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Effects of feeding mode on the performance, life span and greenhouse gas emissions of a vertical flow macrophyte assisted vermifilter

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Effects of feeding mode on the performance, life span and greenhouse gas emissions of a vertical flow macrophyte assisted vermifilter

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This study was conducted to investigate the impact of intermittent feeding on performance, clogging, and gaseous emission on macrophyte assisted vermifiltration (MAVF) based treatment system. Synthetic slaughterhouse wastewater was applied to two different integrated vertical flow based MAVFs. Triplicates were used throughout the study. *Eisenia fetida* earthworms were added to MAVFs, and *Carex muskingmenis* plants were planted. Wastewater was applied to the reactors on 1) intermittent (8 h/day) (IMAVF) and 2) continuous (24 h/day) (CMAVF) basis. The average chemical oxygen demand, total nitrogen, and total phosphorous removals achieved by the IMAVF were 80.2 ± 1.6%, 53.9 ± 1.3% and 66.5 ± 1% respectively, and 68.3 ± 1.3%, 61.2 ± 1.4%, and 60.5 ± 1.4% by the CMAVF, respectively. The diffusion of air to the bedding of IMAVFs during no-flow conditions facilitated higher organics oxidation, adsorption of phosphorous, nitrification, and ammonification. At the end of the study, hydraulic conductivity of IMAVF and CMAVF were found to be 0.036 cm/s and 0.037 cm/s, respectively. CO_2, CH_4 and N_2O emissions from IMAVF were 245.5 ± 38.0 mg C/m^2, 5.0 ± 4.6 mg C/m^2 and 2513.5 ± 2629.9 µg N/m^2 respectively, while CO_2, CH_4 and N_2O emissions from CMAVF were 123.3 ± 14.5 mg C/m^2, 74.8±45.2 mg C/m^2 and 328.4 ± 93.4 µg N/m^2, respectively. Intermittent application of influent could be considered for improving the performance and lifespan of MAVFs, causing lower environmental footprints.

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INTRODUCTION

As global population increases, so does the demand for food, and specifically animal-derived protein, especially in low- and middle-income countries[1]. The Food and Agriculture Organization projects a 15% increase in global meat production for 2026, compared to 2016 baseline data[2], with corresponding increases in greenhouse gas production and the potential for environmental contamination[2]. Therefore, there is an urgent need to identify practices and technologies that can reduce the impact of livestock production[3]. With increased meat production, increased slaughterhouses effluent that is characterized by highly organic components (animal blood and intestinal contents) composed of fats, proteins and complex organic substances[4] can be expected. Furthermore, inorganic compounds are also found in the slaughterhouse wastewaters due to the application of detergents and disinfectants for cleaning and washing. Wastewaters from slaughterhouses are considered industrial wastewaters. In 2004, U.S. EPA developed guidelines for discharge of slaughterhouse effluents[5]. Effective treatments of wastewaters from slaughterhouses involve a variety of engineering practices such as coagulation, dissolved air floatation, advanced oxidation process, and aerobic-anaerobic digestion[6–9]. Where engineering expertise and infrastructure limitations exist, alternative practices to treat slaughterhouse wastewater need to be developed and evaluated.

Macrophyte assisted vermifiltration (MAVF) is gaining increasing acceptance in rural communities due to its low operating cost, user-friendliness, and ease of maintenance[10,11]. In general, MAVFs are soil columns with earthworms and plants. The addition of earthworms aerates the system and adds gut microbes while the plants take up the nutrients[11]. Due to increased intensity of agricultural and industrial production systems, wastewaters are becoming polluted to an even higher degree[10,12], resulting in the need for enhanced performance intensification of MAVFs. Also, adopting a decentralized technology like MAVF is limited due to the significant footprint required[10,13], however, this can be reduced by enhancing the removal processes within the MAVF system.

In order to further intensify the removal performance, artificial aeration has been widely implemented[14,15]. Artificial aeration helps enrich communities by providing an active microbial biomass by making an aerobic ecosystem within the treatment systems, resulting in higher organics oxidation and nitrification[12,16]. However, the provision of artificial aeration requires more energy and investment and adds complexity to the operation, making it more challenging to operate. Other than aeration, partial recirculation of treated effluents and attachment of a pre/post-treatment unit can also be considered[17,18]. For example, membrane bioreactors[19], upflow anaerobic sludge blanket (UASB)[20], anaerobic baffled reactor bed (ABR)[21], and integration of electrolytes[22] have been incorporated as pre/post treatment unit to increase the removal efficiency of wetlands. However, these methodologies would also add more complexities and need skilled human resources to operate and maintain. Intermittent application of influent to the treatment reactor could be adopted as a strategy to enhance the performance of MAVFs, as this method is affordable and easy to apply. The intermittent operation of various treatment filters, including wetlands[23], biofilters[24], and trickling filters[25], have already been investigated.

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For example, Caselles-Osorio and Garcia[26] observed average ammonium removal rates in the effluents from constantly flooded and intermittently flooded wetland of 85.0% and 99.0%, respectively. Similarly, Wang et al. found that the chemical oxygen demand (COD) removal in vermifilters running at 12 h and 6 h of drying time were 64.4 ± 3.6% and 58.7 ± 3.5%, respectively[27]. In intermittent wetlands, bedding, biofilm, and plant roots are exposed to air during no-flow conditions, enhancing microbial degradation of pollutants applied as influent[25]. One drawback to the application of intermittent feeding is a reduction of influent volume treated per day. To further increase the performance of MAVFs, there is a need to further understand the impact of intermittent feeding. In addition to intermittent feeding, performance intensification through a change in the design should also be considered. The research group has already facilitated anoxic denitrification in MAVFs implementing horizontal subsurface flow[11], which was a significant step towards performance intensification as denitrification in MAVFs was limited. However, the provision of horizontal subsurface flow in the developed treatment system[11] required an extensive floor space for its future applications. Therefore, to minimize floor space requirements, investigating the application of anoxic denitrification in the vertical MAVFs is needed. In addition, to the best of our knowledge, greenhouse gas emission from MAVF and impact of intermittent feeding have never been studied.

This study examined the performance intensification by intermittent feeding and design change in the MAVFs in terms of chemical oxygen demand (COD), total nitrogen (TN), and total phosphorous (TP) removals. Specifically, this study carried out a comparative analysis between intermittent and continuously fed MAVFs, in terms of 1) treatment performance, 2) clogging, 3) gaseous emission, and 4) biomass growth.

RESULTS AND DISCUSSION

Dissolved oxygen (DO) and pH

Dissolved oxygen in the influent, which ranged from 4.5 to 5.0 mg/L, decreased in the effluent to <1.5 mg/L (Supplementary Fig. 3a). The decrease in DO was expected with DO utilization in the oxidation of organics and nitrification. The average effluent DO from IMAVFs and CMAVFs was found to be 1.2 ± 0.1 mg/L and 0.9 ± 0.1 mg/L ($p < 0.05$, *t*-test). Lower effluent DO concentrations from CMAVFs, compared to IMAVF is likely attributable to greater potential atmospheric recharge of oxygen to the bedding surface between intermittent wastewater applications and is consistent with previous reports[28,29]. Intermittent recharge reportedly increases oxygen diffusion within the bedding through diffusion and convection[21]. During no flow hours of an intermittently fed MAVF (IMAVF) as the wastewater within the bed media is drained/percolated to the lower layers of MAVF columns creates a negative pressure within the bedding that facilitates the movement of air to the bedding. Caselles-Osorio and Garcia found that the intermittent feeding of influent causes more turbulent conditions, thereby incraesing exposure time or surface for oxygenetation[26]. Supplementary Table 1 has been provided to as a supplemental material to summarise the results obtained.

Influent pH which ranged between 6.4 and 6.6 was lower than the effluent pH which ranged between 6.7and 6.9 for both reactor types (Supplementary Fig. 3b). The difference in the effluent pH between IMAVF and CMAVF was negligible suggesting limited impact of the flow conditions on pH. Similar results have been observed by Singh et al. investigating the development and performance assessment of an integrated MAVF system for the treatment of feedlot runoff[11].

COD removal

COD concentration in both MAVFs was reduced by approximately 70–85% (Fig. 1). The reduction of COD in MAVFs could be attributed to microbial decomposition within the treatment systems[12], specifically the microbes associated plant roots, earthworm guts, and bedding materials[10]. Average COD removal from IMAVFs and CMAVFs were 80.2 ± 1.6% and 68.3 ± 1.3%, respectively, showing higher removal from IMAVFs (*p-value* < 0.05, *t*-test). The difference between the COD removals could be related to higher DO concentration in IMAVFs[21]. Higher DO in the reactor (as stated in Wastewater used) helps create a condition supporting aerobic degradation of organic matter present in the influent[30–32]. The complex organic compounds present in the wastewater are more easily degraded by microbes when oxygen is available. In this process, the complex organic compounds are converted to simpler compounds such as carbon dioxide and water. Removal of COD from slaughterhouse wastewater is quite variable with other treatment systems. According to Mburu et al. wetlands can remove 55% of the influent COD when a five-day retention period was applied[33]. However, in another experiment applying slaughterhouse wastewater to wetland, COD removal of 74% was obtained after 10 days of retention[33]. Similarly, at HRT of 1.16 day in a sequencing batch reactor (SBR), 60% of the COD was removed from the slaughterhouse wastewater[34]. Amuda and Alade estimated a COD removal of 34% after application of coagulation to the slaughterhouse wastewater[35]. The average COD removal from the top, middle and bottom layers of IMAVFs were

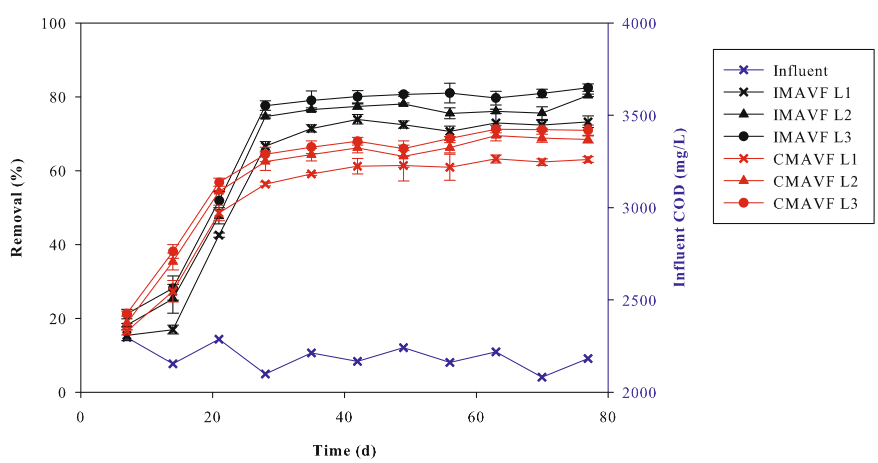

Fig. 1 Effect of intermittent feeding on COD removal. IMAVF Intermittently fed macrophyte assisted vermifiltration, CMAVF Continuously fed macrophyte assisted vermifiltration; Error bars: standard deviation created using replicated ($n = 3$) set-ups for each experimental condition]. Three layers placed at 0–30 cm (L3), 30–60 cm (L2), and 60–100 cm (L1) from the bottom, containing gravel, alum, and soil mix, respectively.

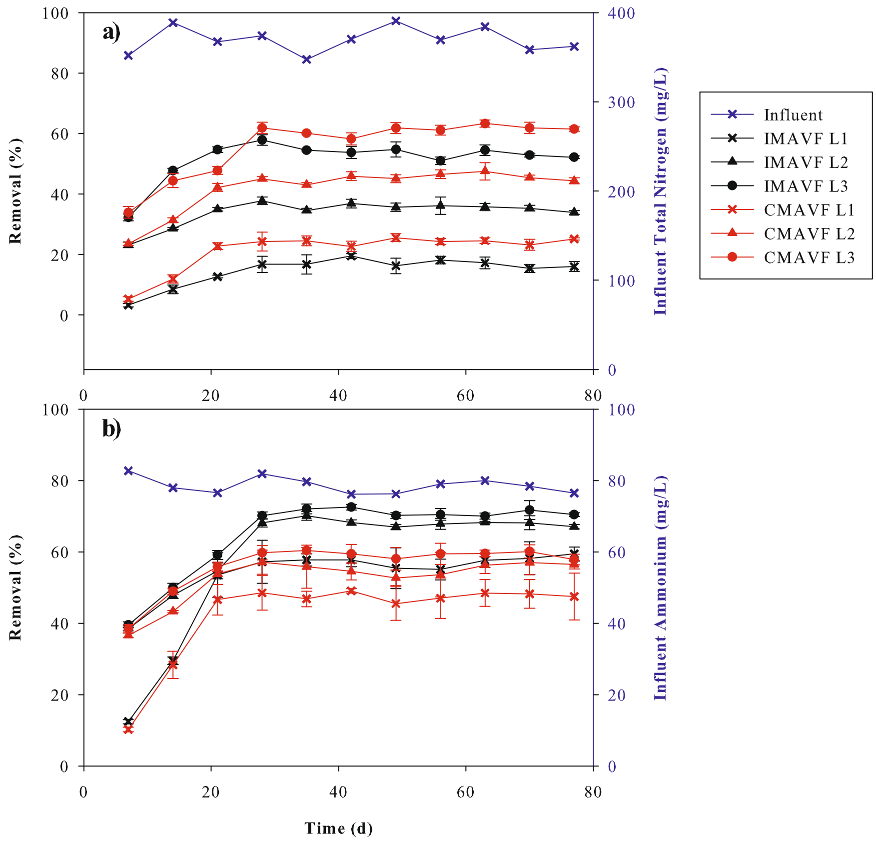

Fig. 2 Intermittent feeding, Total Nitrogen, and Ammonium. Effect of intermittent feeding on (**a**) TN and (**b**) NH_4 removal. IMAVF Intermittently fed macrophyte assisted vermifiltration, CMAVF Continuously fed macrophyte assisted vermifiltration. Error bars: standard deviation created using replicated ($n = 3$) set-ups for each experimental condition]. Three layers placed at 0–30 cm (L3), 30–60 cm (L2), and 60–100 cm (L1) from the bottom, containing gravel, alum, and soil mix, respectively.

71.7 ± 1.6%, 76.8 ± 0.8%, and 80.2 ± 1.6%, respectively, whereas average COD removal from the top, middle, and bottom layers of CMAVFs were found to be 60.9 ± 1.7%, 66.2 ± 1.7%, and 68.3 ± 1.3%, respectively (Fig. 1). The pattern of COD removal top to bottom from both types of treatment systems reveal that up to 60–70% of the removals were obtained within the uppermost layer, while the remaining layers with more anoxic conditions combined for an additional 5–7% removal. The lower COD removal in the middle and bottom layer may be due to the submergence of these layers and restricted diffusion of oxygen into the deeper layers. These conditions possibly favored organics utilization by denitrifying microorganisms[36]. Furthermore, the COD removals at all three depths increased during the initial 3–4 weeks and later a steady state of COD removal was attained. The initial increasing removal rates could be attributed to a gradual buildup of microbial biomass in the treatment systems[37].

Nitrogen removal

The influent TN and NH_4 concentration averaged 369.3 ± 14.2 mg/L and 100.6 ± 2.9 mg/L, respectively. The average TN % removal from the IMAVFs and CMAVFs was 53.9 ± 1.3% and 61.2 ± 1.4%, respectively (Fig. 2a). IMAVFs offered slightly lower TN removal than CMAVFs ($p < 0.05$, *t*-test). The lower TN removal from IMAVF may be attributed to more consistent anaerobic denitrifying environment in the CMAVFs which was maintained through the constant application of the feed water. The constant application of influent limits air/oxygen interaction between the bedding[21], thus, secondary electron acceptors, like nitrate and nitrite, would be utilized by denitrifiers. In these instances, the systems are with low DO and available carbon source would be conducive to denitrification. Complete organics removal (see Reactor details) was not achieved within the first layer and the remaining organics was flushed to the lower layers (L2 and L3), which was later a potential carbon source for denitrification. Comparing TN removal in the three different layers of IMAVFs, TN removal was 17 ± 1.9%, 35.7 ± 1.3%, and 53.9 ± 1.3%, in the top, middle, and bottom layers, respectively. Similarly, TN removal from the top, middle and bottom layers of CMAVFs was 24.3 ± 1.5%, 45.4 ± 1.3%, and 61.2 ± 1.4%, respectively. The analysis of TN removal from different layers indicates that every layer substantially contributed to TN removal, through either the adsorption of ammonia to the bed materials or microbial denitrification.

Average NH_4 removal from IMAVFs and CMAVFs was 70.9 ± 1.2% and 59.3 ± 2%, respectively (Fig. 2b), offering higher NH_4 removal from IMAVF ($p < 0.05$, *t-test*). Higher NH_4 removal from the IMAVF could be attributed to greater air/oxygen penetration into the bedding[29], causing conversion of ammonium to nitrate via nitrification[16]. The nitrifiers (*Nitrosomonos* and *Nitrobactor*) utilize oxygen and convert ammonium to nitrite, followed by conversion to nitrate. In addition, during the no flow hours the grinding activity of earthworms may be increased compared to the hours with flow as submergence of bed media may restrict their activities. Thus, due to intermittent flow, earthworms grind soil particles faster within their gizzard, yielding an increase in specific surface area of the bed media. With greater specific surface area, a higher NH_4 removal from IMAVFs is achieved[37]. The NH_4 removal from the top, middle and bottom layers of IMAVFs were measured at 57.3 ± 3.3%, 68.1 ± 1%, and 70.9 ± 1.2%, respectively, while NH_4 removal of 47.6 ± 4%, 55.4 ± 3.4%, and 59.3 ± 2% was obtained from the top, middle, and bottom layers of CMAVFs. The removal from different layers

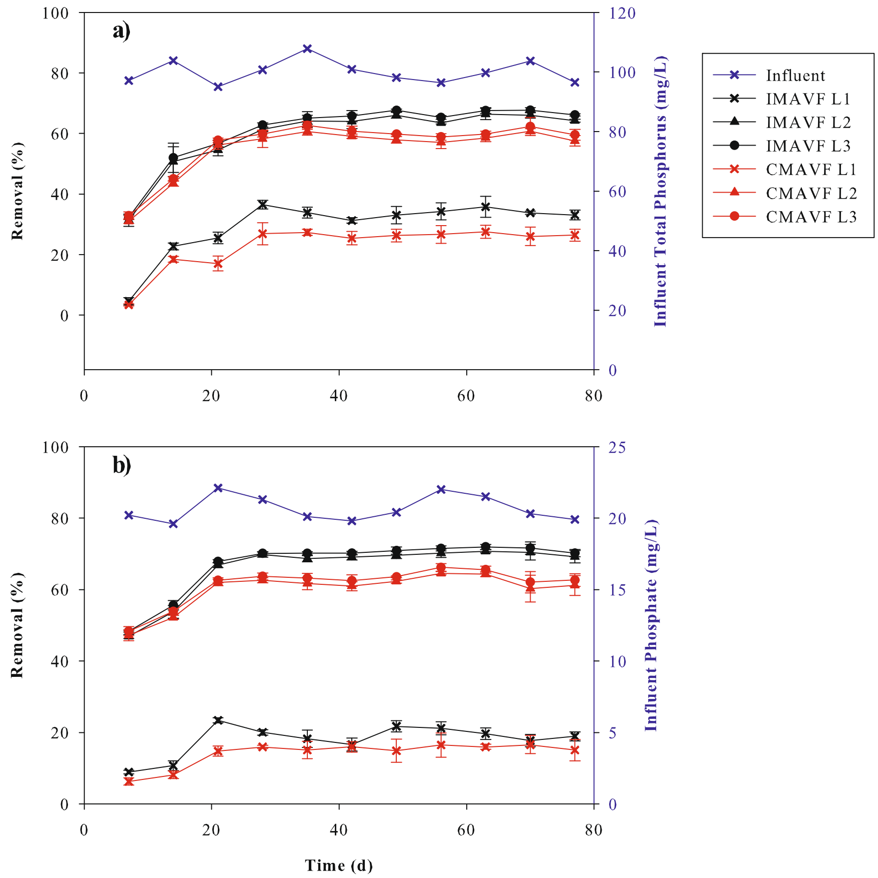

Fig. 3 Intermittent feeding and Total Phosphorous, and Phosphate. Effect of intermittent feeding on (**a**) TP and (**b**) PO_4 removal. IMAVF intermittently fed macrophyte assisted vermifiltration, CMAVF Continuously fed macrophyte assisted vermifiltration. Error bars: standard deviation created using replicated ($n = 3$) set-ups for each experimental condition. Three layers placed at 0–30 cm (L3), 30–60 cm (L2), and 60–100 cm (L1) from the bottom, containing gravel, alum, and soil mix, respectively.

indicates that most of the NH_4 were removed in the top layer of the treatment reactors. Average NH_4 removal from the top layer was comparatively higher in IMAVFs than CMAVFs. These NH_4 removals could be attributed to the presence of aerobic conditions due to earthworm activities[12] and more air diffusion within the bedding during no flow hours promoting nitrification. The NH_4 removals from second layer were also significant, which could be attributed to the presence of alum sludge particles, helping with the adsorption of NH_4[38]. On the other hand, the bottom layer with much lower removal than the top and middle layers, still contributed to overall NH_4 removal and could be due to potential NH_4 adsorption within the bedding material in the bottom layer or to slow microbial biomass accumulation within that anaerobic environment. Removal of ammonium and TN has also been evaluated in different treatment systems including wetlands where after application of slaughterhouse wastewater to a wetland, 39% of the ammonium was removed[33]. Similarly, ammonium and TN removal of 43% and 46% were obtained after the application of slaughterhouse wastewater to wetlands[39]. Gürel & Büyükgüngör obtained a TN removal of 44% after slaughterhouse wastewater was applied to membrane bioreactor[40].

Influent NO_3 averaged to be 0.9 ± 0.2 mg/L. Nitrate concentrations within the top, middle and bottom layers of IMAVFs averaged 93 ± 1.8 mg/L, 16.7 ± 0.8 mg/L, and 3.0 ± 0.2 mg/L, respectively (Supplementary Fig. 4). The effluent concentrations from the top, middle, and bottom layers of CMAVFs were 81.6 ± 2.2 mg/L, 13 ± 1 mg/L, and 2.4 ± 0.2 mg/L, respectively. Elevated NO_3 concentrations within the layers suggests the top layer of these reactors were actively converting NH_4 to NO_3 via nitrification[41]. The lower NO_3 concentration in layer 2 can be attributed to the removal of nitrate in the submerged and more anaerobic environment which was conducive to denitrification[42]. The concentration of NO_3 was further reduced in the bottom (L3) layer of the treatment reactors, also indicative of active denitrification within this layer.

Phosphorous removal

The average concentration of TP and PO_4 in the influents were 100 ± 3.9 mg/L and 20.7 ± 0.9 mg/L, respectively. After achieving steady state COD removal, average TP % removal from IMAVF and CMAVF were $66.5 \pm 1.0\%$ and $60.5 \pm 1.4\%$, respectively (Fig. 3a), showing higher TP removal from IMAVFs ($p < 0.05$, *t-test*). Average PO_4 removal was slightly higher than TP percentage and calculated to be $70.8 \pm 0.8\%$ and $63.7 \pm 1.4\%$ from IMAVF and CMAVF, respectively (Fig. 3b) ($p < 0.05$, *t-test*). The comparatively higher removal rate of TP and PO_4 from IMAVF may be attributed to higher phosphorous uptake by new plant biomass and possibly the increased activity of earthworms grinding up particulate matter thus exposing more surface area for microbial enzymatic phosphatase activity and also exposing more potential PO_4 adsorption sites within the substrate. However, the impact of intermittent feeding on the activity of earthworms and its relationship to bedding moisture content is speculative but promising as a future investigation. Removal of TP and phosphate from other systems ranges widely compared to the MAVF system. For example, after application of slaughterhouse wastewater to wetland, 63% of TP removal was observed[33], while only 34% of influent TP was removed after coagulation[35].

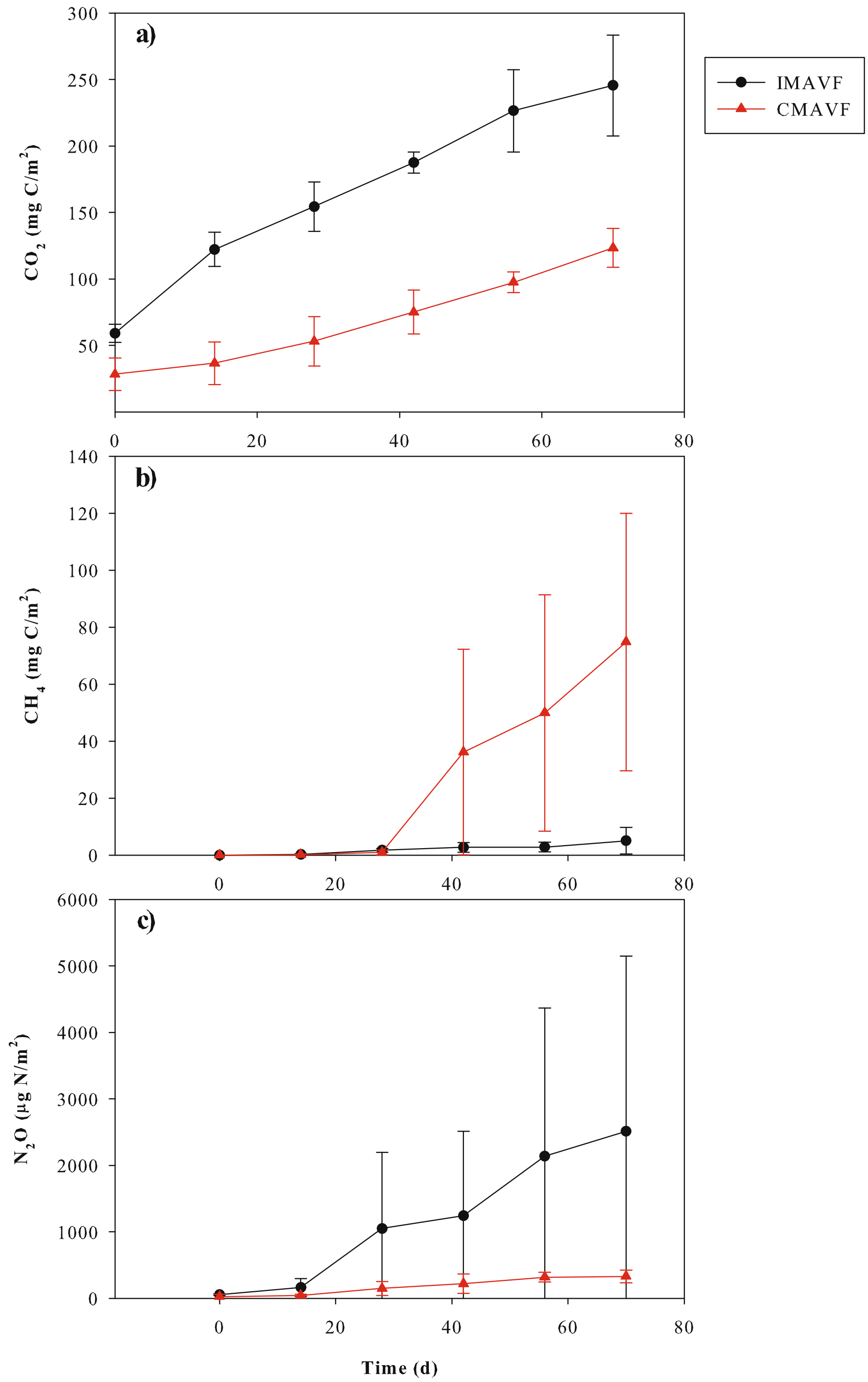

Fig. 4 Intermittent feeding and gaseous emission. Effect of intermittent feeding on (**a**) CO_2, (**b**) CH_4 and (**c**) N_2O emission. IMAVF Intermittently fed macrophyte assisted vermifiltration, CMAVF Continuously fed macrophyte assisted vermifiltration; Error bars: standard deviation created using replicated ($n = 3$) set-ups for each experimental condition.

Comparing the potential for PO_4 removal at various levels, the average TP removal from the first layer of IMAVF was 34 ± 1.9%, while the middle layer removed an additional 30%, and the bottom layer only removed an additional 2%. Similarly, the average TP removal from the top layer of CMAVF was 26.6 ± 2.4%, while the middle and bottom layers removed an additional 32% and 2%, respectively. Thus, the top and middle layers offer substantially higher PO_4 removal than the bottom layer, which removed just 1–3%. The presence of earthworms and plants led to considerable P loss, while the alum sludge within the middle layer promotes PO_4 adsorption due to aluminum compounds that react with and precipitate phosphates. The bottom (L3) layer only contains unreactive limestone gravels, as bedding, so little PO_4 is removed.

Gaseous emission

All gas emissions continued to increase throughout the trial and never reached a steady state (Fig. 4), but patterns of gas emissions were consistent with a variety of microbial processes in the bioreactors; CO_2 emissions were consistent with aerobic decomposition, CH_4 emissions consistent with anaerobic decomposition, and N_2O emissions consistent with aerobic and anaerobic nitrogen transformations (nitrification and denitrification, respectively). Considerable variation between columns for daily CH_4 and N_2O flux rates indicates that local variation within the columns (perhaps density and depth of worm channels) existed between replicates.

The analysis of CO_2 emission from treatment reactors was most consistent and shows that the IMAVFs and CMAVFs emitted an average cumulative flux of 245.5 ± 38.0 and 123.3 ± 14.5 mg C/m^2, respectively (Fig. 4a). Higher CO_2 emissions from IMAVFs are indicative of more aerobic conditions and relatively higher COD degradation[16] as mentioned in Reactor details. Average cumulative CH_4 emission from IMAVF and CMAVFs were 5.0 ± 4.6 and 74.8 ± 45.2 mg C/m^2, respectively, but highly variable within the CMAVF replicate columns (Fig. 4b). CH_4 in wastewater treatment systems is produced under anaerobic conditions[16], and the higher CH_4 emissions from CMAVFs is consistent with a more stable anaerobic condition compared to the fluctuating, partially aerobic environment in the IMAVFs.

N_2O emission from IMAVFs and CMAVFs produced an average cumulative flux of 2513.5 ± 2629.9 and 328.4 ± 93.4 µg N/m^2, respectively (Fig. 4c). Considerable variation was observed between the IMAVF replicates. N_2O is produced microbially during both aerobic (nitrification) or anaerobic processes (denitrification). The observed higher N_2O flux from the IMAVFs is consistent with the observations available in the literature[43]. In the experiments presented here, the potentially large N_2O emissions may be attributed to a combination of both nitrification and denitrification in the top (L1) layer. When water drains from the bed, it becomes more aerobic as air infiltrates the medium. L1 NH_4 is slowly oxidized via nitrification to NO_2 and NO_3 (producing a little byproduct N_2O). Then when water is applied, oxygen flux into the bed is eliminated, DO is quickly consumed, and any NO_2 and NO_3 formed during the previous aerobic cycle is then denitrified to N_2 gas (with a little byproduct N_2O produced) in the now anaerobic bed environment. Any NO_2 and NO_3 remaining may also flow deeper into the perpetually anaerobic L2 and L3 layers that do not experience the fluctuating aerobic/anaerobic environment in L1, and remaining NO_3 is completely reduced through microbial denitrification to N_2 gas. Given ample carbon availability (DOC), the denitrification reaction is complete within L2 and L3 with very little N_2O escaping to the surface. An isotopic analysis of the emitted N_2O relative to isotopic composition of NH_4 and NO_3 in the various layers would provide sound evidence of the source of the N_2O and an interesting follow up experiment.

Accounting for the difference in wastewater volume treated, the relative impact of intermittent versus continuous feeding on greenhouse gas emissions becomes even more important. Using the values of cumulative flux detailed above and assuming three times as many IMAVF would be needed to treat the same flow a CMAVF processed, emissions from the IMAVF would be three times greater. Thus, cumulative CO_2 and CH_4 emissions from IMAVF would be 736.5 and 15 mg C/m^2 compared to 123.3 and 74.8 mg C/m^2 for the CMAVF. For N_2O, the IMAVF and CMAVF would be 7540.5 and 328.4 µg N/m^2, respectively. Based upon a 25x and 250x conversion of CH_4 and N_2O to CO_2 greenhouse gas equivalents (based upon lifespan in the atmosphere and potential to adsorb heat radiation), emissions for these experiments would be 2996 and 2075 mg C(equivalents)/m^2 of MAVF. Note that these MAVF were not at equilibrium with regard to gas emission, so the results only reflect the initial 70 days of operation and should be further refined both to account for the total lifetime of the MAVF and better account for the reasons behind the large range of emission variation between columns.

Earthworm and plant biomass analysis

The biomass analysis of the earthworms increased for both reactor designs (Supplementary Fig. 5). The initial numbers of earthworms (125) in IMAVF decreased to 119, while the number of earthworms in CMAVF increased to 126 (Supplementary Fig. 5a). Similarly, the average weight of earthworms in the IMAVF and CMAVF increased to 41.97 g and 47.64 g, respectively, from an initial average weight of 39.05 g (Supplementary Fig. 5b). The higher average number and weight of earthworms present in the CMAVFs indicate that more substrate is the key characteristic in the growth and multiplication of earthworms over the moisture, along with the prevalence of more consistent wet environment within the CMAVF. However, impact of substrate availability on the growth of earthworm biomass still needs to be considered as a future scope of study.

The average stem height of plants in the IMAVFs and CMAVFs was seen to increase from 4.7 cm (during inoculation) to 46.5 cm and 35.4 cm, respectively (Supplementary Fig. 5c), and the average number of plant stems in the IMAVFs and CMAVFs increased from 15 (during inoculation) to 43 and 34, respectively (Supplementary Fig. 5d). The increases indicate favorable conditions for plant growth in both systems, but the higher numbers and height of stems in the IMAVFs indicate that higher moisture in CMAVFs might somewhat reduce potential plant growth, a surprising finding for the selected wetland plant species. Additional research on the impact of fluctuating moisture on the growth of particular wetland plant species in MAVFs needs further investigation.

Clogging analysis

The analysis of hydraulic conductivity within the IMAVFs and CMAVFs reveals that hydraulic conductivity gradually decreases over the experimental period in both treatment systems. The hydraulic conductivity on the first day of both reactor designs averaged 0.038 cm/s while hydraulic conductivity of 0.037 cm/s and 0.036 cm/s were measured at the end of the trial for IMAVFs and CMAVFs, respectively (Supplementary Fig. 6a). An opposite trend in head loss was observed compared to the hydraulic conductivity. Over the 70-day trial, head loss of the CMAVFs and IMAVFs increased from 0.81 to 0.84 and 0.83 cm, respectively (Supplementary Fig. 6b). The comparatively higher hydraulic conductivity and lower head loss from IMAVFs could be attributed to the higher aerobicity due to the additional DO recharge during the no-flow phase[44]. Higher availability of DO in IMAVFs enhances degradation of organics which may reduce the hydraulic conductivity of the bedding by blocking the pores through which infiltration of wastewater occurs[45,46]. High DO encourages an aerobic ecosystem with more active aerobic microorganisms within the bedding, resulting in a more rapid degradation of organics or solids. The reduction in hydraulic conductivity can also be associated to the formation of gas bubbles due to many ongoing mechanisms (denitrification, anaerobic degradation, aerobic degradation)[47]. It should also be noted that intermittent application of influent to improve the performance and lifespan of MAVFs may increase the capital costs of setting up a treatment system. Thus, a cost-benefit estimation needs to be done for each situation to determine the best practice (intermittent versus continuous application) to treat wastewater.

Next steps

A cost-benefit analysis and additional experiments to identify the mechanisms behind the impact of intermittent feeding on biomass growth and phosphorous removal are necessary before recommending this strategy to be applied at larger scales. Based on the results obtained the proposed strategy can be implemented for the slaughterhouse wastewater treatment after scaling up according to the amount of wastewater generated from the premises. Thus, results from the present study indicate that intermittent application of influent to MAVF based treatment systems enhances DO in IMAVF, significantly increasing their performance, life span, and emission characteristics.

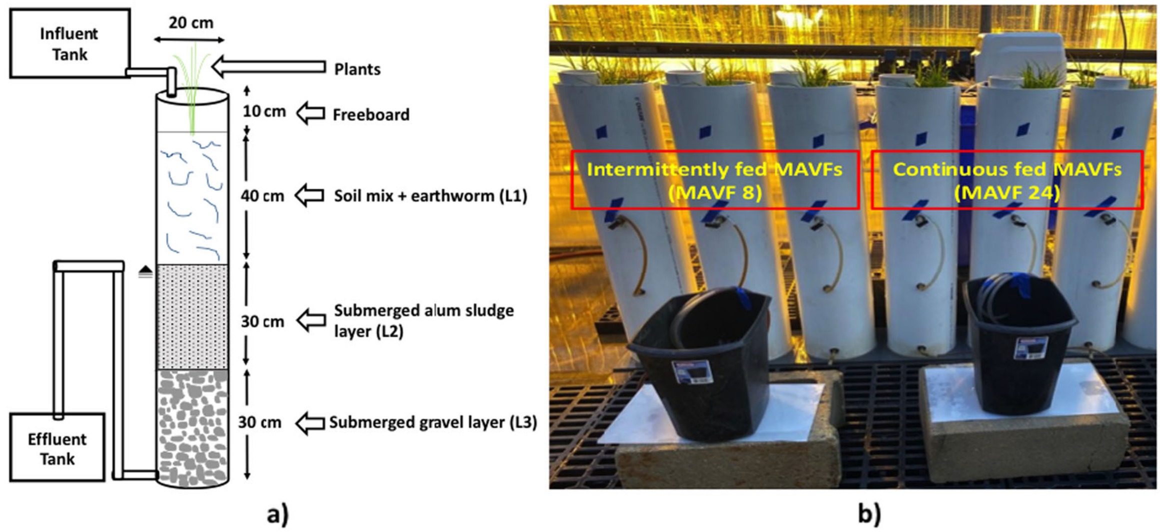

Fig. 5 Experimental setup. a Schematic. **b** Actual treatment setup. IMAVF Intermittently fed macrophyte assisted vermifiltration. CMAVF Continuously fed macrophyte assisted vermifiltration]. Three layers placed at 0–30 cm (L3), 30–60 cm (L2), and 60–100 cm (L1) from the bottom, containing gravel, alum, and soil mix, respectively.

METHODS

Wastewater used

Synthetic slaughterhouse wastewater, prepared daily according to Bustillo-Lecompte et al.[48], was used as the feed water throughout the study. Briefly, commercial meat extract power (1950 mg/L), glycerol (0.2 ml/L), ammonium chloride (360 mg/L), sodium chloride (50 mg/L), potassium dihydrogen orthophosphate (30 mg/L), calcium chloride (24 mg/L), and magnesium chloride (7.5 mg/L) were utilized to prepare one liter of synthetic slaughterhouse wastewater. All chemicals were purchased from Thermo Scientific (Waltham, MA, USA) with the exception of the meat extract power (HiMedia, West Cester, PA, USA) and glycerol (Cole-Parmer, Vernon Hills, IL, USA).

Reactor details

PVC columns (internal diameter: 20 cm and length: 110 cm) were used to fabricate MAVFs utilized in this study. Each PVC column had three sampling ports (internal diameter: 1.27 cm) located 0 cm, 30 cm, and 60 cm from the bottom (Fig. 5). The fabricated MAVF columns were placed in a greenhouse which was being operated on a 12 h light basis at temperature ranging between 24 °C and 28 °C. Each column contained three layers placed at 0–30 cm (L3), 30–60 cm (L2), and 60–100 cm (L1) from the bottom, containing gravel, alum, and soil mix, respectively (Fig. 5a). The soil mix used in this experiment was prepared by mixing compost with garden soil to a ratio of 4:1 in accordance with suggested guidelines[49]. Alum sludge is a by-product of water treatment plants derived after the addition of alum for the coagulation of solids[38]. Phosphorous reaction with aluminium hydroxide $Al(OH)_3$, forms insoluble aluminium phosphate $AlPO_4$ and has been shown to a life span of 3–4 years when subjected to animal wastewater with high phosphorous concentration[50]. Alum sludge used in this study was brought from a water treatment plant based in Florence, Nebraska. *Carex muskingmenis* (palm-sedge), a native wetland plant to the Midwestern USA and tolerant to harsh weather conditions was used during the study. The species of the earthworms added to the reactors was *Eisenia fetida*, an epigeic species surviving on the detritus material present in the top layer[12].

After placing layers, earthworms, and plants in the different MAVFs, the drainpipes were attached to the bottom-most effluent port of the column, and the draining arrangement of the effluent was placed at the height of 60 cm from the bottom drain port, resulting in the complete submergence of layer two and layer three (Fig. 5b). A multi-channel peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) was used to simultaneously deliver equal amount of the feed water to the different MAVFs.

Operation protocol

The total duration of the study was 77 days. During the initial seven days, only tap water was applied to maintain moisture for bacterial growth and gradually acclimatize the inoculated earthworms to the new ecosystem within the bedding[49]. During acclimatization some nutrient and COD leaching from the compost was possible and could have contributed to initial low gas and nutrient fluxes. For the acclimatization, 2 L of tap water was sprinkled over the bedding twice a day. After the successful acclimatization, synthetic wastewater was applied to the bedding to achieve a hydraulic retention time (HRT) of 4d. Based on the feeding pattern, MAVFs were classified as constant (CMAVF) and intermittent (IMAVF). CMAVF continuously received synthetically prepared wastewater throughout the study, while IMAVF received for only 8 h a day. CMAVFs and IMAVFs were fed 7.83 L and 2.61 L of synthetic wastewater per day, respectively. It should be noted that the 5.54 mL/min in both types of MAVF was kept same during the hours of influent application.

Physicochemical analysis

The dissolved oxygen (DO) and pH were measured using probes on weekly basis. Analysis of chemical oxygen demand (COD), total nitrogen (TN), total phosphorous (TP), ammonium (NH_4), nitrate (NO_3), and orthophosphate (PO_4) were analyzed according to Baird et al.[51]. Weekly samples were collected in triplicates and analyzed to determine influent and effluent water quality from the developed MAVFs. The calculated removal rates of the different analytes after IMAVF and CMAVF were statistically evaluated using *t-test* (SigmaPlot, Systat Software, Inc.) with a 0.05 confidence interval.

Greenhouse gas analysis

Flux chambers used to collect greenhouse gases from each MAVFs were constructed from PVC end caps (internal diameter: 7.62 cm) equipped with a rubber septum. On the day of gas collection, the flux chambers were mounted onto an existing, smaller PVC pipe (external diameter: 7.62 cm) within the larger MAVF PVC column which was placed 15 cm deep within the first layer of bedding at the beginning of the study (Supplementary Fig. 1) (the smaller PVC pipe was used to limit the potential effects that repeatedly installing and removing flux chambers from the soil bed would have had on plants growing in the MAVFs). After placing the flux chamber atop the PVC tube and at 10, 20, and 30 min, 25 mL of headspace air was withdrawn from the chamber using a 30 mL plastic syringe and immediately transferred into 12 mL pre-evacuated, Exetainer gas collection vials (Labco, Lampeter, UK). Greenhouse gas (CH_4, CO_2, and N_2O) concentrations were analyzed in the vials using a Varian gas chromatograph equipped with thermal conductivity, flame ionization, and electrical conductivity detectors against a suite of standard gas mixes as previously described[52]. Gas flux samples were collected every fourteen days until the end of the experiment.

Biomass analysis

After successful completion of experimental duration, the reactors were dismantled to analyze the biomass growth of earthworms and plants incorporated into the system. Both the weight and number of earthworms were determined. Similarly, the plant stems and height of the plant stems were also recorded. The biomass measurements of plants and earthworms was conducted only before and after the experiment.

Assessment of clogging

Clogging of each MAVFs was estimated by determining hydraulic conductivity following Pedescoll et al. every 14 days[53] (Supplementary Fig. 2). The falling head method was used to determine the hydraulic conductivity of the MAVFs. The determined hydraulic conductivity was later utilized to determine the head loss.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Godfray, H. C. J. et al. Meat consumption, health, and the environment. *Science* **361**, eaam5324 (2018).
2. OECD-FAO. *OECD "Meat"*. (OECD Publishing, 2017).
3. Du, Y., Ge, Y. & Chang, J. Global strategies to minimize environmental impacts of ruminant production. *Annu. Rev. Anim. Biosci.* **10**, 227–240 (2022).
4. Bazrafshan, E., Kord Mostafapour, F., Farzadkia, M., Ownagh, K. A. & Mahvi, A. H. Slaughterhouse wastewater treatment by combined chemical coagulation and electrocoagulation process. *PloS One* **7**, e40108 (2012).
5. EPA, U. S. Effluent limitations guidelines and new source performance standards for the meat and poultry products, point source category. *Fed. Register* **69**, 54475–54555 (2004).
6. Adou, K. E. et al. Anaerobic mono-digestion of wastewater from the main slaughterhouse in Yamoussoukro (Côte d'Ivoire): Evaluation of biogas potential and removal of organic pollution. *J. Environ. Chem. Eng.* **8**, 103770 (2020).
7. Dassey, A. J. & Theegala, C. S. Evaluating coagulation pretreatment on poultry processing wastewater for dissolved air flotation. *J. Environ. Sci. Health, Part A* **47**, 2069–2076 (2012).
8. Davarnejad, R. & Nasiri, S. Slaughterhouse wastewater treatment using an advanced oxidation process: Optimization study. *Environ. Pollut.* **223**, 1–10 (2017).
9. Del Nery, V. et al. Long-term operating performance of a poultry slaughterhouse wastewater treatment plant. *Resour., Conserv. Recycling* **50**, 102–114 (2007).
10. Samal, K., Dash, R. R. & Bhunia, P. Performance assessment of a *Canna indica* assisted vermifilter for synthetic dairy wastewater treatment. *Process Saf. Environ. Prot.* **111**, 363–374 (2017).
11. Singh, R. et al. Development and performance assessment of an integrated vermifiltration based treatment system for the treatment of feedlot runoff. *J. Clean. Prod.* **278**, 123355 (2021a).
12. Jiang, L. et al. The use of microbial-earthworm ecofilters for wastewater treatment with special attention to influencing factors in performance: A review. *Bioresour. Technol.* **200**, 999–1007 (2016).
13. Singh, R., Samal, K., Dash, R. R. & Bhunia, P. Vermifiltration as a sustainable natural treatment technology for the treatment and reuse of wastewater: A review. *J. Environ. Manag.* **247**, 140–151 (2019a).
14. Park, J. B., Sukias, J. P. & Tanner, C. C. Floating treatment wetlands supplemented with aeration and biofilm attachment surfaces for efficient domestic wastewater treatment. *Ecol. Eng.* **139**, 105582 (2019).
15. Rous, V., Vymazal, J. & Hnátková, T. Treatment wetlands aeration efficiency: A review. *Ecol. Eng.* **136**, 62–67 (2019).
16. Tchobanoglous, G. *Wastewater engineering: Treatment and resource recovery*. Vol. 2 (McGraw-Hill, 2014).
17. Arias, C. A., Brix, H. & Marti, E. Recycling of treated effluents enhances removal of total nitrogen in vertical flow constructed wetlands. *J. Environ. Sci. Health* **40**, 1431–1443 (2005).
18. He, L., Liu, H., Xi, B. & Zhu, Y. Effects of effluent recirculation in vertical-flow constructed wetland on treatment efficiency of livestock wastewater. *Water Sci. Technol.* **54**, 137–146 (2006).
19. Kong, L. et al. A combination process of DMBR-IVCW for domestic sewage treatment. *Fresenius Environ. Bull.* **22**, 665–674 (2013).
20. El-Khateeb, M. & El-Gohary, F. Combining UASB technology and constructed wetland for domestic wastewater reclamation and reuse. *Water Sci. Technol.: Water Supply* **3**, 201–208 (2003).
21. Ye, C., Li, L., Zhang, J. & Yang, Y. Study on ABR stage-constructed wetland integrated system in treatment of rural sewage. *Procedia. Environ. Sci.* **12**, 687–692 (2012).
22. Ju, X., Wu, S., Huang, X., Zhang, Y. & Dong, R. How the novel integration of electrolysis in tidal flow constructed wetlands intensifies nutrient removal and odor control. *Bioresour. Technol.* **169**, 605–613 (2014).
23. Liu, F.-F. et al. Intensified nitrogen transformation in intermittently aerated constructed wetlands: Removal pathways and microbial response mechanism. *Sci. Total Environ.* **650**, 2880–2887 (2019).
24. Zinger, Y., Prodanovic, V., Zhang, K., Fletcher, T. D. & Deletic, A. The effect of intermittent drying and wetting stormwater cycles on the nutrient removal performances of two vegetated biofiltration designs. *Chemosphere* **267**, 129294 (2021).
25. Zhang, J., Huang, X., Liu, C., Shi, H. & Hu, H. Nitrogen removal enhanced by intermittent operation in a subsurface wastewater infiltration system. *Ecol. Eng.* **25**, 419–428 (2005).
26. Caselles-Osorio, A. & García, J. Impact of different feeding strategies and plant presence on the performance of shallow horizontal subsurface-flow constructed wetlands. *Sci. Total Environ.* **378**, 253–262 (2007).
27. Wang, L. et al. The effect of vermifiltration height and wet: Dry time ratio on nutrient removal performance and biological features, and their influence on nutrient removal efficiencies. *Ecol. Eng.* **71**, 165–172 (2014).
28. Decezaro, S. T. et al. Influence of hydraulic loading rate and recirculation on oxygen transfer in a vertical flow constructed wetland. *Sci. Total Environ.* **668**, 988–995 (2019).
29. Saeed, T. & Sun, G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.* **112**, 429–448 (2012).
30. Singh, R., Bhunia, P. & Dash, R. R. Impact of organic loading rate and earthworms on dissolved oxygen and vermifiltration. *J. Hazard., Toxic., Radioact. Waste - ASCE* **23**, 04019001 (2019b).
31. Singh, R., Bhunia, P. & Dash, R. R. Optimization of bio-clogging in the vermifilters: A statistical approach. *J. Environ. Manag.* **233**, 576–585 (2019c).
32. Singh, R., Bhunia, P. & Dash, R. R. Optimization of organics removal and understanding the impact of HRT on treatment of brewery wastewater using vermifilter. *Sci. Total Environ.* **651**, 1283–1293 (2019d).
33. Mburu, C., Kipkemboi, J. & Kimwaga, R. Impact of substrate type, depth and retention time on organic matter removal in vertical subsurface flow constructed wetland mesocosms for treating slaughterhouse wastewater. *Phys. Chem. Earth, Parts A/B/C.* **114**, 102792 (2019).
34. Aziz, A., Basheer, F., Sengar, A., Khan, S. U. & Farooqi, I. H. Biological wastewater treatment (anaerobic-aerobic) technologies for safe discharge of treated slaughterhouse and meat processing wastewater. *Sci. Total Environ.* **686**, 681–708 (2019).
35. Amuda, O. & Alade, A. Coagulation/flocculation process in the treatment of abattoir wastewater. *Desalination* **196**, 22–31 (2006).
36. Lu, S., Hu, H., Sun, Y. & Yang, J. Effect of carbon source on the denitrification in constructed wetlands. *J. Environ. Sci.* **21**, 1036–1043 (2009).
37. Kumar, T., Bhargava, R., Prasad, K. H. & Pruthi, V. Evaluation of vermifiltration process using natural ingredients for effective wastewater treatment. *Ecol. Eng.* **75**, 370–377 (2015).
38. Jo, J.-Y., Kim, J.-G., Tsang, Y. F. & Baek, K. Removal of ammonium, phosphate, and sulfonamide antibiotics using alum sludge and low-grade charcoal pellets. *Chemosphere* **281**, 130960 (2021).
39. Michael, S., Paschal, C., Kivevele, T., Rwiza, M. J. & Njau, K. N. Performance investigation of the slaughterhouse wastewater treatment facility: a case of Mwanza City Slaughterhouse, Tanzania. *Water Pract. Technol.* **15**, 1096–1110 (2020).
40. Gürel, L. & Büyükgüngör, H. Treatment of slaughterhouse plant wastewater by using a membrane bioreactor. *Water Sci. Technol.* **64**, 214–219 (2011).
41. Singh, R., D'Alessio, M., Meneses, Y., Bartelt-Hunt, S. & Ray, C. Nitrogen removal in vermifiltration: Mechanisms, influencing factors, and future research needs. *J. Environ. Manag.* **281**, 111868 (2021b).
42. Qi, W.-K. et al. An anoxic/oxic submerged constructed wetlands process for wastewater treatment: Modeling, simulation and evaluation. *Ecol. Eng.* **67**, 206–215 (2014).
43. Jia, W. et al. Nitrous oxide emissions from surface flow and subsurface flow constructed wetland microcosms: effect of feeding strategies. *Ecol. Eng.* **37**, 1815–1821 (2011).

44. Grace, M. A., Healy, M. G. & Clifford, E. Performance and surface clogging in intermittently loaded and slow sand filters containing novel media. *J. Environ. Manag.* **180**, 102–110 (2016).
45. Hua, G., Zhu, W., Zhao, L. & Huang, J. Clogging pattern in vertical-flow constructed wetlands: Insight from a laboratory study. *J. Hazard. Mater.* **180**, 668–674 (2010).
46. Wang, D. B. et al. Effects of earthworms on surface clogging characteristics of intermittent sand filters. *Water Sci. Technol.* **61**, 2881–2888 (2010).
47. Matos, M. et al. Key factors in the clogging process of horizontal subsurface flow constructed wetlands receiving anaerobically treated sewage. *Ecol. Eng.* **106**, 588–596 (2017).
48. Bustillo-Lecompte, C. F., Mehrvar, M. & Quiñones-Bolaños, E. Combined anaerobic-aerobic and UV/H2O2 processes for the treatment of synthetic slaughterhouse wastewater. *J. Environ. Sci. Health, Part A* **48**, 1122–1135 (2013).
49. Singh, R., Bhunia, P. & Dash, R. R. COD removal index—a mechanistic tool for predicting organics removal performance of vermifilters. *Sci. Total Environ.* **643**, 1652–1659 (2018).
50. Zhao, Y. Q., Babatunde, A. O., Zhao, X. H. & Li, W. C. Development of alum sludge-based constructed wetland: An innovative and cost-effective system for wastewater treatment. *J. Environ. Sci. Health - Part A Toxic./Hazard. Substances Environ. Eng.* **44**, 827–832 (2009).
51. Baird, R. B., Eaton, A. D., Rice, E. W. & Bridgewater, L. *Standard methods for the examination of water and wastewater*. Vol. 23 (American Public Health Association, 2017).
52. Parkin, T. et al. Chamber-based trace gas flux measurement protocol. *USDA-ARS GRACEnet*, 1–28 (2003).
53. Pedescoll, A. et al. Practical method based on saturated hydraulic conductivity used to assess clogging in subsurface flow constructed wetlands. *Ecol. Eng.* **35**, 1216–1224 (2009).

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AUTHOR CONTRIBUTIONS

Writing – Original Draft: R.S.; Visualization: R.S., M.D'A.; Writing – review & editing: R.S., C.R., D.N.M., L.M.D., Y.M., S.B.-H., M.D'A.; Supervision: C.R., M.D'A.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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