

RESEARCH ARTICLE



Biodegradation of Ammonia from Polluted Water using a Biofilters Inoculated with *Klebsiella pneumoniae* and *Bacillus cereus*: A Case Study of the Musi River, Indonesia

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ABSTRACT

High concentrations of ammonia in rivers can cause water pollution through eutrophication. It is necessary to reduce ammonia in an environmentally friendly manner using biofilters, such as zeolite. Water samples from the Musi River, South Sumatra, were collected from six stations, namely Palembang City (Gandus, AMPERA, PUSRI) and Banyuasin Regency (Marga Sungsang, Sungsang II, Sungsang IV). The biofilter was designed to combine physical filtration using filter media such as activated carbon, anthracite, ferrolite, silica sand, and zeolite with biodegradation by nitrifying bacteria. The controls were treated in the same manner as the other samples, without the addition of the bacterial combination. The best decomposing bacteria were determined based on the reduction in ammonia and nitrite levels in the water. Over 14 days, the system achieved a 68.92% reduction in ammonia concentration. The nitrite levels were effectively maintained below 0.063 mg/L throughout the process. Evaluation of the optimal bacterial consortium (Sample C2) showed a significant improvement in key water quality parameters: pH decreased from 8.1 to a more neutral 6.5, while dissolved oxygen increased significantly from 5.4 to 9.42 mg/L, indicating improved aerobic conditions. Molecular identification revealed *Klebsiella pneumoniae* and *Bacillus cereus* as the ammonia- and nitrite-degrading bacteria, respectively. The biofiltration system can reduce ammonia pollution and improve river water quality at the laboratory scale.

Introduction

Issues surrounding water include water security, water scarcity, water footprint, water sustainability, and water pollution control in surface water [1]. The United Nations has designated clean water and sanitation as one of its 17 sustainable development goals (SDGs) for 2030. Water pollution in rivers remains a crucial issue for countries that use river water as their main source of water [2]. The competition between water needs for domestic life, industry, and ecosystems is becoming increasingly intense [3]. Indonesia, with a population of around 270 million, produces domestic wastewater, which is one of the largest contributors to waste generated by human activities [4]. Water pollution in rivers mostly comes from domestic sources [5], which contain nutrients [6]. In addition to domestic waste that contributes to rivers, there is industrial waste [7]. The agricultural industry is growing rapidly, and improper waste disposal, fertilizers, and pesticides are also contributing factors [8]. The use of chemicals such as fertilizers can release nutrients into runoff streams [9,10], and nitrogen reaches rivers from surface runoff of household pollution or industrial waste inputs [11]. Agricultural ammonia emissions in cities show an upward trend [12], some of them may come from fertilizers that usually enter surface water [13].

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The Musi River flows through one of Indonesia's largest cities, Palembang. Rubber, timber, fertilizer, ceramics, detergents, oil, gas, electroplating, and fabric dyeing are some of the industries that are located along the Musi River. Adequate wastewater treatment facilities are lacking in the majority of these enterprises [2]. The primary sources of pollutants and nutrients that are released into the Musi River are thought to be the industrial, urban, and agricultural operations that surround it [14]. Even after crossing the Musi River, Palembang City will flow into the sea, which is also influenced by the activities of the residents of Sungsang. The increasing intensity of anthropogenic activities in the Musi River Basin, such as the growth of residential areas, industrial activities, and agricultural activities, can affect the quality of river water. Rivers undergo physical, chemical, and biological changes as a result of various activities along the river, producing both organic and inorganic contaminants that ultimately lead to pollution [15].

Water quality degradation leads to other serious problems related to water quality degradation and public health risks [16] due to the high level of environmental pollution caused by industry [17]. As many forms of pollution are produced by urban and industrial activity, reducing water pollution is becoming more and more problematic [18]. Despite the fact that the Musi River provides water for drinking, bathing, washing, transportation, rice field irrigation, livestock needs, and leisure [2]. There is a phenomenon of eutrophication as a result of domestic waste [4] and industry [19] containing nitrogen compounds then entering the river basin [20]. Eutrophication is a form of pollution that occurs when nutrient levels exceed normal values [4,21] and have a negative impact on water resources [22]. An excessive amount of ammonia in aquatic environments can disrupt the natural equilibrium [23]. Ammonia (NH_3) is a well-known contaminant that seriously harms aquatic habitats and is challenging to treat [24].

Some microorganisms use ammonia as nitrogen for cell nutrition [25]. Aerobic bacteria and other microorganisms aid in nitrification, which is the chemical process by which ammonia undergoes an oxidation reaction to form nitrite, which is subsequently converted to nitrate [26]. The microbes that restore the microbial oxidation process of ammonia to nitrite and subsequently nitrate in the natural environment are largely unknown [14]. The use of bacterial isolates to remove nitrogen from contaminated water is rarely reported [27]. The DNA sequence analysis technique that encodes the 16S rRNA gene and is used to assess the originality of bacterial isolates found in nature can be utilized to learn more about this report [28]. To alter water pollution control measures, several physical and biological research studies are required in addition to the identification of nitrifying bacteria. Bacterial decomposition and physical filtering techniques utilizing sand and membranes offer a combined treatment methodology for efficient water pollution remediation [21].

One promising green wastewater treatment method for getting rid of many kinds of pollutants is biofiltration [29]. Biological filtration can be used to remove ammonium from water through the nitrification process [30–32]. Research on nitrate content in the Musi River in Palembang City has been conducted [2]; research on ammonia and nitrite levels and the application of biofilters to try to lower ammonia pollution levels hasn't been done, though. Determining the amount of ammonia, nitrite, and nitrate is very important, and if pollution has occurred, remediation efforts such as biofiltration are needed. This study aims to develop a biofilter that can reduce ammonia and nitrite levels and improve water quality parameters. In addition, this study uses degrading bacteria, so the objective of this study is to identify ammonia-degrading bacteria and nitrite-degrading bacteria isolated from the downstream waters of the Musi River in South Sumatra, Indonesia.

Materials and Methods

Research Area

This study was conducted from July to November 2024, utilizing samples from the Musi River in Palembang City and the Musi River Estuary in Banyuasin Regency, South Sumatra. Water sampling in the Musi River in Palembang City consists of three research stations, namely the Gandus station located near residential areas and rice fields, the AMPERA station located near market activities and residential areas, and the PUSRI station located near the fertilizer industry. Water sampling at the Musi River estuary in Banyuasin Regency also consists of three stations located in river flows close to residential areas, including the Marga Sungsang station, which is still heavily influenced by river water; the Sungsang II station, which is close to a large market serving five villages; and the Sungsang IV station, which is influenced by seawater and mangroves. The research locations of the Musi River in Palembang City and the Musi River Estuary in Banyuasin Regency can be seen in Figure 1.

Water quality and biodegradation research at the Marine Bioecology Laboratory, Department of Marine Science, Sriwijaya University. Isolation and morphological observation at the Microbiology Laboratory, Department of Biology, Sriwijaya University. Molecular identification at the Genetics and Biotechnology Laboratory, Department of Biology, Sriwijaya University, and sequencing at PT Genetika Science Indonesia. Ammonia, nitrite, and nitrate levels were measured at the Palembang Health Laboratory Center, Ministry of Health of the Republic of Indonesia. The research area and equipment are carried out in sterile conditions according to applicable standards in the laboratory.

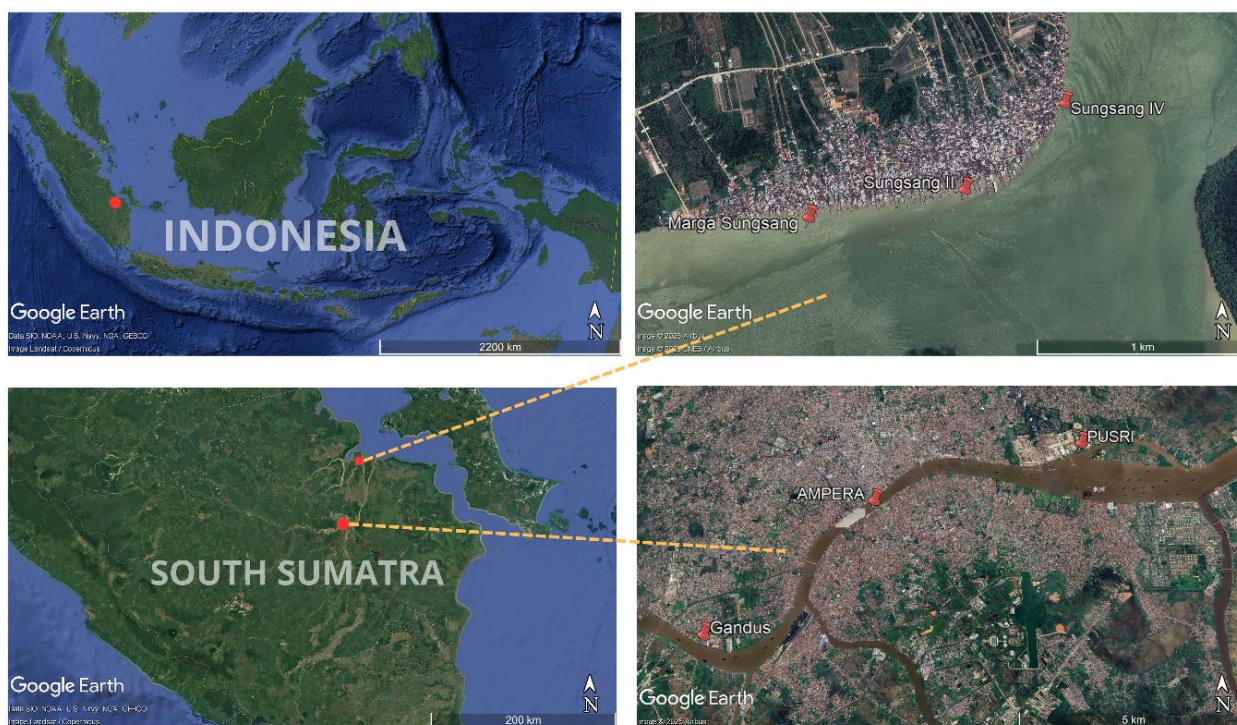


Figure 1. Map of research locations located on the Lower Musi River, South Sumatra, Indonesia. The freshwater research location is located in the Musi River in Palembang City, consisting of the Gandus, AMPERA, and PUSRI stations. Meanwhile, the estuarine location is in the Musi River in Banyuasin Regency, consisting of the Marga Sungsang, Sungsang II, and Sungsang IV stations.

Sampling

Purposive sampling is used to determine the sampling locations. According to Cash et al. [33], samples that are thought to be representative of the region under study are taken using the purposive sampling technique. Water samples were collected from each station for each surface water sample, with 250 mL collected in glass bottles for bacterial isolation, 1 liter in jerry cans to measure odor, taste, color, suspended residue, salinity, and turbidity before filtering, 500 mL in dark plastic bottles to measure ammonia, nitrite, and nitrate before biofiltration, and 20 liters in plastic jerry cans to measure ammonia, nitrite, and nitrate after biofiltration. Water samples were taken directly from the water surface [34]. The samples were placed in bottles, labeled, and put into a cool box.

Water Quality Measurement

Environmental parameters were measured directly at each sampling site to determine the water quality suitable for bacterial growth. Environmental parameter measurements were also conducted in the laboratory on biofiltration water samples. Environmental parameters were measured using a Multiparameter (Hanna Instruments Inc., USA) to measure temperature, pH, and dissolved oxygen in the water. The sensor was dipped into the water sample three times, and the average value was calculated.

Measurement of Ammonia, Nitrite, and Nitrate

Ammonia measurement based on SNI method number 06-6989.30 [35]. The steps for measuring ammonia are as follows: 25 mL of water sample is pipetted and placed in an Erlenmeyer flask, then 1 mL of phenol

solution is added and homogenized. After that, 1 mL of sodium nitroprusside solution is added and homogenized again, then 2.5 mL of oxidizing solution is added and homogenized. The Erlenmeyer flask is then closed and left for 1 hour for the color formation process. After the incubation time is complete, the solution is measured using a spectrophotometer at a wavelength of 640 nm.

Nitrite measurement based on SNI method number 06-6989.9 [36]. The steps for measuring nitrite are as follows: 50 mL of water sample is pipetted and placed in a 200 mL beaker while being filtered using filter paper. Next, 1 mL of sulfanilamide solution is added, then the mixture is homogenized and left for 2 to 8 minutes. After that, 1 mL of NED dihydrochloride solution is added, homogenized, and left for 10 minutes. The sample is then measured using a spectrophotometer at a wavelength of 543 nm.

The nitrate measurement used in this study is based on SNI method number 06-2480 [37]. The steps for measuring nitrate are as follows: 5 mL of water sample is taken and placed in an Erlenmeyer flask, then 0.5 mL of brucine solution, 0.05 mL of sodium arsenite solution, and 5 mL of sulfuric acid solution are added. The mixture is then homogenized and left to cool. After that, the solution is measured using a spectrophotometer at a wavelength of 410 nm.

Isolation and Identification of Bacteria

Serial dilutions were performed by adding 1 mL of sample to 9 mL of sterile distilled water, then taking 1 mL and adding 9 mL of sterile distilled water, and so on until a dilution of 10^{-5} was achieved. The media was prepared by dissolving a specific media that had been homogenized, then sterilized in an autoclave at a temperature of 121 °C with a pressure of 15 lbs for 15 minutes. Bacteria-specific media ammonia decomposer consists of distilled water (H_2O), ammonium sulfate ($(NH_4)_2SO_4$), di-potassium hydrogen phosphate (K_2HPO_4), sodium chloride (NaCl), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), calcium carbonate ($CaCO_3$), red phenol, and agar with a pH of 7 [38]. The specific medium for nitrite-degrading bacteria consists of distilled water (H_2O), sodium nitrite ($NaNO_2$), dipotassium hydrogen phosphate (K_2HPO_4), sodium chloride (NaCl), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), sodium carbonate (Na_2CO_3), and agar with a pH of 7.

Then inoculate using the pour plate method, which involves preparing 5 test tubes containing 9 mL of sterile water. Take a 1 mL sample of water using a micropipette and place the sample into the test tube with a dilution of 10^{-1} . Then vortex for 10–15 minutes. Take 1 mL of water from the vortexed test tube into a test tube with a dilution of 10^{-2} . Repeat the same procedure for the other test tubes up to a dilution of 10^{-5} . Take 1 mL from the test tube with a dilution of 10^{-3} into each petri dish. Repeat the same procedure for dilutions of 10^{-4} and 10^{-5} into each petri dish. Wait until the medium hardens, then cover the edges of the petri dishes with plastic wrap (to prevent contamination). Lastly, it was incubated at 37 °C for 24 hours.

Following inoculation, bacteria are separated from the culture and transferred to a new media using a streak culture technique. Until a pure culture is achieved, each bacterial colony displaying traits including shape, edges, color, elevation, and surface texture is chosen and introduced to the new medium. The bacterial culture was rejuvenated in slanted NA medium by heating the inoculating needle over a Bunsen burner until it glowed, then aseptically taking 1 inoculating needle of the purified bacterial culture. The inoculating needle was then scraped onto the slanted NA medium in a zig-zag motion. The bacterial culture in the NA medium was incubated in an incubator for 24 hours at a temperature of 37 °C.

The combination of bacteria that can lower the highest levels of nitrite and ammonia from the biofiltration process will be the ones that are molecularly identified. DNA extraction, agarose gel electrophoresis, PCR (polymerase chain reaction), and sequencing are the steps involved in 16S rRNA analysis for bacterial identification. This step uses forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1392R (5'-GGT TAC CTT GTT ACG ACT T-3'). Sequencing data was obtained by sending samples to PT Genetika Science Indonesia sequencing services with a standard sample size of 100 bp and a sample concentration of 10 ng/ μ L. Data processing for bacterial gene identification through sequencing results to search for sequence similarities with available databases using basic local alignment search tool (BLAST) on the server online (www.ncbi.nlm.nih.gov accessed on 22 April 2025).

Biofiltration

The stages carried out in biofiltration (Figure 2) include: (a) providing contaminated water marked with a brownish color. Then (b) sedimentation is carried out [39]; larger and heavier particles will fall to the bottom [40,41]. Next (c – d), the sand is washed before use, which is then filtered using a slow sand filter [42], which relies on gravitational force [43]. Media filters such as anthracite sand [29], silica sand and zeolite rock [44],

and anthracite are added. The addition of coconut shell-derived activated carbon [45]. The media filter is arranged and coated with foam to prevent mixing, and the bottom layer is covered with a porous ceramic bioiring and a plain ceramic bioiring. Water passes (e) through the membrane filter [46] with a size of 10 micrometers. Whatman filter paper with a pore size of 45 microns is used to filter contaminants as effectively as possible [47]. After the physical filtration process, the next step is (f) the degradation of various organic pollutants. Bacteria in water samples that are not research objects will be killed in water that is flowed under UV (ultraviolet) light radiation for 100 minutes [48] at a distance of 15 cm [49] and illuminated by a 15-watt UV lamp with a wavelength of 253.7 nm [50]. Killing bacteria in this step is so that there are no living bacteria in the water sample before adding the working object in the form of isolated bacteria from this research.

Furthermore, (g) the isolated bacteria were placed in a plastic box containing dirty water and aerated. Increased aeration will increase dissolved oxygen concentrations, which in turn will improve the efficiency of biological nitrogen removal [51]. Since biological aeration filters (BAF) have often been used as tertiary units in upgraded systems to further reduce organic residues and suspended solids in secondary effluent [52]. A technique known as "trickling filter" involves attaching microorganisms that are receptive to a solution to an existing material [53]. This study uses bio porous material as a place for bacteria to attach and based on the result by Sambu et al. [54] cites 7 days for the nitrification rate with a decrease in ammonia of 0.09 mg/L, which is a small value. The bacteria increased significantly on day 7 and decreased on day 14 [55]. Thus, 14 days were chosen in this investigation to allow for the bacterial breakdown of ammonia, nitrite, and nitrate. After the isolated bacteria are used in the water samples, the water samples are again illuminated to UV light to kill the bacteria used as research objects. However, it is different in the control sample which was not given bacteria. The result (h) is clear water with a reduced ammonia content.

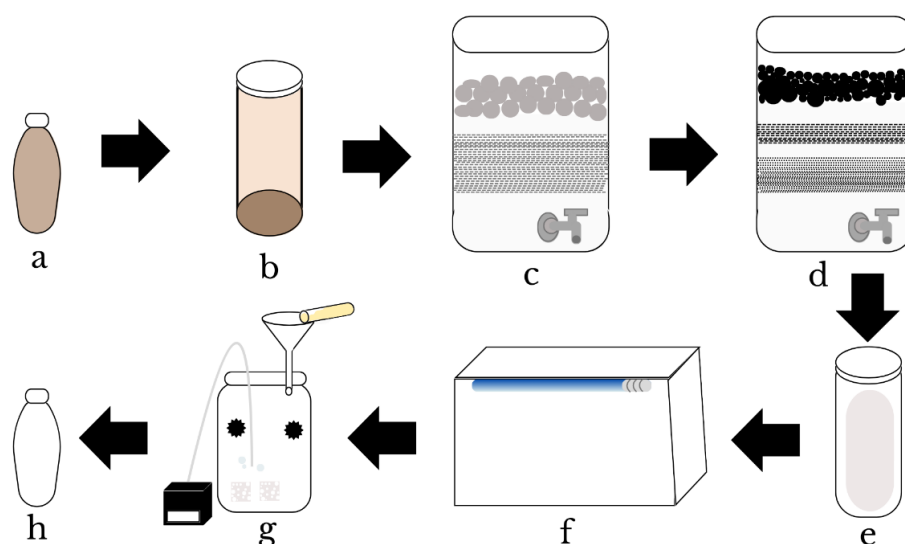


Figure 2. Biofiltration scheme for converting dirty water (a) into clean water (h) through physical and biological stages. The physical stage consists of sedimentation (b) and uses several filter media (c–e). The biological stage uses biodegradation by adding bacterial isolates (g). Before and after the biodegradation process, UV irradiation is carried out for sterilization (f).

Results and Discussion

Results

Ammonia, Nitrite, and Nitrate Concentration

The highest concentrations of ammonia, nitrite, and nitrate among the six research stations were found at the PUSRI station, with ammonia levels of 65.5 mg/L, nitrite levels of 7.56 mg/L, and nitrate levels of 15.66 mg/L, as shown in Figure 3. Meanwhile, the other five stations had low ammonia values of less than 1, with Gandus at 0.38 mg/L, AMPERA at 0.54 mg/L, Marga Sungsang at 0.171 mg/L, Sungsang II at 0.285 mg/L, and Sungsang IV at 0.566 mg/L. The nitrite values were < 0.036 mg/L at Gandus, 0.176 mg/L at AMPERA, 0.546 mg/L at Marga Sungsang, 1.217 mg/L at Sungsang II, and 0.592 mg/L at Sungsang IV. The nitrate values at the Gandus were 2.746 mg/L, AMPERA 3.694 mg/L, Marga Sungsang 6.27 mg/L, Sungsang II 7.492 mg/L, and Sungsang IV 6.554 mg/L.

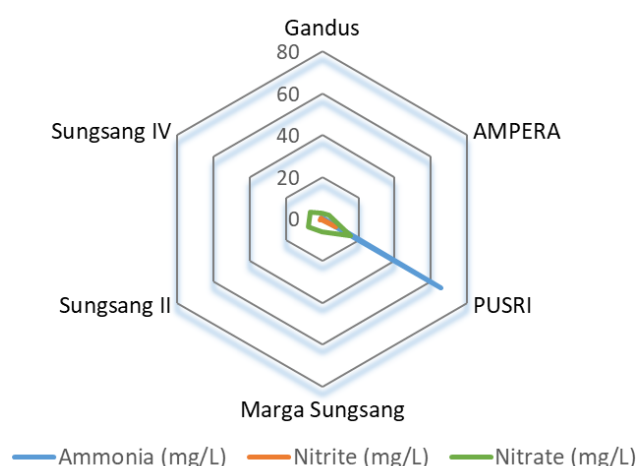


Figure 3. Concentrations of ammonia, nitrite, and nitrate at six monitoring stations (Marga Sungsang, Sungsang II, Sungsang IV, Gandus, AMPERA, and PUSRI) on the Musi River, South Sumatra. Of the six monitoring stations, only one the PUSRI station showed high levels of ammonia, nitrite, and nitrate. These concentration values are used as baseline data to identify water that requires treatment via biofiltration.

The second sampling was conducted only on water samples from the PUSRI station for further testing using a biofilter. The results of the study showed a 3.44% reduction in ammonia, from 7.25 mg/L before filtration to 7 mg/L after physical filtration. The ammonia values can be seen in Table 1. After physical filtration, biodegradation by bacteria over 14 days reduced ammonia by 67.81% from 7 to 2.25 mg/L in sample C2, or 4.84% per day. Overall, the reduction in ammonia using a biofilter consisting of physical filtration and biodegradation was 68.92%. Nitrite-degrading bacteria also worked, with values in C2 remaining low at < 0.063 mg/L, as shown in Table 1. A value of < 0.063 mg/L for nitrite is the instrument's limit of detection. There was a decrease in ammonia and nitrite after biofiltration, but nitrate increased.

Table 1. Concentrations of ammonia, nitrite, and nitrate in water at the PUSRI station. Water sample A was at the beginning before treatment, water sample B after physical filtration treatment, and water sample C after biodegradation. Sample C0 is the control and Samples C1–C20 were obtained from a total of 20 bacterial combinations consisting of 5 ammonia-degrading bacteria and 4 nitrite-degrading bacteria.

Sample	Ammonia degrading bacteria	Nitrite degrading bacteria	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
A			7.25	< 0.063	58.15
B			7	< 0.063	70.075
C0			7	< 0.063	70.07
C1	NA 1.1	NB 3.1.2	2.346	< 0.063	98.05
C2*	NA 1.1	NB 3.2	2.253	< 0.063	99.4
C3	NA 1.1	NB 4.1	2.801	< 0.063	94.7
C4	NA 1.1	NB 3.1.1	2.879	< 0.063	96.95
C5	NA 1.2	NB 3.1.2	4.805	< 0.063	86.3
C6	NA 1.2	NB 3.2	3.925	< 0.063	85.3
C7	NA 1.2	NB 4.1	5.08	< 0.063	79.95
C8	NA 1.2	NB 3.1.1	4.685	< 0.063	81.5
C9	NA 3.2	NB 3.1.2	3.79	0.145	80.5
C10	NA 3.2	N.B 3.2	7.105	< 0.063	73.7
C11	NA 3.2	NB 4.1	8.1	< 0.063	73.35
C12	NA 3.2	NB 3.1.1	7.34	< 0.063	68.85
C13	NA 2.1	NB 3.1.2	4.595	< 0.063	92.15

Sample	Ammonia degrading bacteria	Nitrite degrading bacteria	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
C14	NA 2.1	NB 3.2	4.605	< 0.063	95.35
C15	NA 2.1	NB 4.1	5.545	< 0.063	94.75
C16	NA 2.1	NB 3.1.1	5.715	< 0.063	95.75
C17	NA 3.1	NB 3.1.2	5.64	< 0.063	85.35
C18	NA 3.1	NB 3.2	5.69	< 0.063	100.65
C19	NA 3.1	NB 4.1	6.99	0.125	82.45
C20	NA 3.1	NB 3.1.1	8.325	< 0.063	88.05

A: initial water sample; B: water sample after filtration (physical filtration); C0: control water sample; C1–C20: water samples after biofiltration with a combination of bacteria; *: sample with the highest degradation capacity.

Physical-Chemical Water Parameters

The biofiltration process improved key water quality parameters (Table 2). The initial water from the PUSRI station was alkaline (pH 8.1). Physical filtration alone reduced the pH to 6.3. Following biological treatment with the best consortium (C2), the pH stabilized at 6.5, moving towards a more neutral condition. Dissolved Oxygen (DO) levels showed a marked improvement. Initial DO was 5.40 mg/L. Physical filtration increased to 7.74 mg/L, and biofiltration with aeration further elevated DO to 9.42 mg/L in sample C2. Water temperature decreased slightly from the initial 31.5 °C to a stable range of 28.6–28.8 °C during the controlled laboratory treatment.

Table 2. Results of measurements of physical and chemical parameters of water at the PUSRI station, Musi River, South Sumatra. The physical-chemical parameters of water measured were pH, DO, and temperature, which were measured in three stages A, B, and C.

Sample	pH	DO (mg/L)	Temperature (°C)
A	8.1	5.4	31.5
B	6.3	7.74	28.8
C0	6.3	9.68	27.8
C1	6.5	9.65	28.7
C2	6.5	9.42	28.6
C3	6.5	9.63	28.6
C4	6.5	8.85	28.6
C5	7	8.62	28.8
C6	6.7	7.93	28.7
C7	7.1	8.15	28.8
C8	6.9	8.57	28.7
C9	7.1	8.48	28.8
C10	7.2	8.15	28.7
C11	7.3	8.15	28.7
C12	7.4	8.45	28.7
C13	7.1	8.44	28.8
C14	7	8.14	28.8
C15	7.1	8.19	28.8
C16	7.4	8.04	28.8
C17	7.3	8.13	28.8
C18	7	7.66	28.6
C19	7.4	8.89	28.7
C20	7.4	8.13	28.7

A: initial water sample; B: water sample after filtration (physical filtration); C0: control water sample; C1–C20: water samples after biofiltration with a combination of bacteria.

Molecular Identification of Key Bacterial Isolates

Genomic DNA was extracted from the two most efficacious bacterial isolates in the optimal consortium (C2): the ammonia-degrading isolate (designated NA 1.1) and the nitrite-degrading isolate (designated NB 3.2). The 16S rRNA gene was amplified with universal primers 27F and 1392R. Subsequent Sanger sequencing and analysis utilizing the BLAST on the NCBI (National Center for Biotechnology Information) database yielded conclusive taxonomic identification. The findings indicated that isolate NA 1.1 exhibited a 99.61% sequence similarity to *Klebsiella pneumoniae* strains present in the GenBank database. The high homology substantiates its classification within this species, designating it as the principal agent accountable for the observed ammonia degradation in the biofilter system. Isolate NB 3.2 exhibited a marginally reduced, yet definitive, sequence similarity of 92.46 to 94.82% with multiple *Bacillus cereus* strains. This degree of homology unequivocally categorizes the isolate within the *Bacillus cereus* group, designating it as the principal bacterium accountable for sustaining low nitrite levels throughout the treatment process. The effective molecular identification of the indigenous isolates, *Klebsiella pneumoniae* (NA 1.1) and *Bacillus cereus* (NB 3.2), establishes a scientific foundation for their distinct roles in bioremediation, facilitating focused future research and application.

Discussion

The PUSRI station had an ammonia level of 65.5 mg/L, which is far higher than what was found at other stations and much higher than the previously reported range of 0.022 to 7.134 mg/L for the Musi River [2]. The station's proximity to a fertilizer business appears to directly correlate with the elevated concentration. This aligns with findings indicating that industrial activities, particularly those associated with nitrogen fertilizer manufacturing, can generate wastewater containing ammonia concentrations of 1,500–3,000 mg/L [56]. The initial reduction of ammonia by 3.44% through physical filtration, albeit minor, aligns with the principles of adsorption on porous media [18,41,57]. The filter media utilized, such as natural zeolite and coconut shell activated carbon, are recognized for their adsorptive capabilities. Zeolite has shown ammonia removal efficiencies between 39.93% and 96.67% across different studies [58–60]. In contrast, coconut shell activated carbon, characterized by its high carbon content (~77.4%) and uniform small particle size, can achieve reductions of 98.26 to 98.82% [45,61,62]. The substantial initial load and the specific configuration of the multimedia filter are responsible for the observed reduced efficiency during this initial phase.

The following biological treatment phase, employing native bacteria, achieved significant removal, degrading 67.81% of the residual ammonia over a period of 14 days. The synergistic effect of combined physical and biological filtration achieved a total removal efficiency of 68.92%, indicating a significant enhancement and aligning closely with other wastewater treatment studies that report reductions near 80.76% [63]. The efficacy of the biological stage can be credited to the introduced microbial consortium. The transformation of ammonia into nitrate, as indicated by the reduction in ammonia and nitrite levels coupled with a rise in nitrate, exemplifies the nitrification process, during which ammonia is oxidized first to nitrite and subsequently to nitrate [26,64]. Nitrite undergoes rapid conversion, leading to nitrate becoming the predominant stable nitrogen form in water, which is significantly less toxic than its precursors [58,65].

The notable enhancement in water quality metrics following treatment highlights the comprehensive effectiveness of the system. The reduction in pH from an alkaline 8.1 to a near-neutral 6.5 following physical filtration is significant, as elevated pH levels promote the toxic unionized form of ammonia and may impede its elimination [58,66]. The observed decrease in pH can be linked to the application of activated carbon, which has the capacity to reduce pH levels as a result of the presence of mineral and carbonic acids [67,68]. The final pH of 6.5 is within an acceptable range for aquatic organisms, distancing itself from the severely polluted levels of below 5.0 or above 9.0 [68]. Simultaneously, the enhancement in dissolved oxygen (DO) from 5.4 to 9.42 mg/L was accomplished via active aeration. Such an improvement is an essential element for the success of biofiltration, as aerobic nitrifying bacteria necessitate adequate dissolved oxygen for optimal performance and biofilm formation [31,51]. Improved aeration significantly boosts the efficiency of biological nitrogen removal, as evidenced by research indicating that systems with aeration reached a removal rate of 97.3%, in contrast to 85.8% for those without it [69]. The observed reduction in temperature from 31.5 to 28.6 °C, falling below the 32 °C threshold, likely fostered a more conducive environment for microbial activity and enhanced dissolved oxygen saturation.

The molecular identification technique confirmed that the main functional agents in the biofilter were the natural bacteria *Klebsiella pneumoniae*, which helps break down ammonia, and *Bacillus cereus*, which helps lower nitrite levels. The results of this investigation indicate that *K. pneumoniae* achieved 67.81% ammonia degradation over a 14-day period, aligning with its established function in heterotrophic nitrification. Diverse

strains have shown efficiencies of 83.26% over 30 days [51] and 34.5% within 120 hours [70] and had the capacity to acclimate to heightened ammonia levels of 125 mg/L, with specific strains capable of eliminating 157.18 mg/L of ammonium in under 12 hours [23,71]. This genus enables the aerobic oxidation of ammonia to nitrite [72,73]. *B. cereus* effectively maintained nitrite concentrations below 0.063 mg/L throughout the experiment. This aligns with its documented nitrite-degrading capabilities, with studies demonstrating efficiencies of $68.2 \pm 0.71\%$ over a 5-day duration [74] and rapid degradation noted in biocatalytic systems [75,76]. The successful implementation of the consortium demonstrates the importance of employing regionally adapted, indigenous microbial populations for bioremediation in certain contaminated areas, such as the Musi River. In summary, the hybrid biofilter system successfully addressed ammonia-contaminated water by utilizing a blend of physical adsorption and improved biological nitrification facilitated by a specifically designed indigenous microbial consortium.

Conclusions

Biofiltration can improve water quality through ammonia reduction mechanisms and environmental improvements. Ammonia reduction of up to 68.92% over 14 days, accompanied by consistently low nitrite levels (< 0.063 mg/L), indicates that the biodegradation process is running optimally. The best performance was demonstrated by the combination of C2 bacteria, which not only reduced ammonia but also stabilized water quality parameters by lowering the pH from alkaline to neutral and increasing dissolved oxygen to levels that support aquatic life. The identification of *Klebsiella pneumoniae* as an ammonia-degrading bacterium and *Bacillus cereus* as a nitrite-degrading bacterium indicates the specific role of microorganisms in supporting biofiltration effectiveness. Overall, biofiltration is influenced by the combination of bacteria and the duration of the process, so further research needs to focus on optimizing biodegradation time and daily monitoring of ammonia, nitrite, nitrate, and water quality parameters to achieve maximum treatment efficiency and determine the rate of biodegradation. In addition, biofilm stability and the number of bacterial cells within 14 days need to be observed. This research was conducted on a laboratory scale and did not include data replication. For future studies, data replication should be conducted to allow for statistical testing. If the development will be carried out directly at the river location, biofiltration needs to be adjusted in terms of the amount of filter media, equipment size, and sterile bacterial conditions, which must be directly proportional to the amount of river water without causing negative effects from the use of *Klebsiella pneumoniae* bacteria, which are pathogenic. It is recommended that research isolate non-pathogenic bacteria by identifying them prior to biofiltration testing to avoid the large-scale use of pathogenic bacteria in nature.

Author Contributions

JM: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft; **M:** Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Review & Editing; **HW:** Methodology, Supervision, Data Curation, Writing - Review & Editing; **R:** Methodology, Supervision, Writing - Review & Editing; and **RA:** Conceptualization, Formal Analysis, Writing - Review & Editing.

AI Writing Statement

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Conflicts of interest

The authors declare that they have no conflict of interest.

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