



Article

Field Evaluation of Covered and Open Dairy Lagoons: Microbial Biomass Degradation, Pathogen Reduction, and Solids Stabilization

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Abstract

Commercial-scale dairy farms produce large volumes of manure, posing environmental, animal health, and public health risks due to persistent pathogens and the accumulation of organic pollutants. This field-scale study evaluated the effects of covered anaerobic lagoons (CLs) and open facultative lagoons (OLs) on microbial reduction, solids stabilization, and manure biogeochemical characteristics on commercial dairy farms in California's Central Valley during the summer months. Manure samples collected from lagoon inlets, outlets, and secondary lagoons were analyzed for *Escherichia coli*, total solids (TS), volatile solids (VS), and genomic DNA degradation. CL systems achieved substantially greater *E. coli* reductions (98.38%; 1.82 log) than OL systems (54.88%; 0.35 log), indicating enhanced pathogen suppression under anaerobic conditions. Progressive declines in genomic DNA concentrations and electropherogram signal intensities across treatment stages further demonstrated microbial biomass degradation during storage. In the CL system, TS concentrations decreased from approximately 0.985% at the inlet to 0.485% at the outlet, representing a 50.7% reduction, with an additional 56% reduction observed in the secondary lagoon. For moisture content, both CL and OL systems exhibited increases of approximately 0.37–0.5% from their respective inlets to outlets, with only marginal increases observed in the secondary lagoon. Comparable trends were observed in the OL system. Significant differences in TS, VS, moisture content, pH, electrical conductivity, and major cations (Na⁺, K⁺, Ca²⁺) occurred between CL and OL systems and among treatment stages. The secondary lagoon further enhanced microbial and solids reductions in both systems. Overall, the findings show that CL systems provide superior pathogen reduction and support biogas recovery, whereas OL systems demonstrate stronger VS sta-

Academic Editors: Muhammad Sultan, Yuguang Zhou, Redmond R. Shamshiri and Muhammad Imran

Received: 28 April 2026

Revised: 2 July 2026

Accepted: 4 July 2026

Published: 9 July 2026

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bilization. These results offer practical, field-based insights to inform improved manure management and pathogen mitigation strategies for commercial dairy operations.

Keywords: open and closed dairy lagoon; *Escherichia coli* reduction; volatile solids stabilization; anaerobic lagoon treatment; manure physicochemical characteristics

1. Introduction

Large-scale dairy farms generate substantial quantities of manure, creating environmental, animal health, and public health concerns associated with pathogen persistence, greenhouse gas (GHG) emissions, and organic pollutant accumulation [1,2]. Effective management of dairy manure therefore requires improved treatment and storage strategies capable of reducing microbial contamination and environmental emissions. Manure from large-scale animal feeding operations can contaminate surface water due to the presence of pathogens such as *Escherichia coli*, excess nutrients (nitrogen and phosphorus), and antibiotic resistance-associated microbial biomass [3–6]. In addition, livestock manure from ruminants such as cattle is a major source of methane emissions, contributing to climate change [7,8]. Consequently, manure management on dairy farms is complex and regulated, requiring producers to adopt practices that minimize environmental and public health risks while maintaining operational efficiency [9–11]. At the same time, dairy manure remains an important nutrient-rich soil fertilizer and/or amendment widely applied to cropland [12–15].

In California dairy systems, liquid manure is commonly stored in OLs and CLs following liquid–solid separation. These lagoon systems function as biological reactors where microbial activity drives organic matter degradation, pathogen growth and decay, and gas production [16–18]. Covered lagoons (CLs) are increasingly adopted for methane capture and renewable energy generation; however, field-scale comparisons between CL and OL systems remain limited, particularly regarding microbial biomass degradation, pathogen reduction, solids stabilization, and physicochemical transformations under commercial dairy conditions. Genomic DNA degradation may serve as an additional indicator of microbial biomass transformation and biological stabilization during manure treatment. Existing studies are largely laboratory-based, short-term, or focused on individual treatment parameters, limiting understanding of how lagoon configuration and oxygen regime collectively influence pathogen attenuation, solids stabilization, and microbial biomass transformation under commercial dairy farm conditions.

CL and OL systems operate under distinct oxygen and microbial regimes, which can substantially influence pathogen survival, microbial biomass degradation, VS stabilization, and GHG production [19–24]. Many commercial dairy systems incorporate SL to further stabilize manure and reduce microbial and organic loads prior to land application. Covered lagoons generally maintain sustained anaerobic conditions that favor methanogenic activity and pathogen suppression, whereas OLs may support facultative and surface-aerobic processes due to atmospheric exposure. These differences can alter microbial dynamics and manure treatment performance under field conditions. In addition, lagoon systems function as biological treatment reactors, where microbial degradation of organic matter under anaerobic conditions contributes to pathogen reductions, VS reduction, and the production of methane and other gaseous emissions [25–29]. These biological and physicochemical interactions and lagoons have increased interest in understanding the treatment performance of lagoon systems under commercial dairy conditions.

Previous studies have demonstrated that dairy manure lagoon systems influence pathogen persistence, organic matter degradation, methane production, and nutrient

changes during manure storage [11,19–24]. Covered lagoons and anaerobic digesters have increasingly been adopted to reduce GHG emissions through methane capture and renewable energy generation, whereas OLs remain widely used for manure stabilization and storage in commercial dairy farms [8,14,15]. Earlier research has also shown that lagoon conditions can affect microbial survival, VS degradation, and manure physicochemical properties [20–29].

However, most existing studies have been laboratory-based, short-term, or focused on individual treatment parameters such as pathogen reduction, methane emissions, or nutrient changes independently. Limited information is available regarding the simultaneous evaluation of microbial reduction, genomic DNA degradation, solids stabilization, and physicochemical transformations across CL and OL systems under commercial dairy production conditions. In particular, field-scale comparisons examining how lagoon configuration influences microbial biomass transformation and manure treatment performance remain scarce.

The objective of this study was to evaluate how CL and OL systems influence microbial reduction, solids stabilization, genomic DNA degradation, and physicochemical changes in commercial dairy farms located in Central Valley, California. We hypothesized that the CL system promotes greater microbial inactivation and genomic DNA degradation due to sustained anaerobic conditions, whereas the OL system would favor enhanced VS degradation through facultative and surface-aerobic processes.

2. Materials and Methods

2.1. Study Site and Lagoon Systems

This field-scale study was conducted in commercial dairy farms located in Central Valley, California, United States, during warm summer months. The selected dairy farms utilized flush-based manure management systems, in which manure generated from free-stall barns was periodically removed using recycled water and transported through a liquid–solid separation system prior to lagoon storage (Figure 1). The evaluated farms had comparable herd sizes (approximately 2000–2200 cows). Similar handling practices allow for a direct comparison of OL and CL treatment performance for microbial load, physicochemical properties, and nutrients under commercial operational dairy farm conditions. Figure 1 illustrates the overall manure management workflow, lagoon configurations, treatment stages, and sampling locations evaluated in this study.

Following liquid–solid separation, the liquid manure fraction was directed into either covered primary CL (anaerobic digesters) or OL systems for storage and biological treatment, whereas separated solids were stockpiled for subsequent land application. Covered lagoon systems were designed (hydraulic retention time (HRT) of 35–40 days) to maintain anaerobic conditions for methane capture and renewable energy generation, while OL systems remained exposed to atmospheric conditions and supported facultative and surface-aerobic processes. Many dairy operation systems also incorporate SL to further stabilize manure and reduce microbial and organic loads prior to reuse or land application.

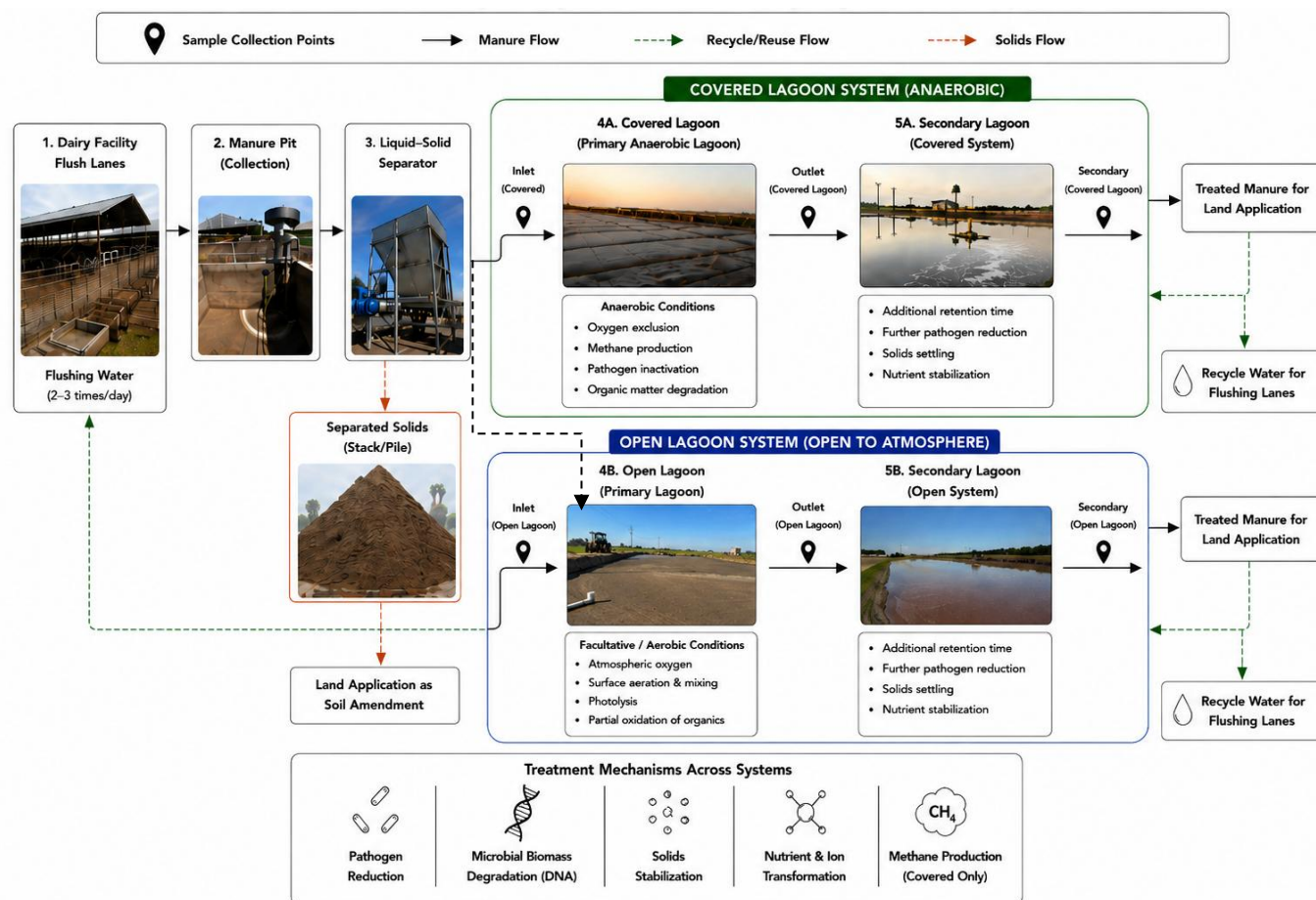


Figure 1. Schematic representation of CL and OL manure management systems, including manure collection, liquid–solid separation, primary lagoon treatment, and SL stages. Arrows indicate manure flow and treatment progression, while sampling locations for microbiological, physicochemical, and genomic DNA analyses are highlighted throughout the treatment systems.

2.2. Sampling Design and Sample Collection

A field-based comparative sampling design was used to evaluate microbial, genomic DNA, solids, and physicochemical transformations across CL and OL systems in commercial dairy farms at the end of 45 days of storage. Six biological replicates were collected for *E. coli*, TS, VS, and related nutrient analysis. Three biological replicates were collected from each sampling location for microbial, genomic DNA, and physicochemical analyses.

Manure samples were collected from multiple treatment stages within each lagoon system, including the inlet of primary lagoons, outlet of primary lagoons, and SL. Sampling locations included the covered lagoon inlet (CLI), covered lagoon outlet (CLO), open lagoon inlet (OLI), open lagoon outlet (OLO), secondary lagoon for covered lagoon systems (SL for CL), and secondary lagoon for open lagoon systems (SL for OL). These locations were selected to assess progressive biological and physicochemical changes occurring during manure storage and treatment across different lagoon configurations.

Sampling was conducted under operational dairy farm conditions using standardized collection procedures. Lagoon manure samples were collected directly from representative locations within each treatment stage using sterile polypropylene sampling containers. To minimize localized variability, samples were collected from well-mixed liquid zones and multiple points within each lagoon section whenever accessible. Collected samples were immediately stored in insulated coolers containing ice packs and transported to the laboratory for subsequent microbiological, genomic DNA, and physicochemical anal-

yses. Sample processing and microbial analyses were initiated shortly after collection to minimize potential biological changes during storage and transport. Biological replicates were collected from each sampling location to evaluate variability among lagoon treatment stages and lagoon systems.

2.3. Microbial Analysis

Microbial analysis was conducted to evaluate the reduction in *E. coli* across CL and OL treatment systems. Enumeration of *E. coli* was performed using the modified mTEC agar method following U.S. Environmental Protection Agency (EPA) Method 1603 [25]. This method is widely used for selective detection and quantification of thermotolerant *E. coli* in environmental and wastewater samples.

Prior to analysis, lagoon manure samples were homogenized by gentle mixing to ensure representative distribution of microbial populations. Samples were filtered through sterile 0.45 µm membrane filters (47 mm in diameter; Hach Company, Loveland, CO, USA) under vacuum filtration to retain bacterial cells on the membrane surface. Membranes were handled aseptically using sterile forceps and pre-rinsed with phosphate-buffered saline (PBS). Approximately 100 µL of manure sample was filtered for microbial enumeration. Following filtration, membranes were transferred onto modified mTEC agar plates and incubated at 44 ± 0.5 °C for 16–18 h. After incubation, magenta or red colonies were identified and counted as *E. coli*. Colony counts were recorded as colony-forming units (CFU) and used to evaluate microbial reductions across lagoon treatment stages.

Modified mTEC agar plates were prepared using sterile Fisherbrand™ polystyrene petri dishes (Thermo Fisher Scientific, Waltham, MA, USA). The selective medium contained chromogenic substrates and inhibitory compounds designed based on the standard methodology [25]. Sterilized media were dispensed into petri dishes under aseptic conditions and allowed to solidify prior to use. All microbial analyses were performed in biological replicates to ensure analytical consistency and reproducibility across lagoon systems and treatment stages. Results were expressed as colony-forming units (CFU mL⁻¹) and log-transformed prior to statistical analysis.

2.4. Genomic DNA Extraction and Analysis

2.4.1. Genomic DNA Extraction

Genomic DNA was extracted from lagoon manure samples to evaluate microbial biomass transformation and DNA degradation across CL and OL treatment systems [26]. DNA extraction was performed using the Quick-DNA™ Fecal/Soil Microbe Microprep Kit (Zymo Research, Irvine, CA, USA). Prior to extraction, manure samples were homogenized to obtain representative subsamples for analysis. The extraction procedure included mechanical bead-beating cell disruption, chemical lysis, column-based purification, removal of PCR inhibitors such as humic substances and polyphenols, and final elution of purified genomic DNA according to the manufacturer's protocol. Purified DNA samples were stored at 4 °C prior to analysis.

2.4.2. Genomic DNA Quantification and Integrity Analysis

Genomic DNA concentration and integrity were evaluated using two complementary analytical approaches: fluorometric quantification using the Qubit™ dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and automated capillary electrophoresis using the Agilent TapeStation 4200 system (Agilent Technologies, Santa Clara, CA, USA). The Qubit dsDNA HS assay was used for sensitive and selective quantification of double-stranded DNA. The assay included reagent preparation, dilution of DNA samples, calibration using supplied standards, and fluorometric meas-

urement using the Qubit fluorometer following manufacturer guidelines (Supplementary Table S1)

DNA fragment distribution and integrity were further characterized using the Agilent TapeStation 4200 system with Genomic DNA ScreenTape assays. The TapeStation system employs a microfluidic capillary electrophoresis platform capable of analyzing genomic DNA fragment profiles over a broad size range (approximately 100–60,000 bp) while minimizing sample volume and cross-contamination. Approximately 1 µL of purified DNA was used for each analysis. Electropherograms, gel images, genomic DNA concentrations, and DNA integrity numbers (DINs) were generated using the Agilent TapeStation 5.2 software platform. Variations in DNA concentration, electropherogram peak intensity, and DNA integrity were used to evaluate microbial biomass degradation and biological stabilization across lagoon treatment stages. The combined use of fluorometric quantification and capillary electrophoresis enabled simultaneous evaluation of DNA concentration and integrity across lagoon treatment stages [30,31].

2.5. Physicochemical Analysis

Physicochemical analyses were conducted to evaluate solids stabilization, moisture dynamics, and nutrient transformations across CL and OL treatment systems. Both VS and TS measurements based on wet basis were determined following U.S. Environmental Protection Agency (EPA) Method 1684 [32], which is commonly used for characterization of biosolids and manure-derived materials. Subsequently, VS values were transformed into VS as %TS. Prior to analysis, manure samples were thoroughly homogenized to obtain representative subsamples. For TS analysis, well-mixed samples were transferred into pre-weighed dishes and dried at 103–105 °C until constant weight was achieved. The remaining dry mass after moisture removal was recorded as TS. VS were subsequently determined by igniting the dried samples at 550 °C for 2 h in a muffle furnace. The mass loss during ignition was attributed to VS, representing the organic fraction of manure susceptible to biological degradation.

Additional physicochemical parameters, including moisture content (MC), pH, EC, sodium (Na⁺), potassium (K⁺), and calcium (Ca²⁺), were measured to assess nutrient transformations and manure stabilization during lagoon treatment. The EC and pH were measured using handheld LAQUAtwin sensors (HORIBA Scientific, Kyoto, Japan) following the manufacturer's guidelines. All measurements were performed in biological replicates to evaluate variability among lagoon systems and treatment stages. Variations in solids content, moisture, EC, and nutrient ion concentrations were used to assess physicochemical changes associated with manure treatment, microbial activity, and organic matter degradation within CL and OL systems. A summary of methods used for the analysis of various parameters in dairy manure samples is given in Supplementary Table S1.

2.6. Statistical Analysis

Statistical analyses were conducted to evaluate differences in microbial reduction, genomic DNA degradation, solids stabilization, and physicochemical properties across CL and OL systems. Data organization and preliminary calculations were carried out in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), where regression analyses—including model fitting, evaluation of goodness-of-fit metrics, and examination of residual behavior—were performed. Subsequent statistical analyses and data visualization were conducted using DATAtab (v2024).

Descriptive statistics, including means, standard deviations, minimum values, and maximum values, were calculated for all measured parameters. Data distribution and additional statistics were performed using histograms, normality plots, and quantile–quantile (Q–Q) analyses prior to statistical testing. One-way analysis of variance (ANOVA) was

used to evaluate differences among lagoon systems and treatment stages for microbial, genomic DNA, solids, and physicochemical parameters. Correlation analysis and linear regression were performed to examine relationships among measured variables and to compare analytical methods used for genomic DNA quantification. Statistical significance was evaluated at a probability level of $p < 0.05$. When ANOVA indicated significant differences, Tukey's honestly significant difference (HSD) test was used for pairwise comparisons among lagoon treatment stages. *E. coli* concentrations were log-transformed prior to statistical analyses to improve normality and variance homogeneity.

3. Results

3.1. Microbial Reduction Across Lagoon Systems

In CL systems, *E. coli* concentrations decreased from 4.24 log (± 0.04) at the inlet (CLI) to 2.45 log (± 0.18) at the outlet (CLO), corresponding to a reduction from approximately 17,500 CFU mL⁻¹ to 283 CFU mL⁻¹ (98.38%; 1.79 log reduction) (Figure 2). Further reductions occurred in the SL for CL, where *E. coli* concentrations declined to 2.12 log, resulting in an overall reduction of 99.14% relative to inlet concentrations. In OL systems, *E. coli* concentrations decreased from 2.88 log (± 0.05) at the inlet (OLI) to 2.53 log (± 0.13) at the outlet (OLO), representing a reduction from 758 CFU mL⁻¹ to 342 CFU mL⁻¹ (54.88%; 0.35 log reduction). Additional treatment in the SL for OL further reduced *E. coli* concentrations to 2.13 log (134 CFU mL⁻¹), corresponding to an overall reduction of 82.32% relative to inlet concentrations.

One-way ANOVA indicated significant differences ($p < 0.05$) in *E. coli* concentrations among lagoon treatment stages, including the CLI, CLO, OLI, OLO, and SL for CL, and SL for OL (Figure 2). The CL system demonstrated greater microbial reduction efficiency than the OL system, likely due to sustained anaerobic conditions, enhanced microbial degradation, and longer retention periods during manure storage. Higher inlet concentrations observed in the CL system may also reflect differences in solids loading and manure characteristics entering the systems. Despite these differences, outlet concentrations in both lagoon systems were substantially reduced following treatment. Secondary lagoons contributed additional microbial reductions in both systems, highlighting the importance of multi-stage lagoon treatment for improving the microbiological quality of dairy manure under commercial farm conditions.

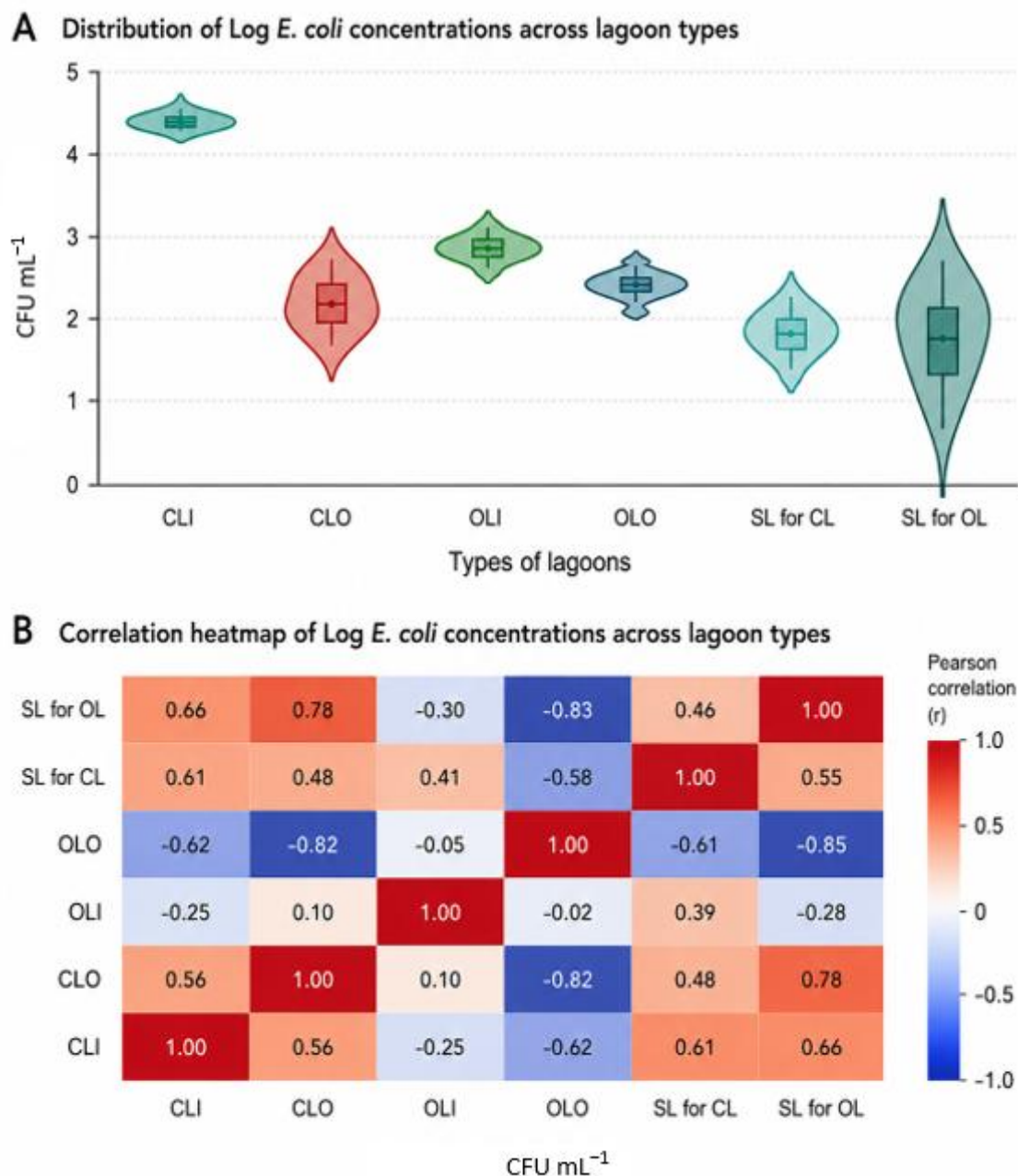


Figure 2. (A) Distribution and correlation of log *E. coli* concentrations across lagoon treatment systems. (A) Violin plots illustrate the distribution and variability of log *E. coli* concentrations among the CLI, CLO, OLI, OLO, SL for CL, and SL for OL ranges; (B) Pearson correlation heatmap showing relationships among lagoon treatment stages based on log *E. coli* concentrations. Positive correlations are shown in red and negative correlations are shown in blue, with color intensity corresponding to correlation strength.

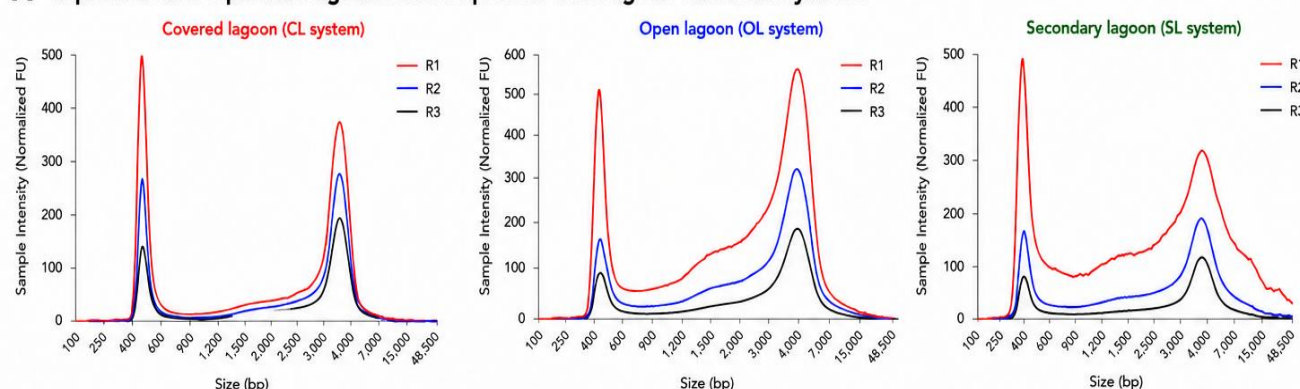
3.2. Genomic DNA Degradation and Microbial Biomass Transformation

In addition to *E. coli* enumeration, genomic DNA analysis was conducted to further evaluate microbial biomass transformation across CL and OL systems. Electrophoresis profiles and electropherograms of lagoon-derived genomic DNA are presented in Figure 3. In both CL and OL systems, inlet samples exhibited the strongest band intensity and highest electropherogram peak signals, whereas outlet and SL samples showed progressively weakened bands and reduced peak intensity. These trends indicate progressive microbial biomass degradation and biological stabilization during lagoon treatment.

At the CLI, electropherogram peak intensity reached approximately 500 fluorescence units (FU), with additional reductions observed in SL. Similar trends were observed in OL systems. DNA integrity numbers (DIN > 5) indicated the persistence of moderately intact genomic DNA despite reductions in signal intensity and DNA abundance across lagoon stages.

Fluorometric quantification further supported these observations (Table 1). Average genomic DNA concentrations at lagoon inlets were 56.03 (± 14.68) ng μL^{-1} for the CLI and 60.33 (± 14.46) ng μL^{-1} for the OLI. Concentrations decreased to 18.60 (± 1.21) ng μL^{-1} at the CLO and 45.53 (± 2.98) ng μL^{-1} at the OLO, with further reductions observed in SL. Closed lagoons exhibited a 66.80% reduction in DNA concentration, whereas OLs showed a 24.53% reduction. Secondary lagoon further enhanced DNA reduction, resulting in overall reductions of 76.02% and 88.32% in CL and OL systems, respectively.

A Representative TapeStation genomic DNA profiles from lagoon treatment systems



B Automated gel electrophoresis of extracted genomic DNA from lagoon treatment systems

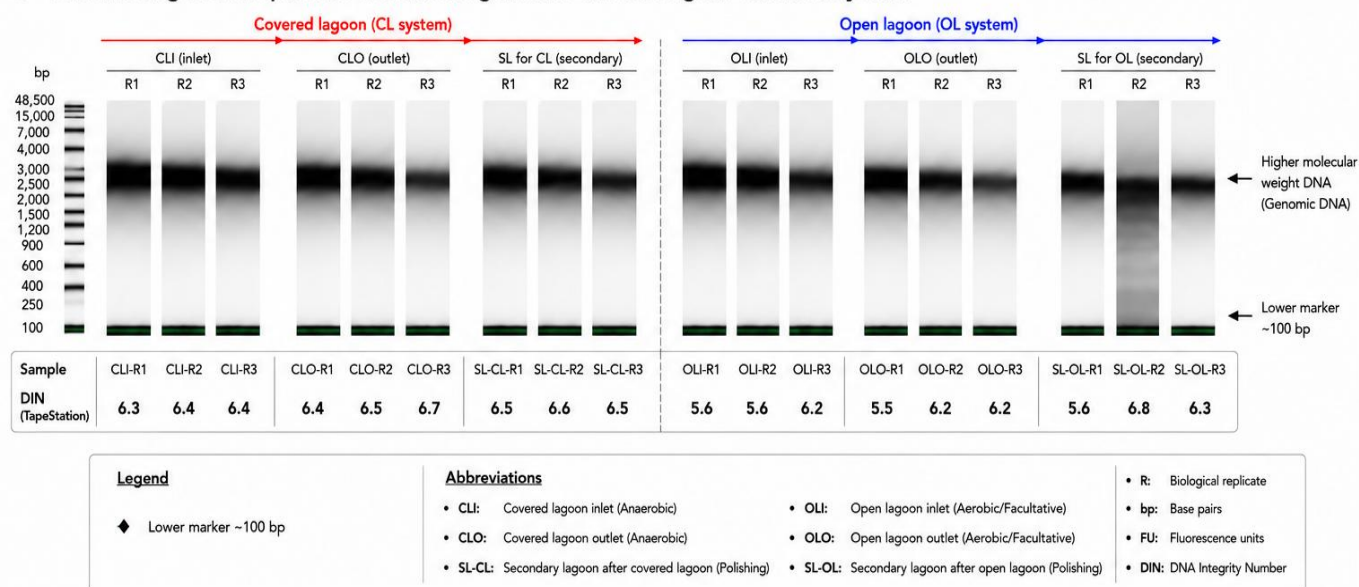


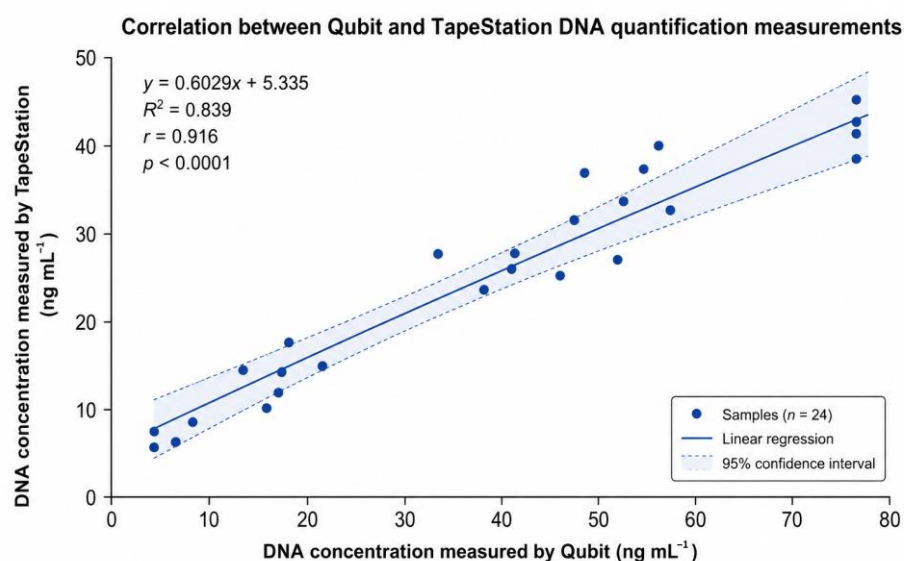
Figure 3. Representative TapeStation genomic DNA profiles and automated gel electrophoresis of extracted genomic DNA from covered and open lagoon treatment systems. (A) Electropherograms showing genomic DNA integrity patterns across covered lagoon (CL), open lagoon (OL), and secondary lagoon (SL) systems for biological replicates (R1–R3). (B) Automated gel electrophoresis illustrating genomic DNA distribution and integrity across lagoon treatment stages, including the covered lagoon inlet (CLI), covered lagoon outlet (CLO), secondary lagoon after covered lagoon (SL for CL), open lagoon inlet (OLI), open lagoon outlet (OLO), and secondary lagoon after open lagoon (SL for OL). DNA integrity number (DIN) values are shown below each sample lane. Higher-

molecular-weight DNA bands indicate preserved genomic DNA integrity, while smearing patterns indicate DNA fragmentation and degradation during lagoon treatment processes.

The DNA values presented in Table 1 are whole genomic DNA quantity and represented total DNA extractable from manure samples, rather than DNA of *E. coli*. In order to retain data integrity, DNA data were not normalized. Genomic DNA concentrations measured using the Qubit fluorometer and Agilent TapeStation showed strong agreement ($R^2 = 0.84$) (Figure 4), confirming consistency between analytical methods. Overall, reductions in DNA concentration, electropherogram intensity, and band sharpness were consistent with declining *E. coli* concentrations (in most cases), indicating progressive microbial attenuation and manure stabilization during lagoon treatment.

Table 1. Genomic DNA concentrations of manure estimated based on two approaches.

Type of Lagoons	Qubit-Based DNA	DNA by TapeStation
Covered lagoon inlet (CLI)	43.10	32.60
	53.00	39.70
	72.00	44.90
Covered lagoon outlet (CLO)	19.30	14.30
	19.30	14.90
	17.20	9.99
Open lagoon inlet (OLI)	53.00	34.90
	77.00	39.50
	51.00	33.40
Covered lagoon outlet (OLO)	48.90	25.80
	44.50	27.80
	43.20	15.50
Secondary lagoon for covered lagoon systems (SL for CL)	13.50	8.83
	13.00	12.10
	13.80	10.50
Secondary lagoon for open lagoon systems (SL for OL)	6.31	8.74
	5.86	4.97
	9.13	6.45



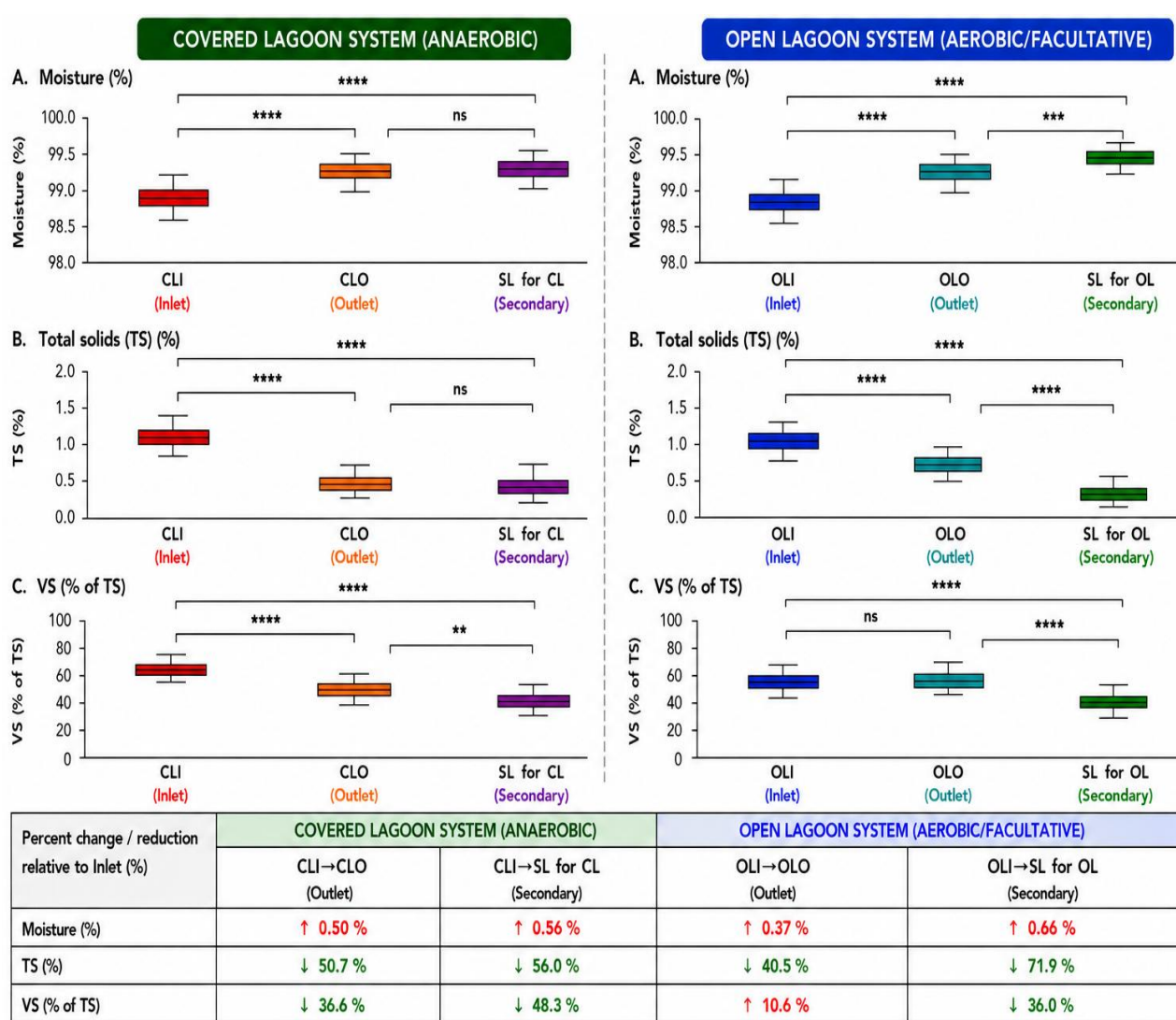
TapeStation: Agilent 4200 TapeStation system; Qubit: Invitrogen Qubit fluorometer.
 DNA was extracted from lagoon samples across treatment stages (inlet, outlet, secondary lagoons).

Figure 4. Correlation between Qubit and TapeStation DNA quantification measurements across lagoon treatment systems. The scatter plot shows the relationship between DNA concentrations measured using the Invitrogen Qubit fluorometer and the Agilent 4200 TapeStation system for lagoon-

derived samples collected from the CLI, CLO, OLI, OLO, SL for CL, and SL for OL. A strong positive correlation was observed between the two analytical methods ($R^2 = 0.839$; $r = 0.916$; $p < 0.0001$), supporting the reliability and consistency of genomic DNA quantification across lagoon treatment stages.

3.3. Moisture, Total Solids, and Volatile Solids Reduction Across Lagoon Treatment Stages

Reductions in MC, TS, and VS across lagoon treatment stages are presented in Figure 5. Statistical analysis using one-way ANOVA indicated significant differences ($p < 0.05$) among lagoon stages for moisture content, TS, and VS (% of TS) in both CL and OL systems. Moisture content remained consistently high (>98%) across all treatment stages, reflecting the highly diluted nature of liquid dairy manure in lagoon systems, which reflects the daily water management used in the farms with three flushings. Marginal increases in MC from inlet to outlet stages were associated with reductions in suspended and settleable solids during lagoon storage and treatment.



ns $P \geq 0.05$ * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ **** $P < 0.0001$ (one-way ANOVA with Tukey's post hoc test)

Figure 5. Comparison of MC, TS, and VS, expressed as % of TS across treatment stages in the CL and OL systems. Panels (A–C) show boxplots for moisture (%), TS (% wet basis), and VS (% of TS), respectively, for samples from the inlet, and outlet, and SL of the CL and OL systems. The covered lagoon system exhibited substantial reductions in TS and VS across treatment stages (maybe due to solids degradation), while the OL system showed moderate TS reductions and variable VS. Percent change or reduction relative to inlet concentrations is summarized in the table below the box-

plots. Boxplots represent the median (center line), interquartile range (box), and range (whiskers); $n = 6$. Statistical significance was evaluated using one-way ANOVA with Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns — not significant).

Table 2 presents the MC, TS, and VS values for manure samples collected from the primary and secondary lagoons of both the CL and OL systems. The MC of the samples increased from the primary lagoon to the secondary lagoon, which resulted in a corresponding decrease in TS values. For the CL system, the initial MC at the inlet was about 99.01%, increasing to about 99.51% (w.b.) at the outlet (an increase of approximately 0.5%). At the SL for the CL system, there was a marginal additional increase of about 0.05%. For the OL system, the MC at the inlet was about 99.09%, increasing to about 99.46% at the outlet (an increase of approximately 0.37%). At the SL for the OL system, the MC further increased to about 99.74%, representing an increase of about 0.28% from the OL system outlet.

TS decreased from approximately 0.985% at the CLI to 0.485% at the CLO, corresponding to a 50.7% reduction. Additional treatment in the secondary lagoon further reduced TS by 56% relative to inlet concentrations. The VS (as a percentage of TS) decreased by about 36.6% and 48.3% from the CLI to the CLO and the CLI to the SL, respectively. Similar trends were observed in the open lagoon system, where TS decreased from the OLI to the OLO and from the OLI to the SL, representing reductions of 40.5% and 71.9%, respectively. For VS (as a percentage of TS) in the open lagoon system, the values increased by about 10.6% from the OLI to the OLO, while from the CLI to the SL they decreased by about 36%. This temporary increase in VS/TS from the OLI to the OLO may reflect changes in organic matter composition during facultative treatment processes.

Statistical evaluation confirmed the robustness of the dataset. Normality testing indicated that the data did not significantly deviate from normal distributions, with p -values of 0.09 for *E. coli*, 0.52 for TS, and 0.84 for VS. This observation needs further data analysis with more replications and more measurements. These results support the validity of subsequent parametric analyses. Distribution characteristics were further examined using histograms and quantile–quantile plots (Supplementary Figure S1), which visually confirmed the consistency of the data across measured variables.

Overall, both CL and OL systems contributed to solids reduction and manure stabilization, although the CL system exhibited greater reductions in VS and stabilization between inlet and outlet stages. Secondary lagoon further enhanced solids reduction in both systems, highlighting the importance of multi-stage lagoon treatment for improving manure quality and reducing organic loading in dairy wastewater management systems. In general, TS decreased by 56% in the CL system and 71.9% in the OL system. The VS in the CL and OL systems was reduced by 48.3% and 36.0%, respectively.

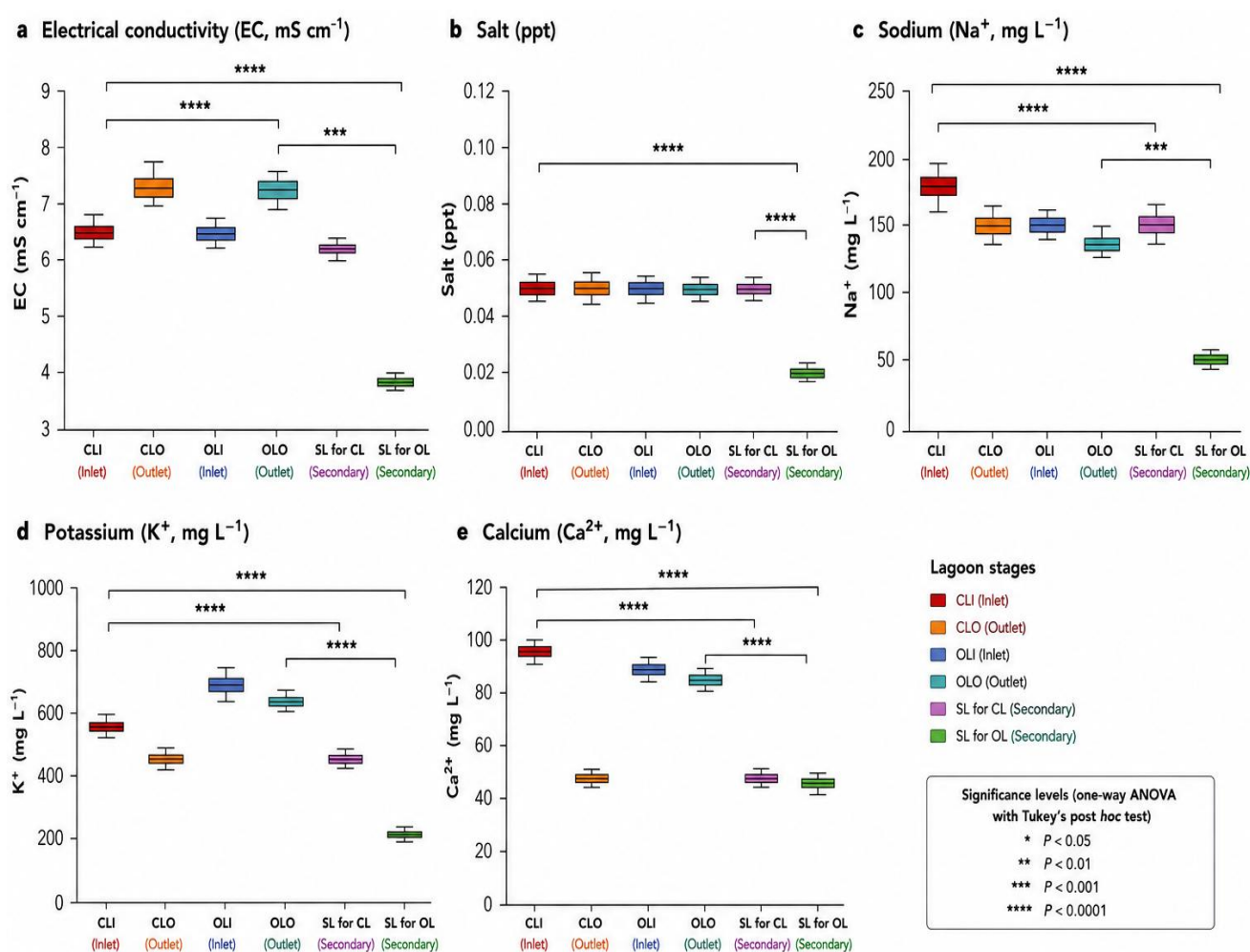
Table 2. Moisture, TS, and VS changes in the covered and open lagoon systems across different treatment stages in commercial dairy farms.

Parameter	CLI	CLO	SL for CL	OLI	OLO	SL for OL
Moisture (%)	99.01 ± 0.06	99.51 ± 0.06	99.57 ± 0.02	99.09 ± 0.10	99.46 ± 0.10	99.74 ± 0.03
Total solids (%)	0.985 ± 0.06	0.485 ± 0.06	0.433 ± 0.02	0.911 ± 0.10	0.542 ± 0.10	0.256 ± 0.03
Volatile solids (% of TS)	64.327 ± 1.21	40.788 ± 2.50	33.279 ± 4.84	55.714 ± 7.21	61.598 ± 11.12	35.635 ± 6.86

3.4. Inorganic and Physicochemical Characteristics Across Lagoon Treatment Stages

Figure 6 shows the boxplot comparison of EC, salt concentration, (Na^+), (K^+), and (Ca^{2+}) across lagoon treatment stages, while the average values and descriptive statistics are included in Table 3. Significant differences ($p < 0.05$) were observed among lagoon stages (CLI, CLO, OLI, OLO, SL for CL, and SL for OL) for several chemical parameters, including Na^+ , Ca^{2+} , EC, and pH.

In general, inorganic constituent concentrations were highest at CL and OL lagoon inlets and decreased through the CL and OL outlets and SL stages. This trend indicates dilution (due to increase in MC), settling, and biological transformation during manure treatment. Average sodium concentrations were approximately 182 mg L^{-1} at the CLI and 150 mg L^{-1} at the CLO, while sodium concentrations at the SL for CL were about 152 mg L^{-1} . Sodium concentrations at the OLI were about 152 mg L^{-1} and at the OLO were about 138 mg L^{-1} . Secondary lagoons in the open lagoon system showed a substantial reduction in sodium concentrations (final concentration: approximately 50 mg L^{-1}).



Boxplots show median (center line), interquartile range (box), and range (whiskers; $n = 5$).
 CLI, covered lagoon inlet; CLO, covered lagoon outlet; OLI, open lagoon inlet; OLO, open lagoon outlet;
 SL for CL, secondary lagoon for covered lagoon; SL for OL, secondary lagoon for open lagoon.

Figure 6. Boxplot comparison of EC, salts, Na^+ , K^+ , and Ca^{2+} across lagoon treatment stages. Panels (a–e) show distributions of physicochemical parameters for the CLI, CLO, OLI, OLO, SL for CL, and SL for OL. Boxplots represent median, interquartile range, and whisker-defined variability ($n = 5$). Statistical significance among treatment stages was evaluated using one-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Distinct physicochemical trends and ion redistribution patterns were observed across lagoon treatment stages.

Electrical conductivity averaged 6.35 mS cm^{-1} at the CLI and 6.90 mS cm^{-1} at the OLI, while outlet values were 7.50 mS cm^{-1} for the CLO and 7.36 mS cm^{-1} for the OLO. Lower EC values were observed in the SL, especially the SL for OL (3.76 mS cm^{-1}). Salt concentrations ranged from approximately 0.02% to 0.06% across lagoon systems.

Calcium concentrations also declined across treatment stages. The pattern was similar across systems, but the reduction from the CLI to the CLO was greater. Average Ca^{2+} concentrations were 95 mg L^{-1} at the CLI and 89 mg L^{-1} at the OLI, decreasing to 46 mg L^{-1} at the CLO and 85 mg L^{-1} at the OLO. Secondary lagoons further reduced Ca^{2+} concentrations to 48 ppm in the SL for CL and 45 ppm in the SL for OL. Similar trends were observed for K^{+} (Table 3), indicating progressive stabilization and settling of dissolved and particulate constituents during lagoon storage.

The pH values ranged from approximately 6.8 at the CLI and 7.1 at the OLI, while outlet values averaged approximately 7.1 for the CLO and 7.3 for the OLO. These near-neutral to slightly alkaline conditions are consistent with previous reports for liquid dairy manure and may influence microbial activity and gaseous emissions during storage.

Overall, reductions in inorganic constituents, EC, and solids across lagoon treatment stages indicate progressive manure stabilization during storage and treatment. These findings demonstrate that multi-stage lagoon systems help improve manure quality and may support strategies aimed at reducing environmental emissions and enhancing nutrient management in commercial dairy operations.

Table 3. EC, nutrients, and pH of the manure samples collected from CL and OL systems across different treatment stages in commercial dairy farms.

Parameter	CLI	CLO	SL for CL	OLI	OLO	SL for OL
EC (mS cm^{-1})	6.35 ± 0.25	7.50 ± 0.05	6.19 ± 0.06	6.90 ± 0.35	7.36 ± 0.05	3.76 ± 0.03
Salt (ppt)	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.02 ± 0.00
Na^{+} (mg L^{-1})	182 ± 4	150 ± 0	152 ± 4	152 ± 4	138 ± 4	50 ± 1
K^{+} (mg L^{-1})	552 ± 8	452 ± 8	453 ± 13	692 ± 15	633 ± 10	213 ± 5
Ca^{2+} (mg L^{-1})	95 ± 4	46 ± 1	48 ± 1	89 ± 2	85 ± 2	45 ± 1
pH	6.8 ± 0.05	7.1 ± 0.08	7.6 ± 0.0	7.1 ± 0.04	7.3 ± 0.08	7.2 ± 0.04

4. Discussion

This field study provides insights into the microbial and physicochemical dynamics of lagoon-based manure management under commercial dairy farm conditions. Both CL and OL systems functioned as active bioreactors that promoted solids reduction and microbial transformation during manure storage. Reductions in VS are particularly important because anaerobic degradation of organic matter produces GHG in dairy manure systems [13,14,22,23]. Concurrent reductions in *E. coli* further demonstrate the role of lagoon treatment in reducing microbial risks associated with dairy effluents [18,33–38].

California's Central Valley is a major dairy-producing region where large manure volumes are linked with air, water, and soil quality [18,39–43]. Lagoon storage remains one of the most practical and widely adopted manure management approaches for large-scale dairy farms. In recent years, CL systems have increasingly been implemented to capture methane for renewable energy production and reduce GHG emissions [9,22,44]. The present research findings indicate that lagoon configuration substantially influences manure characteristics and treatment performance under field conditions.

One more factor which can influence the manure characteristics is temperature. This study captured manure characteristics during warm summer conditions typical of California ($30\text{--}40 \text{ }^{\circ}\text{C}$), when microbial activity and anaerobic digestion are enhanced [11,20,45,46]. Elevated temperatures promote biological degradation processes and may increase methane production from stored manure [13,47–49]. Under these conditions, re-

ductions in VS observed in both lagoon systems likely reflect active microbial degradation of organic matter. The closed lagoon system, which maintained sustained anaerobic conditions, showed greater reductions in VS and *E. coli*, indicating enhanced organic matter stabilization under anaerobic treatment. Restricted oxygen availability in CL systems may further favor anaerobic microbial communities involved in organic matter conversion and methane generation [13,15,50–53].

Physicochemical properties such as pH, EC, salts, and nutrient ions also varied across lagoon stages and may influence microbial processes and gaseous emissions. Electrical conductivity observed in this study was consistent with previously reported ranges for liquid dairy manure [36,40]. Together, these findings suggest that lagoon chemistry and solids stabilization are closely linked to environmental performance in manure management systems.

Overall, the results demonstrate that lagoon design plays an important role in regulating microbial reduction, solids stabilization, and potential emissions from dairy manure storage systems. The closed lagoon system offers additional advantages where there is potential to capture methane which can be further used as renewable energy, while SL further enhances manure stabilization and reduction in microbial loads. These findings provide practical field-based evidence supporting the use of multi-stage lagoon systems for more sustainable and biosecure dairy manure management.

5. Conclusions

This study demonstrated that dairy manure lagoons can substantially reduce bacterial loads and promote manure stabilization prior to land application, although complete bacterial elimination was not achieved. Covered lagoons were more effective in reducing *E. coli*, likely because anaerobic conditions promoted stronger biological treatment. Both CL and OL systems reduced *E. coli*, TS, and VS. The degradation was substantial, particularly when combined with SL systems. Both systems contributed to manure stabilization and improvement in manure quality during storage. Further, the results suggest that electropherograms peak intensity and genomic DNA concentrations declined substantially across lagoon treatment stages in both covered and open lagoon systems, indicating progressive degradation of microbial genetic material during treatment. Despite these reductions, DNA integrity numbers ($DIN > 5$) and strong agreement between Qubit and TapeStation measurements ($R^2 = 0.84$) demonstrated the persistence of moderately intact genomic DNA, while overall decreases in DNA abundance were generally consistent with declining *E. coli* concentrations and increasing manure stabilization. Overall, this field-scale study provides practical information for improving existing understanding of CL and OL systems, associated pathogen reduction, solids stabilization, and changes in physicochemical properties in dairy manure. Future long-term studies across different seasons will enhance this study further to better understand how environmental conditions influence lagoon treatment performance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriengineering8070284/s1>, Figure S1. Distribution diagnostics and normality assessment for log *E. coli*, total solids (TS), and volatile solids (VS) datasets. Histograms with fitted density curves (left panels) and quantile–quantile (Q–Q) plots (right panels) were used to evaluate data distribution and normality assumptions prior to statistical analyses. Overall, the datasets showed approximate normal distributions with minor deviations at distribution tails, supporting the application of parametric statistical methods, including one-way ANOVA.; Table S1: Summary of methods applied for the analysis of various parameters in dairy manure samples.

Author Contributions: P.P. (Pramod Pandey).; Conceptualization, P.P. (Pramod Pandey)., A.C. and N.S.-d.-R.; methodology, A.P., J.H., J.S.T. and P.P. (Pramod Pandey).; software, A.P., P.P. (Pramod Pandey)., N.C., J.H. and P.P. (Prachi Pandey).; validation, A.P., P.P. (Pramod Pandey), W.L. and P.P. (Prachi Pandey); formal analysis, A.P., W.L., J.S.T. and P.P. (Pramod Pandey); investigation, P.P. (Prachi Pandey); resources, P.P. (Pramod Pandey), A.C., J.S.T. and N.S.-d.-R.; writing—original draft preparation, P.P. (Pramod Pandey), A.P. and J.H.; writing—review and editing, P.P. (Pramod Pandey)., A.C., B.C.M., J.S.T., N.C., W.L., N.S.-d.-R. and W.L.; visualization., A.P., J.S.T. and P.P. (Pramod Pandey); supervision, P.P. (Pramod Pandey) All authors have read and agreed to the published version of the manuscript.

Funding: No external funding for this work.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data used in the study are included in the manuscript.

Acknowledgments: Special thanks go to Trudy Van Beek for her invitation to visit the dairy farm with covered lagoons and manure management systems, which allowed us to collect samples from an operational dairy digester system and enhance our understanding of covered lagoon systems. Authors also acknowledge the support of Tim Van Beek and Doug Van Beek (Van Beek Brothers Dairy, Tipton CA) and Dennis Da Silva (Da Silva Dairy Farms, LP, Escalon, CA) for facilitating the samples collection and providing crucial information about dairy manure management in commercial farms. The authors gratefully acknowledge the support provided by the University of California Agriculture and Natural Resources (UC ANR) and the School of Veterinary Medicine at the University of California, Davis. Their institutional support facilitated field access, research coordination, and laboratory analyses that were essential to this study.

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

Abbreviations

ANOVA	analysis of variance
CFU	colony-forming units
CL	covered lagoon
CLI	covered lagoon inlet
CLO	covered lagoon outlet
DIN	DNA integrity number
DNA	Deoxyribonucleic Acid
<i>E. coli</i>	<i>Escherichia coli</i>
EC	electrical conductivity
EPA	Environmental Protection Agency
GHG	greenhouse gas
MC	moisture content
mTEC	Modified Thermotolerant <i>Escherichia coli</i> Medium
OL	open lagoon
OLI	open lagoon inlet
OLO	open lagoon outlet
PBS	phosphate-buffered saline
Q-Q Plot	quantile–quantile Plot
R^2	coefficient of determination
SL for CL	secondary lagoon for covered lagoon systems
SL for OL	secondary lagoon for open lagoon systems
SL	secondary lagoon
TS	total solids
VS	volatile solids

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