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Advancing Resilient Agricultural Systems: Adapting to and Mitigating Climate Change

Content will focus on resilience to climate change in agricultural systems, exploring the latest research investigating strategies to adapt to and mitigate climate change. Innovation and imagination backed by good science, as well as diverse voices and perspectives are encouraged. Where are we now and how can we address those challenges? Abstracts must reflect original research, reviews and analyses, datasets, or issues and perspectives related to objectives in the topics below. Authors are expected to review papers in their subject area that are submitted to this virtual issue.

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- Emissions and Sequestration
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  - » Prediction of climate change impacts
  - » Physiological changes
- Soil Sustainability
  - » Threats to soil sustainability (salinization, contamination, degradation, etc.)
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- Strategies for Water and Nutrient Management
  - » Improved cropping systems
- Plant and Animal Stress
  - » Protecting germplasm and crop wild relatives
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Climate Change Virtual Issue

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Please contact Jerry Hatfield at jerryhatfield67@gmail.com with any questions.

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Core Ideas

- The system reduced wastewater gas emissions (NH_3=100%; CH_4=100%; CO_2=82%).
- The system reduced potential GHG emissions from dairy wastewater (by up to 100%).
- Temperature significantly increased CH_4 (56%) and NH_3 (53%) emissions from untreated wastewater.
- The vermifilter system's carbon footprint was significantly low (< 2 kg CO_2-e d^{-1}).

Efficacy of a vermifilter at mitigating greenhouse gases and ammonia emissions from dairy wastewater

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ABSTRACT

Dairy effluent is a potential source of gaseous pollutants associated with global warming and soil acidification. Mitigating such emissions during handling and storage requires substantial financial and labor input. This study evaluated a low-cost technology for mitigating gaseous emissions from dairy wastewater. For nine months, a pilot-scale vermifilter system installed on a commercial dairy farm was studied. Bimonthly samples of the dairy wastewater influent and effluent from the vermifilter system were collected. These samples' potential gas emissions (ammonia—NH_3, methane—CH_4, carbon dioxide—CO_2, & nitrous oxide—N_2O) were measured using a closed-loop dynamic flux chamber method. Results indicated the following reductions in emissions of these gases by the vermifilter system: 84 to 100% for NH_3; 58 to 82% for CO_2; and 95 to 100% for CH_4. Nitrous oxide emissions were mainly below our instrument detection limits and were thus not reported. The vermifilter showed the potential of reducing the GWP from the dairy wastewater by up to 100%. This study further indicated that higher ambient temperatures led to higher emissions of CH_4 ($R^2 = 0.56$) and NH_3 ($R^2 = 0.53$) from untreated dairy wastewater. Overall, the vermifilter system has potential to mitigate gaseous emissions from dairy wastewater.

Keywords: *Vermifiltration, Eisenia fetida, gas emissions, dairy wastewater, mitigation*

INTRODUCTION

Greenhouse gases (GHG) have attracted much attention due to their association with global climate. Agriculture contributes 24% of global GHG emissions (IPCC, 2019). Livestock agriculture, in particular, has received more attention for its contribution to GHG emissions and other environmental impacts (Rotz, 2018). Reducing emissions is crucial for enhancing agricultural carbon and nitrogen conservation. The primary sources of emissions on dairy farms are fuels for farm operations, soil-crop management, enteric fermentation, and manure management, with significant emissions being methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) (Ba et al., 2020; IPCC, 2019; USEPA, 2018). According to Aguirre-Villegas and Larson (2017), excreted manure is the second only to enteric CH_4 emissions in GHG emissions on a dairy farm. Inadequate manure handling releases contaminants into the air and water resources (NRCS-USDA, 2009).

Manure management systems gather, transport, treat, store, and apply manure (NRCS-USDA, 2009). Regarding treatment and storage, most dairy farms in the USA use a solids-liquid separation method to manage liquid or slurry dairy manure (Rico et al., 2012). Manure solids are composted and utilized to produce crops or as bedding. Liquids are usually treated anaerobically in lagoons or storage ponds pending recycling as flush water or for irrigation of pasture and crops. The anaerobic conditions in lagoons provide appropriate environments for methanogens, which produce CH_4. Anaerobic lagoons are consequently connected with odors and CH_4 emissions (Kupper et al., 2020). Besides the preceding aspects, dairy wastewater is also a potential source of NH_3 emissions, contributing to soil acidification and particulate matter formation in the atmosphere (Hristov et al., 2009). Lagoon emissions must thus be mitigated while monetizing the savings into carbon credits. Carbon credits are quantifiable and verifiable emission reductions (Maraseni et al., 2021) that discourage GHG emissions to reduce climate change risk, encourage low-carbon innovation, and raise new public revenue.

Recent shifts in dairy production systems have spurred interest in quantifying and mitigating gaseous emissions. Dairy farmers can earn carbon credits by lowering gas emissions, odors, and other volatile organic compounds. Approaches to mitigate gas emissions during manure treatment and storage include proper design and sizing of storage structures (Aboltins et al., 2017), use of lagoon covers (Dougherty et al., 2017; VanderZaag et al., 2008), avoiding aeration and agitation (Chastain & Henry, 2010; Owusu-Twum & Sharara, 2020), energy recovery (Cantrell et al., 2008; Yarberry et al., 2019), use of chemical processes, such as NH_3 stripping and struvite precipitation (Arogo et al., 2006; Vendramelli et al., 2017), segregating manure and urine to reduce the contact of enzymes in the feces with the urea (Ndegwa et al., 2008), and acidifying of the manure (Cao et al., 2020; Sokolov et al., 2019). Approaches like proper design and sizing, avoiding aeration and agitation, and acidifying manure cannot solely mitigate emissions and should be used in combinations. Other approaches like covering lagoons are associated with high investment costs and the risk of gas leaks and explosions (Tauseef et al., 2013). A low-cost, sustainable system is needed to reduce gas emissions from dairy wastewater storage.

Vermifiltration is a low-cost, environmentally sustainable technology for the treatment of wastewater (Singh et al., 2019a; Singh et al., 2021). The technology is an aerobic system using microorganisms and earthworms in a filter bed media (Singh et al., 2017). The earthworms enhance biochemical reactions in the system by ingesting and digesting wastewater organics and nutrients (Sinha et al., 2007) and creating aerobic environments for microbial growth. The low cost of the system is due to the simple structures and fewer accessories as compared to other technologies. This technology could be crucial in mitigating dairy wastewater emissions. A study by Lai et al. (2018) reported that vermifiltration reduced the emission of NH_3 from dairy wastewater by 90% but reported an increase in N_2O, CH_4, and CO_2 emissions on the vermifilter surface system. Additional studies are needed to examine this technology's efficacy in mitigating GHG and NH_3 emissions from dairy wastewater. In this study, we: (1) examined the potential mitigation of GHG and NH_3 from dairy wastewater treatment in vermifilter systems and (2) estimated the system's greenhouse footprint.

MATERIALS AND METHODS

Site of the Study

This study was conducted on a commercial dairy farm in Yakima County, Washington state. The dairy operates free-stall barns and manure-flush systems coupled with screw-press solids-liquid separators. A side stream of the liquid from the solids-liquid separators was used in the study. Solids separated wastewater was used as the vermifiltration system works best with low solids in the liquid-stream. Although this is a potential limitation of the vermifilter system, but most diaries, operating a manure-flush system have some form of solids-liquid separation. Supplementary Table 1 shows the initial characteristics of the dairy wastewater.

This study used a pilot-scale vermifilter Biofiltro BIDA®, installed on-site and operated under the prevailing conditions. The study spanned from April to December 2019, with a three-month acclimation period. The vermifilter system was then studied for six months spanning three seasons (summer, autumn, and winter). While the six-month study period was mainly dictated by resource availability, it was also deemed a sufficient period to collect adequate data to evaluate this system statistically. During the 6-months, ambient temperatures ranged between 0 and 30 °C (10 to 29 °C, and 0 to 10 °C in the first three and last three months respectively), the area received between 30.5 to 243.8 mm of rain monthly, wind speeds ranged from 0 to 17 ms^{-1}, and humidity levels were between 15 to 100%. The first three months were generally hotter, received less rainfall, and had lower humidity than the last three months. The vermifilter system was covered, and hence no interference from rain and runoff.

The Vermifilter System Design

The vermifilter system used was a vertical subsurface flow type with an influent-holding tank, a pump station, a filter bed, and an effluent reservoir. The same vermifilter was as used in Miito et al. (2021). Figure 1a shows a schematic of this system, while Figure 1b shows a photo of the actual unit. The vermifilter-bed was constructed from an ordinary shipping container (L x W x H: 6.1 m x 2.4 m x 2.6 m) with polyethylene linings. The top layer (0.2 m of a mixture of wood shavings and chips) was inoculated with approximately 300 kg of *Eisenia fetida* to obtain an earthworm density of approximately 12,000 worms m^{-3}. Previous studies recommend using the earthworm species *Eisenia fetida* and earthworm densities between 10,000 to 15,000 worms m^{-3} (Samal et al., 2017; Singh et al., 2021).

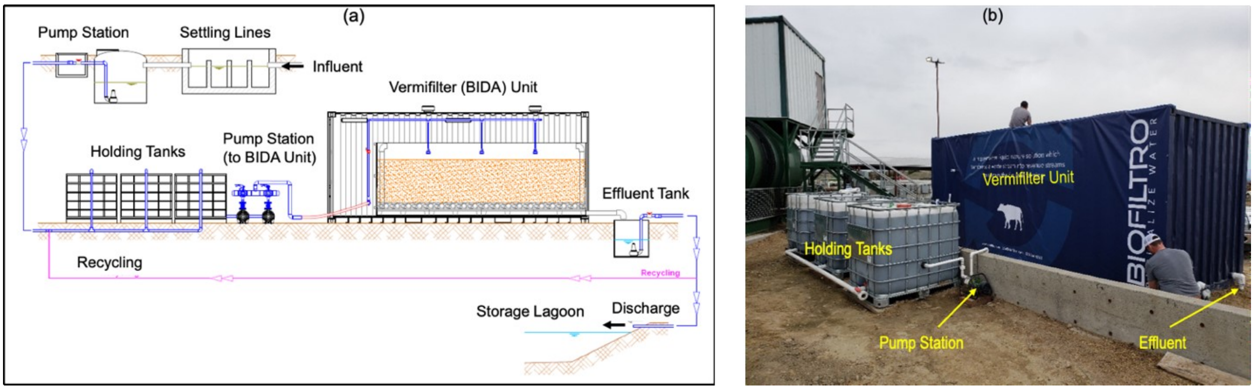

Figure 1. Schematic (a) and photo (b) of the vermifiltration system used in this study (Miito et al., 2021).

The top organic layer (depth 0.50 m) provided a medium for forming a biofilm, consisting of microbes and bacteria that feed off the organic matter and nutrients in the wastewater. Layers of finely crushed stones (depth 0.35 m) and cobblestones (depth 0.35 m) were also incorporated into the unit for improved filtration. A bottom drainage basin was also included, which consisted of thick pallets lining the floor of the vermifilter. During the 3-month acclimation period, dairy wastewater was added to enable biofilms and microbial growth in the vermifilter. After the acclimation period, wastewater from the holding tank was intermittently irrigated uniformly from the top of the vermifilter-bed via rotary head sprinklers every 30 min. The holding tank ensured an uninterrupted wastewater flow into the vermifilter unit and was constantly refilled. A wireless telemetry monitoring and control platform (RF-C1 WiseConn, Fresno, CA) was also installed to monitor the pH, flow rate, oxidation-reduction potential (ORP), and dissolved oxygen (DO) in the system. The telemetry system had sensors embedded in the vermifilter bed and the wastewater pipes. A digital thermometer (B07JBW8VPX, BTMETER, Zhuhai, China) measured the ambient temperature. The wastewater characteristics, specifications, and design parameters of the vermifilter system are presented in Table 1, with the hydraulic, organic, solids, and nitrogen loading rates computed using standard wastewater treatment design equations.

Table 1. Wastewater characteristics & technical specifications of the pilot vermifilter system

Specifications	Values
Chemical oxygen demand (g L^{-1})	2.9 ± 0.5
Total solids (g L^{-1})	7.7 ± 1.4
Total suspended solids (g L^{-1})	2.5 ± 0.8
Total nitrogen (mg/L)	858 ± 247.0
Total ammonia nitrogen (mg L^{-1})	322 ± 69.2
Organic Nitrogen (mg L^{-1})	233 ± 42.8
Nitrate nitrogen (mg L^{-1})	203 ± 93.3
Total Phosphorus (mg L^{-1})	92.4 ± 27.7
Orthophosphates (mg L^{-1})	24.0 ± 7.2
Length x Width x Depth (m)	4.9 x 2.4 x 1.2
Flow rate (m^3 d^{-1})	5.7
Earthworm density (worms m^{-3})	12,000
Total volume (m^3)	0.9
Surface area (m^2)	11.9
Hydraulic loading rate (m^3 m^{-2} d^{-1})	0.5
Organic loading rate (kg COD m^{-2} d^{-1})	1.4 ± 0.2
Solids loading rate (kg TSS m^{-2} d^{-1})	1.2 ± 0.5
Nitrogen loading rate (kg TN m^{-2} d^{-1})	0.4 ± 0.1
Hydraulic retention time (h)	4

Emissions Measurements

The vermifilter systems' influent and effluent dairy wastewater streams were monitored for gaseous emissions. The gases measured included NH_3, CO_2, N_2O, and CH_4, which are typical in dairy operations. Custom-made flux chambers (1 per sample per replicate) were used. Briefly, the lids of eleven airtight 7.6-liter plastic pails (0.24-m diameter x 0.14-m depth, 0.05 m^{-2} footprint) were drilled and fitted with two 2.5-cm airtight hose barbs (Swagelok, Kent WA) on which (2.5 cm diameter, 0.5 m length) Perfluoro alkoxy alkane (PFA) tubing (Swagelok, Kent WA) were connected. The tubing was connected to the multi-point sampler and the Innova gas analyzer assembly. A CAI multi-point sampler (CAI 700 Multi-Point Sample Sequencer) and a photoacoustic IR analyzer (Innova 1412, LumaSense Technologies Inc., Ballerup, Denmark) were used. The INNOVA 1412 analyzer was fitted with NH_3, CO_2, N_2O, $CH_{4,}$ MeOH, and H_2O filters to enable such internal cross compensation among these gases using an internal cross compensation algorithm. According to the manual, the instrument had minimum detection limits of 1.5 ppm for CO_2, 0.4 ppm for CH_4, 0.03 ppm for N_2O, and 1.0 ppm for NH_3.

The vermifilter system's upstream (influent wastewater) and downstream (effluent wastewater) were sampled bimonthly eleven times. For each event, 3.8 L of representative wastewater grab samples were each obtained at the inlet holding tank (N = 3) and outlet tank (N = 3) and loaded into separate dynamic flux chambers, which were sealed off immediately. Air samples from the headspaces of the chamber were drawn into the analyzer to determine concentrations of the gases in the headspace air. The headspace air samples were then returned to the headspace after the analysis. Effectively, cumulative total concentrations of each gas were determined over a 32-min period. In general, the concentrations of these gases would be saturated (steady state) in the headspace within this time. All emission measurements were done in triplicates, separate flux chambers, and on-site. Previous studies have used this flux chamber method to measure manure emissions (Joo et al., 2012; Park et al., 2010; Peterson et al., 2020; Sun et al., 2014).

Three sampling events (start, middle, and end of the six months) were performed to determine surface emissions from the vermifilter. Plastic pail flux chambers (0.3-m diameter x 0.43-m depth, 0.07 m^{-2} footprint) were used. The chambers were buried 0.05 m deep in the filter bed to create an airtight seal and connected to the INNOVA 1412 analyzer. Air samples were drawn from the chamber's headspaces, and gaseous concentrations of NH_3, CO_2, N_2O, and CH_4 were recorded until steady-state conditions were observed. For this scenario, steady-state conditions were observed after 20 minutes. All measurements were done in triplicates at randomized positions on the vermifilter surface.

The wastewater samples were also analyzed for total nitrogen (TN), chemical oxygen demand (COD), total solids (TS), and volatile solids (VS). The TN concentration was determined using the persulfate method and the COD concentration using the HACH Reactor Digestion Method 8000). For TS and VS analysis, unfiltered samples were placed in an oven at 105 °C overnight and then in a muffle furnace at 550 °C for 1 hour, and mass loss was measured. System nitrogen, organic and solid loading rates were then calculated using these properties.

Emissions Computations

The measured gas concentrations in the flux chambers were truncated to exclude the first five readings of each sample period, ensuring that only well-defined transient conditions were considered. The gas fluxes were then calculated by linear regressions of concentration versus time plots during the transient conditions, the dynamic chamber headspace volume (0.01 m^3), and the chamber's emitting surface area (0.05 m^2). The emission fluxes were then computed on the emitting surface area basis using Eq. (1).

$$flux\left(\frac{mg}{m^2\ day}\right) = \frac{ppm}{min} \times \frac{M}{22.4} \times \frac{273}{(273+T)} \times \frac{P}{1013} \times \frac{V}{A} \qquad (eq\ 1)$$

Where: M = molecular weight of gas; T = Ambient temperature (°C); P = atmospheric pressure (1013 hPa); V = headspace chamber volume (m^3); and A = emitting-surface area (m^2).

For each event, computed upstream and downstream emissions fluxes were used to calculate or estimate the respective emission mitigation efficacies, according to Eq. (2).

$$Eff = \left(\frac{E_i - E_o}{E_i}\right) \times 100 \qquad (eq\ 2)$$

Where: Eff = Reduction efficiency (%); E_i = Emissions from Influent samples ($g\ m^{-2}\ d^{-1}$); E_o = Emissions from Effluent tank discharge ($g\ m^{-2}\ d^{-1}$).

Carbon Credits Analysis

A theoretical framework for a market-based approach to emission control has been established (Dumanski, 2004). In this framework, all GHGs are converted to CO_2 equivalents, which are then traded on carbon markets. This market operates similarly to financial markets, and carbon credits are the currency used in these markets. In this study, the GWPs (CO_2 equivalents) were computed using the 100-year GWP emission factors of 25 for CH_4 and 298 for N_2O (IPCC, 2019). Using Eq. (3), the total GWP emissions of the dairy wastewater streams were computed:

$$GWP\ (CO_2\ Equivalent) = 25CH_4 + 298\ N_2O \qquad (eq\ 3)$$

Furthermore, a dairy farm that has already fully implemented the vermifilter technology was used for scenario analysis. The dairy farm in question has 10,000 dairy cows, produces 19,000 L d^{-1} of dairy wastewater, and operates a 186 m^{-2} vermifilter. Total CO_2 equivalents for this dairy wastewater were computed using the global warming potentials from the sampling events. Using the World Bank (2020) approach, theoretical carbon credits were then calculated for the various sampling events using the USA's California cap-and-trade price of 16.89 US\$ $tCO_2\text{-}eq^{-1}$. These carbon credits were then used as estimates of carbon credit savings if a dairy farm incorporated a vermifilter system on their wastewater handling train.

Material flow analysis

The material flow analysis method was used to analyze current nutrient flows (TN and TP), organics (COD), solids (TS), and emissions (NH_3, CH_4, CO_2, N_2O) into and out of the vermifilter system. Biomass growth was considered as retention. The objective was to determine material retention and loss in a continuous vermifiltration system. The MFA is a technique for analyzing material and substance flows into, within, and out of a system with defined spatial and temporal boundaries. STAN 2.6 (subSTance flow ANalysis) software was used to calculate the MFA for this study. STAN software was selected since it uses a graphical interface for implementing MFA. In the software, the standard errors of all measured parameters were used as uncertainties. This analysis used a one-day temporal boundary and the vermifilter system as the spatial boundary. The study's mass/substance flows were all based on wet weight.

Data Analysis

The mean and standard deviation of the gas fluxes, reduction efficiencies, and GWPs were calculated. ANOVA was used to compare mean responses of emission fluxes and GWPs, with post-hoc analysis using Tukey's HSD test, where ANOVA revealed significant differences. $P \leq 0.05$ denoted significant differences between group means. This study used spearman's correlation to examine the correlations between ambient temperatures, emission fluxes, and reduction efficiencies. Further linear regression studies were undertaken to assess the effect of temperature and influent parameters on the vermifilter performance. The R square was used to evaluate the regression models.

RESULTS AND DISCUSSIONS

Gas Emissions

Figure 2 and supplemental Figure S1 indicated that dairy wastewater emits NH_3 (10 to 118 mg m^{-2} d^{-1}), CH_4 (440 to 1870 mg m^{-2} d^{-1}), and CO_2 (5864 to 21663 mg m^{-2} d^{-1}). However, N_2O concentrations were mainly below our instrument's detectable limits, resulting in considerable N_2O–flux measurements variability (-0.9 to 1.1 mg m^{-2} d^{-1}). Most past studies on dairy wastewater emissions focused on anaerobic lagoons and other manure storage systems (Leytem et al., 2017; Leytem et al., 2011; VanderZaag et al., 2010). Leytem et al. (2011) and VanderZaag et al. (2010) reported emission values of 2 g m^{-2} d^{-1} for NH_3, 103 g m^{-2} d^{-1} for CH_4, 637 g m^{-2} d^{-1} for CO_2, and 0.49 g m^{-2} d^{-1} for N_2O. Harper et al. (2009) and Leytem et al. (2011) also reported emission fluxes in ranges of 2 to 6.8 g m^{-2} d^{-1} for NH_3, 22 to 60 g m^{-2} d^{-1} for CH_4, and 0.2 to 0.9 g m^{-2} d^{-1} for N_2O, from dairy wastewater in anaerobic ponds. Because vermifilter emissions have not been studied, studies from other manure systems were used as a reference.

The use of fresh unseparated manure and the emission contribution of biological reactions in wastewater ponds led to higher emission fluxes in prior studies (Harper et al., 2009). The current investigation used wastewater that has been sedimented and screw-pressed to separate solids, yielding 3.4 g L^{-1} volatile solids (~50% TS) in the liquid-stream. The lower volatile solids (VS) content, in the liquid-stream, may explain the current study's lower emissions than reported by Leytem et al. (2017) in anaerobic lagoons and storage tanks (with VS levels of up to 6.2 g L^{-1}).

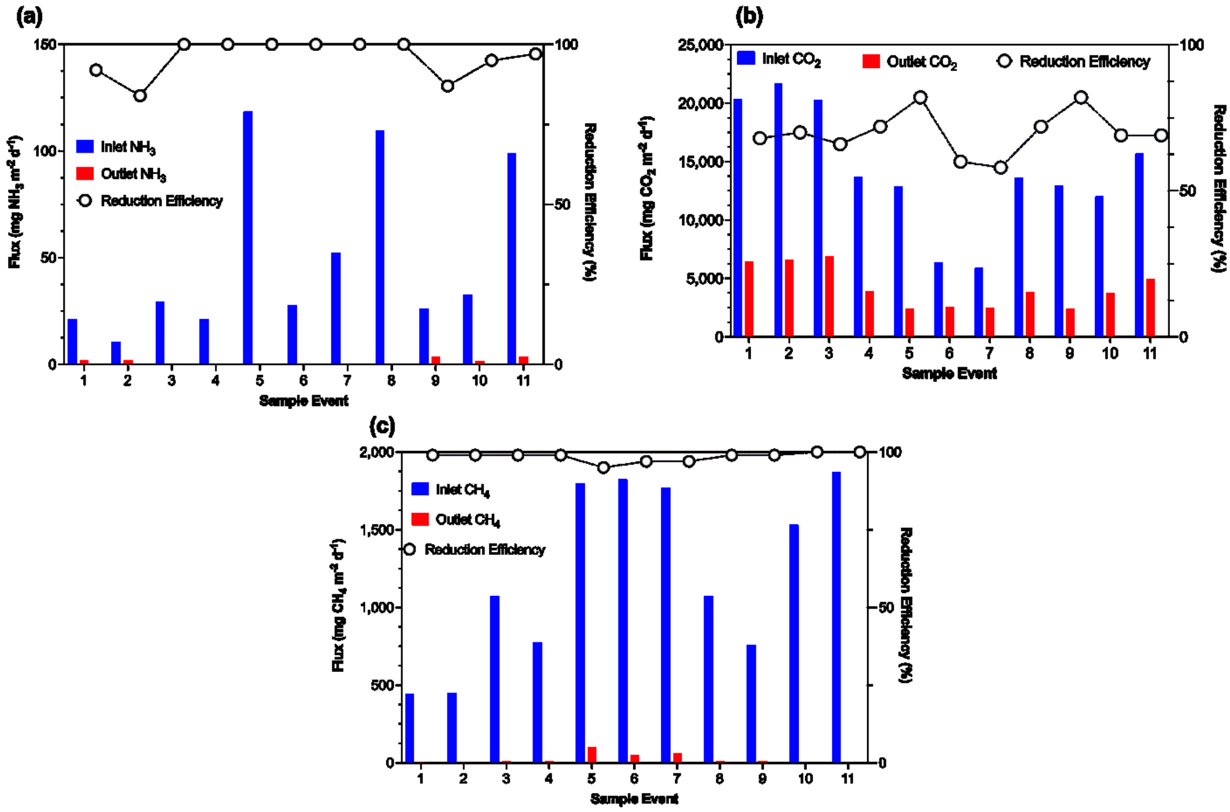

Figure 2. Potential emissions of untreated (influent) and treated (effluent) wastewater in the vermifilter and the efficacies of the system at mitigating emissions of: (a) ammonia, (b) methane, and (c) carbon dioxide.

The main source of gas emissions is the microorganism-mediated biological transformation of organics in dairy effluent. Dairy wastewater produces ammonia when organic nitrogen species are mineralized to ammonium and combined with NH_3 and ammonium nitrogen in manure (Ba et al., 2020). Carbon dioxide is produced by microbial respiration activities in wastewater. On the other hand, CH_4 emissions come from methanogenic processes in the anaerobic layers of dairy wastewater (Laubach et al., 2015).

The effluent wastewater gas emissions were much lower than the influent wastewater emissions, indicating a net reduction by the system's treatment. The vermifilter system resulted in 84 to 100% emissions reduction for NH_3, 95 to 100% for CH_4, and 58 to 82% for CO_2 (Figure 2). During the vermifiltration process, the total solids concentrations of the wastewater also declined by 21 ± 7.0%, while the volatile solids concentration decreased by 53 ± 3.0%. The emissions reductions through the system are thus attributed to organics and solids reduction in the wastewater. In a similar study, Lai et al. (2018) reported a reduction of up to 90% in NH_3 emissions. For reductions of CH_4 and CO_2 emissions, however, the results of this study differed from those reported by Lai et al. (2018), who reported increases of CH_4 emissions by 84.4% and CO_2 emissions by 6.1% in the effluent of a vermifilter system treating dairy wastewater. Limited information is given in Lai et al. (2018) on the organic characteristics of the dairy wastewater and the oxygen transfer rates in the vermifilter. These factors may have contributed to more anoxic conditions in the vermifilter, thus higher emissions.

However, from the telemetry station monitoring the vermifilter system, the pH of influent dairy wastewater varied between 7.5 ± 0.3, while the effluent pH ranged between 6.6 ± 0.4. According to Hristov et al. (2011), lower pH ranges in wastewater imply a shift of the equilibrium to more NH_4^+ than NH_3 in solution, and hence less ammonia emissions were observed from the effluent. The reduction in wastewater pH through the system is attributed to the aerobic degradation of organics, resulting in the generation of acidic compounds, such as carbonic acid. Also, in the vermifilter system, the ORP ranged between 100 to 200 mV, and the DO ranged between 2 to 4 mg L^{-1} during the treatment. These ORP and DO ranges show that the vermifilter system was mainly aerobic, favoring complete oxidation and nitrification.

Some other studies have reported on the effect of vermifilters on emissions from other wastewater streams. Luth et al. (2011) reported that vermifilters could reduce NH_3 emissions by up to 40% and CH_4 by 25% following piggery wastewater treatment. Li et al. (2008) also observed a reduction in NH_3 emission of about 50% in piggery wastewater treated in a vermifilter. The reductions in NH_3 and N_2O were attributed to nitrification-denitrification processes (Luth et al., 2011; Samal et al., 2017), aided by the mechanical activities of earthworms (Singh et al., 2019b). Earthworm activities such as tunneling and burrowing promote aeration in the system, thus enhancing nitrification (Wang et al., 2011; Zhao et al., 2010) and reducing CH_4 production in the systems (Arbeli et al., 2006).

Material Flow Analysis

Results (Figure 3) revealed the following reductions in the wastewater characteristics through the vermifilter: total solids (21 ± 7.0%), volatile solids (53 ± 3.0%), COD (45 ± 4.0%), total nitrogen (77 ± 7.9%), and total phosphorus (47 ± 5.6%). The vermifilter further emitted 1.1 ± 0.2 kg [CO_2] d^{-1}, 2.7 ± 1.9 g [NH_3] d^{-1}, 21.6 ± 16.2 g [CH_4] d^{-1}, 4.9 ± 1.7 g [N_2O] d^{-1} and accumulated 19.2 kg [biomass] d^{-1}. The assumption was that all the water that entered the system was expelled and that the losses in TS, COD, TN, and TP were attributable to emissions or biomass growth and retention. Our research did not determine which percentage went to biomass increase and which proportion could be attributed to the system's retention. Supplemental Figure S2 presents more details of emissions from the exposed vermifilter surface. Because the literature on vermifilter emissions is limited, this study compared vermifilter emissions to dairy wastewater storage system emissions. According to Leytem et al. (2013), wastewater ponds and lagoons treating dairy wastewater contribute 96 to 2464 kg [NH_3] d^{-1}, 471 to 8281 kg [CH_4] d^{-1}, and 5 to 108 kg [N_2O] d^{-1}. The vermifilter surface emissions in this study were significantly lower than emissions from typical wastewater ponds and lagoons.

The TN mass balance analysis showed that 24 to 28% of initial TN load was released into the effluent, a negligible 0.16 to 0.3% as vermifilter surface emissions (NH_3 and N_2O), while the rest (>65%) was assumed as either mineralized or utilized for biomass growth and possible retention in filter media. Biomass growth (earthworms) and possible mass retention by adsorption on the filter surfaces are thus major nitrogen sinks during vermifiltration. Singh et al. (2021) also reported biomass growth during vermifiltration of feedlot runoff. Biomass growth and retention are further justified since castings and earthworms need to be harvested after a given period.

Due to ammonification, nitrification, and denitrification of the nitrogen forms occurring in the vermifilter, the fractions of these species vary significantly. Previous studies have, however, reported little ammonium or ammonia accumulation in the system, as it is either fully mineralized to nitrate-nitrogen or lost as dinitrogen gas. As a result, this nitrogen sink is projected to emit little or no NH_3. Regarding the organic matter, 54 to 58% of initial COD was released into the effluent. The remainder was assumed to be either stabilized or humified in the system, generating carbon dioxide. Because CO_2 emissions are biogenic (from natural oxidation), they do not add to GHG emissions (Alonso-Moreno et al., 2018). Generally, our findings indicate that surface emissions from the vermifilter system are minimal, implying that the system reduces greenhouse gas emissions from dairy wastewater without increasing surface emissions from the vermifilter. A complete material balance should, however, be performed to indicate further sources, sinks, and transformations mechanisms in the system.

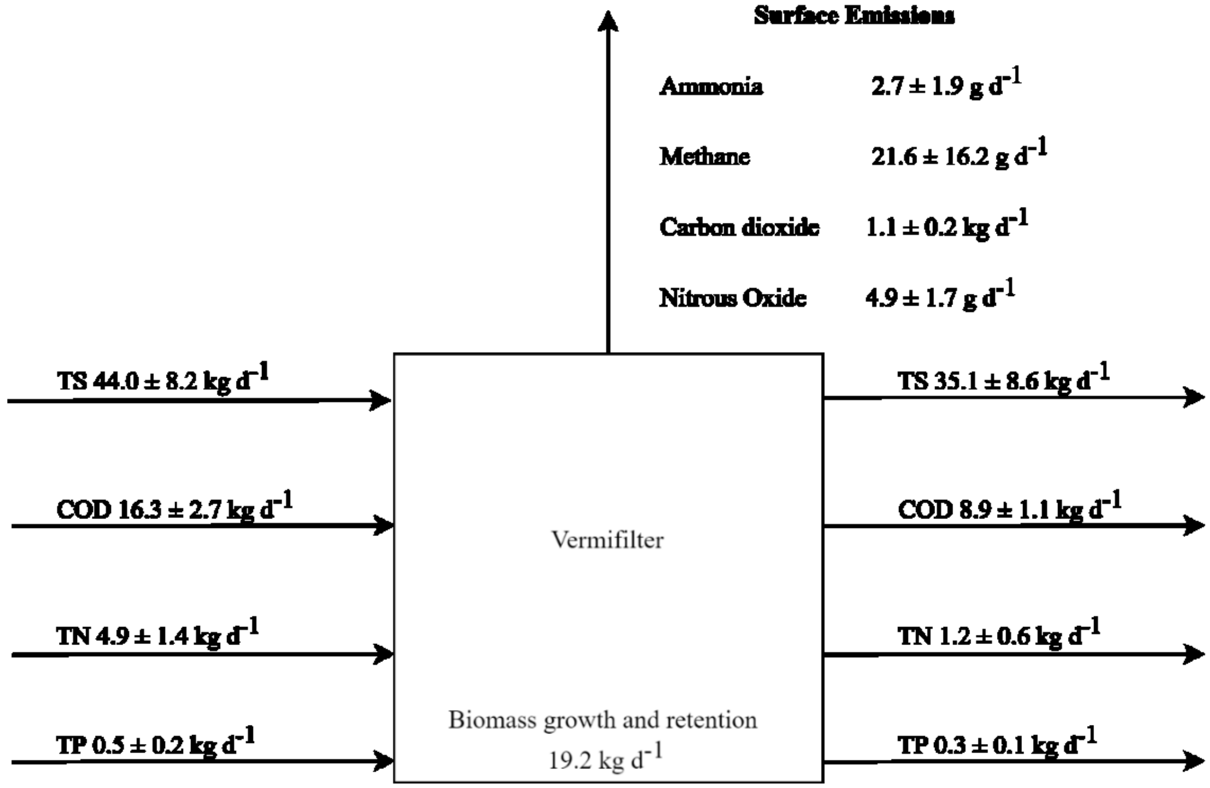

Figure 3. Material flow in the vermifiltration system.

Net GHG and Carbon Trading

The GWPs of the untreated wastewater ranged between 11.2 to 47.1 g CO_2-e m^{-2} d^{-1} (Figure 4). Computations of GWP from the emission flux values reported by Leytem et al. (2017) indicate that storage ponds for similar wastewater can contribute up to 2.5 kg CO_2-e m^{-2} d^{-1}. The range of values from Leytem et al. (2017) is typical of a dairy wastewater lagoon. The current study utilized wastewater from a solids-liquid separator, partly explaining the lower GHGs. The vermifiltration system reduced the dairy wastewater stream's existing GWP (Figure 4) by 94 to 100%. The reduction in GWP is due to decreased existing CH_4 and N_2O emissions from the dairy wastewater. The presence of volatile solids in the effluent dairy wastewater stream, on the other hand, implies the potential of more emissions after treatment from this more recalcitrant fraction of volatile solids.

In the vermifilter system, CH_4 and N_2O emissions reduction is partly attributed to the synergistic action of earthworms and microorganisms that enhance aeration in the system. The increased aeration in the vermifilter leads to more aerobic decomposition of the wastewater's organics, thus reducing CH_4 and N_2O emissions (Lubbers et al., 2013). Hence, it is postulated that employing a vermifilter to treat dairy wastewater before storage in lagoons would significantly lower the GWP of the wastewater during the storage period. This, however, is dependent on how much volatile solids the vermifiltration system can remove.

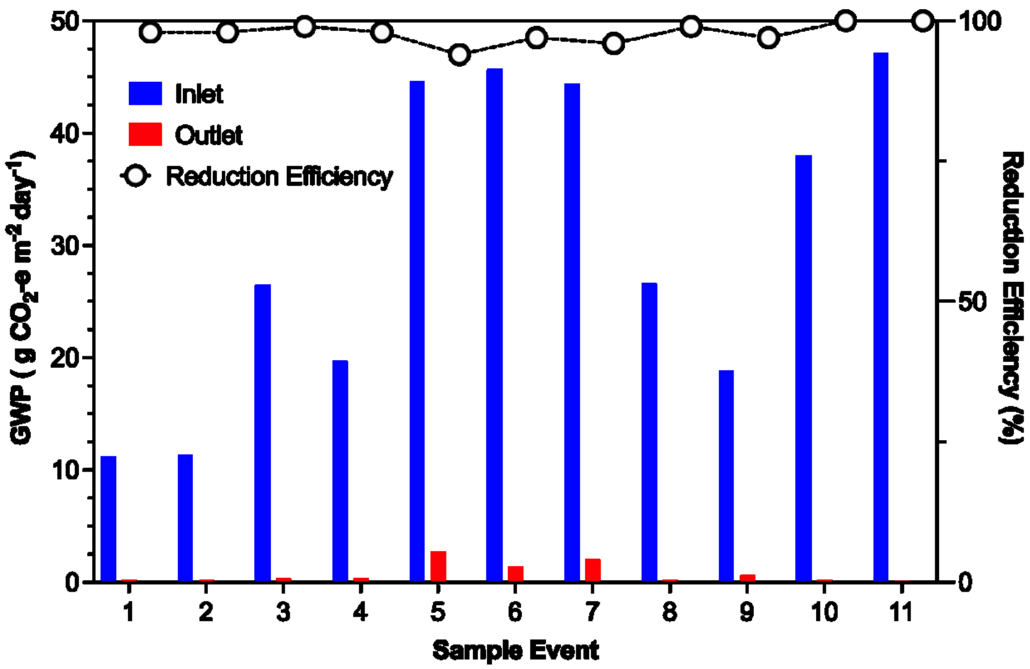

Figure 4. GWP of untreated (inlet) and treated (outlet) dairy wastewater and reductions in the vermifiltration system.

The carbon credit analysis reveals the significant potential of carbon credits from reduced gas emissions using the vermifilter system treating dairy wastewater on a dairy farm. Our study (Table S1) suggests that dairy farms that adopt this technology, therefore, could receive back significant annual carbon credits. These profits could be used to finance supplementary technologies for manure management (such as composting, lagoon covering, etc.) to further nutrient removal and recovery. However, additional considerations should be made on the capital costs, maintenance, and operation costs of the system, as the carbon savings may be nullified by these costs of the vermifilter system. There is thus a need for a more comprehensive economic analysis of the system vs. the carbon credits generated.

Despite the reported potential for carbon credits to dairy farmers, this theoretical analysis is solely based on emissions reduction in dairy wastewater streams and not on the entire manure management chain. Other emissions from further biotransformation of wastewater, separated solids, manure application, feeds are needed to determine the system's carbon credits.

Ambient Temperature and Influent Conditions

The correlation analysis (Supplemental Figure S3) indicated significant ($p < 0.05$) and strong correlations between CH_4 and CO_2 emissions and the prevailing ambient temperature ($R_{CH4} = 0.7$ and $R_{CO2} = -0.8$). Specifically, CH_4 emissions positively correlated with temperature, while CO_2 emissions were negatively correlated with temperature. The reduction of NH_3 emissions in the vermifilter system was also significant and positively correlated ($R = 0.7$) with ambient temperature. A strong positive correlation between influent NH_3 and CH_4 emission fluxes ($R = 0.6$) was also observed. However, emissions of CH_4 and CO_2 from untreated wastewater had a significantly strong negative correlation ($R = -0.7$). The results showed a significantly strong positive correlation between ammonia reduction efficiencies and methane emissions from untreated wastewater ($R = 0.6$). The reduction efficiency of N_2O was also positively correlated with its emission from untreated wastewater ($R = -0.6$). These results agree with previous studies by Leytem et al. (2017) and Arndt et al. (2018), who both reported higher emissions in lagoons and dairy wastewater storage ponds during higher ambient-temperature conditions.

Mathematical regression models (Table 2) showed that untreated wastewater CH_4 emissions increased with ambient temperature ($R^2 = 0.54$), while untreated wastewater CO_2 emissions decreased ($R^2 = 0.65$). The NH_3 emissions reduction efficiencies were also significantly higher at higher ambient temperature conditions ($R^2 = 0.53$). There was also a considerable increase in untreated wastewater NH_3 emissions with increased treated wastewater CH_4 emissions ($R^2 = 0.31$) and increased treated wastewater CH_4 emissions with a decrease in treated wastewater CO_2 emissions ($R^2 = 0.48$). There was also a significant increase in NH_3 emissions reduction efficiency with increased treated wastewater CH_4 emissions ($R^2 = 0.40$) and increased N_2O emissions reduction efficiency with a decrease in treated wastewater N_2O emissions ($R^2 = 0.31$).

Table 2. Showing Linear regression analysis of Emissions and Ambient Temperature

Parameters	Model	Goodness of Fit
Influent CH_4 emissions vs. Temperature	$CH_4 = -1111 + 45.2\,T$	$R^2 = 0.54$
Influent CO_2 emissions vs. Temperature	$CO_2 = 37722 - 459\,T$	$R^2 = 0.65$
NH_3 reduction vs. Temperature	$NH_3 = 72.0 + 0.47\,T$	$R^2 = 0.53$
Influent NH_3 vs. Influent CH_4	$NH_3 = 817.13 + 7.97\,CH_4$	$R^2 = 0.31$
Influent CH_4 vs. Influent CO_2	$CH_4 = 21890 - 6.41 CO_2$	$R^2 = 0.48$
NH_3 Reduction vs. Influent CH_4	$NH_3 = -4678 + 61.4\,CH_4$	$R^2 = 0.40$
N_2O Reduction vs. Influent N_2O	$N_2O = 0.001 - 0.08\,N_2O$	$R^2 = 0.31$

Our findings demonstrate increased CH_4 emission at higher temperatures, which is attributed to enhanced methanogenic activity. According to Barbera et al. (2019), thermophilic methanogens thrive well between 4 °C to 100 °C but are more active at higher temperatures. These results are consistent with Leytem et al. (2018), who observed higher methane emission fluxes in warmer than cooler periods. They attributed the higher fluxes to higher microbial activity in the wastewater systems. Although higher temperatures are expected to boost microbial activity in wastewater, the observed decrease in upstream CO_2 emissions was surprising. This phenomenon requires more examination.

Higher NH_3 reductions at higher ambient temperatures in the vermifilter are attributed to increased microbial metabolism. According to Singh et al. (2017), higher temperatures (25 to 30 °C) boost the metabolic activity of earthworms. Thus, higher temperatures promote nitrification-denitrification, which reduces downstream emissions. Ambient temperature, therefore, positively influences methane emissions from dairy wastewater and favors the mitigation of ammonia emissions during treatment in the vermifilter system.

The strong association between untreated wastewater CH_4 and NH_3 emissions is attributed to increased microbial activities, surface diffusion, and convection rates in the wastewater. The increase in methanogenic activities in the wastewater influences the methanogenesis rates, hence higher methane emissions (Sommer et al., 2013). On the other hand, higher ammonia emissions rates are due to higher ammonization, diffusion, and convection rates on the emitting surfaces (Sommer et al., 2013) of dairy wastewater. The strong association between ammonia and methane emissions implies that the methanogenic and ammonization processes occur in parallel in this system. The strong negative association of influent CH_4 and influent CO_2 emission fluxes is due to hydrogenotrophic methanogenesis of CO_2, yielding more methane during anaerobic respiration in the wastewater (Xu et al., 2019). Overall, gas emissions and ambient temperature are strongly related. Thus, when evaluating the emission reduction potential of vermifiltration, the ambient temperature should be considered.

Conclusion and Future Directions

This study assessed the potential of mitigating gas emissions from dairy wastewater using a vermifiltration system. Based on the results, the system significantly reduced NH_3, CO_2, and CH_4 emissions. The vermifilter system consequently reduced the GHG of the dairy wastewater, providing savings in carbon-credits credits for dairy farms adopting this technology while maintaining low surface emissions. Results obtained in this study also indicated that emissions of CH_4 and CO_2 from untreated wastewater and NH_3 reduction efficiency in the vermifilter had strong associations with the prevailing ambient temperature. Therefore, it is imperative to consider the prevailing ambient temperature in the operation of vermifilters for emissions reduction. Overall, our findings indicate that vermifilter systems have a high potential for economically mitigating gaseous emissions from dairy wastewater, thus alleviating accruing GWP and generating carbon credits for dairy farmers. Future studies should focus on optimizing hydraulic retention times, hydraulic loading rates, and organic loading rates of vermifilters for emission reduction and performing financial and carbon credit analysis on full-scale vermifilter systems.

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SUPPLEMENTAL MATERIAL

A supplemental material document with pertinent tables and figures is included.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aboltins, A., Priekulis, J., Aboltina, B., Melece, L. 2017. Effect of slurry lagoon redesign on reduction of ammonia emission during livestock manure storage. *Agronomy Research*, **15**(5), 1822-1830.

Aguirre-Villegas, H.A., Larson, R.A. 2017. Evaluating greenhouse gas emissions from dairy manure management practices using survey data and lifecycle tools. *Journal of Cleaner Production*, **143**, 169-179.

Alonso-Moreno, C., Garde, J.J., Zafrilla, J.E., Canales-Vázquez, J., Gueddari, A., García-Yuste, S. 2018. The Carbon Dioxide-Rumen Fermentation Processes-strategy, a proposal to sustain environmentally friendly dairy farms. *Journal of Cleaner Production*, **204**, 735-743.

Arbeli, Z., Brenner, A., Abeliovich, A. 2006. Treatment of high-strength dairy wastewater in an anaerobic deep reservoir: Analysis of the methanogenic fermentation pathway and the rate-limiting step. *Water research*, **40**(19), 3653-3659.

Arndt, C., Leytem, A., Hristov, A., Zavala-Araiza, D., Cativiela, J., Conley, S., Daube, C., Faloona, I., Herndon, S. 2018. Short-term methane emissions from 2 dairy farms in California estimated by different measurement techniques and US Environmental Protection Agency inventory methodology: A case study. *Journal of dairy science*, **101**(12), 11461-11479.

Arogo, J., Westerman, P.W., Heber, A.J., Robarge, W.P., Classen, J.J. 2006. Ammonia emissions from animal feeding operations.

Ba, S., Qu, Q., Zhang, K., Groot, J.C.J. 2020. Meta-analysis of greenhouse gas and ammonia emissions from dairy manure composting. *Biosystems Engineering*, **193**, 126-137.

Barbera, A.C., Vymazal, J., Maucieri, C. 2019. Greenhouse Gases Formation and Emission. in: *Encyclopedia of Ecology (Second Edition)*, (Ed.) B. Fath, Elsevier. Oxford, pp. 329-333.

Cantrell, K.B., Ducey, T., Ro, K.S., Hunt, P.G. 2008. Livestock waste-to-bioenergy generation opportunities. *Bioresource technology*, **99**(17), 7941-7953.

Cao, Y., Wang, X., Liu, L., Velthof, G.L., Misselbrook, T., Bai, Z., Ma, L. 2020. Acidification of manure reduces gaseous emissions and nutrient losses from subsequent composting process. *Journal of Environmental Management*, **264**, 110454.

Chastain, J.P., Henry, S. 2010. Management of lagoons and storage structures for dairy manure.

Dougherty, B., Gray, M., Johnson, M.G., Kleber, M. 2017. Can biochar covers reduce emissions from manure lagoons while capturing nutrients? *Journal of environmental quality*, **46**(3), 659-666.

Dumanski, J. 2004. Carbon sequestration, soil conservation, and the Kyoto protocol: summary of implications. *Climatic change*, **65**(3), 255-261.

Harper, L.A., Flesch, T.K., Powell, J.M., Coblentz, W.K., Jokela, W.E., Martin, N.P. 2009. Ammonia emissions from dairy production in Wisconsin1. *Journal of Dairy Science*, **92**(5), 2326-2337.

Hristov, A.N., Hanigan, M., Cole, A., Todd, R., McAllister, T.A., Ndegwa, P.M., Rotz, A. 2011. Ammonia emissions from dairy farms and beef feedlots. *Canadian journal of animal science*, **91**(1), 1-35.

Hristov, A.N., Zaman, S., Vander Pol, M., Ndegwa, P., Campbell, L., Silva, S. 2009. Nitrogen losses from dairy manure estimated through nitrogen mass balance and chemical markers. *Journal of environmental quality*, **38**(6), 2438-2448.

IPCC. 2019. Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. IPCC.

Joo, H.-S., Ndegwa, P.M., Harrison, J., Whitefield, E., Heber, A.J., Ni, J.Q. 2012. Emissions of ammonia and greenhouse gases (GHG) from anaerobically digested and undigested dairy manure. *2012 Dallas, Texas, July 29-August 1, 2012*. American Society of Agricultural and Biological Engineers. pp. 1.

Kupper, T., Häni, C., Neftel, A., Kincaid, C., Bühler, M., Amon, B., VanderZaag, A. 2020. Ammonia and greenhouse gas emissions from slurry storage - A review. *Agriculture, Ecosystems & Environment*, **300**, 106963.

Lai, E., Hess, M., Mitloehner, F.M. 2018. Profiling of the Microbiome Associated With Nitrogen Removal During Vermifiltration of Wastewater From a Commercial Dairy. *Frontiers in Microbiology*, **9**, 13.

Laubach, J., Heubeck, S., Pratt, C., Woodward, K., Guieysse, B., Van Der Weerden, T., Chung, M., Shilton, A., Craggs, R. 2015. Review of greenhouse gas emissions from the storage and land application of farm dairy effluent. *New Zealand Journal of Agricultural Research*, **58**(2), 203-233.

Leytem, A.B., Bjorneberg, D.L., Al Rotz, C., Moraes, L.E., Kebreab, E., Dungan, R.S. 2018. Ammonia emissions from dairy lagoons in the western US. *Transactions of the ASABE*, **61**(3), 1001-1015.

Leytem, A.B., Bjorneberg, D.L., Koehn, A.C., Moraes, L.E., Kebreab, E., Dungan, R.S. 2017. Methane emissions from dairy lagoons in the western United States. *J Dairy Sci*, **100**(8), 6785-6803.

Leytem, A.B., Dungan, R.S., Bjorneberg, D.L., Koehn, A.C. 2011. Emissions of ammonia, methane, carbon dioxide, and nitrous oxide from dairy cattle housing and manure management systems. *Journal of Environmental Quality*, **40**(5), 1383-1394.

Leytem, A.B., Dungan, R.S., Bjorneberg, D.L., Koehn, A.C. 2013. Greenhouse gas and ammonia emissions from an open-freestall dairy in southern idaho. *J Environ Qual*, **42**(1), 10-20.

Li, Y.S., Robin, P., Cluzeau, D., Bouche, M., Qiu, J.P., Laplanche, A., Hassouna, M., Morand, P., Dappelo, C., Callarec, J. 2008. Vermifiltration as a stage in reuse of swine wastewater: Monitoring methodology on an experimental farm. *Ecological Engineering*, **32**(4), 301-309.

Lubbers, I.M., Van Groenigen, K.J., Fonte, S.J., Six, J., Brussaard, L., Van Groenigen, J.W. 2013. Greenhouse-gas emissions from soils increased by earthworms. *Nature Climate Change*, **3**(3), 187-194.

Luth, Robin, P., Germain, P., Lecomte, M., Landrain, B., Li, Y.S., Cluzeau, D. 2011. Earthworm effects on gaseous emissions during vermifiltration of pig fresh slurry. *Bioresource Technology*, **102**(4), 3679-3686.

Maraseni, T., An-Vo, D.-A., Mushtaq, S., Reardon-Smith, K. 2021. Carbon smart agriculture: An integrated regional approach offers significant potential to increase profit and resource use efficiency, and reduce emissions. *Journal of Cleaner Production*, **282**, 124555.

Miito, G.J., Ndegwa, P., Alege, F., Coulibaly, S.S., Davis, R., Harrison, J. 2021. A vermifilter system for reducing nutrients and organic-strength of dairy wastewater. *Environmental Technology & Innovation*, 101648.

Ndegwa, P.M., Hristov, A.N., Arogo, J., Sheffield, R. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems engineering*, **100**(4), 453-469.

NRCS-USDA. 2009. *Agricultural Waste Management Field Handbook*, https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/technical/ecoscience/mnm/?cid=stelprdb1045935.

Owusu-Twum, M.Y., Sharara, M.A. 2020. Sludge management in anaerobic swine lagoons: A review. *Journal of Environmental Management*, **271**, 110949.

Park, K.-H., Wagner-Riddle, C., Gordon, R.J. 2010. Comparing methane fluxes from stored liquid manure using micrometeorological mass balance and floating chamber methods. *Agricultural and Forest Meteorology*, **150**(2), 175-181.

Peterson, C.B., El Mashad, H.M., Zhao, Y., Pan, Y., Mitloehner, F.M. 2020. Effects of SOP lagoon additive on gaseous emissions from stored liquid dairy manure. *Sustainability*, **12**(4), 1393.

Rico, C., Rico, J.L., García, H., García, P.A. 2012. Solid–liquid separation of dairy manure: distribution of components and methane production. *Biomass and Bioenergy*, **39**, 370-377.

Rotz, C.A. 2018. Modeling greenhouse gas emissions from dairy farms. *Journal of Dairy Science*, **101**(7), 6675-6690.

Samal, K., Dash, R.R., Bhunia, P. 2017. Treatment of wastewater by vermifiltration integrated with macrophyte filter: A review. *Journal of Environmental Chemical Engineering*, **5**(3), 2274-2289.

Singh, R., Bhunia, P., Dash, R.R. 2017. A mechanistic review on vermifiltration of wastewater: Design, operation and performance. *Journal of Environmental Management*, **197**, 656-672.

Singh, R., Bhunia, P., Dash, R.R. 2019a. Optimization of organics removal and understanding the impact of HRT on vermifiltration of brewery wastewater. *Science of the Total Environment*, **651**, 1283-1293.

Singh, R., D'Alessio, M., Meneses, Y., Bartelt-Hunt, S.L., Woodbury, B., Ray, C. 2021. Development and performance assessment of an integrated vermifiltration based treatment system for the treatment of feedlot runoff. *Journal of Cleaner Production*, **278**.

Singh, R., Samal, K., Dash, R.R., Bhunia, P. 2019b. Vermifiltration as a sustainable natural treatment technology for the treatment and reuse of wastewater: A review. *Journal of Environmental Management*, **247**, 140-151.

Sinha, R.K., Bharambe, G., Bapat, P. 2007. Removal of high BOD and COD loadings of primary liquid waste products from dairy industry by vermifiltration technology using earthworms. *Indian Journal of Environmental Protection*, **27**(6), 486.

Sokolov, V., VanderZaag, A., Habtewold, J., Dunfield, K., Wagner-Riddle, C., Venkiteswaran, J.J., Gordon, R. 2019. Greenhouse gas mitigation through dairy manure acidification. *Journal of environmental quality*, **48**(5), 1435-1443.

Sommer, S.G., MLC, T.S., Jensen, L.S. 2013. *Animal manure recycling: treatment and management*. John Wiley and Sons.

Sun, F., Harrison, J., Ndegwa, P., Johnson, K. 2014. Effect of manure treatment on ammonia emission during storage under ambient environment. *Water, Air, & Soil Pollution*, **225**(9), 2094.

Tauseef, S., Premalatha, M., Abbasi, T., Abbasi, S. 2013. Methane capture from livestock manure. *Journal of environmental management*, **117**, 187-207.

USEPA. 2018. Inventory of US greenhouse gas emissions and sinks. in: *Greenhouse Gas Emissions*, Vol. 2021, USEPA.

VanderZaag, A., Gordon, R., Burton, D., Jamieson, R., Stratton, G. 2010. Greenhouse gas emissions from surface flow and subsurface flow constructed wetlands treating dairy wastewater. *Journal of environmental quality*, **39**(2), 460-471.

VanderZaag, A., Gordon, R., Glass, V., Jamieson, R. 2008. Floating covers to reduce gas emissions from liquid manure storages: a review. *Applied Engineering in Agriculture*, **24**(5), 657-671.

Vendramelli, R.A., Vijay, S., Yuan, Q. 2017. Mechanism of nitrogen removal in wastewater lagoon: a case study. *Environmental technology*, **38**(12), 1514-1523.

Wang, L.M.A., Zheng, Z., Luo, X.Z., Zhang, J.B.A. 2011. Performance and mechanisms of a microbial-earthworm ecofilter for removing organic matter and nitrogen from synthetic domestic wastewater. *Journal of Hazardous Materials*, **195**, 245-253.

World Bank. 2020. Carbon Pricing Dashboard, (Ed.) T.W. Bank, Vol. 2021. https://carbonpricingdashboard.worldbank.org/map_data.

Xu, R., Xu, S., Florentino, A.P., Zhang, L., Yang, Z., Liu, Y. 2019. Enhancing blackwater methane production by enriching hydrogenotrophic methanogens through hydrogen supplementation. *Bioresource technology*, **278**, 481-485.

Yarberry, A., Lansing, S., Luckarift, H., Diltz, R., Mulbry, W., Yarwood, S. 2019. Effect of anaerobic digester inoculum preservation via lyophilization on methane recovery. *Waste Management*, **87**, 62-70.

Zhao, L.M., Wang, Y.Y., Yang, J., Xing, M.Y., Li, X.W., Yi, D.H., Deng, D.H. 2010. Earthworm-microorganism interactions: A strategy to stabilize domestic wastewater sludge. *Water Research*, **44**(8), 2572-2582.