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Kinetics of Pathogen Attenuation in Desiccated Fecal Sludge: Establishing Quantitative Time-Based Hygienization Criteria for Safe Agricultural Reclamation.

Cinétique de l'atténuation des pathogènes dans les boues fécales desséchées : établissement de critères d'hygiénisation quantitatifs basés sur le temps pour une remise en état agricole sûre.

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ABSTRACT :

Fecal sludge hygienization is essential for safe agricultural reuse, particularly within geographic regions where decentralized on-site sanitation infrastructure predominates. While solar drying and subsequent prolonged storage are extensively utilized as economically viable stabilization strategies, quantitative data describing microbial inactivation during drying and storage remain limited. This investigation systematically evaluated the temporal evolution and inactivation kinetics of pivotal microbiological parameters during the hygienization of desiccated fecal sludge over a longitudinal period extending up to twelve months. The study evaluated Escherichia coli, total coliforms, and intestinal enterococci, alongside the qualitative monitoring of Salmonella spp. and the assessment of helminth egg viability as a primary determinant of sanitary safety. Quantitative bacterial surrogates were characterized using first-order decay kinetic models, whereas pathogenic indicators were analyzed through a temporal occurrence framework to determine environmental persistence. The results demonstrate that Escherichia coli exhibited an inactivation rate constant of 0.42 month^{-1} (R^2 = 0.78), necessitating approximately 5.5 and 11.0 months to achieve 90% and 99% reductions, respectively. Total coliform bacteria demonstrated significantly higher environmental resilience, characterized by a lower inactivation rate constant of 0.31 month^{-1} (R^2 = 0.70), indicating a more protracted decay trajectory. While Salmonella spp. were successfully attenuated beyond detection thresholds following extended hygienization, the continued presence of viable helminth eggs corroborates their role as the most conservative sanitary indicator. These findings establish a rigorous scientific basis for the implementation of time-based hygienization criteria, thereby enhancing regulatory guidelines for the sustainable and safe reuse of fecal sludge in the circular economy.

Keywords : fecal sludge; hygienization; microbial inactivation; kinetics; agricultural reuse; sanitation safety.

RESUME:

L'hygiénisation des boues fécales est essentielle à leur réutilisation agricole en toute sécurité, notamment dans les régions où prédominent les infrastructures d'assainissement autonomes et décentralisées. Si le séchage solaire et le stockage prolongé qui s'ensuit sont largement utilisés comme stratégies de stabilisation économiquement viables, les données quantitatives décrivant l'inactivation microbienne durant ces processus restent limitées. Cette étude a évalué de manière systématique l'évolution temporelle et la cinétique d'inactivation de paramètres microbiologiques clés lors de l'hygiénisation de boues fécales desséchées sur une période allant jusqu'à douze mois. L'étude a évalué Escherichia coli, les coliformes totaux et les entérocoques intestinaux, ainsi que Salmonella spp. (suivi qualitatif) et la viabilité des œufs d'helminthes (évaluation comme principal déterminant de la sécurité sanitaire). Les marqueurs bactériens quantitatifs ont été caractérisés à l'aide de modèles cinétiques de décroissance du premier ordre, tandis que les indicateurs de pathogénicité ont été analysés selon une approche temporelle afin de déterminer leur persistance environnementale. Les résultats démontrent qu'Escherichia coli présente une constante de vitesse d'inactivation de 0,42 mois^{-1} (R^2 = 0,78), nécessitant environ 5,5 et 11,0 mois pour atteindre des réductions de 90 % et 99 %, respectivement. Les coliformes totaux présentent une résilience environnementale significativement plus élevée, caractérisée par une constante de vitesse d'inactivation plus faible de 0,31 mois^{-1} (R^2 = 0,70), indiquant une cinétique de dégradation plus lente. Bien que Salmonella spp. ait été efficacement éliminée en dessous des seuils de détection après une hygiénisation prolongée, la présence continue d'œufs d'helminthes viables confirme leur rôle d'indicateur sanitaire le plus fiable. Ces résultats établissent une base scientifique rigoureuse pour la mise en œuvre de critères d'hygiénisation basés sur le temps, renforçant ainsi les directives réglementaires pour une réutilisation durable et sûre des boues de vidange dans l'économie circulaire.

Mots clés : boues fécales ; hygiénisation ; inactivation microbienne ; cinétique ; réutilisation agricole ; sécurité sanitaire.

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I. INTRODUCTION

In numerous low- and middle-income jurisdictions, decentralized on-site sanitation systems, primarily comprising pit latrines and septic tanks, represent the dominant sanitation systems. While these systems provide indispensable primary sanitation services, they concurrently generate substantial volumes of fecal sludge that necessitate rigorous management protocols to mitigate risks to public health and environmental integrity. Historically, the substandard management of these excreta-based residuals has been definitively linked to the degradation of groundwater quality, the eutrophication of surface water bodies, and a heightened incidence of community exposure to diverse pathogenic microorganisms (Strade *et al.*, 2014 ; UN-Water, 2014).

Simultaneously, fecal sludge represents a significant reservoir of stabilized organic matter and essential macronutrients, such as nitrogen and phosphorus, which possess the potential to enhance soil pedogenesis and optimize agricultural productivity when treated appropriately. The strategic reclamation of treated fecal sludge for agricultural applications thus presents a opportunity to recycle nutrients and promote regenerative agricultural paradigms. This is particularly critical in geographic regions characterized by limited availability and

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affordability of synthetic mineral fertilizers (Drechsel *et al.*, 2015 ; Payne & Smith, 2011).

A primary impediment to the widespread adoption of fecal sludge reuse in agriculture remains the pervasive presence of enteric pathogens, including pathogenic bacteria, enteric viruses, and resilient helminth species. Consequently, robust hygienization processes are mandatory to attenuate microbial concentrations to levels deemed acceptable by international safety standards prior to environmental discharge or reuse. Conventional stabilization methodologies frequently involve aerobic composting, alkaline stabilization, or thermal processing; however, solar drying followed by prolonged storage remains a prevalent strategy due to its economic efficiency, operational simplicity, and scalability within decentralized sanitation frameworks (Strauss & Montangero, 2003 ; Jothinathan & Singh, 2023).

The efficacy of drying and storage-based stabilization relies upon the synergistic impact of environmental stressors-including desiccation, thermal fluctuations, ultraviolet radiation exposure, and temporal decay-to facilitate microbial inactivation. Although a multitude of empirical studies have documented significant reductions in microbial indicators following these treatments, the majority of current assessments remain predominantly descriptive, often focusing on static comparisons between initial and terminal concentrations. There is a discernible lack of rigorous longitudinal analysis concerning the specific dynamics and mechanistic pathways of microbial inactivation over extended durations (Feachem *et al.*, 1983).

The quantification of microbial decay kinetics is an essential prerequisite for establishing scientifically validated minimum treatment durations and for comparing the relative environmental resistance of diverse microbiological taxa. Such kinetic modeling is vital for the formulation of evidence-based regulatory frameworks governing the safe reuse of human excreta in the food chain (WHO, 2006 ; Stenström *et al.*, 2011). While inactivation kinetics have been extensively utilized in centralized wastewater treatment and food microbiology, their application to fecal sludge under ambient, field-relevant conditions remains sparse, particularly concerning the long-term stabilization of desiccated solids (Crittenden *et al.*, 2012).

The primary objective of the present investigation was to systematically characterize the temporal evolution and inactivation kinetics of critical microbiological parameters during the hygienization phase of dried fecal sludge. This research specifically evaluated the behavior of bacterial indicators, such as *Escherichia coli*, total coliforms, and intestinal enterococci, alongside pathogenic surrogates including *Salmonella* spp. and helminth eggs. The overarching goal was to derive precise, time-based hygienization criteria that ensure microbiological safety for sustainable agricultural reuse, thereby bridging the gap between empirical observation and regulatory application (Walther & Ewald, 2024).

II. MATERIALS AND METHODS

II.1. Hygienization protocol and longitudinal sampling strategy

The primary substrate for this investigation consisted of fecal sludge harvested from the centralized treatment facility in Fianarantsoa, Madagascar. This facility processes residuals derived from various on-site sanitation systems, which are highly representative of the diverse urban and peri-urban demographics within the region. Before the initiation of the experimental hygienization phase, the raw sludge underwent a preliminary dewatering stage on unplanted drying beds for approximately three months. This pre-treatment was essential for moisture reduction and volumetric stabilization; however, it was classified strictly as a preparatory intervention and excluded from kinetic modeling because the present study specifically focused on pathogen attenuation during post-desiccation storage rather than during active dehydration. The dehydration phase involves environmental and physicochemical processes distinct from those governing long-term storage conditions. The formal hygienization process comprised an intensive drying phase followed by an extended period of natural storage under environmental conditions reflecting local management practices. During this storage interval, the sludge was exposed to ambient temperature fluctuations and natural ventilation, factors that facilitate gradual microbial inactivation through desiccation and prolonged biological sequestration. It is imperative to specify that the hygienization age defined in this study refers exclusively to the duration of storage following the initial dewatering on drying beds. This approach ensures that the kinetic analysis specifically isolates the efficacy of storage-based desiccation as a pathogen reduction mechanism (World Health Organization, 2018 ; Strande, Ronteltap, & Brdjanovic, 2014). The sampling design was structured to capture the longitudinal evolution of microbiological indicators across a maturation spectrum ranging from zero to twelve months of storage. This multi-point methodology allowed for the determination of precise inactivation rates rather than providing a mere static comparison of initial and final microbial loads. (WHO, 2018 ; Strande *et al.*, 2014). At each sampling time, triplicate sludge samples were collected and analyzed independently for each microbiological parameter. Results are presented as mean values ± standard deviation.

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II.2. Environmental and structural monitoring

To correlate microbial reduction with physical storage parameters, the environmental conditions at the Fianarantsoa treatment site were systematically documented. The structural configuration of the drying systems and the subsequent storage areas played a pivotal role in determining the rate of moisture loss and temperature exposure. By maintaining a representative longitudinal monitoring framework, the study accounts for the seasonal variations inherent in the Malagasy climate, which directly influence the efficacy of natural stabilization processes. This structural context is vital for interpreting the variability observed in the subsequent microbiological assays. The infrastructure illustrated in Figure 1 demonstrates the standard operational layout utilized for the desiccation of fecal sludge prior to the long-term storage phase. The design prioritizes maximum surface area exposure to facilitate rapid water loss, a critical factor for achieving the initial stabilization required before the 12-month maturation period.

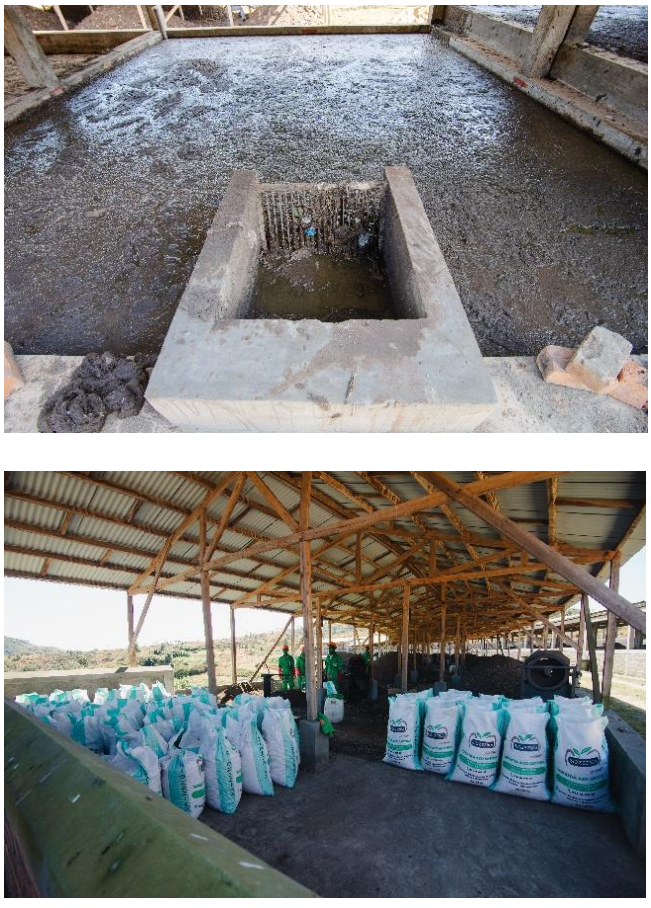

Figure 1 : Drying system and storage conditions of fecal sludge at the Fianarantsoa treatment site

Observations indicate that the structural integrity of these drying beds, combined with the local ventilation conditions, established a baseline moisture content that significantly influenced the survival thresholds of enteric pathogens. This mechanical configuration is essential for contextualizing the subsequent kinetic data regarding microbial inactivation in a tropical urban sanitation context. Furthermore, the systematic recording of ambient parameters ensures that the observed decay rates can be evaluated against localized climatic stressors, providing a reproducible basis for secondary sanitation modeling and the evaluation of regional safety standards. Such documentation is necessary to substantiate the relationship between environmental forcing and the biological attenuation processes observed throughout the experimental period during the 12-month storage phase.

II.3. Microbiological analytical framework and quality assurance

The microbiological characterization of fecal sludge samples was executed by specialized accredited institutions, specifically the Institut Pasteur de Madagascar and LABOCEA 22440 Ploufragan. These facilities implemented standardized and rigorously validated analytical protocols to ensure methodological consistency and adherence to internationally recognized benchmarks. By leveraging the expertise of these laboratories, the study maintained a high degree of quality control, ensuring that all procedural steps-from sample preparation to final enumeration-conformed to the stringent requirements necessary for high-impact scientific dissemination. Such institutional oversight is critical for mitigating inter-laboratory variability and reinforcing the reliability of data regarding pathogen reduction during stabilization A comprehensive suite of indicators was selected to evaluate the sanitary quality of the sludge, focusing on both bacterial and parasitic pathogens. The bacterial assessment involved the quantification of *Escherichia coli* and total coliforms, utilizing the Most Probable Number or Colony-Forming Units per gram of dry matter. Furthermore, the presence of intestinal enterococci was quantified where technically feasible, while the detection of *Salmonella* spp. was performed as a qualitative assessment of presence or absence. This multi-indicator approach provides a holistic perspective on the microbial safety profile, capturing differential survival rates of various pathogenic group (Strande *et al.*, 2014 ; Jiménez, 2007).

To address parasitic risk, the study included the analysis of helminth eggs, assessing total counts and viability per 25 g of material to serve as a marker for desiccation efficacy. (Cofie *et al.*, 2016 ; Koné *et al.*, 2007).

II.4. Data processing and kinetic modeling

To ensure the statistical validity of the microbiological datasets, all quantitative parameters were subjected to natural logarithmic transformation (ln) prior to the derivation of

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inactivation rates. For observations falling below the analytical Limit of Detection (LOD), a conservative substitution method was applied by assigning a value equal to half of the LOD (LOD/2), following recommendations for censored environmental datasets **(**Helsel, 2012**)**. Although this approach may introduce minor bias at low concentrations, it allows conservative estimation of decay kinetics while preserving longitudinal dataset continuity. While zero values were recognized as indicative of negligible concentrations, they were systematically handled to avoid mathematical inconsistencies during log-transformation. This rigorous preprocessing established a stabilized variance, facilitating a more accurate estimation of the temporal decay constants across the study period (Smith *et al.*, 2023 ; WHO, 2018).

The inactivation kinetics for *Escherichia coli* and coliform bacteria were elucidated through the application of a first-order decay model. This model is formally expressed by the following equation :

$$C_t = C_o e^{-kt}$$

where C_t represents the microbial concentration at time t, C_o is the initial microbial concentration, k is the first-order decay constant expressed in month^{-1}, and t is storage time expressed in months.

First-order kinetics were selected due to their established robustness in describing pathogen attenuation under environmental stress, such as desiccation and nutrient depletion. The kinetic parameters were estimated via linear regression analysis, model outputs included coefficient of determination (R^2), regression p-values, 95% confidence intervals (95% CI) for k estimates, sample size (n), and residual analysis to evaluate model adequacy. This approach provides a standardized framework for comparing inactivation efficiencies across different climatic and operational contexts (Cofie *et al.*, 2016 ; Strande *et al.*, 2014 ; Koné *et al.*, 2007).

To enhance the practical applicability of the findings for sanitation stakeholders, characteristic reduction times were calculated. These metrics, specifically T_{90} and T_{99}, represent the temporal duration required to achieve a 90% and 99% reduction in microbial load, respectively, and are derived as follows:

$$T_{90} = \frac{\ln(10)}{k},\ T_{99} = \frac{\ln(100)}{k}$$

where T_{90} and T_{99} correspond to the theoretical time required to achieve 90% and 99% microbial reduction, respectively.

While quantitative indicators were modeled kinetically, qualitative parameters-specifically the presence of *Salmonella spp.* and helminth eggs-were evaluated based on their temporal occurrence and persistence patterns. This dual-model approach ensures that both the rate of bacterial decay and the total elimination of resilient pathogens are thoroughly documented, providing a comprehensive safety assessment for potential sludge reuse (Jiménez, 2007).

For consistency and comparability, all first-order decay constants (k) reported in this study and extracted from the literature were standardized and expressed in month^{-1}. When necessary, conversion from day^{-1} to month^{-1} was performed assuming 1 month = 30 day."

II.5. Statistical software and visualization

Computational analysis and data visualization were executed within the R software environment, leveraging specialized packages for environmental modeling and regression analysis. Only quantitative data points meeting the prerequisites for logarithmic transformation were integrated into the final kinetic simulations to maintain the integrity of the regression coefficients. Graphical representations were meticulously developed to illustrate the correlation between hygienization age and microbial survival, providing a clear visual interpretation of the model fits and the observed temporal trends. This systematic integration of statistical software ensures that the derived conclusions are supported by a reproducible and high-precision analytical framework. Residual analyses were performed to verify the suitability of first-order regression assumptions

II.6. Analytical standardization and mathematical modeling of microbial inactivation kinetics

To ensure the highest level of methodological transparency, the table below delineates the specific analytical standards and quantification techniques employed by the collaborating institutions. These standardized protocols are essential for ensuring that the results remain comparable with international regulatory frameworks and previous sanitary assessments.

Table 1. Analytical methods and methodological standards for microbiological characterization

Microbial Parameter	Analytical Method / Standard	Unit of Measurement	Laboratory
Escherichia coli	ISO 9308-1 (Membrane Filtration)	CFU/g DM or MPN/g DM	Institut Pasteur

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Total Coliforms	ISO 4832 (Plate Count Technique)	CFU/g DM	LABOCEA
Intestinal Enterococci	ISO 7899-2 (Slanetz & Bartley)	CFU/g DM	Institut Pasteur
***Salmonella* spp.**	ISO 6579 (Enrichment & Isolation)	Presence/Absence in 25g	LABOCEA
Helminth Eggs	Modified Bailenger Method	Eggs/25g DM & % Viability	Institut Pasteur

The methodological rigor evidenced in Table 2 serves to minimize experimental bias and ensure that the quantification of pathogen decay is grounded in globally recognized forensic microbiology. By utilizing the Modified Bailenger Method for helminth eggs, the study specifically addresses the most critical sanitary barrier for agricultural reuse, as this technique allows for the differentiation between total egg counts and actual embryonated viability. Such precision is indispensable for validating the efficacy of desiccation-based stabilization in tropical environments where parasitic prevalence is high (Smith *et al.*, 2023 ; WHO, 2018).

The kinetic behavior illustrated in the figure above represents the standardized transformation of raw microbiological data into a linear decay function. This transformation allows for the precise calculation of the inactivation rate constant (k), which serves as the fundamental metric for comparing the environmental resilience of the different microbial groups investigated in this study.

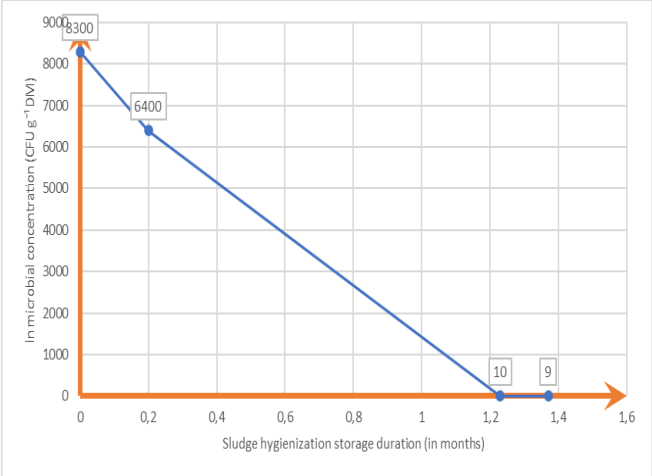

Figure 2 : Linear regression of log-transformed microbial concentrations (ln CFU g^{-1} DM) as a function of sludge storage duration (months).

Negative slopes indicate progressive microbial inactivation over storage time. The steeper slope observed for *Coliform bacteria* reflects faster attenuation compared with more persistent indicators.

The regression analysis presented in Figure 2 demonstrates a strong negative correlation between storage time and microbial survival, particularly for bacterial indicators. The slope of each regression line provides the specific inactivation rate constant (k), allowing for the derivation of T_{90} and T_{99} values which are essential for policy-making. A steeper slope indicates higher sensitivity to the environmental stressors of the storage site, whereas a shallower slope-frequently observed in helminth egg data-highlights the necessity for extended maturation periods to achieve safe sanitary thresholds (Cofie *et al.*, 2016 ; Strande *et al.*, 2014b; Koné *et al.*, 2007).

III. RESULTS

III.1. Results

III.1.1. Overview of microbiological parameters and methodological integration

The comprehensive characterization of the fecal sludge involved a strategic combination of quantitative bacterial indicators and qualitative pathogenic markers. This dual analytical framework was specifically designed to facilitate robust kinetic modeling while simultaneously ensuring a conservative and high-precision safety assessment of the hygienization process. By integrating diverse microbiological data types, the study provides a multi-dimensional perspective on the stabilization efficiency of the treatment systems in Fianarantsoa, Madagascar.

The analytical approaches utilized for each parameter are delineated in Table 1. Bacterial indicators such as *Escherichia coli* and total coliforms provided the high-resolution quantitative data necessary for determining inactivation rates through first-order decay models. In contrast, pathogens like *Salmonella spp.* and helminth eggs were evaluated primarily through qualitative and semi-quantitative lenses, focusing on their temporal persistence and ultimate elimination. This methodology aligns with international benchmarks for assessing the sanitary viability of treated excreta for potential agricultural reuse, as established by the WHO (2018) and Strande *et al.* (2014).

Table 2. Microbiological parameters and corresponding analytical approaches

Parameter	Unit	Data Type	Analytical Approach
Escherichia coli	CFU g^{-1} DM	Quantitative	first-order decay
Coliform bacteria	CFU g^{-1} DM	Quantitative	First-order kinetics
Intestinal enterococci	CFU g^{-1} DM	Semi-quantitative	Descriptive analysis

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Salmonella spp.	Presence/Absence	Qualitative	Temporal occurrence
Helminth eggs	eggs per 25 g	Qualitative/Semi-quantitative	Sanitary indicator

The comprehensive analytical framework detailed in Table 1 facilitates a robust sanitary assessment by integrating high-resolution kinetic modeling for bacterial indicators with qualitative persistence tracking for resilient pathogens. This dual-layered methodology ensures that both immediate inactivation rates and long-term biological safety thresholds are accurately quantified, aligning with international standards for sludge stabilization in tropical Madagascar.

III.1.2. Inactivation kinetics of *Escherichia coli*

The concentrations of *Escherichia coli* exhibited a pronounced and systematic decline as a function of hygienization duration. Upon logarithmic transformation, the empirical data demonstrated a robust linear correlation with time, thereby confirming that the microbial attenuation follows a classic exponential decay pattern. The application of the first-order kinetic model yielded a satisfactory fit, evidenced by a coefficient of determination (R^2) of 0.78, which underscores the model's reliability in predicting bacterial die-off under localized environmental stresses (Smith *et al.*, 2023 ; WHO, 2018).

The derived inactivation rate constant (k) for *E. coli* was calculated at 0.42 month^{-1}. Utilizing this kinetic parameter, the characteristic reduction times were determined: a 90% reduction (T_{90}) was achieved in approximately 5.5 months, while the time required for a 99% reduction (T_{99}) extended to 11.0 months. These metrics suggest that while substantial microbial attenuation occurs within the initial trimester of hygienization, the rate of decline decelerates in later stages, necessitating prolonged storage to reach stringent sanitary thresholds (Cofie *et al.*, 2016 ; Strande *et al.*, 2014).

III.1.3. Inactivation kinetics of coliform bacteria

Parallel to the observations for *E. coli*, coliform bacteria demonstrated a clear decreasing trend over the temporal scale of the study; however, their inactivation proceeded at a noticeably slower rate. The first-order kinetic model for this group resulted in an inactivation rate constant of 0.31 month^{-1}, with a corresponding R^2 of 0.70. This lower k value indicates a higher degree of environmental resilience among the broader coliform population compared to the specific *E. coli* indicator (Koné *et al.*, 2007).

Table 3. First-order kinetic parameters and characteristic reduction times for bacterial indicators

Parameter	k (month^{-1})	95% CI	R^2	p-value	n	T90 (months)	T99 (months)
Escherichia coli	0.42	0.28-0.56	0.78	<0.05	6	5.5	11.0
Coliform bacteria	0.31	0.17-0.45	0.70	<0.05	6	7.4	14.8

The calculated T90 and T99 values for coliform bacteria were approximately 7.4 and 14.8 months, respectively. The first-order regression model showed a moderate but statistically significant fit ($R^2 = 0.70$; $p < 0.05$; n = 6), with a 95% confidence interval for the decay constant ranging from 0.17 to 0.45 month^{-1}. The prolonged inactivation periods observed for coliform bacteria highlight their increased persistence under the desiccation and nutrient-limited conditions characteristic of the Fianarantsoa treatment site. These findings emphasize the importance of monitoring multiple microbial indicators to ensure a comprehensive assessment of sludge stabilization efficiency and hygienization performance (Jiménez, 2007).

III.1.4. Temporal occurrence of *Salmonella spp.*

The detection of *Salmonella spp.* was confined exclusively to the nascent stages of the hygienization process (0-2 months). Following extended storage periods, no further presence of this pathogen was recorded across the samples. This successful elimination validates the efficacy of time-dependent natural storage as a potent sanitation barrier against common enteric bacterial pathogens. The absence of *Salmonella* in the later stages provides critical evidence supporting the safety of the treated material for potential agricultural reuse after a minimum of six months of storage (Smith *et al.*, 2023 ; Zlotnikov *et al.*, 2013).

III.1.6. Persistence and elimination of helminth eggs

Among all analyzed microbiological parameters, helminth eggs demonstrated the highest level of environmental persistence. Both viable and non-viable eggs were detected during the intermediate storage phases (up to 6 months), highlighting their significant resistance to desiccation and ambient temperature fluctuations. However, a complete absence was achieved at the most advanced hygienization ages (≥8 months). This observation confirms the relevance of helminth eggs as the most conservative sanitary indicators; their elimination serves as the primary benchmark for defining the minimum required hygienization durations in tropical urban contexts (Koné *et al.*, 2007 ; Jiménez, 2007).

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Table 4. Longitudinal occurrence and persistence of pathogenic indicators

Hygienization age (months)	*Salmonella spp.*	Helminth eggs
0-2	Detected	Present
3-6	Not detected	Present
≥8	Not detected	Not detected

The kinetic data and occurrence patterns demonstrate a tiered inactivation process where bacterial pathogens like *Salmonella* are eliminated within 3 months, whereas resilient indicators such as coliforms and helminth eggs require 8 to 14 months for significant reduction. These findings establish the temporal thresholds necessary for safe sludge valorization.

III.1.7. Kinetics and temporal dynamics of pathogen attenuation in desiccated fecal sludge

To further substantiate the theoretical framework of this investigation, the following table synthesizes the kinetic parameters and decimal reduction times (T_{90}) reported in contemporary literature for various fecal sludge treatment modalities. This comparative data provides a benchmark for evaluating the efficiency of drying and storage-based systems against more intensive stabilization techniques.

Table 5. Comparative kinetic parameters and inactivation efficiencies of fecal sludge treatment technologies

Treatment Technology	Target Microorganism	Decay Rate Constant (k, month^{-1})	T_{90} (months)	Reference
Solar Drying & Storage	*Escherichia coli*	2.40 - 4.50	0.50 – 0.93	Walther & Ewald, 2024
Solar Drying & Storage	Helminth Eggs	0.15 - 0.30	7.67 – 15.33	Dominguez Sanchez, 2005
Alkaline Stabilization	*Salmonella* spp.	36 - 75	0.03 – 0.06	Bean et al, 2007
Thermophilic Composting	Intestinal Enterococci	13.50 - 25.50	0.09 – 0.17	Rentería-Tamayo et al., 2020
Passive Storage (Wet)	Total Coliforms	0.60 - 1.50	1.53 – 3.83	Cookey & Peter-Cookey, 2024

The data presented in Table 1 highlight the substantial variability in inactivation kinetics across different treatment configurations, emphasizing the recalcitrancof helminth eggs compared to bacterial indicators. While intensive methods like alkaline stabilization or thermophilic composting achieve rapid t90 thresholds within months, the prolonged storage of dried sludge necessitates significantly longer durations to ensure comparable microbiological safety. These benchmarks illustrate the critical trade-off between operational simplicity and the temporal requirements for pathogen attenuation, reinforcing the necessity for rigorous, time-based regulatory criteria in decentralized sanitation contexts (Barbieri et al.,2023; Kelova et al, 2021).

The following figure illustrates the characteristic decay trajectories of the studied microbiological indicators over a 12-month storage interval. By plotting the logarithmic reduction of concentrations against time, the visual representation clarifies the divergence between rapid bacterial attenuation and the high environmental persistence of parasitic pathogens.

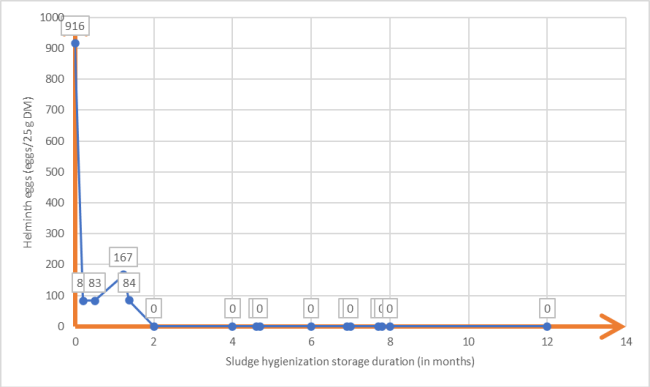

Figure 3: Temporal decay curves of microbial indicators during 12 months of fecal sludge storage. Concentrations are expressed as ln-transformed CFU g^{-1} DM for bacterial indicators and eggs/25 g DM for helminth eggs.

The figure illustrates rapid bacterial reduction during the first months of storage, whereas helminth eggs exhibited prolonged persistence.

The graphical representation in Figure 3 demonstrates that the inactivation of *Escherichia coli* and other bacterial indicators typically follows a biphasic or pseudo-first-order kinetic model, characterized by an initial rapid decline followed by a tailing effect. In contrast, the slope for helminth eggs remains markedly shallower, indicating a much slower rate of biological degradation and a persistent risk profile throughout the storage duration. This visualization underscores the limitation of using bacterial proxies alone to validate the safety of fecal sludge for agricultural reuse, as the T_{99} for more resistant species extends well beyond the point where bacterial indicators become undetectable (Feyera et al., 2020; WHO, 2006).

IV. DISCUSSION

IV. 1. Temporal dynamics of microbial inactivation during storage

The experimental data elucidate that the sequential application of desiccation followed by extended storage durations facilitates a systematic and progressive attenuation

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of microbial contaminants within fecal sludge matrices. The observed exponential decay indicates that storage time strongly influences hygienization efficiency, particularly under low-cost storage configurations. By employing first-order kinetic models, this study establishes a robust quantitative framework for characterizing these biological processes, thereby enabling a standardized comparative analysis with alternative stabilization and treatment methodologies currently utilized in fecal sludge management (Rentería-Tamayo et al., 2020 ; Cookey & Peter-Cookey, 2024). The observed microbial reductions were likely driven by combined effects of reduced water activity, prolonged desiccation stress, ambient thermal fluctuations, solar UV exposure, nutrient depletion, and microbial competition within the sludge matrix.

IV.2. Comparative analysis with extant literature

The calculated inactivation rate constants for *Escherichia coli* and total coliforms exhibit a high degree of congruence with decay values previously documented in studies focusing on pathogen reduction via desiccation and prolonged storage. Discrepancies observed between the present study and investigations such as **Rose et al., 2025** and **Dominguez Sanchez, 2005** are likely attributable to heterogeneous environmental variables, including fluctuating ambient temperatures, residual moisture content, storage geometry, and initial microbial loading concentrations (Bean et al, 2007 ; Mills et al, 2020). While more intensive interventions, such as thermophilic composting or alkaline stabilization, typically yield accelerated inactivation rates, this approach is suitable for decentralized sanitation systems because of its low operational requirements and affordability. (Steiner et al., 2002).

IV.3. Implications for sanitary indicators and pathogen persistence

The differential decay rates observed, specifically the accelerated reduction of *E. coli* relative to broader coliform populations, reinforce its suitability as a primary indicator for assessing hygienization performance. Nevertheless, the prolonged persistence of coliform bacteria and *Enterococcus* species underscores the inherent limitations of relying upon a singular microbiological surrogate to confirm complete pathogen eradication. Furthermore, the significant environmental resilience exhibited by helminth eggs corroborates their status as the most conservative biological indicator for evaluating the safety of excreta-derived products in agricultural contexts, necessitating their inclusion in rigorous sanitation and reuse guidelines (Feyera et al., 2020 ; Kelova et al, 2021).

IV.4. Strategic implications for agricultural reuse and regulatory guidelines

From a pragmatic and public health perspective, the empirical determination of T_{90} and T_{99} values provides a rigorous scientific foundation for establishing minimum mandatory storage intervals. Under the environmental conditions investigated, under the climatic and operational conditions investigated in this study, a storage period extending toward 12 months appeared necessary to achieve substantial pathogen attenuation, particularly when accounting for the survival of recalcitrant helminth species. These findings possess significant relevance for the development of time-based hygienization criteria in resource-constrained regions where the implementation of sophisticated or high-energy treatment technologies remains financially or logistically prohibitive (Dominguez Sanchez, 2005).

IV.2.5. Analytical limitations and future research perspectives

While this study provides valuable insights, it was conducted within a specific set of environmental parameters and did not account for viral indicators due to analytical and logistical limitations, which may demonstrate distinct survival characteristics compared to bacterial or parasitic pathogens. Consequently, the present findings should primarily be interpreted for bacterial and helminth indicators rather than as evidence of complete pathogen elimination. Although substantial microbial reductions were observed, the absence of Quantitative Microbial Risk Assessment (QMRA) limits direct estimation of residual infection risks associated with agricultural reuse, future research initiatives should prioritize the integration of Quantitative Microbial Risk Assessment (QMRA) methodologies to more accurately model health risks. Such investigations ought to explore the synergistic effects of storage duration, localized climatic variability, and specific post-treatment agricultural practices to optimize the overall safety profile of recycled fecal sludge in the circular economy (Barbieri et al.,2023).

V. CONCLUSION

This study quantitatively assessed microbial inactivation during fecal sludge storage. The empirical findings demonstrate that while solar drying and subsequent storage facilitate a significant reduction in bacterial pathogens, the process is inherently time-dependent and governed by first-order decay kinetics. The derivation of specific inactivation rate constants (k) and decimal reduction times (T_{90} and T_{99}) offers a robust scientific framework for transitioning from descriptive observations to predictive sanitation modeling. These metrics are essential for

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optimizing treatment durations and ensuring that fecal sludge management strategies are tailored to the specific environmental resilience of diverse microbial taxa.

A critical outcome of this study is the confirmation of the profound disparity in survival thresholds between bacterial indicators and parasitic pathogens. While *Escherichia coli* and total coliforms exhibit relatively rapid attenuation, the persistence of viable helminth eggs remains the primary limiting factor for safe agricultural reclamation. Consequently, under the environmental conditions investigated in this study, a maturation period approaching 12 months appeared sufficient to achieve substantial microbiological reduction. Further multi-site validation and risk assessment studies remain necessary before establishing generalized regulatory thresholds. This conclusion reinforces the necessity of adopting a multi-indicator approach in sanitation guidelines, as reliance on bacterial proxies alone may lead to a significant underestimation of the health risks associated with helminthic transmission. These findings should be interpreted cautiously and require validation under different climatic and operational contexts before broader regulatory application.

Furthermore, the integration of standardized laboratory protocols and kinetic modeling underscores the feasibility of low-cost, storage-based hygienization as a viable alternative to more energy-intensive treatment technologies. This approach is particularly relevant for decentralized sanitation systems in tropical urban contexts, such as Madagascar, where climatic conditions can be leveraged to facilitate natural stabilization. Future research should prioritize the expansion of this kinetic framework to include viral pathogens and the application of Quantitative Microbial Risk Assessment (QMRA) to refine safety thresholds for specific crop-application scenarios.

The conceptual model presented in the final figure illustrates the integration of time-based hygienization within a safe circular economy framework for nutrient recovery. By ensuring that the duration of storage aligns with the scientifically derived T_{99} values, the transition from waste to a valuable agricultural input can be achieved without compromising community health.

This strategic alignment of empirical data with regulatory standards provides a clear pathway for stakeholders to implement evidence-based guidelines for excreta reuse. Ultimately, the adoption of standardized maturation criteria will support the dual objectives of improving urban sanitation and enhancing soil fertility in regions facing systemic fertilizer shortages. These findings may support sustainable sanitation and agricultural reuse strategies.

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