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Performance of Zinc Oxide-Enhanced Pineapple-Derived Nanocellulose Nanofilter for Bacterial Reduction in River Water

M Rafly Aditya Sunoto[a], Alfi Nur Rusydi[a], Nico Rahman Caesar[a*], Dewangga Paramanandana Raharjo[a], Muhamad Amin[b]

[a] Department of Aquatic Resources Management, Brawijaya University, Malang, **Indonesia**
[b] Environmental and Life Sciences Programme, Faculty of Science, Universiti Brunei Darussalam, **Brunei Darussalam**
*Corresponding email: nicocaesar@ub.ac.id

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Abstract
The Porong River is a main river in Sidoarjo, Indonesia, which indicates pollution due to domestic wastewater discharge, industrial activities, and sediment transport associated with the Lapindo mudflow. The pollution leads to microbiological contamination, including pathogenic bacteria. This study aims to investigate the performance of a ZnO-enhanced cellulose-based nanofilter made from pineapple biowaste to reduce the abundance of bacteria in river water. The methods include fabrication of a nanofilter using nanocellulose derived from pineapple peel waste and reinforced with ZnO nanoparticles at concentrations of 0%, 0.25%, 0.5%, 0.75%, and 1%. Water samples were collected from three sampling stations along the river. Bacterial abundance was counted using the Total Plate Count method. Water quality, including temperature, total suspended solids, total dissolved solids, dissolved oxygen, pH, ammonia, nitrate, phosphate, CO_2, and total organic matter, was also analyzed. Results indicate that the water river detected the presence of Bacillus sp., Enterobacter sp., and Salmonella sp., with abundance ranging from (3.67–3.97) × 10^3 CFU/mL. One-way analysis of variance indicates that water filtration using a ZnO-reinforced nanofilter significantly reduces bacterial concentrations ($F = 10.21$, $p < 0.05$, $n = 3$). The highest filtration effectiveness was observed at 0.25% ZnO, with an effectiveness of 52.89±11.93%. Increasing ZnO content reduces the effectiveness of the nanofilter, but Tukey analysis indicates that the effectiveness reduction is not different compared to optimal filtration after. It shows that a low concentration of ZnO can be applied to develop a nanofilter with better results. ZnO-enhanced nanofilters are a promising bio–nano-based material for sustainable water treatment and reducing bacterial contamination control in aquatic systems.

Keywords: *ZnO nanoparticles; pineapple-derived nanocellulose; nanofiltration; bacterial reduction; river water; Porong River*

1. Introduction

River waters constitute an essential component of ecological systems and human life; Not only supply water for domestic and agricultural needs, but also serve as transportation routes and sites. Currently, the water quality of the river is degraded due to human activities along river basins from the discharge of domestic, industrial, and agricultural waste. Higher levels of organic matter stimulate the proliferation of microorganisms, including pathogenic bacteria. Many pathogenic bacteria have a negative effect on aquatic ecosystems and the health of organisms in environments [1]. So, water sources, including rivers, should be managed by continuous monitoring and the development of effective water treatment technologies.

The Porong River is one of the branches of the Brantas River system in Sidoarjo Regency, East Java. This river experienced environmental pressure caused by anthropogenic activities. Porong River has identified a decline in water quality due to the influx of multiple pollution sources, such as domestic wastewater, industrial effluents, and the discharge of Lapindo mud, as the contributors to contamination in this river system. So, the Porong River is categorized as a polluted river [2]. These environmental conditions promote the growth of certain bacteria, including *Bacillus* sp., *Enterobacter* sp., and *Salmonella* sp., which typically thrive in waters with high levels of organic pollution [3,4].

As concentrations of organic matter and nutrients increase, heterotrophic bacterial populations tend to proliferate because these substances serve as nutrient sources for microbial growth [5]. Therefore, efforts to identify and control bacterial contamination in the Porong River are crucial for maintaining environmental water quality. One important technology is the use of nanofilters to reduce many contaminants. Nanofilters operate through semipermeable membranes with extremely small pore sizes, enabling the removal of microscopic particles, including bacteria and certain organic compounds [6]. This technology offers several advantages, such as high selectivity, efficient filtration performance, and flexibility in membrane material design [7]. So, nanofilter technology is a promising method for reducing bacterial contamination in polluted river water.

Pineapple (*Ananas comosus* (L.) Merr) waste can be utilized as a source of bacterial cellulose [8]. Bacterial cellulose produced through fermentation possesses and it can be a promising material for filtration applications because of its strong adsorption capacity toward both organic and inorganic compounds [9]. To enhance filtration performance, nanofilter membranes can be combined with nanoparticles possessing strong antibacterial properties, such as zinc oxide (ZnO). At the nanoscale, ZnO particles possess a large surface area, which enhances interactions with microorganisms and increases the effectiveness of bacterial inactivation processes [10]. These characteristics have led to their extensive use in various applications, including water treatment technologies and antibacterial materials [11]. Therefore, integrating ZnO nanoparticles into biomaterial-based nanofilter membranes is expected to improve filtration efficiency while providing stronger antibacterial effects. So, this study aims to analyze the effectiveness of a nanofilter made from pineapple waste with ZnO nanoparticles reinforcement in reducing the total number of bacteria in the waters of the Porong River, Sidoarjo. This study also attempts to identify bacterial species present in the Porong River and evaluate the relationship between water quality parameters and bacterial abundance.

2. Materials and Methods

2.1. Research Design

The research design used a randomized block design with independent variables that were the concentration of ZnO nanoparticles (0.25%, 0.5%, 0.75%, and 1%) added into the nanofilter membrane, and the dependent variable was the total number of bacteria present and the effectiveness of filtration. Water quality parameters were also measured to understand the relationship between environmental conditions and bacterial populations in the river. These parameters included temperature, total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen (DO), pH, carbon dioxide (CO_2), phosphate, ammonia, total organic matter (TOM), and nitrate.

2.2. Location, Time, and Sampling Technique

The study was done from December 2024 to February 2025. Sampling location was in the Porong River, located in Sidoarjo Regency, East Java, Indonesia. The Porong River was known to flow through community housing, industrial areas, and the Lapindo mud, which flows, resulting in the river having many potentials to get pollution. The sampling of the water used a purposive sampling with 3 stations for water sampling, representing variations in conditions within the river. Sampling was conducted with three replications at each station.

2.3. Bacterial Cellulose Production

Pineapple peel waste obtained from plantations in the Blitar region of East Java, Indonesia. The fermentation medium to produce bacterial cellulose consisted of a mixture of 1 L pineapple peel extract, 5.0 g ammonia, and 100 g glucose as nutrient sources for bacterial growth. The fermentation medium was adjusted to a pH of 4.5 by adding acetic acid (CH_3COOH). The fermentation process was initiated by inoculating 100 mL of *Acetobacter xylinum* culture (10% of medium volume) into the prepared medium, followed by static incubation at 25–30°C. After ten days of fermentation, the bacterial cellulose pellicle was floated over the medium and harvested, and washed with distilled water until a neutral pH was achieved [9].

2.4. Nanocellulose Production and Nanofilter Membrane Synthesis

The bacterial cellulose pellicle was immersed in 6% NaOH solution for two hours at 90°C. The treated pellicle was then rinsed with water and cut into small portions. 300 g of pellicle was soaked in distilled water and crushed using a blender at 20,000 rpm. The resulting suspension was entered in a high-pressure homogenizer to produce bacterial nanocellulose slurry at 150 bar for five cycles. The suspension was subsequently filtered using a vacuum filtration system to obtain a more homogeneous nanocellulose [9]. In another place, ZnO nanoparticles with particle sizes of 20–30 nm were dispersed in distilled water by sonication for 25 minutes at 400 watts for each concentration of 0.25%, 0.5%, 0.75%, and 1% from 5 g dry bacterial nanocellulose. The suspension was added by dispersing ZnO nanoparticles, then stirred using a magnetic stirrer for two hours to ensure uniform nanoparticle distribution. The suspension was poured into glass molds and dried in an oven at 60°C for 24 hours until a solid nanofilter membrane was formed. The resulting membranes were stored under dry conditions before being used in the filtration experiments.

2.5. Nanofilter Application and Filtration Testing

The nanofilter membranes were tested in the laboratory using water samples collected from the Porong River. Water samples were tested with a syringe containing a membrane. A water sample of 5 mL was sucked into the syringe barrel, and water was manually pressed to pass the membrane [12]. The filtrate was collected and analyzed to determine the total bacterial count. Variations in ZnO nanoparticle concentration within the membrane were used to evaluate the effect of nanoparticle addition on bacterial filtration effectiveness.

2.6. Microbiological Analysis

The total bacterial count was done using the total plate count (TPC) method. The water samples were diluted then a portion of the diluted sample was poured into petri dishes containing nutrient agar medium. The medium was then incubated for 24–48 hours at 25–30°C [13]. The number of colonies formed was counted under a microscope.

2.7. Data Analysis

The data obtained from the measurement of total bacterial counts and water quality parameters were analyzed using statistical methods. One-way analysis of variance (ANOVA) was applied, followed by Tukey analysis with a significance level of 95%.

3. Results and Discussion

3.1. Water Quality of Porong River

Field observations inform water quality as presented in Table 1. It shows that the river is strongly influenced by anthropogenic activities along its watershed. The water temperature ranges from 29–30 °C across the sampling stations, with the highest and lowest temperatures recorded at Station 3 (30.83 °C) and at Station 1 (29.83 °C), respectively. These temperatures are within the standard of 25–32 °C for tropical water [14]. The difference in temperature among stations seemed to be influenced by surrounding environmental conditions. Station 1 is located in the upstream area, so it exhibited lower temperatures due to the presence of vegetation that reduced direct solar radiation on the water surface. Station 3 shows a higher temperature due to its downstream location, with the accumulation of organic waste from upstream areas. Higher sediment enhances microbial decomposition processes and contributes to increased water temperature [15].

Table 1. Water quality parameters before and after filtration

Water Quality Parameter	Unit	Before Filtration	After Filtration
Temperature	^{O}C	30.83 ± 0.47	29.83 ± 0.37
TSS	mg/L	81.5 ± 4.44	61.8 ± 2.98
TDS	mg/L	445.67 ± 30.74	249 ± 13.0
pH	-	8.01 ± 0.08	7.92 ± 0.08
Ammonia	mg/L	0.15 ± 0.02	0.11 ± 0.01
Nitrate	mg/L	2.08 ± 0.02	1.21 ± 0.25
Phosphate	mg/L	0.23 ± 0.038	0.22 ± 0.019
DO	mg/L	5.5 ± 0.20	5.4 ± 0.30
TOM	mg/L	24.9 ± 3.51	23.6 ± 3.41

TSS of water before being filtered ranged from 81.5 to 111.4 mg/L and reduced to 69.7–106.7 mg/L after filtration. It indicates that the nanofilter effectively removed suspended particles from the water. The highest and the lowest TSS concentrations were detected at Station 3 and Station 1, respectively, caused by different sediment accumulation and pollutant inputs transported from upstream areas. The most effective TSS reduction occurred after filtration using the nanofilter containing 0.25% ZnO. ZnO facilitates the aggregation and removal of suspended particles [16]. At higher concentrations, ZnO nanoparticles tend to reduce effective TSS filtration due to agglomeration.

TDS of water before and after filtration were in the range from 445–618 mg/L and 376–592 mg/L, with the highest and lowest values recorded at Station 3 and Station 1, respectively. However, measured TDS is still below the water quality standards with a limit of 1000 mg/L[17]. Elevated TDS levels downstream indicate the accumulation of dissolved minerals and anthropogenic pollutants. The optimal TDS reduction was observed with 0.25% ZnO nanoparticle. At this concentration, nanoparticle dispersion is relatively good, so they are able to enhance the adsorption of dissolved ions in water [18].

DO concentrations measured in the range from 5.29 to 5.5 mg/L. This value is over the minimum threshold of 3 mg/L established by Indonesian Government Regulation No. 22 of 2021. It indicates that oxygen levels in the Porong River are generally sufficient to support aquatic organisms. Variations in DO among stations were influenced by environmental factors such as vegetation cover, water temperature, and biological activity. Vegetation contributes to oxygen production through photosynthesis and supports higher DO of water [19].

The pH values of the river water measured in the range from 8.01–8.24 before filtration and decreased to 7.5–7.96 after filtration. Upon decrease, pH values are still within an acceptable range of 6–8.5 for river water quality standards [20]. The decrease in pH after filtration suggests that the ZnO nanoparticles in the nanofilter may influence ionic equilibrium of the water, though modify ion composition and consequently alter pH levels [21].

The concentration of dissolved CO_2 was measured in the range from 11.6 to 18.63 mg/L. This value is above CO_2 concentration that influences the aquatic organism [22]. Higher CO_2 levels indicate intensive organic matter decomposition within the river system. Degradation of organic materials by bacteria consumes oxygen and releases CO_2 as a metabolic by-product, so CO_2 concentrations are increased, potentially reducing overall water quality [23].

TOM concentrations measured in the range from 24.9 to 34.39 mg/L before filtration and decreased to 15.1–31.75 mg/L after treatment, with the highest TOM levels recorded at Station 3. It means Station 3 has the highest accumulation of organic pollutants from domestic and industrial sources. After filtration, TOM is reduced, which demonstrates the capability of the nanofilter to adsorb organic compounds from the water. ZnO concentration of 0.25% shows the most effective adsorption efficiency for organic contaminants [24].

The phosphate and nitrate concentrations also reduced after filtration. The structure of the nanocellulose membrane effectively traps suspended particles. Phosphate ions are adsorbed onto the hydroxyl groups of cellulose [25]. Phosphate and nitrite ions were adsorbed onto the surface of ZnO nanoparticles [26,27]. Although nitrate is more soluble and less prone to adsorption, the partial reduction is allegedly associated with the retention of organic-bound nitrogen and limited surface interactions with ZnO. The filtration system contributes not only to bacterial removal but also to partial nutrient reduction.

3.2. Bacterial Identification in the Porong River

The water quality of the river is related to the bacterial communities. Polluted waters increase the pathogenic bacteria, whereas healthier ecosystems tend to support beneficial microbial populations [28]. Bacterial identification from water samples is shown in Fig. 1 and Table 2, and the effect of ZnO concentration on total bacterial count is shown in Fig. 2.

Bacterial species dominant obtained from the river are *Salmonella* sp, *Bacillus* sp, and *Enterobacter* sp. After filtration, each station demonstrated a different bacterial count. Water control contains total bacteria of 3.81±0.15 (CFU/mL). After filtration using membranes with ZnO nanoparticle content of 0.25%, 0.50%, 0.75% and 1.0%, total bacteria count reduced to 1.81±0.51, 1.92±0.54, 1.91±0.37, and 2.07±0.54 (CFU/mL), respectively.

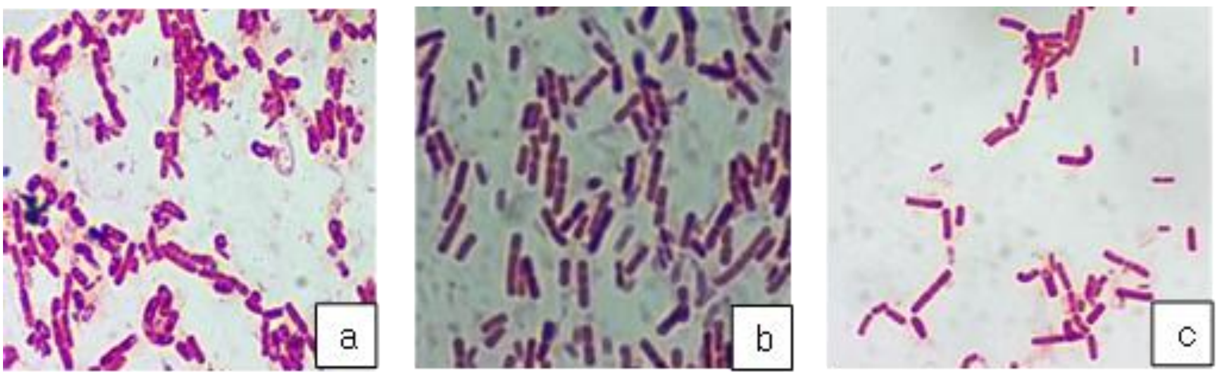

Fig. 1. Bacterial species identified in the Porong River: (a) *Bacillus* sp., (b) *Salmonella* sp., and (c) *Enterobacter* sp.

Bacterial species dominant obtained from the river are *Salmonella* sp, *Bacillus* sp, and *Enterobacter* sp. After filtration, each station demonstrated a different bacterial count. Water control contains total bacteria of 3.81±0.15 (CFU/mL). After filtration using membranes with ZnO nanoparticle content of 0.25%, 0.50%, 0.75% and 1.0%, total bacteria count reduced to 1.81±0.51, 1.92±0.54, 1.91±0.37, and 2.07±0.54 (CFU/mL), respectively. The one-way ANOVA analysis indicates a significant difference in total bacterial counts among the treatments ($F = 10.21$, $p < 0.05$, $n = 3$). The ZnO concentration significantly affected bacterial removal performance. Compared to the control, all ZnO-treated nanofilters showed a reduction in bacterial counts. However, Post-hoc Tukey HSD analysis confirmed that the differences among ZnO nanoparticle concentrations appear relatively small, suggesting that increasing ZnO nanoparticle content does not proportionally enhance filtration effectiveness. No significant differences were observed among the different ZnO nanoparticle concentrations (0.25–1.00%) with $p < 0.05$ (Fig. 2). This phenomenon could be attributed to nanoparticle agglomeration at higher concentrations, which reduces the available active surface area for interaction with bacterial cells.

Table 2. Bacterial species and total plate count in the Porong River

Location	Treatment	Bacterial Species	Total Plate Count (CFU/mL)
1	Control nanofilter	*Salmonella* sp	$(3.67 \pm 0.83) \times 10^3$
	ZnO 0.25%	*Bacillus* sp	$(1.24 \pm 1.52) \times 10^3$
	ZnO 0.50%	*Salmonella* sp	$(1.33 \pm 1.45) \times 10^3$
	ZnO 0.75%	*Salmonella* sp	$(1.41 \pm 1.47) \times 10^3$
	ZnO 1.00%	*Enterobacter* sp	$(1.49 \pm 1.47) \times 10^3$
2	Control nanofilter	*Salmonella* sp	$(3.80 \pm 1.04) \times 10^3$
	ZnO 0.25%	*Bacillus* sp	$(1.92 \pm 2.21) \times 10^3$
	ZnO 0.50%	*Salmonella* sp	$(2.04 \pm 2.35) \times 10^3$
	ZnO 0.75%	*Salmonella* sp	$(2.15 \pm 1.47) \times 10^3$
	ZnO 1.00%	*Bacillus* sp	$(2.18 \pm 1.42) \times 10^3$
3	Control nanofilter	*Salmonella* sp	$(3.97 \pm 1.26) \times 10^3$
	ZnO 0.25%	*Bacillus* sp	$(2.26 \pm 1.35) \times 10^3$
	ZnO 0.50%	*Bacillus* sp	$(2.40 \pm 1.51) \times 10^3$
	ZnO 0.75%	*Bacillus* sp	$(2.16 \pm 1.69) \times 10^3$
	ZnO 1.00%	*Enterobacter* sp	$(2.54 \pm 1.59) \times 10^3$

The one-way ANOVA analysis indicates a significant difference in total bacterial counts among the treatments ($F = 10.21$, $p < 0.05$, $n = 3$). The ZnO concentration significantly affected bacterial removal performance. Compared to the control, all ZnO-treated nanofilters showed a reduction in bacterial counts. However, Post-hoc Tukey analysis confirmed that the differences among ZnO nanoparticle concentrations appear relatively small, suggesting that increasing ZnO nanoparticle content does not proportionally enhance filtration effectiveness. No significant differences were observed among the different ZnO nanoparticle concentrations (0.25–1.00%) with $p < 0.05$ (Fig. 2). This phenomenon could be attributed to nanoparticle agglomeration at higher concentrations, which reduces the available active surface area for interaction with bacterial cells.

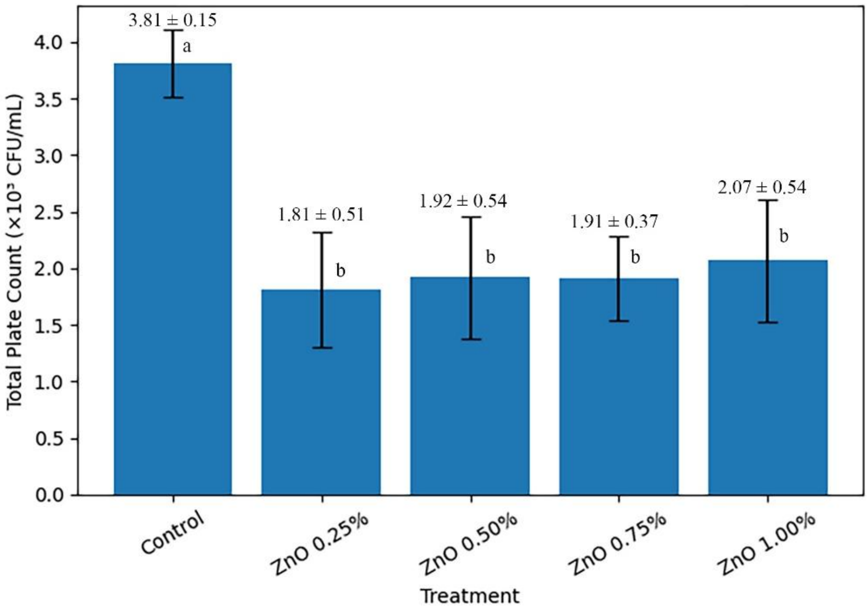

Fig. 2. The effect of ZnO concentration on total bacterial count

3.3. Effectiveness of Filtration

The antibacterial performance of the ZnO nanofilter was evaluated through three experimental replicates at three sampling stations with five treatment variations. Water samples from the Porong River were filtered using nanofilters containing different concentrations of ZnO nanoparticles, and the percentage of bacterial removal was calculated.

The effectiveness of bacterial removal under different ZnO concentrations is presented in Fig. 3. The highest effectiveness was observed at 0.25% ZnO (52.89 ± 11.93%), followed by 0.50%, 0.75%, and 1.00%. The filtration effectiveness decreases with the increase in ZnO nanoparticle concentration. This decrease is allegedly caused by nanoparticle agglomeration and aggregation [29], which reduces the effective surface area available for antibacterial interactions. Therefore, higher ZnO concentrations do not necessarily lead to improved filtration performance. A similar result was also reported by Yanuhar et al. [9], where ZnO concentrations of about 0.25% shows optimal mechanical properties of nanofilter membranes that enhance structural stability and maintain filtration efficiency. Conversely, ZnO nanoparticle concentrations more than 0.5% (0.5% to 1%) indicated a gradual decline in filtration performance. Combining ZnO with other nanomaterials can improve filtration effectiveness. Carbon nanotube, combined with ZnO in bacterial cellulose membranes, reduces *E. coli* from 57% to 91% depends on concentration and route of membrane synthesis [30]. Bacterial cellulose acetate membrane with Fe_3O_4 nanoparticle of 0.75-1.0% can reduce bacterial abundance by about 50% [31]. Magnetic nanomaterial in the cellulose membrane adsorbed bacteria from the wastewater of rivers with an effective value ranging from 39.1% to 67.4% [32].

Increasing ZnO concentrations to 0.5%, 0.75%, and 1% did not significantly improve bacterial removal efficiency. This phenomenon is associated with nanoparticle agglomeration at higher concentrations. Similar findings have been reported in previous nanocomposite studies, where excessive nanoparticle concentrations resulted in reduced antibacterial efficiency due to particle aggregation. Physical filtration is influenced by membrane pores, but bacterial reduction is also influenced by the antimicrobial properties of ZnO nanoparticles. Antimicrobial activity capable of disrupting bacterial cell membranes and inhibiting microbial metabolic processes [33]. Interactions between ZnO nanoparticles and bacterial cells can damage essential cellular components such as proteins, lipids, and DNA [24,34].

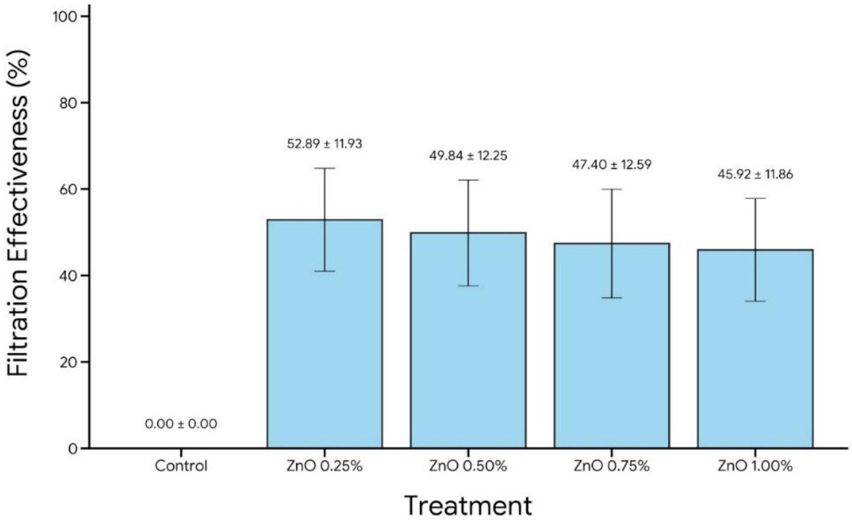

Fig. 3. The effect of ZnO concentration on filtration effectiveness

4. Conclusions

The results of this study demonstrate that the Porong River in Sidoarjo is contaminated by the presence of bacterial species such as *Bacillus* sp., *Enterobacter* sp., and *Salmonella* sp. Microbial contaminant was supported by elevated water quality parameters associated with organic and nutrient pollution, including TSS, TDS, ammonia, nitrate, phosphate, and TOM. Total bacterial counts reach about (3.67–3.97) × 10^3 CFU/mL in control samples. These parameters indicate that the water in the Porong River can be classified as polluted. Filtration using bacterial cellulose membrane from pineapple peel waste with nanoparticle reinforcement is effective in reducing bacterial contamination in the river water. Among the tested treatments, the nanofilter containing 0.25% ZnO exhibited the highest antibacterial performance, achieving bacterial removal efficiencies of 52.89±11.93%. At higher ZnO nanoparticle concentrations (0.5–1%), the removal efficiency decreased slightly. A limitation of this method is that the process is still in a small-scale or laboratory scale to reduce the abundance of bacteria, so it needs further study to generate an industrial scale for filtering many wastewaters in the river. In the future, this nanofilter system represents a promising and sustainable approach for reducing bacterial contamination in polluted river water, particularly in aquatic environments affected by anthropogenic pollution, such as the Porong River.

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Author Declaration

Data availability statement: Data will be made available on request.

CRediT authorship contribution: MRAS: Investigation, Data Curation. ANR: Methodology, Supervision, Validation. NRC: Resources, Project Administration, Funding Acquisition. DPR: Formal Analysis. MA: Writing – Review & Editing.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests.

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