

Article

Experimental Investigation of a Modified Tower Bio-Rack Constructed Wetland System for Domestic Wastewater Treatment

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Abstract

The growing urbanisation of India is a major contributor to the production of 72,368 million litres of wastewater daily. Unfortunately, not even 28 to 31% of the generated wastewater receives proper treatment before disposal, putting public health, water quality, and ecological conditions at risk. Traditional wastewater treatment technologies have been proven effective, but they cannot be applied in decentralised settings due to excessive initial investment costs, continuous power needs, and the need for expert supervision. Constructed wetlands (CWs) provide an efficient and environmentally friendly option for decentralised treatment, but these systems suffer from a gradual loss of effectiveness associated with the problem of media-clogging in traditional setups. This research investigates the functioning and efficiency of the Modified Tower Bio-rack Constructed Wetland (MTBRCW) technology designed specifically to mitigate media-clogging issues. The MTBRCW is tested on the basis of its performance under continuous operating conditions for thirteen months (January 2025 to January 2026), as well as on the effectiveness of the treatment at eight different hydraulic retention times (days 1 to 8). A pilot-scale MTBRCW system was monitored through two periodic sampling events (S1 and S2) conducted during each month of operation. The pilot-scale MTBRCW unit is made up of an inlet storage tank (volume 0.099 m³) followed by two wetland containers (volume 0.034 m³ each) planted with *Typha angustifolia* and *Chrysopogon zizanioides* (vetiver grass). In continuous testing mode, influent–effluent paired samples are collected for eight days at each HRT (totalling eighty samples), and samples are analysed according to APHA Standard Methods for pH, BOD, COD, TN, and TP. In continuous testing mode, the MTBRCW exhibits high removal efficiencies at the levels of 89.8% for BOD, 87.5% for COD, 78.2% for TN, and 74.4% for TP. The BOD/COD of the effluent was within the prescribed CPCB discharge limits for all thirteen months of the study, and the TN levels were adhered to in 12 out of 13 months, with one non-compliance event recorded only in July 2025 (effluent TN = 10.8 mg/L), coinciding with the peak monsoon hydraulic loading rate of 0.28 m³/m²·d. TP remained within CPCB limits in all thirteen months. In batch testing mode, removal efficiencies are 94.9% for BOD and 89.9% for COD by day 8. In addition, there were no indications of clogging or any reduction in hydraulic performance during the entire period of the tests through the use of visual inspections and measurement of the outlet flows, but this can only be seen as an observation in a field operation, and not as proof of the hydraulic performance of the system, since no tracer test or measurement of hydraulic conductivity was conducted.



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Keywords: Modified Tower Bio-rack Constructed Wetland (MTBRCW); media-free constructed wetland; domestic wastewater treatment; *Typha angustifolia*; *Vetiver zizanioides*; BOD removal; COD removal; nutrient removal; decentralised wastewater treatment; nature-based solutions

1. Introduction

Water resources are increasingly threatened by rapid urbanisation, population growth, and inadequate wastewater management. The gap between wastewater generation and treatment remains a major environmental and public health challenge, particularly in developing countries [1,2]. In India, approximately 72,368 MLD of municipal wastewater is discharged without adequate treatment into receiving water bodies [3]. This contributes to water pollution, degradation of aquatic ecosystems, and increased public health risks.

Traditional technologies used for wastewater treatment, including the activated sludge process, sequencing batch reactor, and membrane bioreactors, may be impractical for use in decentralised systems in developing countries due to high initial costs, operating expenses, energy consumption, and maintenance challenges [4,5]. Therefore, decentralised and cost-effective wastewater treatment systems based on natural methods have been widely recognised.

Constructed wetlands (CW) have become an ideal solution for the treatment of domestic sewage because of their efficiency and sustainability. CWs utilise the synergistic effect of aquatic plants, bacteria, and filtration materials to eliminate organic waste, suspended matter, pathogens, and nutrients from domestic wastewater. The simple operation, low energy demand, and environmental benefits associated with CWs have made their use popular in many parts of Europe, North America, Asia, and Sub-Saharan Africa [6–8].

Although there are some significant benefits, one of the major problems with traditional CW systems that use gravel or sand filter beds is the problem of clogging of the filter bed, which limits the hydraulic conductivity to a degree of 40% after 3–5 years of installation [9]. This leads to poor water treatment capacity and high costs of maintenance that make such systems unsuitable for decentralised solutions [10]. Moreover, there is an additional problem of low oxygen supply due to filter bed saturation [11,12].

The MTBRCW has been introduced as an alternative wetland that addresses the issues of clogging associated with traditional CWs. Unlike conventional systems that rely on porous granular media, the MTBRCW uses bio-racks arranged in vertical positions, where the inert media offer vast surface area for microbial colonisation without restricting the passage of water [13–15]. There is substantial evidence regarding the efficiency of bio-racks in removing up to 85% of BOD [13,14,16].

The choice of plants is very important for the performance of CWs because of the variation in the root structure, oxygen transfer capability, and nutrient uptake ability of plants [17,18]. Amongst the wetland plants used, *Typha angustifolia* and *Chrysopogon zizanioides* (vetiver grass) have shown great potential in terms of their ability to treat pollutants and produce high biomass [19]. The application of both of these plants in a dual-stage MTBRCW provides an efficient mechanism for treating strong influent in the initial stage and polishing nutrients in the second stage [18,20].

The current research work is the first pilot-scale experimental study for a duration of 13 months concerning the application of the MTBRCW system to treat real household wastewater at Indian institutions, along with an eight-level Batch Mode HRT optimisation exercise. The objectives of this research work are to: (i) assess the removal efficiency of the system for BOD, COD, TN, and TP in practical Continuous Flow Mode operation; (ii) deter-

mine the effect of HRT on treatment efficacy in a Batch Mode operation; (iii) investigate the hydraulics and resistance to clogging throughout the entire experimentation period; and (iv) test the feasibility of using the MTBRCW process as a sustainable and decentralised option to treat household wastewater in the Indian perspective, according to CPCB discharge criteria and SDG 6 goals. The main contributions of the current study include the following: (i) it is the first pilot-scale experimental analysis on the MTBRCW system conducted over a period of 13 months under actual domestic sewage conditions in India; (ii) it is the first quantitative optimisation of HRT parameters for the MTBRCW system based on eight-level experiments in Batch Mode; (iii) it shows effluents in compliance with CPCB criteria in terms of BOD and COD over all 13 months, TN in 12 out of 13 months (one non-compliance event in July 2025 only), and TP in all 13 months, without any hydraulic clogging over the entire 13 months of its operation; and (iv) it presents a unique arrangement of two sequential plants—*T. angustifolia* at the inlet high-load container followed by *C. zizanioides* (*vetiver grass*) in the polishing container—thereby creating a stratified aerobic/anoxic condition using one gravity-fed and media-free treatment tower. Table 1 quantitatively compares the present study with other bio-rack systems and media-free CWs found in the literature.

1.1. Evolution of Constructed Wetlands for Wastewater Treatment

The scientific justification for the CW treatment process can be attributed to initial research work carried out at the Max Planck Institute in the 1990s on the pollutant removal potential of wetland macrophytes in controlled reed beds [21,22]. Further evolution of HSSF and VSSF CW types provided better control of hydraulic conditions and lessened odour issues related to open water systems [11]. The reviews conducted by Vymazal over decades showed that BOD reduction rates of 70–90% and TN removal rates of 40–80% were observed in various CW systems using different plant types under different climatic regions [2,10]. As for the major parameters influencing the CW treatment efficiency, Kadlec and Wallace stated that hydraulic retention time and organic loading rate were key parameters to be considered [17,18]. CWs have been applied in India since the beginning of the 2000s in residential and industrial facilities; however, their implementation within national sanitation policy is still insufficient [23–25].

1.2. Limitations of Conventional Constructed Wetland Systems

Although CWs have several advantages, there is a well-known problem related to the structural failure of the CWs themselves, which consists of clogging of the filter bed made of porous media. The main causes of clogging in subsurface flow CWs include the deposition of suspended solids, microbial biofilm accumulation, and chemical precipitation, all of which progressively restrict hydraulic flow through the granular substrate [12]. A dramatic decrease in hydraulic conductivity of the porous media in just 3–5 years after starting full-scale operation was noted by Aiello et al. [9], thereby substantially decreasing the economic feasibility of such CW structures. Another issue linked with clogging is oxygen limitation. According to Fu et al. [13], clogging prevents the formation of the aerobic zone beneath the upper layer in the filter bed. Bassi et al. [26] highlighted that the long-term ecological and functional sustainability of wetland systems in India is severely compromised by progressive substrate degradation and loss of hydraulic functionality, reinforcing the need for media-free design alternatives. Karungamye [27] showed that oxygen released in the roots of macrophytes is not enough to maintain the aerobic zone at regular loading rates. Jones et al. [28] stated that maintenance requirements due to media clogging and aeration costs eliminate any cost-effectiveness of the conventional CW approach.

1.3. Bio-Rack Structures and Media-Free Constructed Wetland Designs

The idea of designing CWs without using any media stemmed from the understanding that, despite serving as an excellent support medium and surface for the growth of microorganisms, the substrate also presents the most challenging technical difficulties in traditional CWs. Sathe and Munavalli [15] proposed and experimentally tested the new bio-rack wetland approach, showing that specially designed perforated panels can substitute for gravel media, resulting in removal efficiencies of greater than 85% BOD and 80% COD. Most importantly, no evidence of hydraulic clogging occurred within the experimental period in their CW systems.

Koli and Munavalli [16] took the investigation to the field scale by evaluating baffled/bio-rack CW configurations and found that the incorporation of bio-racks along with the presence of *Typha* as the key macrophyte consistently resulted in BOD and total nitrogen removal efficiencies of more than 75% despite variable loading rates, which is a condition that typically reduces media-based CW system efficiencies because of the increased clogging problem. More recently, Gujar and Munavalli [14] evaluated bio-rack CW systems using *Typha angustifolia* plants and demonstrated efficient removal of BOD levels higher than 80% and total phosphorus levels of close to 70%.

In the study of Fernández et al. [29] and Hassan et al. [30], media-free and bio-rack CW designs were systematically reviewed, showing that the absence of the substrate changes the focus of treatment from media-surface biofilm to root zone microbes and open water, improving oxygen levels, gas transfer, and hydraulic uniformity. Similarly, Liu et al. [19] showed that bio-racks could also be used alongside additional treatment options, in this case, an electrolysis process, which resulted in over 90% nitrogen removal and 85% phosphorous removal, indicating that bio-racks can accommodate process intensification.

Finally, the hydraulic benefits of media-free CWs have been shown in the study of Hassan et al. [31]. Using computational fluid dynamics modelling, it was proven that CWs modified to include bio-racks had substantially higher flow uniformity than conventional gravel-bed systems. In addition, some researchers showed that plant types and surface loading rates were essential factors determining hydraulic efficiency in shallow subsurface CWs, where more efficient hydraulic flow was observed in dense root systems without solid media [32].

1.4. Role of Macrophyte Species in Constructed Wetland Performance

The choice of macrophyte species in a CW system is crucial for its treatment efficacy due to several mechanisms by which the plants contribute to the treatment processes. Plant roots provide an essential habitat for the bacteria participating in most of the biological processes in the CW system, as they offer an environment for attachment, release oxygen into the rhizosphere, and release organic matter that enhances bacterial growth [8,32]. The above-ground biomass of the plant contributes to creating a thermal microclimate in the treatment zone, protecting it from extreme temperatures, reducing evapotranspiration, and stabilising the temperature regime [33]. Oxygen transport capabilities of macrophyte roots are a key factor for aerobic treatment in CWs because the radial oxygen loss (ROL) rate varies from one macrophyte species to another [6,34].

A number of studies have shown that *Typha angustifolia* is a highly productive plant for CW systems receiving a high organic load. Ansari et al. [35] demonstrated high rates of ROL in the roots of *Typha*, which allowed the bacteria in the rhizosphere to conduct nitrification despite the strong anaerobic conditions caused by the organic load. According to Kumar and Singh [36], *Typha* has a significant role in nitrogen and phosphorus removal from subsurface CWs, where the contribution of nitrogen removal by *Typha* could be up to 30% under ideal conditions.

Chrysopogon zizanioides (vetiver grass) has been receiving much attention from researchers with regard to CWs and phytoremediation due to its unique deep root system, productivity, and resistance to extreme pH, temperature, salinity, and pollutant concentrations [36]. According to Truong et al., vetiver roots have been observed to grow to depths beyond 3 m, allowing the plant to access nutrients from deep pools in the soil and create treatment potential through the entire soil column depths, which far exceed those achieved by any common macrophyte species used for CW construction [17]. Ansari et al. [35], who studied vetiver and *Typha* in their respective horizontal flow CWs treating domestic wastewater, found that the use of both plants allowed for a removal efficiency of nutrients that was 80% greater than that of the control systems; the higher efficiency was attributed to the complementary rooting systems of both plant types. Further field-based confirmation on the usefulness of *T. angustifolia* was provided by Gujar & Munavalli [14] in their study on CW systems in India; the authors showed that the plant maintained its effectiveness in removing nutrients despite seasonal variations in temperature and influent loads [37].

1.5. Performance Summary of Relevant Studies

Table 1 summarises the treatment performance and key conclusions reported across relevant constructed wetland studies considered in the present work, encompassing bio-rack systems, hybrid configurations, media-free designs, and conventional CW reviews.

Table 1. Summary of relevant studies on bio-rack and constructed wetland systems for domestic wastewater treatment.

Authors and Year	Wetland Category	Removal Efficiency	Conclusion	Novelty vs. Present Study
Sathe & Munavalli (2019) [15]	Modified Bio-rack Wetland	BOD: 85%, COD: 80%	Bio-rack structures effectively replace gravel media without loss of treatment performance	Lab-scale only; no long-term monitoring; single plant species; no HRT optimisation; and no CPCB compliance assessment
Koli & Munavalli (2021) [16]	Baffled and Bio-rack Hybrid CW	BOD + N: >75%	<i>Typha angustifolia</i> significantly enhances pollutant removal under fluctuating loads	Hybrid baffled configuration; shorter monitoring duration; no systematic Batch Mode HRT experiments; and no mass balance
Greenway (2007) [32]	Macrophytes in Nutrient Removal via CWs	N: 60–80%, P: 50–70%	Macrophyte species selection critically determines nutrient uptake efficiency and overall CW treatment performance	Review study only; no original experimental data; no media-free bio-rack design; no HRT optimisation; and no Indian regulatory compliance assessment
Hassan et al. (2021) [30]	Media-Free CW Review	BOD: 80–90%, N: 70–80%	Bio-rack systems eliminate clogging and improve gas diffusion	Review study only; no original experimental data; no pilot monitoring; and no HRT optimisation experiments
Hu, S. et al. (2023) [37]	Modified CW Systems	COD: >85%, P: 90%	Modifications significantly improve treatment capacity and reliability	Modified CW with mechanical components; no media-free bio-rack design; and no 13-month continuous monitoring dataset
Soares et al. (2025) [38]	Bio-rack Wetland HRT Study	BOD: 70–90%, N: 50–85%	HRT is a key determinant of bio-rack CW performance in tropical climates	Single HRT study; no long-term continuous monitoring; no CPCB compliance assessment; and no mass balance
Vymazal (2011) [2]	CW Review (5 decades)	BOD: 85%, COD: 92%, N: 75%	CWs represent sustainable, cost-effective solutions for decentralised treatment	Review of conventional gravel-bed CWs; no media-free design; clogging risk inherent; and no batch HRT data

Table 1. Cont.

Authors and Year	Wetland Category	Removal Efficiency	Conclusion	Novelty vs. Present Study
Kumar et al. (2025) [39]	Bio-electrochemical and Tidal Flow CW Review	Performance improved with advanced CW configurations	Advanced bio-electrochemical and tidal flow CW modifications significantly improve nitrogen and organic removal	Review study only; no original experimental data; no media-free bio-rack design; and no Indian regulatory compliance
Xia et al. (2025) [40]	Nitrogen Removal Mechanisms in CW (Critical Review)	Improved hydraulic uniformity	Key mechanisms of nitrogen removal from rural domestic wastewater in CWs are identified	Review of nitrogen mechanisms only; no pilot-scale experimental data; no media-free design; and no Indian regulatory compliance
Gomes, P. et al. (2026) [41]	CW Characterisation Overview	BOD: 70–90%, N: 50–80%	CWs are safe and sustainable solutions for water resource treatment across diverse applications	Overview/review study only; no original experimental data; no media-free bio-rack design; no HRT optimisation; and no Indian regulatory compliance assessment
Aiello et. al. (2016) [9]	CW Clogging Review	Hydraulic conductivity reduced 40%+	Clogging is the primary long-term failure mechanism in media-based CWs	Clogging review of conventional systems; no media-free solution presented; and no treatment performance data
Present Study (MTBRCW)	Pilot-scale Media-free Bio-rack CW	BOD: 89.8%, COD: 87.5%, TN: 78.2%, TP: 74.4%	MTBRCW achieves CPCB-compliant treatment with no clogging over 13 months under Indian institutional conditions	A 13-month pilot-scale monitoring study; 8-level batch HRT optimisation; two-species sequential configuration; no clogging observed; 11/13 months CPCB compliance; Indian decentralised context; and mass balance analysis

1.6. Research Gap

A critical review of the existing literature highlights a number of areas where there is a need for further research, which will be effectively addressed by the current experimental study. Firstly, traditional CW technologies depend on porous media like gravel or sand as the main substrate for biofilm formation and pollutant removal, yet media clogging is extensively reported as the leading mode of failure in traditional CWs [13,25], resulting in decreasing hydraulic conductivity and efficiency over time. The MTBRCW solves this major issue by functioning as a completely media-less constructed wetland where bio-rack units replace porous media, removing the fundamental reason behind clogging without compromising any other essential requirements for biological treatment. Nevertheless, there is still no detailed long-term investigation that studies media-less bio-rack CW technology with a sole focus on proving the zero clogging behaviour of these CWs. Firstly, although the existence of MTBRCW has been shown experimentally through the work of Gujar and Munavalli [14], Sathe and Munavalli [15], and Koli and Munavalli [16], no systematic and prolonged experimental evaluation of the performance of this system under both continuous and Batch Modes of operation, along with multiple parameter measurements and high-frequency testing according to CPCB discharge standards, has been reported before. Secondly, the effect on the performance of using the two wetland plants *Typha angustifolia* and *Chrysopogon zizanioides* (vetiver grass) in sequential two-container MTBRCW systems has not been sufficiently understood. Thirdly, the relation between the hydraulic loading rate, organic loading rate, and hydraulic retention time in an MTBRCW system has not been studied using a Batch Mode HRT experiment like that reported here. Lastly, the climatic conditions of the Western Maharashtra region of India, marked by the variability

in loadings during monsoons, have not been examined for their impact on the performance of bio-rack systems.

2. Methodology

2.1. Experimental Setup and Design

Experimental testing was conducted at the School of Civil and Environmental Sciences, JSPM University, Pune, Maharashtra, India. The MTBRCW system employed in the experiment was made using the pilot-scale design containing three units connected in series: the inlet wastewater storage tank of dimensions $0.55\text{ m} \times 0.60\text{ m} \times 0.30\text{ m}$ (total volume = 0.099 m^3), followed by two units of wetland treatment tanks each of dimensions $0.38\text{ m} \times 0.36\text{ m} \times 0.25\text{ m}$ (total volume = 0.034 m^3 each), making a total system volume of around 0.167 m^3 (167 L) (Figure 1). The system has a total height of 1.6 m, which is placed in a stacked tower formation, from which the name was derived. Inlet wastewater enters the elevated inlet tank from where it is discharged under gravity through the two wetland units joined by PVC pipes, having control valves installed in each connection to control flow rates. This system works through a media-free subsurface constructed wetland wherein bio-racks made of readily available and chemically stable HDPE material act as the only physical substrate necessary for the growth of plants and biofilm formation without utilising any granular, porous medium like gravel, sand, or soil. The use of a media-free subsurface in this system specifically means the lack of granular porous media, which are the principal source of clogging in conventional CWs. Bio-rack panels are non-porous and consist of vertically perforated panels that offer sufficient biofilm attachment surfaces while ensuring unimpeded open flow channels, making this system inherently different from granular media-based systems [14–16,31].

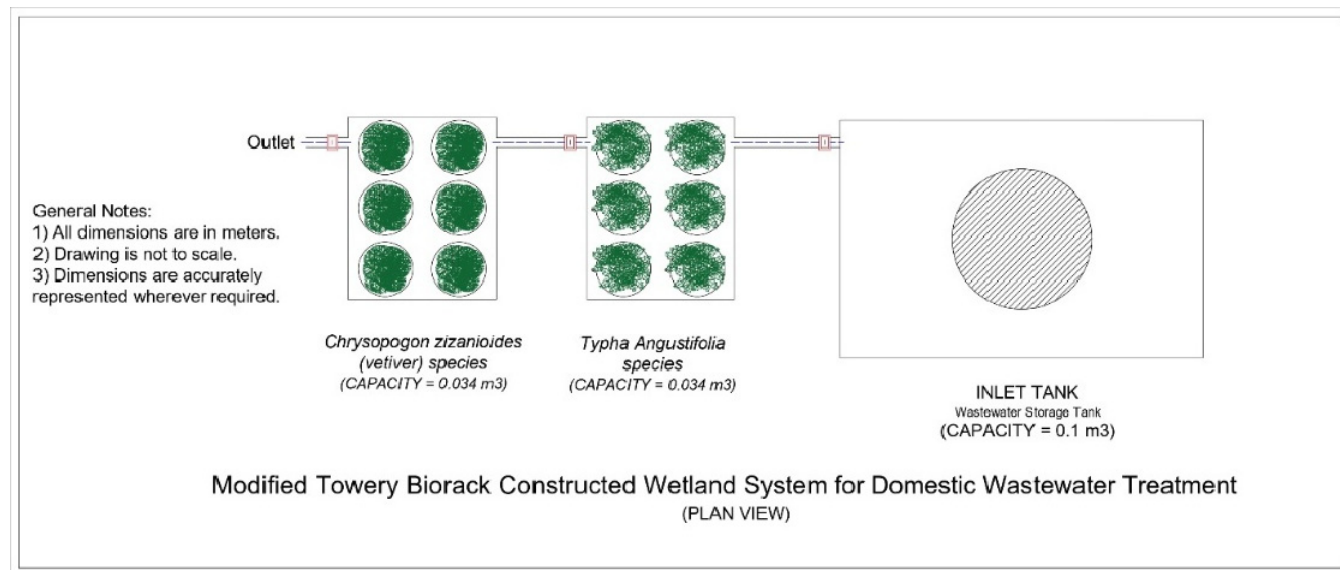


Figure 1. Plan view of the MTBRCW system showing the inlet tank (0.1 m^3), wetland containers with *Typha angustifolia* (Container 1) and *Chrysopogon zizanioides* (vetiver grass) (Container 2), and the outlet arrangement.

Bio-rack structures were designed using perforated panels installed vertically, with regular spacing between panels in each container within the wetland setup. The design helped create a large surface area for biofilm formation without posing any problems of blockage because the flow channels remained open. Each container had six slots for planting plants in a 3×2 arrangement pattern, as illustrated in Figure 2 (plan view of the wetland setup). Wastewater enters the container via the top connecting pipe fitted with a

control valve. After flowing through the root matrix, it was drained out via the outlet at the bottom of the lower container.

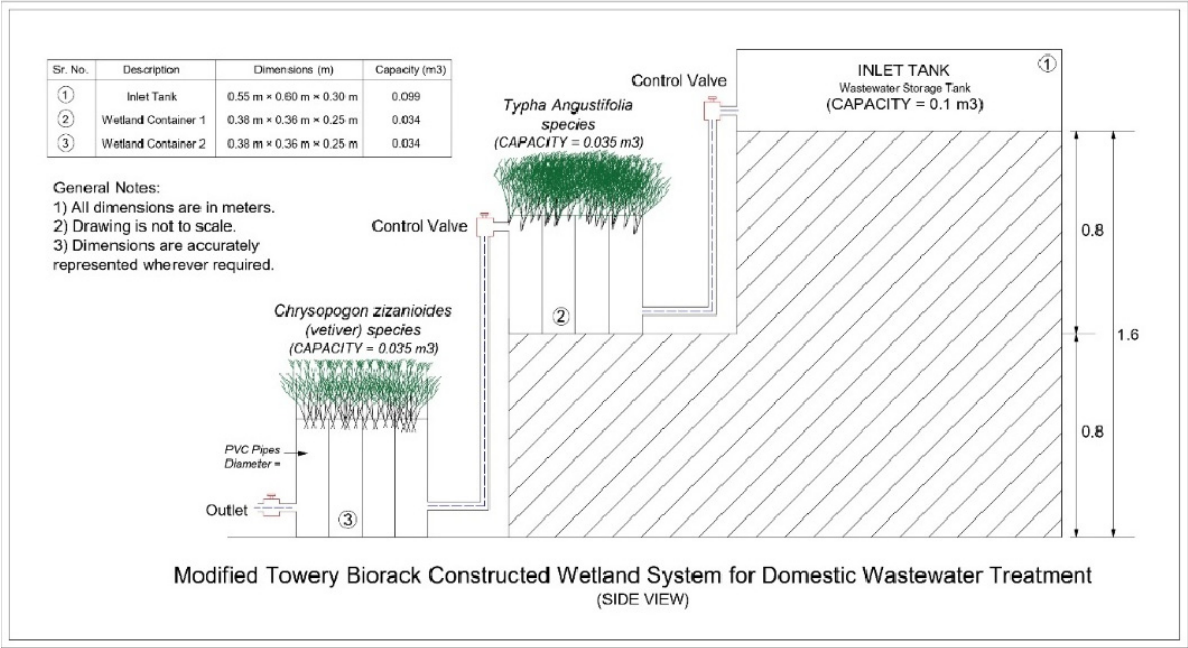


Figure 2. Side view of the MTBRCW system showing the gravity-flow configuration, tower height (1.6 m), PVC connecting pipes, control valves, and component dimensions.

The developed pilot-scale MTBRCW system is depicted in Figure 3, illustrating the integrated treatment process with the inclusion of the influent equalization tank, bio-rack wetlands reactors and gravity-based hydraulic flow connections. The first treatment step was populated with *Typha angustifolia*, and the development of its roots in the bio-rack reactor matrix is illustrated in Figure 4, whereas the second step was populated with *Chrysopogon zizanioides* (vetiver grass) as illustrated in Figure 5. Well-developed root systems of both macrophytes formed a dense rhizosphere network (Figure 6) that provided a large surface area for biofilm formation and development, aiding filtration, sedimentation, increased oxygen delivery to the rhizosphere and microbial degradation and plant uptake of organic substances and nutrients.

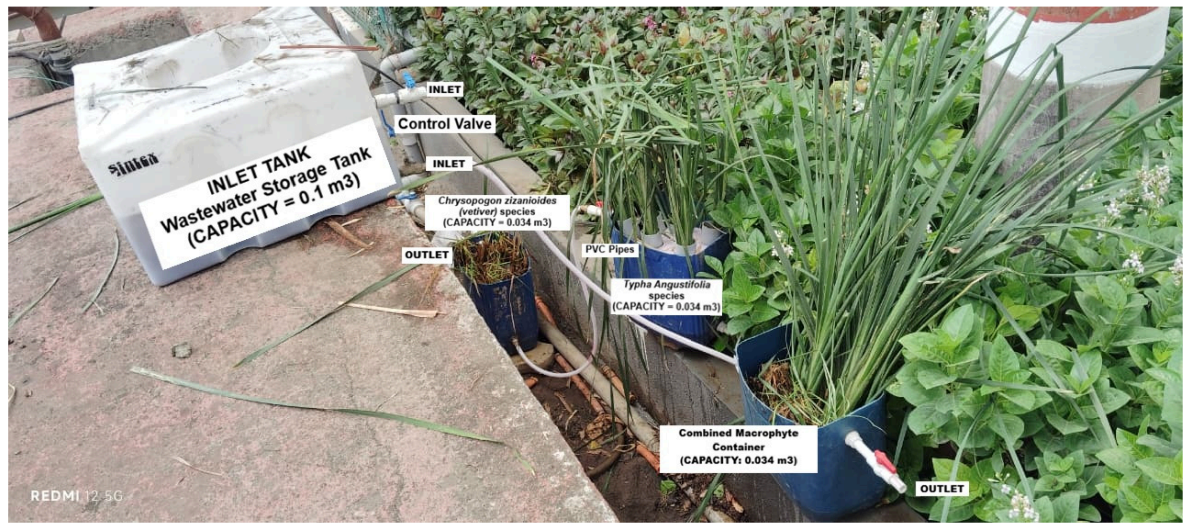


Figure 3. Photographic view of the experimental MTBRCW setup showing the inlet tank, two-stage wetland containers, and gravity-flow PVC connections.



Figure 4. *Typha angustifolia* established in Container 1 (first stage) of the MTBRCW system, showing the bio-rack planting arrangement and the developed root matrix.



Figure 5. *Chrysopogon zizanioides* (vetiver grass) established in Container 2 (second stage) of the MTBRCW system.



Figure 6. Combined root zone of *Typha angustifolia* and *Chrysopogon zizanioides* (vetiver grass) showing the dense root matrix developed within the bio-rack container.

2.2. Plant Species Selection and Establishment

The MTBRCW system consists of two macrophyte species with excellent track records in CW operations: *Typha angustifolia* (cattails) and *Chrysopogon zizanioides* (vetiver grass). The choice of these plants was guided by their root characteristics, which complement each other. Specifically, *Typha* is characterised by an extremely dense lateral root system that thrives under high load conditions at the inlet point. Meanwhile, vetiver's highly fibrous nature makes it an ideal species for removing any remaining pollutants along the way through its ability to polish the water. Other advantages of the two species include their excellent nutrient uptake rate, ability to tolerate extreme conditions, and effectiveness in analogous systems [42–44]. In the MTBRCW system, the *Typha* plant was introduced into Container 1, which handles the raw influent and has the highest organic and hydraulic load. The *vetiver*, on the other hand, was planted in Container 2 because of its fine root structure, which removes residual contaminants further downstream.

The plant samples were collected from a nearby nursery and were acclimated in diluted institutional sewage for four weeks prior to initiating the experiment. The acclimation process provided adequate time for the root system to attach itself to the bio-rack modules, and the formation of the first microbial films was initiated in this phase. Each container contained six plants arranged in a 3×2 grid pattern, which was equivalent to an effective surface area of $0.38 \text{ m} \times 0.36 \text{ m}$ (0.137 m^2).

2.3. Wastewater Source and Characterisation

Domestic wastewater in its crude form was used to supply the CW. It comprised a mixture of grey water and black water from an Indian institution. The qualities of the wastewater were in conformity with those specified in CPCB guidelines [1]. The wastewater samples were taken and tested for all target parameters. Here, S1 and S2 represent two periodic influent samples collected from the same MTBRCW system during the first and second halves of each month, respectively. As illustrated in Figure 7, the raw BOD of influent wastewater (S1 and S2) ranged approximately between 210 and 305 mg/L over the monitoring period from January 2025 to January 2026, with S1 generally recording slightly higher BOD values than S2. Similarly, Figure 8 depicts the raw total phosphorus (TP) levels in the influent, which varied between roughly 5.7 and 10.5 mg/L across both sites, with S2

occasionally exceeding S1, particularly during the monsoon and post-monsoon months. Average values for various qualities of the influent are presented in Table 2 below.

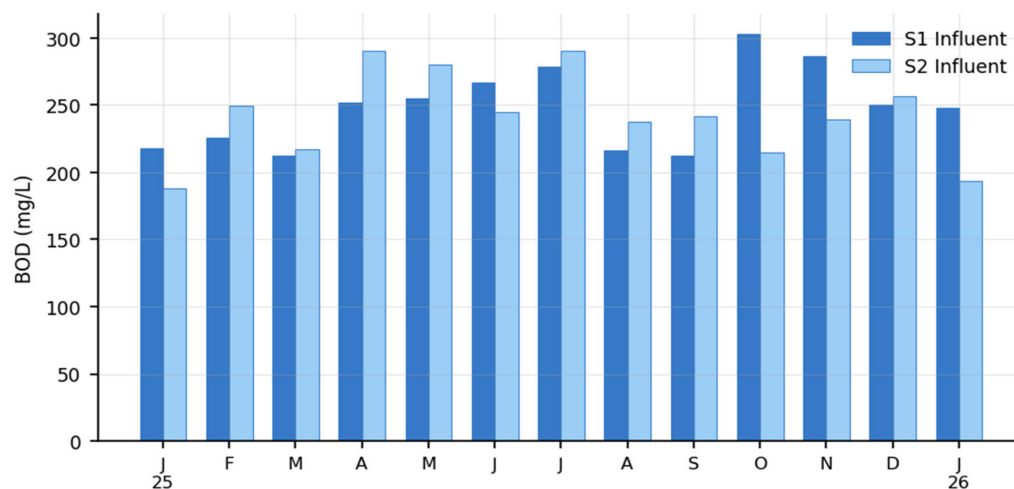


Figure 7. Raw BOD: S1 vs. S2 influent comparison across 13 months (mg/L).

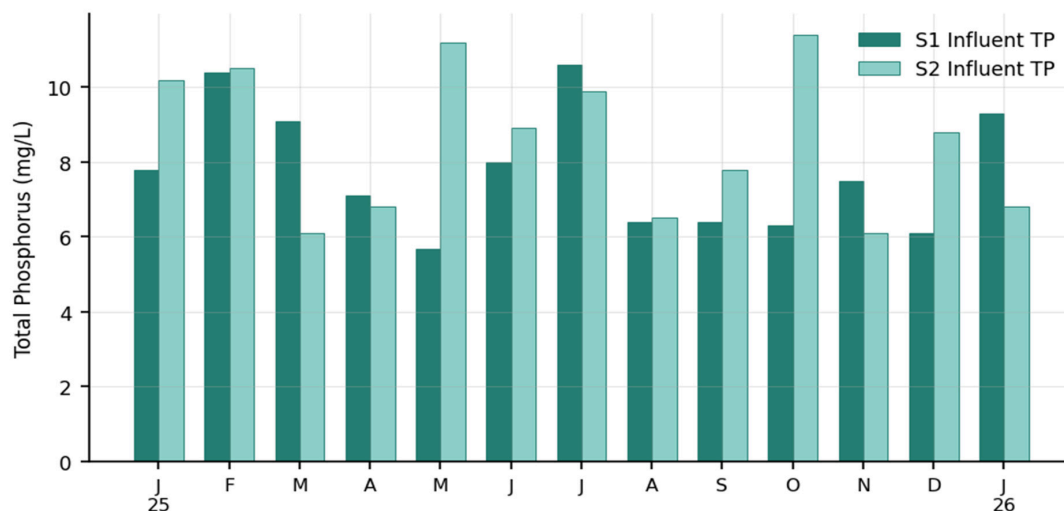


Figure 8. Raw TP: S1 vs. S2 influent comparison across 13 months (mg/L).

Table 2. Average influent domestic wastewater characteristics and CPCB discharge standards.

Parameter	Unit	Influent Range	Average Influent	CPCB Standard
pH	-	6.8–7.8	7.2	6.5–8.5
BOD	mg/L	180–280	235	≤30
COD	mg/L	420–680	540	≤250
TSS	mg/L	150–310	225	≤100
TDS	mg/L	520–880	710	≤2100
Total Nitrogen (TN)	mg/L	28–48	38	≤10
Total Phosphorus (TP)	mg/L	4.5–9.8	7.1	≤5
Ammonia (NH ₃ -N)	mg/L	22–44	33	≤50
Faecal Coliform	MPN/100 mL	10 ⁴ –10 ⁶	~10 ⁵	≤1000

Note: The CPCB standards stated in Table 2 above refer to the “General Standards for Discharge of Environmental Pollutants” in Schedule VI (Part A), which applies to discharge into inland surface water bodies, issued under the Environment (Protection) Rules, 1986 (amended), as per the provisions of the Environment (Protection) Act, 1986. The standards have been laid down by the CPCB of India, which apply to treated domestic sewage effluent discharged into inland water bodies through decentralised treatment systems.

2.4. Experimental Protocol and Loading Conditions

The experiment was conducted in two modes, viz., Continuous Flow Mode and Batch Mode, over an observation period of thirteen months between January 2025 and January 2026. For the Continuous Flow Mode, the domestic wastewater was introduced into one pilot-scale MTBRCW reactor using gravity flow from the inlet storage tank. Two samples (S1 and S2) were obtained from this reactor every sampling month during two different times to reflect the natural variation in influent wastewater quality on that day. Thus, the S1 and S2 samples are temporal replicates within one MTBRCW unit. There were 26 pairs of raw samples over the course of the 13-month period. Both influent and effluent samples were collected simultaneously at each sampling time point and were evaluated for their pH value, BOD, COD, TN, and TP. Average monthly data were calculated based on the results obtained from the two samples and were used for performance analysis. As shown in Figure 9, the hydraulic loading rate in the Continuous Flow Mode varied between $0.18 \text{ m}^3/\text{m}^2\cdot\text{d}$ and $0.28 \text{ m}^3/\text{m}^2\cdot\text{d}$ throughout the monitoring period, with the highest rates recorded in July and August of 2025, reflecting increased inflow during the monsoon season. The corresponding organic loading rate (OLR) ranged from about 0.037 to $0.080 \text{ kg BOD}/\text{m}^3\cdot\text{d}$ throughout the course of experimentation. Batch Modes were performed for eight HRTs, namely, 1, 2, 3, 4, 5, 6, 7, and 8 days, while maintaining all other parameters constant. It must be noted that each individual HRT was assessed only by a single batch cycle ($n = 1$), rather than with independently replicated batch cycles, thereby representing an inherent drawback of the batch test design. Since no replicates were carried out for the various levels of HRT, it is not possible to statistically segregate the influence of HRT from other biological variations on a daily basis. The above discussion implies that the present batch test results can only serve as an indicator of HRT-dependent performance trends. However, despite the aforementioned limitation of the batch test, the systematic increase in efficiency with HRT at all eight HRT levels, as well as the correlation between HRT performance and first-order kinetics [3,11], suggests that the trends observed can be relied upon with some degree of assurance.

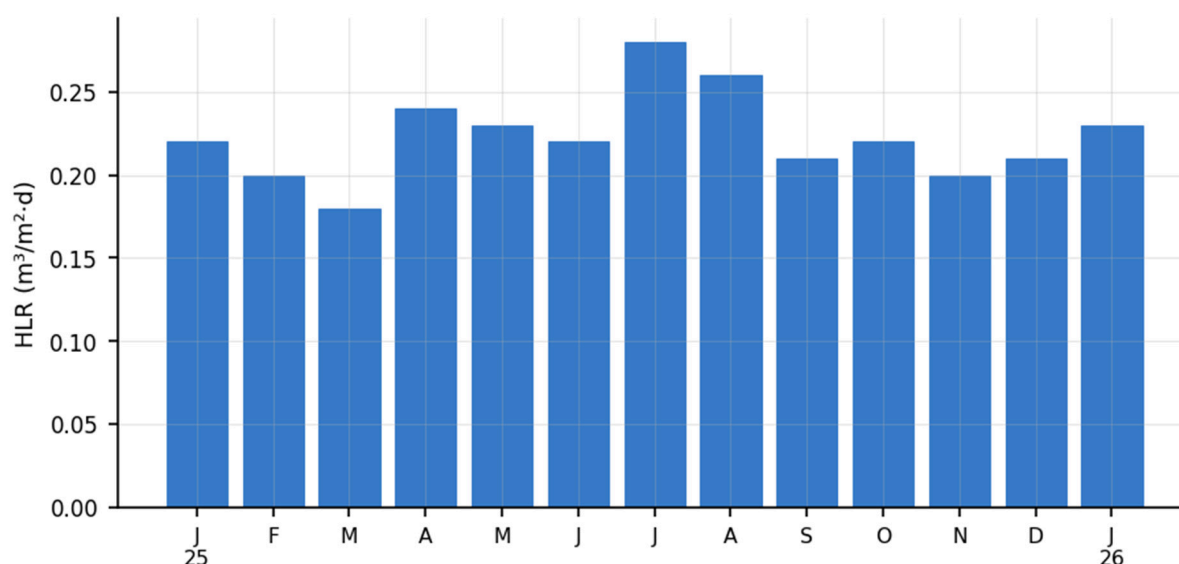


Figure 9. Monthly hydraulic loading rate (HLR) variation during Continuous Flow Mode operation ($\text{m}^3/\text{m}^2\cdot\text{d}$).

Hydraulic Characterisation

The hydraulic characterisation of the MTBRCW process system was performed using the data obtained from the following set of measurements and calculations. The biological

treatment volume of the MTBRCW comprises the two wetland containers only, with a combined liquid capacity of 68 L (0.068 m^3 ; 0.034 m^3 per container). The inlet storage tank (0.099 m^3) functions solely as a feed reservoir and does not contribute to biological treatment; it was therefore excluded from HRT calculations. The total system volume, including the storage tank, is 167 L (0.167 m^3), but only the wetland volume of 68 L is used for treatment HRT determination. The surface area of each wetland tank amounted to 0.137 m^2 ($0.38 \text{ m} \times 0.36 \text{ m}$). The water depth in each tank was kept at the design level of 0.25 m throughout the duration of monitoring, as verified weekly by visual observation of the water level.

The actual flow rate was determined weekly at the outlet of the system by using calibrated volumetric measurements, i.e., timed water collection in a graduated cylinder. Average monthly flow rates varied between 24.7 L/d and 38.4 L/d, depending on the monitored range of HLR of $0.18\text{--}0.28 \text{ m}^3/\text{m}^2\cdot\text{d}$. The nominal HRT of the Continuous Flow Mode process is calculated as follows:

$$\text{HRT (days)} = \text{Effective liquid volume (L)} \div \text{Flow rate (L/d)}$$

Using the wetland-only biological treatment volume of 68 L (excluding the inlet storage tank), and the observed flow rate range of 24.7–38.4 L/d, the corrected continuous operation HRT is:

$$\text{HRT (minimum)} = 68/38.4 = 1.77 \text{ days (at peak HLR of } 0.28 \text{ m}^3/\text{m}^2\cdot\text{d, July 2025)}$$

$$\text{HRT (maximum)} = 68/24.7 = 2.75 \text{ days (at minimum HLR of } 0.18 \text{ m}^3/\text{m}^2\cdot\text{d)}$$

The corrected Continuous Flow Mode HRT range is therefore approximately 1.8 to 2.8 days. The Figure 10 shows the actual variations of HLR and OLR of the present study.

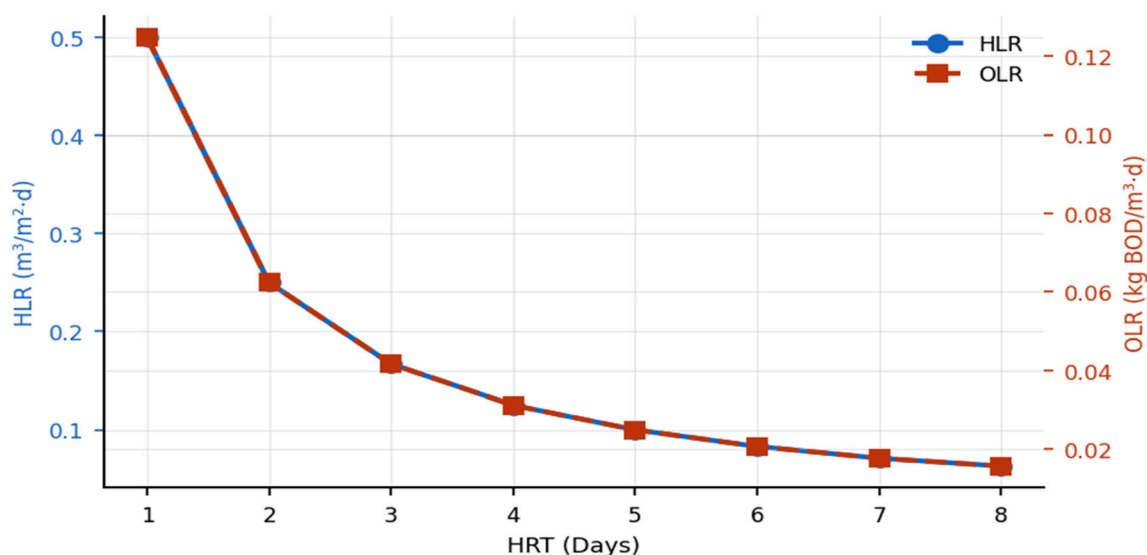


Figure 10. Batch mode: HLR and OLR variation with increasing HRT (days 1–8).

As the MTBRCW system does not use granular porous media for the bio-rack panels, the effective porosity of the wetland boxes would be approximately 1.0, which means that the full volume of the box can act as a water retention space for calculating HRT, negating the need for the porosity correction factor often considered in CW hydraulics.

As for short-circuiting and dead zone evaluation, no tracer study was carried out in this pilot-scale experiment. The natural downward gravity-fed flow through sequentially stacked vertical bio-rack panels makes short-circuiting unlikely in this case, as per the

computational fluid dynamics simulation carried out by Hassan et al. [31], where the bio-rack design showed much better performance than a free-surface CW regarding flow distribution uniformity. The uniform quality of effluents from both sample points S1 and S2 over 13 months is further proof of good flow distribution. A future study for formal hydraulic efficiency indices should employ the residence time distribution analysis method using tracers.

The samples were then transferred to the laboratory in cold storage and tested within 6 h of sample collection to ensure there was no biological degradation. The analysis of all water quality parameters was carried out using the Standard Methods for water and wastewater (APHA, 2017) [43].

2.5. Analytical Methods

All the tests for water analysis were carried out according to APHA's "Standard Methods for the Examination of Water and Wastewater" [43] (2017). The BOD₅ test was carried out in the 5-day incubation method at 20 °C; the COD test was conducted using closed reflux colorimetry; the total suspended solids (TSS) test was conducted gravimetrically using membrane filtration; the total dissolved solids (TDS) test was carried out using a calibrated conductivity meter; total nitrogen was analysed using the persulfate digestion test and then by spectrophotometry; the total phosphorus test was conducted using the ascorbic acid method after persulfate digestion; ammonia nitrogen was tested using the indophenol blue spectrophotometric test; and faecal coliform was counted using the multiple tube fermentation most probable number (MPN) method. The pH and dissolved oxygen (DO) tests were conducted using calibrated portable meters in situ. DO was measured at two vertical positions within each wetland container, near the water surface (0.05–0.10 m depth) and near the container base (0.20–0.25 m depth), which is consistent with the maximum design water depth of 0.25 m per container. These two measurement points capture the upper aerobic zone and the lower treatment zone within each container, respectively. All in situ measurements were taken consistently between 09:00 and 10:00 h (IST) on each sampling day to minimise diurnal variability associated with photosynthetic oxygen production and nocturnal respiration of the macrophytes. While it is acknowledged that DO concentrations may vary between daytime and night-time due to plant photosynthesis and microbial respiration cycles, single time-point morning measurements are consistent with standard monitoring practice in comparable CW studies [16,31] and represent a conservative assessment, as DO concentrations tend to be lower in the morning than midday peak values.

2.6. Statistical Analysis

The removal efficiencies were estimated as follows: $RE (\%) = [(C_{in} - C_{out}) / C_{in}] \times 100$, where C_{in} and C_{out} are the concentrations of the influent and effluent, respectively. This formula was applied to BOD, COD, TN, and TP only. pH is not a concentration parameter, and removal efficiency does not apply to it; instead, pH shift ($\Delta pH = \text{Influent pH} - \text{Effluent pH}$) was used to characterise the change in pH across the system. The measures of central tendency (mean, standard deviation, and coefficient of variation) were computed for all parameters throughout the experiment. First Table 3 shows the full monitoring data then standard deviation values of the duplicate samples at different times (S1 and S2) are presented in Table 4 in the case of effluent concentration and removal efficiency, respectively, by calculating $SD = |S1 - S2| / \sqrt{2}$. A one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test was applied to evaluate statistically significant differences ($p < 0.05$) in monthly removal efficiencies across seasonal loading phases, using the thirteen monthly average values ($n = 13$) as the unit of

analysis. For this analysis, months were grouped into four seasonal categories consistent with the Indian climate: winter (Jan–Feb), summer (Mar–May), monsoon (Jun–Sep), and post-monsoon (Oct–Jan). The degrees of freedom for the between-group effect = 3 and the degrees of freedom for the within-group error = 9. This grouping provided sufficient replication at the seasonal level to satisfy the analytical requirements of ANOVA. Correlation studies were carried out using IBM SPSS Statistics software version 26. Prior to pooling the two replicate MTBRCW units (S1 and S2) for monthly average calculations, a paired-samples *t*-test was performed in IBM SPSS Statistics v26 to evaluate whether statistically significant differences existed between the two units across all monitored parameters. No significant inter-unit differences were detected for BOD ($t = -0.40$, $p = 0.698$), COD ($t = -1.55$, $p = 0.148$), TN ($t = -0.89$, $p = 0.392$), or TP ($t = 0.30$, $p = 0.769$) at the 95% confidence level ($p > 0.05$ in all cases), confirming that the two units performed equivalently and that pooling of monthly average values was statistically justified.

Table 3. Raw monitoring data—individual samples S1 and S2 for all parameters across 13 months (Jan 2025–Jan 2026). BOD, COD, TN, and TP in mg/L.

Sr.	Month and Year	Sample	pH Bf.	pH Aft.	BOD Bf. (mg/L)	BOD Aft. (mg/L)	COD Bf. (mg/L)	COD Aft. (mg/L)	TN Bf. (mg/L)	TN Aft. (mg/L)	TP Bf. (mg/L)	TP Aft. (mg/L)
1	Jan-25	S1	7.4	6.5	218	18.2	616.5	77.5	49.5	5.8	7.8	1.0
2	Jan-25	S2	7.0	6.9	188.2	17.8	594.0	71.2	34.1	9.1	10.2	0.9
3	Feb-25	S1	7.6	7.2	225.8	17.0	673.9	61.2	31.1	5.8	10.4	2.4
4	Feb-25	S2	7.6	7.2	249.3	32.5	523.4	71.5	48.1	9.3	10.5	2.3
5	Mar-25	S1	7.5	6.5	212.3	19.5	445.7	56.2	31.3	7.0	9.1	1.8
6	Mar-25	S2	7.2	6.7	217.0	31.8	593.5	74.2	32.9	10.0	6.1	1.8
7	Apr-25	S1	7.8	7.1	251.8	27.0	644.1	82.2	34.3	5.4	7.1	1.6
8	Apr-25	S2	7.4	7.0	290.2	20.0	595.4	64.0	50.0	8.2	6.8	1.5
9	May-25	S1	7.4	6.8	255.2	31.1	528.8	55.5	51.9	8.6	5.7	1.0
10	May-25	S2	6.9	6.8	280.0	22.0	441.5	63.3	51.9	8.7	11.2	3.1
11	Jun-25	S1	6.8	6.7	266.8	24.2	494.4	75.8	31.6	8.1	8.0	3.3
12	Jun-25	S2	7.7	6.8	245.1	17.4	662.3	86.8	35.9	9.4	8.9	1.3
13	Jul-25	S1	7.6	7.0	278.4	24.1	425.1	60.6	29.4	11.3	10.6	3.0
14	Jul-25	S2	7.1	6.6	290.4	32.0	447.3	68.3	30.6	10.2	9.9	1.2
15	Aug-25	S1	7.3	7.0	216.8	30.6	535.0	55.2	41.4	10.0	6.4	1.7
16	Aug-25	S2	7.8	7.1	237.6	23.8	456.5	55.8	36.8	9.1	6.5	1.5
17	Sep-25	S1	6.9	6.8	212.5	31.2	648.5	48.4	34.5	9.6	6.4	1.2
18	Sep-25	S2	7.7	7.1	241.7	28.9	634.9	54.1	31.2	8.0	7.8	2.1
19	Oct-25	S1	7.5	7.2	303.1	15.9	529.7	61.3	48.8	6.8	6.3	2.0
20	Oct-25	S2	7.2	6.8	215.0	31.5	540.2	86.3	41.7	5.5	11.4	3.0
21	Nov-25	S1	7.8	7.4	286.8	17.2	551.3	55.3	38.2	5.6	7.5	3.4
22	Nov-25	S2	7.1	6.9	239.6	22.0	673.9	92.8	41.8	9.9	6.1	1.6
23	Dec-25	S1	7.8	7.1	250.1	28.2	439.9	73.0	40.6	10.8	6.1	3.3
24	Dec-25	S2	6.9	6.7	256.4	26.8	486.2	50.8	49.5	6.8	8.8	2.4
25	Jan-26	S1	7.2	7.1	247.7	31.8	478.1	79.4	34.5	7.8	9.3	1.6
26	Jan-26	S2	7.1	6.8	193.7	22.7	684.6	92.8	30.7	6.6	6.8	3.2
CPCB	Standard	—	—	6.5–8.5	—	≤30	—	≤250	—	≤10	—	≤5

Note: S1 and S2 = two temporal replicate samples collected from the single MTBRCW unit on each monthly sampling day at different time points to capture daily variability. Inf. = influent; Eff. = effluent. CPCB = Central Pollution Control Board discharge standards for inland surface water.

Table 4. Monthly average influent and effluent quality—continuous flow mode (Jan 2025–Jan 2026).

Sr.	Month and Year	pH Inf.	pH Eff.	BOD Inf. (mg/L)	BOD Eff. (mg/L)	COD Inf. (mg/L)	COD Eff. (mg/L)	TN Inf. (mg/L)	TN Eff. (mg/L)	TP Inf. (mg/L)	TP Eff. (mg/L)	BOD Eff. SD	COD Eff. SD	TN Eff. SD	TP Eff. SD
1	Jan-25	7.20	6.75	203.1	18.0	605.3	74.4	41.8	7.5	9.00	0.95	0.28	4.45	2.33	0.07
2	Feb-25	7.60	7.20	237.6	24.8	598.7	66.4	39.6	7.6	10.45	2.35	10.96	7.28	2.47	0.07
3	Mar-25	7.35	6.60	214.7	25.7	519.6	65.2	32.1	8.5	7.60	1.80	8.70	12.73	2.12	0.00
4	Apr-25	7.40	7.25	271.0	23.5	619.8	73.1	42.2	6.8	6.95	1.55	4.95	12.87	1.98	0.07
5	May-25	7.15	6.95	267.6	26.6	485.2	59.4	51.9	8.7	8.45	2.05	6.43	5.52	0.07	1.48
6	Jun-25	7.25	7.00	256.0	20.8	578.4	81.3	33.8	8.8	8.45	2.30	4.81	7.78	0.92	1.41
7	Jul-25	7.35	6.80	284.4	28.1	436.2	64.5	30.0	10.8	10.25	2.10	5.59	5.44	0.78	1.27
8	Aug-25	7.55	7.05	227.2	27.2	495.8	55.5	39.1	9.6	6.45	1.60	4.81	0.42	0.64	0.14
9	Sep-25	7.30	7.10	227.1	30.1	641.7	51.3	32.9	8.8	7.10	1.65	1.63	4.03	1.13	0.64
10	Oct-25	7.35	7.00	259.1	23.7	535.0	73.8	45.3	6.2	8.85	2.50	11.03	17.68	0.92	0.71
11	Nov-25	7.45	7.35	263.2	19.6	612.6	74.1	40.0	7.8	6.80	2.50	3.39	26.52	3.04	1.27
12	Dec-25	7.35	6.90	253.3	27.5	463.1	61.9	45.1	8.8	7.45	2.85	0.99	15.70	2.83	0.64
13	Jan-26	7.15	7.00	220.7	27.3	581.4	86.1	32.6	7.2	8.05	2.40	6.43	9.48	0.85	1.13
—	Overall Avg.	7.34	7.00	245.0	24.8	551.8	68.2	39.0	8.2	8.14	2.05	Note: SD values represent the standard deviation between the two replicate samples (S1 and S2) calculated as SD = S1 – S2 / √2 for each monthly sampling event.			
	CPCB	6.5–8.5		≤30		≤250		≤10		≤5					

Note: Values represent monthly averages of two samples (S1 and S2). Inf. = influent; Eff. = effluent. All concentration values are in mg/L except pH.

3. Results and Discussion

3.1. System Start-Up and Stabilisation

Following a four-week plant adaptation period, the MTBRCW system began delivering progressively improving pollutant removal performance as the biofilm formed on the surface of the bio-rack and as colonisation of the roots of the macrophytes in the structural supports of each reactor vessel progressed. The start-up process described above is in agreement with the general pattern of start-up observed for media-less CW and bio-rack CW systems [15,30,31], wherein the duration of biofilm establishment is greater compared to gravel-bed systems owing to the lack of any initial microbial population within the matrix.

The analysis of dissolved oxygen data collected during the initiation of the monitoring phase (January 2025) showed that the oxygen penetration was much deeper in the media-free configuration of the bio-rack wetland than in comparable gravel-bed CWs found in the literature sources [6,13,27]. The average DO content recorded near the surface (0.05–0.10 m depth) of the wetland containers varied from 3.2 to 5.8 mg/L, while the DO measurements taken near the container base (0.20–0.25 m depth, corresponding to the maximum design water depth of 0.25 m per container) always exceeded the threshold value of 1.0 mg/L. Such results correspond well to both the predictions put forward by Lee, B.H. [8] about the dependence between media porosity and the depth of oxygen diffusion and to the experimental data obtained by Koli and Munavalli [16], who proved the existence of increased DO contents in bio-rack CWs in comparison with those of gravel beds. It should be noted that such high DO contents are especially beneficial to the process of nitrification because the main nitrifiers, namely, *Nitrosomonas* and *Nitrospira*, need dissolved oxygen concentrations of around 0.5 mg/L and higher to ensure oxidative processes.

3.2. BOD and COD Removal Performance

Table 3 illustrates the full monitoring data in terms of 26 individual samples taken over 13 months, starting from January 2025 to January 2026. Two samples (S1 and S2) were obtained every monthly sampling day from the only MTBRCW unit to account for the natural variation in the daily influent wastewater quality and treatment efficiency.

As such, S1 and S2 can be considered temporal replicates for that single MTBRCW unit and not independent unit replicates. In the case of S1, the BOD varied between 15.9 and 31.8 mg/L, whereas S2 exhibited 17.8–32.5 mg/L. The comparison between S1 and S2 using a paired-sample *t*-test indicated the absence of any statistically significant difference in the treatment efficiency at 95% confidence level in terms of BOD ($t = -0.40$, $p = 0.698$), COD ($t = -1.55$, $p = 0.148$), TN ($t = -0.89$, $p = 0.392$), and TP ($t = 0.30$, $p = 0.769$).

The MTBRCW showed highly consistent removal efficiency for BOD throughout the year at 86.8–92.6% in the monthly average range, with 89.8% mean BOD removal efficiency (Table 4). As clearly illustrated in Figure 11, the effluent BOD remained consistently well below the CPCB discharge limit of ≤ 30 mg/L across all thirteen months of monitoring, with an annual average effluent BOD concentration of 24.8 mg/L, demonstrating strong compliance with regulatory criteria despite the influent BOD fluctuating considerably in the monthly average range of 203–284 mg/L. The stark contrast between the tall blue influent bars and the uniformly low green effluent bars in Figure 11 visually reinforces the robustness and stability of the treatment system across varying seasonal conditions. The system performance for BOD removal is even better than the performance range (70–90%) shown by mature CW systems around the world, as reported by Vymazal [2,10]. It is worth mentioning here that the BOD removal efficiency of the system was highly consistent during monitoring of the year, despite significant variations in the BOD of the wastewater in the monthly average range of 203–284 mg/L (Table 3). This indicates the effectiveness of the MTBRCW system for BOD removal in highly variable domestic wastewater.

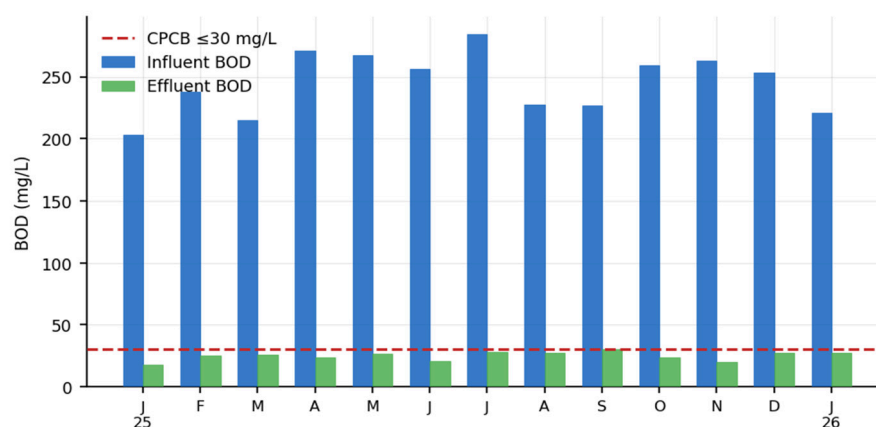


Figure 11. BOD—monthly influent vs. effluent (mg/L) with CPCB limit.

To test the significance of the variation in the monthly removal of BOD (86.8% to 92.6%), a Mann–Whitney U test was carried out on monsoon month values (June–September 2025, $N = 4$, mean = 89.20%) and non-monsoon month values (January–May 2025, October 2025–January 2026, $N = 9$, mean = 90.06%). The outcome of the test was not significant ($U = 13.5$, $p = 0.537$), which shows that the difference between the BOD removal efficiencies for both categories of months was not significant at a 95% confidence level. The above-mentioned result strengthens the previous one, according to which the MTBRCW plant showed stable BOD removal during all seasons, with only ~6% variation due to changes in influent.

However, a deeper analysis of system performance during peak periods of organic loading shows the importance of safety margins. The highest hydraulic loading rate ($0.28 \text{ m}^3/\text{m}^2 \cdot \text{d}$) observed during the peak month of monsoon flow in July 2025 corresponded to an average effluent BOD of 28.1 mg/L, which was below the CPCB limit of ≤ 30 mg/L but with a lower safety margin of about 1.9 mg/L. Specifically, sample S2 had an effluent BOD of 32.0 mg/L in July (Table 3), thus exceeding the limit marginally on one occasion. It is therefore clear that even though the MTBRCW system satisfies the regulatory

effluent BOD requirement under conditions of higher monsoon flows, it operates close to its treatment capacity during high flow and high influent BOD situations. For improved safety margins during the monsoon period, it would be necessary to either reduce the flow rate of water entering the system by 10–15% or treat the raw wastewater prior to entering through a primary settling tank before implementation at a larger scale. These results are similar to those presented by Vymazal [10] and Hu et al. [38].

The Batch Mode HRT investigation offers a mechanistic understanding of the time-varying kinetics of BOD removal within the MTBRCW system (Table 5). As shown in Figure 12, at an influent BOD concentration of 249.5 mg/L, the effluent BOD decreased sharply from 185.0 mg/L on day 1 (efficiency of 25.9%) to 12.8 mg/L on day 8 (efficiency of 94.9%), following a steep declining curve that gradually flattens with increasing HRT. The CPCB effluent limit of ≤ 30 mg/L was attained on day 5 (effluent BOD of 32.0 mg/L) and maintained consistently thereafter, with effluent BOD dropping further to 22.5 mg/L on day 6 onwards at 91.0% efficiency. A parallel trend was observed for COD as depicted in Figure 13, where the effluent COD declined from 420 mg/L at HRT = 1 day to 55 mg/L at HRT = 8 days, crossing the CPCB limit of ≤ 250 mg/L between day 3 and day 4 and remaining well within compliance from day 4 onwards. Figure 14 further consolidates these findings by presenting the progressive improvement in both BOD and COD removal efficiencies with increasing HRT, where BOD removal efficiency consistently outpaced COD removal across all HRT values, with both curves rising steeply between days 1 and 4 before gradually plateauing beyond day 5. The removal rate exhibited a typical first-order kinetics decline profile observed in CWs by Metcalf & Eddy [3] and Kadlec and Wallace [11], showing the highest rate of increase in performance between days 1 and 4, followed by progressively lower efficiencies thereafter. This is consistent with the shift from substrate-controlled to diffusion-controlled biodegradation after consumption of readily degradable organics and increasing recalcitrance of BOD [13].

A linear regression ANOVA test was conducted on HRT and the effect on removal efficiency in Batch Mode based on the eight levels of HRT for days 1–8. This indicated a very significant role of HRT in BOD removal efficiency ($F = 29.6$, $r^2 = 0.832$, $p = 0.002$) and COD removal efficiency ($F = 35.6$, $r^2 = 0.856$, $p < 0.001$). In this regard, HRT can be considered to have a very significant statistical role in the determination of performance in the MTBRCW process. Comparing HRT in the early phase (days 1–4 with average removal of BOD at 57.1%) to HRT in the late phase (days 5–8 with average removal of BOD at 91.6%) indicated a very significant statistical difference ($t = -2.87$, $p = 0.029$). On the other hand, there is a marginal improvement from day 5 (removal of 87.2%) to day 8 (removal of 94.9%) with a difference of 7.7%. Thus, HRT in days 5–6 can be considered optimal.

It is important to mention here that HRT recommendations based on Batch Mode tests conducted in the present research are unique for a particular value of BOD concentration in the influent, which is equal to 249.5 mg/L and is typical for moderately or strongly polluted domestic wastewater generated in peri-urban and institutional areas of India. In the case of weakly polluted wastewater (BOD < 100 mg/L), as it may happen in rural or decentralised residential units, HRTs lower than those recommended in the present work are sufficient to attain the effluent quality that meets the standards established by the CPCB, considering the lower amount of the organic matter load. For highly polluted wastewater (BOD > 300 mg/L), longer retention time periods are to be taken into account, or the process should include preliminary treatment of the feed. The kinetic constants of first order obtained in the present research can be used as a basis for HRT calculation according to the classical area approach model for first-order reactions [17,44].

Table 5. Batch Mode experiment results—effect of HRT on treatment performance (constant influent load).

Sr.	HRT (Days)	pH Before	pH After	BOD Before (mg/L)	BOD After (mg/L)	BOD Removed (mg/L)	BOD Removal (%)	COD Before (mg/L)	COD After (mg/L)	COD Removed (mg/L)	COD Removal (%)	TN Before (mg/L)	TN After (mg/L)	TP Before (mg/L)	TP After (mg/L)
1	1	7.35	7.3	249.5	185.0	64.5	25.9	543.8	420.0	123.8	22.8	38.7	32.5	8.15	6.80
2	2	7.35	7.2	249.5	132.0	117.5	47.1	543.8	320.0	223.8	41.2	38.7	27.0	8.15	5.60
3	3	7.35	7.1	249.5	85.0	164.5	65.9	543.8	230.0	313.8	57.7	38.7	22.0	8.15	4.50
4	4	7.35	7	249.5	52.0	197.5	79.2	543.8	165.0	378.8	69.7	38.7	17.5	8.15	3.50
5	5	7.35	6.9	249.5	32.0	217.5	87.2	543.8	118.0	425.8	78.3	38.7	13.8	8.15	2.80
6	6	7.35	6.9	249.5	22.5	227.0	91.0	543.8	88.0	455.8	83.8	38.7	10.5	8.15	2.10
7	7	7.35	6.8	249.5	16.5	233.0	93.4	543.8	68.0	475.8	87.5	38.7	8.2	8.15	1.65
8	8	7.35	6.8	249.5	12.8	236.7	94.9	543.8	55.0	488.8	89.9	38.7	6.5	8.15	1.30
CPCB		6.5–8.5		—	≤30	—		—	≤250	—		≤10		≤5	

Note: Constant influent parameters for all 8 batches: pH = 7.35 | BOD = 249.5 mg/L | COD = 543.8 mg/L | TN = 38.7 mg/L | TP = 8.15 mg/L. Only HRT (detention time) was varied. CPCB BOD ≤ 30 mg/L achieved from day 5; COD ≤ 250 mg/L achieved from day 4; TN ≤ 10 mg/L achieved between days 6–7; and TP ≤ 5 mg/L achieved from day 3.

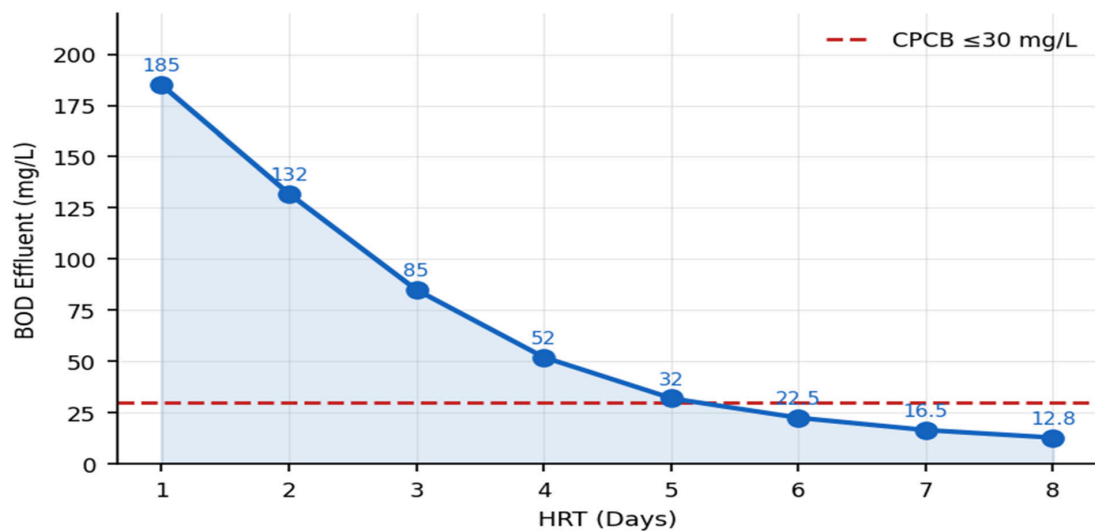


Figure 12. Batch Mode—BOD effluent decay over HRT (days 1–8).

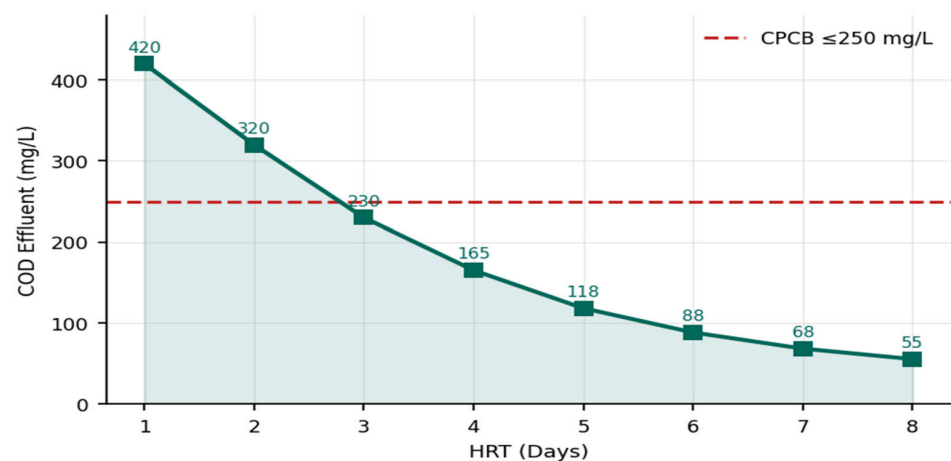


Figure 13. Batch Mode—COD effluent decay over HRT (days 1–8).

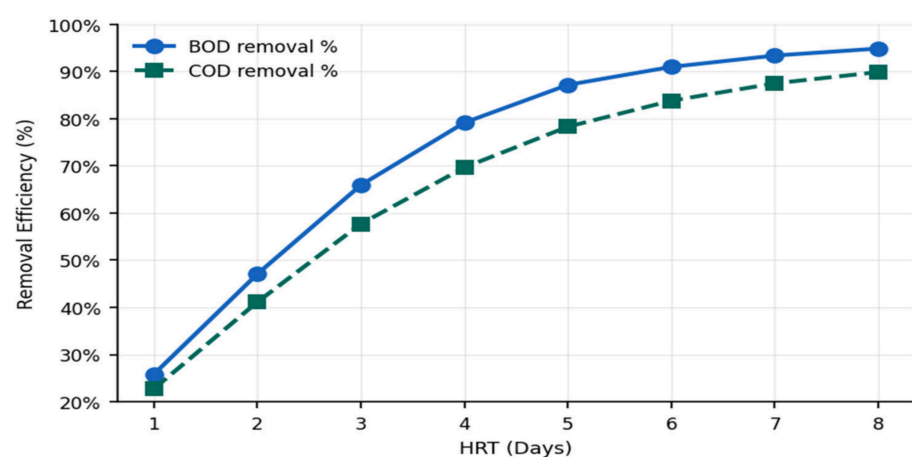


Figure 14. Batch Mode—BOD and COD removal efficiency vs. HRT (%).

The higher efficiency of the BOD removal of the MTBRCWs compared with the CWs presented in the literature may be explained, with inferences being drawn on the basis of the interplay between the following three factors specific to the bio-rack technology. Firstly, it should be pointed out that direct measurements of dissolved oxygen profiles, microbial community composition and activity, biomass concentration, and plant nutrient

uptake were not made during the study, and the following explanations are based on the analysis of DO gradients and the information presented in the literature. Firstly, the absence of porous media prevents oxygen diffusion limitations typical for gravel-bed HSSF systems [13,27]. Thus, there is an assumption that it will be possible to maintain aerobic conditions in a larger part of the water column and, thus, more efficient organic matter degradation will take place. Secondly, the bio-rack structure probably allowed the creation of a greater specific surface area for the microorganism colonisation compared to media-based CWs [31,33]. Finally, oxygen produced by *Typha angustifolia* and *Chrysopogon zizanioides* (vetiver grass) via radial oxygen loss may have been used to increase the level of dissolved oxygen in the environment [34,37]. This is consistent with the results obtained by Sathe & Munavalli [15] and Gujar & Munavalli [14].

The removal of COD was in line with that of BOD, with removal efficiency averages between 85.2% and 92.0% per month, with a mean value of 87.5%. As clearly depicted in Figure 15, the effluent COD remained consistently and substantially below the CPCB discharge limit of ≤ 250 mg/L across all thirteen months, with effluent values ranging between approximately 55 and 85 mg/L despite influent COD fluctuating widely between 436 and 642 mg/L. The monthly removal performance is further captured in Figure 16, which shows that COD removal efficiency remained above 85% in all thirteen months and closely tracked BOD removal trends across the monitoring period. The radar chart in Figure 17 provides a consolidated visual summary of mean annual removal efficiencies, confirming COD removal at 87.5%, BOD at 89.8%, TN at 78.2%, and TP at 74.4%, while the parameter-wise grouped comparison in Figure 18 further reinforces this pattern, showing consistently high and closely tracking removal performance across all four parameters throughout the monitoring period. As further evidenced in Figure 19, the monthly OLR and BOD removal efficiency displayed no consistent inverse relationship, with BOD removal remaining robustly above 86% even during months of elevated organic loading, confirming the system's resilience to organic load fluctuations throughout the monitoring period. It can be noted that the ability to maintain COD removal at levels of over 85% in all thirteen months despite large fluctuations in influent COD level highlights the natural resilience characteristic of the bio-rack CW technology for wastewater treatment. Such behaviour aligns with the conclusions of Hassan et al. [31], who attributed the stable functioning of bio-rack CWs to the higher structural stability of the biofilms formed on solid materials, rather than to the microbial communities formed on heterogeneous particles. Similar results were found by Patyal et al. [38] for CW systems enhanced by their design features, with COD removal consistently maintained at over 85%. In batch testing, COD was removed by 89.9% on day 8, while the target level of CPCB for COD of ≤ 250 mg/L was attained since day 3.

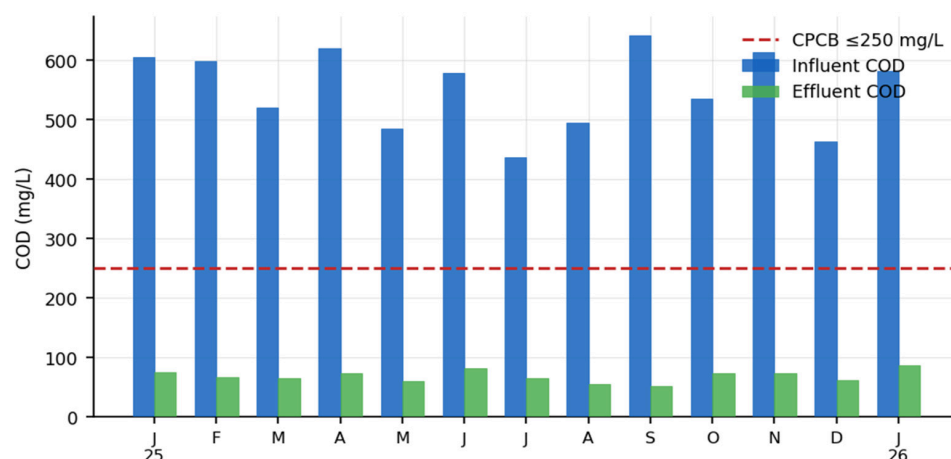


Figure 15. COD—monthly influent vs. effluent (mg/L) with CPCB limit.

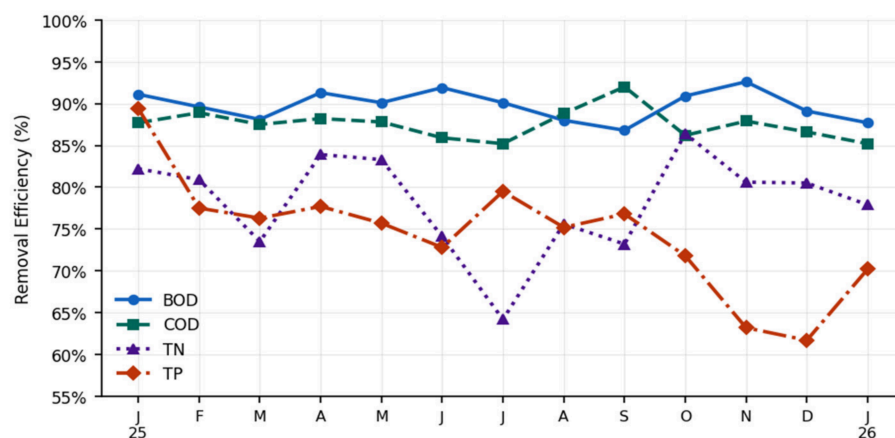


Figure 16. Monthly removal efficiency trends for BOD, COD, TN, and TP (%)—Continuous Flow Mode.

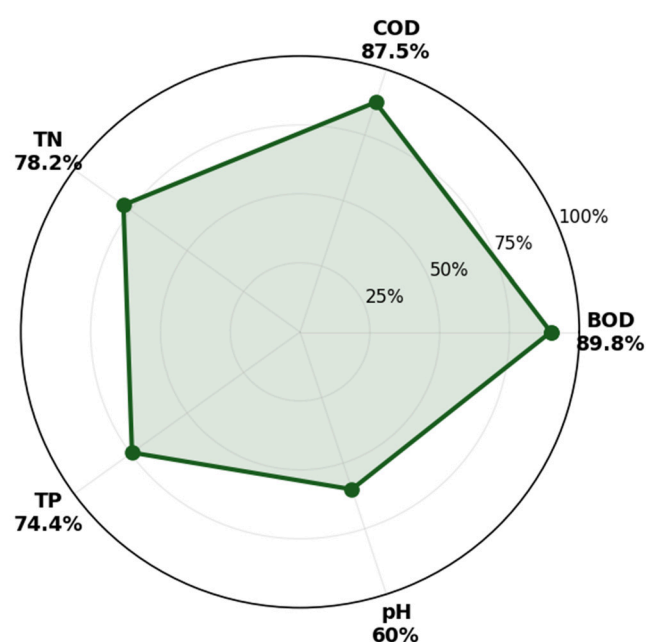


Figure 17. Overall average removal efficiency—radar chart (%).

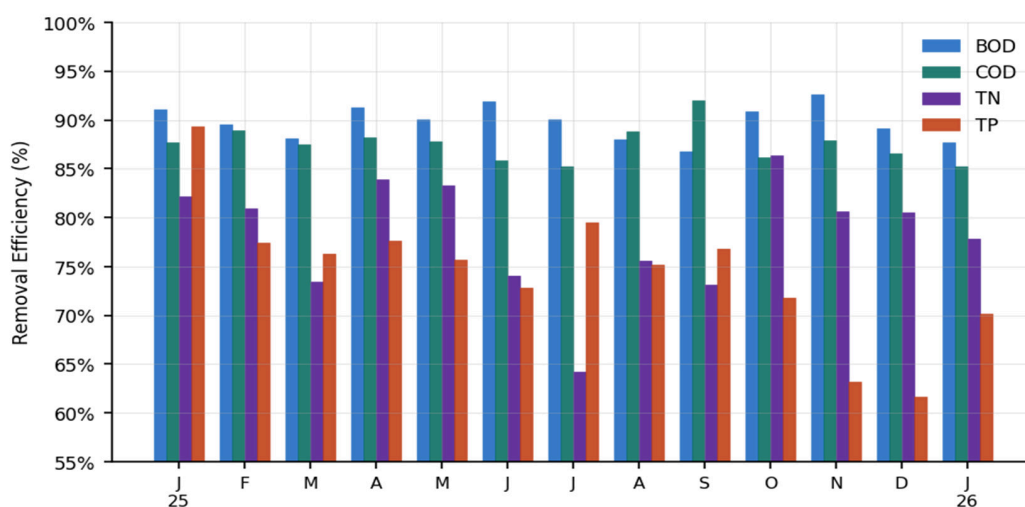


Figure 18. Grouped removal performance—monthly comparative bar chart (%).

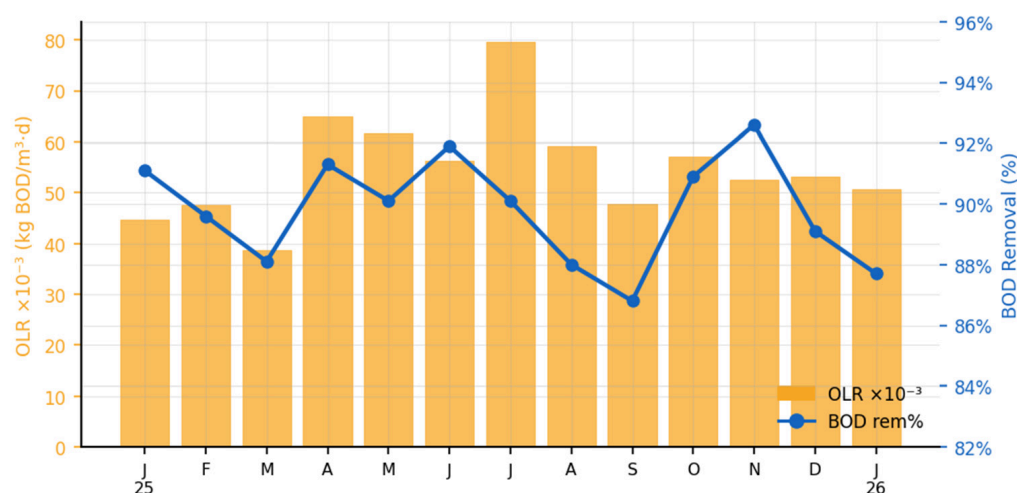


Figure 19. Monthly OLR vs. BOD removal efficiency—dual-axis (Continuous Flow Mode).

3.3. Total Suspended Solids and Total Dissolved Solids Removal

TSS removal efficiency remained consistently high throughout the study period, a direct benefit of the media-free design, which eliminates internal solid accumulation that causes progressive clogging in conventional systems from the influent flow. The physical straining of solids through macrophyte roots growing in bio-racks and sedimentation within the slow-moving sections of the containers, coupled with biofilm-induced uptake and enzymatic breakdown of suspended solids, served to remove the particulate fraction. These processes are well studied in CWs and have been found to perform consistently regardless of variations in HRT and loading rate [6,11,17]. The concentration of suspended solids in the effluents was within the acceptable CPCB limit of 100 mg/L throughout the study period, corroborating the work conducted by Vymazal, J. [22], who reviewed TSS removal mechanisms for fifty-two CW systems and recorded an average efficiency of 70–90%. As predicted, the TDS removal efficiency for a media-less biological treatment process was relatively low at 28–35%, and all TDS concentrations in the effluents were well below the permitted CPCB limit of ≤ 2100 mg/L [11]. This was consistent with the selectivity of CW treatment processes toward organic and nutrient pollutants rather than inorganic dissolved pollutants [17].

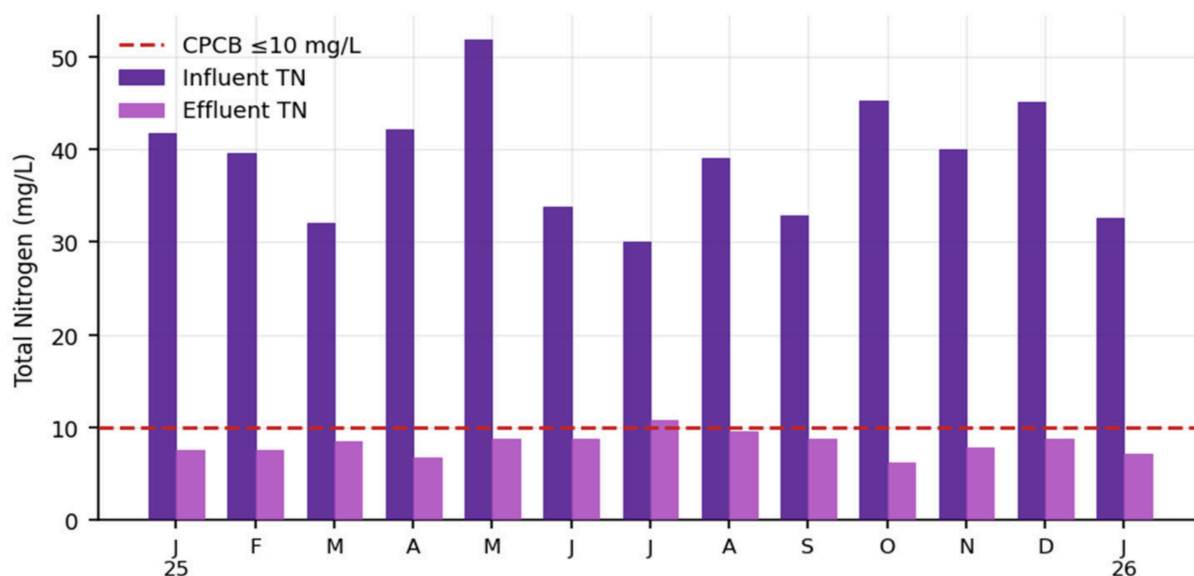
3.4. Nutrient Removal: Nitrogen and Phosphorus

TN removal efficiency ranged between 64.2% and 86.4%, averaging 78.2% over the total monitoring period of thirteen months (Table 6). As illustrated in Figure 20, the effluent TN remained consistently below the CPCB discharge limit of ≤ 10 mg/L across nearly all months of monitoring, despite the influent TN varying considerably between approximately 30 and 52 mg/L, underscoring the system's reliable nitrogen removal capacity throughout the year. The lowest monthly removal rate for TN was obtained in July 2025 (64.2%) during the peak of the monsoon season, at which point the hydraulic loading rate had also peaked at its highest level ($\text{HLR} = 0.28 \text{ m}^3/\text{m}^2 \cdot \text{d}$), reducing the hydraulic retention time of the system to its lowest level, which is also visually reflected in Figure 20 by the relatively higher effluent TN bar observed in July compared to other months. Conversely, the maximum TN removal rate of 86.4% occurred in October 2025, following the monsoon season and when a lower HLR, together with more stable conditions in terms of effluent composition, allowed for more constant microbial activity. Seasonality has been identified as an important variable in previous studies: HRT has been found to be the principal factor governing nitrogen removal in CWs by Vymazal [10,11] and Kadlec [23], due to the minimum time required for nitrification and denitrification processes.

Table 6. Monthly pollutant removal efficiency (%)—Continuous Flow Mode (Jan 2025–Jan 2026).

Sr.	Month and Year	Δ pH (Shift) *	BOD Removal (%)	COD Removal (%)	TN Removal (%)	TP Removal (%)	BOD RE SD	COD RE SD	TN RE SD	TP RE SD	CPCB Compliance
1	Jan-25	6.3	91.1	87.7	82.2	89.4	0.14	0.45	2.51	0.53	✓
2	Feb-25	5.3	89.6	88.9	80.9	77.5	5.47	0.74	2.65	5.30	✓
3	Mar-25	10.2	88.1	87.5	73.5	76.3	4.28	1.32	2.29	0.00	✓
4	Apr-25	2.0	91.3	88.2	83.9	77.7	1.27	1.35	2.13	0.72	✓
5	May-25	2.8	90.1	87.8	83.3	75.7	3.35	0.57	0.08	11.33	✓
6	Jun-25	3.4	91.9	85.9	74.1	72.8	2.56	0.82	0.98	10.84	✓
7	Jul-25	7.5	90.1	85.2	64.2	79.5	2.15	0.55	0.84	5.64	✗
8	Aug-25	6.6	88.0	88.8	75.6	75.2	2.31	0.04	0.69	1.10	✓
9	Sep-25	2.7	86.8	92.0	73.2	76.8	0.55	0.44	1.2	4.91	✓
10	Oct-25	4.8	90.9	86.2	86.4	71.8	3.03	1.84	1.00	5.45	✓
11	Nov-25	1.3	92.6	87.9	80.6	63.2	1.05	2.86	3.27	11.49	✓
12	Dec-25	6.1	89.1	86.6	80.5	61.7	0.35	1.62	3.05	5.59	✓
13	Jan-26	-0.7	87.7	85.2	77.9	70.2	2.69	0.99	0.91	8.66	✓
—	Overall Average	+0.35	89.8	87.5	78.2	74.4	2.25	1.05	1.66	5.50	12/13 Compliant

Note: * Δ pH = Influent pH – Effluent pH (absolute shift). pH is not a concentration parameter; removal efficiency does not apply. Positive values indicate a decrease in pH through the system; negative values indicate an increase. ✓ = all parameters within CPCB discharge limits; ✗ = one or more parameters exceed CPCB limits. SD values for monthly removal efficiencies calculated as $SD = |RE(S1) - RE(S2)| / \sqrt{2}$, where RE(S1) and RE(S2) are removal efficiencies computed from individual temporal replicate samples S1 and S2.

**Figure 20.** Total nitrogen—monthly influent vs. effluent (mg/L) with CPCB limit.

The efficiency of nitrogen removal is also highly dependent on the HRT in the TN Batch Mode case study. At a constant TN concentration in the influent, which is 38.7 mg/L, the effluent TN decreased from 32.5 mg/L (on the 1st day of the test; removal efficiency 16.0%) to 6.5 mg/L on the 8th day (removal efficiency 83.2%). According to the CPCB guidelines of TN concentration not exceeding 10 mg/L, this level was achieved within the period of day 6 to day 7 (TN concentration on day 6—10.5 mg/L, on day 7—8.2 mg/L). It can therefore be concluded that for batch operation, the minimum HRT required for efficient and reliable CPCB-compliant TN removal (≤ 10 mg/L) is about 7 days at the influent TN concentration used in this case study. As for the Continuous Flow Mode operation, the biological HRT is calculated using the wetland treatment volume only (68 L, excluding the inlet storage tank), with HLR values of 0.18–0.28 $\text{m}^3/\text{m}^2\cdot\text{d}$ yielding flow rates of 24.7–38.4 L/d. The corrected Continuous Flow Mode HRT range is therefore

approximately 1.8 to 2.8 days. This is considerably shorter than the minimum 7-day Batch Mode HRT identified for reliable TN compliance. It accounts for the reason that July 2025, the month with the highest HLR, recorded the lowest TN removal efficiency (64.2%) and the only case of TN concentration surpassing the permissible limits of CPCB, which was at 10.8 mg/L. There are practical lessons from this observation that during periods of peak water flow rates, the HRT in the Continuous Flow Mode could be below the critical threshold needed for efficient nitrification–denitrification. The relatively better TN removal capability of MTBRCW as opposed to many horizontal subsurface flow constructed wetlands documented in scientific studies [11] is due to the stratification of the aerobic and anoxic microenvironment achieved using the dual-container planting method. The oxygenating capability of *Typha* plants with spreading roots in the inlet region facilitates ammonia oxidation, while the deep rooting of vetiver plants provides the anoxic conditions with adequate organic carbon for denitrification. The nitrification–denitrification one-step system is a natural advantage of the sequential bio-rack system, as reported in the work by Kadlec [6]. It is important to recognise that all mechanistic interpretations presented in this section remain inferential. No direct measurements of microbial community composition (e.g., 16S rRNA gene sequencing), biomass accumulation on bio-racks, or plant nutrient uptake through tissue analysis were performed in this study. The proposed nitrification–denitrification pathway is inferred from DO gradient data and analogous findings in the published literature. Such direct experimental data would be required to confirm the proposed treatment mechanisms, and this constitutes a key limitation of the present study.

It is acknowledged that, under the present experimental setup, the contribution of the individual species of plants used in nutrient removal is not isolated from the two-stage sequential container setup. Single-species control units, as well as control bio-racks without any plants, have not been incorporated into this experiment. This, therefore, represents one of the limitations of this particular study. It has, however, already been proved that the roles of *T. angustifolia* and *C. zizanioides* in CW systems are complementary in their respective ways. The former supplies oxygen in the rhizosphere during inlet load conditions that allow nitrification, while the latter, through its dense root network, provides conditions conducive to denitrification in the polishing section of CW systems [35–37]. The DO gradient observed between the two containers in this study, with the DO content being higher in Container 1 and relatively lower in Container 2, is, therefore, in conformity with this mechanism. It is suggested that further studies using unplanted control setups, as well as setups involving different individual species, be conducted.

The average phosphorus removal efficiency rate was 74.4%, while effluent phosphorus concentrations varied from 0.95 to 2.85 mg/L over the entire duration of the thirteen months under consideration, staying far below CPCB phosphorus discharge standards (5 mg/L) (Table 7 and Figure 21). Although the influent TP concentrations showed significant variations around 6.5–10.5 mg/L, phosphorus removal performance in this treatment plant remained fairly constant irrespective of seasonality. The best phosphorus removal efficiency of 89.4% was achieved in January 2025, while the worst removal efficiency of 61.7% occurred in December 2025 when the effluent TP concentrations were relatively high. Such a phosphorus removal efficiency decline in December is probably associated with macrophyte senescence in constructed wetlands [34,38,42]. The removal of phosphorus in constructed wetlands is mainly dependent on factors such as plant uptake, assimilation of phosphorus into microbe biomasses, and chemical adsorption of phosphorus on soil particles. However, since the MTBRCW lacks adsorptive materials such as calcium-rich gravels, the latter process is negligible in the system, thereby leaving the former two methods as the main contributors to phosphorus removal. In Batch Mode, as depicted in Figure 22, the effluent TP declined steadily with increasing HRT, crossing below the CPCB

limit of ≤ 5 mg/L from day 3 onward (4.5 mg/L) and reaching 1.3 mg/L by day 8 (84.0% removal), while effluent TN simultaneously followed a near-linear declining trajectory, crossing the CPCB limit of ≤ 10 mg/L between day 6 and day 7, which is consistent with the phosphorus removal kinetics reported by Brix, H. [7], for plant-dependent CW systems.

Table 7. Monthly total phosphorus (TP) removal performance—Continuous Flow Mode (Jan 2025–Jan 2026).

Sr.	Month and Year	TP Influent (mg/L)	TP Effluent (mg/L)	TP Removal (%)	CPCB Limit (mg/L)
1	Jan-25	9.00	0.95	89.4	≤ 5
2	Feb-25	10.45	2.35	77.5	≤ 5
3	Mar-25	7.60	1.80	76.3	≤ 5
4	Apr-25	6.95	1.55	77.7	≤ 5
5	May-25	8.45	2.05	75.7	≤ 5
6	Jun-25	8.45	2.30	72.8	≤ 5
7	Jul-25	10.25	2.10	79.5	≤ 5
8	Aug-25	6.45	1.60	75.2	≤ 5
9	Sep-25	7.10	1.65	76.8	≤ 5
10	Oct-25	8.85	2.50	71.8	≤ 5
11	Nov-25	6.80	2.50	63.2	≤ 5
12	Dec-25	7.45	2.85	61.7	≤ 5
13	Jan-26	8.05	2.40	70.2	≤ 5
—	Average	8.14	2.05	74.4	≤ 5

Note: CPCB discharge standard for total phosphorus = ≤ 5 mg/L (inland surface water). All effluent values are below the CPCB limit throughout the monitoring period.

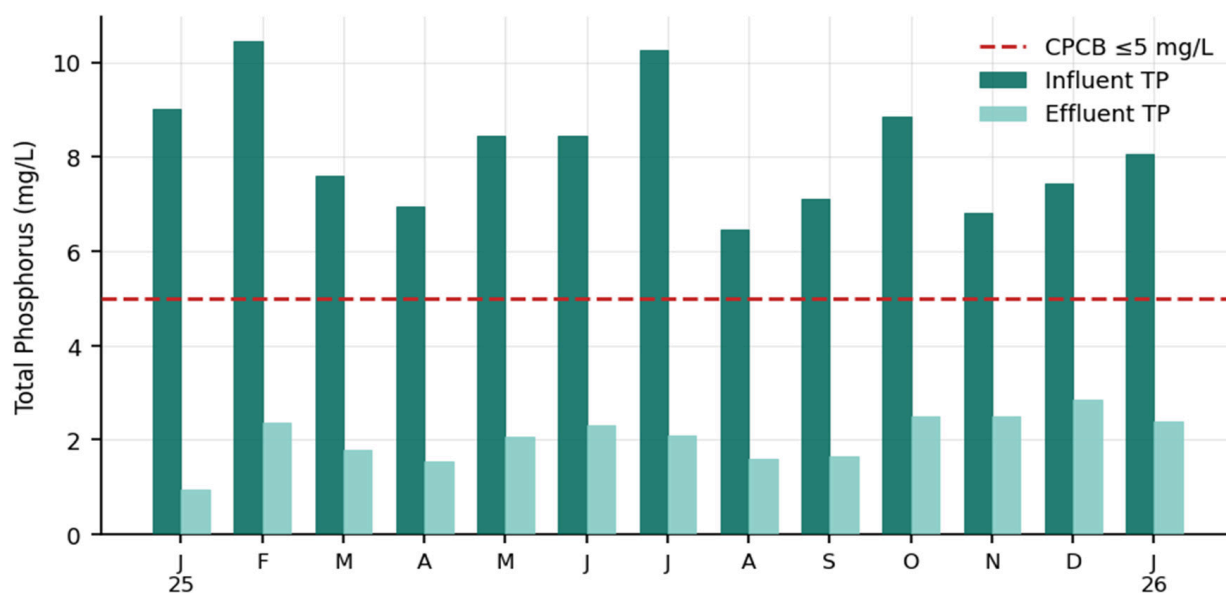


Figure 21. Total phosphorus—monthly influent vs. effluent (mg/L) with CPCB limit.

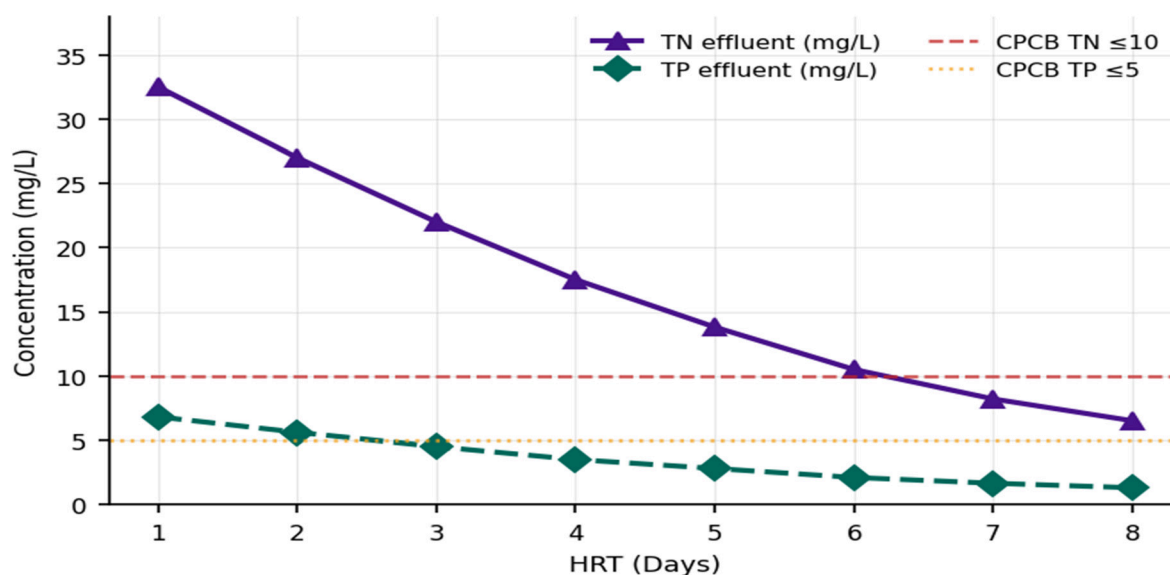


Figure 22. Batch Mode: TN and TP effluent reduction over HRT with CPCB thresholds.

3.5. Faecal Coliform Removal

Faecal coliform removal was efficient throughout the monitoring period at the MTBRCW treatment facility, as summarised in Table 8. Faecal coliform concentration levels varied between 1.8×10^5 and 8.2×10^5 MPN/100 mL in the influent samples during the monitoring period, in line with the total concentration ranges of influents of 10^4 to 10^6 MPN/100 mL documented in Table 2 above. Effluents were consistent at 200–800 MPN/100 mL, meeting CPCB's standard limit of ≤ 1000 MPN/100 mL in every single month without fail. The log reductions in each month were between 2.8 and 3.4 across the monitoring period. There was a marked seasonality trend evident in the faecal coliform removal, with $\log_{10}$ reductions of 3.3–3.4 achieved in the summer period (March–May 2025), as a result of increased irradiance and temperatures that helped in the inactivation process, and reductions of only 2.8 in the monsoon period (June–September 2025), which reflected low solar irradiance and increased hydraulic loading in dilution. Regardless of the seasonality, CPCB compliance was achieved in all of the thirteen months of monitoring. Faecal coliforms were measured in multiple tube fermentation using the Most Probable Number (MPN) method as per APHA Standard Methods (2017) [43]. The pathogens are removed from the MTBRCW system due to several simultaneous processes: UV killing on the surface of the open water, sedimentation and filtration through the root matrix and biofilm layers, protozoan and bacteriophage predation, the production of antibacterial agents by the wetland macrophytes, and drying in the dry periods [7,22,43]. The surface character of the MTBRCW system and the large amounts of aerial biomass of *Typha angustifolia* in Container 1 were very favourable for UV killing and pathogen predation. The achieved reductions are in line with the data obtained for surface flow CWs running in tropical areas (2.5–3.8 log) [7,43], indicating the same efficiency of media-free bio-rack CW systems.

The open-surface nature of the MTBRCW process distinguishes the pathogen removal methods of the MTBRCW from those of traditional HSSF-CW processes. Due to their nature, subsurface flows do not allow for any significant UV exposure, which is the most effective natural method of disinfection available in CW systems. With the use of an open container system, the MTBRCW allows for direct sunlight exposure in each stacked container, thus providing constant UV inactivation through the course of the treatment process. This is clearly evident by the nature of the seasonality found in this study, where $\log_{10}$ reduction peaks were found to be between 3.3 and 3.4 during high-irradiance summer periods and

lowered to 2.8 during low-irradiance monsoon months, which is clearly indicative of UV-based disinfection. Furthermore, the media-free system prevents the formation of anaerobic zones in the gravel matrix, which could shelter pathogens in a traditional system. Moreover, the constantly aerobic state of the water column due to DO levels exceeding 1.0 mg/L in all depth profiles makes conditions inhospitable for enteric pathogens. The presence of root–matrix filtration by *Typha angustifolia* and *Chrysopogon zizanioides* provides additional filtering and predation of pathogens [7,22,43]. Additional disinfection may be required depending on the desired pathogen standards.

Table 8. Monthly faecal coliform concentrations and log₁₀ reductions in the MTBRCW system (January 2025–January 2026).

Sr. No.	Month and Year	Influent FC (MPN/100 mL)	Effluent FC (MPN/100 mL)	Log ₁₀ Reduction	CPCB Limit (MPN/100 mL)	Compliance
1	Jan-25	4.2×10^5	580	2.9	≤ 1000	✓
2	Feb-25	3.8×10^5	480	2.9	≤ 1000	✓
3	Mar-25	6.5×10^5	310	3.3	≤ 1000	✓
4	Apr-25	8.2×10^5	326	3.4	≤ 1000	✓
5	May-25	7.4×10^5	280	3.4	≤ 1000	✓
6	Jun-25	2.8×10^5	443	2.8	≤ 1000	✓
7	Jul-25	1.8×10^5	285	2.8	≤ 1000	✓
8	Aug-25	2.1×10^5	332	2.8	≤ 1000	✓
9	Sep-25	3.2×10^5	507	2.8	≤ 1000	✓
10	Oct-25	4.8×10^5	420	3.1	≤ 1000	✓
11	Nov-25	5.6×10^5	380	3.2	≤ 1000	✓
12	Dec-25	4.9×10^5	440	3.0	≤ 1000	✓
13	Jan-26	4.1×10^5	560	2.9	≤ 1000	✓

(Note: ✓ indicates the influent and effluent values follows the CPCB Standards).

3.6. Dissolved Oxygen Dynamics and Hydraulic Performance

The dissolved oxygen profiles recorded during the operation of the MTBRCW process contribute significantly towards understanding the treatment processes explained in previous sections. Dissolved oxygen was measured at two depth positions within each wetland container: near the surface (0.05–0.10 m) and near the container base (0.20–0.25 m), consistent with the design water depth of 0.25 m per container. Measurements consistently exhibited a vertical concentration gradient, with higher DO values near the surface (average 4.1 mg/L) decreasing to lower but aerobically sufficient values at the container base (average 1.4 mg/L). These gradients are typical of biological systems where the source of oxygen originates mainly from atmospheric diffusion and the release of oxygen in the root zone, as demonstrated by Hassan et al. [31] and Liu et al. [6] for bio-rack and media-free CW systems, respectively. The ability to sustain DO levels greater than 1.0 mg/L in the water column becomes critical for nitrification efficiency because it is below this level that *Nitrosomonas* bacteria can no longer survive [6]. Even though the DO gradient data suggest the presence of aerobic and anoxic conditions in the system, a more definitive validation of nitrogen conversion processes via microbial community analysis (such as 16S rRNA sequencing of *Nitrosomonas* and *Nitrospira* communities) and determination of nitrogen and phosphorus accumulation by plants was not undertaken as part of the current pilot-scale study. The importance of further studies in this regard has been mentioned explicitly. As shown in Figure 23, the effluent COD values from both S1 and S2 remained closely comparable across all months, ranging between approximately 48 and 92 mg/L, reflecting the uniformity and reproducibility of treatment performance across the two

sampling points and reinforcing the reliability of the average monthly values used for system performance analysis. A similar degree of consistency between the two sampling points was observed for effluent pH: Figure 24 presents the individual sample-wise effluent pH values for both S1 and S2 across the monitoring period, confirming that both sampling points yielded effluent pH values consistently within the CPCB acceptable band, with only marginal month-to-month variation. Building on this, Figure 25 shows that the effluent pH remained within the CPCB permissible range of 6.5–8.5 throughout all thirteen months of monitoring, with a mean pH shift (ΔpH) of +0.35 units (influent pH decreasing slightly through the system), tracking closely below the influent pH and indicating that the biological treatment processes within the system did not cause any adverse shift in pH, which is essential for sustaining microbial activity across nitrification and denitrification pathways.

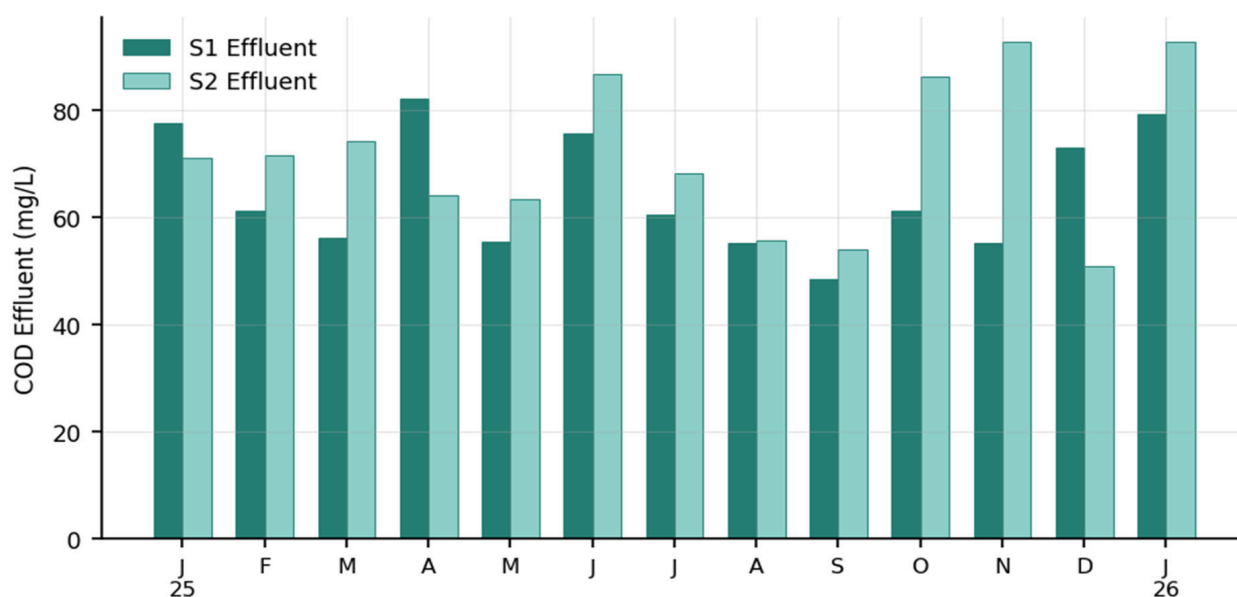


Figure 23. Raw COD effluent: S1 vs. S2 comparison across 13 months (mg/L).

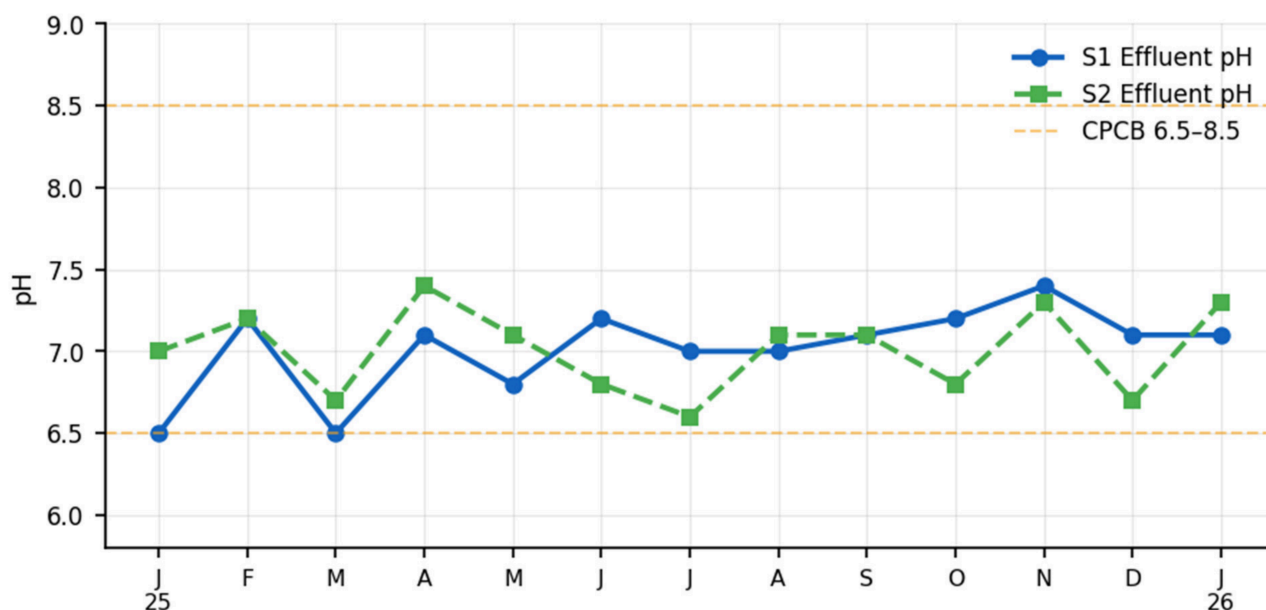


Figure 24. Raw pH effluent: S1 vs. S2 comparison across 13 months.

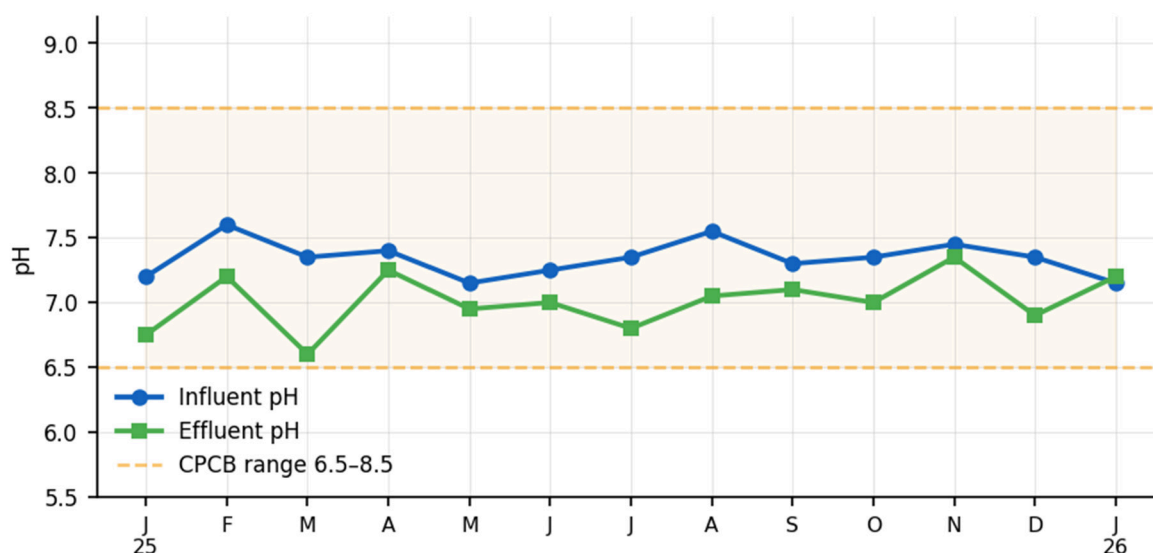


Figure 25. pH variation—monthly average influent vs. effluent within CPCB range.

The correlation analysis carried out with the use of IBM SPSS Statistics v26 identified critical correlations between operational parameters and treatment efficiencies during the 13-month monitoring period. A moderately inverse relationship was found between HLR and the efficiency of TN removal ($r = -0.66$, $p = 0.015$), proving that higher values of hydraulic loading, especially in monsoon months, were responsible for a lower rate of nitrogen removal due to a reduced effective hydraulic retention time for the nitrification–denitrification process. At the same time, there was no statistically significant correlation between HLR and the efficiency of BOD removal ($r = -0.08$, $p = 0.795$). This means that the ability of MTBRCWs to remove BOD was not sensitive to variation in hydraulic loading in the range of $0.18\text{--}0.28\text{ m}^3/\text{m}^2\cdot\text{d}$. Furthermore, no statistically significant correlation was detected between ambient temperature and the efficiency of TN removal ($r = 0.06$, $p = 0.847$). The result indicated that the ability of the MTBRCW to remove nitrogen was buffered enough due to an aerobic–anoxic microenvironment created as a result of the sequential bio-racks. There was no statistically significant correlation between OLR and the efficiency of TP removal ($r = 0.06$, $p = 0.837$).

The hydraulic performance was continuously monitored for the 13-month operation period. Weekly measurements of flow rates were carried out at the inlet and outlet through the calibrated volumetric method (timed water volume in the graduated cylinder). There was no reduction in the flow rate at the outlet point compared to the flow rate at the inlet at any of the monitoring periods. There was also no reduction in the flow rate at the outlet from the periodic inspections, which demonstrated that there was no hydraulic restriction due to clogging of the system. Visual observations of water levels in the two tanks showed a constant operating water level throughout the entire experimental period. The absence of surface water pooling, excess flow, or water level increase, which are some of the key symptoms of clogging in traditional CW systems, was clearly evident in the two monitoring tanks. Upon concluding the experiment, direct observations were made in both tanks, showing clear flow channels in the bio-racks without any solid formation that would cause hydraulic restriction. Even though no tracer experiments or calculations regarding hydraulic conductivity have been carried out in the current pilot-scale experiment, the constant water flows and water level curves represent hydraulic stability observable from the field. However, it can be noted that these measurements should be conducted for more robust quantified evidence of hydraulic stability, which is recommended as an important step for future implementations. The results of this experiment are in agreement with the

hydraulic stability findings of Hassan et al. [31] and Koli and Munavalli [16] on bio-rack CW systems. Figure 26 shows that the efficiency of BOD removal has demonstrated an evident inverse dependency on HLR for all eight HRT days, with an efficiency growing rapidly from ~26% at the maximum HLR of $0.5 \text{ m}^3/\text{m}^2\cdot\text{d}$ on day 1 to >94% at the minimum HLR on days 7 and 8, which means that low hydraulic loading always provides more efficient treatment. An almost identical dependency can be seen in Figure 27, where the BOD removal efficiency inversely correlated with the OLR value, ranging from ~26% at the maximum OLR value of $0.125 \text{ kg BOD}/\text{m}^3\cdot\text{d}$ on day 1 to >94% at minimum OLR values on days 7 and 8. However, it must be pointed out that this result contradicts sharply with the rapid degradation of the hydraulic properties reported by Fu, G. et al. [12] in relation to gravel-bed CW systems as well as the decrease in their performance due to clogging observed by Aiello et al. [9] and Gujar & Munavalli [14] in relation to HSSF systems, considering they possessed similar organic loads. In fact, the functioning of the MTBRCW system without visible clogging for 13 months despite the high loads of organic matter in July–August and its hydraulic performance at $0.18\text{--}0.28 \text{ m}^3/\text{m}^2\cdot\text{d}$ loading rates is in line with the inherent strength of the media-less bio-rack concept. These results, nonetheless, should only be viewed as a demonstration of operational experience rather than hydraulic performance since tracer tests and hydraulic conductivity measurements were not carried out in the current pilot-scale study.

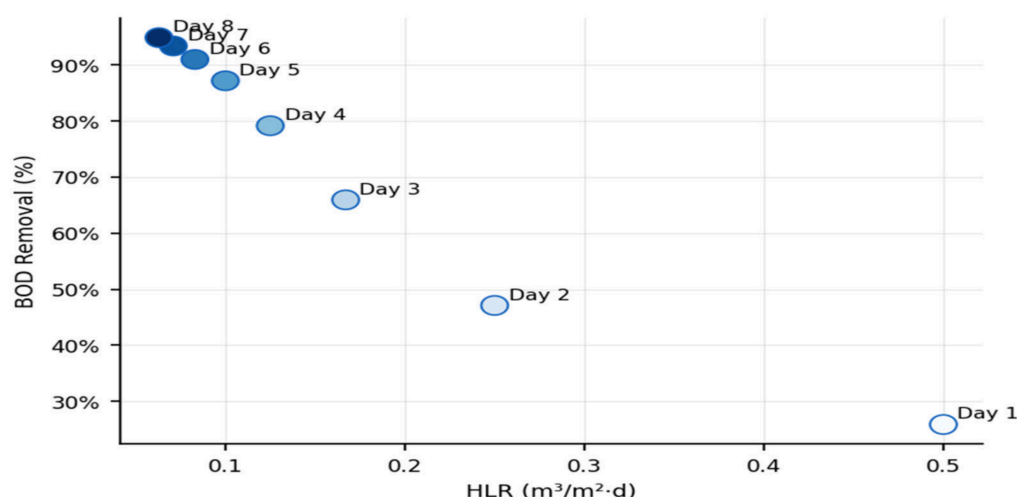


Figure 26. Batch Mode—HLR ($\text{m}^3/\text{m}^2\cdot\text{d}$) vs. BOD removal efficiency (%).

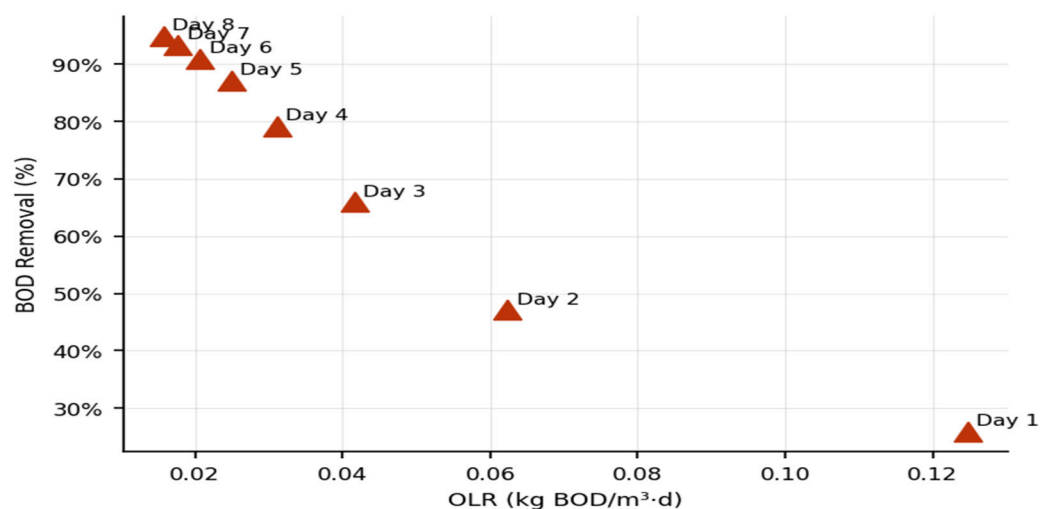


Figure 27. Batch Mode—OLR ($\text{kg BOD}/\text{m}^3\cdot\text{d}$) vs. BOD removal efficiency (%).

3.7. Comparative Performance and Operational Advantages

The overall efficiency of BOD removal (89.8%), COD (87.5%), TN (78.2%), and TP (74.4%) in Continuous Flow Mode, and BOD (94.9%) and COD (89.9%) removal efficiencies during the maximum batch hydraulic retention time can be considered quite comparable with CW systems described in the relevant literature in various climatic zones and setups. As noted earlier, the comprehensive reviews by Vymazal [10,11,20] on CW performance around the world show median BOD removal of 75–90% from mature HSSF systems, with the Continuous Flow Mode MTBRCW performing near the higher end of this range. The results reported by Patyal et al. [38] in their assessment of structurally enhanced CWs show COD removals of 85–92% in different systems, which is similar to the variation seen in MTBRCW's COD removal rates of 85.2–92.0%. Comparable outcomes were obtained in Africa and South America when removing BOD at rates between 75% and 88%, as well as TN at rates between 60% and 80%, using HSSF treatment processes designed for tropical climates to treat domestic wastewater [6,7]. In comparison, the MTBRCW process showed comparable or better pollutant removal capabilities, while also exhibiting stable hydraulic characteristics.

In discussing the advantages of the MTBRCW process compared to traditional HSSF-CW systems, it is important to examine three perspectives that can be seen as preliminary in their nature. Those include hydraulic stability, ease of operation, and capital cost indicators. It needs to be emphasised that the economic evaluation proposed here should not be viewed as conclusive since there was no formal cost–benefit analysis carried out or calculation of the treatment cost per cubic metre of sewage water in the current study. The non-occurrence of clogging during 13 months of operation in contrast to the hydraulic failure within 3–5 years in gravel-bed systems [9,12,25] implies the possibility of having maintenance benefits in the long term as reported by Brix [7] and Hu et al. [38], where they observed maintenance costs associated with clogging problems making up more than 40% of the overall life cycle operational costs in conventional CWs. But in the present pilot-scale study, no cost evaluation in terms of labour, inspection, plant harvesting, and other repair works was carried out. While constructing the pilot-scale MTBRCW, the majority of the structural elements were obtained as salvage materials from JSPM University, Pune, and only the HDPE bio-rack plates and macrophytes required procurement. Thus, the material costs involved in the construction of the MTBRCW system could be considerably lower than those of conventional HSSF-CWs, owing to the non-use of granular filter media like gravel and sand, which form the major material cost in conventional systems. The capital cost comparison between conventional HSSF-CWs and the MTBRCW system on a component-wise basis, considering the current material prices in Maharashtra state, India, is presented in Table 9, showing savings in capital costs by 30–35%.

The operational cost is extremely low since the system uses no energy input due to its gravity-fed media-less design and has no need for media replacement or chemical addition, thus removing the two main recurring costs associated with conventional CWs. Maintenance is restricted to yearly plant harvesting and inspections of connections between PVC pipes only, thus involving little labour. This combination renders the MTBRCW a truly inexpensive and low-maintenance system that can be applied in decentralised mode in developing countries like India.

The system maintained CPCB-compliant BOD and COD effluent quality consistently throughout both monsoon and post-monsoon periods, including during peak hydraulic stress in July and August, demonstrating strong climatic resilience. According to Hu et al. [38], there was a marked difference in the seasonality of operation in CW systems under temperate climates; however, in tropical monsoon climates, there was no significant fluctuation in the efficiency of the process throughout different seasons [39]. This was

consistent with the case of the MTBRCW system that operated successfully during an approximate 55% increase in HLR from 0.18 to 0.28 m³/m²·day, thus demonstrating the high hydraulic adaptability of media-less CW systems [31].

Table 9. Component-wise capital cost comparison—conventional HSSF-CW vs. the MTBRCW system (estimated per m² of wetland surface area, Indian market rates).

Sr.	Component	Conventional HSSF-CW (INR/m ²) [15,29]	MTBRCW (INR/m ²)	Remarks
1	Gravel/sand filter media	1800–2200	0	Completely eliminated in MTBRCW
2	HDPE bio-rack panels	0	600–800	Replaces granular media as the structural substrate
3	Structural tank/container	1200–1500	800–1000	Repurposed salvage materials used at the pilot scale
4	Macrophyte plants	300–400	300–400	Same species and cost for both systems
5	PVC pipes, valves, and fittings	400–500	400–500	Similar for both systems
6	Labour and installation	600–800	400–500	Simpler installation due to the absence of media filling
Total (estimated)		4300–5400	2500–3200	An ~30–35% lower capital cost for MTBRCW

Note: Conventional HSSF-CW cost estimates are indicative figures based on the published literature [15,29] and local contractor quotations in Maharashtra, India (2024–2025). MTBRCW costs reflect actual material procurement for the pilot-scale system constructed at JSPM University, Pune. Formal techno-economic accounting was not conducted in this study; these figures should be treated as preliminary estimates only.

3.8. Mass Loading and Mass Balance Analysis

In addition to the removal efficiencies obtained based on concentration levels for the parameters studied, as shown in Sections 3.2–3.5, a mass loading analysis was conducted for the BOD and TN, which were the two most important parameters in CPCB compliance assessment. The mass loadings can be estimated using the formula below:

$$\text{Mass load (g/d)} = \text{Concentration (mg/L)} \times \text{Flow rate (L/d)} \times 10^{-3}$$

where the monthly flow rates were calculated based on the HLR values measured and the wetland surface area of 0.137 m².

On average, the influent BOD mass loading was 7.18 g BOD/day, whereas the effluent BOD mass loading was 0.73 g/day, resulting in an average mass removal rate of 6.45 g BOD/d, equal to 89.8% mass removal of BOD, which is similar to the concentration-based BOD removal efficiency of 89.8% as shown in Section 3.2. On the other hand, the annual average influent mass loading of TN was 1.13 g TN/day and effluent mass loading was 0.24 g TN/d, leading to 0.88 g TN/d mass removal or 78.4% TN removal efficiency.

The maximum BOD influent mass loading occurred in July 2025 when the HLR was 0.28 m³/m²·d and the flow rate was 38.4 L/d, where BOD mass loading was 10.91 g/d. This value was close to double the minimum monthly BOD mass loading, which occurred in March 2025, at 5.29 g/d. While there was a large discrepancy between maximum and minimum mass loading in terms of almost doubling, the effluent BOD mass export in July (1.08 g/d) was low, indicating that the MTBRCW system efficiently managed increased mass loading levels. A comparison of mass-based and concentration-based removal efficiencies for all 13 monitored months showed consistent results.

4. Limitations and Future Research Directions

However, despite the absence of signs of clogging or inefficiency of the hydraulic functioning of the system within 13 months of its functioning, the obtained result should be treated as an operational one, not a hydraulic performance result quantitatively proven. Indeed, no tracer tests were conducted; no measurements of hydraulic conductivity of the filter media were performed during the experiment. It is also important to emphasise that clogging in the case of conventional CWs develops gradually within 3–5 years [9,12]. Thus, the duration of the current experiment can be said to provide enough information about short- and medium-term hydraulic efficiency, but it is not sufficient for making conclusive statements about the clogging potential after several years of operation. It is recommended that further studies involve monitoring periods of 3 years or longer, regular measurement of hydraulic conductivity, and post-operation inspection of the structure of the bio-rack. Finally, as there was no parallel CW system used for comparison purposes, the results obtained here should be considered indirect evidence of its performance; a control experiment will be more appropriate for providing direct information in the future. Lastly, one should note the lack of a true domestic wastewater source for this study, with institutional campus waste being a relatively predictable alternative. A test under actual residential settings is suggested before implementing the system at the community scale. However, irrespective of this, it can be seen from Table 2 that the characteristics of the influent are in agreement with the quality parameters of medium-strength domestic wastewater, according to CPCB guidelines [1], i.e., BOD = 180–280 mg/L and TN = 28–48 mg/L [1–3].

5. Conclusions

The purpose of this experiment was very simple: to investigate the possibility of a wetland system without any media to provide effective, regulation-compliant processing of domestic wastewater on a sustained basis, thereby addressing the age-old issue of clogging associated with this type of technology. This question is conclusively answered by the monitoring data spanning 13 months of sustained use, and the findings from a Batch Mode analysis conducted using eight different hydraulic retention times. The MTBRCW system exhibited average removal efficiency rates for BOD, COD, total nitrogen, and total phosphorous of 89.8%, 87.5%, 78.2%, and 74.4%, respectively. The effluent BOD and COD rates were maintained within permissible limits prescribed by CPCB during all thirteen months of operation. TN was found in accordance with the prescribed limits in twelve out of thirteen months, with the single non-compliance event occurring in July 2025 (effluent TN = 10.8 mg/L), coinciding with the peak monsoon HLR of $0.28 \text{ m}^3/\text{m}^2\cdot\text{d}$, as recorded in Figure 9 and discussed in Section 3.4. TP remained within CPCB limits in all thirteen months. September 2025, while also a monsoon month, recorded compliant TN (8.8 mg/L) and TP (1.65 mg/L) effluent values, as shown in Table 6. First-order removal kinetics of BOD and COD were confirmed through Batch Mode experiments. CPCB-compliant BOD effluents were achieved after day 5 of retention, and total nitrogen effluents were achieved after day 7 of retention, allowing identification of an optimal operating range of 6 to 7 days. There were no clogs, hydraulic degradation, or a drop in treatment efficiency observed throughout the entire course of the experiment, all of which are indicative of the structural strength inherent in swapping out the conventional porous material for open bio-racks, which provide for microbial attachment surfaces while maintaining fluid passage. By strategically placing *Typha angustifolia* in the high-flow container followed by *Chrysopogon zizanioides* (vetiver grass) in the polishing container, we constructed a microenvironment suitable for aerobic and anaerobic conditions necessary for nitrification and denitrification, respectively, resulting in efficient nitrogen removal that neither plant can independently deliver on its own. Thus, the MTBRCW system provides an integrated treatment facility

that is effective in treatment and easy to operate, stable hydraulically, and not prone to climate variations. It has been observed initially that the cost of implementation of such a system would be relatively low compared to the traditional HSSF-CW system, but still, a techno-economic analysis needs to be carried out. Future adaptations involving modified bio-rack configurations and locally available macrophyte species, combined with supplementary pathogen removal processes where necessary, represent a promising avenue for expanding access to safe sanitation in communities not served by centralised treatment infrastructure.

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