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Chapter 3
Design and Performance Assessment of Subsurface Constructed Wetlands for Pollutant Removal

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1. Introduction

In this chapter, my focus will be on the design and performance assessment of CWs for pollutant removal. I will discuss the constraints of using wetlands, their advantages, and how we can overcome their disadvantages through wetland implementation. The structure of today's presentation comprises six parts: (1) Introduction to CWs and the customized design of Horizontal Flow Constructed Wetlands (HFCWs) focusing on organic removal, (2) Optimization of nitrogen and phosphorus removal in wastewater deficient in organics using HFCWs, (3) Optimization of HFCWs design through Machine Learning, (4) Metagenomics analysis of NIH Roorkee HFCWs, (5) Optimization of filler media depth in HFCWs to maximize removal rate coefficients of targeted pollutant(s), and (6) Importance of Deep HFCWs. Firstly, we will take a look at CWs, which replicate natural wetland processes and offer cost-effectiveness and ease of operation. However, a major drawback or limitation is the substantial land area required, which limits their widespread adoption. In my country, India, there are currently no guidelines for constructing wetlands, making it essential for our research group to work towards providing design and performance assessment guidelines. Wetlands play a crucial role in pollutant removal, both kinetically and dynamically. The advantages of using wetlands include their capability, particularly when constructed as deep and HFCWs, to facilitate improved pathways for antibiotic removal, and nutrient removal, anaerobic conditions for sulfate reduction, as well as reduced land area requirements. These systems represent energy-sustainable solutions. CWs can be categorized into three types: traditional, hybrid, and enhanced (as shown in Figure 1).

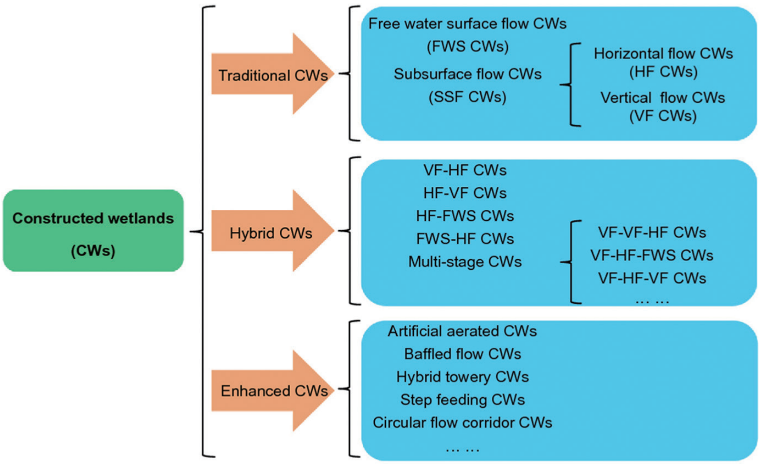

Figure 1: CWs classification.
Source: Wu et al. 2015

Traditional CWs can be categorized into subsurface and surface systems. In this chapter, we will specifically focus on subsurface HFCWs and VFCWs. These subsurface systems are designed to treat wastewater by promoting the removal of various pollutants through natural biological processes. Subsurface CWs are particularly effective in removing organic material, suspended solids, and nitrogen. The primary mechanisms involved include autotrophic denitrification and nitrification. Autotrophic denitrification is a process where bacteria convert nitrogen compounds into nitrogen gas under low oxygen conditions, effectively reducing nitrogen levels in the water. Nitrification, on the other hand, involves the conversion of ammonia into nitrate by aerobic bacteria, which is then followed by denitrification. The advantage of subsurface systems lies in their ability to maintain a more controlled environment for microbial activity, which enhances the efficiency of these biological processes. The water flows horizontally or vertically through a porous medium, such as gravel or sand, where microorganisms colonize and form biofilms. These biofilms play a crucial role in breaking down pollutants.

Currently, significant research is focused on optimizing CWs for not only nitrogen removal but also for phosphorus and pathogen removal. Phosphorus is a critical nutrient that can cause eutrophication in water bodies, leading to excessive growth of algae and deterioration of water quality. Pathogen removal is essential for ensuring that treated water is safe for discharge or reuse. Studies are exploring various configurations and operational strategies to enhance the removal of these contaminants, making CWs more versatile and effective in different environmental conditions. By understanding and improving the design and operation of subsurface HFCWs and VFCWs, researchers aim to develop more efficient and sustainable wastewater treatment solutions that can be widely adopted to address various water pollution challenges.

(1) Problems Associated with HFCWs

In a previous study conducted by Rampuria et al. (2020; 2021), deep CWs were assessed for treating both domestic and hospital sewage over an extended period. These systems were primarily designed to meet organic removal requirements specified in Indian standards. The study found a linear relationship between biological oxygen demand (BOD) removal and loading rates of Aakanksha, with linearity observed up to very high loading rates (as shown in Figure 2). Additionally, it was observed that the existing CWs fell well below the curve's flattening point (Figure 2), indicating highly underloaded systems in terms of organic loading (Soti et al. 2024). This highlighted an opportunity to address a major drawback of such systems, namely the area requirement.

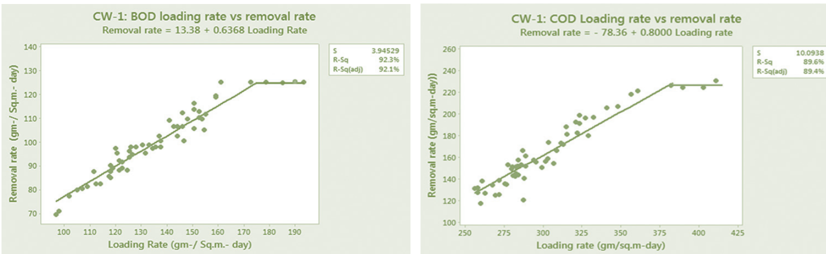

Figure 2: BOD and COD loading rate and removal rate.
Source: Rampuria et al. 2020

As shown in Figure 2, in the first curve, the x-axis represents the loading rate, which is the areal loading rate, and the y-axis represents the removal rate. As the loading rate increases up to a certain level, the removal rate also increases. However, beyond a certain point, increasing the loading rate in terms of organic loading does not result in a proportional increase in the removal rate. Instead, the removal rate reaches a plateau. This indicates that the system has reached an optimum condition, where further increases in the loading rate do not significantly improve system efficiency.

To find the maximum removal efficiency of the system, we increased the loading rates. We utilized the P-k-C* approach to customize the design of CWs and minimize area requirements. The areal loading rate coefficient values were calculated using the P-k-C* approach as shown in the equation as follows:

$$k = \frac{PQi}{A}[\left(\frac{Ci - C*}{Co - C*}\right)^{\frac{1}{P}} - 1] \qquad \text{(Eq1)}$$

where Co, Ci, and C* (mg/L) are outlet, inlet concentration, and background concentration, respectively. k (m/d) is the first-order areal rate coefficient. P represents apparent number of tanks in series (TIS) dimensionless, Qi (m^3/d) is influent flow rate.

First-order areal removal rate coefficient (K, m/d) at any temperature (T°) was calculated by:

$$k_{T°} = k_{20°}(1.047)^{(T°-20°)} \qquad \text{(Eq2)}$$

Essentially, in Equation 1, we observed that the area of the CWs depends on the first removal rate coefficient. Therefore, higher values of k, which represent the first removal rate coefficient and indicate the degradability of organics, result in higher removal efficiencies of the system. We will discuss removal rates for BOD, TN, TK, and TP.

(2) Customized Design of HFCWs Based on Removal of Organics

In this study, our primary objective was to reduce the area required for the CWs. Initially, we aimed to achieve this by optimizing the k-values. We collected data and assessed the k-values using the P-k-C* approach and the plug flow equation, subsequently optimizing them. Then, we classified the CWs based on the organic loading rate (OLR), temperature, and depth, as these parameters vary according to the k-values in different geographical conditions. Next, we conducted area calculations using the P-k-C* approach, aiming to determine judicious land use for CWs. The optimization approach is described in Figure 3.

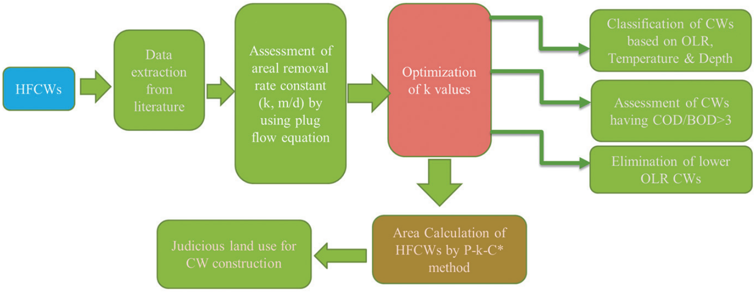

Figure 3: Optimization approach.
Source: Singh et al. 2022a

We calculated the k-values based on organic loading rate, temperature, and depth, categorizing loading into low organic, medium organic, and high organic systems (Figure 4). As depicted in Figure 4A, the higher the loading rate, the higher the organic loading rate, and consequently, the higher the k-values. This indicates that the removal of organics is more efficient under conditions of higher removal rates. We evaluated 111 VFCWs and HFCWs and found that most wetlands are underloaded, as the actual area of the wetland exceeds the calculated area using the P-k-C*approach. These systems can enhance removal efficiency by increasing the depth of the media or increasing the loading rate.

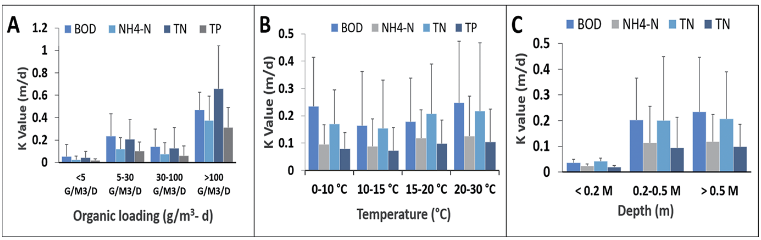

Figure 4: Variation in $K_{20°C}$ values at different OLRs (A), temperatures (B), and depths (C) computed using the Plug flow equation.
Source: Singh et al. 2022a

Similarly, we have determined the k-values through area calculation (refer to Figure 5). Negative values indicate that the systems are underloaded, while positive values indicate overloaded systems. Therefore, efficiency is highest in normally loaded or overloaded systems.

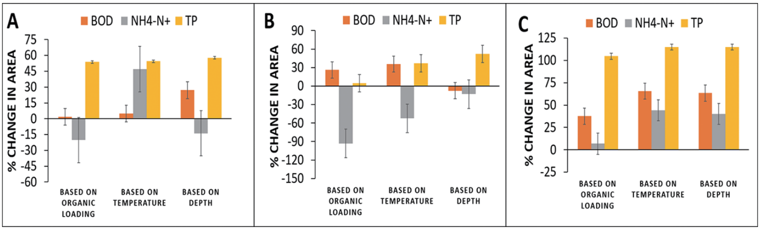

Figure 5: The area percentage error bars in column plots of BOD removal using Plug flow equation for (A) Lab-scale, (B) Pilot-scale, and Full-scale HFCWs.
Source: Singh et al. 2022a

This study concludes that the k-values, calculated based on the P-k-C* equation for different pollutants, exhibited wide variations (ranging from 0.006 to 0.40 m/day), influenced by both environmental factors and operational conditions. The error bars in the column plot highlight the importance of subclassifying the CWs based on loading, environmental conditions (such as temperature), and compliance norms. This subclassification would lead to more meaningful k-values, facilitating customized design approaches. Reducing the standard deviation of the k-value could be achieved by categorizing datasets according to inlet conditions, such as depth and substrate loading rates, for different pollutants. Furthermore, the calculation of HFCWs based on required discharge standards revealed that the actual area of existing CWs exceeds the calculated area, indicating significant underloading of these systems. The areal rate constants derived in this study could provide valuable support for designing HFCWs to optimize land use more effectively, ensuring better pollutant removal and system efficiency.

2. Optimization of Nitrogen and Phosphorous Removal in Wastewater Deficient in Organics of HFCWs

In the first phase, we assessed 74 HFCWs and classified them into higher-loading systems and lower-loading systems. Higher-loading systems showed higher k-values and better removal efficiency, indicating that they are more effective at treating pollutants. Conversely, lower-loaded organic systems can also be beneficial in achieving maximum removal efficiency in HFCWs by optimizing conditions for microbial activity. Deeper wetland systems, in particular, create an anoxic zone in the bottom layer. This anoxic environment is conducive to important biochemical processes such as nitrification and denitrification, which are crucial for nitrogen removal. Additionally, the anoxic conditions support the activity of phosphorus-solubilizing bacteria, which helps in the effective removal of phosphorus. By facilitating these processes, deeper HFCWs can enhance the overall pollutant removal efficiency, making them a valuable option for wastewater treatment.

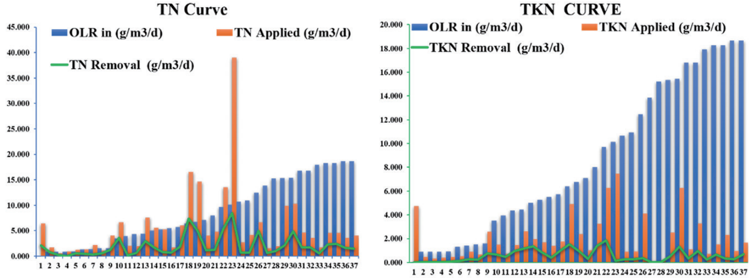

Figure 6: The behavior of organic loading rate (OLR) with TN and total kjeldahl nitrogen (TKN).
Source: Singh et al. 2022a

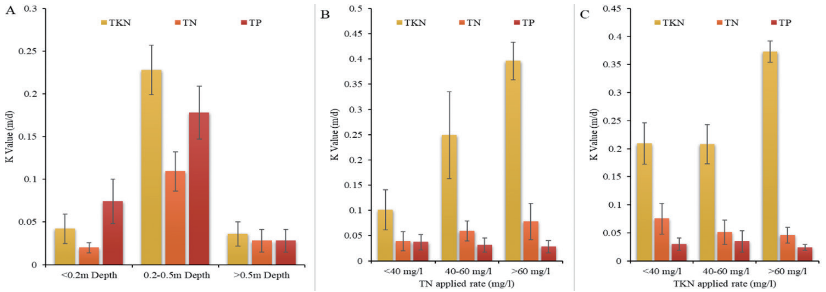

Figure 7: Optimization of k-values through depth classification of HFCWs (A) and optimization curve of k-based on TN (B) and TKN (C) applied rates.
Source: Singh et al. 2022a

Curves were plotted to observe the behavior of biological reactions, as shown in Figure 6. These curves demonstrated that increasing the organic loading rate along the x-axis initially resulted in higher removal efficiency of total nitrogen. However, beyond a certain point, further increases in the organic loading rate did not improve removal efficiency, indicating that the system had reached optimum conditions.

Figure 7 shows the optimization of k-values through depth classification of HFCWs, and the optimization curve of k based on TN and TKN applied rates. It is evident from these curves that the k-values in low-organic systems varied with depth and the applied TN and TKN loading rates. This variation highlights the importance of considering these factors when optimizing HFCWs for maximum removal efficiency.

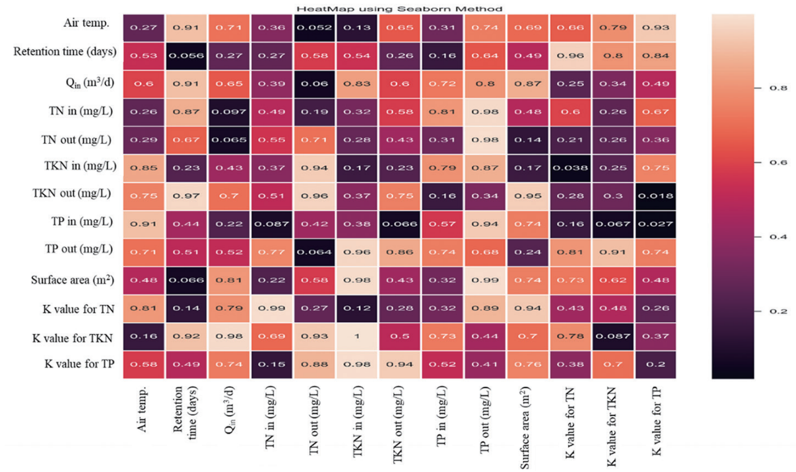

Figure 8: Pairwise correlation Heatmap of influent and effluent parameters.
Source: Singh et al. 2022b

It has been identified that the k-value of the system is not solely dependent on any empirical relationship. Factors such as retention time, discharge, and air temperature significantly influence removal efficiency, with surface area and depth playing crucial roles in maximizing the efficiency of constructed wetlands. Therefore, relying solely on empirical relationships is insufficient to accurately predict wetland removal efficiency. To address this, we applied machine learning techniques. Initially, we utilized 12 parameters to identify and predict k-values using both multi-linear regression and artificial neural networks (ANN) approaches. The correlation between influent and effluent parameters is illustrated in the heatmaps (Figure 8).

Table 1: Comparison between the root mean square error (RMSE) and Standard deviations (SDs) of k-values

Prediction output	P-k-C* Approach		SVR on unseen datasets		RF on unseen datasets		ANN on unseen datasets	
	R^2	SDs	R^2	RMSE	R^2	RMSE	R^2	RMSE
k_{TKN}	0.657	33.80%	0.512	9.70%	0.635	8.70%	0.768	6.70%
k_{TN}	0.711	36.95%	0.6	8.50%	0.701	6.20%	0.835	4.30%
k_{TP}	0.637	34.86%	0.429	16.50%	0.596	13.10%	0.723	8.70%

(SVR: Support vector regression, RF: Random Forest, R2: Coefficient of determination).
Source: Singh et al. 2022b

Table 1 displays the root mean square error (RMSE) and standard deviations (SDs) of k-values. The results indicate that the ANN algorithm achieved a higher R^2 and lower RMSE compared to other methods. Initially, actual k-values were calculated using the P-k-C* approach, resulting in an SD of 33.80%, which was deemed unacceptable. Additionally, the R^2 of these k-values was relatively low. To enhance system efficiency and accuracy, we implemented a machine learning approach. The ANN demonstrated superior performance in terms of accuracy, with errors consistently below 6.7% variation compared to the actual k-values. Figure 9 shows the real data and predicted data of the k-values curve for TKN, TN, and TP by using various machine learning algorithms.

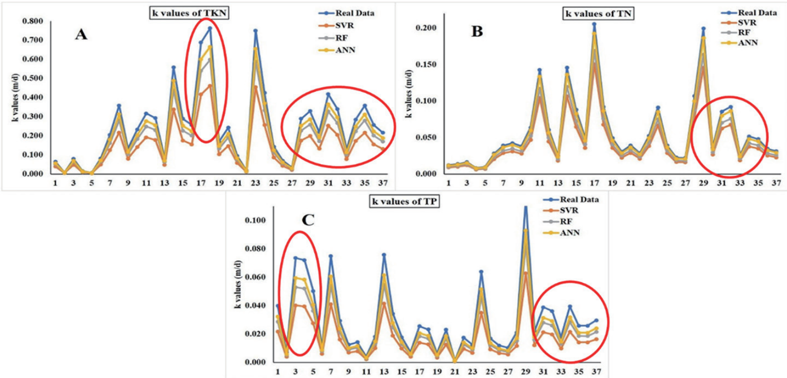

Figure 9: Real data and predicted data of k-values Curve for TKN (A), TN (B), and TP (C).
Source: Singh et al. 2023a

Overall, a strong direct correlation was observed between TN applied (R^2 0.77) and TKN applied (R^2 0.83). The initial large variations (ranging from 0.01 to 0.54 m/day; SD of 83.41%) observed in k-values calculated by the reverse P-k-C* approach could be reduced through dataset classification. Classification based on wetland depth, COD/TN, COD/TKN, TN application rate, and TKN application rate led to a reduction in SD to 30.32%, 38.21%, 39.21%, 34.83%, and 21.98%, respectively. HFCWs with COD/TN and COD/TKN ratios of 12 and 16.6 demonstrated the highest nitrogen removal efficiency of 84.45%. This study contributes to the customized design of HFCWs for TN, TKN, and TP pollutant removal from low-organic wastewater, with minimal area requirements.

3. Optimization of HFCWs design through Machine Learning

This study aims to assist in the design process by analyzing the treatment dynamics of 111 HFCWs using secondary datasets comprising 1232 data points. Essentially, wastewater biological reactions exhibit non-linear behavior, often characterized by exponential variations. Machine learning tools prove highly valuable in identifying fundamental relationships between input variables and k-values, and for understanding these k-values vary in response to input variables. Table 2 shows the R^2 for SVR and multiple linear regression (MLR) algorithm.

Table 2: The R^2 of k-values for the SVR and MLR-based model

Effluents \ R^2	**R^2 of k_1-Values (effluent in mg/l)**		**R^2 of k_2-Values (effluent in ($g/m^3/d$)**	
	MLR	SVR	MLR	SVR
BOD	0.921	0.982	0.911	0.954
COD	0.901	0.968	0.885	0.948
NH_4^+-N	0.782	0.845	0.626	0.782
TN	0.810	0.889	0.722	0.819
TP	0.592	0.612	0.369	0.518

Source: Singh et al. 2023a

Among the two models, SVR resulted in better predictions of all effluent concentrations, measured in mg/L as well as in $g/m^3/d$. The prediction of NH_4^+-N ($g/m^3/d$) and TN ($g/m^3/d$) achieved the highest accuracy, with R^2 and RMSE values of 0.847 and 0.44%, and 0.947 and 0.18%, respectively. The areal removal rate (k, m/d) values computed by the reverse P-k-C* approach, using the predicted effluent concentrations, exhibited high correlation with actual k-values of BOD, COD, and TN, with R^2 values of 0.954, 0.948, and 0.819, respectively. The SVR algorithm demonstrates promising potential for predicting k-values for BOD, COD, and TN with high confidence, thereby aiding in optimizing the design of HFCWs in terms of area requirements for organics and nitrogen removal. This enhanced predictive capability is crucial for developing more efficient and effective CW designs that maximize pollutant removal while minimizing land use.

4. Metagenomics Analysis of NIH Roorkee HFCWs

Building upon previous findings, this study conducted a metagenomics analysis of HFCWs at the National Institute of Hydrology (NIH) in Roorkee, India, referred to as NIH Roorkee HFCWs. At NIH Roorkee, eight cells were constructed in a decreasing order of depth, from 1.5 m in cell 1 to 0.8 m in cell 8. The arrangement and depths of these HFCWs are illustrated in Figure 10.

Figure 10: Diagram of HFCWs (A) and actual site photographs (BandC). Source: Singh et al. 2023b

These studies identified the role of media depth in pollutant removal within HFCWs. The focus was on HFCWs due to their effectiveness in removing nitrogen through denitrification, as well as phosphorus and other nutrients. In these systems, the anaerobic zone at the bottom aids in the removal of these pollutants. Wastewater samples were collected from both deep and shallow cells of the sequential CW for physicochemical analysis. Subsequently, the areal rate coefficients (k) were calculated, followed by metagenomic analysis. Tables 3 and 4 present the performance assessment results of the NIH Roorkee wetlands and the areal removal rate coefficients in HFCWs.

Table 3: Performance assessment results of NIH Roorkee HFCWs

HSSFCW	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8
Temperature (ºC)	24.24	24.04	23.66	23.71	23.17	22.96	22.8	22.64
Surface area (m^2)	35	35	35	35	35	35	35	35
Wetland depth (m)	1.5	1.4	1.3	1.2	1.1	1	0.9	0.8
Volume (m^3)	52.5	49	45.5	42	38.5	35	31.5	28
Sewage inflow (m^3/d)	32	32	32	32	32	32	32	32
BOD_{in} (ppm)	99	47	37	17	14	14	13	11
BOD_{out} (ppm)	29	21	17	14	14	13	11	10
COD_{in} (ppm)	137	61	36	38	25	23	20	16
COD_{out} (ppm)	49	36	28	25	23	20	16	15
NO_3-N_{in} (ppm)	4.1	2.29	1.68	1.53	1.51	1.37	1.42	1.2
NO_3-N_{out} (ppm)	2.29	1.68	1.53	1.51	1.37	1.42	1.2	1.12
TKN_{in} (ppm)	25.8	18	16	15	14.4	13.3	12	11
TKN_{out} (ppm)	18	16	15	14.4	13.3	12	11	8.5
$Phosphate_{in}$ (ppm)	10.23	9.29	9.41	8.41	7.22	6.46	6.21	5.34
$Phosphate_{out}$ (ppm)	9.29	9.41	8.41	7.22	6.46	6.21	5.34	4.78
DO_{in} (ppm)	0.37	0.66	0.8	0.77	0.93	1.21	1.1	1.49
DO_{out} (ppm)	0.66	0.8	0.77	0.93	1.21	1.1	1.49	3.85

Source: Singh et al. 2023b

Table 4: Areal removal rate coefficients in NIH Roorkee HFCWs, CW1 and CW2

Wetland	k_{BOD} (m/d)	k_{TKN} (m/d)	k_{TN} (m/d)	k_{NH4-N} (m/d)	k_{TP} (m/d)
NIH-CW Cell 1	0.627	0.155	0.166	NA	0.148
NIH-CW Cell 2	0.483	0.051	0.059	NA	0.048
NIH-CW Cell 3	0.522	0.028	0.028	NA	0.026
NIH-CW Cell 4	0.208	0.018	0.016	NA	0.016
NIH-CW Cell 5	0.000	0.033	0.034	NA	0.031
NIH-CW Cell 6	0.099	0.043	0.037	NA	0.04
NIH-CW Cell 7	0.339	0.036	0.04	NA	0.033
NIH-CW Cell 8	0.476	0.109	0.099	NA	0.097
CW1 (2.2m)	0.35	0.27	0.21	0.15	NA
CW2 (2.51m)	0.41	0.25	0.16	0.13	NA

(NA: not available).
Source: Singh et al. 2023b

We calculated the k-values and observed that the k-values of the Cell 1 system are notably high, given the system's depth of 1.5 m. As the depth of the media decreases, the k-values also decrease accordingly. Our findings suggested that increasing the depth of media enhances pollutant removal efficiency, but only up to a certain level (1.5 m in Cell 1). We also conducted a similar study on wastewater field-scale HFCWs for treating residential wastewater (CW1) and hospital wastewater (CW2) to compare with NIH Roorkee HFCWs. The depth of these wetlands was 2.2 m and 2.51 m, respectively. The k-values of NIH Roorkee, CW1, and CW2 are presented in Table 4. The results indicated that the organic loading rates (OLR) for Cell 1, CW1, and CW2 fall within the range of 30-100 g/m^3/d. The customized k_{BOD} value for this OLR was 0.395 ± 27.74%. Therefore, the k_{BOD} value for the experimental data exceeded the range of customized k-values according to the organic loading classification. This could be attributed to the considerably greater depth of the experimental wetlands (1.5 m, 2.2 m, and 2.51 m) compared to the wetlands data available in the literature used for customization. In deep wetlands, there is a possibility of developing anoxic zones at the bottom, which could aid both denitrification and anammox processes. The former process would facilitate BOD removal (Rampuria et al. 2020). OLR values for Cell 2 to Cell 8 lie within the range of 5–30 g/m^3/d. The k-values for this OLR were 0.268 ± 29.11%. Consequently, the k_{BOD} value for experimental data falls within the range of customized k-values according to the organic loading classification (Singh et al. 2022a). The k_{TN} and k_{TP} values for all cells fall within the range of customized k-values according to their organic loading classification (Singh et al. 2022a).

In the metagenomics analysis, we conducted 16S rRNA amplicon sequencing on samples collected from the inlet and outlet of both deep and shallow cells. The genera present in the three samples are shown in Figure 11A. The bacterial diversity was found to be significantly different among the samples. The bacterial diversity was considerably reduced from 164 species at the inlet to 76 species at the outlet, and there were 114 species in the deep-cell wetlands. The inlet sample had 59 unique genera, while the deep cell and shallow cell consisted of 11 and 7 unique genera, respectively. The reduction in bacterial diversity during the CW treatment indicates the selective nature of the system. In addition, in the phylum level analysis (Figure 11B), we also identified Bacteroidetes, Firmicutes, and Proteobacteria, which play crucial roles in nutrient removal.

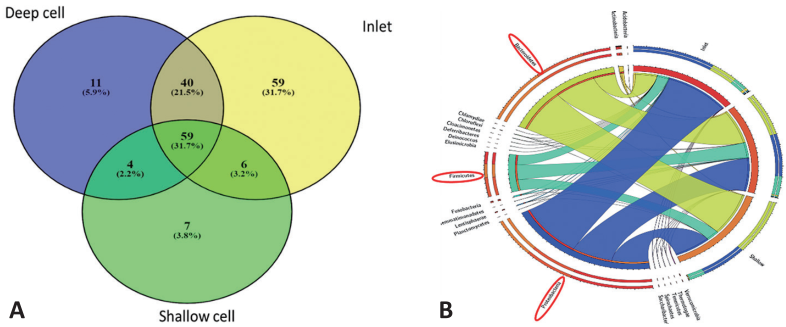

Figure 11: (A) Venn diagram of the genera present in the three samples, (B) Phylum level identification. Source: Singh et al. 2023b

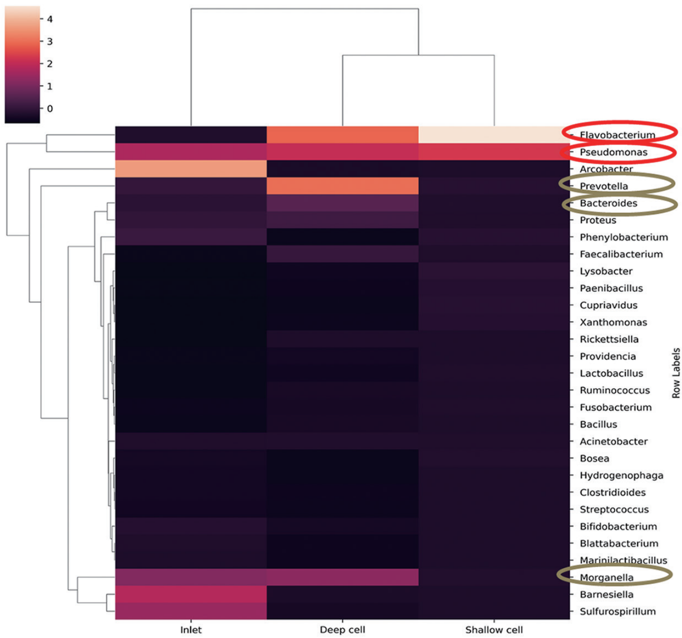

Figure 12: Relationship between the inlet and outlet samples in both deep and shallow cells at the Genus level. Source: Singh et al. 2023b

Figure 12 depicts the relationship between three samples at the genus level. The inlet sample appears relatively distantly related to the deep and shallow cell samples in terms of the relative abundance of different bacterial genera observed. *Pseudomonas*, *Flavobacterium*, *Prevotella*, *Morganella*, and *Bacteroidetes* are present in deep systems. The functions of these genes are beneficial for heterotrophic nitrification, anaerobic denitrification, and phosphate accumulation. *Flavobacterium* contributes to aerobic denitrification, while *Prevotella* genes aid in anaerobic generation, which helps reduce phosphorus solubilizing bacteria (PSB). *Morganella* is found in both environments, often associated with animals and exhibiting high antibiotic resistance. *Bacteroidetes* are responsible for the catabolism of complex carbohydrates. Deep cells exhibit a significant presence of *Bacteroidetes*, correlating with better removal efficiency compared to shallow cells.

Overall, full-scale HFCWs utilizing sequential shallower depths was found to efficiently remove organics and nutrients. The areal removal rate coefficients of different cells in this system were higher for organics and within the expected range for nitrogen and phosphate, compared to the optimized removal rate coefficients calculated using secondary data. Metagenomic analysis supports the removal of organics and nutrients, as indicated by the presence of specific bacterial genera. The abundance of heterotrophic nitrifiers, aerobic denitrifiers and phosphate-accumulating bacteria demonstrates the effectiveness of such deep HFCW systems for combined organics and nutrient removal.

5. Optimization of Depth of Filler Media in HFCWs for Maximizing Removal Rate Coefficients of Targeted Pollutant(s)

In this study, the optimal depths for the removal of various organic compounds were identified. To determine the depth of media required for maximum removal rate coefficients, curves were plotted to show the relationship between increasing media depth and k-values using 111 secondary data points from HFCWs (as presented in Figure 13).

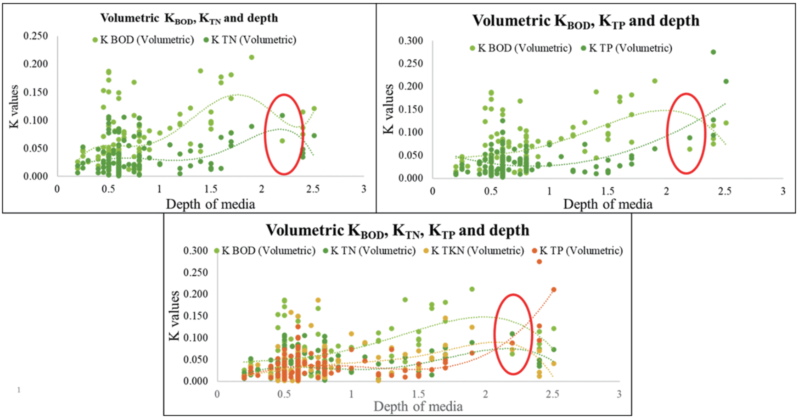

Figure 13: The optimum depth of media for the removal of combinations of pollutants.
Source: Singh et al. 2024

Observations indicated that as the media depth increases up to a certain level, typically around 2 m or 2.2 m, the k-values reach their maximum, with the peak occurring at 2 m. Beyond this depth, the k-values start to decrease. This suggests that for effective removal of both BOD and TN from wastewater, the ideal depth of the wetland should be around 2 m. Similarly, we found a common depth point for BOD, TN, and TP removal. If we aim to remove BOD, TN, and TP simultaneously, the depth should be 2.2 m. For exclusive BOD removal, the depth should be 1.6 m.

The optimal depths for BOD, TN, TKN, and TP pollutants are found to be 1.6 m, 2.2 m, 1.9 m, and 2.4 m, respectively, when the correlation curve is plotted between volumetric loading rate (k, 1/day) and depth of media. This data was validated using Grey Wolf Optimization (GWO) in MATLAB software, which was run 1,000 times to determine the maximum depth for removing BOD and TN. Initially, GWO analysis was employed within MATLAB software. Subsequently, a novel equation was developed for predicting the depth of BOD and TN removal in terms of k, as k is a critical parameter for determining the maximum k-values to optimize the removal rate coefficients.

The equations of correlation were obtained using the curve as shown in equations (i) to (iv).

$$f(x) = (depth)_{BOD} = 0.203k_{BOD}^4 + 0.114k_{BOD}^3 - 0.095k_{BOD}^2 + 0.101k_{BOD} + 0.073 \quad (i)$$

$$f(x) = (depth)_{TN} = 0.081k_{TN}^4 + 0.354k_{TN}^3 - 0.041k_{TN}^2 + 0.193k_{TN} - 0.022 \quad (ii)$$

$$f(x) = (depth)_{TKN} = -0.042k_{TKN}^4 + 0.195k_{TKN}^3 + 0.297k_{TKN}^2 + 0.156k_{TKN} - 0.018 \quad (iii)$$

$$f(x) = (depth)_{TP} = 0.013k_{TP}^4 + 0.038k_{TP}^3 - 0.108k_{TP}^2 + 0.159k + 0.088 \quad (iv)$$

After that, these equations were further used to run simulations in MATLAB software 1,000 times, and then we derived an equation to find the optimum depth for pollutant removal using GWO. The equation of depth of media was forecasted using the removal rate coefficients for nutrient removal by MLR model as shown in equation (v).

$$f(x) = \text{Depth} = -0.8216(k_{BOD}) + 6.170\,(k_{TN}) - 2.011(k_{TKN}) + 0.927(k_{TP}) + 0.952 \quad (v)$$

The computed optimal depths were 1.48 m and 1.71 m for TKN removal, 1.91 m for TN removal, and 2.14 m for TP removal.

With an in-depth explanation using the correlation curve, it was observed that BOD increased with the increasing depth of media up to 1.6 m. This phenomenon occurs due to substrate and oxygen limitations for heterotrophic denitrification. Similarly, concerning the removal of TN, the depth of media increased up to 2.2 m, where the removal efficiency peaked at 2 m, and after 2.2 m, the rate did not increase further. This could be attributed to the generation of an anoxic zone in the deep stretches of the wetland, which may favor heterotrophic denitrification and anammox processes. Regarding PSB, an anaerobic bottom zone in deep wetlands may provide favorable conditions for their activity. PSBs play a vital role in solubilizing inorganic and organic insoluble phosphorus to form soluble phosphorus, which can then be assimilated by other phosphate-accumulating bacteria.

Metagenomic analysis provided further insights. The removal of organic matter in HFCWs primarily occurs in aerobic zones, where heterotrophic bacteria play a dominant role, typically up to a depth of 1.48 m. For TKN removal, the ideal depth of media is around 1.7 m. This removal process involves heterotrophic bacteria facilitating hydrolysis, alongside aerobic nitrifying microorganisms. However, increasing the depth of the media beyond approximately 1.908 m leads to the formation of an anoxic zone due to dissolved oxygen depletion. Within this anoxic zone, heterotrophic denitrification and anammox processes become predominant pathways, facilitating complete nitrogen removal in the form of nitrogen gas. Likewise, at greater depths, such as 2.14 m, anaerobic conditions may develop, potentially harboring phosphorus-accumulating organisms and phosphorus-solubilizing bacteria. This environment contributes to phosphorus removal within the CWs. Thus, understanding the interplay between media depth and microbial activity is crucial for optimizing pollutant removal in constructed wetlands.

6. Importance of Deep HFCWs

The deep HFCWs offer significant advantages over VFCWs, including reduced space requirements and enhanced removal of nitrogen, phosphorus, and sulfur compounds. This system features anaerobic zones at the bottom layer, facilitating efficient removal of phosphorus and nitrogen within the wetland. Additionally, deep-CWs exhibit a more pronounced redox gradient. The pollutant removal pathways in deep-CWs are summarized in Figure 14.

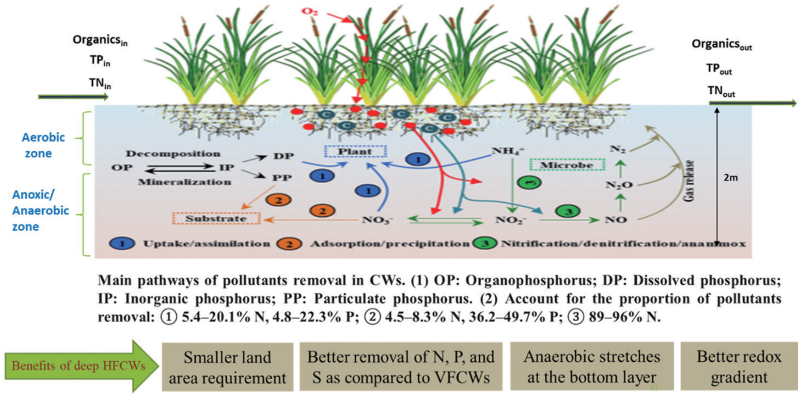

Figure 14: Pollutant removal pathways in deep-CWs.
Source: Soti et al. 2022; 2023

In general, the calculation of HFCWs based on required discharge standards reveals that the actual area of existing CWs exceeds the calculated area, indicating that HFCWs are significantly underloaded systems. The areal rate constants derived in this work may provide excellent design support for efficient land use planning. Deep-CWs reduce land requirements and offer effective organic removal efficiencies, along with improved nitrogen removal. Deep wetlands provide an optimal environment for the in situ growth and coexistence of diverse microbial populations, supporting contaminant and nitrogen removal, particularly anammox bacteria (Rampuria et al. 2021). Over a 20-year period, HFCWs demonstrate superior life cycle costs compared to VFCWs for pollutant removal.

Table 5. Comparison of k20 for BOD, TKN, TN and TP in different types of CWs

Field scale HFCWs	Type of wastewater	k_{BOD}	k_{TKN}	k_{TN}	k_{TP}
Shallow 0.5m MNIT HFCW	Primary	0.345	0.086	0.103	0.080
Shallow 0.8m NIH HFCW	Primary	0.476	0.099	0.109	0.089
Deep 1.5m NIH HFCW	Primary	0.627	0.166	0.155	0.072
Deep 2.51m Rajnish Hospital HFCWs	Primary	0.361	0.157	0.122	0.097
Shallow 0.85m VFCW	Secondary	0.183	0.152	0.120	0.148
Deep 2m Vishakhapatanam VFCW	Primary	0.314	0.220	0.140	0.088

Source: Author

Table 5 provides a comparative analysis of various field-scale VFCWs and HFCWs, detailing their depths, types of wastewater treated, and removal rate coefficients (k-values) for BOD, TKN, TN, and TP. The Shallow 0.5m MNIT HFCW, used for primary wastewater treatment, shows moderate removal efficiencies with k-values of 0.345 for BOD, 0.086 for TKN, 0.103 for TN, and 0.080 for TP. This system's moderate depth limits its capacity for nitrogen and phosphorus removal compared to deeper systems. In comparison, the Shallow 0.8m NIH HFCW, also used for primary treatment, exhibits higher removal rates with k-values of 0.476 for BOD, 0.099 for TKN, 0.109 for TN, and 0.089 for TP. The slight increase in depth enhances its efficiency in removing organic and nitrogenous compounds. The Deep 1.5m NIH HFCW, another primary treatment system, demonstrates the highest removal efficiencies for BOD (0.627), TKN (0.166), and TN (0.155), although its TP removal (0.072) is slightly lower compared to other systems. The greater depth allows for more effective denitrification and organic matter breakdown, but phosphorus removal remains less efficient. Meanwhile, the Deep 2.51m Rajnish Hospital HFCWs, treating primary wastewater from a hospital, show balanced removal efficiencies with k-values of 0.361 for BOD, 0.157 for TKN, 0.122 for TN, and 0.097 for TP. While effective, this system does not outperform the 1.5m NIH HFCW for BOD and TN removal, possibly due to the complex nature of hospital wastewater.

In the case of secondary treatment, the Shallow 0.85m VFCW demonstrates lower k-values for BOD (0.183) but shows comparable efficiencies for TKN (0.152), TN (0.120), and higher TP removal (0.148). This suggests that VFCWs, even at shallower depths, can be quite effective for nutrient removal when treating secondary effluent. Lastly, the Deep 2m Vishakhapatnam VFCW used for primary treatment presents k-values of 0.314 for BOD, 0.220 for TKN, 0.140 for TN, and 0.088 for TP, indicating effective removal rates, particularly for TKN. This depth allows for the development of anoxic zones conducive to nitrogen removal processes.

These observations highlight the impact of depth and type of wastewater on the pollutant removal efficiencies of constructed wetlands. Deeper systems generally offer better performance for nitrogen removal due to enhanced denitrification conditions, while the type of wastewater influences the efficiency of BOD and TP removal. This information is crucial for designing and optimizing HFCWs and VFCWs for specific wastewater treatment needs.

7. Conclusion

This study presents a comprehensive analysis of various field-scale HFCWs and VFCWs. Shallow HFCWs, such as the 0.5m MNIT HFCW, show moderate removal efficiencies, which improve with a slight increase in depth, as seen in the 0.8m NIH HFCW. Deeper HFCWs, like the 1.5m NIH HFCW, achieve the highest removal efficiencies for

BOD, TKN, and TN, although TP removal is slightly lower compared to shallower systems. This indicates that deeper wetlands enhance the removal of organic and nitrogen compounds due to the development of anoxic zones that favor denitrification and other nutrient removal processes. The 2.51m Rajnish Hospital HFCWs demonstrate balanced removal efficiencies, supporting effective nitrogen and phosphorus removal due to prolonged contact time and enhanced microbial activity. Secondary treatment VFCWs, such as the 0.85m VFCW, show lower BOD removal but are effective for TKN, TN, and particularly TP removal, likely due to aerobic conditions promoting phosphorus-accumulating organisms. The 2m Vishakhapatnam VFCW, used for primary treatment, shows effective removal rates, especially for TKN, with its depth creating a gradient of aerobic to anaerobic conditions, optimizing various pollutant removal processes. These observations underscore the importance of designing constructed wetlands with appropriate depths to maximize their treatment efficiency for different types of wastewater. Deeper systems generally provide better removal efficiencies for nitrogen compounds due to anoxic conditions, while phosphorus removal effectiveness varies depending on specific bacterial presence and treatment phase. This study contributes to the development of effective designs, helping to avoid the creation of under-loaded CWs, thereby conserving land areas. These findings offer valuable insights into customizing CW design and configuration to notably improve nutrient removal efficiency in both domestic and industrial wastewater treatment contexts. Additionally, the study provides optimization strategies tailored specifically for constructing wetlands within the Indian context. Notably, it emphasizes the advantages of deeper wetlands, which can facilitate in situ growth, minimize land requirements, and achieve superior removal efficiencies.

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Chapter 3. Design and Performance Assessment of Subsurface Constructed Wetlands for Pollutant Removal

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