because it was capable of rapidly measuring mean size (Z-average) and the polydispersity index (PDI) of nanobubble populations in aqueous suspension and automated system that accurately follows the equation for accurate results.

Procedure

1. Five liters of deionized (DI) water were measured and collected in a clean container.
2. Then the sterilized DI water was filtered by using a 0.22 μm membrane filter to remove any residual particulates and impurities.
3. Then the nanobubble generator nozzle was placed in the filtered DI water produce nanobubbles under controlled conditions.
4. After the release of moderate level of nanobubble in the DI water was then gently mixed to ensure uniform dispersion and allowed to stabilize before sampling.
5. A clean, dust-free quartz cuvette was rinsed with the filtered DI water and then filled with the nanobubble containing DI water. The cuvette was sealed carefully to prevent contamination and air exposure.
6. The Malvern Particle Analyzer was powered on and allowed to equilibrate to the temperature (25 °C).

7. The sample cuvette was placed in the analyzer to ensure that the outer surface was wiped clean and free from fingerprints or dust.
8. Measurement parameters such as temperature (25 °C), equilibration time (120 s), measurement duration (30-60 s), and scattering angle (90°) were set according to instrument specifications.
9. The measurement was performed, and intensity fluctuations of scattered light were recorded for multiple runs to ensure reproducibility.
10. The DLS software analyzed the autocorrelation data to determine the Z-average particle diameter and the polydispersity index (PI).
11. The final data were saved and exported for analysis. Average bubble size and PI values were reported as mean $\pm$ standard deviation from replicate measurements.



Fig 2.2: Malvern Particle Analyzer for Nano bubble size measurement

2.4.3 Types of Equipment

- Incubator
- Shaker incubator
- Autoclave machine
- Vortex machine
- Microscope
- Laminar air flow cabinet
- Petri dishes, spreader, metallic loop, Bunsen burner
- Glass pipettes, micropipettes, test tubes, and an electric balance
- Glassware, pH meter, Whatman filter paper

2.4.4 Water Sample Collection and Processing

Water samples were collected from three locations in Dhaka: Hatirjheel, Aftabnagar and Banani in the city. 10 liters of water was collected from each site and the water samples were gathered in sterilized, autoclaved bottles with proper labeling on it. For transportation, the bottles were kept in a large ice box to maintain sample integrity and ensure accurate results. Upon arrival, all samples were immediately processed in the Biotechnology laboratory to obtain all the experiments.

2.5 Collection of Bacteria

Before isolating the bacteria from the water samples, pre filtration of each sample was done using a 0.4 mm Whatman filter paper. Afterwards, 2 ml of the filtered water sample was diluted with 8 ml of LB (Luria Broth) medium in an 15ml autoclaved Falcon tube for enrichment. The mixture was then incubated at 37°C in a shaker incubator for four hours to promote optimal bacterial growth. Following pre-incubation, a serial dilution was performed by adding 1 ml of the culture to 9 ml of sterile 0.9% sodium chloride (NaCl) solution. The dilution process was continued across five autoclaved Falcon tubes up to a 10^{-5} dilution. In addition, 100 µl of the original (undiluted) sample was inoculated into the culture medium.

2.6.1 Culture Media Used for Bacterial Isolation

The selection of culture media was based on the microorganisms' type that are being grown and most commonly found in the urban lake water samples along with the available laboratory

resources. Since different bacterial species have distinct nutritional and environmental requirements, it is essential to select media that meet these specific needs (*Technology Networks, 2020; PubMed, 2017*).

A total of five differential and selective media were used throughout this study: TCBS agar, XLD agar, MacConkey agar, MSA agar and nutrient agar to allow for the growth of different groups of bacteria and for overall representation of the range of bacterial presence. The samples were plated on these media using the spread and streak plate method after serial dilution and incubated at 37°C for 18–24 hours. At 24 hours of incubation, colony-forming units (CFUs) were counted for each dilution, and distinct bacterial colonies were purified once more by the streak plate method on the same media.

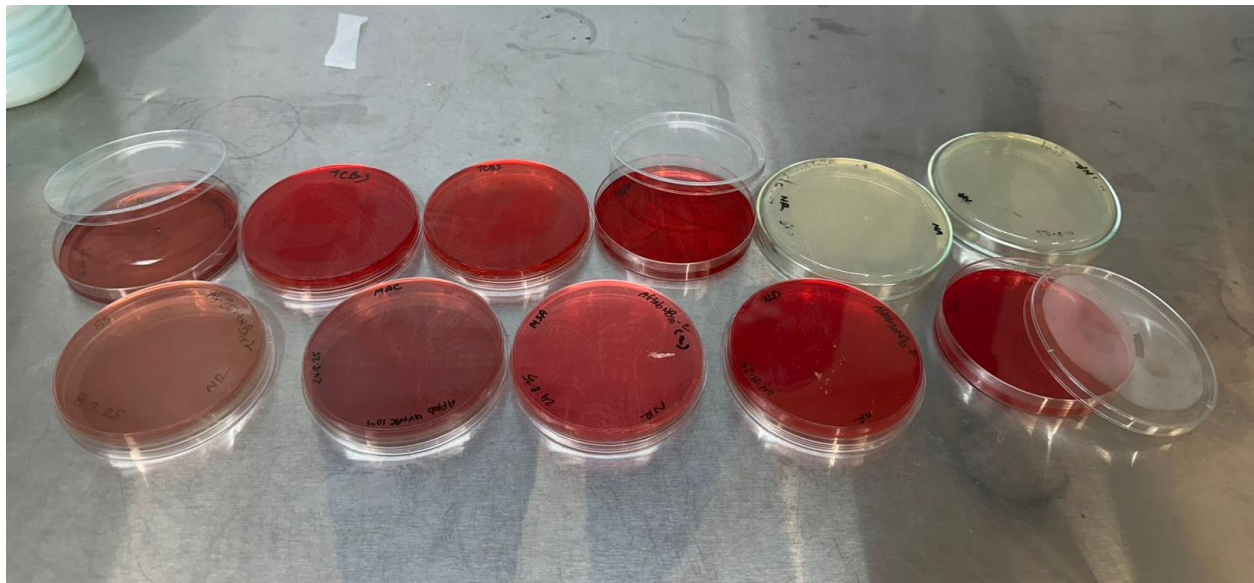


Fig 2.3: Media poured in plates under laminar flow

2.6.2 Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar

TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) agar is a selective medium specifically formulated for the isolation of *Vibrio cholerae* and other pathogenic *Vibrio* species. Its high alkalinity, together with thiosulfate and sodium citrate, suppresses the growth of *Enterobacteriaceae*, while ox-bile and sodium cholate inhibit Gram-positive bacteria, including *enterococci*.



Fig 2.4: Colonies observed on TCBS agar after the spread plate method

Vibrio species that ferment sucrose can produce acid, which changes the bromothymol blue and thymol blue pH indicators to yellow. Sodium chloride in the medium supports the metabolic activity and growth of halophilic *Vibrio* strains (Aryal, 2023). TCBS agar is widely used for cultivating *Vibrio cholerae*, *Vibrio fluvialis*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* (HiMedia Laboratories, n.d.). To prepare TCBS agar, 89.08 grams of the medium powder is dissolved in one liter of distilled water, boiled, and poured into Petri dishes for solidification without autoclaving.

2.6.3 Xylose Lysine Deoxycholate (XLD)

Xylose Lysine Deoxycholate (XLD) agar is a selective and differential medium used for the isolation and identification of enteric pathogens, particularly *Salmonella* and *Shigella* species. It contains yeast extract as a source of nutrients and vitamins, and sodium deoxycholate as a selective agent that inhibits the growth of Gram-positive bacteria. The medium includes xylose (0.1%), lactose (1%), sucrose (1%), lysine, and beef extract. Most enteric bacteria ferment xylose, turning the medium yellow, except *Shigella* spp., which remains red due to the absence of xylose fermentation. *Salmonella* ferments xylose initially but later decarboxylates lysine, reverting the pH to alkaline and producing red colonies, distinguishing it from non-pathogenic coliforms. Lactose and sucrose are added to prevent reversion in coliforms by maintaining acid production. The phenol red indicator detects pH changes, turning yellow in acid and red in alkaline conditions. Sodium thiosulfate and ferric ammonium citrate act as an H₂S indicator system, can turn into



Fig 2.5: Colonies observed on XLD agar after the spread plate method

black-centered colonies when hydrogen sulfide is produced, a characteristic feature of *Salmonella*. Thus, XLD agar efficiently differentiates *Salmonella* (red colonies with black centers) and *Shigella* (red colonies) from other enteric bacteria (Aryal, 2018). To prepare XLD agar, 55 grams of the medium powder were dissolved in one liter of distilled water, heated according to the instructed boiling temperature to ensure complete dissolution, and then poured into Petri dishes to solidify before use (HiMedia Laboratories, n.d.).

2.6.4 MacConkey Agar

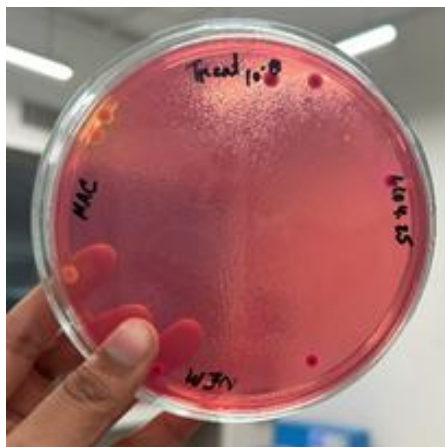


Fig 2.6: Colonies observed on MacConkey agar after the spread plate method

MacConkey agar serves as both a selective and differential medium widely used for the isolation and identification of Gram-negative enteric bacteria. Its selective property arises from the presence

of bile salts and crystal violet, which inhibit the growth of most Gram-positive bacteria, allowing the selective recovery of Gram-negative organisms. As a differential medium, MacConkey agar distinguishes bacteria based on their ability to ferment lactose. Lactose-fermenting organisms, such as *Escherichia coli*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*, produce acid during fermentation, which lowers the pH and results in pink to red colonies due to the neutral red pH indicator. In contrast, non-lactose fermenters, including *Salmonella* and *Shigella* species, do not produce acid and therefore form colorless or transparent colonies. This visual differentiation enables rapid distinction between coliforms and non-coliforms (Aryal, 2022). Common bacteria cultured on MacConkey agar include *E. coli*, *Enterobacter*, *Klebsiella*, *Salmonella*, and *Shigella* species (HiMedia Laboratories, n.d.). To prepare MacConkey agar, 50 grams of the dehydrated medium powder are dissolved in one liter of distilled water, the solution is heated until completely dissolved, and then autoclaved at 121 °C for 15 minutes. The sterilized medium is poured into sterile Petri dishes and allowed to solidify before inoculation.

2.6.5 Mannitol Salt Agar (MSA) Media

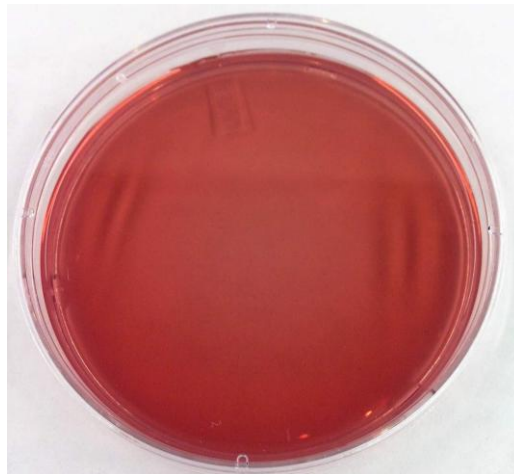


Fig 2.7: Colonies observed on MSA agar after the spread plate method

Mannitol Salt Agar (MSA) serves as both a selective and differential medium in microbiology. Its selective nature is due to the high concentration of sodium chloride (7.5%), which inhibits the growth of most Gram-negative bacteria and many Gram-positive bacteria, allowing *Staphylococcus* species to grow preferentially. As a differential medium, MSA distinguishes

bacteria based on their ability to ferment mannitol. Strong mannitol fermenters, such as *Staphylococcus aureus*, produce acid, leading to a yellow coloration of the medium, whereas non-mannitol fermenters, such as *Staphylococcus epidermidis*, do not produce acid and therefore the medium remains pink or red, allowing clear differentiation among *Staphylococcus* species (HiMedia Laboratories, n.d.). Common organisms cultured on MSA include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Micrococcus* species. To prepare MSA, 111 grams of the medium powder are dissolved in one liter of distilled water, boiled to dissolve completely, and poured into Petri dishes for solidification.

2.7.1 Bacterial Inoculation Methods

In this study, bacterial inoculation was carried out by first performing serial dilution, followed by the spread plate method for culturing organisms, and finally using the streak plate method to obtain isolated bacterial colonies.

2.7.2 Spread Plate Method

The spread plate technique is a widely used microbiological methodology for isolating and counting viable microorganisms in liquid samples. This method is accomplished by taking a known volume of sample and systematically spreading it on the surface of a cooled solid media in order to allow for distinct, spaced colonies to develop after incubation and ultimately count them as colony-forming units per milliliter. After incubation on LB media for enrichment, serial dilution was completed to achieve the desired concentration range of 20–300 CFUs/mL, the concentration range that is appropriate and accurate for enumeration. After the diluted samples had achieved the desired range, 100 μ L of each diluted sample was pipetted onto the agar plates, and spread evenly with a sterile spreading stick for accurate colony counts post-incubation. Ultimately, colonies were counted and were used to determine the microbial load of each sample.

2.7.3 Streak Plate Method

The streak plate method is one of the necessary microbiological technique used for selective media growth identification and is used to obtain pure bacterial cultures and well-isolated colonies from

mixed microbial communities. This method consists of gradually decreasing the bacterial density across the surface of an agar plate, ensuring that the final streaks contain only a few cells to grow pure colonies. This method is particularly useful for identifying bacterial species and isolating single-species cultures from mixed microbial communities (Dahal, 2024).

In this study, colonies produced from the spread plate method were selected for streaking. Using a sterile inoculating loop, a small portion of the colony was transferred to one side of the agar plate to create an initial streak. The loop was then streaked in a defined pattern across the plate to progressively dilute the bacterial sample. The plates were incubated at 37°C for 18–24 hours, after which isolated colonies developed, allowing the recovery of pure bacterial cultures for subsequent morphological and characterization analyses.



Fig 2.8: Isolating single colony from selective MSA media following streak plate method

2.8.1 Possible Identifications

As part of this study, HiMedia analysis was employed to gain a clearer understanding of the bacterial species that were isolated and examined. The identification process was primarily based on the observable characteristics and colony morphology of the isolates grown on different culture media.

2.8.2 HiMedia Analysis

After microscopic examination, bacterial isolates were streaked onto various culture media plates for further analysis. Species identification was carried out by observing colony growth patterns, pigmentation, and morphology, following the guidelines provided in the HiMedia publication.

Based on these criteria, colonies grown on TCBS, XLD, MSA and MacConkey agar were evaluated for their distinctive color and appearance. Using this approach, *Salmonella* spp., *Escherichia coli*, and *Vibrio cholerae* were successfully identified (HiMedia Laboratories, n.d.).

Table 2.1: HiMedia Analysis (HiMedia Laboratories, n.d.)

Bacterial Species	Selective Plate & Differential Plate	Colony Colour	Reason for Colour	Identity
<i>Vibrio Cholerae</i>	Thiosulfate-Citrate-Bile-Sucrose(TCBS)	Yellow	The yellow color results from sucrose fermentation, producing acid.	Positive for <i>Vibrio</i> spp.
<i>Escherichia Coli/ Klebsiella</i> spp.	MaccOnkey	Pink	The pink colony indicates	Positive for <i>E. coli</i>
<i>Salmonella</i> spp. / <i>Shigella</i>	XLD	Yellow, Red	Yellow colony indicates Red colony/ with black dots indicates	Positive for <i>E. coli</i> Positive for <i>Shigella</i> / <i>Salmonella</i>
<i>Staphylococcus</i> spp.	MSA	Yellow or Pink	Yellow colony indicates	Positive for <i>Staphylococcus</i>

2.9.1 Triple Sugar Iron (TSI) Agar

Triple Sugar Iron (TSI) agar is one of the common differential medium used in microbiology to the differentiation of Gram-negative enteric bacilli on the basis of carbohydrate fermentation (glucose, lactose, and sucrose) as well as hydrogen sulfide (H₂S) and gas production. The medium contains 0.1% glucose, 1% lactose, and 1% sucrose as fermentable carbohydrates in addition to ferrous sulfate and sodium thiosulfate to detect H₂S. The phenol red indicator allows for visualizing color changes due to acid production, which is the result of carbohydrate fermentation.

The TSI agar has peptone and yeast extract as nutrient sources, and the agar serves as a solidifying medium. The slant and butt (created by tilting the solid medium in a test tube) allows differentiation between aerobic and anaerobic metabolic activity. Interpretation of differential reactions on TSI agar is based on the following observations of pH indicator color changes and gas or H₂S production:

- Glucose fermentation only: Red (alkaline) slant / Yellow (acid) butt (K/A)
- Glucose + lactose and/or sucrose fermentation: Yellow slant / Yellow butt (A/A)
- No carbohydrate fermentation (peptone catabolism): Red slant / Red butt (K/K)
- H₂S production: Black precipitate in the butt
- Gas production: Cracks, bubbles, or lifting of the agar

Common organisms tested on TSI agar include *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, and *Proteus spp.* (Aryal, 2022c; HiMedia Laboratories, n.d.).

Preparation of Medium

A total of 65 grams of TSI agar powder was dissolved in one liter of distilled water and heated to boiling until the medium was completely dissolved. Approximately 10 mL of the prepared medium

was dispensed into test tubes and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the tubes were cooled in a slanted position to create a butt and slant configuration.

Inoculation

A pure colony of the test organism was picked using a sterile inoculating needle. The medium was stabbed straight down the center of the agar butt to near the bottom and then streaked on the surface of the slant in a zigzag motion to ensure both aerobic and anaerobic regions were inoculated.

Incubation

The inoculated tubes were incubated at 35–37°C for 24 hours.

Observation and Interpretation

After incubation, the tubes were examined for color changes, gas formation, and blackening due to hydrogen sulfide (H₂S) production. The reactions were recorded as combinations of A (acid), K (alkaline), G (gas), and H₂S (black precipitate) indicators, for example, *A/A*, *G*, *H₂S*+

2.9.2 Catalase Test

The Catalase Test is a biochemical test used to determine the ability of bacteria to produce the enzyme catalase, which breaks down hydrogen peroxide (H₂O₂) into water (H₂O) and oxygen (O₂). This reaction protects bacterial cells from the toxic effects of hydrogen peroxide, a byproduct of aerobic metabolism. The test is primarily employed to differentiate catalase-positive organisms, such as *Staphylococcus* and *Micrococcus* species, from catalase-negative organisms, such as *Streptococcus* and *Enterococcus* species (Aryal, 2022e).

When hydrogen peroxide is added to a bacterial colony, the presence of catalase enzymes leads to the rapid release of oxygen bubbles, indicating a positive result. Therefore, the organism's ability to survive in oxygenated environments by neutralizing reactive oxygen species.

Catalase-positive bacteria, such as *Staphylococcus* and *Micrococcus*, are typically aerobic or facultative anaerobes, capable of producing the enzyme to counteract oxidative stress. In contrast, catalase-negative bacteria, such as *Streptococcus* and *Enterococcus*, are generally aero tolerant anaerobes, lacking this enzyme and relying on alternative mechanisms for peroxide detoxification.

In contrast, the absence of bubble formation denotes a negative reaction. This test plays a key role in the identification of Gram-positive cocci and helps in distinguishing staphylococci from streptococci in clinical microbiology.

Reagents Used

- 3% Hydrogen Peroxide (H₂O₂):
Serves as the substrate for the catalase enzyme. When catalase is present, hydrogen peroxide is decomposed into water and oxygen, producing visible effervescence.
- Glass Slide or Test Tube:
Used as the surface for the reaction. The slide test is preferred for rapid detection, while the test tube method is used for stronger catalase producers or when dealing with cultures that may cause splattering.

Preparation

A clean glass slide was labeled and sterilized. A small amount of bacterial culture was transferred onto the slide using a sterile inoculating loop or wooden applicator stick. (Iron loops were avoided, as they can produce false-positive reactions by reacting with hydrogen peroxide.)

Addition of Reagent

A drop of 3% hydrogen peroxide was placed onto the bacterial smear using a sterile dropper.

Observation

The slide was observed immediately for bubble formation. The appearance of brisk effervescence indicated a positive catalase reaction, whereas the absence of bubbles indicated a negative reaction.

Interpretation:

- Catalase Positive: Immediate bubble formation (*e.g., Staphylococcus aureus, Micrococcus luteus*).
- Catalase Negative: No bubble formation (*e.g., Streptococcus pyogenes, Enterococcus faecalis*).

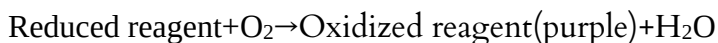
2.9.3 Oxidase Test

The Oxidase Test is a biochemical test used to determine the presence of the enzyme cytochrome c oxidase in bacteria. This enzyme plays a crucial role in the electron transport chain, transferring electrons from cytochrome c to molecular oxygen and forming water. The test is primarily used to differentiate oxidase-positive organisms, such as *Pseudomonas*, *Neisseria*, and *Vibrio* species, from oxidase-negative organisms, such as members of the *Enterobacteriaceae* family (Aryal, 2022f).

The oxidase test is significant in bacterial identification and classification, particularly for distinguishing between aerobic and facultatively anaerobic Gram-negative bacilli. A positive reaction is indicated by the rapid development of a dark purple or blue color, signifying the presence of cytochrome c oxidase, while no color change denotes a negative result.

The oxidase test is based on the oxidation of a chromogenic reducing agent (such as tetramethyl-p-phenylenediamine dihydrochloride) by the enzyme cytochrome c oxidase. When present, this enzyme facilitates the transfer of electrons from the reagent to molecular oxygen, producing water and a deep purple indophenol compound.

Reaction:



The formation of the purple color within 10–30 seconds indicates a positive reaction, confirming the organism's ability to perform oxidative phosphorylation via the cytochrome oxidase system. In contrast, oxidase-negative bacteria lack this enzyme and therefore do not produce any color change upon exposure to the reagent.

Reagents Used

- 1% Tetramethyl-p-phenylenediamine dihydrochloride (Oxidase Reagent):
Serves as the chromogenic reducing agent that acts as an artificial electron donor for cytochrome c oxidase. Upon oxidation, it forms a deep purple (indophenol) compound in

positive organisms.

- Filter Paper or Oxidase Test Strip:

Provides a surface for the reaction. The reagent can be freshly prepared or impregnated into oxidase test discs or strips for convenience.

- Sterile Applicator or Wooden Stick:

Used for transferring bacterial colonies to the reagent-impregnated surface. Metal loops are avoided, as they can cause false-positive results.

Test Procedure

Preparation:

A clean piece of filter paper was placed on a Petri dish or glass slide, and a few drops of 1% oxidase reagent were added to moisten the surface. Alternatively, a commercial oxidase test strip was used directly.

Inoculation:

A fresh bacterial colony (18–24 hours old) was picked using a sterile wooden stick and rubbed onto the moistened reagent area of the filter paper or oxidase disc.

Observation:

The spot was observed for 30 seconds to 1 minute for the appearance of a purple or dark blue coloration.

Interpretation:

- Oxidase Positive: Development of a dark purple color within 10–30 seconds (*e.g.*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Vibrio cholerae*).
- Oxidase Negative: No color change or delayed color development (*e.g.*, *Escherichia coli*, *Klebsiella pneumoniae*).

2.10.1 Buffer Preparation

Buffer solutions play a vital role in maintaining a stable pH during biological and molecular processes. To ensure optimal conditions for DNA isolation and related molecular biology experiments, several buffers were prepared for this study.

1. 10X TBE Buffer:

- 150 ml of distilled water was added to a clean beaker.
- 27.3 g of Tris base, 14.3 g of Boric acid, and 2.329 g of EDTA were added.
- The mixture was placed on a magnetic stirrer for 3–5 minutes until fully dissolved.
- The final pH was adjusted to 8.3. If the pH exceeded 8.3, HCl (Hydrochloric acid) was added; if lower, NaOH (Sodium hydroxide) pellets were added.
- The final volume was brought to 250 ml with distilled water.
- The pH was rechecked to confirm accuracy after autoclave.

2. Running Buffer

- 50 ml of 10X TBE buffer was measured and added to a beaker.
- 700 ml of distilled water was added.
- The pH was adjusted to 8.3. If higher than 8.3, HCl was added; if lower, NaOH pellets were added.
- The final volume was adjusted to 1 liter with distilled water.
- The pH was verified again after adjustment.

3. 1x TE Buffer

- 10 mM of Tris-HCl and 1 mM of EDTA were added.
- 10 ml of distilled water was poured into the beaker.
- The pH was adjusted to 8.3, using HCl if above 8.3 or NaOH if below.
- The final volume was made up to 20 ml with distilled water.
- The pH was checked once more to ensure accuracy.

2.10.2 DNA Isolation Using the Boiling Method

The boiling method is a quick and straightforward approach for extracting bacterial DNA. It relies on heat treatment to lyse bacterial cells and release their genetic material. The procedure was carried out as follows:

- Bacterial colonies were cultured on agar plates and incubated for 18–24 hours at 37°C.
- Selected colonies were suspended in 500 µl of TE buffer or sterile distilled water in Eppendorf tubes.
- The samples were centrifuged at 13,000 rpm for 5 minutes.
- The supernatant was discarded, and the pellet was resuspended in TE buffer or sterile distilled water.
- The tubes were centrifuged again at 13,000 rpm for 5 minutes.

- After discarding the supernatant, the pellet was resuspended in 500 µl of TE buffer or sterile distilled water.
- The suspensions were heated at 100°C for 10 minutes to lyse the cells and release DNA into the solution.
- Following boiling, the samples were immediately cooled on ice for 10 minutes to induce cold shock, then stored at –20°C.
- The tubes were centrifuged once more at 13,000 rpm for 5 minutes to pellet cell debris.
- The supernatant containing the extracted DNA was transferred into fresh microcentrifuge tubes for further use (Green & Sambrook, 2012).

The isolated DNA was subsequently used for downstream molecular applications such as PCR and gel electrophoresis to confirm the presence of the target bacterial species.

2.11.1 PCR Method

Studying isolated DNA fragments can be difficult because molecular and genetic analyses require large amounts of DNA. The Polymerase Chain Reaction (PCR), often called *molecular photocopying*, is a conventional method used to amplify (replicate) small quantities of DNA or RNA from a sample. PCR has numerous applications in research, clinical, and forensic laboratories. Forensic scientists use it to analyze DNA evidence from crime scenes, while clinical laboratories apply it for diagnosing viral infections. In pharmaceutical research, PCR assists in analyzing and duplicating genetic material for drug and vaccine development.

2.11.2 Steps of PCR

1. Initialization Step

This step is required for DNA polymerases that need heat activation, as in *hot start PCR*. The reaction mixture is heated to 94°C–96°C and maintained for 1–9 minutes to activate the enzyme.

2. Denaturation

The reaction is then heated to 94°C–98°C for 20–30 seconds. During this stage, hydrogen bonds between complementary DNA bases are broken, separating the double-stranded DNA into single strands, which serve as templates for replication.

3. Annealing

The temperature is reduced to 50°C–65°C for 20–40 seconds to allow primers to bind (anneal) to their complementary sequences on the single-stranded DNA templates. The temperature must be optimized, generally 3–5°C below the melting temperature (T_m) of the primers to ensure specific binding. If the temperature is too low, nonspecific binding may occur; if too high, primers may fail to anneal. Stable hydrogen bonds between primer and template form only when sequences are complementary.

4. Extension Step

This step depends on the DNA polymerase used. For Taq polymerase, the optimal temperature is around 72°C (ranging from 75°C to 80°C). The enzyme synthesizes new DNA strands by adding deoxynucleoside triphosphates (dNTPs) to the 3' end of each primer, extending the strand in the 5' to 3' direction. Typically, DNA polymerase adds about 1,000 bases per minute at optimal temperature. Under ideal conditions, the DNA amount doubles with each cycle, resulting in exponential amplification of the target fragment.

5. Final Elongation

After the last PCR cycle, a final elongation step is carried out at 70°C–74°C for 5–15 minutes to ensure that any remaining single-stranded DNA is fully extended.

6. Final Hold

For short-term storage, the reaction can be held at 4°C–15°C indefinitely to preserve the amplified DNA.

2.11.3 PCR Components

Template DNA

The template DNA serves as the sample that contains the target sequence to be amplified. To begin the reaction, the two strands of the double-stranded DNA molecule are separated by exposing them to high temperatures, allowing the process to initiate.

Primers

Primers are short, single-stranded DNA sequences (typically **18–25 nucleotides long**) that bind, or hybridize, to specific regions on the template DNA. This provides a starting point for the DNA polymerase to attach and begin synthesizing new DNA strands during the polymerization process.

DNA Polymerase

DNA polymerase is a specialized enzyme responsible for synthesizing new DNA strands that are complementary to the target sequence in the template DNA.

Mg²⁺ (Magnesium ions)

Magnesium ions act as essential cofactors for the DNA polymerase enzyme. They are vital for the enzyme's proper activity and for achieving optimal DNA synthesis efficiency.

Buffer

A buffer solution is included in the PCR mixture to maintain the optimal **pH** and **ionic strength** required for DNA polymerase activity. It ensures enzyme stability, preserves reaction components, and prevents DNA degradation, which is crucial for the accuracy of any enzymatic reaction.

dNTPs (Deoxynucleoside Triphosphates)

dNTPs serve as the building blocks for new DNA strand synthesis. Each dNTP provides the nucleotides needed for elongation. If the dNTP concentration is insufficient, the PCR process will halt midway, preventing complete amplification.

2.11.4 PCR Equipment

Thermocycler Machine

The thermocycler precisely regulates the temperature required for the three main PCR stages—denaturation, annealing, and extension. Its ability to cycle through these temperatures accurately and repeatedly allows multiple rounds of amplification, which is why it is referred to as a “cycler.” The number of cycles is determined by the size and nature of the target gene.

PCR Tubes

Specially designed PCR tubes are used to contain the reaction mixture during amplification. These tubes are optimized to prevent evaporation under high temperatures and to enhance reaction efficiency, ensuring consistent and reliable PCR results.

PCR Reaction Preparation:

Table 2.2: PCR Component

Component	Volume per sample
Nuclease-free water (NF)	1µl
Master mix	10µl
Isolated DNA	5µl
Forward primer (Fp)	2µl
Reverse Primer (Rp)	2µl
	Total volume: 20µl

2.12.1 PCR Run

1. To start the software, firstly, the F2 button was pressed.
2. The machine was configured with the temperature and amount of time needed for each stage.
3. The thermocycler's final volume, 20 μ l, was chosen.
4. The "35 cycles" option was selected by pressing the F1 button.
5. The PCR tubes were placed once the cover was opened.
6. The F1 button was pressed to start the procedure.

2.12.2 Thermal cycle

Table 2.3: Steps of Thermal Cycle

Initial denaturation	95 °C (5 min)
Denaturation	95 °C (30 sec)
Annealing	<i>E. coli</i> : 57 °C (1 min) <i>Vibrio</i> : 58 °C (1 min) <i>Klebsiella</i> : 53.2 °C (1 min)
Elongation	72 °C (1 min)
Final elongation	72 °C (5 min)
Storage	4 °C (short) and –20 °C (long)

2.12.3 Primer condition

Table 2.4: Condition of Primers

Primer	Primer Sequence
<i>E.coli</i> Forward	5'-GACCTCGGTTTAGTTCACAGA-3'
<i>E.coli</i> Reverse	5'-CACACGCTGACGCTGACCA-3'
<i>Vibrio</i> Forward	5'-ATAATGGCTCACCAAGAAGG-3'
<i>Vibrio</i> Reverse	5'-TTAGAACTTATAACCACC-3'
<i>Klebsiella</i> Forward	5'-TGCAGATAATTCACGCCCAG-3'
<i>Klebsiella</i> Reverse	5'-ACCCGCTGGACGCCAT-3'

2.13 DNA Visualization by Gel Electrophoresis

After DNA amplification, gel electrophoresis was carried out to confirm the presence of DNA to visualize them. The procedure involved the following steps:

2.14.1 Agarose Gel Preparation

To prepare 100 ml of 1% agarose solution:

- 1 g of agarose powder was measured into a flask.
- 90 ml of distilled water and 10 ml of 10X TBE buffer were added.
- The mixture was heated on a hot plate or in a microwave until the agarose completely dissolved and the solution became clear.
- After cooling slightly, 4 µl of ethidium bromide (EtBr) was added.

- A comb was positioned in the gel casting tray.
- The molten agarose was poured into the tray, ensuring no air bubbles formed. The comb was placed about 1 inch from one end and positioned vertically so that the teeth were 1-2 mm above the tray surface. Once the gel had solidified, the comb was carefully removed.

2.14.2 Agarose Gel Preparation for Gel Electrophoresis

For PCR products:

- Agarose gel was prepared as described above, with the comb removed after solidification.
- The gel tray was placed in the electrophoresis chamber and covered with a 1X TBE buffer until the wells were submerged.
- 4-5 μ l of PCR products were loaded into each well.
- Electrophoresis was run at 100 V for 35 minutes.
- DNA bands were observed under short-wave UV light.

Chapter 3

Result

3.1 Labeling the isolated colonies

The water samples were collected from three sources: one from the lake situated at Badda Hatirjheel, Banani lake in the heart of Dhaka city and the other one from Aftabnagar Lake situated at Aftabnagar in Dhaka city.. The samples were collected from two spots of each source, twice from both spots. Therefore, samples were collected four times in total from each source throughout the course of 4 months.

According to the samples, the isolated bacterial colony stocks were named as:

- ‘Hati’ For Hatirjheel
- ‘Moha’ for Banani lake because this sample was mostly taken from the Mohakhali’s side of the lake.
- ‘Af’ for Aftabnagar Lake water samples was written.

The samples from the first spot of the source were named as ‘S1’,

The samples from the second spot of the source were named as ‘S2’

The samples from the third spot of the source were named as ‘S3’

Before Treatment as ‘UT’, After 1-hour Treatment as ‘NB’

3.2.1 Bacterial Colony Count Results on Nutrient Agar (NA) Media Before and After Treatment

Samples were treated with the Nano bubble generator for one hour without interruption and were aseptically transferred to sterile Falcon tubes. Two milliliters of treated and corresponding untreated water samples were incubated for 4 hours in a shaker incubator at 37 °C. After incubation, serial ten-fold dilutions were prepared in sterile 0.9% normal saline (w/v NaCl) down

to 10^{-5} . From each selected dilution, 100 μL (0.1 mL) was spread onto Nutrient Agar plates and incubated at 37 °C for 24 hours. After incubation, single, well-isolated colonies on the most appropriate countable plate (i.e., the plate yielding 30–300 colonies where possible) were enumerated. All steps were performed under aseptic conditions.

Colony enumeration equation:

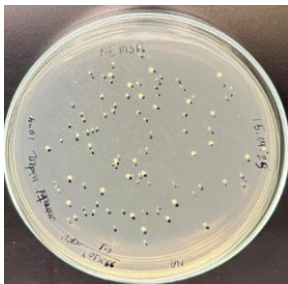
$$\text{CFU/mL} = (\text{N} \times \text{DF}) / \text{V}$$

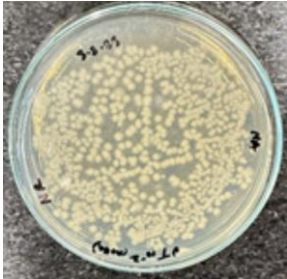
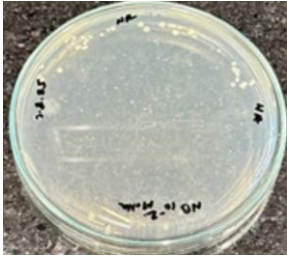
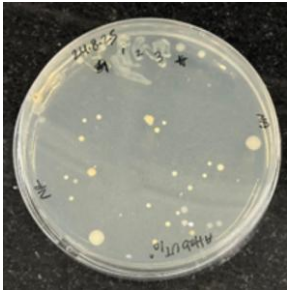
Where,

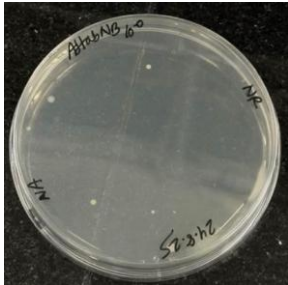
- N = number of colonies counted on the plate,
- DF = dilution factor (reciprocal of the plated dilution; for a 10^{-2} plated dilution DF = 10^2 = 100),
- V = plated volume in mL (here, 0.1 mL).

3.2.2 Results table (counts, dilution used, and percent reduction)

Table 3.1: Colony count of treated and untreated water samples in Nutrient agar media & overall reduction percentage

Sample site	Observed CFU/mL (as calculated)	Dilution Factor(DF) used for count	Colony Reduction Percentage (%)
Hatirjheel	Untreated: 9.90×10^2 (990) 	10^0 (undiluted, DF = 1)	64.65%

Hatirjheel	Treated: 3.50×10^2 (350) 	10^0 (undiluted, DF = 1)	64.65%
Banani Lake	Untreated: TNTC (Too Numerous to Count) 	No countable plate obtained at plated dilutions (TNTC)	93.6%
Banani Lake	Treated: 3.20×10^4 (32,000) 	10^{-2} (DF = 100)	93.6%
Aftab Nagar Lake	Untreated: 8.60×10^2 (860) 	10^0 (undiluted, DF = 1)	86.05%

Aftab Nagar Lake	Treated: 1.20×10^2 (120) 	10^0 (undiluted, DF = 1)	86.05%
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3.3.1 Selective Media Growth Observation of Treated and Untreated Water Samples on TCBS, MacConkey, XLD, and MSA Plates

All observations were made on countable plates under aseptic laboratory conditions, demonstrating the efficiency of the nanobubble treatment in eliminating *Vibrio*, *E. coli*, *Pseudomonas spp*, *Staphylococcus spp*, *Enterobacter*, etc. contamination, particularly in samples with initially high microbial loads because these bacteria are mostly responsible for causing lethal and dangerous disease to humans and animals, significantly in Asian regions.

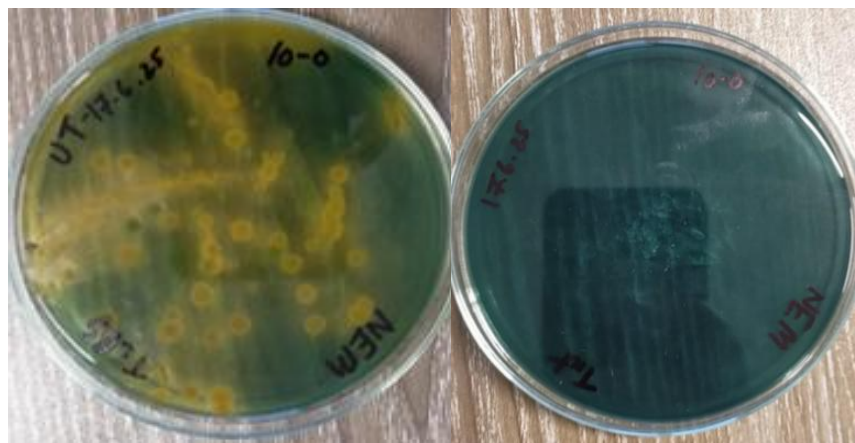


Fig 3.1: Hatirjheel treated and Untreated water samples' colonial growth observed on TCBS agar after the spread plate method

Growth Observation on **Thiosulfate Citrate Bile Salts Sucrose** (TCBS) agar, in the Hatirjheel sample, the untreated 10^0 plate exhibited **numerous yellow colonies**, characteristic of *Vibrio*

cholerae, whereas the treated sample (10⁰ dilution) showed no visible colony formation, indicating complete suppression of *Vibrio* growth following nanobubble treatment. For the Banani Lake sample, no *Vibrio cholerae* colonies were detected in either the treated or untreated 10⁰ plates, suggesting the absence of *Vibrio cholerae* contamination at the time of sampling.

Fig 3.2: Aftabnagar lake Untreated water sample; *Vibrio* growth observed on red TCBS agar following streak plate method

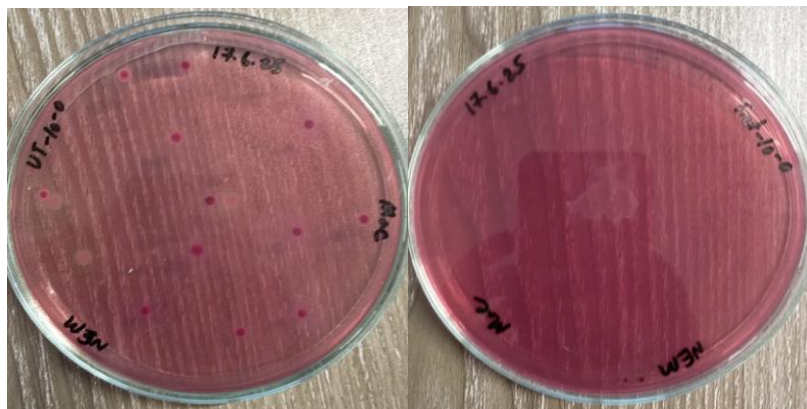


Fig 3.3: Pink colony growth on MacConkey agar indicating *Enterobacter*, *E. coli* & *Klebsiella*

MacConkey agar, a selective and differential medium formulated for the isolation of Gram-negative, lactose-fermenting enteric bacteria, was used to assess the bacterial population in the treated and untreated water samples collected from Hatirjheel, Banani Lake, and Aftab Nagar. Growth was observed from the 10^0 dilution plates after 24 hours of incubation at 37 °C.

In the Hatirjheel sample, it was observed that the untreated 10^0 plate developed approximately 48 **pink colonies**, which are typically indicative of lactose-fermenting bacteria such as *Escherichia coli*, *Klebsiella spp.*, or *Enterobacter spp.* The pink coloration was expected due to acid production from lactose fermentation. In contrast, the treated Hatirjheel sample (10^0 dilution) showed no visible colonies, indicating that no viable enteric bacteria were detected following nanobubble treatment.

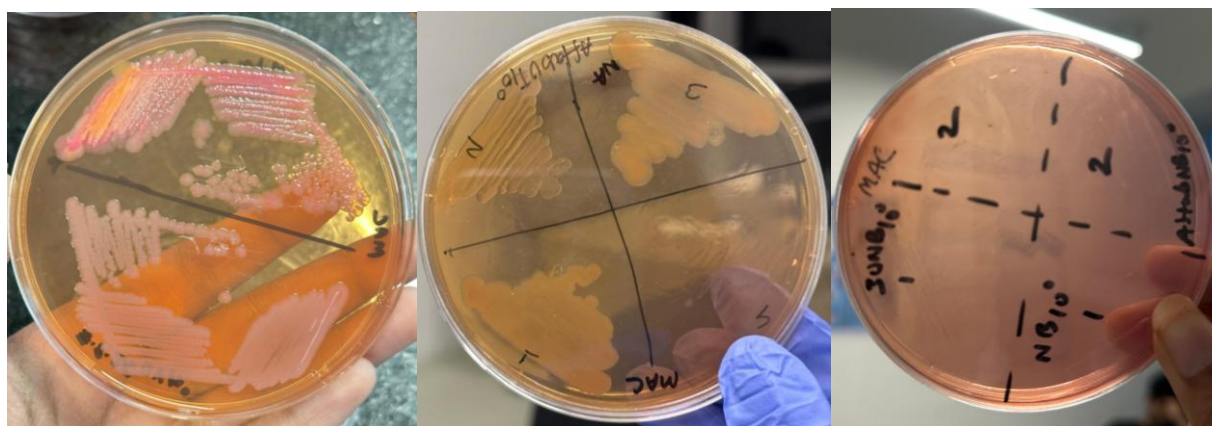


Fig 3.4: Untreated water sample showed various pink colonies, whereas treated sample contains no growth of bacteria in the MacConkey agar

Similarly, in both Banani Lake and Aftab Nagar samples, the untreated plates showed moderate growth of pink colonies, which was consistent with the presence of coliform-type bacteria commonly found in contaminated surface water. However, the treated plates from both sites showed no observable bacterial growth, confirming the complete suppression of bacterial species capable of growing on MacConkey agar after treatment.

Xylose Lysine Deoxycholate (XLD) agar was used as a selective and differential medium to identify and distinguish enteric pathogens such as *Salmonella* and *Shigella* from other Gram-negative intestinal bacteria. Growth observations were made from the 10^0 dilution plates after 24

hours of incubation at 37 °C for both treated and untreated water samples collected from Hatirjheel, Banani Lake, and Aftab Nagar.

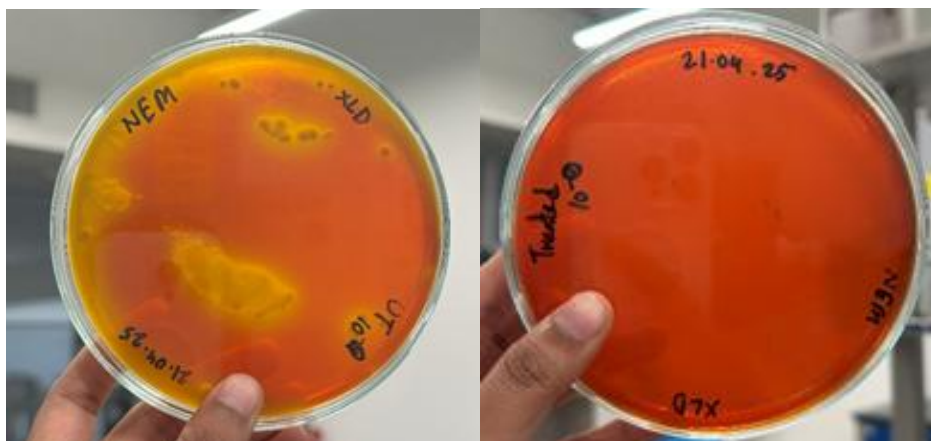


Fig 3.5: Untreated water sample showed various yellow colonies confirms fermentation, whereas treated sample contains no growth of bacteria in the XLD agar

In the Hatirjheel sample, the untreated 10⁰ plate showed moderate growth of **yellow colonies**, indicative of **lactose- or xylose-fermenting** bacteria such as *Escherichia coli* or *Enterobacter spp.* No black-centered colonies were seen, suggesting the absence of *Salmonella* or *Proteus* species. The treated Hatirjheel sample showed no visible colonies, reflecting the complete inactivation of enteric bacteria after nanobubble treatment.

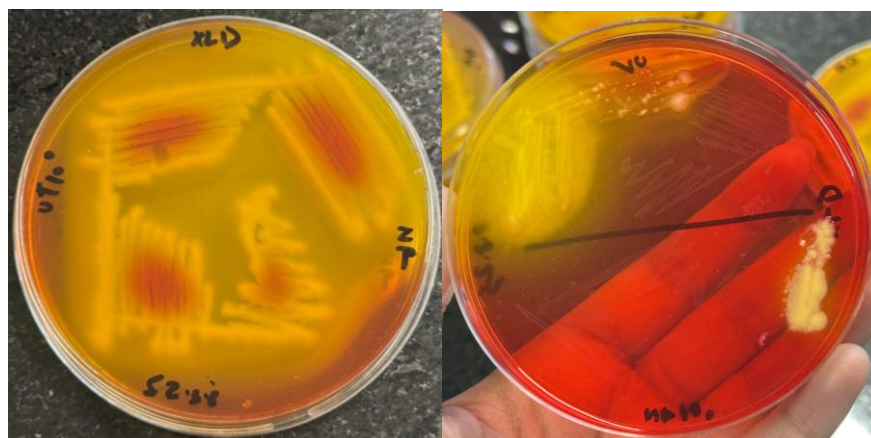


Fig 3.6: Untreated water sample showed various yellow colonies with red center, whereas treated sample contains slight growth of bacteria in the XLD agar

For the Banani Lake sample, the untreated plate exhibited yellow colonies with red centers, which are typically associated with *Enterobacter aerogenes* or other moderately fermentative coliforms that re-alkalize the medium after initial acidification. The treated Banani sample showed only slight initial yellow growth near the streak line, which disappeared further along the plate, indicating a significant reduction in viable bacterial load after treatment.

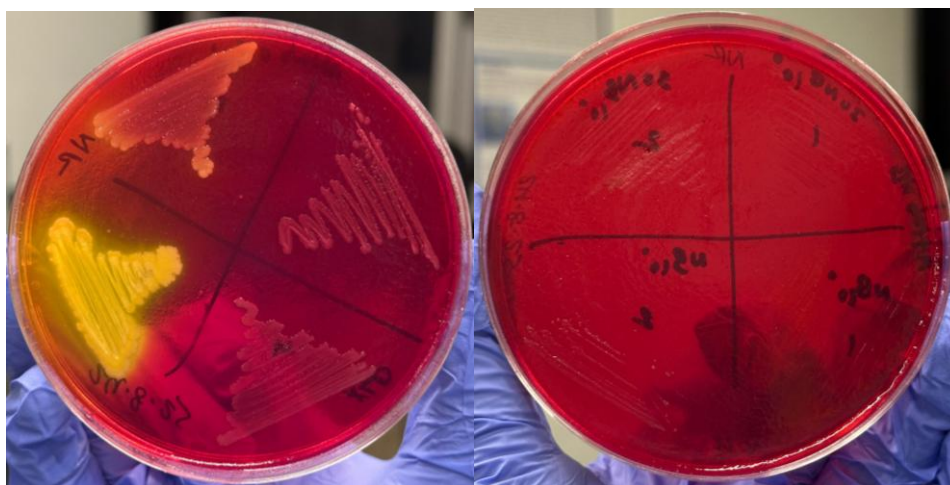


Fig 3.7: Untreated water sample showed various red and yellow colonies, whereas treated sample contains no growth of bacteria in the XLD agar

Both yellow and red colonies were visible on the untreated 10^0 plate in the Aftab Nagar sample, indicating a mixed population of lactose-fermenting coliforms and non-fermenting enteric bacteria like *Shigella spp.* However, there was no discernible growth on the treated plate, indicating that the nanobubble treatment had successfully eliminated these bacteria.

The absence of black colonies in any of the samples suggests that neither the treated nor untreated samples contained any hydrogen sulfide-producing bacteria, such as *Salmonella* or *Proteus* species. The disinfection potential of nanobubble treatment in contaminated surface water was supported by the overall **growth patterns on XLD agar**, which showed that it significantly decreased or eliminated Gram-negative enteric bacteria.

Staphylococcus species were identified and distinguished using **Mannitol Salt Agar (MSA)** as a selective and differential medium based on their ability to ferment mannitol and tolerate high salt concentrations. Growth observations were made from the 10^0 dilution plates after 24 hours of

incubation at 37 °C for both treated and untreated water samples collected from Hatirjheel, Banani Lake, and Aftab Nagar. In the **Hatirjheel sample**, the untreated 10⁰ plate **showed yellow colonies**, indicative of mannitol-fermenting *Staphylococcus aureus*. This suggested the presence of pathogenic *Staphylococcus* capable of acidifying the medium.



Fig 3.8: Untreated water sample showed various yellow and red colonies, whereas treated sample contains no growth of bacteria in the MSA agar

For the Banani Lake sample, the untreated plate showed yellow colonies at 10⁰ and 10⁻¹ dilutions, while at 10⁻² dilution, red colonies were observed. The yellow colonies were likely *Staphylococcus aureus*, whereas the red colonies were likely non-mannitol fermenting *Staphylococcus* species, such as *Staphylococcus epidermidis*, reflecting a mixed population.

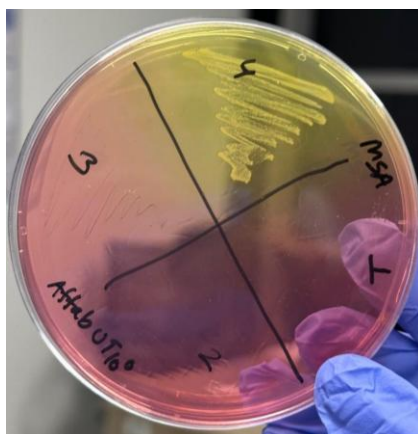


Fig 3.9: Aftabnagar Untreated water sample showed only yellow colonies after streak plate method in the MSA agar

In the **Aftab Nagar sample**, the untreated plate **showed yellow colonies only**, indicating the presence of **Staphylococcus aureus**. From colonies initially isolated on Nutrient Agar, **four morphologically different colonies** were streaked onto MSA, and only the **fourth colony grew**, producing **yellow colonies**, confirming its identity as **Staphylococcus aureus**.

After **1-hour nanobubble treatment**, **no colonies were observed** in any of the samples, indicating that the nanobubble treatment effectively eliminated viable *Staphylococcus* populations from the water.

Overall, growth patterns on MSA demonstrated that nanobubble treatment markedly reduced or completely removed mannitol-fermenting and non-fermenting *Staphylococcus* species from the contaminated surface water.

3.3.2 Probable bacterial presence in Selective Media observation table

Table 3.2: List of Probable identified bacteria from Untreated water samples

Sample	Untreated Water (10 ⁶ plate)	Probable Bacteria
Hatirjheel	TCBS, MacConkey, XLD, MSA showed growth	• <i>Vibrio cholerae</i>, • <i>Escherichia coli</i>, • <i>Klebsiella spp.</i>, • <i>Enterobacter spp.</i>, • <i>Staphylococcus aureus</i>
Banani Lake	TCBS, MacConkey, XLD, MSA showed growth	• <i>Escherichia coli</i>, • <i>Klebsiella spp.</i>, • <i>Enterobacter spp.</i>, • <i>Staphylococcus aureus</i>, • <i>S. epidermidis</i>, • <i>Enterobacter aerogenes</i>
Aftab Nagar Lake	TCBS, MacConkey, XLD, MSA showed growth	• <i>Vibrio cholerae</i>, other <i>Vibrio spp.</i>, • <i>Escherichia coli</i>, • <i>Klebsiella spp.</i>, • <i>Enterobacter spp.</i>, • <i>Shigella spp.</i>, • <i>Staphylococcus aureus</i>

3.4 Triple Sugar Iron (TSI), Catalase, and Oxidase Test Observations

Triple Sugar Iron (TSI) agar was used to differentiate enteric bacteria based on their ability to ferment sugars (glucose, lactose, sucrose), produce gas, and generate hydrogen sulfide (H₂S). Catalase and Oxidase tests were performed to assess the production of the respective enzymes in the isolated bacteria.



Fig 3.10: TSI test showed pink slant, cracked media, yellow butt & slant confirming the presence of *E. coli*, *Klebsiella*, *Vibrio spp.* and others

The inoculated TSI slants showed **pink slant, cracked media, and yellow butt and slant** for the bacteria taken from the water samples in the chosen selective media. These reactions confirmed the presence of **glucose, lactose, and sucrose fermenting bacteria**, including *Escherichia coli*, *Klebsiella spp.*, *Vibrio spp.*, and other enteric bacteria. Gas production was observed as cracks and bubbles in the agar. **H₂S production was negative** for most bacteria, indicating the absence of significant sulfur-reducing species in the tested isolates.



Fig 3.11: All the bacteria showed Catalase test positive

Catalase test: All tested bacterial isolates, including *E. coli*, *Klebsiella spp.*, and *Vibrio spp.*, showed **positive catalase activity**, indicating their ability to decompose hydrogen peroxide into water and oxygen. **Bubbles appeared immediately after adding hydrogen peroxide (H₂O₂) to the bacterial colony.**

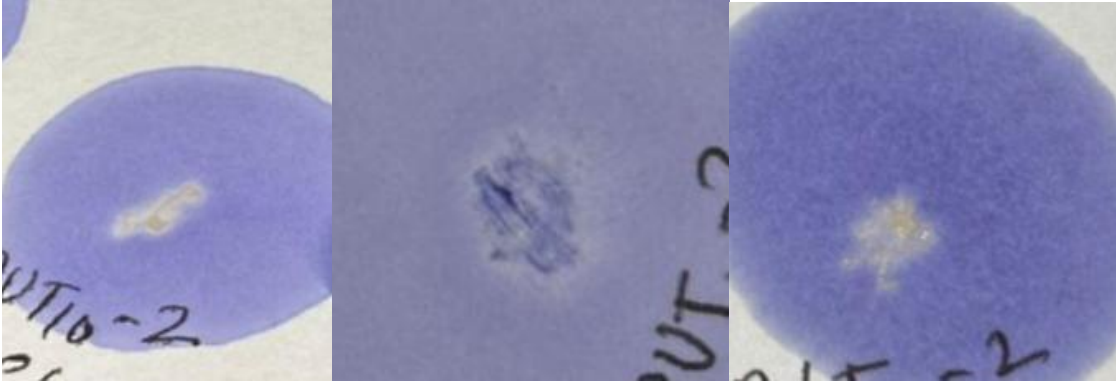


Fig 3.12: Oxidase negative for *E.coli*, positive for *Vibrio cholerae*, negative for *Klebsiella* were detected

- *Escherichia coli* – no color change, **oxidase negative**, indicating the absence of cytochrome c oxidase.
- *Vibrio cholerae* – color changed to purple/blue, **oxidase positive**, confirming its characteristic cytochrome c oxidase activity.
- *Klebsiella spp.* – no color change, **oxidase negative**.

3.5 PCR results checked via Agarose Gel-electrophoresis and confirmation

All gels exhibited good resolution, allowing clear differentiation of DNA fragments of different sizes. However, faint, smeared, or multiple bands observed in 2–3 DNA samples likely resulted from **partial DNA degradation, primer-dimer formation, or nonspecific amplification**, as well as possible issues with sample loading or gel running conditions, which may require optimization.

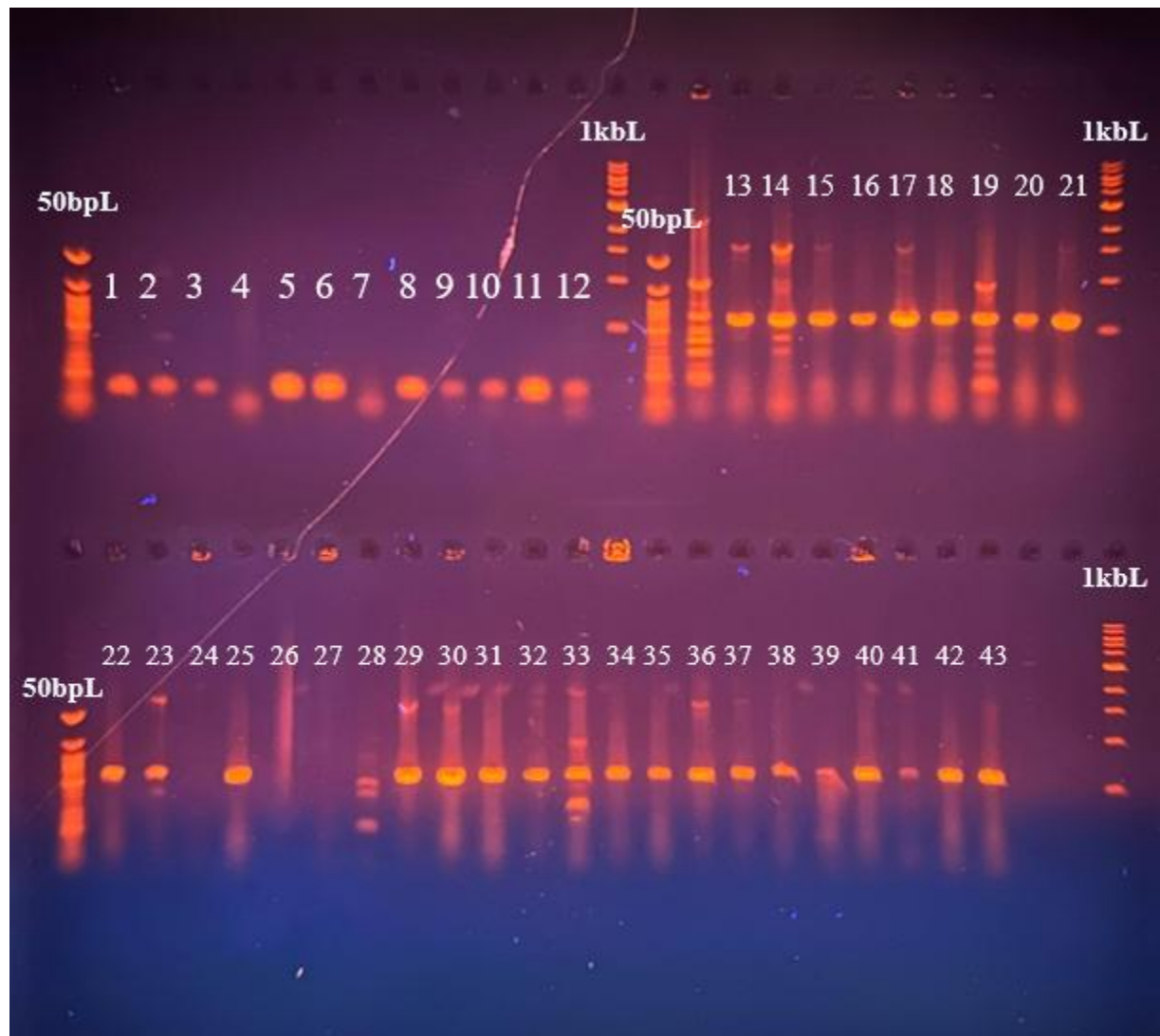


Fig 3.13: PCR result of *Klebsiella pneumoniae* sample from 1-7 (Banani lake), 8-12 (Hatirjheel Lake), *E.coli* 13-21 (Banani lake), 22-28 (Aftabnagar lake), 29-43 (Hatirjheel lake)

- ***Klebsiella pneumoniae* (Untreated water samples)**

In the wells labeled 50 bp L and 1 kb L, 50 bp and 1 kb DNA ladders were used, respectively. The ladder bands appeared sharp and distinct, providing a reliable reference for estimating DNA fragment sizes. In wells 1–3 and 9, 10, and 12, distinct and clear DNA bands were observed at the same position and of similar size, indicating successful amplification with consistent fragment length. Wells 5, 6, and 11 showed slightly larger bands compared to the others but still within the expected range, suggesting minor variation in amplification efficiency. Wells 4 and 7, on the other hand showed faint bands or perhaps smearing which could be a sign of partial template DNA degradation or a lower DNA concentration.

All of the amplified DNA bands which had an approximate size of **100–150 bp**, were visible between the second and third bands from the bottom of the 50 bp ladder and beneath the smallest band of the 1 kb ladder. The observed band sizes are within the expected range, as the PCR amplicon size for *Klebsiella pneumoniae* using the particular primer set is **133 bp**. These findings thus validate the existence of *Klebsiella pneumoniae* in the samples and can be regarded as legitimate.

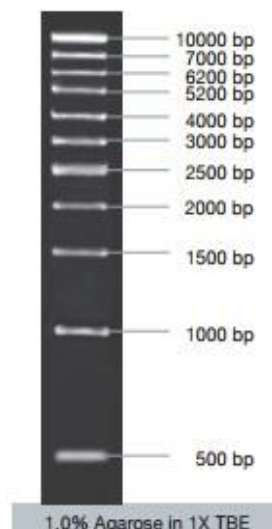


Fig 3.14: Universal 1kb ladder band sizes in 1x TBE buffer with 1% Agarose

- ***E. coli* (Untreated water samples)**

Most samples from lanes 13–21 and 22–43 had DNA bands found in them, suggesting that PCR amplification was generally successful. However, no bands were visible in wells 24, 26, and 27, which may have been caused by either partial degradation of the template DNA, insufficient DNA concentration, or incomplete amplification. The faint and slightly diffused appearance of the bands in wells 38, 39, and 41 was probably caused by uneven loading or inaccurate PCR product dispensing into the wells.

Overall, most lanes showed strong, uniformly sized bands that were well defined, indicating optimal amplification efficiency and good DNA integrity. Multiple bands were visible in wells 14, 17, 19, 23, 28, and 33, though. This could be the consequence of non-specific primer annealing, amplification of non-target sequences, or residual genomic DNA contamination that led to the formation of secondary products. A few wells also showed some smearing, which could have been brought on by too much DNA loading, leftover salts in the PCR mix, or slight DNA degradation during electrophoresis.

All prominent DNA bands migrated between the **500–600 bp region** of the 50 bp ladder and slightly below the **500 bp band of the 1 kb ladder**, corresponding to the expected fragment range. As the target amplicon size for *Escherichia coli* using the specific primer set is **approximately 585 bp**, the observed band sizes align well with the expected outcome. Therefore, the samples displaying strong and distinct bands within this range are confirmed to be **positive for *E. coli***.

- *Vibrio* (Untreated water samples)



Fig 3.15: *Vibrio cholerae* PCR result from 3-9 (Aftabnagar lake), 10-12 (Banani Lake), 1&2 (Hatirjheel lake)

When a 1 kb DNA ladder was loaded into well L, it displayed clear, distinct bands that were a good way to estimate the sizes of the fragments. Clear DNA bands were seen in wells 2, 3, 6, 8, 9, and 11, demonstrating that the target sequence had been amplified successfully. Conversely, wells 1 and 10 displayed no discernible DNA bands, which could be the result of ineffective amplification, low DNA concentration, or the absence of the target gene. The band in well 9 seemed to be the brightest among the positive wells, indicating a higher DNA yield or higher amplification efficiency. In wells 4, 5, and 7, faint bands with smearing were seen, most likely as a result of partial DNA degradation or low template concentration. In contrast, the band in well 2 was weak.

In **wells 6 and 9**, multiple bands appeared along with visible smearing. This may have resulted from **non-specific primer binding**, **suboptimal annealing temperature**, or **residual contamination** that produced secondary amplification products. In **well 12**, the band was completely smeared, which could be attributed to **overloading of the PCR product**, **excess salts or impurities in the sample**, or **thermal degradation of DNA** during electrophoresis.

The amplified fragments were approximately **154 bp** in size, which corresponds to the expected amplicon size for *Vibrio cholerae*. Therefore, according to the observed results, **7 out of 12 samples** displayed successful bands consistent with *Vibrio cholerae* DNA.

3.6 pH Measurements & Water Quality after Nano bubble Treatment



Fig 3.16: After the treatment, treated sample pH 8.15 & untreated sample pH 8.81, significant color change in the water is visible.

After being treated with nanobubbles for an hour, the pH of the Hatirjheel water sample dropped from 8.81 to 8.15, representing a pH reduction of roughly 0.5 to 1 unit. Following treatment, there was also a discernible change in the color of the water; the treated sample became noticeably clearer, less yellowish, and more transparent, while the untreated sample appeared somewhat turbid and yellowish.

3.7 Hydrodynamic diameter (Z-average), Size distribution, Polydispersity Index (PDI) & Stability of the Nano bubbles over Time

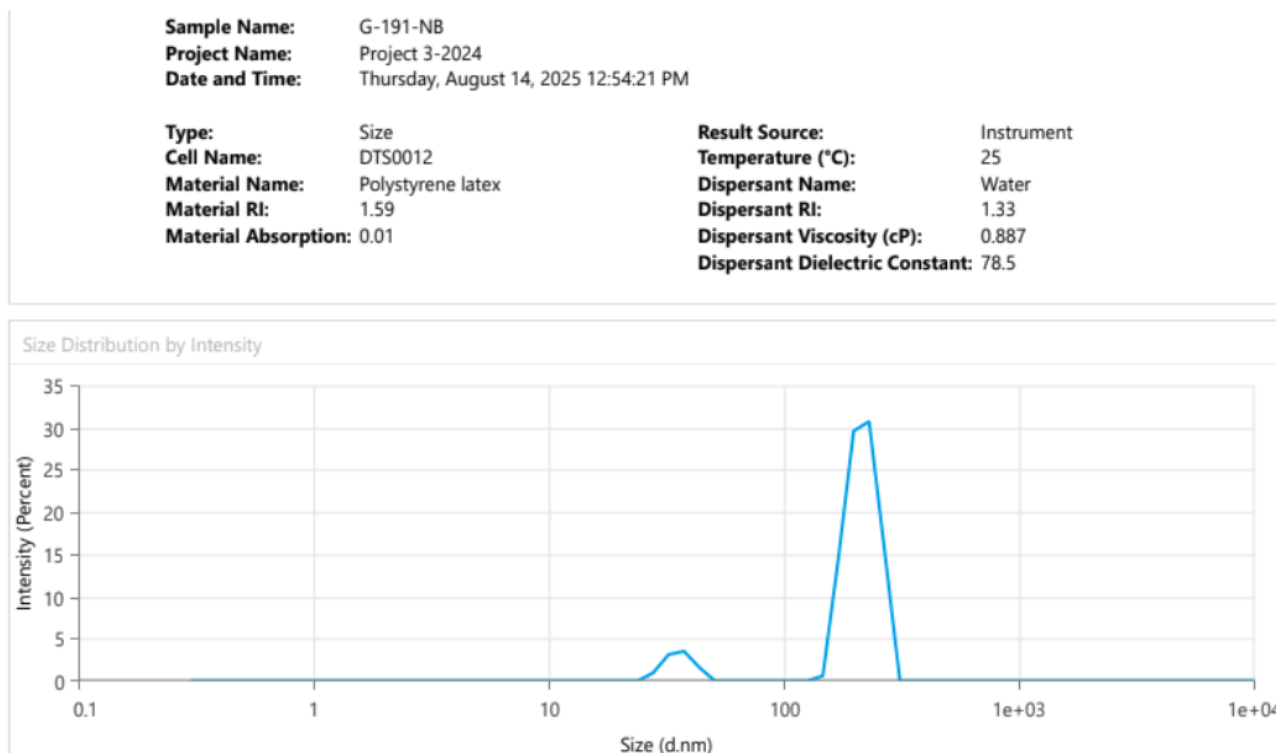


Fig 3.17: Size distribution by intensity of Nano bubbles

The dynamic light scattering (DLS) analysis showed the size distribution of polystyrene latex of Nano bubbles in water by intensity. The major peak occurred at approximately 215 nm, indicating that the majority of the Nano bubbles had diameters around this size. A smaller secondary peak at ~36 nm was also observed, suggesting the presence of a minor population of smaller bubbles or particle aggregates. This bimodal distribution reflects the heterogeneity of the sample.

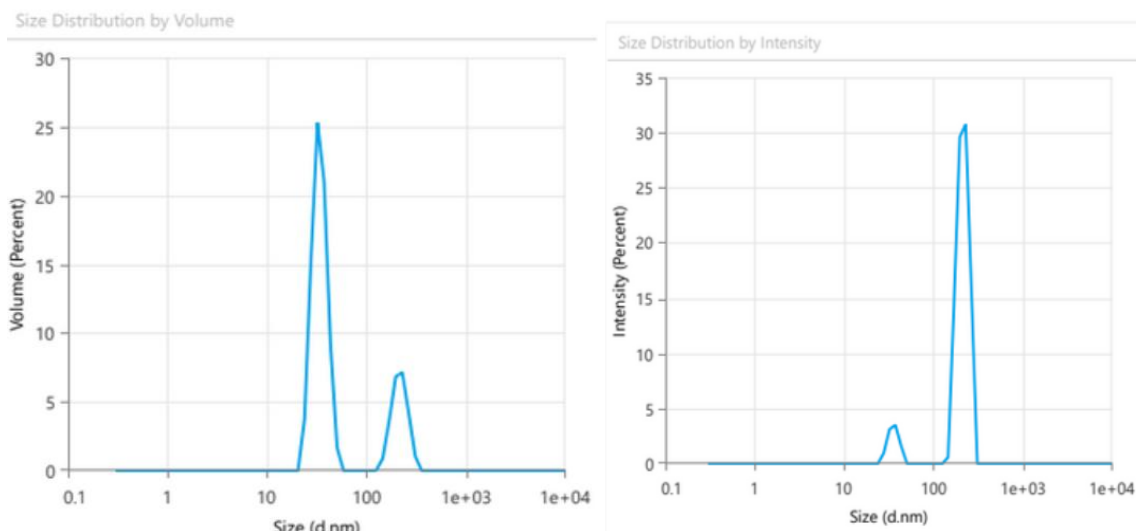


Fig 3.18: Size distribution by volume & intensity

The volume-based size distribution verified that particles ranging from 100 to 200 nm contributed the greatest amount of the sample volume. A tiny portion of the total volume was represented by the minor peak at about 35–40 nm. This suggested that although there were smaller bubbles, larger Nano bubbles made up the majority of the dispersed phase. It was further highlighted by this intensity-weighted size distribution that the ~215 nm population was responsible for the majority of the scattering intensity. The larger size fraction predominated in the analysis because intensity-weighted distributions were more sensitive to larger particles.

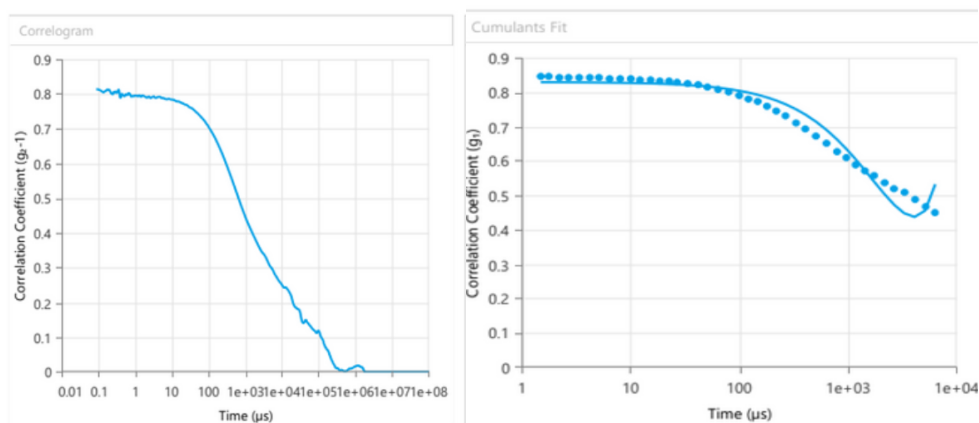


Fig 3.19: Correlogram & Cumulants fit

The correlation coefficient (g_1) was plotted against time in the correlogram. A stable population of Nano bubbles with comparatively uniform Brownian motion was suggested by the correlation

function's smooth decay. Reliable DLS measurements with little aggregation during the measurement period were indicated by the fit quality. By fitting the correlation function, the cumulants analysis produced a Z-average size and polydispersity index (PDI). The solid line displayed the fitted model, and the dotted points represented the experimental data.

Table 3.3: DLS Measurement Parameters and Results

DLS Measurement Parameter	Results
Z Average (nm)	633
Polydispersity Index (PI)	0.7813
In Range (%)	76.51
Peak 1 Mean by Intensity ordered by area (nm)	215.7
Peak 2 Mean by Intensity ordered by area (nm)	35.8

According to the results of the DLS analysis, the nano bubbles' Z-average hydrodynamic diameter is 633 nm, and their high polydispersity index (PDI = 0.7813) suggests a wide size distribution. The measured size range is occupied by about 76.51% of the particles. The findings from the intensity and volume distributions were corroborated by peak analysis, which showed two dominant populations: Peak 1 at 215.7 nm and Peak 2 at 35.8 nm. These findings validated the nano bubble preparation's polydisperse nature and offered quantitative information about the colloidal system's stability and size characteristics.

Chapter 4

Discussion

The primary goal of this study was to assess the bactericidal effectiveness of water produced by nanobubbles on samples of naturally contaminated urban lakes. The total viable count (TVC) decreased by 64.6% in the Hatirjheel water sample and 86.05% in the Aftab Nagar Lake water sample, according to the results. A percent reduction could not be computed for the Banani Lake untreated sample because it was too numerous to count (TNTC); the treated value for Banani was 3.20×10^4 CFU/mL (calculated, DF = 100). Between Hatirjheel and Aftab Nagar, the two locations with countable untreated values, the overall average reduction is 75.3%.

4.1 Evaluation of Microbial Reduction and Confirmed Pathogens

The initial presence of several pathogenic and opportunistic bacterial groups, such as *Vibrio cholerae*, *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Shigella spp.*, *Staphylococcus aureus*, and *S. epidermidis* was validated by selective media characterization. The detection of ***Vibrio cholerae*, *E. coli*, and *Klebsiella pneumoniae*** in untreated lake water samples were further confirmed by PCR amplification demonstrating significant microbial contamination in line with fecal infiltration and anthropogenic pollution. Selective agar plates (TCBS, MacConkey, XLD, and MSA) showed either no growth or significantly decreased colonies after treatment, indicating almost total bacterial inactivation. The absence of *Proteus* or *Salmonella* species in the tested samples was verified by the absence of black-centered colonies on XLD media.

These findings strongly indicate that nanobubble-generated water effectively suppresses both **Gram-negative** and **Gram-positive** bacterial populations, with particular efficacy against enteric and coliform-type pathogens, thereby demonstrating its potential as a non-chemical disinfection technology for contaminated surface waters.

4.2 Mechanistic Insights: Reactive Oxygen Species and Oxidative Stress

The production of reactive oxygen species (ROS) during bubble collapse is the main mechanism by which nanobubble water exhibits bactericidal effects. The formation of radicals like $\cdot\text{OH}$ (hydroxyl radical), O_2^- (superoxide anion) and singlet oxygen ($^1\text{O}_2$) is facilitated by the high

internal Laplace pressure and interfacial electric potential during nanobubble implosion. These ROS cause oxidative stress on proteins, nucleic acids, and bacterial membranes, which results in DNA degradation and cell lysis (*Li et al., 2022; Fan et al., 2020*).

In addition, nanobubbles' extended stability and negative zeta potential (usually between -20 to -35 mV for oxygen or air nanobubbles) improve their ability to interact with microbial cell walls, allowing for localized oxidation and extended contact. The results of the study are in line with the mechanisms put forth by Ohgaki & Akagi (2018) and Suzuki et al. (2016), who found that rapid cell death without the use of chemical additives was caused by ROS-induced membrane peroxidation and elevated intracellular ROS levels.

4.3 pH Reduction and Its Implications

The pH value of Hatirjheel water dropped by approximately 0.5 to 1 unit after an hour of nanobubble treatment from 8.81 to 8.15. According to published research, the dissolution of CO₂ and interfacial oxidation reactions during nanobubble collapse are responsible for this pH decrease. According to Pang et al. (2020) and Wang et al. (2018), nanobubbles continuously release trace amounts of hydrogen ions (H⁺) as a byproduct of ROS generation which slightly acidifies the medium. Moreover, the effectiveness of disinfection is further increased by this pH shift which also increases the oxidative environment and interferes with microbial enzymatic activity and membrane integrity.

4.4 Dynamic Light Scattering (DLS) Characterization and Stability Evaluation

A Z-average diameter of 633 nm was found using Dynamic Light Scattering (DLS) analysis with two prominent intensity peaks at 215.7 nm and 35.8 nm indicating micro–nanobubble coexistence. Moderate to broad size heterogeneity is indicated by the polydispersity index (PI = 0.7813) which is typical of Venturi-based systems that do not use downstream size fractionation. PI values below 0.3 indicate monodisperse systems while values between 0.5 and 0.8 indicate a stable but heterogeneous bubble population per International Organization for Standardization (ISO) standards and literature (Zhou et al., 2019; Gao et al., 2023). Therefore, even though the size of the generated bubbles varies but the obtained PI indicates that their stability and persistence are adequate for functional disinfection. By permitting simultaneous interactions across several bubble

size regimes, this wide distribution may even improve bactericidal efficacy by smaller nanobubbles contributing to Reactive Oxygen Species (ROS) production, while larger microbubbles aid in mixing and oxygenation.

4.5 Confirmed Mechanistic Pathway and Broader Potential

A synergistic mechanism involving ROS generation, enhanced oxygen dissolution, interfacial charge interactions and mild acidification is used by the constructed nanobubble generator to degrade and inactivate pathogenic bacteria according to this thesis which is based on current findings and supporting literature. In a variety of bacteria, this integrated pathway results in membrane rupture, oxidative DNA damage, and metabolic inhibition.

Besides, dissolved heavy metals like Fe^{2+} and Mn^{2+} can be oxidized by nanobubbles to their insoluble higher valence states which makes it easier to remove them later by filtration or precipitation process (Feng et al., 2022; Gao et al., 2023). Therefore, this generator has great potential for thorough water remediation that addresses both biological and chemical contaminants, going beyond microbial disinfection.

4.6 Thesis Statement

Through reactive oxygen species-mediated **oxidative stress, interfacial charge disruption, and microenvironmental acidification**, this study conclusively showed that this custom designed Nano bubble generator can effectively inactivate multiple pathogenic bacteria including *Vibrio cholerae*, *Escherichia coli*, and *Klebsiella pneumoniae* in urban polluted lake water. For **prolonged oxidative activity**, the generated nano bubbles (**Z-average = 633 nm, PI = 0.7813**) show adequate stability. These results supported the use of nano bubble technology as a potential low-cost, chemical-free, and environmentally friendly substitute for heavy metal oxidation and microbial disinfection for Bangladesh and other developing countries' contaminated water systems.

Chapter 5

Conclusion

According to this study, the nanobubble generator is a workable and sustainable system to enhance the microbial contaminated water quality in Dhaka's urban lakes. All of the treatment outcomes confirm that pathogenic microorganisms can be effectively suppressed by nanobubble technology without the need for chemicals which represents a major breakthrough in environmentally friendly water purification.

The generator creates highly stable reactive nanobubbles that can cause oxidative processes that compromise the integrity of microorganisms as confirmed by the observed physicochemical and microbiological results. The oxidative reactions that take place at the gas–liquid interface and improve bactericidal activity are reflected in the concurrent drop in pH following treatment. All of these results offer convincing experimental evidence in favor of using nanobubbles as natural water disinfection systems.

The research outcomes demonstrate how nanobubble technology can be used to address microbial contamination as well as solve more general issues with water quality by verifying the breakdown of pathogenic microbes like *Escherichia coli*, *Vibrio cholerae* and *Klebsiella pneumoniae*. The results also indicate that, along with disinfection nanobubbles may aid in the oxidation of heavy metals and the breakdown of pollutants offering a comprehensive strategy for environmentally friendly water treatment.

To sum up, the constructed nanobubble generator shows great promise as an effective, affordable, and environmentally responsible substitute for water purification and a viable solution for Bangladesh's water crisis. Its effective operation highlights the ability of incorporating nanobubble-based systems into upcoming community-scale treatment solutions, encouraging safer water management techniques in areas with limited resources and high population densities.

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