Constructed Wetlands planted with Lignocellulosic Grass Species for Wastewater Treatment and Biomass Utilisation

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Abstract

The present study evaluated the performance and treatment efficiency of tropical vertical flow constructed wetlands (VFCWs) using five different lignocellulosic grass species suitable to the tropical climate of Kerala to function as decentralized ecological sanitation systems for the treatment and utilization of wastewater. The study also investigated the biomass yield and bioethanol production potential of the grasses harvested from the VFCWs.

The results indicate the significant role of plants and root zone treatment in the removal of contaminants in the VFCW systems. Among the planted VFCWs, the systems using Pennisetum purpureum and Urochloa brizantha achieved high pollutant removal efficiency for TSS, organic matter and nutrients, though there was no significant statistical difference between the two. The VFCW using Pennisetum purpureum obtained mean removal efficiencies of TSS (89.87%), BOD (87.40%), COD (79.30%), NH_4-N (69.70%), Nitrates (68.80%), TN (43.27%) and Phosphates (49.80%). The mean removal efficiencies obtained in the VFCW using Urochloa brizantha were: TSS (88.90%), BOD (85.60%), COD (77.30%), NH_4-N (67.10%), Nitrates (67.07%), TN (42.53%) and Phosphates (48.80%). The biomass production of Pennisetum purpureum was found to be significantly high when compared to other grasses. Ethanol production after 72 hours expressed in volume of ethanol to the volume of the total reaction mixture(v/v) was obtained high for Andropogon gayanus (10.62%) followed by Panicum maximum (7.93%).

Keywords: Vertical flow constructed wetlands, wastewater treatment, lignocellulosic grass species, biomass utilisation, bioethanol production.

Introduction

Conventional sanitation concepts based on centralised treatment facilities are neither ecological nor economical solutions in addressing the water crisis as these systems are capital and energy-intensive with high construction, operation and maintenance costs, the huge requirement of labour, high greenhouse gas (GHG) emissions and daily production of sludge[28,58]. Constructed wetlands (CWs) were developed as an alternative to the centralized systems and can be applied as decentralized small-scale systems for wastewater treatment with less energy and operational requirements[9,15,17,18,36,48].

CWs are the artificial replica of natural wetlands developed to optimize the inherent function of plants, soil and the rhizosphere microbes that occur in the wetlands for the treatment of pollutants in wastewater[36,50,51]. They are increasingly researched as a low-energy green technology particularly in the milieu of growing climate change concerns[26]. Subsurface VFCWs are gaining importance as an ecological wastewater treatment technology and can play an indispensable part in realizing the principles of ecological sanitation[25,27,34,45].

The selection of wetland vegetation is an important aspect in deciding the contaminant removal efficiency and performance of a VFCW[7]. The vegetation influences the oxygen level in the wetland bed, enables filtration, prevents clogging in the CWs and offers a larger surface area for the colonization of microorganisms[8-10]. Constructed wetlands provide an efficient mechanism for the removal of nutrients while facilitating a suitable environment for the cultivation of grasses that can be utilised as possible raw materials for producing bioethanol[60]. The integrated approach for the treatment of wastewater combined with biomass productivity in CWs can realize both pollution control and bioenergy production[35,54]. The biomass produced by plants provides additional values as cattle fodder, biofuel, medicines, raw material for pulp and paper, soil conditioner and compost. Also, CWs provide environmental benefits such as green space, carbon dioxide sequestration, habitat creation and preservation of biodiversity[20,31,33,50,51].

Subsurface flow constructed wetlands are often significant for developing countries in tropical regions with warm and humid weather throughout the year[3,12,13,23]. The possibility of applying constructed wetlands as decentralised “ecological sanitation systems”[55-57] is considerable in India, but the rate of adoption and replication of the technology had been extremely slow[34,36]. Of late, there is a growing interest in the cultivation of lignocellulosic perennial grasses for bioethanol production[59].

Lignocellulosic perennial crops like short rotation coppices and inedible grasses are potential biofuels as they have high yield, low costs, appropriateness for less quality land and less environmental impact. Thus, it is important to recognise native, highly tolerant and valuable perennial plants with potential for contaminant removal along with high biomass productivity. The present study attempts to explore the possibility of VFCWs to perform as ecological sanitation system coupling wastewater treatment with biomass utilisation for bioenergy production.

Material and Methods

Analysis of influent wastewater: The wastewater used in this study was dairy wastewater obtained from the influent tank of the Milma dairy plant located at Ambalathara in Trivandrum district of Kerala. The physical, chemical and biological analyses were conducted as per the standard methods for the examination of water and wastewater[5]. The raw dairy wastewater had high BOD concentrations ranging from 730 mgL^{-1} to 765 mgL^{-1} while the COD values ranged from 1117 mgL^{-1} to 1259 mgL^{-1}. The average BOD/COD ratio was 0.63. The concentration of TSS ranged from 367 mgL^{-1} to 432 mgL^{-1}. The average concentrations of ammoniacal nitrogen, nitrates, TN and phosphates were observed as 52.34 mgL^{-1}, 4.10 mgL^{-1}, 79.64 mgL^{-1} and 14.33 mgL^{-1} respectively. The physicochemical and biological characteristics of raw dairy effluent are presented in table 1.

Design and configuration of experimental VFCW systems: The VFCWs were constructed inside the campus of Mar Baselios College of Engineering and Technology located in the district of Thiruvananthapuram in Kerala. The design aspects were based on the constructed wetlands manual[11,21,45,46]. The efficiency of treatment processes in a vertical flow CW depends on the design and operational criteria including hydraulic load, wastewater source and quality, plant species and substrate materials. Adequate treatment is closely related to a suitable hydraulic load and wastewater flow[14,37,38,40]. Based on the design, the control and experimental VFCWs (labeled VFCW1- VFCW5) were constructed of rectangular plastic containers, each of length 0.65m, width 0.45m and depth 0.45m[36]. A control system without vegetation was constructed to study the effect of macrophytes in the removal of pollutants.

In this study, the substrate materials used as filter media consisted of gravel, coarse sand and coco-peat (coir fiber pith). The characteristics of the substrate materials used as filter media are given in table 2. The slope of the bottom bed was oriented 1% towards the outlet. The schematic representation of the filter bed is illustrated in figure 1.

Table 1
Characteristics of influent dairy wastewater

Parameters	Unit	Average ± SD
pH	-	7.51 ± 0.19
Temperature	℃	26.00 ± 0.28
Turbidity	NTU	224.80 ± 11.46
TSS	$mg\ L^{-1}$	398.00 ± 21.69
BOD	$mg\ L^{-1}$	750.20 ± 10.84
COD	$mg\ L^{-1}$	1200.00 ± 58.74
Ammoniacal Nitrogen	$mg\ L^{-1}$	52.34 ± 5.87
Nitrates	$mg\ L^{-1}$	4.10 ± 0.90
Total Nitrogen	$mg\ L^{-1}$	79.64 ± 3.88
Phosphates	$mg\ L^{-1}$	14.33 ± 1.66
Fecal Coliforms	MPN/100mL	6.20 E8 ± 1.04 E8

Table 2
Characteristics of substrates used as filter media

Substrate for filter bed	Characteristics
Bottom layer, Gravel (10cm)	Porosity = 0.42
Coarse sand (20cm)	Porosity = 0.39 Effective size, d_{10} = 0.30 mm Uniformity coefficient = 4
Coco-peat (8 cm)	pH = 6.20 Bulk density = 0.09 gcm^{-3} Electrical conductivity = 0.16 $mScm^{-1}$ Porosity = 0.65
Top layer, Gravel (2 cm)	Porosity = 0.42

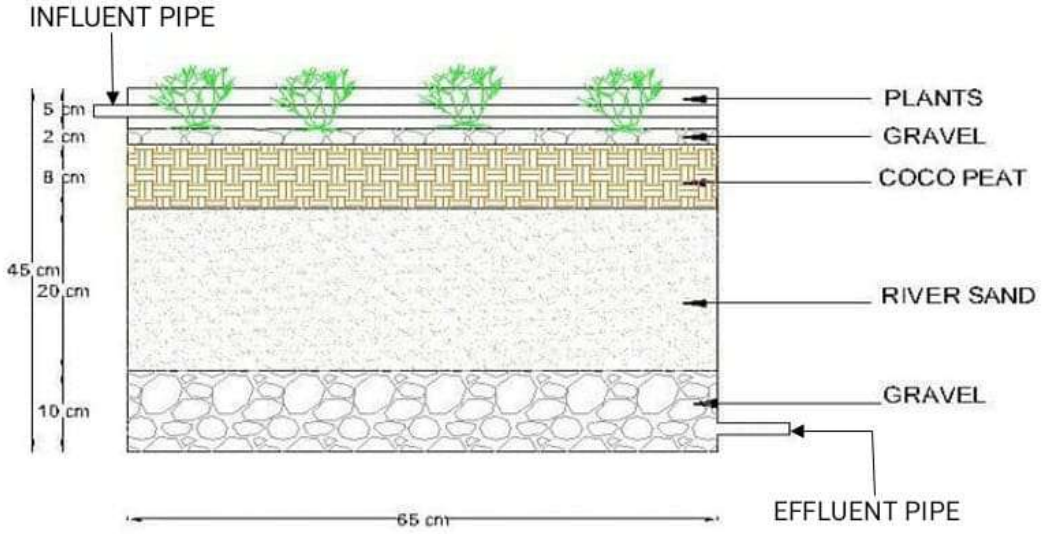


Figure 1: Schematic representation of the filter bed profile in the VFCW mesocosms

Wetland vegetation: Five different plant species were identified and selected for the study, all belonging to the grass family Poaceae with a fibrous root system. The plant species used in the study are local, perennial, lignocellulosic species quite adapted to the tropical climatic conditions of Kerala and widely used as fodder crops. All the grass species used in this study were collected from the fodder farm of Kerala Agricultural University (KAU).

The grass species used in the first experimental system (VFCW1) was Cumbu Napier hybrid grass (*Pennisetum purpureum)* which is an interspecific hybrid between fodder Cumbu (*Pennisetum glaucum)* and Napier grass *(P. purpureum Schumach)*. The second experimental (VFCW2) system was planted with Gamba grass *(Andropogon gayanus)* which is a common perennial forage grass of the tropical regions. The grass planted in the VFCW3 system is Guinea grass *(Panicum maximum)*, a common fodder of the tropical regions, well adapted to the climate conditions of Kerala. The fourth experimental system (VFCW4) was planted with Para grass, (*Brachiaria mutica)* popularly referred to as buffalo grass, california grass and water grass. The fifth experimental (VFCW5) system was planted with Palisade grass (*Urochloa brizantha***)** which is a rhizomatous perennial grass and is often used as a forage for livestock.[22,36]

Construction and operation of VFCW systems: The selected grasses were planted in the various VFCW systems with a plant density of 6 plant stems/unit. The wastewater treatment was initiated in the VFCWs after providing the required plant establishment period and thereafter an acclimatization period of one month. The raw wastewater was fed on to the VFCW bed evenly by means of distribution pipes perforated at the bottom. During the treatment period, 6 parallel units were operated simultaneously including 5 planted VFCWs and one control system[36]. Raw influent from the inlet and treated effluent from the outlet of the VFCWs were sampled monthly throughout the treatment period and the various physico-chemical characteristics were analyzed to determine the treatment efficiencies. The general appearance, growth and health of the plants growing in the different VFCW systems were monitored throughout the study period. The above-ground biomass yields obtained from the various planted VFCWs were estimated and assessed for their nutrient uptakes. The harvested biomass was then evaluated for its ethanol production potential.

Estimation of bioethanol yield: Ethanol production from lignocellulosic biomass has different steps which included the collection of lignocellulosic plant biomass, compositional analysis, pre-treatment, enzymatic hydrolysis and ethanol fermentation. The compositional analysis was conducted to determine the percent of constituents such as cellulose, hemicellulose and lignin of the species considered. Pre-treatment is an indispensable process to reduce the recalcitrance of plant biomass and has a significant role in biomass processing[32]. The objective of pre-treatment was to break down the structure of lignin and disrupt the crystalline structure of cellulose for increasing the enzyme accessibility to the cellulose during hydrolysis. The enzymatic hydrolysis involves the procedure that converts polysaccharides to monomeric sugars. The fermentable sugars obtained from hydrolysis can be fermented into ethanol and other products with the help of microorganisms[39,42,43].

The experimental analysis required for the study was carried out at the Microbial Processes and Technology division of CSIR-NIIST in Thiruvananthapuram. The analysis was conducted according to the experimental protocols and laboratory analytical procedure for lignocellulosic biomass compositional analysis, hydrolysis and fermentation, provided by National Renewable Energy Laboratory[30].

Statistical Analysis: Ordinary one-way ANOVA and Tukey's multiple comparison test were used to determine significant statistical differences in the performance and treatment efficiency as well as biomass yield between the different groups of treatments (control system, VFCW1, VFCW2, VFCW3, VFCW4 and VFCW5). All statistical analyses were performed at 0.05 significant levels. The statistical analysis was carried out using the software package Graph Pad Prism 8.2.1.

Results and Discussion

Performance of VFCW systems: The performance and treatment efficiency of subsurface constructed wetlands can be generally expressed in terms of "percent concentration reduction and percent mass removal of the pollutants" [1,41]. In this study, the performance and treatment efficiency of the different VFCWs were analyzed based on the average concentration of the influent and effluent, percent removal of the contaminants and the aerial load reduction (ALR)[40]. ALR is calculated as the difference between the inlet pollutant loading rate (ILR) and the outlet loading rate (OLR) expressed in $gm^{-2}d^{-1}$. The influent and treated effluent was sampled monthly from the inlet and outlet of different VFCWs and analyzed for various physical, chemical and biological parameters.

Treatment of dairy wastewater was carried out in the experimental and control VFCWs for a period of ten months. The systems were operating at a hydraulic loading rate (HLR) of 0.06 m d^{-1} with a detention period of 1 day. During the treatment period, the organic loading rate (OLR)varied between 43.80 and 45.90 g BOD$_5$ $m^{-2}d^{-1}$. Table 3 presents the statistical data of the concentration of various pollutants in the effluent obtained from the different VFCW systems during the treatment period. The effluent concentrations of the various parameters were compared with the effluent standards in India for disposal on land for irrigation as well as the USEPA standards for non-potable reuse[29,47].

Table 3
Statistical data of influent and effluent concentration from the various VFCW units treating dairy wastewater (mean value ± SD)

Parameter	Unit	Influent dairy wastewater	Treated effluent obtained from						Effluent standards	
			Control system	VFCW 1 (Cumbu Napier grass)	VFCW 2 (Gamba grass)	VFCW 3 (Guinea grass)	VFCW 4 (Para grass)	VFCW 5 (Palisade grass)	On land for irrigation (MoEFCC India)	Non-potable reuse (USEPA)
pH	---	7.51 ± 0.19	7.30 ± 0.05	7.10 ± 0.02	7.20 ± 0.11	7.20 ± 0.15	7.00 ± 0.33	7.10 ± 0.12	6-9	6-7
Turbidity	NTU	224.80 ± 11.46	28.78 ± 3.90	3.01 ± 2.00	6.60 ± 2.78	6.12 ± 3.85	7.13 ± 3.64	2.98 ± 2.52	NS	2-5
TSS	mgL^{-1}	398.00 ± 21.69	143.28 ± 14.03	41.18 ± 20.48	84.21 ± 14.25	68.05 ± 24.09	82.15 ± 13.26	44.28 ± 19.76	100	30
BOD$_5$	mgL^{-1}	750.20 ± 10.84	489.88 ± 15.66	94.52 ± 41.67	158.40 ± 44.47	146.28 ± 23.29	182.08 ± 54.07	108.04±25.59	100	30
COD	mgL^{-1}	1200.00 ±58.74	786.20 ± 45.19	249.05 ±66.73	334.13 ± 96.80	325.71 ± 76.24	359.16 ± 89.27	272.66± 57.54	250	90
NH4-N	mgL^{-1}	52.34 ± 5.87	33.01 ± 1.58	15.85 ± 1.18	19.51 ± 1.22	17.95 ± 1.13	20.14 ± 1.24	17.24 ± 1.82	NS	NS
Nitrates	mgL^{-1}	4.10 ± 0.90	3.85 ± 0.86	1.24 ± 0.69	1.68 ± 0.67	1.53 ± 0.65	1.60 ± 0.68	1.31 ± 0.71	NS	10
TN	mgL^{-1}	79.64 ± 3.88	69.37 ± 3.39	45.15 ± 6.81	47.10 ± 7.44	46.10 ± 8.48	47.32 ± 8.96	45.74 ± 8.86	NS	NS
Phosphate	mgL^{-1}	14.33 ± 1.66	13.53 ± 1.50	7.25 ± 1.99	7.68 ± 2.18	7.66 ± 1.85	7.71 ± 2.01	7.38 ± 1.84	NS	NS

Table 4
ILR and ALR for each pollutant in the various VFCW systems treating dairy wastewater (mean values)

Parameter	ILR ($gm^{-2}d^{-1}$)	Areal Reduction Load (ALR) in $gm^{-2}d^{-1}$					
		Control system	VFCW 1 (Cumbu Napier grass)	VFCW 2 (Gamba grass)	VFCW 3 (Guinea grass)	VFCW 4 (Para grass)	VFCW 5 (Palisade grass)
BOD$_5$	45.01	15.62	39.34	35.51	36.24	34.09	38.53
COD	72.00	24.83	57.06	51.95	52.46	50.45	55.64
Nitrates	0.25	0.01	0.17	0.14	0.15	0.15	0.17
TN	4.78	0.62	2.07	1.95	2.01	1.94	2.03
Phosphates	0.86	0.05	0.42	0.39	0.40	0.39	0.41

The concentrations of TSS, BOD and COD were high in the dairy influent and though there was a considerable reduction in the concentration of treated effluent from the planted VFCWs, the level of standards has not been achieved. The concentration of the various pollutants was found to be low in the case of treated effluent from the VFCW1 system planted with Cumbu Napier grass when compared to effluent from other VFCWs.

Table 4 shows the ILR and Areal Load reduction in the various VFCW systems treated with dairy wastewater. For all the parameters analysed, the highest ALR was achieved in the systems planted with Cumbu Napier hybrid grass followed by Palisade grass and Guinea grass. The mean pollutant removal efficiency obtained in the different systems is graphically presented in figure 3.

Removal of Suspended solids: The removal of suspended solids in the various VFCWs with the growth of the plants is shown in figure 2A. The VFCW1 system planted with Cumbu Napier grass had a mean TSS removal of 89.70%, whereas for planted systems with Gamba grass (VFCW2), Guinea grass (VFCW3), Para grass (VFCW4) and Palisade grass (VFCW5), it was observed as 78.90%, 82.90%, 79.4% and 88.90% respectively. In the control system, the removal efficiency observed was 64.10%.

The removal of TSS in CWs can be due to sedimentation, filtration, interception, adsorption and root zone treatment. The voids and media grain structure have a substantial influence on the trapping of the suspended solids during its flow path[6,44]. The substrate materials such as sand, coco-peat and gravel, as well as the roots of the plants, acted as the filters to trap the suspended particles[8,46].

Vertical flow systems are highly effective in removing TSS, provided the bed clogging problems are managed through a "load and rest operation regime"[20]. The removal efficiency obtained from the control and planted systems indicated the positive influence of plants in the removal of suspended solids. This signifies the role of root zone treatment and filtration by impacting suspended solids in the roots as well as stems of the plants in the VFCWs.

Removal of organic matter: The percentage removal of BOD and COD with the growth of the macrophytes is presented in fig. 2B and fig. 2C respectively. In the control unit, BOD and COD removal efficiency observed was less than 40% for the entire operational period.

For planted VFCWs, the mean removal efficiency of BOD observed was 87.40%, 78.90%, 80.50%, 75.70% and 85.60% for systems vegetated with Cumbu Napier Hybrid grass, Gamba grass, Guinea grass, Para grass and Palisade grass respectively. In the VFCW1 using Cumbu Napier hybrid grass, mean COD removal efficiency was observed as 79.30% while the removal efficiency of other systems planted with Gamba grass, Guinea grass, Para grass and Palisade grass was 72.20%, 72.90%, 70.10% and 77.30% respectively. In the planted VFCWs, it was observed that the performance improvement in the removal of organic matter occurred after about 90 days of treatment.

The settleable organic matter in the influent was largely removed by the process of deposition and filtration in the VFCWs whereas attached and suspended bacterial growth was the predominant removal mechanism for soluble organic compounds.

The removal of BOD in the planted VFCW systems occurs due to the biodegradation of organic matter that takes place in the biofilm together with the roots of plants and stems and the surface of the substrate[6,45].

The oxygen needed for aerobic degradation can be provided by the processes of diffusion and convection as well as oxygen seepage from the macrophyte roots into the rhizosphere, based on the wetland design. Therefore, the treatment efficiency in the planted VFCWs for the removal of organic matter greatly depends upon the oxygen availability in the bed, the design of the wetland, the treatment conditions and the characteristics of the substrates of the filter bed media[51-53].

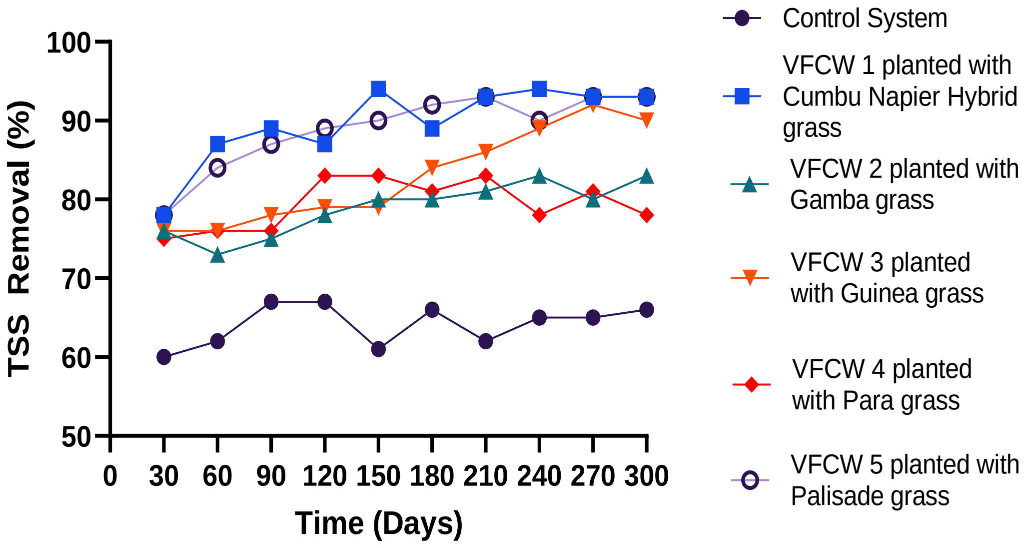
The intermittent flow regime in the VFCWs enables the formation of a vadose zone allowing for diffusion of atmospheric oxygen into the CW media[20].

Furthermore, the presence of macrophytes and their root system provides a favourable environment that promotes the growth of a diverse group of microorganisms. The diversity of roots delayed the percolation of wastewater through the bed increasing the detention time and as a result, increased treatment efficiency.

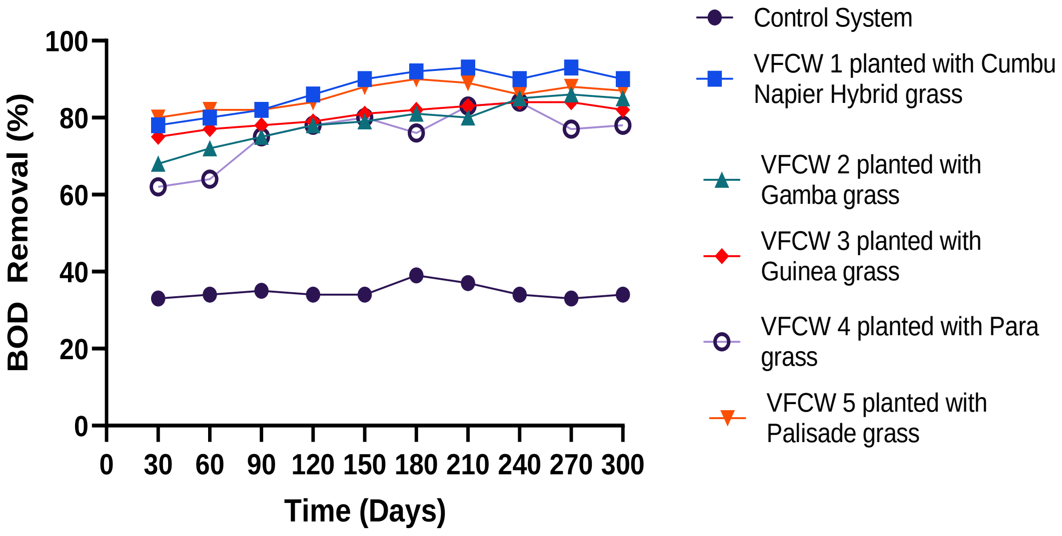
Removal of Nutrients: The results obtained show that the nutrient removals in the planted VFCWs were significantly higher than those in the control system. This indicated the importance of the presence of plants and the uptake of nutrients by them. The mean percentage removal of nitrates, phosphates and TN in the VFCWs during the treatment with dairy wastewater is presented in fig. 2D, 2E and 2F respectively.

The pH values of the effluent from the planted systems were just above 7 which indicated that conditions were suitable for nitrification within the wetland bed. Ammonia gets oxidized to nitrate with the help of nitrifying bacteria in the aerobic zones of the VFCWs.

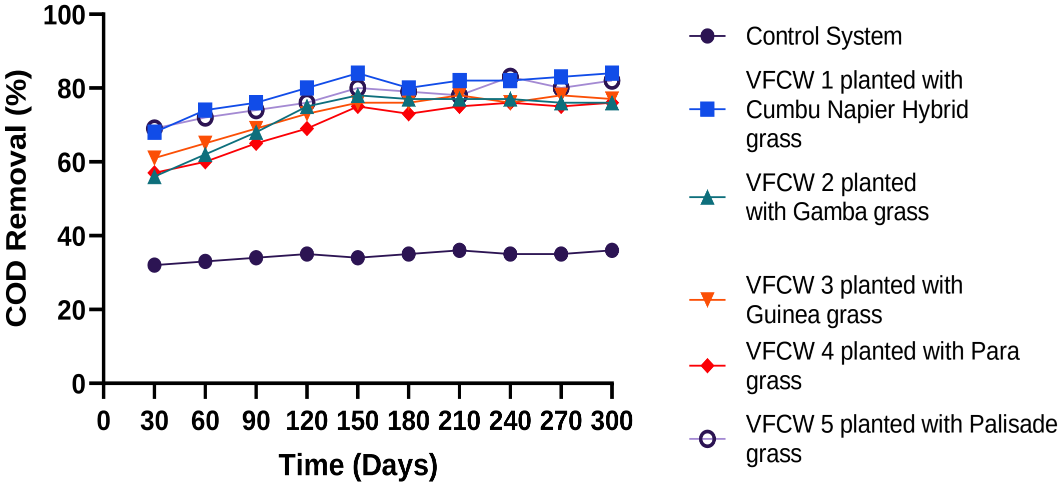
The oxygen essential for nitrification is supplied by atmospheric transmission and leakage from the roots of the plants. In vertical flow constructed wetlands, very high nitrification proceeds but due to the absence of entirely anaerobic conditions in the wetland bed, denitrification is very limited in these systems[16,49,50].


TSS Removal (%)
100
90
80
70
60
50
0
30
60
90
120
150
180
210
240
270
300
Time (Days)
Control System
VFCW 1 planted with Cumbu Napier Hybrid grass
VFCW 2 planted with Gamba grass
VFCW 3 planted with Guinea grass
VFCW 4 planted with Para grass
VFCW 5 planted with Palisade grass

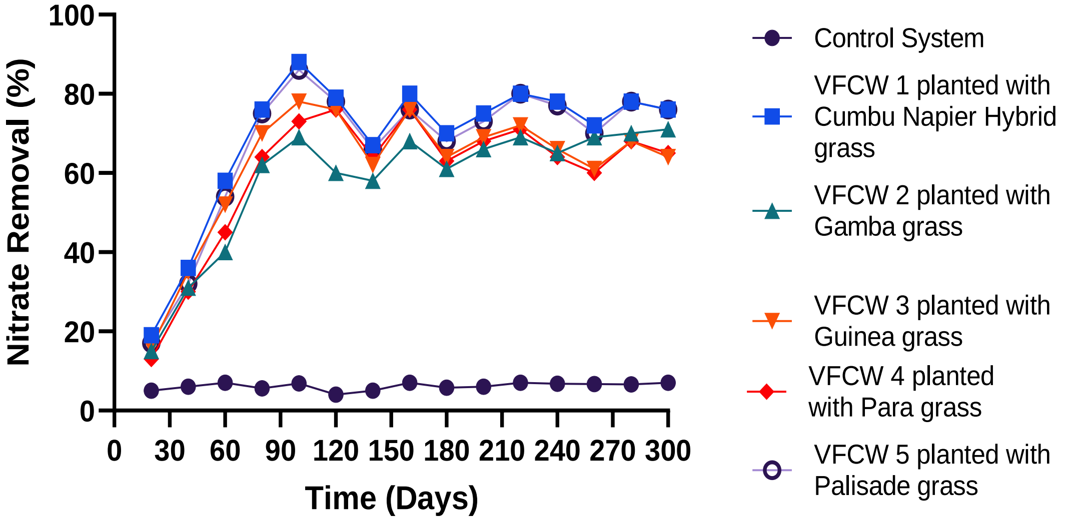
(2A)


BOD Removal (%)
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
270
300
Time (Days)
Control System
VFCW 1 planted with Cumbu Napier Hybrid grass
VFCW 2 planted with Gamba grass
VFCW 3 planted with Guinea grass
VFCW 4 planted with Para grass
VFCW 5 planted with Palisade grass

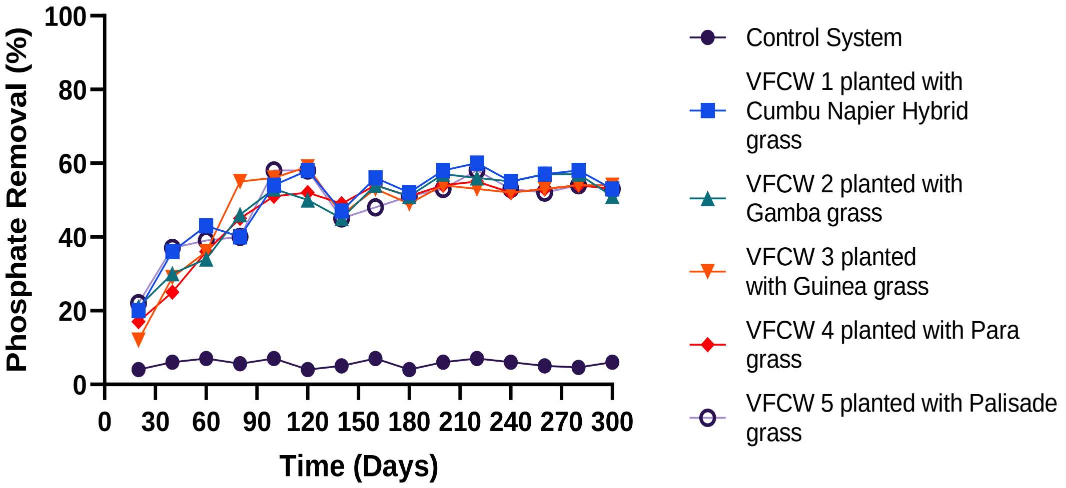
(2B)


COD Removal (%)
100
80
60
40
20
0
0
30
60
90
120
150
180
210
240
270
300
Time (Days)
Control System
VFCW 1 planted with Cumbu Napier Hybrid grass
VFCW 2 planted with Gamba grass
VFCW 3 planted with Guinea grass
VFCW 4 planted with Para grass
VFCW 5 planted with Palisade grass

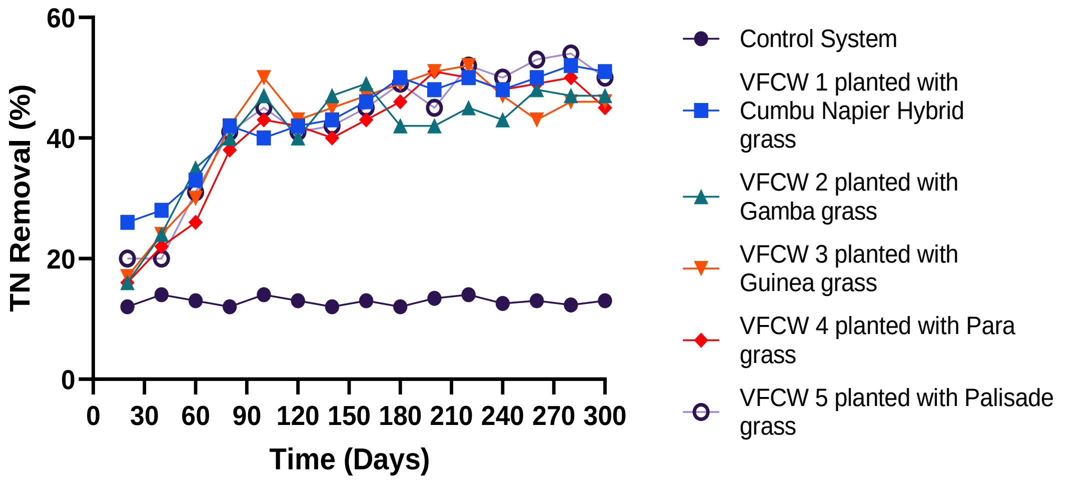
(2C)



(2D)



(2E)



(2F)

Figure 2: Removal of various pollutants in the control and planted VFCW systems during the entire operational period (A) TSS; (B) BOD; (C) COD; (D) Nitrates; (E) Phosphates; (F) TN

The processes for the removal of phosphorus in CWs include "adsorption, complexation and precipitation, storage, plant uptake (with subsequent harvest) and biotic assimilation"[45,50]. The removal efficiency of phosphorus is generally reported to be low in subsurface constructed wetlands unless special media with high sorption capacity are used[49]. The results obtained show that the removal of phosphates is effective in the planted VFCWs when compared to the control system.

In order to study the direct contribution of plants in nutrient removal, the harvested biomass was analysed for nitrogen and phosphorus. The average nitrogen uptake was obtained as 47.42 g m^{-2}, 16.06 g m^{-2}, 20.5 g m^{-2}, 15.56 gm^{-2} and 23.96 gm^{-2} whereas the average phosphorus uptake was 3.46 gm^{-2}, 1.17 gm^{-2}, 1.49 g m^{-2}, 0.52 g m^{-2} and 1.75 gm^{-2} for Cumbu Napier, Gamba, Guinea, Para and Palisade grass respectively. This is supported by the values reported in literature for above-ground nitrogen ranging from 2 - 64 g Nm^{-2} and for phosphorus in the range 0.01- 19 g P m^{-2} [49].

Comparison of treatment efficiency: The VFCWs planted with grasses obtained high pollutant removal efficiency than the control system with unplanted filter bed. The mean removal efficiencies achieved in the control unit were as follows: Turbidity (87.19%), TSS (64.10%), BOD (34.70%), COD (34.50%), NH_4-N (36.90%), Nitrates (6.16%), TN (12.89%) and Phosphates (5.61%).

In the case of planted VFCWs, high pollutant removal efficiency was observed in the VFCW1 planted with *Pennisetum purpureum* followed by VFCW5 planted with *Urochloa brizantha*. Mean removal efficiencies observed in VFCW1 were Turbidity (98.64%), TSS (89.70%), BOD (87.40%), COD (79.30%), NH_4-N (69.70%), Nitrates (68.80%), TN (43.27%) and Phosphates (49.80%). In the VFCW5 system planted with *Urochloa brizantha*, the mean removal efficiencies observed were Turbidity (98.67%), TSS (88.90%), BOD (85.60%), COD (77.30%), NH_4-N (67.10%), Nitrates (67.07%), TN (42.53%) and Phosphates (48.80%). The average pollutant removal efficiency of the experimental VFCWs during the study period is presented in figure 3.

According to statistical analysis for the removal of suspended solids, organic matter and nutrients, there was a significant difference ($p<0.05$) in the treatment between the control system and all the experimental planted VFCW systems. High removal efficiencies were observed in the systems planted with Cumbu Napier hybrid grass (VFCW1) and Palisade grass (VFCW5), though there was no significant statistical difference between them for all the parameters analyzed.

A significant difference was observed between VFCW1 and other planted systems VFCW2, VFCW3, VFCW4 for the removal of TSS, BOD and COD. There was no significant difference amongst the VFCWs planted with different species for the removal of Nitrates, TN and Phosphates.

Biomass production: The grasses planted in the different VFCWs were harvested four times during the treatment period of ten months. The first harvesting was done after 120 days of planting and the subsequent harvests at 60 days interval.

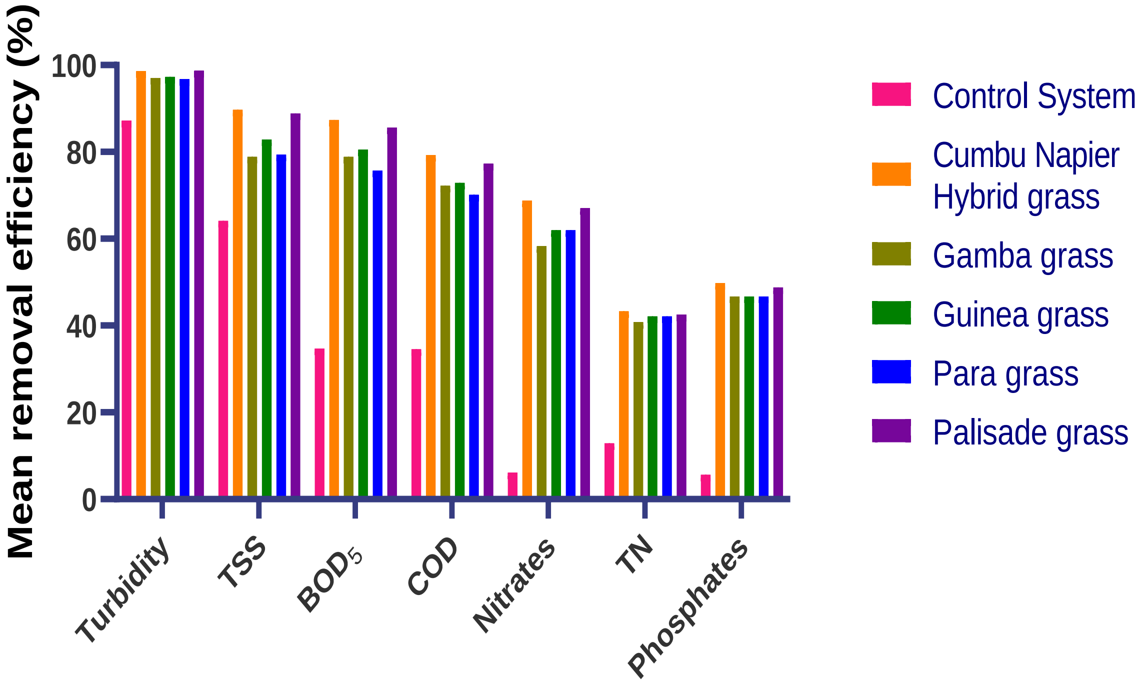


Figure 3: Mean removal efficiency of pollutants in the different VFCW systems treating dairy wastewater

The average green biomass yields obtained from the various VFCWs planted with *Pennisetum purpureum* and*ropogon gayanus, Panicum maximum, Brachiaria mutica* and *Urochloa brizantha* during the entire treatment period were 5.75 kgm^{-2} , 1.78 kgm^{-2}, 2.29 kgm^{-2}, 1.57 kgm^{-2} and 2.55 kgm^{-2} whereas the average dry biomass yields were obtained as 1.92 kgm^{-2}, 0.59 kgm^{-2}, 0.76 kgm^{-2}, 0.52 kgm^{-2} and 0.85 kgm^{-2} respectively. According to statistical analysis, the biomass yield of *Pennisetum purpureum* was found to be significantly ($p < 0.05$) higher than other grasses.

The results obtained shows that there is a decline in the biomass yield of the grasses after each cutting cycle. The reason for this decline can be attributed to the restrictions in the space and availability of nutrients in the VFCW mesocosms as the plant grows. In this study, the grasses were grown in the VFCW systems without applying any external fertilizer, but the plants extracted the required nutrients and water from the influent wastewater[24,34,36].

Bioethanol yield: The various steps involved in the estimation of bioethanol yield included compositional analysis, pre-treatment, saccharification and fermentation. The compositional analysis of the biomass harvested from the different VFCW systems was conducted to estimate the cellulose, hemicellulose, lignin and ash content. Each experiment was replicated thrice and the presented results indicate the mean values obtained from all experiments. Cellulose and hemicellulose were analysed and detected by using High Performance Liquid Chromatography (HPLC). Biomass compositions vary according to topographical locations of materials, methods (procedures) used for analysis, nature and type of biomass, the part of plant biomass used in the analysis and the pre-treatment method adopted.

Table 5 presents the results of the compositional analysis of the five different species studied. From the results obtained, Gamba grass has high cellulosic content (47%) followed by Palisade grass (46.97%), Guinea grass (44.44%), Para grass (41.29%) and Cumbu Napier hybrid grass (37.23%). The results indicated that Gamba grass has the greatest potential for bioethanol production as it contained a high concentration of cellulose which constitutes a good source for obtaining glucose.

In this study, the alkaline pre-treatment process was adopted. Alkaline pre-treatment causes swelling of the lignocellulosic biomass, leading to the decrease of degree of polymerization and crystallinity of cellulose and increase of the surface area to facilitate the enzymatic hydrolysis of cellulose[2]. Treatment with NaOH can increase the porosity of lignocellulosic biomass that subsequently results in improved glucose yield after the enzymatic hydrolysis.

Alkaline pre-treatment also helps in removing acetyl and other acidic substitutions on hemicelluloses that shield cellulose from cellulase attack. The efficiency of alkaline pre-treatment depends on the peculiarities of lignocellulosic biomass and the treatment conditions maintained[2,4]. Alkaline pre-treatment was adopted in this study as it has been found to be more effective in the case of non-woody plants with comparatively low lignin content.

After the pre-treatment of biomass with alkali, pH was adjusted to 7. The solids and liquids were separated and biomass washed with tap water and used for hydrolysis. Biomass loading of 5% and NaOH loading of 0.5% (w/v) produced total residual sugar concentration of 6.34 mg/mL, 20.56 mg/mL,14.79 mg/mL, 8.23 mg/mL and 7.70 mg/mL at 70hrs of incubation for Cumbu Napier, Gamba, Guinea, Para and Palisade grass respectively. Glucose yield at different time intervals is shown in table 6.

Enzymatic digestibility of the cellulosic constituent of pre-treated biomass is very critical in the conversion of lignocellulosic biomasses to bioethanol. The efficiency of sugar yield from the pre-treated biomass is greatly influenced by the nature of hydrolysing enzymes employed in the process. In a study, Sukumaran et al[43] have observed that enzyme loading and duration of hydrolysis will be determined to a great extent by the characteristics of the enzymes, which in turn influences the overall cost of production.

The process of fermentation of hydrolysates obtained from the alkali pre-treated samples was carried out without any “detoxification process”. The wild yeast of “*Saccharomyces cerevisiae* RPP-03N” was used for carrying out the process of fermentation.

Table 5
Compositional analysis of biomass (Mean ± SD)

Plant Species	% Cellulose	% Hemicellulose	% Lignin	% Ash	% Total
Cumbu Napier hybrid grass	37.23 ± 8.90	20.94 ± 2.40	23.70 ± 1.20	1.96 ± 0.20	83.83 ± 12.60
Gamba grass	47.27 ± 7.90	23.72 ± 2.10	25.84 ± 0.60	1.63 ± 0.00	98.47 ± 9.40
Guinea grass	44.44 ± 3.70	19.72 ± 1.20	22.99 ± 0.20	3.37 ± 0.10	90.51 ± 5.20
Para grass	41.29 ± 5.80	19.77 ± 1.20	21.77 ± 0.30	1.72 ± 0.10	84.55 ± 5.20
Palisade grass	46.97 ± 11.00	20.96 ± 0.10	18.94 ± 0.00	1.98 ± 0.00	88.84 ± 10.90

Table 6
Time vs Glucose yield during hydrolysis

Plant species	**Glucose yield (mg/ml)**		
	0th hr	**24th hr**	**70th hr**
Cumbu Napier hybrid grass	1.69	5.58	6.34
Gamba grass	0.33	11.56	**20.56**
Guinea grass	1.85	15.31	14.79
Para grass	0.85	10.78	8.23
Palisade grass	1.70	11.24	7.71

Table 7
Fermentation results of hydrolysates

Plant Species	**Time (hr)**	**Glucose (mg/ml)**	**Ethanol % (v/v)**
Cumbu Napier hybrid grass	0	6.34	0.08
	72	0.11	2.91
Gamba grass	0	20.56	0.12
	72	0.21	**10.62**
Guinea grass	0	14.79	0.04
	72	0.04	7.93
Para grass	0	8.23	0.03
	72	0.13	4.12
Palisade grass	0	7.71	0.07
	72	0.16	3.87

Table 7 shows the fermentation results of hydrolysates. Percentages of ethanol production after 72 hours were expressed in volume of ethanol to the volume of the total reaction mixture(v/v) of Cumbu Napier hybrid grass, Gamba grass, Guinea grass, Para grass and Palisade as 2.91, 10.62, 7.93, 4.12 and 3.87 respectively. The highest ethanol yield was obtained for Gamba grass (10.62%) followed by Guinea grass (7.93%) and Para grass (4.12%).

Conclusion

The present study investigated the potential of VFCWs using local lignocellulosic perennial fodder grasses suitable to the tropical climate of Kerala to function as decentralised ecological sanitation systems for wastewater treatment coupled with biomass utilisation for bioethanol production. The results of the study strongly indicated the positive influence of plants and their root system for treating the contaminants and improving the water quality in VFCWs. The VFCWs planted with *Pennisetum purpureum* and *Urochloa brizantha* obtained high pollutant removal efficiency for TSS, BOD, COD and nutrients, though there was no significant statistical difference between the two. In the case of treatment of dairy effluent, only VFCW1 planted with Cumbu Napier hybrid grass reached the Indian standards.

Therefore, the study suggested that for treating wastewaters with a high organic loading rate, a preliminary treatment has to be provided before feeding into the VFCWs for achieving the desired effluent quality standards. The green and dry biomass yield of *Pennisetum pupureum* were found to be significantly higher when compared to *Andropogon gayanus, Panicum maximum, Brachiaria mutica* and *Urochloa brizantha*. The highest percentage of ethanol production after 72 hours expressed in volume of ethanol to the volume of the total reaction mixture (v/v) was obtained for *Andropogon gayanus* (10.62%) followed by *Panicum maximum* (7.93%).

This study has shown that VFCWs planted with highly productive, low-input, perennial lignocellulosic grasses can perform as eco-san system realising improvement in water quality, enabling water reuse and nutrient reuse and bioethanol production. Further investigation will be needed for optimising the pre-treatment, hydrolysis conditions and fermentation parameters for improving the bioethanol yield of the lignocellulosic species used in the study.

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