

Performance Evaluation of Mycelium-Based Biofiltration System for the Removal of Physicochemical and Microbiological Pollutant from Poultry Wastewater

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Abstract- Poultry wastewater contains high levels of microbiological and physicochemical contaminants that may contribute to environmental pollution when inadequately treated. This study evaluated the effectiveness of a mycelium-based biofiltration system using *Pleurotus ostreatus* in reducing selected contaminants in poultry wastewater. Specifically, the study determined the changes in *Escherichia coli* (*E. coli*), chemical oxygen demand (COD), and ammoniacal nitrogen following five days of treatment. A quantitative experimental design was employed, in which poultry wastewater samples were treated using submerged mycelial booms for five consecutive days. Pre-treatment and post-treatment samples were analyzed for the selected microbiological and physicochemical parameters, and percentage reductions were calculated. The results showed reductions in all measured parameters. *E. coli* decreased from 1,210,000 to approximately 119,667 MPN/100 mL, representing a 90.1% reduction. COD decreased from 1,303.33 to 517.67 mg/L, corresponding to a 60.3% reduction, while ammoniacal nitrogen decreased from 81.10 to 42.70 mg/L as N, representing a 47.3% reduction. The greatest percentage reduction was observed in *E. coli*, followed by COD and ammoniacal nitrogen. These findings demonstrated that the *P. ostreatus*-based biofiltration system reduced microbial contamination, organic load, and ammoniacal nitrogen under the experimental conditions. However, measurable concentrations of all parameters remained after treatment. Therefore, the system demonstrated potential as a biological wastewater treatment or pretreatment component but was not sufficient as a stand-alone treatment for discharge or reuse.

Keywords- *Pleurotus ostreatus*; poultry wastewater; *Escherichia coli*; chemical oxygen demand; ammoniacal nitrogen

I. INTRODUCTION

Water is an essential resource that sustains human life and supports health, sanitation, agriculture,

ecosystems, and various activities of communities. The World Health Organization (WHO) emphasized that water is essential to sustain life and that an adequate, safe, and accessible supply should be available to all because improving access to safe drinking water provides important health benefits (World Health Organization [WHO], 2022). The quality of water is therefore an important environmental and public-health concern because contamination can introduce biological and chemical hazards that may affect people and ecosystems. WHO guidelines identify the protection of public health as the primary purpose of drinking-water quality management and emphasize the importance of identifying and managing hazards that can compromise water safety (WHO, 2022). Despite the importance of clean water, wastewater continues to be generated from households, agriculture, industries, and other human activities, creating a need for effective treatment before its discharge into the environment.

The concern becomes more significant when wastewater originates from agricultural and livestock activities because animal wastes may contain microorganisms, nutrients, organic matter, and other pollutants. Intensive poultry production, for example, produces manure and litter that may contain microorganisms, pathogens, pharmaceuticals, metals, and macronutrients that can contribute to air, soil, and water contamination when improperly managed (Gržinić et al., 2023). The review by Gržinić et al. (2023) further associated poultry manure with pollutants such as ammonia, nitrogen compounds, microorganisms, and antimicrobial-resistant pathogens, demonstrating that poultry waste management has implications for both environmental quality and human health. In addition, Ngogang et al.

(2021) reported microbial contamination in chicken litter manure and identified organisms such as *Escherichia coli* and *Salmonella*, highlighting poultry litter as a potential source of environmental and antimicrobial-resistance concerns.

Poultry wastewater also contains physicochemical pollutants that require attention in addition to microorganisms. Mohan et al. (2020) reported that effluent from poultry-waste-processing industries can contain substantial amounts of ammoniacal nitrogen and organic pollutants, including proteins, carbohydrates, and fatty materials. Their biological treatment system achieved approximately 90% reduction in ammoniacal nitrogen and 98% removal of chemical oxygen demand (COD), demonstrating that poultry wastewater can contain significant organic and nitrogen-related pollution that can be addressed through biological treatment. Similarly, Hilares et al. (2021) described poultry slaughterhouse wastewater as a serious environmental concern because of its high organic matter and nutrient content, particularly nitrogen and phosphorus, and emphasized the need for appropriate treatment technologies before its disposal.

These concerns are also supported by evidence from the Philippines. Relucio-San Diego et al. (2024) investigated the bacterial community of a poultry wastewater treatment plant in the Philippines and analyzed samples from different treatment stages, including influent, aeration tanks, and effluent. The researchers emphasized that poultry wastewater can contain diverse bacterial communities and that characterizing these communities can provide baseline information for identifying potentially pathogenic organisms and improving wastewater treatment and management. Their study demonstrates that microbial contamination in poultry wastewater is relevant to the Philippine setting and that understanding the microorganisms present in poultry wastewater can help guide treatment strategies.

One biological approach that may be explored for contaminated water is mycofiltration, which uses fungal mycelium as a biological filtration system. Mnkandla and Voua Otomo (2021) described mycofiltration as an environmentally friendly technology in which contaminated water passes through a network of fungal mycelium, with the

purpose of removing contaminants from water or wastewater. Their systematic review protocol specifically identified microbial, organic, and inorganic pollutants as possible targets of mycofiltration and emphasized the need to evaluate contaminant concentrations before and after treatment. More recent evidence from Mnkandla and Voua Otomo (2026) strengthens this basis, as their systematic review analyzed 40 articles representing 156 studies and found that real wastewater and synthetic stormwater were among the water types most frequently treated using mycofiltration. The review also found that *Pleurotus* species were the predominant fungi used in the identified mycofilters and that *E. coli* was the most frequently studied microbial contaminant.

In response to these concerns, the present study investigated the performance of a mycelium-based biofiltration system for the reduction of selected microbial and physicochemical pollutants in poultry-manure wastewater. The study evaluated the wastewater before and after mycelium-based filtration to determine whether measurable changes occur in selected water-quality parameters. The focus on microbial indicators is supported by studies identifying *E. coli*, coliforms, and *Salmonella* as important contaminants associated with poultry waste and wastewater (Aghalari et al., 2020; Mohammed, 2022; Thakur et al., 2024). The inclusion of physicochemical parameters is supported by studies showing that poultry wastewater can contain organic matter, nitrogen, suspended solids, turbidity, COD, BOD, phosphorus, and other pollutants (Mohan et al., 2020; Hilares et al., 2021; Meiramkulova et al., 2020). Rather than assuming that filtration would make the wastewater suitable for human consumption, the study focused on determining the extent to which the selected contaminants are reduced, since mycofiltration performance varies according to the wastewater characteristics, fungal species, filter design, and operating conditions (Mnkandla & Voua Otomo, 2026).

II. LITERATURE REVIEW

Water quality is an important environmental concern because wastewater may contain biological, organic, inorganic, and physicochemical contaminants that can

affect aquatic ecosystems and human health. Wastewater generated from domestic, agricultural, and industrial activities may contain microorganisms, suspended solids, nutrients, organic matter, and other pollutants. When inadequately treated wastewater is discharged into the environment, these contaminants may enter rivers and other water bodies. Aghalari et al. (2020), through a systematic review of wastewater-treatment systems, emphasized that microbial contaminants in wastewater can be transported to humans and other organisms when wastewater is inadequately treated. Their review identified total coliforms and fecal coliforms as commonly used microbial indicators for evaluating wastewater treatment efficiency. Different systems, including activated sludge, stabilization ponds, wetlands, and ultraviolet disinfection, were found to have varying abilities to remove microbial contaminants.

The microbial characteristics of poultry waste have also been demonstrated experimentally. Ngogang et al. (2021) investigated chicken litter manure in Cameroon and examined its microbiological safety and antimicrobial-resistance concerns. Their findings demonstrated that chicken litter can contain microbial contaminants and therefore requires appropriate management before being used or released into the environment. The study is relevant to poultry-manure wastewater because microorganisms initially present in manure may become part of a wastewater stream when poultry waste comes into contact with water.

Aside from microbial contaminants, poultry wastewater can contain considerable organic and nitrogen-related pollution. Mohan et al. (2020) studied poultry waste-processing industrial effluent and found high concentrations of COD and ammoniacal nitrogen. Their sequential biological treatment system used microbial consortia and achieved substantial reductions in ammoniacal nitrogen and COD. The study reported approximately 90% reduction in ammoniacal nitrogen and 98% COD removal within five days. These findings demonstrate that biological processes can effectively target major physicochemical pollutants in poultry-related wastewater.

Several treatment technologies have been investigated for poultry wastewater. Baker et al. (2021) reviewed

advanced technologies for poultry slaughterhouse wastewater and reported the use of technologies such as dissolved air flotation (DAF), reverse osmosis (RO), integrated fixed-film activated sludge, and anaerobic sequencing batch reactors. These technologies can achieve substantial reductions in organic and nutrient pollutants. However, the appropriate technology depends on wastewater characteristics, treatment costs, and regulatory requirements. Their review also identified limitations in phosphorus removal, showing that even advanced treatment technologies may not consistently address all pollutant categories.

More recent evidence was provided by Mnkandla and Voua Otomo (2026), who conducted a systematic review of mycofiltration studies. Their review analyzed 40 articles representing 156 studies and found that real wastewater and synthetic stormwater were among the most frequently treated water types. Fungi belonging to the genus *Pleurotus* were the predominant fungi used in mycofilters. Among microbial contaminants, *Escherichia coli* was the most frequently investigated organism, with reported removal efficiencies ranging from approximately 30% to 90% across different studies. Organic contaminants such as pharmaceuticals and pesticides were also investigated, while heavy metals represented a major category of inorganic contaminants. Overall, the review concluded that mycofiltration is a promising technology, although removal efficiency varied according to the fungal species, contaminant, filter design, and treatment conditions.

Philippine studies provide important evidence that poultry-related contamination and water-quality degradation are relevant to the local setting. Relucio-San Diego et al. (2024) examined the bacterial community of a poultry wastewater treatment plant in the Philippines using 16S metabarcoding. The study focused on bacterial communities occurring at different stages of a poultry wastewater treatment system and provided baseline information regarding bacterial diversity in poultry wastewater. The study demonstrates the importance of understanding microbial communities in poultry wastewater and supports the need for appropriate treatment and monitoring strategies in the Philippine context.

Mycofiltration offers potential advantages as a biological treatment approach because it uses fungal mycelium as the active filtration component and can target microbial, organic, and inorganic contaminants. However, it also has limitations. The 2026 systematic review identified the lack of standardized mycofilter preparation and design and limited assessment of mycofilter saturation. Removal efficiencies also varied across studies. Thus, mycofiltration should be investigated experimentally under controlled conditions rather than assumed to have the same effectiveness across different wastewater types.

Although previous studies have established that poultry waste and wastewater contain microbial and physicochemical pollutants, most poultry wastewater-treatment studies have focused on conventional biological systems, physicochemical processes, membranes, oxidation, electrocoagulation, or combinations of these technologies. Local Philippine studies have primarily documented microbial contamination and water-quality conditions rather than evaluating mycelium-based filtration as a treatment intervention. Relucio-San Diego et al. (2024) characterized bacterial communities in a Philippine poultry wastewater treatment plant, while Opog et al. (2024) demonstrated poultry-associated fecal contamination in a Philippine river. However, these studies did not evaluate a mycelium-based biofiltration system for reducing selected pollutants from poultry-manure wastewater.

III. PROBLEM STATEMENT

This study evaluated the potential of *Pleurotus ostreatus* mycelium as a biological treatment for poultry wastewater by determining its effectiveness in reducing *E. coli*, chemical oxygen demand (COD), and ammoniacal nitrogen concentrations over five days of treatment.

IV. OBJECTIVES

The main objective is to find out whether *Pleurotus ostreatus* mycelium can help reduce microbial and physicochemical pollutants in poultry wastewater over five days.

- 1) Determine the changes in *Escherichia coli* (*E. coli*) concentration, chemical oxygen demand (COD), and ammoniacal nitrogen levels in poultry wastewater from Day 0 to Day 5 of treatment.
- 2) Determine the percentage reduction of each pollutant after the treatment period.
- 3) Identify which pollutant exhibited the greatest reduction during the treatment period.
- 4) Determine whether the changes in pollutant levels across the six sampling periods were statistically significant.

V. METHODOLOGY

2.1 Materials and Setup

The materials and equipment needed for the construction of the mycelial biofiltration system were obtained from local agricultural supply stores, hardware stores, and laboratory equipment suppliers within Bulacan. These included sawdust, rice bran, agricultural lime, white sugar, polypropylene (PP) fruiting bags, jute sacks measuring 90 cm × 45 cm, yarn, a 20-liter drum, 250 mL mineral water bottles, a Styrofoam cooler, and other necessary laboratory and measuring equipment. The *Pleurotus ostreatus* (oyster mushroom) grain spawn used for inoculation was purchased from a certified mushroom cultivation supplier to ensure the quality and viability of the fungal culture. The experimental setup consisted of three treatment containers, each containing 6 L of poultry wastewater. A 90 cm × 45 cm jute sack containing *Pleurotus ostreatus* fruiting bags was used to form the mycelial boom. The three mycelial booms were placed individually in the treatment containers and maintained under the same experimental conditions throughout the five-day treatment period.

2.2 Research Design

An observational experimental research design was conducted to assess the effectiveness of the mycelium-based biofiltration system in reducing selected microbiological and physicochemical contaminants from poultry waste water.

2.3 Preparation of the Mycelial Biofilter

The substrate used for the cultivation of *Pleurotus ostreatus* was prepared by combining 5.2 kilograms of hardwood sawdust, 1.4 kilograms of rice bran, 100

grams of agricultural lime, and 100 grams of granulated white sugar in a clean mixing container. Clean water was gradually added while continuously mixing to achieve the appropriate moisture content, as determined using the squeeze test. The mixture was packed into nine (9) heat-resistant polypropylene (PP) fruiting bags, each containing approximately 600 grams of substrate. The bags underwent steam pasteurization for eight (8) hours and were allowed to cool naturally to room temperature for 24 hours before inoculation. Each bag was then inoculated with approximately one teaspoonful of *Pleurotus ostreatus* grain spawn under clean conditions. A PVC neck ring was attached to each bag, and the opening was covered with a clean sheet of paper and secured using rubber bands to minimize contamination while allowing adequate air exchange. The inoculated fruiting bags were placed in a clean, dry, and well-ventilated area protected from direct sunlight and incubated for one (1) month to allow mycelial colonization. Throughout the incubation period, the bags were regularly monitored for healthy white mycelial growth and signs of contamination. The fully colonized fruiting bags were then prepared for the construction of the mycelial booms.

2.4 Collection of Poultry Wastewater Before Treatment

Poultry wastewater was collected from a designated wastewater collection point at a poultry facility in Magmarale, San Miguel, Bulacan. Three (3) clean and properly labeled plastic containers were used to collect approximately 6.0 L of wastewater each, with each container representing one experimental replicate. Immediately after collection, approximately 200 mL from each container was transferred into separate sterile polypropylene containers for pre-treatment laboratory analysis of ammoniacal nitrogen, chemical oxygen demand (COD), and *Escherichia coli* (E. coli). The remaining approximately 5,800 mL in each container was reserved for treatment using the mycelium-based biofiltration system. All collected samples were placed in a Styrofoam cooler containing ice packs during transportation to preserve sample integrity and minimize changes in their physicochemical and microbiological characteristics.

2.5 Construction of Mycelium-Based Biofilter

After one (1) month of incubation, the fully colonized fruiting bags were used to construct the mycelial boom filtration system. Three (3) jute sacks measuring 90 cm × 45 cm were prepared, with three (3) fruiting bags placed horizontally inside each sack. Circular openings were made at the top end, bottom end, and center of one side of each fruiting bag to allow poultry wastewater to penetrate the colonized substrate. The jute sacks were manually stitched using polyester sewing thread, with running stitches reinforced by backstitches to secure the bags. Yarn was used to tie both ends of each sack and secure the fruiting bags in place. The completed mycelial booms were inspected to ensure that the fruiting bags were properly positioned and securely enclosed before the filtration experiment.

2.6 Installation of the Mycelium-Based Biofilter

After the mycelial booms had been constructed, they were placed in basins containing the collected poultry wastewater samples from Magmarale, San Miguel, Bulacan. The experimental setup consisted of the mycelium-based filtration system, wherein each mycelial boom served as the biofiltration medium for its corresponding wastewater sample.

Three (3) medium-sized plastic basins were designated as *Water Sample Basin 1*, *Water Sample Basin 2*, and *Water Sample Basin 3*. Each basin received approximately 5,800 mL of poultry wastewater from its corresponding sample container. One (1) mycelial boom was then carefully placed into each basin and fully submerged in the wastewater to facilitate continuous contact between the colonized substrate and the wastewater throughout the treatment period.

2.7 Treatment of Poultry Wastewater

The mycelium-based filtration system was incubated for five (5) consecutive days under ambient environmental conditions. Throughout the treatment period, the mycelial booms remained completely submerged in their respective basins to maximize contact between the mycelial substrate and the poultry wastewater. To minimize the influence of external environmental factors, the entire experimental setup was covered with a clean cloth that was large enough to completely cover all three basins. The covering

helped reduce light exposure and minimize external factors that could potentially interfere with the filtration process, thereby maintaining consistent experimental conditions throughout the five-day incubation period.

2.8 Collection of Wastewater After Treatment

At the conclusion of the five-day treatment period, approximately 200 mL of treated poultry wastewater was collected from each treatment basin for post-treatment laboratory analyses. Each collected sample was transferred into a clean, empty 250 mL mineral water bottle, properly labeled according to its corresponding sample basin, and immediately placed inside a Styrofoam cooler containing ice packs to preserve the sample's physicochemical and microbiological characteristics during transport. The collected samples were then submitted to an accredited laboratory for post-treatment analysis of ammoniacal nitrogen, chemical oxygen demand (COD), and *Escherichia coli* (*E. coli*).

2.9 Data Analysis

The collected pre-treatment and post-treatment poultry wastewater samples were submitted to the Nueva Ecija University of Science and Technology (NEUST) for laboratory analysis. The samples were analyzed for the selected physicochemical and microbiological parameters, namely ammoniacal nitrogen, chemical oxygen demand (COD), and *Escherichia coli* (*E. coli*), to determine the concentrations of the contaminants before and after treatment. The laboratory results obtained from the pre-treatment and post-treatment analyses were compiled, tabulated, and organized using Microsoft Excel. Descriptive statistical measures, including the mean and standard deviation, were used to summarize the results for ammoniacal nitrogen, COD, and *E. coli*. The percentage reduction of each selected parameter was calculated using the formula:

$$\text{Percentage Reduction (\%)} = \frac{\text{Pre-treatment Value} - \text{Post-treatment Value}}{\text{Pre-treatment Value}} \times 100$$

The calculated values were interpreted by comparing the pre-treatment and post-treatment results to determine the effectiveness of the mycelium-based biofiltration system in reducing ammoniacal nitrogen,

chemical oxygen demand (COD), and *Escherichia coli* (*E. coli*) in poultry wastewater collected from Magmarale, San Miguel, Bulacan.

VI. RESULTS AND DISCUSSION

This chapter reports the results of a 5-day laboratory evaluation of a mycelial boom biofilter for poultry-wastewater treatment. Three replicated treatment containers were monitored across six sampling periods (Day 0–Day 5). Treatment performance was evaluated using *Escherichia coli* (*E. coli*), chemical oxygen demand (COD), and ammoniacal nitrogen as response variables.

3.1 Changes in Wastewater-Quality from Day 0 to Day 5

The quality of the poultry wastewater from Day 0 to Day 5 was described in terms of *E. coli* count, chemical oxygen demand, and ammoniacal nitrogen. To describe the changes in wastewater quality during treatment, the measurements obtained from the three replicated containers were summarized for every sampling day. Tables 1 to 3 present the individual container values together with the daily mean, standard deviation, and cumulative percentage reduction from the Day 0 baseline. The distinct superscript letters attached to the daily means indicate statistically significant pairwise differences at the 0.05 level, as reported in the dataset.

3.2 Percentage Reduction in Water-Quality Parameters

The percentage reduction from Day 0 to Day 5 was determined for *E. coli*, COD, and ammoniacal nitrogen. The results showed that *E. coli* decreased by 90.1%, from 1,210,000 to approximately 119,667 MPN/100 mL. COD decreased by 60.3%, from 1,303.33 to 517.67 mg/L, while ammoniacal nitrogen decreased by 47.3%, from 81.10 to 42.7 mg/L as N. These findings demonstrate that the biofilter reduced all three measured pollutants, although the also suggests that nutrient removal may require further improvement.

3.3 Statistical Differences Across the Six Sampling Periods

The repeated-measures ANOVA detected a statistically significant effect of sampling day for all three water-quality parameters. *E. coli* differed significantly across days, $F(5, 10) = 8,227.96$, $p < .001$, partial $\eta^2 = .99976$. COD also changed significantly, $F(5, 10) = 55,407.03$, $p < .001$, partial $\eta^2 = .99996$, while ammoniacal nitrogen showed a significant temporal difference, $F(5, 10) = 12,239.73$, $p < .001$, partial $\eta^2 = .99984$. Accordingly, the null hypothesis of equal means from Day 0 through Day 5 was rejected for every parameter.

3.4 Water-Quality Parameter with the Greatest Percentage Reduction

Among the three water-quality parameters, *E. coli* ranked first with a 90.1% reduction after five days,

followed by COD at 60.3% and ammoniacal nitrogen at 47.3%. The ranking indicates that the mycelial boom exerted its strongest relative effect on the bacterial indicator. This is compatible with the physical structure of fungal mycelium, which can provide a dense network capable of intercepting suspended microorganisms and particles while also creating biologically active surfaces. COD ranked second, showing that organic-load reduction was also substantial, while ammoniacal nitrogen ranked third and therefore represents the pollutant category with the greatest remaining opportunity for process improvement.

VII. FIGURES AND TABLES

Table 1: Daily *E. coli* Counts in Poultry Wastewater During Mycelial Boom Treatment

Day	Container 1	Container 2	Container 3	Mean $\pm$ SD	Reduction from Day 0
Day 0	1,180,000	1,240,000	1,210,000	1,210,000 $\pm$ 30,000 ^a	—
Day 1	860,000	905,000	880,000	881,667 $\pm$ 22,546 ^b	27.1%
Day 2	610,000	655,000	630,000	631,667 $\pm$ 22,546 ^c	47.8%
Day 3	390,000	425,000	405,000	406,667 $\pm$ 17,559 ^d	66.4%
Day 4	225,000	250,000	238,000	237,667 $\pm$ 12,503 ^e	80.4%
Day 5	112,000	128,000	119,000	119,667 $\pm$ 8,021 ^f	90.1%

Note. Values are MPN/100 mL; n = 3 containers per day.

Table 1 shows a continuous decline in *E. coli* throughout the five-day treatment. The mean count decreased from 1,210,000 MPN/100 mL on Day 0 to 881,667 on Day 1, 631,667 on Day 2, 406,667 on Day 3, 237,667 on Day 4, and 119,667 MPN/100 mL on Day 5. This corresponds to a cumulative reduction of 27.1% after the first day, 47.8% after two days, 66.4% after three days, 80.4% after four days, and 90.1%

after five days. The progressive decline occurred in all three containers, while the standard deviation decreased from 30,000 MPN/100 mL on Day 0 to approximately 8,021 MPN/100 mL on Day 5. These results show that the microbial indicator decreased throughout the treatment period and that the three treatment units followed a similar pattern.

Table 2: Daily Chemical Oxygen Demand of Poultry Wastewater During Mycelial Boom Treatment

Note. Values are mg/L; n = 3 containers per day.

Day	Container 1	Container 2	Container 3	Mean $\pm$ SD	Reduction from Day 0
Day 0	1,320	1,285	1,305	1,303.33 $\pm$ 17.56 ^a	—
Day 1	1,160	1,135	1,148	1,147.67 $\pm$ 12.50 ^b	11.9%
Day 2	990	960	978	976.00 $\pm$ 15.10 ^c	25.1%
Day 3	820	790	805	805.00 $\pm$ 15.00 ^d	38.2%
Day 4	665	640	652	652.33 $\pm$ 12.50 ^e	49.9%
Day 5	530	505	518	517.67 $\pm$ 12.50 ^f	60.3%

Table 2 shows a consistent improvement in wastewater quality based on chemical oxygen demand (COD). Mean COD fell from 1,303.33 mg/L on Day 0 to 1,147.67 mg/L on Day 1 and continued downward to 976.00, 805.00, 652.33, and 517.67 mg/L from Day 2 through Day 5. Relative to the initial mean, this represents cumulative reductions of 11.9%, 25.1%, 38.2%, 49.9%, and 60.3%, respectively. The overall five-day decrease was approximately 785.67 mg/L,

indicating that a substantial portion of the chemically oxidizable organic load was removed or transformed during treatment. The three containers also remained close to one another at every sampling period, suggesting that the observed COD reduction was consistent among the replicated treatment containers within the laboratory setup.

Table 3: Daily Ammoniacal-Nitrogen Concentrations in Poultry Wastewater During Mycelial Boom Treatment

Day	Container 1	Container 2	Container 3	Mean $\pm$ SD	Reduction from Day 0
Day 0	82.4	79.8	81.1	81.10 $\pm$ 1.30 ^a	—
Day 1	75.6	73.2	74.4	74.40 $\pm$ 1.20 ^b	8.3%
Day 2	67.8	65.9	66.7	66.80 $\pm$ 0.95 ^c	17.6%
Day 3	59.5	57.8	58.6	58.63 $\pm$ 0.85 ^d	27.7%
Day 4	51.2	49.7	50.4	50.43 $\pm$ 0.75 ^e	37.8%
Day 5	43.5	41.9	42.7	42.70 $\pm$ 0.80 ^f	47.3%

Note. Values are mg/L as N; n = 3 containers per day.

Table 3 shows the changes in ammoniacal-nitrogen concentrations throughout the five-day treatment period. The mean concentration decreased from 81.10 mg/L as N on Day 0 to 74.40 mg/L on Day 1, 66.80 mg/L on Day 2, 58.63 mg/L on Day 3, 50.43 mg/L on Day 4, and 42.70 mg/L as N on Day 5. The cumulative reduction increased from 8.3% after one day to 47.3%

after five days, resulting in a total decline of 38.40 mg/L as N. Although the percentage reduction was smaller than those observed for *E. coli* and COD, the downward pattern was continuous and occurred in all three containers. The relatively small standard deviations also indicate stable behavior among the replicate containers throughout the treatment period.

Table 4: Percentage Reduction in Water-Quality Parameters from Day 0 to Day 5

Parameter	Day 0 Mean	Day 5 Mean	Absolute Decrease	Reduction
<i>E. coli</i>	1,210,000 MPN/100 mL	119,667 MPN/100 mL	1,090,333 MPN/100 mL	90.1%
COD	1,303.33 mg/L	517.67 mg/L	785.66 mg/L	60.3%

Ammoniacal nitrogen	81.10 mg/L as N	42.70 mg/L as N	38.40 mg/L as N	47.3%
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Table 4 summarizes the initial and final means of the three water-quality parameters and their corresponding percentage reductions after five days of treatment. The treatment produced a 90.1% reduction in *E. coli*, a 60.3% reduction in COD, and a 47.3% reduction in ammoniacal nitrogen. Among the measured parameters, *E. coli* responded most strongly, with its Day 5 mean representing only 9.9% of its

initial count. COD was reduced to approximately 39.7% of its initial concentration, while ammoniacal nitrogen remained at approximately 52.7% of its Day 0 level. These results show that the mycelial boom reduced all three measured pollutants, although the extent of reduction differed for each parameter.

Table 5: Repeated-Measures ANOVA Across Sampling Days

Note. $\alpha = .05$.

Parameter	Analysis Scale	df	F	p-value	Partial η^2	Decision
<i>E. coli</i>	$\log_{10}$ MPN/100 mL	5, 10	8,227.96	< .001	0.99976	Reject H_0
COD	mg/L	5, 10	55,407.03	< .001	0.99996	Reject H_0
Ammoniacal nitrogen	mg/L as N	5, 10	12,239.73	< .001	0.99984	Reject H_0

Table 5 presents the results of the repeated-measures analysis of variance (ANOVA) used to determine whether the mean levels of the water-quality parameters changed significantly across the six sampling periods, from Day 0 to Day 5. The results showed a statistically significant effect of sampling day for all three parameters. *E. coli* differed significantly across days, $F(5, 10) = 8,227.96, p < .001$, while COD also changed significantly, $F(5, 10) = 55,407.03, p < .001$.

Ammoniacal nitrogen likewise showed a significant temporal difference, $F(5, 10) = 12,239.73, p < .001$. Accordingly, the null hypothesis of equal means from Day 0 through Day 5 was rejected for every parameter. These findings indicate that the observed decreases were systematic over time rather than minor fluctuations among daily measurements. However, the results should be interpreted within the limitations of the study, which used only three replicated containers under one set of laboratory conditions.

Table 6: Ranking of Water-Quality Parameters by Day 5 Percentage Reduction

Rank	Parameter	Reduction	Day 5 Mean
1	<i>E. coli</i>	90.1%	119,667 MPN/100 mL
2	COD	60.3%	517.67 mg/L
3	Ammoniacal nitrogen	47.3%	42.70 mg/L as N

Note. Ranking is based only on percentage reduction between the Day 0 and Day 5 means and does not imply that the final concentration met a regulatory or reuse standard.

Table 6 presents the ranking of the three water-quality parameters according to their percentage reduction from Day 0 to Day 5, together with their final mean concentrations. *E. coli* ranked first, with a 90.1% reduction, followed by COD at 60.3% and

ammoniacal nitrogen at 47.3%. This ranking indicates that the mycelial boom exerted its strongest relative effect on the bacterial indicator, while COD also showed a substantial reduction. Ammoniacal nitrogen ranked third, making it the pollutant category with the

greatest remaining opportunity for process improvement. However, the ranking is based only on percentage reduction and does not mean that *E. coli* was completely removed or that the treated wastewater met a regulatory or reuse standard. The results instead

provide a basis for identifying which parameters responded most strongly and which may require further improvement in future biofilter designs.

Figure 1: Cumulative Reduction of Water-Quality Parameters from Day 0 to Day 5

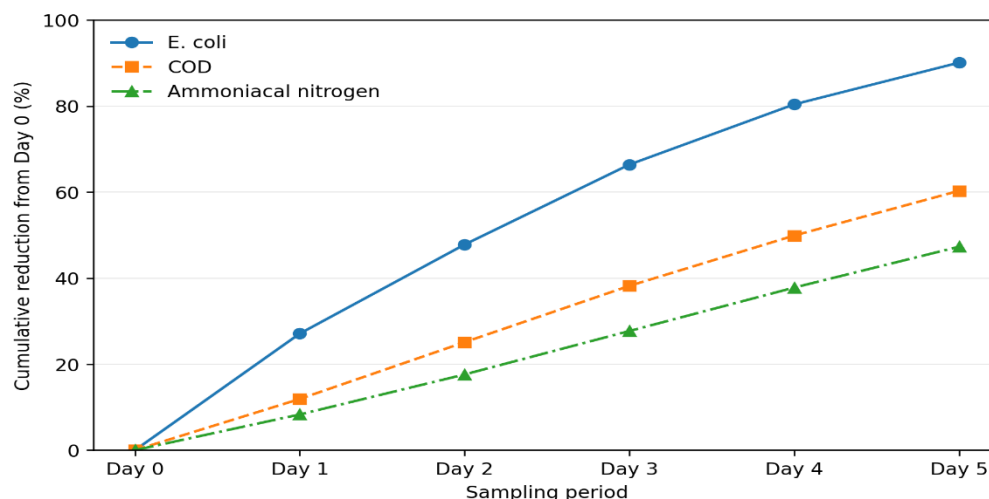


Figure 7 shows the cumulative percentage-reduction trajectories of the three water-quality parameters throughout the five-day treatment period. Expressing all three parameters as percentage change from Day 0 allows direct visual comparison despite their different measurement units. The figure shows that *E. coli* declined fastest, COD showed an intermediate reduction pattern, and ammoniacal nitrogen declined more gradually but continuously. The treatment effect accumulated over time for all three parameters, with the final reductions reaching 90.1% for *E. coli*, 60.3% for COD, and 47.3% for ammoniacal nitrogen. The curves did not plateau within the five-day observation period, indicating that measurable changes were still occurring by Day 5. This finding suggests that future experiments should extend the monitoring period to determine whether the downward trends continue, stabilize, or reach diminishing return.

VIII. CONCLUSION

The study concluded that the mycelium-based biofiltration system using *Pleurotus ostreatus* produced a consistent reduction in all three selected poultry wastewater contaminants after five days of treatment. *E. coli* showed the greatest reduction,

decreasing from 1,210,000 to approximately 119,667 MPN/100 mL, equivalent to a 90.1% reduction. COD decreased from 1,303.33 to 517.67 mg/L, representing a 60.3% reduction, while ammoniacal nitrogen decreased from 81.10 to 42.70 mg/L as N, corresponding to a 47.3% reduction.

The results indicated that the mycelial boom had its strongest relative effect on *E. coli*, followed by COD and ammoniacal nitrogen. The continuous decline observed throughout the treatment period suggested that the system was capable of improving several aspects of poultry wastewater quality under the conditions tested. However, measurable concentrations of all three contaminants remained after treatment; therefore, the findings did not support considering the system a stand-alone treatment capable of guaranteeing discharge or reuse compliance.

Overall, the findings demonstrated the potential of *Pleurotus ostreatus* mycelium as a biological filtration component for poultry wastewater treatment, particularly in reducing microbial contamination and organic load. The lower reduction in ammoniacal nitrogen highlighted an area requiring further

improvement. Extending the treatment period and investigating optimized filter designs or additional treatment stages could help achieve more balanced contaminant removal. Thus, the study provided preliminary evidence that mycelium-based biofiltration could serve as a promising biological treatment or pretreatment approach, while further research would be needed to determine its long-term effectiveness and applicability under different wastewater conditions.

REFERENCES

- [1] Aghalari, Zahra, Hans-Uwe Dahms, Mika Sillanpää, Juan Eduardo Sosa-Hernandez, and Roberto Parra-Saldívar. "Effectiveness of Wastewater Treatment Systems in Removing Microbial Agents: A Systematic Review." *Globalization and Health* 16, no. 1 (2020): 13. [Springer](#)
- [2] Aghalari, Zahra, Hans-Uwe Dahms, Mika Sillanpää, Juan Eduardo Sosa-Hernandez, and Roberto Parra-Saldívar. "Effectiveness of Wastewater Treatment Systems in Removing Microbial Agents: A Systematic Review." *Globalization and Health* 16, no. 1 (2020): 13. [Springer](#)
- [3] B. R. Baker, R. Mohamed, A. Al-Gheethi, and H. A. Aziz, "Advanced technologies for poultry slaughterhouse wastewater treatment: A systematic review," *Journal of Dispersion Science and Technology*, vol. 42, no. 6, pp. 880–899, 2021. [Taylor & Francis](#)
- [4] B. Dalecka, M. Strods, T. Juhna, and G. K. Rajarao, "Removal of total phosphorus, ammonia nitrogen and organic carbon from non-sterile municipal wastewater with *Trametes versicolor* and *Aspergillus luchuensis*," *Microbiological Research*, vol. 241, Art. no. 126586, 2020. [ScienceDirect](#)
- [5] Gržinić, Goran, et al. "Intensive Poultry Farming: A Review of the Impact on the Environment and Human Health." *Science of the Total Environment* 858, no. 3 (2023): 160014. [ScienceDirect](#)
- [6] R. T. Hilares et al., "Promising physicochemical technologies for poultry slaughterhouse wastewater treatment: A critical review," *Journal of Environmental Chemical Engineering*, vol. 9, Art. no. 105174, 2021. [ScienceDirect](#)
- [7] Mohan, Thanmaya, Noorul Shaheen Sheik Farid, Swathi K. V., Sowmya A., and Ramani K. "Sustainable Biological System for the Removal of High Strength Ammoniacal Nitrogen and Organic Pollutants in Poultry Waste Processing Industrial Effluent." *Journal of the Air & Waste Management Association* 70, no. 12 (2020): 1236–1243. [Taylor & Francis](#)
- [8] Ngogang, Marie Paule, et al. "Microbial Contamination of Chicken Litter Manure and Antimicrobial Resistance Threat in an Urban Area Setting in Cameroon." *Antibiotics* 10, no. 1 (2021): 20. [MDPI](#)
- [9] United Nations Human Settlements Programme (UN-Habitat) and World Health Organization. *Progress on the Proportion of Domestic and Industrial Wastewater Flows Safely Treated: Mid-Term Status of SDG Indicator 6.3.1 and Acceleration Needs, with a Special Focus on Climate Change, Wastewater Reuse and Health*. Geneva: World Health Organization, 2024. [WHO](#)