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Sustainable recycling of waste polyethylene terephthalate sponges as biocarriers for domestic wastewater treatment

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ABSTRACT

Recycling of plastic waste has been implemented for tens of years, but reuse in environmental engineering, in particular in wastewater treatment is very limited. This study evaluated the potential of reusing waste polyethylene terephthalate (PET) sponges as bio-carriers for the remediation of domestic wastewater. The test was conducted with domestic wastewater from the University administration building, using a lab-scale fixed bed bioreactor and run for nearly 3 months. Initial results revealed that the COD removal efficiencies were observed within the 70–80% range, yielding a mean effluent concentration of less than 50 mg/L, which adheres to the national standards of QCVN 14:2025/BTNMT, Column A. Ammonia-nitrogen (NH_4-N) reduction efficiencies reached substantial levels of 94–96%, indicative of the robust proliferation of nitrifying microbial assemblages sequestered upon the substrate surface. Metagenon analysis rendered the presence of anaerobic bacterial genera, such as *Romboutsia* and *Clostridium*, together with specialized denitrifying groups, including *Thauera* and *Pseudoxanthomonas*, provides biological evidence of possible simultaneous nitrification-denitrification mechanism, thereby optimizing total nitrogen removal in a single treatment stage. These findings suggest that recycled PET can serve as a viable alternative to conventional, high-cost biocarriers within both aerobic and anaerobic biological treatment configurations.

Keywords: PET plastic, fixed bed biofilm reactor, wastewater, bacterial community.

INTRODUCTION

Reusing plastic waste as a substrate offers many environmental and economic benefits. Environmentally, it contributes to reducing the pressure of landfilling and burning plastics, thereby limiting greenhouse gas and microplastic emissions. Economically, recycled plastics are much cheaper than imported commercial plastics, and help to proactively secure domestic supply. In fact, many studies have shown that plastic wastes can be very effectively used as media or biocarriers for wastewater treatment (Deng et al., 2016; Ali et al., 2023; Anh et al., 2024).

The fixed bed biofilm reactor (FBBR) technology is a biological treatment technology based on the development of microbial biofilms on a fixed media (Izadi et al., 2019). This technology combines the growth of attached and suspended microorganisms. FBBR is different from moving bed biofilm reactor (MBBR) in some factors, including the longer hydraulic retention time and recycle of sludge (Metcalf et al., 2014). The FBBR system integrates three biological processes: suspended activated sludge, facultative denitrification (nitrogen removal), and microbial growth in the form of attached microorganisms on the surface of the media placed in the system. The treatment tank in the FBBR technology uses a fixed media along with a continuous aeration system to increase the number of available microorganisms. These microorganisms play a role in decomposing organic matter; they grow and attach to the surface of the media to further enhance the wastewater treatment process.

Plastic media commonly used in the FBBR or MBBR technologies are typically made from polymers, such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) or polyethylene terephthalate (PET) – materials with high mechanical and chemical strength (Le et al., 2016; Fauzi et al., 2023). For the MBBR tank, the plastic carriers often have smaller density than water, so that they are able to float in water, moving flexibly within the tank. While in the FBBR tank, the carriers tend not to move within the tank due to higher density than water. The media in the FBBR tank can be classified into sponge-type, cylindrical or chip or disc type carriers. The design goal was to optimize the specific surface area (m^2/m^3) to facilitate microbial biomass growth while ensuring smooth flow and preventing clogging. In general, sponge-based carriers have much higher specific surface area than other classic cylindrical carriers, chip or disc type carriers.

Review of the literature revealed that there were few studies using sponge-based carriers, partly due to their high biodegradability (Dang et al., 2020). Specifically, PU sponges, the most commonly-used polymer sponges were easy to degraded after several months (Duc et al., 2014; Zhang et al., 2016; Dacewicz and Lenart-Boroń, 2023). In addition, most of the previous research with sponge-based carriers were conducted with the commercial ones (Trinh et al., 2014; Guo et al., 2019; Kong et al., 2025). The constraints of using commercial sponge-based carriers are the cost and some of them may require skillful operation to avoid leaching (Dang et al., 2020; Li et al., 2023).

The novelty of this study was to test the possibility of reusing waste PET sponges as bio-carriers in the FBBR, which has not been done previously. A hypothesis was made that high pollutant removal efficiencies could be obtained owing to (1) the high surface specific areas of PET sponges; and (2) good durability of this sponge in wastewater. Should the PET waste can be reused as bio-carriers, it represents a cost-effective alternative for the wastewater treatment infrastructure. Implementing such recycled media not only reduces capital expenditure for treatment plants, but also mitigates the environmental burden associated with solid waste management and plastic accumulation.

MATERIALS AND METHODS

Materials

Wastewater was collected from the wastewater discharge pipe into the septic tank of building A1, Hanoi University of Civil Engineering. The wastewater temperature averaged 27.5 ± 2.3 °C, and the pH was 6.9 ± 0.1. The influent ammonium nitrogen ($N\text{-}NH_4^+$) concentrations ranged from 40 to 69 mg/L, while the influent chemical oxygen demand (COD) varied between 168 and 265 mg/L. The ratio C/N was 3.96 ± 0.23. The seeding activated sludge was collected from the Kim Lien wastewater treatment plant, Hanoi, with an average MLSS of 2.257 mg/l.

The biological carrier material for the treatment tank was cut from non-woven fabric PET mattress with a fibrous structure, cut into small pieces measuring 2.5 × 2.0 × 1.2 cm. The carrier material occupied 30% of the reaction tank volume. The characteristics of these carriers were determined as follows (Hong Anh et al., 2024, Dang et al., 2020). The surface area was analyzed by the Brunauer-Emmett-Teller (BET) method using a Gemini VII 2390 V1.02T analyzer (Micromeritics Instrument Corp., Norcross, GA).

Specific surface area

A one-liter glass volumetric beaker was filled with PET sponge pieces and packed as tightly as possible. Then, the number of pieces used to fill the volumetric beaker (N) was counted. Finally, the specific surface area of the PET pieces was calculated based on the following:

$$\begin{aligned} &\textit{Specifiz surface area } (SSA) = \\ &= (\textit{Surface area of } 01 \textit{ piece} \times N)/ \\ &/\textit{Volume of beaker} \end{aligned} \quad (1)$$

where: N – number of PET pieces.

Porosity: using saturation method (water displacement)

First, the total volume of dry carriers was measured (V_{total}). Then, they were submerged in water and agitation was employed to ensure all air is removed from the pores. After that, the volume of water absorbed was measured (V_{void}).

$$n = (V_{void}/V_{total}) \times 100\% \quad (2)$$

Density

The density of PET sponge carriers was calculated using the following equation:

$$\rho = \frac{m}{V} \quad (3)$$

where: m (g) and V (cm^3) are the mass and volume of the loofah sponge.

Surface examination

The pristine and the tested samples were sent for surface examination using the scanning electron microscopy (SEM) method to produce detailed, magnified images of an object by scanning its surface with a focused beam of electrons. This was implemented using the Tabletop Microscope (TM4000plus, Hitachi, Japan).

Testing procedure

The experimental set-up was depicted in Figure 1. Wastewater was introduced into the tank with a flow rate of Q = 18 (l/day). The wastewater was pumped from the feed tank (D = 390 mm, H = 420 mm) to anoxic compartment (L × B × H = 385 × 230 × 285 mm), then flowed by gravity to the oxic compartment (L × B × H = 385 × 230 × 285 mm). The treated water was collected after the settling tank (D = 200 mm, H = 500 mm). The water from the oxic tank was recirculated back to the anoxic tank with a flow rate of Q = 9 (l/day). This ratio needs to ensure sufficient nitrate supply for effective denitrification, but not so high as to disrupt hydraulic conditions or increase pumping energy costs. Sludge was recirculated from the settling tank to the anoxic tank daily. Dissolved oxygen concentration in the tanks was controlled so that DO < 2 mg/L for the anoxic tank and DO > 2 mg/L for the oxic tank. The system retention time is 16 hours. Daily checks were conducted in terms of parameters such as temperature, pH, DO, and activated sludge load. Weekly wastewater samples were taken at four locations: the feed tank, anoxic tank, oxic tank, and the settling tank for analysis of COD, NH_4-N, and SVI30. The test was conducted from April to July, 2025.

Sample analysis

COD and NH_4-N were determined following the standard analytical method (APHA 2005). DO and pH were measured daily using 210-K handheld devices, Horiba, Japan and 1100 LAQUA, Horiba, Japan. NH_4-N was sampled weekly and determined by an IC-8100ST ion chromatograph (Tosoh – Japan).

Biofilm extraction

After the experiment was completed, the carrier samples were analyzed for the microbial population growing on the substrate. First, they were placed in a 0.9% NaCl saline solution and shaken for 5–10 minutes (using a Vortex mixer), then sonicated for 10 minutes at 30 kHz (using a machine). After that, the samples were centrifuged at 4000 rpm at 40 °C for 10 minutes. Finally, the supernatant was decanted, and the sediment at the bottom was stored in a test tube at -20 °C until the sample was used for DNA extraction and PCR amplification.

PCR amplification and sequencing were performed using Oxford nanopore technology

Total DNA was extracted from this solution using the DNeasy PowerSoil Pro kit (Qiagen, USA). DNA concentration was quantified by fluorescence measurement using a Qubit fluorescence

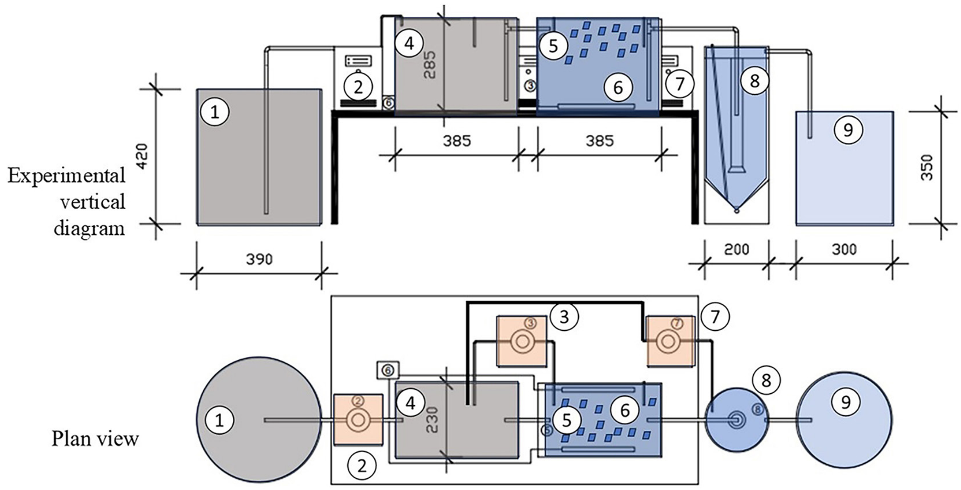


Figure 1. Experimental set-up: 1) feed tank, 2) wastewater pump, Q = 18 L/day, 3) wastewater recirculation pump, Q = 9 L/day, 4) anoxic tank, 5) oxic tank + carriers, 6) air diffuser, 7) sludge recirculation pump, 8) settling tank, 9) treated water tank

spectrometer, and purity was assessed by the optical density ratio (OD260/OD280). Samples were considered suitable for further analysis if DNA concentration ≥ 0.20 ng/μL, total amount ≥ 15.00 ng, and optical density ratio OD260/OD280 ≥ 1.50. Sufficient length 16S rDNA amplicons were generated by PCR amplification using universal primers 27F and 1492R. Sequencing libraries were prepared using a Ligation sequencing kit (ONT, UK) according to the manufacturer's procedure. Library concentrations were determined by Qubit DNA measurement (Thermo Fisher Scientific), with quality control requiring concentrations ≥ 15.00 ng/μL. Finally, sequencing was performed on an on-site nanopore platform using a flow cell dongle.

RESULTS AND DISCUSSION

Morphological analysis of PET plastic carriers

Figures 2a and 2b show images of PET plastic material before and after being used as microbial carriers in the experimental system, respectively. As it was mentioned above, the material was cut from a waste PET mattress with a fibrous structure, and an average size of approximately 2.5 × 2.0 × 1.2 cm, an average porosity of approximately 91.3%, and a specific surface area of approximately 6390 ± 250 m^2/m^3.

Before use, PET material is bright white, with a clean and soft surface. The interwoven plastic fibers form a porous, breathable structure with high porosity and good water permeability. This structure increases the surface area, creating favorable conditions for microorganisms to adhere and form biofilms during the system's startup phase. In addition, the material is chemically inert, durable in water, and does not dissolve or deform under the influence of microorganisms and organic compounds in wastewater. After use in the wastewater treatment system, PET material undergoes a noticeable change in color and morphology. The initial white color turns grayish-brown due to the adhesion and growth of biofilms along with suspended solids and organic matter. Despite variations in color and porosity, the PET material retains its original shape without breaking or deforming, demonstrating high mechanical strength and stability in the operating environment. Overall, morphological observations show that the PET material cut from plastic pads has good microbial adhesion, high durability, and is effective in the treatment of domestic wastewater. The formation of a dense biofilm on the material surface is evidence of the adaptation and vigorous growth of the microbial system, contributing to improved

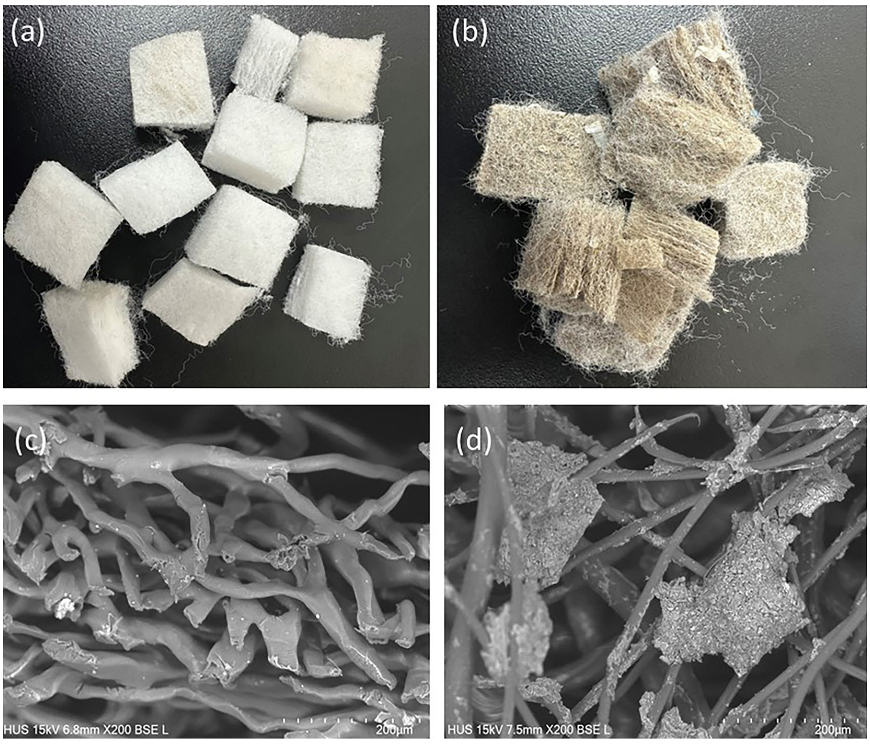


Figure 2. The images of PET carriers and SEM analysis (x200) before (a,c) and after testing (b,d)

efficiency in removing pollutants, such as BOD, COD, and $N–NH_4^+$ during the treatment process.

Further surface scanning using SEM method revealed the clear difference of the two samples (Figure 2c and 2d). Specifically, in Figure 2c, the fibers are highly porous with a three-dimensional network of interconnected channels. The “ribbon-like” or “branching” geometry is specifically engineered to provide a high surface-area-to-volume ratio. As it was mentioned above, surface area is one of critical factor; more area equals more space for bacteria to colonize. In Figure 2d, a significant accumulation of floc-like structures and rough aggregates clinging to the fibers were found. The grainy, irregular masses are a mix of microbial colonies (bacteria) and the “glue” (EPS) they secrete to anchor themselves against the shear forces of flowing water. It should be noted that these bio aggregates are crucial for the treatment of organic and nutrient matters.

In an effort of comparing the physical characteristics of PET carriers produced from this study and others from previous research, it was found that the surface area of PU/PET sponge carriers is much higher than that of HDPE/PP plastic carriers. Compared to some recycled plastic materials, such as plastic straw segments (length 2.5 cm, pore diameter 1.8 cm, porosity 71.8 ± 1.9%, surface area 453 ± 7.6 m^2/m^3), and the commercial substrate WD-f10-4 bioM™ (specific surface area 900 m^2/m^3, density 0.96–0.98 g/cm^3), it can be seen that the specific surface area of PET sponge was about 13 times higher than that of straws (453 m^2/m^3) and about 6–7 times greater than WD-f10-4 (900 m^2/m^3). The PET carriers had characteristics similar to commercial polyurethane sponge substrate DHY-1 with porosity of 92–96% and specific surface area of 6000–12000 m^2/m^3 (Trinh et al., 2014). However, these media exhibited approximately one-third the specific surface area of commercial LEVAPOR carriers. Theoretically, a greater specific surface area facilitates higher biomass immobilization; this increased microbial density enhances the volumetric removal rates of COD and other dissolved substrates within the reactor.

One should be noted that the HDPE caps or shredded pieces only allows microorganisms to form a thin biofilm around the outside. This difference leads to the superior advantage of a PET sponge, since the deep pores in the sponge act as buffer zones protecting microorganisms from mechanical impacts and sudden changes in flow, while also allowing the establishment of different dissolved oxygen DO concentration gradients on the same piece of carrier material.

Another thing worth noting is that the sponges were made of pure PET plastic (as consulted with the producer), thus it would be hardly biodegradable. PET is classified as highly recalcitrant because of its high degree of crystallinity (30–50%), which makes it resistant to natural microbial degradation (Mohanan et al., 2020). In aquatic environments, complete degradation of PET can take between 50 and hundreds of years (Mohanan et al., 2020). In contrast, PU sponges are widely recognized to be more susceptible to microbial interaction and degradation (Dacewicz and Lenart-Boroń, 2023). While some petroleum-derived polyurethanes are also resistant, many PU formulations – especially those used in medical or specific industrial sponges – are designed or found to be biostable yet susceptible to enzymatic hydrolysis and mineralization by specialized bacteria and fungi (Gui et al., 2023; Najam et al., 2025). Figure 2b can confirm the still firm form of these PET sponges after the testing with wastewater.

Experimental conditions

It should be noted that the system was seeding with sludge from the nearby domestic wastewater treatment plant (Kim Lien plant). After that, it was run for about four weeks until MLSS was about 2000 mg/L, then the sampling was started.

The pH and dissolved oxygen (DO) concentration are two environmental parameters that determine the activity of microorganisms, especially nitrifying bacteria attached to the PET carrier material. Throughout the experiment, the input pH value in anoxic tank fluctuated between 6.5 and 8.2 (Figure 3a), which is the optimal range for the growth of nitrifying bacteria (AOB and NOB). Considering the trend, the pH in the aerobic tank tended to decrease slightly (down to 6.5–6.8) compared to the input in the anoxic tank or slightly similar. This is explained by the strong ammonium nitrification process on the PET substrate. According to the reaction equation, for every 1 mg of $N-NH_4$ oxidized to NO_3^-, approximately 7.14 mg of alkalinity ($CaCO_3$ equivalent) is released. The alkalinity concentration in domestic wastewater is quite stable, thus, the pH value in the FBBR tank is always maintained at a neutral level, not dropping below 6.5 which is a level that can inhibit microorganisms, thus ensuring high ammonia removal efficiency.

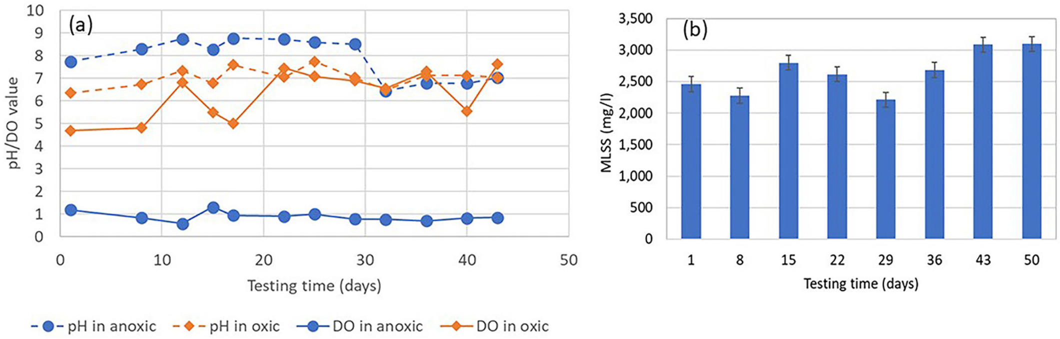


Figure 3. Testing conditions

Dissolved oxygen concentration is maintained through a continuous aeration system to ensure aerobic conditions for biofilm growth. The DO concentration in the tank is controlled at 4.8–7.0 mg/L (Figure 3a). This DO level was more than sufficient for oxygen to diffuse into the outer layer of the biofilm on the PET fibers, providing electrons for the oxidation of organic matter and nitrification. Due to the porous structure with a porosity of more than 90%, the DO concentration was not uniformly distributed, which is a key difference between PET sponge material and HDPE bottle caps. Specifically, the surface layer of PET sponge material was in direct contact with water, resulting in high DO, favorable for aerobic bacteria. While inside the sponge core, oxygen diffusion is limited by the interwoven fiber structure and the thick biofilm covering the outside. This creates micro-anoxic or anaerobic zones within the substrate core even when the DO outside the tank is very high, allowing simultaneous nitrification and denitrification in the same tank, resulting in more efficient removal of total nitrogen. MLSS in the experimental model is presented in Figure 3b. Initially, the sludge taken from the Kim Lien wastewater treatment plant had an MLSS of 3,257 mg/l. After adding sludge to the aerobic tank, MLSS decreased slightly to about 2000 mg/L due to the changes in the culture environment. Throughout the operation of the model, MLSS fluctuated significantly (as shown in Figure 3). The decrease in biomass normally affects the system performance, for instance, it might lead to a decrease in COD and T-N treatment efficiency (Phanwilai et al., 2020). After adjusting and controlling operating conditions, MLSS tends to increase again and continue to grow.

Pollutant removal efficiency

Figure 4a illustrates the temporal fluctuations of COD throughout the operational period using PET plastic media. The influent COD concentrations exhibited moderate variability, ranging from 168 to 265 mg/L, reflecting the inherent stochasticity of domestic wastewater generation. Following treatment, residual COD concentrations were markedly reduced to 6–49 mg/L. This performance corresponds to a removal efficiency of 71–92%, demonstrating the system's effectiveness in

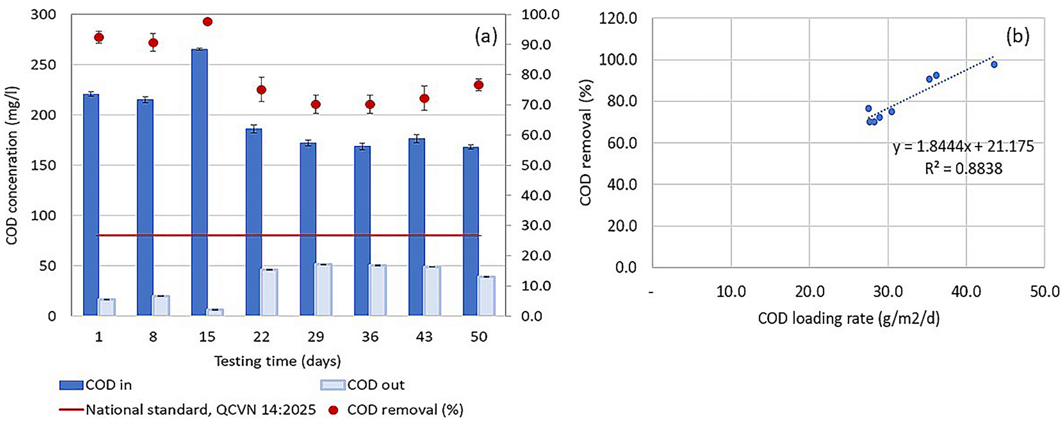
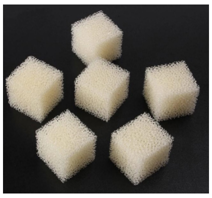


Figure 4. COD removal efficiency and correlation with COD loading rate

stabilizing organic loads. Compared with the Vietnam national technical guideline QCVN 14:2025/BTNMT – Column A (80 mg/L), all treated water samples met the standards for discharge. This result confirms the stable treatment capacity of the system using recycled PET sponges, with high efficiency and good reliability.

In comparison with previous studies using sponge or piece-type carriers (as in Table 1), it can be seen that the organic and nutrient removal efficiencies were comparable. A closer look at the impact of specific surface area (SSA), it can be seen that there was relative and positive correlation between the SSA and COD removal for the same type of bio-carriers, for instance sponge-based carriers. As SSA increases, the capacity for biofilm growth increases. For example, comparing the base PU sponge (2500 m^2/m^3) to the

Table 1. Comparison of some commercial and reused wastes as carriers

Types of plastic carriers	Images	Specific surface area (m^2/m^3)	Density (g/cm^3)	Porosity (%)	COD and Nutrient removal efficiencies (%)	Reference
PU sponge		2500	0.5	90±5%	47% COD 42% NH_4 (NH_4 in = 5–6 mg/L)	Kong et al., 2025
Commercial PU sponges (DHY 1)		6000–12000	0.95	95%	80-92% NH_4 (NH_4 in = 10–12 mg/L)	Duc et al., 2014
Commercial PU sponge (LEVAPOR)		20000	1.04–1.1	85%	84.3% COD 86% NH_4 (NH_4 in = 35–36 mg/L)	Guo et al., 2019
Recycled PET sponge pieces		6390±250	0.96	91.3%	75% COD 95% NH_4 (NH_4 in = 40–70 mg/L)	This study (2026)
Unmodified HDPE caps		255.4±5.3	0.92–0.97	81.6±2.8%	83.5% COD 81.4% NH_4 (NH_4in = 55.8 ± 21.8 mg/L	Hong Anh et al. (2024)
Recycled Shredded Plastics		224	0.92	42.5	72% COD 52% NO3	Abyar et al. (2024)
Recycled PP plastic straw segments		453±7.6	-	71.8 ±1.9	87.7% COD 58.7% NH_4 (NH_4 in = 23 ± 8 mg/L)	Kim et al. (2021)

LEVAPOR commercial PU sponge (20000 m^2/m^3), a massive jump in COD removal from 47% to 84.3% can be seen. No relationship could be found when comparing different types of carriers with different base polymers (Table 1). Different explanation was made about this correlation, probably depending on the type of carriers. Zhu and Miao (2022) using 5 different types (shapes and base polymers) of carriers revealed that a larger SSA does not always translate to higher efficiency due to the "hump" effect. As biomass thickens on high-surface-area carriers, it can restrict the diffusion of nutrients and oxygen to the inner layers, potentially reducing overall metabolic activity. In contrast, Cheng et al. (2025) found that high SSA is particularly critical for slow-growing organisms, such as nitrifying bacteria, therefore, preferred for nitrification and anammox processes (Cheng et al., 2025).

As it is illustrated in Figure 4b, a relative correlation exists between the COD surface area loading rate and removal efficiency. Within the tested range of 27.5–43.5 $g/m^2/d$, the system demonstrated enhanced performance at higher organic loads, peaking at a removal efficiency of approximately 98%. This positive slope suggests that the system has not reached its maximum treatment capacity within the tested range of 27–44 $g/m^2/d$. Compared to the LEVAPOR carriers studied by Guo et al. (2019), which provide a high and stable removal baseline due to their superior specific surface area and protective pore structure, the PET media require higher loading rates to reach peak efficiency. This indicates that while the PET media are effective, their performance is more sensitive to influent organic strength than commercial sponge-type carriers.

The results of monitoring the changes in the $N\text{-}NH_4^+$ content in wastewater through the treatment stages are shown in Figure 5. In general, the influent $N\text{-}NH_4^+$ content ranged from 40–70 mg/L, significantly higher than the permissible limit of national technical guideline QCVN 14-MT:2025/BTNMT (Column A: 4 mg/L, Column B: 8 mg/L). This reflects the characteristics of domestic wastewater with high nitrogen load, requiring the biological treatment process to be appropriately designed to ensure effective ammonia removal. After passing through the treatment tanks, the $N\text{-}NH_4^+$ content decreased sharply. In the anaerobic tank, the $N\text{-}NH_4^+$ concentration decreased to approximately 5–12 mg/L. Although this is not yet a zone of strong oxidation, some $N\text{-}NH_4^+$ may have been converted due to the presence of autotrophic microorganisms active under oxygen-deficient conditions, and local nitrification may have occurred at the boundary between the anaerobic and aerobic zones due to recirculation from the aerobic to the anaerobic compartment.

Notably, in the oxic tank, the $N\text{-}NH_4^+$ content continues to decrease sharply, to only 1–3 mg/L. This is the stage where nitrification occurs vigorously, mainly involving the bacterial groups Nitrosomonas (oxidizing $NH_4^+ \rightarrow NO_2^-$) and Nitrobacter (oxidizing $NO_2^- \rightarrow NO_3^-$). Maintaining a stable dissolved oxygen (DO) concentration in the

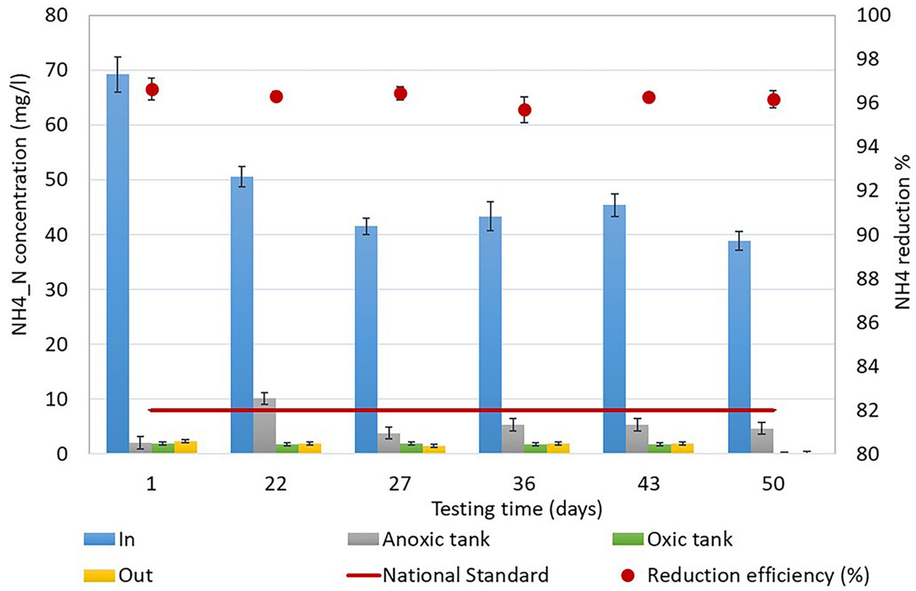


Figure 5. NH4-N removal efficiency

aerobic tank is a decisive factor in the efficiency of this conversion. At the system outlet, the $N\text{-}NH_4^+$ concentration consistently remained below 4 mg/L, meeting the QCVN 14-MT:2025/BTNMT – Column A standard, demonstrating very high ammonia removal efficiency. The NH_4^+ removal efficiency of the entire system ranged from 94–96%, showing the stability and good performance of the microbial system in the biological tank.

Again, in comparison with similar studies (Table 1), it can be found that the recycled PET sponge achieves the highest NH_4-N removal (95%) despite having a moderate SSA (approximately 6400 m^2/m^3). This suggests that while SSA is a baseline requirement, the quality of the surface or the synergy with porosity can sometimes outweigh surface area. For instance, high porosity ensures that nutrients like NH_4-N can actually reach the bacteria living deep inside the sponge. Additionally, when compared with the study of Trinh et al., (2014) with the same type of sponge material but different polymer base, same surface area (about over 6000 m^2/m^3), it was found that the $N\text{-}NH_4^+$ treatment efficiency was also over 90% even though the initial NH_4-N concentration was quite low 10–12 mg/L. This proves the good capability of sponge-type carriers in dealing with different nutrient loadings in wastewater, regardless of whether they are new or recycled sponges.

Figure 6 illustrates a positive correlation between DO concentration and the percentage of NH_4 reduction. As the DO concentration increases from approximately 3.0 mg/L to 7.0 mg/L, the efficiency of NH_4 reduction generally improves, rising from roughly 96% to 98%. In wastewater treatment, specifically within systems like the MBBR, FBBR or integrated fixed-film activated sludge (IFAS) system mentioned in the text, higher DO levels facilitate the aerobic nitrification process better (Dang et al., 2020). Nitrifying bacteria require oxygen to convert ammonia (NH_4) into nitrite and then nitrate.

Figure 7 shows that the treatment efficiency according to TN is not high. After 3 weeks of operation, the T-N treatment efficiency reached an average of about 61% and began to stabilize after 1 month of operation. The C/N ratio in the domestic wastewater from office buildings where the wastewater characteristics mainly contain nitrogen sources and are low in carbon may also be the reason why the TN treatment efficiency of the model is not high (Wang et al., 2018). In this study, the feed wastewater from a septic tank of an office building in the university campus which had the C/N of only 3.96 ± 0.23 was employed. Another possible explanation may lie in the oxygen transfer mechanism within the high-surface-area PET matrix. During the test, the DO in the oxic tank was rather high, ranging from 4.69 mg/L to 7.45 mg/L. According to Fick's Law of Diffusion, these high DO levels create a steep concentration gradient that deeply penetrates porous structures. This observation aligns with Zhu and Miao (2022), who noted that while high specific surface area increases potential removal capacity, the actual efficiency is heavily dictated by the oxygen concentration gradient. In the early stage of your study, the oxygen satisfied the demand for nitrifiers, but inhibited the formation of anoxic micro-niches required for denitrification.

After that, a significant shift occurred as TN reduction reached its peak of nearly 70%. This improvement coincided with a sharp decrease in effluent nitrate to 12.29 mg/L. This transition suggests the maturation of a stratified biofilm despite the sustained high DO levels (reaching 7.62 mg/L

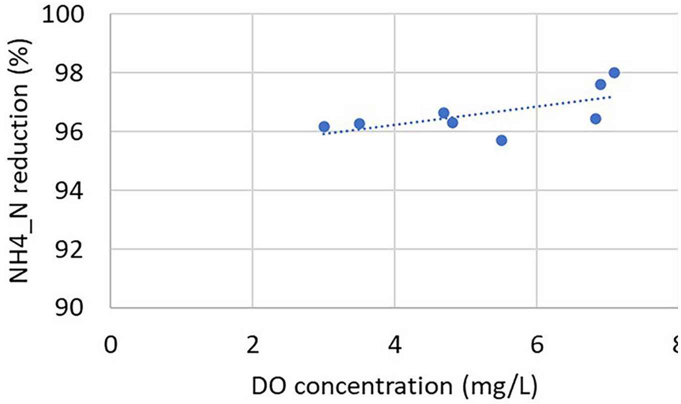


Figure 6. Correlation between DO and NH_4-N concentration

by Day 43). This performance improvement aligns with the research by Ngo et al. (2012), who demonstrated that sponge biological media facilitate higher biomass retention and internal microenvironments, allowing nitrifiers to thrive even under high organic loads, compared to plastic carriers. This stratification allowed for (i) outer layer protection**,** in which dense heterotrophic growth, fueled by high COD loading (28–43 g/m^2/d), acted as a biological barrier, consuming a significant portion of the DO before it could reach the sponge core; (ii) internal anoxic pores in which the increased biofilm thickness restricted oxygen diffusion, creating stable anoxic zones within the PET sponge core. This effectively utilized the low DO environment of the anoxic tank (0.58–1.31 mg/L); and finally (iii) simultaneous nitrification-denitrification (SND) within these shielded internal zones.

The consistently low nitrite (NO_2^-) levels, remaining below 0.13 mg/L in the effluent, confirm that the system maintained a complete and robust nitrification pathway. Furthermore, the structural durability of the PET sponges ensured that the SSA of 6400 m^2/m^3 remained available for this complex microbial stratification. This supports the findings of Chu and Wang (2011b), who studied nitrogen removal in MBBRs and found that high-porosity carriers provide a superior environment for stable simultaneous nitrification-denitrification by protecting slow-growing nitrifiers and providing deep-pore anoxic zones.

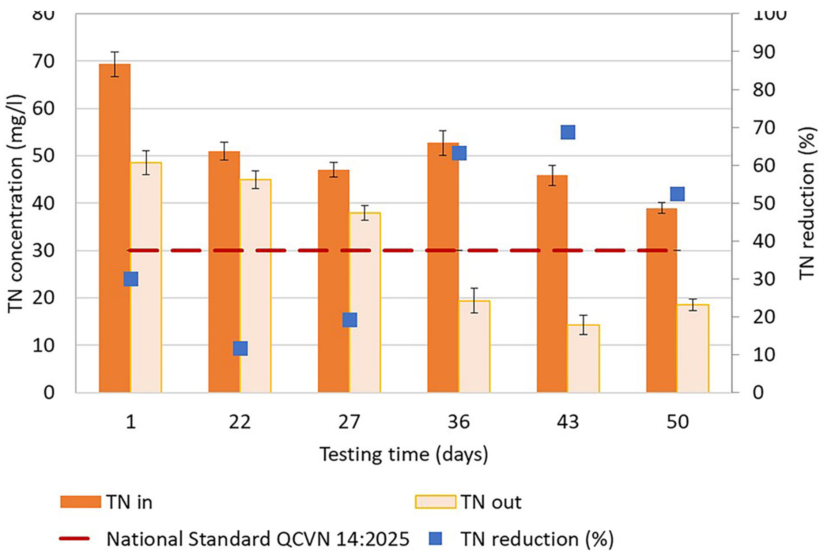


Figure 7. TN reduction efficiency

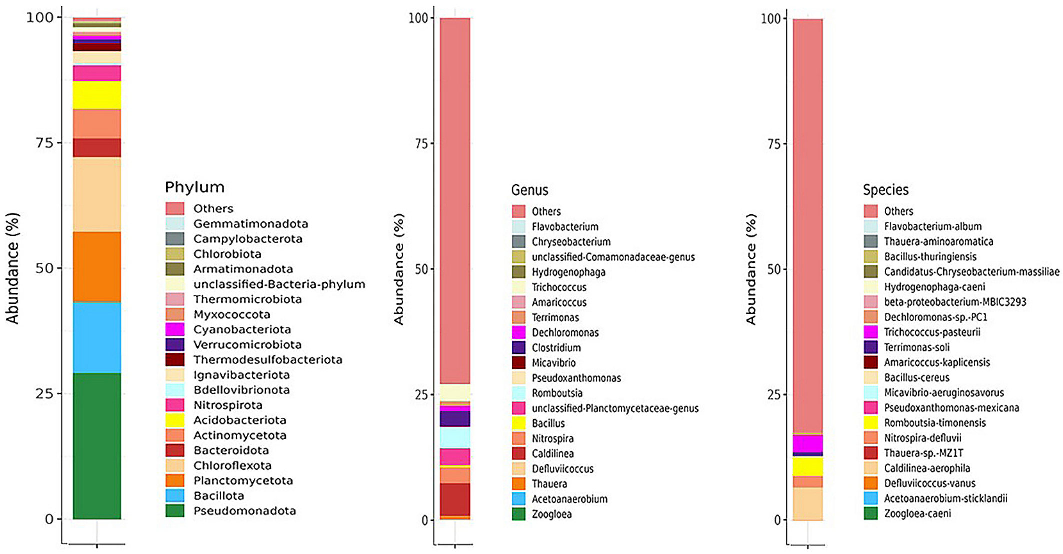


Figure 8. Diversity and Richness of Microbial communities attached to a PET sponge

Microbial community on PET plastic carriers

Figure 8 illustrates the diversity and abundance of the microbial community attached to the PET sponge at three taxonomic levels: Phylum, Genus, and Species. This community forms a highly active biofilm, and these microorganisms have great potential in wastewater treatment, particularly in the degradation of organic pollutants.

At the phylum level, the most dominant phyla are: Pseudomonadota (30%), with flexible metabolic capabilities and the ability to degrade complex organic compounds, including hydrocarbons and plastics, accounting for the majority on the porous MBBR carrier (Zhang et al., 2020); (2) Bacillota (25%), which participate in biotransformation processes, breaking down large molecules and playing an important role in anaerobic degradation processes (Zhu et al., 2018); (3) *Planctomycetota* (25%), with some species performing anaerobic ammonium oxidation (anammox), an extremely efficient process for nitrogen removal in wastewater. In addition, the presence of Nitrospirota (2%) also contributes significantly to aerobic ammonium oxidation, providing a prerequisite for nitrate removal in the anoxic tank (Gao et al., 2020). The study also found a relatively high abundance of ammonium-removing microbial phyla, such as Actinobacteria, Acidobacteria, and Bacteroidetes, in the biofilm samples, consistent with the study by Zhang et al. (2020). Moreover, Bacteroidetes have been reported to be beneficial in the degradation of particulate organic matter (POM) (Zhang et al., 2020). The study by Zheng et al. (2022) also indicated that on sponge carriers, Proteobacteria and Bacteroidetes always account for a large proportion and show apparent differences between the outer and inner biofilm layers (Zheng et al., 2022).

For the groups identified at the genus level, those with a relative abundance of less than 1.0% were grouped as "Others," and the 20 most abundant genera are presented in Figure 7 above. At the genus level, more specific functions emerge: (1) *Zoogloea*: this genus is a typical indicator of activated sludge, with strong EPS-producing ability, facilitating floc formation, which is extremely important for solid settling in wastewater treatment, helping to protect the microbial population from mechanical shear forces and maintaining system stability even when influent pollutant concentrations fluctuate (Hou et al., 2021); (2) *Flavobacterium* (5%), with the ability to degrade various organic compounds, including proteins and carbohydrates; (3) *Romboutsia* and *Clostridium* (5%): the dense presence of anaerobic bacterial genera, such as *Romboutsia* and *Clostridium* (accounting for approximately 5% in total) provides experimental evidence for the existence of deep anaerobic zones within the carrier matrix; (4) *Thauera* (2%), *Nitrospira* (5%), and *Pseudoxanthomonas* (5%), which are particularly important in the denitrification process, converting nitrate into nitrogen gas, a key step in nitrogen removal from wastewater (Shitu et al., 2020). In addition, the genera unclassified-*Planctomycetales* and *Chryseobacterium* also contribute to nitrogen removal processes in wastewater. *Nitrospira* sp. were found to have a more significant impact on nitrification than other nitrifying bacteria, such as *Nitrobacter* and *Nitrosomonadales*. Bartelme et al. (2017) demonstrated complete nitrification by *Nitrospira*, which can directly oxidize ammonium nitrogen to nitrate nitrogen in a single-step process. *Nitrosomonas* was detected in wastewater with high nutrient and ammonium concentrations, which is also consistent with the characteristics of the wastewater investigated in this experiment (Bartelme et al., 2017).

For sponge-type carriers, the interwoven fiber network structure with extremely high porosity (~94.17%) is similar to the characteristics of polymer sponges that have been proven to retain sludge and provide large contact areas in MBBR systems, protecting microbial populations from the mechanical shear of water flow (Zheng et al., 2022). As the biofilm develops and thickens, the diffusion of DO from the bulk water into deeper layers is significantly hindered by the outer EPS layers and bacterial cells, establishing an oxygen concentration gradient decreasing from the fiber surface toward the pore core, allowing anaerobic species to inhabit and function even under vigorously aerated aerobic conditions. In contrast, for solid carriers with rough surfaces, the dense and flat solid surface prevents microorganisms from forming a thick biofilm on the exterior. This entire layer is almost directly exposed to high DO concentrations in the reactor, making it difficult for anaerobic species to establish stable populations.

The differentiation of habitats on a PET sponge leads to the formation of a multifunctional microbial ecosystem, enabling simultaneous nitrification-denitrification, an important characteristic that has been demonstrated on sponge-type biocarriers due to the coordination between internal and external microbial groups within the biofilm (Zheng et al., 2022), especially in the treatment of wastewater with high nitrogen concentrations (Chu and Wang, 2011a). Nitrification occurs in the outer layer of the biofilm on PET fibers, where *Nitrospira* (5%) and groups belonging to *Pseudomonadota* (30%) are present. *Nitrospira* plays a crucial role in the oxidation of nitrite to nitrate, a vital step in the removal of nitrogen. Denitrification occurs in the deep micro-anaerobic zones within the sponge core with the participation of *Thauera* (2%), *Pseudoxanthomonas* (5%), and *Dechloromonas*. Owing to this SND mechanism, a PET sponge achieves a consistently high N–NH_4 removal efficiency (94–96%), outperforming the performance typically observed for solid recycled plastic carriers. The presence of *Zoogloea,* a genus that produces strong EPS, helps protect the microbial population from mechanical shear forces, thereby maintaining system stability even when influent pollutant concentrations fluctuate.

At the species level, the following were detected: (1) *Zoogloea caeni*: a species indicative of good floc-forming ability in activated sludge systems, which is essential for effective separation of treated water from sludge; (2) *Thauera* sp. and *Thauera* sp. MZ1T: the presence of these species indicates a strong denitrification capacity of the biofilm; (3) *Dechloromonas* sp. PC1: This species is capable of perchlorate reduction, and many *Dechloromonas* species are also well known for their denitrification ability. In addition, the presence of species such as *Caldilinea aerophila* and genera belonging to *Flavobacterium* (5%) indicates strong degradation capacity for complex organic compounds (proteins and carbohydrates) (Liu et al., 2018). This combination, with its superior nitrogen removal capability, confirms that a PET sponge is not only a plastic waste recycling solution but also an ideal biocarrier material, creating an optimal habitat for complex microbial ecosystems in domestic wastewater treatment.

Thus, the presence of a diverse and specialized microbial community on PET sponges demonstrates their potential as biocarrier materials in biofilm-based wastewater treatment systems, such as moving bed biofilm reactors or fixed bed biofilm reactors (Dang et al., 2020). A PET sponge provides a large surface area for microbial attachment and the formation of a stable biofilm. The above results indicate that a PET sponge serves as an ideal habitat for a specialized microbial ecosystem that is highly effective in removing key pollutants from wastewater, highlighting its potential as a sustainable and efficient solution for wastewater treatment applications.

CONCLUSIONS

Initial research on recycled PET sponges from waste materials has shown that these sponges have the ability to form and maintain a stable biofilm, playing an active role in wastewater treatment. COD removal efficiency ranged from 70–80%, with an average effluent concentration below 50 mg/L, meeting the requirements of QCVN 14:2025/BTNMT, column A. For ammonia reduction (NH_4^+-N), the treatment efficiency reached a high level, from 94–96%, reflecting the good growth of nitrifying microbial populations adhering to the substrate surface. This result shows that recycled PET plastic can fully meet the requirements for carrier material in aerobic or anaerobic biological systems, replacing traditional materials with higher costs. The presence of anaerobic bacterial genera, such as *Romboutsia* and *Clostridium*, together with specialized denitrifying groups, including *Thauera* and *Pseudoxanthomonas*, provides biological evidence supporting the SND mechanism, thereby optimizing total nitrogen removal in a single treatment stage.

In conclusion, the primary results have shown that reusing plastic waste as biocarriers in wastewater treatment is possible and is a parallel approach to solid waste management and water resource protection. This is not only a technical solution, but also an opportunity to reduce the environmental burden, save costs, and promote the development of green technologies and circular economy. The future study on PET sponges would focus on understanding and solving some limitations of PET sponges, which include their long-term durability and performance, potential clogging, scalability, and material cost to confidently promote this type of media for large scale application in wastewater treatment plants.

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