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Removal of the Most Probable Number of *coli* from Hospital Wastewater Using Nanofiltration Membranes

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ABSTRACT

Hospitals are significant sources of wastewater containing pathogenic microorganisms, particularly enteropathogenic and toxigenic Escherichia coli (E. coli), which pose serious health and environmental risks if left untreated. This study evaluated the effectiveness of nanofiltration (NF) membranes in reducing E. coli concentrations in hospital wastewater using the Most Probable Number (MPN) method. Samples were collected from a type B hospital in Palembang, Indonesia, and treated with NF membranes operated at 60 psi and contact times between 10 and 60 seconds.The NF membrane achieved high removal efficiencies ranging from 99.75% to 100%, with complete elimination of E. coli at retention times of 30 seconds or more. This confirms nanofiltration as an effective tertiary treatment method for improving the microbiological quality of hospital effluent and ensuring compliance with discharge standards. The membrane's performance is attributed to its fine pore size and electrostatic repulsion of bacterial cells.To enhance practical application, future studies should focus on scaling up to full or industrial levels, evaluating long-term performance, fouling behavior, maintenance needs, and economic feasibility. Such efforts are vital to integrating nanofiltration into sustainable hospital wastewater management systems that protect public and environmental health.

Keywords: *Hospital wastewater, Nanofiltration membranes, E. Coli*

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INTRODUCTION

The removal of pathogenic microorganisms such as *Escherichia coli (E. coli)* from hospital wastewater is critical to prevent health risks and environmental contamination. The Most Probable Number (MPN) method is commonly used to quantify E. coli levels in wastewater. Nanofiltration (NF) technology, with pore sizes between 1–10 nanometers, has proven effective in removing these pathogens. Studies have shown that nanofiltration membranes coated with silver nanoparticles significantly enhance the removal efficiency of E. coli compared to conventional membranes (Mangayarkarasi et al., 2012).

Nano-Filtration Membrane Bioreactor (NF-MBR) systems have demonstrated high removal efficiencies for organic contaminants and nutrients in hospital wastewater, achieving up to 92% removal of Chemical Oxygen Demand (COD) and 88% removal of ammonia nitrogen (NH_3-N) (Kootenaei & Rad, 2013). Additionally, membrane bioreactors coupled with disinfection methods such as chlorination or ozonation have shown significant log reductions in coliform bacteria, including *Escherichia coli* (Chiemchaisri et al., 2022).

However, future research should expand beyond laboratory-scale experiments and address the application of NF-MBR systems at full-scale hospital wastewater treatment plants or even industrial-scale facilities. Cost analysis is also crucial yet absent in this study. A comprehensive assessment including capital investment, operational costs, membrane replacement, maintenance, and potential membrane fouling (clogging) is essential, especially considering the financial constraints typically faced by healthcare institutions.

The current study lacks clarity regarding its methodology. It does not specify how many hospitals were included as sampling sites, nor does it define key terms such as "AM" used in the results. Details on sampling procedures, data acquisition methods, and data analysis techniques are also missing. Furthermore, the study was based on a single, short-term observation period, without repeated trials to confirm the consistency and reliability of membrane performance under various environmental conditions. If no direct long-term data is available, it is recommended to compare findings with existing long-term studies to contextualize the results (e.g., Madaeni et al., 2015; Nghiem et al., 2006).

While the tabular data presentation is adequate, the lack of visual aids such as graphs showing trends over time (e.g., contact time vs. MPN values) makes it difficult for readers to interpret contaminant reduction patterns. Graphical representations would improve clarity and reader engagement.

In the discussion section, the findings should be critically compared with previous studies to validate the observed removal efficiencies and provide broader insight into the performance of nanofiltration systems. For instance, the study notes increased effectiveness with longer retention times (10 to 60 seconds), but fails to assess whether such durations are feasible in practical wastewater treatment plants (WWTPs), which typically operate under high flow conditions.

Although nanofiltration is widely recognized for its high efficiency, membrane fouling remains a major operational challenge. Unfortunately, this paper does not address fouling mechanisms, frequency, or mitigation strategies such as backwashing, chemical cleaning, or pretreatment approaches an omission that limits its practical applicability.

The conclusion should directly answer the research objective and offer concrete recommendations for real-world applications. Furthermore, it is advised that future studies expand the microbial scope beyond *E. coli* to include other clinically relevant pathogens such as *Pseudomonas aeruginosa*, *Enterococcus spp.*, and *Clostridium difficile* to ensure a comprehensive microbial risk assessment.

As the standard of living rises, the demand for water supply consistently increases, leading to environmental damage, including harm to water resources, and resulting in water scarcity. Water resources are diminishing due to the prevalence of various types of water pollution stemming from inadequate wastewater management across various industrial sectors. The discharge of wastewater into the environment can degrade it, rendering its utilization as a drinking water source and for various purposes challenging (Panagopoulos & Haralambous, 2020b, 2020a).

Hospitals, in their health service operations for the community, require large amounts of water in various service facilities. According to the World Health Organization (WHO), each inpatient requires 40–60 L/day of water to meet the needs of health facilities. The operating room requires approximately 100 L/intervention (Mehtar, 2018). The utilization of water by hospitals results in the generation of substantial volumes of wastewater. The quantity of wastewater generated by hospitals varies depending on factors such as the capacity or number of available beds, the type and size of health facilities, the availability of technical facilities, services provided (laundry, kitchen, air conditioning), the wastewater management system in the hospital, facility management, and other considerations (Wiafe et al., 2016).

Hospital wastewater is one of the major contributors to environmental contamination due to the presence of pharmaceutical residues, heavy metals, hazardous chemicals, and pathogenic microorganisms such as *Escherichia coli*. An effective method for treating such wastewater is the use of membrane technology, particularly nanofiltration. Several studies by Kurniawan and colleagues have demonstrated the significant effectiveness of hybrid membrane systems in reducing antibiotic concentrations and microbial loads. In a 2017 study, they showed that a combination of nanofiltration and reverse osmosis could substantially reduce antibiotic residues and mitigate the spread of resistant pathogens in hospital effluents (Kurniawan et al., 2017).

Further research by Kurniawan (2018) confirmed that nanofiltration plays a vital role in the removal of ciprofloxacin, a commonly detected antibiotic in hospital wastewater. Additionally, Kurniawan and Nasir (2016) noted that contaminants in hospital wastewater could be predicted based on their initial profiles, making it possible to optimize membrane-based treatment strategies early in the process. One of the main advantages of nanofiltration is its ability to retain extremely small particles, including *E. coli*, which are often used as biological indicators in the Most Probable Number (MPN) method. This comprehensive approach meets the dual need for wastewater treatment technologies that address both chemical and microbiological contaminants, thereby safeguarding public health and aquatic ecosystems.

One of the microbiological parameters utilized for assessing water quality is the detection of coliforms or fecal coliforms in a water sample. Hospital effluents contain numerous bacteria and pathogenic microorganisms, including Escherichia coli (E. coli), Enterococci, thermotolerant coliforms, and fecal coliforms (Majumder et al., 2021). The presence of coliform bacteria or fecal coliforms in water samples indicates the potential presence of enteropathogenic and toxigenic bacteria, posing health risks to individuals.

Nanofiltration membranes exhibit distinctive separation properties, positioned between ultrafiltration and reverse osmosis membranes, featuring a pore size of approximately 1 nm, aligning with a molecular weight limit ranging from 100 to 5000 Da (Oatley-Radcliffe et al., 2017). There is an expectation that nanofiltration will efficiently eliminate various micropollutants from wastewater and facilitate the production of high-quality waste in a more sustainable manner compared to reverse osmosis. This is attributed to its elevated permeate flux and capacity to operate at lower pressures, consequently contributing to reduced energy consumption (Foureaux et al., 2019).

Recent studies employing nanofiltration membranes in tertiary wastewater treatment have demonstrated promising outcomes in terms of the removal efficiency of concerning contaminants, including active pharmaceutical compounds, endocrine disruptors, personal care products, and heavy metals (Cuhorka et al., 2020; Xu et al., 2020). However, limited research has initiated efforts to address elimination of bacteriological factors needs to be done due to the persistent ability of bacteria in the environment. Therefore, this research aimed to Analyzing the rejection capability of the nanofiltration membrane in degrading bacteriological factors E.Coli from hospital wastewater.

METHODS

1. Sampling Site and Sample Description

Wastewater samples were collected from one type B hospital located in Palembang City, Indonesia. This hospital was selected based on accessibility and the presence of an operational wastewater treatment system. The sampling site was the retention pond situated upstream of the hospital's wastewater treatment plant. The pond is located underground, which minimizes the influence of external environmental variables such as rainfall and temperature fluctuations.

Sampling was conducted using a grab sampling method, where five surface samples were collected from different points of the pond and subsequently combined into one composite sample to represent the wastewater quality. Only one hospital was used as the sample source in this study. Samples were labeled as follows:

a. **BM01–BM03**: Untreated (raw) wastewater samples.
b. **AM01–AM06**: Treated samples using nanofiltration membrane under a constant pressure of 60 psi, with varied contact times: 10, 20, 30, 40, 50, and 60 seconds respectively. The prefix "**AM**" refers to "**Air Limbah Membran**" (Membrane-treated Wastewater).

2. Membrane Filtration Setup

The nanofiltration process was conducted using a laboratory-scale filtration unit with a constant operating pressure of 60 psi, which aligns with the membrane manufacturer's optimum specifications for organic and microbial contaminant removal. The contact time was varied from 10 to 60 seconds in 10-second increments to evaluate the impact of retention time on bacterial removal efficiency.

3. Microbial Analysis using MPN Method

The microbial quality of the wastewater was evaluated using the Most Probable Number (MPN) method, following the procedures outlined by APHA Standard Methods for the Examination of Water and Wastewater (APHA, 2017). The analysis was conducted in three stages:

a. **Presumptive Test**: Using Lactose Broth (LB) medium with Durham tubes to detect gas production indicating coliform presence.
b. **Confirmed Test**: Tubes with gas formation were subcultured into Brilliant Green Lactose Bile (BGLB) broth to confirm coliform bacteria.
c. **Completed Test**: Positive BGLB tubes were streaked onto Eosin Methylene Blue (EMB) agar for the identification of *Escherichia coli*, confirmed by metallic green sheen colonies.

Lactose and bile salts in LB and BGLB media inhibit the growth of non-enteric bacteria while promoting the growth of coliforms due to their ability to ferment lactose and produce acid and gas.

4. Data Acquisition and Analysis

MPN values were determined by observing gas formation patterns in serial dilutions and referring to standard MPN tables (McCrady Table).
The independent variable in this study was the MPN value of *E. coli* before and after membrane treatment, while the dependent variable was the efficacy of the membrane in reducing MPN coliform levels.

Data were analyzed by calculating the **log reduction value (LRV)** of coliform bacteria using the formula:

$$Log\ Reduction = \log 10 = \left(\frac{MPN\ untreated}{MPN\ treated}\right)$$

Descriptive statistics were used to summarize the MPN concentrations across different retention times. The results were then interpreted to determine the trend in bacterial removal efficiency with increasing contact time. To improve visualization, it is recommended in future studies to include time vs. MPN graphs to depict reduction trends more clearly.

RESULTS

Testing using the MPN (Most Probable Number) method was conducted through both presumptive and confirmatory tests. Positive indications in the presumptive test were determined by the formation of gas and acid in Durham tubes, along with a visible color change in the inoculated media. Table 1 presents the results for untreated (raw) wastewater samples, while Table 2 displays the results of treated samples after the presumptive test.

Table 1. Sample Inspection Results Before Treatment

Sampel	Presumptive test			Confirmative test of BGLB			MPN/100 mL	Description
	10	1	0.1	10	1	0.1		
BM01	5	5	5	5	5	5	1600	Continued
BM02	5	5	5	5	5	4	1600	Continued
BM03	5	5	5	5	5	4	1600	Continued

Table 2. Results of Water Examination After Treatment in a Presumptive Test

Sampel	Number positive tube			Value MPN/100 mL	Description
	10	1	0.1		
AM01	2	1	1	20	Continued
AM02	2	0	0	9	Not Continued
AM03	0	0	0	0	Not Continued
AM04	0	0	0	0	Not Continued
AM05	0	0	0	0	Not Continued
AM06	0	0	0	0	Not Continued

The confirmatory test was conducted using Brilliant Green Lactose Bile (BGLB) broth as shown in Table 3, where positive tubes confirm the presence of coliform bacteria.

Table 3. Water Inspection Results After Treatment in the Confirmation Test

Sampel	Number positive tube			Value MPN/100 mL	Description
	10	1	0.1		
AM01	2	1	1	20	Continued
AM02	2	0	0	9	Not Continued
AM03	0	0	0	0	Not Continued
AM04	0	0	0	0	Not Continued
AM05	0	0	0	0	Not Continued
AM06	0	0	0	0	Not Continued

The MPN value was determined based on the count of positive tubes showing fermentation after incubation, using the McCrady MPN Table. Results are presented in Table 4.

Tabel 4. Results of Most Probable Number

Sample	Coliform (MPN/mL)	Coliform (MPN/100 mL)	Coliform standard	Description
BM01	16	1600	50	Not Qualify
AM01	0.09	9	50	Qualify
AM02	0.04	4	50	Qualify
AM03	0	0	50	Qualify
AM04	0	0	50	Qualify
AM05	0	0	50	Qualify
AM06	0	0	50	Qualify

Based on Table 4, the most probable coliform numbers are evident in samples processed and unprocessed using the membrane rejection technique with the MPN Test. Notably, only sample BM01 fails to meet the maximum total coliform limit requirements for clean water standard quality (50 MPN/100 mL sample). Specifically, the BM01 sample, which was not processed using the membrane filtration technique, revealed the presence of 1600 MPN/100 mL of coliform bacteria during the MPN test. Among the processed samples, sample AM02 exhibited the lowest total coliform bacteria with a total of 4 MPN/100 mL, followed by sample AM01 with a total of 9 MPN/100 mL.

Samples AM03-AM06 did not exhibit any coliform bacteria in 100 mL. Despite the presence of coliform bacteria in samples AM01-AM06, these results still comply with the standard clean water quality requirements. The results of membrane rejection calculations are detailed in Table 5. The analysis of the nanofiltration membrane rejection performance in eliminating E. coli yielded highly favorable results, approaching perfection throughout the entire process.

Tabel 5. Rejection of Membrane NF

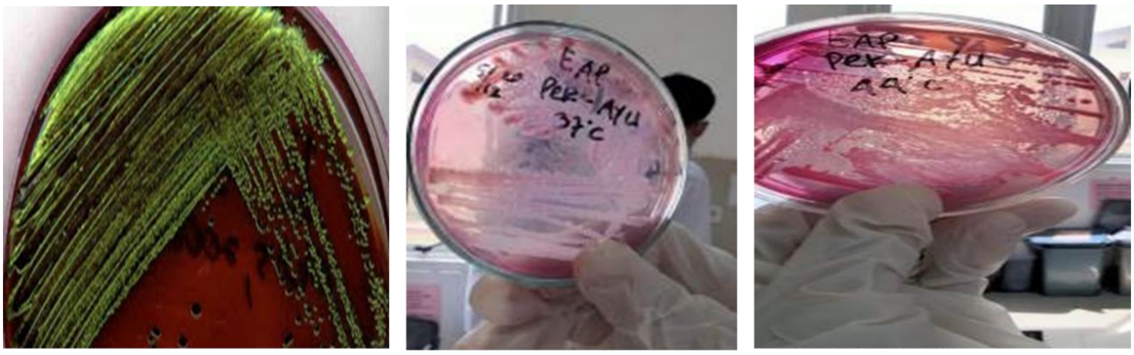
Treatment	% Rejection
AM01	99.43
AM02	99.75
AM03	100
AM04	100
AM05	100
AM06	100

Table 6 indicates that samples AM03-AM04, processed using the NF membrane rejection technique, are free from E. coli bacteria and comply with the specified limits for total E. coli (maximum 0 CFU/100 mL). In the context of water utilized for sanitary hygiene purposes, it is imperative that 100 mL of water does not contain E. coli bacteria. However, sample BM01 did not meet these requirements as it contained E. coli bacteria. The presence of E. coli bacteria in wastewater samples from the WWTP signals the need for additional treatment before the water is discharged to the SPAL. This is of concern due to the pathogenic nature of E. coli, which is often associated with various diseases.

Table 6. Escherichia Coli Identification Results

Sample	BM01	AM01	AM02
Microscopic	Gram Negative Basil	Gram Negative Basil	Gram Negative Basil
Indol	Positive	Negative	Positive
MR	Positive	Positive	Negative
VP	Negative	Positive	Negative
Simon Citrat	Negative	Positive	Negative
Description	*Escherichia coli*	Not contain E. coli	Not 47ontain E. coli

To better understand the experimental setup used in this study, a schematic diagram of the nanofiltration system is presented in Figure 1. This configuration was designed to evaluate the removal efficiency of *Escherichia coli (E. coli)* from hospital wastewater using nanofiltration membranes. The system includes several essential components that work together to ensure optimal filtration performance, including a feed tank, pre-filtration unit, high-pressure pump, nanofiltration membrane module, and separate outlets for permeate and concentrate streams. Each element of the system plays a critical role in supporting the filtration process, from pre-treatment to bacterial separation and discharge. The diagram illustrates the flow of wastewater through the system and highlights the points of bacterial rejection and treated water collection.



(a) (b) (c)

Figure 1. Isolation Form: (a) BM01, (b) AM01, and (c) AM02

DISCUSSION

This study reinforces the significant potential of nanofiltration (NF) membrane technology in removing pathogenic bacteria, particularly *Escherichia coli* and total coliforms, from hospital wastewater. Hospital effluents are a recognized source of various biological and chemical contaminants, including antibiotic-resistant microorganisms, pharmaceuticals, and disinfectants, many of which are difficult to eliminate through conventional wastewater treatment processes. The presence of *E. coli* in untreated hospital wastewater, as revealed through both MPN testing (Table 2 and Table 5) and biochemical identification (Table 7), signals a severe environmental and public health concern, particularly in low-to-middle-income countries where effluent regulation and infrastructure may be limited.

In this study, untreated samples (BM01–BM03) demonstrated coliform concentrations of up to 1600 MPN/100 mL, which significantly exceeds the regulatory threshold of 50 MPN/100 mL established in the Indonesian Ministry of Health Regulation (Permenkes No. 32 Tahun 2017) for clean water quality. The detection of *E. coli* through gram staining and biochemical assays (positive Indole and Methyl Red tests) further confirms the pathogenic nature of the raw effluent. This clearly illustrates the inadequacy of untreated hospital wastewater for direct environmental discharge or any form of reuse.

After applying nanofiltration under controlled laboratory conditions at an operational pressure of 60 psi, a noticeable and consistent reduction in microbial content was observed. As shown in Table 5, even with a short contact time of 10 seconds (sample AM01), the MPN value dropped to 9 MPN/100 mL—already within regulatory limits. With extended contact times of 30 to 60 seconds (samples AM03–AM06), complete removal of coliforms was achieved, as evidenced by 0 MPN/100 mL and the absence of biochemical indicators of *E. coli*. These results were further supported by the calculated membrane rejection rates (Table 6), where samples AM03 through

AM06 achieved 100% bacterial rejection. The findings suggest a direct correlation between retention time and microbial removal efficiency, with longer exposure to the NF membrane enhancing contaminant capture.

The effectiveness of nanofiltration in this context can be attributed to the membrane's small pore size, typically in the range of 0.001–0.01 microns, which is substantially smaller than the average diameter of *E. coli* (0.5–2.0 microns). In addition to size exclusion, surface charge interactions may also play a role in bacterial retention. Many NF membranes possess a negatively charged surface, which can repel similarly charged bacterial cell membranes, thereby enhancing removal efficiency through electrostatic repulsion.

These results are consistent with previous studies that have demonstrated the microbial rejection capabilities of NF membranes. Nghiem et al. (2006) found that nanofiltration could remove over 99% of *E. coli* and other bacteria in hospital and municipal wastewater samples. Madaeni and Ghaemi (2007) also reported complete bacterial removal using nanofiltration membranes under optimized operational conditions. These studies collectively affirm the reliability of NF technology for tertiary treatment and microbial disinfection.

Moreover, the implications of these findings extend beyond compliance with regulatory standards. The treated effluent, meeting clean water quality benchmarks, opens opportunities for non-potable reuse applications such as toilet flushing, irrigation of green areas, or use in hospital cooling towers. In water-scarce regions, such reuse practices could significantly contribute to sustainability and reduce the demand for freshwater resources. Additionally, although not tested directly in this study, the successful elimination of *E. coli* and coliforms suggests that NF membranes could also be effective in reducing or removing antibiotic-resistant bacteria, an emerging environmental threat that often originates in hospital waste streams (Michael et al., 2013).

Despite these promising outcomes, several limitations of this study must be acknowledged. Most notably, the experiment was conducted as a single, short-term laboratory observation and did not include repeated trials over extended timeframes or variable environmental conditions. In real-world applications, operational conditions such as temperature, influent variability, and chemical loading can influence membrane performance. Therefore, the long-term reliability and durability of the NF membrane under such fluctuating conditions remain uncertain.

One of the most critical issues in real-world membrane applications is **membrane fouling**, which refers to the accumulation of organic matter, microorganisms, and other particulates on the membrane surface, leading to clogging and performance deterioration. Fouling increases the frequency of cleaning, shortens membrane lifespan, and raises operational costs. Unfortunately, this study did not investigate fouling behavior, membrane cleaning procedures, or any strategies to prevent clogging. In practice, effective fouling control typically involves pretreatment processes such as sand filtration, coagulation-flocculation, or even primary sedimentation, all of which require further evaluation.

Another challenge lies in contact time optimization. While this study demonstrated improved bacterial rejection with increased retention time (from 10 to 60 seconds), it remains unclear how such durations can be feasibly implemented in full-scale hospital wastewater treatment plants

(WWTPs), which often operate with continuous high flow rates. In large WWTPs, achieving long retention times may require either reduced flow velocities, larger membrane surface areas, or the use of multiple membrane modules each of which has cost and space implications.

Additionally, the study focused solely on bacterial contaminants, particularly coliforms and *E. coli*. However, hospital wastewater may also contain viruses (e.g., norovirus, rotavirus), protozoa (e.g., *Giardia lamblia*, *Cryptosporidium*), fungi, and residual pharmaceuticals, all of which pose unique treatment challenges and require further analysis. Incorporating broader microbial and chemical parameters into future research would provide a more comprehensive understanding of NF membrane performance and its suitability for diverse hospital effluent compositions.

Furthermore, the study lacks data visualization tools such as graphs or trend charts to illustrate the progression of microbial reduction over time. The inclusion of time vs. MPN reduction graphs or log-reduction trend lines would enhance the clarity and impact of the results, enabling readers to better grasp the correlation between operational parameters and treatment outcomes.

CONCLUSIONS

Hospital wastewater contains hazardous pathogenic contaminants, with *Escherichia coli* (E. coli) serving as a key indicator of fecal pollution and microbial risk. If not adequately treated, these pathogens can significantly degrade water quality and pose serious health and environmental threats. The objective of this study was to evaluate the effectiveness of nanofiltration membrane technology in removing E. coli and total coliform bacteria from hospital effluent.

The findings clearly demonstrate that nanofiltration is a highly effective treatment method, achieving up to 100% removal of coliform bacteria under optimal conditions (60 psi and 30–60 seconds contact time). This performance ensures compliance with national clean water standards and confirms nanofiltration as a viable and sustainable option for enhancing hospital wastewater treatment, particularly as a tertiary treatment step.

Moreover, the successful bacterial reduction observed in this study suggests broader potential for nanofiltration in water reuse applications, such as non-potable reuse for irrigation, sanitation, or cooling systems thereby contributing to water conservation efforts and sustainable hospital operations.

However, as this study focused primarily on E. coli and total coliforms, future research should expand the pathogen scope by investigating the membrane’s effectiveness against a wider range of hospital-related contaminants. These include viruses, protozoa, antibiotic-resistant bacteria, pharmaceutical residues, and heavy metals. A more comprehensive understanding of membrane performance across multiple pollutant classes will help develop holistic and resilient hospital wastewater treatment strategies.

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