EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2025, 26(7), 392–402
https://doi.org/10.12912/27197050/205572
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Received: 2025.05.07
Accepted: 2025.06.09
Published: 2025.06.15

Utilizing activated sludge substrates the conduit tofu and slaughterhouse industries to treatment greywater

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ABSTRACT

This study examined the use of various types of activated sludge substrates in greywater treatment. It employed conduit sludge, tofu production waste, and slaughterhouse waste, which were rich in organic compounds, as effective biofiltration agents. These waste materials contained microbial activity capable of degrading pollutants in greywater, making them suitable for pollutant reduction processes. The analysis of waste characteristics in this research aimed to contribute to sustainable wastewater treatment solutions. The objective of this study was to analyze the effect of substrate selection on activated sludge preparation and to assess the impact of activated sludge variations on greywater treatment. The research consisted of two stages. The first stage focused on characterization and cultivation to determine the most viable activated sludge for the subsequent phase. The second stage involved acclimatization and biodegradation, evaluating how the activated sludge adapted to greywater and degraded pollutants. The first stage employed initial characterization followed by cultivation with four treatments: Treatment I, Treatment II, Treatment III, and Treatment IV, with the addition of nutrients. Each sample in each reactor underwent total suspended solids (TSS) analysis at three-day intervals. The best-performing activated sludge was acclimatized in the second stage with greywater under the following conditions: Treatment I contained 300 g of activated sludge and 1,000 mL of greywater; Treatment II contained 300 g of activated sludge and 1,200 mL of greywater; Treatment III contained 300 g of activated sludge and 1,400 mL of greywater, along with a control. At three-day intervals, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), TSS, and ammonia levels were analyzed. The results indicated that Treatment IV yielded the highest microbial growth. This fourth treatment was subsequently acclimatized with greywater, and the findings revealed that Treatment III effectively reduced pollutants compared to Treatment I and Treatment II.

Keywords: activated sludge, greywater, waste treatment.

INTRODUCTION

Domestic wastewater is generated from household activities, including kitchen use, bathing, laundry, and other daily tasks. It consists of 99.9% water and 0.1% solids, which include inorganic components like salts, sediments, and granular materials, as well as organic substances such as proteins, carbohydrates, and lipids. Household wastewater also contains pollutants and suspended particles, making effective treatment necessary. One of its defining characteristics is its biodegradability, allowing for natural decomposition processes (Rifaini and Noerhayati, 2024; Gross, 2015).

Household wastewater is categorized into two types: blackwater, which originates from toilets, and greywater, which is produced by sinks, bathtubs, laundry systems, and kitchens. Greywater contains a variety of chemical and organic pollutants, primarily introduced by detergents, soaps, shampoos, fats, and other substances. Blackwater, on the other hand, is solely generated from toilet waste (Khanam and Patidar, 2022). While kitchen and laundry greywater typically have higher levels of solids, organic carbon, and bacteria, they generally contain fewer chemicals and pathogens compared to blackwater (Kariuki et al., 2011).

A significant portion of greywater is discharged untreated into sewer systems or allowed to infiltrate the ground, contributing to environmental contamination (Maligal et al., 2021; Khanam and Patidar, 2022). Since it comprises roughly 70% of household wastewater, greywater can lead to serious pollution issues. It contains essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), alongside heavy metals, harmful bacteria, and microorganisms (Shankhwar, 2016). The inadequate treatment of domestic wastewater is a major factor in water pollution, particularly in natural water bodies (Zevhiana, 2023).

Several treatment methods have been developed to manage domestic wastewater, with greywater treatment relying on physical, chemical, and biological approaches. Physical treatments include biochar filtration, soil-based filters, sand, bark, membranes, and various filtration techniques (Heshamuddin et al., 2014; Comez, 2024; Dalahmeh et al., 2014). Chemical treatments utilize coagulation, flocculation, granular activated carbon, and photocatalytic oxidation (Razi et al., 2018; Pidou et al., 2008; Gulyas et al., 2013). Biological methods include anaerobic sludge blankets (UASB), rotating biological contactors (RBC), membrane bioreactors (MBR), constructed wetlands, activated sludge processes, and sequencing batch reactors (SBR) (Kraume, 2010; Atanasova et al., 2017; Abdel-Shafy et al., 2009; Baban et al., 2010; Collivignarelli, 2020).

Activated sludge is an effective biological treatment that utilizes aerobic microorganisms to break down organic contaminants. The process involves suspended microbial colonies that oxidize organic matter into carbon dioxide (CO_2), water (H_2O), ammonium (NH_4), and new biomass (Megasari et al., 2012). The activated sludge process comprises three main steps: seeding, acclimatization, and biodegradation (Sudaryati et al., 2012). During the seeding phase, microorganisms are cultivated to form an efficient microbial community for wastewater treatment. The acclimatization stage allows the activated sludge to adapt to specific wastewater conditions before undergoing the biodegradation process (Mukhtar et al., 2017).

Activated sludge consists of microbial communities thriving in suspended formations resembling small clusters of solids that settle easily. These communities include autotrophic and heterotrophic bacteria that require oxygen to grow. Heterotrophic microorganisms rely on organic carbon, while autotrophic bacteria use inorganic carbon as a substrate (Frącz, 2016). Given its biological nature, activated sludge treatment enables decentralized wastewater management by leveraging natural microbial processes for pollutant degradation.

Previous studies have investigated various greywater treatment methods. Comez (2024) demonstrated that biochar filtration significantly improves water quality. Dos Santos et al. (2024) examined oxidation techniques using sodium dodecyl sulfate as a greywater treatment model, while Nazif et al. (2023) developed multilayer filters to optimize COD reduction. Despite these advancements, little research has focused on activated sludge applications for greywater treatment, highlighting a need for further investigation in this area.

Addressing this research gap, the present study evaluates the effectiveness of activated sludge substrate types in greywater treatment. It specifically examines the use of conduit sludge, tofu production waste, and slaughterhouse waste, which serve as biofiltration agents rich in organic compounds. These substrates support microbial activity and facilitate pollutant degradation in greywater, demonstrating their potential for sustainable water purification. The detailed characterization of these materials underscores their relevance in improving greywater treatment processes.

MATERIALS AND METHODS

Materials

Materials from sludge, including sludge from canals, tofu industries, and abattoirs, were used as sources of nutrients and microorganisms. Materials for microorganism nutrients included glucose, KCL fertilizer, urea fertilizer, TSP fertilizer, and distilled water as nutrient sources.

Characterization of sludge

The initial characteristics of sludge were analyzed, including total organic carbon using the TOC method, total nitrogen using the Micro Kjeldahl method, and total phosphorus with the Stannous Chloride method (Fahruddin et al., 2020).

Activated sludge seeding

Three different types of activated sludge—slaughterhouse, tofu industry, and canal—were used to seed the seedlings. The seedlings were placed in plastic containers and were aerated using various treatments.

For Seedling I, 50 g of sludge sediment from a tofu industrial ditch was combined with 100 g of glucose solution, 50 g of urea fertilizer, 250 g of KCL fertilizer, 250 g of TSP fertilizer, and 500 mL of distilled water. In Seedling II, 250 g of mud sediment from a river or canal and 250 g of mud sediment from a tofu factory ditch were mixed with 100 g of glucose solution, distilled water, and nutrients, including 50 g of urea fertilizer, 250 g of KCL fertilizer, and 250 g of TSP fertilizer. The mixture was diluted to 500 mL. For Seedling III, 250 g of sludge sediment from a slaughterhouse and 250 g of sediment from a tofu industrial ditch were combined with 50 g of urea fertilizer, 250 g of KCL fertilizer, 250 g of TSP fertilizer, 10 g of glucose solution, and distilled water. This mixture was also diluted to 500 mL. Lastly, Seedling IV was prepared by combining 125 g of sludge sediment from a river or canal, 250 g of sludge sediment from a tofu industrial ditch, and 250 g of sludge sediment from a slaughterhouse with 50 g of urea fertilizer, 250 g of KCL fertilizer, 250 g of TSP fertilizer, 10 g of glucose solution, and distilled water. This mixture was diluted to 500 mL for aeration.

Observations were made on days 0, 3, 6, 9, and n, including the measurement of total suspended solids (TSS). The gravimetric method was used to measure the total suspended solids. Filter sheets that had been previously weighed were used to filter water samples. At 105 °C, the residue was dried until its weight remained constant. According to Fahruddin and Tanjung (2019), the total amount of suspended particles was represented by the increase in mass on the filter paper. The Equation (1) was used to estimate the total suspended solids value.

$$Total\ suspended\ solids\ (TSS) = \frac{a-b}{c} \times 10^6 \quad (1)$$

where: a − weight of disk+solid (g), b −weight of empty disk (g), c − volume of smple used (mL).

Acclimatization and biodegradation

Greywater effluent was allowed to stand for a day during the initial stabilization phase before being introduced into each bioreactor. An aerator provided air and simultaneously acted as a stirrer to mix the activated sludge of various compositions.

In Treatment I, 300 g of activated sludge was combined with 1000 mL of greywater. Treatment II included 300 g of activated sludge mixed with 1200 mL of greywater, while Treatment III contained 300 g of activated sludge mixed with 1400 mL of greywater. A control setup was also prepared without treatment. Observations were conducted on each day, starting from day 0, 3, and 6, to measure relevant parameters.

Degree of acidity (pH)

A pH meter, which had been calibrated using pH 4 buffer and pH 7 with a 15-minute stabilization time, was used to measure the pH. After being cleaned with distilled water, the electrode was dried and then was immersed in a solution made from the treated water sample. (Fahruddin et al, 2019) then tested the pH value.

Biological oxygen demand (BOD)

Titrating with 0.025 N Na-thiosulphate, aerating for 15 minutes, sampling to 250 mL, titrating the sample in a 250 mL container, watching for color changes, and titrating on the fifth day were the methods used to quantify BOD. Equations 2 and 3 provided the formulas for calculating dissolved oxygen (OT) and BOD, respectively.

$$\frac{V_t \times N_t \times BE\ O_2 \times V_b \times 1000}{V_c \times (V_b \times 2)} \quad (2)$$

where: V_t – volume of titrant (in mL), N_t − normality of the titrant, $BE\ O_2$ – equivalent weight of oxygen, V_c – volume of the sample, V_b – volume of the blank

$$BOD = [(OT_{C0} - OT_{C5}) - \\ - K(OT_{B0} - OT_{B5}) \times fp \quad (3)$$

where: OT_c – dissolved oxygen of the sample, OT_B – dissolved oxygen blank, *fp* – dilution factor, *K* – the correction factor that accounts for changes in the blank (*fp*-1)/*fp*.

Chemical oxygen demand (COD)

The material was homogenized, analyzed, pH adjusters were added, and chemical analysis was carried out as part of this COD measurement. Following that, the outcomes were contrasted with the 0.1 N FAS standard. The COD value was determined based on the Equation 4.

$$COD\left(\frac{\text{mg}}{\text{L}}\right)\text{O}_2 \frac{(A - B) \times N \times 8000}{\text{mL}\ sample} \quad (4)$$

where: *A* – volume of FAS solution required for blank (mL), *B* – volume of FAS solution required for the sample (mL), *N* – normality of FAS solution.

Total suspended solid (TSS)

The gravimetric method was used to measure the total suspended solids. Filter sheets that had been previously weighed were used to filter water samples. At 105 °C, the residue was dried until its weight remained constant. According to Fahruddin and Tanjung (2019), the total amount of suspended particles was represented by the increase in mass on the filter paper. The Equation 1 was used to estimate the total suspended solids value.

Ammonia

SNI 06-6989.30-2005 served as the basis for this ammonia measurement. 25 mL of the test sample was pipetted into a 50 mL Erlenmeyer. 1 mL of the homogenized phenol solution and 1 mL of sodium nitroprusside were added. After adding 2.5 mL of the homogenized oxidizing solution, the Erlenmeyer was covered with paraffin or plastic film. It was left for an hour to allow the color to form. The Equation (5) was used to calculate ammonia.

$$Ammonia\ content\ (\text{mg N/L}) = C \times fp \quad (5)$$

where: *c* – level obtained from measurement (mg/L), *fp* – dilution factor.

RESULT AND DISCUSSION

Characterization of sludge

The Abattoir had the highest potassium content (0.206%), while the Canal had the lowest (0.073%). In contrast to the Canal (0.097%) and Tofu Industry (0.13%), the Abattoir's Organic N concentration (0.844%) was significantly greater. All three sources had relatively consistent organic C values, with the Abattoir having the lowest (1.47%) and the Canal having the highest (1.64%). The levels of phosphorus in the Tofu Industry (1.1%) were much higher than those in the Canal (0.084%) and Abattoir (0.114%), as indicated in Table 1.

Table 1 showed that every sludge had a different content. While the tofu industry contained notable levels of phosphorus, abattoirs had larger levels of potassium and organic nitrogen. Compared to the other two sources, canals often contained fewer nutrients. Given that it made up roughly 70% of all household garbage and contained nutrients like nitrogen (N), phosphorus (P), and potassium (K), as well as heavy logs and harmful germs and microbes, greywater frequently presented a significant problem (Shankhwar, 2016).

Activated sludge seeding

Growth of treatments was observed over several days (0, 3, 6, and 9). The levels of Treatment I were 3500 mg/L on day 0, increased to 5700 mg/L on day 3, rose to 5800 mg/L on day 6, and reached 12000 mg/L on day 9. The levels of Treatment II were 5200 mg/L on day 0, grew to 6100 mg/L on day 3, increased to 8400 mg/L on day 6, and reached 9000 mg/L on day 9. The levels

Table 1. The initial characteristics of activated sludge

Type	Potassium	N organic	C organic	Phosphor
Conduit	0.073%	0.097%	1.64%	0.084%
Slaughterhouse	0.206%	0.844%	1.47%	0.114%
Tofu industry	0.123%	0.13%	1.50%	1.1%

of Treatment III were 4900 mg/L on day 0, rose to 9000 mg/L on day 3, remained at 9000 mg/L on day 6, and increased to 12500 mg/L on day 9. The IV dose for Treatment was 13600 mg/L on day 0, grew to 31200 mg/L on day 3, rose to 35200 mg/L on day 6, and reached 67000 mg/L on day 9. It was evident from this data that the growth patterns of the various treatments differed. Treatment III showed steady growth from days 6 to 9, while Treatment IV displayed the fastest and most notable growth. As seen in Figure 1 dan 2, Treatments I and II grew well, with greater increases on some days.

The TSS value was the lowest for Treatment I's microbial biomass growth rate. This was because the sludge used as seeds had only a small amount of microbial species, as it came from the tofu industry's sewer. The sludge used as seed contained a limited variety of microbial species since it originated solely from tofu factory waste.

The environmental conditions of the biomass source had a significant impact on the development of biomass into activated sludge, and the variety of microorganisms in polluted settings was lower than in less polluted environments. It was observed from Figure 2 that the variety of microbial species and the TSS value increased with the number of sludge types in the sludge composition

This was because the microorganisms found in Treatment IV were a mix of different microorganisms that came from the three sediments in the contaminated water body under different conditions. High quantities of organic matter and, consequently, heterotrophic bacteria that used this organic matter for metabolism were frequently found in contaminated water. Moreover, microorganisms had a comprehensive supply of food (organic matter), which led to a rise in the growth of microbial biomass. Microbes require enough time to proliferate; if the necessary elements are available, they develop quickly. They also require nutrition to grow (Sudaryati et al., 2012).

The breakdown of organic materials in wastewater depends on bacteria. A large number of bacteria are needed for these substances to be broken down. Bacteria proliferate if there is enough food in the environment to sustain steady bacterial growth. The log phase occurs when bacteria grow steadily and slowly at first due to the new atmosphere in the wastewater; following the log phase, bacteria continue to proliferate if there is sufficient food to maintain steady bacterial growth (Sudaryati et al., 2021).

Acclimatization and biodegradation

Degree of acidity (pH)

According to the control's observations of the pH parameter, the pH level stayed steady at 8.18 during the three-day time points (0, 3, and 6). This demonstrated that the pH level remained constant in the absence of any treatment, suggesting that the control condition had no effect on the pH value. On day 0, Treatment I was 8.18, the same as the control, which dropped to 7.30. On day 0, Treatment II was 8.18, which dropped to 7.38. It was observed from Figure 3 that Treatment III was 8.18 and decreased to 7.26 on day 6.

The survival of aquatic organisms is significantly impacted by the level of acidity. Because of

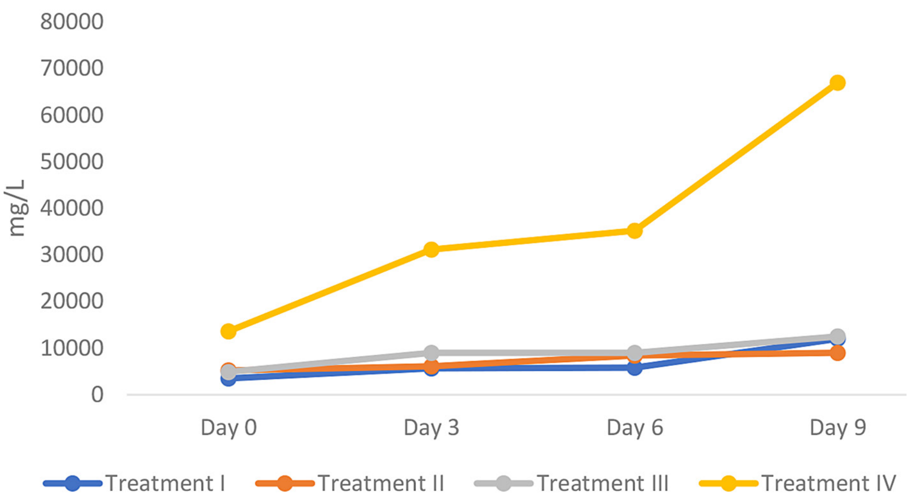

Figure 1. The growth of activated sludge seeding was observed

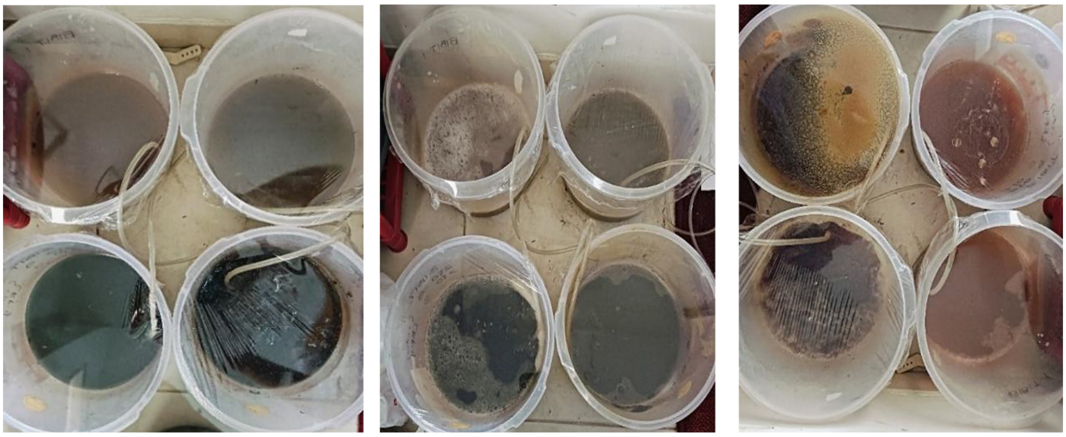

Figure 2. Activated sludge seeding had been performed at (a) day 0, (b) day 3, (c), day 6

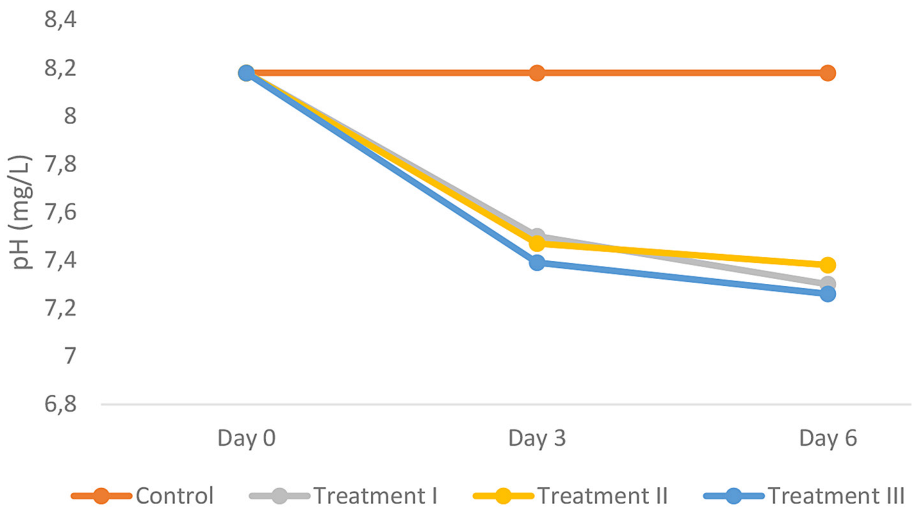

Figure 3. The results of pH observation

this, the pH of water is frequently used to determine its quality. A pH range of 6.0 to 8.0 is ideal for the growth of most bacteria (Sudaryati et al., 2021).

The development of bacteria in biological treatment is also influenced by temperature and pH. The ideal pH range for the growth of degrading bacteria is between 7.5 and 8.5 (Hibban, 2016). The pH of water is commonly used as an indicator of water quality. Since fungi (mold) thrive at low pH (acid) and microorganisms (bacteria) flourish at neutral and alkaline pH, the organic decomposition process becomes more active at these pH levels (Sudaryati, 2021).

Treatment III steadily and consistently reduces pH from 8.18 mg/L to 7.39 mg/L on day three and 7.26 mg/L on day six. The biological process of denitrification in microorganisms from tofu factory waste permits high microbial activity and photosynthesis, resulting in a pH shift from alkaline to neutral. Microorganisms break down organic materials by utilizing oxygen that is released into the environment. Although photosynthesis serves as the primary energy conversion pathway, certain bacteria are capable of decomposing complex organic materials.

Biological oxygen demand

According to the BOD control observation data on days 0, 3, and 6, the value stayed at 153.50 mg/L. In Treatment I, 153.50 mg/L was reduced to 23.38 mg/L. The concentration in Treatment II dropped from 153.50 mg/L to 22.56 mg/L. The concentration in Treatment III decreased from 153.50 mg/L to 18.87 mg/L. It was observed from Figure 4 that Treatment III had experienced the largest drop from day 0 to day 6.

The biological oxygen demand, often referred to as BOD, was the quantity of dissolved oxygen needed by microorganisms to decompose organic matter in aerobic conditions. This comprised practically all dissolved organic compounds as well as certain organic molecules floating in water. The most widely used metric for assessing surface water and wastewater was the BOD measure (Ramadani et al., 2021). An increase in the number of organisms and the pace of decomposition resulted in a decrease in dissolved oxygen. Aquatic biota life was seriously endangered by water with a high BOD value that could not raise dissolved oxygen levels (Fadzry et al., 2020). Between Treatment I and Treatment II, the levels decreased from the control of 153.50 mg/L to 23.38 mg/L, 22.56 mg/L, and 18.87 mg/L, respectively. The aeration process and the growth of microbes were responsible for this change. The BOD value decreased more effectively when more oxygen was present in the water (Rahmawan et al., 2023).

Chemical oxygen demand

According to the COD observation data, the control's COD content stayed steady at 378.27 mg/L between time 0 and time 6. In Treatment I, the COD level decreased from 378.27 mg/L to 58.77 mg/L. The second treatment fell from 378.27 mg/L to 56.67 mg/L. The third treatment's COD concentration dropped from 378.27 mg/L to 48.00 mg/L. It was observed from Figure 5 that Treatment III had undergone the most significant reduction in COD from time 0 to time 6.

According to Figure 4, Treatment III was the most effective since it exhibited the largest drop in COD value from day 0 to day 6, reaching its lowest value of 48.00 mg/L on day 6. Oxygen Chemical Demand, also known as chemical oxygen demand, is the amount of energy required for waste compounds in water to undergo oxidation through chemical reactions. COD originates from various industries, including the manufacture of paper, leather tanning, sugar, meat cutting, fish canning, shrimp freezing, bread, milk, cheese, and butter (Santoso, 2018). COD decreases from 378.27 mg/L in the control to 58.77 mg/L in Treatment I, 56.67 mg/L in Treatment II, and 48.00 mg/L in Treatment III. The activity of microorganisms in breaking down organic substances for their own metabolism causes this decline (Sudaryati, 2012). The amount of organic materials that can break down biologically is indicated by the BOD to COD ratio (Soedjono, 2018). As the BOD to COD ratio decreases, the quantity of organic materials that can be broken down by biological processes in the wastewater also decreases (Pranoto, 2019).

Total suspended solids

According to the TSS observation data, the control stayed at 56 mg/L between time 0 and time 6. In Treatment I, 56 mg/L was reduced to 2.67 mg/L. The second treatment's concentration dropped from 56 mg/L to 6.67 mg/L. The third treatment reduced the levels from 56 mg/L to 1.33 mg/L. TSS decreased the most from time 0 to time 6 in Treatment III. It was observed from Figure 6 that Treatment III had exhibited the greatest decrease in TSS value from day 0 to

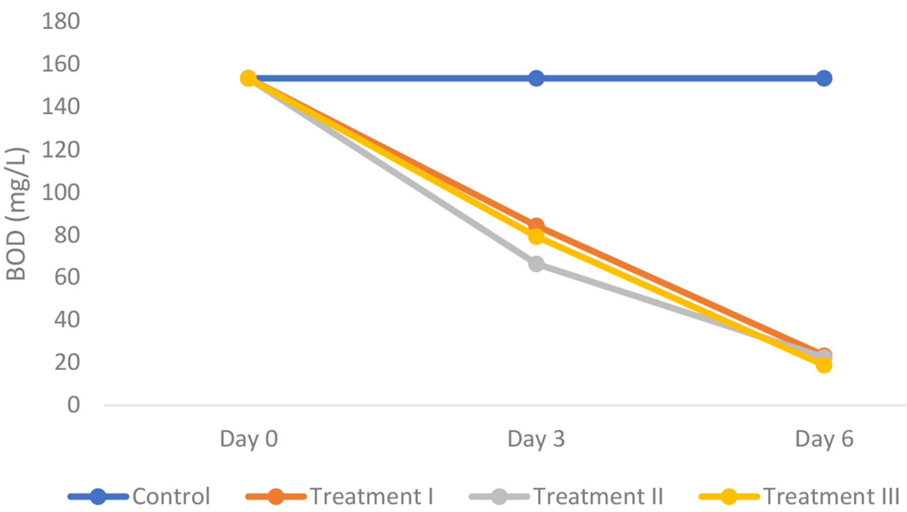

Figure 4. The results of BOD observation

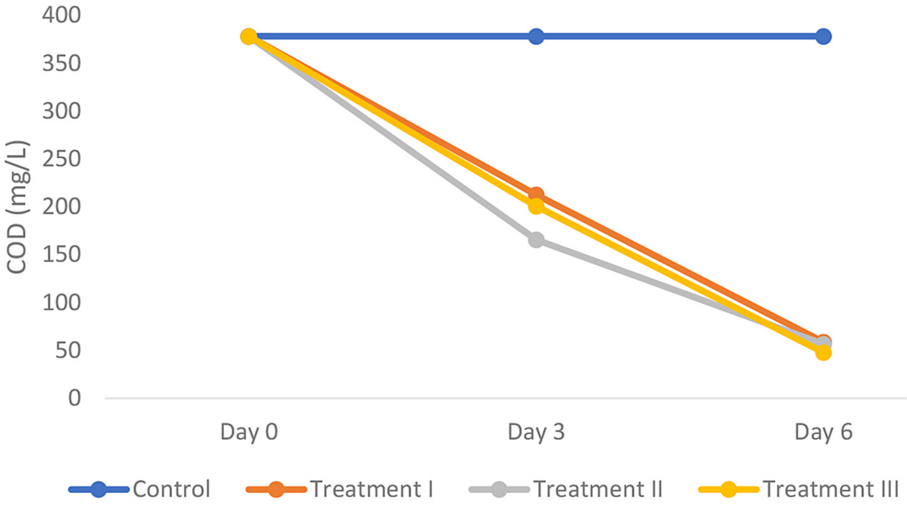

Figure 5. The results of COD observation

day 6, reaching its lowest level of 1.33 mg/L on day 6. Consequently, it was considered the most effective treatment.

Fine particles in water that can be detected by weight after being filtered through 0.042 mm filter paper are known as total suspended solids, or TSS. These particles include mud, organic waste, microorganisms, industrial waste, and home trash (Faradila et al., 2023). The activated sludge technique results in a decrease in TSS in this study. By the sixth day, the concentration of 56 mg/L had decreased to 2.67 mg/L in Treatment I, had dropped to 6.67 mg/L in Treatment II, and had reduced to 1.33 mg/L in Treatment III. This is related to the microorganisms in activated sludge that aid in the breakdown process, making it easier for suspended particles to precipitate and lowering the TSS level in greywater (Khuriyah et al., 2023).

Ammonia

According to the observation data, the control's ammonia (NH_3) concentration was 10.36mg/L from time 0 to time 6. In Treatment I, 10.36 mg/L was reduced to 5.95 mg/L. In Treatment II, 10.36 mg/L was lowered to 4.78 mg/L. In Treatment III, 10.36 mg/L decreased to 3.45 mg/L. From time 0 to time 6, Treatment III's ammonia levels dropped the most. It was observed from Figure 6 that Treatment III had exhibited the most substantial decrease in ammonia concentration from day 0 to day 6, reaching its lowest recorded value of 3.45 mg/L on

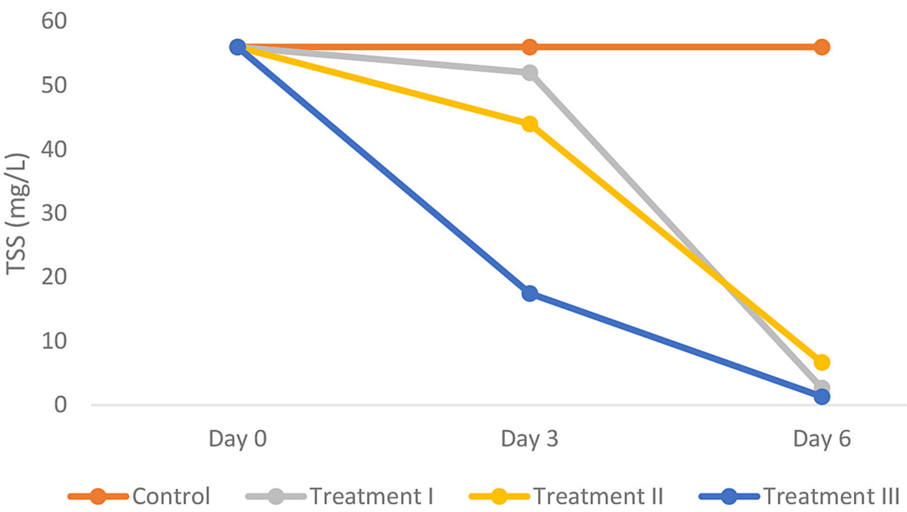

Figure 6. The results of TSS observation

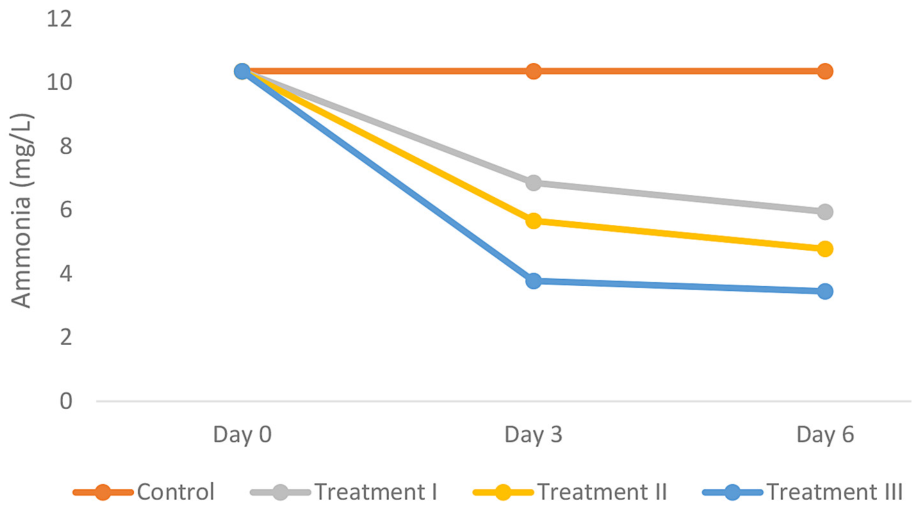

Figure 7. The results of TSS observation

day 6. Consequently, it was regarded as the most effective treatment.

The primary sources of ammonia in surface water had been urine, feces, and the microbial breakdown of organic matter in natural water or wastewater from homes or businesses. Temperature, oxygen content, ammonia's source, and aquatic plants that had used it as a fertilizer had impacted its levels (Guanabara et al., 2014). In Treatment I, the control level of 10.36 mg/L decreased to 5.95 mg/L; in Treatment II, it dropped to 4.78 mg/L; and in Treatment III, it fell to 2.45mg/L. Microorganisms or bacteria that can break down ammonia compounds in wastewater are used in the study to break down the ammonia. Nitrosomonas and Nitrobacter bacteria are responsible for the nitrification process, which is the breakdown of ammonia (Nourmohammadi et al., 2013; Smyk & Ignatowicz, 2017). Despite having the same morphology round form, smooth edges and texture, and convex elevation Nitrosomonas and Nitrobacter bacteria differ in color. According to Kidding et al. (2015), Nitrobacter is yellow and Nitrosomonas is white. Because they are unable to produce the organic materials they require on their own, heterotrophic bacteria must obtain their nourishment from organic materials found in their surroundings (Notowinarto & Agustina, 2015).

CONCLUSIONS

The degradation of organic matter in wastewater depends greatly on bacterial activity. A sufficient nutrient supply accelerates bacterial growth, especially during the logarithmic phase. Contaminated water, rich in organic matter, provides an ideal environment for heterotrophic bacteria, allowing rapid microbial biomass development. This analysis highlights the importance of microbial diversity and nutrient availability in enhancing wastewater treatment efficiency. In this first phase, Treatment IV, with its rich microbial composition, demonstrates the highest effectiveness in promoting organic matter decomposition. Overall, Treatment III in the second phase effectively reduces pH, BOD, COD, TSS, and ammonia levels, demonstrating its potential for efficient wastewater treatment.

Acknowledgements

The authors appreciate the efforts the parents, supervisors, and the authors included in this reference for their assistance with my research.

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