

Assessment of Anaerobic Digestate as a Nutrient Source for Bioponic Production of Lettuce (*Lactuca Sativa*) in a Rooftop NFT System

Abstract Finding sustainable alternatives to mineral fertilizers is essential for improving food production in urban areas. This study evaluated the use of anaerobic digestate (AD), derived from cheese whey and swine wastewater, as a nutrient source for producing lettuce (*Lactuca sativa* “Claribel”) in a rooftop NFT hydroponic system. The study focused on the design and functional validation of a modular hydroponic prototype and on the exploratory evaluation of plant response to four nutrient treatments: raw digestate (AD), nitrified digestate (ND), enriched nitrified digestate (END), and a conventional inorganic control (C). The prototype operated reliably over a 21-day cycle, maintaining stable flow and effective aeration ($>6 \text{ mg/L DO}$), with no clogging. Initial leaks were corrected, and the turbidity of the digestate effectively suppressed algal growth compared with the inorganic control. Significant differences in plant performance were observed across treatments. The inorganic control consistently outperformed all digestate-based treatments in terms of plant height, leaf area, leaf number, and fresh weight ($p \leq 0.05$). END and AD treatments performed similarly, while the ND treatment showed the weakest results across all metrics, though still close to the other digestate treatments. Partial nitrification was achieved, with a maximum conversion efficiency of 40%, indicating that the tested formulations represented an early-stage stabilization scenario rather than a fully optimized nutrient solution. Overall, the results show that the proposed rooftop NFT prototype is a useful platform for testing digestate-based fertilization strategies under urban conditions, while also highlighting that improved nitrification, nutrient balancing, and solution stabilization are necessary before digestate can approach the performance of conventional mineral solutions.

Keywords— Bioponics, digestate, hydroponics, nitrification, plant nutrition.

I. INTRODUCTION

In the current century, the world’s growing population is facing increasing challenges, particularly in rapidly expanding urban areas. Urban growth often worsens food insecurity, with an estimated 1.7 billion people living in urban areas experiencing moderate to severe food insecurity [1]. In response, urban agriculture has gained attention as a means of improving food security, supporting local economies, and enhancing environmental sustainability [2]. Among the different production strategies, hydroponics allows for soilless crop production under controlled conditions and is recognized for its efficient use of water and nutrients, making it a common approach in urban food production systems [3].

At the same time, organic waste generation represents a major global challenge. Organic residues account for a substantial fraction of municipal solid waste and are also widely produced in the agroindustrial sector, including livestock wastewater and food-processing byproducts such as cheese whey [4]. These waste streams often result in significant nutrient losses due to inadequate valorization strategies,

representing a missed opportunity for closing nutrient cycles and improving the sustainability of food systems.

To address these interconnected challenges—sustainable food production in urban environments and the underutilization of organic waste—innovative systems such as bioponics have emerged. Bioponics, an organic form of hydroponics, recycles organic waste into nutrient-rich solutions for plant growth, simultaneously contributing to waste management and food security [3]. Within this framework, the use of liquid anaerobic digestate (AD), a byproduct of the anaerobic degradation of organic matter, has gained attention as a potential nutrient source for hydroponic crop production [5].

Anaerobic digestion is widely accepted as an effective strategy for remediating organic waste while simultaneously producing energy and recovering nutrients [6]. In particular, co-digestion of multiple organic substrates has been shown to enhance nutrient content and improve digestate stability. Most nutrients remain after digestion, resulting in anaerobic digestate (AD) containing essential macro- and micronutrients, although their concentrations vary depending on the feedstock composition [7]. These nutrients are further mineralized by microbial activity, becoming available for plant uptake.

Despite its many benefits, the use of untreated digestate in hydroponic systems presents several challenges. Previous studies have reported inconsistent plant responses, with some crops showing increased yields or higher concentrations of antioxidants and soluble sugars [8], while others exhibited inhibited shoot development or reduced fruiting [9]. A review by Szekely and Jijakli [3] identified key factors contributing to phytotoxicity or growth inhibition, including the presence of undigested residues with high chemical and biochemical oxygen demand, elevated ammonium (NH_4^+) concentrations, and alkaline pH conditions (7–9). At pH values above 7.5, ammonium is increasingly converted to toxic ammonia (NH_3), negatively affecting plant growth.

In alkaline digestate solutions, several nutrients become chemically unavailable to plants. Calcium, magnesium, and phosphorus can precipitate at pH values above 8 as water-insoluble compounds such as carbonates, hydroxyapatite, or struvite, leading to nutrient imbalances and reduced uptake [10]. Unlike soil-based systems, where buffering processes moderate fluctuations in pH and nutrient availability, hydroponic systems expose plants directly to the nutrient solution, making them particularly sensitive to chemical imbalances. Consequently, precise nutrient management is critical when digestate is used in hydroponic settings.

To overcome these limitations, incorporating a nitrification step into digestate treatment has emerged as a reliable approach. Nitrification is a biological process in which ammonia is oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-) by

microorganisms, a nitrogen form generally preferred by plants [11]. In addition to improving nitrogen availability, nitrification has been shown to reduce pathogen loads and lower solution pH, thereby enhancing nutrient solubility and crop suitability [12]. Although plant nutrient uptake can influence pH over time, nitrified digestate has been reported to function as a stable nutrient solution without reducing yields [13]. In some cases, deficiencies in calcium or magnesium have been observed, but these can be corrected through targeted supplementation [14,15].

For this reason, this study explored the use of anaerobic digestate subjected to nitrification and micronutrient supplementation within a rooftop nutrient film technique (NFT) hydroponic system. Specifically, the objectives were: (1) to verify the design and functionality of a rooftop nutrient film technique (NFT) hydroponic system using digestate-based nutrient solutions, and (2) to compare lettuce growth under different digestate-based treatments, using a conventional inorganic nutrient solution as a reference treatment..

II. MATERIALS AND METHODS

A. Study location & system design

The study was conducted at the Bioenergy Laboratory and on the rooftop of the Environmental Science and Development Faculty at Zamorano University, Francisco Morazán, Honduras, between January and June 2025. The site is located at approximately 800 m above sea level, with an average annual temperature of 25 °C.

A rooftop hydroponic system was designed using the Nutrient Film Technique (NFT) to evaluate multiple nutrient treatments under controlled conditions. The system consisted of an 'A'-frame structure supporting modular gullies and independent nutrient reservoirs for each treatment, allowing physical separation of treatments while maintaining uniform environmental conditions. The system layout is shown in Fig. 1.

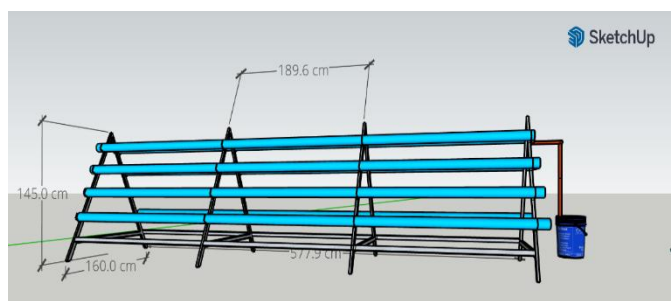


Fig 1. Hydroponic system design in SketchUp

The structure supported eight polyvinyl chloride (PVC) gullies (SDR 26), each 6 m in length and 7.62 cm in diameter. Planting holes (5.5 cm diameter) were spaced at 17.5 cm intervals, allowing up to 30 plants per gully. The gullies were arranged across four vertical levels, with treatment positions mirrored on both sides of the structure.

Each treatment was assigned an independent 40 L reservoir connected to its respective gullies through PVC inlet and outlet lines. Nutrient solutions were delivered using submersible pumps (Little Giant®, 380 GPH, 33 W), providing a flow rate of approximately 4 L/min, suitable for NFT operation and preventing sedimentation [13]. Effluent was returned by gravity to the corresponding reservoir, forming a closed-loop recirculation system.

Aeration was supplied to each reservoir using 8 W air pumps (HITOP®, 320 GPH) to maintain dissolved oxygen concentrations above 3 mg/L. To reduce excessive solar radiation and temperature fluctuations, a 70% shade net (2 × 6 m) was installed approximately 2 m above the system. Prior to crop establishment, the system was operated for 7 days using plain water to verify uniform flow distribution, adequate oxygenation, absence of leaks, and overall operational stability. This pre-operational verification reduced the likelihood that subsequent plant responses would be confounded by hydraulic or aeration failures in the prototype rather than by differences among nutrient treatments.

B. Anaerobic Digestion and Nitrification Processes

Four nutrient treatments were evaluated for hydroponic lettuce cultivation: raw anaerobic digestate (AD) obtained from the co-digestion of cheese whey and swine wastewater; nitrified digestate (ND), produced by biological oxidation of ammonium; enriched nitrified digestate (END), consisting of ND supplemented with micronutrients; and a conventional inorganic nutrient solution used as a control (C). Each solution was prepared independently and supplied as the sole nutrient source in a recirculating NFT system.

Cheese whey was collected from the campus dairy plant, and anaerobic inoculum was obtained from the university swine farm digester. The substrates were mixed at a 1:1 volumetric ratio (100 L each) and introduced into a 200 L vertical batch bioreactor for mesophilic anaerobic digestion (35–45 °C) over a period of 132 days. Feedstocks were analyzed for total solids, volatile solids, ammoniacal nitrogen, pH, and electrical conductivity using standard methods [16].

After digestion, the resulting digestate was characterized for nitrogen species, phosphorus, pH, and electrical conductivity. Based on comparison with recommended nutrient ranges for hydroponic lettuce [17], a 1:4 dilution was selected to adjust electrical conductivity and reduce phosphorus concentration while maintaining adequate nitrogen availability. This solution corresponded to AD in the system. Because digestate dilution was guided primarily by electrical conductivity and phosphorus constraints, rather than by exact nutrient equivalence with the inorganic control, the resulting treatments should be interpreted as feasible digestate preparation scenarios for exploratory testing.

C. Nitrification process and micronutrient enrichment

Nitrification was applied to the diluted digestate to reduce ammonium toxicity and increase nitrate availability. A moving

bed biofilm reactor (MBBR) was operated following the protocol as described in [13]. Digestate was continuously aerated for 24 h using a 10 W air pump, maintaining dissolved oxygen concentrations above 6 mg/L, after which 5 mL of commercial nitrifying bacteria were inoculated into each reactor. Biofilm support media consisted of coarse polyurethane sponges (5 L per reactor) [18]. Nitrate and ammonia concentrations were measured prior to inoculation and at 7-day intervals. Nitrification efficiency was calculated as:

$$NE (\%) = \left(\frac{[NO_3^-] \text{ after nitrification} - [NO_3^-] \text{ before nitrification}}{[NH_3] \text{ before nitrification}} \right) \times 100 \quad (1)$$

Where:

NE = Nitrification efficiency

$[NO_3^-]$ after nitrification = Nitrate concentration in the digestate after the nitrification process (mg/L).

$[NO_3^-]$ before nitrification = Nitrate concentration in the digestate before the nitrification process (mg/L).

$[NH_3]$ before nitrification = Ammonia concentration in the digestate before the nitrification process (mg/L).

This solution corresponded to ND. For the purposes of this study, nitrification was considered successful at conversion efficiencies $\geq 85\%$, corresponding to a nitrate-dominant nitrogen profile ($\geq 75:25 NO_3^-:NH_4^+$) reported as optimal for hydroponic lettuce production [19].

The END treatment was prepared by supplementing ND with micronutrients (Fe, Mn, Zn, B, Cu, and Mo) at concentrations equivalent to those in the control solution, to address reported micronutrient limitations in digestate-based hydroponics [15]. The control solution was prepared using commercial fertilizers (AgriFeed® F1 and F2) according to manufacturer recommendations for hydroponic lettuce production.

The experiment followed a completely randomized design with four treatments (AD, ND, END, and C). Individual lettuce plants served as experimental units and were supplied with nutrient solutions through independent 40 L reservoirs within a shared NFT structure (Fig. 2). Treatment positions were mirrored across the system to minimize positional effects. Six plants per treatment were randomly selected weekly for measurement, resulting in a total sample size of 216 plants.

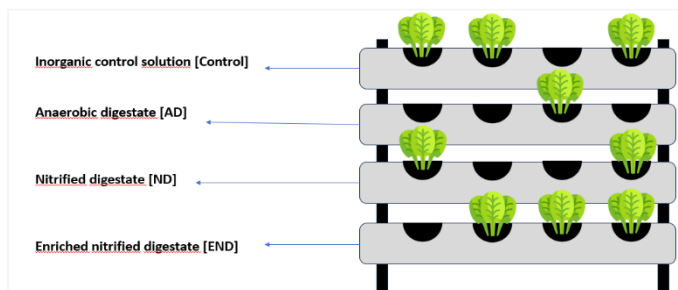


Fig 2. Illustration of the experimental set-up by levels

D. Crop management, measurements, and statistical analysis

Throughout the crop cycle, nutrient solution pH, electrical conductivity (EC), salinity, dissolved oxygen (DO), and temperature were monitored to maintain stable growing conditions. pH was maintained between 5.5 and 6.5 through periodic adjustment using phosphoric acid or sodium carbonate as required. EC was monitored daily and maintained within the range of 1.8–2.5 mS/cm through dilution or nutrient replenishment as needed. Solution temperature ranged between 20 and 30 °C, and dissolved oxygen concentrations were maintained above 6 mg/L through continuous aeration.

Seedlings of lettuce (*Lactuca sativa* ‘Claribel’) were obtained from a local nursery and transplanted into the NFT system 25 days after sowing, once plants had developed three to four true leaves. Each plant was placed in a 6 × 8 cm plastic cup filled with coconut fiber as the growing medium, chosen based on various favorable properties such as high porosity and water holding capacity [20]. The crop cycle lasted 21 days after transplantation. Agronomical variables evaluated included plant height, number of leaves, leaf area, fresh biomass at harvest, and post-transplant mortality. Plant height, leaf number, and leaf area were measured weekly, while fresh weight was determined at harvest.

The experiment followed a completely randomized design with four treatments (AD, ND, END, and C), comprising 54 plants per treatment and a total of 216 experimental units. Data normality was assessed using the Ryan–Joiner test. For normally distributed variables, treatment effects were analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s HSD test for pairwise comparisons. Non-normally distributed data were analyzed using the Kruskal–Wallis test, with Dunn’s test applied when significant differences were detected. Statistical analyses were performed using Minitab® version 21.1, with significance set at $p \leq 0.05$.

III. RESULTS

A. Hydroponic system performance and verification

The NFT hydroponic system operated reliably throughout the 21-day crop cycle under rooftop conditions, supporting its suitability for evaluating digestate-based nutrient solutions. Pump operation remained stable across all gullies, with flow rates maintained between 3 and 4 L/min after manual adjustment to prevent excessive reservoir drainage. Three minor leaks were detected during initial circulation; two were sealed manually, while one self-resolved during continued operation.

Flow distribution was uniform across the NFT channels, with a slight reduction observed in the third row, likely due to its marginally higher elevation. No clogging or sedimentation was observed in any gully, indicating that suspended solids in the digestate did not impair hydraulic performance. Dissolved oxygen concentrations remained above 3 mg/L in all reservoirs, confirming adequate aeration. Algal growth was negligible in

digestate-based treatments and limited to minor biofilm formation near inlets in the inorganic control, likely due to higher solution turbidity and reduced light penetration in digestate treatments. Overall, these observations confirmed that the prototype maintained the hydraulic and aeration conditions required for comparative testing of digestate-based treatments.

B. Physicochemical characterization of feedstocks

The physicochemical properties of cheese whey and swine digestate used as feedstocks are summarized in Table 1. Cheese whey exhibited high total and volatile solids concentrations, reflecting a high organic carbon content, but low concentrations of ammoniacal nitrogen and phosphorus, consistent with its composition as a carbon-rich but mineral-poor byproduct [21]. In contrast, swine digestate contained substantially higher levels of ammoniacal nitrogen and total phosphorus, consistent with typical post-digestion nutrient profiles [7].

TABLE I
PHYSICOCHEMICAL CHARACTERIZATION OF
INDIVIDUAL FEEDSTOCKS (WHEY AND SWINE
DIGESTATE)

Parameter	Whey	Swine Digestate
pH	6.6	7.3
Conductivity (dSm/m)	5.2	7.1
Total Solids (g/L)	65.5	7.6
Volatile Solids (g/L)	60.6	5.1
N - NH ₃ (mg/L)	2.2	133
Total P (mg/L)	6.5	170

These contrasting characteristics supported their selection for co-digestion: whey provided readily degradable carbon to stimulate microbial activity, while swine digestate contributed mineral nutrients and an active microbial inoculum. However, the elevated electrical conductivity observed in both substrates highlighted the need for dilution prior to hydroponic application.

C. Digestate characterization prior to nitrification

After 132 days of mesophilic batch digestion, the resulting digestate exhibited high ammoniacal nitrogen (~546 mg/L), total phosphorus (~285 mg/L), and electrical conductivity (~8 dS/m) (Table 2). These concentrations exceed levels considered suitable for hydroponic lettuce cultivation and are associated with phytotoxicity and ionic stress [22,23,24].

TABLE II
CHARACTERIZATION OF ANAEROBIC DIGESTATE

Parameters	Drum #1	Drum #2	Mixture	Final mixture 1:4
pH	4.91	4.95	4.94	4.94
Conductivity (dSm/m)	7.11	8.81	7.96	2.0
Suspended solids (mg/L)	2836.8	3506.6	3171.7	792.9
Salinity (ppt)	3.85	4.95	4.4	1.1
Ammoniacal nitrogen (N - NH ₃ ; mg/L)	595	497	546	136
Total P (mg/L)	308	292	285	71

Based on these results, a 1:4 dilution was applied, reducing conductivity, salinity, and nutrient concentrations to ranges compatible with hydroponic use while maintaining sufficient nitrogen availability for subsequent nitrification. The low pH of the undiluted digestate (4.9) suggests partial inhibition of methanogenic activity, likely due to the accumulation of volatile fatty acids and limited buffering capacity, which may have contributed to the elevated organic load and strong odors observed prior to system initiation [25].

A noticeable reduction in malodors was observed within 5–6 days of system operation, potentially attributable to continuous aeration and the establishment of microbial biofilms on the coconut fiber substrate. Similar odor attenuation through aeration has been reported in wastewater treatment systems [26,27].

During digestion, larvae of *Hermetia illucens* were observed on the surface of the digestate, feeding on floating organic residues. Although not quantified or manipulated experimentally, their presence suggests additional biological activity within the system that may have contributed marginally to organic matter breakdown. It's worth noting that these larvae were removed prior to introduction to the system.

D. Nitrification process

The target nitrification efficiency of 85% was established to approximate the nitrate-dominant nitrogen profile of the control solution. However, this benchmark was not achieved under the tested conditions. During the first 10 days of operation, ammonia oxidation remained minimal, with conversion efficiencies below 10%. This delayed onset may be attributed to the use of a non-specialized inoculum—commercial nitrifying bacteria intended for aquaria—which are not adapted to the high nutrient loads and organic complexity of digestate-based systems.

Around day 15, a consistent decline in pH was observed across the MBBR-integrated treatments, with the END treatment exhibiting the earliest and most pronounced decrease (Fig. 3). A similar lag phase prior to pH decline and NO₃ accumulation has been reported in digestate-fed systems [13]. The faster pH drop observed in the END treatment may indicate that micronutrient availability supported nitrification activity, potentially by alleviating trace element limitations associated with nitrifying microorganisms. Nitrate measurements confirmed partial system activation, with conversion efficiencies of approximately 40% for END and 28% for ND. These results underscore the influence of inoculum source and trace element availability on biofilter performance and highlight the need for improved acclimatization strategies or dedicated nitrification units in future applications.

E. Behavior of Control Variables Throughout Cultivation

A comparative summary of key nutrient concentrations across treatments is presented in Table 3. The control solution was formulated to meet or exceed recommended nutrient requirements for hydroponic lettuce [17]. All digestate-based

treatments were diluted to achieve comparable EC values (~2 dS/m), ensuring osmotic conditions within acceptable thresholds for lettuce cultivation.

Despite similar EC, nutrient profiles differed markedly among treatments, particularly in nitrogen speciation. The control exhibited a nitrate-dominant nitrogen form, whereas the AD treatment was characterized by high ammonium and minimal nitrate, reflecting the absence of intentional nitrification during digestion. The ND treatment showed a shift toward nitrate following biological nitrification, while the END treatment combined nitrified digestate with supplemental micronutrients to address potential trace element limitations. Compared with the control, digestate-based solutions provided lower total nitrogen concentrations (137 mg/L vs. 218 mg/L) and, except for END, lacked complete micronutrient coverage. While these formulations were sufficient to support moderate plant growth, they did not fully match the optimized nutrient balance of the control solution.

TABLE III
CROP NUTRITIONAL REQUIREMENTS AND
TREATMENT COMPOSITIONS

Parameters	Requirement	Control	AD	ND	END
pH	5.5-6.5	6.5	6.5	6.5	6.5
EC (dS/m)	1.9-2.5	2.1	2	2	2
Salinity (ppt)	<4.5	1.13	1.1	1.1	1.1
Total N (mg/L)	150	218	137	137	137
N - NH ₃ (mg/L)	-	15.2	127	45	75
N - NO ₃ (mg/L)	-	203.7	10	92	62
P (mg/L)	39-48	143	71.25	71.25	71.25
K (mg/L)	216	309	NM*	NM	NM
Ca (mg/L)	139	227	NM	NM	NM
Mg (mg/L)	31-47	21	NM	NM	21
Fe (mg/L)	2.3-3	1.81	NM	NM	1.81
Mn (mg/L)	0.05-0.8	0.62	NM	NM	0.62
Zn (mg/L)	0.11-0.15	0.23	NM	NM	0.23
B (mg/L)	0.38-0.5	0.19	NM	NM	0.19
Cu (mg/L)	0.13-0.15	0.32	NM	NM	0.32
Mo (mg/L)	0.07-0.1	0.1	NM	NM	0.1

Future experiments may explore reduced dilution rates or targeted nutrient enrichment to improve alignment with crop requirements while preserving the sustainability advantages of digestate-based fertilization.

F. Behavior of Control Variables Throughout Cultivation

Throughout the crop cycle, daily measurements of pH, electrical conductivity (EC), and temperature were taken at the treatment reservoirs to monitor the nutrient solution environment and identify potential abiotic stress factors. This monitoring is particularly important in hydroponic lettuce systems because, in the absence of soil buffering, even small fluctuations can directly affect nutrient availability and plant health. Lettuce is sensitive to these parameters: pH must remain near 5.5–6.5 to optimize nutrient uptake, EC values above ~2.5 dS/m can reduce yield, and solution temperatures outside the 20–30 °C range impair oxygen availability and root function [28].

As shown in Fig. 3, pH trends exhibited a characteristic initial sharp increase during the first few days across all digestate-based treatments. This early instability and delayed stabilization mirror dynamics reported in digestate-fed hydroponic systems, where pH shifts have been associated with microbial activity, residual organic compounds, and ammonia presence [13]. Frequent acid additions were required during the initial phase to counteract this increase; however, pH stabilized by day 6 and subsequently required adjustment only every 2–3 days. In contrast, the control treatment displayed a more regular pH cycling pattern, consistent with typical hydroponic systems where nutrient uptake drives gradual pH changes. Notably, a decline in pH was observed in the END treatment after day 9 and again during the final three days of cultivation, likely associated with ongoing nitrification activity.

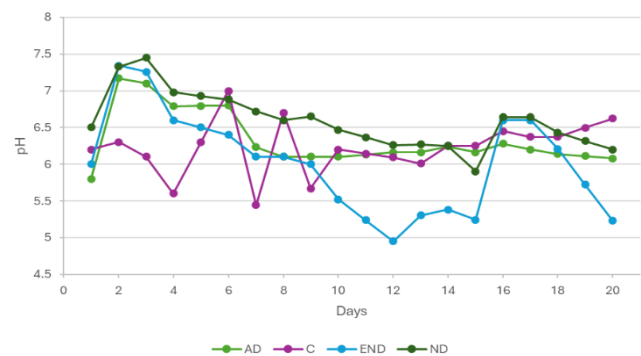


Fig. 3 pH behavior throughout crop cycle

Electrical conductivity (EC) remained within acceptable limits for hydroponic lettuce throughout the experiment, ranging between 1.8 and 2.5 dS/m across all treatments (Fig. 4). In the control solution, EC followed a cyclic depletion–replenishment pattern reflecting periodic nutrient additions. Digestate-based treatments showed more moderate fluctuations, likely due to reduced nutrient uptake associated with higher plant mortality and slower early growth. A marked decline in EC across all treatments was observed after day 13, coinciding with nearly continuous rainfall events that complicated solution management and likely caused unintended dilution of the nutrient solutions. These observations highlight the sensitivity of the system to external environmental inputs, particularly when using partially stabilized organic nutrient sources.

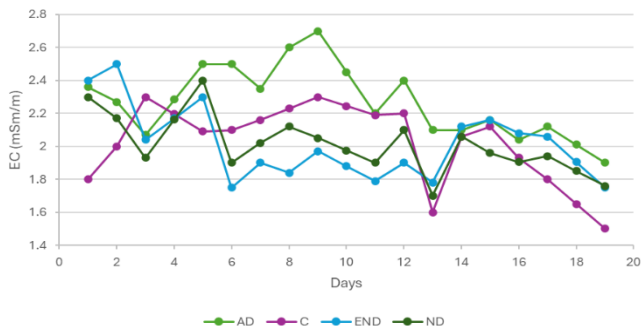


Fig 4. EC behavior throughout crop cycle

As shown in Fig. 5, solution temperature remained relatively stable throughout the experiment, ranging between 22 and 30 °C, with differences among treatments consistently below 1 °C. No extreme deviations were recorded during the cultivation period, indicating that temperature conditions were suitable for lettuce growth and did not confound treatment effects.

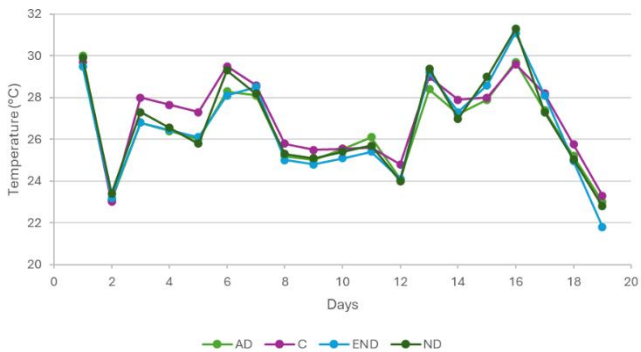


Fig. 5 Temperature behavior throughout crop cycle

Together, these control variables indicate that while the system maintained generally stable conditions across treatments, short-term disturbances—such as early pH instability and rainfall-induced dilution—may have introduced transient stress, particularly in digestate-based systems. Nevertheless, pH, EC, and temperature remained within acceptable hydroponic ranges for most of the crop cycle.

G. Plant Mortality and Treatment Effects

To capture treatment-induced stress during early establishment, plant mortality was monitored during the first days following transplant. As shown in Fig. 6, mortality rates varied noticeably among treatments. Most mortality occurred within the first five days after transplant, after which survival stabilized, indicating that plant losses were primarily associated with early establishment stress rather than prolonged nutrient deficiencies. Digestate-based treatments (AD, ND, and END) exhibited higher mortality rates (16–19%) compared to the control treatment (5%), despite all nutrient solutions being diluted to safe EC levels (~2 dS/m).

Although digestate solutions were aerated for 14 hours prior to use, this period may not have been sufficient to achieve full stabilization. Residual volatile fatty acids (VFAs) and elevated microbial loads can persist for several days, potentially stressing seedlings through localized oxygen depletion. Combined with the initial pH instability observed in digestate treatments, these factors likely contributed to early plant mortality.

Similar responses have been reported in hydroponic systems using organic nutrient solutions with limited pre-aeration, where partial stabilization resulted in increased seedling losses and slower early growth [9]. Furthermore, studies on aeration dynamics indicate that substantial reductions in chemical and biochemical oxygen demand—key indicators of organic stabilization—typically require 48–72 hours of fine-bubble aeration, rather than shorter periods such as the 14 hours applied in this study [26].

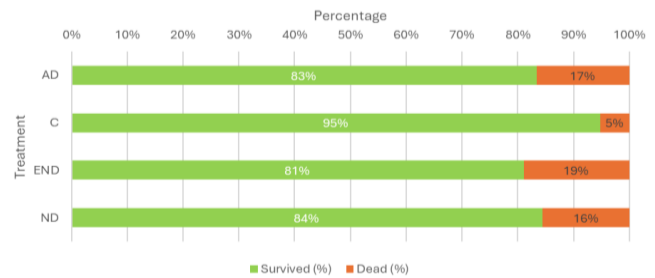


Fig. 6 Mortality across all treatments

H. Effect of Treatment on Plant Growth Variables

A two-way ANOVA was initially conducted to evaluate the influence of treatment and replicate position (gully side) on plant height, leaf number, and leaf area. In all cases, treatment had a statistically significant effect ($p < 0.05$), while neither replicate nor the treatment $\times$ replicate interaction was significant ($p > 0.27$), indicating that plant development was driven by nutrient source rather than system positioning. Consequently, data from both sides of the structure were pooled for subsequent analyses.

Across all measured growth variables, the control treatment (inorganic nutrient solution) consistently outperformed digestate-based treatments, while differences among digestate treatments were more nuanced. To avoid redundancy, growth dynamics are presented using plant height and leaf number over time, complemented by leaf number and fresh weight at harvest as integrative performance indicators.

I. Plant height

Plant height increased progressively throughout the cultivation period in all treatments (Fig. 7). The control treatment exhibited consistently greater shoot elongation from the first week onward, finishing the cycle with the tallest plants. Digestate-based treatments followed similar temporal trends but remained shorter throughout the cycle, with ND showing the lowest final height. These results indicate that while

digestate formulations supported vegetative growth, their capacity to sustain shoot elongation was inferior to that of the inorganic control.

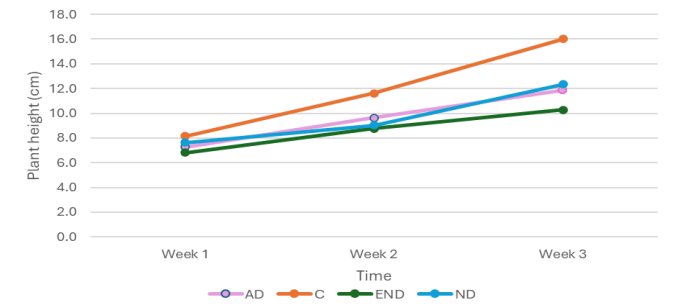


Fig. 7 Plant height (cm) though cultivation period

J. Leaf number

Leaf number increased steadily across all treatments during the cultivation period (Fig. 8), reflecting ongoing foliar development. The control treatment consistently produced the highest number of leaves, while END and AD tracked closely together at intermediate levels. ND remained lowest throughout the cycle. At harvest (Fig. 9), statistical analysis confirmed a significant treatment effect on leaf number ($p = 0.013$), with the control and END grouped together, AD showing intermediate values, and ND exhibiting significantly fewer leaves (Table 4).

These results suggest that micronutrient supplementation in END partially mitigated the limitations associated with digestate-based nutrition, allowing foliar development closer to that of the inorganic control, whereas ND remained constrained despite partial nitrification.

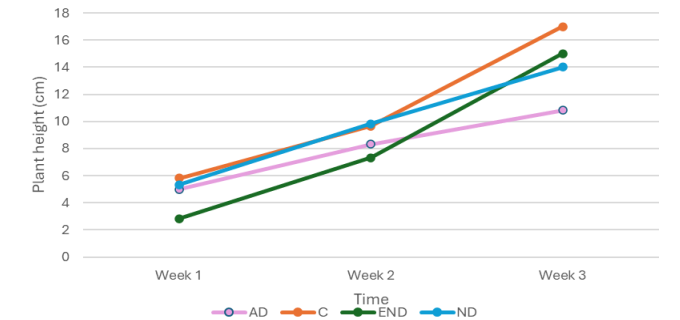


Fig. 8 Leaf number (#) though cultivation period

Treatment	Mean (leaves)	SD	Tukey Group
C	14.50	1.38	A
END	12.50	2.51	A/B
AD	11.00	2.37	A/B
ND	10.00	2.45	B

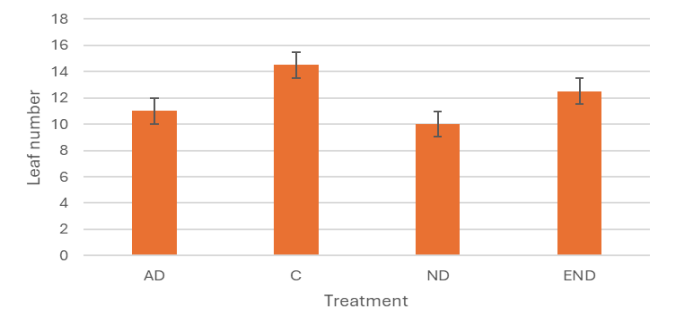


Fig. 9 Average leaf number at harvest

K. Fresh weight

Fresh weight at harvest integrated the cumulative effects of nutrient availability, uptake efficiency, and biomass allocation (Fig. 10). The control treatment achieved the highest biomass, while ND consistently produced the lowest fresh weight. AD and END exhibited intermediate and overlapping distributions, though with greater variability. A Kruskal–Wallis test revealed a significant treatment effect ($p = 0.017$), with post hoc analysis indicating that only the control differed significantly from ND ($p = 0.013$), while differences among digestate-based treatments were not statistically significant (Table 5).

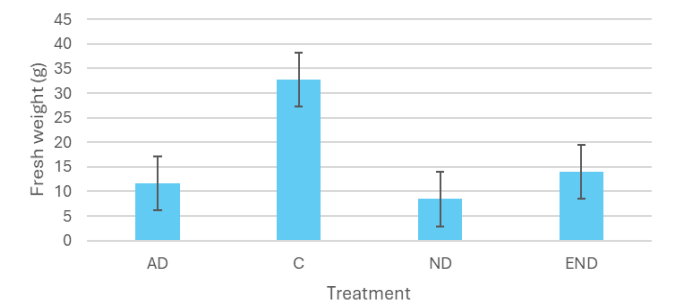


Fig. 10 Average fresh weight (g) at harvest

Treatment	Median (g)	Mean Rank	Dunn Group
C	36.15	19.8	A
END	12.30	12.5	A/B
AD	12.85	10.3	A/B
ND	6.35	7.3	B

L. Integrated interpretation of growth responses

The reduced vegetative performance observed in digestate-based treatments was consistent across all retained growth indicators. Compared with the control, digestate-fed plants were shorter, produced fewer leaves, and accumulated substantially less fresh biomass. These patterns reflect a combination of factors, including nitrogen form, early pH instability, and incomplete stabilization of the digestate nutrient solutions.

Unlike the nitrate-dominant control, digestate treatments—particularly AD and ND—contained higher proportions of ammonium-N, which is assimilated less efficiently and may become phytotoxic under fluctuating pH conditions [9,13]. Early pH rises observed in these treatments likely increased the proportion of free ammonia, inhibiting root function and shoot expansion. The partial improvement observed in END supports the role of micronutrient availability in alleviating some of these constraints, consistent with findings that reported moderate biomass recovery following trace element supplementation in digestate-fed systems [8,14].

Fresh weight results consolidate these trends. Although ND underwent biological nitrification, the process was only partially effective, resulting in nitrate concentrations well below those of the control. Fully nitrified digestates can achieve yields comparable to mineral solutions when pH and nitrogen form are adequately controlled [13]. In this study, incomplete nitrification, coupled with limited pre-aeration (14 h), likely compounded ammonium stress and reduced early vigor, contributing to persistently lower biomass accumulation.

Interestingly, qualitative observations following the experimental period indicated continued growth in ND and END plants during an additional week, partially narrowing the gap with the control. This behavior aligns with reports that digestate-fed crops may exhibit delayed but sustained growth once early stress subsides and microbial transformations stabilize [13]. These findings suggest that longer cultivation cycles and extended preconditioning steps—such as 48–72 h aeration or complete nitrification—could improve the performance of digestate-based nutrient solutions in future trials.

IV. CONCLUSIONS

The custom-designed rooftop NFT hydroponic prototype proved to be a robust, innovative system capable of 24/7 continuous operation under urban conditions. Its modular structure with independent nutrient reservoirs, stable flow rates and effective aeration, supported its use as an exploratory platform for controlled comparative testing of digestate-based nutrient solutions. The system therefore provides a useful and replicable framework for future research on nutrient recovery and urban bioponic production.

Of the tested nutrient solutions, the conventional inorganic control performed better than all digestate-based treatments in terms of plant height, leaf number, leaf area, and fresh weight. Although END and AD supported moderate lettuce growth, the digestate-based treatments were constrained by incomplete nitrification, lower nutrient balance relative to the control, and early instability during establishment. These findings indicate that anaerobic digestate has potential for use in rooftop bioponic systems, but that improved nitrification, nutrient balancing, and stabilization are required before its performance can approach that of conventional mineral solutions.

V. RECOMMENDATIONS

Future studies should evaluate digestates from diverse feedstocks and apply dilution strategies based on target nutrient composition rather than electrical conductivity alone, in order to improve comparability with conventional hydroponic solutions.

Further work should optimize the nitrification stage by testing dedicated nitrification units, alternative biocarriers, and inocula better adapted to digestate-based systems. Longer preconditioning periods and extended crop cycles should be assessed to determine whether improved stabilization and delayed nutrient mineralization enhance plant establishment and biomass accumulation.

Incorporate post-harvest microbiological testing of the lettuce to verify its safety for consumption and determine whether additional processing steps are necessary.

As a complementary line of research, future studies may also examine whether whey- and swine-derived digestates could support *Hermetia illucens* production within a broader integrated bioresource management strategy.

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