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CONSTRUCTED WETLANDS FOR NITROGEN REMOVAL AND SIMULTANEOUS REUSE OF DOMESTIC SEWAGE

WETLANDS CONSTRUÍDOS PARA REMOÇÃO DE NITROGÊNIO E REUSO SIMULTÂNEO DE ESGOTOS DOMÉSTICOS

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Abstract

This study evaluated the performance of a vertical flow constructed wetland (VFCW) planted with irrigated rice (Oryza sativa L., species BRS-GO Guará) and a vertical flow soil filter without plants (VFSF-CTR) as a control, both on a reduced experimental scale. The primary objective was to remove and recover nitrogen (N) from domestic sewage using soil red-yellow latosol (RYL) mixed with medium sand (modified RYL) as the support medium. The VFCW and VFSF-CTR systems were operated over ten months and two rice crop cycles, utilizing 3 (three) different hydraulic loading rates (HLR): 4.0, 8.0, and 15.0 cm d^{-1}. Each treatment had three repetitions, totaling 18 (eighteen) experimental units. For the VFCW, the removal efficiencies for total Kjeldahl nitrogen (KTN) and ammonium nitrogen ($N\text{-}NH_4^+$) ranged from 86% to 95%, while for the VFSF-CTR, they ranged from 79% to 94%. The presence of the rice crop enhanced KTN removal efficiencies, independent of the HLR. Both systems demonstrated tertiary treatment removal efficiency in a single stage, with minimal variations in removal efficiencies when operated at an HLR of 15 cm d^{-1}. There was substantial accumulation of KTN and mineral N ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) in the soil, particularly in the 0-5 cm layer, which can be utilized for fertilizing other crops. Additionally, nitrogen was immobilized in microbial biomass (MBN). This rapid fertilization improved soil quality in the short term (10 (ten) months of sewage application) and enabled the production of grains and vegetable biomass for human and animal consumption. The VFCW presented good performance under both low and high hydraulic loads, demonstrating larger operational flexibility.

Keywords: nitrogen mineralization**;** nutrient removal**;** rice**;** sewege treatment**;** soil fertilization.

Resumo

Este estudo avaliou o comportamento de *wetland* construído de fluxo vertical (WCFV), plantado com arroz irrigado (Oryza sativa L.), espécie BRS-GO Guará, e um filtro de solo de fluxo vertical sem planta (FSFV-CTR), como controle, ambos em uma escala experimental reduzida. O objetivo foi a remoção e recuperação de N de esgoto doméstico, utilizando-se Latosolo Vermelho-Amarelo (LVA) misturado com areia média (LVA modificado) como meio suporte. A duração da operação de WCFV e FSFV-CTR de dez meses e dois ciclos de cultivo do arroz, utilizou 3 (três) diferentes taxas de aplicação hidráulica - TAH (4,0, 8,0 e 15,0 cm d^{-1}); com três repetições, totalizando 18 (dezoito) unidades experimentais. Para o WCFV, as eficiências de remoção de nitrogênio total Kjeldahl (NTK) e $N\text{-}NH_4^+$ variaram de 86% a 95%; e, para FSFV -CTR, de 79% a 94%; com a cultura do arroz favorecendo maiores eficiências na remoção de NTK, independente da TAH. Ambos os sistemas apresentaram eficiência de remoção de tratamento terciário em um único estágio; além disso, as variações nas eficiências de remoção foram mínimas quando operados com HLR de 15 cm d^{-1}. Houve grande acúmulo de NTK e N mineral (N-NH_4^+ e N-NO_3^-) no solo, principalmente na camada de 0-5 cm, que pode ser utilizado para adubação de outras culturas. Houve também imobilização do N na biomassa microbiana (NBM). Essa rápida adubação melhorou a qualidade do solo em curto prazo 10 (dez) meses de aplicação de esgoto) e proporcionou a produção de grãos e biomassa vegetal para alimentação humana e animal. O WCFV apresentou bons resultados, seja operado com baixas ou altas cargas hidráulicas, indicando maior flexibilidade operacional.

Palavras-chaves: mineralização do nitrogênio**;** remoção de nutrientes**;** arroz**;** fertilização do solo

1 INTRODUCTION

Constructed wetlands (CW) are among the various treatment technologies capable of removing nitrogen (N) from sewage. They function as biofilters where bacteria grow, adhering to a filter bed or support medium in the presence of vegetation. These systems are simple to implement, operate, and maintain; they are hightly cost-effective, and their efficiency is comparable to advanced sewage treatment technologies. Compared to conventional technologies, which require intensive energy use (Li et al., 2018), CW systems are especially attractive for rural areas.

In Brazil, CWs are crucial for sewage treatment in rural areas due to inadequate sewage treatment and disposal. According to Brasil (2019a), in 2017, 48.6% of the 5.4 million rural households used rudimentary pits for sewage disposal, and 11.7% disposed of sewage into ditches, rivers, lakes, seas, and other water bodies.

CWs operate on the principle of the circular economy by treating sewage, removing and recovering nitrogen, and simultaneously producing effluents of sufficient quality for discharge into water bodies and irrigation of new crops. The treatment process involves the percolation of sewage through the CW filter bed, during which plant roots absorb nutrients, primarily carbon (C), nitrogen (N), and phosphorus (P). Nutrient absorption by plants reduces the pollutant load in the sewage. Additionally, other physical, chemical, and biological processes occur during percolation to remove the remaining pollutants.

In CW systems, N removal is typically variable (Wu et al., 2015a) and depends on several factors, including plant species, support medium, temperature, pH, dissolved oxygen, influent C/N ratio, applied loads, and hydraulic detention time (Saeed; Sun, 2012; Wu et al., 2015b). Organic nitrogen (Norg) is mineralized by the soil complex (support medium and plant-microorganisms) through ammonification and nitrification processes, involving enzymatic hydrolysis produced by microbial activity and other organic matter degradation processes (Zhi et al., 2015). Ammonification is performed by heterotrophic microorganisms (fungi and bacteria), and nitrification by bacteria Nitrosomonas, Nitrosococcus, and Nitrosopira, which oxidize N-NH4+ to N-NO2-, and Nitrobacter and Nitrospira, which oxidize N-NO2- to N-NO3- (Abou-Elelaa et al., 2013). In the N mineralization process, inorganic nitrogen ions (N-NH4+ and N-NO3-) are released into the soil solution. The mineralized fraction depends on the aforementioned factors, water availability, and the quantity and quality of plant residues present in the soil (Chen et al., 2014).

Total N removal in CWs occurs through mechanisms such as adsorption to soil particles (support medium), precipitation, plant absorption, ammonia volatilization and immobilization in microbial biomass, denitrification, anaerobic ammonia oxidation (ANAMMOX), biological nitrogen fixation, and nitrate leaching (Vymazal, 2007). The ANAMMOX process involves partial nitrification (Dong; Sun, 2007), simultaneously removing ammonium and nitrite and converting them to gaseous nitrogen (Zhang et al., 2008) through the action of anaerobic bacteria of the phylum Planctomycetes and genera: Candidatus Kuenenia, Candidatus Brocadia, Candidatus Jettenia, Candidatus Anammoxoglobus, and Candidatus Scalindua (Oshiki; Satoh; Okabi, 2016). The ANAMMOX process occurs simultaneously with denitrification, which can potentially enhancenitrogen removal (Zhi; Ji, 2014). According to these authors, when the C/N ratio is >6.0, ANAMMOX becomes another primary mechanism for removing NH4+. Other factors influencing the occurrence of ANAMMOX include an optimal temperature of 40±3°C (Scheeren et al., 2011).

In CW systems, total N removal generally depends on complete nitrification, with N-NO3- being permanently removed by denitrification (Saeed; Sun, 2012). However, insufficient organic carbon sources often limit these anaerobic and heterotrophic microbial processes (Li et al., 2018). To maintain optimal denitrification, the C/N ratio should be close to 1.5 (Krishna Mohan et al., 2016). For C/N ratios lower than this value, denitrification does not occur (Zoppas; Bernardes; Meneguzzi, 2016). According to Zoppas, Bernardes and Meneguzzi (2016), the most representative genera of denitrifying heterotrophic bacteria are Alcaligenes, Paracoccus, Pseudomonas, Thiobacillus, and Thiosphaera, with an appropriate pH range of 6.5-8.0. During sewage treatment by CW, if labile carbon increases in the soil/support medium, it can enhance both denitrification, contributing to N loss from the soil (Chen et al., 2014), and immobilization of soil microbial biomass nitrogen (MBN) (Caravaca et al., 2005), thereby increasing the soil N reserve (Yang et al., 2010).

Most CW studies in Brazil analyze N removal based on the difference between influent and effluent N concentrations. They rarely consider the C/N ratio and soil immobilization evaluation. Studies generally show N removal results in CW by the nitrification/denitrification process. However, with denitrification, N is lost and cannot be recovered.

Regarding the support medium for CWs (horizontal and vertical flow), the most traditionally employed are crushed gravel and coarse sand. Commonly used plants include Juncus spp (Junco), Echinochloa polystachya (capim mandante / capim Canarana), and Typha species such as Typha domingensis, or Typha latifolia (Taboa). Various authors report significant variations in N removal efficiencies with other types of support medium and plants (Feng et al., 2020; Cui et al., 2015; Cui et al., 2012).

This study evaluated the performance of vertical flow constructed wetlands (VFCW) and a vertical flow soil filter as a control (VFSF-CTR), both on a reduced experimental scale, for N removal and recovery from domestic sewage. red-yellow latosol (RYL) mixed with medium sand (modified RYL) was used as a soil support medium, and rice crops were utilized to aid the treatment process. The VFCW was operated with different hydraulic loading rates (HLR) to investigate N removal and recovery from domestic sewage and measure the production of rice grain and vegetable biomass during sewage treatment.

2 MATERIALS AND METHODS

2.1 Experimental setup

The experiment setup consisted of three small-scale systems, each with six units: three VFCW and three VFSF-CTR (Figure 1). The experimental units were cylindrical plastic containers (drums) with a capacity of 200 L, a height of 0.87 m, an internal diameter of 0.60 m, and a surface area of 0.26 m². Holes of 10 mm and 40 mm were drilled in the drums, into which metal nozzles were inserted to connect the piezometers used for measuring soil head losses and the drainage pipes for the treated sewage.

The experimental design was based on random blocks with three repetitions, arranged in a 3 x 2 factorial scheme. This involved three different hydraulic loading rates (HLR) of sewage (4.0, 8.0, and 15.0 cm d-1) and two treatments: one with a rice crop (VFCW) and the other without a rice crop (VFSF-CTR).

The support medium was red-yellow latosol (RYL) mixed with medium sand (modified RYL) in a 1:1.5 ratio to ensure adequate hydraulic conductivity necessary for sewage treatment. After mixing, the soil had a sandy texture with small amounts of silt and clay, and an average hydraulic conductivity of 1.98×10^{-3} cm s-1, considered suitable for sewage treatment (Table 1).

The soils of the VFCW units were planted with rice (Oryza sativa L.) previously sown in a seedbed and transplanted twenty-five days after sowing.

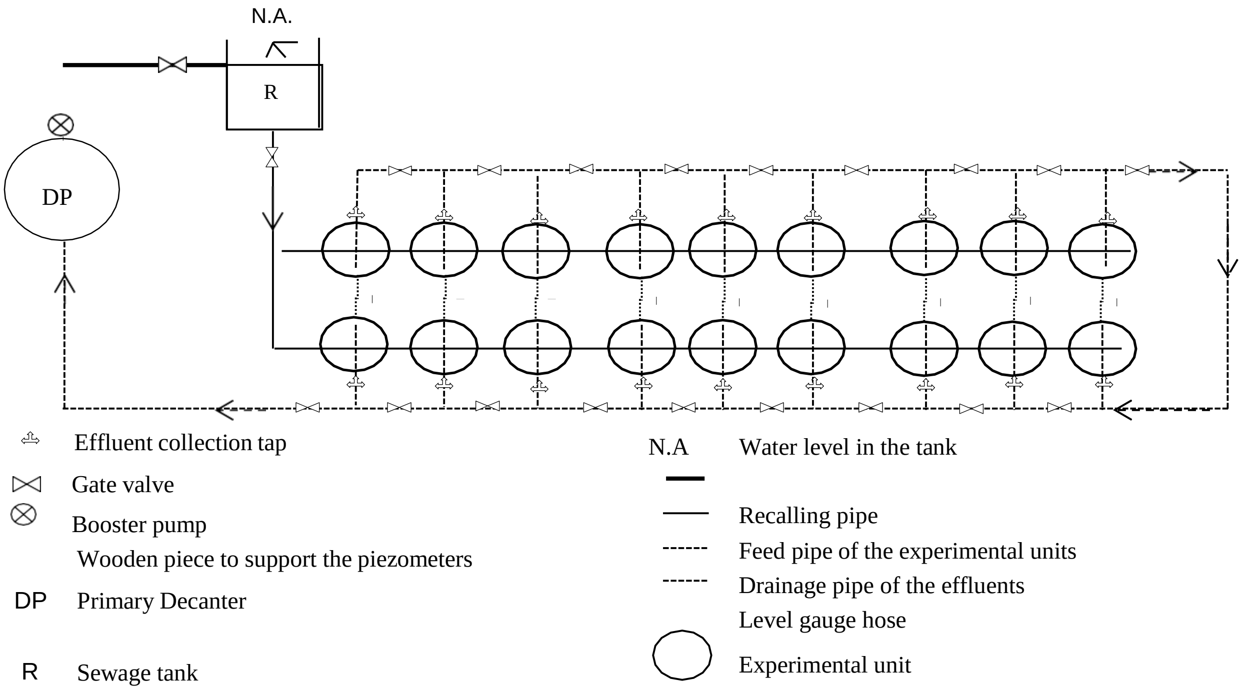
The operational conditions included HLRs of 4.0, 8.0, and 15.0 cm d-1 with alternating intermittent feeding (Monday, Wednesday, and Friday) over ten months of operation. The intermittent sewage application favored aeration, which is necessary for both nitrification and control of soil clogging.

2.2 Systems performance analysis

To evaluate the system performance for N removal, soil samples were taken before sewage application and at the end of each rice cycle. Additionally, influent and effluent samples from the sewage treatment were collected every fifteen days.

The analyzed parameters in the influent and effluent sewage included biochemical oxygen demand (BOD), total Kjeldahl nitrogen (KTN), ammonium (N-NH4+), nitrate (N-NO3-), dissolved oxygen (DO), hydrogen potential (pH), and others. The procedures for collecting, preserving, and analyzing influent and effluent sewage samples followed the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WPCF, 1995).

Figure 1 - Configuration of the experimental system on a reduced scale



Source: created by the authors.

Table 1 - Soil characteristics of experimental units VFCW and VFSF-CTR

Soil texture	**Value/concentration**
Medium gravel (%)	2.70
Fine gravel (%)	12.40
Coarse sand (%)	15.30
Medium sand (%)	31.60
Fine sand (%)	29.80
Silt (%)	3.10
Clay (%)	5.00
Chemical characteristics	
Organic carbon (g kg^{-1})	4.30
Organic Matter (g kg^{-1})	7.40
Cation Exchange Capacity (cmol dm^{-3})	3.92
Total nitrogen (mg kg^{-1})	340.00
Phosphorus (mg kg^{-1})	0.50
pH	5.30

Source: created by the authors.

At the end of the first rice crop cycle, the aerial parts of the rice plants were harvested, and soil samples were collected from the 0-5 cm layer of the VFCW and VFSF-CTR. At the end of the second rice cycle, the entire rice plants were harvested, and soil samples were collected from the 0-5 cm and 5-20 cm layers of the VFCW and VFSF-CTR.

Various soil analyses were performed, including total organic carbon (TOC) (Walkley and Black, 1934), cation exchange capacity (CEC), total nitrogen (N total) by the Kjeldahl method, N-NO3- and N-NH4+ after extraction with 1 mol L-1 KCl, soil microbial biomass nitrogen (MBN) by the fumigation and extraction method (Brookes, 1985), microbiological activity by soil basal respiration (Alef and Nannipiere, 1995), and pH (Hanna pH meter).

2.3 Statistical analysis

Statistical analysis of the data was conducted using SISVAR software (Ferreira, 2000), applying the Tukey test (p-value <0.05).

3 RESULTS AND DISCUSSION

Table 2 presents the characteristics of the sewage influent to the VFCW and VFSF-CTR experimental units. The concentration of KTN (Norg and N-NH4+) is 44.87 mg L-1, including 31.62 mg L-1 of N-NH4+ (90.5%). As mentioned by Holzschuh et al. (2011), the primary source of nitrogen for rice cultivation is N-NH4+, which facilitates immediate removal through the absorption process.

Table 2 - Characteristics of sewage influent in the experimental units during the two rice cycles (BOD: biochemical oxygen demand; KTN: total Kjeldahl nitrogen and pH)

Parameter	**1st rice cycle**				**2nd rice cycle**			
	Min	**Mean**	**Max**	**Standard deviation**	**Min**	**Mean**	**Max**	**Standard deviation**
BOD (mg L^{-1})	113.00	**150.45**	199.00	30.30	87.00	**152.00**	188.00	35.21
KTN (mg L^{-1})	31.11	**44.87**	52.30	6.62	-	**-**	-	-
$N\text{-}NH_4^+$ (mg L^{-1})	17.30	**31.62**	45.70	7.82	34.80	**39.72**	44.20	3.62
$N\text{-}NO_3^-$ (mg L^{-1})	0.00	**0.19**	0.36	0.16	0.00	**0.00**	0.00	0.00
pH	6.81	**7.13**	7.40	0.20	7.17	**7.29**	7.38	0.08

Source: created by the authors.

3.1 KTN concentrations and removal

Figure 2 presents the average KTN concentrations in the influent (dots) and effluent (bars), as well as the percentage efficiency of KTN removal in the two treatments (VFCW and VFSF-CTR) during the first rice cycle, considering the different HLRs.

By comparing the percentage efficiency of KTN removal in the two treatments in Figure 2, it is evident that the average KTN removal efficiency ranged from 93-94% for VFCW and 88-92% for VFSF-CTR, indicating slightly lower performance for the latter.

There was no significant difference in KTN removal for VFCW between operations with HLRs of 15 cm d^{-1} and 4 cm d^{-1} (both labeled 'b'), demonstrating that VFCW has flexible operation. However, KTN removal was higher at 15.0 cm d^{-1} (indicated by a lower bar).

For VFSF-CTR, KTN removal was similar across all three HLRs (all labeled 'a') and showed lower efficiency compared to VFCW. However, there was a significant difference in KTN removal between VFCW and VFSF-CTR at an HLR of 15 cm d^{-1} (labeled 'A' and 'B'). The VFSF-CTR performance was still satisfactory (88-92%) due to the presence of silt and clay in the support medium (Table 1), which contributed to greater accumulation of KTN in the soil. As noted by Cui et al. (2012), finer soil porosity enhances pollutant removal compared to larger soil porosity.

From Table 3, the following observations regarding KTN accumulation in soil can be made:

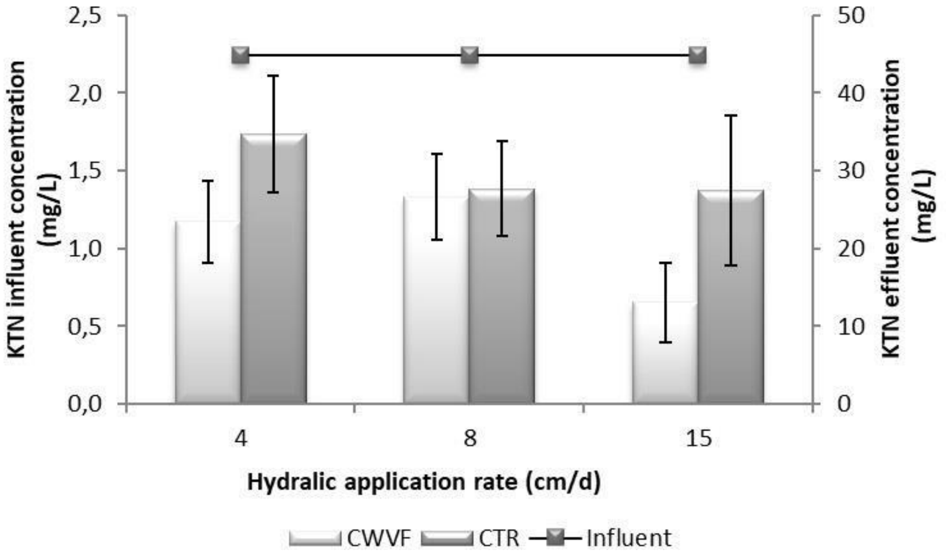
i) There is a significant increase in both soil layers, for both treatments, and across both rice cycles, when the HLR is increased. However, the VFCW operated at an HLR of 15 cm d^{-1} accumulated significantly greater amounts of KTN compared to smaller HLRs (4 and 8 cm d^{-1}).

ii) KTN accumulation is significantly higher in the VFCW than in the VFSF-CTR in the first rice cycle, in the 0-5 cm soil layer, for all HLRs. This is likely due to biological nitrogen fixation (Chi *et al*., 2005), the exudation of organic compounds released by living roots (Coskun *et al*., 2017), and enhanced soil microbial activity (Silva; Bernardes; Ramos, 2015).

iii) There is no significant difference between VFCW and VFSF-CTR among the soil layers during the second rice cycle, despite the VFCW higher amounts of KTN. This may be due to the reduced influence of the plant on accumulation, likely because cutting the rice plant for regrowth reduces BNF and the exudation of organic compounds by the roots, as the BRS-GO Guará rice species does not develop well under this type of management (oral information from an EMBRAPA researcher).

iv) There is greater KTN accumulation in the 0-5 cm layer of both VFCW and VFSF-CTR, which is consistent with findings by Mander *et al*. (2008) who observed lower accumulation in deeper soil layers (30-40 cm and 40-60 cm).

Figure 2 - Average KTN concentrations and removal efficiencies (%) during the first rice cycle for VFCW and VFSF-CTR systems at different HLRs



Source: created by the authors.

Note: Using the Tukey-test (p-Value<0.05), uppercase letters compare the two treatments for the same HLR, and the lowercase letters compare the different HLRs within the same treatment.

From Table 3, the following observations regarding KTN accumulation in soil can be made:

v) There is a significant increase in both soil layers, for both treatments, and across both rice cycles, when the HLR is increased. However, the VFCW operated at an HLR of 15 cm d^{-1} accumulated significantly greater amounts of KTN compared to smaller HLRs (4 and 8 cm d^{-1}).

vi) KTN accumulation is significantly higher in the VFCW than in the VFSF-CTR in the first rice cycle, in the 0-5 cm soil layer, for all HLRs. This is likely due to biological nitrogen fixation (Chi *et al*., 2005), the exudation of organic compounds released by living roots (Coskun *et al*., 2017), and enhanced soil microbial activity (Silva; Bernardes; Ramos, 2015).

vii) There is no significant difference between VFCW and VFSF-CTR among the soil layers during the second rice cycle, despite the VFCW higher amounts of KTN. This may be due to the reduced influence of the plant on accumulation, likely because cutting the rice plant for regrowth reduces BNF and the exudation of organic compounds by the roots, as the BRS-GO Guará rice species does not develop well under this type of management (oral information from an EMBRAPA researcher).

viii) There is greater KTN accumulation in the 0-5 cm layer of both VFCW and VFSF-CTR, which is consistent with findings by Mander *et al*. (2008) who observed lower accumulation in deeper soil layers (30-40 cm and 40-60 cm).

Table 3 - KTN accumulated in the soil layers (0-5 and 5-20 cm) of VFCW and VFSF-CTR systems at the end of the rice cycles

HLR (cm d^{-1})	**KTN accumulation in soil (mg N kg$_{soil}^{-1}$)**	
	VFCW	**VFSF-CTR**
	1st rice cycle (0-5 cm)	
4	366.17 aC	275.86 bC
8	479.06 aB	396.28 bB
15	584.43 aA	448.96 bA
	2nd rice cycle (0-5 cm)	
4	531.75 aB	381.23 aB
8	667.21 aB	531.75 aB
15	885.47 aA	727.42 aA
	2nd rice cycle (5-20 cm)	
4	358.65 aB	238.23 aB
8	569.38 aA	418.86 aA
15	569.38 aA	441.43 aA
Before sewage application		**340.00**

Source: created by the authors.

Note: Using the Tukey-test (p-Value<0.05), uppercase letters compare the two treatments for the same HLR, and the lowercase letters compare the different HLRs within the same treatment.

Indeed, the presence of rice cultivation in the VFCW contributed to a higher accumulation of KTN in the soils. Godoy et al. (2015) reported KTN accumulation 3.2 times higher than the amounts observed in this study after three years of cultivation. They noted 1.90 g kg-1 in the 0-10 cm layer of a Red Acrisol cultivated with rice using traditional fertilizer. It is worth noting that in this study, the sewage application lasted only ten months, and the soil layer thickness was 0-5 cm of mixed RYL soil. Thus, the mixed RYL soil demonstrated rapid fertilization in the short term.

Mander et al. (2008) studied a constructed wetland with horizontal flow (CWHF) using a sand support medium cultivated with Typha latifolia and observed that after three years of operation, only 245 mg N kgsoil-1 accumulated in the 0-10 cm layer. In contrast, the present study showed higher KTN accumulation (Table 3), probably due to the influence of sewage characteristics, support medium, cultivar species, and type of flow.

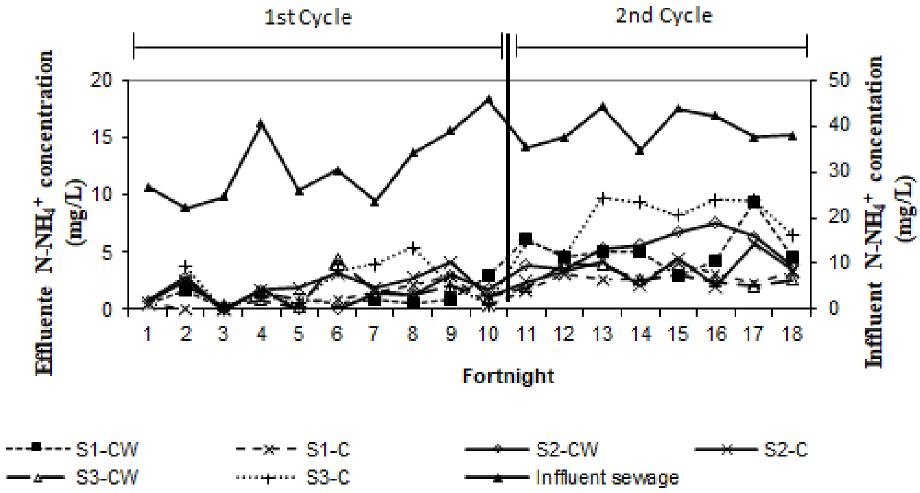
3.3 Ammonium removal

The variability of N-NH4+ concentrations in the effluents of the VFCW and VFSF-CTR was lower during the first rice cycle but increased over time, reaching the largest variations during the second rice cycle (Figure 3). However, for the VFCW with an HLR of 15 cm d-1, the overall variation was lower.

In the second rice cycle, the data from Table 5 and Table 6 indicate a larger drop in pH values, which helps explain the reduction in N-NH4+ removal efficiencies for both VFCW and VFSF-CTR. Data in Table 7 draw attention to the increase in cation exchange capacity (CEC) which is due to the adsorption of higher-valence cations, contributing to the reduction in N-NH4+ removal efficiencies. Additionally, in the VFCW, cutting the rice for regrowth reduced the uptake of N-NH4+ by the plants.

In the second rice cycle, the data from Table 5 and Table 6 indicate a larger drop in pH values, which helps explain the reduction in N-NH4+ removal efficiencies for both VFCW and VFSF-CTR. Data in Table 7 draw attention to the increase in cation exchange capacity (CEC) which is due to the adsorption of higher-valence cations, contributing to the reduction in N-NH4+ removal efficiencies. Additionally, in the VFCW, cutting the rice for regrowth reduced the uptake of N-NH4+ by the plants.

Figure 3 - N-NH$_4^+$ concentrations in influents and effluents for the VFCW and VFSF-CTR systems



Source: created by the authors.

Table 4 - N-NH$_4^+$ average concentrations and average removal efficiency in influents and effluents, for the VFCW and VFSF-CTR systems during the two rice cycles

HLR (cm d^{-1})	1st cycle				2nd cycle			
	VFCW		VFSF-CTR		VFCW		VFSF-CTR	
	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}	%
4	1.48 ± 1.73	**95.32**	2.35 ± 1.63	**88.80**	5.11 ± 2.04	**87.13**	3.04 ± 0.55	**92.35**
8	1.54 ± 1.30	**95.12**	2.01 ± 1.18	**93.64**	5.57 ± 1.49	**85.98**	3.63 ± 1.29	**90.86**
15	1.56 ± 1.19	**95.06**	2.67 ± 2.14	**91.55**	2.96 ± 0.75	**92.55**	8.23 ± 1.91	**79.28**
Influent (mg L^{-1})	**31.62±7.82**				**39.72±3.62**			

Source: created by the authors.

Table 5 - Average pH values in the soil layers (0-5 and 5-20 cm) for VFCW and VFSF-CTR systems during the two rice cycles

HLR (cm d^{-1})	1st rice cycle		2nd rice cycle			
	0-5cm		0-5cm		5-20cm	
	VFCW	VFSF-CTR	VFCW	VFSF-CTR	VFCW	VFSF-CTR
4	4.12	3.64	3.77	3.71	3.70	3.75
8	4.15	3.79	3.99	3.69	3.65	3.66
15	4.39	3.87	3.87	4.09	3.66	3.74
Before sewage application			**5.30**			

Source: created by the authors.

Table 6. Average pH values of influents and effluents for VFCW and VFSF-CTR systems during the two rice cycles.

HLR (cm d^{-1})	1st rice cycle		2nd rice cycle	
	VFCW	VFSF-CTR	VFCW	VFSF-CTR
		pH effluent		
4	5.08 ± 0.47	4.92 ± 0.42	4.52 ± 0.28	4.69 ± 0.32
8	4.88 ± 0.54	4.27 ± 0.58	4.49 ± 0.25	3.77 ± 0.05
15	4.88 ± 0.71	4.92 ± 0.52	3.82 ± 0.10	4.90 ± 0.31
pH influent	**7.13 ± 0.20**		**7.29 ± 0.08**	

Source: created by the authors.

Table 7 - Cation Exchange Capacity (CEC) in the soil layers (0-5 and 5-20 cm) for VFCW and VFSF-CTR systems during the two rice cycles

HLR (cm d^{-1})	CEC (cmol dm^{-3})					
	1st rice cycle		2nd rice cycle			
	0-5 cm		0-5 cm		5-20 cm	
	VFCW	VFSF-CTR	VFCW	VFSF-CTR	VFCW	VFSF-CTR
4	3.27	3.49	4.37	4.58	4.12	4.28
8	3.87	4.09	4.92	5.22	4.66	4.37
15	3.92	3.56	4.92	4.26	4.58	4.21
Soil CEC before sewage application (cmol dm^{-3})				**3.92**		

Source: created by the authors.

Regarding the concentration of mineralized nitrogen (N-NH4+ + N-NO3-) in the soil and nitrogen accumulation, the following observations can be highlighted:

I. At the end of the first rice cycle, the amounts of N-NH4+ in the 0-5 cm layer of both treatments (VFCW and VFSF-CTR) were similar, regardless of HLR (indicated by letters aA in the 1st and

4th columns). The amount of N-NO3- was significantly higher in VFCW and directly proportional to HLR, which contributed to significantly higher amounts of mineral nitrogen (N-NH4+ and N-NO3-)

II. At the end of the second rice cycle, in the 0-5 cm layers, the significant increase of N-NH4+ in VFCW contributed to significantly higher amounts of mineral nitrogen (N-NH4+ and N-NO3-) compared to VFSF-CTR, demonstrating a substantial impact from the rice crop.

III. At the end of the second rice cycle, in the 5-20 cm layers, the contributions of N-NH4+ and N-NO3- varied for both VFCW and VFSF-CTR. The amounts of mineral nitrogen (N-NH4+ + and N-NO3-) were similar (except for HLR=15 cm d-1) for the two HLRs and both treatments, with higher accumulation of mineral nitrogen.

The accumulation of mineral N (N-NH4+ and N-NO3-) in both rice cycles, in the 0-5 cm layers (Table 8), is attributed to the plant's contribution to rapid nitrogen cycling, higher microbial activity (Silva; Bernardes; Ramos, 2015), and the presence of rhizosphere microorganisms (Boer et al., 2007). In this study, soil aeration facilitated by intermittent sewage application, an unsaturated operation regime, and oxygen transfer by the rice crop aerenchyma (VFCW system) enhanced nitrification. In Table 9, Comparing the average DO values from the influent (0.11 mg L-1) with the effluent values (1.34-1.52 mg L-1) demonstrates that soil oxygenation was sufficient to support the growth of nitrifying bacteria. Furthermore, the small difference between the DO concentrations in VFCW and VFSF-CTR emphasizes the predominant effect of intermittent sewage application on soil oxygenation.

3.4 N-NO$_3^-$ leaching into effluents

Figure 4 shows the N-NO$_3^-$ concentrations influents and effluents from the VFCW and VFSF-CTR systems, measured fortnightly over ten months, for the two rice cycles and the three HLR (4.0; 8.0; and 15.0 cm/d).

Table 8 - Concentration of N mineralized in the soil layers (0-5 and 5-20 cm) for VFCW and VFSF-CTR systems during the two rice cycles

HLR (cm d^{-1})	N mineralized (mg N/kg of soil)					
	1st cycle (0-5 cm)					
	VFCW			VFSF-CTR		
	N-NH$_4^+$	N-NO$_3^-$	Total (N-NH$_4^+$ + N-NO$_3^-$)	*N-NH$_4^+$*	N-NO$_3^-$	Total (N-NH$_4^+$ + N-NO$_3^-$)
	1st cycle (0-5 cm)					
4	11.96 aA	32.68 aC	44.64 aB	9.97 aA	14.58 bB	24.56 bB
8	14.53 aA	42.06 aB	57.02 aC	10.68 aA	22.06 bA	32.74 bC
15	12.60 aA	64.14 aA	76.75 aA	11.37 aA	26.63 bA	38.00 bA
	2nd cycle (0-5 cm)					
4	18.54 aC	31.66 aA	50.19 aC	9.90 bA	17.56 bB	27.46 bB
8	21.42 aB	32.78 aA	66.26 aB	18.54 aB	25.03 aB	44.65 bC
15	40.14 aA	44.84 aA	72.91 aA	19.61 bB	42.32 aA	60.86 bA
	2nd cycle (5-20 cm)					
4	5.57 bA	26.27 aB	31.85 aB	1.25 aC	30.59 bA	31.85 aB
8	9.90 aA	21.95 aB	31.85 aB	9.90 aB	21.95 aC	31.85 aB
15	9.90 aA	34.75 bA	44.64 aA	22.88 bA	18.00 aB	41.14 aA

Source: created by the authors.

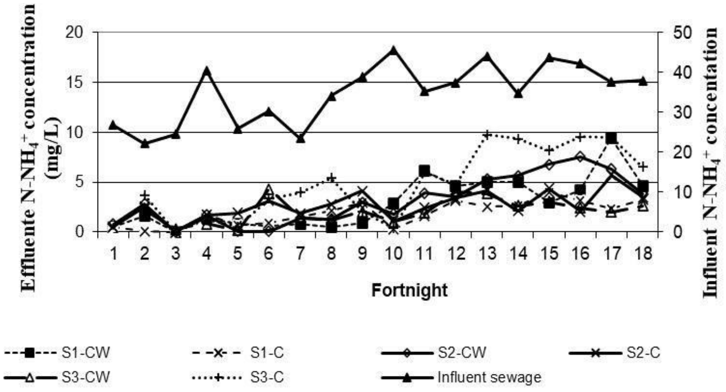
Note: Using the Tukey test (p-Value<0.05), lowercase letters in the rows compare the two treatments (VFCW e VFSF-CTR), and the uppercase letters in the columns compare the HLR.

Table 9 - Average dissolved oxygen values in influents and effluents for VFCW and VFSF-CTR systems during the two rice cycles

HLR (cm d^{-1})	1st cycle		2nd cycle	
	VFCW	CTR	VFCW	CTR
Influent DO (mg L^{-1})	0.11 ± 0.02		0.11 ± 0.02	
Effluent Average DO (mg L^{-1})				
4	1.50 ± 0.16	1.44 ± 0.18	1.52 ± 0.14	1.34 ± 0.19
8	1.51 ± 0.08	1.51 ± 0.18	1.47 ± 0.17	1.43 ± 0.19
15	1.46 ± 0.15	1.43 ± 0.13	1.47 ± 0.12	1.36 ± 0.16

Source: created by the authors.

Figure 4 - N-NO_3^- concentrations in influents and effluents for the VFCW and VFSF-CTR



Source: created by the authors.

In the first cycle, during the first month (two fortnightly measurements), there was increased nitrate leaching (N-NO3-) in the effluents of both VFCW and VFSF-CTR. In the second cycle, the reduction in N-NO3- leaching was attributed to a slight increase in soil acidity (Table 5), which favored its retention in the soil through adsorption. To enhance N-NO3- leaching from the soil and thereby nitrify the effluent for reuse in irrigation, it is necessary to raise the soil pH.

From Table 10, comparing the average N-NO3- concentrations in the influent (0.00 - 0.19 mg L-1) with the effluent values (0.02 - 0.48 mg L-1), it is evident that there was a slight increase in the N-NO3- concentrations in the effluent from both treatments (VFCW and VFSF-CTR), due to the leaching of N-NO3- accumulated in the soil (Table 8). For both rice cycles and both treatments, all N-NO3- effluent concentrations remained below 10 mg L-1, complying with the limits for water bodies classified as Classes 1, 2, and 3 (Brasil, 2005). Additionally, the N-NO3- concentrations in the effluent did not exceed the superior threshold value for irrigation of 5 mg L-1 (Almeida, 2010).

3.5 N immobilized in the microbial biomass

The main observations arising from Table 11, related to MBN immobilization in soil, are:

i) For the 0-5 cm layer in both rice cycles, the MBN was similar for VFCW and VFSF-CTR, with immobilization significantly higher at 15 cm d-1 when comparing the HLRs.

ii) In the second rice cycle, in the 5-20 cm layer, MBN immobilization was similar across all HLRs and both treatments. For VFCW, MBN immobilization was significantly higher at HLRs of 8 and 15 cm d-1.

In comparison with previous studies, the values (27.91 and 22.3 mg N/kg soil) for the second rice cycle, 0-5 cm layer, at 15 cm d-1, measured over nine months, were about half of the average obtained by Wu et al. (2017).

These authors reported a value of 46 mg N/kg, equivalent to 3.60% of soil KTN, for soil fertilized with NPK in the 0-20 cm layer under rice cultivation over 20 years. This indicates that, in a shorter duration of wastewater application, the results obtained in this study were very promising for improving soil quality in a short period. Thus, in soils receiving sewage, MBN immobilization can surpass that in traditionally cultivated soils. Additionally, from Table 11, the percentage of MBN relative to KTN (3.15 and 3.07%) falls within the recommended range (1-5%) by Moreira and Siqueira (2002), indicating improved soil quality (Coser et al., 2016). The higher the percentage of MBN relative to KTN, the greater the improvement in soil organic matter quality (Yang et al., 2010).

Table 10 - Average N-NO_3^- concentrations in influents and effluents for VFCW and VFSF-CTR during the rice cycles

HLR (cm d^{-1})	**1st cycle**				**2nd cycle**			
	VFCW		**CTR**		**VFCW**		**CTR**	
	mg L^{-1}	**% NIT**	**mg L^{-1}**	**% NIT**	**mg L^{-1}**	**% NIT**	**mg L^{-1}**	**% NIT**
Effluent N-NO_3^- concentrations								
4	0.27 ± 0.54	**42.11**	0.29 ± 0.44	**52.63**	0.04 ± 0.02	**4.00**	0.36 ± 0.55	**36.00**
8	0.41 ± 0.87	**115.79**	0.37 ± 0.76	**94.74**	0.14 ± 0.15	**14.00**	0.06 ± 0.05	**6.00**
15	0.28 ± 0.66	**47.37**	0.48 ± 1.15	**152.63**	0.02 ± 0.02	**2.00**	0.06 ± 0.07	**6.00**
Influente (mg L^{-1})	**0.19±0.16**				**0.00±0.00**			

Source: created by the authors.

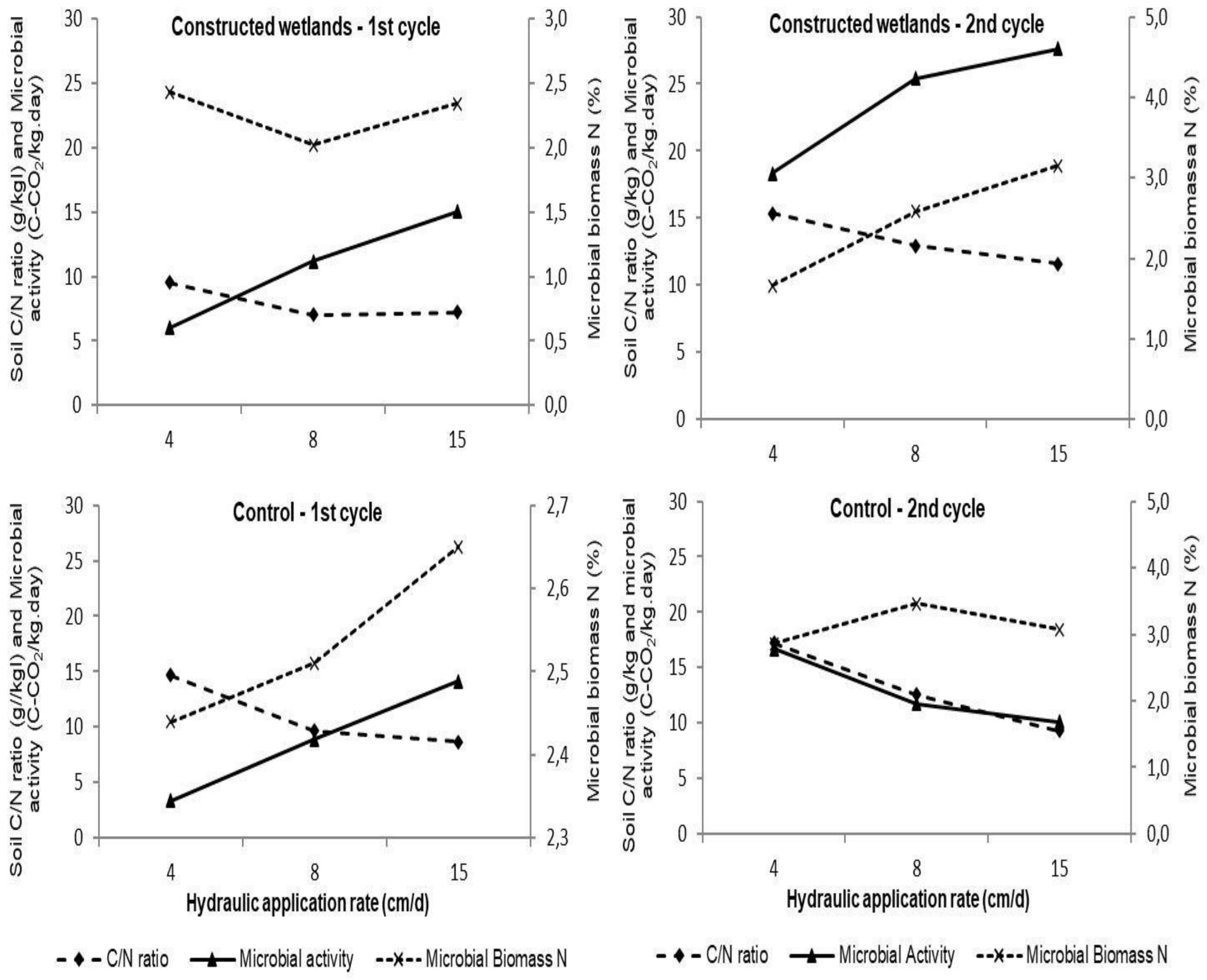
Table 11 - MBN in the soil layers (0-5 and 5-20 cm) of the VFCW and VFSF-CTR, at the end of the two rice cycles

(cm d^{-1})	**MBN (mg N/kg soil)**			
	1st rice cycle (0-5 cm)			
	VFCW	**% of soil KTN**	**VFSF-CTR**	**% of soil KTN**
4	8.89 aB	**2.43**	6.72 aB	**2.44**
8	9.70 aB	**2.02**	6.00 aB	**1.51**
15	13.66 aA	**2.34**	11.89 aA	**2.65**
	2nd rice cycle (0-5 cm)			
4	8.84 aB	**1.66**	10.92 aB	**2.86**
8	17.20 aB	**2.58**	18.38 aA	**3.46**
15	27.91 aA	**3.15**	22.30 aA	**3.07**
	2nd rice cycle (5-20 cm)			
4	9.38 aA	**2.62**	7.73 aA	**3.24**
8	14.32 aA	**2.52**	4.96 bA	**1.18**
15	13.39 aA	**2.35**	6.65 bA	**1.51**

Source: created by the authors.

Note: Using the Tukey test (p-Value<0.05), lowercase letters in the rows compare the two treatments (**VFCW e VFSF-CTR)** and the uppercase letters in the columns compare the HLR.

Figure 5 - C/N ratio, microbial activity, and N immobilization in the soil layer (0–5 cm) for VFCW and VFSF-CTR during the two rice cycles, at HLR of 4.0, 8.0, and 15.0 cm d^{-1}



Source: created by the authors.

Figure 5 shows correlation between the C/N ratio and microbial activity with MBN immobilization. The C/N ratio decreased with the increase in HLR. This likely occurred due to the increase of nitrogen in the soil as the HLR increased. As for microbial activity, it increased with HLR in the VFCW for both cycles and increased for VFSF-CTR only in the 1st cycle. The decrease in microbial activity for VFSF-CTR in the 2nd cycle occurs because this system, without rice crops, cannot handle the excess organic load with the increase in HLR.

Regarding MBN immobilization, Figure 5 shows the following aspects: i) the MBN slightly decreases with the increase in HLR, probably due to the rice crop competing for nitrogen in the first cycle; ii) The VFSF-CTR (1st cycle) exhibited the same behavior as VFCW (2nd cycle) because there was no rice growth after cutting. However, VFCW had higher microbial activity and greater MBN immobilization; iii) For the VFSF-CTR (2nd cycle), the behavior was different; MBN immobilization slightly increased, while both the C/N ratio and microbial activity decreased. In summary, the increase in HLR is beneficial for the VFCW system, as it enhances microbial activity, promotes organic matter degradation, and improves MBN immobilization, thereby enhancing the soil's nutritional quality. The dynamics of MBN immobilization for soils receiving chemical fertilizers are different because immobilization is directly proportional to the C/N ratio and inversely proportional to the decomposition rate of organic matter.

3.6 Production of rice grain and vegetable mass

The amounts, by weight, of rice grain and vegetative mass produced in the VFCW (reduced experimental scale, cross-sectional area of 0.26 m², for one cycle, or a 5-month experiment) and the estimated production considering an area of 1 hectare (ha), and a one-year time frame, are presented in Table 12.

Table 12 - Rice grain and vegetable mass productivity in the VFCW (reduced experimental scale) and in the estimated area of 1 hectare

HLR (cm d^{-1})	Productivity for VFCW (g 0.26 m^{-2} per cycle)			Productivity Estimate (t ha^{-1} year^{-1})		
	Grains in husk	Leaves	Leaves+panicles+ Grains husk	Grains in husk	Leaves	Leaves+panicles+ Grains husk
4	181.16 B	126.90 B	188.59 B	6.97	4.88	7.25
8	233.98 A	184.91 A	250.55 A	8.99	7.11	9.64
15	254.55 A	202.56 A	273.51 A	9.79	7.79	10.52

Source: created by the authors

Note: Using the Tukey test (p-Value<0.05), uppercase letters in the columns compare the HLRs.

From Table 12, it is evident that a significant increase in rice grain and leaf production occurred only when the HLR was doubled from 4.0 to 8.0 cm d- 1 (p-Value < 0.01, different uppercase letters). There was no significant difference in production between systems operated with HLRs of 8 and 15 cm d-1. Therefore, higher HLRs do not significantly impact rice crop productivity. Based on the estimated rice crop productivity (Table 12), the results are approximately 1.15 to 1.8 times higher than the average rice harvest in Brazil (years 2019/2020) of 6.27 t ha^{-1} year^{-1} (Brasil, 2019b). The estimated rice productivity from this study could meet the annual demand of 214 to 309 individuals, considering the per capita rice consumption in Brazil is 34 kg/year (Planeta Arroz, 2021). Given the significant productivity of rice grain in husk (Table 12), the rice husk could be reused as an adsorbent for clarifying residual frying oil used in biodiesel production (Santos; Junger; Soares, 2014).

4 CONCLUSIONS

The use of soil (modified YRL) as a support medium, with rice crops (VFCW) or without (VFSF-CTR), presented the following benefits: (i) efficiently removed nitrogen from primary domestic sewage, achieving tertiary treatment levels in a single stage; (ii) adsorbed and accumulated nearly all the nitrogen, thereby preventing atmospheric losses; (iii) produced effluent of acceptable quality for reuse in irrigation of new crops and for discharge into lotic (rivers) or lentic (lakes) environments.

The use of rice crops demonstrated additional benefits: (i) achieved higher efficiencies in total Kjeldahl nitrogen (KTN) removal, independent of HLR; (ii) exhibited lower variation in nitrogen removal efficiencies at higher HLR (15.0 cm d-1); (iii) facilitated higher accumulation of nitrogen in mineral, organic, and microbial forms, initiating the fertilization process and improving soil quality; (iv) produced substantial grain and vegetative mass, contributing to food and feed supply; (v) provided greater operational flexibility, accommodating both low and high hydraulic loads.

Nitrogen removal in both VFCW and VFSF-CTR systems was predominantly based on retention and adsorption, primarily in the 0–5 cm soil layer. Microbial biomass nitrogen (MBN) removal by microbial activity and nitrogen uptake by the rice plants occurred efficiently. This nitrogen recovery process prevented nitrogen losses to the atmosphere, mitigating contributions to the greenhouse effect.

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