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Design optimisation of five pilot-scale two-stage vertical flow-constructed wetlands for piggery wastewater treatment

Q1 Karan N[a], Jayanta Kumar Gogoi[a], Anasuya Ganguly[a], António Guerreiro de Brito[b],
Q2 Claudia S. C. Marques dos Santos Cordovil[c], Diego Correa[d], Luisa Gouveia[d,e] and Srikanth Mutnuri[a,*]

[a] Water Sanitation and Hygiene Laboratory, Department of Biological Sciences, Birla Institute of Technology and Science, K K Birla Goa Campus, NH 17 B, Zuarinagar, Goa 403 726, India
[b] School of Agriculture, University of Lisbon, LEAF, Tapada da Ajuda, Lisboa 1349-017, Portugal
[c] Instituto Superior de Agronomia (ISA/ULisboa), Tapada da Ajuda, Lisboa 1349-017, Portugal
[d] LNEG, National Laboratory of Energy and Geology I.P., Bioenergy and Biorefineries Unit, Estrada do Paço do Lumiar 22, Lisbon 1649-038, Portugal
[e] GreenCoLab, Green Ocean Technologies and Products Collaborative Laboratory, University of Algarve, Campus de Gambelas, Faro 8005-139,
Q3 Portugal
Q4 *Corresponding author. E-mail: srikanth.mutnuri@gmail.com; jayantagogoi200@gmail.com

SM, 0000-0001-6956-0143

ABSTRACT

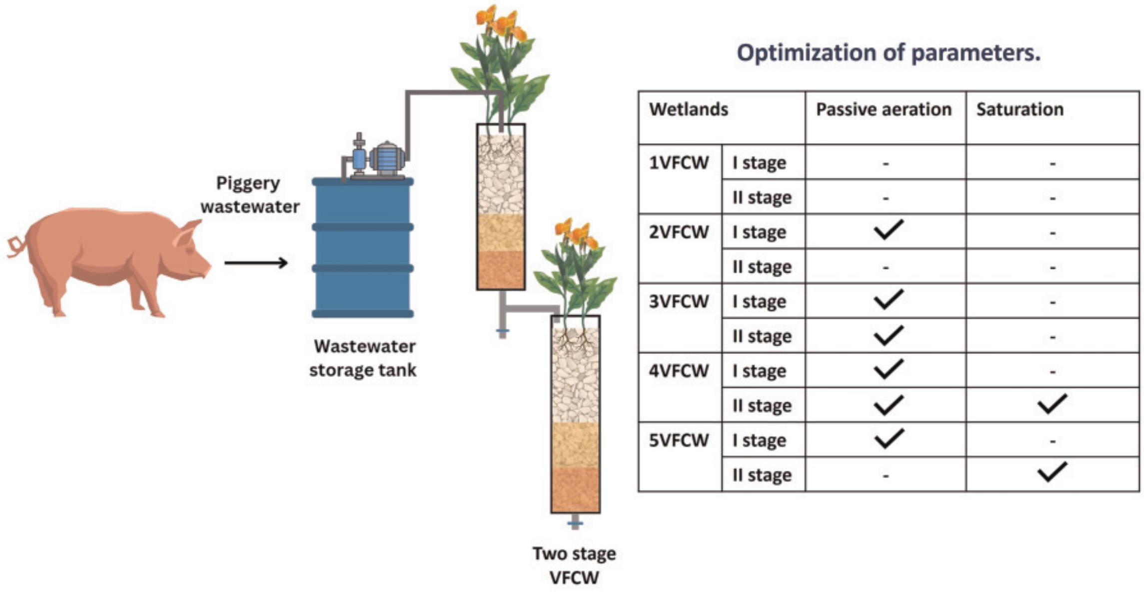
With growing pig farming, sustainable piggery wastewater treatment methods are essential for environmental protection. This study evaluated five pilot-scale two-stage vertical flow-constructed wetlands (VFCWs) with varying configurations of aeration, plantation, and saturation zones. Three VFCW configurations (1VFCW, 2VFCW, and 3VFCW) were unsaturated, while 4VFCW and 5VFCW were saturated in the second stage (up to 60 and 90 cm, respectively). The 5VFCW featured a stacked configuration with no space between its two stages. Passive aeration was selectively applied in 2VFCW, 3VFCW, 4VFCW, and 5VFCW, while plants were present in most configurations except the control. Saturated 4VFCW achieved the highest removal efficiency for TN (77.03 ± 16.24%) and NO_3^- (46.06 ± 45.96%), while the stacked 5VFCW showed the highest removal for chemical oxygen demand (COD) (94.17 ± 4.85%) and Total ammoniacal nitrogen (TOC) (86.35 ± 6.78%). Unsaturated 1VFCW excelled in TAN removal (98.89 ± 0.33%), and the control system (C) showed the highest removal efficiency for PO_4^{3-} (90.38 ± 6.52%) and TOC (87.52 ± 9.83%). Overall, 4VFCW emerged as the most balanced and effective system, supported by an optimal combination of aerobic and anaerobic conditions that facilitated sequential nitrification and denitrification, along with an extended hydraulic retention time due to saturation. Q5

Key words: aeration, piggery wastewater, plantation, saturation, sustainable, vertical flow constructed wetland

HIGHLIGHTS

- Evaluated five VFCWs with varying aeration, saturation zone, and plantation for piggery wastewater treatment.
- The saturated VFCWs showed better treatment for COD, NO_3^-, TN, PO_4^{3-}, and total ammoniacal nitrogen (TOC) than unsaturated VFCWs.
- The unsaturated VFCWs (1VFCW, 2VFCW, and 3VFCW) showed higher TAN removal than the saturated VFCWs.
- The optimal redox gradient of 4VFCW and 5VFCW types favoured simultaneous nitrification and denitrification.

GRAPHICAL ABSTRACT



Wetlands		Passive aeration	Saturation
1VFCW	I stage	-	-
	II stage	-	-
2VFCW	I stage	✓	-
	II stage	-	-
3VFCW	I stage	✓	-
	II stage	✓	-
4VFCW	I stage	✓	-
	II stage	✓	✓
5VFCW	I stage	✓	-
	II stage	-	✓

1. INTRODUCTION

Wastewater (WW) generation is escalating due to population growth, urbanisation, industrialisation, agriculture, and livestock activities. Recycling and reusing wastewater are vital strategies to alleviate water scarcity while protecting the environment. Conventional wastewater treatment (WWT) involves processes that are costly, energy-intensive, and labour-demanding. Additionally, these processes contribute to greenhouse gas (GHG) emissions (Ferreira *et al.* 2019). Therefore, exploring sustainable and cost-effective alternatives is particularly important for small and under-resourced communities.

Animal husbandry and livestock sectors are pivotal for rural livelihoods and global economic development, particularly in regions like India. Pigs offer significant economic potential among livestock species due to their high fecundity, efficient feed conversion, early maturity, and short generation interval. Although pig farming requires relatively low investments in infrastructure and equipment, it uses large amounts of water and generates highly polluted wastewater. Piggery wastewater is characterized by extremely high concentrations of organic matter, nitrogen (N), phosphorus (P), and pathogenic contaminants, making it one of the most challenging agricultural waste streams to treat. It also contains residual antibiotics (e.g., sulfonamides, tetracyclines) and antibiotic-resistant bacteria (ARB) (Tang *et al.* 2023). High chemical oxygen demand (COD) (~5,000 mg/L) and BOD (~2,000 mg/L) along with the high amount of ammonia and phosphorus levels present in piggery wastewater can heavily burden traditional treatment methods, not optimized for the removal of organic compounds and nutrients (Li *et al.* 2013; Gogoi *et al.* 2024b). Therefore, treating piggery wastewater remains a significant challenge, necessitating sustainable, low-cost, yet highly efficient solutions such as vertical flow constructed wetlands (VFCWs) where the removal of organic compounds and nutrients can be combined through optimal design.

Constructed wetlands (CWs) are gaining widespread attention as a sustainable alternative sewage treatment technology due to their ability to efficiently treat various types of wastewater, including domestic wastewater, Q6 stormwater, leachate, polluted rivers, rural runoff, and industrial effluents (Abou-Elela & Hellal 2012; Saeed Q7 & Sun 2013; Sharma *et al.* 2022; Gogoi *et al.* 2024a). CWs provide a natural, straightforward, and eco-friendly solution for WWT. They are artificially engineered systems that utilise plants, microbes, and filter media to Q8 remove pollutants from wastewater (Nagarjun *et al.* 2019; Sharma *et al.* 2022; Gogoi & Mutnuri 2024). Key benefits include low construction and maintenance costs and minimal operational expertise requirements (Abbasi *et al.* 2019; Sharma *et al.* 2022; Gogoi *et al.* 2024a). CWs operate on renewable energy sources such as solar, wind, or gravity, making them particularly viable for small farms in rural communities (Khan & Khalil 2017; Yadav *et al.* 2018; Abbasi *et al.* 2019).

Among the types of CWs, VFCWs are the most widely used sub-surface flow systems due to their high treatment efficiency and compact space requirements (Stefanakis *et al.* 2014; Sharma *et al.* 2022). VFCWs are typically filled with filter media like coarse sand or gravel, which support plant growth, act as filters, and accumulate organic matter, phosphorus, sulphate, arsenate, and pathogens (Stanković 2017). Macrophytes with rhizome root systems are commonly used in CWs, providing a large rhizosphere surface for microbial growth. Commonly used plants include *Canna indicana and Canna glauca* (Yadav *et al.* 2018; Karungamye 2022).

CWs remove pollutants through three key mechanisms: physical, chemical, and biological processes. The physical process involves sedimentation, where suspended particles in wastewater settle, leading to contaminant removal. This sedimentation process also aids in the removal of coliform bacteria (Dotro *et al.* 2015; Gogoi *et al.* 2024a). Adsorption is another critical mechanism, particularly for phosphorus removal (Saeed *et al.* Q9 2012). The use of zeolite media results in substantial phosphorus elimination (Liu *et al.* 2009). Biological processes play a prominent role in WWT within CWs. These include ammonification, denitrification, biological phosphorus removal (BPR), and aerobic and anaerobic organic degradation (Sharma *et al.* 2022; Gogoi & Mutnuri 2024). Ammonification is the conversion of organic nitrogen to ammoniacal nitrogen (NH_4-N), which occurs more rapidly under aerobic conditions. Nitrification follows a two-step process, where ammonium is first transformed to nitrite and then to nitrate. This process is facilitated by microorganisms such as *Nitrosomonas, Nitrosococcus, and Nitrosospira* (Gogoi & Mutnuri 2024). Denitrification, on the other hand, occurs under strictly anaerobic conditions, converting nitrate to nitrogen gas. This process relies on bacteria like *Bacillus, Enterobacter, Micrococcus, Pseudomonas, and Spirillum* (Lee *et al.* 2009; Abou-Elela *et al.* 2013). The enhanced biological phosphorus removal (EBPR) is carried out by a group of organisms that can take up extracellular orthophosphate and store it as a polyphosphate reserve. This process is favoured by the sequential exposure of anaerobic to aerobic conditions and involves bacteria such as *Acinetobacter*, *Corynebacterium*, *Pseudomonas*, etc. (Kim & Lenz 2001; Yuan *et al.* 2012). Q10

The efficiency of CWs in treating wastewater is influenced by various factors, like hydraulic retention time (HRT), hydraulic loading rate, soil and substrate types, vegetation, living organisms, pollutant loading rates, climatic conditions, temperature, pH, and oxygen availability (Khanijo 2002; Abbasi *et al.* 2019; Sharma *et al.* 2022). Advancements in CW design configurations offer significant potential for improving treatment performance.

Studies have demonstrated the efficacy of various CW designs in improving pollutant removal efficiencies. For instance, vertical-flow CWs with *Typha spp.* achieved notable COD and phosphate removal (33 and 45%, respectively) while supporting nitrification (49%) over 280 days of operation in Brazil (Sezerino *et al.* 2003). Similarly, a hybrid CW system in Italy effectively reduced COD (79%), total nitrogen (TN) (64%), and phosphorus (61%), underscoring its potential in nutrient management (Borin *et al.* 2013). The role of vegetation in CW systems has been extensively investigated. Napier grass demonstrated high removal efficiencies for BOD (94%) and COD (64%) in vertical subsurface flow CWs, while providing biomass yields suitable for agricultural applications (Klomjek 2016). In Mexico, in-series constructed wetlands using *Typha* sp. and *Scirpus* sp. achieved COD removal efficiencies of up to 80% under extended hydraulic retention times (HRTs), meeting irrigation water quality standards for lower pollutant loads (De La Mora-Orozco *et al.* 2018). CWs have shown high removal efficiencies for various antibiotics, including sulfamethoxazole (34–84%), tetracycline (9–97%), ciprofloxacin (5–98%), and doxycycline (43–70%), making them a promising low-cost alternative for WWT (Sanjrani *et al.* 2021). Additionally, VFCWs have been shown to contain diverse microbial communities (*Proteobacteria*, *Firmicutes*) that actively degrade antibiotics, removing up to 83% of sulfadiazine (Guo *et al.* 2023). These findings highlight the potential of constructed wetland systems as cost-effective and environmentally friendly technologies for managing piggery wastewater.

Despite the widespread adoption of CWs for WWT, there is a lack of clarity on the optimal configuration for treating high-strength piggery wastewater, particularly in the Indian context, where resource constraints and climatic variability play significant roles. Existing studies primarily focus on general wastewater or specific configurations without systematically addressing the interplay of parameters such as aeration, saturation, and wetland depth. Most studies have concentrated on horizontal flow-constructed wetlands (HFCWs) or single-stage VFCWs for domestic and agricultural wastewater, demonstrating moderate COD and nitrogen removal efficiencies. However, they often fail to address the unique challenges posed by piggery wastewater, which has higher concentrations of organic matter, nitrogen, and phosphorus.

This study builds on previous research by adopting a multi-parameter optimization approach, combining passive aeration, saturation zone depth variation, and plantation in two-stage VFCWs to enhance treatment efficiency. Unlike prior studies, this research systematically evaluates the interaction of these factors to create optimal redox zonation for simultaneous nitrification and denitrification. The current study aims to address this gap by designing five configurations of two-stage VFCWs for piggery WWT at a pilot scale. The VFCWs configuration varied regarding aeration, saturation, and plantation to investigate optimal treatment performance efficiency. Then, the comparative treatment efficiency of each type of VFCW was studied in reference to parameters like COD, TOC, NO_3^-, PO_4^{3-}, TAN, TN, and pH.

2. MATERIALS AND METHODS

2.1. Site description and study design

Five pilot-scale two-stage VFCW systems for piggery WW treatment were demonstrated at BITS Pilani, KK Birla Goa campus (15.394° N, 73.880° E), India, as shown in Figure 2. The treatment system consisted of a 100 L storage tank sequenced with 2-stage VFCWs. The wetland systems were provided with a roof made of transparent polyvinyl chloride (PVC) sheets. The wastewater (WW) was collected from a pig farm in Majorda, Goa, India (15.311° N, 73.929° E) and was stored in a 100 L plastic tank before being pumped to the wetlands daily. The fresh piggery WW was collected every 2 days for the treatment. The study was documented for ~2 months.

2.2. Design and optimisation of 2-stage CWss

The effect of passive aeration, saturation, and plantation on VFCW treatment efficiency was investigated through five different configurations of two-stage VFCWs, namely, 1VFCW, 2VFCW, 3VFCW, 4VFCW, and 5VFCW, along with control (C). PVC pipes with a diameter of 15 cm and height of 100 cm were used for the VFCWs demonstration, as shown in Figure 2. Each stage of the VFCWs was packed with river gravels arranged in three distinct layers: the top filter layer, middle transition layer, and bottom drainage layer. The gravel sizes and layer depths were optimized for filtration and pollutant removal. Detailed information on the gravel sizes and height profiles for each VFCW configuration and the control system (C) is provided in Table 1. The filter media distribution and its sizes, organic loading rate, and hydraulic loading rate were selected based on our previous study (Gogoi *et al.* 2024a).

2.2.1. Saturation

The C, 1VFCW, 2VFCW, and 3VFCW remained unsaturated in both stages. In contrast, 4VFCW and 5VFCW were partially saturated in the second stage, with 4VFCW saturated up to 60 cm and 5VFCW up to 90 cm (Figure 1). The saturation levels were regulated by adjusting the height of the drainage pipes, creating controlled anaerobic conditions in the saturated zones. The 5VCFW had a stacked configuration, with the first-stage VFCW directly mounted on the second-stage VFCW, as shown in Figure 1. The varying saturation configuration was designed to assess its impact on the removal of different wastewater parameters and to achieve optimal redox gradient zones for simultaneous NO_3^- and TAN removal. The saturated zones create anaerobic conditions, while the unsaturated zones promote better gas exchange (Gogoi & Mutnuri 2024; Gogoi *et al.* 2024b). The geometry of unsaturation and saturation zonations was done in reference to previous studies on VFCW (Gogoi *et al.* 2022; Gogoi & Mutnuri 2024; Gogoi *et al.* 2024a, b).

2.2.2. Passive aeration

Different passive aeration configurations were implemented using 1-inch plastic pipes to study their effect on treatment (Figures 1 and 2). Passive aeration was applied to the first stages of the 2VFCW and 5VFCW (both up to 80 cm), both stages of the C and 3VFCW (both up to 80 cm), and both stages of the 4VFCW (first stage up to 80 cm and second stage up to 30 cm). No aeration was applied to either stage of the 1VFCW.

2.2.3. Plantation

Two *Canna indica* plants were planted in both stages of the 1VFCW, 2VFCW, 3VCFW, and 4VFCW wetlands. The control wetland had no plants, and in the 5VFCW, plants were only planted in the first stage due to the stacked configuration (Figures 1 and 2). The plant density was in reference to the study done by Gogoi *et al.* (2024a). The *Canna indica* was used due to its perennial growth type, rhizome root type, and adaptability to the soggy and Goa's tropical climate conditions (study field site).

2.2.4. Wetland operation

The Piggery wastewater was pumped to the first stage of the wetlands twice a day at 1 L/day using a peristaltic pump (NFP-03, Flowtech, India). The active volume capacity of the saturated wetlands (4VFCW and 5VFCW)

Table 1 | Filter layers profile of a 2-stage VFCWs

	I Stage							**II Stage**						
	C	**1VFCW**	**2VFCW**	**3VFCW**	**4VFCW**	**5VFCW**		**C**	**1VFCW**	**2VFCW**	**3VFCW**	**4VFCW**	**5VFCW**	
Filter layers	**Layer depth (cm)**						**Gravel size (mm)**	**Layer depth (cm)**						**Gravel size (mm)**
Top layer	40	40	40	40	20	40	10	40	40	40	40	40	40	02–08
Middle layer	20	20	20	20	20	20	10–20	20	20	20	20	40	40	10–15
Bottom layer	20	20	20	20	20	20	20–40	20	20	20	20	20	20	20–30

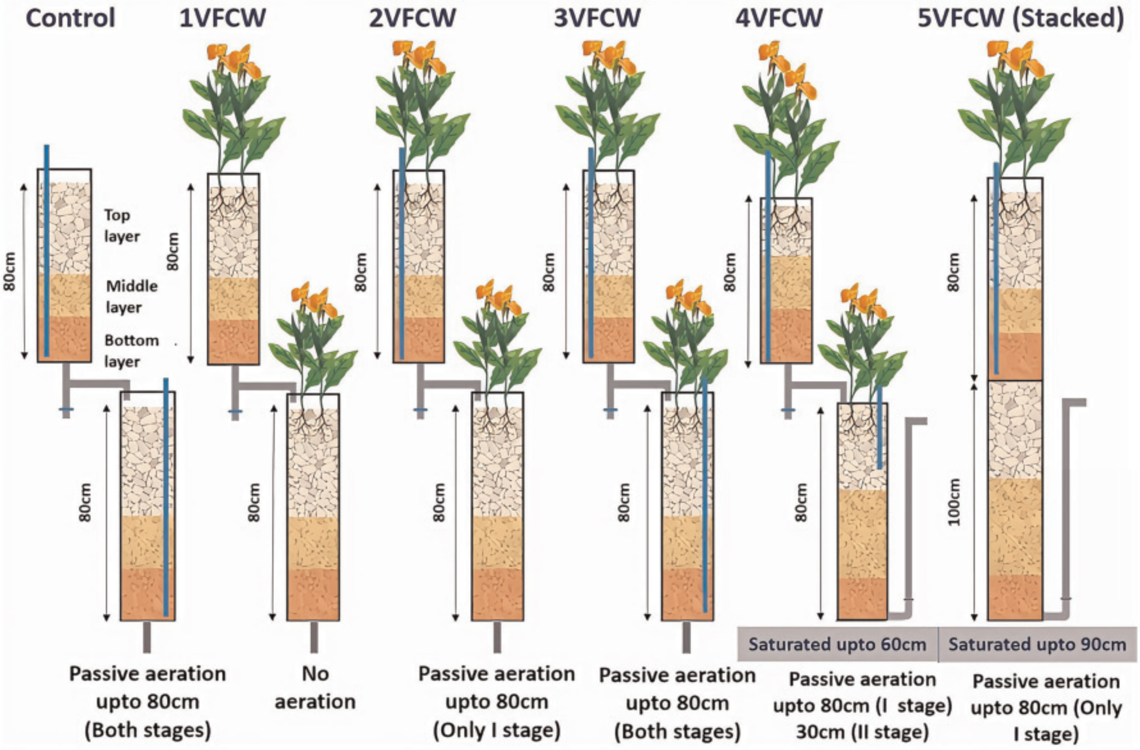


Figure 1 | Schematic diagram of six pilot-scale wetland systems employed in this study.

Figure 2 | Pilot-scale demonstrated 2-stage vertical flow constructed wetland types.

was determined by calculating the water-holding capacity as the difference between input and output volumes. The wetlands' HRTs were then calculated based on the daily wastewater feed flow rate relative to the wetland volume capacity.

2.3. Sampling protocol

Samples were collected twice weekly from both stages of each VFCW and analyzed within 24 h to ensure data accuracy and reliability.

2.4. Analytical methods

Key wastewater parameters were selected based on their significance in assessing the treatment efficiency and environmental impact of piggery wastewater. Chemical oxygen demand (COD) was measured using the **5220**

D closed reflux colorimetric method to quantify the organic load, which is crucial for evaluating the oxidation process and overall organic pollutant levels. Total ammoniacal nitrogen (TAN) was determined with the **Spectroquant® Prove 100 spectrophotometer kit (1.00683.0001)** as it serves as an indicator of nitrogenous compounds, which are essential for monitoring nutrient removal performance and potential toxicity to aquatic ecosystems. Ortho-phosphate (PO_4^{3-}) was measured by the **Vanado-molybdophosphoric acid colorimetric method** to track phosphorus concentrations, an important parameter in controlling eutrophication. Nitrate (NO_3^-) was analyzed using a **LAQUAtwin-NO3-11-S040 nitrate meter** to assess the oxidation of nitrogen compounds, providing insights into nitrification efficiency. Total organic carbon (TOC) and TN were analyzed with a **Shimadzu TOC-TNM-L ROHS analyzer** to offer a comprehensive understanding of the carbon and nitrogen content in the samples, reflecting overall pollutant levels and treatment efficacy. Finally, pH was measured using an **Oakton pH 510 Series Meter (P/N 54X002608)** to monitor the acidity or alkalinity of the wastewater, which directly influences microbial activity and treatment performance (APHA/AWWA/WEF 2005).

2.5. Statistical analysis

Q11 The raw data were analyzed using Microsoft Excel 2021 (Microsoft Corporation, 2021) to obtain graphs with
Q12 mean and standard deviations. The averages of removal efficiencies for each parameter in the different
Q13 VFCWs were subjected to ANOVA using R software (**version 4.4.1**; R Core Team 2023), followed by Tukey's
post-hoc test to compare means using $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Wastewater characteristics of raw piggery wastewater and VFCWs effluent

The analysis of piggery wastewater shown in Table 2 revealed the high levels of various pollutants in the raw effluent, highlighting the significant environmental impact of such effluents and the respective values after each treatment in both stages of the wetlands. The COD was recorded at 5,232.50 ± 2,572.43 mg/L, indicating a high concentration of oxidisable organic matter. This elevated COD can significantly decrease the water's dissolved oxygen (DO), risking aquatic life. TOC was determined to be 967.30 ± 448.54 mg/L. This further depletes the DO in the process of its oxidation. TAN and nitrate (NO_3^-) levels were 69.50 ± 17.68 mg/L and 60.80 ± 33.09 mg/L, respectively. TN concentration was 81.30 ± 39.03 mg/L, indicating a substantial nitrogen load. PO_4^{3-} concentrations were 65.10 ± 4.57 mg/L, indicating significant phosphorus content. This high nitrogen and phosphorous concentration can result in considerable eutrophication, threatening aquatic lifeforms. The treated effluents generally showed improved water quality, with varying efficiencies across different wetlands (Table 2).

3.2. Two-stage VFCW's treatment efficiency

The HRTs for the second-stage 4VFCW and 5VFCW were 68 h 28 min and 76 h 48 min, respectively. HRT is critical as it influences the contact time between wastewater and the media, which is essential for effective treatment. The chosen flow rate ensured a balance between HRT and the wetlands' treatment capacity to optimize pollutant removal, as longer HRTs can enhance performance but may reduce the volume of water treated, affecting overall system efficiency. Studies on similar systems suggest that an HRT of 2–4 days is effective for pollutant removal (Sezerino *et al.* 2003; Bustillo-Lecompte *et al.* 2016; Gogoi *et al.* 2024b). The HRTs in this study fall within this range, supporting efficient COD removal and nitrogen removal via denitrification.

Furthermore, the selected HRTs were optimal concerning both pollutant removal and the volume of wastewater that must be treated. Figure 3 provides a comprehensive comparison of the removal efficiencies for COD, TAN, NO_3^-, PO_4^3, TN, and TOC across the five different configurations of two-stage VFCWs, including the control. The following sections discuss the removal performance for each parameter in detail.

3.2.1. Chemical oxygen demand

5VFCW, the stacked-type configuration with passive aeration and plants in the first stage, demonstrated the highest COD removal efficiency of 94.17 ± 4.85%. This might be due to longer HRT compared to other wetlands, as the compact design and no space between the stages facilitate more effective interaction between wastewater and microbial communities, leading to better degradation of organic pollutants. Research has shown that longer
Q14 HRTs and partially saturated wetlands are more effective at removing COD (Shruthi & Shivashankara 2022;
Viveros *et al.* 2022). The remaining wetlands, including the control, showed almost similar removal efficiencies

Table 2 | Characteristics of piggery wastewater and treated effluent of both the stages of VFCWs

	Average reduction observed						
Samples	**COD (mg/L)**	**TAN (mg/L)**	**NO_3^- (mg/L)**	**TN (mg/L)**	**PO_4^{3-} (mg/L)**	**TOC (mg/L)**	**pH**
Raw Sample	5,232.50 ± 2,572.43	69.50 ± 17.68	60.80 ± 33.09	81.30 ± 39.03	65.10 ± 4.57	967.30 ± 448.54	5.79 ± 0.61
1-C	1,581.75 ± 1,503.41	24.75 ± 1.77	102.80 ± 49.85	51.76 ± 24.61	55.54 ± 25.70	572.12 ± 296.04	6.14 ± 0.50
2-C	326.50 ± 201.50	4.20 ± 2.40	103.00 ± 41.39	18.07 ± 7.47	6.41 ± 4.68	112.05 ± 64.94	6.95 ± 0.31
1-1VFCW	1,531.75 ± 1,418.63	5.70 ± 0.85	86.00 ± 49.54	60.06 ± 27.28	58.89 ± 10.29	683.94 ± 228.74	5.68 ± 0.33
2-1VFCW	483.50 ± 266.83	0.80 ± 0.42	110.20 ± 76.30	20.56 ± 8.89	19.65 ± 6.56	184.91 ± 130.29	6.82 ± 0.28
1-2VFCW	1,436.75 ± 1,309.76	8.80 ± 1.98	79.80 ± 39.09	51.18 ± 20.20	44.42 ± 2.59	624.68 ± 289.74	5.72 ± 0.25
2-2VFCW	508.50 ± 250.44	1.30 ± 0.71	110.60 ± 69.07	24.75 ± 10.81	18.95 ± 1.32	169.55 ± 82.24	6.79 ± 0.09
1-3VFCW	1,715.25 ± 1,925.77	8.15 ± 1.77	81.20 ± 44.18	60.32 ± 20.61	40.46 ± 12.71	572.48 ± 246.22	5.98 ± 2.99
2-3VFCW	442.00 ± 188.39	1.25 ± 1.48	97.40 ± 59.52	23.30 ± 12.03	12.17 ± 8.67	154.71 ± 54.60	7.06 ± 0.41
1-4VFCW	2,180.50 ± 2,792.98	4.60 ± 0.85	58.40 ± 36.60	69.64 ± 30.44	39.57 ± 1.20	826.78 ± 386.77	5.62 ± 0.66
2-4VFCW	412.00 ± 246.83	8.20 ± 0.85	22.00 ± 10.07	13.92 ± 2.66	7.44 ± 0.26	156.80 ± 86.38	6.95 ± 0.42
5VFCW	246.20 ± 100.56	50.94 ± 30.67	42.88 ± 9.56	57.00 ± 21.67	7.72 ± 2.89	124.54 ± 43.56	6.41 ± 0.22

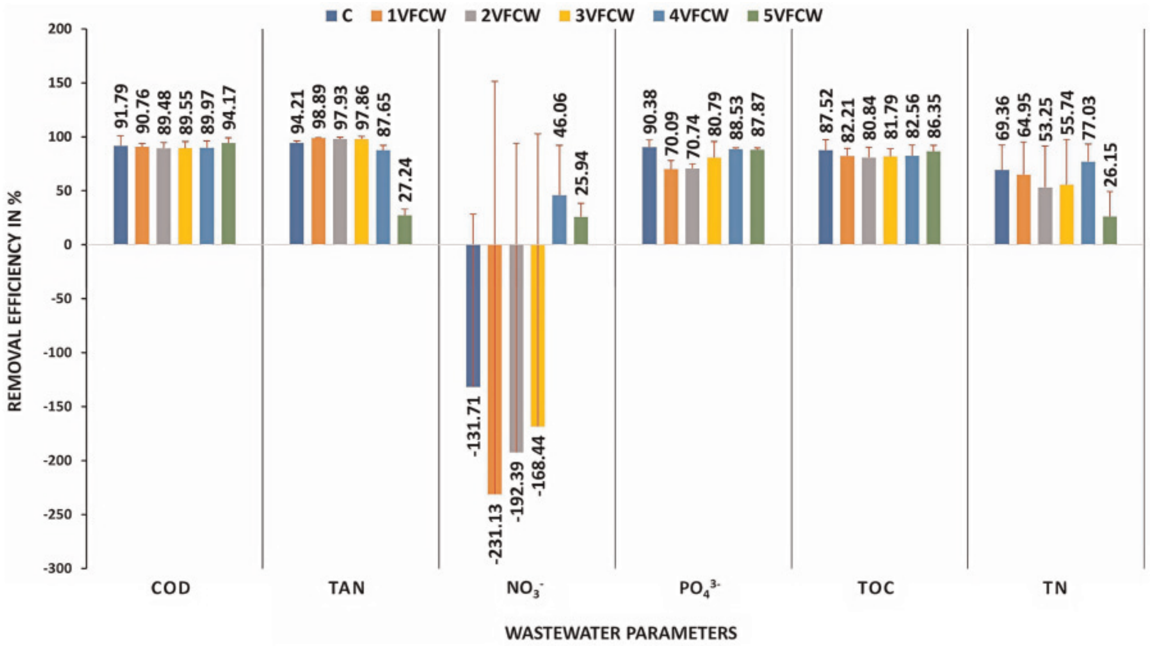


Figure 3 | Piggery wastewater treatment removal efficiency by various configured two-stage VFCWs.

in the range of 90%. The post-hoc test following ANOVA revealed no statistically significant differences ($p > 0.05$) in COD removal efficiencies between any pair of wetland configurations. While the stacked 5VFCW showed the highest mean COD removal, its difference was not statistically significant compared to other configurations, including the control. This lack of significant differences could indicate that the overall treatment processes for COD removal were robust across all configurations, with minor enhancements observed in 5VFCW due to its stacked design and prolonged HRT.

3.2.2. Total ammoniacal nitrogen

All unsaturated VFCW types – 1VFCW, 2VFCW, and 3VFCW – demonstrated higher TAN removal efficiency than the saturated VFCW types (4VFCW and 5VFCW). The presence of plants in both stages of 1VFCW, 2VFCW, and 3VFCW likely contributed to this improved TAN removal. In contrast, the saturated zones in 4VFCW and 5VFCW created anaerobic conditions that do not support nitrification. The higher TAN removal in unsaturated wetlands is primarily attributed to the activity of aerobic nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter*, which require oxygen to convert ammonium to nitrite and nitrate (Gogoi & Mutnuri 2024). Passive aeration and plant-mediated oxygen transfer in these unsaturated systems likely enhanced microbial nitrification, leading to more efficient TAN removal (Shelef *et al.* 2013; Wiessner *et al.* 2013). The highest removal efficiency observed in 1VFCW suggests that aeration can be effectively compensated by plant uptake. The lowest TAN removal was recorded in 5VFCW (29.28%). The second stage of the 5VFCW system was saturated and lacked vegetation, creating anaerobic conditions unfavorable for nitrification. This finding underscores the role of oxygen availability as a key factor in microbial community composition – unsaturated systems with aeration favor nitrifiers, whereas saturated systems support anaerobic microbial populations involved in denitrification. The post-hoc test following ANOVA revealed significant differences in TAN removal efficiency, with 5VFCW exhibiting significantly lower removal compared to the control (C) and other configurations ($p < 0.05$). However, the control (C), 1VFCW, 2VFCW, and 3VFCW showed no significant differences in TAN removal from one another, indicating similar efficiencies. These results suggest that saturated and stacked configurations are less effective in TAN removal than unsaturated systems.

3.2.3. Nitrate (NO_3^-)

The highest NO_3^- removal efficiency was observed in the 4VFCW wetland at $46.06 \pm 45.96\%$, followed by 5VFCW at $26.83 \pm 12.46\%$. This positive nitrate removal in 4VFCW and 5VFCW can be attributed to the saturated, anaerobic conditions in their second stages, which provided an environment conducive to denitrification. Denitrification is a biological process in which nitrate is reduced to nitrogen gas, occurring exclusively under anaerobic conditions with sufficient organic carbon as an electron donor (Vymazal 2007). Facultative anaerobic bacteria such as *Pseudomonas*, *Bacillus*, and *Paracoccus* drive denitrification by using nitrate as an electron acceptor in the absence of oxygen, converting it to nitrogen gas (Abou-Elela *et al.* 2013). The controlled saturation zone in 4VFCW likely optimized conditions for these bacteria, leading to improved nitrate removal compared to other configurations. In contrast, the control wetland and 1VFCW, 2VFCW, and 3VFCW exhibited negative nitrate removal efficiencies, indicating an increase in nitrate levels. These unsaturated configurations lacked the anaerobic conditions necessary for denitrification, allowing nitrates to accumulate due to active nitrification. Additionally, aeration in some of these wetlands promoted aerobic nitrifying bacteria, which converted ammonium to nitrate without enabling subsequent nitrate reduction. Saturated configurations, such as 4VFCW and 5VFCW, created distinct redox gradients that supported sequential nitrification and denitrification. The anaerobic zones in their second stages facilitated denitrification, significantly improving nitrate removal compared to unsaturated wetlands. This highlights the microbial shift associated with varying oxygen levels – nitrifiers dominate in aerated, unsaturated environments, while denitrifiers thrive in saturated zones with limited oxygen. The superior nitrate removal in 4VFCW compared to 5VFCW may be due to root exudates secreted by plants in the second stage of 4VFCW, which provide organic carbon essential for denitrifying bacteria (Zhai *et al.* 2013). These findings underscore the importance of incorporating saturation zones in wetland configurations to enhance nitrate removal by fostering anaerobic conditions. The post-hoc test following ANOVA revealed significant differences in NO_3^- removal between saturated and unsaturated wetland configurations. The control (C), 2VFCW, 3VFCW, and 4VFCW showed no significant differences in nitrate removal from one another, with large p-values ($p > 0.05$) indicating minimal variability. However, the stacked wetland configurations (4VFCW and 5VFCW) exhibited significantly higher nitrate removal, suggesting that these designs are more effective in reducing nitrate levels.

3.2.4. Phosphate (PO_4^{3-})

The highest PO_4^{3-} removal of 90.38 ± 6.52% was observed in the control wetland than in the planted VFCWs (Figure 3). The main reason for less phosphorus removal in all the planted VFCWs compared to the unplanted control might be due to the decaying of a plant's root and fallen leaves, resulting in the release of phosphorus into the wetland. Further, the organic acids released in the root exudates mobilise or solubilise the rhizosphere-bound phosphorus, which can also leach out in the wetlands effluent (Richardson & Simpson 2011; Ma *et al.* 2022). Furthermore, the planted VFCWs enhance the aeration in the VFCWs media column, which improves the oxidation of organic and inorganic phosphorous to phosphates (Gogoi *et al.* 2024b). Thus, the phosphorus removal in control can be majorly due to microbial remediation and then the adsorption in the filter media.

The saturated wetlands 4VFCW and 5VFCW also showed high phosphate removal rates comparable to control. The high HRTs in the wetlands leading to increased contact between the water and wetland filter media might have led to better phosphate adsorption to the media (Minakshi *et al.* 2022). The least PO_4^{3-} removal was observed in 1VFCW, closely followed by 2VFCW. The post-hoc test following ANOVA revealed that the control group (C) showed significantly better phosphate removal compared to 1VFCW and 2VFCW ($p < 0.05$). No significant differences were observed between the control group and other configurations (e.g., 3VFCW, 4VFCW, 5VFCW), nor between the different VFCWs themselves. These results suggest that the control group performed better in phosphate removal, while the remaining VFCW configurations showed similar or less effective performance in comparison. This might be due to unsaturation and significantly less HRT, which leads to reduced adsorption.

3.2.5. Total organic carbon

The control showed slightly more TOC removal of 87.52 ± 9.83%, followed closely by 5VFCW (86.35 ± 6.78%) (Figure 3). All the VFCWs' TOC removal efficiency was closely related (ranging from 81 to 86%) and comparable differences with control (Figure 3). The higher removal in 5VFCW was likely due to the second stage, which had longer HRT and was devoid of plants. The main reason for slightly less TOC removal in all the planted VFCWs compared to control might be due to the decaying of a plant's root and fallen leaves, resulting in the release of organic carbon into the wetland. Further, the plants in the wetland system secrete root exudates, which are a complex mixture of organic compounds that can also contribute to TOC (Shelef *et al.* 2013). Plant roots may release up to 20% of their photosynthesis products into the soil (Haichar *et al.* 2008). This might be the reason for the better performance of the control compared to VFCWs with plants in both stages. The post-hoc test following ANOVA revealed no statistically significant differences ($p > 0.05$) in TOC removal efficiencies between any pair of configurations. While 5VFCW showed a slight increase in TOC removal compared to other VFCW, this difference was not statistically significant. Thus, all VFCW configurations showed effective TOC removal, wherein the control showed slightly more TOC removal than the 5VFCW (highest TOC removal VFCW configuration) by 1.17% (Figure 3).

3.2.6. Total nitrogen

The highest TN removal was observed in 4VFCW (77 ± 16.24%). In this system, the second stage was saturated up to 60 cm, creating a balance between aerobic and anaerobic conditions. This balance is crucial for nitrogen removal, as it enables sequential nitrification and denitrification. The first stage, with better oxygen availability, likely supported nitrifying bacteria, while the saturated second stage facilitated denitrification by anaerobic bacteria. The lowest TN removal was recorded in 5VFCW, which can be attributed to its lower TAN removal efficiency. The post-hoc test following ANOVA confirmed that 5VFCW had significantly lower ($p < 0.05$) TN removal compared to all other configurations, indicating that it was the least effective treatment for TN removal. The second stage of 5VFCW was saturated up to 90 cm, creating predominantly anaerobic conditions that were less favorable for nitrification. Additionally, aeration in the first stage was limited to 80 cm, which may have provided insufficient oxygen for effective nitrification, ultimately reducing overall nitrogen removal. These findings further emphasize the importance of microbial community structuring in VFCWs, where maintaining an optimal balance between aerobic and anaerobic zones is essential for efficient nitrogen removal.

3.2.7. Overall WWT comparison of the 2-stage VFCWs

Each wetland configuration demonstrated unique advantages, making them suitable for different treatment applications. Overall, the saturated VFCWs (4VFCW and 5VFCW) showed better treatment efficiency than the unsaturated VFCWs (1VFCW, 2VFCW, and 3VFCW). The 4VFCW configuration showed the highest treatment efficiency for PO_4^{3-} (88.53% ± 1.21), NO_3^- (46.06 ± 45.96%), and TN (77 ± 16.24%), whereas the 5VFCW showed the highest removal efficiency for COD (94.17 ± 4.85%). The 1VFCW configuration showed the highest removal efficiency for TAN (98.89 ± 0.33%).

Moreover, the control (C), devoid of plants, exhibited the highest removal efficiency for PO_4^{3-} (90.38 ± 6.52%) and TOC (87.52 ± 9.83%). This can be attributed to the sand and gravel filtration along with the inhabited microbial remediation. Overall, the 4VFCW configuration was found to be the most optimal design of the other VFCWs for piggery WWT, showing high removal efficiencies across multiple parameters.

3.3. Comparison with other studies

The performance of the two-stage VFCWs in this study shows significant improvements compared to other studies, primarily due to optimized configurations that balance aeration, saturation, and redox zonation. The COD removal efficiency, particularly in 5VFCW (94.17 ± 4.85%), surpasses that of horizontal subsurface flow constructed wetlands (44.85%) (Udom *et al.* 2018) and three-stage surface flow constructed wetlands (79.0–82.7%) (Luo *et al.* 2018b). The higher efficiency in this study can be attributed to the stacked design of 5VFCW, which facilitates longer HRT and better contact between wastewater and microbial communities, enhancing organic matter degradation. In comparison, horizontal and surface flow systems often experience limited oxygen diffusion and shorter retention times, reducing treatment efficiency. Additionally, the higher influent COD (5,232.50 ± 2,572.43 mg/L) in this study may have provided greater substrate availability for microbial degradation, contributing to higher removal efficiency.

For TAN removal, all VFCWs except 5VFCW exceeded the 87.7–97.9% efficiency reported in three-stage surface flow wetlands treating lagoon-pretreated swine wastewater (Luo *et al.* 2018a) and outperformed zoning tidal flow wetlands with 74.13% TAN removal (Han *et al.* 2019a). The superior TAN removal in unsaturated VFCWs (1VFCW, 2VFCW, and 3VFCW) is likely due to better oxygen diffusion and root-mediated oxygen transfer from *Canna indica*, which supports nitrification. In contrast, tidal and surface flow systems often struggle with inconsistent aeration, limiting nitrification efficiency. The longer HRT in this study (68–76 h in saturated wetlands) also provided more time for biological nitrogen transformations, improving treatment performance.

Phosphate removal in this study was also notably high, significantly exceeding the 45% removal reported in VFCWs using sand as a filter medium for swine WWT in Brazil (Sezerino *et al.* 2003). Although a dolomite-based system achieved 94.8% removal (Zibiene *et al.* 2015), its reliance on specialized dolomite chippings limits scalability in resource-constrained areas. The high phosphate removal observed in the control and saturated VFCWs can be attributed to prolonged HRT and enhanced adsorption by the gravel-based media used in this study, which is more practical and affordable for small-scale applications.

The 4VFCW configuration achieved a TN removal efficiency of 77 ± 16.24%, surpassing the 57.41% reported for partially saturated wetlands treating anaerobically digested swine wastewater (Han *et al.* 2019b). This improved performance is due to the optimal redox zonation created by saturation up to 60 cm in the second stage, which promotes simultaneous nitrification and denitrification. These controlled saturation levels ensured the presence of both aerobic and anaerobic zones, enhancing nitrogen removal. Partially saturated and fully saturated systems often lack this balance, resulting in incomplete nitrogen removal.

Overall, the combination of passive aeration, saturation control, and planting in the VFCWs used in this study provides a more robust and efficient design compared to the systems reported in other studies, making it particularly suitable for treating high-strength piggery wastewater in resource-constrained regions.

4. CONCLUSION

This study evaluated the performance of five different configurations of two-stage VFCWs along with a control (C) for treating piggery wastewater. Among the VFCWs, 4VFCW achieved the highest treatment efficiency for PO_4^{3-}, NO_3^-, and TN, 5VFCW for COD and TOC, and 1VFCW for TAN. The control system showed the highest removal of PO_4^{3-} and TOC compared to the planted VFCWs. Overall, 4VFCW with saturation up to 60 cm in the second stage emerged as the most balanced and effective system, combining high efficiencies across multiple parameters, particularly TN and NO_3^- removal, due to its optimal redox zonation and HRTs.

The saturated systems (4VFCW and 5VFCW) offer ecological benefits by promoting denitrification and phosphorus removal in anaerobic zones, reducing the risk of waterway eutrophication and nitrate pollution. Their longer HRTs enhance treatment performance, making them well suited for regions with high nutrient loads. Unsaturated systems (1VFCW, 2VFCW, and 3VFCW), on the other hand, support better oxygen diffusion, facilitating efficient nitrification and TAN removal. These systems are particularly beneficial for areas where nitrogen control is a priority.

Economically, all VFCW configurations provide a low-cost, sustainable alternative to conventional treatment methods. The use of locally available materials such as gravel and plants reduces construction costs, while the passive aeration system and reliance on natural processes minimize operational expenses and energy consumption. The modular design allows for easy scalability and customization, making these systems highly adaptable for small-scale pig farms or decentralized wastewater management in rural or resource-constrained regions.

Future research will focus on utilizing diverse macrophytes, such as *Phragmites australis* and Vetiver grass, to identify species with enhanced pollutant uptake capabilities and resilience to local climatic conditions. Investigating alternative filter media, such as biochar, zeolite, and coconut coir could enhance nutrient adsorption and promote microbial activity. VFCWs can also be integrated with microbial fuel cells (MFCs) to generate bioelectricity during the treatment process, contributing to economic value and energy sustainability. Additionally, combining VFCWs with photobioreactors could improve nutrient removal while simultaneously producing valuable microalgal biomass for applications like biofuels, animal feed, or biofertilizers.

The scalability and long-term performance of the 4VFCW configuration for full-scale piggery WWT needs to be assessed. Its ability to achieve high pollutant removal efficiency with minimal energy input and reliance on natural processes makes it ideal for decentralized wastewater management. These advancements aim to develop a comprehensive approach to enhancing WWT efficiency and resource recovery, with particular benefits for rural areas in India.

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AUTHOR CONTRIBUTIONS

KN and JKG completed the 2-stage VFCW optimisation work and laboratory analysis. All the authors were involved in manuscript writing and revising. Prof. Srikanth Mutnuri supported us with his technical expertise throughout the study.

ETHICS APPROVAL

We know of no conflicts of interest in this publication.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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