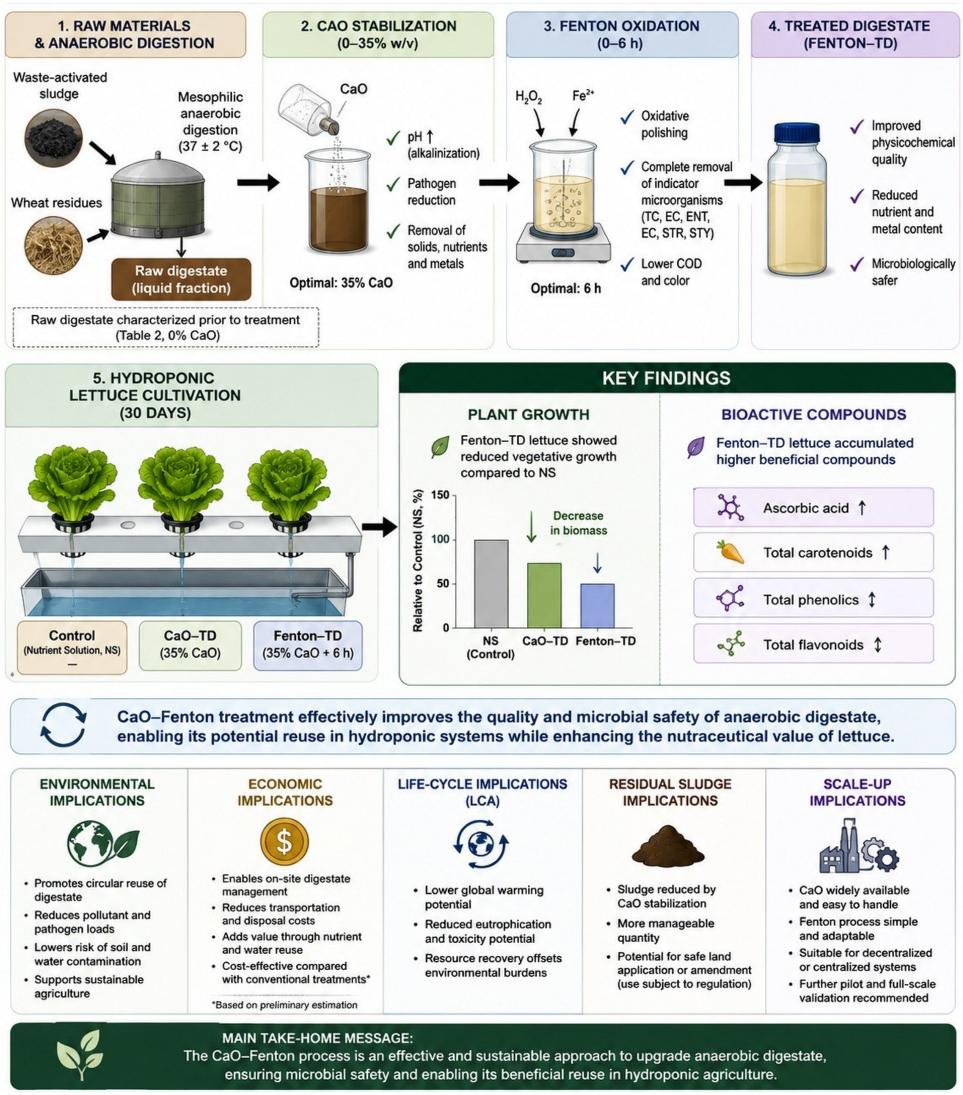
From Waste to Resource: Integrated CaO–Fenton Treatment of Digestate for Nutrient Recovery and Hydroponic Lettuce Cultivation

Raid Alrowais,[a,]* Mahmoud M. Abdel-Daiem,[b,]* Rania Saber Yousef,[c] Osama konsowa Ahmed,[c] Dalia Ahmed,[b] and Noha Said [b]

** Corresponding authors: rnalrowais@ju.edu.sa; mmabdeldaiem@eng.zu.edu.eg*

DOI: 10.15376/biores.21.4.10734-10776

GRAPHICAL ABSTRACT



From Waste to Resource: Integrated CaO–Fenton Treatment of Digestate for Nutrient Recovery and Hydroponic Lettuce Cultivation

Raid Alrowais ,[a,]* Mahmoud M. Abdel-Daiem ,[b,]* Rania Saber Yousef,[c]
Osama konsowa Ahmed,[c] Dalia Ahmed,[b] and Noha Said [b]

An integrated CaO–Fenton waste-to-resource strategy was evaluated for converting anaerobic sewage digestate into a microbiologically safe and nutritionally functional solution for hydroponic lettuce (*Lactuca sativa* L.) cultivation. Lime stabilization at dosages up to 35% achieved near-complete pathogen inactivation. Beside substantial decreases in total solids (TS), total volatile solids (TVS), chemical oxygen demand (COD), NH_4^+ ions, and heavy metals. Subsequent Fenton oxidation using hydrogen peroxide (H_2O_2) and Fe^{2+} ensured complete microbial sterilization and further improved effluent quality, achieving additional reductions in TS (87.7%), TVS (100%), COD (52.5%), and BOD (52.4%). When applied in a deep-water culture (DWC) hydroponic system, CaO–Fenton-TD supported plant growth with moderate biomass reductions but significantly increased the accumulation of bioactive compounds with potential nutritional value, including ascorbic acid, total carotenoids, total phenolics, and total flavonoids, relative to the control. One-way ANOVA followed by Tukey's HSD confirmed that both CaO stabilization and Fenton oxidation had significant effects ($P \leq 0.05$) on the physicochemical and microbial properties of the digestate. Overall, the results demonstrated the feasibility of using CaO–Fenton-treated anaerobic digestate in hydroponic systems. The proposed approach supports circular economy by promoting nutrient recovery and beneficial reuse of digestate, thereby reducing waste-disposal burdens and dependence on conventional mineral fertilizers.

DOI: 10.15376/biores.21.4.10734-10776

Keywords: Advanced oxidation processes; CaO–Fenton treatment; Anaerobic digestate stabilization; Hydroponic cultivation; Waste-to-resource conversion; Circular economy; SDG 9: Industry; Innovation and Infrastructure

Contact information: a: Department of Civil Engineering, College of Engineering, Jouf University, Sakakah 72388, Saudi Arabia; b: Environmental Engineering Department, Faculty of Engineering, Zagazig University, Zagazig, 44519, Egypt; c: Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt;
** Corresponding authors: rnalrowais@ju.edu.sa; mmabdeldaiem@eng.zu.edu.eg*

INTRODUCTION

Agricultural production remains one of the most environmentally intensive sectors worldwide, exerting substantial pressure on natural resources through excessive water consumption, nutrient depletion, and the generation of large volumes of organic waste (Ingrao *et al.* 2023; Said *et al.* 2026). Agriculture currently accounts for 72% of global freshwater withdrawals, stressing water systems already burdened by climate change and population growth (UN-WWDR 2023). Moreover, the increasing use of fertilizers, declining soil quality, and waste mismanagement continue to exacerbate pollution and degrade ecosystem services (Çakmakçi *et al.* 2025). These mounting enhance resource efficiency, strengthen circular economy practices, and mitigate environmental degradation (Bhatia and Sindhu 2024).

In this context, soilless cultivation, particularly hydroponic systems, has emerged as an efficient alternative to traditional agriculture (Bergstrand *et al.* 2020). Hydroponics allow precise nutrient management, support higher planting density, and shorten crop cycles. Additionally, it reduces space requirements by up to 80% and water consumption by approximately 95% compared to traditional soil-based cultivation (Chatterjee *et al.* 2025). Hydroponic systems can also integrate treated wastewater as a source of water and nutrient, improving resource-use efficiency and reducing the environmental footprint of food production (Moncada *et al.* 2020; Rajendran *et al.* 2024; Mathe *et al.* 2024).

Anaerobic digestion (AD) remains widely adopted for managing sewage sludge, agricultural residues, and industrial organic wastes because it generates renewable biogas and nutrient-rich digestate (Ahmed *et al.* 2025; Alrowais *et al.* 2025). Although digestate contains valuable macronutrients (N, P, K) and organic matter, its direct use poses risks as it often carries pathogens, antibiotic-resistant bacteria, heavy metals, and persistent organic pollutants (Alrowais *et al.* 2024). Consequently, the safe reuse of digestate, particularly in food production systems, requires effective stabilization, sanitation, and conditioning processes (Pelayo Lind *et al.* 2021).

Recent reviews emphasize that sustainable digestate management should extend beyond direct land application toward integrated post-treatment and resource-recovery strategies that improve nutrient recovery, reduce environmental risks, and convert digestate into higher-value agricultural products (Chojnacka and Moustakas 2024; Guan *et al.* 2024). Such approaches are particularly important where digestate is intended for controlled cultivation systems, as its composition and potential contaminants require appropriate conditioning before reuse.

Existing treatment methods, such as composting, lime stabilization, thermal drying, and ammonia-based sanitation, can reduce hazards but are often energy-intensive, costly, or insufficient to remove recalcitrant contaminants (Bergstrand *et al.* 2020; Kechasov *et al.* 2021; Pelayo Lind *et al.* 2021; Mathe *et al.* 2024; Chaves *et al.* 2025). Accordingly, advanced and hybrid treatment approaches have received increasing attention (Garrido-Cardenas *et al.* 2020; Adetunji and Olaniran 2021; Alrowais *et al.* 2023). Recent studies further indicate that digestate treatment should extend beyond nutrient recovery to simultaneously address microbial safety, metal contamination, organic matter, and nutrient imbalance, particularly when hydroponic reuse is intended (Pelayo Lind *et al.* 2021; Mathe *et al.* 2024; Chaves *et al.* 2025).

Advanced oxidation processes (AOPs), particularly the Fenton reaction, have demonstrated strong potential for degrading persistent organic pollutants and achieving microbial inactivation through the reaction of ferrous iron (Fe^{2+}) with hydrogen peroxide (H_2O_2), which generates highly reactive hydroxyl radicals (OH•) (Bao *et al.* 2021; Wang *et al.* 2024; Lee *et al.* 2025; Wu *et al.* 2025). Fenton-based treatments are effective against pharmaceuticals, phenols, dyes, and emerging contaminants in wastewater and sludge (Akbay *et al.* 2022; Cheng *et al.* 2024; Silva 2025). Yet, large-scale applications remain limited due to the high cost of reagents and the production of iron-rich sludge that requires disposal or recovery. Integrating lime stabilization (CaO) with the Fenton process offers a promising solution, as CaO enhances pH adjustment, coagulation, heavy metal precipitation, and pathogen reduction, while the Fe^{2+}–H_2O_2 Fenton reaction provides powerful oxidative polishing (Yuan *et al.* 2019; Tang *et al.* 2020). Despite these complementary functions, the application of sequential CaO–Fenton treatment to digestate intended for agricultural reuse remains insufficiently investigated (Ayoub 2022; Wang *et al.* 2025a).

Lettuce (*Lactuca sativa* L.), a globally consumed leafy vegetable, is particularly suitable for evaluating the reuse of treated digestate (TD) in hydroponic systems (Moncada *et al.* 2020; Ntinas *et al.* 2021). Lettuce has a short growth cycle and high nutrient sensitivity and, because it is commonly consumed raw, represents a stringent test crop for which microbial safety and chemical purity are critical concerns (Santos *et al.* 2024; Feng *et al.* 2025). Several studies report that hydroponically grown lettuce effectively removes nitrogen and phosphorus from nutrient-enriched wastewater (Ntinas *et al.* 2021; Santos *et al.* 2024), but evidence regarding food safety, pathogen risks, heavy metal uptake, and biochemical responses. Recent studies have increasingly explored the nexus between digestate management, advanced treatment processes, and soilless food production. For instance, biogas digestate can partially replace mineral fertilizers in hydroponic nutrient solutions (NSs) (Bergstrand *et al.* 2020). Successful nutrient recovery, however, does not necessarily imply a balanced hydroponic NS. Digestate-based solutions may exhibit inappropriate nutrient ratios, including NH_4^+ dominance and insufficient K, potentially affecting root development, nutrient uptake, and crop performance (Bergstrand *et al.* 2020; Pelayo Lind *et al.* 2021).

A recent study showed that aerobic polishing of liquid digestate can produce a hydroponic fertilizer with substantial organic carbon removal and minimal NH_4^+ loss, confirming the technical feasibility of digestate-based nutrient recycling in controlled systems (Mathe *et al.* 2024). Other studies have evaluated digestate-derived extracts as biostimulants and nutrient sources in crop production, highlighting their agronomic potential while also emphasizing the need for improved sanitization and contamination control (Bergstrand *et al.* 2020; Pelayo Lind *et al.* 2021; Chaves *et al.* 2025). More recently, digestate has been recognized as a potential source of biostimulant compounds, including humic substances, phytohormones, protein hydrolysates, and other bioactive components that may promote plant growth, nutrient uptake, and stress tolerance (Sani *et al.* 2025).

Despite these potential benefits, variability in digestate composition and the presence of potential contaminants remain challenges for standardized agricultural application. In parallel, several studies have reported the successful growth of lettuce and other crops in hydroponic systems fed with treated municipal or agro-industrial wastewater, while emphasizing the importance of monitoring pathogens, heavy metals, and organic micropollutants for safe reuse (Moncada *et al.* 2020; Ntinas *et al.* 2021; Santos *et al.* 2024).

Beyond plant growth, responses to wastewater- or digestate-derived NSs may involve trade-offs between biomass production and biochemical composition. Moderate changes in salinity, ionic composition, or nutrient availability have been associated with increased accumulation of phenolics, flavonoids, carotenoids, and ascorbic acid in leafy vegetables, sometimes accompanied by reduced growth (Moncada *et al.* 2020; Ntinas *et al.* 2021; Chatterjee *et al.* 2025). However, such biochemical responses should not necessarily be interpreted as improved nutritional quality, as they may also reflect physiological responses to an altered nutrient environment.

Collectively, previous studies demonstrate the potential of digestate valorization, advanced oxidation, and wastewater-based hydroponics; however, their integration remains insufficiently investigated. In particular, limited information is available on whether sequential CaO–Fenton treatment can simultaneously improve the microbiological and physicochemical quality of anaerobic digestate while producing a nutrient stream suitable for hydroponic cultivation. Moreover, the resulting nutrient balance, plant growth response, and accumulation of bioactive compounds require integrated evaluation. These gaps provide the rationale for the present study.

To address these gaps, this study investigates the sequential CaO–Fenton treatment of anaerobic digestate and comprehensively evaluates its performance in terms of pathogen inactivation, contaminant removal, nutrient preservation, and hydroponic applicability. In addition to treatment efficiency, the growth and biochemical responses of hydroponically cultivated *Lactuca sativa* L. are evaluated to assess the agronomic potential of the TD within a circular resource recovery framework. By integrating digestate treatment with hydroponic lettuce cultivation, this study contributes to sustainable waste valorization and the development of resource-efficient nutrient recycling strategies for agricultural applications.

MATERIALS AND METHODS

Methodology

Figure 1 shows a flowchart of the methodology used in this study. It provides a structured overview of the integrated treatment and evaluation framework, illustrating how anaerobic digestate is transformed from a raw waste material into a TD. It begins with the collection and comprehensive characterization of the digestate, establishing its baseline physicochemical and microbiological properties. The first treatment stage, CaO stabilization, acts as a crucial preconditioning step, elevating pH to inactivate pathogens and promote solid–liquid separation. The clarified liquid fraction is then subjected to Fenton oxidation, an advanced process that targets recalcitrant organic pollutants through the controlled generation of •OH.

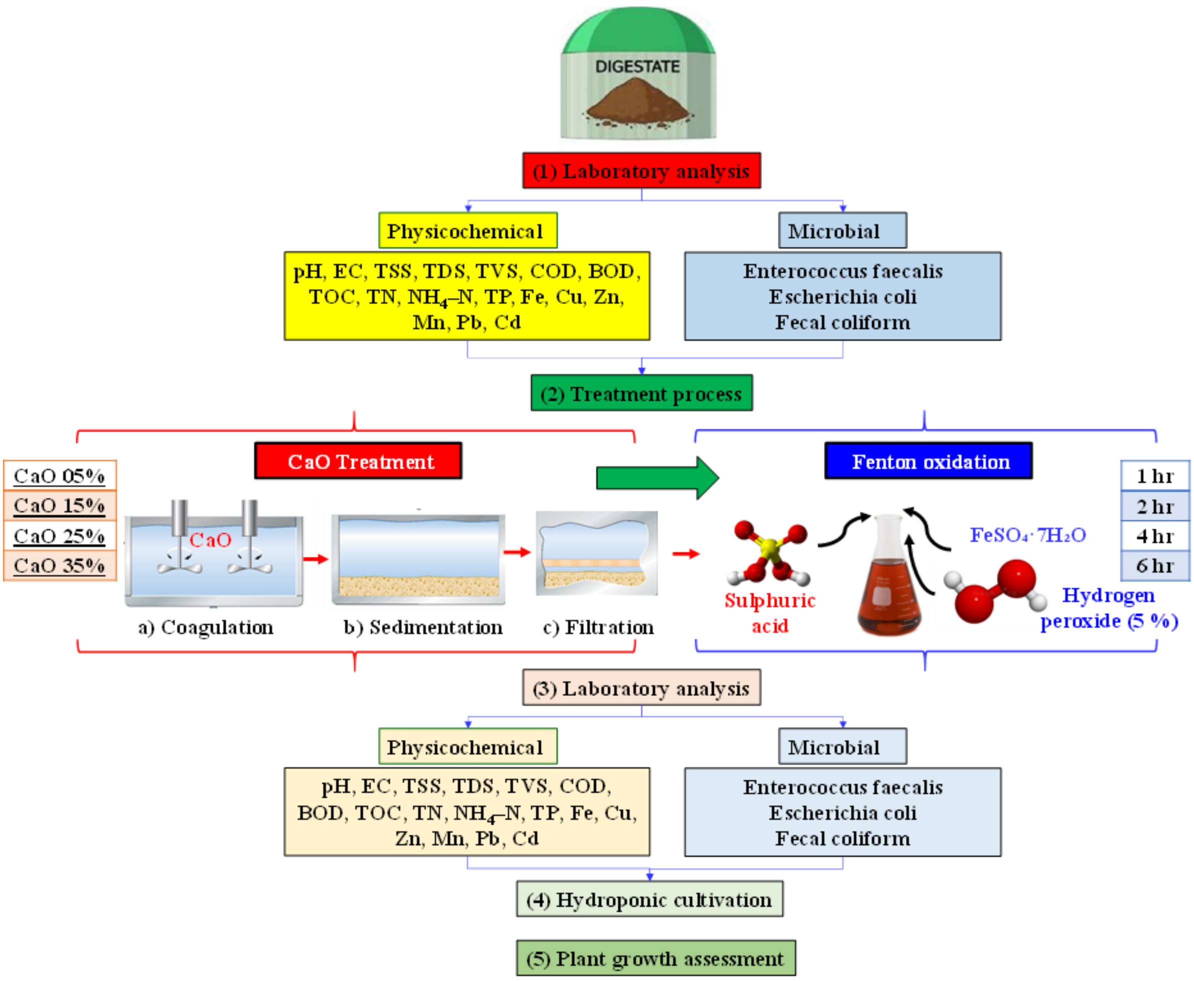


Fig. 1. Flowchart methodology of the current study

Following these sequential treatments, the flowchart outlines the post-treatment analyses used to assess nutrient retention, contaminant removal, and microbiological quality of the TD. The final sections of the diagram highlight the application of the TD in a deep-water culture (DWC) hydroponic system, followed by systematic monitoring of plant growth and nutritional traits. Overall, the flowchart effectively visualizes the full waste-to-resource pathway, demonstrating a circular approach that integrates advanced waste treatment, agricultural reuse, and multidisciplinary performance assessment within a coherent experimental framework.

Material Source and Lab Analysis

Materials source and Characterization

The anaerobic digestate used in this study was generated in a laboratory-scale batch mesophilic AD system operated at 35 ± 1 °C in a 5 L reactor. The system was designed to stabilize waste-activated sludge (2.5 kg) supplemented with wheat crop residues (6% w/w) to achieve an optimized carbon-to-nitrogen ratio of 25. The digestion continued for 100 days to obtain a well-stabilized digestate, yielding approximately 12 L of biogas per kilogram of substrate. Biogas production was monitored periodically and remained negligible for several consecutive days toward the end of the digestion period. Thus, the digestate used in this study was produced under controlled laboratory conditions to ensure experimental reproducibility and minimize variability in feedstock composition.

The resulting digestate was filtered through a fine-mesh coffee filter to separate undigested solid biomass from the liquid fraction. The filtrates were pooled, thoroughly homogenized, transferred into sterile airtight polypropylene containers, and stored at 4 °C until further treatment and analysis. Prior to treatment, the homogenized liquid digestate was characterized to establish its baseline composition. The characterization included physicochemical properties, nutrients, metals, solids and organic-load parameters, and microbiological indicators. The initial composition of the raw digestate is summarized in Table 1 and was used as the baseline for evaluating the effects of subsequent CaO stabilization and Fenton oxidation.

Lab analysis

The solution pH and electrical conductivity (EC) were measured by using a portable meter (HI 98107). Total solids (TS), total dissolved solids (TDS), total volatile solids (TVS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC), total nitrogen (TN), ammoniacal nitrogen (NH_4–N), and phosphorus (PO_4–P) were determined following standard methods (APHA 2005).

Concentrations of potassium (K) and heavy metals, including iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), lead (Pb), and cadmium (Cd), were determined by atomic absorption spectrophotometry (AAS) using a Fisher Scientific ICE 3000 instrument. Prior to analysis, 5 mL of each homogenized liquid sample was subjected to microwave-assisted acid digestion using 10 mL of concentrated trace-metal-grade HNO_3 (65%) and 2 mL of H_2O_2 (30%) at 180 °C for 20 min, following a procedure adapted from EPA Method 3015A (U.S. EPA 2007). Following digestion, the samples were cooled and diluted to a final volume of 50 mL with deionized water. Calibration standards were prepared in matched acid matrices to minimize matrix interference. Potassium and metal concentrations were reported as mg/kg wet weight (WW) of the liquid digestate fraction, approximately equivalent to mg/L on a volumetric basis given the measured digestate density of 1.002 ± 0.005 g/mL. Phenolic compounds were quantified using the Folin–Ciocalteu colorimetric assay (Medina 2011). Microbial quality was assessed by enumerating *Enterococcus faecalis*, *Escherichia coli*, and fecal coliforms following standard procedures (APHA 2005; Cheng *et al.* 2024). Serial tenfold dilutions of each sample were prepared using sterile 0.85% (w/v) NaCl solution and plated onto selective media: m-Enterococcus agar for *E. faecalis* and Chromocult Coliform Agar for *E. coli* and fecal coliforms. Plate counts were performed in triplicate and expressed as $\log_{10}$ CFU/mL. These baseline measurements provided essential benchmarks for evaluating purification efficiency following CaO–Fenton treatment.

Table 1. Baseline Characterization of the Raw Anaerobic Digestate Used in this Study

Category	Parameter	Raw Digestate
Physicochemical	pH	7.60 ± 0.46
	EC (mS/cm)	1.87 ± 0.11
Nutrients	TOC (g/kg)	2.31 ± 0.14
	PO_4–P (g/kg)	1.24 ± 0.07
	NH_4–N (mg/kg)	340.00 ± 20.40
	K (mg/kg)	163.00 ± 9.78
Metals	Cd (mg/kg)	9.08 ± 0.54
	Pb (mg/kg)	5.24 ± 0.31
	Fe (mg/kg)	521 ± 31.26
	Zn (mg/kg)	67.28 ± 4.04
	Cu (mg/kg)	7.91 ± 0.47
	Mn (mg/kg)	49.87 ± 2.99
Solids/organic load	TS (g/L)	10.30 ± 0.46
	TDS (g/L)	5.26 ± 0.27
	TVS (g/L)	3.72 ± 0.22
	COD (g/L)	28.90 ± 0.98
	BOD (g/L)	15.84 ± 1.05

Note: Values are presented as mean ± SD (n = 3). EC, electrical conductivity; TOC, total organic carbon; TS, total solids; TDS, total dissolved solids; TVS, total volatile solids; COD, chemical oxygen demand; BOD, biochemical oxygen demand

CaO Pre-treatment of Anaerobic Digestate

Calcium oxide was employed as an alkaline stabilization agent to suppress the growth of pathogenic microorganisms and facilitate the initial flocculation and precipitation of organic matter. It was gradually added to the homogenized liquid digestate at 5%, 15%, 25%, and 35% (w/v), where % (w/v) represents the mass of CaO added per unit volume of digestate (g CaO per 100 mL of digestate), under continuous stirring (250 rpm) to ensure uniform mixing and prevent localized heat generation during hydration. Accordingly, these dosages correspond to 50, 150, 250, and 350 g CaO/L of digestate, respectively. The initial TS content of the digestate was 10.30 g/L. Based on this measured value, the corresponding CaO dosages were approximately 4.85, 14.6, 24.3, and 34.0 g CaO/g TS, respectively.

During CaO hydration, temperature increased by 8 to 18 °C above ambient conditions (25 ± 2 °C), reflecting the exothermic nature of the reaction (≈65 kJ/mol). The pH rose rapidly from 7.6 to above 12 within 15 to 20 min across all dosages and remained stable throughout the 2 h reaction period, creating conditions favorable for pathogen inactivation. The mixture was maintained under ambient conditions for 2 h to ensure complete conversion of CaO to $Ca(OH)_2$ and sustained high alkalinity. Subsequently, the slurry was left to settle for 12 h to allow solid-liquid separation. The clarified supernatant was collected by gentle siphoning, stored at 4 °C for subsequent Fenton oxidation, and analyzed for EC without dilution.

Post-CaO treatment samples were analyzed using the same physicochemical and microbiological protocols applied to the raw digestate, allowing evaluation of the effectiveness of this initial stabilization step.

Fenton Oxidation of CaO-TD

After CaO stabilization, the separated supernatant was subjected to Fenton oxidation, an advanced oxidation processes (AOP) widely recognized for its effectiveness in degrading persistent organic contaminants. The Fenton reaction was conducted at an initial pH of 3.0 because acidic conditions favor hydroxyl-radical generation (Wang *et al*. 2021; Akbay *et al.* 2022; Ayoub 2022). At pH values below 3, reduced hydroxyl radical generation diminishes Fenton oxidation efficiency, whereas at pH values above 3, iron precipitates as ferric hydroxide, thereby limiting process performance (Ayoub 2022).

The Fenton oxidation was performed through the reaction of Fe^{2+} with H_2O_2 at a molar ratio of 1:10. For each experiment, 1.0 L of the clarified supernatant was transferred into a reaction beaker. The reagents were added sequentially as follows: first, 1.390 g of $FeSO_4\cdot 7H_2O$ was added and mixed using a magnetic stirrer until completely dissolved. The pH was then adjusted to 3.0 using dilute H_2SO_4. Subsequently, 5.11 mL of commercial H_2O_2 solution (30% w/w), corresponding to approximately 1,700 mg of active H_2O_2, was gradually added to initiate the Fenton reaction. During the reaction period, the solution pH was continuously monitored using a digital pH probe and maintained at 3.0 ± 0.2 by periodic addition of small volumes of 0.1 M H_2SO_4 or 0.1 M NaOH, as required. These conditions were selected to ensure effective oxidation while controlling reagent consumption. Four reaction durations (1, 2, 4, and 6 h) were tested to find the influence of oxidation time on treatment efficiency.

After each reaction period, the pH was adjusted to 7.0 using 1 M NaOH to terminate Fenton activity. This neutralization step also promoted the precipitation of iron species and co-precipitation of associated heavy metals. The treated solution was then allowed to settle for 6 h to facilitate separation of iron sludge and oxidation by-products, followed by filtration through 0.45 μm membrane filters. The filtration step was used to separate ferric hydroxide precipitates, particulate-bound contaminants, and insoluble organic matter from the treated liquid, thereby minimizing the potential for clogging during subsequent hydroponic application. Accordingly, the concentration measured after Fenton treatment represents the post-filtration liquid fraction and may reflect the combined effects of oxidation, precipitation/co-precipitation, settling, and filtration. All filtrates were stored at 4 °C and subsequently analyzed for physicochemical parameters, nutrient composition, residual contaminants, and microbial indicators to evaluate treatment performance.

Hydroponic System Setup and Plant Cultivation

Hydroponic cultivation of Lettuce seedlings was carried out in a greenhouse that was covered with transparent polyethylene sheeting and equipped with side vents to facilitate natural ventilation during warm periods. All experimental units were placed on a shaded workstation covered with a protective screen to limit excessive solar exposure and maintain stable microclimatic conditions. Plant growth trials were conducted during August to September 2025 under greenhouse air temperatures ranging from 28 to 35 °C, an average relative humidity of approximately 45%, a 13-h photoperiod, and an average photosynthetic photon flux density (PPFD) of approximately 900 μmol m^{-2} s^{-1}. A DWC system without recirculation was employed to evaluate the suitability of TD as a hydroponic growth medium. Each treatment consisted of three independent opaque 10-L plastic reservoirs, which served as biological replicates (n = 3), with three plants grown in each reservoir. Each reservoir was equipped with a perforated floating board to support the plants while minimizing light penetration into the NSs. (Santos *et al.* 2024). A compressed-air pump supplied continuous aeration through diffusers to ensure sufficient dissolved oxygen (DO) levels.

Three distinct hydroponic water treatments were evaluated in this study: a standard NS adapted from previous research serving as the positive control; a CaO-TD derived from the supernatant following 35% CaO stabilization and solid-liquid separation; and a Fenton-TD obtained after a 6-h oxidation reaction (Carvalho *et al.* 2018). Plant morphological performance was evaluated by measuring the number of leaves, plant height (cm), and root length (cm) during the cultivation period. Plant height and root length were measured using a calibrated digital caliper with a precision of ±0.01 cm. Measurements from the three plants within each reservoir were averaged to obtain a single value for each biological replicate, and the results were expressed as mean ± standard deviation (SD) of the three independent reservoirs (n = 3). All treatments were cultivated for the same duration, and all plants were harvested simultaneously when the control plants reached marketable size. Thus, differences among treatments were evaluated at the same cultivation endpoint. This approach is consistent with common practice in digestate-based hydroponic systems (Ntinas *et al.* 2021; Santos *et al.* 2024) and reflects agronomic performance within a commercially relevant timeframe.

Lettuce Cultivation and Growth Monitoring

Lactuca sativa L seedlings (selected based on rapid growth and high nutrient uptake efficiency) were germinated on sterilized rockwool cubes moistened with distilled water. Once seedlings reached the two-leaf stage, uniform individuals were transplanted into hydroponic units containing the respective NSs. Plants were cultivated for a full growth cycle, and their development was monitored at regular intervals. Parameters, including plant height, root length, and number of leaves were recorded. Physiological characteristics, including chlorophyll *a* and *b*, and carotenoids were determined spectrophotometrically. These measurements enabled a detailed comparison of plant performance among the different hydroponic nutrient sources.

Photosynthetic Pigment and Biochemical Analyses

Chlorophyll and carotenoid contents were quantified following the spectrophotometric method (Wellburn 1994). Briefly, 0.25 g of fresh leaf tissue was homogenized in 50 mL of 80% acetone using a mortar and pestle, and the extract was centrifuged to obtain a clear supernatant. Absorbance readings were recorded at 663 and 645 nm to determine chlorophyll *a* and chlorophyll *b* contents, respectively, and at 470 nm to quantify total carotenoids. Pigment concentrations were calculated using the following equations:

$$\text{Chlorophyll } a\ (C_a) = 12.7 \times A_{663} - 2.69 \times A_{645} \quad (1)$$

$$\text{Chlorophyll } b\ (C_b) = 22.9 \times A_{645} - 4.68 \times A_{663} \quad (2)$$

$$\text{Total carotenoids} = (1000 \times A_{470} - 1.82 \times C_a - 85.02 \times C_b) / 198 \quad (3)$$

All pigment contents were expressed as mg per g of fresh weight (FW, and total chlorophyll was computed as the sum of chlorophyll *a* and *b*. Total phenolic content was determined using the Folin–Ciocalteu colorimetric method (Medina 2011). Fresh plant tissue (0.5 g) was extracted with 80% methanol, mixed with 10% Folin–Ciocalteu reagent and 7.5% Na_2CO_3, incubated at 40 °C for 30 min, and the absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE)/g FW. Total flavonoid content was determined using a colorimetric assay with slight modifications (Salerno *et al.* 2014). Briefly, 25 µL of sample or catechin standard was mixed with 100 µL of water and 7.5 µL of 5% $NaNO_2$, followed by 7.5 µL of 10% $AlCl_3$. After 6 min, 50 µL of 1 M NaOH was added, followed by 60 µL of water. After mixing, absorbance was measured at 510 nm against a prepared blank. Results were expressed as catechin equivalents. L-Ascorbic acid (vitamin C) was determined according to Khadka and Pathak (2023). Ascorbic acid was oxidized to dehydroascorbic acid using bromine water in an acetic acid medium at 37 °C and subsequently reacted with 2,4-dinitrophenylhydrazine (DNPH). The resulting product formed a red-colored complex following the addition of 85% H_2SO_4, and absorbance was measured at 530 nm. The composition of the NS used for the control treatment is summarized in Table 2.

Table 2. Composition of the NS Used for the Control Treatment (Carvalho *et al*. 2018)

Component	Concentration (mg/L)
Calcium nitrate ($Ca(NO_3)_2·4H_2O$)	900
Potassium nitrate (KNO_3)	134
Potassium sulfate (K_2SO_4)	280
Magnesium sulfate ($MgSO_4·7H_2O$)	495
Potassium chloride (KCl)	138
Monoammonium phosphate ($NH_4H_2PO_4$)	142
Ferric chloride ($FeCl_3·6H_2O$)	11.97
Manganese sulfate ($MnSO_4·H_2O$)	3.39
Boric acid (H_3BO_3)	2.92
Zinc sulfate ($ZnSO_4·7H_2O$)	0.49
Copper sulfate ($CuSO_4·5H_2O$)	0.08
Sodium molybdate ($Na_2MoO_4·2H_2O$)	0.12
EDTA-disodium ($C_{10}H_{14}N_2O_8Na_2·2H_2O$)	16.42

Statistical Analysis

Statistical analyses and data presentation were conducted using SPSS version 27, GraphPad Prism version 9.0.2, JMP Pro 16, and Microsoft Excel 365. Three independent reservoirs per treatment were used as biological replicates (n = 3), while chemical, physiological, and microbiological analyses were performed in triplicate (n = 3 technical replicates). The results are presented as mean ± SD. Statistical significance was evaluated at a 95% confidence level ($P \leq 0.05$).

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to identify significant differences between treatments at $P \leq 0.05$ (95% confidence level). Correlation analysis and principal component analysis (PCA) were applied as exploratory approaches to visualize relationships among variables. Correlation patterns were presented using heatmaps, while PCA scree plots and biplots were used to summarize data variance and illustrate associations among the parameters studied.

RESULTS AND DISCUSSION

CaO-treatment of Anaerobic Digestate

Effect of CaO-treatment on microbial characteristics

CaO treatment markedly reduced the concentrations of all monitored pathogenic indicators in the anaerobic digestate, with the degree of inactivation increasing progressively as the CaO dosage increased (Fig. 2). The 0% CaO treatment represents untreated digestate (UTD) control. Among the tested microorganisms, *Enterococcus faecalis*, *Escherichia coli*, and fecal coliforms all exhibited substantial reductions, confirming the effectiveness of alkaline stabilization for microbial inactivation. The UTD showed high initial concentrations of 751 CFU/100 mL (*E. faecalis*), 372 CFU/100 mL (*E. coli*), and 561 CFU/100 mL (fecal coliforms), consistent with typical pathogen loads reported for sewage-derived digestates (López *et al*. 2020; Franchitti *et al*. 2024; Zheng *et al*. 2024). These high baseline counts confirm the hygienic risks associated with raw digestate and the necessity of effective stabilization prior to agricultural reuse.

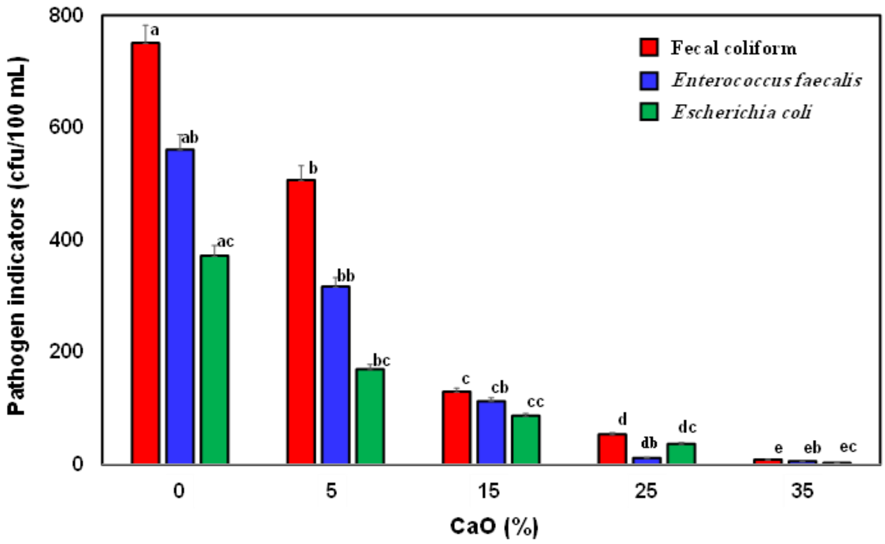


Fig. 2. Effect of CaO treatment on pathogenic indicators in anaerobic digestate. The 0% CaO condition represents UTD (control). Bars show the mean values with error lines indicating the SD. Values with the same letter do not differ significantly at $P \leq 0.05$ (95% confidence level).

The bacterial species *Enterococcus faecalis* is widely recognized as one of the most resilient fecal indicator organisms because of its thick peptidoglycan cell wall, strong environmental tolerance, and ability to survive substantial pH fluctuations. Nonetheless, CaO treatment induced substantial reductions. A low dose of 5% CaO reduced *E. faecalis* by 32.5%, indicating an early inactivation effect. Increasing the CaO concentration produced progressively greater reductions, with 82.7% removal at 15% CaO. Near-complete inactivation (98.8%) was achieved at the highest concentration (35% CaO). Therefore, *Enterococcus* spp. Requires strongly alkaline conditions for effective inactivation, as elevated pH levels (>12) disrupt cellular enzymatic activity and cause irreversible damage to the cell membrane, as reported in previous research (Reza *et al.* 2025; Wang *et al.* 2025b).

The effectiveness of CaO in reducing pathogenic indicators can be attributed to multiple synergistic mechanisms. The elevation of pH to values exceeding 12 leads to irreversible denaturation of microbial proteins and enzymes, disruption of cell membranes, and loss of osmotic regulation (Chojnacka and Moustakas 2024; Senevirathne and Kaparaju 2025). In addition, the exothermic hydration of CaO (65 kJ/mol) generates localized heat, creating a thermally hostile microenvironment (Tonanzi *et al*. 2021). Furthermore, elevated concentrations of Ca^{2+} and OH^{-} ions increase osmotic stress and interfere with essential metal cofactors in microbial enzymatic systems (Chen *et al.* 2023). These combined effects explain the observed inactivation efficiency, as well as the differential susceptibility between Gram-negative bacteria such as *E. coli* and Gram-positive bacteria such as *E. faecalis*, which possess a thicker peptidoglycan cell wall (Xu *et al.* 2020).

Both fecal coliforms and *Escherichia coli* exhibited even greater sensitivity to CaO treatment than *Enterococcus faecalis*. For fecal coliforms, reductions reached 43.5% at 5% CaO and nearly 80% at 15% CaO, while higher doses resulted in dramatic inactivation, achieving 97.9% at 25% CaO and 98.9% at 35% CaO. This rapid decline reflects their vulnerability to the combined effects of elevated pH, increased ionic strength, and heat generated during CaO hydration. In comparison, *E. coli* showed even higher sensitivity, with a 54.3% reduction at 5% CaO, substantially exceeding the reduction observed for *E. faecalis* at the same dose, consistent with its lower resistance to alkaline stress and rapid loss of viability at pH > 12. The inactivation efficiency of *E. coli* increased to 76.6% at 15% CaO and exceeded 99% at 35% CaO. These findings are consistent with previous studies reporting high *E. coli* removal efficiencies in lime-stabilized sludge, where CaO hydration simultaneously elevates pH and releases heat, creating a hostile environment for Gram-negative bacteria (Floriano da Silva *et al.* 2023; Pasoń and Stańczyk-Mazanek 2023; Wang *et al.* 2025b).

Statistical analysis further confirmed the effectiveness of CaO stabilization. Relative to the UTD, CaO-treated samples exhibited a significant reduction in all pathogenic indicators, as confirmed by Tukey's HSD test. The control treatment consistently showed the highest pathogen concentrations, indicating that no substantial inactivation occurred in its absence. At low CaO doses, pathogen concentrations were reduced; however, in some cases these reductions were not significantly different from the control, as indicated by shared Tukey letters. In contrast, higher CaO doses (25% and 35%) resulted in significantly lower pathogen levels than UTD, demonstrating the strong inhibitory effect of alkaline stabilization.

Following the evaluation of microbial inactivation, the influence of CaO treatment on the physiochemical properties of the digestate was investigated to assess its suitability for subsequent oxidation and resource recovery.

Effect of CaO-treatment on physiochemical characteristics

Progressive addition of CaO substantially altered the physicochemical characteristics of the anaerobic digestate (Table 3). Increasing CaO dosage promoted alkaline stabilization, nutrient precipitation, and organic matter removal, resulting in clear dose-dependent changes across all measured parameters.

The digestate pH rose sharply from 7.6 in the UTD to 12.3 at 35% CaO. This reflects the complete hydration of CaO to $Ca(OH)_2$ and the rapid increase in hydroxide ion concentration. High pH is critical for inactivating pathogens, denaturing cellular components, and destabilizing colloidal organic matter. Recent investigations reported similar pH outcomes, confirming effective stabilization (Yuan *et al.* 2019; Xu *et al.* 2020; She *et al.* 2021; Wang *et al.* 2025b).

EC showed a dramatic decline, from 1.87 mS/cm in the UTD to only 0.61 mS/cm at 35% CaO, corresponding to approximately a 67.4% reduction. The observed decrease in EC is attributed to the net removal of dissolved ions under high-pH conditions through extensive precipitation of $CaCO_3$, $Ca_3(PO_4)_2$, and metal hydroxides, which outweighs the ionic contribution from added Ca^{2+} and OH^- (Chojnacka and Moustakas 2024; Mathe *et al.* 2024; Senevirathne and Kaparaju 2025). The EC was measured in the clarified supernatant after 12 h of sedimentation, without dilution. This reduction is especially significant because high salinity is one of the major limitations preventing the direct reuse of digestate in sensitive irrigation systems or hydroponic cultivation (Reza *et al.* 2025; Wang *et al.* 2025b). The substantial EC reduction demonstrates the effective removal of dissolved ions and supports the feasibility of using lime-TD in hydroponic systems (Xu *et al*. 2020). Similarly, TOC decreased significantly from 2.31 g/kg in the control to 0.87 g/kg at 35% CaO, corresponding to a 62% reduction. This reduction is associated with CaO-induced adsorption, coagulation, and precipitation of soluble and colloidal organic fractions, along with alkaline conditions that promote cell lysis, protein denaturation, and the aggregation of organic constituents (Tang *et al.* 2020; Zheng *et al.* 2021).

Table 3. Effect of CaO Dosage on the Physicochemical, Nutrient, and Metal Characteristics of Anaerobic Digestate

Parameter		CaO Dose (%)				
		0	5	15	25	35
Physio-chemical	**pH**	7.60 ± 0.46[d]	9.60 ± 0.58[c]	10.50 ± 0.63[bc]	11.40 ± 0.68[ab]	12.30 ± 0.74[a]
	EC (mS/cm)	1.87 ± 0.11[a]	1.67 ± 0.10[a]	1.27 ± 0.08[b]	0.90 ± 0.05[c]	0.61 ± 0.04[d]
Nutrients	PO_4–P (g/kg)	1.24 ± 0.07[a]	1.02 ± 0.06[b]	0.92 ± 0.06[b]	0.67 ± 0.04[c]	0.39 ± 0.02[d]
	NH_4-N (mg/kg)	340.00 ± 20.40[a]	315.00 ± 18.90[a]	217.00 ± 13.00[b]	197.00 ± 11.80[b]	74.00 ± 4.44[c]
	K (mg/kg)	163.00 ± 9.78[a]	120.00 ± 7.20[b]	94.00 ± 5.64[c]	71.00 ± 4.26[d]	57.00 ± 3.42[d]
Metals	Cd (mg/kg)	9.08 ± 0.54[a]	6.48 ± 0.39[b]	5.72 ± 0.34[b]	2.07 ± 0.12[c]	1.43 ± 0.09[c]
	Pb (mg/kg)	5.24 ± 0.31[a]	2.67 ± 0.16[b]	2.04 ± 0.12[c]	1.58 ± 0.09[d]	0.37 ± 0.02[e]
	Fe (mg/kg)	521 ± 31.26[a]	480 ± 28.80[ab]	437 ± 26.22[bc]	376 ± 22.56[c]	271 ± 16.26[d]
	Zn (mg/kg)	67.28 ± 4.04[a]	50.49 ± 3.03[b]	46.11 ± 2.76[bc]	38.77 ± 2.33[c]	27.62 ± 1.65[d]
	Cu (mg/kg)	7.91 ± 0.47[a]	6.48 ± 0.39[b]	5.00 ± 0.30[c]	4.16 ± 0.25[c]	2.48 ± 0.15[d]
	Mn (mg/kg)	49.87 ± 2.99[a]	3.61 ± 0.22[b]	3.00 ± 0.18[b]	2.43 ± 0.15[b]	2.07 ± 0.12[b]
Solids and organic load	TS (g/L)	10.30 ± 0.46[a]	9.24 ± 0.51[b]	7.34 ± 0.28[c]	6.34 ± 0.39[c]	3.59 ± 0.18[d]
	TDS (g/L)	5.26 ± 0.27[a]	4.65 ± 0.19[b]	3.28 ± 0.22[c]	1.67 ± 0.06[d]	1.00 ± 0.06[e]
	TVS (g/L)	3.72 ± 0.22[a]	2.50 ± 0.08[b]	2.15 ± 0.10[bc]	2.04 ± 0.14[c]	0.64 ± 0.03[d]
	TOC (g/kg)	2.31 ± 0.14[a]	0.98 ± 0.06[b]	0.93 ± 0.06[b]	0.90 ± 0.05[b]	0.87 ± 0.05[b]
	COD (g/L)	28.90 ± 0.98[a]	27.62 ± 1.74[a]	21.08 ± 1.05[b]	18.75 ± 1.07[b]	12.62 ± 0.49[c]
	BOD (g/L)	15.84 ± 1.05[a]	13.51 ± 0.59[b]	11.34 ± 0.67[c]	9.34 ± 0.35[d]	6.49 ± 0.34[e]

Note: Values are presented as mean ± SD (n = 3). EC, electrical conductivity; TOC, total organic carbon; TS, total solids; TDS, total dissolved solids; TVS, total volatile solids; COD, chemical oxygen demand; BOD, biochemical oxygen demand. Different superscript letters within the same row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$), whereas values sharing at least one superscript letter are not significantly different. The 0% CaO Condition Represents UTD (Control).

Macronutrient concentrations also changed substantially following CaO treatment. Phosphate concentrations decreased from 1.24 g/kg to 0.39 g/kg at a CaO dosage of 35%, corresponding to a removal efficiency of approximately 68%. This reduction is attributed to the high pH conditions, which promote the precipitation of stable calcium phosphate phases, such as hydroxyapatite and tricalcium phosphate, consistent with previous studies (Elgarahy *et al.* 2024; Wang *et al.* 2024; Kacprzak *et al.* 2025). The NH_4^+ concentration decreased from 340 mg/kg to 74 mg/kg at 35% CaO, corresponding to an approximate removal efficiency of 78.2%. This reduction is primarily attributed to the volatilization of ammonia gas (NH_3) under highly alkaline conditions (pH > 12), as well as the possible incorporation of NH_4^+ into calcium-based precipitates, as reported in previous studies (Wang *et al.* 2024, 2025b). Potassium concentrations decreased from 163 mg/kg to 57 mg/kg at 35% CaO, corresponding to approximately 65% removal, and likely due to co-precipitation with organic flocs, consistent with observations reported in previous research (da Silva *et al.* 2022).

CaO stabilization also produced substantial reductions in heavy metal concentrations within the liquid fraction of the digestate. The removal efficiencies increasing proportionally with the CaO dosage. Initially, the UTD exhibited elevated levels of metals, including Fe (521 mg/kg), Zn (67.3 mg/kg), and Mn (49.9 mg/kg), as well as trace metals such as Pb (5.24 mg/kg) and Cd (9.08 mg/kg). These concentrations reflect the typical composition of sewage-derived digestate, which often contains metals accumulated from domestic wastewater, pipeline corrosion, and industrial discharges (Xu *et al.* 2020; Reza *et al*. 2025; Silva 2025). Following CaO addition, however, all metal concentrations decreased significantly, illustrating the strong metal-immobilizing capacity of lime treatment, as reported in previous studies (Xu *et al.* 2020; da Silva *et al.* 2022; Floriano da Silva *et al.* 2023).

A distinct dose-dependent response was observed across all metals. At low CaO levels (5%), moderate reductions were observed, such as 49.0% for Pb, 28.6% for Cd, and 25.0% for Zn. These early reductions can be attributed to rapid pH elevation, which promotes hydroxide precipitation and enhances coagulation–flocculation of fine particles (Chatterjee *et al.* 2025). However, the most pronounced improvements emerged at CaO dosages ≥25%. For instance, Pb concentration declined from 5.24 to 1.58 mg/kg (69.8% removal) at 25% CaO and further to 0.37 mg/kg (92.9%) at 35% CaO. Similarly, Cd removal increased dramatically at higher alkalinity, reaching 84.2% at 35% CaO. Similar observations have been reported by previous studies (Xu *et al.* 2020; Zheng *et al.* 2021; da Silva *et al.* 2022).

The relatively high initial Fe concentration (521 mg/kg WW) represents the UTD and reflects the total Fe content measured in the liquid digestate fraction. Iron and Zinc showed moderate removal efficiencies, 48.0% and 58.9%, respectively, at the highest CaO level. This behavior aligns with their known partial resistance to complete precipitation, especially when present in organically bound or colloidal forms (Xu *et al.* 2020). The progressive decline in Fe from 521 to 271 mg/kg is beneficial for downstream Fenton processes, since excessive Fe can lead to sludge formation and operational inefficiencies (Tang *et al.* 2020). Meanwhile, the reduction in Zn and Cu (up to 68.6%) contributes to lowering phytotoxic risks while retaining trace amounts that may remain beneficial for plant micronutrient nutrition in hydroponic systems (Floriano da Silva *et al.* 2023; Chatterjee *et al.* 2025).

Among all metals, Mn exhibited the most remarkable response, with its concentration decreasing from 49.9 mg/kg to only 2.07 mg/kg, a removal efficiency of 95.9% at 35% CaO. Even at the lowest CaO dose (5%), Mn removal reached 92.8%. This rapid and near-complete removal is highly advantageous since Mn toxicity is known to impair root development in hydroponic crops at concentrations significantly lower than other metals (Floriano da Silva *et al.* 2023; Reza *et al.* 2025).

CaO addition raises the pH above 12, creating highly supersaturated conditions that drive extensive precipitation of dissolved ions as $CaCO_3$, calcium phosphates (*e.g.*, hydroxyapatite), and metal hydroxides, thereby removing a substantial fraction of ionic species from solution (Zhang *et al.* 2023). Under these alkaline conditions, both homogeneous and heterogeneous nucleation processes are enhanced, with carbonate preferentially binding Ca^{2+} through $CaCO_3$ formation, while heavy metals concurrently precipitate as hydroxides due to reduced solubility (Masindi *et al.* 2023).

During the subsequent 12 h settling period, coagulation and flocculation further decrease the ionic load *via* sweep-floc mechanisms, whereby metal hydroxide precipitates entrap colloidal and fine particulate matter (Nagargoje *et al.* 2025). Consequently, the reduction in EC reflects the overall decrease in dissolved ionic content resulting from precipitation and phase separation, with EC effectively indicating the concentration of remaining free ions in the treated sample (Mortadi *et al.* 2020). This combined effect explains the apparent decline in EC despite the initial addition of ions from CaO treatment.

In addition to nutrient and metal removal, CaO treatment markedly improved the solids characteristics of the digestate. These reductions demonstrate the strong coagulation and stabilization effects of alkaline conditioning. The UTD exhibited relatively high concentrations of solids and organic load indicators such as COD and BOD, reflecting the typical characteristics of raw sewage-derived digestate. However, the gradual addition of CaO from 5% to 35% led to a marked decline in both particulate and dissolved fractions, confirming the strong coagulation, flocculation, and stabilization effects associated with alkaline conditioning (Kacprzak *et al.* 2025).

A significant reduction in TS was observed as the CaO dose increased, declining from 10.3 g/L in the UTD to 3.59 g/L at 35% CaO, corresponding to an overall reduction of approximately 65.10%. This trend reflects the ability of $Ca(OH)_2$ to neutralize colloidal charges, promote aggregation, and facilitate rapid sedimentation of suspended particles. Similar reductions have been reported in lime-treated sewage sludge and digestate in recent studies, demonstrating that lime stabilization consistently improves particle removal efficiency (Zheng *et al.* 2021; Wang *et al.* 2024). Likewise, TDS decreased dramatically, by more than 80% at the highest CaO dose, indicating the precipitation of soluble ions, primarily as calcium carbonates, phosphates, and hydroxides.

TVS, which represents the biodegradable organic fraction, also declined sharply with CaO addition. The TVS removal reached 82.8% at 35% CaO, demonstrating substantial stabilization of the organic matrix and reduction of putrescible materials. This outcome aligns with findings showing reductions in TVS in lime-treated biosolids (Yuan *et al.* 2019; Xu *et al.* 2020; Zheng *et al.* 2021). The reduction in TVS is particularly relevant to hydroponic applications, as residual biodegradable organic matter may promote microbial regrowth and compromise nutrient-solution quality. Reductions in COD and BOD were likewise substantial, with COD decreasing from 28.90 to 12.62 g/L (56.30%) and BOD from 15.84 to 6.49 g/L (59%). These results reflect the dual effect of CaO in removing both particulate and soluble organic matter. High pH also promotes chemical oxidation and hydrolysis of certain organic compounds, thereby destabilizing them (She *et al*. 2021; Wang *et al*. 2024; Kacprzak *et al*. 2025). The observed reduction in organic matter indicates that CaO pretreatment improves effluent quality prior to AOPs (*e.g.*, Fenton treatment), thereby reducing downstream chemical demand and enhancing overall sustainability. Comparable improvements have been documented in previous research (Yuan *et al.* 2019; Zheng *et al.* 2021; Wang *et al.* 2025b).

Overall, the results indicate that CaO dosages of 25% to 35% yield the most effective improvements in digestate quality, resulting in substantial reductions in solids, organic load, and salinity. These findings reinforce the suitability of CaO treatment as an efficient, low-cost, and scalable pretreatment method for improving the physicochemical quality of anaerobic digestate prior to further purification and reuse. Moreover, the substantial reductions in solids and organic matter improve the physicochemical quality of the TD, enhancing its potential for reuse in circular agricultural applications, including hydroponic systems.

One-way ANOVA showed that CaO dose had a statistically significant effect on all measured digestate parameters ($P \leq 0.05$). Relative to the UTD, increasing CaO addition resulted in a progressive increase in pH and decrease in EC. Indicators of organic matter, including TOC, TS, TDS, TVS, COD, and BOD, generally decreased with increasing CaO dose; however, the magnitude and statistical separation among doses varied by parameter. Notably, TOC showed a marked reduction following CaO addition, with no significant differences among the CaO-treated groups (5 to 35%). Nutrient concentrations (PO_4–P, NH_4^+, and K) also declined with CaO treatment. Similarly, the concentrations of heavy metals (Cd, Pb, Fe, Zn, Cu, and Mn) were significantly reduced with CaO treatment, with the strongest reductions generally observed at higher CaO doses. Overall, these results indicate a significant effect of CaO dosage on digestate characteristics, with the 35% CaO treatment generally producing the greatest changes among the evaluated doses.

The effect of CaO stabilization and subsequent Fenton oxidation on selected key physicochemical and contaminant parameters are further illustrated in Fig. S1, providing a graphical overview of the treatment response.

Fenton Oxidation of CaO-TD

Based on the optimal CaO stabilization conditions identified above, the clarified digestate was subsequently subjected to Fenton oxidation at different reaction times (0 to 6 h) to evaluate its additional effects on physicochemical and microbiological quality.

Effect of Fenton oxidation on microbial characteristics

The Fenton oxidation process achieved complete inactivation of all three bacterial indicators, *Enterococcus faecalis*, *Escherichia coli*, and fecal coliforms, across all applied reaction times (1, 2, 4, and 6 h). In the CaO-stabilized digestate before Fenton treatment, residual microbial loads were still detectable (9, 3, and 6 CFU/100 mL, respectively), indicating that although high-dose lime treatment substantially reduced microbial contamination, trace survival of pathogens remained possible. This highlights the limitation of lime stabilization as a stand-alone disinfection strategy when extremely stringent microbial thresholds are required, such as in hydroponic systems where plant roots are in continuous contact with the treated effluent (Wang *et al.* 2025a).

Following Fenton oxidation, however, all microbial indicators became non-detectable regardless of reaction duration. This demonstrates the high efficiency of OH• generated from the Fe^{2+}/H_2O_2 catalytic system, which rapidly oxidize cell membranes, intracellular components, and nucleic acids (Akbay *et al.* 2022; Kazimierowicz *et al.* 2023). Complete inactivation at even the shortest treatment time (1 h) indicates that Fenton-driven disinfection occurs rapidly and does not require prolonged exposure. The fact that *Enterococcus faecalis*, typically more resistant due to its thick peptidoglycan cell wall, was entirely removed under all conditions further underscores the strong biocidal capability of the Fenton process. These findings closely align with recent studies reporting the high antimicrobial performance of Fenton oxidation in various wastewater streams (Li *et al.* 2023; Ferri and Bolelli 2025; Fiorentino *et al.* 2025).

The synergy between lime stabilization and Fenton oxidation creates an effective multi-barrier treatment approach that enhances the physicochemical and microbiological quality of the digestate. CaO treatment elevates pH and removes solids, reducing microbial loads and enhancing accessibility of Fenton-generated radicals to remaining bacteria (Wang *et al.* 2025a). The Fenton process further enhances microbial reduction through oxidative reactions, substantially improving the microbiological quality of the treated effluent (Wang *et al.* 2022). The observed elimination of the monitored bacterial indicators demonstrates the effectiveness of the integrated CaO–Fenton process in improving the microbiological quality of the TD for potential soilless cultivation applications (Yuan *et al.* 2019; Tang *et al.* 2020).

Fenton oxidation effects on physiochemical characteristics

Table 4 summarizes the changes in EC, organic carbon, nutrients, heavy metals, solids, and organic-load indicators during Fenton treatment. The 0 h condition corresponds to CaO-TD without Fenton oxidation and serves as the baseline for assessing the effects of Fenton reaction time. The temporal evolution of the major physicochemical, nutrient, solids, and metal parameters during Fenton oxidation is further illustrated in Figure S2 to provide a clearer visualization of treatment kinetics.

Fenton oxidation produced progressive changes in the physicochemical characteristics of the CaO-treated digestate, with the magnitude of the response generally increasing with reaction time. EC declined steadily from an initial 0.61 mS/cm in the CaO-treated control to 0.24 mS/cm at 6 h, corresponding to a 60.7% reduction. The reaction between Fe^{2+} and H_2O_2 generates highly reactive OH•, while iron-mediated coagulation and precipitation may further contribute to contaminant removal (Bao et al. 2021; Wang et al. 2024; Lee et al. 2025). Together, these oxidative and coagulative mechanisms improved the quality of the TD for potential agricultural reuse (Bergstrand *et al.* 2020).

Minimization of organic carbon is necessary to enhance nitrification and limit microbial growth in a closed hydroponic system (Mathe *et al.* 2024). A more pronounced effect was observed in the removal of TOC, where values decreased from 0.88 g/kg in the control to 0.47 g/kg at 6 h, representing 46.3% TOC reduction. The stepwise decline, 22.3% at 1 h, 39.4% at 2 h, and 42.9% at 4 h, indicates rapid oxidation of readily degradable organics, followed by slower mineralization of more recalcitrant compounds. This behavior is consistent with the kinetics of hydroxyl radical (OH•) generation in Fenton systems (Tang *et al.* 2020; Kazimierowicz *et al.* 2023; Silva 2025).

Phosphorus showed parallel improvements, with PO_4–P decreasing from 0.39 g/kg to 0.25 g/kg by 4 to 6 h (≈35.9% removal). These removals exceed typical phosphorus reductions from lime treatment alone, demonstrating the added benefit of Fenton-generated Fe(III) species, which react with phosphate to form insoluble $FePO_4$ (Gholikandi *et al.* 2018; Kwon *et al.* 2021; Li *et al.* 2025). CaO pretreatment also contributed residual Ca^{2+}, which can precipitate phosphate as $Ca_3(PO_4)_2$. This combined Fe–Ca–P precipitation mechanism aligns well with studies showing that hybrid lime-Fenton systems outperform single-step treatments in removing phosphate from digestate (Gholikandi *et al.* 2018; Tang *et al.* 2020; Yuan *et al*. 2020).

Table 4. Effect of Fenton Treatment Time (1 to 6 h) on the Physicochemical, Nutrient, and Heavy-metal Characteristics of CaO-TD

Parameter		Fenton Treatment Time (h)				
		0	1	2	4	6
Physiochemical	EC (mS/cm)	0.61 ± 0.04[a]	0.57 ± 0.04[a]	0.34 ± 0.02[b]	0.31 ± 0.02[bc]	0.24 ± 0.01[c]
Nutrients	PO_4–P (g/kg)	0.39 ± 0.02[a]	0.27 ± 0.02[b]	0.27 ± 0.01[b]	0.25 ± 0.02[b]	0.25 ± 0.02[b]
	NH_4^+-N (mg/kg)	74.00 ± 4.44[a]	76.00 ± 3.40[a]	80.00 ± 3.10[ab]	87.00 ± 3.90[b]	87.00 ± 4.20[b]
	K (mg/kg)	57.00 ± 3.42[a]	56.00 ± 1.60[a]	54.00 ± 1.80[a]	54.00 ± 1.50[a]	53.00 ± 1.70[a]
Metals	Cd (mg/kg)	1.43 ± 0.09[a]	1.08 ± 0.08[b]	0.97 ± 0.06[b]	0.34 ± 0.03[c]	0.25 ± 0.02[c]
	Pb (mg/kg)	0.37 ± 0.02[a]	0.20 ± 0.01[b]	0.19 ± 0.01[b]	0.10 ± 0.01[c]	0.10 ± 0.01[c]
	Fe (mg/kg)	271.00 ± 16.26[a]	260.00 ± 13.00[a]	257.00 ± 12.00[a]	211.00 ± 11.00[b]	189.00 ± 10.00[b]
	Zn (mg/kg)	27.60 ± 1.65[a]	25.30 ± 0.90[ab]	23.50 ± 1.30[bc]	20.20 ± 1.50[c]	20.00 ± 1.20[c]
	Cu (mg/kg)	2.48 ± 0.15[a]	2.31 ± 0.17[ab]	2.00 ± 0.13[b]	1.95 ± 0.15[b]	1.94 ± 0.16[b]
	Mn (mg/kg)	2.07 ± 0.12[a]	2.00 ± 0.09[a]	1.93 ± 0.14[a]	1.37 ± 0.12[b]	1.00 ± 0.08[c]
Solids and organic load	TS (g/L)	3.59 ± 0.18[a]	3.31 ± 0.17[a]	2.18 ± 0.11[b]	1.37 ± 0.08[c]	0.44 ± 0.03[d]
	TDS (g/L)	1.00 ± 0.06[a]	0.97 ± 0.05[a]	0.77 ± 0.04[b]	0.48 ± 0.03[c]	0.15 ± 0.01[d]
	TVS (g/L)	0.64 ± 0.03[a]	0.41 ± 0.02[b]	0.32 ± 0.02[c]	0.13 ± 0.01[d]	0.00 ± 0.00[e]
	TOC (g/kg)	0.87 ± 0.05[a]	0.68 ± 0.04[b]	0.53 ± 0.03[c]	0.50 ± 0.03[c]	0.47 ± 0.03[c]
	COD (g/L)	12.62 ± 0.49[a]	10.34 ± 0.52[b]	9.35 ± 0.47[bc]	8.94 ± 0.45[c]	6.00 ± 0.30[d]
	BOD (g/L)	6.49 ± 0.34[a]	5.54 ± 0.29[b]	4.38 ± 0.22[c]	3.57 ± 0.18[d]	3.09 ± 0.16[d]

Note: The 0 h sample represents the CaO-TD baseline without Fenton oxidation. Values are presented as mean ± SD (n = 3). EC, electrical conductivity; TOC, total organic carbon; TS, total solids; TDS, total dissolved solids; TVS, total volatile solids; COD, chemical oxygen demand; BOD, biochemical oxygen demand. Different superscript letters within the same row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$), whereas values sharing at least one superscript letter are not significantly different.

The NH_4^+ concentration did not decrease but instead increased from 74 to 87 mg/kg after 6 h. This is expected, as hydroxyl radical–mediated oxidation promotes the mineralization of organic nitrogen into NH_4^+ rather than removing nitrogen from the system. Similar increases in NH_4^+ concentrations have been reported following advanced oxidation treatments of digestate and sludge effluents in previous research (Feki *et al.* 2019). From a reuse perspective, this outcome is advantageous, as organic nitrogen is reduced while plant-available nitrogen remains in solution, thereby preserving the fertilizer value of the TD (Bergstrand *et al.* 2020; Bhatia and Sindhu 2024). Potassium (K), a highly soluble macronutrient, remained largely stable during treatment, decreasing only from 57 mg/kg to 53 mg/kg (7% reduction). This minimal loss is consistent with K^+ not participating in precipitation reactions under Fenton conditions, indicating that the process maintains the agronomic nutrient potential of the treated effluent (Gholikandi *et al.* 2018).

Overall, the results demonstrate that Fenton oxidation provides a strong polishing effect by effectively reducing salinity, organic matter, and phosphorus while preserving essential nutrients, particularly nitrogen and potassium, which is an essential combination for producing a hydroponic-compatible nutrient medium. Compared with recent literature, the CaO–Fenton system applied in this study achieves performance at the upper end of reported ranges, especially with respect to TOC and phosphorus removal. Consistent with the functional requirements of European Union (EU) regulation 2019/1009 and established digestate management standardization parameters (Chojnacka and Moustakas 2024; Senevirathne and Kaparaju 2025). This dual-stage treatment improves key physicochemical and microbiological quality attributes, enhances nutrient-use efficiency and product stability, and preserves the agronomic value of the digestate. These characteristics highlight its potential as a scalable approach for producing nutrient-rich treated effluents for further evaluation in circular hydroponic and resource-recovery systems.

Fenton post-treatment, applied after CaO stabilization, is highly effective in reducing heavy metals in the digestate, with a clear dependence on reaction time. The combined CaO–Fenton system achieves substantial reductions in heavy metals, particularly at extended reaction times. Lead (Pb) decreases from 0.37 mg/kg in control to 0.10 mg/kg at 4 to 6 h, giving a 73% removal. Cadmium (Cd) is reduced from 1.43 mg/kg to 0.25 mg/kg at 6 h (82.5% removal), with a particularly sharp drop after 4 h (76.2% removal). These high removal ratios suggest that Pb and Cd are effectively scavenged by iron hydroxide flocs and co-precipitated with Ca/Fe-phosphate and carbonate phases formed during CaO and Fenton treatment (Bergstrand *et al.* 2020; Qiu *et al.* 2021; Floriano da Silva *et al.* 2023). Previous studies on Fenton-assisted sludge and digestate treatment have reported similar removal rates of Pb and Cd (Azhdarpoor *et al.* 2018; Yuan *et al.* 2020).

Iron (Fe), present both as a native metal and as added Fe^{2+} in the Fenton reagent, showed more modest removal, with residual concentrations decreasing from 271 mg/kg to 189 mg/kg at 6 h (30.3% removal). This limited reduction may be associated with the continuous cycling of Fe^{2+} and Fe^{3+} during the Fenton reaction, followed by the formation of iron hydroxide/oxide phases during neutralization (Bao *et al.* 2021; Akbay *et al.* 2022). Similar Fe removal has been reported in Fenton-treated sludge and leachates (Blumfelde *et al.* 2024; Wang *et al.* 2025a).

It should be noted that the reported post-Fenton concentrations represent the filtered liquid fraction. Therefore, the observed decreases in Fe and other heavy metals may reflect not only Fenton-mediated transformations but also precipitation and co-precipitation during neutralization, followed by the removal of particulate-bound species through settling and 0.45 μm filtration. These mechanisms are consistent with the reported role of iron-hydroxide flocs and solid-phase partitioning in the removal of metals during Fenton-based treatment (Yuan *et al.* 2020; Qiu *et al.* 2021; Floriano da Silva *et al*. 2023).

The micronutrients Zn, Cu, and Mn exhibited a clear but more gradual decline with increasing Fenton reaction time. Zinc decreased from 27.6 mg/kg to 20.0 mg/kg at 6 h (≈27.60% removal), copper from 2.48 to 1.94 mg/kg (≈21.8% removal), and manganese from 2.07 to 1.00 mg/kg (≈51.70% removal). The stronger reduction of Mn relative to Zn and Cu suggests that Mn is more prone to oxidation and precipitation under Fenton conditions, likely forming Mn oxides/hydroxides that settle with the sludge (Qiu *et al.* 2021; Taghavi *et al.* 2021). The partial removal of Zn and Cu, while not as dramatic as for Pb and Cd, is beneficial for reducing potential phytotoxicity while still retaining sufficient micronutrient content for plant growth (Floriano da Silva *et al*. 2023). Comparable partial removals of Zn and Cu have been reported in Fenton- and photo-Fenton-TD and agricultural effluents, where these metals partition between soluble, colloidal, and solid phases (Azhdarpoor *et al.* 2018; Yuan *et al.* 2020; Qiu *et al.* 2021).

The Fenton treatment, particularly when applied following CaO stabilization, proved highly effective in reducing heavy metal concentrations, especially cadmium and lead, to levels well below the maximum permissible limits set by EU fertilizer regulations (Senevirathne and Kaparaju 2025). Although the untreated material exhibited cadmium concentrations close to regulatory thresholds, treatment durations of 4 h or longer ensured full compliance. Essential micronutrients such as Fe, Zn, Cu, and Mn remained at concentrations below regulatory concern, indicating that the CaO–Fenton sequence effectively reduced contaminant levels while preserving agronomically important nutrients without evidence of secondary metal contamination.

TS declined sharply from 3.59 g/L in control to just 0.44 g/L after 6 h of Fenton treatment, corresponding to an 87.7% reduction. Similar observations were found in earlier studies (Almomani *et al*. 2019; Akbay *et al.* 2022). A similar trend is observed for TDS, which decrease from 1.00 g/L to 0.15 g/L (85% removal). These results indicate that Fenton oxidation not only degrades soluble organic matter but also promotes the coagulation and removal of colloidal and particulate fractions, likely aided by residual iron flocs formed during the oxidation reaction (Feki *et al.* 2019; Bao *et al.* 2021). TVS, a key indicator of biodegradable organic matter, shows the most dramatic reduction, from 0.64 g/L to complete removal (100%) after 6 h, demonstrating that Fenton is highly efficient in destroying recalcitrant organic fractions remaining after AD and CaO stabilization (Kazimierowicz *et al.* 2023; Silva 2025).

Organic load parameters show similarly strong improvements. COD drops from 12.62 to 6.00 g/L (52.5% reduction), while BOD decreases from 6.49 to 3.09 g/L (52.4% removal). The reaction kinetics followed a pseudo-first-order trend, characterized by rapid hydroxyl radical generation during the initial 30 to 60 min, which explains the considerable COD reductions of 18% to 26% already achieved within the first 1 to 2 h. This behavior is consistent with previous studies (Silva 2025; Wu *et al*. 2025). Furthermore, the highest COD removal efficiency was attained at an optimal pH of approximately 3, in accordance with the conditions employed in the present study and as documented by previous research (Wang *et al.* 2021; Ayoub 2022). These observations are consistent with recent reports showing COD reductions of 35%–70% in digestates, leachates, and agro-industrial effluents subjected to Fenton processes (Almomani *et al.* 2019; Akbay *et al.* 2022; Ayoub 2022; Hammam *et al.* 2022; Wang *et al.* 2025a).

Overall, the progressive improvements in TS, TDS, TVS, COD, and BOD with reaction time reinforce that Fenton oxidation is an effective polishing step following CaO stabilization, capable of transforming anaerobic digestate into a cleaner and more stable effluent. These findings are consistent with recent literature advocating integrated AOPs with digestate management to improve efficiency, enhance nutrient recovery, and reduce environmental impacts associated with waste treatment (Feki *et al.* 2019; Tang *et al.* 2020; Akbay *et al.* 2022).

ANOVA showed that Fenton treatment time had a statistically significant effect on most physicochemical properties of the CaO-TD ($P \leq 0.05$), where 0 h represents the CaO-TD without Fenton oxidation. The only exception was K, for which no significant effect of treatment time was detected ($p > 0.05$). Compared with the control, Fenton-treated samples showed significant changes in most parameters. EC and TOC decreased significantly compared with the control, although the extent and timing of statistical separation differed between the two parameters. Phosphate (PO_4–P) concentrations were significantly lower than the CaO-only control after Fenton application, with no additional significant differences beyond 1 h, suggesting rapid stabilization through oxidation and precipitation. In contrast, NH_4^+ concentrations generally increased with treatment time, with significantly higher concentrations observed at 4 to 6 h than in the CaO-treated control, likely due to oxidative mineralization of organic nitrogen. K concentrations showed only a slight numerical decrease and did not differ significantly among treatment times.

Heavy metals (Cd, Pb, Fe, Zn, Cu, and Mn) generally decreased with increasing Fenton treatment time. The strongest reductions were generally observed after 4 to 6 h, supporting the role of Fenton oxidation in enhancing metal immobilization. Indicators of solids and organic load (TS, TDS, TVS, TOC, COD, and BOD) were significantly reduced relative to the control, with generally progressive reductions as treatment time increased. Overall, using the CaO-TD without Fenton treatment (control) as the control, the ANOVA results demonstrate that Fenton oxidation provides a significant additional stabilization step, with most physicochemical improvements becoming significant within substantial improvements occurring within the first 2 to 4 h and further reductions in several parameters at 6 h.

The present study focused on conventional physicochemical and microbial parameters in accordance with established standards for wastewater reuse. However, it is recognized that digestate derived from wastewater sources may contain organic micropollutants, including pharmaceuticals, endocrine-disrupting compounds, and antibiotic resistance genes (ARGs). Previous studies have shown that Fenton oxidation can achieve substantial removal of many pharmaceutical compounds (>70% to 90%) and can contribute to partial degradation of ARGs through oxidative damage to DNA and disruption of cell membranes (Wang *et al.* 2019; Zhang *et al.* 2023). Nevertheless, complete mineralization is not always achieved, and transformation products (*e.g.*, hydroxylated intermediates) may persist. These compounds were not characterized in the present study and warrant further investigation in future research.

Plant Growth and Quality under TD

Following the physicochemical and microbiological evaluation of the TD, its agronomic performance was assessed through hydroponic lettuce cultivation. Table 5 summarizes the morphological and biochemical responses of lettuce grown using the control NS, CaO-TD (35%), and CaO–Fenton-TD (6 h). Plant growth parameters included the number of leaves, plant height, and root length, while biochemical parameters comprised ascorbic acid, chlorophyll a, chlorophyll b, total carotenoids, total phenolics, and total flavonoids.

Table 5. Effects of CaO- and Fenton-TD on Plant Growth and Biochemical Characteristics under Hydroponic Cultivation

Parameter		Control (NS)	CaO (35%)	Fenton (6 h)
Growth	Number of leaves	15 ± 1[a]	13 ± 1[a]	10 ± 1[b]
	Plant height (cm)	19.00 ± 0.75[a]	16.00 ± 0.68[b]	11.00 ± 0.55[c]
	Root length (cm)	21.00 ± 0.95[a]	18.00 ± 0.82[b]	14.00 ± 0.70[c]
Photo-synthetic pigments	Chlorophyll a (mg/100 g FW)	21.60 ± 1.15[a]	23.18 ± 1.30[a]	22.10 ± 1.05[a]
	Chlorophyll b (mg/100 g FW)	9.15 ± 0.48[a]	9.20 ± 0.42[a]	9.11 ± 0.39[a]
	Total carotenoids (mg/100 g FW)	11.00 ± 0.62[a]	12.20 ± 0.74[ab]	13.95 ± 0.88[b]
Bioactive compound	Ascorbic acid (mg/100 g FW)	4.50 ± 0.28[a]	5.34 ± 0.31[a]	7.50 ± 0.42[b]
	Total phenolics (mg/g FW)	18.67 ± 1.10[a]	27.43 ± 1.35[b]	28.91 ± 1.52[b]
	Total flavonoids (mg/g FW)	7.64 ± 0.42[a]	8.69 ± 0.48[ab]	9.17 ± 0.51[b]

Note: NS, nutrient solution; FW, fresh weight. Values are presented as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences among treatments according to Tukey's HSD test ($p \leq 0.05$), whereas values sharing at least one superscript letter are not significantly different.

There was a clear divergence between vegetative growth performance and nutritional/functional quality of plants grown with CaO-treated and CaO–Fenton-TD relative to the control NS. From a morphological standpoint, plants supplied with the conventional NS (control) exhibited the most vigorous growth, with 15 leaves, a height of 19 cm, and root length of 21 cm. When CaO-TD was used, these parameters declined moderately to 13 leaves, 16 cm height, and 18 cm root length. The strongest reduction was observed under the Fenton 6 h treatment, where plants developed only 10 leaves, with height decreasing to 11 cm and root length to 14 cm. These results indicate that, although CaO–Fenton-TD supports plant growth, it induces a growth penalty compared with the fully balanced mineral NS.

Despite the reduction in biomass, photosynthetic pigment contents were maintained or slightly enhanced, suggesting that the functional capacity of the photosynthetic apparatus was not severely compromised. Chlorophyll increased from 21.6 mg/100 g FW in the control to 23.18 mg/100 g FW under CaO treatment and remained relatively high (22.10 mg/100 g FW) under Fenton 6 h. Chlorophyll *b* was remarkably stable across treatments (~9.1 to 9.2 mg/100 g FW), indicating that chlorophyll composition and the light-harvesting complex were largely preserved. β-carotene showed a more pronounced positive response, rising from 11.0 mg/100 g FW in control to 12.2 mg/100 g FW with CaO-TD and further to 13.95 mg/100 g FW following Fenton treatment. This stepwise increase in carotenoid content may reflect a physiological response to the altered nutrient environment, potentially involving mild eustress and enhanced synthesis of photoprotective pigments, as reported in hydroponic lettuce and leafy vegetables subjected to moderate abiotic stress conditions (*e.g.*, salinity, reclaimed water use, or oxidative elicitors) (Bergstrand *et al.* 2020; Ntinas *et al.* 2021; Bhatia and Sindhu 2024; Chatterjee *et al.* 2025).

The most remarkable changes were observed in antioxidant and nutraceutical traits, which improved substantially under digestate-based treatments. Ascorbic acid (vitamin C) increased from 4.5 mg/100 g FW in the control to 5.34 mg/100 g FW with CaO-TD and almost doubled to 7.5 mg/100 g FW under Fenton 6 h. Total phenolic content showed an even stronger response, rising from 1867 mg/100 g FW in the control to 2743 mg/100 g FW in the CaO treatment and 2891 mg/100 g FW with Fenton-TD. Similarly, total flavonoids increased from 764 mg/100 g FW (control) to 869 mg/100 g FW (CaO) and 917 mg/100 g FW (Fenton). These results indicate that CaO–Fenton–TD not only preserves but enhances the functional quality of the harvested biomass by stimulating the biosynthesis of antioxidant secondary metabolites. This behavior aligns with numerous hydroponic studies showing that moderate abiotic stress, whether from salinity, altered ionic composition, or wastewater-derived nutrients, often leads to increased phenolics, flavonoids, carotenoids, and vitamin C in lettuce and other leafy greens, even when total yield is slightly reduced. In other words, plants become smaller but more nutritionally dense (Moncada *et al.* 2020; Ntinas *et al.* 2021; Chatterjee *et al.* 2025; Ferri and Bolelli 2025).

The contrasting responses between plant growth and biochemical quality suggest that CaO–Fenton treatment altered the nutrient balance of the digestate rather than simply improving or impairing its overall suitability for hydroponic cultivation. The lower leaf number, plant height, and root length observed with TD may be associated with nutrient imbalance, particularly the relatively low K availability, NH_4^+ dominance, excess phosphorus, and elevated concentrations of some micronutrients compared with conventional hydroponic requirements (Bergstrand *et al.* 2020; Pelayo Lind *et al.* 2021; Chojnacka and Moustakas 2024). In contrast, the increases in ascorbic acid, carotenoids, phenolics, and flavonoids may represent a physiological response to the altered nutrient environment. Similar increases in antioxidant compounds have been associated with moderate abiotic stress in hydroponic crops; however, this mechanism cannot be confirmed here because specific stress markers were not measured (Moncada *et al.* 2020; Ntinas *et al.* 2021; Chatterjee *et al.* 2025). Thus, the TD supported plant development but increased the accumulation of antioxidant and bioactive compounds despite reduced vegetative growth, indicating that further nutrient adjustment is required before its use as a complete hydroponic NSs.

ANOVA showed that treatment significantly affected most plant growth and biochemical parameters ($p \leq 0.05$). Plant height, root length, and leaf number generally decreased, with the greatest reductions under Fenton treatment. Conversely, ascorbic acid, β-carotene, total phenolics, and total flavonoids generally increased, with Fenton treatment producing the highest values. Chlorophyll a and b were not significantly affected ($p > 0.05$). Overall, the treatments reduced plant growth while enhancing several biochemical quality attributes.

Taken together, these findings indicate a quantitative trade-off: the nutrient control solution maximized morphological growth, whereas CaO-TD and, particularly, Fenton-TD increased the accumulation of antioxidant and bioactive compounds at the expense of some biomass production. These biochemical changes may reflect potential nutritional benefits and/or a physiological response to the altered nutrient environment. From a circular bioeconomy and functional-food perspective, this trade-off can be advantageous. The CaO–Fenton process converts a problematic waste stream (anaerobic digestate) into a sanitized, nutrient-bearing hydroponic solution that produces crops with elevated health-promoting compounds. These findings are in line with recent work on digestate-based hydroponics and reclaimed-water cultivation, but the current study adds novelty by demonstrating this effect specifically for CaO–Fenton-treated sewage digestate, and by concurrently evaluating morphological traits, pigments, vitamin C, phenolics, and flavonoids in a single integrated experimental framework. The reduction in plant height and root length, together with the increased accumulation of ascorbic acid, carotenoids, phenolics, and flavonoids, indicates a contrasting morphological and biochemical response to CaO–Fenton-TD. These responses may be consistent with physiological adaptation to the altered nutrient and metal environment, as increased accumulation of antioxidant compounds has been reported in plants exposed to moderate abiotic stress conditions (Moncada *et al.* 2020; Ntinas *et al.* 2021; Chatterjee *et al.* 2025). Therefore, the increased bioactive compound levels should not be interpreted solely as improved nutritional quality, as they may partly reflect a stress-induced adaptive response. The maintenance or slight increase in chlorophyll a further suggests that photosynthetic pigment levels were not substantially impaired despite the reduction in vegetative growth (Bergstrand *et al.* 2020; *Ntinas et al.* 2021). However, these morphological and biochemical responses provide only indirect evidence of physiological stress and cannot confirm a eustress or hormetic mechanism. Specific stress biomarkers, such as proline, malondialdehyde (MDA), and antioxidant enzyme activities, should therefore be evaluated in future studies to validate the proposed stress-response hypothesis and distinguish stress-related responses from nutritional benefits.

Nutrient Balance and System Performance

To evaluate the nutritional suitability of the TD for hydroponic application, its physicochemical and nutrient composition was compared with standard hydroponic NS ranges reported in the literature. Table 6 summarizes the concentrations observed in TD across Fenton treatment times (0 to 6 h) and their corresponding assessment relative to recommended hydroponic ranges. In addition, the performance of the integrated CaO–Fenton system was compared with alternative digestate and sludge treatment technologies to provide a broader assessment of its treatment efficiency and potential applicability.

Table 6. Comparison of TD Nutrient Composition with Standard Hydroponic NS Requirements

Parameter		TD (0 to 6 h)	Hydroponic NS Range	Assessment	References
Physiochemical	**EC (mS/cm)**	0.24–0.61	2.0–2.5	Low	(Bergstrand *et al.* 2020)
Nutrients	**PO_4–P (mg/L)**	250–390	2.1–38	Excess	(Bergstrand *et al.* 2020)
	NH_4^+–N (mg/L)	74–87	13–61	High	(Bergstrand *et al.* 2020)
	K (mg/L)	53–57	120–200	Deficient	(Bergstrand *et al.* 2020)
Metals	**Cd (mg/L)**	0.25–1.43	<0.01	Elevated	(Ntinas *et al.* 2021)
	Pb (mg/L)	0.10–0.37	<0.042	Elevated	(Ntinas *et al.* 2021)
	Fe (mg/L)	189–271	0.28–1.68	Excess	(Bergstrand *et al.* 2020)
	Zn (mg/L)	20–27	0.01–0.72	Excess	(Bergstrand *et al.* 2020)
	Cu (mg/L)	1.94–2.48	0.05–0.13	Excess	(Bergstrand *et al.* 2020)
	Mn (mg/L)	1.00–2.07	0.07–0.57	High	(Bergstrand *et al.* 2020)
Solids and organic load	**TOC (mg/L)**	470–870	<631	Moderate–High	(Bergstrand *et al.* 2020)

Note: Values in mg/L are considered approximately equivalent to mg/kg for liquid digestate.

Reported heavy metal removal efficiencies for thermal hydrolysis (40% to 60% for Pb and Cd), electrocoagulation (50% to 80%), bioleaching (60% to 90%, albeit with slower kinetics), and UV/H_2O_2 processes (30% to 60%) indicate moderate effectiveness across methods (Tonanzi *et al*. 2021; Chen *et al*. 2023; Al-Qodah *et al*. 2024). In contrast, the CaO–Fenton system achieved higher removal efficiencies, reaching up to 92.9% for Pb and 82.5% for Cd, indicating performance comparable to or, in some cases, higher than that reported for alternative treatment technologies. Furthermore, the TD complied with EU Regulation 2019/1009 limits for heavy metals at Fenton reaction times ≥4 h (Chojnacka and Moustakas 2024; Senevirathne and Kaparaju 2025). Importantly, essential micronutrients (Zn, Cu, Mn, and Fe) were only partially reduced, suggesting that the process can achieve substantial contaminant reduction while retaining agronomically relevant nutrients.

Other AOP configurations may offer complementary advantages over conventional Fenton treatment. Photo-Fenton can enhance Fe^{3+} photoreduction to Fe^{2+} and hydroxyl-radical generation through irradiation, potentially improving oxidation efficiency, whereas electro-Fenton enables *in situ* H_2O_2 generation and Fe^{2+} regeneration. UV/H_2O_2 avoids the direct addition of iron and associated iron-sludge generation but requires an irradiation source and may be less favorable in complex wastewater matrices (Rueda-Márquez *et al.* 2020). Electro-Fenton has also demonstrated effective sludge disintegration compared with conventional treatment approaches (Feki *et al.* 2019). Thus, although the CaO–Fenton approach offers operational simplicity and combines stabilization with oxidative polishing, comparative studies considering treatment efficiency, energy demand, reagent consumption, sludge generation, and scalability are required to identify the most suitable AOP for digestate reuse.

Despite this favorable balance from an environmental perspective, the comparison with hydroponic NS ranges reveals substantial deviations in both macro- and micronutrient composition, indicating that TD does not provide a nutritionally balanced solution for direct hydroponic application. The K concentrations remained relatively stable during treatment (53 to 57 mg/L); however, these values are significantly below the recommended hydroponic range (120 to 200 mg/L) (Bergstrand *et al.* 2020). This indicates that stability does not equate to adequacy, and insufficient K supply may constrain plant growth, reduce yield, and impair nutrient uptake efficiency over extended cultivation periods (Chojnacka and Moustakas 2024).

In contrast, ammonium nitrogen (NH_4^+–N) concentrations increased during treatment (74 to 87 mg/L) and exceeded typical hydroponic levels (Bergstrand *et al.* 2020). The predominance of NH_4^+ over NO_3^- represents a critical imbalance, given that nitrate is the preferred nitrogen form in hydroponic systems (Pelayo Lind *et al.* 2021). Elevated NH_4^+ can adversely affect root development, alter rhizosphere pH, and limit the uptake of essential cations such as Ca^{2+} and K^+ (Carvalho *et al.* 2018; Pelayo Lind *et al.* 2021), thereby weakening overall nutrient balance despite sufficient TN availability.

Phosphate concentrations (250 to 390 mg/L) were also considerably higher than hydroponic requirements (Bergstrand *et al.* 2020), which may promote nutrient antagonisms and reduce micronutrient availability (Carvalho *et al.* 2018). This effect is further intensified by elevated levels of Fe, Zn, and Cu, which exceed typical hydroponic ranges and may disrupt nutrient uptake or induce phytotoxic responses. Although heavy metals such as Cd and Pb were substantially reduced during treatment, their concentrations remained above recommended hydroponic limits, indicating that additional optimization and validation are required prior to practical hydroponic implementation (Ntinas *et al.* 2021; Chojnacka and Moustakas 2024).

In long-term or recirculating hydroponic systems, residual dissolved organic matter (470 to 870 mg/L TOC) may further influence system performance by acting as a substrate for microbial growth (Mathe *et al*. 2024). This can lead to nutrient competition, pH fluctuations, and oxygen depletion (Bergstrand *et al.* 2020). In addition, surviving microorganisms may colonize plant roots and establish a rhizosphere microbiome that influences nutrient dynamics and overall system performance (Ntinas *et al.* 2021). While some of these microorganisms may be beneficial, the presence of opportunistic or biofilm-forming species cannot be excluded, potentially contributing to system instability over time. Accordingly, monitoring root-associated microbial communities with advanced molecular tools, in combination with microbiological assessment of plant tissues, is recommended to support long-term system stability and provide a more comprehensive evaluation of microbial quality in hydroponic applications.

Overall, the reduction in plant biomass relative to mineral nutrient control may be associated with nutrient imbalance rather than nutrient deficiency. The combined effects of NH_4^+ dominance, potassium deficiency, excess phosphorus, and elevated micronutrient levels disrupt optimal plant nutrition (Chojnacka and Moustakas 2024; Senevirathne and Kaparaju 2025). Similar trends have been reported in hydroponic systems using digestate or reclaimed water, where imbalances in nutrient composition, rather than absolute concentrations, limit plant performance (Bergstrand *et al.* 2020; Moncada *et al.* 2020; Ntinas *et al.* 2021; Santos *et al*. 2024). Therefore, the TD should be regarded as a nutrient-rich amendment that requires further conditioning and validation, rather than a complete hydroponic NSs. Based on these nutrient imbalances, the TD could be further conditioned before hydroponic application by promoting nitrification to convert excess NH_4^+ to NO_3^- and supplementing deficient nutrients, particularly K, to approach recommended hydroponic concentrations (Bergstrand *et al.* 2020; Pelayo Lind *et al.* 2021). Such targeted adjustment may improve vegetative growth while retaining the observed accumulation of antioxidant and bioactive compounds. However, the optimal supplementation strategy should be experimentally validated to determine whether these biochemical responses can be maintained after nutrient rebalancing.

From a commercial perspective, the observed growth–quality trade-off suggests that direct use of TD may reduce marketable biomass and overall productivity. Nevertheless, the enhanced accumulation of bioactive compounds may provide added value for quality-focused production. Therefore, nutrient rebalancing of TD should focus on improving biomass yield while preserving the enhanced bioactive compound content.

Multivariate Statistical Analysis

Correlation analysis of physicochemical and plant parameters

The Pearson correlation heatmap reveals strong and systematic relationships among physiochemical parameters, heavy metals, microbial indicators, and plant traits, indicating that the studied system is governed by tightly interconnected chemical, biological, and physiological processes, as indicated in Fig. 3. The magnitude and consistency of several correlation coefficients highlight the presence of common sources of contamination and shared response mechanisms.

Strong positive correlations were observed among conventional water quality parameters. COD and BOD were almost perfectly correlated ($r \approx 0.99$), indicating that organic pollution in the system is predominantly biodegradable. EC exhibited very high correlations with TS ($r > 0.90$), confirming that TS are major contributors to ionic strength. Similarly, nutrients such as PO_4–P and NH_4^+ showed strong positive correlations with COD and TOC ($r \approx 0.85$ to 0.95), suggesting that nutrient enrichment is closely linked to organic loading.

Heavy metals showed near-perfect positive correlations among themselves, for example, Pb–Cd, Cd–Zn, and Fe–Mn ($r \approx 0.95$ to 1.00), strongly suggesting a common origin and similar environmental behavior. These metals were also highly correlated with organic pollution indicators, such as COD and TOC ($r > 0.90$), indicating that organic matter likely enhances metal mobility and bioavailability through complexation and adsorption processes. This coupling between organic pollution and metal contamination is environmentally significant, as it increases the potential for metal uptake by plants.

Microbial indicators, including *Escherichia coli*, *Enterococcus faecalis*, and fecal coliforms, showed strong positive correlations with organic pollution parameters, particularly COD and BOD ($r \approx 0.90$ to 0.98). These relationships indicate that microbial contamination increases under conditions of high organic load, a characteristic of wastewater-impacted environments. In addition, microbial indicators were positively correlated with several heavy metals ($r > 0.85$), suggesting that chemical and biological contamination co-occur and may pose combined ecological and health risks.

In contrast, plant physiological traits exhibited predominantly negative correlations with pollution-related variables. Photosynthetic pigments, especially chlorophyll-*a* and chlorophyll-*b*, were inversely correlated with heavy metals and microbial indicators ($r \approx -0.80$ to -0.95), indicating that elevated pollution levels adversely affect photosynthetic efficiency. Likewise, plant growth parameters such as root length and plant height showed negative correlations with COD and metal concentrations ($r \approx -0.70$ to -0.90), reflecting growth inhibition under polluted conditions.

Antioxidant-related traits displayed a different pattern. Ascorbic acid, total phenolics, and total flavonoids were strongly and positively correlated with each other ($r > 0.95$), demonstrating the coordinated activation of antioxidant defense mechanisms.

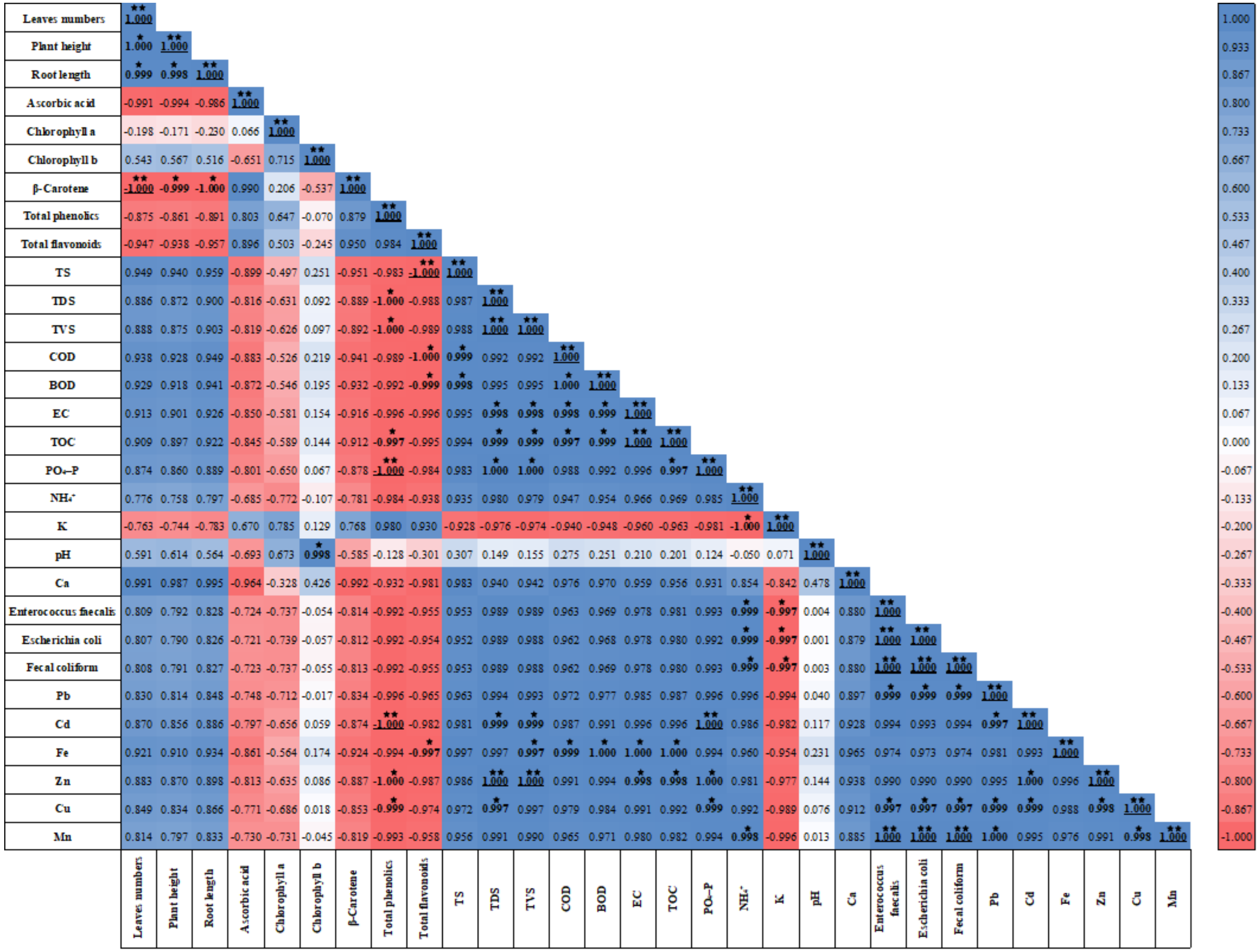


Fig. 3. Pearson correlation matrix among plant growth parameters, physiochemical parameters, microbial indicators, and heavy metals. Values represent Pearson correlation coefficients (r). **Bold** (*) and **bold and underline** (**) indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

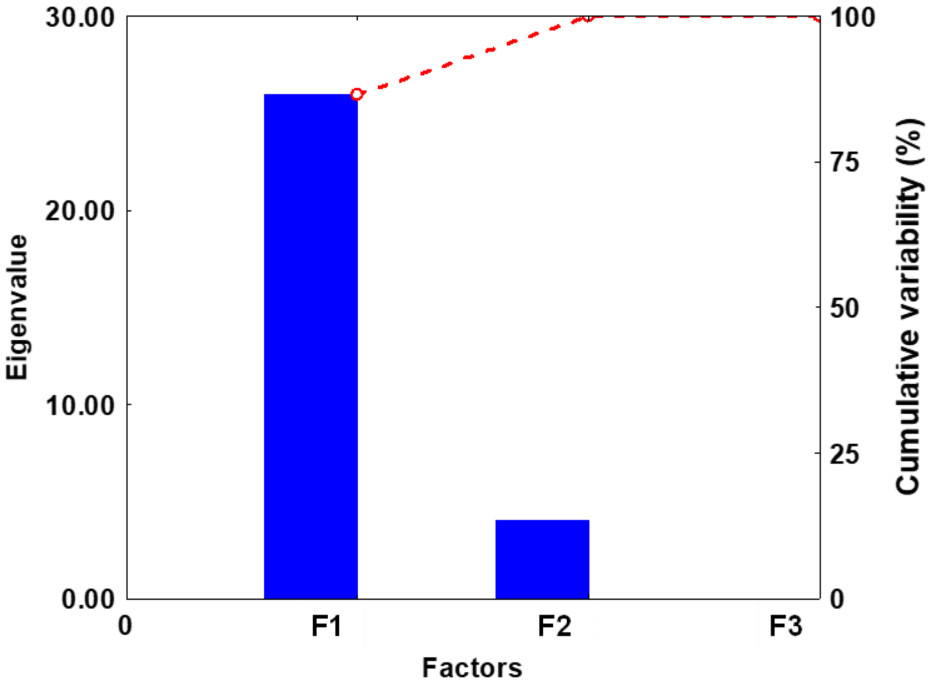
These compounds showed weaker or variable correlations with pollution indicators, suggesting that antioxidant responses may depend on stress intensity rather than pollutant concentration alone. Moderate stress conditions may stimulate antioxidant synthesis, whereas severe stress could suppress metabolic activity.
Overall, the Pearson correlation heatmap demonstrates a clear separation between a pollution-dominated domain, characterized by strong positive correlations among water quality parameters, heavy metals, microbial indicators, and a plant response domain, where physiological and biochemical traits are often negatively correlated with pollution variables. These patterns indicate that increasing environmental contamination exerts a strong negative influence on plant performance, while simultaneously triggering adaptive biochemical responses.

Principal component analysis of treatment performance

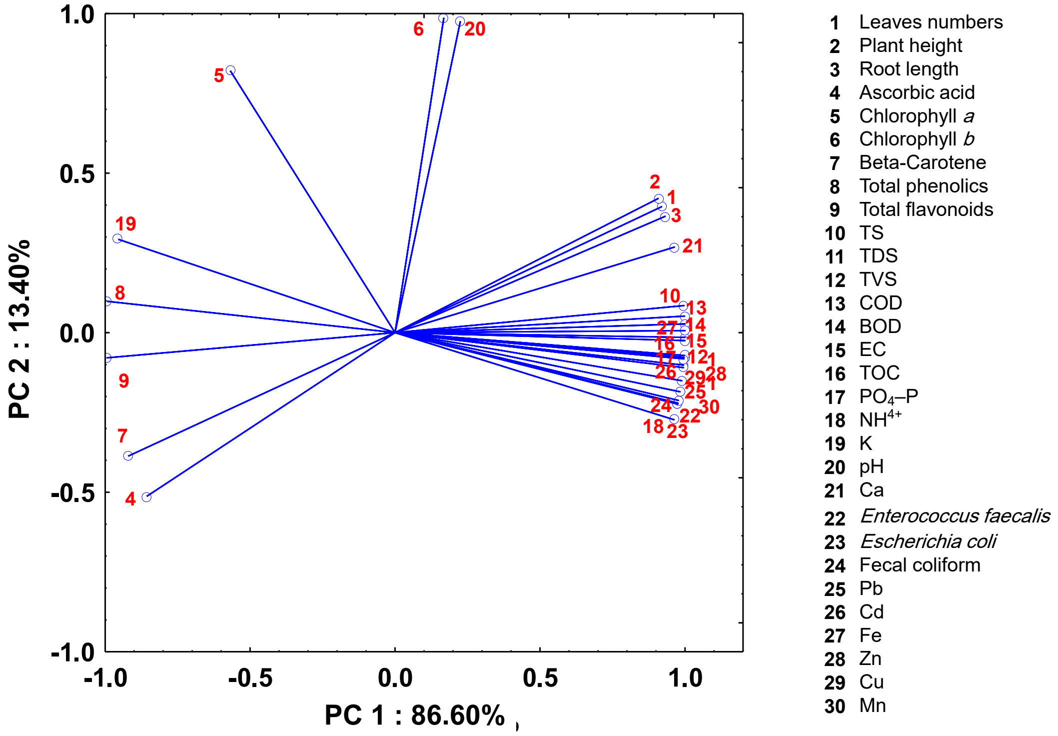
PCA was employed to elucidate the multivariate structure of the dataset and to identify the main factors governing the relationships among plant growth parameters, physiological and biochemical traits, water quality indicators, microbial indicators, and heavy metals, as shown in Fig. 4. Although 30 variables were included, the PCA was based on three treatment conditions; therefore, after mean-centering, the data matrix had a maximum rank of $n-1=2$, allowing only two non-zero principal components. Figure 4a presents the PCA scree plot and corresponding eigenvalues, with PC1 and PC2 exhibiting eigenvalues of approximately 25.97 and 4.03, respectively, while the remaining components had eigenvalues approaching zero. Accordingly, PC1 and PC2 explained 86.6% and 13.4% of the total variance, respectively, resulting in a cumulative explained variance of 100%. Figure 4b presents the PCA biplot, illustrating the relationships among the studied variables and treatment conditions.

The PC1 was strongly and positively associated with most water quality parameters, including TS, TDS, TVS, COD, BOD, EC, TOC, PO_4–P, NH_4^+, K, and Ca, as well as with heavy metals (Pb, Cd, Fe, Zn, Cu, and Mn) and microbial indicators (*Enterococcus faecalis*, *Escherichia coli*, and fecal coliforms). The close alignment of these variables along PC1 suggests that they are controlled by common processes and likely originate from similar anthropogenic sources. This pattern supports the interpretation of PC1 as a composite pollution gradient, integrating organic loading, nutrient enrichment, salinity, microbial contamination, and metal accumulation. The simultaneous loading of metals and organic parameters further implies that organic matter plays a critical role in enhancing metal mobility and bioavailability through complexation and adsorption mechanisms.

In contrast, PC2 was primarily associated with plant physiological and biochemical traits, including chlorophyll *a*, chlorophyll *b*, ascorbic acid, β-carotene, total phenolics, and total flavonoids. The separation of these variables from the pollution-dominated axis indicates that PC2 reflects internal plant responses rather than direct environmental contamination levels. The strong contribution of antioxidant compounds and photosynthetic pigments suggests that PC2 represents a physiological adjustment and defense axis, capturing plant strategies to cope with environmental stress through modulation of photosynthetic activity and activation of antioxidant systems. Plant growth parameters, such as number of leaves, plant height, and root length, occupied an intermediate position between PC1 and PC2. This spatial distribution indicates that both external environmental stressors and internal physiological regulation influence plant growth. The partial association with PC1 reflects the inhibitory effects of pollution on growth performance. At the same time, the alignment with PC2 suggests that physiological and biochemical adaptations may mitigate some of the negative impacts of environmental stress, allowing plants to maintain growth under adverse conditions.



a)



b)

Fig. 4. PCA (a) relationships between factor number and Eigenvalues, and (b) principal component biplot chart. The blue lines indicate the direction and magnitude of variable contributions, with numerical loading values (PC1, PC2) shown for each variable.

Overall, the PCA results reveal a clear functional separation between pollution-related variables and plant response traits. The strong dominance of the pollution gradient PC1 highlights water quality deterioration as the primary driver of system variability. In contrast, PC2 reflects variations associated with plant physiological and biochemical responses to environmental conditions. When interpreted together with the Pearson correlation analysis, the PCA serves as an exploratory multivariate tool that integrates chemical, biological, and physiological variables, providing insights into potential relationships between digestate properties and plant responses.

While the integrated CaO–Fenton treatment substantially improved physicochemical quality and eliminated detectable bacterial indicators, additional investigations are required to evaluate residual oxidants, pharmaceutical residues, ARGs, emerging contaminants, plant tissue contamination, and long-term hydroponic performance before food-production applications can be fully validated. Given the limited number of treatment conditions, the PCA findings should be interpreted primarily as an exploratory representation of multivariate patterns and differences among the Control, CaO (35%), and Fenton (6 h) treatments.

Comparison with Previous Studies

To contextualize the performance of the proposed treatment, the integrated CaO–Fenton approach was compared with recent sludge and digestate treatment strategies and alternative AOPs reported in the literature (Table 7). The comparison considers treatment performance, major limitations, and relevance to resource recovery and hydroponic reuse.

The treatment approaches investigated differ considerably in their mechanisms, treatment objectives, and resource-recovery potential. Fenton pretreatment primarily improves sludge solubilization and subsequent AD, although chemical consumption and process control remain important constraints (Akbay *et al.* 2022). Ca-based treatments provide sludge stabilization and microbial control but may create highly alkaline conditions and alter sludge and nutrient characteristics, requiring further conditioning before reuse (Mortadi *et al.* 2020; Pasoń and Stańczyk-Mazanek 2023; Wang *et al.* 2025b). Bioleaching combined with Fenton-like treatment can enhance the mobilization and removal of sludge-associated heavy metals, although acidic conditions and treatment time may limit its direct application (Qiu *et al.* 2021). Similarly, thermal hydrolysis coupled with AD can modify heavy-metal behavior and associated risks but requires considerable energy and may redistribute rather than remove metals (Chen *et al.* 2023). Alternative AOPs, including photo-Fenton, electro-Fenton, and UV/H_2O_2, offer complementary treatment options but differ in their operational requirements and applicability to complex digestate matrices (Rueda-Márquez *et al.* 2020).

From a resource-reuse perspective, aerobic polishing and controlled nitrification are more directly applicable to hydroponic systems because they improve liquid digestate quality, nitrogen form, and pH management (Pelayo Lind *et al.* 2021; Mathe *et al.* 2024). Compared with these previous approaches, the sequential CaO–Fenton process investigated in this study provides a multi-barrier treatment combining alkaline stabilization and oxidative polishing, simultaneously improving microbial control, reducing organic load, and decreasing heavy metal concentrations. However, the remaining nutrient imbalance and need for pH adjustment indicate that further conditioning is required before direct hydroponic application. Thus, the proposed process represents a promising treatment strategy for improving digestate quality and facilitating subsequent nutrient recovery and hydroponic reuse.

Table 7. Comparison of the Present CaO–Fenton Approach with Previous Sludge and Digestate Treatment Strategies

Treatment approach	Main reported outcome	Main limitation	Relevance to hydroponic/ resource reuse	References
Sequential CaO–Fenton (present study)	High microbial control, organic-load reduction, and heavy-metal immobilization	Chemical demand, pH adjustment, and nutrient imbalance	Directly evaluated for hydroponic lettuce cultivation; nutrient conditioning required	Present study
Fenton pretreatment	Improves sludge solubilization and subsequent AD	Chemical consumption and process control	Mainly a pretreatment	(Akbay *et al*. 2022)
Ca-based treatment	Improves sludge stabilization and microbial control	High alkalinity and changes in sludge/nutrient characteristics	Requires further conditioning	(Mortadi *et al*. 2020; Pasoń and Stańczyk-Mazanek 2023; Wang *et al*. 2025b)
Bioleaching + Fenton-like	Enhances removal/mobilization of sludge-associated metals	Requires acidic conditions and control of H_2O_2 dosage and reaction conditions	Requires post-treatment	Qiu *et al.* 2021
Thermal hydrolysis + AD	Alters heavy metal behavior and associated risks	Energy demand; metals may be redistributed	Primarily relevant to sludge treatment and resource recovery	Chen *et al.* 2023
Aerobic polishing	Improves liquid digestate for hydroponic fertilizer preparation	Requires additional aeration and biological-process control	Directly relevant to hydroponics nutrient reuse	Mathe *et al.* 2024
Controlled nitrification	Improves N form and pH management	Requires biological-process control	Improves digestate suitability for hydroponic application	Pelayo Lind *et al.* 2021
Photo-Fenton	Light-assisted Fe^{2+} regeneration enhances •OH generation and contaminant oxidation	Requires irradiation and suitable optical conditions; efficiency can be matrix-dependent	Potential polishing option, but requires evaluation for digestate matrices	Rueda-Márquez *et al.* 2020
Electro-Fenton	Enables in situ H_2O_2 generation and electrochemical regeneration of Fe^{2+}	Electrical-energy demand, electrode requirements, and greater process complexity	Potential advanced polishing option; scale-up and cost require evaluation	Rueda-Márquez *et al.* 2020
UV/H_2O_2	Generates •OH through H_2O_2 photolysis and can provide effective oxidation/ disinfection	UV-energy requirement and reduced performance in highly absorbing/turbid matrices	Potentially suitable after adequate clarification	Rueda-Márquez *et al.* 2020

Sustainability, Economic, Environmental, and Scale-Up Implications of CaO–Fenton Treatment

Treatment sustainability and resource-recovery implications

The CaO treatment should be considered primarily as a hygienisation and conditioning step rather than a standalone fertilization strategy. While high-pH conditions are effective for pathogen inactivation, the treatment is conducted under controlled conditions and the effluent is subsequently neutralized to near-neutral pH (6.5 to 7.5) prior to hydroponic application, thereby avoiding direct exposure of plant roots to alkaline conditions (Senevirathne and Kaparaju 2025). More generally, appropriate processing of digestate is necessary to improve its agronomic value while minimizing potential environmental and safety concerns associated with direct application (Chojnacka and Moustakas 2024; Senevirathne and Kaparaju 2025).

However, this approach involves several trade-offs that may influence the overall sustainability and efficiency of digestate reuse. From a nutrient perspective, reductions in ammonium may be beneficial by limiting toxicity and volatilization losses; nevertheless, the concurrent removal or immobilization of essential nutrients such as phosphorus and potassium, potentially through precipitation and association with the generated solids, can reduce plant-available nutrient fractions and overall fertilizer value (Carvalho *et al.* 2018; Bergstrand *et al.* 2020). These precipitated forms represent a potential nutrient sink but could be recovered and reused as slow-release amendments to improve nutrient efficiency (Chojnacka and Moustakas 2024). In addition, high CaO dosages are associated with increased chemical consumption, generation of calcium-rich residual solids requiring appropriate management, and ammonia volatilization under alkaline conditions (Senevirathne and Kaparaju 2025). These factors highlight the need to balance disinfection efficiency with nutrient preservation and to integrate nutrient recovery or supplementation strategies to maintain agronomic performance and long-term sustainability (Pelayo Lind *et al.* 2021).

Compared with other lime-based approaches, CaO provides rapid alkalization through hydration to $Ca(OH)_2$, thereby facilitating pathogen inactivation, coagulation, and precipitation processes (Zheng *et al.* 2021; Zhang *et al.* 2023; Wang *et al.* 2024). However, lime-based treatment alone may alter nutrient availability and may not achieve the degree of microbial and physicochemical polishing required for subsequent reuse (Bergstrand *et al.* 2020; Pelayo Lind *et al.* 2021; Senevirathne and Kaparaju 2025). In the present study, the subsequent Fenton step provided additional oxidative polishing and complete inactivation of the monitored microbial indicators, thereby overcoming some limitations of CaO treatment alone. Nevertheless, nutrient rebalancing may still be required before hydroponic application because the sequential treatment altered N, P, K, and micronutrient concentrations.

Economic implications

From an economic perspective, assessment of the proposed treatment should consider the complete CaO–Fenton treatment train rather than CaO consumption alone. A preliminary operating-cost assessment was therefore conducted for the maximum stabilization condition (35% w/v CaO), considering CaO, $FeSO_4 \cdot 7H_2O$, H_2O_2, pH adjustment, electricity, labor and maintenance, and sludge management (Table 8). Unit-cost assumptions were informed by published economic assessments of Fenton-based and wastewater treatment processes (Minella *et al.* 2018; Dobrosz-Gómez *et al.* 2024), supplemented by scenario-based assumptions where directly applicable literature values were unavailable. Based on these assumptions, the estimated gross operating expenditure (OPEX) was 78.18 USD/m³, with CaO representing the largest cost component (42.00 USD/m³; 53.7%), followed by H_2O_2 (19.72 USD/m³; 25.2%) and

$FeSO_4 \cdot 7H_2O$ (5.70 USD/m³; 7.3%). Potential resource-recovery credits associated with reclaimed water and NPK nutrients were assumed at 14.25 USD/m³ for the preliminary scenario, reducing the preliminary net OPEX to 63.93 USD/m³. Resource recovery and digestate valorization may therefore partially offset the costs associated with treatment and residual management (Wang and Lee 2021; Chojnacka and Moustakas 2024). Nevertheless, the present assessment is based on laboratory-scale reagent consumption, literature-derived unit costs, and scenario assumptions. Further optimization of CaO and Fenton reagent dosages, together with pilot-scale mass and energy balances and site-specific costs, is required to establish full-scale economic feasibility (Chojnacka and Moustakas 2024; Mancuso *et al.* 2024).

Table 8. Preliminary Operating-cost Assessment of the Sequential CaO–Fenton Treatment Process at 35% (w/v) CaO

Cost Component	Consumption	Unit cost (USD/unit)	Cost (USD/m³)
CaO (35% w/v)	350 kg/m³	0.120/kg	42.00
$FeSO_4 \cdot 7H_2O$	1.39 kg/m³	4.10/kg	5.70
H_2O_2 (30% w/w)	5.11 L/m³	3.86/L	19.72
pH adjustment (H_2SO_4/NaOH)	~18.50 kg/m³	0.150/kg	2.78
Electricity	8.50 kWh/m³	0.100/kWh	0.85
Labor and maintenance	—	—	3.50
Sludge management	~110 kg/m³	0.033/kg	3.63
Gross OPEX	—	—	78.18
Resource-recovery credits	Water and NPK recovery	—	−14.25
Net OPEX	—	—	63.93

Note: Reagent consumption was scaled from the 1.0-L bench-scale protocol to 1 m³ of digestate. Costs may vary with treatment scale, location, market conditions, reagent procurement, and process optimization. Therefore, the estimated OPEX should be considered preliminary and subject to validation under pilot- and full-scale operating conditions.

Residual-solid management and valorization

Residual-solid management represents another important consideration for practical implementation. The sequential CaO–Fenton process is expected to generate distinct solid residual streams. CaO treatment may produce a Ca-rich precipitated fraction through alkaline precipitation, coagulation, and co-precipitation, whereas Fenton treatment followed by neutralization may generate Fe-rich sludge (Mortadi *et al.* 2020; Li *et al.* 2025). Importantly, reductions in metals observed in the treated liquid should not necessarily be interpreted as destruction of these elements, as they may partly represent transfer from the liquid phase to the separated solids. Consequently, the resulting residual fractions require appropriate characterization and management. Rather than considering these residuals solely as wastes requiring disposal, their potential valorization should be investigated in accordance with circular approaches to sewage sludge and biosolids management (Elgarahy *et al.* 2024; Zheng *et al.* 2024). Depending on their composition, contaminant concentrations, stability, leaching behavior, and regulatory compliance, potential pathways may include evaluation of the Ca-rich fraction as a liming or mineral material and recovery or reuse of iron from the Fe-rich sludge. However, these valorization pathways were not experimentally investigated in the present study; therefore, detailed physicochemical characterization, leaching assessment, and environmental-safety evaluation are required before beneficial reuse can be recommended (Zhang *et al.* 2023; Kacprzak *et al.* 2025).

Life-cycle and broader environmental implications

From a life-cycle perspective, the potential environmental benefits of digestate valorization should be balanced against the upstream and operational burdens associated with chemical production and transportation, energy consumption, pH adjustment, solid-liquid separation, and residual-solid management. Conversely, beneficial reuse of TD may provide potential environmental benefits through nutrient recovery, reduced demand for conventional fertilizers, avoided waste disposal, treated-water reuse, and improved water-use efficiency in hydroponic cultivation (Chojnacka and Moustakas 2024; Rajendran *et al*. 2024). Therefore, treatment efficiency alone cannot establish the overall environmental sustainability of the proposed process. Since formal life-cycle and techno-economic assessments were not conducted in the present study, future studies should quantify chemical and energy inputs, greenhouse-gas emissions, transportation, residual-solid management, nutrient-substitution credits, water-reuse benefits, and avoided waste-management burdens to determine whether resource-recovery benefits offset the additional treatment burdens (Zheng *et al.* 2024; Said *et al.* 2026).

Scale-up and regulatory implications

From a scalability perspective, the sequential CaO–Fenton process demonstrated technical potential for treatment of the high-strength digestate investigated in this study. The CaO dosages of 25 to 35% (w/v) were evaluated to establish a dose-response relationship and identify conditions providing effective microbial reduction and physicochemical stabilization. However, the relatively high CaO requirement represents an important constraint for scale-up because increasing chemical dosage also increases reagent consumption and residual-solid production. Therefore, the laboratory-scale condition providing the highest treatment performance should not necessarily be interpreted as the economically optimal full-scale condition. Pilot-scale optimization should determine the minimum CaO and Fenton reagent dosages required to achieve the target microbiological and physicochemical quality while minimizing chemical consumption, energy requirements, and residual-solid generation.

For practical implementation, further studies should evaluate the process under continuous or semi-continuous pilot-scale operation, including optimization of CaO and Fenton reagent dosages, mixing, pH control, reaction time, solid-liquid separation, energy consumption, sludge production, and long-term process reliability. Regulatory compliance should also be established before TD is recommended for commercial hydroponic application. Depending on the jurisdiction and intended use, regulatory assessment may need to consider feedstock origin, microbiological safety, potentially toxic elements, nutrient composition, organic contaminants, stability, and other relevant product- or reuse-quality requirements. Therefore, the reductions in microbial indicators and metals demonstrated in the present study should be interpreted as evidence of treatment effectiveness rather than confirmation of regulatory compliance for unrestricted commercial hydroponic reuse.

Limitations and Future Work

The use of a single laboratory-generated digestate, one crop species (lettuce), a single growth cycle, and a non-recirculating DWC system limits the broader generalization of the findings. In addition, plant nutrient uptake, heavy-metal accumulation in edible plant tissues, physiological stress markers, microbial regrowth and pathogen occurrence over time, residual H_2O_2, phytotoxicity, DO, sensory quality, taste, consumer acceptability, and emerging contaminants, including pharmaceuticals, microplastics, and ARGs, were not evaluated. Future studies should address these limitations using digestates obtained from full-scale anaerobic wastewater treatment facilities, diverse crop species, multiple growth cycles, and recirculating pilot-scale systems. Plant tissue analysis should assess actual nutrient uptake patterns and heavy-metal bioaccumulation to confirm food safety, while supplemented TD with targeted nutrient adjustment should be evaluated to improve plant growth while retaining bioactive compound accumulation. Physiological stress biomarkers (e.g., proline, MDA, and antioxidant enzyme activities) should be measured to validate the proposed stress-response hypothesis and distinguish stress-related responses from nutritional benefits. Residual H_2O_2 quenching, real-time DO monitoring, oxidation–reduction potential (ORP) monitoring before and after Fenton reagent addition, and systematic pH monitoring across all CaO dosages should also be evaluated under pilot-scale conditions to support process control and scale-up assessment. Characterization of the Ca-rich precipitate should also be performed to confirm $CaCO_3$ formation following CaO hydration and carbonation. Time-series monitoring of microbial regrowth and comprehensive analysis of emerging contaminants should also be considered.

CONCLUSIONS

1. The integrated CaO–Fenton treatment of digestate demonstrated high effectiveness as a dual-stage treatment system for improving the physicochemical and microbiological quality of anaerobic sewage digestate and enhancing its potential for hydroponic nutrient recovery and reuse.
2. High-dose CaO (≥25%) resulted in >98% pathogen removal, >65% salinity reduction, >60% TOC removal, and >80% NH_4^+ reduction, while immobilizing more than 90% of hazardous metals such as Pb, Cd, and Mn. Subsequent Fenton oxidation achieved complete inactivation of residual microbial indicators and further reduced TVS (100%), TS (87.7%), COD (52.5%), and BOD (52.4%), while largely preserving essential macronutrients.
3. Hydroponic application to lettuce (*Lactuca sativa* L.) revealed a trade-off between biomass production and quality, characterized by reduced plant growth, but significant increases in vitamin C, phenolics, flavonoids, and β-carotene.
4. One-way ANOVA followed by Tukey's HSD indicated that both CaO stabilization and Fenton oxidation had statistically significant effects ($P \leq 0.05$) on the physicochemical and microbial properties of the digestate, highlighting the effectiveness of the CaO–Fenton approach in improving digestate quality and its potential for nutrient recovery and reuse in hydroponic systems.

ACKNOWLEDGMENTS

This work was funded by the Deanship of Graduate Studies and Scientific Research at Jouf University under grant No. (DGSSR-2025-02-01313).

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing and improving the clarity and organization of the text. The authors reviewed and edited the content and take full responsibility for the scientific accuracy and integrity of the work.

REFERENCES CITED

Adetunji, A. I., and Olaniran, A. O. (2021). "Treatment of industrial oily wastewater by advanced technologies: A review," *Applied Water Science* 11(6), 98. https://doi.org/10.1007/s13201-021-01430-4

Ahmed, D. S., Nasef, B. M., and Said, N. (2025). "Application of bokashi for enhancing anaerobic digestion and sustainable biogas production: recurrent neural network (RNN) modeling implementation," *Environmental Science and Pollution Research* 32(51), 29037-29053. https://doi.org/10.1007/s11356-025-37176-8

Akbay, H. E. G., Dizge, N., and Kumbur, H. (2022). "Evaluation of electro-oxidation and Fenton pretreatments on industrial fruit waste and municipal sewage sludge to enhance biogas production by anaerobic co-digestion," *Journal of Environmental Management* 319, article 115711. https://doi.org/10.1016/j.jenvman.2022.115711

Al-Qodah, Z., Al-Shannag, M., Hudaib, B., and Bani-Salameh, W. (2024). "Enhancement of dairy wastewater treatment efficiency in batch chemical-assisted solar-powered electrocoagulation-adsorption system," *Case Studies in Chemical and Environmental Engineering* 9, article 100760. https://doi.org/10.1016/j.cscee.2024.100760

Almomani, F., Bhosale, R. R., Khraisheh, M. A. M., and Shawaqfah, M. (2019). "Enhancement of biogas production from agricultural wastes via pre-treatment with advanced oxidation processes," *Fuel* 253, 964-974. https://doi.org/10.1016/j.fuel.2019.05.057

Alrowais, R., Abdel-Daiem, M. M., Nasef, B. M., Metwally, A. A., and Said, N. (2025). "Sustainable management of wastewater sludge through co-digestion, mechanical pretreatment and recurrent neural network (RNN) modeling," *Sustainability* 17(20), article su17209323. https://doi.org/10.3390/su17209323

Alrowais, R., Said, N., Mahmoud-Aly, M., Helmi, A. M., Nasef, B. M., and Abdel daiem, M. M. (2024). "Influences of straw alkaline pretreatment on biogas production and digestate characteristics: artificial neural network and multivariate statistical techniques," *Environmental Science and Pollution Research* 31(9), 13638-13655. https://doi.org/10.1007/s11356-024-31945-7

Alrowais, R., Yousef, R. S., Ahmed, O. konsowa, Mahmoud-Aly, M., Abdel daiem, M. M., and Said, N. (2023). "Enhanced detoxification methods for the safe reuse of treated olive mill wastewater in irrigation," *Environmental Sciences Europe* 35(1), article 95. https://doi.org/10.1186/s12302-023-00797-2

APHA. (2005). "Standard methods for the examination of water and wastewater," *American Public Health Association (APHA)*, Washington, DC, USA, 21.

Ayoub, M. (2022). "Fenton process for the treatment of wastewater ef fl uent from the edible oil industry," *Water Science and Technology* 86(6), 1388-1401. https://doi.org/10.2166/wst.2022.283

Azhdarpoor, A., Hoseini, R., and Dehghani, M. (2018). "Investigation of combined fenton / bioleaching and bio-acidification / fenton-like processes in the removal of sludge-borne metals," *HealthScope* 7(4), article e61211. https://doi.org/10.5812/jhealthscope.61211.Research

Bao, Y., Yan, Q., Ji, J., Qiu, B., Zhang, J., and Xing, M. (2021). "Graphene-based photo-fenton catalysts for pollutant control," *Transactions of Tianjin University* 27(2), 110-126. https://doi.org/10.1007/s12209-020-00276-2

Bergstrand, K.-J., Asp, H., and Hultberg, M. (2020). "Utilizing anaerobic digestates as nutrient solutions in hydroponic production systems," *Sustainability* 12(23), article 10076. https://doi.org/10.3390/su122310076

Bhatia, T., and Sindhu, S. S. (2024). "Sustainable management of organic agricultural wastes: contributions in nutrients availability, pollution mitigation and crop production," *Discover Agriculture* 2(1), article 130. https://doi.org/10.1007/s44279-024-00147-7

Blumfelde, M., Dēliņa, A., Puzane, K., Bartkevičs, V., and Muter, O. (2024). "Disinfection of activated sludge by combination of the fenton reagent and physical treatment," *Agronomy* 14(12), article 14123037. https://doi.org/10.3390/agronomy14123037

Çakmakçi, R., Çakmakçi, S., and Çakmakçi, M. F. (2025). "Principles of environmentally sustainable agriculture for building resilient and resource-efficient food systems," *Turkish Journal of Biology* 49(5), 550-584. https://doi.org/10.55730/1300-0152.2764

Carvalho, R. da S. C., Bastos, R. G., and Souza, C. F. (2018). "Influence of the use of wastewater on nutrient absorption and production of lettuce grown in a hydroponic system," *Agricultural Water Management* 203, 311-321. https://doi.org/10.1016/j.agwat.2018.03.028

Chatterjee, A., Ghosh, P., Winkler, B., Debnath, S., Cichocki, J., Trenkner, M., Vanicela, B., Riethmueller, C., Walz, M., and Chandra, S. (2025). "Demystifying the integration of hydroponics cultivation system reinforcing bioeconomy and sustainable agricultural growth," *Scientia Horticulturae* 341, article 113973. https://doi.org/10.1016/j.scienta.2025.113973

Chaves, B., Salomez, M., Sambusiti, C., Thévenin, N., Vieublé-Gonod, L., and Richard-Molard, C. (2025). "Digestate as a source of biostimulants for winter rye growth," *Bioresource Technology Reports* 29, article 102057. https://doi.org/10.1016/j.biteb.2025.102057

Chen, Q., Zhao, B., Zhang, Y., Zhu, F., Wang, H., Wang, J., and Fu, X. (2023). "The function of 'Cambi® thermal hydrolysis+ anaerobic digestion' on heavy metal behavior and risks in a full-scale sludge treatment plant based on four seasons investigation," *Journal of Hazardous Materials* 445, article 130579. https://doi.org/10.1016/j.jhazmat.2022.130579

Cheng, L., Lu, Z., Liu, J., Liu, J., Zhao, Y., Ni, Z., Lin, Q., Zhu, R., Chen, X., and Lin, W. (2024). "Novel heterogeneous Fenton catalysts for promoting carbon iron electron transfer by one-step hydrothermal synthesization," *Journal of Colloid and Interface Science* 656, 597-608. https://doi.org/10.1016/j.jcis.2023.11.150

Chojnacka, K., and Moustakas, K. (2024). "Anaerobic digestate management for carbon neutrality and fertilizer use: A review of current practices and future opportunities," *Biomass and Bioenergy* 180, article 106991. https://doi.org/10.1016/j.biombioe.2023.106991

Dobrosz-Gómez, I., Salazar-Sogamoso, L.-M., Castaño-Sánchez, J.-C., Salazar-López, D.-O., and Gómez-García, M.-Á. (2024). "Environmental and economic evaluation of the sequential combination of coagulation–flocculation with different electro-fenton-based configurations for the treatment of raw textile wastewater," *Water*, 16(15), article 2154. https://doi.org/10.3390/w16152154

Elgarahy, A. M., Eloffy, M. G., Priya, A. K., Yogeshwaran, V., Yang, Z., Elwakeel, K. Z., and Lopez-Maldonado, E. A. (2024). "Biosolids management and utilizations: A review," *Journal of Cleaner Production* 451, article 141974. https://doi.org/10.1016/j.jclepro.2024.141974

Feki, E., Sayadi, S., Loukil, S., Dhouib, A., and Khoufi, S. (2019). "Comparison between thermo-alkaline and electro-Fenton disintegration effect on waste activated sludge anaerobic digestion," *BioMed Research International* 2019(1), article 2496905. https://doi.org/10.1155/2019/2496905

Feng, H., Zhang, Y., Fang, K., Hu, Y., and Cheng, H. (2025). "Investigating the toxic impacts of various arsenic compounds on hydroponics lettuce (Lactuca sativa L.): Biomass, arsenic accumulation and species distribution," *Water, Air, and Soil Pollution* 236(5), article 287. https://doi.org/10.1007/s11270-025-07933-z

Ferri, E. N., and Bolelli, L. (2025). "Wastewater remediation treatments aimed at water reuse: Recent outcomes from pilot- and full-scale tests," *Applied Sciences* 15(5), article app15052448. https://doi.org/10.3390/app15052448

Fiorentino, A., Manoli, K., Karaolia, P., Proto, A., Mantzavinos, D., and Fatta-Kassinos, D. (2025). "Eco-friendly and cost-effective Fe-IDS Fenton processes for the inactivation of antibiotic-resistant bacteria in MBR effluents," *Journal of Environmental Chemical Engineering* 13(5), article 2118678. https://doi.org/10.1016/j.jece.2025.118678

Floriano da Silva, L. C., Vinhas Ítavo, L. C., Martins Santos, R., Brandão Ferreira Ítavo, C. C., Zirondi Longhini, V., Menezes Dias, A., dos Santos Difante, G., Moreira Arcanjo, A. H., Santos Santana, J. C., Gurgel, A. L. C., and de Oliveira Scarpino van Cleef, F. (2023). "Urban sewage sludge stabilization by alkalization-composting-vermicomposting process: Crop-livestock residue use," *Plos One* 18(9), article 2e0289362. https://doi.org/10.1371/journal.pone.0289362

Franchitti, E., Pedullà, M., Madsen, A. M., and Traversi, D. (2024). "Effect of anaerobic digestion on pathogens and antimicrobial resistance in the sewage sludge," *Environment International* 191, article 2108998. https://doi.org/10.1016/j.envint.2024.108998

Garrido-Cardenas, J. A., Esteban-García, B., Agüera, A., Sánchez-Pérez, J. A., and Manzano-Agugliaro, F. (2020). "Wastewater treatment by advanced oxidation process and their worldwide research trends," *International Journal of Environmental Research and Public Health* 17(1), article 2170. https://doi.org/10.3390/ijerph17010170

Gholikandi, G. B., Nili Ardakani, M., and Moradi, F. (2018). "Fered-Fenton technology for efficient waste-activated sludge stabilization: Determination of the main specifications and optimization of the energy consumption," *Journal of Environmental Chemical Engineering* 6(1), 1546-1557. https://doi.org/10.1016/j.jece.2018.01.071

Guan, D., Zhao, J., Wang, Y., Fu, Z., Zhang, D., Zhang, H., Xie, J., Sun, Y., Zhu, J., and Wang, D. (2024). "A critical review on sustainable management and resource utilization of digestate," *Process Safety and Environmental Protection*, 183, 339-354. https://doi.org/10.1016/j.psep.2024.01.029

Hammam, R. A., Shaltout, Fatma Mohamed Hassan, Mansour, S. A. M., and Hassanain, A. M. (2022). "Chemical Treatment of industrial wastewater using fenton reaction," *Engineering Research Journal* 173, C16-C27. https://doi.org/10.21608/erj.2022.223166

Ingrao, C., Strippoli, R., Lagioia, G., and Huisingh, D. (2023). "Water scarcity in agriculture: An overview of causes, impacts and approaches for reducing the risks," *Heliyon* 9(8), article 2e18507. https://doi.org/10.1016/j.heliyon.2023.e18507

Kacprzak, M., Baran, J., and Fijalkowski, K. (2025). "Emerging sewage sludge treatment technologies for land carbon sequestration: A comprehensive review," *Journal of Material Cycles and Waste Management* 27(5), 2866-2886. https://doi.org/10.1007/s10163-025-02369-3

Kazimierowicz, J., Dębowski, M., and Zieliński, M. (2023). "Effect of pharmaceutical sludge pre-treatment with Fenton/Fenton-like reagents on toxicity and anaerobic digestion efficiency," *International Journal of Environmental Research and Public Health* 20(1), article ijerph200110271.

https://doi.org/10.3390/ijerph20010271
Kechasov, D., Verheul, M. J., Paponov, M., Panosyan, A., and Paponov, I. A. (2021). "Organic waste-based fertilizer in hydroponics increases tomato fruit size but reduces fruit quality," *Frontiers in Plant Science* 12, article 680030. https://doi.org/10.3389/fpls.2021.680030
Khadka, D., and Pathak, K. (2023). "Spectrophotometric determination of total vitamin c content in different fruits and vegetables consumed in tansen, palpa," *Tribhuvan Journal* 1(1), article 2079. https://doi.org/10.3126/tribj.v1i1.53497
Kwon, G., Kang, J., Nam, J.-H., Kim, Y.-O., and Jahng, D. (2021). "Struvite production from anaerobic digestate of piggery wastewater using ferronickel slag as a magnesium source," *Environmental Technology* 42(3), 429-443. https://doi.org/10.1080/09593330.2019.1631390
Lee, J., Weon, S., Lee, S. S. S., Yun, E., Chung, M. W., Kim, C., Wang, H., and Fortner, J. D. (2025). "Microwave-enhanced catalytic degradation of organic compounds with silica-coated iron oxide nanocrystals *via* Fenton-like reaction pathway," *NPJ Clean Water* 8(1), article 25. https://doi.org/10.1038/s41545-025-00449-3
Li, G.-Q., Wang, C.-Y., Chen, T., Zhang, Y.-Y., Dou, M., and Wan, H.-Y. (2023). "Inactivation of Escherichia coli and Enterococcus faecalis by sulfate radical and hydroxyl radical using Fe(II) based advanced oxidation systems," *Desalination and Water Treatment* 284, 205-215. https://doi.org/10.5004/dwt.2023.29219
Li, X., Li, L., Wang, Z., Mei, W., Gao, Q., Wei, L., Zhao, Q., and Jiang, J. (2025). "Revisiting Fenton process for enhanced dewatering of waste activated sludge: Transformation in spatial distribution and chemical speciation of phosphorus," *Chemical Engineering Journal* 525, article 170109. https://doi.org/10.1016/j.cej.2025.170109
López, A., Rodríguez-Chueca, J., Mosteo, R., Gómez, J., and Ormad, M. P. (2020). "Microbiological quality of sewage sludge after digestion treatment: A pilot scale case of study," *Journal of Cleaner Production* 254, article 120101. https://doi.org/10.1016/j.jclepro.2020.120101
Mancuso, G., Habchi, S., Maraldi, M., Valenti, F., and El Bari, H. (2024). "Comprehensive review of technologies for separate digestate treatment and agricultural valorisation within circular and green economy," *Bioresource Technology* 409, article 131252. https://doi.org/10.1016/j.biortech.2024.131252
Masindi, V., Foteinis, S., Renforth, P., and Chatzisymeon, E. (2023). "Wastewater treatment for carbon dioxide removal," *ACS Omega* 8(43), 40251-40259. https://doi.org/10.1021/acsomega.3c04231
Mathe, L. O. J., Ramsumer, S., Brink, H. G., and Nicol, W. (2024). "Aerobic polishing of liquid digestate for preparation of hydroponic fertiliser," *Sustainability* 16(10), article 4077. https://doi.org/10.3390/su16104077
Medina, M. B. (2011). "Simple and rapid method for the analysis of phenolic compounds in beverages and grains," *Journal of Agricultural and Food Chemistry* 59(5), 1565-1571. https://doi.org/10.1021/jf103711c
Minella, M., De Bellis, N., Gallo, A., Giagnorio, M., Minero, C., Bertinetti, S., Sethi, R., Tiraferri, A., and Vione, D. (2018). "Coupling of nanofiltration and thermal Fenton reaction for the abatement of carbamazepine in wastewater," *ACS Omega* 3(8), article 9407. https://doi.org/10.1021/acsomega.8b01055
Moncada, A., Vetrano, F., and Miceli, A. (2020). "Alleviation of salt stress by plant growth-promoting bacteria in hydroponic leaf lettuce," *Agronomy* 10(10), article 1523. https://doi.org/10.3390/agronomy10101523
Mortadi, A., Elmelouky, A., Chahid, E. G., Essajai, R., Naserllah, H., Chahbi, M., Ghyati, N., Zaim, S., Cherkaoui, O., and El Moznine, R. (2020). "Effects of

calcium oxide on the surface properties of industrial sludge by analyzing rheological and electrical properties," *Journal of Environmental Chemical Engineering* 8(3), article 103764. https://doi.org/10.1016/j.jece.2020.103764

Nagargoje, D. R., Mahajan, M., Patole S., and Zade D.B (2025). "Research and reviews in material and chemical science," *Bhumi Publishing* India, https://doi.org/10.5281/zenodo.18109019

Ntinas, G. K., Bantis, F., Koukounaras, A., and Kougias, P. G. (2021). "Exploitation of liquid digestate as the sole nutrient source for floating hydroponic cultivation of baby lettuce (Lactuca sativa) in greenhouses," *Energies* 14(21), article 7199. https://doi.org/10.3390/en14217199

Pasoń, Ł., and Stańczyk-Mazanek, E. (2023). "Effect of sewage sludge liming on drug-resistant bacteria of the Enterobacteriaceae family," *Desalination and Water Treatment* 301, 135-142. https://doi.org/10.5004/dwt.2023.29591

Pelayo Lind, O., Hultberg, M., Bergstrand, K.-J., Larsson-Jönsson, H., Caspersen, S., and Asp, H. (2021). "Biogas digestate in vegetable hydroponic production: pH dynamics and pH management by controlled nitrification," *Waste and Biomass Valorization* 12(1), 123-133. https://doi.org/10.1007/s12649-020-00965-y

Qiu, C., Xie, S., Liu, N., Meng, K., Wang, C., Wang, D., and Wang, S. (2021). "Removal behavior and chemical speciation distributions of heavy metals in sewage sludge during bioleaching and combined bioleaching/Fenton-like processes," *Scientific Reports* 11(1), article 14879. https://doi.org/10.1038/s41598-021-94216-2

Rajendran, S., Domalachenpa, T., Arora, H., Li, P., Sharma, A., and Rajauria, G. (2024). "Hydroponics: Exploring innovative sustainable technologies and applications across crop production, with Emphasis on potato mini-tuber cultivation," *Heliyon* 10(5), article e26823. https://doi.org/10.1016/j.heliyon.2024.e26823

Reza, M., Tehrani, F., Sayedbarzani, M., and Bagheri, H. (2025). "Evaluating treated sewage sludge as a sustainable fertilizer for enhanced tomato growth," *Journal of Plant Biology and Crop Research* 9(1), article 117.

Rueda-Márquez, J. J., Levchuk, I., Manzano, M., and Sillanpää, M. (2020). "Toxicity Reduction of Industrial and Municipal Wastewater by Advanced Oxidation Processes (Photo-Fenton, UVC/H_2O_2, Electro-Fenton and Galvanic Fenton): A Review," *Catalysts* 10(6), article ctal10060612. https://doi.org/10.3390/catal10060612

Said, N., Abdel-Daiem, M. M., Almoshawah, Y. A., Metwally, A. A., and Mostafa, N. (2026). "Environmental and energy performance of rice straw–based energy pathways in Egypt: Life cycle assessment and supply chain optimization," *Sustainability* 18(9), article 4426. DOI 10.3390/su18094426

Salerno, L., Modica, M. N., Pittalà, V., Romeo, G., Siracusa, M. A., Di Giacomo, C., Sorrenti, V., and Acquaviva, R. (2014). "Antioxidant activity and phenolic content of microwave-assisted Solanum melongena extracts," *The Scientific World Journal* 2014, article 719486, 6 pages. https://doi.org/10.1155/2014/719486

Sani, M. N. H., Amin, M., Bergstrand, K.-J., Caspersen, S., Prade, T., and Yong, J. W. H. (2025). "Harnessing biostimulants from biogas digestates for high-value resource recovery: a review," *Environmental Chemistry Letters* 23(1), 139-164. https://doi.org/10.1007/s10311-024-01801-8

Santos, O., Vaz, D., Sebastião, F., Sousa, H., and Vieira, J. (2024). "Wastewater as a nutrient source for hydroponic production of lettuce: Summer and winter growth," *Agricultural Water Management* 301, article 108966. https://doi.org/10.1016/j.agwat.2024.108966

Senevirathne, N., and Kaparaju, P. (2025). "Enhancing the agronomic value of anaerobic digestate: A review of current vs. Emerging technologies, challenges and future directions," *Agriculture* 15(20), article 2108. https://doi.org/10.3390/agriculture15202108

She, Y., Wei, W., Ai, X., Hong, J., and Xin, X. (2021). "Synergistic pretreatment of CaO and freezing/thawing to enhance volatile fatty acids recycling and dewaterability of waste activated sludge via anaerobic fermentation," *Chemosphere* 280, article 130939. https://doi.org/10.1016/j.chemosphere.2021.130939

Silva, J. A. (2025). "Advanced oxidation process in the sustainable treatment of refractory wastewater: A systematic literature review," *Sustainability* 17(8), 3439 https://doi.org/10.3390/su17083439

da Silva, W. R., Fracetto, G. G. M., Fracetto, F. J. C., da Silva, Y. J. A. V., de Souza, A. A. B., and do Nascimento, C. W. A. (2022). "The stabilization method of sewage sludge affects soil microbial attributes and boosts soil P content and maize yield in a sludge-amended soil in the field," *Journal of Soil Science and Plant Nutrition* 22(2), 1267-1276. https://doi.org/10.1007/s42729-021-00729-0

Taghavi, K., Naghipour, D., Ashrafi, S. D., and Salehi, M. (2021). "The removal of heavy metals from the leachate of aged landfill: The Application of the fenton process and nanosilica absorbent.," *Environment and Natural Resources Journal* 19(6), 427-434. https://doi.org/10.32526/ennrj/19/202100051

Tang, S., Wang, Z., Yuan, D., Zhang, C., Rao, Y., Wang, Z., and Yin, K. (2020). "Ferrous ion-tartaric acid chelation promoted calcium peroxide fenton-like reactions for simulated organic wastewater treatment," *Journal of Cleaner Production* 268, article 122253. https://doi.org/10.1016/j.jclepro.2020.122253

Tonanzi, B., Gallipoli, A., Annesini, M. C., La Penna, C., Gianico, A., and Braguglia, C. M. (2021). "Pre-treatments and anaerobic hydrolysis as strategical key steps for resource recovery from sludge: the role of disintegration degree in metals leaching," *Journal of Environmental Chemical Engineering* 9(1), article 104649. https://doi.org/10.1016/j.jece.2020.104649

U.S. EPA. (2007). Method 3015A (SW-846): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts, Revision 1. U.S. Environmental Protection Agency, Washington, DC.

UN-WWDR. (2023). *UN World Water Development Report, Partnerships and Cooperation for Water*.

Wang, H., Hasani, M., Wu, F., and Warriner, K. (2022). "Pre-oxidation of spent lettuce wash water by continuous Advanced Oxidation Process to reduce chlorine demand and cross-contamination of pathogens during post-harvest washing," *Food Microbiology* 103, article 103937. https://doi.org/10.1016/j.fm.2021.103937

Wang, L., Liang, L., Xu, J., Wang, Y., Yan, B., Chen, G., Li, N., and Hou, L. (2025a). "Pilot-Scale Fenton-like system for wastewater treatment using iron mud carbon catalyst," *Applied Sciences* 15(15), article 8210. https://doi.org/10.3390/app15158210

Wang, L., Liu, Y., and Chen, H. (2019). "A steam-explosion-based hydrolysis and acidification technology for cornstalk bioconversion," *BioEnergy Research* 12, 103-111. https://doi.org/10.1007/s12155-018-9945-9

Wang, Y., Fang, W., Li, H., Wang, R., Wang, X., Wang, X., Zhou, L., and Zheng, G. (2025b). "CaO pretreatment enhances fecal pollution indicator removal during mesophilic anaerobic digestion of sewage sludge," *NPJ Clean Water* 8(1), article 72. https://doi.org/10.1038/s41545-025-00506-x

Wang, Y., Wang, K., Zhou, X., Dai, B., and Du, D. (2024). "Calcium oxide enhances the anaerobic co-digestion of excess sludge and plant waste: Performance and mechanism," *Water Science and Technology* 90(4), 1267-1279. https://doi.org/10.2166/wst.2024.268

Wang, Z., Liu, T., Duan, H., Song, Y., Lu, X., Hu, S., Yuan, Z., Batstone, D., and Zheng, M. (2021). "Post-treatment options for anaerobically digested sludge: Current status and future prospect," *Water Research* 205, article 117665. https://doi.org/10.1016/j.watres.2021.117665

Wellburn, A. R. (1994). "The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution," *Journal of Plant Physiology* 144(3), 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2

Wu, Y., Wang, C., Wang, L., and Hou, C. (2025). "Recent advances in iron oxide-based heterojunction photo-fenton catalysts for the elimination of organic pollutants," *Catalysts* 15(4), article 391. https://doi.org/10.3390/catal15040391

Xu, Q., Huang, Q.-S., Wei, W., Sun, J., Dai, X., and Ni, B.-J. (2020). "Improving the treatment of waste activated sludge using calcium peroxide," *Water Research* 187, article 116440. https://doi.org/10.1016/j.watres.2020.116440

Yuan, D., Zhang, C., Tang, S., Li, X., Tang, J., Rao, Y., Wang, Z., and Zhang, Q. (2019). "Enhancing CaO fenton-like process by Fe (II)-oxalic acid complexation for organic wastewater treatment," *Water Research* 163, article 114861. https://doi.org/10.1016/j.watres.2019.114861

Yuan, J., Zhang, W., Xiao, Z., Zhou, X., and Zeng, Q. (2020). "Efficient dewatering and heavy-metal removal in municipal sewage using oxidants," *Chemical Engineering Journal* 388, article 124298. https://doi.org/10.1016/j.cej.2020.124298

Zhang, B., Zhou, X., Ren, X., Hu, X., and Ji, B. (2023). "Recent research on municipal sludge as soil fertilizer in China: A review," *Water, Air, and Soil Pollution* 234(2), article 119. https://doi.org/10.1007/s11270-023-06142-w

Zheng, M., Hu, Z., Liu, T., Sperandio, M., Volcke, E. I. P., Wang, Z., Hao, X., Duan, H., Vlaeminck, S. E., Xu, K., *et al*. (2024). "Pathways to advanced resource recovery from sewage," *Nature Sustainability* 7(11), 1395-1404. https://doi.org/10.1038/s41893-024-01423-6

Zheng, M., Ping, Q., Wang, L., Dai, X., Li, Y., and Snyder, S. A. (2021). "Pretreatment using UV combined with CaO for the anaerobic digestion of waste activated sludge: Mechanistic modeling for attenuation of trace organic contaminants," *Journal of Hazardous Materials* 402, article 123484. https://doi.org/10.1016/j.jhazmat.2020.123484

Article submitted: June 28, 2026; Peer review completed: July 19, 2026; Revised version received: August 20, 2026; Accepted: September 1, 2026; Published: September 17, 2026.
DOI: 10.15376/biores.21.4.10734-10776

APPENDIX

Supplementary Information

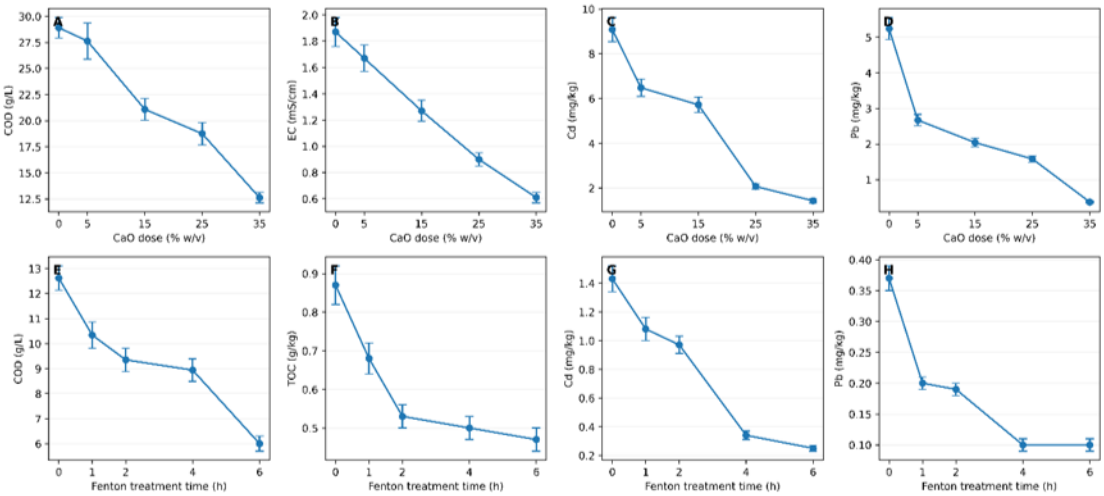


Fig. S1. Effects of CaO stabilization and subsequent Fenton oxidation on selected physicochemical and contaminant parameters of TD. Panels (A–D) show the effects of increasing CaO dosage (0 to 35% w/v) on COD, EC, Cd, and Pb, respectively, whereas panels (E–H) show the effects of Fenton treatment time (0 to 6 h) on COD, TOC, Cd, and Pb, respectively. The 0 h Fenton condition represents the CaO-TD baseline without Fenton oxidation. Values are presented as mean ± SD (n = 3).

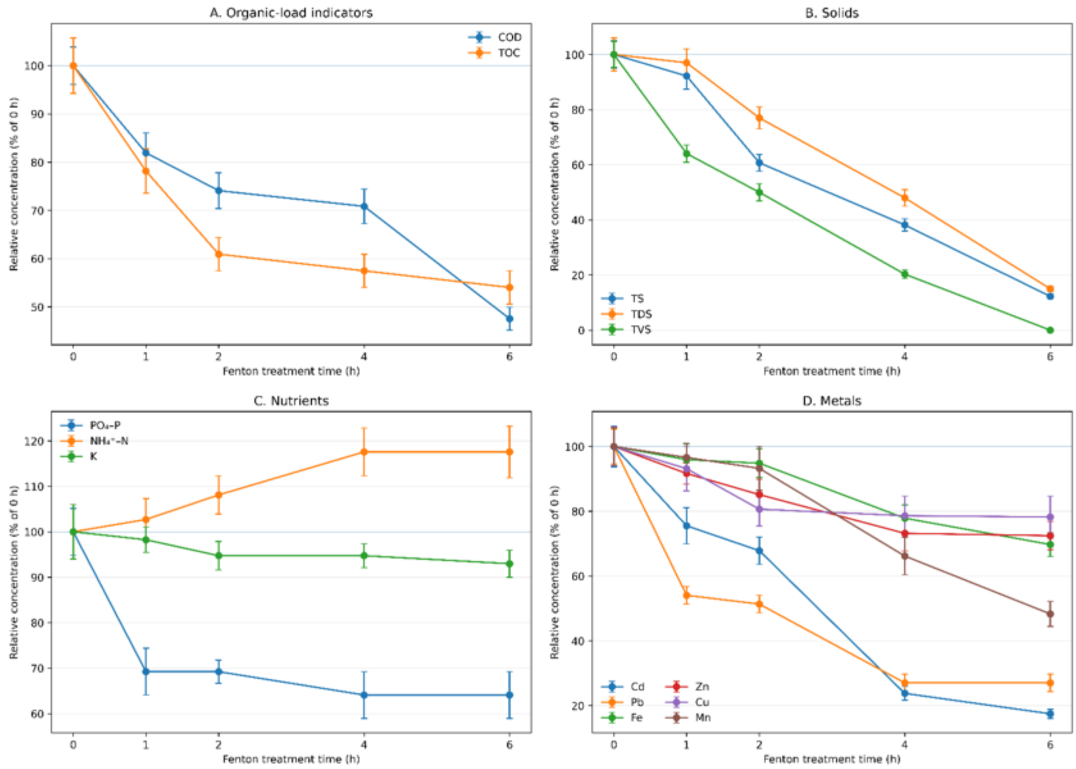


Fig. S2. Temporal evolution of selected parameters during Fenton treatment of CaO-TD. Changes in (A) organic-load indicators (COD and TOC), (B) solids (TS, TDS, and TVS), (C) nutrients (PO_4–P, NH_4^+–N, and K), and (D) metals (Cd, Pb, Fe, Zn, Cu, and Mn) are expressed as relative concentrations with respect to the 0 h baseline (100%). The 0 h condition represents CaO-TD without Fenton oxidation. Values are presented as mean ± SD (n = 3).