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Anaerobic Digestion Coupled with Anammox Treatment for Nutrient Retention and Water Quality Improvement

John H. Loughrin [1,*], Stacy W. Antle [1], Philip J. Silva [1], Michael D. Bryant [1], Jason R. Simmons [1], Matias B. Vanotti [2], Ariel A. Szogi [2] and Karamat R. Sistani [1]

[1] Food Animal Environmental Systems Research Unit, Agricultural Research Service, United States Department of Agriculture, 2413 Nashville Road, Suite B5, Bowling Green, KY 42101, USA; stacy.antle@usda.gov (S.W.A.); phil.silva@usda.gov (P.J.S.); michael.bryant@usda.gov (M.D.B.); jason.simmons@usda.gov (J.R.S.); karamat.sistani@usda.gov (K.R.S.)

[2] Coastal Plains Soil, Water and Plant Research Center (USDA-ARS), 2611 West Lucas Street, Florence, SC 29501, USA; matias.vanotti@gmail.com (M.B.V.); aaszog@gmail.com (A.A.S.)

* Correspondence: john.loughrin@usda.gov; Tel.: +1-202-480-5438

Abstract

Confined animal feeding operations often use pit recharge waste management systems, where water is recycled from treatment lagoons to flush waste from animal housing. This practice has been linked to reduced animal performance and excess mortality due to the presence of pathogens and ammonia. As a project to improve the quality of flush water, the effluents from two anaerobic digesters were treated in aeration–recirculation systems, one inoculated with an anammox (anaerobic ammonia oxidation) consortium and the other left uninoculated. The effluent from the aeration tanks was sent to a shared constructed wetland planted with cattails and duckweed and hence to a lagoon. Water from the lagoon was recycled back to the digesters, where it was reused to suspend waste for anaerobic digestion. Fresh water was used to suspend feed to the system for the first six months, whereas lagoon water was recycled into the system for the last year and a half. The inoculated aeration tank reduced ammonium concentrations more efficiently than the uninoculated tank and also performed nitrification more effectively. Ammonia oxidation and denitrification continued in the wetland and the lagoon, and ammonium concentrations in the recycled water were reduced by over 98 percent compared to its concentration in the digesters. Phosphate concentrations in the lagoon averaged only 5 percent that of its concentration in the digesters over the two-year evaluation period. Inductively coupled plasma spectroscopy of wastewater sludge and plants showed that chemical precipitation of phosphate and utilization by duckweed and cattails accounted for phosphorus reduction by the system. Wastewater pH, conductivity, chemical oxygen demand and oxidation reduction potential were steady during the study period.

Keywords: ammonia; anaerobic digestion; anammox; eutrophication; nitrate; nitrite; phosphate; phosphorus; wastewater treatment

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1. Introduction

Water use in livestock operations in 2015 consumed approximately 2×10^9 gallons of water in the United States each year, with the largest consumers being Texas, California, Iowa, Nebraska, and Kansas [1]. This is problematic, especially in the western US, where the intensity and duration of periodic droughts have been increasing [2].

Animal waste in large rearing facilities is typically treated using pit recharge systems, where wastewater is flushed from housing and treated in facultative lagoons until reused for flush waste from subfloor pits. Pathogens may persist in these lagoons, especially in winter, and ammonia is lost to volatilization, especially in summer [3,4]. Recirculating wastewater through animal housing has been tied to decreased animal performance and greater mortality [5], stressing the need for improved and healthier waste management in animal rearing facilities.

Anaerobic digesters have many advantages for the treatment of wastewater from concentrated animal feeding operations (CAFOs), as opposed to pit recharge systems. The waste is high-strength, which makes aeration impractical for technical and economic reasons, as does the volume of waste produced in these facilities. Digestion can reduce wastewater strength and solid content and reduce offensive odors and potentially pathogenic organisms while possibly adding revenue in the form of biogas [6]. However, the removal of pathogens in wastewater is limited by the presence of resistant-spore-forming pathogens such as *Clostridium* spp., usually requiring thermophilic digestion to aid in pathogen reduction [7].

A recent estimate places the amount of animal manure produced by CAFO at approximately 9.8 billion livestock, producing 1.4 billion tons per annum in the United States alone [8]. Most of this waste receives inadequate treatment in facultative lagoons, which leads to air and ground water contamination even prior to land application [8,9]. Anaerobic digestion coupled with biogas harvesting can aid in reducing wastewater organic strength, reducing methane emissions, hydrolyzing organic forms of phosphate, and mineralizing nitrogen facilitating their removal from wastewater [10].

Without some means of removing phosphate and nitrogen, the discharge of post-digestion wastewater to the environment is still problematic, either directly for cropland or after storage in facultative lagoons. Although the nitrogen-to-phosphorus ratio is not ideal in digester effluent for use as fertilizer, if it is stored in a facultative lagoon prior to crop application, much of the nitrogen is lost to ammonia volatilization, which results in an even more unfavorable nitrogen-to-phosphate ratio when applied to cropland [11].

Wastewater from CAFOs might be more desirable as an irrigant for cropland if it were applied strictly for meeting crop phosphate requirements and supplementing with inorganic nitrogen. In this way, phosphorus water contamination might be mitigated, which seems to be more important for driving eutrophication [12,13]. Reducing both the nitrogen and phosphorus contents of the wastewater is critical for accomplishing this.

Reducing the nitrogen and phosphorus content of wastewater can be facilitated by aeration. However, aeration of animal wastewater is impractical due to its high strength and large volume. The treatment of municipal wastewater is usually performed by sequencing batch reactors (SBRs), which accomplish chemical oxygen demand removal (COD), as well as nitrogen and phosphorus removal [14]. While the aeration of municipal wastewater is energy-intensive, even for efficient SBR [15], the effluent of anaerobic digesters has COD concentrations comparable to those of a municipal sewage influent. For instance, Barker et al. found that the effluents from six different anaerobic digester designs averaged 872 ± 942 mg L^{-1} of total COD and 342 ± 571 mg L^{-1} of soluble COD [16]. Municipal wastewater effluents, in comparison, might typically have COD concentrations of 600–900 mg L^{-1} [17].

Recently, anaerobic ammonia oxidation consortia (anammox) have been used as an alternative to SBR for the treatment of municipal wastewater. Compared to SBR, anammox has advantages that include less aeration, fewer organic carbon requirements and reduced sludge production [18,19]. Due to their lower costs and other advantages, anammox treatment systems have achieved widespread adoption, especially in Europe, China, Japan, Australia, and Brazil [20].

Anammox consortiums (anaerobic-ammonia-oxidizing), comprising bacteria belonging to the phylum Planctomycetota and other commensal phyla, have been adapted to high-strength wastewater typical in agriculture and food industry wastes [21–24]. We recently presented the initial testing of a dual anerobic digestion system in which the effluent of one was treated in a reactor by such a consortium developed by the Agricultural Research Service in Florence, SC [22], and the effluent of the other digester was treated in an uninoculated reactor [25].

The effluents from the two reactors were sent to a sand filter and hence to a constructed wetland planted with cattails (*Typha latifolia* L.) and duckweed (*Lemna gibba* L.). Treated wastewater passed into a lagoon also containing duckweed. In the previous paper [25], the water from the wetland was not passed on to the lagoon, and fresh water was used to mix feed sent to the digesters. In the study presented here, fresh water was used to feed the digesters for the first six months, and lagoon water was recycled into the digesters for the last 18 months. While the previous manuscript reported the details of system construction and the initial-water quality parameters, this paper describes the operation of this system over a two-year period and performance characteristics of the system in terms of nitrogen and phosphate removal and other wastewater quality considerations.

2. Materials and Methods

2.1. Waste Treatment System Description

A diagrammatic illustration of the digester and treatment systems is presented in Figure 1. These systems have been described in detail in a previous article [25]. In brief, two anaerobic digesters and anammox aeration tanks were housed in a building erected on a concrete pad. The digesters had a diameter of 2.44 m and height of 2.77 m, a volume of 11,360 L, and a slurry/supernatant volume of 9085 L.

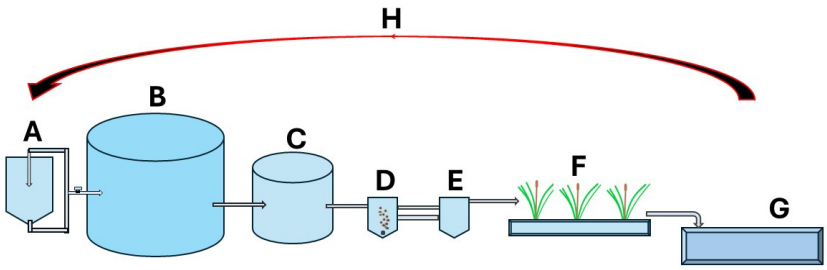


Figure 1. Schematic representation of wastewater treatment system. A. Waste mixing/feed tank; B. digester; C. wastewater holding tank; D. annamox/aeration tank; E. bacterial biomass retention/recirculation tank back to aeration tank; F. wetland; G. lagoon; H. wastewater return. Tanks B through E are duplicates: one of each is dedicated to a system with an uninoculated aeration tank, while the other ones are inoculated with an anammox consortium. Not included in the diagram are two indoor holding tanks holding feed leading from tanks C to tanks D.

The building floors measured 9.1 by 5.2 m, with walls of 4.9 m, and the building had a peak height of 5.9 m. A single wastewater feeding tank, the two anaerobic digesters, two aeration tanks, and two settling tanks were also housed in the building, as well as two digester effluent tanks, which were gravity-fed from two effluent-holding tanks outside the building. In addition, the concrete pad also quartered a constructed wetland with dimensions of 3.6 m^2, filled with Crider soil with an average depth of 0.3 m and a water depth of 0.3 m. Broadleaf cattails (*T. latifolia*) were planted in the wetland to aid in the remediation of the wastewater fed from the overflow of the settling tanks inside the building. The wetland discharge led to a lagoon with a plastic liner, with dimensions of 10 by 5 m and an average depth of 4.75 m with a slope of about 30°, providing a roughly estimated capacity

of 125 m^3. The lagoon supported a thick cover of *L. gibba* during warm seasons and a lesser cover during the cool seasons. In addition, two planters with a volume of 110 L filled with Crider soil were located at the shallow end of the lagoon at a depth of about 0.3 m. One was planted with cattails and the other with Indian lotus (*Nelumbo nucifera* Gaertn).

The system was fed by an AMT model 316B-95 self-priming 230 V single-phase centrifugal pump (Gorman-Rupp, Mansfield, OH, USA). Wastewater was fed to the pump from a cone-bottom feed tank with a capacity of 946 L. Feed was routed to individual digesters by means of a ball valve connected to a 5.08 cm polyvinyl chloride (PVC) pipe. After initially seeding each digester with 95 L of digestate from a commercial anerobic digester along with 13.6 kg of dairy manure, each digester was fed identical amounts of feed weekly consisting of 22.7 to 34.1 kg of chicken litter collected from a commercial poultry-rearing facility, or cracked corn in 757 L lagoon water. After feeding the feeding tank, lines were rinsed with approximately 189 L of lagoon water. Monthly amounts of feed can be found in the Supplemental Material, Table S1.

Digestate levels were measured by means of a clear 5.08 cm-diameter PVC pipe with a length of 30 cm (McMaster-Carr, Aurora, OH, USA) connected to the side of the digester. The equivalent volume fed to each tank was drained prior to feeding by opening a ball valve and emptied into a 3028 L HDPE effluent holding tank located outside of the building. The digester was vented though a 1.27 cm diameter PVC pipe connected to an EKM model EKM-PGM-075 pulse output diaphragm gas meter, which recorded 1.0-ft^3 (28.3 L) per pulse (EKM Metering, Santa Cruz, CA, USA). From the meter, the pipe led to a condensate trap constructed from a 10.2 cm-diameter pipe with a length of 0.3 m, buried at a depth of 90 cm. Prior to feeding digesters, they were vented by opening a three-way valve on the gas line to avoid excess pressure or vacuum in the system.

From the outdoor holding tanks, water was gravity-fed to an effluent-holding tank indoors. Wastewater was then fed via a peristaltic pump to a 208 L cone bottom HDPE tank filled with 38 L of polypropylene bioballs at a rate of 93 mL min^{-1}. Aeration was supplied at 6 L min^{-1} to these tanks. Water from the aeration tank was fed at 188 mL min^{-1} to the bottom of a settling tank. This water was pumped back to the aeration tank at a rate of 93 mL min^{-1}, and the overflow from the settling tank was gravity-fed to a sand filter located in an aluminum gutter system at the head of the wetland. The overflow from the settling tank to the sand filter was fitted via a wire screen to prevent biomass from escaping the system. The volumes of the anammox/aeration tanks and biomass retention/recirculation tanks were maintained at 204 and 193 L, respectively.

Aeration was supplied to the sand filter three times per day at a rate of 4 L min^{-1} in 15 min intervals every 6 h starting from 12 March 2024. Water from the sand filter was trickle-fed to the wetland and the overflow of the wetland was trickle-fed to the lagoon for further treatment and reuse by the digester/wastewater treatment complex. The volumes of the aeration tanks and settling tanks were maintained at 204 and 193 L, respectively. The aeration tank system of system A was installed prior to system startup in June 2024 and left uninoculated, whereas the aeration tank of system B became operational on 19 November 2024 and was inoculated with 5 L of an anammox consortium on 3 December 2024, with an additional 7 L of consortium added on 14 May 2025. The anammox consortium [26] contained a mixture of anammox sludge [27] and high-performance nitrifying sludge [28] in a volumetric proportion of 4:1.

2.2. Sample Collection

For the digesters, samples for wastewater and microbiome analyses were taken from a port approximately 1.0 m from the bottom of the tanks. Other wastewater quality samples were taken from near the surface of the anammox tanks, the wetland, and the lagoon.

Wastewater analysis was performed once weekly from June 2024 through June 2026 and the results were averaged for each month except during October 2025, when a government shutdown occurred.

2.3. Wastewater Analysis

Weekly samples were collected prior to feeding the digesters. Water temperature, pH, conductivity, and oxidation–reduction potential (ORP) was measured using a YSI ProDSS water-quality meter (YSI Inc., Yellow Springs, OH, USA). All other water-quality parameters were measured on samples that had been stored at −4 °C prior to analysis using APHA standard methods [29]. For nitrate and ammonium analyses, samples were filtered through syringe filters with a 0.25 um pore size and then analyzed on a SEAL AQ400 discrete spectrophotometric analyzer (SEAL Analytical, Inc., Mequon, MN, USA) using methods AGR-232-C Rev.1 and EPA-153-A Rev. 2 for nitrate/nitrite and ammonia, respectively [30,31].

For ion determinations, 2 mL samples were filtered through 0.2 µm pore-size filters prior to analysis. Anions and cations were determined by injecting 25 µL samples into a Dionex ICS-6000 HPIC system (Thermo Fisher Scientific, Waltham, MA, USA). An AS22 reagent was used as the isocratic mobile phase for anion analysis at 1.0 mL min^{-1} through a 50 mm × 4.0 mm IonPac AG22 guard column and 250 mm by 4.0 mm IonPacAS22 Analytical column held at 25 °C with a model 061830 conductivity detector. Cations were determined using methanesulfonic acid as the mobile phase on a 50 mm × 4.0 mm IonPac CG12A Guard and 250 mm × 4.0 mm IonPac CS12A Analytical column on an isocratic run at 1.2 mL min^{-1} at 25 °C using a model 061830 conductivity detector (Thermo Fisher Scientific, Waltham, MA, USA) and a Cation Dynamically Regenerating Suppressor (Thermo Fisher Scientific, Waltham, MA, USA). Bicarbonate was analyzed as described previously [32].

Acid digestion was used to analyze metals in dried sludge samples and plant samples. A total of 9 mL of HNO_3 and 3 mL HCl was mixed with 2.0 g of sludge or plant samples in microwave digestion vessels and predigested in a fume hood for 60 min [33]. Plant samples were dried for a minimum of 7 h at 65 °C, passed through a grinder mill and sieved through a 2 mm sieve prior to analysis.

Plant samples of 0.2 g were ashed in a muffle furnace at 500 °C for 4 h and then extracted with 1 mL of 6 M HCl for 1 h, followed by a 40 mL acid solution (0.0125 M H_2SO_4 and 0.05 M HCl) for 1 h [34]. The digested sludge and plant ash samples were filtered through quantitative grade filter paper (Q2, Fisher Scientific, Hampton, NH, USA) before analysis using an inductively coupled plasma optical emission spectrophotometer (ICP-OES) (5110 VDV; Agilent Technologies, Inc., Santa Clara, CA, USA).

2.4. Statistical Analysis

Statistical analysis was performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). Mean comparisons of water chemistry and ion concentrations were analyzed in stages of the inoculated and uninoculated systems using a general linear model procedure and Duncan's multiple range tests to highlight and separate significant changes in their levels or concentrations. The data analyzed covered the dates from 1 June 2024 to 1 July 2026, inclusive for the digesters, wetland and lagoon, and the uninoculated aeration tank, and 1 December 2024 to 1 July 2026 inclusive for the inoculated aeration tank, recirculation tanks and system effluents.

3. Results and Discussion

3.1. Digester Performance During the Evaluation Period

Biogas quality and production measurements started in April 2025 when both digesters consistently reached stable production, reliable gas meters were installed, and tank leaks had been sealed. From April 2025 to June 2026, biogas production averaged 10.1 ± 0.73 m^3 wk^{-1} for the uninoculated digester complex and 10.4 ± 0.80 m^3 wk^{-1} for the system that received the anammox consortium in its aeration tank (mean of 55 determinations ± s.e.m). This represented gas production of roughly 0.3 m^3 of biogas per kg of volatile solids. Carbon dioxide concentrations averaged 514,000 ± 25,000 µg L^{-1} for the unseeded system and 496,000 ± 31,000 µg L^{-1} for the inoculated system. Methane concentrations averaged 463,000 ± 28,200 µg L^{-1} for the uninoculated system and 422,400 ± 22,300 µg L^{-1} for the inoculated system.

Gas production averaged 13.2 ± 1.1 m^3 wk^{-1} and 15.1 ± 1,4 m^3 wk^{-1} for the uninoculated and inoculated digester complexes, respectively, in summer, and 3.2 ± 1.4 and 3.3 ± 0.54 m^3 wk^{-1} for the uninoculated and inoculated digester complexes, respectively, in winter. This was a consequence of the building temperature being set at 21 °C in winter and using cold lagoon water to mix with the feed, resulting in digester water temperatures averaging 27.4 °C in summer and 18.6 °C in winter. Both systems used sound systems employing underwater speakers in place of mixing systems to improve digester performance as has been described previously [25]. As the effluent from each system complex fed into the same wetland and lagoon and thus used the same water to mix feed, differences in biogas production were not expected and none were observed.

3.2. Environmental Conditions and Wastewater Quality of the System

Figure 2 presents the average soil temperature at a depth of 30 cm at the farm and air temperatures during the study period. Air and soil temperatures averaged around 26.5 °C during the months of June, July and August, whereas air and soil temperatures averaged around 1.2 and 9.6 °C, respectively, during the months of December, January and February. During the same periods, digester, anammox, wetland and lagoon water temperatures averaged 24.8, 26.9, 23.7 and 26.8 °C during summer, while they averaged about 17.9, 17.3, 6.0, and 6.9 °C in winter.

Despite the variation in temperature, the system performance was stable throughout the evaluation period. Table 1 presents the average water-quality parameters from June 2024 to July 2026. The pH in the digesters averaged 6.7, but all subsequent stages of both complexes were alkaline, ranging from about 7.2 for the recirculation tank effluent to 7.96 for the uninoculated anammox tank. The dissolved oxygen concentration was low and stable throughout the system, with the notable exception of the lagoon, which averaged 6.2 mg L^{-1} of O_2. However, DO averaged 9.8 ± 0.80 mg L^{-1} before wastewater addition to the lagoon and 4.80 ± 1.20 mg L^{-1} thereafter. Dissolved oxygen was also higher in the cooler months, averaging 3.2 mg L^{-1} during the winter of 2025–2026 and 0.4 mg L^{-1} in the spring of 2026. Duckweed also acted strongly to reduce the dissolved oxygen concentration: during the summer of 2024, before waste was added to the lagoon and duckweed cover was low, DO averaged 9.3 mg L^{-1}, while, during the second summer with waste being directed to the lagoon and causing high duckweed coverage, it averaged 0.2 mg L^{-1}.

Oxidation–reduction potential (ORP) was low throughout the system, ranging from averages of −197 mV for the digesters to −54 mV for the anammox tanks. It was also low in the wetland and lagoon, averaging about −110 mv for both, though the lagoon averaged −22 mV before wastewater addition and −136 mV thereafter. This was likely not only due to the wastewater addition, but also due to the high duckweed cover limiting oxygen exchange at the water surface. The chemical oxygen demand (COD) in the lagoon averaged

150 mg L^{-1} before wastewater addition and 185 mg L^{-1} afterwards. ORP exhibited seasonal variation related to that of DO in the lagoon, decreasing in summer when water temperatures were higher and duckweed cover was greater (Supplemental Material, Figure S2).

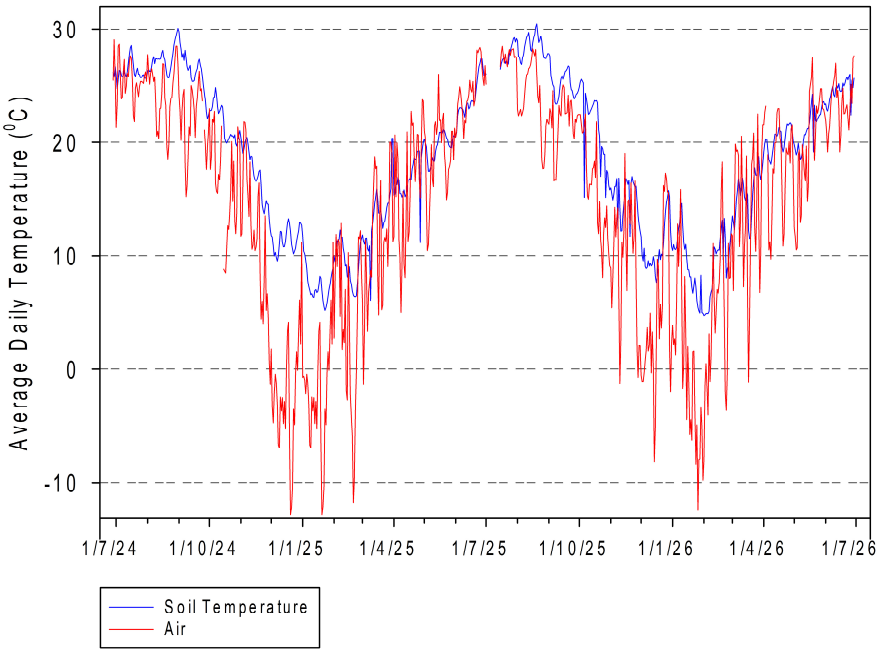


Figure 2. Air and soil temperatures at the Western Kentucky University farm during the study period. Soil temperatures were recorded at hourly intervals, whereas air temperatures represent the average daily temperature recorded at five-minute intervals by the Kentucky Mesonet station located at the same farm as the digester complex.

Table 1. Selected parameters of system wastewater covering June 2024 to June 2026.

	pH	Oxidation Reduction Potential (mV)	Dissolved Oxygen (mg L^{-1})	Conductivity (mS cm^{-1})	Chemical Oxygen Demand (mg L^{-1}) [1]
Treatment Stage		Uninoculated System			
Digester [2]	6.70 ± 0.021 d	−197 ± 10.2 d	2.27 ± 0.30 b	3440 ± 233 a	1180 ± 176 a
Feed Tank [2]	7.76 ± 0.148 a	−165 ± 14.0 cd	2.39 ± 0.28 b	3380 ± 148 ab	935 ± 96.1 ab
Aeration Tank [2]	7.96 ± 0.042 a	−56.2 ± 12.2 a	2.87 ± 0.29 b	3090 ± 198 abcd	729 ± 91.1 bc
Recirculation Tank [2]	7.91 ± 0.039 a	−81.0 ± 10.1 ab	0.72 ± 0.13 b	3130 ± 200 abc	716 ± 87.3 bc
System Effluent [2]	7.21 ± 0.030 cd	−81.9 ± 11.8 ab	2.97 ± 0.28 b	2560 ± 136 de	697 ± 102 bc
		Inoculated System			
Digester [2]	6.71± 0.038 d	−197.13 ± 10.2 d	1.40 ± 0.19 b	3550 ± 249 a	1180 ± 168 a
Feed Tank [2]	7.86 ± 0.030 a	−133 ± 18.1 bc	2.76 ± 0.32 b	3300 ± 179 ab	924 ± 115 ab
Aeration Tank [2]	7.79 ±0.078 a	−52.3 ± 7.25 a	2.70 ± 0.80 b	2680 ± 264 cde	737 ± 128 bc
Recirculation Tank [2]	7.87 ± 0.066 a	−74.0 ± 11.1 ab	1.36 ± 0.35 b	2850 ± 232 bcd	679 ± 129 bc
System Effluent [2]	7.17 ± 0.034 bcd	−61.3 ± 12.4 a	2.80 ± 0.24 b	2490 ± 214 e	605 ± 111 c
		Wetland and Lagoon			
Wetland [2]	7.62 ± 0.095 abc	−109 ± 15.5 abc	1.36 ± 0.27 b	2610 ± 207 cde	5200 ± 83.5 c
Lagoon [2]	7.68 ± 0.153 ab	−110 ± 16.2 bcd	6.23 ± 0.99 a	630 ± 75.6 f	174 ± 23.3 d

[1] For COD analyses, data represents the means of 19 determinations. [2] Data represents the means of 23 averaged monthly determinations for digesters, feed tanks, the uninoculated aeration tank, wetland and lagoon and 17 determinations for the inoculated aeration tank and both systems' effluents. Within columns, means followed by the same letter are not significantly different, using a general linear model procedure with Duncan's multiple-range test for mean separations at $p = 0.05$.

The chemical oxygen demand was lowered by 85 percent in the lagoon as compared to the average of the digesters and 81 percent as compared to the average of the feed tanks from January 2025 to June 2026. Using a ratio of 0.41 BOD5/COD [35], a plausible average BOD_5 of approximately 70 mg L^{-1} could be estimated for the lagoon. The system averaged the addition of approximately 4.7 ± 0.1 kg wk^{-1} of volatile solids (VSs) for both systems combined during the study period, or approximately 520 mg L^{-1} wk^{-1} VS for each digester. The use of sound to accelerate digestion rather than mechanical mixing acted to retain the solids in the digesters by reducing the suspension of solids and hence reducing wastewater strength in the wetland and lagoon, while high nutrient levels and vegetation in the wetland and lagoon acted to increase COD.

The COD values in the lagoon were comparable to those of other highly vegetated eutrophic waters [36,37] and COD concentrations remained relatively constant throughout the system after July 2025, likely due to the maturation of microbial populations in the digesters through the wetland. The most notable finding of the wastewater treatment system in regard to water quality was that the system achieved a good deal of stability after the effluent from the wetland was directed to the lagoon starting from January 2025. Conductivity, COD, pH, ORP, and pH all exhibited less variation than expected, although DO and ORP in the lagoon did show previously noted slight seasonal variations (Supplemental Material, Figures S1–S3).

3.3. Ionic Composition of the Wastewater System

Table 2 presents the concentrations of major ions in the treatment systems, wetland and lagoon. The concentrations of these ions were similar in the system inoculated with the anammox consortium and the uninoculated system. For instance, chloride averaged 62.5 mg L^{-1} across all five stages measured in both the uninoculated system and the inoculated system, while potassium averaged 2360 mg L^{-1} in the uninoculated system and 2480 mg L^{-1} in the inoculated system. The concentration of magnesium in the system effluent increased by approximately 41 and 49 percent in the uninoculated and inoculated treatment systems compared to its concentrations in the digesters, while calcium concentrations decreased from the digesters to the effluent by about 33 and 23 percent in the uninoculated and inoculated treatment systems, respectively. This was probably due to increased pH of the feed, anammox, and recirculation tanks compared to that of the digester tanks and the resulting higher alkalinity, since these conditions favor the precipitation of carbonate salts of calcium over that of magnesium [38]. The pH increased from an average of 6.7 in the digesters to 7.9 in the recirculation tanks, while bicarbonate buffering decreased from an average of 370 mg L^{-1} (6.1 mM) in the digesters to 215 mg L^{-1} (3.5 mM) in the recirculation tanks. In that the anammox process does not directly consume bicarbonate as conventional nitrification–denitrification wastewater treatment does, we did not expect great decreases in bicarbonate concentrations, though the presence of bicarbonate can improve anammox activity [39]. These conditions likely favored the precipitation of carbonate, bicarbonate and phosphate salts of calcium over those of magnesium [40].

Concentrations of all the ions excluding bicarbonate dropped by an average of 49 percent in the wetland compared to their concentrations in the systems' effluents and by an average of 65 percent from the wetland to the lagoon. This was likely mostly accounted for by the uptake from plants in the lagoon and wetland, particularly by the macrophytes *Typha latifolia* and *Lemna gibba*, as well as some degree of mineral precipitation in the wetland and the lagoon.

Bicarbonate concentrations were the highest in the digesters and feed tanks and decreased in the anammox and recirculation tanks. Bicarbonate is not consumed to the same degree in the anammox pathway as it is during denitrification in SBR; it is still consumed

by anammox bacteria coupling the reduction of carbon dioxide with the oxidation of nitrite to nitrate [41,42]. It is interesting that lower concentrations of bicarbonate were found in the inoculated anammox and recirculation tanks than in their uninoculated counterparts.

Table 2. Ion concentrations in the system wastewater covering the period from June 2024 to June 2026.

	Cl^-	SO_4^{2-}	Mg^{2+}	Ca^{2+}	Na^+	K^+	HCO_3^-
Treatment Stage	Concentration (mg L^{-1}) [1]						
Uninoculated System							
Digester	62.9 a	20.0 abc	62.5 abcd	132 a	109 b	402 c	372 a
Feed Tank	59.0 ab	25.6 ab	57.1 bcd	122 ab	113 ab	415 bc	312 bc
Aeration Tank	55.0 ab	27.1 ab	41.8 de	74.1 bc	106 b	376 c	296 dc
Recirculation Tank	66.6 a	28.8 a	78.7 ab	97.1 abc	155 a	599 a	268 de
System Effluent	68.8 a	28.0 ab	88.6 abc	87.8 bc	152 a	570 ab	nm [2]
Inoculated System							
Digester	62.8 a	15.9 bc	62.7 abcd	128 a	113 b	404 c	367 a
Feed Tank	56.5 ab	25.4 ab	48.4 bcd	108 abc	103 b	365 c	344 ab
Aeration Tank	68.1 a	35.0 a	85.4 ab	106 abc	153 a	571 ab	240 e
Recirculation Tank	69.6 a	36.8 a	86.0 ab	101 abc	153 a	568 ab	161 f
System Effluent	67.5 a	34.1 a	93.8 a	98.5 abc	155 a	577 ab	nm
Wetland and Lagoon							
Wetland	41.9 b	15.4 bc	52.1 bcd	62.2 cd	98.7 b	314 c	147 f
Lagoon	12.9 c	3.40 c	17.6 e	26.9 d	40.5 c	112 d	55.5 g

[1] Data represents the mean of 32 determinations for the digesters and anammox A; 31 for the feed tanks, wetland and lagoon; 17 for anammox B, and recirculation tanks, 15 for system effluent. Bicarbonate analyses represent the mean of 10 determinations. Within columns, means followed by the same letter are not significantly different, using a generalized linear model with Duncan's multiple-range test for mean separations at $p = 0.05$. [2] nm = not measured.

Much of the reduction in ion concentration in the lagoon could be due to dilution, as it holds more water than the rest of the system. The lagoon had an approximate volume of 125,000 L, while, during the course of the evaluation period, approximately 200,000 L of wastewater were fed to the system, of which about 140,000 L was recycled water fed to the lagoon starting from January 2025. The lagoon was lowered by about 30 cm in July 2025 to repair a leak in its liner, and fresh water was added to bring the water level back up. In addition, significant precipitation events occurred on 30 January, 15 February, 15 March and 3 April of 2025, which caused some water to flow out of the shallow end of the lagoon. We calculated that roughly 76 m^3 of fresh water was added by precipitation during the study period. Thus, approximately 136,000 L of fresh water was added to the system, not counting roughly 2000–4000 L added to the lagoon after the leak that occurred in July 2025.

The concentrations of Na, K, Mg, and Ca started increasing in the lagoon during the spring of 2025 (Supplemental Material, Figure S5), undoubtedly due to wastewater recycling. Increasing conductivity or concentration of ions would signal when consideration could be given to applying wastewater to cropland for irrigation.

3.4. Wastewater Phosphate Analyses

Phosphate concentrations in the treatment systems, the wetland and the lagoon are presented in Figure 3. In both the inoculated and uninoculated systems, there was a drop in the concentration of phosphate of over 20 percent after the anaerobic digestion tanks, indicating phosphate precipitation in the feed tank. Although phosphate concentrations in

the unseeded system were lower on average than that of the system, this difference was not pronounced.

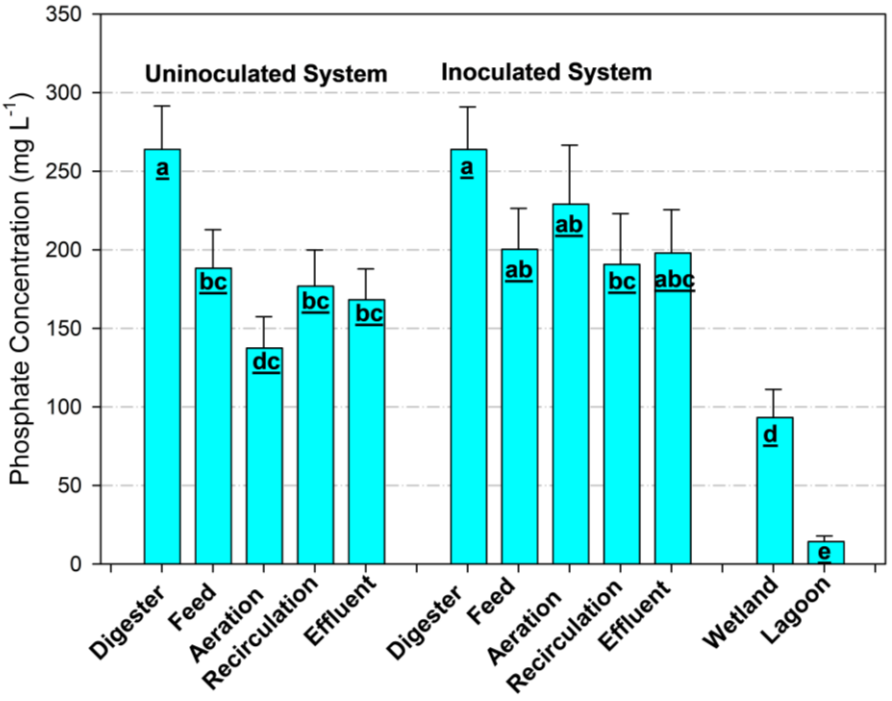


Figure 3. Average phosphate concentrations in digester complex not inoculated with anammox consortium and the complex inoculated with anammox consortium, as well as the wetland and the lagoon shared by both complexes. Data represents the mean of 32 determinations ± standard error of the mean for digesters, uninoculated anammox reactor, wetland and lagoon, 26 determinations for feed tanks, 18 determinations for recirculation tanks and system effluent and 17 determinations for anammox reactor inoculated with the anammox consortium. Means followed by the same letter are not significantly different, using a generalized linear model with Duncan's multiple-range test for mean separations at $p = 0.05$.

Phosphate concentration dropped by an average of 50 percent in the wetland relative to the average concentration in the effluent from the uninoculated and inoculated effluents. Cattails were planted in the wetland in the autumn of 2023 and exhibited strong growth from the spring of 2024 and throughout most of the evaluation period. However, the cattails were infested with corn aphids (*Rhopalosiphum maidis* Fitch) in the summer of 2025, which required insecticide spraying and resulted in reduced growth. Duckweed was not deliberately added to the system and appeared in 2025, likely introduced with the cattails or introduced to the system by mallard ducks that visited the site in the springs of 2023–2026.

Duckweed cover was much heavier in the lagoon than the wetland due to less competition from the cattails. The phosphate concentration in the lagoon was only 15 percent of that of the wetland, dropping from an average of 93.2 ± 18.0 mg L^{-1} to 14.3 ± 3.6 mg L^{-1}. The drops in the concentrations in other ions were less pronounced. Sodium, potassium, magnesium and calcium dropped by 36, 45, 36, and 33 percent in the wetland relative to the system effluent, and 59, 64, 66, and 57 percent in the lagoon compared to the wetland.

Over the course of this two-year study, the system was supplied with 0.96 MT of poultry litter and 1.5 MT of cracked corn, representing a rough estimate of 47.8 kg of phosphate supplied to a system with a total water capacity of around 105,000 L (Supplemental Material, Table S1). This would represent a phosphate concentration of 455 mg L^{-1} of phosphate throughout the system. Phosphate is highly liable to precipitation in wastewater treatment systems of course, spurring the development of processes for its recovery in wastewater

treatment systems, i.e., [10,40], many of which entail its recovery as magnesium, aluminum, iron or calcium salts [43,44].

In this system, some phosphate was lost due to leaks and several precipitation events that caused overflow in the lagoon. The majority of phosphate reduction in the digesters and aeration tanks was likely due to chemical precipitation. ICP analysis of the digester sludges, wetland sand filter, and wetland showed concentrations of phosphorus of 1180 ± 223, 717 ± 131, 2370 ± 1570, and 688 ± 176 mg kg^{-1} of dry weight in the uninoculated and inoculated digesters, sand filter and wetland, respectively. It was not possible to measure the quantity of sludge in the digesters or in other parts of the system and therefore we could not determine the magnitude of phosphorus precipitation in the system, but these values indicate points where phosphorus precipitation occurred. Phosphate concentrations in wetland water dropped by an average of 50 percent compared to the two systems' effluents (Figure 3). It dropped by a further 85% in the lagoon compared to the wetland. This undoubtedly was largely accounted for by uptake by the cattails and duckweed in the wetland and lagoon, but what was striking was the relatively low concentration of phosphate in the lagoon even during the winter when duckweed cover was low.

While phosphate precipitation could account for much of the reduction in phosphorus, the majority of the reduction seemed more likely to be due to uptake by the duckweed and cattails. Table 3 presents ICP analyses of plants collected from the wild and from the wetland and the lagoon.

Table 3. Inductively coupled plasma analysis of selected metal concentrations in cattail tissues collected from the wild and from the wastewater treatment system, duckweed and sludge collected from the wastewater lagoon [1].

Metal	Wild Cattail (Stem/Leaf)	Wild Cattail (Root)	Treatment System Cattail (Stem/Leaf)	Treatment System Cattail (Root)	Live Duckweed [2]	Lagoon Sludge [2]
			Concentration (Milligrams per kilogram of dry weight)			
Ca	11,400	4780	8060	9760	10,800	39,200
Cu	4.34	6.08	4.31	6.74	44.1	3.51
Fe	315	4185	256	1020	737	3420
K	12,500	8190	19,600	24,200	68,000	9940
Mg	1037	1413	4140	4100	109	111
Mn	466	66.7	140	112	491	149
Na	826	1990	2070	4230	1940	1620
P	1230	760	3700	7660	16,400	6440
Zn	16.1	38.4	10.0	15.4	21.0	109
Total	27,800	21,400	38,000	51,100	98,500	39,200

[1] Data represents the average of duplicate determinations. [2] Data is from one determination.

Results of these analyses indicated that both cattails and duckweed were capable of accumulating considerable metals and especially the plant nutrients, potassium and phosphorus. In the case of phosphorus, cattail stems and leaves accumulated three times as much as the wild cattails we sampled, and cattail roots and rhizomes collected ten times as much. Duckweed accumulation of potassium and phosphorus was even higher than that of the cattails.

The sludge from the lagoon, which seemed to contain a large proportion of decaying duckweed, contained relatively high concentrations of calcium, iron and phosphorus. In that duckweeds have an average lifespan of about 30 days [45], it is plausible to conclude that much of the phosphorus in the lagoon sludge consists of a mixture of calcium and iron (II) phosphates, given the low ORP of the lagoon.

3.5. Wastewater Ammonium and Nitrate

Ammonium concentrations in the digesters and feed tanks averaged over 200 mg L^{-1} (Figure 4). In the uninoculated aeration tank, ammonium decreased by 15 percent relative to the feed tank while in the inoculated aeration tank ammonium dropped by 48 percent relative to its feed tank. Nitrate production in the aeration tank was dramatically higher than in the uninoculated tank, increasing from 0.6 mg L^{-1} in the feed tank to 10 mg L^{-1} in the uninoculated system and from 0.5 mg L^{-1} to 58 mg L^{-1} in the inoculated aeration tank. Ammonium and nitrate averaged 168 and 14 mg L^{-1}, respectively, in the uninoculated system's effluent and 133 and 35 mg L^{-1} in the system's effluent.

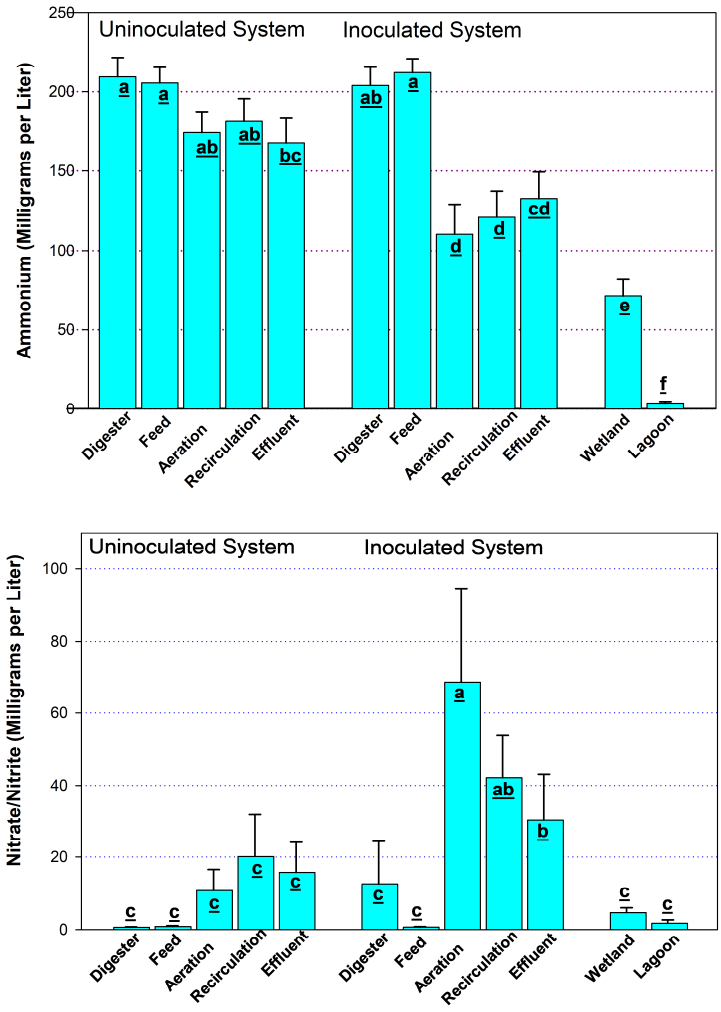


Figure 4. (**Top**), average ammonium concentrations in digester complex not inoculated with anammox consortium and with anammox consortium as well as wetland and lagoon shared by both complexes. (**Bottom**), average nitrate/nitrite concentrations in digester complex not inoculated with anammox consortium and inoculated with anammox consortium as well as wetland and lagoon shared by both treatment systems. Data represents the mean of 28 determinations ± standard error of the mean for digesters, unseeded anammox reactor, wetland and lagoon; 26 determinations for feed tanks, 18 determinations for recirculation tanks and system effluent and 19 determinations for anammox reactor inoculated with anammox consortium. Means followed by the same letter are not significantly different, using a generalized linear model with Duncan's multiple-range test for mean separations at $p = 0.05$.

The concentrations of both ammonium and nitrate decreased in the wetland, dropping by over 50 percent for ammonium and by over 65 percent for nitrate. More dramatic reductions in their concentration occurred in the lagoon, with ammonium and nitrate

averaging less than 4 and 2 mg L^{-1}, respectively, in the lagoon during the two-year study period. While there was a considerable reduction in the ammonium concentration in the aeration tanks, especially the one inoculated with the anammox consortium, most of the nitrogen loss occurred in the wetland and the lagoon. Utilization by cattails, duckweed and algae undoubtedly accounted for much of this, though both ammonium and nitrate concentrations remained low in the lagoon throughout the year, and no seasonal variation was noted in either ammonium or nitrate concentration, even though the winters were quite cool (Figures S6 and S7, Supplemental Material). This suggests that a large part of nitrogen removal was not due to utilization by plants alone but also by ammonia-oxidizing bacteria (AOB) and/or archaea (AOA). Denitrifiers present in the soil of the wetland and lagoon also likely played a role in that an environment with a complex ORP profile supported by organic matter exuded by roots and released by decaying matter favors denitrification [46,47]. This is similar to other studies, where high denitrification activity was noted in constructed wetlands treating swine waste [45]. It has been suggested that the ammonia removal in cattail-planted wetlands occurs more in the water column than in the wetland soil [48], though the interactions between plants, microbes, organic and inorganic matter, and ORP are complex [48].

Although ammonia removal was not as optimal in the inoculated aeration tank as envisioned—either due to overloading of the consortium, loss of microbial biomass, or perhaps unsuitability of the consortium to environmental conditions in the wastewater—the relatively high concentrations of nitrate vis à vis ammonium in the wetland and lagoon (Figure 4) indicate high ammonia-oxidizing activity continuing post-aeration tank. Whether this was because of the aeration tanks seeding the wetland and the lagoon with escaped biomass or, more likely, because of the development of niches that favored the development of AOB and AOA is unknown and will require 16S microbial sequencing. In addition, comparison of the uninoculated and inoculated aeration tanks showed concentrations of 21.4 and 23.1 percent VS, respectively, indicating more biomass in the inoculated aeration tank. Therefore, the inoculated tank may have had more ammonia removal capability due to its greater biomass or because of the qualitative differences in its microbiome. Again, microbial sequencing will be required to address this question.

Nevertheless, results obtained in this study show that minimal aeration of anaerobic digester effluent coupled with solid retention and a minimally sized wetland and lagoon are potentially able to recycle wastewater back to animal housing over an indefinite period of time. A 16S analysis of the recycled wastewater will also help to determine if this recycled wastewater is likely to be healthier for animals than that of typical facultative lagoons.

Both the wetland and the lagoon are complex systems where ORP, DO, and pH are likely to vary at small scales. Variations in these and other environmental conditions are likely to favor nitrification and denitrification, leading to nitrogen remediation of the wastewater.

4. Conclusions

The digester coupled with wastewater system exhibited remarkable stability over a two year period in which, for the last year and a half, no water was added except for that provided by precipitation. Sound excitation of the digesters eliminated the need for mechanical mixing, thereby retaining solids in the digesters. Solid retention, in turn, reduced effluent strength and enabled the use of low levels of aeration, coupled with further treatment by a constructed wetland and lagoon, to recycle water back into the treatment system. While the aeration tank inoculated with an anammox consortium showed greater nitrification activity and ammonia-removal efficiency than the uninoculated aeration tank did, results indicated that a system like this was capable of remediating wastewater nitrogen

if inoculated with an anammox consortium, due to continued nitrification and denitrification in the wetland and the lagoon.

High phosphate removal was exhibited by the system due to a combination of chemical precipitation and utilization by aquatic vegetation. The system may be capable of remediating nitrogen and phosphate indefinitely, especially if the cattails and/or duckweed are harvested to ensure continued phosphate removal from the wastewater. The retention of P, K, Fe and other nutrients within the system facilitates their recovery instead of losing them to leaching and run-off. In the meantime, the lagoon, although anoxic, has sufficiently good water quality to support insect life and frogs, which we believe to be green or bronze frogs (*Lithobate clamitans* Latreille). In terms of reduced COD and ammonium, as well as increased ORP, the wastewater from this system should be more suited to land application and pit-recharge systems. While this study was limited by its lack of replication and both systems sharing a constructed wetland and lagoon, it did indicate that anerobic digestion coupled with affordable wastewater treatment can be a means of improving water quality for use in irrigation and animal housing management. The development of affordable sustainable wastewater treatment systems for animal-rearing operations will help ensure profitability and environmental stewardship for agriculture.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/environments13110599/s1, Table S1: Feeding schedule of digesters; Figure S1: pH of waste treatment system during the study period; Figure S2: Oxidation-Reduction Potential in digesters, anammox tanks, wetland and lagoon; Figure S3: Conductivity in digesters, anammox tanks, wetland and lagoon; Figure S4: Chemical oxygen demand of waste treatment system during the study period; Figure S5: Average sodium, potassium, magnesium and calcium concentrations in the wetland and lagoon; Figure S6: Ammonium in the system that was not inoculated with the anammox consortium (system A) and that was inoculated with the anammox consortium (system B); Figure S7: Nitrate/nitrite in the system that was not inoculated with the anammox consortium (system A) and that was inoculated with the anammox consortium (system B); Figure S8: Phosphate concentrations in waste treatment system during the study period; Figure S9: Treatment system April 2025; Figure S10: Treatment system September 2025; Figure S11: lagoon August 2026.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Anammox	Anaerobic Ammonia Oxidizing
AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
BOD_5	5 day Biological Oxygen Demand
CAFO	Confined Animal Feeding Operation
COD	Chemical Oxygen Demand
ICP	Inductively Coupled Plasma Spectrometry
ORP	Oxidation Reduction Potential
SBR	Sequencing Batch Reactor
VS	Volatile Solids

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