Research article

Phytoremediation as a Sustainable Alternative for Organic Matter Removal From Slaughterhouse Wastewater Pretreated by Immediate One-Step Lime Precipitation

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Abstract.
In this study, slaughterhouse wastewater previously treated by immediate one-step lime precipitation was treated using a vertical flow constructed wetland (VFCW). A VFCW pilot scale planted with *Vetiveria zizanioides* in light expanded clay aggregates was used to study the influence of the organic load applied (3 to 212 g m^{-2} d^{-1}) and the bed depth of the VFCW (0.35 and 0.70 m) on the organic matter removal (COD). Two VFCWs operated in parallel under continuous flow, and a hydraulic load of around 80 L m^{-2} d^{-1} was used. The results indicated that an increase in the organic load decreased COD removal efficiency. The bed depth of the VFCW had a significant influence on the removal of organic matter, with greater removal at high bed depths. For organic loads applied up to 9.5 g m^{-2} d^{-1}, COD removal efficiencies of 71.4 ± 4.0% and 85.2 ± 3.4% were observed for lower and higher VFCW bed depth, respectively, which met the requirements for water reuse for irrigation. Throughout the tests, *Vetiveria zizanioides* did not show signs of toxicity, and its growth was substantial.

Keywords: slaughterhouse wastewater, vertical flow constructed wetlands, *Vetiveria zizanioides,* organic matter removal, immediate one-step lime precipitation

1. Introduction

The wastewater reuse has been strongly driven by the European Commission and also by the United Nations, according to the Sustainable Development Goals (SDGs), as a need to address global water scarcity [1]. Slaughterhouse is one of the sectors in which the treated wastewater recover is practically non-existent, since most industries look at effluent as a waste, choosing to discharge effluent into the municipal collection system or a water line [2]. Depending on the company's productivity, large amounts of slaughterhouse wastewater are expected due to slaughtering process and cleaning

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of facilities [3]. These volumes of water could be used for other purposes (e.g., for irrigation). Thus, slaughterhouse should be encouraged for wastewater recover.

Slaughterhouse wastewater (SWW) recover has proved to be a challenge for researchers due to its high organic content (e.g., 5000 and 3000 mg O_2 L^{-1} for chemical oxygen demand (COD) and biochemical oxygen demand (BOD), respectively), nutrients (e.g., 450 and 50 mg L^{-1} for total nitrogen and total phosphorus, respectively), oils and fats, suspended solids (e.g., 3000 mg L^{-1}) and bacterial contamination [3]. Currently, conventional wastewater treatments processes have been used to treat SWW. Despite the high efficacy, these treatment processes have some drawbacks. For example, the coagulation-flocculation process produces sludges rich in Fe^{3+} or Al^{3+} salts, which are difficult to apply on agricultural land and their disposal is landfill [4], presenting a cost to the company. Activated sludges have considerable energy consumption and are poorly tolerant to load and flow variations [5]. In this way, different SWW treatment processes have been investigated, namely chemical (e.g., electrochemical oxidation [6], acid precipitation [7] and lime precipitation [8]) and biological treatments (e.g., wetlands [9]), advanced oxidation processes [10] and combined processes [10].

Lime precipitation and wetlands are low-cost treatment processes. High organic matter removals (ca. 93%) with lime precipitation process have been reported by some authors [8]. However, it is not enough to guarantee legal disposal specifications (e.g., discharge into environment or reuse for irrigation). Constructed wetland (CW) has emerged as a sustainable alternative in the treatment of various types of effluents, including those previously treated by immediate one-step lime precipitation (e.g., explosives [11]), compared to conventional processes, due to its low cost, low maintenance and high efficiency in removing contaminants.

The effectiveness of CW in the treatment of slaughterhouse effluents has been investigated by some authors. Mburu et al. [12] studied the effect of depth, time and substrate variation on organic matter removal from slaughterhouse wastewater, using vertical sub-surface flow constructed wetlands (without plants). These authors observed that vertical sub-surface flow constructed wetlands operated at a longer retention time of 5 days, with intermittent wastewater feeding, facilitated over 50% removal of organic matter in slaughterhouse wastewater. High organic matter removals (>70%) were achieved using constructed wetland planted with *C. papyrus* and *P. mauritianus*, in the slaughterhouse wastewater treatment [9]. Manh et al. [13] used constructed wetlands planted *Vetiveria zizanioides L* to treat slaughterhouse wastewater from a biodigester. The results showed that the concentrations of BOD_5 and COD can be reduced by 59.8% and 60%, respectively. Recently, the interest in *Vetiveria zizanioides* plant in constructed wetlands to treat effluents has increased [11,14], however, studies of its use in slaughterhouse wastewater treatment are still few.

Thus, this work aims to understand the effect of the organic load applied and the bed depth from vertical flow constructed wetland (VFCW) planted with *Vetiveria zizanioides* on the organic matter removal from a slaughterhouse wastewater pretreated by immediate one-step lime precipitation process.

2. Material and methods

SWW was collected from a slaughterhouse (with a production capacity of 700 m^3/day of wastewater) located in Portugal. This SWW include effluents from the slaughtering process (cattle, pigs, goats, sheep and ratite) and the cleaning facilities. SWW composition was pH 7.2±0.2, conductivity 3.1±0.3 mS cm^{-1}, COD 5430±1646 mg O_2 L^{-1}, BOD_5 2813±208 mg O_2 L^{-1} and ammonium nitrogen (NH_4^+) 88±1 mg N-NH_4^+ L^{-1}. Before constructed wetland treatment, SWW was previously pretreated by immediate one-step lime precipitation process, according to Madeira et al. [15].

From February to April, two VFCW (Figure 1), VFCW 1 (0.40m x 0.60m x 0.35m) and VFCW 2 (0.40m x 0.60m x 0.70m), planted with *Vetiveria zizanioides* in light expanded clay aggregates (Leca®NR 10/20), were used in continuous mode. Table 1 shows the applied operation to the VFCW. For each VFCW, three types of SWW were tested (trials A to C in Table 1). Hydraulic retention times used were 3.6±0.5 and 7.1±0.9 hours, for VFCW 1 and VFCW 2, respectively.

During this experiment, wastewater samples were collected daily from the inlet and outlet of the bed. The influent and effluent of both VFCW were analysed to pH, dissolved oxygen (DO), redox potential, electrical conductivity, ammonium nitrogen, COD, and flow rate. Physical-chemical parameters were analysed according to the standard analytical methods defined by Baird et al. [16]. Air and bed temperature were also monitored.

Vetiveria zizaniodes plants were visually inspected on a weekly basis for toxicity signals. The biomass composition was analysed at the beginning and at the end of the experiments, in terms of calcium, magnesium, sodium, potassium, total nitrogen and phosphorus [17-19].

Two-Way ANOVA was used to compare the means, using the Least Significance Difference (LSD) test, at 95% confidence level.

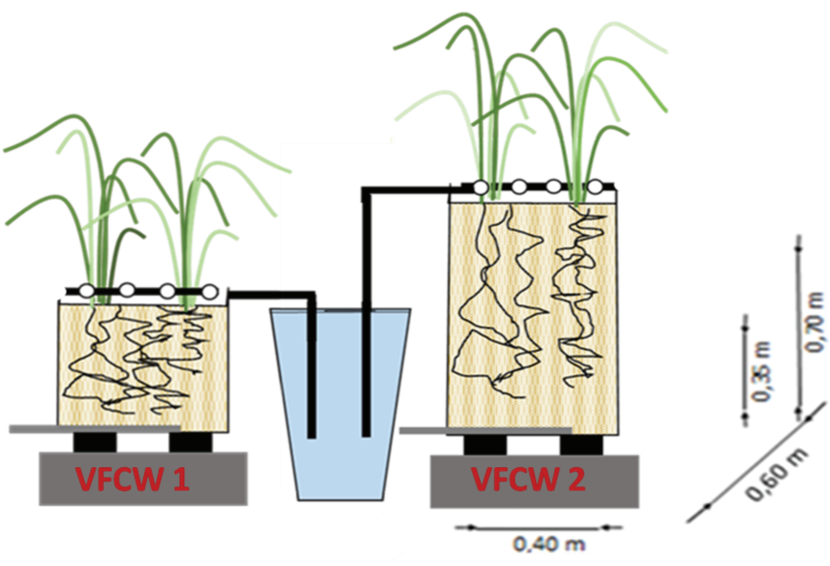

Figure 1: Schematic representation of the VFCW.

Table 1: Conditions applied to VFCW 1 and VFCW 2. Mean ± Standard Deviation, calculated for a 95% confidence level and number of determinations (n≥10).

		VFCW 1			VFCW 2		
Parameters / Trials		A1	B1	C1	A2	B2	C2
COD	($g\ m^{-2}\ d^{-1}$)	4.1±0.5	9.5±2.2	211.8±26.4	3.2±1.1	9.4±2.5	211.8±26.4
NH_4^+-N load	($g\ m^{-2}\ d^{-1}$)	0.3±0.1	0.5±0.05	3.9±0.5	0.3±0.1	0.5±0.06	3.9±0.5
Hydraulic load	($L\ m^{-2}\ d^{-1}$)	81±10			80±10		

3. Results and discussion

Figure ?? shows the inlet and outlet results for pH, redox potential, conductivity and DO, in VFCW 1 and VFCW 2 for the three trials.

Fig. 2. pH, redox potential, conductivity and DO results, in inlet and outlet of VFCW 1 (a, b, c and d, respectively) and VFCW 2 (e, f, g and h, respectively), for the different trials (A, B and C). Box and whisker plots showing median, 25th and 75th percentile (box), and range (whiskers).

No significant difference ($p<0.05$) between VFCW and inlet and outlet were observed for pH (Figure ?? a and e), indicating that the wastewater had sufficient alkalinity to act as a buffer. The same behavior was observed for redox potential (Figure ?? b and f), conductivity (Figure ?? c and g) and DO (Figure ?? d and h), without significant differences ($p<0.05$). However, an increase in COD load from 9.5 to 211 g m^{-2} d^{-1}

and ammonium nitrogen load from 0.5 to 3.9 g m^{-2} d^{-1} (trials B to C), provided a significant oxygen consumption ($p<0.05$), reaching zero values of DO in the effluent of both VFCWs (Figure ?? d and h). Dissolved oxygen consumption is mainly due to aerobic degradation of organic matter rather than nitrification.

Figure ?? shows the COD and ammonium nitrogen removal efficiencies obtained with different organic loads in the two VFCW, VFCW 1 (Figure ??a and 3b) and VFCW 2 (Figure ??c and 3d). This figure shows that the highest COD removal efficiencies were obtained with the smallest COD loads applied, in both VFCW. When the COD load was 211.8 g m^{-2} d^{-1}, COD removal did not occur in VFCW 1, and decreased a lot in VFCW 2, reaching values between 60 and 70%. In fact, an increase in the depth of the bed allows for a greater distribution of plant roots and, consequently, a greater distribution of microorganisms in the bed, contributing to a greater removal of organic matter.

Ammonium nitrogen removal efficiency was highest for the lower loads (0.3±0.1 g m^{-2} d^{-1}) and occurred in presence of organic matter and with low dissolved oxygen, reaching a maximum value of 86% for both VFCWs (Figure ??b) and d)). However, this value decreased to 58 % (trial C) in VFCW 2 when a maximum load removed is reached, since this occurred with very low dissolved oxygen. Like COD, deeper VFCW had greater removal of ammonium nitrogen. Almeida et al. [20] also obtained greater ammonium nitrogen removal in deeper VFCW when the authors used VFCW planted with *Vetiveria zizanioides* in the treatment of synthetic effluent (68± 3 to 290± 8 mg L^{-1} NH_4^+-N). In this way, deep root system seems to favor the creation of zones with different oxidations conditions that allow the ammonium nitrogen and organic matter removals [20].

Fig. 3. Organic and ammonium load values in inlet and outlet of VFCW 1(a and b, respectively) and VFCW 2 (c and d, respectively), and the respective COD and ammonium removal efficiencies for the different trials (A, B and C). Box and whisker plots showing median, 25th and 75th percentile (box), and range (whiskers).

Regarding the leaves biomass composition (Table 2), the deeper VFCW had a significative ($p<0.05$) greater accumulation of nutrients (calcium, potassium, total nitrogen, and phosphorus) compared to the less deep VFCW, since the beginning of the experiments. Signs of toxicity or chlorosis in the leaves were never detected in *Vetiveria zizanioides*.

Table 2: Leaves biomass composition.

Parameter (mg g^{-1} DW)	**Leaves biomass composition**		
	Beginning	**Ending**	
		VFCW 1	**VFCW 2**
Ca^{2+}	1.20±0.05	2.00±0.09	2.30±0.01
Mg^{2+}	0.04±0.01	0.06±0.01	0.07±0.02
Na^{+}	0.45±0.01	0.30±0.01	0.36±0.02
K^{+}	0.47±0.02	3.14±0.02	4.0±0.02
TN	10.5±0.5	15.9±0.2	17.6±0.2
P	1.02±0.01	0.20±0.03	0.44±0.03

4. Conclusions

The organic matter and ammonium nitrogen removal efficiencies are influenced by VFCW depth as well as the applied ammonium nitrogen and organic load. For organic loads applied up to 9.5 g m^{-2} d^{-1}, removal efficiencies of 71.4±4 and 85.2±3.4 % were obtained respectively for the lowest and highest depth VFCW studied. For both VFCW, the COD concentrations (about 33.5 mg L^{-1} and 17.36 mg L^{-1} for the lowest and highest depth VFCW) obtained by these removals meet the requirements for water reuse for irrigation (≤40 CBO_5 (mg/L O_2[21]). In this way**,** immediate one-step lime precipitation followed by VFCW is a good solution for slaughterhouse wastewater reuse.

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