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Domestic wastewater treatment using modified tripikon-S with pumice filters as support media

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ABSTRACT

Research on tripikon-S has been conducted to reduce organics and nutrients in domestic wastewater. This study aims to analyze the performance of modified tripikon-S with the addition of pumice as a support media in domestic wastewater treatment. The tripikon-S used is a modification of three pipes of different sizes arranged concentrically. In the middle pipe, aerobic processes occur, the central section serves as a flow path, and the lower section is where anaerobic processes take place. Two tripikon-S units were tested between reactors with and without pumice stone, operated in batch mode with a retention time (td) of 2 days. The results showed that pumice has the ability to attach microorganisms. This can be analyzed based on the results of the SEM test which shows changes in the biofilm surface until the seeding ends. In addition, the attached VSS formed was 2.817 mg/L. This value indicates that biofilm formed on pumice has entered the maturation phase and continued by running using artificial wastewater with concentration variations. Variations in treatment with COD concentrations of 500 mg/L, 750 mg/L, and 1000 mg/L resulted in COD removal in the reactor with pumice stone > 85%, TN > 48%, and TP > 67%. This shows that the modification of tripikon-S with pumice supporting media is able to reduce organics and nutrients in wastewater. This result is reinforced by the identification of microorganisms using Oxford Nanopore Technology, where in this study identified microorganisms that are able to degrade organic and nutrients in wastewater. So, this system should be considered as an alternative to domestic wastewater treatment, especially in suburban areas.

Keywords: biofilm, domestic wastewater treatment, pumice, support media, tripikon-S.

INTRODUCTION

Rivers in residential areas are often the direct recipients of domestic wastewater from residential activities that have the potential to pollute rivers and disrupt aquatic ecosystems (Fitriana et al., 2021; Hikmat and Juwana, 2019). The toilet system used by residents, which involves direct disposal into the river, significantly contributes to water pollution due to problems such as decreased water quality, unpleasant odors, and unattractive appearance (Putri et al., 2016). This problem is exacerbated by population density along the riverbanks and the lack of adequate wastewater treatment infrastructure. River water plays a significant role in life. Treating wastewater from riverside settlements requires an extraordinary approach that considers the condition of local settlement buildings (Anh et al., 2023; Ryanti et al., 2018). Domestic wastewater contains various pollutants, including organic matter, suspended solids, nitrogen, phosphorus, and pathogenic microorganisms. The design of a wastewater treatment system must consider fluctuations in discharge and the characteristics of the wastewater (Firmansyah and Razif, 2016).

Effective domestic wastewater treatment for households located along sustainable river banks is needed. Treatment is available for households above the river, such as biofilter tanks (Machineni, 2019). However, the house's location with tidal conditions can seep into the biofilter reinforcement/pavement system made of concrete and variations with floating gardens (Borges Pedro et al., 2020) but are vulnerable to being carried away by tidal current (Benvenuti et al., 2018). Another floating garden application was found in Cambodia (Chakraborty et al., 2012), where the floating media used was not durable. Vulnerability to tidal water and seepage is a concern when determining the technology. Domestic wastewater treatment with tripikon-S has developed as a viable solution for sustainable wastewater management, particularly for coastal communities and stilt dwellings. Tripikon-S, a black water home waste treatment system, has been implemented in various Indonesian cities, including Yogyakarta, Lampung, Pontianak, and South Kalimantan. This system's septic pipe design is concentric, which improves decomposition and waste treatment efficiency. In the medium pipe, an aerobic process takes place; the middle part is the trajectory, and the lower part is where the anaerobic process occurs(Normasari et al., 2017). The small pipe functions to insert waste where the inlet part is connected to the goose-necked toilet, the medium pipe functions as a place for the process to take place, while the large pipe functions as a place for further aeration and precipitation where there is a mechanism for the separation of suspended material and the decomposition of organic substances by microorganisms (Marlisa et al., 2015; Noor, 2011; Rachman, 2018). The biological processing in the center pipe is anaerobic, similar to what occurs in conventional septic tanks, while the separation of suspended material occurs in a turbulent-free environment. Therefore, in the design, the dimensions of the central and outer pipes are important points to enable the physical settling mechanism with a residence time of two to four days, as is the case in septic tanks (Maheng et al., 2016).

Specifically, the tripikon-S installation facilitates the anaerobic degradation of organic matter, providing a viable alternative to traditional septic tanks in areas with specific geographical and environmental conditions, such as riverbanks and swamps (Adicita et al., 2020; Rachman, 2018). The advantage of this technique is that it is intended to withstand environmental issues associated with high groundwater levels, which are common in coastal locations with stilt buildings. It works vertically, so it doesn't need a lot of space. There is no need for a soil infiltration system, and the materials used are easy to find and apply, resulting in low investment, operational, and maintenance costs (Vindriani et al., 2022; Noor and Soewondo, 2018). Research on modifying the tripikon-S has been carried out extensively to improve waste disposal efficiency. Marlisa et al. (2015) in their research added a biological treatment mechanism using bioballs as a buffer medium with an HRT of 48 hours, resulting in a COD removal efficiency of 66% for a COD load of 1.500 mg/L and 64% for a COD load of 2.000 mg/L. Another modification with a venturi-shaped chamber resulted in a 67% COD removal for a COD load of 1.500 mg/L and 65% for a COD load of 2.000 mg/L at an HRT of 48 hours (Putri et al., 2016). Recent research with bioballs and fiber filters by Karmilia et al. (2024) shows better results with a COD removal efficiency of 67.76%. Meanwhile, an elimination efficiency of 87.5% was obtained for the modified reactor for the TSS parameter. In research with the addition of sand filter media, the highest BOD_5 removal efficiency of 57% was achieved (Maheng et al., 2016). Another modification is related to the use of trichlorfon to treat industrial tofu waste, which results in a COD removal efficiency of 70.41% within 30 days, a BOD removal of 74.83%, and a pH of 31.25% (Sardi et al., 2021).

Previous studies have utilized various media to support treatment with tripikon-S; however, pumice stone has not been used as an additional filter medium and surface for bacterial growth to enhance removal with tripikon-S. Adding a pumice filter as an extra filter media and a surface for bacterial growth to adhere to is one way to improve tripikon-S's efficacy. This study will employ pumice stone, which is relatively easy to find and prepare (Samat et al., 2021). Which has been widely used as a filtration medium to enhance performance and reduce the harmful effects of organic and chemical pollutants (An et al., 2023; Md Anawar and Chowdhury, 2020). The novelty of this study is expected to improve quality through physical and biological filtration mechanisms (Ratnawati and Ulfah, 2020) by placing pumice stones in large Tripikon-S pipes. The high porosity of pumice allows it to retain suspended particles, and the more excellent aeration creates an optimal environment for its role in the biodegradation of organic pollutants (de Rozari et al., 2021). Thus, pumice can be utilized in wastewater treatment as a filtration medium in biofilm reactors to treat various liquid wastes (Khelladi et al., 2022) because biofilms can effectively degrade organic pollutants and facilitate nutrient removal (Narendra et al., 2017). Integrating natural materials and simplified treatment processes presents a promising treatment alternative for achieving sustainable wastewater management (Samat et al., 2021).

A workable, economical, and effective wastewater treatment solution is required, and one way to achieve this is by modifying current technology. Tripikon is a treatment design modification that uses a vertically built septic tank with three pipes used concentrically. Because of their varying sizes, the three pipes that are utilized are referred to as small, medium, and large pipes. This study aims to analyze the effect of tripikon-S modification with the addition of pumice as a growth medium for microorganisms on the reduction of organic matter and nutrients. This study also observes the surface morphology of the biofilm using SEM analysis and identifies the microorganisms that play a role in wastewater treatment using Oxford Nanopre Technology.

MATERIALS AND METHODS

Laboratory design

In this study, we innovatively designed the tripikon-S using PVC pipes. The tripikon-S features a small 2-inch diameter pipe that also functions as an inlet, a medium 4-inch diameter pipe placed in the middle section, and a large pipe as the outermost section with a diameter of 8 inches and a height of one meter. Another innovative modification was made by adding a 2-inch pipe at 85 cm from the bottom, with a horizontal length of 50 cm, which was then connected to a 1.3-meter vertical pipe, the lower end of which was used as an outlet and provided with fiber as a final filter. Two reactors were prepared to compare the conditions with and without modification, namely reactors with and without additional modification of a pumice filter and fiber. The pumice stones were placed in a supported space on the large tripikon-S pipe at 55 cm from the bottom of the large pipe, with a height of 20 cm, so that approximately 45 pumice stones with a volume size of 5–10 cm^3 could be filled. The pumice stones had been washed and dried in the hot sun. The design described above can be seen in Figure 1. Tripikon-S without pumice and fiber is referred to as reactor 1 and the reactor with the modified pumice and fiber filter is referred to as reactor 2.

Operation setting in the laboratory

The research was conducted under batch and anaerobic conditions to determine the optimal conditions for domestic wastewater treatment. Artificial blackwater was used in this study was

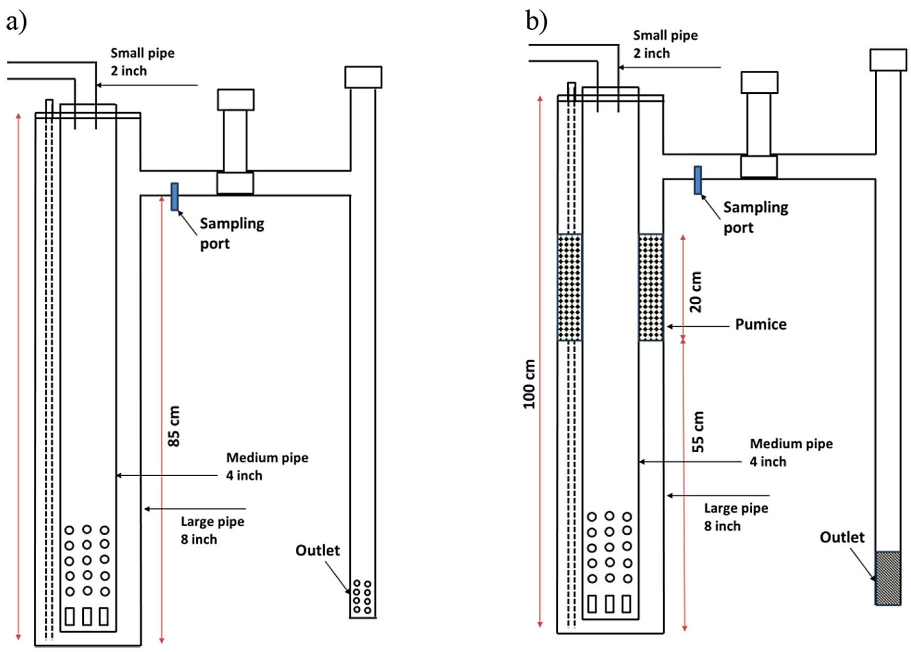

Figure 1. Design of tripikon-S (a) reactor 1 without modification and (b) reactor 2 modified pumice and fiber filter

produced based on target COD values and C: N:P ratios to be achieved. When the target COD value is 500 mg/L COD and the C:N:P ratio is 250:5:1, then 16.5 g of glucose ($C_6H_{12}O_6$), 150 mg ammonium chloride (NH_4Cl), and 87.62 mg potassium dihydrogen phosphate (KH_2PO_4) mixed with kaolin ($Al_2Si_2O_5(OH)_4$) as the total solid component, totaling 101 mg, to achieve a concentration of 185 mg/L TSS. Distilled water was used for dilution, specifically bottled drinking water, Amidis. The influent was adjusted to have a C:N:P ratio of 250:5:1 under anaerobic conditions with three variations of COD load, namely 500 mg/L, 750 mg/L, and 1000 mg/L.

The Communal Wastewater Treatment Plant provided the bacterial inoculum for this investigation. Six liters of WWTP sludge were added to the reactor at the start of the operation (using a computation of 20% of the total reactor volume). The reactor uses the pumice stones as a growing medium for biofilms. A total of 45 pumice stones were washed with a diameter, dried in the hot sun, and placed in a 20 cm high buffer chamber. 10 cm thick filter of fiber and gravel is placed at the end of the outlet pipe. A 50-liter bucket is added, and a submersible pump is positioned at the bottom of the bucket to guarantee anaerobic conditions for batch operation. Water is drained from the effluent collection bucket to the inflow pipe via a ¾-inch hose that is attached to the pump. The operational setup design and reactor conditions during operation can be seen in Figure 2. In this plan, nitrogen gas is pushed into the reactor for ± 10 minutes to create anaerobic conditions, and all reactor holes are sealed to keep outside air out of the system.

Sample analysis and testing

The parameters tested were COD as a parameter describing substrate degradation, total nitrogen (TN), total phosphate (TP), pH, temperature, and DO to assess bacterial environmental conditions. COD was tested using the SMWW 5220D-Closed Reflux Colorimetric Method, pH using a pH meter, temperature using SMWW 2550 Temperature B, and DO using a DO meter. TN was analyzed using the method described in "Methods and Quality Assurance for CBP Water Quality Monitoring Programs" and total phosphate was analyzed using EPA-NERL:365.1. The screening test results for organic load parameters COD, TN, and TP in the batch phase were conducted to test the ability of tripikon-S to treat the generated waste. Initially, tests were performed on input concentrations, where the COD input was the same as the COD load variations, namely 500 mg/L, 750 mg/L, and 1000 mg/L. Meanwhile, the initial concentrations were recalculated for the TN and TP parameters to ensure the C: N: P ratio. The measured input concentrations were as follows: TN: 120 mg/L, 250 mg/L, and 550 mg/L; TP: 0.5 mg/L, 1 mg/L, and 1.5 mg/L.

SEM analysis and microorganism identification

The surface morphology of the biofilm was examined using a Hitachi SU3500 scanning electron microscope (SEM) with a magnification of 65 to 1000 times, according to the conditions of the surface morphological information that can be

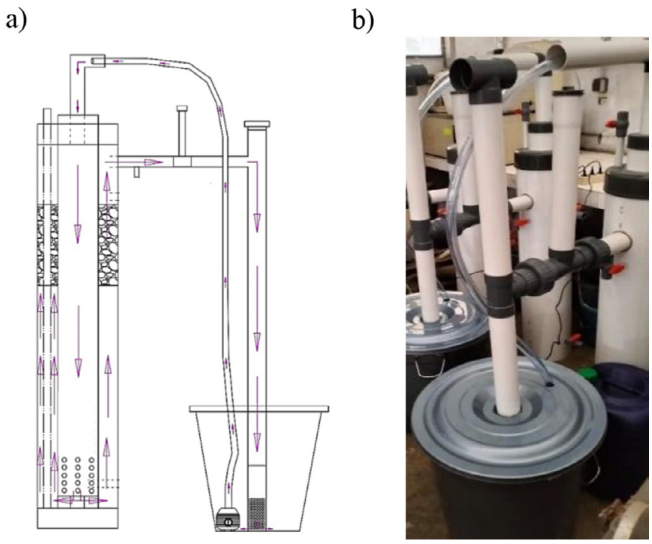

Figure 2. (a) Design of operational settings and (b) existing conditions of the reactor when operated

seen on the pumice surface. The sample was made by removing one pumice stone from a modified Tripikon-S reactor. The pumice stone's moisture content was then lowered by placing it in a heating oven and heating it for 24 hours at 70 degrees Celsius. The dried sample is sealed and transported directly to the test site. Oxford Nanopore Technologies (ONT) uses the WF-16S approach to identify the bacterial community. WF-16S examines bacterial populations by sequencing their 16S rRNA gene. To prepare samples for bacterial community identification, a pumice stone from a modified tripikon-S reactor is used. The biofilm layer attached to the pumice stone is then removed using a spatula and 10 mL of distilled water, placed in a sample tube and taken directly to the test site.

RESULTS AND DISCUSSIONS

Reactor condition evaluation

The condition of the reactor when the batch process is carried out is tested by looking at temperature changes, DO and pH conditions measured in the first 8 hours on the first day for the three COD input variations (Figure 3.), where the conditions show that reactor temperature 2 is in the range of 23.3 °C to 26.1 °C, while the temperature measurement results for reactor 1 range from 23.3 °C to 26.5 °C. There is an increase in temperature, which indicates the activity of microorganisms, but it is still within a range that is not very different, which means that the temperature conditions are mesophilic (Isa et al., 2020). So, in the tripikon-S system, the type of bacteria that is often found in mesophilic bacteria. So, it can be concluded that stable environmental conditions support the degradation process. The optimum temperature for bacterial activity is within the range of 25–35 °C.

The measurement of the DO parameter shown in Figure 4 shows that the DO value for the modified reactor is in the range of 1.8 mg/L to 2.7 mg/L, while for the DO measurement results in the control reactor, it ranges from 1.7 mg/L to 2.7 mg/L. The DO data in reactor 2 and reactor 1 show that the reactors are transitioning from anoxic to aerobic conditions. The DO value in the modified reactor tends to be higher than in the control reactor, which could indicate a more favorable environment for aerobic microorganisms. The rise and fall of the measured DO value

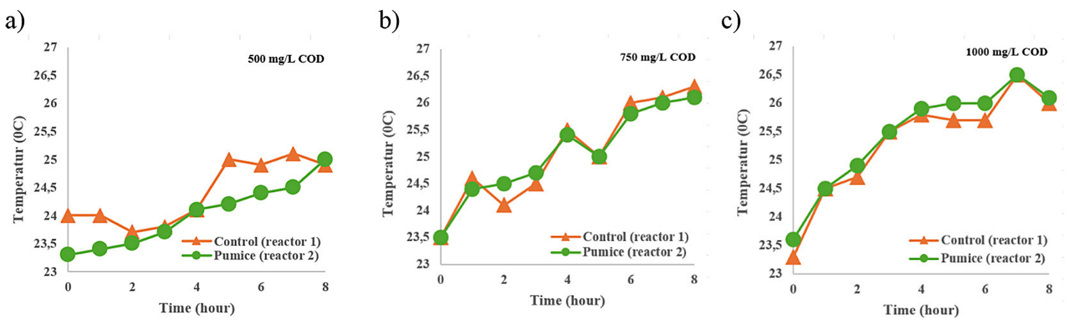

Figure 3. Comparison of changes in temperature in the tripikon-S reactor 1 (control) and reactor 2 (pumice) in the first 8 hours of treatment at COD doses (a) 500 mg/L; (b) 750 mg/l and (c) 1000 mg/l

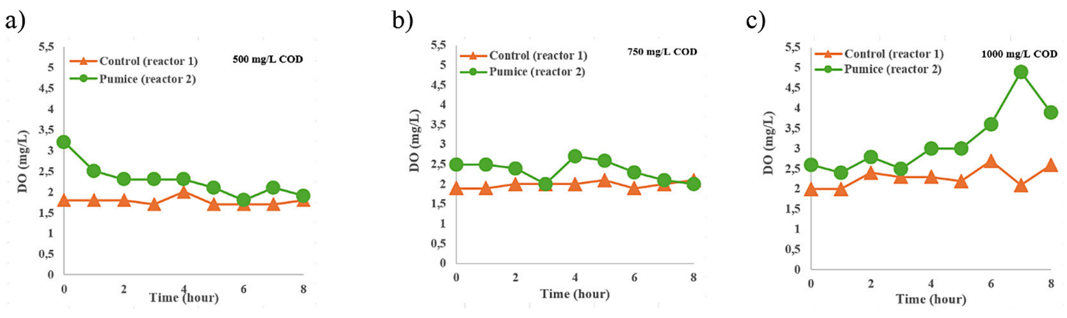

Figure 4. Comparison of changes in DO in tripikon-S reactor 1 (control) and reactor 2 (pumice) in the first 8 hours of treatment at COD doses (a) 500 mg/L; (b) 750 mg/l and (c) 1000 mg/l

indicates the activity of microorganisms. Microorganism activity increases when the DO value decreases. The high activity of microorganisms causes a decrease in oxygen conditions (Prambudy et al., 2019). Increased DO concentration affects biofilm growth and bacterial density in the biofilm layer. In the Figure 4., it is shown that anaerobic conditions had not yet occurred at the beginning of data collection in the reactor. This reactor's conditions are unsuitable for anaerobic processes and can cause inhibition of anaerobic processes. Because the anaerobic process cannot run properly, the rate of liquid waste treatment decreases. Waste may not decompose optimally, and the parameters of outgoing water quality, such as BOD, COD, or TSS, can be higher than desired (Bajpai, 2017).

Reactor 1's pH measurement results vary from 7.13 to 4.38, while reactor 2's pH falls within the initial neutral range of 6.13 to 3.71, according to Figure 5's results for the three experimental variants. Although all of them tend to fall closer to pH 4, the tripikon-S settings in both reactors demonstrate that they function by adhering to the acidity conditions of bacterial growth. The first drop in pH during the batch process is a sign of the fast bacterial growth that aids in the hydrolysis of organic molecules into volatile fatty acids and the complex acidogenesis of complex monomers. This condition is caused by the acidogenesis stage by acid-forming bacteria that convert organic materials in leachate into volatile acids. Another possibility is that the pH decreases due to the formation of acids that come from the decomposition of materials, such as carbohydrates and fats that become acetic acid and other acids, such as propionate, butyrate, and valerate that are formed in the hydrolysis-acidogenesis process (Hartati et al., 2011; Kahar et al., 2017). The effect of a decrease in pH in anaerobic reactors includes inhibition of aerobic microorganisms where, if the pH drops to become too acidic, microbial activity can be inhibited. A decrease in pH is often caused by the accumulation of organic acids in the reactor. This can lead to an imbalance in the process, where microbes cannot break down these acids as quickly as they are formed. When the pH drops and the temperature rises, the rate of waste treatment can decrease significantly. The decomposition of organic matter may not occur as efficiently as usual, increasing the concentration of polluting parameters such as BOD, COD, and TSS in the effluent.

Evaluation of the effect of modification with contaminant removal

The results of the parameter removal tests for organic load COD, TN, and TP in the batch phase were conducted to assess tripikon-S's ability to treat the generated wastewater. For the COD parameter (Figure 6), with input concentration ratios of 500 mg/L:750 mg/L; 1000 mg/L, the average removal rates in the first tripikon-S reactor (control) were 81.9%:90.1%:84.4%, and the average removal rates in the second reactor were 98.5%: 93.9%:85.9%. These results suggest a promising potential for tripikon-S to improve the removal efficiency at higher COD concentrations. Pores in expanded clay with a large surface area can enhance the growth and development of bacteria that aid in COD removal (Shehab et al., 2021). To determine whether Reactor 2 provides more effective removal compared to the control reactor, a two-tailed paired t-test was conducted. The results showed that the COD removal had a P-value of 0.04621 (smaller than α 0.05), indicating a significant difference.

For the TN parameter (Figure 6b), the input concentration ratio is 120 mg/L TN:250

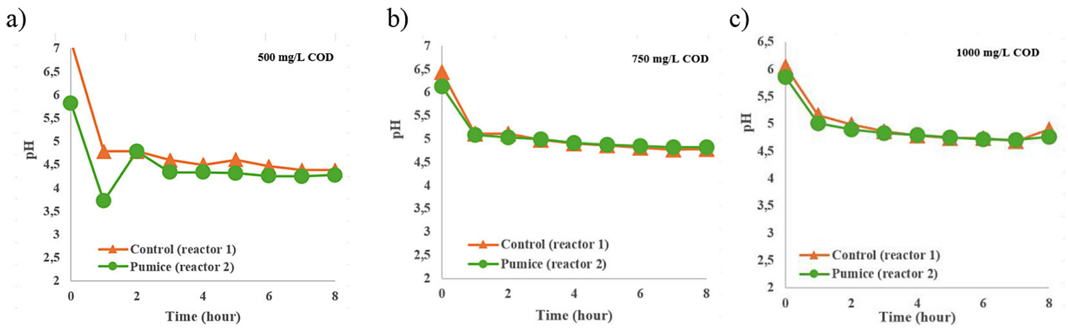

Figure 5. Comparison of changes in pH in the tripikon-S reactor 1 (control) and reactor 2 (pumice) in the first 8 hours of treatment at COD doses (a) 500 mg/L; (b) 750 mg/l and (c) 1000 mg/l

mg/L TN;550 mg/L TN. In reactor 1, the average removal efficiency with tripikon-S is 46.1%: 23.9%:17.3%, whereas in reactor 2 (pumice), the average removal efficiency was higher, with a percentage ratio of 50.2%:48.4%:75.4%. These data clearly show an increase in removal efficiency for the TN parameter in reactor 2, demonstrating that the upgraded tripikon-S reactor, which includes expanded clay, can remove nitrogen loads more successfully. It is vital to remember that DO levels play an important role in the nitrogen elimination process. Nitrification occurs under oxygen-deficient conditions, and the presence of expanded clay, with its physical properties and ability to support microbial activity, also functions as a medium for microbial colonization, supporting the biological degradation of nitrogen compounds (Indah et al., 2022). The porous structure of expanded clay allows for increased water retention, which in turn promotes the growth of denitrifying bacteria, which is crucial in reducing total nitrogen levels (Çifçi and Meriç, 2016).

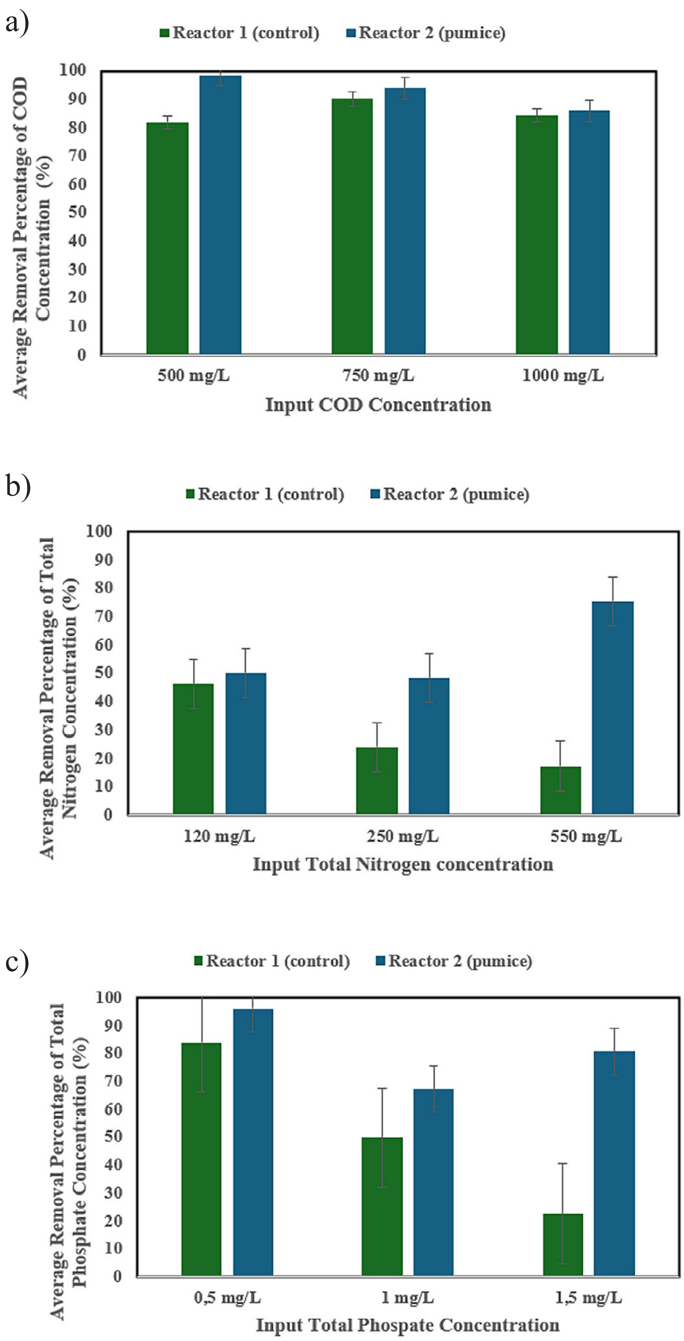

Figure 6. Comparison of average concentration of removal of (a) COD, (b) total nitrogen and (c) total phosphate in tripikon-S between reactor 1 (control) and reactor 2 (pumice)

For the total phosphate parameter (Figure 6c), the input concentration ratio is 0.5 mg/L TP: 1 mg/L TP; 1.5 mg/L TP, the average removal efficiency with Tripikon-S in reactor 1 is 83.9%:49.9%: 22.6%, while in reactor 2 (pumice), the average removal efficiency was better, with a percentage ratio of 96.2%:67.3%:80.7%. This study demonstrates that pumice can remove phosphorus from wastewater, with pumice significantly absorbing phosphate (Fetene and Addis, 2020). This efficiency is a crucial part of managing nutrient pollution in domestic wastewater discharge. When related to COD removal, pumice has proven effective in reducing its content in treated wastewater, indicating its ability to facilitate biological processes leading to nitrogen and phosphorus removal (Janyasupab and Jampeetong, 2022). This microbial activity also implies the role of pumice by enhancing the overall performance of the treatment system due to the availability of suitable niches for biofilm formation. Pumice's ability to handle multiple pollutants simultaneously makes it a highly advantageous component in integrated treatment systems designed for wastewater treatment. In another study, it was noted that extended hydraulic retention time could further enhance contaminant removal efficiency (Janyasupab and Jampeetong, 2022).

When compared to other research, the performance of this system has the advantage of being able to reserve more COD. The use of PET used plastic bottle media in an FBR system can minimize COD by approximately 85% (Fauzi, Soewondo, Nur, et al., 2023). This value is lower than in this study, which reached 98%. This PET has a consistent biofilm development rate (Nur et al., 2021). However, the TN system has a smaller allowance as compared to PET media. This study is also not significantly different from others that use supportive medium in wastewater treatment. The type of media utilized influences biofilm formation on supportive media (Fauzi et al., 2025; Setiyawan et al., 2023). As a result of the changes in the media utilized, the organic and nutrient removal efficiency varies (Fauzi et al., 2023). The efficiency of this allowance depends on the properties of wastewater, such as the C/N and C:N:P ratios (Soewondo et al., 2025; Soewondo et al., 2025).

Identification of microorganisms and biofilm morphology

Testing the growth of attached microbes on pumice media is rather difficult because the pumice is located inside the reactor and requires draining to obtain pumice samples. On the other hand, draining the reactor cannot be done in a short period because the draining process can affect the DO concentration and substrate concentration in the reactor. Therefore, the calculation of the attached medium concentration was performed after three repetitions of the running process for each COD load variation. Microbes began to experience an increase in concentration, with a significant increase occurring up to day 95, during which adhering microorganisms grew, forming colonies that caused the biofilm layer to thicken. Following this is a continuous decrease in bacterial VSS, as shown in Figure 7. During the initial phase, adaptation and growth occur, as indicated by a significant increase in VSS concentration from 0 to approximately 1.100 mg/L. This indicates the initial growth of microorganisms and the formation of primary biofilm on the surface of the medium. Under these conditions, the substrate continues to diffuse into the biofilm,

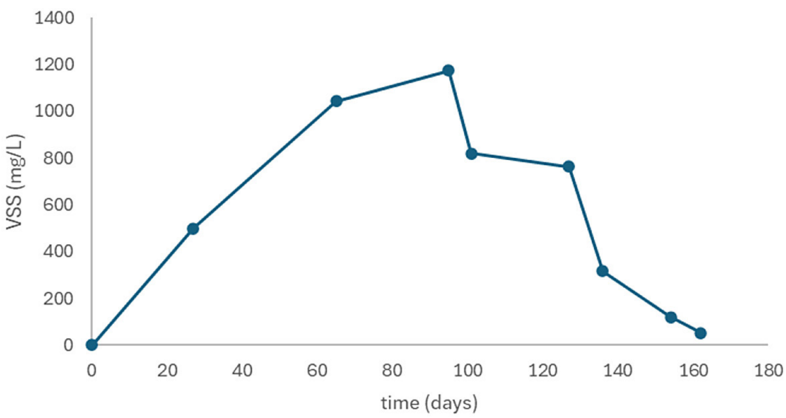

Figure 7. Bacterial growth (mg/L VSS) on pumice

allowing microorganisms to continue growing until a steady state is reached where the substrate can no longer penetrate the inner layers (Kilic, 2025). Subsequently, when VSS reaches a peak value of approximately 1.200 mg/L, it indicates that the biofilm is mature and actively degrading organic compounds. The reactor under these conditions demonstrates stability and optimal performance in treating domestic wastewater. Following this, activity and cell lysis decrease, leading to a sharp drop in VSS from 1.200 mg/L to approximately 100 mg/L. The decreasing VSS value after day 95 indicates that the maximum thickness penetrable by the substrate has been reached. This is also caused by microorganisms undergoing release and forming new colonies or dissolving in the liquid and dying. This release occurs because the biofilm layer begins to erode when the incoming liquid exceeds the biofilm's capacity. Under these conditions, the system no longer operates efficiently (Bas et al., 2017; Fauzi et al., 2025).

Additionally, using SEM, the development of biofilms on pumice will be observed, highlighting the advantages of pumice in optimizing the interaction of microbial communities that are important for domestic wastewater treatment applications. Visualization using SEM of four biofilm samples taken at four different time points is shown in Figure 8. On day 0, no bacteria were observed adhering to the pumice. On day 108, significant adhesion began to form on the pumice. By day 151, the attachment decreases further, and by day 162, the attachment decreases again. This indicates that the pumice has reached the lag phase, where attached bacteria die and are carried away with the effluent flow. The biofilm developing on the pumice stones can exhibit distinct structural characteristics that are crucial for its ecological role. As seen in Figure 8, strong growth patterns were observed using SEM, highlighting the importance of substrate characteristics, such as those exhibited by the pumice stones, in shaping biofilm architecture. Exploration of oxygen penetration and structural growth of biofilms supported by particulate materials demonstrates that the physical properties of pumice can influence biofilm maturation and the rate of nutrient and oxygen diffusion through the biofilm matrix,

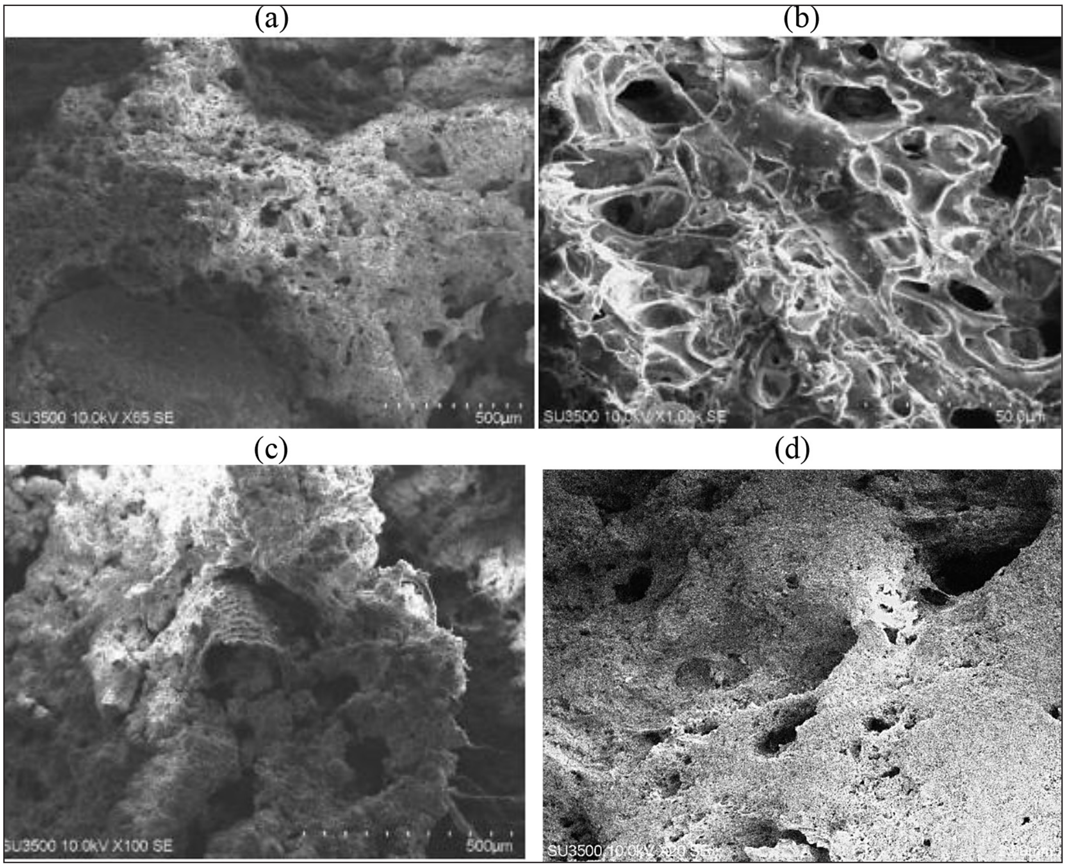

Figure 8. Biofilm surface on pumice observed using SEM microscope on (a) day 0; (b) day 108; (c) day 151; (d) day 162

Table 1. Microbial communities obtained from biofilm samples on pumice tripikon-S

Phylum	Class	Order	Family	Genus	Species	Total
Proteobacteria	Gammaproteobacteria	Pseudomonodales	Pseudomonaceae	Pseudomonas	*Pseudomonas jinjuensis*	40
					Pseudomonas khazarica	26
		Aeromonadales	Aeromonadaceae	Tolumonas	*Tolumonas auensis*	41
		Pasteurellales	Pasteurellaceae	Actinobacillus-rossii	-	7
	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Niveibacterium	*Niveibacterium umoris*	10
		Burkholderiales	Alcaligenaceae	Derxia	*Derxia gummosa*	7
	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	-	-	23
		Hyphomicrobiales	-	-	-	8
	Deltaproteobacteria	Myxococcales	Labilitrichaceae	Labilithrix	*Labilithrix luetola*	10
Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium	*Clostridium manihotivorum*	7
	Negativicutes	Selenomonadales	Sporomusaceae	Anaeroarcus	*Anaeroarcus burkinensis*	25
	Bacili	Lactobacillales	Aerococcaeae	Eremococcus	*Eremococcus coleocola*	3
Acidobacteria	Acidobacteriia	Bryobacterales	Bryobacteraceae	Paludibacilum	*Paludibacilum fermentans*	11
	Holophagae	Holophagales	Holophagaceae	Geothrix	*Geothrix fermentans*	6
Bacteroidota	Bacteroidia	Bacteroidales	Paludibacteraceae	Paludicbacter	*Paludibacter jiangxiensis*	13
Actinobacteria	Actinomycetia	Nakamurellales	Nakamurellaceae	Nakamurella	-	4
Verrumicrobia	Opitutae	Opitutales	Opitutaceae	Oleiharenicola	-	5
Cyanobacteria	Nostocales	Symphyonemataceae	Loriellopsis	Loriellopsis cavernicola	-	5
	Pseudanabaenales	Oculatellaceae	Oculatella	Oculatella mojaviensis	--	4
Planctomycetota	Planctomycetia	Gemmatales	Gemmataceae	Gemmata	*Gemmata obscuriglobus*	2
Candidatus	Vampirovibrionales	Vampirovibrio	Vampirovibrio chlorellavorus	-	-	3
Unclassified	-	-	-	-	-	4

ultimately affecting metabolic activity (Boessmann et al., 2004).

To identify the types of bacteria attached to pumice, a microbial community analysis was conducted using comprehensive microbial community sequencing from phylum to species level with 0.5% readout, employing 162-targeted metagenomics on biofilm samples using the Zymobiomics DNA Miniprep kit from Oxford Nanopore Technologies. The microbial community analysis revealed 432 (Table 1) bacterial species attached to the pumice stones, with the five most abundant species being *Tolumonas auensis*, a bacterium found in anoxic freshwater lake sediments. These bacteria are thermophilic bacteria that can grow under both aerobic and anoxic conditions and play a role in producing tolena. Next is *Pseudomonas jinjuensis*, these bacteria belong to the Pseudomonadota phylum, Gammaproteobacteria class, which are commonly found in wastewater treatment processes. *Pseudomonas khazarica* is a bacterium found in Lake Khazar in Iran. *Anaeroarcus burkinensi* is an anaerobic bacterium isolated from farmland soil and belongs to the mesophilic bacteria. *Paludibacter jiangxiensis* is a mesophilic bacterium isolated from decaying rice stems in rural rice fields. Acidobacteria are involved in phosphorus removal and have the potential to utilize various organic compounds, including glucose, xylose, acetate, and fatty acids (Godzieba et al., 2022). The dominance of the phyla Proteobacteria, Bacteroidetes, and Acidobacteria is commonly found in active sludge systems (Ai et al., 2019). The phylum Firmicutes consists of autotrophic bacteria found in the annamox process (anaerobic ammonium oxidation) (Yue et al., 2024).

CONCLUSIONS

Domestic wastewater treatment with modified tripikon-S and pumice as a biofilm growth medium has been shown to be effective in removing COD, TN, and TP under batch conditions with significant removal rates, with the best COD removal rate averaging 98.5% at a COD concentration of 500 mg/L, TN removed by 75% at a COD concentration of 1000 mg/L, and TP removed by 92% at a COD concentration of 500 mg/L. The establishment of biofilm, the presence of diverse microorganisms, and changes in environmental parameters (DO, temperature, and pH) in the reactor with expanded clay all point to the presence of microbial activity that has eliminated organic contaminants from residential wastewater. The elimination of organic and nutritional waste is quite effective because there are numerous types of microorganisms capable of grading contaminants. As a result, this system can be one of the options for home wastewater treatment, particularly in riverside locations.

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